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Original Research Article

Effects of high-fat diet on plasma profiles of eicosanoid metabolites in mice

Weicang Wang[a,1], Jun Yang[b,1], Haixia Yang[a], Katherine Z. Sanidad[a,c], Bruce D. Hammock[b], Daeyoung Kim[d], Guodong Zhang[a,c,*]

[a] Department of Food Science, University of Massachusetts, Amherst, MA, 01003, USA
[b] Department of Entomology and Comprehensive Cancer Center, University of California, Davis, CA, 95616, USA
[c] Molecular and Cellular Biology Graduate Program, University of Massachusetts, Amherst, MA, 01003, USA
[d] Department of Mathematics and Statistics, University of Massachusetts, Amherst, MA, 01003, USA

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ABSTRACT

Obesity is a serious health problem in the US and is associated with increased risks of various human diseases. To date, the mechanisms by which obesity increases the risks of a wide range of human diseases are not well understood. Here we used a LC–MS/MS-based lipidomics, which can analyze >100 bioactive lipid mediators produced by cyclooxygenase, lipoxygenase, and cytochrome P450 enzymes, to analyze plasma profiles of lipid mediators in high-fat diet induced obesity in C57BL/6 mice. Our results show that the plasma concentrations of epoxyoctadecenoic acids (EpOMEs, also termed as leukotoxins) are significantly increased in plasma of high-fat diet-fed mice, in addition, EpOMEs are among the most abundant lipid mediators detected in mouse plasma. Since substantial studies have shown that EpOMEs and their metabolites have a large array of detrimental effects on health, enhanced levels of EpOMEs could contribute to the pathology of obesity.

1. Introduction

Obesity is growing at an alarming rate in the US: currently, more than 35% adults and nearly 17% children are obese [1,2]. Individuals with obesity have significantly increased risks of developing various diseases, including cardiovascular diseases, lung and respiratory diseases, diabetes, hypertension, and certain types of cancers [3]. To date, the mechanisms by which obesity increases the risks of such diverse range of human diseases are not well understood. While most previous mechanistic research of obesity has focused on proteinous mediators [4], the roles of non-proteinous regulators, such as eicosanoids and associated lipid mediators (LMs), are largely unknown.

Eicosanoids and associated LMs are enzymatic metabolites of polyunsaturated fatty acids (PUFAs) produced by cyclooxygenase (COX), lipoxygenase (LOX), and cytochrome P450 (CYP) enzymes [5–7]. The enzymatic metabolism of PUFAs leads to formation of several major classes of LMs, including prostaglandins produced by COX enzymes, leukotrienes and hydroxyl fatty acids produced by LOX enzymes, and epoxy and dihydroxy fatty acids produced by CYP enzymes [5–7]. Multiple PUFAs, including linoleic acid (LA, 18:2ω-6), α-linolenic acid (ALA, 18:3ω-3), dihomo-γ-linolenic acid (DGLA, 20:3ω-6), arachidonic acid (ARA, 20:4ω-6), eicosapentaenoic acid (EPA, 20:5ω-3), and docosahexaenoic acid (DHA, 22:6ω-3), are efficient substrates of these PUFA metabolizing enzymes [7,8]. Together, this leads to formation of a large array of LMs, which have diverse chemical structures and biological actions. Previous studies have shown that LMs play central roles in regulating inflammation and hemostasis, and play important roles in the pathology of obesity [9–14]. Most previous studies of eicosanoids in obesity have focused on profiles of LMs in adipose tissues, few studies have characterized the profiles of LMs in plasma. Since the circulating LMs could play an important role in systematic biological responses, here we used a LC–MS/MS-based lipidomics to analyze the plasma profiles of LMs in high-fat diet (HFD)-induced obesity in mice (see list of LMs covered by our LC–MS/MS method in Supplemental Table S1) [15].

* Corresponding author at: Department of Food Science, University of Massachusetts, Amherst, MA, USA.
E-mail address: guodongzhang@umass.edu (G. Zhang).
[1] W.W. and J.Y. contributed equally to this work.

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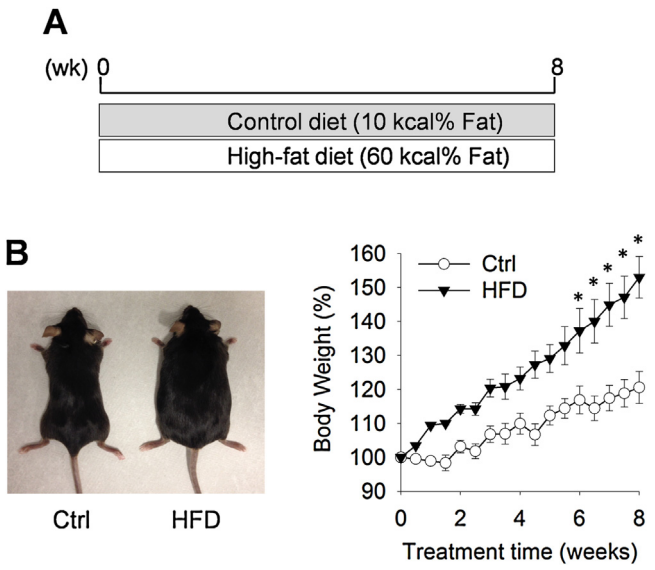

Fig. 1. HFD-induced obesity in C57BL/6 mice. (*A*) Scheme of animal experiment. (*B*) Body weight of mice maintained on control diet and HFD diet. n = 12 mice per group, the results are mean ± SEM, *P < 0.05.

2. Materials and methods

2.1. Obesity experiment

C57BL/6 male mice (6-week age, purchased from Charles River) were maintained on a high-fat diet (60% kcal% fat, purchased from Research Diet Inc., catalog # D12492) and a control diet (10 kcal% fat, catalog # D12450J from Research Diet Inc.) for 8 weeks. After 8 weeks of dietary feeding, the mice were sacrificed and the plasma was collected for LC–MS/MS analysis. The animal experiment was conducted in accordance with the protocols approved by the Institutional Animal Care and Use Committee of the University of Massachusetts Amherst.

2.2. Extraction of lipid metabolites from plasma and colon tissues

To extract LMs from mouse plasma, ~250 μL mouse plasma was mixed with deuterated internal standards (500 nM of d_4-6-keto PGF_{1a}, d_4-TXB_2, d_4-PGE_2, d_4-LTB_4, d_{11}-14,15-DHET, d_4-9-HODE, d_8-5-HETE, d_{11}-11,12-EET), then loaded onto pre-washed Waters® Oasis solid phase extraction cartridges, washed with 95:5 water/methanol with 0.1% acetic acid, the analytes were eluted with methanol and ethyl acetate, dried using a centrifugal vacuum evaporator, then reconstituted in methanol for LC–MS/MS analysis, as we described [15].

2.3. LC–MS/MS measurement of lipid metabolites

The LC–MS/MS analysis was carried out on an Agilent 1200SL HPLC system (Agilent, Santa Clara, CA) coupled to a 4000 QTRAP MS/MS (AB Sciex, Foster City, CA) as described in our previous report [15]. The peaks were identified according to the retention time and specific multiple reaction monitoring transitions of the standards of lipid metabolite. The concentrations of the lipid metabolites were calculated against the calibration curve with standards.

2.4. Data analysis

Data are expressed as mean ± standard error of the mean (SEM). For the comparison between control group and HFD group, Shapiro-Wilk test was used to verify the normality of data. When data were normally distributed, statistical significance was calculated using two-side *t*-test; otherwise, significance was determined by Mann-Whitney *U* test. All of these data analysis was performed by using SigmaPlot software (San Jose, CA). P values less than 0.05 are reported as statistically significant.

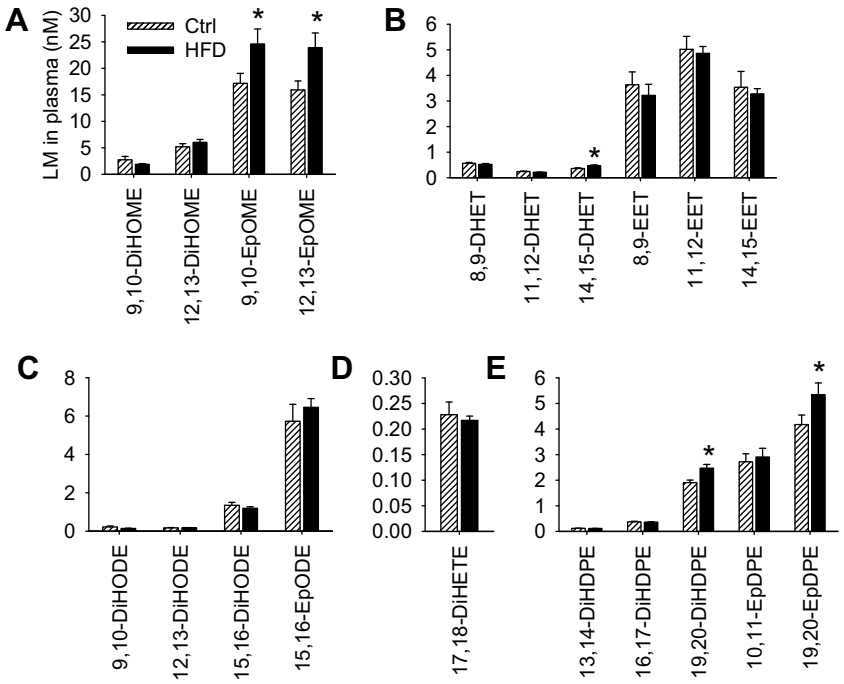

Fig. 2. Effects of HFD on plasma profiles of CYP-derived LMs, including fatty acid epoxides and diols derived from LA (*A*), ARA (*B*), ALA (*C*), EPA (*D*), and DHA (*E*). n = 12 mice per group, the results are mean ± SEM, * P < 0.05.