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Neonatal isoflavone exposure interferes with reproductive system of female Wistar rats

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Soy based infant formulas (SBIF) are used in many western countries as one alternative to breast feeding. Recently there is increasing concern about possible adverse effects of such diets. Soy contains high amounts of isoflavones (ISO), which are hormonal active substances. To give SBIF to infants leads to extremely high serum concentrations of ISO in a sensitive phase of child development. Particularly there are concerns that ISO may affect the development and physiological function of reproductive tract later in life.
The aim of our study was to investigate effects of ISO on the development of the reproductive tissues in different exposure scenarios with major focus on neonatal exposure in a Wistar rat model.
In our study we address and simulate four different exposure scenarios. Exposure to two different diets started with parental animals one week prior mating, continued during *in utero* period, maintained through adolescence into adulthood. Animals were exposed to: A, a ISO depleted diet (IDD) and B, same diet enriched with an ISO extract (IRD; 508mg ISO/kg). Pups of each group were randomized into subgroups and fed daily by pipette with ISO-suspension [(32 mg ISO/kg BW) ISO+] and placebo from postnatal day (PND) 1 until PND 23. Rats were sacrificed at PND 23 and 80.
Body weight and food intake were not affected by ISO+, lifelong IRD diet increased both by tendency. Interestingly, visceral fat mass was significantly reduced in IRD groups.
Vaginal opening (VO), a marker for puberty onset, occurred significantly earlier in animals through ISO+ independently whether the animals were kept on IDD (9.4 days earlier) or IRD (5.5 days earlier). At PND70 we observed an irregular estrus cycle in ISO+ rats.
Relative uterine weight at PND23 and 80 and rel. ovarian weight at PND80 were not significantly affected by any diet. Ovary weight at PND23 was lowest in IRD groups however ISO+ had no additional influence.
A significant increase of vaginal epithelial height was observed in ISO+ on PND23.
In summary, our results indicate that ISO intake during weaning period has an estrogenic effect on prepubertal rats indicated by increased vaginal epithelial heights and earlier VO. In addition the exposure of rats during the period of weaning to ISO resulted in estrus cycle irregularities. That indicates that SBIF may result in adverse effects on reproductive tract even later in life. Toxic and molecular mechanisms inducing these effects need to be characterized in future studies.

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ß-Tanycytes in the regulation of the hypothalamus-pituitary-thyroid-axis

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The hypothalamic network plays an important role in the regulation of different endocrine and metabolic functions. Largely unknown players in this network are the so-called tanycytes. The cell bodies of these specialized ependymal glial cells contact the cerebrospinal fluid in the wall of the 3rd ventricle and send processes into the hypothalamic nuclei and the median eminence. Our recent findings indicated that tanycytes are chemosensors responding to a number of circulating signals such as thyrotropin-releasing hormone (TRH) and the analog taltirelin with intracellular calcium waves only in ß-tanycytes. This suggested a possible role in regulating of the hypothalamus-pituitary-thyroid (HPT) axis. However, the cellular signalling pathway and the physiological functions of tanycytes are unknown.
To investigate the role of tanycytes in regulation of the HPT axis we systematically studied the calcium response with pharmacological and genetic tools by measuring intracellular calcium in brain slices of mice. Here we show that the TRH-R1 receptor stimulated the calcium response in ß-tanycytes via a Gq/11 signalling pathway. To clarify the impact of tanycytes on the TRH release in the median eminence we transduced TRH-positive neurons with an adeno-associated virus to express a mutated muscarinergic receptor which was exclusively activated by clozapine-N-oxide. With this technique it became possible to selectively activate TRH-neurons, which led to a temporal increase of energy expenditure and TSH plasma levels. In animals with a tanycytic-specific as well as a glial knockout of Gq/11 the basal levels of TSH were reduced.
In summary, ß-tanycytes are active signalling cells within the brain that respond to TRH and modulate the release of hormones of the HPT-axis.

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Selenium restores endothelial dysfunction and metabolic profile in type 2 diabetic rats

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Aim: Endothelial dysfunction is responsible for diabetic vascular complications and develops as a result of oxidative stress and its pathological process (1). In this study, we investigated the effects of selenium on endothelial dysfunction and oxidative stress in type 2 diabetic rats.
Methods: The rats were divided into 5 groups as control rats, untreated diabetic rats and diabetic rats treated with different doses of selenium (180, 300, 500 mcg/kg/day). In order to develop of the type 2 diabetic rat model, the diabetic rats were fed the high-fat diet until the end of study and control rats were fed regular chow. The diabetic rats were injected intraperitoneally with low dose streptozotocin. Endothelium-dependent and -independent relaxations were determined in rat thoracic aorta. Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and endothelial nitric oxide synthase (eNOS) mRNA expressions were analyzed by RT-PCR. Fasting blood glucose, lipid profile, lipid peroxidation, insulin and nitric oxide were tested in blood samples of rats. Malondialdehyde, superoxide dismutase, catalase and glutathione peroxidase levels were measured in rat liver samples.
Results: RT-PCR results showed that selenium reversed the increased NADPH oxidase expression while decreasing the eNOS expression back to control levels, and improved the impairment of endothelium-dependent vasorelaxation in diabetic aorta. Selenium treatment significantly decreased blood glucose, cholesterol, trigliserid levels and enhanced antioxidant status in diabetic rats.
Conclusions: The present study confirmed antioxidant and ameliorative effects of selenium in the treatment of type 2 diabetic rats induced by a high- fat diet combined with streptozotocin injection. Because of insulin resistance and insulin deficiency, the combination of high- fat diet and multiple low- dose of STZ could be an experimental animal model for type 2 diabetes and pharmacological screening (2). Our findings indicate that selenium restores metabolic profile and ameliorates vascular responses and endothelial dysfunction in diabetes via regulation of antioxidant enzymes and nitric oxide release.

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Influence of bile acids on the viability of pancreatic beta-cells and islet granularity

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Question:
Type-2 diabetes mellitus (T2DM) is not only characterized by hyperglycemia but also by hyperlipidemia. These diabetes-associated alterations also affect the physiological bile acid pool. Bile acids (BAs) are known to influence regulation of pancreatic beta-cells. As excessive substrate supply is one main cause for malfunction and loss of islet cells in T2DM, we investigated whether BAs can conserve beta-cell viability and islet morphology in the presence of elevated glucose and lipid concentrations.
Methods:
Islets were isolated from C57Bl/6 mice. Cell death was measured by TUNEL assay. For culture, standard (11.1 mM glucose), glucotoxic (25 or 33 mM glucose) or glucolipotoxic (33 mM glucose/10 µM of the LXR-agonist TO-901317) conditions were used. Histological analysis of islet granularity as a measure for insulin content was performed by image digitalization and processing.
Results:
Ursodeoxycholic and lithocholic acid (UDCA, LCA, 500 nM) had no effect on cell viability of beta-cells cultured in standard medium with the respective BA for 7 d (n=4 independent preparations). Culture in glucolipotoxic milieu increased apoptotic cell death (7 d, 2.3±0.4 vs. 9.7±1.3 %, n=10, p≤0.001). In the presence of UDCA this harmful effect was markedly attenuated by approximately 45 % (n=6, p≤0.05). Importantly, UDCA lost its protective influence in combination with LCA (n=4). To investigate whether BAs and/or nutrient excess affect pancreatic islet histology alterations in islet granularity were determined. Glucotoxicity decreased islet granularity after 1 d of culture (7.7±0.2 vs. 6.3±0.3 px/µm^2, n=33-34 islets, p≤0.001). The effect was even more pronounced after 7 days (n=36-39, p≤0.001). UDCA protected against the loss of islet granularity induced by 25 mM glucose (n=30, p≤0.01). In standard medium (11 mM glucose) LCA significantly reduced islet granularity (3 d, 6.7±0.3 vs. 5.0±0.3 px/µm^2, n=31, p≤0.001) whereby co-incubation with UDCA prevented this effect (n=34, p≤0.001).
Conclusion:
BAs influence beta-cell viability and islet morphology differently. The hydrophilic UDCA attenuates the effect of elevated nutrient supply on both parameters. By contrast, the lipophilic LCA lowers islet granularity *per se*. Of particular interest, UDCA and LCA