The Effects of TCDD in a Murine Model of Type II Diabetes

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Introduction

Several epidemiological studies report alterations in glucose metabolism severe enough to lead to increased incidence of diabetes (1-3). Studies in experimental animals demonstrate that TCDD causes hypoglycemia (4). Diabetes has a genetic component in both experimental animals and humans (5). It is possible that the difference between the human and rodent data maybe that TCDD induces hyperglycemia in humans predisposed to diabetes, while experimentals studies have not examined the effects of TCDD in a strain or species susceptable to developing diabetes. In mice, type II diabetes can be induced by placing the mice on a high fat high simple carbohydrate diet for 3-4 months (5). While previous studies of TCDD in rodents demonstrate that TCDD decreases serum glucose concentrations, the effects of TCDD in a rodent model of diabetes have not been examined. The present study examines the dose-response and time course effects of TCDD in a murine model of type II diabetes.

Methods and Animal Treatment

TCDD was obtained from ChemSyn (purity >98%). TCDD was dissolved in acetone and the resulting acetone solution was diluted with corn oil. The acetone was removed from the corn oil solution by evaporation in a Speed Vac SVC100 (Savant Instruments, Farmingdale, NY). All other chemicals were obtained from Sigma Chemical Co. and were of the highest grade available.

Male C57BL/6J mice (4 weeks old) were placed on either a normal diet (Purina Rodent Chow) or a diabetic diet for 2 weeks prior to exposure to TCDD by oral gavage. The diabetic diet was a high fat high simple carbohydrate (HFHSC) diet consisting of 36% lard, 35% simple carbohydrates and 20.5% protein (diet #1850 BioServ, Flemington NJ). In an acute study, mice were treated with a single dose of either 0, 1, 3, 10, 30, or 60 ug TCDD/kg and the study was terminated 3 days after dosing. In addition, subchronic studies and time course studies were also performed. Mice were exposed to either 0, 1.5 or 150 ng TCDD/kg/d for 4, 8, or 12 weeks. An extended dose-response study was performed on mice receiving either 0, 1.5, 4.5, 15, 45 or 150 ng TCDD/kg/d for 16 weeks. Three days after the

last dose mice were killed and blood and livers were collected. Serum glucose and cholesterol were determined on a Cobas Fara II (Hoffmann LaRoche). Serum glucose was determined in both resting and fasting animals. Livers were homogenized, microsomal fractions were prepared and EROD activity was determined (6).

Results

Increases in serum glucose and cholesterol were observed in mice on the HFHSC diet after 14 and 6 weeks, respectively. The magnitude of these changes and the time course were similar to those observed by Surwit et al., (5). In the acute study, TCDD did not alter either serum glucose or cholesterol in mice on either diet (data not shown). In mice treated with corn oil alone, the HFHSC diet decreased EROD activity compared to animals on the normal diet. However, the induction of EROD activity by TCDD was not altered by the HFHSC diet.

TCDD did not consistently alter serum glucose at any of the doses or times examined. After 16 weeks on the diet, resting serum glucose concentrations were higher in animals on the diet but were unaltered by TCDD (Figure 1). In addition, measuring serum glucose or cholesterol in resting or fasting mice did not alter the response to TCDD. TCDD consistently decreased serum cholesterol in a dose-dependent manner in animals on the HFHSC diet but did not alter cholesterol in animals on the normal diet. The magnitude of this effect is presented after 16 weeks of exposure (Figure 2). The diet also altered the effects of TCDD on the liver. TCDD increased liver weights only in animals on the high fat diet (Table 1). The increased liver weight was associated with visual alterations in liver color. Livers from animals on the HFHSC diet and receiving TCDD were pale in color, suggesting fatty livers.

Conclusions

A diet of high fat and high simple carbohydrates induces signs of type II diabetes in male C57BL/6J mice, as evidenced by increases in resting serum glucose and cholesterol. The effects of this diet are only moderately altered by TCDD. TCDD decreases serum cholesterol concentrations only in animals on the HFHSC diet. The HFHSC diet decreased EROD activity in control animals but did not alter the TCDD induction of hepatic EROD activity. The TCDD induced decreases in serum cholesterol were associated with increased liver weight and accumulation of lipid in hepatic tissue.

These initial studies compared the interactions of TCDD with animals on a HFHSC diet to examine whether TCDD alters the time of onset or severity of the diet-induced alterations in serum glucose. While the results of these studies do not indicate that TCDD exacerbates the effects of the diet on serum glucose, these results must be viewed cautiously. The murine model of type II diabetes used in these experiments has not been fully described and the interactions of TCDD with this diet require further examination. In this model, not only are the amounts of fat and carbohydrates altered but the types of fats and carbohydrates are different between the two diets. It is possible that the present diet induces the maximum effects on glucose metabolism and TCDD cannot further exacerbate the response. If other diets were used in this protocol with different concentrations of fat, it is possible that TCDD adminsitration may decrease the fat content required to induce hyperglycemia. Future studies are required in which the diet is modified in order to verify

whether TCDD alters the time of onset or severity of type II diabetes in these animals.

References

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Table 1. The effects of diet on TCDD-induced increases in liver/body weight ratios.

Treatment	Normal Diet	HFHSC Diet
Control	0.049 ± 0.005	0.052 ± 0.006
1.5 ng TCDD/kg/d	0.050 ± 0.007	0.055 ± 0.005
150 ng TCDD/kg/d	0.052 ± 0.005	0.066 ± 0.006*

* - significantly different from control $p < 0.05$

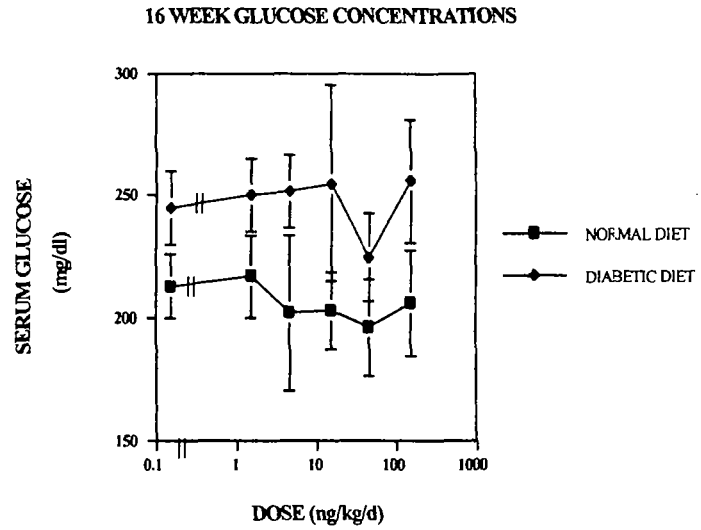

Figure 1

The effects of TCDD on resting serum glucose concentrations following 16 weeks of exposure.

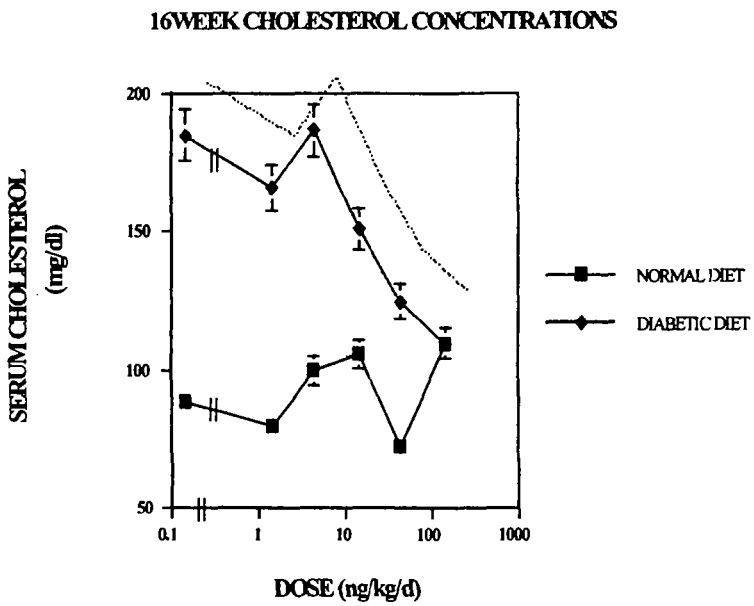

Figure 2

The effects of TCDD on resting serum cholesterol following 16 weeks of exposure