

#59: Antisense oligonucleotide-mediated knockdown of Mpzl3 protects from the metabolic consequences of a high-fat, energy-dense diet in mice

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In recent decades, obesity has become a major health concern in the United States and many other parts of the world. It is associated with a variety of medical conditions including heart disease, high blood pressure, diabetes, respiratory deficiencies, and stroke. In the United States from 2011-2014, the prevalence of obesity was just over 36% in adults and 17% in adolescents. These values continue to rise despite public health programs and initiatives to promote a healthier lifestyle. Body weight regulatory mechanisms are also poorly understood, contributing to a lack of clinically efficient pharmacological agents for weight loss. Thus, there is an unmet medical need to identify metabolic pathways regulating body weight. It was previously shown that mice deficient in the gene encoding myelin protein zero-like 3 (Mpzl3-KO) had reduced body weight and fat mass, increased energy expenditure, improved glycemic control, and reduced hepatic lipid synthesis (Czyzyk et al. 2013. *AJPEM* 305(2):E282-92). In this study, we examined the effects of antisense oligonucleotide (ASO)-mediated knockdown of Mpzl3. Male six-week-old C57BL6/N mice were placed on a high-fat, energy-dense diet for seven weeks, followed by placement into scrambled control or Mpzl3 ASO treatment groups via block randomization by body weight. ASOs were administered IP twice weekly at a dose of 50mg/kg. Measurement of food intake after two weeks treatment showed no difference in consumption. After three weeks of treatment, mice receiving Mpzl3 ASO injections showed significantly reduced body weight ($p < 0.05$) and fat mass ($p < 0.01$) compared to control animals. Serum analyses at this time point revealed reductions in circulating cholesterol levels by 17% ($p < 0.05$) and triglycerides by 43% ($p < 0.001$). Indirect calorimetry experiments after four weeks treatment showed a marked increase in activity levels in Mpzl3 ASO treated mice compared to controls, but no change in energy expenditure. Furthermore, the respiratory exchange ratio (a measure of CO₂/O₂ production) was significantly reduced in the Mpzl3 ASO group indicating an increase in whole body fat metabolism ($p < 0.01$) in these mice. Additional serum collected at the time of sacrifice (seven weeks treatment) showed further reductions in circulating cholesterol by 38% ($p < 0.0001$) and triglycerides by 24.5% ($p < 0.001$). Tissue was harvested from liver, brown (BAT) and white adipose (WAT), intestine, skeletal muscle, and hypothalamus. RT-PCR analysis revealed significant reductions in relative mRNA levels of Mpzl3 in the metabolically active tissues: liver, BAT, intestine, and skeletal muscle; WAT trended towards a reduction. Knockdown was not seen in the hypothalamus. We have therefore shown that ASO-mediated knockdown of Mpzl3 in mice lowered body weight and fat mass, increased whole body fat oxidation leading to reduction in circulating cholesterol and triglycerides, and increased activity level. Furthermore, these data suggest that reduction of MPZL3 might have therapeutic potential for obesity and comorbid conditions.