



Bisphenol A and

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Introduction:

The organic compound bisphenol A (BPA), with an annual production of 2-3 million tons, is a fundamental building block in the synthesis of plastics and epoxy resins. Suspected to be harmful to humans since the eighties, the concerns about the use of BPA attracted the interest of media only in 2008, when many governments

stimulated studies on its safety and banned the sale of products containing BPA, especially baby bottles and toys.



BPA is classified as an endocrine disruptor, it is involved in the development of several diseases affecting heart, reproductive system, prostate and breast. The USA National Institute of Environmental Health Sciences produced in April 2008 a draft on risk assessment for human health following to BPA exposure through food, use of products contaminated by BPA and the environment. There are several evidences about the endocrine effects played by BPA and it is possible to define a maximum tolerated daily dose (TDI) of 0.05 mg/kg bw.

Infants and children fed with liquid food are among the most exposed, and those fed with food stored in polycarbonate bottles can introduce up to 13 micrograms of BPA per kg_{bw} per day. In 2009, a study found that drinking from polycarbonate bottles causes an increase in urinary levels of BPA, from 1.2 to 2 µg/g creatinine. Consequently, serious concerns arise about the risk of long-term effects on endocrine, reproductive and neurobehavioral systems following exposure in utero and/or during childhood.

Obesity: urinary levels in

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Materials and methods:

Urine samples, from patients aged between 6 and 14 years, were provided by the Department of Pediatric of the Second University of Naples. All samples were stored at -80°C prior to use.



The following demographic and clinical data were collected with a standardized questionnaire at baseline: sex, age, physical activity, socioeconomic status, previous diseases. Weight and height were measured while the subjects were fasting overnight and wearing only underwear. Body Mass Index (BMI) was calculated as weight (kg) divided by height (m^2). Waist circumferences were measured using a plastic tape meter at the level of the umbilicus.

Blood pressure, lipids, insulin and glucose were, also, measured.

In each subject, the degree of insulin resistance was estimated at the baseline by HOMA according to the method described by Matthews et al. In particular, an insulin resistance score (HOMA-IR) was computed with the formula: fasting plasma glucose (mmol/L) times fasting serum insulin (mU/L) divided by 22.5. Low HOMA-IR values indicate high insulin sensitivity, whereas high HOMA-IR values indicate low insulin sensitivity (insulin resistance).

Serum concentration of adiponectin and resistin were evaluated by commercially available ELISA kits.



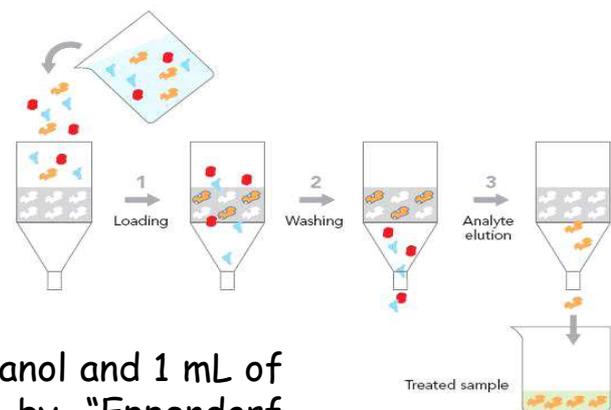
obese children

Mita D.G. (1), Diano N. (1)

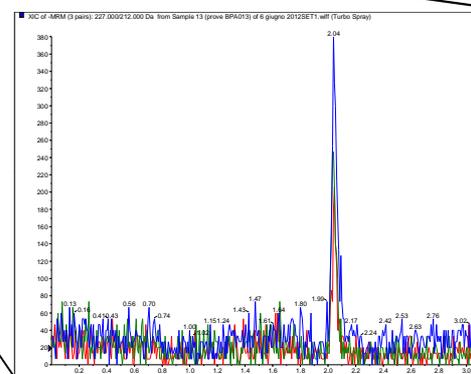
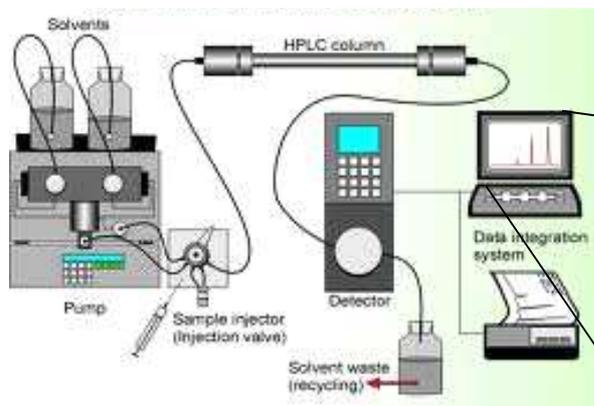


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A solid phase extraction of the samples using cartridges Chromabond HR-X, a porous resin-based polystyrene-divinylbenzene, was conducted. The cartridge is conditioned previously with 5 mL of methanol and 5 mL of water.



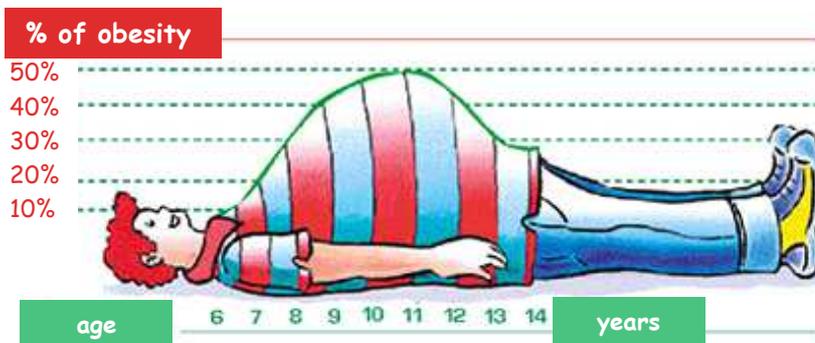
The BPA was recovered by 2 mL of methanol and 1 mL of water, and subsequently concentrated by "Eppendorf Concentrator" up to a final volume of about 1 mL.



Finally, the recovered samples were filtered through filters of MCE and analyzed by HPLC and/or LC/MS-MS. To correct for urinary dilution, we measured the urinary creatinine, following usual practice.

Aim:

Recent studies focus on the correlation between prolonged exposure to BPA during adolescence and an increased risk of obesity.



Starting from this evidence, we carried out a study involving more than 100 children residing in the Regione Campania, which has a high percentage, about 48.8%, of overweight young people, aged from 6 to 14 years. Aim of this study was to estimate the amount of BPA in the urine of normal weight and overweight children, to evaluate a possible correlation between this endocrine disruptor and childhood obesity.

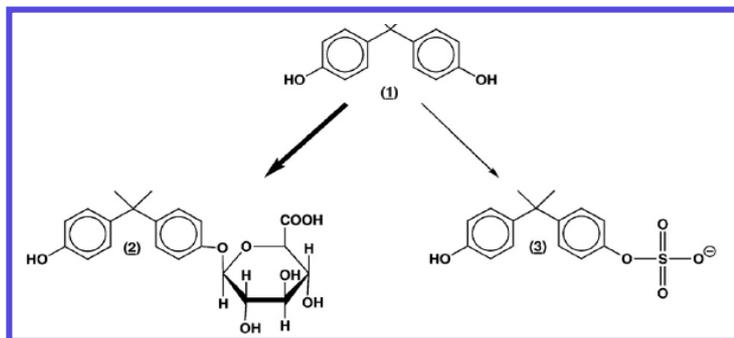
RESULTS

	<i>overweight</i>	<i>normal weight</i>
<i>Number</i>	90	15
AGE	10.66	10.55
SEX M/F	50/40	9/6
HEIGHT (cm)	147.80	151.16
BMI	30.93	25.07
BMI z score	3.84	1.41
HOMA-IR	2.41	1.82
Cir. (cm)	89.86	79.75
SBP-SDS	0.9	0.9
DBP-SDS	0.6	0.4
Triglycerides (mg/dL)	115.70	95.12
HDL-C (mg/dL)	49	46
Adiponectin (ng/mL)	4.8	5.6
Resistin (ng/mL)	2.1	1.5

The demographic and clinical characteristics are shown in Table 1.

There were no significant differences in maternal education, physical activity, socio-economic status and diet. We therefore assumed all participants to have normal physical activity and categorized them as having "excessive" caloric intake based on the daily caloric guidelines set for children with normal physical activity. Because BMI varies widely by age and sex, age and sex standardized BMI z scores have been commonly used. Overweight and obese were categorized as BMI z score of 2.0 or greater.

The urine samples were treated to transfer the analyte of interest from the biological matrix to a solution, more concentrated, compatible with the appropriate detection techniques, sensitive and selective. In particular, the method involved two stages: the enzymatic de-conjugation of BPA-glucuronide/sulfate and the solid phase extraction with recovery in pure solvent.



The β -glucuronidase (EC number 3.2.1.31), used for this study, is extracted from bovine liver (10.000U/mg) and is supplied by Sigma. The sulfatase (EC number 3.1.6.1) is extracted from *Helix pomatia*.

The urine samples after the addition of an internal standard, control for extraction yield, were treated with 300 μ L of enzyme solution (for 50 mL of urine) in acetate buffer 0.1 M at pH 5.0. The enzymatic reaction is carried out for 2 hours in thermostatic bath at 37 °C.

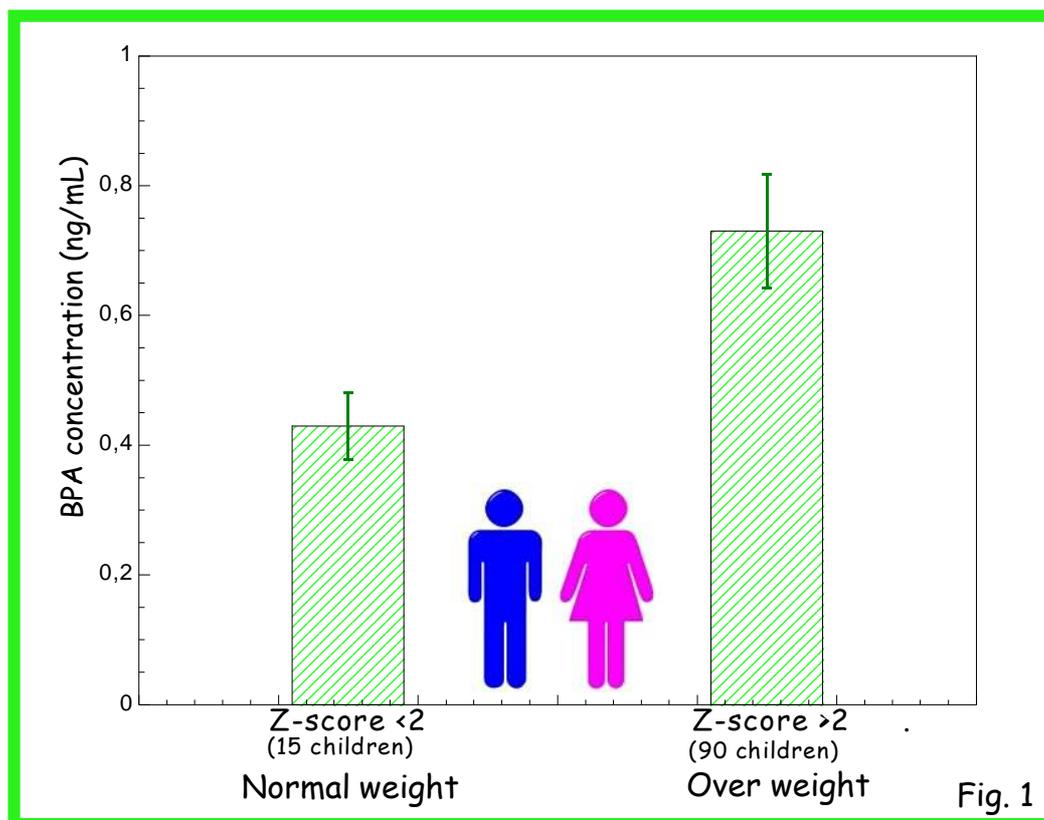
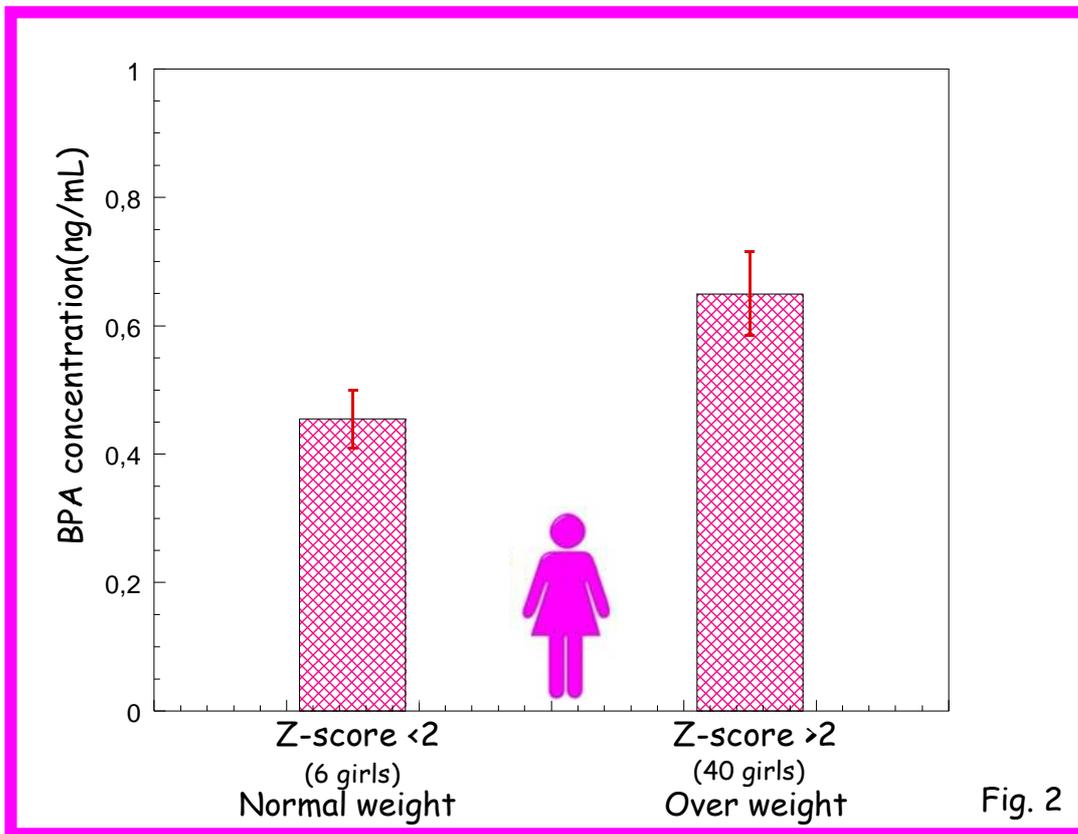


Fig. 1

BPA was detected in 95% of the samples (from normal and obese patients) with total concentration ranging from 0.2 ng/mL to 1.45 ng/mL.

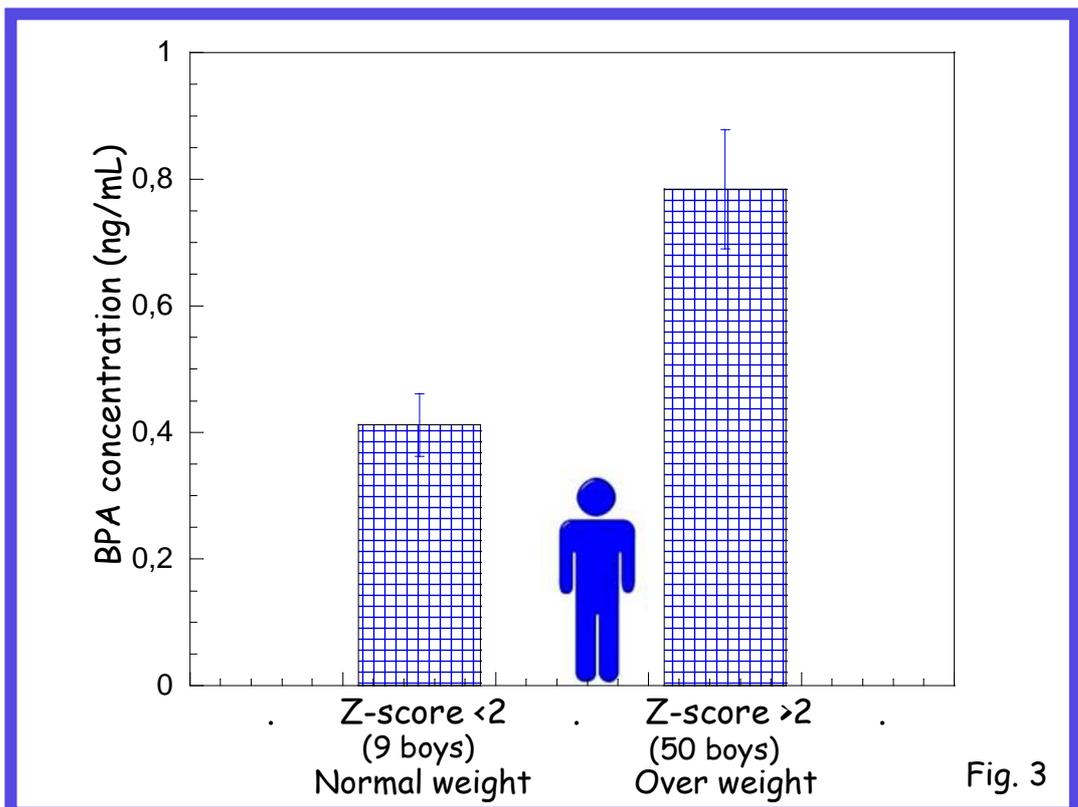
From the analysis made, by referring to the value of the Z-score of BMI, the mean value of concentration of BPA in the urine of obese children is found to be equal to 0.78 ng/mL while, for the normal weight, average value is found to be equal to 0.44 ng/mL. (Fig.1).

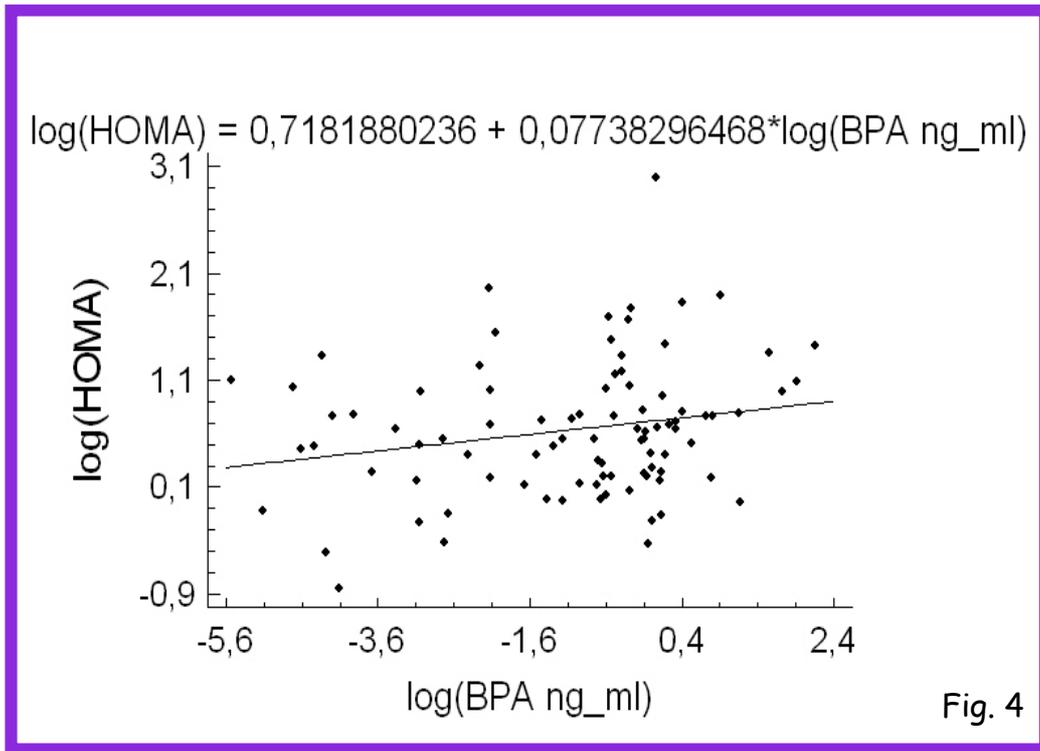


Analyzing the data in relation to the sex difference, the average concentration of BPA in overweight girls is 0.65 ng/mL and is 41% greater than that of normal weight (0.46 ng/mL) (Fig .2).

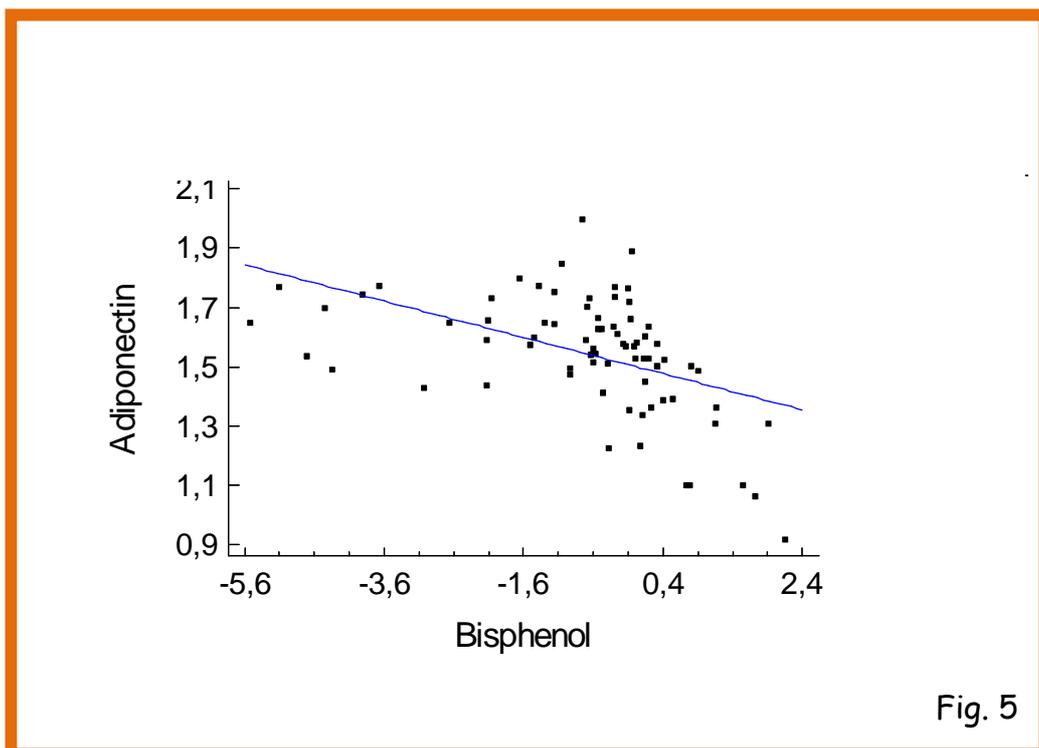
The average concentration of BPA in overweight boys is 0.79 ng/mL and it is 92% greater than that of normal weight (0.41 ng/mL) (Fig .3).

The concentrations of BPA in overweight boys, also, exceed more than 20% the corresponding values in overweight girls. Indeed, for normal weight children, the difference in BPA concentration in the two sexes is irrelevant.



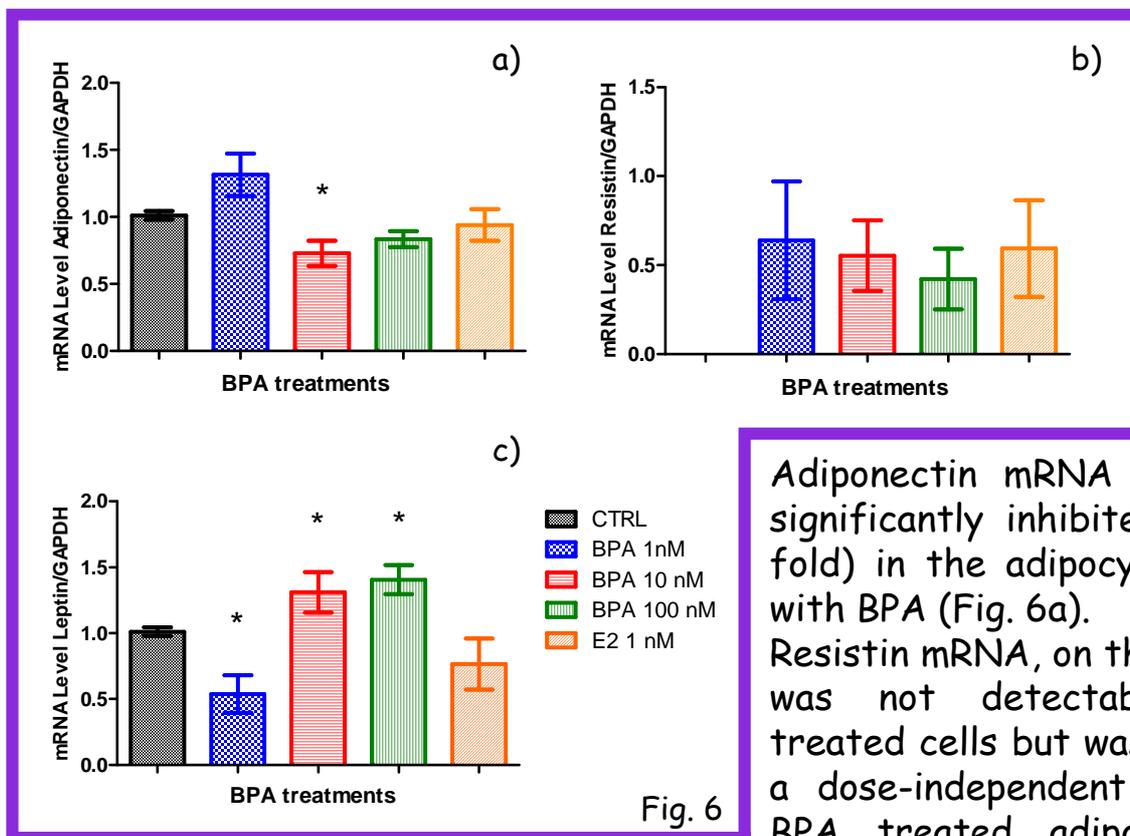


No significant statistical correlation were found between BPA and waist circumference, lipids and blood pressure. A GLM showed a significant positive correlation between BPA concentration and HOMA ($p < 0.03$), adjusting for waist circumference, age and sex (Fig. 4). A statistically significant inverse correlation ($p < 0.01$) was found between BPA levels and adiponectin serum concentration, adjusting for waist circumference, age and sex (Fig. 5).



.... Working in Progress

Preadipocytes, obtained from subcutaneous abdominal adipose tissue, were differentiated to mature adipocytes and were treated for 24 h with increasing doses of BPA (1, 10, 100 nM). To evaluate the effect of BPA on adiponectin, resistin and leptin expression, a Real-Time PCR was performed.



Adiponectin mRNA levels were significantly inhibited (nearly 4 fold) in the adipocytes treated with BPA (Fig. 6a).

Resistin mRNA, on the contrary, was not detectable in not treated cells but was present, in a dose-independent manner, in BPA treated adipocytes (Fig. 6b).

Leptin mRNA levels were significantly hyper-expressed in the adipocytes treated with BPA (Fig. 6c).

To evaluate the influence of exposure to BPA on fat accumulation, we have calculated the average lipidic area per cell using Red Oil staining.

To assess glucose metabolism and feeding behavior, *in vivo* experiments on mouse models exposed to BPA will be carried out.

Conclusions

In experimental studies, BPA exposure has been shown to disrupt multiple metabolic mechanisms, suggesting that it may increase body mass in environmentally relevant doses and therefore contribute to obesity in humans. This possibility has recently been explored in adults.

Our aim has been to evaluate a correlation between this endocrine disruptor and childhood obesity. For this reason, BPA concentration has been measured in the urine of normal weight and overweight children. Compared to blood collection, urine collection is thought to be more suitable for assessment of BPA exposure because of its less invasiveness.



BPA is rapidly excreted in urine, with a half-life in the range of 4 to 43 Hours. However, BPA has been detected in fat, suggesting that the compound accumulates in fat and other physiologic compartments. Even if urinary BPA concentration does not solely reflect recent consumption, it is likely to be a "noisy" indicator of chronic exposure.

We do not know how the exposure occurs, whether the sources of calories consumed differ between obese and normal weight children. Obese children may drink more canned or bottled beverages, or eat more canned food, and thus have higher urinary BPA levels.



The results from this pilot analysis suggest that children residing in Campania (Italy) have positive urinary concentrations of BPA. In particular, obese children have higher urinary concentration of BPA compared to normal weight children.

Moreover, BPA may represent one of the factors able to modulate insulin resistance in childhood obesity. This action appears to be due to a combined effect of BPA on adipose tissue, consisting both in adiponectin expression inhibition and leptin and resistin expression stimulation.