The Stimulation of Tumor Necrosis Factor and Inhibition of Glucose Transport and Lipoprotein Lipase in Adipose Cells by 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin

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2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is found throughout the environment in industrialized countries, and most people have had some exposure. TCDD has very high lipid solubility and is concentrated in adipose tissue. Because an epidemiologic association between TCDD exposure and diabetes has been described, we examined the effects of TCDD in adipocytes. The addition of TCDD to 3T3-F442a cells, both at the initiation of differentiation and after cells were fully differentiated, resulted in a 2-fold increase in the secretion of tumor necrosis factor (TNF). When added during differentiation, there was also a 25% decrease in lipid accumulation. In addition to the stimulation of TNF, TCDD affected glucose transport and lipoprotein lipase (LPL) activity. When added to cultures of cells that were undergoing differentiation, TCDD inhibited total 2-deoxyglucose transport in a dose-dependent fashion, with 50% inhibition of glucose transport when added to cultures for 48 hours at 5 nmol/L TCDD. In addition, when cells were exposed to 50 nmol/L TCDD for 48 hours, there was a 40% reduction in LPL activity. Thus, the addition of TCDD to adipocyte cultures resulted in an increase in TNF secretion and a decrease in glucose transport and LPL activity. Because TCDD is concentrated in adipose tissue, these studies provide a possible physiologic mechanism for epidemiologic studies that link dioxin to diabetes.

2,3,7,8-TETRACHLORODIBENZO-*p*-dioxin (TCDD) is usually referred to as "dioxin" and is a byproduct of many industrial processes. Most people living in industrialized countries have been exposed to TCDD through the food supply, and many Vietnam veterans were exposed to TCDD through exposure to the herbicide, "Agent Orange." TCDD is highly soluble in lipid and has a half-life in humans estimated to be between 7 and 9 years.[1,2]

Because of its high-lipid solubility, TCDD is concentrated in adipose tissue, and several studies have examined the effects of TCDD on adipocytes in vitro. The addition of TCDD to adipocytes resulted in a decrease in adipocyte differentiation and a decrease in the expression of several adipocyte differentiation markers[3,4]. In addition, rats fed TCDD demonstrated less adipose tissue and lower levels of lipoprotein lipase (LPL) and glycerol-3-phosphate dehydrogenase than pair-fed controls, suggesting inhibited adipocyte differentiation.[5] In mice, a single injection of TCDD led to decreased glucose transporter activity.[6]

Whether these effects in adipose tissue are a direct result of TCDD or due to the stimulation of some other product is not clear. One adipocyte secretory product that is capable of inducing many of the previously observed effects of TCDD is tumor necrosis factor α (TNFα), which is known to inhibit LPL, and glucose transport in adipocytes.[7-9] Indeed, the increased expression of TNF by adipose tissue has been implicated in the insulin resistance of obesity.[10-13] Furthermore, a number of epidemiologic studies have suggested a link between TCDD exposure and diabetes or insulin resistance.[14,15]

This study in the 3T3-F442a adipocyte cell line was intended to determine whether the addition of TCDD would induce the secretion of TNF and produce changes in LPL and glucose transport. The data show that TNF secretion was increased in the cells, along with decreases in LPL and glucose transport, suggesting a possible mechanistic link between TCDD exposure and abnormal adipose tissue metabolism.

MATERIALS AND METHODS

Cells and Tissue Culture

3T3-F442A cells were obtained from Dr Howard Green (Harvard Medical School, Boston, MA).[16] 3T3-F442A cells were maintained in 75-cm^2 flasks in Dulbecco's modified Eagles Medium (DMEM) (GIBCO BRL, Invitrogen, Grand Island, NY) supplemented with 10% calf serum, penicillin, and streptomycin. For experiments, they were subcultured in 6-well (35-mm) dishes. Confluent cultures were allowed to differentiate by adding DMEM containing 10% fetal bovine serum and 100 nmol/L insulin for 3 to 5 days. To maintain the differentiated state, cells were then cultured in DMEM containing 10 nmol/L insulin after switching to the differentiation medium.

TCDD was obtained from Cambridge Isotopes (Andover, MA), and the concentration and purity were verified by analysis (Radian International, Austin, TX). Pure TCDD powder (0.75 mg) was initially dissolved in 1 mL acetone and then diluted further in 5 mL dimethylsulfoxide (DMSO). This stock solution was used to add TCDD to cultured adipocytes, and each experiment involved control cultures to which an equal portion of DMSO:acetone was added.

Measurements and Assays

TNF was measured using a commercial kit from R & D Systems (Minneapolis, MN). LPL activity was measured as heparin released LPL activity in the cells, as described previously.[17] To measure heparin releasable LPL, the medium was aspirated, and the cells were treated with 10 U/mL heparin in DMEM for 45 minutes at 37°C, followed by the measurement of LPL catalytic activity using an emulsified [^{3}H]-triolein substrate with human serum as a source of apolipoprotein (apo) CII. LPL activity was expressed as nmol of free fatty acid released/h/dish. Cell protein content per 35 mm dish was constant (± 7%).

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Glucose transport was measured using the nonmetabolizable analog 2-deoxy(^{3}H)glucose (DOG), as described previously.[17] Prior to the glucose transport assay, the cells were incubated with 1 mL of serum-free DMEM for 2 hours. The cells were then washed with Krebs-Ringer phosphate buffer, which was used for the glucose transport assay. Because insulin is present in the differentiation medium of 3T3-F442A cells and is also used to maintain the adipocyte phenotype, glucose transport was measured in the presence of 10 nmol/L insulin. Hence, the data in these cells represent maximal glucose transport. Data were expressed per milligram cell protein (BioRad reagent, Richmond, CA), and noncarrier-mediated glucose transport was determined in the presence of 10 μmol/L cytochalasin.

Statistics

All data were expressed as the mean ± SEM. Statistical analysis was performed using the Student's *t* test.

RESULTS

The effects of TCDD were examined in 3T3-F442A cells both during and after differentiation. We examined the effects of TCDD on TNF secretion and the effects of TCDD on glucose transport and LPL activity.

TNF Secretion in Response to TCDD

Insulin was added to 3T3-F442A cells to induce differentiation. At the time of addition of insulin, TCDD was added at a concentration of 50 nmol/L. As shown in Fig 1, cells exposed to TCDD demonstrated increased TNF secretion into the medium. This increase was significant 3 hours after addition of TCDD. During these 7 days, cells continued to accumulate lipid. Although there was no change in total cell protein, there was a decrease in total adipocyte lipid accumulation. After 48 hours in culture, total triglyceride content in TCDD-exposed 3T3-F442A cells was 25% lower than in control cells ($P <$ .05). Similar effects of TCDD were observed when it was added to fully differentiated 3T3-F442A adipocytes. When 50 nmol/L TCDD was added to adipocytes that were fully differentiated, there was a significant 2-fold increase in medium TNF (Fig 2), although no change in adipocyte triglyceride content was observed in cells that were fully differentiated.

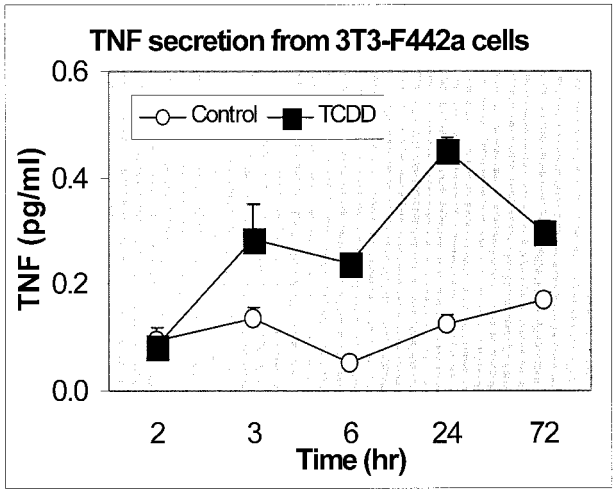

Fig 1. Effect of TCDD on TNF secretion. TCDD (50 nmol/L) was added to cultures of 3T3-F442A cells at the time of differentiation, and TNF was measured in the medium at the indicated times. The changes at 3, 6, 24, and 72 hours are all statistically significant ($P <$ .05, n = 6 for each point).

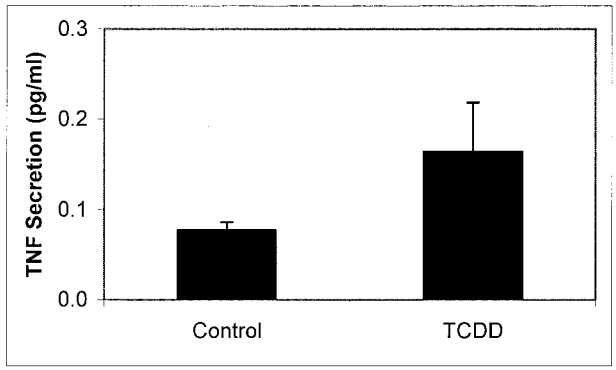

Fig 2. Effects of TCDD in mature 3T3-F442A adipocytes. After cells had differentiated (7 days), TCDD 50 nmol/L was added for 6 hours, and TNF was measured in the medium. This effect was statistically significant ($P <$ .05, n = 5).

Effect of TCDD on Glucose Transport

Glucose transport was measured in 3T3-F442A preadipocytes during differentiation into adipocytes. To assess the dose-response effect, different concentrations of TCDD were added to 3T3-F442A preadipocytes at the time of addition of differentiation medium, and glucose transport measured after 48 hours. As shown in Fig 3, there was a dose-related inhibition in glucose transport, which was significant at a TCDD concentration of 5 nmol/L. To determine the time-course of glucose transport inhibition, 50 nmol/L TCDD was added to preadipocytes at the time of differentiation. As shown in Fig 4, there was no inhibition of glucose transport until 48 hours of TCDD exposure in culture, and this effect was prolonged for up to 7 days despite medium changes into medium that did not contain additional TCDD.

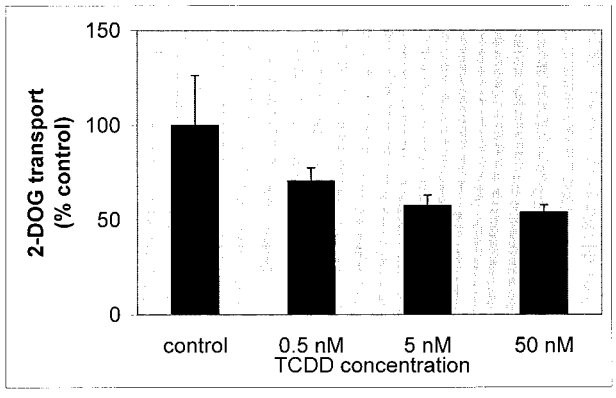

Fig 3. Dose response of TCDD on glucose transport. Increasing concentrations of TCDD were added to 3T3-F442A preadipocytes at the time of addition of differentiation medium, and glucose transport was measured after 48 hours. The changes at 5 and 50 nmol/L were statistically significant ($P <$ .05, n = 6 for each point).

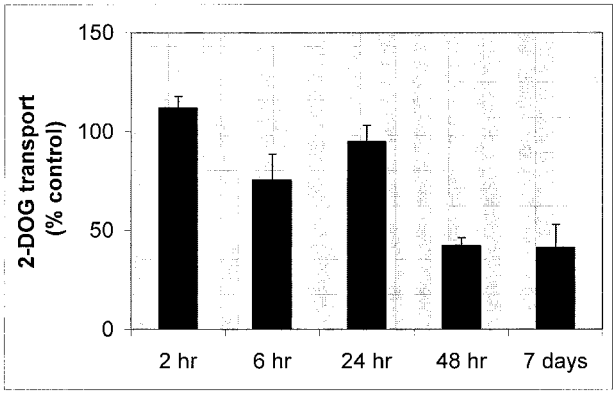

Fig 4. Time course of effect on glucose transport. A total of 50 nmol/L TCDD was added to preadipocytes at the time of differentiation, and 2-DOG transport was measured at the times indicated, and total transport was expressed as a percent of control (no TCDD added) cells. Medium was changed every 2 days, and TCDD was not present in the medium changes. The changes at 48 hours and 7 days are statistically significant ($P < .05$, n = 6 to 9 for each point).

Effect of TCDD on LPL Activity

To determine the effect of TCDD on LPL activity, TCDD was added to cultures of 3T3-F442A preadipocytes at the time of differentiation. LPL was then measured as heparin-released activity during the differentiation process. As shown in Fig 5, cells exposed to TCDD demonstrated lower levels of LPL after 24 hours in culture. This effect to decrease LPL activity was only found in cells undergoing differentiation. When TCDD was added to either fully differentiated 3T3-F442A adipocytes or to primary cultures of rat adipocytes, no changes in LPL activity were observed.

DISCUSSION

TCDD is part of a family of halogenated polyaromatic hydrocarbons that are known to be concentrated in adipose tissue[1] and bind to a cytosolic, high-affinity receptor known as the aryl hydrocarbon (Ah) receptor.[18] The binding of TCDD to this receptor leads to multiple changes in gene expression and cellular metabolism in different tissues. Previous studies have suggested that some of the toxic effects of TCDD occur through the expression of TNF in several different cell types.[19,20] For example, administration of anti-TNFα antibody resulted in less TCDD-induced oxidative stress in hepatic nuclei,[21] and anti-TNF antibodies have also been found to reduce TCDD-mediated mortality in mice.[22] Because no previous study has examined the relationship between TCDD and TNF in adipocytes, we examined TNF expression along with the effects of TCDD on several insulin responsive processes in adipocytes.

The addition of TCDD to cultures of 3T3-F442a preadipocytes at the time of differentiation resulted in an increase in TNF secretion into the medium. This increase in TNF secretion occurred within 3 hours and persisted up to 72 hours. TNF is known to have multiple effects on adipocytes, and previous studies have demonstrated that the addition of TNF resulted in decreases in insulin receptor phosphorylation, glucose transport, and LPL activity.[7-9] Hence, we measured glucose transport and LPL in the 3T3-F442a cells following the addition of TCDD. As shown, there was a dose-related decrease in glucose transport, which persisted for up to 7 days, and a decrease in LPL activity. Because the changes in glucose transport and LPL are known effects of TNF, we suggest that these TCDD-mediated changes were due to increased TNF secretion, although it is possible that these changes are direct effects of TCDD.

In previous studies, TCDD induced a decrease in adipose tissue mass and changes in adipose tissue differentiation and lipid accumulation.[5,6,23] Rats fed TCDD demonstrated less adipose tissue and lower levels of differentiation enzymes (lipoprotein lipase and glycerol-3-phosphate dehydrogenase) than pair-fed controls, suggesting inhibited adipocyte differentiation.[5] A single intraperitoneal injection of TCDD in mice led to decreased glucose transporter activity, including insulin-responsive transporters (GLUT 4, found in adipose and muscle), and non–insulin-responsive transporters (GLUT 1, found in the blood-brain barrier).[6] When TCDD was added to cultures of 3T3-L1 cells, there was a decrease in adipogenesis, a decrease in the induction of enzymes of differentiation, and a decrease in expression of a number of adipogenic transcription factors, including CCAAT/enhancer binding protein α (C/EBPα) and peroxisome proliferator-activated receptor-γ (PPARγ).[3,23,24] These above effects of TCDD have also been described following the addition of TNF to adipocyte cultures,[25] suggesting that TNF may be the cellular mediator of the pleomorphic effects of TCDD in adipocytes.

The relationship between TCDD, TNF, and insulin action may have considerable clinical significance. Several previous studies have reported an epidemiologic association between TCDD exposure, usually from high industrial exposure, and some component of impaired carbohydrate metabolism.[26-28] In a 20-year follow-up study of Air Force veterans of Vietnam, subjects with high blood TCDD levels demonstrated a higher incidence of diabetes and a shorter time to onset of diabetes

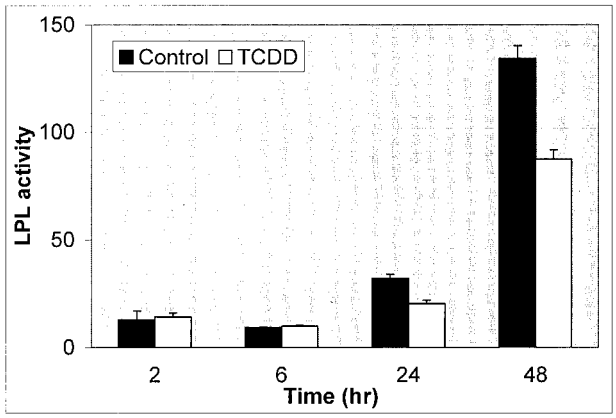

Fig 5. Effect of TCDD on LPL. TCDD (50 nmol/L) was added to 3T3-F442A cells at the time of differentiation, and LPL activity was measured at the times indicated. LPL activity was expressed as nEq FFA released/hr. The decrease in LPL in the TCDD treated cells at 48 hours was statistically significant ($P < .05$, n = 3 to 6 experiments for each point).

when compared with Vietnam veterans with low blood TCDD levels.[14] This elevated risk of developing diabetes was also present among subjects with "background" TCDD levels, suggesting that the persistence of TCDD in the environment may exacerbate other risk factors for type 2 diabetes.[29] In addition, nondiabetic subjects with high blood TCDD levels demonstrated hyperinsulinemia, both fasting and postoral glucose load, which would suggest that subjects with high TCDD levels were insulin-resistant.[14,30,31]

An epidemiologic association between TCDD and diabetes is incomplete without a possible mechanism to explain such an association. The induction of adipocyte TNF secretion by TCDD may provide such a mechanistic link. Since the initial description of adipocyte TNF expression,[10] the increased secretion of TNF by adipocytes of obese subjects and rodents has been an important advance in the understanding of the insulin resistance of obesity.[32,33] Because of the widespread presence of TCDD exposure in our environment, further studies are needed to determine the extent to which TCDD alters adipocyte metabolism in humans.

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