GLOBAL METABOLOMIC REVEAL METABOLITE PERTURBATIONS ASSOCIATED WITH DIOXIN EXPOSURE IN A CHINESE MALE WORKER COHORT

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Introduction

Dioxins are a range of polychlorinated aryl hydrocarbons belonging to two families, namely polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzo-furans (PCDFs)[1]. Dioxins are persistent organic pollutants (POPs) that are extremely stable with half-lives of 7-11 years[2] and the first POPs to be included in the Stockholm Convention. They are ubiquitous in environment and thus cause widespread exposure of humans and animals. Dioxins can cause a variety of health hazards to human beings by interfering with the aryl hydrocarbon receptor (AhR), including inducing cancer, triggering disorders of immune, reproductive development and endocrine system, etc[3]. However, previous dioxin toxicity studies primarily focused on the activation of AhR with signaling pathways at gene and protein levels. The links between dioxin-medicated AhR activation and physiological effects of toxicity remain largely unknown and need to be further identified through the evaluation of downstream metabolic processes. Mass spectrometry (MS)-based metabolomics that measures changes in small molecule downstream of the genome, transcriptome and proteome can captures the terminal dynamic alteration of metabolites in response to external stressors[4]. This technique has been expanded quickly and used to investigate biological effects of many environmental contaminants in humans[5]. Therefore, it is worthy conducting a global metabolomics study to investigate metabolic perturbations and toxic mechanisms possibly related to dioxin exposure in humans. In this study, metabolic profiles were investigated on human serum samples with the hope to understand the underlying mechanisms of adverse health risks associated with dioxin exposure.

Materials and Methods

Serum samples of 215 male workers from a waste incineration power plant in Shenzhen were collected. To begin with, dioxin quantification was conducted based on the US EPA method 1613 with some minor modifications using high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS). Then, 48 and 47 healthy participants with dioxin exposure concentration of 75% or more and of 25% or less based on quartile distribution of the serum dioxin concentrations were further selected for metabolomics analysis. After a simple protein precipitation, the metabolic profiling analysis of the serum samples were performed using an ultrahigh-performance liquid chromatography system coupled to a QExactive focus hybrid quadrupole-orbitrap mass spectrometer.

Results and discussion

Demographic characteristics of the two groups are listed in Table 1. The serum TEQ values were statistically significant between the two groups ($p < 0.001$). As a result, we presumed serum TEQ values to be the key factors leading to the potential health effects. It was found that there were significant differences in smoking and education levels between the two groups. Therefore, the relationship between metabolites and serum dioxin exposure levels was investigated by using partial correlation analysis adjusted by age, BMI, smoking status, drinking frequency and education level.

Table 1. Demographic characteristics of the two groups for metabolomics study.

Characteristics	High dioxin exposure group (n=48)	Low dioxin exposure group (n=47)	*p*-value
TEQ value (pg TEQ/g lipid)	48.71 (29.49-765.35) [a]	8.16 (3.29-9.94) [a]	<0.001
Age (years)	27.54 (4.47) [b]	27.81 (4.72) [b]	0.731
Height (cm)	171.35 (3.91) [b]	170.79 (5.69) [b]	0.773
Weight (kg)	65.85 (14.06) [b]	66.19 (12.62) [b]	0.720
BMI (kg/m^2)	22.36 (4.84) [b]	22.85 (3.86) [b]	0.207
Cigarette Smoking			
Never	38	17	
Past	0	3	<0.001
Current	10	27	
Alcohol Drinking			
Never	45	40	0.263
Once/twice a week	2	6	

More than 3 times a week	1	1	
Education Level			
Less than high school	6	16	
High school	14	16	<0.05
More than high school	28	15	

a Expressed as medium (min-max).
b Expressed as mean (SD); SD: standard deviation.

The obtained data was imported into software SIMCA-P (Version 14.1, Umetrics, Umea, Sweden) for multivariable statistical analysis. A partial least-squares discriminant analysis (PLS-DA), a supervised pattern recognition approach, was used for group differentiation between the high dioxin exposure group and low dioxin exposure group following unit variance scaling (Figure 1A and B). The results indicated that dioxin exposure may significantly perturb small molecule metabolisms. The developed PLS-DA models were further validated by using the 500-time permutation test (Figure 1C and D). The results showed that the PLS-DA models were not overfitting.

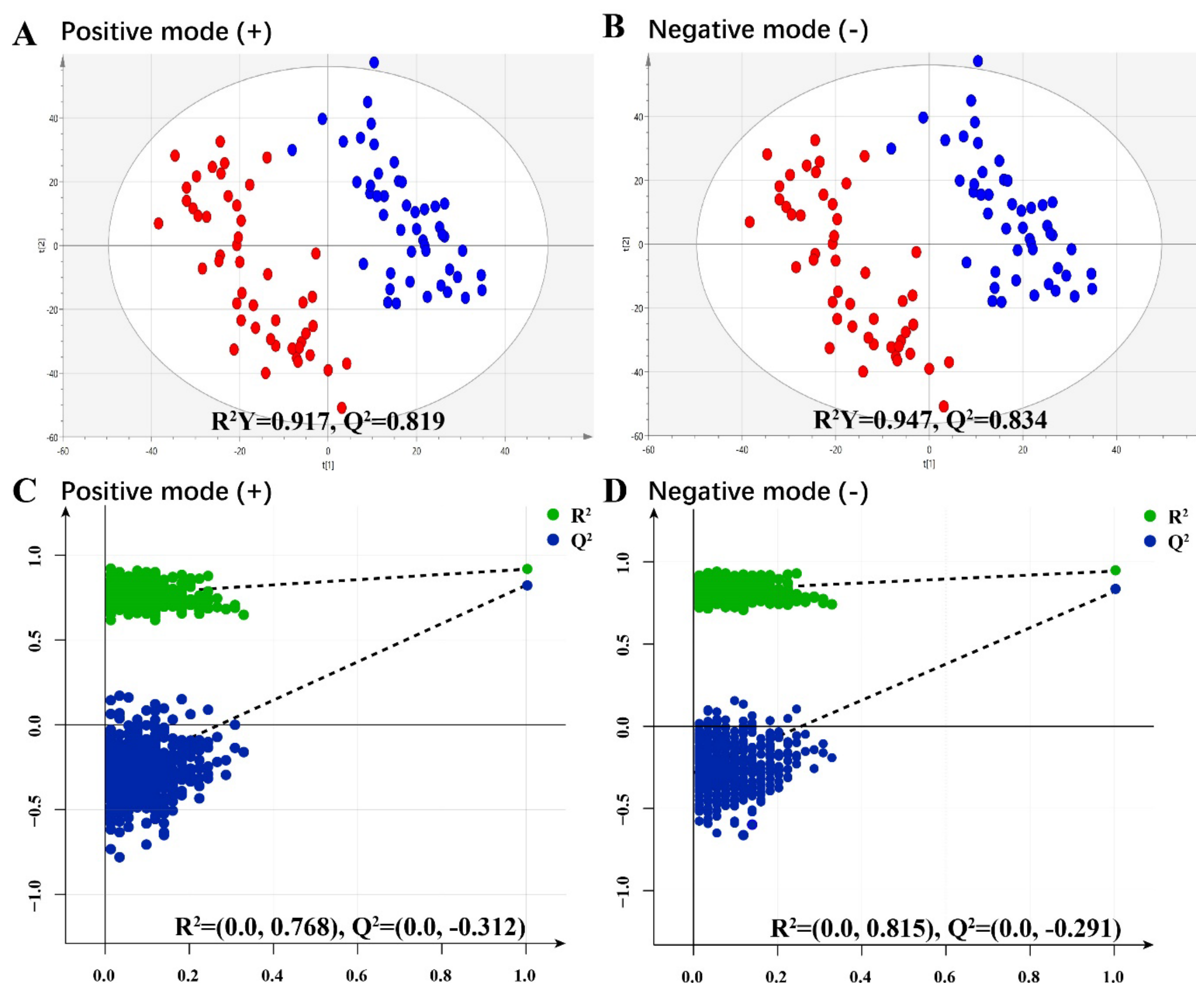

Figure 1. Scatter plots obtained from PLS-DA models: LC-MS-ESI positive-ion mode (A) and LC-MS-ESI negative-ion mode (B). The blue triangles and green triangles indicate for low dioxin exposure group and high dioxin exposure group, respectively. Validation of the developed PLS-DA models using the 500-time permutation tests: validation plot for positive-ion mode (C) and negative-ion model (D). R^2 = the goodness of fit (green circle); Q^2 = the predictive capability (blue circle).

The differential metabolites between the two groups were selected based on three criteria: (1) the variable importance in projection (VIP) scores in PLS-DA models >1; (2) the p-values in Mann-Whitney U test between the two groups <0.05; (3) the p-values in partial correlation analysis after adjustment by age, BMI, smoking status, drinking frequency and education level <0.05. After the screening and identification with standard materials, a total of 20 metabolites were identified as potential biomarkers, including acylcarnitines, fatty acids and derivatives, glycerophospholipids, etc. (Table 2). The identified metabolites changed linearly along with the TEQ values (Figure 2).

Table 2. Summary of the 20 potential biomarkers.

No	Metabolites [a]	FC [b]	*p*-value [c]	VIP	Class	Related pathway
1	Tetradecanoylcarnitine [a]	1.86	1.06E-04	1.46	Acylcarnitines	β-Oxidation of fatty acid
2	Decanoylcarnitine [a]	2.10	1.84E-04	1.47	Acylcarnitines	β-Oxidation of fatty acid
3	L-Palmitoylcarnitine [a]	1.58	1.67E-03	1.44	Acylcarnitines	β-Oxidation of fatty acid
4	Palmitamide	2.56	2.43E-08	2.28	Fatty acids and conjugates	β-Oxidation of fatty acid
5	3-Hydroxycapric acid [a]	0.58	2.15E-04	1.67	Fatty acids and conjugates	β-Oxidation of fatty acid
6	Prostaglandin H_2 (PGH_2)	3.27	1.17E-10	2.40	Fatty acids and conjugates	Arachidonic acid metabolism
7	Arachidonic acid (AA)	0.87	1.86E-03	1.40	Fatty acids and conjugates	Arachidonic acid metabolism
8	Stearidonic acid	1.28	8.27E-03	1.27	Fatty acids and conjugates	α-Linolenic acid metabolism
9	9-OxoODE	0.55	1.35E-05	2.23	Fatty acids and conjugates	Linoleic acid metabolism
10	Octadecanamide	2.95	7.37E-09	2.35	Fatty acids and conjugates	/
11	Glycerophospho-N-palmitoyl ethanolamine (GP-NPEA)	1.50	5.07E-05	2.05	Fatty acids and conjugates	/
12	N-Oleoylserine	1.34	4.01E-07	2.06	Fatty acids and conjugates	/
13	PC (18:1/18:1)	0.34	4.47E-04	1.74	Glycerophospholipids	Glycerophospholipid metabolism
14	LPC (16:0/0:0)	1.44	4.34E-04	1.75	Glycerophospholipids	Glycerophospholipid metabolism
15	LPE (16:0/0:0)	0.60	2.70E-05	1.53	Glycerophospholipids	Glycerophospholipid metabolism
16	Sphingosine-1-phosphate (S1P) [a]	1.55	3.97E-04	1.45	Sphingolipids	Sphingolipid metabolism
17	Adenosine monophosphate (AMP) [a]	1.67	7.92E-08	2.57	Purines and purine derivatives	Purine metabolism
18	Xanthine [a]	0.59	2.61E-03	1.04	Purines and purine derivatives	Purine metabolism
19	Indolelactic acid [a]	1.88	4.37E-02	1.33	Tryptophan metabolites	Tryptophan metabolism
20	Aspartic acid [a]	0.90	3.38E-02	1.13	Amino acids	Aspartic acid metabolism

a The metabolite was identified by authentic standard. b FC (fold change) represented the ratio of peak intensity of high dioxin exposure group to low dioxin exposure group. c *p*-value was obtained by Mann-Whitney U test between peak intensities of the metabolite in the two groups.

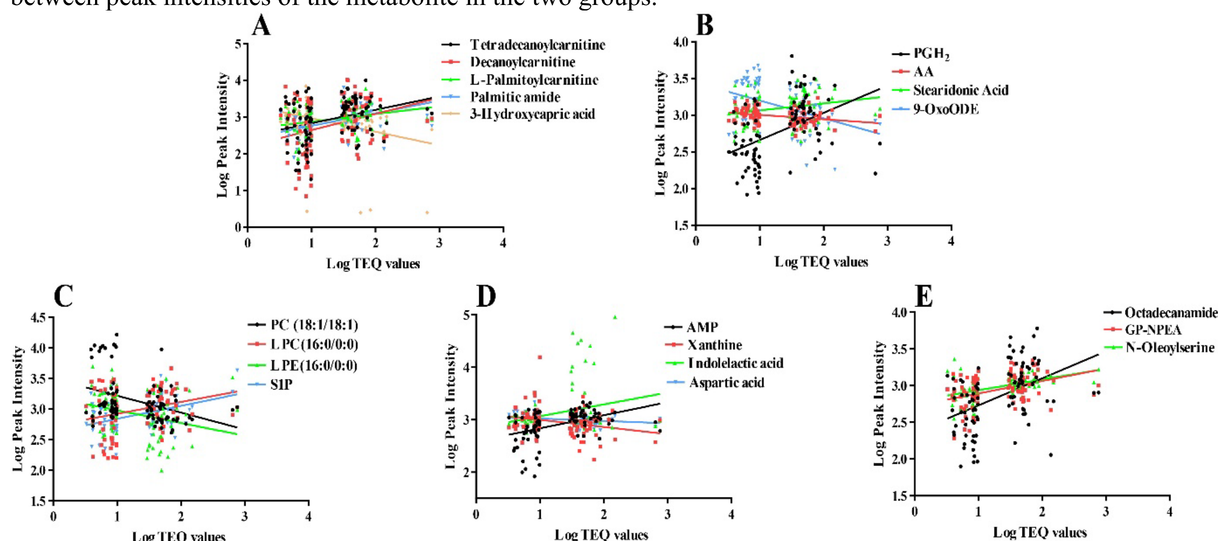

Figure 2. Linear fit graphs of log TEQ value and log peak intensity of the 20 potential biomarkers.

Pathway analysis based on the identified metabolites was carried out using MetaboAnalyst according to the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database (www.genome.jp/kegg/). These results suggested metabolic pathways such as fatty acid β-oxidation, essential fatty acid metabolism, arachidonic acid metabolism, glycerophospholipid and sphingolipid metabolism and purine metabolism were disturbed after dioxin exposure (Figure 3).

Because the fatty acid β-oxidation plays a key role in energy homeostasis in organs especially in the liver[6], the noticed downregulation of fatty acid β-oxidation suggested that people with high dioxin exposure may be at risk of liver diseases. The significant up-regulations of PGH_2, LPC (16:0/0:0), LPE (16:0/0:0) and sphingosine-1-phosphate implied that high level of dioxin exposure may be associated with inflammation. These findings implied the associations between dioxin exposure and potential adverse health risks including liver diseases and inflammation. This study provides important evidence for the health hazards of dioxins.

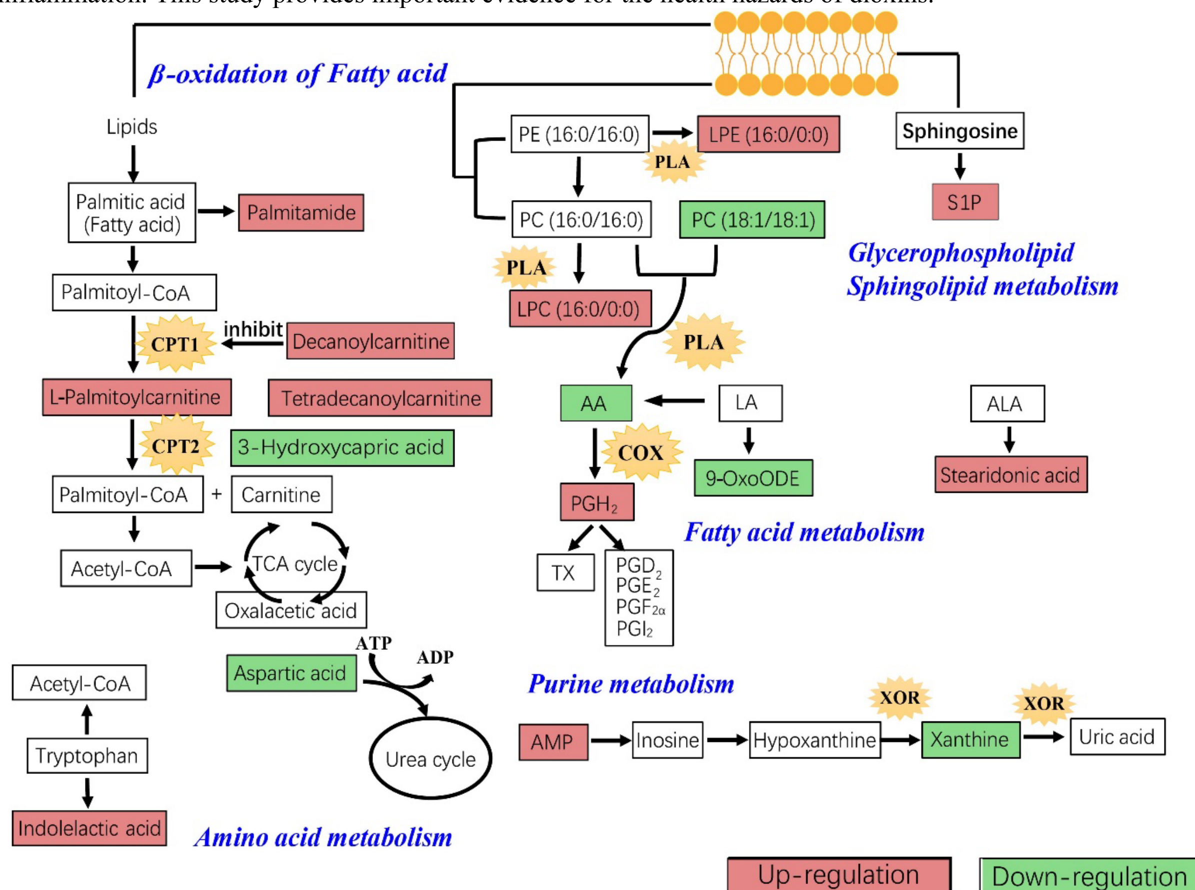

Figure 3. Disturbed signaling pathway.

Acknowledgements

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