PERINATAL PROGRAMMING OF OBESITY BY THE ENDOCRINE DISRUPTOR BISPHENOL A IN A MOUSE MODEL

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Introduction
Human exposure to endocrine disrupting compounds (EDCs) is implicated in the worldwide pandemic of obesity. Following the Developmental Origins of Health and Disease (DOHaD) principle[1], exposure to EDCs during the sensitive perinatal period can program the developing organism towards increased susceptibility to develop obesity later in life[2,3]. In the EU-FP7 project OBELIX, experimental studies that test the potential of EDCs to program the organism to develop the obese phenotype are designed to provide evidence to support epidemiological associations between exposure to major EDCs and obesity later in life. Here we report results for male offspring of the first EDC tested, bisphenol A (BPA).

Materials and methods
Female C57BL/6J mice were mated with FVB males. Dams were exposed to BPA via the diet (dose range 0 – 3000 µg/kg BW) during the perinatal period (Table 1). Exposure of offspring was terminated at weaning. F1 animals of litters smaller than 10 pups were then assessed for obesity and metabolic disease related parameters, serum endocrine and lipid levels, and histopathology of key tissues. Tissues were also collected for DNA methylation profiling.

Table 1 – Experimental design

maternal exposure			F1 unexposed
2w	*3w*	*3w*	*18w*
premating	*gestation*	*lactation*	*juvenile / adult*

Results and discussion:
Throughout the study, male progeny showed a dose-dependent increase of body weight, in Fig. 1 shown at 21 weeks of age. Individual responses were variable (compare top dose in blue oval with unexposed control). The red box represents >90th percentile overweight animals (n=7). Female progeny showed a dose-dependent decrease of body weight (not shown), and further results in females are not reported here. This sex-defined difference supports the specificity of the effect.
Body weight in male animals was better correlated to parameters of fat mass (weight of fat pads) than to parameters of body size (body length, femur length) (Table 2), supporting that the increased body weight indeed was due to increased body mass.

Fig. 1 – Body weight dose response in F1 males

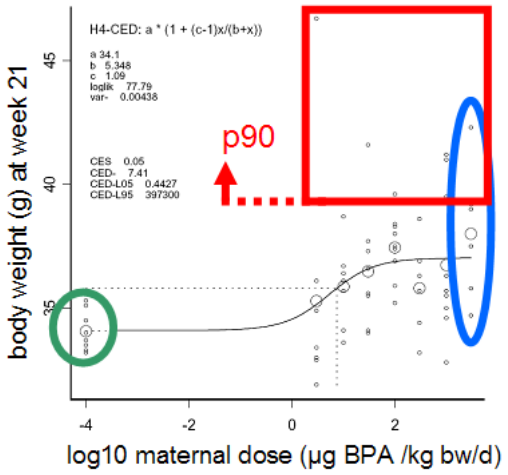


Table 2 – Correlation of selected physical parameters with body weight in F1 males

	r
epididymal adipose tissue	**0.67**
interscapular adipose tissue	**0.64**
sum fat pads*	**0.61**
body length (nose – tail base)	0.32
right femur length	0.11

n= 60, all dose groups included
*sum fat pads includes rostral and caudal subcutaneous, epididymal, perirenal, mesenterial, and interscapular fat pads

Increased fat mass was further supported by the observed hyperplasia in both white (perirenal) and brown (interscapular) fat pads of overweight male animals compared to controls (Fig. 2). This effect was even more prominent in >90th percentile animals than in top dose animals (Table 3).

Fig. 2 – Adipose tissue histopathology

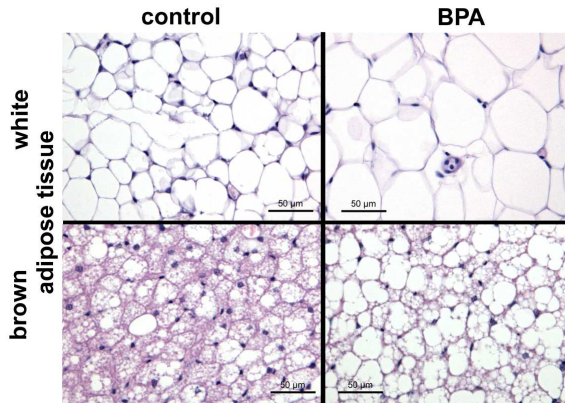


Table 3 – Distribution of adipocyte hyperplasia

BAT adipose cell lipid accumulation in interscapular fat

	normal	*slight*	*strong*	
controls	8	0	0	
top dose group overweight males (>p0.9 BW)	4	4	0	*
	1	2	4	**

WAT adipose cell diameter in perirenal fat

	diameter (µm)	
controls	40.2 ± 6.0	
top dose group overweight males (>p0.9 BW)	47.4 ± 8.9	*
	53.6 ± 8.4	**

*,** P<0.05, <0.01; Fisher's exact test for brown adipose tissue (BAT), T-test for white adipose tissue (WAT)

Overweight can be due to increased energy intake or decreased energy expenditure. Energy intake was tested by measuring food intake in top dose males, and appeared to be increased (Fig. 3a). Energy expenditure was monitored by measuring physical activity (Laboras System) in top dose males, which appeared to cover a shorter distance compared to control males (Fig. 3b).

Fig. 3a – Food consumption in F1 males

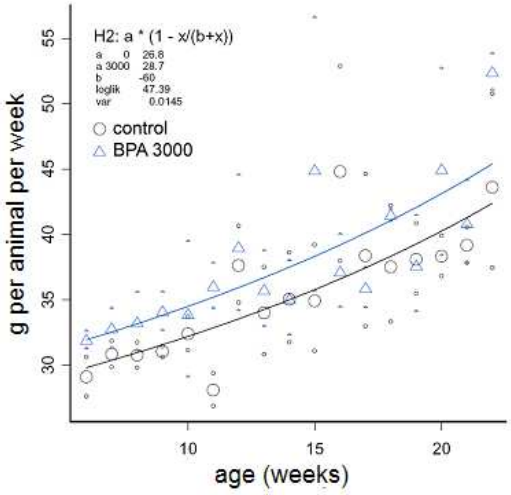
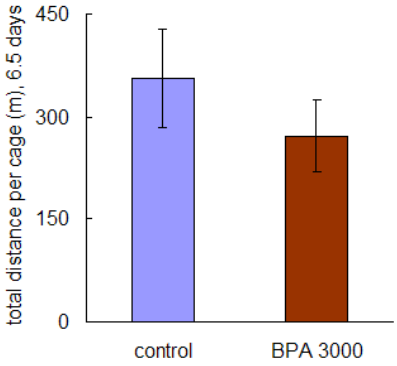


Fig. 3b – Physical activity in F1 males



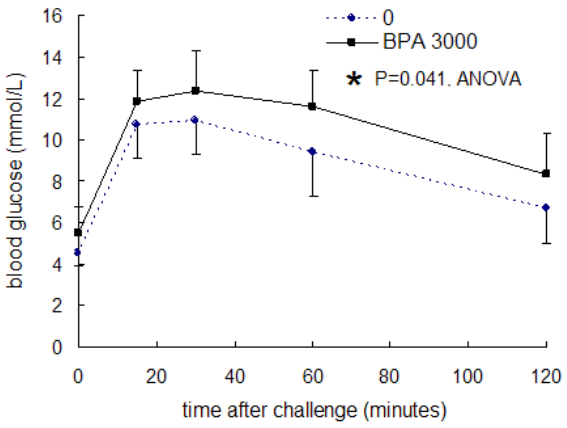
In male progeny, all measured serum lipid parameters showed a significant correlation with body weight (Table 4). Of these, only free fatty acids also showed a significant dose-response. In male progeny, leptin and insulin were positively, and adiponectin negatively correlated to body weight (Table 4). Insulin also showed a dose-dependent increase, and log-transformed glucagon a dose-dependent decrease. In a glucose tolerance test, top dose males also showed a delayed clearance of intraperitoneally injected glucose, as compared to controls (Fig. 4), suggesting perturbed glucose homeostasis. Together, these results suggest that the increased fat mass is accompanied by metabolic disruption.

Table 4 – Effects in serum parameters in male progeny

	dose response	*r*
lipid profile		
cholesterol	ns	0.40
triglycerides	ns	0.40
HDL-c	ns	0.37
FFA	increase	0.35
endocrine profile		
adiponectin	ns	-0.20
leptin	ns	0.72
ghrelin	nd	nd
PYY	nd	nd
insulin	increase	0.52
glucagon	decrease*	ns

*after log transformation; ns, not significant, nd, not detectable; n = 60

Fig. 4 – Glucose clearance in F1 males



Thus, in our model, BPA acts as a programming factor resulting in overweight and metabolic disbalance in later life of perinatally exposed male mice, confirming previous observations[4]. Epigenetic analysis is ongoing to elucidate underlying mechanisms of perinatal programming. Other EDCs will be tested in the same model.

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References:

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