

**GHRELIN, NITRITE AND PARAOXONASE/ARYLESTERASE  
CONCENTRATIONS IN CEMENT PLANT WORKERS****KONCENTRACIJE GRELINA, NITRITA I PARAOKSONAZE/ARILESTERAZE  
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**Summary:** Occupational cement dust exposure has been associated with an increased risk of liver abnormalities, pulmonary disorders, and carcinogenesis. Decreased antioxidant capacity and increased plasma lipid peroxidation have been posed as possible causal mechanisms of disease. Accordingly, this study examined the serum paraoxonase (PON1) arylesterase (AE), ghrelin, HDL-C, LDL-C and serum nitrite (NOx) levels in cement dust exposed workers. Twenty-eight volunteer male cement plant workers and 30 volunteer control male workers, aged 29–54 years, participated. The concentrations of serum PON1, AE, NOx, ghrelin, and HDL-cholesterol and LDL-cholesterol were measured in both groups. PON-1, AE, ghrelin and HDL-cholesterol were lower in the cement plant workers than in controls. Serum nitrite (NOx), and LDL-C levels in cement plant workers were higher ( $p < 0.05$ ) than in the control group workers. No correlation was observed between the serum levels of HDL-cholesterol and PON1 and between HDL-cholesterol and ghrelin. A weak negative correlation was detected between the serum ghrelin and NOx. The study results strongly suggest that HDL-paraoxonase, AE, ghrelin, HDL-C, and high NOx, and LDL-C levels may have a role in disease involving oxidative damage. However, some studies are necessary to address the association between occupational dust exposure and respiratory symptoms.

**Keywords:** cement dust, occupational exposure, paraoxonase, nitrite, ghrelin

**Kratka sadržaj:** Izlaganje cementnoj prašini na radnom mestu povezano je s povećanim rizikom od promena na jetri, plućnih bolesti i nastanka kancera. Mogući mehanizmi koji uzrokuju bolesti su smanjeni antioksidativni kapacitet i povišena lipidna peroksidacija u plazmi. U skladu s tim, u ovoj studiji proučavani su nivoi paraoksonaze (PON1) – arylesteraze (AE), grelina, HDL-C, LDL-C i nitrita (NOx) u serumu kod radnika izloženih cementnoj prašini. U studiji je učestvovalo dvadeset osam radnika u cementari i 30 dobrovoljaca (kontrola), starosti 29–54 godine. Koncentracije PON1, AE, NOx, grelina i HDL-holesterola i LDL-holesterola u serumu merene su u obe grupe. PON-1, AE, grelin i HDL-holesterol bili su niži kod radnika u cementari nego u kontrolnoj grupi. Nivoi nitrita i LDL-C u serumu kod radnika u cementari bili su viši ( $p < 0,05$ ) nego u kontrolnoj grupi radnika. Nije primećena korelacija između nivoa HDL-holesterola i PON1 u serumu, kao ni između HDL-holesterola i grelina. Slaba negativna korelacija otkrivena je između grelina i NOx u serumu. Rezultati studije snažno ukazuju na to da HDL-paraoksonaza, AE, grelin, HDL-C i visoki nivoi NOx i LDL-C verovatno igraju ulogu u bolesti koja uključuje oksidativna oštećenja. Međutim, potrebna su dalja istraživanja kako bi se ispitala veza između izlaganja prašini na radnom mestu i respiratornih simptoma.

**Ključne reči:** cementna prašina, izlaganje na radnom mestu, paraoksonaza, nitrit, grelin

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**Introduction**

Cement, in particular portland cement, is widely used by the construction industry as a building material (1). Worldwide demand for cement, especially in developing countries, has increased substantially over the last 20 years. The accompanying growth in world wide cement production has consequently increased

the number of workers exposed to airborne cement dust. Cement dust contains many constituents of varying toxicity including: calcium oxide (62–66%), silicon oxide (19–22%), aluminum trioxide (4–8%), iron oxide (2–5%), magnesium oxide (1–2%), crystalline silica, sulphur oxide and other alkali oxides (1). In addition, trace metals such as chromium are typically found in small concentrations (1). Worker exposure occurs primarily through the respiratory route, but dermal exposure can also result in adverse health effects. There are numerous reports on the adverse health effects of cement dust in both animals (2), and humans (3–9). Liver abnormalities (4), pulmonary disorders (2), carcinogenesis (5–8), decreased antioxidant capacity and increased plasma lipid peroxidation have been reported among cement factory workers (9).

There is considerable evidence for the harmful effects of cement dust in living systems (2–9). The altered generation and metabolism of NO<sub>x</sub>, PON1, and ghrelin may potentially play a role in the etiology of diseases that have been found to be associated with occupational cement dust exposure. However, no studies have been published examining the high density lipoprotein-cholesterol (HDL-C), and ghrelin levels of workers exposed to cement dust.

Lipid oxidation is believed to occur in response to increased oxidative stress or a deficiency of endogenous antioxidants. Human serum paraoxonase (PON1), a lipophilic antioxidant, is linked with HDL-C (10). Tobacco consumption is an environmental exposure which has been reported to depress PON1 activity and concentration (11, 12). PON1 is believed to play an important role in low density lipoprotein (LDL) protection against oxidation *in vivo*. Also, it has been recently reported that the interaction between the immobilized form of ghrelin and HDL-C was also inhibited by paraoxan, a substrate for the esterase PON1 (12).

Human ghrelin (hunger hormone) is a novel peptide hormone, which is predominantly synthesized in the stomach (10, 12, 13), but it is also synthesized in several other tissues including the central nervous system, kidney, heart, parathyroid glands, the small and large intestines,  $\alpha$ -cells of the pancreatic islet (14), placenta (15), and salivary gland (16). It stimulates growth hormone (GH) secretion and acts as a signal for the regulation of food intake, energy expenditure and fat accumulation, and body weight gain (12, 17).

Increased oxidative stress impairs endothelial function and is thought to mediate vascular disease (18). Vascular function is dependent upon the balance of oxidant and antioxidant mechanisms, which determines endothelial function. Endothelial function is usually defined as nitric oxide (NO) production and/or bioavailability. Nitric oxide is a potent vasodilator and cytostatic agent (18, 19). Also, it has been

reported that LDL, especially ox-LDL, is a potent inhibitor of endothelial function (19, 20). The mechanisms by which LDL inhibits endothelial-derived NO activity include the down-regulation of endothelial NO expression (20, 21), decreased receptor-mediated NO release (20), and NO inactivation via increases in O<sub>2</sub><sup>-</sup> production (22, 23). Indeed, endothelial dysfunction is a hallmark of hypercholesterolemia and is rapidly improved by cholesterol reduction (24).

The objective of this study was to evaluate serum arylesterase (AE), PON1, ghrelin, HDL-C, LDL-C, and NO<sub>x</sub> in male, non-smoking cement factory workers employed in the profession for at least 10 years.

## Materials and Methods

### Subjects and Sample Collection

Sera were from Dr. Aydin's biological fluids archives. The institutional ethics committee approved the study protocol. The exposed group was comprised of 28 non-smoking males, 29 to 54 years of age, who had been occupationally exposed to cement dust for at least 10 years. It was assumed that all workers in the cement plant were exposed to cement to the same extent (survey data). The control group included 30 non-smoking males of the same age group and socioeconomic status as the exposed subjects. Control group men were not occupationally exposed to cement dust or any known physical or chemical agent. The experimental design controlled for other potential confounding factors such as diet or physical exercise.

Intravenous blood samples were collected into tubes from all exposed and control subjects. All blood samples were collected at the same time (10:00 am), as Cummings et al. (25) found that plasma ghrelin levels rose by an average of 78% 1–2 h before the onset of each meal and fell to trough levels within 1 h after food was first consumed. Subjects were asked to remain fasting until blood was drawn. Thus, we examined serum total ghrelin level roughly before breakfast was eaten. HDL-C and LDL-C were determined using an automatic analyzer (Olympus AU600) in the Biochemistry Laboratory of Firat Medical Center, Elazig.

### Serum nitrite levels (NO<sub>x</sub>)

Serum nitrite levels were determined using a colorimetric method (26). For this assay, the following reagents were added to 100  $\mu$ L of serum in an assay tube: 250  $\mu$ L of 100 mmol/L potassium phosphate buffer (pH 7.4), 50  $\mu$ L of distilled water, 50  $\mu$ L of 0.2 mmol/L FAD, and 10  $\mu$ L of 12 mmol/L  $\beta$ -NADPH. The enzymatic reaction was initiated through the addition and subsequent mixing of 50  $\mu$ L

of 500 U/L nitrate reductase. After mixing, the reaction was allowed to develop in the dark because FAD is photo labile. After a 45 minute reaction period conducted at room temperature, the absorbance at a wavelength of 340 nm was measured with a Techcomp, 8500 II spectrophotometer. Absorbance was converted to concentration using a calibration curve prepared with sodium nitrite in distilled water. Serum nitrate was measured as nitrite after enzymatic reduction by an improved method (26–28). Values obtained by this procedure represent the sum of nitrite and nitrate. Nitrate concentration was obtained by subtracting nitrite concentration from total nitrite + nitrate (26, 27).

#### Assay of paraoxonase activity

PON1 assays were performed without additional NaCl (baseline activity) and with 1 mol/L NaCl included in the assay buffer (salt-stimulated activity), by following the formation of p-nitrophenol by its absorbance at 405 nm. Assay buffer was 0.125 mol/L Tris-HCl pH= 8.5, 1.25 mmol/L CaCl<sub>2</sub>, and 1 mol/L NaCl with a pH 8.5 (11). For each set of assays, a 6 mmol/L freshly prepared paraoxan (O,O-diethyl-o-p-nitrophenylphosphate; Sigma Chemical Company) substrate solution of 120 mmol/L paraoxan in acetone diluted with 0.125 mmol/L Tris-HCl was used. Paraoxon stock solution was handled very cautiously with protective measures. The assay tube contained 750 µL Tris buffer, 50 µL sera (1:2 diluted with water) and 200 µL, 6 mmol/L paraoxan. The reaction was initiated at 37 °C by the addition of the substrate solution, and absorbance was continuously monitored at 412 nm, 25 °C, using a Techcomp, 8500 II spectrophotometer. The paraoxonase unit was defined as the enzyme quantity that disintegrates 1 µmol paraoxan substrate in one minute (11, 19). The percent stimulation of PON1 was calculated as follows:

$$\frac{[(\text{Paraoxonase activity with 1 mol/L NaCl}) - (\text{basal activity})] / \text{basal paraoxonase activity}}{\times 100}$$

#### Assay of arylesterase activity

Arylesterase (AE) activity, which is not affected by the presence of salt, was measured with phenyl acetate as a substrate as described earlier (10, 30). The assay tube contained 750 µL 0.1 mol/L Tris-HCl (pH=8.5), 1 mmol/L CaCl<sub>2</sub> and 125 µL 12 mmol/L phenyl acetate and 125 µL serum (1:10 diluted with water). The absorbance was continuously monitored at 270 nm at 37 °C. Units were expressed as millimoles of phenyl acetate hydrolyzed per minute.

#### Hormone assay of serum ghrelin

Serum immunoreactive ghrelin levels were measured in duplicate using a commercial radioimmunoassay (RIA) that uses <sup>125</sup>I-labeled bioactive ghrelin

as a tracer and a rabbit polyclonal antibody raised against full-length octanoylated human ghrelin (Phoenix, Europe, Karlsruhe, Germany), as described previously (25, 31). The assay detects both octanoyl-ghrelin and des-octanoyl-ghrelin. Ghrelin concentrations were calculated from standard curves generated in the same assay with ghrelin, which is synthetically made with solid phase synthesis. Radioactivity of the prepared samples was measured with a gamma counter (Multigamma 1261; LKB Wallac, Turku, Finland).

#### Chemicals

Ghrelin was purchased from Phoenix-Germany. Other chemicals were purchased from Sigma-Aldrich.

#### Statistical analysis

Student's t test was used for comparing results from the exposed and control groups. The data are expressed as arithmetic means ± standard deviation (S.D.). P values less than 0.05 were considered significant. Pearson correlation coefficients were used to test the correlation between each of the two biochemical variables.

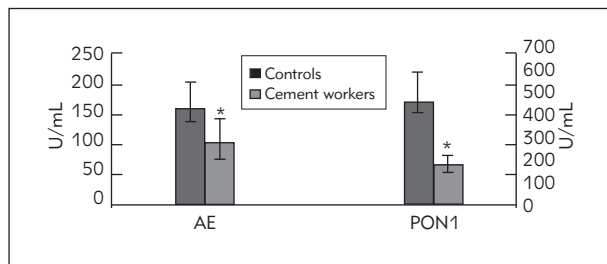
## Results

The demographic characteristics and glucose data of the study groups are given in Table I. BMI (kg/m<sup>2</sup>) of subjects were as follows (Table I): Control: 23.93 ± 2.07; Cement workers: 24.07 ± 2.39 (P= 0.814). Blood serum concentrations of NOx, PON1, AE, ghrelin, HDL-C and LDL-C for the exposed and control study groups are presented in Figure 1–3. The concentrations of NOx and LDL-C in workers occupationally exposed to cement dust were higher (p<0.05) than those of the control group (Figure 2 and 3), whereas, PON-1, AE, ghrelin, and HDL-C were lower in the cement plant workers than in controls (Figure 1–3). No correlation was observed between the serum levels of HDL-cholesterol and PON1 and between HDL-cholesterol and ghrelin. A weak negative correlation was detected between the serum ghrelin and NOx.

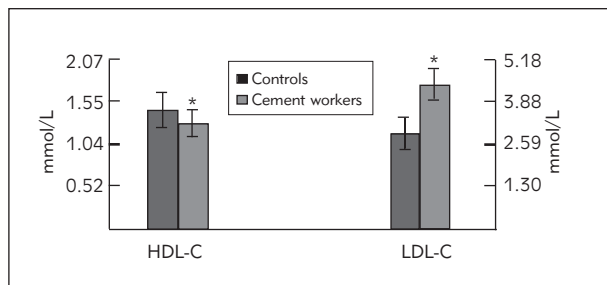
**Table I** Demographic features and glucose levels of the subjects (n:58).

Parameters	Control (n:30)	Cement workers (n:28)
Age (year)	46 ± 8	47 ± 9
BMI (kg/m <sup>2</sup> )	23.93 ± 2.07	24.07 ± 2.39
Glucose, mmol/L	4.8 ± 0.5	5.8 ± 0.7

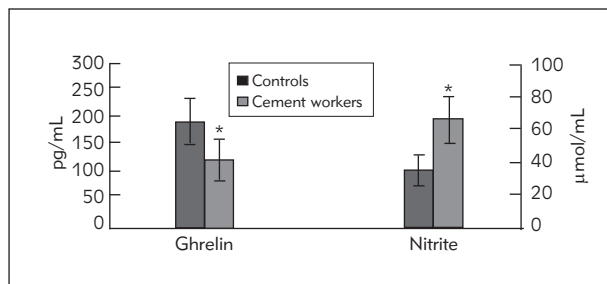
BMI, body mass index (as kg/m<sup>2</sup>)



**Figure 1** Serum PON1/AE levels of groups,  $p < 0.05$  for cement workers vs. normal. AE: Arylesterase, PON1: Paraonase. Values are given as mean  $\pm$ SD. All data points are the average of 28–30 data point.



**Figure 2** Serum HDL-C and LDL-C levels of groups,  $p < 0.05$  for cement workers vs. normal. HDL-C: High density lipoprotein cholesterol, LDL-C: Low density lipoprotein cholesterol. Values are given as mean  $\pm$ SD. All data points are the average of 28–30 data point.



**Figure 3** Serum ghrelin and NOx levels of groups,  $p < 0.05$  for cement workers vs. normal. Values are given as mean  $\pm$ SD. All data points are the average of 28–30 data point.

## Discussion

In previous studies, airborne cement dust exposure has been shown to be the likely causative agent for several different adverse health effects observed in lab animals (3) and humans (2, 4–9). Higher incidences of respiratory symptoms (2), carcinogenesis (5–8), and liver abnormalities (4) have been reported among cement factory workers. Cement dust exposure has also been associated with both increased oxidative stress and decreased antioxidant capacity, which in turn can promote lipid peroxidation (9). Therefore, we examined the effect of occupational cement dust exposure on serum

paraonase, an HDL-cholesterol-associated lipophilic antioxidant.

PON1 is continuously generated in physiologic conditions, and has been studied extensively in relation to cardiovascular diseases (32, 33). In the current study, PON1, ghrelin and AE activity were found to be significantly lower ( $p < 0.05$ ) in workers occupationally exposed to cement dust than in the control groups. This could be related to enhanced lipid peroxidation, (9) since oxidized lipids are reported to inhibit PON1 activity (34, 35). Decreased PON1 and AE blood serum levels observed in the cement dust exposed subjects could also have been caused by a change in HDL secondary levels or decreased protein expression as a result of tissue damage.

Although PON1 levels may be normal, serum PON1 activity would be lowered as a result of a change in synthesis or secretion of the HDL secondary. Also, it has been reported that the binding of HDL to ghrelin is inhibited approximately 50% by 1 mmol/L paraoxan, resulting in a  $K_m$  that is proximal to that of PON1 for paraoxan (12). Therefore, it can be suggested that either ghrelin binds to the same active site as paraoxan or that the binding of paraoxan causes an alteration to an allosteric site where ghrelin interacts.

Alternatively, decreased PON1 levels in the exposed workers may have been a result of damaged cells expressing this protein at a considerably lower rate. Supporting this putative function is the report of inhibited microsomal PON1 activity in rats that were chronically administered  $CCl_4$  (36). Moreover, the decreased lipophilic antioxidant PON1 activity and ghrelin level observed in this study are consistent with an earlier study examining serum antioxidant levels in workers exposed to cement dust (9). PON1 is believed to play a central role in the inhibitory effect of HDL on lipid peroxidation. Lipid oxidation is believed to occur in response to increased oxidative stress or deficiency of endogenous antioxidants.

Although ghrelin is expressed in almost all tissues, its expression is highest in the stomach, where its secretion from A-like cells is up-regulated during fasting and hypoglycemia (17). The role of ghrelin in regulating the long-term energy balance in workers exposed to cement dust has not been investigated. In the present study, we observed a lower concentration of ghrelin in cement workers. Based on job descriptions, the exposed subjects are more physically active in their occupation than the control subjects. Ghrelin not only fulfills a hormonal function in the body, but also is an endogenous antioxidant (37). Therefore, ghrelin may have been used up by the body in order to eliminate the inflammation (18) and oxidative stress caused by physical activity and cement dust, and this may have caused a decline in the amount of ghrelin. Additio-

nally, increases in glucose are accompanied by decreases in ghrelin. Increased glucose level observed in this study may also have brought about a decrease in ghrelin levels. Possibly, decreased ghrelin levels may be associated with energy regulation. All these reasons may account for the decrease in ghrelin amount either individually or in combination. Consequently, it is plausible that the exposed subjects' metabolism is not compensating for changes in energy homeostasis and a concomitant decrease in ghrelin production.

The significantly higher NO<sub>x</sub> levels observed in the exposed subjects' serum in this study may be the result of a generalized increase in NO<sub>x</sub> synthesis throughout the body; alternatively, increased NO<sub>x</sub> production could have been promoted by oxidative stress (18, 20). The latter explanation is consistent with an earlier study where significantly lower serum antioxidant levels were observed in workers exposed to cement dust (9). Long term cement dust exposure may enhance the endogenous production of superoxide anion as evidenced by increased serum levels of malondialdehyde, and decreased superoxide dismutase activity (9).

Excessive NO production has been shown to be associated with the progression of respiratory disease such as asthma in response to occupational dust

exposure (19, 38). Respiratory exposure to crystalline silica, organic dusts, and asbestos induces iNOS activity and the production of NO (19, 39). Up-regulation of NO production has also been reported in a silica inhalation study with rats (38). Furthermore, NO can react with superoxide anion (O<sub>2</sub><sup>-</sup>) to form a reactive species, peroxynitrite (19, 39–41), a potent oxidant. In this study, the observed cogeneration of multiple reactive species confirms our previously reported high level of reactive oxygen species (ROS) in the cement workers (9). The overall observations of the study clearly indicate that serum LDL-C and NO<sub>x</sub> levels were significantly higher in workers exposed to cement dust as compared to the control groups. This finding is of significance, as NO is a highly unstable molecule and causes lipid peroxidation (39). The PON1 and ghrelin levels in cement dust exposed workers were found to be significantly lower than in the controls, suggesting that exposure to cement dust causes a reduction in the HDL-cholesterol-associated lipophilic antioxidant. Finally, the reduction in PON1 levels suggests a possible link between HDL-C and ghrelin interaction (42) which is consistent with results from a previous study where serum levels of antioxidant enzymes and plasma vitamins C and E in this occupationally cement dust exposed cohort were found to be significantly lower than those of an unexposed control group.

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