Is childhood obesity a result of toxic exposure to cadmium or malathion? 
An observational pilot Egyptian study

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ABSTRACT
Nowadays, exposures to some environmental chemicals may contribute to obesity in children. The aim of the current work is to assess the association between the environmental pollutants cadmium, malaoxon and malathion dicarboxylic acid (MDCA) and obesity in children. Authors conducted a case-control study on 80 children. We recruited 40 obese children and 40 normal-weight children. For each child, we measured urinary concentrations of cadmium (by ICP), malaoxon (by LC/MS/MS), and MDCA (by LC/MS/MS). Results: Malaoxon concentrations were slightly higher among non-obese group B children (median = 0, IQR 0 to 10.29 mg/g) than in obese group A children (median = 0, IQR = 0 to 2.14). There were no significant differences in creatinine-adjusted MDCA or Cadmium.

Abbreviations: MDCA: Malathion dicarboxylic acid; Cd: Cadmium; ED: Endocrine disruptor; AASD: The Asian Association for the Study of Diabetes; MUCH: Mansoura University Children Hospital; BMI: body mass index; LC/MS/MS: Liquid Chromatography/Mass Spectrometry/Mass Spectrometry; ICP-MS: Inductive coupled plasma-Mass spectrometry; ROC: Receiver operating curve

KEYWORDS
Cadmium; malaoxon; malathion dicarboxylic acid; obesity; children

Introduction
Obesity has become an epidemic metabolic disease throughout the world and children are among the fastest rising groups in developing obesity [1]. This epidemic may be due to increased consumption of calorie-rich foods coupled with decreased physical activity. However, these factors alone fail to explain fully the rapidity of rising rates of obesity, especially in infants and toddlers [2].

Chronic exposure to environmental pollutants, including pesticides and heavy metals, via airborne particles, soil, water and subsequently food, is nearly unavoidable on a daily basis [3,4]. In Egypt, the Delta Valley of the River Nile occupies approximately 2% of Egypt’s landmass, holding around 41% of the population and 63% of the agricultural land. It is heavily contaminated with such chemicals and responsible for the risk of spread [4].

Both cadmium, which is found in wastewater, and malathion, a commonly used pesticide, have reported endocrine disrupting effects [2,5,6]. Both may be contributors to the obesity epidemic [2,7,8].

Thus, the aim of the current work is to estimate concentrations of cadmium and malathion metabolites (malaoxon and MDCA), as environmental chemical pollutants, and investigate their association with obesity in a sample of obese Egyptian children.

Material & methods
We conducted an observational analytical case-control study on eighty children from Pediatric Endocrinology and Diabetes Unit, Children Hospital (MJCH), Mansoura University, Egypt; at their primary hospital visits. We obtained ethical approval for conducting the study from Mansoura University Institutional Research Board (R.18.03.88) according to Declaration of Helsinki. Parents provided informed consent for study participation.

We included children aged 2-18 years old of both sexes in the study. We excluded children with hepatic, renal, endocrine, genetic disorders. We also excluded...
children with history of intra-uterine growth retardation, on corticosteroid therapy, born to diabetic mothers. Then, we calculated each child’s body mass index (BMI) from measured height and weight according to Wang [9]. Then we divided them into two groups: Group A consisted of 40 obese child with BMI >95th percentile and Group B consisted of 40 normal-weight children (BMI 5th–85th percentile). For control group we selected siblings of children obese children or children attending our clinic for general medical complaints unrelated to endocrine or nutritional disorders.

From each child, we collected five mL urine in polyethylene containers that were cleaned using multistep acid leaching and then samples were frozen immediately at −20 °C until analysis of urinary cadmium, malafoxon and MDCA. We stored all samples in Mansoura Toxicology Lab, Forensic Medicine and Clinical Toxicology Department, and then we transferred them to the Main Defense Chemical Warfare Laboratories of The Ministry of Defense in an icebox using ice chips to preserve them for measurement of urinary cadmium, malafoxon, and MDCA.

Material

We obtained cadmium, malafoxon, and MDCA analytical standards (>99.9%) from Sigma-Aldrich, Co. Egypt. We obtained acetonitrile, high purity concentrated nitric acid and formic acid from Merck.

Methods

We used high purity concentrated nitric acid to digest samples for detection of cadmium according to Sakata [10]. We quantified the concentrations of cadmium in urine samples using ICPMS 7700 Agilent. We quantified malafoxon, and MDCA using LC/MS/MS (Agilent 6460 Series Triple Quadrupole LC/MS; Agilent 1260 infinity LC system) according to manufacture instructions. Then, we performed quantification with three-point calibration curve for malafoxon and four-point calibration curve for MDCA.

Adjustment to urinary creatinine concentration

We adjusted Cd and malafoxon metabolite concentrations by creatinine concentration to correct for the urine volume.

| Table 1. Sociodemographical characteristics and body mass indices of the studied groups of children (n=80). |
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| Age (years) | Group A (n=40) | Group B (n=40) | p-value |
| Male | 31 (78.0%) | 26 (65.0%) | 0.217 |
| Female | 9 (23.0%) | 14 (35.0%) | 0.329 |
| Urban | 10 (25.0%) | 14 (35.0%) | 0.329 |
| Rural | 30 (75.0%) | 26 (65.0%) | 0.217 |
| Height (cm) | 145.35 ± 16.56 | 158.25 ± 10.14 | ≤0.001* |
| Weight (kg) | 72.27 ± 22.21 | 44.92 ± 7.24 | ≤0.001* |
| BMI (kg/m²) | 33.34 ± 5.37 | 17.78 ± 1.11 | ≤0.001* |

BMI: body mass index; kg: kilograms; cm: centimeters; m: height; Group A: 40 obese child; Group B: 40 normal-weight children; * p value is significant.

Statistical analysis

We analyzed data using the Statistical Package of Social Science (SPSS) program for Windows (Standard version 24). We first tested the normality of data with one-sample Kolmogorov-Smirnov test. We described qualitative data using number and percent. Association between categorical variables was tested using Chi-square test. We calculated mean ± SD (standard deviation) for parametric data and median and interquartile range (IQR) for nonparametric data. We compared the two groups with Student’s t-test for parametric data and Mann-Whitney test for non-parametric data with level of significance at p < 0.05.

Results

In the present study, groups were similar with regards to age and sex (p = 0.217, 0.329 respectively), and significantly different in BMI measurements (p ≤ 0.001), as anticipated (Table 1).

Although malafoxon concentrations approached zero, they were slightly higher among non-obese group B children (median = 0, IQR 0 to 10.29 mg/g) than in obese group A children (median = 0, IQR = 0 to 2.14). Adjusted urinary MDCA and Cd concentrations were similar between groups. There were no significant differences in creatinine-adjusted MDCA or Cd (Table 2).

Discussion

In the past few decades, obesity and diabetes have reached epidemic rising proportions with no evident sign of decline in any country worldwide. Metabolic diseases are multifactorial diseases. Genetic susceptibility, life-style risk factors, and sedentary behavior are major contributors. However, environmental exposure to chemical pollutants is part of this
equation, especially those interfering with hormonal action, so-called endocrine disruptors [11].

Malathion is a commonly used pesticide with well-declared endocrine disruptor effects. MDCA and malaoxon were chosen as malathion biomarkers for this study, as it was proposed that malathion toxicity depends mainly on its metabolic bioactivation to its toxic oxon metabolite malaoxon [12,13].

Results showed an unexpectedly significant increase in malathion biomarkers in non-obese children despite both groups reporting a median of zero. On correlating the urinary concentrations of studied toxins with other parameters, only MDCA showed significant positive correlation with age and BMI. These findings are not consistent with Laverda, who conducted a large longitudinal study to examine the association between weight gain and pesticides [14]. They studied the relationship between different pesticides classes and total cumulative days of exposure in relation to BMI. They declared no relation between malathion exposure and obesity. Meanwhile, these results go in accordance with that of Raafat, who found positive correlation between malathion blood concentration and waist circumference in farmers exposed to pesticides [15]. According to the Agency for Toxic Substances and Disease Registry ranking, cadmium is the 7th most toxic heavy metal which humans or animals may get exposed to at work or in the environment [16]. It is a highly toxic non-essential heavy metal with detrimental effects on the enzymatic systems of cells and oxidative stress [17]. In the present work, we found no significant differences in urinary concentrations or correlation to other study parameters.

These findings conflict with findings of Park et al. [18]. They reviewed the epigenetic effect of cadmium in association to obesity risks especially in children either through researches that conducted during prenatal period or early life exposure either in animal or human studies. They found that cadmium is significantly associated with low birth weights that is often followed by rapid adiposity gain as well, increase fat mass and altered adipocyte differentiation. Park et al. found that some but not all human studies showed a positive association between cadmium and obesity [18]. On the other hand, our results are similar to those of Barregard et al. [19]. They found no association between cadmium exposure and increased risk of type 2 diabetes.

Our sample size was small since the cost needed for the analytical part was expensive. Thus, we recommend a larger sample size in future research. Another limitation for this study that some of the children recruited as control group were siblings for the study cases who may have similar dietary and environmental exposures. This factor may conceal a difference between both groups. Lastly, the number of non-obese children was similar to obese children.

In conclusion, the study revealed that urinary concentrations of the malathion metabolite, malaoxon, were lower among obese children than among children of normal weight. Neither MDCA nor cadmium urinary concentrations correlated with obesity. Malathion metabolites and cadmium appear not to explain childhood obesity in this small sample.

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Disclosure statement

The authors have no conflicts of interest to declare.

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