Abstract

In view of the worldwide obesity pandemic, also in women of childbearing age, the detrimental consequences of obesity socioeconomically challenge the healthcare system. Importantly, maternal obesity is linked to metabolic issues as well as alterations in brain development in the offspring, possibly exerting dysfunctions of hypothalamic neuroplasticity. The influence of the fetal environment on multiple, if not all organs shortly before and after birth is summarized by the concept of perinatal programming. Regarding pathophysiological mechanisms, it has been established that maternal obesity is associated with increased interleukin 6 (IL-6) signaling in the offspring. With research supporting the beneficial effects of exercise on weight management and an effect on IL-6 signaling, we set out to examine the effects of maternal exercise on offspring's neuroplasticity. In addition, we separately regarded the role of so-called IL-6 trans signaling (IL-6ts) in the brain, since brain-specific IL-6ts potentially collides with the proinflammatory effect on neuroplasticity of obesity and its consequences. Thus, besides the use of wild type (WT) mice, we examined a transgene mouse model expressing an IL-6ts inhibitor (glial fibrillary acid protein (GFAP) induced soluble glycoprotein 130 crystallizable fragment (sgp130FC) in the central nervous system.

We challenged WT and transgenic ^{GFAPsgp130}dams with a western-style diet (WSD) to generate diet-induced obesity. Two weeks prior mating, WT lean and overweight dams were offered voluntary exercise that was executed throughout pregnancy (SD-RUN^{dams} and WSD-RUN^{dams}) as the main intervention alternatively to the transgenic model, while others remained sedentary (CO: SD^{dams} and WSD^{dams}). To examine short- and long-term effects of maternal obesity and potential improvement through maternal exercise or brain-restricted IL-6ts inhibition with and without an obesogenic environment, offspring was analyzed on postnatal day (P) 21 and P120. At P56, offspring was challenged with the western-style diet themselves, a so-called 2nd hit, to evaluate long-term influence on maternal obesity in a metabolically challenging condition. Systemic glucose tolerance was assessed with intraperitoneal glucose and insulin tolerance tests (ipGTT, ipITT) at P21 and P120, and food intake and preference was monitored from P56 to P60. At P21 and P120, the hypothalamus, blood, epigonadal white adipose tissue (egWAT), and the quadriceps (at P21 only) was withdrawn from offspring. Organs were prepared and analyzed with proteomics, targeted protein analysis, methylation analysis, and mRNA expression analysis.

All dams on WSD weighed more than 23 g entering pregnancy and revealed disturbed glucose tolerance. Interestingly, transgenic ^{GFAPsgp130}mice in general were lighter than WT mice on both diets. In offspring, maternal obesity placed a burden on body fat percentage (%egWAT) that could not be extinguished by maternal interventions at P21, neither by maternal exercise, nor brain-restricted IL-6ts. However, elimiation of maternal obesity-induced increase in %egWAT was achieved at P120 through maternal exercise as well as the transgenic alteration. This

observation was paired with persistent glucose intolerance through maternal obesity at P21, which was increased in WSD-RUN to WSD. However, a disturbed insulin tolerance derived from ipITT at P21 was only present in WSD to SD. At P120, maternal exercise was able to reduce the glucose tolerance dysregulation in offspring with 2nd hit, especially in offspring from lean exercised dams. Transgenic offspring remained to display a tendency in disturbed glucose tolerance worsened by maternal obesity.

Serum analysis at P21 revealed elevated inflammatory and metabolically relevant markers (IL-6, sIL-6R, leptin, insulin, and fibroblast growth factor 21 (FGF-21)) through maternal obesity in WSD to SD. Maternal exercise was able to reduce IL-6, sIL-6R, as well as FGF-21 in WSD-RUN, while leptin and insulin remained elevated in WSD-RUN to SD-RUN, in alignment with %egWAT and ipGTT at P21. ^{GFAPsgp130}WSD to ^{GFAPsgp130}SD showed elevation of FGF-21 and a reduction in monocyte chemoattractant protein 1 (MCP-1). At P120, there was no difference in IL-6 and MCP-1 in WT offspring with 2nd hit, but a strong elevation of both inflammatory markers through maternal obesity in transgenic ^{GFAPsgp130}WSD to ^{GFAPsgp130}SD offspring.

Regarding the influence of maternal obesity and maternal exercise on the hypothalamus as the center of energy homeostasis, hypothalamic proteome analysis from WT offspring (SD, WSD, SD-RUN, WSD-RUN) at P21 presented an influence of maternal exercise on the hypothalamic proteome, particularly in WSD-RUN. Maternal obesity induced altered protein expression in WSD to SD offspring of prostaglandin synthase 1 (PTGS1) and junctophillin-4 (JPH4), both partaking in synaptic regulations as well as inflammatory processes. Maternal obesity in the offspring of exercised dams (WSD-RUN to SD-RUN) presented various altered proteins associated with synapse organization. Altered proteome between WSD-RUN and WSD revealed an influence of maternal exercise on, amongst others, improved dendritic spine formation, supported by the upregulation of pAKT/AKT by maternal exercise in targeted protein analysis (RUN-effect). Targeted protein analysis further revealed an upregulation by maternal exercise of various kinases (janus kinase (pJNK/JNK), mitogen-associated kinase p38 (pP38/P38), extracellular signal-regulated kinase (pERK/ERK)), which have been associated with increased hypothalamic insulin and leptin sensitivity during the critical window of perinatal programming. Proteome analysis from transgenic offspring revealed, amongst other regulations, an altered regulation of proteins associated with the establishment of the blood-brain barrier, an effect not seen in WT offspring.

Proteome analysis at P120 from WT offspring with 2nd hit revealed alterations in mitogen-activated protein kinase (MAPK1/3, or ERK1/2) signaling as well as an adjustment of post synapse organization. MAPK1/3 alterations were supported by targeted protein expression, displaying an influence of maternal obesity on pERK/ERK, reducing expression in WSD and WSD-RUN (diet-effect). pAKT/AKT remained to be positively influenced by maternal exercise.

The egWAT at P21 from WT offspring revealed to be altered primarily by maternal obesity (downregulation of oxidoreductase activity-associated protein cytochrome P450 2F2 (CYP2F2) and peroxisome-proliferator-activated receptor gamma coactivator 1α (*Pgc1*α), elevated *Il-6* and *Mcp-1*) and only marginal reduction in inflammation marker *Il-1β* through maternal exercise. ^{GFAPsgp130}WSD to ^{GFAPsgp130}SD equally revealed a reduction in CYP2F2, in addition to an altered cholesterol metabolism.
The quadriceps was not greatly affected by maternal obesity nor maternal interventions, but targeted protein analysis at P21 revealed an upregulation of pAKT/AKT through maternal exercise in WT offspring, in alignment to observations seen in the hypothalamus at P21 and P120.

All in all, we were able to reveal a positive effect of maternal exercise on offspring, presented by a protection from increased %egWAT at P120 and numerous effects on synaptic plasticity as well as systemic regulation of inflammatory markers. Regarding the ^{GFAPsgp130}transgenic model, the influence of maternal obesity on P120 %egWAT was also diminished. Glucose metabolism in offspring was affected by maternal obesity, displaying insulin resistance in WSD, while impaired glucose tolerance was present in WSD-RUN and ^{GFAPsgp130}WSD. Maternal exercise seemed to exert a long-term protection on glucose metabolism at P120, especially by maternal exercise of lean dams. Brain-restricted IL-6ts however revealed no positive impact on glucose homeostasis. Effects displayed in serum analyses indicated a strong effect of maternal obesity on offspring' systemic metabolically relevant and inflammatory markers that can largely be diminished by maternal exercise. Brain-restricted IL-6ts seemed to induce compensatory upregulation of inflammation markers long-term. An influence of maternal obesity on the hypothalamic proteome was apparent in our study and should be studied further in specific hypothalamic knock-out models. A greater alteration in the proteome of WSD-RUN to SD-RUN than WSD to SD argued for an interaction of maternal obesity and maternal exercise, in which maternal exercise derived changes are only initiated in an obesogenic environment through maternal obesity. The transgenic alteration was not able to completely diminish hypothalamic alterations by maternal obesity. Effects of maternal obesity on egWAT were only moderately influenced by maternal exercise and the quadriceps was only mildly affected by maternal obesity and/or maternal exercise. Brain-restricted IL-6ts inhibition also displayed no great influence on egWAT or quadriceps derived alterations by maternal obesity. The regulation of important metabolic and synaptic markers at P21 were shown to be relevant for the phenotype at P120 within the meaning of perinatal programming.