

Global Metabolomic Profiling Reveals an Association of Metal Fume Exposure and Plasma Unsaturated Fatty Acids

Yongyue Wei^{1,2}*, Zhaoxi Wang¹, Chiung-yu Chang¹, Tianteng Fan¹, Li Su¹, Feng Chen², David C. Christiani¹*

1 Department of Environmental Health, Harvard School of Public Health, Harvard University, Boston, Massachusetts, United States of America, **2** Department of Epidemiology and Biostatistics, Key Laboratory of Modern Toxicology of Ministry of Education, School of Public Health, Nanjing Medical University, Nanjing, Jiangsu, China

Abstract

Background: Welding-associated air pollutants negatively affect the health of exposed workers; however, their molecular mechanisms in causing disease remain largely unclear. Few studies have systematically investigated the systemic toxic effects of welding fumes on humans.

Objectives: To explore the effects of welding fumes on the plasma metabolome, and to identify biomarkers for risk assessment of welding fume exposure.

Methods: The two-stage, self-controlled exploratory study included 11 boilermakers from a 2011 discovery panel and 8 boilermakers from a 2012 validation panel. Plasma samples were collected pre- and post-welding fume exposure and analyzed by chromatography/mass spectrometry.

Results: Eicosapentaenoic or docosapentaenoic acid metabolic changes post-welding were significantly associated with particulate (PM_{2.5}) exposure ($p < 0.05$). The combined analysis by linear mixed-effects model showed that exposure was associated with a statistically significant decline in metabolite change of eicosapentaenoic acid [$\hat{\beta}$ (95% CI) = $-0.013(-0.022 \sim -0.004)$; $p = 0.005$], docosapentaenoic acid n₃ [$\hat{\beta}$ (95% CI) = $-0.010(-0.018 \sim -0.002)$; $p = 0.017$], and docosapentaenoic acid n₆ [$\hat{\beta}$ (95% CI) = $-0.007(-0.013 \sim -0.001)$; $p = 0.021$]. Pathway analysis identified an association of the unsaturated fatty acid pathway with exposure ($p_{\text{Study-2011}} = 0.025$; $p_{\text{Study-2012}} = 0.021$; $p_{\text{Combined}} = 0.009$). The functional network built by these fatty acids and their interactive genes contained significant enrichment of genes associated with various diseases, including neoplasms, cardiovascular diseases, and lipid metabolism disorders.

Conclusions: High-dose exposure of metal welding fumes decreases unsaturated fatty acids with an exposure-response relationship. This alteration in fatty acids is a potential biological mediator and biomarker for exposure-related health disorders.

Citation: Wei Y, Wang Z, Chang C-y, Fan T, Su L, et al. (2013) Global Metabolomic Profiling Reveals an Association of Metal Fume Exposure and Plasma Unsaturated Fatty Acids. PLoS ONE 8(10): e77413. doi:10.1371/journal.pone.0077413

Editor: Manlio Vinciguerra, University College London, United Kingdom

Received: August 6, 2013; **Accepted:** September 10, 2013; **Published:** October 15, 2013

Copyright: © 2013 Wei et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This work was supported by US NIH grants ES009860 and ES00002. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: dchris@hsph.harvard.edu

These authors contributed equally to this work.

Introduction

The impact of welding-associated air pollutants on the health of exposed workers remains a major concern in occupational medicine and public health [1]. Exposure to fine particles (PM_{2.5}) in ambient air increases the risk for acute disorders [2–5] and chronic diseases [6–10]. Ultra-fine and fine welding particles often contain various metals as a result of combustion; these include iron, nickel, sulfur, copper, vanadium, cadmium, and their oxides [11–13]. Metal particles may induce diverse biological effects, including activation of mitogen-activated protein kinase [14], DNA damage and cell apoptosis [5,15,16], lipid peroxidation

[16,17], and alteration of gene expression [18]. Further, fine metal particles inhaled into the respiratory tract translocate to blood circulation, which ultimately deposits particles in other organs and produces systemic toxic effects.

Recently, metabolomics has attracted increasing interest in the field of toxicology because it is a fast and reproducible method that directly reflects biological events [19,20]. Metabolomics uses a non-targeted approach to obtain an accurate representation of the metabolome, including small molecules involved in highly complex biochemical networks; global changes in the metabolome may relate to particular diseases or phenotypes [21,22]. The use of peripheral fluids, such as plasma or urine, for metabolomic

analysis makes it an attractive method for studying the toxic effects of various exposures such as welding fumes. To date, however, few studies have investigated the systemic toxic effects of welding fumes on humans.

Taking advantage of our well-established occupational cohort of male boilermaker (welder) construction workers, we performed an exploratory metabolomics study using a two-stage, self-controlled design to explore the systemic toxic effects of welding fumes measured as metabolic changes. This study identified biological biomarkers for health risk assessment of welding fume exposure.

Materials and Methods

Ethics Statement

The Institutional Review Board at the Harvard School of Public Health approved the study protocol, and informed written consent was obtained from each adult prior to participation.

Study Design and Population

Eleven non-smoking, non-diabetic boilermakers at an apprentice welding school (Union Local 29, Quincy, MA) were recruited in 2011 to participate in a discovery study (Study-2011); five of the same subjects from Study-2011 and three new subjects were recruited in 2012 (Study-2012) for validation. All participants were selected from the well-characterized occupational cohort of male boilermaker construction workers in Eastern Massachusetts as previously described [23]. The welding school consists of a large, temperature-controlled room outfitted with ten workstations where the welders receive instruction and perform welding, cutting, and grinding techniques. Boilermakers primarily per-

formed shielded metal arc (stick) and gas metal arc welding (TIG), using base metals of mild steel (manganese alloys) and stainless steel (manganese, chromium, and nickel alloys) with electrodes composed mainly of iron with variable amounts of manganese (1~5%). The same breakfast and lunch including turkey, chicken, ham sandwiches and vegetables was provided for each participant during workshop to control the inter-subject variability.

Data Collection

Peripheral blood samples were collected before (pre) and immediately after the ~5 h welding workshop (post) from all subjects. Samples were inventoried and immediately stored at -80°C . Samples of personal, integrated, gravimetric particulate matters with an aerodynamic diameter of ≤ 2.5 μm ($\text{PM}_{2.5}$) were collected over the duration of the welding workshop. A self-administered questionnaire collected information on medical history, medication use, demographics, and smoking history as previously described [13].

Metabolite Profiling

Sample preparation. Frozen samples were sent to Metabolon, Inc. (Durham, NC) and accessioned into the Metabolon LIMS system by a unique identifier associated with the original source only. Samples were prepared using the automated MicroLab STAR[®] system (Hamilton Company, Reno, NV). Recovery standards were added prior to the first step in the extraction process for quality control purposes. Sample preparation used a proprietary series of organic and aqueous extractions to remove proteins while allowing maximum recovery of small molecules. The resulting extract was divided into two fractions:

Table 1. Characteristics of the study population.^a

Characteristic	Study-2011 (n = 11)	Study-2012 (n = 8) ^b
PM _{2.5} total exposure	74.2 ± 27.4	114.9 ± 59.7
Age (years) ^c	49.1 ± 10.2	46.1 ± 11.7
BMI (kg/m ²)	26.5 ± 2.9	28.1 ± 3.7
Male	11	8
Race		
White	9	6
Black	1	0
Hispanic	1	2
Medical history	3	4
Diabetes	0	0
Asthma	0	1
High blood pressure	1	1
High cholesterol hyperlipidemia	2	2
Medication use	4	4
Simvastatin for high cholesterol	2	1
Omeprazole for acid reflux	1	1
Lisinopril for high blood pressure	0	1
Fexofenadine for allergy	1	1
Ibuprofen for headaches	1	0
Albuterol for asthma	0	1
Oxycodone for fractured back	0	1

^aValues presented either as mean ± SD or n;

^bFive subjects participated in both studies;

^cAt study entry.

doi:10.1371/journal.pone.0077413.t001

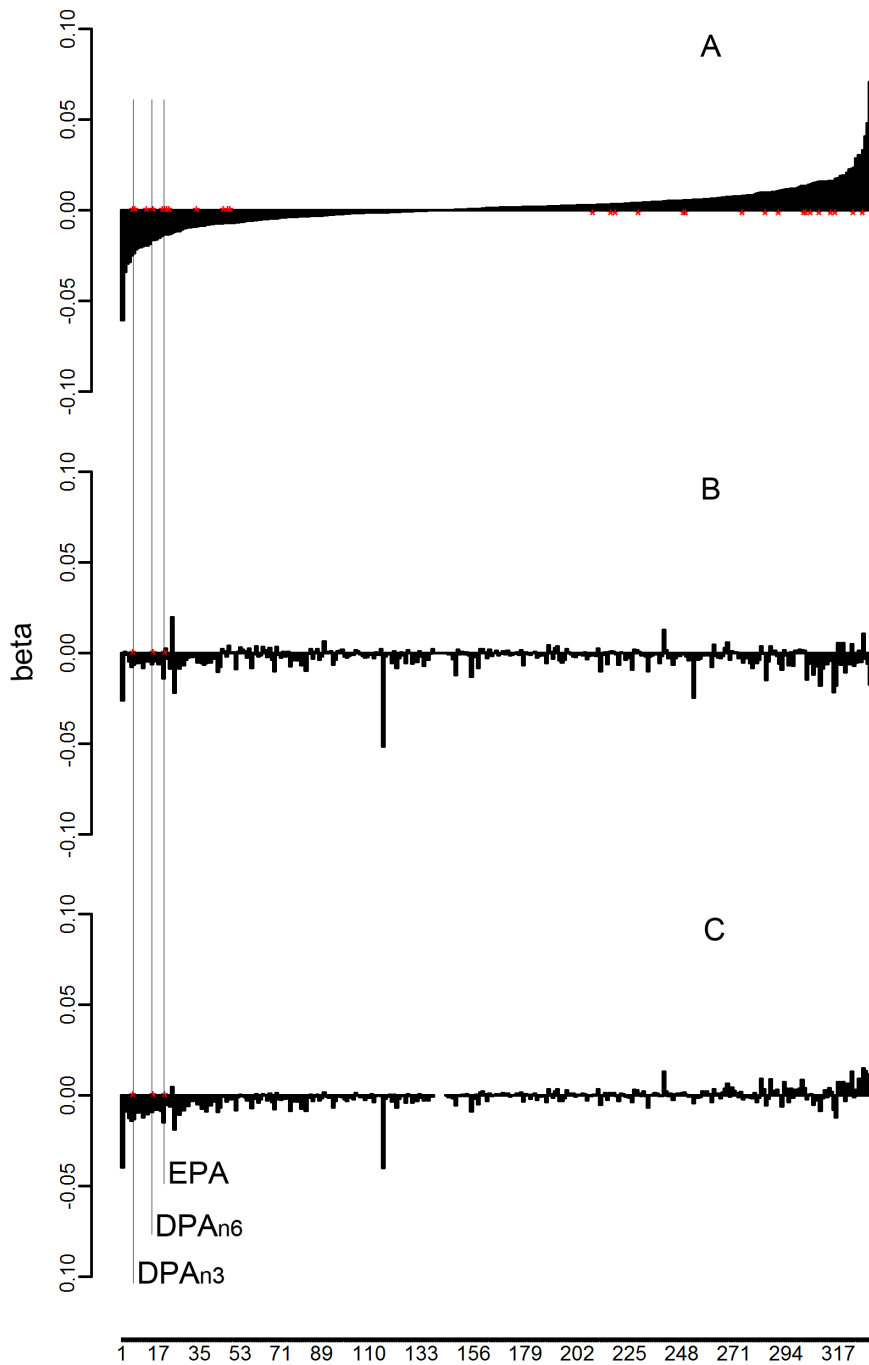


Figure 1. Flowchart for single-compound analysis. Metabolite changes (post-welding workshop – pre-welding workshop) for 333 compounds were analyzed by regression with $PM_{2.5}$ total exposure in Study-2011 (A) and Study-2012 (B), and by linear mixed-effects model in the combined dataset of both studies (C). The y-axis represents the coefficients of exposure in regression models. The x-axis represents the metabolites ordered by the effects of exposure from discovery analysis. Thirty biochemical metabolic changes significantly associated with exposure ($p < 0.05$) in Study-2011. Three of the thirty changes were validated in Study-2012 ($p < 0.05$) and remained significant in combined analysis: eicosapentaenoic acid (EPA) and docosapentaenoic acids (DPA_{n3} , DPA_{n6}).
doi:10.1371/journal.pone.0077413.g001

one for analysis by liquid chromatography (LC), and one for analysis by gas chromatography (GC). Samples were placed briefly on a TurboVap® (Zymark, Westborough, MA) to remove organic solvents, frozen, and dried under vacuum. Extracted samples were split into equal parts for analysis by gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/mass spectrometry (LC/MS) platforms. Several technical replicate samples

were created from a homogeneous pool containing a small amount of each sample.

Instrument variability control. Instrument variability was determined by calculating the median relative standard deviation (RSD) for internal standards added to each sample prior to mass spectrometer injection (RSD = 5%). Overall process variability was determined by calculating the median RSD for all endogenous

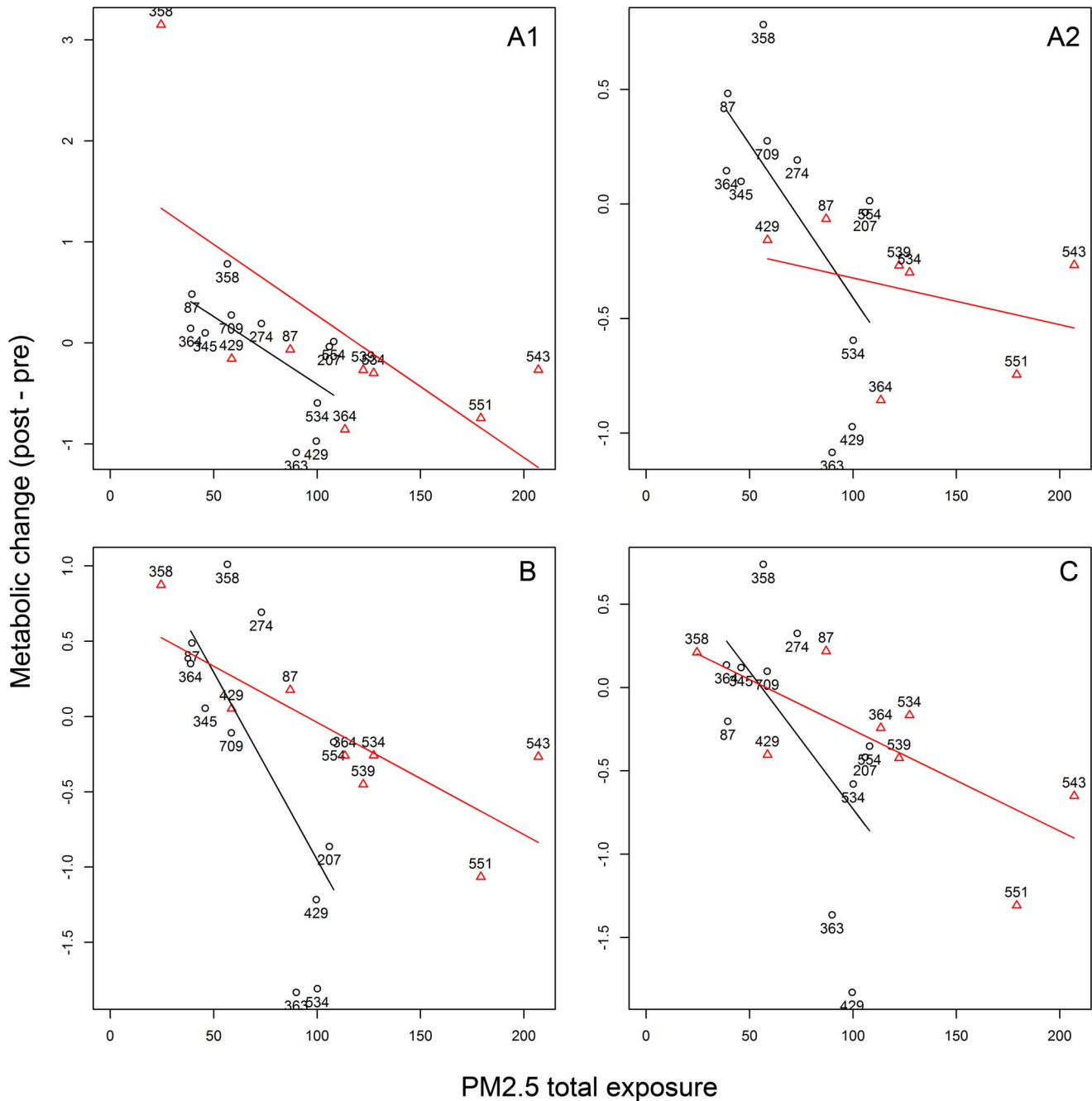


Figure 2. Scatter plots of metabolic changes by exposure of EPA and DPA. Scatter plots illustrate the biochemical compounds that had a metabolic change significantly associated with welding fume exposure: A1) eicosapentaenoic acid (EPA); A2) EPA after removal of a potential outlier (subject ID: 358); B) docosapentaenoic acid n_3 (DPA_{n3}); and C) docosapentaenoic acid n_6 (DPA_{n6}). The x-axis represents total PM_{2.5} exposure during the welding workshop, while the y-axis represents biochemical metabolic change (post-welding workshop – pre-welding workshop). Black circles represent data from Study-2011; red triangles represent data from Study-2012. Each mark is labeled with the subject ID. doi:10.1371/journal.pone.0077413.g002

metabolites (i.e., non-instrument standards) present in pooled technical replicates (RSD = 14%). Both RSD values meet Metabolon's acceptance criteria.

Data extraction and quality assurance. Raw MS data files were loaded into a relational database. Peaks were identified using Metabolon's proprietary peak integration software, and component parts were stored in a separate and specifically designed complex data structure.

Compound identification. Compounds were identified by comparison to library entries of purified standards or recurrent unknown entities. Identification of known chemical entities was based on comparison to the over 1,000 commercially available, purified standard compounds registered in LIMS for distribution to both LC and GC platforms.

Normalization. For studies spanning multiple days, data normalization was performed to correct variation resulting from instrument inter-day tuning differences. Each compound was

Table 2. Metabolites associated with PM_{2.5} metal welding fume exposure.

Metabolite	Baseline		Δ (post - pre)	
	Study-2011	Study-2012	Study-2011	Study-2012
Eicosapentaenoate (EPA; 20:5n3)	1.06±0.44(0.45~1.89)	1.42±0.67(0.70~2.88)	-0.06±0.58(-1.08~0.78)	0.06±1.28(-0.86~3.15)
Docosapentaenoate (DPA _{n3} ; 22:5n3)	1.29±0.71(0.45~2.68)	1.09±0.29(0.65~1.48)	-0.31±0.98(-1.83~1.01)	-0.15±0.56(-1.07~0.87)
Docosapentaenoate (DPA _{n6} ; 22:5n6)	1.32±0.60(0.52~2.4)	1.10±0.33(0.70~1.67)	-0.30±0.75(-1.83~0.74)	-0.35±0.49(-1.31~0.22)

*Values presented as mean ± SD (min~max).
doi:10.1371/journal.pone.0077413.t002

corrected in run-day blocks by equalizing the medians to 1.00 and normalizing each data point proportionately. Normalized metabolites were used for analysis.

Statistical Analysis

Demographics were presented by frequencies for categorical variables and means ± standard deviation (mean ± SD) for continuous variables. The change of each metabolite during welding day (Δ = post-pre) was calculated for each subject. The permutation test with 10,000 perturbations was used to test global metabolic changes between post- and pre-welding workshop samples.

To investigate the association between metabolic change and PM_{2.5} exposure, univariate linear regression was used in Study-2011 (Eq. 1). Compounds with $p \leq 0.05$ were further validated in Study-2012 using the same statistical model, followed by combined analysis of both studies using linear mixed-effects model (LMM) given a random slope of exposure for each study and autoregressive correlation structure (Eq. 2). The multiple comparison was adjusted by false discovery rate (FDR) [24]

$$\Delta_i = \beta \cdot PM_{2.5} total_exposure_i + e_i \quad (1)$$

where $i = 1, \dots, 11$ in Study-2011 and $i = 1, \dots, 8$ in Study-2012.

$$\Delta_{ij} = \alpha + \beta_1 \cdot PM_{2.5} total_exposure_{ij} + \beta_2 \cdot age_{ij} + \beta_3 \cdot medication_use_{ij} + e_{ij} \quad (2)$$

where $i = 1, \dots, 14$ to represent each subject; $j = 1$ or 2 to denote each study (1 = Study-2011; 2 = Study-2012).

For pathway-level association analysis, principal component analysis (PCA) was performed to generate the first principal component (PC₁) from all metabolites of each pathway. PC₁ was used as the response to perform linear regression with exposure in Study-2011 and Study-2012 separately, and perform LMM for combined analysis with adjustment for age and medication use. LMM analysis was performed by R “nlme” package. All statistical analyses were performed using R Version 15.0 (<http://cran.r-project.org>).

The functional network was built using MetaCore™ online software (GeneGo Inc., Carlsbad, CA) to illustrate potential biological connections of significant compounds and interactive genes. Enrichment analysis for disease-associated genes was conducted for each network in MetaCore™. The p value for enrichment analysis was calculated using Eq. 3:

$$p = \frac{R!n!(N-R)(N-n)!}{N!} \sum_{i=\max(r, R+n-N)}^{\min(n, R)} \frac{1}{i!(R-i)!(N-R-n+i)!} \quad (3)$$

where N denotes the total number of genes that were causally associated with all diseases and disorders in MetaCore™; n denotes the total number of genes that were causally associated with the intersecting disease of interest (e.g., lipid metabolism disorders); R denotes the total number of genes/proteins visible in the network; and r denotes the total number of genes/proteins visible in the network intersecting with the disease of interest.

Table 3. Association of PM_{2.5} metal welding fume exposure with metabolite change.

Metabolite	Association analysis ^a				Combined analysis ^{a,b}					
	Study-2011		Study-2012		Univariate analysis			Multivariate analysis		
	Δ95% CI	p	Δ95% CI	p	Δ95% CI	p	q^c	Δ95% CI	p	q^c
Eicosapentaenoate (EPA; 20:5n3)	-0.013(-0.026, -0.001)	0.038	-0.014(-0.027, -0.001)	0.033	-0.013(-0.022, -0.004)	0.005	0.249	-0.014(-0.023, -0.006)	0.003	0.229
Docosapentaenoate (DPA _{n3} ; 22:5n3)	-0.025(-0.044, -0.005)	0.018	-0.007(-0.013, -0.002)	0.017	-0.010(-0.018, -0.002)	0.017	0.313	-0.010(-0.019, -0.001)	0.020	0.398
Docosapentaenoate (DPA _{n6} ; 22:5n6)	-0.016(-0.032, -0.0002)	0.048	-0.006(-0.012, -0.0005)	0.038	-0.007(-0.013, -0.001)	0.021	0.313	-0.021(-0.013, -0.001)	0.029	0.418

^aTotal PM_{2.5} exposure as predictor, metabolite change as response;

^bLinear mixed-effects model was used with/without adjustment for age and medication use;

^c q represented FDR adjusted p value using Benjamini & Hochberg method.

doi:10.1371/journal.pone.0077413.t003

Table 4. Association of PM_{2.5} metal welding fume exposure with metabolite pathways.

Pathway	N ^a	N ₂ ^b	N ₃ ^c	Study-2011 ^d		Study-2012 ^d		Combined analysis ^f		
				VPC ₁ ^e	p	VPC ₁ ^e	p	VPC ₁ ^e	p	p _{adj}
Unsaturated fatty acid	7	7	6	0.84	0.025	0.81	0.021	0.77	0.009	0.013
Phenylalanine & tyrosine metabolism	13	8	1	0.29	0.013	0.36	0.547	0.24	0.425	0.480

^aNumber of biochemical compounds within the same pathway;

^bNumber of biochemical compounds that have the same coefficient direction when regressed by exposure both in Study-2011 and Study-2012;

^cNumber of biochemical compounds with association $p < 0.05$ in combined dataset analyzed by linear mixed model with adjustment of age and medication use;

^dFirst principal component (PC₁) was used as response, with PM_{2.5} total exposure as predictor in linear regression model or linear mixed-effects model;

^eProportion of variance explained by PC₁;

^fPC₁ was used as response in the linear mixed model with random slope with or without adjustment of age and medication use.

doi:10.1371/journal.pone.0077413.t004

Results

Study-2011 consisted of eleven male boilermakers with a mean age of 49.1±10.2 years, average body mass index (BMI) of 26.5±2.9, and of whom 82% were white (Table 1). Three participants reported medical history, two with hyperlipidemia and one with hypertension; four participants reported medication use for high cholesterol, seasonal allergy, asthma, etc. Eight boilermakers were recruited in Study-2012 for validation; the characteristics of this cohort did not significantly differ from Study-

2011 (Table 1). No participants reported metal fever during welding workshop.

Single Biochemical Analysis

Plasma samples were collected from each participant prior to and after a welding workshop for metabolomic profiling. We identified 333 compounds of known biochemical identity that comprised the analysis dataset. In Study-2011, 30 of 333 detected compounds were significantly associated with a metabolic change during welding workshop (Δ) with total PM_{2.5} exposure ($p \leq 0.05$);

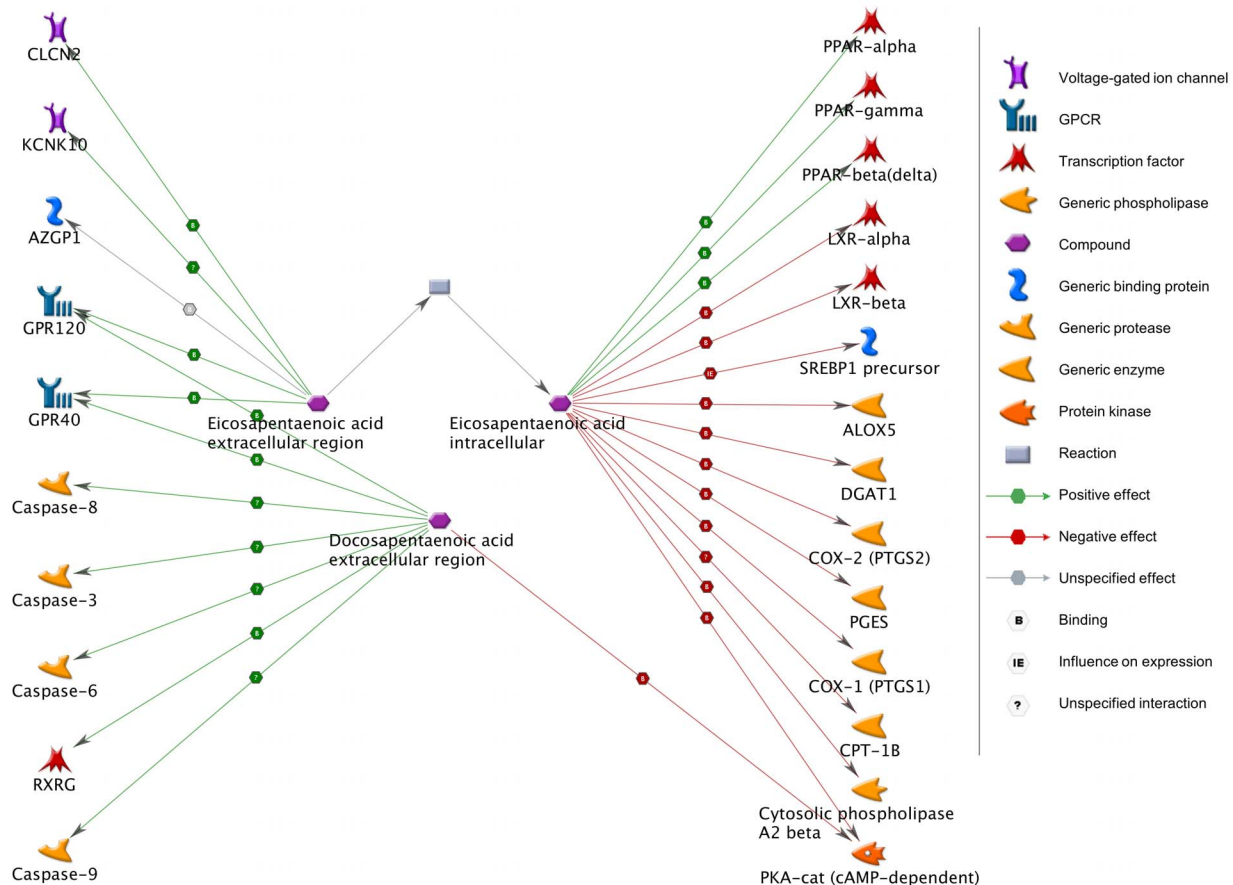


Figure 3. Functional network for EPA and DPA. Network analysis revealed that intracellular and extracellular eicosapentaenoic acid (EPA) interact with 19 genes, while docosapentaenoic acid (DPA) interacts with 7 genes; EPA, DPA_{n3}, DPA_{n6}, and 24 regulated genes were used to build the illustrated functional network. The green line represents activation; the red line represents inhibition; the gray line represents unspecified effects. doi:10.1371/journal.pone.0077413.g003

however, only three passed validation in Study-2012 by the criteria of consistent effect direction of exposure and $p \leq 0.05$ (Figure 1, Table 2). The combined LMM analysis showed that total $PM_{2.5}$ exposure associated with a statistically significant ($p < 0.05$) decline in metabolic change of eicosapentaenoic acid (EPA) ($\hat{\beta} = -0.013/\mu\text{g}/\text{m}^3$; 95% CI = $-0.022 \sim -0.004$; $p = 0.005$; $q = 0.249$), docosapentaenoic acid n_3 (DPA_{n3}) ($\hat{\beta} = -0.010/\mu\text{g}/\text{m}^3$; 95% CI = $-0.018 \sim -0.002$; $p = 0.017$; $q = 0.313$), and docosapentaenoic acid n_6 (DPA_{n6}) ($\hat{\beta} = -0.007/\mu\text{g}/\text{m}^3$; 95% CI = $-0.013 \sim -0.001$; $p = 0.021$; $q = 0.313$) (Figure 2). Adjustment for age and medication use did not affect significance (Table 3). LMM application with random intercepts for each study provided consistent results; however, the model did not reach convergence when random intercept and random slope were simultaneously considered.

In Study-2012, one subject (ID: 358) exhibited EPA metabolic changes much larger than others. These data were therefore removed for subsequent sensitivity analysis, which displayed a consistent trend while losing statistical significance ($p = 0.447$); the combined analysis remained statistically significant in univariate analysis ($p = 0.030$) and multivariate analysis ($p = 0.041$) (Figure 2-A2).

The five subjects who participated in both studies were also separately assessed by sensitivity analysis using an LMM with random slopes for each subject. Borderline statistical significance remained for EPA ($p_{\text{Study-2011}} = 0.070$; $p_{\text{Study-2012}} = 0.088$; $p_{\text{Combined}} = 0.042$; $p_{\text{Combined}} = 0.012$ with adjustment for age and medication use) and DPA_{n3} ($p_{\text{Study-2011}} = 0.041$; $p_{\text{Study-2012}} = 0.031$; $p_{\text{Combined}} = 0.003$; $p_{\text{Combined}} = 0.066$ with adjustment for age and medication use). LMM with random intercepts for each subject yielded similar results.

Conversely, sensitivity analysis was performed excluding the five subjects from Study-2011 who participated in both studies; this analysis included six subjects for Study-2011 and eight subjects for Study-2012. Combined analysis remained significant or borderline significant for EPA ($p = 0.046$), DPA_{n3} ($p = 0.064$), and DPA_{n6} ($p = 0.020$), even after adjustment for age and medication use.

Pathway Analysis

EPA, DPA_{n3} , and DPA_{n6} participate in the biosynthesis of unsaturated fatty acids (KEGG entry: map01040). To explore whether this pathway connects metabolites associated with $PM_{2.5}$ metal fume exposure, we performed PCA to integrate multiple biochemicals within each pathway. While two of 63 pathways significantly associated with PM exposure ($p \leq 0.05$) in Study-2011, only the unsaturated fatty acids pathway was replicated in Study-2012 ($p_{\text{Study-2011}} = 0.025$; $p_{\text{Study-2012}} = 0.021$) and appeared in the combined analysis ($p = 0.009$, $q = 0.145$; $p_{\text{adj}} = 0.013$, $q = 0.204$) (Table 4). Seven biochemical molecules were detected in the unsaturated fatty acids pathway for which metabolic change negatively associated with exposure dosage; six of the seven molecules were statistically significant by combined analysis of both studies (Table S1).

Functional Network Analysis

To explore interactions of EPA and DPA with specific genes, we performed a functional network analysis. EPA extracellular and intracellular metabolites interacted with 19 genes; DPA interacted with 7 genes. All metabolite compounds and 24 interactive genes were then used to build a network (Figure 3). Using *a priori* knowledge, the top five diseases that associated with the most interactive genes within the network (21/24 genes) were endocrine system diseases ($p = 1.6 \times 10^{-60}$), neoplasms ($p = 2.0 \times 10^{-59}$), lung

disease ($p = 2.0 \times 10^{-59}$), respiratory tract diseases ($p = 2.0 \times 10^{-59}$), and digestive system diseases ($p = 2.0 \times 10^{-59}$). Additionally, 16 of 24 genes associated with cardiovascular disease ($p = 1.3 \times 10^{-41}$), and 9 of 24 genes associated with lipid metabolism disorders/hyperlipidemias ($p = 2.2 \times 10^{-27}$). These disease enrichment profiles remained significant when all seven compounds were used to build a network by the same approach.

Discussion

A challenge to understand the adverse effects of welding fume exposure is the complex nature of environmental and behavioral influences. Metabolic profiles are greatly affected by diet, smoking behavior, medical history, or even time [25–29]. Kuo *et al.* recruited non-smoking, non-alcohol drinking welders for a metabolomic study, but intra-subject variation was not considered [30]. Here, we excluded smoking or diabetic boilermakers to reduce such confounding effects. A self-controlled design eliminated intra-subject variation, and a two-stage design avoided chance findings in our limited sample size.

The main finding of this study is that unsaturated fatty acids are consistently reduced by respirable welding fume exposure, with a significant negative exposure-response relationship. To date, many studies have investigated the beneficial effects of EPA and DHA in neural function [31], tumor suppression [32], cardiovascular event risk reduction [33], diabetes mellitus [34,35], anti-inflammatory activity [36], and blood lipid reduction [37]. Although the roles of DPA have not yet been systematically examined because of the lack of available pure DPA, there is some evidence that DPA has stronger beneficial health effects than EPA and DHA *in vitro* [38]. We also detected a global metabolite change, which may be explained by diet during work shift, circadian variation [30], or $PM_{2.5}$ exposure.

Surprisingly, the functional network built from unsaturated fatty acids includes 24 genes that showed very intense biological functions related to various diseases, including those involving endocrine system diseases, respiratory diseases, neoplasms, cardiovascular diseases, and lipid metabolism disorders. Intracellular EPA activates peroxisome proliferator-activated receptors (PPARs) [39], which are implicated in the pathology of numerous diseases including obesity, diabetes, atherosclerosis, and cancer (RefSeq, Jul 2008). EPA also inhibits *COX-1/COX-2* [40], which encode proteins that may promote cell proliferation during tumor progression (RefSeq, Sep 2012). DPA activates *RXR α* [41], a member of the retinoid X receptor family that is involved in mediating the anti-proliferative effects of retinoic acid and is down-regulated in several types of human cancers, including lung cancer [42]; DPA also activates cysteine-aspartic acid protease (Caspase) family members, which are involved in cancer cell apoptosis [43]. The functional network analysis strengthens the hypothesis that the decline of unsaturated fatty acids is a potential mediator of multiple health disorders in boilermakers.

Previous studies demonstrate the association of welding fume exposure on decline of heart rate variability (HRV), an important indicator of cardiovascular disease morbidity [4,44,45]. Interestingly, several recent studies show that supplementation of EPA and DPA benefit HRV in healthy adults, cardiovascular disease risk populations, or in cardiovascular disease patients [46–50]. These results strongly indicate that the metabolic change of unsaturated fatty acids is an important biological mediator of exposure-related decreases in HRV.

Despite these findings, we recognize limitations to the study: we lack metabolite information from a non-welding day, and p values were not small enough to pass multiple comparison correction due

to small sample size. However, pathway-based analysis showed an acceptable FDR ($q < 0.2$), and the two-stage, self-controlled study design helped reduce chance findings.

In summary, this exploratory study shows evidence that high dose exposure of metal welding fumes decreases unsaturated fatty acids with an exposure-response relationship. The metabolic change in unsaturated fatty acids is a potential biomarker for exposure-related health disorders for which further studies are encouraged.

Supporting Information

Table S1 Association analysis for metal welding fume exposure and metabolic change of biochemical compounds of unsaturated fatty acid pathway.

References

- Hobson A, Seixas N, Sterling D, Racette BA (2011) Estimation of particulate mass and manganese exposure levels among welders. *Ann Occup Hyg* 55: 113–125.
- Antonini JM, Krishna Murthy GG, Brain JD (1997) Responses to welding fumes: lung injury, inflammation, and the release of tumor necrosis factor- α and interleukin-1 β . *Exp Lung Res* 23: 205–227.
- Hassaballa HA, Lateef OB, Bell J, Kim E, Casey L (2005) Metal fume fever presenting as aseptic meningitis with pericarditis, pleuritis and pneumonitis. *Occup Med (Lond)* 55: 638–641.
- Cavallari JM, Eisen EA, Fang SC, Schwartz J, Hauser R, et al. (2008) PM_{2.5} metal exposures and nocturnal heart rate variability: a panel study of boilermaker construction workers. *Environ Health* 7: 36.
- Mukherjee S, Palmer LJ, Kim JY, Aeschliman DB, Houk RS, et al. (2004) Smoking status and occupational exposure affects oxidative DNA injury in boilermakers exposed to metal fume and residual oil fly ash. *Cancer Epidemiol Biomarkers Prev* 13: 454–460.
- Szram J, Schofield SJ, Cosgrove MP, Cullinan P (2012) Welding, longitudinal lung function decline and chronic respiratory symptoms: a systematic review of cohort studies. *Eur Respir J*.
- Manette A, Brennan P, Zaridze D, Szeszenia-Dabrowska N, Rudnai P, et al. (2012) Welding and lung cancer in Central and Eastern Europe and the United Kingdom. *Am J Epidemiol* 175: 706–714.
- Keskinen H, Kalliomaki PL, Alanko K (1980) Occupational asthma due to stainless steel welding fumes. *Clin Allergy* 10: 151–159.
- Ibft E, Bonde JP, Hansen J (2010) Exposure to metal welding fume particles and risk for cardiovascular disease in Denmark: a prospective cohort study. *Occup Environ Med* 67: 772–777.
- Hauser R, Eisen EA, Pothier L, Lewis D, Bledsoe T, et al. (2002) Spirometric abnormalities associated with chronic bronchitis, asthma, and airway hyperresponsiveness among boilermaker construction workers. *Chest* 121: 2052–2060.
- van der Wal JF (1986) Further studies on the exposure of welders to fumes, chromium, nickel and gases in Dutch industries: plasma welding and cutting of stainless steel. *Ann Occup Hyg* 30: 153–161.
- van der Wal JF (1985) Exposure of welders to fumes, Cr, Ni, Cu and gases in Dutch industries. *Ann Occup Hyg* 29: 377–389.
- Kim JY, Hauser R, Wand MP, Herrick RF, Amarasiwardena CJ, et al. (2003) The association of expired nitric oxide with occupational particulate metal exposure. *Environ Res* 93: 158–166.
- Tessier DM, Pascal LE (2006) Activation of MAP kinases by hexavalent chromium, manganese and nickel in human lung epithelial cells. *Toxicol Lett* 167: 114–121.
- Antonini JM, Leonard SS, Roberts JR, Solano-Lopez C, Young SH, et al. (2005) Effect of stainless steel manual metal arc welding fume on free radical production, DNA damage, and apoptosis induction. *Mol Cell Biochem* 279: 17–23.
- Chuang CH, Huang CE, Chen HL (2010) DNA strand breakage and lipid peroxidation after exposure to welding fumes in vivo. *Mutagenesis* 25: 71–76.
- Geleskul Iu F, Gorbani LN, Baida NA, Voitkevich VG, Kornuta NA (1987) [Lipid peroxidation in the tissues of animals as an index of the biological aggressiveness of welding aerosols]. *Vrach Delo*: 108–110.
- Wang Z, Neuburg D, Li C, Su L, Kim JY, et al. (2005) Global gene expression profiling in whole-blood samples from individuals exposed to metal fumes. *Environ Health Perspect* 113: 233–241.
- Dieterle F, Schlotterbeck G, Ross A, Niederhauser U, Senn H (2006) Application of metabolomics in a compound ranking study in early drug development revealing drug-induced excretion of choline into urine. *Chem Res Toxicol* 19: 1175–1181.
- Robertson DG, Watkins PB, Reily MD (2011) Metabolomics in toxicology: preclinical and clinical applications. *Toxicol Sci* 120 Suppl 1: S146–170.
- Stewart JD, Bolt HM (2011) Metabolomics: biomarkers of disease and drug toxicity. *Arch Toxicol* 85: 3–4.
- Nicholson JK, Connelly J, Lindon JC, Holmes E (2002) Metabonomics: a platform for studying drug toxicity and gene function. *Nat Rev Drug Discov* 1: 153–161.
- Chen JC, Cavallari JM, Stone PH, Christiani DC (2007) Obesity is a modifier of autonomic cardiac responses to fine metal particulates. *Environ Health Perspect* 115: 1002–1006.
- Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society Series B* 57: 12.
- Sulochana G, Arunagiri R (1978) Effect of smoking on excretion of tryptophan metabolite in urine-3-hydroxy anthranilic acid. *Indian J Exp Biol* 16: 507–508.
- Xu T, Holzapfel C, Dong X, Bader E, Yu Z, et al. (2013) Effects of smoking and smoking cessation on human serum metabolite profile: results from the KORA cohort study. *BMC Med* 11: 60.
- Saleem F, Ametaj BN, Bouatra S, Mandal R, Zebeli Q, et al. (2012) A metabolomics approach to uncover the effects of grain diets on rumen health in dairy cows. *J Dairy Sci* 95: 6606–6623.
- Wang-Sattler R, Yu Z, Herder C, Messias AC, Floegel A, et al. (2012) Novel biomarkers for pre-diabetes identified by metabolomics. *Mol Syst Biol* 8: 615.
- Ang JE, Revell V, Mann A, Mantele S, Otway DT, et al. (2012) Identification of human plasma metabolites exhibiting time-of-day variation using an untargeted liquid chromatography-mass spectrometry metabolomic approach. *Chromatogr Int* 29: 868–881.
- Wang KC, Kuo CH, Tian TF, Tsai MH, Chiung YM, et al. (2012) Metabolomic characterization of laborers exposed to welding fumes. *Chem Res Toxicol* 25: 676–686.
- Dyall SC, Michael GJ, Michael-Titus AT (2010) Omega-3 fatty acids reverse age-related decreases in nuclear receptors and increase neurogenesis in old rats. *J Neurosci Res* 88: 2091–2102.
- Tsuji M, Murota SI, Morita I (2003) Docosapentaenoic acid (22: 5, n-3) suppressed tube-forming activity in endothelial cells induced by vascular endothelial growth factor. *Prostaglandins Leukot Essent Fatty Acids* 68: 337–342.
- von Schacky C (2012) Meta-analysing randomised controlled trials with omega-3 fatty acids in cardiovascular disease. *Evid Based Med*.
- Poole CD, Halcox JP, Jenkins-Jones S, Carr ES, Schifflers MG, et al. (2013) Omega-3 Fatty acids and mortality outcome in patients with and without type 2 diabetes after myocardial infarction: a retrospective, matched-cohort study. *Clin Ther* 35: 40–51.
- Wu JH, Micha R, Imamura F, Pan A, Biggs ML, et al. (2012) Omega-3 fatty acids and incident type 2 diabetes: a systematic review and meta-analysis. *Br J Nutr* 107 Suppl 2: S214–227.
- Calder PC (2012) The role of marine omega-3 (n-3) fatty acids in inflammatory processes, atherosclerosis and plaque stability. *Mol Nutr Food Res* 56: 1073–1080.
- Jacobson TA, Glickstein SB, Rowe JD, Soni PN (2012) Effects of eicosapentaenoic acid and docosahexaenoic acid on low-density lipoprotein cholesterol and other lipids: a review. *J Clin Lipidol* 6: 5–18.
- Kaur G, Cameron-Smith D, Garg M, Sinclair AJ (2011) Docosapentaenoic acid (22: 5n-3): a review of its biological effects. *Prog Lipid Res* 50: 28–34.
- Desvergne B, Wahli W (1999) Peroxisome proliferator-activated receptors: nuclear control of metabolism. *Endocr Rev* 20: 649–688.
- Ringbom T, Huss U, Stenholm A, Flock S, Skattebol L, et al. (2001) Cox-2 inhibitory effects of naturally occurring and modified fatty acids. *J Nat Prod* 64: 745–749.
- Goldstein JT, Dobrzyn A, Clagett-Dame M, Pike JW, DeLuca HF (2003) Isolation and characterization of unsaturated fatty acids as natural ligands for the retinoid-X receptor. *Arch Biochem Biophys* 420: 185–193.
- Picard E, Seguin C, Monhoven N, Rochette-Egly C, Siat J, et al. (1999) Expression of retinoid receptor genes and proteins in non-small-cell lung cancer. *J Natl Cancer Inst* 91: 1059–1066.

43. Fiandalo MV, Kyprianou N (2012) Caspase control: protagonists of cancer cell apoptosis. *Exp Oncol* 34: 165–175.
44. Tsuji H, Larson MG, Venditti EJ, Jr., Manders ES, Evans JC, et al. (1996) Impact of reduced heart rate variability on risk for cardiac events. The Framingham Heart Study. *Circulation* 94: 2850–2855.
45. Scharrer E, Hessel H, Kronseder A, Guth W, Rolinski B, et al. (2007) Heart rate variability, hemostatic and acute inflammatory blood parameters in healthy adults after short-term exposure to welding fume. *Int Arch Occup Environ Health* 80: 265–272.
46. Mozaffarian D, Stein PK, Princeas RJ, Siscovick DS (2008) Dietary fish and omega-3 fatty acid consumption and heart rate variability in US adults. *Circulation* 117: 1130–1137.
47. Ninio DM, Hill AM, Howe PR, Buckley JD, Saint DA (2008) Docosahexaenoic acid-rich fish oil improves heart rate variability and heart rate responses to exercise in overweight adults. *Br J Nutr* 100: 1097–1103.
48. Romieu I, Tellez-Rojo MM, Lazo M, Manzano-Patino A, Cortez-Lugo M, et al. (2005) Omega-3 fatty acid prevents heart rate variability reductions associated with particulate matter. *Am J Respir Crit Care Med* 172: 1534–1540.
49. Sauder KA, Skulas-Ray AC, Campbell TS, Johnson JA, Kris-Etherton PM, et al. (2013) Effects of omega-3 Fatty Acid supplementation on heart rate variability at rest and during acute stress in adults with moderate hypertriglyceridemia. *Psychosom Med* 75: 382–389.
50. Villa B, Calabresi L, Chiesa G, Rise P, Galli C, et al. (2002) Omega-3 fatty acid ethyl esters increase heart rate variability in patients with coronary disease. *Pharmacol Res* 45: 475.