Response

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Investigating Susceptibility to Diabetes Using Features of the Adipose Tissue in Response to *In Utero* Polycyclic Aromatic Hydrocarbons Exposure (*Diabetes Metab J* 2016;40:494-508)

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We appreciate your interest and comments on our article entitled "Investigating susceptibility to diabetes using features of the adipose tissue in response to *in utero* polycyclic aromatic hydrocarbons exposure" that was published in *Diabetes & Metabolism Journal* [1].

The ultimate goal of the study was to assess whether *in utero* exposure to polycyclic aromatic hydrocarbon (PAH) in the form of 2-aminoanthracene will produce vulnerability in the pups towards diabetes development using parameters of animal weight gain, serum glucose levels, adipose tissue (AT) histology and size, cd68+ specific staining and inflammatory gene expression in the AT. To assess the effect of *in utero* 2AA exposure, the weights of the rats were monitored. Animal weights were monitored for almost 3 months prior to the introduction of moderate high fat diet. The animals in all treatment groups begin with same initial weights at week 1, but over time they start to disperse from each other and demonstrate increment in weight over time. Moreover, low dose group shows the highest weight findings over time, while high dose group at the bottom, and control group in between over time. Similarly for the older rats by week 5, the low dose group whether on regular or moderate high fat diet, showed greater body weight. These measurements were not statistically significant. This is a trend we have noted previously while investigating broad gene expression of the pancreas of 3 to 4 weeks old Fisher-344 rats [2]. In this particular study, we found 0 and 50 mg/kg group did not show any differences in weight gain. However the 100 mg/kg group showed slight reductions in body weight gain.

To understand the role of insulin resistance in the current study, CD68 positive cells was determined in both the young and older animals. CD68 has been previously employed as a marker for macrophages [3]. The presence of CD68+ cells in AT in the 2 weeks old rats were not significantly different. Nonetheless, the low dose animals showed slightly higher levels of CD68+ cells while the high dose group indicated slightly fewer CD68+ cells. In older rats that were exposed to 2AA *in utero* and also consumed moderate high fat diet, the quantity of CD68+ cells were slightly greater, though not that significant, than control groups. For instance, the low dose male high fat, high dose male high fat, and high dose female regular diet animals showed similarly-high levels of CD68+ cells in the AT. However, when comparing between the younger and the older groups, all young pups had macrophages between adipocytes that occasionally form loose aggregates around capillaries. This feature was not present in the older pups, such that it likely represents a normal aging change in the distribution of these resident macrophages in AT.

Your observation that obesity and adipocyte inflammation are

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critical drivers of diabetes is accurate. It has been established in in vitro experiments that increased adipose cell size correlates with serum insulin concentrations, insulin resistance, and increased risk of developing type 2 diabetes [4]. Enlarged AT and adipocytes produce a variety of hormones involved in glucose metabolism, inflammation, macrophage activation, and fibrinolysis. Dysfunctional adipocytes provide a critical link between obesity and insulin resistance leading to abnormal fat storage and mobilization [5,6]. However the goal our study should be kept in mind. We were not necessarily interested the animals developing full blown diabetes. We were more interested in observing trends toward diabetes, that is whether *in utero* exposure to a PAH. The diet contains approximately ~42% fat and induces less severe obesity [7] and further, the time of dietary ingestion was relative short (5 weeks over all). It appears the duration of feeding moderate high fat diet has minimized the intensity of diabetic effects. In fact the non-fasting serum glucose levels at sacrifice though significant was much lower than in diabetic animals. Diabetic animals have fasting glucose levels in the range of 200 mg/dL [8]. The mRNA expression of adiponectin and other inflammatory proteins examined are consistent with susceptibility towards diabetes.

The other important issue you raised was whether the low dose (50 mg/kg) or high dose (100 mg/kg) exposure leads to the most severe outcomes. This is actually a challenge we have had so far. Unfortunately, we have not been able to delineate clearly which treatment will likely result in greater diabetic outcomes. Probably using additional tools such as global gene expression analysis and extending the duration of exposure and dietary treatments will enable us accurately answer this particular question. We would like to thank Dr. Choi for the interest in our study and for your thoughtful comments.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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