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Deoxynivalenol-induced weight loss in the diet-induced obese mouse is reversible and PKR-independent

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HIGHLIGHTS

- Mice were fed a high-fat diet to induce obesity and increase body weight.
- Diet-induced obese mice lost weight when fed deoxynivalenol (DON).
- Weight suppression was reversed in when DON was removed from diet.
- DON's weight suppression was not dependent on the stress kinase PKR.

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ABSTRACT

The trichothecene deoxynivalenol (DON), a potent ribotoxic mycotoxin produced by the cereal blight fungus *Fusarium graminearum*, commonly contaminates grain-based foods. Oral exposure to DON causes decreased food intake, reduced weight gain and body weight loss in experimental animals – effects that have been linked to dysregulation of hormones responsible for mediating satiety at the central nervous system level. When diet-induced obese (DIO) mice are fed DON, they consume less food, eventually achieving body weights of control diet-fed mice. Here, we extended these findings by characterizing: (1) reversibility of DON-induced body weight loss and anorexia in DIO mice and (2) the role of double-stranded RNA-activated protein kinase (PKR) which has been previously linked to initiation of the ribotoxic stress response. The results demonstrated that DON-induced weight loss was reversible in DIO mice and this effect corresponded to initiation of a robust hyperphagic response. When DIO mice deficient in PKR were exposed to DON, they exhibited weight suppression similar to DIO wild-type fed the toxin, suggesting the toxin's weight effects were not dependent on PKR. Taken together, DON's effects on food consumption and body weight are not permanent and, furthermore, PKR is not an essential signaling molecule for DON's anorectic and weight effects.

1. Introduction

Deoxynivalenol (DON), a trichothecene mycotoxin produced by *Fusarium graminearum* during cereal head blight, frequently contaminates grain products worldwide. Acute DON exposure in experimental animals is associated with gastrointestinal illness and vomiting, while sub-chronic, low-level exposure results in anorexia, decreased growth rate and body weight loss (Amuzie and Pestka, 2010; Flannery et al., 2011; Hughes et al., 1999; Trenholm et al., 1984). These latter effects have been linked to dysregulation of hormones that mediate satiety in the hypothalamus of the brain (Girardet et al., 2011; Kobayashi-Hattori et al., 2011).

As of 2011, 36% of the United States' adult population over 20 years of age was considered obese (USDHHS, 2012). The obese state increases risk of developing metabolic disease, high blood pressure, heart disease, particular types of cancer and potentially increases susceptibility to food and environmental toxicants (Bianchini et al., 2002; Bray, 2004; Brewer and Balen, 2010; Lin et al., 2010; Manson et al., 1990; Meigs et al., 1997; Must et al., 1999). The diet-induced obese (DIO) mouse, an animal model widely employed to investigate mechanisms and implications of obesity, can be used to study the effects of toxicant exposure during the obese state. Our laboratory has recently determined when DIO mice are exposed to DON via diet, they consume less food and eventually achieve body

Abbreviations: ANOVA, Analysis of Variance; CCK, cholecystokin; SOCS3, suppressor of cytokine signaling 3; DON, deoxynivalenol; DIO, diet-induced obese; IL-1β, interleukin-1β; IL-6, interleukin-6; LFD, low fat diet; PYY, peptide YY; PKR-KO, PKR-knockout; PCR, polymerase chain reaction; RSR, ribotoxic stress response; PKR, RNA-activated protein kinase; TNF-α, tumor necrosis factor-α; WT, wild type.

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weights of control diet-fed mice (Amuzie et al., 2011; Kobayashi-Hattori et al., 2011). Two questions arising from studies of DON's effects on the DIO model relate to (1) the reversibility of the weight loss when the toxin is removed from the diet and (2) the underlying mechanisms for the toxin's attenuating effects.

DON is a potent activator of the innate immune response in experimental animals. It can evoke robust induction of proinflammatory cytokines such as interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) within a few hours of administration (Amuzie et al., 2009). Although these responses are seemingly paradoxical for a translational inhibitor, the effects appear to be driven by mRNA stabilization and transcription of these proinflammatory genes (Chung et al., 2003; Jia et al., 2006; Pestka et al., 2004). This upregulation could mediate DON's effect on food intake and body weight in at least two ways. First, it is well-known that proinflammatory gene expression is associated with a sickness response in many species that include anorexia (Johnson, 1998). Second, recent evidence from mouse models suggests a mechanistic link between DON-induced proinflammatory cytokine stimulation and its negative weight effects through the action of suppressor of cytokine signaling 3 (SOCS3) on growth hormone signaling (Amuzie and Pestka, 2010; Amuzie et al., 2009).

DON's capacity to robustly induce the innate immune response has been linked in mononuclear phagocyte models to the activation of the mitogen activated protein kinase (MAPK) signaling cascade – a process known as the ribotoxic stress response (RSR) (Pestka, 2010). Studies in the murine RAW 264.7 macrophage and human U937 monocyte models have revealed that double-stranded RNA-activated protein kinase (PKR), a ribosome-associated signaling molecule, is a key initiator of DON-induced RSR (Bae et al., 2010; Zhou et al., 2003). Notably, PKR activation occurs within 5 min upon DON exposure in these cultures and both pharmacologic inhibition of PKR inhibitors and its genetic ablation suppress DON-induced MAPK signaling cascade and downstream sequelae in vitro (Zhou et al., 2003, 2005).

The aims of this study were to address the aforementioned questions about response longevity and mechanisms by testing two hypotheses. The first hypothesis, addressed in Studies 1 and 2, was that dietary DON's weight suppressive effects will be reversed once the toxin was removed from the food of DIO mice. The second hypothesis, addressed in Study 3, was that PKR-knockout (PKR-KO) DIO mice will be recalcitrant to DON-induced weight suppression as compared to wild type (WT) DIO mice. The results presented herein demonstrate DON's weight suppressive effects in DIO mice were reversible but independent of PKR.

2. Materials and methods

2.1. Mice and diets

The Institutional Animal Care and Use Committee at Michigan State University approved all experiments involving mice. For Studies 1 and 2 assessing reversibility, adult C57BL6 (10–11 wk) mice were purchased from Charles River Breeding (Portage, MI). For Study 3 on the role of PKR, C57BL6 adult female wild-type (WT) and PKR-KO were provided by Dr. Randal Kauffman (University of Michigan, Ann Arbor, MI) and bred by Van Andel Institute (Grand Rapids, MI). The WT and PKR-KO mice genotypes were verified using polymerase chain reaction (PCR) (Baltzis et al., 2002).

Upon arrival, mice were acclimated to a 12 h light/dark cycle and kept at a constant temperature (21–24° C) and humidity (40–55%). All animals were housed in polycarbonate cages containing aspen bedding, nestlets and wire-top lids. Mice were allowed free access to food and water for the duration of experiments. To induce obesity, mice were fed a pelleted very high fat diet (VHFD; D12492) containing 60% kcal from fat to which DON had been added by a cold extrusion method 0, 10 or 20 ppm (Research Diets Inc., New Brunswick, NJ). DON was obtained from *F. graminearum* cultures, extracted, isolated and purity verified according to our previously published method (Clifford et al., 2003). Control mice were fed a pellet low fat diet (LFD) containing 10% kcal from fat (D12450B; Research Diets Inc.).

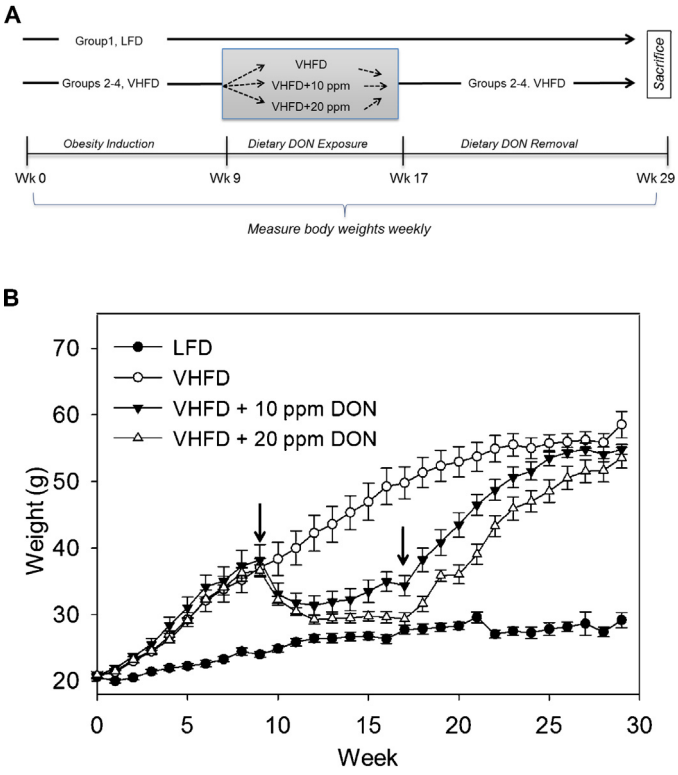

Fig. 1. Effect of long term inclusion and removal of, dietary DON on body weights of DIO mice. (A) Study 1 experimental design (B) Obesity was induced in 3 groups of mice with 2 groups being switched from VHFD to VHFD + 10 ppm DON or VHFD + 20 ppm DON at 9 wk as indicated by the first arrow. At 17 wk mice fed DON were switched back to unamended VHFD as indicated by the second arrow. Mice fed 10% kcal from fat served as a low-fat control group. Weights were assessed weekly. Data are mean ± SEM (n = 8/gp).

2.2. Experimental design

Study 1. The goal of this experiment was to characterize body weight changes that occurred upon the addition and removal of DON from VHFD. Four different groups of C57BL6 mice were employed (n = 8/gp; 3/cage) (Fig. 1A). One control group was fed LFD diet and the other three groups given VHFD to induce obesity. At wk 9, two of the VHFD groups were switched to either VHFD + 10 ppm DON or VHFD + 20 ppm DON for 8 wk. Then, at wk 17, the two VHFD + DON-fed groups were switched back to VHFD. Body weights were recorded weekly throughout the experiment.

Study 2. The purpose of this experiment was to relate food intake to body weight changes during the addition and subsequent removal of DON from VHFD (Fig. 2A). Briefly, mice were fed VHFD for 8 wk to induce obesity and then split into two groups (n = 9–10/group). One group was continued on VHFD while the other group was fed VHFD + 10 ppm DON for 5 wk. At wk 13, mice fed VHFD + 10 ppm were switched back to unamended VHFD. Daily food intake and weights were measured as described previously (Kobayashi-Hattori et al., 2011) from 52 to 66 d to capture the transition period from VHFD to VHFD + 10 ppm DON and from 88 to 96 d to encompass the transition from VHFD + 10 ppm DON back to VHFD.

Study 3. The goal of this study was to determine whether PKR was essential for DON-induced weight suppression (Fig. 3A). Upon arrival, PKR-KO and wild-type (WT) mice were randomized into the following groups (n = 5–6/gp): (1) WT mice fed VHFD, (2) WT mice fed VHFD + 10 ppm DON, (3) PKR-KO mice fed VHFD and (4) PKR-KO mice fed VHFD + 10 ppm DON. Mice were housed 2–3 per cage and allowed unrestricted access to food and water. Mice were weighed weekly for 15 wks and then euthanized using 50 mg/kg bw sodium pentobarbital.

2.3. Statistics

Statistical comparisons between two diet groups were made for each time point using a Student's t-test unless normality failed and a Mann–Whitney Rank Sum Test was executed. Multiple diet groups at a given time point were analyzed for statistical significance using a One-way Analysis of Variance (ANOVA) with Holm–Sidak multiple comparison procedure and when normality failed a One-way ANOVA on Ranks with a Tukey test was utilized. Statistical significance was obtained when $p < 0.05$.