Supplementary Methods

Animals and study design:

Upon receipt of mice from Jackson Laboratory, they were separated into individual cages and fed the same HFD *ad-libitum* with free access to water for a five week acclimatization period. During this period, body weight (BW) was monitored on a weekly basis.

20% weight reduction was achieved by feeding mice 50% of their *ad-libitum* daily caloric intake until they reached 80% of initial value (defined as BW immediately before the start of CR phase). Subsequently, mice were switched to ~80% of their *ad libitum* daily food intake and the number of calories provided were adjusted (when % of initial BW of a mouse deviated from 80% by more than 1%, the daily amount of food intake was adjusted by 0.1g = 0.524 kcal) on a daily basis in order to weight stabilize each mouse at 80% ±1% of their original weight. WR mice were provided with food twice daily, 1/3 of the total daily calories in the morning (08:00-8:30h) and 2/3 in the evening (18:00-18:30h). BW was monitored daily or weekly in WR and AL groups, respectively. WR mice were determined to be weight stable at 80% of their initial weight when their BW did not deviate by more than ±1% of the value reported on the previous day (with no changes in food given) for 4 consecutive days at which point the value for their daily provision of HFD was fixed for the remaining of the CR phase.

The doses of the MC4R agonist in this study were chosen based on previous studies in DIO mice showing a ~10% BW loss with the drug *vs*. vehicle over a 2-w infusion period (AstraZeneca data on file).

During the course of the experiment, some of the mice developed skin lesions which were treated with topical antibiotics. Skin lesions were primarily found in the AL group of mice as a likely consequence of *ad libitum* access to the HFD. To reduce the progression of skin lesions and development of new ones, all mice were washed in warm water with a pet shampoo (Ferret Glow) twice during the drug treatment.

Mini-pump implantation

The mini-pumps (ALZET Model 2004) held 200 µL and release the solution at a nominal rate of 0.25 µl/hr over 28 days. Mini-pumps were filled with solution sterilely and soaked in PBS at 37°C for 40 hrs prior to surgery according to the manufacturer's recommendations. Animals were anesthetized with inhaled isoflurane (2-3.5%) in oxygen. Skin was shaved and wiped with betadine and alcohol pads, followed by a small incision (0.5-1 cm) in the interscapular region. The pump was inserted into a small pocket that was formed by spreading the subcutaneous connective tissue apart with a hemostat. The pump was positioned with the flow moderator facing caudally. Care was taken not to disrupt BAT tissue. The incision was closed with two wound clips followed with application of triple antibiotic to minimize the risk of infections. During the second and third mini-pump surgeries (weeks 17 and 21), the existing pumps were explanted and replaced with a fresh pump.

Serum and plasma collection

Blood was obtained by retro-orbital bleed following a 4.5-h fast at several time points. Blood from the first two bleeds was allowed to clot for 2 h at room temperature, centrifuged at 4°C for 20 min at 2,000 x g, and serum was collected and frozen at −80°C until time of assay. For the remaining three bleeds, plasma was collected on ice using heparinized capillary tubes (Fisherbrand) in order to protect the integrity of the MC4R agonist. A protease inhibitor (Protease inhibitor cocktail set 1, Calbiochem, USA) was added to the aliquots (1 part to10 parts plasma of the 50x solution) used for assaying the drug concentration. Plasma was obtained by centrifugation for 10 min at 1,500 x g at 4°C and frozen at −80°C until time of assay.

Indirect calorimetry

Measurements of oxygen (O_2) and carbon dioxide (CO_2) concentrations were taken every 26 minutes during a 72 hour period while mice had AL access to HFD and water. To diminish the effects of stress associated with exposure of mice to new environment data points collected during the last 48 hours were used to calculate the average total 24-hour energy expenditure (TEE; kcal/24hr) and respiratory exchange ratio (RER = VCO_2/VO_2) [3]. Resting energy expenditure (REE; kcal/24hr) was defined as the lowest one hour period of energy expenditure, coinciding with the lowest one hour of total ambulatory activity during the 48-hour period; this value was extrapolated to 24 hours. Non-resting energy expenditure (NREE; kcal/24hr) was calculated as the difference between TEE and REE over the 48 hour period.

Physical activity was measured by an infrared beam system integrated with the LabMaster system. Total activity (infrared beam breaks) in X, Y, and Z axis was recorded every 26 minutes. The system is designed to differentiate between fine motor movement (a single X or Y axis beam break), ambulatory movement (simultaneous breaking of two adjacent X or Y beams), and rearing (Z axis beam break).

Sacrifice

After a 5-6 hour fast on the day of sacrifice, mice were anesthetized with isoflurane, the last mini-pump was removed, and the body composition was measured immediately prior to sacrifice. Mice were sacrificed by carbon dioxide asphyxiation and had their blood collected by cardiac puncture.

Statistical analysis

Data are expressed as means ± SEM. Statistical analysis was performed using JMP and Statistica software. Where appropriate, two-way ANOVAs were performed using drug treatment and state as grouping variables. Student t-tests (2-tailed) were conducted to compare treatment effects between different groups. P alpha < 0.05 was taken as significant.