THE EFFECT OF 2378-TCDD ON THE PLASMA LEVELS OF LEPTIN, TRYPTOPHAN, AND LIPID PARAMETERS

Heldur Hakk and Gerald L. Larsen

United States Department of Agriculture, Agricultural Research Service, Biosciences Research Laboratory, Box 5674 State University Station, Fargo, ND 58105, USA

Introduction

One of the hallmark features of acute dioxin toxicity is a body weight loss that results in death if lethal doses are involved. The so-called "wasting syndrome" is a result of hypophagia[1], and is manifested in animals exposed to lethal doses of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2378-TCDD) as an immediate, chronic body weight loss. Rats that were administered a sublethal dose of 2378-TCDD also showed an initial decrease in body weight, but displayed an eventual rise in weight. However, dioxin treated rats never fully recover to the weight of a paired control. The explanation for these results is that 2378-TCDD causes a decrease in the body weight set point, presumably by interfering with the signaling in the hypothalamus[2].

Recent interest in the hormone leptin has been spurred by its ability to modulate appetite and control body weight, and the discovery of a leptin receptor[3]. The purpose of the present study is to investigate how levels of various plasma components in male rats, most significantly the hormone leptin, are affected after exposure to sublethal doses of 2378-TCDD, and thereby hypothesize about the role of leptin in the wasting syndrome.

Materials and Methods

Chemical: Unlabelled 2378-TCDD was synthesized in-house by accepted methods[4]. A dose of 25 µg/kg 2378-TCDD in 0.5 ml peanut oil, was prepared and administered by gavage.

Animals: Animals used in the study were male Sprague-Dawley rats (Taconic, Germantown, NY, USA), and ranged from 215-254g. Rats were housed in stainless steel metabolism cages that allowed for the separate collection of urine and feces.

Treatment: In the time-course study, 21 rats received the 2378-TCDD dose solution, while 21 control rats received vehicle alone. Feed consumption, including spilled feed, and body weights were measured each day. At days 1, 2, 3, 7, 14, 21, and 28, three treated and three control animals were sacrificed by dorsal vein exsanguination. Liver and thymus were removed, weighed, and frozen immediately in liquid nitrogen.

In the pair-feeding study, rats were given 0.5 ml peanut oil at t=0h, and received the same amount of feed that the 2378-TCDD treated rats consumed on the same day of the experiment. Body weights were measured daily and the rats were sacrificed as above, i.e., at 1, 2, 3, 7, 14, 21, and 28 days.

Analyses: Plasma was separated from the red blood cells by centrifugation at 4000 x g for 20 min. Plasma was analyzed for leptin hormone levels using a mouse leptin ELISA kit (Crystal Chem Inc., Chicago, IL) that displayed greater than 90% cross reactivity with rat leptin. Triglyceride and cholesterol levels were measured colorimetrically with lipid-specific enzyme kits (Johnson &

Johnson Diagnostics, Inc. Rochester, NY). Tryptophan was isolated from plasma according to Unkila[5], characterized by GC/MS, and quantitated using a standard curve. Comparison of individual data points were made by using a two-tailed Student's t-test with a preselected significance level of $P < 0.05$ (SigmaPlot, Jandel Scientific, San Rafael, CA).

Results and Discussion

The results from the time course study demonstrated that control rats gained 57% of their original body weight during the 28 days of the experiment, and 2378-TCDD treated rats only gained 4% of their original body weight during the same period (Figure 1). The body weights of 2378-TCDD treated rats paralleled that observed in pair-fed rats, indicating that feed consumption was the primary means of maintaining body weights. In previous studies, it had been shown that the acute toxicity of 2378-TCDD lead to a displacement of the body weight set point, so that these rats chronically maintained a body weight at a reduced percentage of the level that is normal for their age[2]. Pair-fed rats, however, demonstrated a slow rate of body weight gain after ad libitum feeding was resumed, until body weights became the same as controls.

Total plasma cholesterol was elevated ($P < 0.05$) in 2378-TCDD treated rats during the first 3 days when compared to pair-fed and control rats, but after day 3, no significant differences were observed (Figure 2A). After day 14, the plasma triglyceride levels were significantly lower ($P < 0.05$) in pair-fed rats when compared to both control and 2378-TCDD treated rats (data not shown). Therefore, based on the plasma lipid data, the metabolic response of male Sprague-Dawley rats to dioxin exposure and pair-feeding was different both in the lipids affected and the timing of the response.

Leptin levels were substantially lower following the administration of 2378-TCDD (Figure 2B). At day 1, less than 30% of control levels of leptin were present in 2378-TCDD rats. This difference was shown in a separate experiment to be concentration dependant (data not shown). The levels of plasma leptin in 2378-TCDD treated rats remained low (200-300 pg/ml) until 21 days, when they began to rise and approach those observed in controls (700 pg/ml). Similar low plasma levels of leptin were detected in pair-fed rats over the time-course study (200-275 pg/ml; Figure 2B), correlating leptin declines with a decrease in body weight, regardless of the cause of the body weight loss.

Leptin is a 146 amino acid hormone produced in adipocytes[6], and serves as a lipostat to regulate appetite by informing the hypothalamus of total body fat. Under normal conditions, decreased fat reserves in adipocytes would lead to decreased release of leptin into the blood, which would be interpreted by the hypothalamus as a signal to increase feeding[7]. In the 2378-TCDD treated rats, low leptin levels did not result in increased feed consumption, but rather, a chronic, low level of feed intake was maintained (data not shown). The pair-fed rats, however, consumed all the feed provided each day. The cascade of events controlling eating behavior is complex, and 12 neurotransmitters in the brain are already known to be involved. A leptin receptor is known to exist in the arcuate nucleus region of the hypothalamus[3]. The low sensitivity to decreased leptin levels in the treated rats may be due to 2378-TCDD serving as an antagonist toward the leptin receptor. However, no data was obtained to implicate 2378-TCDD as a competitive ligand for the leptin receptor.

Melanocortin receptors (MCR) also exist in the arcuate nucleus of the hypothalamus, and have recently been shown to be involved in the appetite response[8]. In particular, MCR-4 agonists have been shown to suppress feeding in mice[9], even in response to injections of neuropeptide-Y

(NPY), a potent appetite-stimulating neurotransmitter, whose production is suppressed by leptin. 2378-TCDD may serve as a persistent agonist of MCR-4 in the hypothalamus, lowering the appetite, and thereby causing a permanent depression of the body weight set point. Future studies will be needed to establish a role for 2378-TCDD in the leptin hormone cascade.

A concomitant increase in plasma tryptophan ($P < 0.05$) was observed in 2378-TCDD treated rats when compared to controls (Figure 2C). Pair-fed rats, however, showed a slight decrease in plasma tryptophan. Circulating tryptophan levels serve as a reliable indicator of brain tryptophan and serotonin concentrations[10]. Serotonin has long been known to help regulate food intake and body weight[11], and serves to suppress feed intake. 2378-TCDD is known to inhibit tryptophan pyrrolase, the key hepatic enzyme in tryptophan catabolism[12]. Therefore, an increased level of plasma tryptophan following a 2378-TCDD dose, would be interpreted in the hypothalamus as signal to reduce feed intake. Stahl[13] has shown, however, that depletion of brain serotonin with the neurotoxin 5,7-dihydroxytryptamine did not alleviate TCDD-induced wasting syndrome. These data indicate that increased plasma tryptophan and brain serotonin may be a secondary effect of 2378-TCDD exposure, or that 2378-TCDD operates through an alternate neural pathway to exert its toxic effects in body weight maintenance.

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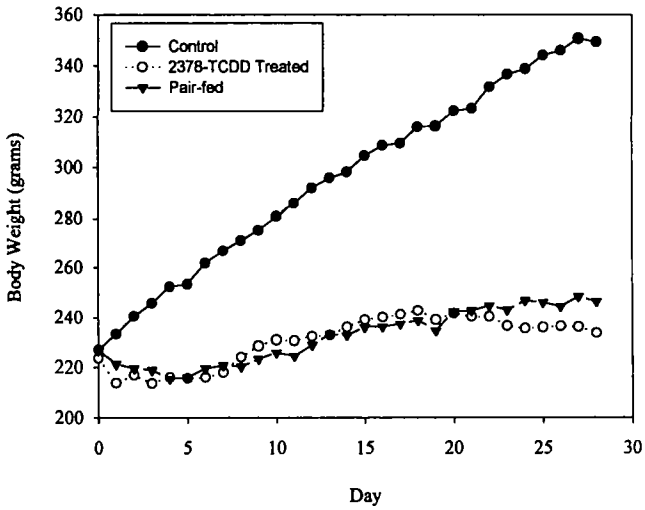

Figure 1. Daily body weights of control, 2378-TCDD treated, and pair-fed male Sprague-Dawley rats during a 28 day time course study (n=3).

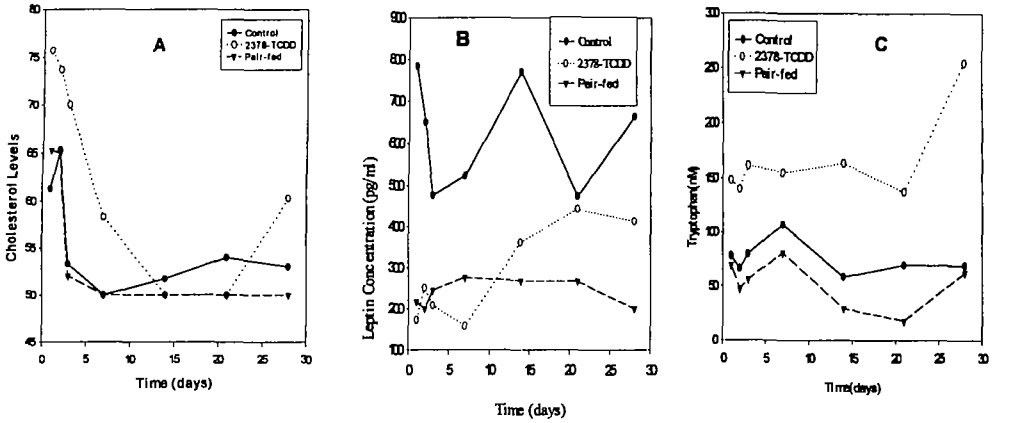

Figure 2. Changes in plasma levels at various times from control, 2378-TCDD treated, and pair-fed male Sprague-Dawley rats of (A) cholesterol, (B) leptin, and (C) tryptophan (n=3).

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