EFFECTS ON PLASMATIC ANDROSTENEDIONE IN FEMALE WORKERS EXPOSED TO URBAN STRESSORS

G. TOMEI, A. SANCINI[1], D. CERRATTI[1], M. FIASCHETTI[1], M.F. ANZANI[1], M. CIARROCCA[1], M.V. ROSATI[1], E. TOMAO[2], R. GIUBILATI[1], B. PIMPINELLA[1] and F. TOMEI[1]

Department of Psychiatric Science and Psychological Medicine, "Sapienza" University of Rome; [1]Department of Occupational Medicine, "Sapienza" University of Rome; [2]General Office for Military Health, Rome, Italy

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The purpose of this study is to investigate whether occupational exposure to urban stressors can cause alterations on androstenedione plasma levels and related diseases in female traffic police compared to a control group. The research was carried out on an initial sample of 468 female workers (209 traffic police and 259 controls). After excluding the subjects with confounding factors, 192 female subjects: 96 traffic police and 96 controls were included in the study. Traffic police and controls were matched by age, length of service, body mass index, alcohol consumption and cigarette smoking habits, habitual consumption of Italian coffee, and habitual intake of soy and liquorice in diet. The results show that the percentage of subjects with fertility and mental health disorders were no different between traffic police and controls. Mean androstenedione values were significantly higher in female traffic police compared to controls. The distribution into classes of androstenedione values in traffic police was statistically significant. The percentage of traffic police with fertility and mental health disorders were not significant compared to controls. Our results suggest that the occupational exposure to urban stressors could alter plasma androstenedione levels in female traffic police. According to our previous research all the hormonal parameters studied, including androstenedione, could be used as early biological markers of chronic exposure to urban stressors, usable in occupational sets.

The human body continually tries to preserve a dynamic equilibrium with its environment through the system which regulates the response to any kind of stressor. Many environmental and occupational stressors can affect endocrine and reproductive functions. In this regard, growing scientific evidence supports the hypothesis that such alterations may have serious consequences for these human systems.

The subjects examined in this study were female traffic police in Rome exposed to physical (i.e. noise), chemical (i.e. air pollutants) and psycho-social urban stressors (1-3). In our previous studies on traffic police we observed the effects of urban pollutants on the following neuro-immune-endocrine parameters, adrenocorticotropic hormone (ACTH), cortisol (CORT), insulin as indicators of the early neuro-immune-endocrine system response to urban

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Mailing address:
Prof. Francesco Tomei,
Via Monte delle Gioie, 13
00199 Rome, Italy
Tel: ++39 06 49912541
Fax: ++39 06 49912554
e-mail: francesco.tomei@uniroma1.it

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stressors (4-6).

In addition, it is well-known that personal exposure to pollutants from direct exposure to traffic fumes, as experienced by some outdoor workers such as traffic police, may be considered higher than that of indoor workers (particularly in Italian cities) (7). In our previous research, we evaluated the following parameters in traffic police: urinary S-phenylmercapturic acid, trans-trans muconic acid, pyrenol acid; environmental and blood benzene; sister chromatid exchanges (SCE) and micronuclei (MN) in peripheral blood lymphocytes; and antibodies to the benzo(a)pyrene (BaP) diol epoxide-DNA adducts in sera. In particular, we found that exposure dosage to benzene (time weight average, TWA) (mean and SD 10.7±12.9 $\mu g/m^3$ in traffic police and 3.6±1.6 $\mu g/m^3$ in controls) and toluene (mean and SD 40.7±44.6 $\mu g/m^3$ in traffic police and 13.5±9.4 $\mu g/m^3$ in controls) was significantly higher among traffic police than indoor workers (8-13).

Furthermore, during the period March-April 2004 the mean values in air pollutants in Rome were: benzo(a)pyrene (BaP) (0.48 ng/m^3), polycyclic aromatic hydrocarbons (PAHs) (4.45 ng/m^3), lead (Pb) (22 ng/Nm^3), nickel (Ni) (9 ng/Nm^3), carbon monoxide (CO) (1.4 mg/m^3), nitrogen dioxide (NO_2) (77.5 $\mu g/m^3$), ozone (O_3) (49.4 $\mu g/m^3$), benzene (4.35$\mu g/m^3$), PM_{10} (50 $\mu g/m^3$) and sulphur dioxide (SO_2) (4.7 ng/m^3) were monitored (14). In that same period blood samples from each worker were taken. Since we have already measured the environmental and biological levels of the main pollutants in our working population and it is known that levels in traffic police workers are significantly higher than in controls, we did not repeat the exposure dosage study in this work.

Studies in literature suggest that exposure to urban chemical pollutants, such as carbon monoxide (CO) and dimethylbenz(a)anthracene (15-17), could cause alterations of synthesis, secretion and/or action of androstenedione. Some authors observed female reproductive health disorders following exposure to benzene, styrene, toluene, gasoline, hydrogen sulphide (H_2S), and to PM_{10} and NO_2 (18-21). Furthermore, alterations in plasma androstenedione levels were observed in psychiatric disorders which affect the efficiency and performance of the worker (22), such as depression (23), manic-depressive or psychotic symptoms (24), anxiety disorders (25). Traffic police are also exposed to psycho-social stressors that could be harmful to their health (2). Through the compilation and elaboration of a questionnaire, our previous study has ascertained stronger subjective stress in traffic police compared to a control group. Sources of psycho-social stressors for the traffic police may be the relations with the public, exposure to episodes of criminality, and the need to maintain high levels of services in various contexts (26). Moreover, psycho-social stressors may affect the reproductive–endocrine axis (27). The purpose of this study is to investigate whether occupational exposure to urban stressors can cause alterations also on androstenedione plasma levels and disorders linked to its exposure, in another group of female traffic police compared to a control group.

MATERIALS AND METHODS

The research was carried out on an initial sample of 468 female Municipal Police employees (259 municipality administrative staff (controls) and 209 traffic police). Each subject's medical history and current medical condition were recorded on a questionnaire by a physician. Items referred to the history of reproductive health (data on menarche, pregnancy, childbirth, menstrual cycle, menopause, previous and/or current gynaecological and endocrinological disease), as well as others referred to depression, manic-depressive or psychotic symptoms, collected according to a binary method (yes or no), were in the questionnaire.

In order to avoid the influence of confounding factors, the subjects who had stated to be in menopause, perimenopause or postmenopause, to be using hormone agents (hormone replacement therapy and oral contraceptives), to have undergone hysterectomy, to be pregnant or breast-feeding, to be in amenorrhoea, to have menstrual cycle disturbances for quantity and/or for duration (< 21 days or >35 days) and to have current or previous gynaecological and/or endocrinological pathologies (hypothalamic, pituitary, thyroid gland disease, polycystic ovary syndrome), or to have ovarian cancers were excluded from the study. Subjects who referred playing competitive or not competitive sport, along with those who referred being exposed to solvents, paints and pesticide during leisure activities were excluded from the study.

Two groups were studied: traffic police exposed to urban pollutants who worked in shifts on parking, patrols, keeping passage-ways free, controling traffic at crossings and on roads with intense flows of vehicles; municipal administrative staff who carried out indoor activities, such as administrative and bureaucratic tasks, with a slighter level of exposure were used as control group. Both traffic police and the control group worked seven hours per day for at least five days per week.

Female traffic police were matched with controls (by mean, standard deviation and distribution in classes) by age (28), length of service, body mass index (BMI) (kg/m^2) (29), alcohol drinking habits (number of glasses of wine/beer per day and number of glasses of spirits per week) (30), cigarette smoking habits (number of cigarettes per day, years of smoking habit) (31), habitual intake of soy and liquorice in diet (32), and habitual consumption of Italian coffee (number of cups per day) (33).

One hundred and ninety-two female subjects were included in this study: 96 traffic police and 96 controls. The characteristics of the studied population are shown in Table I. A 10 ml sample of venous blood was taken from each subject between 8 and 10 a.m., in fasting condition during the period March 2004-April 2004. Samples were kept in a refrigerator at +4°C until they were transferred (by means of a container held at the same temperature) to the laboratory, where they were immediately centrifuged and the serum was stored at –20°C until analysis (within 3 days). A laboratory technician carried out the androstenedione dosage on venous blood samples by enzyme immunometric assay (EIA). Our normal laboratory values were between 0.5-5.4 ng/ml for female sex. The analytical sensitivity of the assay was calculated from the mean minus two standard deviations of twenty (20) replicate analyses of Standard 0 and was found to be 0.019 ng/ml. The inter-assay coefficients of variation (CVs) were 9.6, 12.1 and 8.8% for quality control sera containing 0.2, 2.3 and 4.4 ng/ml, respectively. The intra-assay coefficients of variation (CVs) were 9.1, 5.6 and 4.7% for quality control sera containing 0.3, 2.6 and 4.7 ng/ml, respectively. The laboratory staff did not know which samples came from the group of traffic police and which from the control group, although both the physicians in charge and the technicians knew a study was in progress.

The study was conducted in accordance with the principles set out in the Declaration of Helsinki. All participants were informed of the nature of the screening and all signed a consent for the questionnaire and laboratory analysis. Statistical analysis of the data was based on the calculation of the mean, standard deviation, distribution, max. and minimal values, and frequency according to the nature of the single variables.

The unpaired t-test or $\chi 2$ test was used to analyze differences in values or ratios between groups. The differences were considered significant when the *p* values were <0.05. The statistical analysis was carried out using the statistical program Solo-BMDP^{TM} Statistical Software.

RESULTS

The mean androstenedione values were significantly higher in female traffic police compared to controls ($p=0.029$) (Table I). The distribution into classes of androstenedione values in traffic police workers was statistically significant ($p= 0.04$) (Fig. 1). The number of traffic police and controls with Androstenedione values outside the upper and lower normal limit of our laboratory was not significant compared to controls ($p>0.05$). The percentage of traffic police with fertility disorders (20.9% *vs* 21.9% in traffic police and controls, respectively; $p>0.05$) and mental health disorders (depression, anxiety, panic attacks) (10.0% *vs* 9.8% in traffic police and controls, respectively; $p>0.05$) was not significantly different compared to controls.

Several authors over the years have investigated the effects of the agents present in urban air on the hypothalamus-pituitary-gonadal axis; literature research on the effects on androstenedione were conducted *in vivo* on experimental animals, or *in vitro*, there are few studies on urban pollution carried out on humans and no study on the possible alterations of plasma androstenedione levels has been carried out on municipal police workers exposed to pollution.

Androstenedione is an androgenic hormone which is secreted by adrenal and ovarian glands in women, under the control of ACTH and LH, respectively, which plays an important role as a precursor for peripheral conversion in Testosterone (T), Dihydrotestosterone (DHT) and Estrone (E_1). There are few studies in the literature on the exposure to chemical urban stressors and effects on androstenedione (15-17). Two studies have shown that the aromatization of androstenedione in normal estrogen biosynthesis was altered by CO (15-16). Also Dimethylbenz(a)nthracene (DMBA), chemical carcinogen, induced effect on serum steroid levels. DMBA exposure determines increases of androstenedione, testosterone and estradiol levels (17).

Table I. *Age, length of service and androstenedione values in female traffic police and controls.*

	Traffic police N° 96	Controls N° 96
Age (yr)		
Mean (SD)	38.7 (4.3)	39.8 (4.0)
min-max	26-48	28-49
Length of service (yr)		
Mean (SD)	7.4 (5.2)	6.6 (4.7)
min-max	1-20	1-21
Androstenedione (ng/ml)		
Mean (SD)	2.5 (0.9)*	2.2 (1.0)
min-max	0.6-5	0.5-4.9

**p=0.029 in female traffic police vs controls*

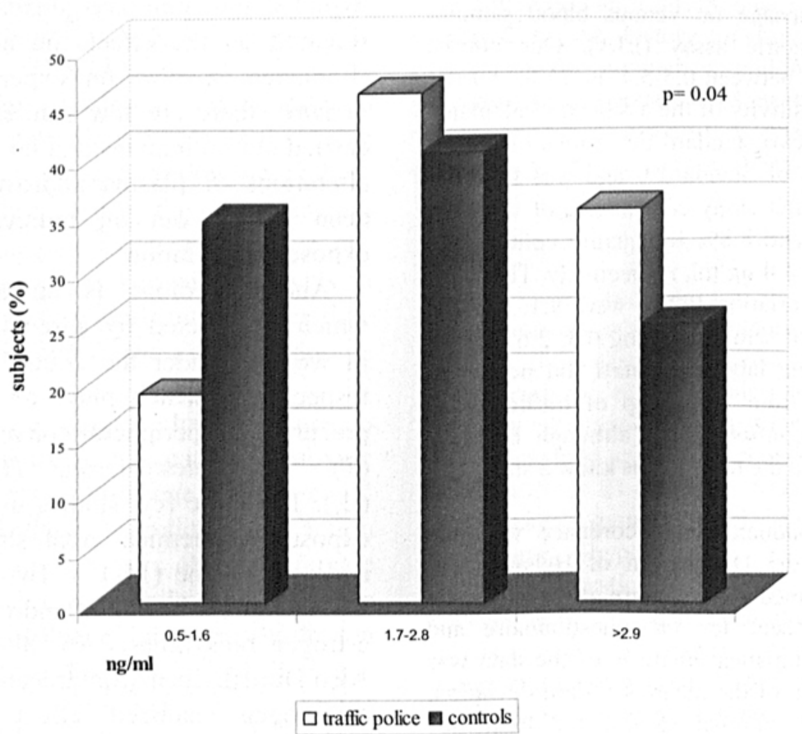

Fig. 1. *Distribution of Androstenedione values (ng/ml) in female traffic police officers and controls. p= 0.04*

In our study, we observed a significant increase of androstenedione plasma levels in female traffic police compared to controls. These results suggest that a chronic working exposure to urban stressors could be able to increase androstenedione production. From the analysis of the literature data, the evidence that female reproductive health may be affected by exposure to urban pollutants emerges (18-20).

In female workers, a weak relative risk of spontaneous abortion associated with the exposure to benzene, gasoline and H_2S was found (18). In women exposed to benzene, a high percentage of oligomenorrhea/amenorrhea cases were observed in a study carried out on 3,000 women who worked in a large petrochemical company in Beijing (China), and an abnormal menstrual cycle length was observed (19). Cho et al. showed an increased frequency of oligomenorrhea in women occupationally exposed to benzene, toluene, xylene and styrene. In female animals exposed to PM_{10} and NO_2 an increased number of implantation failures were observed (20).

Data in the literature could support the hypothesis that reproductive health and related systems represent a target of urban stressors, including physical, chemical and psycho-social stressors. The results of two studies highlighted that such physical agents influence the levels of plasmatic concentrations of some hormones, in particular dehydroepiandrosterone (34) and testosterone (35). According to the literature data in our study, respectively 20.9% and 21.9% of traffic police and controls referred fertility disorders, but this difference was not significantly different. Besides the chemical and physical urban stressors mentioned above, psycho-social stressors may also affect the reproductive–endocrine axis (27).

Androstenedione plays an important role in female neurobiology and some authors have observed alterations of androstenedione plasma levels in some psychological disorders (22-25). Data in the literature suggest that androstenedione may be identified as neurosteroids with neurobiologic effects (22); neurosteroids are able to modify neural activities, and bind and modulate different types of membrane receptors. The involvement of neurosteroids, such as androstenedione on major depression (23), manic depressive or psychotic symptoms (24), anxiety disorders (25) and stress response to different stimuli are reviewed and critically evaluated.

According to the literature data in our study, traffic police and controls (10% and 9.8%, respectively) referred mental health disorders (depression, anxiety, panic attacks) but this difference was not significantly different. The results of this should be cautiously interpreted since chemical pollutants were not determined in the examined subjects. The absence of literature studies on the possible alterations of plasma androstenedione levels in municipal police workers exposed to pollution gives this original manuscript an interest in the field of environmental and occupational exposure to noxious agents.

In conclusion, our results suggest that the occupational exposure to low doses of chemical stressors, interacting with and adding to the psychosocial ones, could alter androstenedione plasma concentrations in female traffic police.

These results may depend on a chronic working exposure to urban stressors. According to our previous research on other neuro-immune-endocrine markers, all the hormonal parameters studied, including androstenedione, could be used as early biological markers of chronic exposure to urban stressors, usable in occupational sets.

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