SSAT inducer as a potential treatment for obesity-related heart failure

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Introduction: Obesity is a growing health problem worldwide, especially in developed countries. It increases the risks of heart disease and type-2 diabetes, and is associated with impaired cardiac function. While effects of obesity can be ameliorated through life-style change and exercise, pharmacological intervention remains another important potential approach. Recently, a correlation between fat metabolism and polyamine catabolism has been reported (1). Spermidine/spermine acetyl transferase (SSAT), is the key regulatory enzyme of polyamine catabolism. It catalyzes addition of acetyl groups to the α-amino N atoms of polyamines, which triggers their extra-cellular transport, and is the rate-limiting step of polyamine catabolism. Mice overexpressing the SSAT gene have decreased adipose tissue mass, increased levels of fatty acid and glucose oxidation, elevated basal metabolic rates, increased insulin sensitivity, and improved glucose tolerance (2). Since high SSAT activity shows apparent benefits in obesity, we thus propose to use chemical SSAT inducers to treat obesity and its cardiac complications. Here, we have employed high-field MRI to examine the influence of $N_{1,}N_{11}$-diethylnorspermine (DENS), a potent SSAT inducer, on body fat content and cardiac function in obese *ob/ob* mice. We hypothesised that increasing SSAT would decrease fat content and improve cardiac function in obese mice.

Methods: C57Bl/6 wild-type mice and matched ob/ob mice with leptin deficiency (*ob/ob*) were used as animal models. One control and one treatment group were established for each genotype (each comprised 6-8 mice). DENS (40 mg/kg-bodyweight), was administrated from immediately after weaning through i.p. injection for three times a week throughout the 24-week experimental period. Body fat percentage of mice at 0, 8, 16 and 24 weeks after weaning, and cardiac function at 24 weeks after weaning, were measured using a Varian (Palo Alto, CA, USA) 4.7-T horizontal-bore magnet controlled with a Unity Inova spectrometer. A72-mm ID circularly-polarized bird-cage coil (M2M Imaging, Cleveland, OH, USA) was used to measure fat content, and a 40mm ID Millipede™ coil from Varian Inc. (Palo Alto, CA, USA) for cardiac function measurements. Throughout each MRI scan, animals were anaesthetized with 4% isoflurane administered via air flow, and core body temperatures were maintained between 35 and 38°C. ECGs, respiration rates and core body temperatures were monitored using a Small Animal Monitoring System (SAII, Stony Brook, NY). For fat content, a respiration-gated MRI scan using the three-point Dixon fat/water imaging protocol (3) spanning the whole body was applied. (TR = 1000 msec; TE was adjusted according to the separation of the fat and water peaks to obtain in-phase and opposed-phase images, flip angle = 60°, thickness = 2 mm, averages = 4, matrix = 128x128, numbers of slices and FOV were adjusted to cover the whole body from the anterior to posterior, see Figure 1). Total body volume was determined from regions of interest that had been manually selected by combining water and fat signals using ImageJ. Total fat volume was determined from threshold-selected regions in 'fat signal only' images and fat volume percentage derived. For cardiac function, an ECG and respiration-gated gradient-echo sequence MRI scan in the cardiac short axis orientation, spanning from the apex to the base of the left ventricle, was carried out. (TR = 2* R-R interval, ~ 280-360 msec, TE = 1.8 msec, cardiac phases = 10, flip angle = 60°, slices = 12, thickness = 1 mm, averages = 4, FOV = 40 x 40 mm, matrix = 128x128). End-diastolic volume (EDV), end-systolic volume (ESV), ejection fraction (EF) and left ventricular mass (LVM) were determined manually from regions of interest in the cine images using ImageJ. All groups of mice completed body fat and cardiac MRI scans.

Results: Obese mice were significantly heavier than C57 mice throughout the 24-week period ($p < 0.001$; linear mixed-effects model, LME). DENS-treatment significantly lowered bodyweight in *ob/ob* ($p < 0.001$) but not C57 wt mice (Figure 2B). DENS-treated mice had significantly lower body-fat content (obese mice: 61.8% ± 3.8%, C57 mice 36.9% ± 3.5%) than matched controls within the same genotype (obese mice: 68.1% ± 4.9%, C57 mice 41.8% ± 2.2%) at the 24- week time point ($p < 0.05$) but not at any of the previous time points (Figure 2A). Cardiac hypertrophy developed in obese control mice, where average LVM was 95 ± 2 mg, which was significantly higher than that of C57 control mice (65 ± 12 mg, $p < 0.001$). DENS treatment in obese mice significantly lowered the cardiac mass (to 68 ± 5 mg, $p < 0.001$, Figure 2C). Obese mice treated with DENS have higher cardiac output (CO, 0.44 ± 0.05 ml/min/g) than that of untreated obese mice (0.35 ± 0.05 ml/min/g), and it is similar to that of C57 mice (0.45 ± 0.23 ml/min/g).

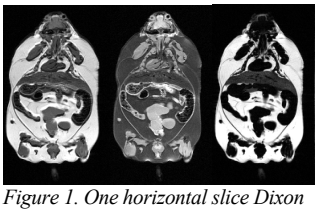

Figure 1. One horizontal slice Dixon images of a 6-month-old mouse, from left to right: image from water and fat signals combined, image from water signal only, and image from fat signal only.

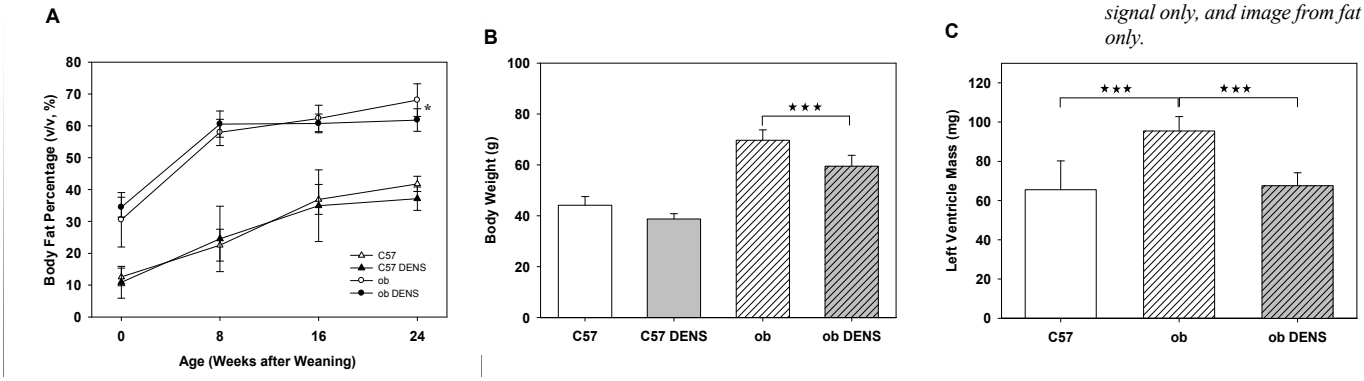

*Figure 2. Physiological changes with DENS treatment in mice. C57: C57Bl/6 mice group, C57 DENS: C57Bl/6 mice with DENS treatment group, ob: ob/ob mice group, ob DENS: ob/ob mice with DENS treatment group. (A) Repeated measurements of body fat percentages at four time points. (B) Body weights at 24 weeks after weaning. (C) Left ventricular mass at 24 weeks after weaning (***, $p < 0.001$).*

Discussion: This is the first study to show that DENS, a potent SSAT inducer, has beneficial effects in a mouse model of obesity and its cardiac complications. We showed that DENS treatment decreased body-fat volume and lowered elevated cardiac mass in obesity. Mature *ob/ob* mice typically have bodyweights about double those of age- and sex-matched C57 mice. They also develop cardiac complications, including hypertrophy, similar to those in obese humans. We showed here that DENS decreased body fat in both ob/ob and C57 mice, after 24 weeks' treatment. DENS treatment significantly lowered body-fat content in *ob/ob* but not C57 mice, perhaps because of the higher body-fat content in the former. This effect was manifest only at 24 weeks and not the two previous time points. DENS also prevented *ob/ob* mice from developing left ventricular hypertrophy and cardiac dysfunction. At 24 weeks, *ob/ob* controls mice had significantly higher LVM and lower CO, indicative of left ventricular hypertrophy and cardiac dysfunction. DENS-treated *ob/ob* mice had similar LVM and CO to that of C57 mice, which were significantly different from those of obese mice. In conclusion, the SSAT inducer DENS had significant beneficial effects in obesity and associated left ventricular hypertrophy and cardiac dysfunction. Therefore, SSAT might serve as a potential target for design and development of drugs to improve obesity and its cardiac complications.

Reference: 1) Jell et al., J Biol Chem. 282:8404-8413. 2007. 2) Pirinen et al., Mol Cell Biol. **27**:4953-4967. 2007. 3) Dixon, Radiology, 153: 189-94. 1984.

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