

Weight Gain Associated with Chronic Exposure to Chlorpyrifos in Rats

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ABSTRACT

Objective: This work exposed rats to low levels of the organophosphate insecticide chlorpyrifos and monitored for toxic effects, including weight gain.

Methods: Rats received either a subcutaneous injection of chlorpyrifos, 5 mg/kg/day, or an equal volume of vehicle daily for 4 months. Subjects were observed for 30 minutes after injection for signs of acute toxicity. Body weights were recorded at baseline, 2 months, 3 months, and 4 months. At the end of the experiment, the weights of hearts, medial lobe of the livers, peri-nephric fat pads, and gastrocnemius muscles were recorded. Effects of chlorpyrifos on adipocyte differentiation in culture were studied. Results were compared using RMANOVA.

Results: No signs of acute cholinergic toxicity were observed after injections in any subject. Rats in the 5 mg/kg group were significantly heavier than those in the control group by 2 months (335.7 ± 16.7 g vs. 318.6 ± 15.8 g; $p = 0.034$). This difference increased at 3 months (350.1 ± 16.4 g vs. 322.3 ± 21.3 g $p = 0.006$) and 4 months (374.4 ± 22.2 g vs. 340.2 ± 25.2 g $p = 0.006$). At 4 months, the weights of the perinephric fat pads were significantly increased in the chlorpyrifos group relative to controls ($2.867 + 0.516$ vs. $1.130 + 0.171$, $p = 0.0039$). The two groups showed no weight differences between hearts, livers, and gastrocnemius muscles. Chlorpyrifos did not affect adipocyte differentiation in tissue culture.

Conclusions: Chronic exposure to chlorpyrifos at 5 mg/kg/day caused an increase in rat body weight when compared to controls. This increase was in adipose tissue. Chlorpyrifos did not induce differentiation of adipocytes in culture.

INTRODUCTION

Obesity has increased in the United States over the past decade and affects children, adolescents, and adults. As many as 16.5% of children aged 6 to 19 years of age are classified as overweight, another 31.5% are considered at risk, and over 11% are obese [1,2]. Attributing the rapid and recent increase in obesity to overeating, inactivity, and genetics does not adequately explain the current trends [3]. A possible contributing cause is exposure to environment chemicals with biological activity.

Our population is chronically exposed to a host of environmental chemicals. Investigations by the Environmental Health

Laboratory at the Centers for Disease Control verify that human populations of the United States have detectable blood and urine levels of chemicals, including organophosphate insecticides [4]. Sources of exposure to these chemicals include residues on food and treatment of commercial and residential buildings for pest control.

Chronic exposure to organophosphate insecticides has been associated with neuropsychological conditions and has more recently been shown to have specific endocrine effects [5–7]. We are reporting the results of the effect of prolonged low-dose exposure to chlorpyrifos, an organophosphate insecticide, in young rats that gained weight. We did not observe any acute toxic

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effects after dosing. Chlorpyrifos was chosen due to its history of extensive use and anecdotal reports of chronic problems after low level exposures in indoor settings. In addition, the effect on chlorpyrifos on adipocyte differentiation was assessed as a potential mechanism of the weight gain.

METHODS

The Institutional Animal Care and Use Committee of East Carolina University approved all experiments. This placebo controlled study used female Long-Evans rats about 6 months of age. Chlorpyrifos was obtained from SigmaAldrich (St. Louis, MO) and dissolved in DMSO then diluted with normal saline just prior to injection, for a final concentration of 4.0 mg/mL. Each rat received daily subcutaneous injection of chlorpyrifos, 5 mg/kg/day, $n = 10$, or an equal volume of vehicle ($n = 10$). Subjects were observed for 30 minutes after injection for signs of acute cholinergic or other toxicity, with atropine and pralidoxime available for treatment as needed. Expected and observed acute signs of toxicity in rats are fasciculations, muscle weakness, muscle paralysis, respiratory distress, seizures, coma, and moribund state. This protocol was carried out for 4 months. After the start of injections, weights were recorded at baseline, 2 months, 3 months, and 4 months. Weights were compared within each group using RMANOVA. Weights of two groups were compared at each time point using ANOVA followed by a Fisher PSD post-hoc analysis. A p-value of less than 0.05 was considered statistically significant. At the end of the study period, rats were euthanized by carbon dioxide inhalations. At necropsy, we removed and weighed hearts, livers, perinephric fat pads, and gastrocnemius muscles. 3T3-L1 preadipocytes were placed in cell culture and stimulated to differentiate using:

- 1) a mixture of methyl-isobutylxanthine (MIX), dexamethasone (DEX) and insulin (MDI; standard protocol);
- 2) 10 ul of dimethyl sulfoxide (DMSO), or
- 3) increasing concentrations of chlorpyrifos (.002 mg/ml; .004 mg/ml; .006 mg/ml and .008 mg/ml) dissolved in 10 ul of DMSO. Cells from all groups were observed 24 hours and 6 days after stimulation, and descriptive findings of cell growth, fat accumulation, acceleration of growth, and number of cells are reported.

RESULTS

We did not observe any signs of acute cholinergic toxicity in any rat at any time after injection. One rat in the group that received 5 mg/kg/day of chlorpyrifos was euthanized during the second month of exposure due to the development of a mammary tumor, leaving a total of 9 animals in this group for analysis.

Both groups gained significant amounts of weight between baseline and 2 months ($p < 0.0001$) as well as between 3 and 4 months. After exposure was initiated (335.7 ± 16.7 g vs. 318.6 ± 15.8 g; $p = 0.034$), rats in the exposed group were significantly heavier than those in the control group by month 2. This difference

increased at 3 months (350.1 ± 16.4 g vs. 322.3 ± 21.3 g $p = 0.006$) and 4 months (374.4 ± 22.2 g vs. 340.2 ± 25.2 g $p = 0.006$) (Figure 1). At the end of the experiment, weights of the perinephric fat pads were significantly increased in the chlorpyrifos group relative to controls (2.867 ± 0.516 vs. 1.130 ± 0.171 , $p = 0.0039$, mean difference = -1.737 , 95% confidence interval of the difference of the means -2.835 , -0.638). Heart weights were 1.456 ± 0.240 vs. 1.200 ± 0.359 , with $p = 0.0859$. Liver weights were 4.444 ± 1.657 vs. 3.590 ± 0.415 , $p = 0.1322$, mean difference = -0.845 , 95% confidence interval of the mean difference = -1.994 to 0.286 . These results are depicted in Figure 2.

When pre-differentiated adipocytes were treated in culture with up to .008 ug/ml of chlorpyrifos or with 10 ul DMSO, normal cell growth was observed, with evident fat accumulation, but without acceleration of growth or increased numbers of cells. The chlorpyrifos treatment did result in an increased number of dead cells compared to the MDI-treated group.

DISCUSSION

The adverse health effects of obesity are enormous. Obese children are more likely to have increased risk factors for cardiovascular diseases, including elevations of blood pressure, cholesterol, lipids, and C-reactive protein [8,9]. Type 2 diabetes mellitus is also increased in obese children, and an association exists between childhood obesity and asthma with body mass index being a significant predictor of childhood asthma [10–12]. Fatty liver and cholecystitis are of increased incidence and prevalence in obese children [13,14].

A possible cause contributing to the obesity epidemic is the introduction of chemicals with biological activity into the environment. These chemicals may result in obesity in susceptible individuals. Arguments to support this hypothesis include the rapidity with which obesity rates are increasing and the fact that environmental chemicals can act as 'endocrine disrupters' [15,16]. Pesticides may be one of these environmental chemicals. Organochlorine, organophosphate, and carbamate pesticides have been shown to have endocrine effects that can result in weight gain [17–23]. The World Health Organization states "there is no segment of the general population that is sheltered from exposure to pesticides and potentially serious health effects." Exposures may come in the form of residues on foods, pesticide drift, and insecticide treatment of houses, schools, and yards. The role of environmental chemicals in the epidemic of childhood and adolescent obesity has not been explored.

There is no doubt that human populations of the United States are chronically exposed to a host of environmental chemicals and have blood levels that indicate such levels. The Environmental Health Laboratory at the Centers for Disease Control has conducted laboratory investigations of blood and urine levels in human populations of the United States for a host of environmental chemicals. These investigations have established population-wide levels of organophosphate, carbamate, and pyrethroid insecticides, herbicides, as well as phthalates,

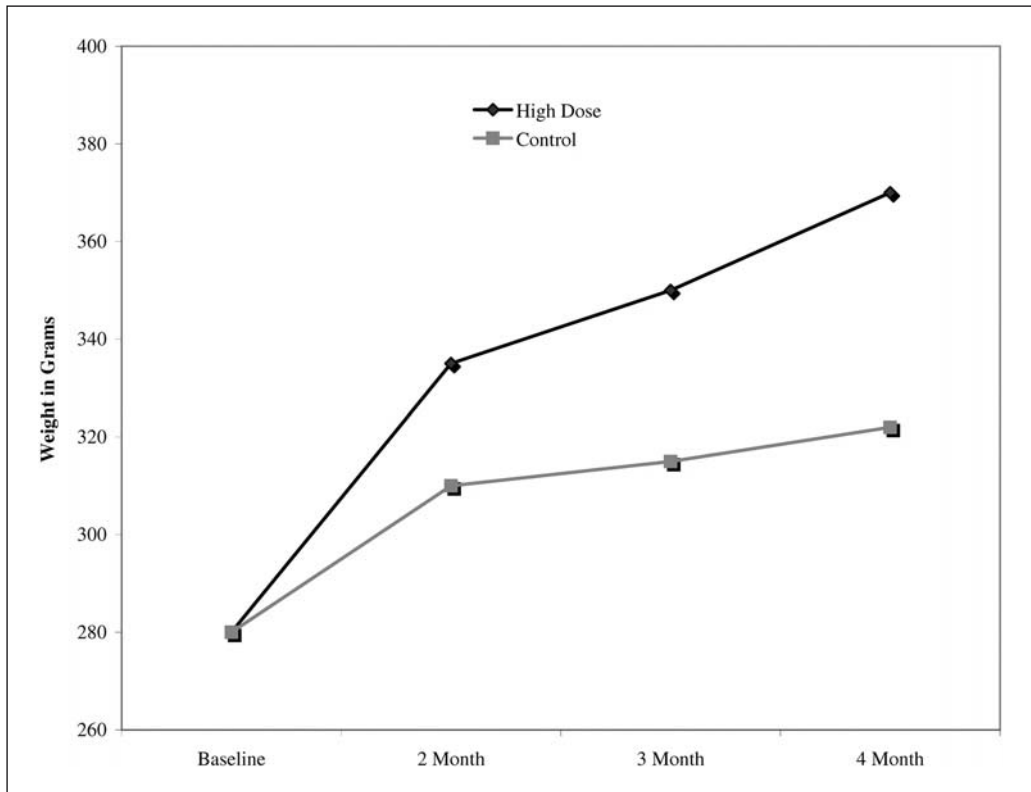


Figure 1: A Comparison of Total Body Weights in Grams of Rats Exposed to Chlorpyrifos 5 mg/kg/day to Control Rats

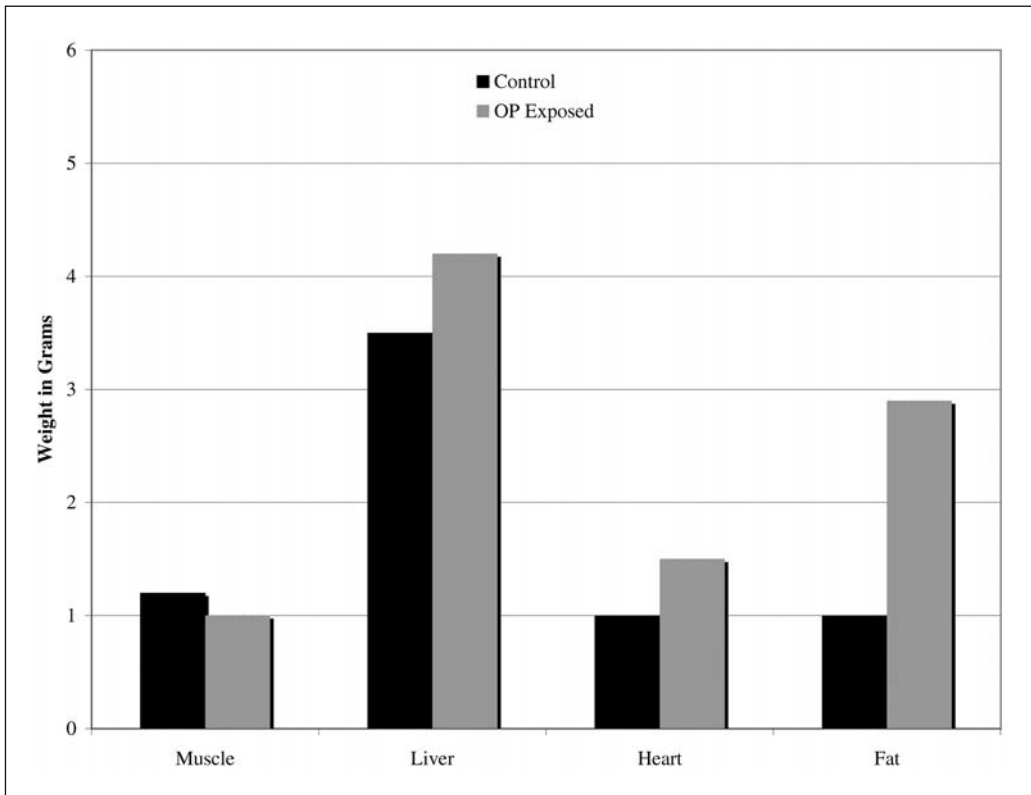


Figure 2: A Comparison of Gastrocnemius Muscle, Heart, Medial Lobe of Liver, and Peri-nephric Fat Pad Weights in Grams of Rats Exposed to Chlorpyrifos 5 mg/kg/day to Control Rats

polyaromatic hydrocarbons, polychlorinated biphenyls, phytoestrogens, and heavy metals [4]. Any of these substances are possible candidates for playing a role in the obesity epidemic.

Organophosphate insecticides have a variety of actions beyond acute cholinergic toxicity that leads to autonomic hyperactivity and muscle weakness. This pilot study of long-term exposure to an organophosphate insecticide in young rats revealed a progressive increase in weight in those rats exposed to 5 mg/kg/day of chlorpyrifos, but not in the control group. Peri-nephric fat pad weight is used as a marker of total body fat. There was a significant increase in the weight of the peri-nephric fat pad but not in muscle, heart, or liver. The weight increase represents an increase in adipose tissue. Rats did not have any evidence of acute toxic effects during the course of the experiment.

Organophosphate pesticides may induce accelerated differentiation of immature adipocytes into mature fat cells. This is one potential mechanism of weight gain. When this theory was tested in culture, it was not substantiated. When pre-differentiated adipocytes were treated with a dose of chlorpyrifos that was not toxic to the cells, the number of cells that differentiated from the controls was not noticeable.

LIMITATIONS

The specific mechanism for weight gain in exposed animals is not elucidated. This will be a direction for future research. Possible mechanisms that need further examination include endocrine and metabolic effects. Other investigators have shown that organophosphates are capable of inducing hypothyroidism and euthyroid syndrome, both states that would result in abnormal weight gain [7]. The sample size is small, but it was large enough to obtain a statistically significant result.

Further investigations are also needed to determine if the result seen with chlorpyrifos generalizes to other insecticides, other classes of environmental chemicals, other age groups, and other species. Generalizations to humans cannot be made at this time. But with a remarkable increase in obesity in human populations chronically exposed to environmental chemicals, this area of study becomes an important one in terms of potential impact on human health.

The authors have no potential financial conflicts of interest to report.

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