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An environmental wide association study (EWAS) approach to the metabolic syndrome☆

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ABSTRACT

Background: Environmental contaminants have previously been linked to components of the Metabolic Syndrome (MetS). However, exposure to environmental contaminants is in part determined by various lifestyle factors.
Objective: Using an "Environmental Wide Association Study" (ELWAS) integrating environmental contaminants and lifestyle factors, we aimed to evaluate a possible additive role of both contaminants and lifestyle factors regarding MetS.
Methods: 1016 subjects aged 70 years were investigated in the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study. 43 environmental contaminants were measured in the circulation. Dietary records were used to evaluate 21 nutrients and the proportions of 13 fatty acids were determined in serum cholesterol esters to further quantify fat quality intake. Adding 5 other important lifestyle factors yielded together 76 environmental and lifestyle factors. MetS was defined by the NCEP/ATPIII-criteria.
Results: 23% had MetS. Using cross-validation within the sample, fourteen environmental contaminants or lifestyle factors consistently showed a false discovery rate <0.05. When the major variables entered a multiple model, only *p*, *p*′-DDE levels (positive), PCB209 (inverse) and exercise habits (inverse) were together with a fatty acid pattern, with high levels of palmitic acid and oleic acid and low levels of linoleic acid, related to MetS ($p<0.002$ for all variables).
Conclusion: Using a cross-sectional EWAS approach, certain environmental contaminants and lifestyle factors were found to be associated with prevalent metabolic syndrome in an additive fashion in an elderly population.

1. Introduction

A number of environmental contaminants of different kinds, e.g. persistent organic pollutants (POPs), plastic associated chemicals (PACs) and heavy metals, have been linked to different risk factors for cardiovascular disease (CVD) such as obesity, diabetes, hypertension and dyslipidemia (Hatch et al., 2010; Lee et al., 2010, 2011; Lind et al., 2012a,b, 2013; Olsen et al., 2012a; Rignell-Hydbom et al., 2010; Ronn et al., 2011; Roos et al., in press; Ruzzin et al., 2010; Sergeev and Carpenter, 2010; Stahlhut et al., 2007). These cardiometabolic risk factors are commonly aggregating in the same individual, a high-risk state known as The Metabolic syndrome (MetS) (Alberti and Zimmet, 1998; Anonymous, 2001; Lind et al., 1988; Reaven, 1988). Some recent studies have shown POPs to be linked to this syndrome (Lee et al., 2007; Uemura et al., 2009).

However, an important confounder regarding the relationship between environmental pollutants and MetS is the lifestyle of the individual. Several lifestyle factors, such as diet, smoking, social group, and occupation, are linked to the exposure to different environmental pollutants. Thus, a comprehensive evaluation of the links between environmental contaminants should account for lifestyle factors as well.

Recently, Chirag J. Patel and co-workers presented an attractive way to present associations between multiple measured environmental contaminants and diabetes using data from the National Health and Nutrition Examination Survey (NHANES) study (Patel et al., 2010). They used a format commonly used in genetic studies, Genetic Wide Association Study (GWAS), and then applied this format to environmental factors, Environmental Wide Association Study (EWAS). These authors have later applied this format also in other EWAS studies (Patel et al., 2012; Tzoulaki et al., 2012). An advantage of this format is the systematic use of all environmental factors evaluated, since this approach will limit the

Abbreviations: BDE, bromated diphenyl ether; CVD, cardiovascular disease; *p*,*p*′-DDE, 1,1-dichloro-2,2-bis(4-dichlordiphenyl) ethylene; a, metabolite to DDT; EWAS, Environmental Wide Association Study; GWAS, Genetic Wide Association Study; HCB, hexachlorobenzene; OCDD, octachlorodibenzo-p-dioxin; MetS, Metabolic Syndrome; NHANES, National Health and Nutrition Examination Survey; PACs, plastic associated chemicals; PCBs, polychlorinated biphenyls; PIVUS, Prospective Investigation of the Vasculature in Uppsala Seniors; POPs, persistent organic pollutants; TCDD, tetrachlorodibenzo-p-dioxin; TEQ, toxic equivalents; TNC, trans-nonachlordane.

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number of false positive findings induced by selective reporting of significant results, as previously been discussed by Ioannidis and others (Blair et al., 2009; Boffetta et al., 2008; Fallin and Kao, 2011; Ioannidis, 2005, 2008; Ioannidis et al., 2009; Young, 2010).

In the original ELWAS format, Patel and co-workers mainly used lifestyle factors, such as diet, exercise habits, socio-economic status, smoking etc., as confounders in the analysis. Since many of these lifestyle factors could well be an integrated part of the load of environmental factors, we propose an extension of the EWAS concept to also include major lifestyle factors in the main analysis. We used cross-sectional data from the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study (Lind et al., 2005) in which we in a systematic way used data on a large set of environmental contaminants, as well as dietary records, measurement of fatty acids in cholesterol esters to further explore the quality of fat intake in more detail in addition to history of major lifestyle factors. We hypothesized that environmental contaminants and lifestyle factors are related to prevalent MetS in an additive fashion.

2. Material and methods

2.1. Subjects

Eligible subjects were all aged 70 and lived in the City of Uppsala, Sweden, a City with 170,000 inhabitants. The subjects were randomly chosen from the register of community living. A total of 1016 subjects participated, giving a participation rate of 50.1%. Only one immigrant (from China) was included in the sample.

The study was approved by the Ethics Committee of Uppsala University, and all the participants gave their informed consent prior to the study.

All subjects were investigated in the morning after an over-night fast. No medication or smoking was allowed after midnight. The participants were asked to answer a questionnaire about their medical history, smoking habits and regular medication.

Blood pressure was measured by a calibrated mercury sphygmomanometer in the non-cannulated arm to the nearest mm Hg after at least 30 min of rest and the average of three recordings was used. Lipid variables and fasting blood glucose were measured by standard laboratory techniques.

All environmental and lifestyle variables measured in the PIVUS study with <5% missing values were included in the present evaluation.

Basic characteristics are given in Table 1.

Approximately 10% of the cohort reported a history of coronary heart disease, 4% reported stroke, and 9% reported diabetes mellitus. Almost half the cohort reported some sort of cardiovascular medication (45%), with antihypertensive medication being the most prevalent (32%). Fifteen percent reported the use of statins, while insulin and oral antiglycemic drugs were reported in 2 and 6%, respectively — see reference (Lind et al., 2005) for details.

2.2. Chemical analysis

2.2.1. Metals

All 11 metal elements in this study were determined in whole blood. The analysis was performed using inductively coupled plasma-sector field mass spectrometry, ICP-SFMS, after microwave-assisted digestion with nitric acid (Rodushkin and Axelsson, 2003) according to a method accredited for 10 of the 11 metal elements tested, with Al being unaccredited. Further details can be found elsewhere (Olsen et al., 2012a).

2.2.2. Bisphenol A and phthalates

Human serum was analyzed for levels of bisphenol A (BPA) and ten phthalate metabolites (mono-[2-ethyl-5-hydroxyhexyl] phthalate [MEHHP]; mono [2-ethyl-5-oxohexyl] phthalate [MEOHP]; mono-[2-ethylhexyl] phthalate [MEHP]; monobenzyl phthalate [MBZP]; monocyclohexyl phthalate [MCHP]; monoethyl phthalate [MEP]; monoisobutyl phthalate [MIBP]; monoisononyl phthalate [MINP]; monomethyl phthalate [MMP]; and mono-n-octyl phthalate [MOP]) at ALS Canada following the general procedures presented by the Centers for Disease Control and Prevention Detectable levels were found in almost all subjects for four phthalate metabolites, MEHP, MEP MIBP and MMP. Further details can be found elsewhere (Olsen et al., 2012b).

2.2.3. Persistent organic pollutants POPs

POPs were measured in stored plasma samples collected at baseline. Analyses of POPs were performed using a Micromass Autospec Ultima (Waters, Milford, MA, USA) high resolution chromatography coupled to high resolution mass spectrometry (HRGC/HRMS) system based on the method by Sandau et al. (Sandau et al., 2003) with some modifications. All details on POP analyses including quality control are provided in elsewhere (Salihovic et al., 2012). A total of 23 POPs were measured: 16 polychlorinated biphenyl (PCB) congeners, 5 organochlorine (OC) pesticides, 1 octachlorodibenzo-*p*-dioxin (OCDD), and 1 polybrominated diphenyl ether (PBDE) congener. Among the 23 POPs measured, 2 OC pesticides (*trans*-chlordane and *cis*-chlordane) with detection rates <10% were not included in the final statistical analyses; the minimum detection rate was 72%, and was observed for BDE47. Plasma concentrations were lipid adjusted and are expressed as ng/g lipid.

2.3. Biomarkers of dietary fat quality

Fatty acid composition was measured in serum as an objective marker of the average dietary fatty acid intake during the preceding weeks (Boberg et al., 1985; Ohrvall et al., 1996). Serum was extracted with a hexane-isopropanol solution and cholesterol esters were separated from the extract by thin-layer chromatography before interesterification with acidic methanol was performed. Free cholesterol that had been liberated in the reaction was removed by aluminum oxide to avoid contamination of the column. The percentage composition of methylated fatty acids from 14:0 to 22:6 was determined by gas chromatography (25-m NB-351 silica capillary column) with a flame ionization detector and helium as carrier gas. The CV varied between 0.2% and 5.0% in successive gas chromatography runs. The relative amount of fatty acids is expressed as the percentage of the total amount of fatty acids.

2.4. Definition of the metabolic syndrome

MetS was defined by the NECP/ATP III criteria (2001). Three of the following five criteria should be fulfilled: Blood pressure >130/85 mm Hg or antihypertensive treatment, fasting blood glucose >5.6 mmol/l, serum triglycerides >1.7 mmol/l, waist circumference >102 cm in men and >88 cm in women, HDL-cholesterol <1.0 mmol/l in men and <1.3 in women.

2.5. Definition of lifestyle factors

Educational level was divided into three groups: <9 years, 9–12 years and >12 years of education. Exercise habits were divided into four groups: <2 times light exercise (no sweat) per week, ≥2 times lightexercise per week, 1–2 times heavy exercise (sweat) per week, >2 times heavy exercise (sweat) per week. The dietary intakes of total calories, macronutrients, alcohol and intake of vitamin and trace elements were assessed by use of 7-day food diary and computer software. The subjects were told to give the number of close friends and relatives as a measure of their social network. They were also asked how many hours they usually slept during the night and if they were current smokers or not.

Table 1
Means and medians given for the environmental contaminants and lifestyle factors included in the analysis. The abbreviations given in parenthesis are the abbreviations used in the figures.

Category	Variable	N	Mean (SD)	Median (25th and 75th percentile)
Persistent organic pollutants (POPs)				
	2,4,4′,5-Tetrachlorobiphenyl (PCB74) ng/g lipid.	988	15.1 (7.5)	13.9 (10, 18.8)
	2,2′,4,4′,5-Pentachlorobiphenyl (PCB99) ng/g lipid	988	15.7 (9.6)	13.8 (9.5, 19.4)
	2,3,3′,4,4′-Pentachlorobiphenyl (PCB105) ng/g lipid	988	5.7 (4.1)	4.9 (3.3, 7)
	2,3′,4,4′,5-Pentachlorobiphenyl (PCB118) ng/g lipid	987	34.1 (20.1)	30 (21.6, 41.7)
	3,3′,4,4′,5-Pentachlorobiphenyl (PCB126) ng/g lipid	982	8.4 (7.5)	6.2 (3.3, 10.8)
	2,2′,3,4,4′,5′-Hexachlorobiphenyl(PCB138) ng/g lipid	988	135.4 (58.9)	124.9 (95.3, 168.2)
	2,2′,4,4′,5,5′-Hexachlorobiphenyl (PCB153) ng/g lipid	988	229.9 (91.1)	217 (166.8, 279.8)
	2,3,3′,4,4′,5-Hexachlorobiphenyl (PCB156) ng/g lipid	988	24.9 (9.7)	23.6 (18.2, 29.9)
	2,3,3′,4,4′,5′-Hexachlorobiphenyl (PCB157) ng/g lipid	988	4.8 (2.8)	4.3 (3.2, 5.5)
	3,3′,4,4′,5,5′-Hexachlorobiphenyl (PCB169) ng/g lipid	982	27.7 (11.3)	26 (20.1, 34.2)
	2,2′,3,3′,4,4′,5-Heptachlorobiphenyl(PCB170) ng/g lipid	988	80 (31.3)	74.8 (59.6, 96.3)
	2,2′,3,4,4′,5,5′-Heptachlorobiphenyl (PCB180) ng/g lipid	988	189.2 (83.5)	176.3 (139.3, 229)
	2,3,3′,4,4′,5,5′-Heptachlorobiphenyl (PCB189) ng/g lipid	988	4.6 (9.5)	2.9 (2.2, 3.9)
	2,2′,3,3′,4,4′,5,5′-Octachlorobiphenyl (PCB194) ng/g lipid	988	19.1 (9.7)	18.4 (13.6, 24.3)
	2,2′,3,3′,4,4′,5,5′,6-Nonachlorobiphenyl (PCB206) ng/g lipid	988	4.4 (1.9)	4.2 (3.1, 5.4)
	Decachlorobiphenyl (PCB209) ng/g lipid	988	4.3 (1.9)	4.1 (3, 5.3)
	Octachlorodibenzo-p-dioxin(OCDD) ng/g lipid	983	0.5 (0.3)	0.4 (<LOD, 0.6)
	Hexachlorobenzene (HCB) ng/g lipid	988	43 (26.6)	38 (29, 50.2)
	Trans-nonachlordane(TNC) ng/g lipid	988	24.6 (15.4)	20.8 (14.1, 31.6)
	1,1-bis-(4-chlorophenyl)-2,2-dichloroethene (*p*, *p*′-DDE) ng/g lipid	988	423.4 (436.7)	290.6 (158.1, 538.4)
	2,2′,4,4′-tetra-bromodiphenyl ether (BDE47) ng/g lipid	988	4.1 (18.2)	1.9 (1.5, 2.9)
Plastic associated chemicals (PACs)				
	Bisphenol A (BPA) ng/mL	1003	4.9 (4.2)	3.8 (2, 6.5)
	Mono-2-ethylhexyl phthalate (MEHP) ng/mL	1003	19.6 (43.7)	4.5 (2, 15.5)
	Mono-ethyl phthalate (MEP) ng/mL	1003	16 (16.7)	11.6 (7.2, 17.5)
	Mono-isobutyl phthalate (MIBP) ng/mL	1003	49.1 (131.9)	13.5 (9.3, 29.3)
	Mono-methyl phthalate (MMP) ng/mL	1003	3.3 (4.8)	1.5 (0.8, 3.1)
Metals				
	Aluminum (Al) umol/L	1000	0.7 (0.5)	0.6 (0.5, 0.8)
	Cadmium (Cd) nmol/L	1000	3.4 (3.3)	2.4 (1.7, 3.7)
	Cobolt (Co) nmol/L	998	2.5 (7.6)	1.4 (1.1, 1.9)
	Chromium (Cr) nmol/L	998	15.9 (14.2)	11.8 (9.6, 16)
	Copper (Cu) nmol/L	1000	12.9 (1.9)	12.8 (11.6, 14.1)
	Mercury (Hg) nmol/L	1000	11.1 (8.2)	8.9 (5.9, 13.7)
	Manganese (Mn) nmol/L	1000	143.7 (43.6)	137.5 (115, 165)
	Molybdenum (Mo) nmol/L	998	11.1 (6.1)	9.7 (8.1, 11.8)
	Nickel (Ni) nmol/L	999	144 (175.2)	90.6 (68.3, 109)
	Lead (Pb) umol/L	1000	0.093 (0.055)	0.083 (0.059, 0.11)
	Zink (Zn) umol/L	1000	95.9 (12.7)	96 (87.7, 104)
Dietary factors				
	Energy intake (Energy) (kcal/day)	861	1890 (505.2)	1832.6 (1547.2, 2165.4)
	Vitamin A (VitA) (ug/day)	861	912.8 (648.4)	711.3 (475.6, 1136.3)
	Beta-caroten (Caroten) (ug/day)	861	1906.4 (1204.4)	1644.3 (1042.1, 2497.7)
	Vitamin D (VitD) (ug/day)	861	5.8 (2.2)	5.5 (4.2, 7)
	Vitamin E (VitE) (mg/day)	861	7.2 (2.3)	6.9 (5.6, 8.3)
	Vitamin C (VitC) (mg/day)	861	83.1 (46.7)	74.8 (50.9, 106.3)
	Thiamin (mg/day)	861	1.2 (0.3)	1.1 (.9, 1.4)
	Riboflavin (mg/day)	861	1.7 (0.5)	1.7 (1.4, 2)

(continued on next page)

Table 1 (*continued*)

Category	Variable	N	Mean (SD)	Median (25th and 75th percentile)
Dietary factors				
	Niacin (mg/day)	861	15.4 (4)	15.0 (12.6, 17.8)
	Vitamin B6 (VitB6) (mg/day)	861	1.8 (0.5)	1.8 (1.5, 2.1)
	Vitamin B12 (VitB12) (ug/day)	861	6.7 (3.4)	6 (4.6, 7.8)
	Calcium (mg/day)	861	968 (323.5)	940.2 (730.7, 1160.3)
	Phosphorous (mg/day)	861	1368.1 (352.3)	1341.5 (1122.1, 1579.2)
	Iron (mg/day)	861	10.3 (3.1)	9.8 (8.3, 11.8)
	Magnesium (mg/day)	861	319.2 (77.9)	315 (267.7, 366.4)
	Sodium (g/day)	861	2.98 (0.83)	2.87 (2.42, 3.42)
	Potassium (g/day)	861	3.18 (0.78)	3.16 (2.65, 3.68)
	Alcohol (g/day)	861	2.5 (3.0)	1.6 (0.4, 3.7)
	Protein (% of total energy intake)	861	16.3 (2.1)	16.2 (14.8, 17.7)
	Carbohydrates (% of total energy intake)	861	48.7 (5.6)	49 (45.3, 52.4)
	Fat (% of total energy intake)	861	32.2 (4.9)	32.2 (29.4, 35.2)
Serum fatty acid composition				
	Myristic acid (FA140) (% of total fatty acids in CE)	942	0.9 (0.2)	0.9 (0.8, 1.0)
	Pentadecanoic acid (FA150) (% of total fatty acids in CE)	938	0.2 (0.1)	0.2 (0.2,0.3)
	Palmitic acid (FA160) (% of total fatty acids in CE)	942	11.8 (.8)	11.7 (11.2, 12.2)
	Palmitoleic acid (FA161) (% of total fatty acids in CE)	942	3.5 (1.2)	3.3 (2.7, 4.2)
	Stearic acid (FA180) (% of total fatty acids in CE)	942	0.8 (0.2)	0.8 (0.7, 0.9)
	Oleic acid (FA181) (% of total fatty acids in CE)	942	22.4 (1.9)	22.3 (21.1, 23.5)
	Linoleic acid (FA182) (% of total fatty acids in CE)	942	48 (4.2)	48.1 (45.4, 50.9)
	Gamma-linolenic acid (FA1836) (% of total fatty acids in CE)	941	0.8 (0.3)	0.8 (0.6, 1)
	Alpha-linolenic acid (FA1833) (% of total fatty acids in CE)	942	1 (0.2)	0.9 (0.8, 1.1)
	Dihomo-gamma-linolenic acid (FA203) (% of total fatty acids in CE)	941	0.7 (0.2)	0.7 (0.6, 0.8)
	Arachidonic acid (FA204) (% of total fatty acids in CE)	942	6.2 (1.3)	6 (5.3, 6.8)
	Eicosapentanoic acid (FA205) (% of total fatty acids in CE)	942	2.4 (1.1)	2.1 (1.6, 2.8)
	Docosahexaenoic acid (FA226) (% of total fatty acids in CE)	940	1.0 (0.3)	0.9 (0.8, 1.1)
Other lifestyle related factors				
	Number of friends	926	15.8 (22.3)	10.0 (6.0, 20)
	Hours slept per night (Sleep)	959	7.1 (1.1)	7.0 (6.5, 8.0)
	Education level	1005	<10 years: 57% 10–12 years:18% >12 years: 25%	
	Current smoking	1015	11%	
	Exercise habits	989	Sedentary: 12% Light: 59% Moderate: 22% Heavy: 7%	

2.6. Statistical analysis

All variables were evaluated regarding non-normality and variables with a skewed distribution were ln-transformed, such as most environmental contaminants. All continuous variables were thereafter transformed to a SD-scale to facilitate comparability between variables. Some lifestyle factors were on an ordinal scale (exercise 4 levels, education 3 levels).

As a first step, a principal component factor analysis with an orthogonal varimax rotation procedure was used to investigate the interrelationships between the 76 environmental and lifestyle factors.

As a second step, differences between prevalences of diseases and medications between subjects with and without the MetS were evaluated by the chi-square test.

As the third step, one logistic regression model for each of the 76 environmental or lifestyle factors was analyzed with adjustment for

Table 2
Proportions of cardiovascular diseases and medications (given in %) in subjects with and without the metabolic syndrome (MetS).

	MetS present (n = 235)	MetS absent (n = 781)	p-Value
Antihypertensive treatment	51	26	0.0001
Myocardial infarction	9.2	7.3	0.20
Stroke	6.1	3.3	0.031
Diabetes	24	4.2	0.0001
Statin treatment	23	12	0.0001

gender and with prevalent MetS as the dependent variable. We performed this analysis using a cross-validation within the sample in the way that we divide that sample in quintiles of the id-number. We run the analysis 5 times using only 80% of the sample size in each run. In the first run, the first quintile of the sample was excluded. In the second run, the second quintile was excluded, etc. Only the environmental or lifestyle factors that with a false discovery rate (FDR) <5% in all 5 runs were regarded as valid.

The FDR is the expected proportion of false positives among all significant hypotheses. In this study, a FDR <5% was considered as significant when the Benjamini–Hochberg step down method was used to calculate FDR.

As a fourth step, variables with a FDR<5% in all of the 5 runs in the cross-validation procedure described above were considered as potential independent variables together with gender in a multiple logistic regression model with prevalent MetS as the dependent variable in the total sample. However, only variables not closely related to each other in the initial factor analysis entered the model.

The fifth step was a sensitivity analysis excluding subjects with major cardiovascular disorders or medications from the multiple logistic regression model described above. We also performed a multiple logistic regression model as above when adjusting for these 5 potential confounders.

STATA 11 (STATA Inc., College Station, Texas, USA) was used for calculations.

3. Results

Means and medians for the investigated environmental contaminants and lifestyle factors are given in Table 1.

3.1. Relationships between the environmental and lifestyle factors

A factor analysis of the 76 environmental and lifestyle factors disclosed 9 factors with an eigenvalue >2.0 (see Supplementary Tables 1 and 2 for details). The first factor mainly consisted of nutritional variables, the second and third factors consisted of persistent organic pollutants, the fourth factor was mainly loaded by n = 3 PUFAs, saturated fatty acids (with a negative contribution of linoleic acid) were the main components of factor 5, factor 6 was loaded by a number of environmental contaminants, factor 7 was driven by the dietary intake of fat or carbohydrates, factor 8 mainly consisted of phthalate metabolites, while factor 9 was mainly loaded by n = 6 PUFAs. To note is that none of the lifestyle factors were an important contributor to any of the 9 factors.

3.2. Prevalence of the MetS and related disorders and medications

MetS was present in 23% of the sample. Subjects with the MetS showed significantly increased prevalence of antihypertensive treatment, treatment for diabetes and statin use than subjects without the MetS. A history of stroke or myocardial infarction was more common in subjects with the MetS, although this was only significant for stroke (see Table 2 for details).

3.3. Relationships between the MetS and the 76 environmental and lifestyle factors

Relationships for 76 environmental contaminants or lifestyle factors with prevalent MetS in an EWAS in the total sample are shown in Fig. 1 as the p-values on the −Log10 scale. Fig. 2 shows the OR and 95% CI for these 76 analyses adjusted for gender. Using the cross-validation procedure with 5 runs disclosed altogether 32 variables with a FDR <5% in any of the 5 runs. However, only 14 environmental of lifestyle factors showed a FDR<5% in all of the 5 runs, and were therefore consider as consistent findings (FA 16:0, FA 16:1, FA 18:2, n6, FA 18:3, n6, FA 20:3, FA 20:4, DDE, exercise habits, PCB105, PCB118, PCB194, PCB206, PCB209, and TNC).

Of these 14 environmental or lifestyle factors, we used only 7 in a logistic regression model with MetS as dependent variable due to close relationships between some of the variables as disclosed by the factor analysis, and because some of the fatty acids were derivatives of others. Thus, since PCB105 and PCB118 are highly correlated we used only PCB105 in the model. Also PCB194, PCB206 and PCB209 were highly correlated and therefore only PCB209 entered the model. Palmitoleic acid is a desaturation product of palmitic acid and is only found in low amounts in the Swedish diet, and was therefore not included. Similarly,

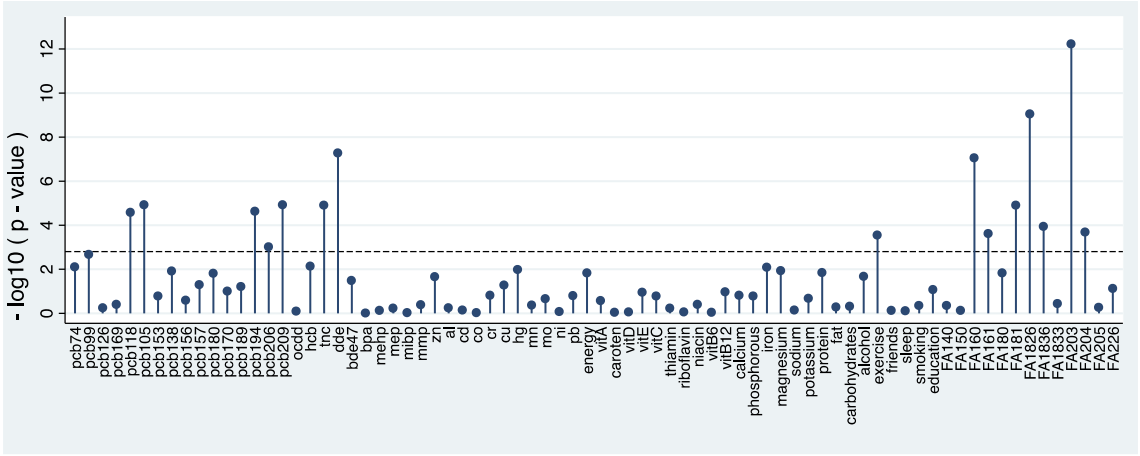

Fig. 1. "Manhattan" type plot showing the p-values (expressed at the −log10 scale) in the total sample for 76 environmental contaminants or lifestyle factors vs. prevalent metabolic syndrome (MetS) in an "Environmental and Lifestyle Wide Association Study," ELWAS. The broken line denotes variables with a false discovery rate (FDR) <5%, except for FA 18:0 which was not significant following internal cross-validation. For abbreviations of variables, see Table 1.

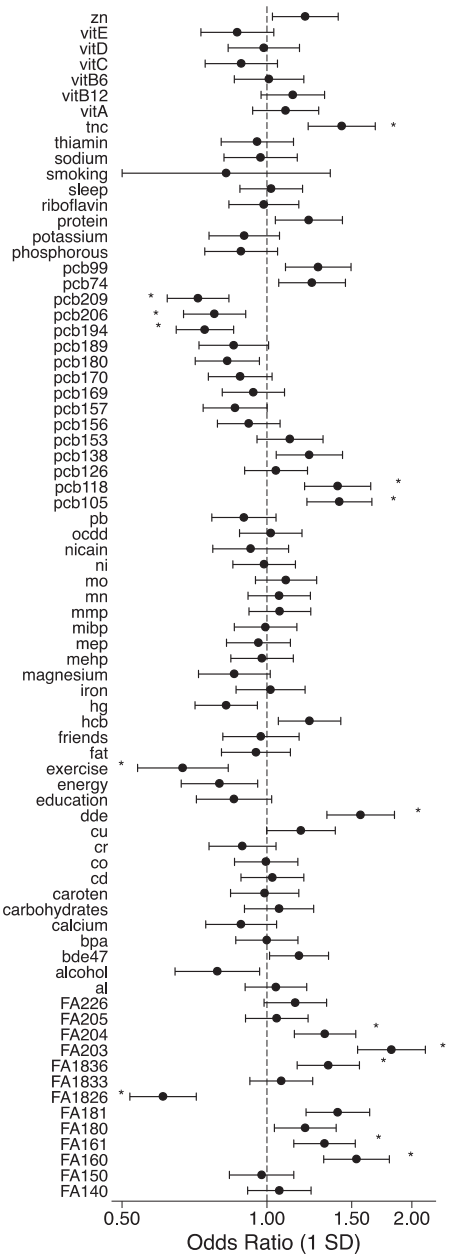

Fig. 2. Forest plot showing the OR and 95%CI for the associations between 76 environmental contaminants or lifestyle factors vs. prevalent metabolic syndrome (MetS) in an "Environmental Wide Association Study," EWAS. A star before or after the CI denotes variables with a false discovery rate (FDR)<5%. For abbreviations of variables, see Table 1.

the fatty acids gamma-linolenic acid, dihomo-gamma-linolenic acid and arachidonic acid were endogenously produced derivatives of other fatty acids and also found in very low amounts in the diet, and thus not included in the model. Thus, PCB105, PCB209, TNC, DDE, exercise, and the major fatty acids palmitic acid and linoleic acid were entered as independent variables together with gender.

Since linoleic acid is highly correlated to palmitic acid in an inverse manner (factor 5 described above), we used the factor scores of this fatty acid factor 5, as we have described previously (Ohrvall et al., 1996), to substitute palmitic acid and linoleic acid in a secondary model.

In the first model including the two fatty acids, only *p*, *p*′-DDE levels (positive), PCB209 (inverse) and exercise (inverse) were highly significantly related to occurrence of MetS in an independent manner, while TNC, and the two fatty acids were of borderline significance (p = 0.05–0.01, see Table 3 for details). When the three major fatty acids were substituted by the factor number 5 scores, also this fatty acid factor was highly significant (p<0.0001, positive). Nagelkerke R^2 was 0.22 and 0.23 for the two logistic regression models described above when only the environmental or lifestyle factors (not gender) were included in the models.

Table 3
Logistic regression analysis with prevalent Metabolic Syndrome (MetS) as dependent variable with gender and the eight environmental or lifestyle factors that consistently showed FDR <5% as independent variables in the left-hand side of the table. In the right-hand side we have substituted the three major fatty acids with "Fatty acid factor" created by a factor analysis that catches the main variance in the two fatty acids used in the former model. All variables are transformed to a SD scale to simplify comparison, except exercise being a trend test with four groups.

	Model with three fatty acids		Model with "Fatty acid factor"	
Variable	OR (95% CI)	p-Value	OR (95% CI)	p-Value
p,*p*′-DDE	1.46 (1.20, 1.78)	0.00019	1.05 (1.23, 1.83)	<0.0001
TNC	1.30 (1.05, 1.61)	0.017	1.33 (1.07, 1.66)	0.010
PCB209	0.57 (0.47, 0.69)	<0.0001	0.58 (0.48, 0.70)	<0.0001
PCB105	1.17 (0.92, 1.49)	0.19	1.14 (0.90, 1.45)	0.28
Exercise	0.71 (0.55, 0.90)	0.0047	0.67 (0.53, 0.86)	0.0014
Linoleic acid	0.77 (0.62, 0.95)	0.015	–	–
Palmitic acid	1.30 (1.06, 1.59)	0.010	–	–
Fatty acid factor	–	–	1.59 (1.34, 1.88)	<0.0001

PCB = polychlorinated biphenyls; TNC = trans-nonachlor; *p*,*p*′-DDE = 2, 2-Bis (4-chlorophenyl)-1, 1-dichloroethene.

3.4. Sensitivity analysis

In a sensitivity analysis, we excluded subjects with myocardial infarction, stroke, antihypertensive treatment, antidiabetic treatment and statin medication in five different logistic regression models as above. Only when subjects on antihypertensive treatment were excluded (n = 284) any major deviation in results compared to the models given in Table 3 was seen, since in this case exercise habits were not longer significant (now p = 0.15).

Adjustment for these 5 potential confounders did only marginally change the results given in the logistic regression models in Table 3.

Neither did further adjustment for glomerular filtration rate change the results presented in Table 3 in any major way. No case with hepatic failure was included in the sample.

4. Discussion

The role of environmental contaminants in MetS is unclear. This study has used the EWAS approach to determine the associations between MetS and environmental contaminants and lifestyle factors. It was found that both environmental contaminants and lifestyle factors were related to MetS in an additive way. This EWAS approach is a data-driven approach and should mainly be used as a way to create hypothesis to be tested in future studies. Furthermore, since a cross-sectional approach was used in the present study, and as this approach is prone to reverse causation problems, future validations should ideally be performed in a prospective fashion.

4.1. Comparison with the literature

Only a few studies have investigated the relationship between environmental contaminants and MetS. Using data from the NHANES 1999–2002 survey, Lee and co-workers showed that organochlorine (OC) pesticides was most strongly related to MetS of the five subclasses of POPs evaluated (Lee et al., 2011). PCBs were also related to MetS. Those data on OC pesticides are consistent with the present study where *p*, *p*′-DDE levels were a strong independent environmental factor being directly related to prevalent MetS. In contrast to that previous

study, we found PCB209 to be strongly and independently related to MetS. PCB209 was not evaluated as a single compound in NHANES, but there were no indications that the non-dioxin-like PCBs, including PCB209, would be inversely related to MetS.

We have previously shown that PCB209 and other highly chlorinated PCBs (substituted with 6–10 chlorine atoms) are inversely related to obesity in the PIVUS study, either as evaluated by body fat at Dual-energy X-ray absorptiometry (Ronn et al., 2011), or as waist circumference (Lee et al., 2012). Since abdominal obesity is a key driver of MetS, it was not surprising that PCB209 levels were inversely related also to MetS. Although it cannot be excluded that this PCB might have actions that are opposite of the less chlorinated PCBs (substituted with 4–5 chlorine atoms), it seems more likely that this inverse relationship is due to the high lipophilicity of this highly chlorinated compound. As shown by Wolff et al. (Wolff et al., 2007), circulating levels of POPs will show an inverse relationship with obesity for a substantial number of years following the time of main exposure due to the accumulation in fat tissue. This period will be longer for the highly chlorinated PCBs due to the longer half-life. Any recurrent exposure will also prolong the period during which the relationship between the specific POP and obesity will be negative. Despite the fact that all PCBs were subjected to the same ban back in the 1970s in Sweden, the exposure to the highly chlorinated PCBs with long half-lives is more likely be to prolonged when compared to less chlorinated PCBs with shorter half-lives. Thus, it is likely that the observed negative relationship between PCB209 is due to the kinetic properties of this contaminant. An inverse relationship between highly chlorinated PCBs and obesity has also been documented by other investigators (Dirinck et al., 2011).

Since the POPs are stored in fat tissue it would have been desirable to adjust for obesity in the present study. Unfortunately, only 7% of the subjects with the MetS are normal-weight so such an adjustment is not meaningful. Furthermore, since it is believed that obesity is the main driver of the MetS, adjustment for obesity would be to adjust for a factor on the causal pathway. We have therefore in the present study used lipid-normalized values for the POPs to mimic the body burden of these highly lipid soluble compounds.

In a nation-wide survey of > 1300 Japanese subjects, circulating levels of Dioxins and PCBs were related to occurrence of MetS when expressed as toxic equivalents (TEQ) (Uemura et al., 2009). In the present study no association between MetS and TEQ was found (OR 1.11, 95%CI 0.89 to 1.40, $p = 0.33$). The Japanese study furthermore showed that several of the PCB congeners, like PCB105 and PCB118 were related to MetS, as in the present study. However, PCB209 was not measured in that study.

It is well known that exercise could influence several of the components of MetS in a beneficial way. It is therefore not surprising that exercise habits were an important factor in this analysis.

A specific pattern of fatty acids in cholesterol esters, with high proportions of saturated (and some monounsaturated) fatty acids in combination with low levels of linoleic acid has previously consistently been associated with MetS both in cross-sectional and prospective studies (Warensjo et al., 2005, 2006). This analysis of fatty acids mainly reflects the daily intake of these fatty acids during the last weeks, and thereby provides a complimentary tool to the dietary records to further quantify the quality of fat intake. Using factor analysis, we show that this pattern with high proportions of palmitic acid, palmitoleic acid, and gamma-linolenic acid, and low proportions of and linoleic acid is related to MetS also in this population of elderly subjects. As shown in controlled feeding studies, such serum fatty acid pattern partly reflects a high intake of saturated fat and low intake in unsaturated vegetable fats (Warensjo et al., 2008). It should also be noted that oleic acid in cholesterol esters mainly reflects a high intake of saturated fat from animal fats in most Western populations including US and the current Swedish population (Ma et al., 1995; Warensjo et al., 2008). It is important to note, however, the fact that the environmental contaminants *p,p′*-DDE and PCB209 were related to MetS independently of this fatty acid pattern, suggesting that a possible effect of environmental contaminants on MetS is not due to a specific dietary fatty acid composition pattern.

4.2. The EWAS approach

The present study used the EWAS concept originally developed by Patel et al. (2010). The advantage of the present approach is that major lifestyle factors are also taken into account in order to analyze a large number of major external influences on the organism. It also allows us to evaluate if the influence of environmental contaminants is additive to the influence of the lifestyle factors. Furthermore, the use of Manhattan and Forest plots illustrates the relationships in a comprehensive way.

One problem with data-driven research, such as performed with the EWAS approach, is the validation of the positive findings. While genetic studies using the GWAS approach mainly have used commonly measured phenotypes like diabetes, BMI or lipids, not many studies with a fair amount of observations have a large number of environmental contaminants measured. Thus, while big consortia could be formed around the GWAS of common phenotypes for the sake of both discovery and validation, this approach is not applicable when dealing with environmental contaminants. We therefore used internal cross-validation in 5 iterations in the present sample. Despite this approach, our positive findings have to be replicated in a totally independent sample before considered validated.

Since this a mainly a hypothesis-generating study, we did use the FDR approach to adjust for multiple comparisons rather than the Bonferroni adjustment used to adjust for correction for multiple hypothesis testing. Interestingly, using FDR and internal cross-validation resulted in almost identical results as when using Bonferroni without cross-validation.

4.3. Limitation of the study

The present sample is limited to 70-year old Caucasians living in Sweden. Caution should therefore be used in drawing conclusions regarding other ethnic and age groups. Furthermore, the exposure to environmental contaminants might be different in other counties.

The present study had a moderate participation rate. Therefore, we carried out an evaluation of cardiovascular disorders and medications in 100 consecutive subjects who declined to participate (Lind et al., 2005). History of myocardial infarction, coronary revascularization and the use of cardiovascular drugs, antihypertensive medication, statins and insulin treatment were similar to those in the investigated sample, while the prevalence of diabetes, congestive heart failure and stroke tended to be higher among the non-participants.

Clearly not all aspects of lifestyle were covered since we do not have very sensitive instruments to in a good way quantify possible important lifestyle factors, such as stress and friendship. We did however include a number of lifestyle factors that are considered to be of importance for a healthy life, such as diet, smoking, exercise, sleep. However, residual confounding due to not measured lifestyle dimensions is likely to exist.

Furthermore, data on occupational exposure to contaminants are unfortunately lacking in the PIVUS study, so this important lifestyle factor is not included in the EWAS.

4.4. Conclusion

Using an EWAS approach, in this cross-sectional study certain environmental contaminants and lifestyle factors were found to be associated with prevalent metabolic syndrome in an additive fashion in an elderly population.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.envint.2013.01.017.

References

Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabetic medicine: a journal of the British Diabetic Association 1998;15(7):539–53.

Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). JAMA: The Journal of the American Medical Association 2001;285(19):2486–97.

Blair A, Saracci R, Vineis P, Cocco P, Forastiere F, Grandjean P, et al. Epidemiology, public health, and the rhetoric of false positives. Environmental Health Perspectives 2009;117(12):1809–13.

Boberg M, Croon LB, Gustafsson IB, Vessby B. Platelet fatty acid composition in relation to fatty acid composition in plasma and to serum lipoprotein lipids in healthy subjects with special reference to the linoleic acid pathway. Clinical Science (London, England) 1985;68(5):581–7.

Boffetta P, McLaughlin JK, La Vecchia C, Tarone RE, Lipworth L, Blot WJ. False-positive results in cancer epidemiology: a plea for epistemological modesty. Journal of the National Cancer Institute 2008;100(14):988–95.

Dirinck E, Jorens PG, Covaci A, Geens T, Roosens L, Neels H, et al. Obesity and persistent organic pollutants: possible obesogenic effect of organochlorine pesticides and polychlorinated biphenyls. Obesity (Silver Spring) 2011;19(4):709–14.

Fallin MD, Kao WH. Is "X"-WAS the future for all of epidemiology? Epidemiology 2011;22(4):457–9. [discussion 467–458].

Hatch EE, Nelson JW, Stahlhut RW, Webster TF. Association of endocrine disruptors and obesity: perspectives from epidemiological studies. International Journal of Andrology 2010;33(2):324–32.

Ioannidis JP. Why most published research findings are false. PLoS Medicine 2005;2(8):e124.

Ioannidis JP. Why most discovered true associations are inflated. Epidemiology 2008;19(5):640–8.

Ioannidis JP, Loy EY, Poulton R, Chia KS. Researching genetic versus nongenetic determinants of disease: a comparison and proposed unification. Science Translational Medicine 2009;1(7). [7 ps8].

Lee DH, Lee IK, Porta M, Steffes M, Jacobs Jr DR. Relationship between serum concentrations of persistent organic pollutants and the prevalence of metabolic syndrome among non-diabetic adults: results from the National Health and Nutrition Examination Survey 1999–2002. Diabetologia 2007;50(9):1841–51.

Lee DH, Steffes MW, Sjodin A, Jones RS, Needham LL, Jacobs Jr DR. Low dose of some persistent organic pollutants predicts type 2 diabetes: a nested case–control study. Environmental Health Perspectives 2010;118(9):1235–42.

Lee DH, Steffes MW, Sjodin A, Jones RS, Needham LL, Jacobs Jr DR. Low dose organochlorine pesticides and polychlorinated biphenyls predict obesity, dyslipidemia, and insulin resistance among people free of diabetes. PloS One 2011;6(1):e15977.

Lee DH, Lind L, Jacobs Jr DR, Salihovic S, van Bavel B, Lind PM. Associations of persistent organic pollutants with abdominal obesity in the elderly: The Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study. Environment International 2012;40:170–8.

Lind L, Jakobsson S, Lithell H, Wengle B, Ljunghall S. Relation of serum calcium concentration to metabolic risk factors for cardiovascular disease. BMJ 1988;297(6654):960–3.

Lind L, Fors N, Hall J, Marttala K, Stenborg A. A comparison of three different methods to evaluate endothelium-dependent vasodilation in the elderly: the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study. Arteriosclerosis, Thrombosis, and Vascular Biology 2005;25(11):2368–75.

Lind PM, Zethelius B, Lind L. Circulating levels of phthalate metabolites are associated with prevalent diabetes in the elderly. Diabetes Care 2012a;35(7):1519–24.

Lind PM, Roos V, Ronn M, Johansson L, Ahlstrom H, Kullberg J, et al. Serum concentrations of phthalate metabolites are related to abdominal fat distribution two years later in elderly women. Environmental health: a global access science source 2012b;11:21.

Lind PM, Lee DH, Jacobs DR, Salihovic S, Bavel B, Wolff MS, et al. Circulating levels of persistent organic pollutants are related to retrospective assessment of life-time weight change. Chemosphere 2013;90(3):998-1004.

Ma J, Folsom AR, Shahar E, Eckfeldt JH. Plasma fatty acid composition as an indicator of habitual dietary fat intake in middle-aged adults. The Atherosclerosis Risk in Communities (ARIC) Study Investigators. The American Journal of Clinical Nutrition 1995;62(3):564–71.

Ohrvall M, Berglund L, Salminen I, Lithell H, Aro A, Vessby B. The serum cholesterol ester fatty acid composition but not the serum concentration of alpha tocopherol predicts the development of myocardial infarction in 50-year-old men: 19 years follow-up. Atherosclerosis 1996;127(1):65–71.

Olsen L, Lind PM, Lind L. Gender differences for associations between circulating levels of metals and coronary risk in the elderly. International Journal of Hygiene and Environmental Health 2012a;215(3):411–7.

Olsen L, Lampa E, Birkholz DA, Lind L, Lind PM. Circulating levels of bisphenol A (BPA) and phthalates in an elderly population in Sweden, based on the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS). Ecotoxicology and Environmental Safety 2012b;75(1):242–8.

Patel CJ, Bhattacharya J, Butte AJ. An Environment-Wide Association Study (EWAS) on type 2 diabetes mellitus. PloS One 2010;5(5):e10746.

Patel CJ, Cullen MR, Ioannidis JP, Butte AJ. Systematic evaluation of environmental factors: persistent pollutants and nutrients correlated with serum lipid levels. International Journal of Epidemiology 2012;41(3):828–43.

Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. Diabetes 1988;37(12):1595–607.

Rignell-Hydbom A, Elfving M, Ivarsson SA, Lindh C, Jonsson BA, Olofsson P, et al. A nested case–control study of intrauterine exposure to persistent organochlorine pollutants in relation to risk of type 1 diabetes. PloS One 2010;5(6):e11281.

Rodushkin I, Axelsson MD. Application of double focusing sector field ICP-MS for multielemental characterization of human hair and nails. Part III. Direct analysis by laser ablation. The Science of the Total Environment 2003;305(1–3):23–39.

Ronn M, Lind L, van Bavel B, Salihovic S, Michaelsson K, Lind PM. Circulating levels of persistent organic pollutants associate in divergent ways to fat mass measured by DXA in humans. Chemosphere 2011;85(3):335–43.

Roos V, Ronn M, Salihovic S, Lind L, Bavel BV, Kullberg J, et al. Circulating levels of pesistent organic pollutants in relation to visceral and subcutaneous adipose tissue by abdominal MRI. Obesity (Silver Spring) in press. http://dx.doi.org/10.1038/oby.2012.123 [Epub ahead of print, PMID: 22627916].

Ruzzin J, Petersen R, Meugnier E, Madsen L, Lock EJ, Lillefosse H, et al. Persistent organic pollutant exposure leads to insulin resistance syndrome. Environmental Health Perspectives 2010;118(4):465–71.

Salihovic S, Mattioli L, Lindstrom G, Lind L, Lind PM, van Bavel B. A rapid method for screening of the Stockholm Convention POPs in small amounts of human plasma using SPE and HRGC/HRMS. Chemosphere 2012;86(7):747–53.

Sandau CD, Sjodin A, Davis MD, Barr JR, Maggio VL, Waterman AL, et al. Comprehensive solid-phase extraction method for persistent organic pollutants. Validation and application to the analysis of persistent chlorinated pesticides. Analytical Chemistry 2003;75(1):71–7.

Sergeev AV, Carpenter DO. Exposure to persistent organic pollutants increases hospitalization rates for myocardial infarction with comorbid hypertension. Primary prevention insights 2010;2:1–9.

Stahlhut RW, van Wijngaarden E, Dye TD, Cook S, Swan SH. Concentrations of urinary phthalate metabolites are associated with increased waist circumference and insulin resistance in adult U.S. males. Environmental Health Perspectives 2007;115(6):876–82.

Tzoulaki I, Patel CJ, Okamura T, Chan Q, Brown IJ, Miura K, et al. A nutrient-wide association study on blood pressure. Circulation 2012;126(21):2456–64.

Uemura H, Arisawa K, Hiyoshi M, Kitayama A, Takami H, Sawachika F, et al. Prevalence of metabolic syndrome associated with body burden levels of dioxin and related compounds among Japan's general population. Environmental Health Perspectives 2009;117(4):568–73.

Warensjo E, Riserus U, Vessby B. Fatty acid composition of serum lipids predicts the development of the metabolic syndrome in men. Diabetologia 2005;48(10):1999–2005.

Warensjo E, Sundstrom J, Lind L, Vessby B. Factor analysis of fatty acids in serum lipids as a measure of dietary fat quality in relation to the metabolic syndrome in men. The American Journal of Clinical Nutrition 2006;84(2):442–8.

Warensjo E, Riserus U, Gustafsson IB, Mohsen R, Cederholm T, Vessby B. Effects of saturated and unsaturated fatty acids on estimated desaturase activities during a controlled dietary intervention. Nutrition, metabolism, and cardiovascular diseases: NMCD 2008;18(10):683–90.

Wolff MS, Anderson HA, Britton JA, Rothman N. Pharmacokinetic variability and modern epidemiology–the example of dichlorodiphenyltrichloroethane, body mass index, and birth cohort. Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 2007;16(10):1925–30.

Young SS. Acknowledge and fix the multiple testing problem. International Journal of Epidemiology 2010;39(3):934. [author reply 934–935].