

## Di- (2ethylhexyl) Phthalate is Associated with Impairments of Glucose Metabolism and Inflammatory Process in Glucose Homeostatic Organs in Mice Fed Fat Diet: The Role of the Antioxidant Vitamin E

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**Abstract:** We evaluate the effects of di (2-ethylhexyl) phthalate (DEHP) on glucose metabolism, and inflammatory process; and the ability of vitamin E to suppress these deleterious effects in white adipose tissue (WAT), liver, and pancreas of mice fed high fat diet (HFD). Mice were treated with 500 mg/kg body weight DEHP while receiving an HFD. After 4 weeks, a group of mice received an oral dose of vitamin E. Ten weeks later, blood glucose, plasma insulin were assessed and then, mice were sacrificed and concentrations of tumor necrosis factor (TNF)- $\alpha$ , interleukin (IL)-1 $\beta$ , and interferon (IFN)- $\gamma$  from WAT, liver, and pancreas were measured. HFD-fed mice decreased weight gain following DEHP and vitamin E supplementations. HFD-fed mice expressed high levels of glucose and insulin. However, HFD-fed, DEHP treated mice expressed much higher level of glucose and low insulin concentration; decreased glucose, and increased insulin levels following vitamin E supplementation. TNF- $\alpha$ , IL-1 $\beta$ , and IFN- $\gamma$  levels decreased in WAT and liver following DEHP administration. However, TNF- $\alpha$ , and IL-1 $\beta$  levels increased in all organs while the IFN- $\gamma$  level decreased in WAT and pancreas following HFD-fed and DEHP treatment. Vitamin E decreased cytokine levels in HFD-fed mice, in WAT and the liver, and did not affect cytokine levels in the pancreas.

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### 1. Introduction

The prevalence of obesity and its associated metabolic complications has dramatically increased during the past decades in the United States and Europe, as well as developing countries. However, well-established risk factors, such as diet, lifestyle, and genetics, cannot fully explain this phenomenon (Lin et al., 2011). It has been suggested that this could, to some extent, be linked to the exposure to environmental pollutants, which coincidentally increased during the same period (Lin et al., 2011; Feige et al., 2010).

Among the environmental pollutants, di-(2-ethylhexyl) phthalate (DEHP) commonly used as a plasticizer in variety of products, including lubricants, perfume, hairsprays, and cosmetics, construction materials, wood finishers, adhesives, floorings and paints, medical devices such as containers, bags, and tubing, food packaging, and its propensity to leach can lead to high levels of human exposure.

The diet is believed to be the main source of DEHP exposure in the general population, with higher concentrations found in high-fat foods (e.g., dairy, poultry, and oils). Furthermore, because they are not covalently bound into plastic when used as plasticizers, DEHP was reported to leach or migrate from polyvinyl chloride (PVC)-containing items into

the air, dust, water, soil, and sediment (Kwak et al., 2009).

Several experimental studies showed that the macronutrient composition of a diet is an important environmental determinant of insulin action and elevated blood lipid levels associated with excess dietary fat can lead to the development of several disorders (Ferreira et al., 2011). In experimental animals, high fat diet results in impaired glucose tolerance and the impairment is associated with decreased basal and insulin-stimulated glucose metabolism, and insulin sensitivity (Lichtenstein et al., 2000).

Earlier animal studies revealed that short-term exposure to high doses of DEHP was hepatotoxic and animals treated with relatively high doses of phthalates such as DEHP typically display decreased body weight and fat mass (Thayer et al., 2012). Prolonged intake of a high-fat diet (HFD) is harmful to the pancreas by causing pancreatic endocrine and exocrine abnormalities and increasing inflammatory cytokine expression in the pancreas (Yan et al., 2012). Although the adverse effects of dietary fats are well known, it is currently thought that abnormally high chemokine levels, as released from the expanding adipose tissue in obesity, activate monocytes and increase the secretion of proinflammatory adipokines (Badawi et al., 2010). Several

proinflammatory cytokines, including tumor necrosis factor (TNF), interleukin (IL)-1 $\beta$ , and interferon (IFN)- $\gamma$  that are secreted by adipose tissue and other tissues, can cause insulin dysfunction in white adipose tissue (WAT), skeletal muscle, liver and pancreas by inhibiting insulin signal transduction and causing  $\beta$  cell failure. Proinflammatory cytokines, including TNF, can attenuate insulin-induced suppression of hepatic glucose production, enhance hepatic production of triglycerides and free fatty acids, and may inhibit insulin-stimulated glucose uptake (Byrne & Sarah, 2005).

It was recently demonstrated that proinflammatory cytokines, such as TNF- $\alpha$ , IFN- $\gamma$ , and IL-1 $\beta$ —the main cytokines involved in type 1 and type 2 diabetes—have different effects in insulin-sensitive organs, including WAT, liver, and pancreas. Notably, these molecules mediate inflammatory processes and  $\beta$  cell failure, ultimately resulting in overt metabolic disorders (Kim H & Kim K, 2007). IL-1 $\beta$  plays an important role in lipid metabolism by regulating insulin levels and lipase activity under physiologic conditions (Mojtaba et al., 2011). TNF impairs insulin signaling in hepatocytes and adipose tissue; reduces fatty acid oxidation in hepatocytes, which is accompanied by the accumulation of bioactive lipids (Galic et al., 2010); and stimulates adipocyte lipolysis (Moldes et al., 2001). IFN- $\gamma$ , which is produced by T cells that infiltrate human islets, as well as macrophage-derived IL-1 $\beta$  and TNF, may have cytotoxic effects on  $\beta$  cells in vivo (Khazai et al., 2007; Ablamuniths et al., 1998).

Increasing the intake of antioxidants could prevent diseases and may have beneficial effects on health. Antioxidants, such as vitamin E, are capable of quenching free radicals and prevent them from causing cell damage (Sen et al., 2010). Vitamin E is the most effective chain-breaking, peroxy radical scavenger that protects biologic membranes against lipid peroxidation. Dietary vitamin E supplementation has a variety of effects on the immune system, including enhancing humoral and cellular immune reactions, regulation of the cytokine balance in the immune system, T cell differentiation, proliferative responses of lymphocytes to mutagens, and the suppression of anti-inflammatory reactions (Hsieh & Lin, 2005).

Because HFD, and DEHP are well known to affect insulin sensitivity and proinflammatory cytokine expression in WAT, pancreas, and liver; our objective was to evaluate the combined effects of di-(2-ethylhexyl) phthalate (DEHP) and an HFD on insulin resistance, the inflammatory process in white adipose tissue (WAT), liver, and pancreas, and to determine the ability of the antioxidant vitamin E to suppress these deleterious effects.

## 2. Material and Methods

### *Chemicals and Diets*

DEHP (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in fish oil (99.5% pure; Shanghai Solvent Factory, Shanghai, China) and vitamin E ( $\alpha$ -tocopherol) (Sigma Aldrich) was dissolved in Tween 80 (AMRESKO LLC, Solon, OH, USA). HFD (31% lard, 3% soybean, 12% sucrose, and 54% rodent chow diet [RCD]) was prepared according to Harlan Laboratories, Inc, 2008 formula (TD.06414) with little modification. The diet was pelleted and dried before being provided to the mice. Both diets were purchased from Hubei Experimental Animal Center (Wuhan, China). 2.5g of D-glucose was dissolved in 10 ml of saline and the solution was prepared the day before glucose ingestion for further efficiency. 4g EDTA was dissolved in 100 ml of 0.88% saline solution.

### *Mice and Feeding Study*

Ten-week-old male Balb/c Mice ( $n = 30$ ) were acclimatized for 1 week, during which time they were fed rodent chow diet (RCD). After this period, the mice were randomly divided into four groups of mice. One groups of mice ( $n=12$  mice) were fed the high fat diet (HFD) (31% lard, 3% soybean, 12% sucrose and 54% normal diet powder ) and were orally treated with 500 mg/kg body weight DEHP (Sigma-Aldrich) every day for 10 weeks. After 4 weeks of treatment, this group was subdivided into two subgroups ( $n=6$  mice per group); one subgroup continued their diet and DEHP treatment, while the other subgroup also received daily an oral dose of vitamin E ( $\alpha$ -tocopherol) (Sigma-Aldrich) (30 IU/day/kg body weight).

A further two groups of mice ( $n=6$  mice per group) were fed the RCD, HFD, without DEHP supplementation for 10 weeks. The remaining six mice were fed the RCD with DEHP supplementation. All mice were weighted once a week. At week 10, mice were sacrificed and WAT, liver, and pancreas were quickly removed, homogenized, and centrifuged. The homogenate was then stored at  $-20^{\circ}\text{C}$  until analysis. The current study protocol was approved by the Ethics Committee for Animal Care and Use, Central China Normal University, China (March 26, 2010; CCNU-SKY-2010-005).

### *Oral Glucose Tolerance Test (OGTT), Insulin Assay and Homeostasis Model Assessment Insulin Resistance (HOMA-IR)*

Blood samples were drawn from each mouse's tail at the end of the experimental period (10 weeks). An oral glucose tolerance test was performed before necropsy after 10 weeks following an overnight fast (16h). The mice were administered glucose at a level of 0.25 g/kg body weight. Samples

of whole blood (2-3µl each) were collected from a tail-clip bleed before and immediately 30, 60, and 120 minutes after glucose administration. Blood glucose levels were measured using a Freestyle Glucose Monitoring system. Additional samples of whole blood (30 µl) were collected from tail for the determination of serum insulin level after glucose ingestion. The blood was allowed to clot on ice for 15 minutes and then centrifuged (200Xg) at 4°C for 10 minutes. Serum was analyzed using the Rat/Mouse Insulin ELISA kit from e-Bioscience Inc (San Diego, CA, USA) according to the manufacturer's instructions. The fasting blood glucose (FBG) and the fasting stimulated insulin (FSI) concentrations were used to calculate the Homeostasis Model Assessment - Insulin Resistance (HOMA-IR) value:

$$HOMA - IR = \frac{FSI \left( \frac{FE}{\mu l} \right) \times FBG \left( \mu \frac{mol}{ml} \right)}{22.5}$$

### Tissue Homogenization and Pro-inflammatory Cytokine Enzyme-Linked Immunosorbent Assays

WAT, liver, and pancreas (200–300 mg) samples were homogenized in 0.5 ml of ice-cold PBS (pH 7.4) using a Potter–Elvehjem homogenizer. An aliquot of the homogenate was centrifuged for 15 min at 14,000×g. The supernatant was retained and concentrations of TNF-α, IL-1β, and IFN-γ were measured using ELISA commercially available murine assay kits from e-Bioscience Inc. (San Diego, CA, USA) as suggested by the manufacturer. All measurements were performed in duplicate and TNF, IL-1β, and IFN-γ concentrations in each sample were calculated using a standard curve based on the optical density of concentration standards provided in each kit.

### Statistical Analysis

All data were analyzed using origin V6.10.52 software and the results are presented as means ± standard error. One-way analysis of variance (ANOVA) was used to compare the means, and values of P<0.05 were considered statistically significant. The figure 1 represents the overview of our study.

## 3. Results

### Effects of DEHP in Combination with the HFD, and Vitamin E on Mice body weight gain

The initial body weights of mice, prior to feeding with experimental diets, did not significantly differ. As shown on figure 2, at week 4, however, significant increases in the body weights of HFD-fed and RCD-fed control mice were observed compared to mice in addition treated with DEHP. Additionally, at week 8 and 10, the DEHP exposed mice fed an HFD supplemented with vitamin E significantly decreased body weight comparable to the level of

mice fed HFD with or without DEHP treatment. These results indicate that DEHP and vitamin E had suppressive effects on body weight gain especially following HFD consumption in mice.

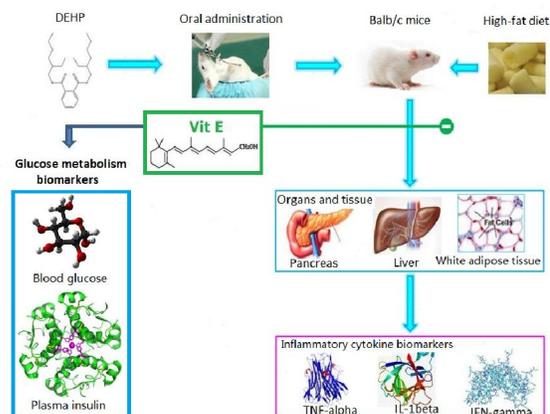


Figure 1 Study overview

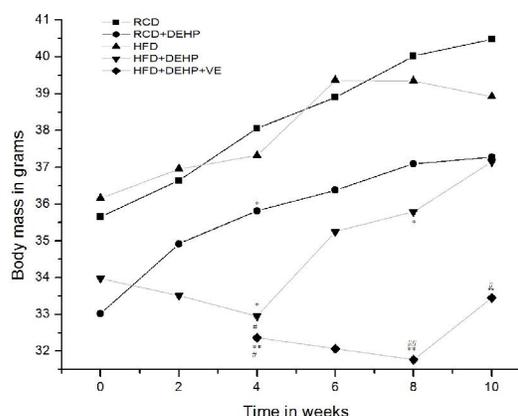
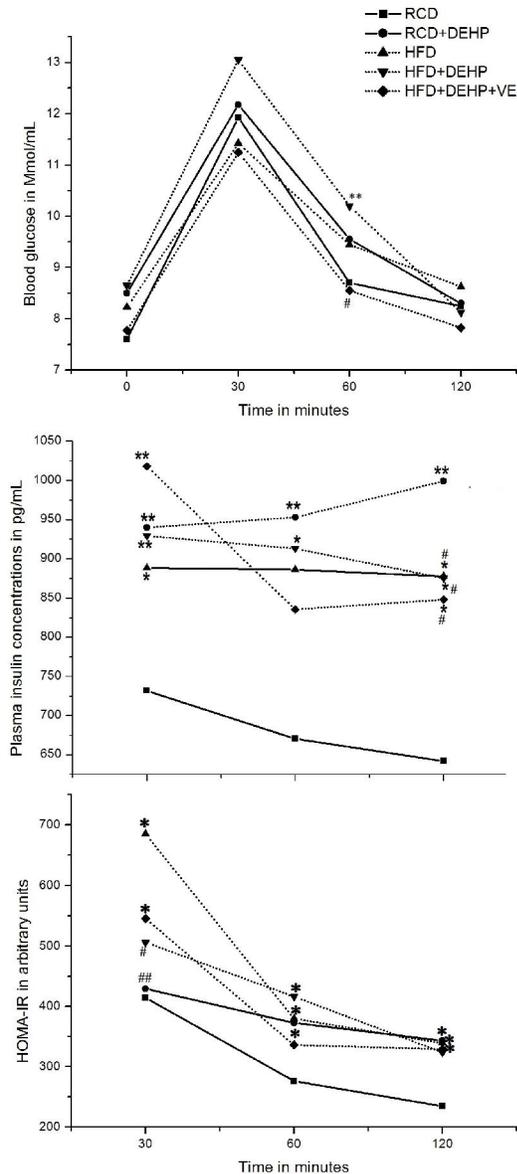


Figure 2 Effects of DEHP, and vitamin E on body weight gain in mice fed fat die. \* P< 0.05 or \*\*P< 0.01 for the high fat diet (HFD), rodent chow diet (RCD), HFD DEHP, or vit E treated mice effects mice body mass compared to the RCD mice model effect. # P< 0.05 or ### P< 0.01 for the RCD, HFD, or vitamin E treated mice effects mice body mass compared to the HFD mice model effect.

### Effects of DEHP in Combination with the HFD, and vitamin E on Blood glucose levels, Insulin Concentrations, and HOMA-IR values

As shown on figure 3, DEHP exposed mice fed HFD expressed high levels of glucose at 30 and 60 minutes after glucose ingestion followed by the levels of mice fed HFD compared to the levels of mice fed RCD with or without DEHP supplement (fig 3A). In contrast, the high levels of insulin and HOMA-IR values were observed at 60 and 120

minutes after glucose ingestion in HFD-fed mice followed by levels of HFD-fed mice with DEHP supplement compared to the levels of mice fed RCD with or without DEHP supplement (fig 3B & fig 3C).



**Figure 3** Effects of DEHP, and vitamin E on glucose metabolism in HFD-fed mice. Data are means  $\pm$  SE. \*  $P < 0.05$  or \*\* $P < 0.01$  for HFD, RCD, HFD DEHP, or vitamin E treated mice effects on blood glucose level, plasma insulin concentrations, and HOMA-IR value compared to RCD mice model effect. #  $P < 0.05$  or ##  $P < 0.01$  for the RCD, HFD, or vitamin E treated mice effects on blood glucose level, plasma insulin concentrations, and HOMA-IR value compared to the HFD mice model effect.

As shown on figure 3, HFD-fed mice, exposed to DEHP with vitamin E supplement expressed low level of glucose at 30, 60, and 120 minutes and HOMA-IR at 60, and 120 minutes after glucose ingestion compared with those for mice fed HFD without vitamin E supplement (fig 3A & fig 3C). In contrast, HFD-fed mice, exposed to DEHP with vitamin E supplement expressed high level of insulin at 30 minutes, and it significantly decreased at 60, and 120 minutes after glucose ingestion (fig 3B).

#### **Effects of DEHP in Combination with the HFD, and vitamin E on Pro-inflammatory Cytokines Expression in Target Organs**

As shown on figure 4, proinflammatory cytokines were differently expressed in target organs involved in glucose homeostasis. In WAT, TNF- $\alpha$  level significantly decreased in HFD-fed, and RCD-fed, DEHP exposed mice compared with those in RCD-fed mice model. However, TNF- $\alpha$  level significantly increased in HFD-fed, DEHP exposed mice compared with those in HFD-fed and RCD-fed, DEHP exposed mice. In pancreas, and the liver, TNF- $\alpha$  level did not show any change following diet feeding. However, TNF- $\alpha$  level decreased or increased significantly in HFD-fed, DEHP exposed mice respectively in pancreas or the liver compared to that in RCD-fed, DEHP exposed mice (fig 4A).

In WAT and pancreas, IL-1 $\beta$  level significantly increased in HFD-fed mice compared with that in RCD-fed mice. Additionally, IL-1 $\beta$  level significantly increased in HFD-fed, and RCD-fed mice following DEHP administration and regardless of food supplement. In liver, IL-1 $\beta$  level significantly decreased in HFD-fed mice compared with that in RCD-fed mice. Furthermore, the level of this cytokine significantly increased in HFD-fed, DEHP exposed mice compared with that in RCD-fed, DEHP exposed mice (fig 4B).

In WAT, IFN- $\gamma$  level significantly increased in RCD-fed, DEHP exposed mice compared with those in RCD-fed and HFD-fed mice; however, IFN- $\gamma$  level decreased in HFD-fed, DEHP exposed mice compared with that in RCD-fed, DEHP exposed mice. In pancreas, HFD-fed, and DEHP exposed mice expressed significantly low level of IFN- $\gamma$  compared with that in RCD-fed mice; additionally, HFD-fed, DEHP exposed mice decreased significantly IFN- $\gamma$  level in pancreas compared with that expressed in RCD-fed, DEHP exposed mice. In liver, IFN- $\gamma$  level increased significantly in HFD-fed mice compared with that in RCD-fed mice and decreased significantly following DEHP administration; otherwise, IFN- $\gamma$  level increased significantly in HFD-fed, DEHP exposed mice compared with that in RCD-fed, DEHP exposed mice (fig 4C).

As shown on figure 4, the antioxidant vitamin E had several effects on WAT, liver, and pancreas in HFD-fed, DEHP treated mice. In WAT, TNF- $\alpha$  level decreased significantly in HFD-fed, DEHP treated mice with vitamin E supplement compared with that in HFD-fed, DEHP exposed mice without that antioxidant supplement. In pancreas, there was no change of TNF- $\alpha$  level in HFD-fed, DEHP exposed mice with or without vitamin E supplement. In liver, TNF- $\alpha$  level decreased in HFD-fed, DEHP treated mice with vitamin E supplement compared with those in HFD-fed, and HFD-fed, DEHP exposed mice. However, the difference between TNF- $\alpha$  level in HFD-fed, DEHP exposed mice with or without vitamin E supplement was not significant (fig 4A).

In WAT and liver, IL-1 $\beta$  level decreased significantly in HFD-fed, DEHP exposed mice with vitamin E supplement compared with those in HFD-fed and HFD-fed, DEHP exposed mice. In pancreas, there was no change of IL-1 $\beta$  level in HFD-fed, DEHP exposed mice with or without vitamin E supplement (fig 4B).

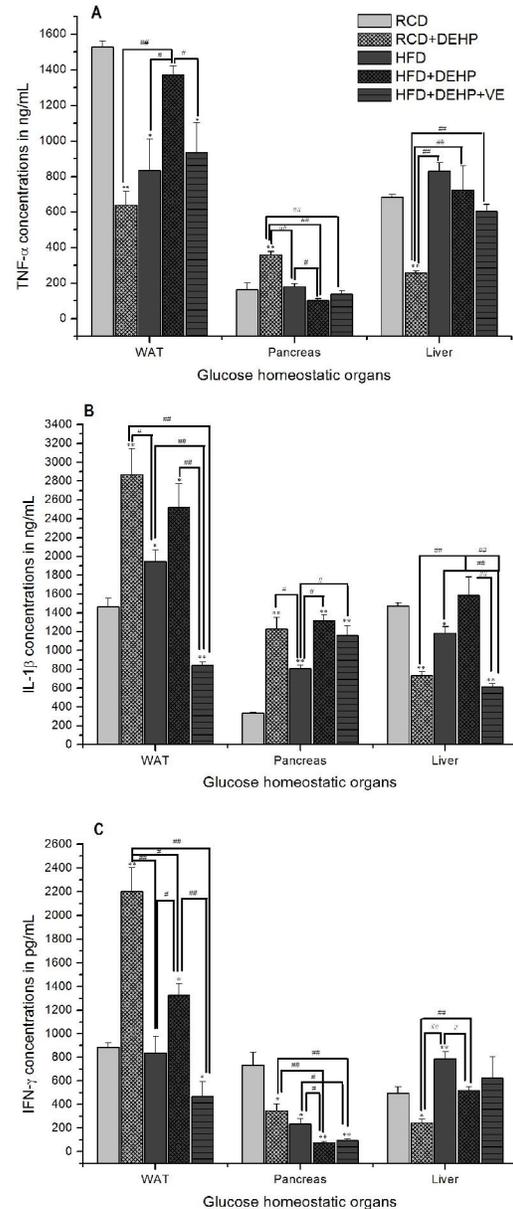
In WAT and pancreas, IFN- $\gamma$  level decreased significantly in HFD-fed, DEHP exposed mice with vitamin E supplement compared with that in HFD-fed, DEHP exposed without that antioxidant supplement. In liver, the difference between IFN- $\gamma$  level in HFD-fed, DEHP exposed mice with or without vitamin E supplement was not significant (fig 4C).

As shown on figure 4, the antioxidant vitamin E had several effects on WAT, liver, and pancreas in HFD-fed, DEHP treated mice. In WAT, TNF- $\alpha$  level decreased significantly in HFD-fed, DEHP treated mice with vitamin E supplement compared with that in HFD-fed, DEHP exposed mice without that antioxidant supplement. In pancreas, there was no change of TNF- $\alpha$  level in HFD-fed, DEHP exposed mice with or without vitamin E supplement. In liver, TNF- $\alpha$  level decreased in HFD-fed, DEHP treated mice with vitamin E supplement compared with those in HFD-fed, and HFD-fed, DEHP exposed mice. However, the difference between TNF- $\alpha$  level in HFD-fed, DEHP exposed mice with or without vitamin E supplement was not significant (fig 4A).

In WAT and liver, IL-1 $\beta$  level decreased significantly in HFD-fed, DEHP exposed mice with vitamin E supplement compared with those in HFD-fed and HFD-fed, DEHP exposed mice. In pancreas, there was no change of IL-1 $\beta$  level in HFD-fed, DEHP exposed mice with or without vitamin E supplement (fig 4B).

In WAT and pancreas, IFN- $\gamma$  level decreased significantly in HFD-fed, DEHP exposed

mice with vitamin E supplement compared with that in HFD-fed, DEHP exposed without that antioxidant supplement. In liver, the difference between IFN- $\gamma$  level in HFD-fed, DEHP exposed mice with or without vitamin E supplement was not significant (fig 4C).



**Figure 4** Effects of DEHP, and vitamin E on cytokine levels in WAT, liver, and pancreas in HFD-fed mice. \*  $P < 0.05$  or \*\*  $P < 0.01$  for the high fat diet (HFD), rodent chow diet (RCD) or HFD DEHP or Vitamin E treated mice effects on cytokine expression level compared to the rodent chow diet (RCD) mice model effect in the three target organs. Other groups were then compared one by one at #  $P < 0.05$  or ##  $P < 0.01$ .

#### 4. Discussions

In this study, we examined the combined effects of an HFD and DEHP, on glucose metabolism, and on the expression of proinflammatory cytokines (TNF, IL-1 $\beta$ , and IFN- $\gamma$ ) in three organs involved in glucose homeostasis. We also examined whether treatment with vitamin E, an antioxidant, could suppress these effects following DEHP treatment and fat diet feeding.

Our study showed that the body weights of HFD-fed and RCD-fed mice did not differ along the time of exposure. However, DEHP-exposed mice decreased weights compared to non-exposed mice, especially in HFD-fed mice. There were no differences in the average body weight between HFD-fed and RCD-fed group of mice until 14 weeks of age (Akagari et al., 2008) and DEHP exposure protected mice for weight gain under both a regular diet and a diet containing high fat content and it strongly protected mice from HFD-induced obesity without affecting food intake by inhibiting adipocyte hypertrophy and hepatic droplet accumulation (Feige et al., 2010). Thus, increased FA release from adipocytes together with decreased FA uptake from the circulation might, at least in part, explain the loss of fat (Xie et al., 2002).

Notably, we found that HFD-fed, DEHP treated mice with vitamin E supplement decreased weights compared to those in the same group did not receive vitamin E supplement. The body weight decreased when the dosage was more than 100 IU/kg per day, and all mice died within 3 days at 400 IU/kg per day (Yasunage et al., 1982).

We also found that HFD-fed mice expressed high levels of glucose and insulin while HFD-mice with DEHP supplement expressed much higher level of glucose with low insulin concentrations and HOMA-IR values. The high fat diet increased in plasma levels of glucose, insulin and insulin resistance and fasting insulin concentrations and HOMA-IR, which are indices of insulin resistance did not change during the feeding with a high fat diet for 8 weeks, but significantly increased in 22-week old mice fed a high fat diet for 18 weeks (Choi et al., 2003), due to the pancreatic infiltration which characterized by a heavier pancreas due to more pancreatic fat, consisting of a high composition of triglyceride and FFAs, as well as increased cytokine production, leading to increased basal insulin release and impaired glucose-stimulated insulin secretion by beta-cells (Fraulob et al., 2010). Insulin levels were significantly lower in mice treated with 1,000 mg/kg b.w day DEHP, whereas glycemia was not affected (2). DEHP induced deleterious effects like degenerative changes in the brain, and thyroid decreased in the level of serum insulin; it

significantly decreased insulin receptor mRNA expression suggesting that DEHP downregulates the transcription of the gene (Rajesh et al., 2013).

HFD-fed, DEHP exposed mice with vitamin E supplement expressed high levels of plasma insulin and low levels of serum glucose and HOMA-IR value. These results suggest that mice with vitamin E supplement well regulate blood glucose compared to those without vitamin E supplement. Vitamin E improves the free radicals defense system potential and insulin sensitivity of rats fed with high fructose diet (Rajesh et al., 2013).

Our findings also yield potentially impact of a high fat diet in combination with orally exposure to DEHP on proinflammatory cytokine expression in glucose homeostatic organs. TNF- $\alpha$  level decreased following DEHP administration in WAT, liver, except in pancreas. Furthermore, fat diet did not have any effect of TNF- $\alpha$  expression in pancreas, and the liver but decreased level of that cytokine in WAT. This is consistent with earlier findings of Betanzos et al., that the expression levels of TNF- $\alpha$  decreased only in adipose tissue of mice fed with an HFD, while in other tissues there was no change in the expression level of this cytokine (Betanzos et al., 2012). Recent studies have indicated that adipocytes are important physiological regulators of the immune responses in fat deposits via Toll-like receptor (TLR) signaling cascades. Adipocytes may also inhibit visceral adipogenesis by suppressing the galanin-mediated adipogenesis cascade and may attenuate cytokine production in adipose tissue by repressing proinflammatory signaling cascades involving TLR3 and TLR4 in HFD-fed mice (Betanzos et al., 2012). Additionally, a short period of high-fat feeding failed to stimulate diet-induced thermogenesis and mice showed increased mRNA and protein expression of lipase (Rippe et al., 2003), suggesting that dietary fat is capable of transcriptionally regulating lipase expression to protect the pancreas and adipose tissue against inflammation. In our study, mice were treated with DEHP for 6 weeks and each diet for 10 weeks. It was reported that PPARs can protect the liver from obesity-induced inflammation in animals fed an HFD for 6 months (Abdelmegeed et al., 2011), and the enzymes responsible for fatty catabolism were increased in rodents exposed to DEHP at levels as high as 1,500%.

IL-1 $\beta$  level increased in WAT and pancreas following high fat diet supplement and independently with DEHP exposure. However, in the liver, IL-1 $\beta$  decreased following fat diet feeding and increased following DEHP administration. The receptor for IL-1 $\beta$  is highly expressed in the  $\beta$ - cells; this may explain the high sensitivity of the  $\beta$ - cells to IL-1 $\beta$  (Ardestani et al., 2011) and a number of studies have

described a positive association between IL-1 $\beta$  gene polymorphisms and obesity, suggesting the protein has functional roles in regulating fat mass, fat metabolism, and body mass (Mojtaba et al., 2011; Novo et al., 2013). Phthalates including DEHP are regulators of important transcriptional factors in homeostasis in the liver, i.e. peroxisome proliferator-activated receptors (Ichiro, 2013), which are also widely involved in obesity status in adipose tissue.

IFN- $\gamma$  level increased following DEHP administration; furthermore, the high fat diet supplement decreased level of that cytokine in white adipose tissue. IFN- $\gamma$  expression in adipose tissue remained below detection limits in obese mice but was significantly induced 6 h after LPS administration (Poulain et al., 2013) suggesting that DEHP can induce IFN- $\gamma$  expression in WAT with fat diet feeding. In contrast, in liver and pancreas, IFN- $\gamma$  level decreased following DEHP administration and the high fat decreased and increased the level of that cytokine in pancreas and the liver, respectively.

As expected, vitamin E had antioxidant effects by decreasing the proinflammatory cytokine levels in WAT and the liver in HFD-fed, DEHP-treated mice.

Vitamin E supplement decreased levels of TNF- $\alpha$  and IL-1 $\beta$  in WAT and liver but did not affect IFN- $\gamma$  expression in liver in mice fed high fat diet and exposed to DEHP. In HFD-induced obesity, 6 weeks of  $\alpha$ -tocopherol supplementation reduced serum levels of TNF in patients with metabolic syndrome (Lira et al., 2002). Vitamin E inhibits the activation of NF- $\kappa$ B by multiple stimuli, including IL-1 $\beta$ , and TNF- $\alpha$  (Rajesh et al., 2013). In the pancreas, vitamin E did not affect proinflammatory expression. An earlier study revealed that vitamin E did not have beneficial effects on advanced  $\beta$  cell autoimmunity, but vitamin E may have protective effects in the pancreas, possibly by acting together with other antioxidants (Pazdro & Burgess, 2010).

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