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Associations of organochlorine pesticides and polychlorinated biphenyls in visceral vs. subcutaneous adipose tissue with type 2 diabetes and insulin resistance

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HIGHLIGHTS

- Concentrations of PCBs were much higher in visceral fat than subcutaneous fat.
- Organochlorine pesticides showed inconsistent patterns between the two fat beds.
- POPs in visceral or subcutaneous fat were significantly associated with diabetes.
- POPs in visceral or subcutaneous fat were significantly associated with insulin resistance.

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ABSTRACT

Background exposure to organochlorine (OC) pesticides and polychlorinated biphenyls (PCBs) has been linked to type 2 diabetes. As OC pesticides and PCBs mainly accumulate in adipose tissue and there are physiological and clinical differences between visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT), we explored if there were associations of OC pesticides and PCBs in VAT or SAT with type 2 diabetes and insulin resistance. Participants were 50 patients with or without type 2 diabetes who underwent surgery for either cancer or benign liver or gallbladder lesions. We analyzed 14 OC pesticides and 22 PCB congeners in both VAT and SAT. Insulin resistance was estimated using homeostasis model assessment (HOMA). Although concentrations of OC pesticides and PCBs were strongly correlated between VAT and SAT, absolute concentrations differed substantially between them. In particular, concentrations of all PCBs were consistently about 5–10 times higher in VAT than SAT, but these patterns were independent of diabetes status. Some OC pesticides or PCBs, such as dichlorodiphenyltrichloroethanes (DDTs), chlordanes, and PCBs with 5 or less chlorides showed significant associations with diabetes or insulin resistance. For example, when tertiles of concentration-based summary measures were used, adjusted ORs were 1.0, 2.3, and 9.0 (P trend = 0.02) for DDTs in VAT and 1.0, 2.1, and 5.7 (P trend = 0.08) for PCBs with 5 or less chlorides. This study generally confirmed previous findings using serum concentrations. It would be useful to study pharmacodynamics of POPs in VAT and SAT further.

1. Introduction

The background exposure to persistent organic pollutants (POPs), in particular organochlorine (OC) pesticides and polychlorinated biphenyls (PCBs) have been linked to obesity-related metabolic dysfunction such as insulin resistance and type 2 diabetes (Lee et al., 2006, 2007a,b; Airaksinen et al., 2011; Grandjean et al., 2011). Following strong cross-sectional associations

Abbreviations: CHLs, chlordanes; DDTs, dichlorodiphenyltrichloroethanes; HCB, hexachlorobenzene; HCHs, hexachlorocyclohexanes; HOMA–IR, homeostasis model assessment–insulin resistance; LOD, limit of detection; OC pesticides, organochlorine pesticides; ORs, odds ratios; PCBs, polychlorinated biphenyls; POPs, persistent organic pollutants; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.

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observed in general populations, several prospective studies (Rignell-Hydbom et al., 2009; Turyk et al., 2009; Lee et al., 2010, 2011a) also demonstrated that POPs can predict the future risk of insulin resistance and/or type 2 diabetes. Furthermore, recent experimental animal studies have reported that the exposure to POP mixtures through contaminated fish oil induced severe impairment of whole-body insulin action and it contributed to the development of abdominal obesity, dyslipidemia, and hepatosteatosis (Ruzzin et al., 2010; Ibrahim et al., 2011).

Although POPs accumulate mainly in adipose tissue, collecting adipose tissue is difficult in practice. Therefore most epidemiological studies of POPs have used circulating concentrations of POPs as a marker of body burden of POPs because circulating POPs concentrations are reportedly strongly correlated to those of POPs in adipose tissue (Rusiecki et al., 2005).

One recent small scale study with 7 study subjects measured OC pesticides and PCBs in various tissues including visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) and demonstrated a complicated distribution of OC pesticides and PCBs in various lipid compartments (Yu et al., 2011). However, the interpretation of the findings was limited due to small sample size and lack of measurement of the lipid content of adipose tissues. In fact, the capillary filtration coefficient in VAT has been reported to be two times the value found in SAT (Ballard and Rosell, 1971). As a higher capillary filtration coefficient indicates that the surface area over which filtration occurs is larger in VAT than SAT, the difference in capillary filtration coefficient between VAT and SAT can be associated with the different rates of chemical uptake by these tissues (Street and Sharma, 2011).

It is well-known that there are anatomical, cellular, molecular, physiological, clinical and prognostic differences between VAT and SAT. VAT adipocytes are more metabolically active, more sensitive to lipolysis and more insulin-resistant than SAT adipocytes (Ibrahim, 2010). To further understand the role of POPs in VAT or SAT in relation to type 2 diabetes and insulin resistance, we compared concentrations of OC pesticides and PCBs of VAT with SAT and their relations with type 2 diabetes or insulin resistance were examined using adipose tissue obtained during elective surgery.

2. Materials and methods

2.1. Study subjects

We recruited 50 Korean patients who underwent elective surgery for either cancer or benign lesions in pancreas, liver, or gallbladder. Among them, 26 subjects (13 males and 13 females) had liver or gallbladder cancer, 8 subjects (4 males and 4 females) had gallbladder or intra-hepatic duct stones, 13 subjects (7 males and 6 females) had both cancer and stones, and 3 subjects (3 females) had other diseases (chronic cholecystitis, pancreatic pseudocyst, and xanthogranulomatous cholecystitis). Surgeons excised samples of 5 grams or more from VAT and SAT. All samples were stored at −70 °C until analyses. All patients signed informed consent and this study was approved by the Ethics Committee of the Kyungpook National University Institutional Review Board.

2.2. Measurements

Demographic characteristics and health-related information were collected using standardized questionnaires. Blood was drawn after at least 12 h overnight fasting. Fasting glucose was determined by enzymatic methods using ADVIA 1650 (Bayer Inc., New York, USA) and insulin was measured by a radioimmunoassay with a Packard gamma counter (GMI Inc., Minnesota, USA). Participants were considered to have type 2 diabetes if 1) their fasting plasma glucose was $\geqslant$126 mg dL^{-1} or 2) they reported history of physician-diagnosed diabetes. Insulin resistance was estimated using the homeostasis model assessment (HOMA) method calculated by the following equation: (fasting insulin [mU L^{-1}] $*$ fasting glucose [mmol L^{-1}]/22.5).

We analyzed 14 OC pesticides and 22 PCB congeners in 50 VAT samples and 50 SAT samples. OC pesticides were classified into dichlorodiphenyltrichloroethanes (DDTs), chlordanes (CHLs), hexachlorocyclohexanes (HCHs), and hexachlorobenzene (HCB). DDTs included p,p′-DDT, o,p′-DDT, p,p′-DDE, p,p′-DDD, and o,p′-DDD; CHLs included oxy-chlordane, trans-chlordane, cis-chlordane, trans-nonachlor, and cis-nonachlor; and HCHs included α-, β- and γ-HCH. Measured PCB congeners were 8, 18, 28, 29, 44, 52, 87, 101, 105, 110, 118, 128, 138, 153, 170, 180, 187, 194, 195, 200, 205 and 206. Tissue samples were homogenized with anhydrous Na_2SO_4 and extracted for 20 h in 400 mL of a 3:1 mixture of dichloromethane (DCM) and hexane using a Soxhlet apparatus. Prior to extraction, surrogate standards PCBs 103, 198, and 209 were spiked into the samples. Aliquots of extracted samples were sub-sampled for lipid measurement. Lipids were removed from the extracts by gel permeation chromatography using Bio-beads S–X3 (Bio-Rad Laboratories, Hercules, CA, USA). The columns were eluted with a mixture of 50% DCM in hexane (flow rate 5 mL min^{-1}). The first 100 mL of eluant was discarded and the following 150 mL fraction, which contained OC pesticides and PCBs, was collected and passed through a cartridge packed with 0.5 g of silica gel (neutral, 70–230 mesh, GL Sciences, Tokyo, Japan). The eluant was concentrated to 10 mL and spiked with internal standards, ^{13}C-labeled OCPs (DDTs, CHLs, HCHs and HCB; ES-5349, Cambridge Isotope Laboratories, Andover, MA, USA) and ^{13}C-labeled PCBs (CBs 28, 52, 118, 153, 180, 202, and 209; EC- 9605-SS, Wellington Laboratories, Guelph, ON, Canada), were spiked into the samples. The extracts were cleaned by passage through a multi-layer silica gel column with 150 mL of 15% DCM in hexane by the Dioxin Cleanup System (DAC695/DPU8; GL Sciences). The eluant was concentrated to approximately 1 mL, and was then evaporated at room temperature to 100 μL. The residues were dissolved in 100 μL of *n*-nonane for instrumental analysis. A high-resolution gas chromatography/mass spectrometer (HRGC/HRMS; JMS 800D, Jeol, Tokyo, Japan) was used for the identification and quantification of OC pesticides and PCBs, based on the relative response factors of individual compounds. The HRMS was operated in the electron ionization mode, and ions were monitored by selected ion monitoring. OC pesticides and PCB congeners were quantified by a DB5-MS capillary column (30 m length, 0.25 mm inner diameter, 0.25 μm film thickness; J & W Scientific, Palo Alto, CA, USA). The respective recoveries of PCBs 103, 198 and 209, spiked into all samples before extraction, ranged from 86 ± 9% (average ± SD), 87 ± 10%, and 86 ± 11%. Recoveries of ^{13}C-labeled OC pesticides and PCBs were 92 ± 11% and 93 ± 14%, respectively. The calculated limits of detection (LOD; signal-to-noise ratio = 3) were 0.5–4 pg g^{-1} for individual PCB congeners, 1.5 pg g^{-1} for DDTs, 1.0 pg g^{-1} for CHLs, 2.0 pg g^{-1} for HCHs, and 1.0 pg g^{-1} for HCB. Detection rates (proportion of subjects with concentrations above the LOD) of all POPs were more than 90%, except trans-chlordane (detection rate: 26% in SAT and 82% in VAT), which we excluded from analysis. All POPs concentrations were adjusted to the lipid content of tissues, using an adjusting method that is described in detail elsewhere (Moreno Frias et al., 2004). One third of LOD value of each chemical was assigned to subjects with below the limit of detection.

2.3. Statistical analyses

The concentrations of individual OC pesticides or PCBs in adipose tissue were categorized into tertiles for use as predictors in logistic regression to test the associations with type 2 diabetes. Relations of OC pesticides or PCBs with log-transformed HOMR–IR