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Short term exendin-4 treatment reduces markers of metabolic disorders in female offspring of obese rat dams

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ABSTRACT

Objectives: Maternal obesity imposes significant health risks in the offspring including diabetes and dyslipidemia. We previously showed that the hypoglycaemic agent exendin-4 (Ex-4) administered from weaning can reverse the maternal impact of 'transmitted disorders' in such offspring. However daily injection for six-weeks was required and the beneficial effect may lapse upon drug withdrawal. This study aimed to investigate whether short term Ex-4 treatment during suckling period in a rodent model can reverse transmitted metabolic disorders due to maternal obesity.
Methods: Maternal obesity was induced in female Sprague Dawley rats by high-fat diet feeding for 6 weeks, throughout gestation and lactation. Female offspring were treated with Ex-4 (5 μg/kg/day) between postnatal day (P) 4 and 14. Female offspring were harvested at weaning (P20). Lipid and glucose metabolic markers were measured in the liver and fat. Appetite regulators were measured in the plasma and hypothalamus.
Results: Maternal obesity significantly increased body weight, fat mass, and liver weight in the offspring. There was an associated inhibition of peroxisomal proliferator activated receptor gamma coactivator 1α (PGC1α), increased fatty acid synthase (FASN) expression in the liver, and reduced adipocyte triglyceride lipase (ATGL) expression. It also increased the plasma gut hormone ghrelin and reduced glucagon-like peptide-1. Ex-4 treatment partially reversed the maternal impact on adiposity and impaired lipid metabolism in the offspring, with increased liver PGC1α and inhibition of FASN mRNA expression. Ex-4 treatment also increased the expression of a novel fat depletion gene a2-zinc-glycoprotein 1 in the fat tissue.
Conclusion: Short term Ex-4 treatment during the suckling period significantly improved the metabolic profile in the offspring from the obese mothers at weaning. Long-term studies are needed to follow such offspring to adulthood to examine the sustained effects of Ex-4 in preventing the development of metabolic disease.

1. Introduction

The global obesity epidemic is associated with a dramatic rise in the rate of childhood obesity. Epidemiological and animal studies have established that maternal obesity plays a significant role in the increased incidence of childhood obesity and the future risk of obesity and obesity related chronic diseases, including diabetes and cardiovascular disorders (Bayol et al., 2005, 2010; Samuelsson et al., 2007; Chen and Morris, 2009).

The hypothalamic neuronal systems that regulate appetite and energy homeostasis are not mature in newborn rats, with development continuing until weaning (Pinto et al., 2004; Bouret and

Abbreviations: AGRP, agouti-related protein; ATGL, adipocyte triglyceride lipase; ARC, arcuate nucleus; AZGP1, α(2)-zinc-glycoprotein 1; CPT-1, carnitine palmitoyltransferase-1; Ex-4, exendin-4; FASN, fatty acid synthase; FOXO1, forkhead box protein O 1; GLP-1, glucagon-like peptide-1; HFD, high-fat diet; IUGR, intrauterine growth restriction; MC4R, melanocortin 4 receptor; NPY, neuropeptide Y; NPY Y1R, Neuropeptide Y1 receptor; Ob-Rb, active lepton receptor; P, postnatal day; POMC, pro-opiomelanocortin; PGC1α, peroxisome proliferator activated receptor gamma coactivator 1α; PPARγ, peroxisome proliferator-activated receptor γ; SREBP, sterol regulatory element binding protein; SOCS, suppressor of cytokine-signaling; STAT, signal transducer and activator of transcription.

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Simerly, 2006). Specifically between postnatal days (P) 4–14, there is a marked increase in serum levels of the hormone leptin (called the 'leptin surge'). This is believed to support the post-natal development and maturation of hypothalamic neuronal projections between nuclei involved in the regulation of energy homeostasis (Ahima et al., 1998; Bouret et al., 2004). Leptin binds to its active form of the receptor Ob-Rb, followed by a phosphorylation of its downstream signal transducer and activator of transcription (STAT) 3 leading to nuclear gene transcription. Any homeostatic disturbances during the 'leptin surge' period can alter the neural development resulting in metabolic disorders (*e.g.*, obesity, hyperlipidemia), as observed in leptin-deficient *ob/ob* mice or pups of obese dams with an abnormally high level of blood leptin (Pinto et al., 2004; Yura et al., 2005; Bouret and Simerly, 2006; Kirk et al., 2009). We have shown that maternal obesity prior to gestation changes the hypothalamic appetite stimulator neuropeptide Y (NPY) expression (Chen et al., 2008), and impairs NPY production in response to both hypo- and hyperglycaemia (Chen and Morris, 2009; Chen et al., 2014a Chen et al., 2014a). Dysregulation of NPY production is closely linked to hyperphagia during the suckling period and obesity at weaning in such offspring (Chen and Morris, 2009). Additional nutrient influx during the suckling period exacerbates metabolic disorders (Chen et al., 2008, 2009), which can subsequently impact on the second generation (Rajia et al., 2010). The replenishment of blood leptin in *ob/ob* mice during the time of the leptin surge induces synaptic reorganization and may reverse their abnormal phenotype (Pinto et al., 2004). This suggests that an intervention during the suckling period, especially during the leptin surge period, may reduce obesity and metabolic derangements in the offspring. The obese phenotype in pups from obese rats has been believed to be partially due to the composition of breast milk (Gorski et al., 2006) as the birth weight of pups from lean and obese dams are similar. However, nutritional approaches that use breast milk from lean rats have been shown to be ineffective in changing the obese phenotype in offspring of obese mothers (Gorski et al., 2006).

We have previously used the hypoglycaemic drug exendin-4 (Ex-4), a glucagon-like peptide-1 (GLP-1) receptor analogue, to treat offspring of obese dams for 6 weeks starting at weaning and have shown a reversal of the maternal impact on the propensity of the offspring to high-fat diet (HFD)-induced obesity (Chen et al., 2014b). However, such treatment needs to be administered daily and requires 6 weeks to exert significant effects to normalize glucose homeostasis. In a previous study, Ex-4 was injected daily into neonate rats with intrauterine growth restriction (IUGR) from P0-6 (Stoffers et al., 2003). Such short term Ex-4 treatment in early life permanently reversed the diabetic phenotype in the IUGR rats, *via* promoting the development of the pancreas, in particular insulin generating β-cells (Stoffers et al., 2003). Since maternal obesity causes insulin resistance at a very young age lasting until adulthood (Chen et al., 2008, 2009), we propose that short-term Ex-4 treatment during the early postnatal period may improve the metabolic profile in such offspring.

We have recently demonstrated this in post-weaning rats (Chen et al., 2014b), but through mechanisms that are independent of pancreatic function. Since early postnatal neural development plays a critical role in determining the future risk of lipid and glucose metabolic disorders, we hypothesized that short-term Ex-4 treatment during P4-14 (leptin surge period) could improve the metabolic profile at weaning in the offspring of obese mothers. In this study we treated female rat pups with daily Ex-4 between P4 and P14, and measured body weight, adiposity, blood lipid levels and hypothalamic expression of regulators for energy homeostasis. In addition, we also measured mRNA expression of lipid and glucose metabolic regulators, such as adipose triglyceride lipase (ATGL), lipid oxidative regulator carnitine palmitoyltransferase-1 (CPT-1)α, peroxisome proliferator activated receptor gamma coactivator 1-alpha (PGC-1α) and its downstream signaling pathway, as well as the novel 'fat depletion gene' α(2)-zinc-glycoprotein 1 (AZGP1), in the fat and the liver.

2. Animal and methods

2.1. Modeling maternal obesity and treatment

The study was approved by the Animal Care and Ethics Committees of University of New South Wales and University of Technology Sydney. Maternal obesity was modeled as previously described (Chen et al., 2012, 2014b). Briefly, female Sprague Dawley breeders (8 weeks, Animal Resource Centre Pty. Ltd, WA, Australia) were housed under the standard conditions (Chen et al., 2012, 2014b). Maternal obesity was induced by consuming a pellet high-fat diet (HFD, 20 kJ/g, 43.5% calorie as fat, SF03-020, Specialty Feeds, WA, Australia, $n = 8$) for 6 weeks (Chen et al., 2014b), while the control rats were fed standard chow (11 kJ/g, 14% calorie as fat, Gordon's specialty Stockfeeds, New South Wales, Australia, $n = 8$). Then females were mated with lean male rats (8 weeks) from the same source. Litter size was adjusted to 10/litter (sex ~1:1). The same control or HFD diet was continued in the dams until the pups reached P20.

From P4-14, 2–3 female pups from every litter were injected with saline daily to act as the control group (CS: chow offspring + saline, HS: HFD offspring + saline), while the other females were injected with Ex-4 (5 μg/kg/day i.p., Auspep, VIC, Australia. CE: chow offspring + Ex-4, HE: HFD offspring + Ex-4).

2.2. Leptin sensitivity test

In a sub-cohort of offspring, leptin (15 μg/g, i.p., Sigma–Aldrich Pty Ltd, NSW, Australia) was injected into P10 pups ($n = 3$–4, 1 per litter), with saline-injected into littermates to act as the control group as previously described (Kirk et al., 2009; Glavas et al., 2010). The whole hypothalamus was then harvested 45 min post-injection for the measurement of the protein levels of the downstream signaling of the leptin receptor, including STAT3 and its phosphorylated form p-STAT3 by western blotting. Blood glucose level was measured using a glucose meter (Accu-Chek®, Roche, Nutley, NJ) in saline treated pups before harvesting the brain.

2.3. Sample collection

At weaning (P20), female offspring were deeply anesthetised with sodium thiopental (Pentothal®, 0.1 mg/g, i.p, Abbott Australasia, NSW, Australia), immediately after being taken away from their mothers. Body weight and naso-annual length were measured prior to the blood collection by cardiac puncture, and blood glucose levels were measured using a glucose meter (Accu-Chek®, Roche, Nutley, NJ). Serum was immediately separated by centrifugation at 4 °C (12,000 g, 8 min) and stored at −20 °C in DNAse and RNAse free Eppendorf tubes for later measurement of hormones (ghrelin, GLP-1 and leptin) and lipids (triglyceride and non-esterified fatty acid). Pups were then killed by decapitation and the liver and fat pads (retroperitoneal, mesenteric fat and gonadal fat) were weighed. The hypothalamus was micro-dissected into regions containing arcuate nucleus (ARC) and paraventricular nucleus (PVN) as previously described (Chen et al., 2009). The brain, liver and retroperitoneal fat were then snap-frozen for mRNA measurement.

2.4. Plasma lipid and hormone assays

Liver lysis ($n = 8$) was homogenized using chloroform:methanol mixture (2:1), and air-evaporated. Absolute ethanol (250 μl) was