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Polycyclic aromatic hydrocarbons are associated with insulin receptor substrate 2 methylation in adipose tissues of Korean women

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are highly lipid soluble and are an increasing concern for general populations given their various adverse health effects, including obesity-related metabolic dysfunction. DNA methylation can act as a downstream effector for the biological effects of environmental exposures, but whether PAHs influence DNA methylation is unclear. To test for possible adverse effects of PAHs on adipose tissue (AT), we determined the promoter methylation status of 12 genes involved in glucose and lipid metabolism (*CS, GLUT4, IR, IRS1, IRS2, LIPIN1, MCAD, PCK1, PCK2, PPARGC1*B, *SDHA,* and *SREBP1*) in visceral AT of Korean women by using methylation-specific PCR (MSP). *IRS2* methylation alone was significantly associated with concentrations of individual PAH chemicals. When the PAH summary measure was used, the odds ratios of *IRS2* hypermethylation across quartile of the PAH summary measure were 1, 1.7, 2.0, and 11.2 (95% confidence interval: 1.5–84.0) after adjusting for age and BMI (P trend=0.02). The strength of association between PAH summary measure and *IRS2* hypermethylation was as similar as that of BMI. Collectively, these results suggested that lipophilic PAHs might be contributing factors to the pathogenesis of insulin resistance through methylation-mediated suppression of the *IRS2* gene. However, further studies with large sample size are needed to confirm our findings.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are widespread environmental contaminants with common sources such as cigarette smoking, automobile exhaust, diesel combustion, and diet. Environmental exposure to PAHs has been known to increase the risk of various diseases, including cancer and reproductive and cardiovascular diseases (Baird et al., 2005; Lewtas, 2007), and PAHs have been recently linked to obesity and obesity-related metabolic functions (Kim et al., 2014; Scinicariello and Buser, 2014).

PAHs are highly lipid soluble and accumulate in adipose tissue (AT). AT is not merely an energy reservoir, but also has a dynamic role in maintaining normal carbohydrate and lipid levels as well as in regulating metabolic and inflammatory functions (Galic et al., 2010). Importantly, recent evidence has demonstrated that AT appears to be a major player in the toxicological response to lipophilic chemicals such as persistent organic pollutants (La Merrill et al., 2013). In this regard, AT could serve as an internal source for chronic PAH exposure and as a target of PAH toxicity.

Several studies have reported the occurrence and accumulation profiles on PAHs in human AT (Guerranti et al., 2009; Moon et al., 2012; Wang et al., 2015), but none have evaluated their corresponding biological effects. Even though the causative mechanisms of PAH-related health effects on the molecular level are not yet completely understood, PAHs exposure induced hypo- and hypermethylation at multiple promoter regions, suggesting that epigenetic modulation of genes may be a key mechanism (Alegria-Torres et al., 2013; Duan et al., 2013; Hew et al., 2015; Pavanello et al., 2009; White et al., 2016; Yang et al., 2012). Therefore, in this study, we evaluated if concentrations of PAHs in visceral AT were related to the methylation status of 12 key genes involved in glucose and lipid metabolism by using methylation-specific PCR (MSP). Compared to subcutaneous AT, visceral AT is highly metabolically active, more susceptible to lipolysis, and strongly linked to many obesity-related diseases (Ibrahim, 2010; Karadag et al., 2011). The genes analyzed were citrate synthase (*CS*), glucose

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transporter 4 (*GLUT4*), insulin receptor (*IR*), insulin receptor substrate 1 (*IRS1*), insulin receptor substrate 2 (*IRS2*), lipin1 (*LIPIN1*), medium chain acyl CoA dehydrogenase (*MCAD*), peroxisome proliferator-activated receptor gamma coactivator 1β (*PPARGC1B*), phosphoenolpyruvate carboxykinase 1 (*PCK1*), phosphoenolpyruvate carboxykinase 2 (*PCK2*), sterol regulatory element binding transcription factor 1 (*SREBP1*), and succinate dehydrogenase (*SDHA*).

2. Materials and methods

2.1. Sample collection

Fifty-three non-smoking female patients who had myoma and were undergoing laparoscopy-assisted surgery at Kyungpook National University Hospital (KNUH) consented to provide a small amount of visceral AT for our study. Between May 2007 and May 2008, we obtained AT from patients' omental fat tissue. All samples were stored at −70 °C until our analyses. The donors' age, body mass index (BMI), and extractable lipid content are summarized in Table 1. The use of human subjects in this study was approved by the ethics committee of the Institutional Review Board at KNUH, Korea.

2.2. Sample preparation and instrumental analyses

Sixteen PAHs were analyzed in human AT using previously described methods (Moon et al., 2012). Briefly, approximately 1–2 g fat samples were homogenized with anhydrous Na_2SO_4 and extracted with mixed solvents of DCM and hexane using a Soxhlet apparatus. Prior to extraction, 100 ng of deuterated-PAHs was spiked into an aliquot of the extract (10 mL). The extract was then concentrated to 11 mL, and a 1 mL aliquot was used for gravimetric determination of lipid content. The extracts were subjected to gel permeation chromatography using Bio-Beads S–X3 (Bio-Rad, Hercules, CA, USA) and connected sequentially to a cartridge packed with 0.5 g of silica gel. A mixture of 50% hexane in DCM was used as a mobile phase at a flow rate of 5 mL/min. The first 100 mL of the fraction was discarded, and the following 100 mL fraction, which contained PAHs, was collected. The eluents were then concentrated to approximately 1 mL, and were dissolved in 1 mL *n*-nonane for instrumental analysis. The aromatic fraction was analyzed by a gas chromatograph (Agilent 6890, Wilmington, DE, USA) coupled with a mass spectrometer detector (Agilent 5973N). The capillary column used was a DB5-MS column (30 m length, 0.25 mm inner diameter, 0.25 μm film thickness; J & W Scientific, Palo Alto, CA, USA). The mass spectrometer was operated in the selective ion monitoring (SIM) mode using molecular ions of individual PAHs. The calculated limits of quantification (LOQs) for individual PAHs were set at five times the limits of detection (LODs), which were calculated as a signal to noise ratio of three. The LOQ for PAHs ranged from 1.0 to 2.5 ng/g lipid weight.

Table 1.
Characteristics of study subjects and concentrations (ng g^{-1} lipid weight) of polycyclic aromatic hydrocarbons (PAHs) in adipose tissue.

	Mean ± SD	Median	Range	Detection frequency (%)
Age (years)	47 ± 6	47	40–68	
BMI (kg m^{-2})[a]	23 ± 3	23	18–31	
Lipid (%)	73 ± 1	73	68–83	
PAHs				
Naphthalene	76 ± 83	41	< 1.0–300	77
Acenaphthylene	1.9 ± 1.7	1.5	< 1.0–7.6	96
Acenaphthene	5.4 ± 6.0	4.1	< 1.0–24	64
Fluorene	4.6 ± 3.1	4.1	< 1.0–12	94
Phenanthrene	11 ± 8.4	9.7	< 1.0–31	85
Anthracene	0.41 ± 0.42	0.3	< 1.0–1.7	70
Fluoranthene	1.8 ± 1.3	1.5	< 1.0–5.5	89
Pyrene	1.9 ± 1.1	1.7	< 1.0–5.0	98
Benzo[*a*]anthracene	< 1.0			0
Chrysene	< 1.0			0
Benzo[*b*] fluoranthene	< 1.5			0
Benzo[*k*] fluoranthene	< 1.5			0
Benzo[*a*]pyrene	< 1.5			0
Indeno[1,2,3-*c*,*d*] pyrene	< 2.5			0
Dibenzo[*a*,*h*] anthracene	< 2.5			0
Benzo[*g*,*h*,*j*]perylene	< 2.5			0

[a] Body mass index.

2.3. Genomic DNA extraction, bisulfite treatment and methylation analysis

Genomic DNA was extracted using the QIAamp DNA Mini Kit (Qiagen, Valencia, CA, USA). Methylation of the promoter CpG islands (CGIs) of *CS, GLUT4, IR, IRS1, IRS2, LIPIN1, MCAD, PPARGC1B, PCK1, PCK2, SREBP1,* and *SDHA* was determined by sodium bisulfite treatment of genomic DNA followed by MSP. Bisulfite modification of genomic DNA was carried out using the EZ DNA Methylation-Gold Kit (Zymo Research, Orange, CA, USA) according to manufacturer's instructions. Unmethylated cytosines, but not methylated cytosines, were converted to uracils and amplified using primers specific for either methylated or unmethylated alleles. The primer sequences used are listed in Supplementary Table 1. All PCR amplifications were performed in a PTC-100 thermal cycler (MJ Research, Watertown, MA, USA) using reagents supplied in the GeneAmp DNA Amplification Kit with AmpliTaq Gold DNA polymerase. PCR products were analyzed on 2% agarose gel, stained with ethidium bromide (EtBr), and visualized under UV light. To assess the validity of results and the repeatability of the MSP assay, three internal quality controls were included in each set of PCR, namely, water as the method blank, CpGenome™ Universal methylated DNA (Chemicon, Temecula, CA, USA) as positive control, and CpGenome™ Universal unmethylated DNA (Chemicon) as negative control. All MSP experiments were done in duplicate.

2.4. Cell culture, RNA isolation, and reverse transcription-polymerase chain reaction (RT-PCR)

Three human non-small cell lung cancer (NSCLC) cell lines, that is, H1299, A549, H23, were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cell lines were propagated according to the instructions from the ATCC. The cells were treated with 20 μM 5-azadeoxycytidine (5-Aza-dC) for 3 days and the culture medium was changed daily. Total RNA was then extracted using TRIzol (Invitrogen, Mt Waverly, VIC, Australia) according to the manufacturer's instructions. Residual genomic DNA was digested with RNase-free DNase (Invitrogen). First strand cDNA was reverse-transcribed from 2 μg of total RNA in a total volume of 20 μl using oligo(dT) and a SuperScript preamplification kit (Invitrogen). The resulting cDNA was amplified by forward (5′-AGATCTGTCTGGCTTTATCACCAGGA-3′) and reverse (5′-CCTAG-CATGCGAGGGTTATATCTGC-3′) primers under conditions described previously (Dunaif et al., 2001). Amplified products were electrophoresed in 2% agarose gels, visualized with EtBr, and then photographed.