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Urinary levels of the acrolein conjugates of carnosine are associated with inhaled toxicants

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Abstract

Objective: The inhalation of air-borne toxicants is associated with adverse health outcomes which can be somewhat mitigated by enhancing endogenous anti-oxidant capacity. Carnosine is a naturally occurring dipeptide (β -alanine-L-histidine), present in high abundance in skeletal and cardiac muscle. This multi-functional dipeptide has anti-oxidant properties, can buffer intracellular pH, chelate metals, and sequester aldehydes such as acrolein. Due to these chemical properties, carnosine may be protective against inhaled pollutants which can contain metals and aldehydes and can stimulate the generation of electrophiles in exposed tissues. Thus, assessment of carnosine levels, or levels of its acrolein conjugates (carnosine-propanal and carnosine-propanol) may inform on level of exposure and risk assessment.

Methods: We used established mass spectroscopy methods to measure levels of urinary carnosine (n=605) and its conjugates with acrolein (n=561) in a subset of participants in the Louisville Healthy Heart Study (mean age = 51 ± 10 ; 52% male). We then determined association between these measures and air pollution exposure and smoking behavior using statistical modeling approaches.

Results: We found that higher levels of non-conjugated carnosine, carnosine-propanal, and carnosine-propanol were significantly associated with males ($p < 0.02$) and those of Caucasian

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Disclosure of Interest

The authors have nothing to disclose.

ethnicity ($p < 0.02$). Levels of carnosine-propanol were significantly higher in never-smokers ($p = 0.001$) but lower in current smokers ($p = 0.037$). This conjugate also demonstrated a negative association with mean-daily particulate air pollution (PM_{2.5}) levels ($p = 0.01$).

Conclusions: These findings suggest that urinary levels of carnosine-propanol may inform as to risk from inhaled pollutants.

Keywords

carnosine; PM_{2.5}; smoking; acrolein; biomarker

INTRODUCTION

A large body of epidemiological and experimental evidence has identified associations between exposure to air pollution and adverse health outcomes (1–3). Air pollution is a complex mix of particles, gases, and metals. High levels of these components are found in vehicle exhausts, factory emissions, and the combustion of carbonaceous material. Involuntary exposure to these ubiquitous air-borne toxicants contributes to local and systemic oxidative stress, and the subsequent *in vivo* generation of oxidized lipids (e.g. aldehydes) and electrophilic intermediates. Indeed, it has been shown that exposure to fine particulate matter (PM_{2.5}) air pollution results in the generation of lipid peroxidation products (4) and that some the adverse outcomes of PM_{2.5} exposure can be mitigated by measures taken to reduce oxidative stress (5–7). Humans are also directly exposed to existing, environmental sources of aldehydes. Cigarette smoke, for instance, is estimated to contain over 5000 compounds and among the most abundant of these constituents, are several highly reactive aldehydes including acrolein (8, 9). High levels of reactive aldehydes are similarly generated during the use of electronic nicotine delivery devices (ENDs) (10). As with PM_{2.5} inhalation, some of the adverse health consequences attributed to the use of cigarettes are likewise believed to result from exposure to aldehydes and other toxic compounds. Regardless of their source, efforts taken to curtail the production or impact of oxidized lipids would seem to mitigate the adverse health impacts of exposure.

Carnosine is a naturally occurring dipeptide (β -alanine-L-histidine) found in abundance in tissues with a high metabolic rate, such as skeletal muscle, heart, and brain (11). It is structurally and functionally related to a series of other naturally occurring dipeptides including homocarnosine (γ -amino-butyric acid-histidine), anserine (β -alanyl-L1-methylhistidine), and balenine (β -alanyl-L3-methylhistidine), as well as their N-acetylated forms (12). These histidyl peptides have been evolutionarily conserved for over 500 million years and are present across all animal species (11). Although their specific physiological role remains unclear, it is believed that, because they contain an imidazole ring, histidyl dipeptides buffer intracellular pH and thereby prevent tissue acidification by buffering lactic acid, particularly during periods of high glycolytic activity or ischemia (13). In humans, the levels of carnosine increase after high intensity training (14), and carnosine or its precursor, β -alanine, are widely used by athletes as performance enhancing supplements (11). However, carnosine can be physiologically protective for other reasons as well. Due to a highly reactive nucleophilic amine, carnosine and related peptides can also bind to a wide variety of lipid peroxidation products such as 4-hydroxy *trans*-2-nonenal (HNE), acrolein,

and other endogenous aldehydes (15, 16). Carnosine also quenches singlet oxygen and chelates metals, and has been shown to form stable conjugates with some reactive carbonyl species generated from oxidized lipids (16, 17), which are then excreted in the urine (18, 19). Because of these diverse properties, carnosine is believed to play a generally protective role, in part by neutralizing reactive biomolecules that contribute to tissue injury in several disease states (20–22).

Direct evidence of a protective role for carnosine derives from several supplementation studies, which have shown that oral carnosine supplementation increases the excretion of urinary carnosine-aldehyde conjugates, improves glucose uptake in obese humans (23, 24), and improves renal function in obese Zucker rats (25). It has also been recently reported that perfusion of isolated mice hearts with carnosine protects against ischemia-reperfusion injury (26) and that apolipoprotein E null mice supplied with carnosine-containing drinking water had decreased atherosclerosis (27). Additionally, it has been found that carnosine supplementation increases the excretion of advanced glycation end products (AGEs) in obese diabetic patients (23). Collectively, this evidence supports the notion that carnosine protects against oxidative stress and tissue injury associated with increased production of electrophilic species.

As carnosine levels are lower in the lungs than in other tissues (11), it is unclear if it can neutralize and protect against the electrophilic intermediates acquired from or generated by inhalation exposures in humans. In support of this idea, we have recently shown that some of the adverse effects of PM_{2.5} exposure on bone marrow stem cells in mice were mitigated in those animals drinking water supplemented with carnosine (18). Nevertheless, it remains unclear whether the urinary levels of carnosine or its conjugates reflect tissue levels of carnosine, total oxidative load, or on-going tissue injury. Although carnosine readily reacts with and forms conjugates with a range of aldehydes, previous work has shown that its conjugates with acrolein (carnosine-propanal and carnosine-propanol) are particularly abundant in mouse urine (19). Given that acrolein is generated by lipid oxidation (28) and inflammatory reactions (29), we hypothesized that the urinary levels of carnosine-propanal and/or carnosine-propanol are reflective of exposure to PM_{2.5} or smoking behavior which both contribute to extensive tissue and lipoprotein oxidation (30), and chronic, unresolved inflammation (31). Thus, we measured the urinary levels of carnosine and its propanal and propanol conjugates in a cohort with mild-to-high cardiovascular disease (CVD) risk and examined associations with tobacco use and PM_{2.5} exposures.

MATERIALS AND METHODS

Study cohort and measurements

The study utilized a previously described cohort (32). These participants were recruited from the University of Louisville Hospital and affiliated clinic system from October of 2009 through May 2018. They were older than 18 years of age and presented with mild-to-high CVD risk as assessed using Framingham Risk scores (FRS). Patients visiting the clinics were pre-screened through medical records to exclude those who did not meet the enrollment criteria. Those unwilling or unable to provide informed consent or with significant and/or severe comorbidities were excluded. These included significant chronic lung, liver, kidney, hematological, or neoplastic disease, chronic neurological or psychiatric

illness, acute infections or unhealed wounds, chronic infectious disease such as HIV or hepatitis, severe coagulopathies, drug/substance abuse, and chronic cachexia. Other exclusions included pregnant women, prisoners, and other vulnerable populations, those with conditions known to effect peripheral blood cell counts and bone marrow function, and those with a history of malignancies, organ transplant, untreated thyroid disease, and anemia. Subjects on hormone replacement therapy or medications affecting bone marrow function or peripheral blood cell counts were also excluded from the study.

Samples of blood and urine were collected from participants who met the enrollment criteria and gave written consent. Demographic information, including age, sex, ethnicity, residential address, smoking status and history, secondhand smoke exposure (verified by measurement of urinary cotinine concentrations), and body mass index (BMI; calculated from self-reported height and weight) was obtained by using a questionnaire. A diagnosis of diabetes and medication usage was obtained from chart review. The study was approved by the University of Louisville Institutional Review Board (IRB 09.0174).

To assess air pollution exposure, average daily PM_{2.5} values were obtained from all US Environmental Protection Agency-validated monitors in the Louisville Metropolitan Area. These average values varied little between the monitors; thus they accurately represented PM_{2.5} exposure levels throughout the larger residential area of the cohort. From these data, PM_{2.5} levels on the day prior to participant enrollment were used. Distance to major roadway (defined as any roadway segment traversed by > 5000 vehicles per day as measured by the Kentucky Transportation Cabinet) was calculated using Geographic Information Systems as previously described (33).

Carnosine Measurements

The measurement of carnosine and its propanal- and propanol-conjugates was accomplished as previously described (18). In brief, urine samples were diluted in a solution of 75% acetonitrile:25% water containing 30 nM ¹³C₉ carnosine as an internal standard. Samples were separated and carnosine and its conjugates were identified using a Waters ACQUITY ultra performance liquid chromatography H-Class System (BEH hydrophilic interaction liquid chromatography column equipped with an in-line frit filter unit) coupled with a Xevo TQ-S micro triple quadrupole mass spectrometer. The analytes were eluted using a binary solvent system consisting of 10 mM ammonium formate, 0.125% formic acid in 50% acetonitrile: 50% water for mobile phase A and 10 mM ammonium formate, 0.125% formic acid in 95% acetonitrile: 5% water for mobile phase B at a flow rate of 0.55 mL/min. Initial conditions were 0.1: 99.9 A: B ramping to 99.9: 0.1 A:B over 5 min then quickly ramping to 0.1:99.9 A:B over 0.5 min (Supplemental Figure 1). Aldehyde conjugates were quantified using the peak ratio of histidyl-dipeptide and ¹³C₉ carnosine internal standard, interpolated using a standard curve and expressed as nmole/mg creatinine.

The precision of this liquid chromatography/mass spec/mass spec method was validated by replicate analysis of samples with highest and lowest concentrations of the analytes. Urine (500ul each) was pooled from the ten lowest and highest carnosine-propanol concentration samples. Five aliquots from each sample were processed and analyzed each day for 3 consecutive days. Relative variability was calculated from the coefficient of variation of

replicates within a single sample analysis and between multiple sample analysis as described (34). The inter- and intra-assay variabilities were: carnosine low: 8.95%; carnosine high: 5.65%; carnosine-propanal low: 18.97%; carnosine-propanal high: 6.34%; carnosine-propanol low: 16.45%; carnosine-propanol high 5.08%. The lower limits of quantification were: carnosine: 5 nM; carnosine-propanal: 181 nM; carnosine-propanol: 131 nM. Urinary levels of cotinine (35), the acrolein metabolite 3-hydroxypropyl mercapturic acid (3-HPMA) (36), and muconic acid (37) were measured using GC/MS (Agilent Technologies) methods and normalized to urinary levels of creatinine.

Statistical evaluation

Statistical analyses were performed using R scripts (version 3.5.2). Associations between non-conjugated carnosine (Table 1) or its conjugates (Table 2, 3) with categorical variables including some population demographics (gender and race), and smoking status were evaluated using chi-square test. The study population was stratified into those with low (0.04–0.29 nmole/mg creatinine), middle (0.30–0.58 nmole/mg creatinine) and high (0.59–6.73 nmole/mg creatinine) levels of non-conjugated carnosine (Table 1) or carnosine propanol (Table 2). As the upper limit of carnosine propanal was much smaller, stratification was only into low (0.0–0.14 nmole/mg creatinine) and high (0.15–1.01 nmole/mg creatinine) levels (Table 3). A p-value less than 0.05 was used to indicate that the association between each of the categorical variables and the conjugates was statistically significant. Associations between non-conjugated carnosine (Table 1) or its conjugates (Tables 2, 3) with continuous variables were analyzed by one-way ANNOVA with a linear regression model. The association between carnosine propanol, as binary dependent variable, and smoking status, adjusted by age, gender, race, diabetes, body mass index (BMI), average mean PM_{2.5} and medications (beta blocker, aspirin and diuretics), was evaluated using a logistical regression method with a fixed effect approach. Adjustment for FRS was also done using a logistic model (Supplementary Table 1).

RESULTS

Levels of carnosine, carnosine-propanal, and carnosine-propanol and demographic characteristics

Initially we examined associations with patient demographics and stratified, urinary levels of non-conjugated carnosine (Table 1). We found that these levels were significantly lower in females, Blacks, and those with advanced age. Carnosine reacts with acrolein to form carnosine-propanal, which is further reduced by the enzyme aldose reductase to form carnosine-propanol (19). These two conjugates are present in urine in higher abundance than most other carnosine-aldehyde conjugates (14, 19, 38). Hence, as an approximation of total carnosine-derived conjugates, we measured the urinary levels of carnosine-propanal and carnosine-propanol and examined their association with cohort demographics. This analysis showed that stratified levels of carnosine propanal had significant associations with sex, ethnicity, and age (Table 3). With regards to carnosine propanol, levels of this conjugate were significantly higher in males than females, and higher in Caucasians and Hispanics than Blacks (Table 2). Low levels of carnosine-propanol were associated with increasing age (Table 2).

Associations between carnosine, carnosine-propanal, and carnosine-propanol and smoking

As smoking is a major source of exposure to acrolein and other highly reactive chemicals (39), we further examined the association between smoking status and levels of carnosine and its acrolein-conjugates in both unadjusted and adjusted models. There was no significant association between urinary levels of non-conjugated carnosine (Table 1) or carnosine-propanal (Table 3) in those who are current smokers or those who are never smokers. However, we found significant associations between carnosine propanol and current smokers and self-reported never smokers (Table 2). The association with never smokers ($p < 0.001$) was greater than that for current smokers ($p = 0.037$) in the unadjusted model. Consistently, high levels of cotinine, and the acrolein metabolite, HPMA were also associated with low carnosine propanol levels (Table 2). The summary statistics and regression coefficients for analyzed associations between carnosine propanol and smoking status, after adjustment by age, gender, race, diabetes, BMI, daily mean $PM_{2.5}$ and medications are listed in Table 4. Using a logistical regression model, we found that current smoking had an inverse relationship (regression coefficient: 0.555; $p = 0.008$) with levels of carnosine propanol. This inverse relationship remained significant, even after adjustment for FRS (Supplementary Table 1). In contrast, a history of never smoking had a positive relationship (regression coefficient: 1.57; $p = 0.039$) with levels of carnosine propanol. These results are depicted in the box plots of Fig 1A and 1B. In addition, we looked at associations with carnosine-propanol:carnosine-propanal ratios (Supplementary Table 2). Using logarithm-transformed ($\log_2$) values in a linear regression model, we found significant associations of these ratios with race, $PM_{2.5}$ levels, and some medication use.

Associations between carnosine, carnosine-propanal, and carnosine-propanol and air pollution exposure

Exposure to $PM_{2.5}$ contributes to adverse health outcomes including cardiovascular morbidity and mortality (3, 40, 41), and current models of $PM_{2.5}$ -induced toxicity suggest that this may result from the induction of oxidative stress and the production of secondary oxidation products (e.g. aldehydes such as acrolein). Thus, we next examined the relationships between carnosine and its acrolein-conjugates with daily mean $PM_{2.5}$ levels as measured 24h prior to blood sample collection. In unadjusted models, daily $PM_{2.5}$ was associated with lower levels of non-conjugated carnosine (Table 1) and its propanol conjugate (Table 2). The summary statistics and regression coefficients for analyzed associations between carnosine propanol and $PM_{2.5}$ levels adjusted by age, gender, race, and beta-blocker use are listed in Table 5. We found that levels of this conjugate had a small but significant inverse association with $PM_{2.5}$ when using a logistic regression model (regression coefficient = 0.960; $p = 0.02$). These results are depicted in the box plots of Fig1C.

DISCUSSION

The direct inhalation of reactive aldehydes (e.g. contained in cigarette smoke), or of airborne toxins which promote oxidative stress and lipid peroxidation *in vivo*, and the systemic delivery of these reactive products, are believed to underlie the pathologies associated with $PM_{2.5}$ and cigarette smoke exposure. The endogenous histidyl-di-peptide carnosine

neutralizes oxidized lipids and, in this way, may protect against the deleterious effects of inhaled toxins. In this study we show that urinary levels of carnosine-propanol are inversely associated with current smoking but positively associated with never smoking (Table 2). Levels of this carnosine conjugate demonstrated a similar, inverse association with contemporaneous levels of ambient PM_{2.5}, but we found no association with other determinants or indicators of pollution exposure such as distance to major roadways, traffic level, or levels of the benzene metabolite, muconic acid (Table 2).

Tissue oxidative stress leads to oxidation of unsaturated fatty acids in the plasma membrane. The oxidation of these lipids generates unsaturated aldehydes such as HNE and acrolein. Previous work has shown that in most tissues both acrolein and HNE are readily detoxified via multiple metabolic pathways (42). In tissues such as liver and kidney, these aldehydes readily form conjugates with glutathione, a reaction that is catalyzed by glutathione-S-transferases, which protects against cardiovascular injury (43). In addition, these aldehydes can be directly reduced by aldo-keto reductases or oxidized by aldehyde dehydrogenases (44). Glutathione conjugates of aldehydes, after hydrolysis and acetylation via the mercapturic pathway, is excreted in the urine as mercapturic acids (39, 45). In the present study we detected high levels of the mercapturic acid metabolite of acrolein – HPMA, in the urine and these levels were inversely associated with carnosine-propanal and carnosine-propanol, suggesting that carnosine and glutathione represent complementary pathways for the metabolism and detoxification of unsaturated aldehydes. The glutathione-linked pathway is likely to predominate in tissues such as liver and kidney. However, the carnosine-linked pathway is likely to provide added protection to tissues with high metabolic activity such as the brain, heart and skeletal muscle. Hence, measurements of the urinary carnosine-propanal and carnosine-propanol may provide additional information, reflective of the level of oxidative stress in these tissues. Therefore, measurements of these conjugates are likely to provide more tissue-specific estimates of oxidative stress than the levels of glutathione-derived mercapturic conjugates, which likely originate from a much larger number of organs and tissue sites.

We observed that levels of carnosine propanol were higher in never smokers (Table 2) and had a positive association with never smoking in the adjusted, logistic regression model (Table 4), while there were lower levels of this conjugate in current smokers (Table 2) and there was an inverse association with current smokers in the adjusted model (Table 4).

While the basis of these associations are uncertain, factors such as poor nutrition, or lack of physical activity in smokers may contribute to this observation. Furthermore, because acrolein is highly reactive, it is likely that even in smokers it is readily detoxified by glutathione-linked metabolism in the lungs and other tissues and that there is little direct exposure of the heart, skeletal muscle or the brain to inhaled acrolein. This view is in agreement with the observation that carnosine-propanal and carnosine-propanol were not sensitive to roadway exposure or exposure to other air pollutants such as benzene (Tables 2, 3). We also cannot rule out the possibility that smoking decreases the levels of carnosine in the skeletal muscle or that it inhibits carnosine metabolism thereby leading to a net decrease in conjugate formation. It is also notable that this inverse association remains significant after adjustment for FRS (Supplementary Table 1). Thus, it is smoking behavior per se and

not other risk factors impacting cardiovascular health which appear to drive the association of carnosine propanol with smoking. The levels of carnosine-propanol were also associated with exposure to PM_{2.5} (Table 2), but as with smoking, the association was negative, which may be reflective of generalized oxidative stress induced by PM_{2.5} exposure. Inhalation of PM_{2.5} elicits systemic toxicity, leading to insulin resistance and inflammation in the skeletal muscle (7), which may lead to a decrease in carnosine levels in this tissue.

With regards to demographic variables, we found that levels of urinary, non-conjugated carnosine, carnosine-propanal, and carnosine-propanol were higher in males than females (Table 1–3). This is consistent with previous work showing that the levels of carnosine in the skeletal muscle are generally higher in post-pubescent males than females (11), and that females generally have higher levels of the carnosine-degrading enzyme, carnosinase (46). This observation suggests that the measurements of urinary carnosine-propanal and carnosine-propanol are reflective not only of the level of tissue oxidative stress, but also the levels of tissue carnosine. This is further reinforced by the observation that increasing age was associated with lower levels of carnosine and the acrolein-conjugates (Tables 1–3), an observation which is concordant with previous work showing that aging is not only associated with a decrease in carnosine levels in the skeletal muscle (47, 48), but also with a general loss of muscle mass with age (49). Finally, we observed that Caucasians and Hispanics had generally higher levels of non-conjugated carnosine, carnosine-propanal and carnosine-propanol than Blacks (Tables 1–3). Little is known about ethnic determinants of carnosine levels, which may be dependent upon the balance of carnosine-synthesizing and degrading enzymes. Carnosine levels in skeletal muscle are also sensitive to general nutrition and physical activity because carnosine is present in high amounts in meat and its levels are increased by exercise training (11). However, whether lower levels of carnosine-propanal and carnosine-propanol in Blacks are reflective of differences in such life style factors requires further investigation.

This study is the first of its kind analysis assessing the association of non-conjugated carnosine, and levels of its acrolein conjugates with two risk factors for CVD, smoking and exposure to PM_{2.5}. A large number of participants of diverse demographics and presenting with variable CVD risk were studied. A weakness of this study is that only a single urine sample was collected. Thus, carnosine, and the conjugate levels, might be reflective of only the most recent dietary or physical conditions. However, given our interest in relationships to chronic outcomes, it is likely that variability due to diet and physical conditions would bias the result towards null. It is also noteworthy that we only measured only two conjugates of carnosine (propanol and propanal). While these are the major metabolites of acrolein, an abundant environmental and endogenous aldehyde associated with the incidence of CVD, there may be other, minor conjugates of carnosine (e.g., with 4-hydroxy-trans-2-nonenal), which may also be associated with CVD risk, but were not detected in our study.

In summary, we have found significant, inverse associations between measured levels of carnosine-propanol and exposure to cigarette smoke or air pollution. Additional longitudinal studies are required to fully assess the clinical utility of measuring urinary carnosine-propanal and carnosine-propanol and to establish their ability to predict disease severity and progression.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Association between urinary levels of carnosine propanol and sources of inhaled toxicants.

Illustrated are boxplots depicting the relationships between carnosine-propanol levels (nmole/mg creatinine) and smoking status (A, B). Also illustrated is a boxplot depicting the relationship between daily mean PM_{2.5} levels ($\mu\text{g}/\text{m}^3$) and stratified levels of carnosine propanol (C).

Table 1.

Characteristics of Study Participants Stratified by Urinary Levels of Carnosine

	Total n=605	Low (0.04 : 0.29) n=202	Middle (0.30 : 0.58) n=202	High (0.59 : 6.73) n=201	P
Categorical Variable n (%)					
Gender ***					0.002
Female	322 (53.5)	126 (62.7)	106 (53.0)	90 (44.8)	
Male	280 (46.5)	75(37.3)	94 (47.0)	111 (55.2)	
Ethnicity *					0.021
Black	202 (33.6)	82 (41.0)	54 (26.9)	66 (33.0)	
Caucasian	369 (61.4)	111 (55.5)	138 (68.7)	120 (60.0)	
Smoking Status					
Current smoker	202 (37.8)	68 (38.2)	71 (42.0)	63 (33.5)	0.251
Never smoker	169 (37.1)	48 (31.4)	56 (39.9)	68 (40.2)	0.192
Former smoker	130 (28.5)	48 (31.1)	30 (22.6)	52 (30.8)	0.195
Environmental smoke	41 (32.5)	16 (32.0)	8 (22.2)	17 (42.5)	0.186
Continuous Variable mean (SD)					
Age ***	50.8 (11.4)	54.0 (10.2)	49.6 (11.1)	48.8 (12.1)	<0.001
Creatinine *** (mg/dL)	130 (85.3)	119 (91.9)	129 (98.9)	140 (63.3)	<0.001
Muconic Acid (nm/mg creatinine)	0.19 (0.27)	0.15 (0.22)	0.20 (0.23)	0.22 (0.35)	0.060
HPMA (ng/mg creatinine)	818 (1141)	772 (1173)	802 (1115)	877 (1138)	0.280
Cotinine (µg/g creatinine)	571 (1200)	639 (1377)	573 (1098)	504 (1113)	0.291
Road 50 meters	110 (49.3)	105 (50.7)	107 (45.1)	117 (50.7)	0.300
Distance to major road (m)	562 (624)	531 (590)	620 (662)	542 (623)	0.435
Vehicle meters (m per million)	8.8 (13.6)	7.5 (10.8)	10.1 (15.8)	9.0 (13.9)	0.491
Daily mean PM2.5 *	13.8 (5.7)	14.8 (5.6)	12.9 (5.5)	13.6 (5.8)	0.011

The p-values for categorical values were obtained by a chi-square test, * p<0.05; **p<0.01; ***p<0.001. The p-values for continuous variables were obtained using one-way ANOVA.

*
p<0.05;

**
p<0.01;

p<0.001

Table 2:

Characteristics of Study Participants Stratified by Urinary Levels of Carnosine-Propanol

	Total n=561	Low (0.04 : 0.29) nm/mg creatinine n=187	Middle (0.30 : 0.58) nm/mg creatinine n=187	High (0.59 : 6.73) nm/mg creatinine n=187	P value
Categorical Variable n (%)					
Gender ***					<0.001
Female	294 (52.7)	140 (74.9)	84 (45.2)	70 (37.8)	
Male	264 (47.3)	47 (25.1)	102 (54.8)	115 (62.2)	
Ethnicity ***					<0.001
Black	191 (34.2)	89 (47.9)	61 (32.6)	41 (22.1)	
Caucasian	337 (60.3)	89 (47.9)	120 (64.2)	128 (68.8)	
Hispanic	31 (5.5)	8 (4.3)	6 (3.2)	17 (9.1)	
Smoking Status					
Current smoker *	181 (36.3)	70 (41.7)	62 (38.5)	49 (28.8)	0.037
Never smoker **	169 (37.1)	47 (30.5)	44 (31.7)	78 (48.2)	0.001
Former smoker	129 (28.3)	42 (27.3)	46 (32.8)	41 (25.3)	0.328
Environmental smoke	40 (32.0)	16 (34.0)	14 (29.2)	10 (33.3)	0.864
Continuous Variable mean (SD)					
Age ***	51.3 (11.0)	53.7 (10.7)	50.4 (11.0)	49.7 (10.9)	<0.001
Creatinine *** (mg/dL)	129.6 (85.3)	119 (80.3)	115 (82.9)	141 (83.9)	<0.001
Muconic Acid (nm/mg creatinine)	0.19 (0.27)	0.18 (0.22)	0.16 (0.20)	0.24 (0.38)	0.403
HPMA * (ng/mg creatinine)	820.0 (1141)	976 (1317)	770 (1117)	713 (946)	0.032
Cotinine *** (µg/g creatinine)	571.4 (1199)	883 (1528)	518 (1188)	314 (643)	<0.001
Road 50 meters	110.1 (49.1)	107 (46.9)	111 (48.7)	112 (51.8)	0.681
Distance to major road (m)	563.1 (623.1)	603 (625)	526 (584)	560 (660)	0.285
Vehicle meters (m per million)	8.8 (13.6)	9.5 (13.5)	7.5 (10.6)	9.5 (16.0)	0.292
Daily mean PM2.5 ** (ug/m³)	13.8 (5.7)	14.8 (5.6)	13.2 (5.6)	13.3 (5.8)	0.010

The p-values for categorical values were obtained by a chi-square test, * p<0.05; **p<0.01; ***p<0.001. The p-values for continuous variables were obtained using one-way ANOVA.

*
p<0.05;

**
p<0.01;

p<0.001

Table 3:

Characteristics of Study Participants Stratified by Urinary Levels of Carnosine-Propanal

	Total n=561	Low (0.0 : 0.14) nm/mg creatinine n=281	High (0.15 :1.01) nm/mg creatinine n=280	P
Categorical Variable n (%)				
Gender ***				<0.001
Female	294 (52.7)	191(69.0)	103 (37.2)	
Male	264 (47.3)	90 (32.0)	174 (61.9)	
Ethnicity ***				0.010
Black	191 (34.2)	112 (40.0)	79 (28.3)	
Caucasian	337 (60.3)	156 (55.7)	181 (64.9)	
Hispanic	31 (5.5)	12 (4.3)	19 (6.8)	
Smoking Status				
Current smoker	181 (36.3)	99 (39.8)	82 (32.8)	0.128
Never smoker	169 (37.1)	73 (33.0)	96 (41.0)	0.096
Former smoker	129 (28.3)	60 (27.2)	69 (29.4)	0.674
Environmental smoke	40 (32.0)	22 (33.3)	18 (31.5)	1.000
Continuous Variable mean (SD)				
Age ***	51.3 (11.0)	52.6 (11.3)	49.9 (10.5)	0.003
Creatinine *** (mg/dL)	129.6 (85.3)	104 (80.3)	154 (82.7)	<0.001
Muonic Acid (nm/mg creatinine)	0.19 (0.27)	0.19 (0.24)	0.19 (0.30)	0.916
HPMA (ng/mg creatinine)	820.0 (1141)	895 (1256)	744 (1008)	0.343
Cotinine *** (µg/g creatinine)	571.4 (1199)	767 (1412)	375 (897)	<0.001
Road 50 meters	110.1 (49.1)	108 (48.5)	112 (49.7)	0.351
Distance to major road (m)	563.1 (623.1)	563 (604)	564 (642)	0.291
Vehicle meters (m per million)	8.8 (13.6)	8.2 (11.4)	9.4 (15.3)	0.760
Daily mean PM2.5 (ug/m³)	13.8 (5.7)	13.7 (5.6)	13.8 (5.8)	0.764

The p-values for categorical values were obtained by a chi-square test, * p<0.05; **p<0.01; ***p<0.001. The p-values for continuous variables were obtained using one-way ANOVA.

*
p<0.05;

**
p<0.01;

p<0.001

Table 4.

Associations of Carnosine-Propanol with Smoking Status (n=441)

Variable	Exp (Coefficient)	P
Current Smoker *	0.555	0.008 **
Age *	0.975	0.018 *
Female *	0.350	<0.001 ***
Race-Caucasian	1.472	0.072
Mean-PM _{2.5}	0.969	0.090
Diabetes	1.533	0.070
BMI *	1.028	0.043 *
Beta Blocker	0.847	0.461
Aspirin	0.663	0.074
Diuretics *	0.569	0.017 *
Never Smoking *	1.571	0.039 *
Age *	0.979	0.042 *
Female *	0.360	<0.001 ***
Race-Caucasian	1.494	0.061
Mean-PM _{2.5}	0.967	0.068
Diabetes	1.519	0.077
BMI *	1.031	0.025 *
Beta Blocker	0.895	0.627
Aspirin	0.667	0.078
Diuretics *	0.565	0.015 *

Calculated a logistic regression model (low and high). Associations were adjusted by age, gender, race, diabetes, BMI, daily mean PM_{2.5} and medications,

* p,0.05;

** p<0.01;

*** p<0.001.

Table 5.Association of Daily Mean PM_{2.5} Levels with Urinary Levels of Carnosine Propanol (n=447)

	Variable	Exponential (Coefficient)	P-value
Carnosine propanol (447)	Mean- PM _{2.5} *	0.960	0.020 *
	Age *	0.972	0.004 **
	Female *	0.390	<0.001 ***
	Race-Caucasian	1.484	0.052
	Beta Blocker	0.744	0.154

Calculated using a logistic regression model (low and high). Associations were adjusted for age, gender, race and beta blocker usage.

*
p<0.05;

**
p<0.01;

p<0.001.