

# EFFECTS OF POST-WEANING ICV IL-6 ON FOOD INTAKE AND BODY WEIGHT

Based on the evidence of the ability of 3 weeks post-weaning exercise to permanently decrease body weight gain and adiposity in rats and the association between 3 weeks post-weaning exercise and an increase in plasma interleukin-6 (IL-6), our hypothesis was that early onset exercise would lower body weight due to an increase in IL-6. To test this hypothesis, we predicted that injecting IL-6 intracerebroventricularly (icv) during the 2nd–3rd week post-weaning in sedentary rats would mimic the effects of exercise in preventing the development of obesity in DIO rats fed a high energy diet. Conversely, we postulated that icv injections of anti-IL-6 antibody during the 2nd–3rd week post-weaning would prevent the exercise-induced inhibition of obesity in these rats. As a preliminary study, we injected a nonspecific antibody or saline into the lateral ventricle of juvenile DIO rats and compared the results to rats that were left undisturbed to ascertain that the antibody or the injection process itself did not alter body weight gain in a non-specific way. These preliminary studies were critical controls for the IL-6 and anti-IL-6 studies.

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## BACKGROUND

Obesity has become an increasing health problem in the industrialized world.<sup>1</sup> Many individuals are unable to maintain normal energy homeostasis. The brain regulates food intake and body weight and the hypothalamus is an important region that facilitates the sensing of metabolic and hormonal signals (leptin, glucose, fatty acids, IL-6, etc) sent throughout the body to regulate food intake and energy expenditure. Under normal homeostatic conditions, these signals are able to tightly regulate energy balance where intake = expenditure + storage. When intake exceeds expenditure, however, excess calories are stored, mainly as fat. Scientists in the laboratory of Barry Levin assess the role of neurons which can monitor and change their activity in response to these signals in the regulation of energy homeostasis. Utilizing rats selectively bred for their propensity to develop diet-induced obesity (DIO) when fed a 31% fat high energy (HE) diet,<sup>2</sup> one of the main goals of the lab is to examine manipulations of the post-natal environment and assess their ability to prevent obesity in these obesity prone rats. Studies by Christa Patterson showed that 3 weeks of post-weaning voluntary exercise prevents DIO rats fed a HE diet from becoming obese even after exercise cessation. It is possible that post-weaning exercise alters the predisposition of DIO rats to become obese since hypothalamic pathways that control energy homeostasis continue to develop after birth. Additionally, such exercise raises plasma

interleukin-6 (IL-6) levels and IL-6 is known to enter the brain where it can decrease food intake and increase energy expenditure.<sup>3</sup> IL-6 is generated by muscle and adipose tissue and increases in the plasma and brain following exercise. In the brain, IL-6 acts on similar pathways as leptin and can modulate food intake.<sup>4,5</sup> Leptin is a hormone that is released from white adipose tissue where it circulates in the plasma and enters the brain in proportion to overall body fat.<sup>6</sup> Leptin acts in the hypothalamus and other brain areas as a negative feedback signal which decreases food intake and increases energy expenditure.<sup>7,8</sup> Based on the ability of 3 weeks post-weaning exercise to permanently decrease body weight gain and adiposity and the association between 3 weeks post-weaning exercise and an increase in plasma IL-6, we hypothesized that early onset exercise lowers body weight due to an increase in IL-6 which permanently alters pathways involved in energy homeostasis.

## METHODS

For the preliminary and IL-6 studies, selectively bred DIO rats were weaned and individually housed with *ad libitum* access to 31% fat HE diet and water on a reverse 12 hour:12 hour light dark cycle (on at 7:00 pm and off at 7:00 am). Prior to 4 weeks of age, stereotaxic surgery was performed on all rats to implant a 28 gauge cannulae into the left lateral ventricle (LV). After recovery, 10 ng of angiotensin II in saline was administered to test for

correct cannula placement which was confirmed by greater than 2 mL of water intake over 1 hour. Rats with correct placement were randomly divided into 5 groups ( $n=8/\text{grp}$ ) for the IL-6 study and 3 groups for the preliminary study ( $n=4/\text{grp}$ ). The preliminary study group was divided into: 1) undisturbed-remained sedentary and uncannulated (UD); 2) remained sedentary and were injected with artificial cerebrospinal fluid (aCSF); and 3) remained sedentary and were injected icv with non-specific rabbit anti-goat IgG (AB). Additionally, the IL-6 study group was divided into: 1) remained sedentary and were injected icv with non-specific rabbit anti-goat IgG (Sed/IgG); 2) remained sedentary and were injected icv with aCSF (Sed/aCSF); 3) remained sedentary and injected icv with recombinant rat IL-6 reconstituted in aCSF (Sed/IL-6); 4) given voluntary access to a running wheel placed in their home cages for 3 weeks post-weaning and injected icv with aCSF (Ex/Sed/aCSF); and 5) given voluntary access to a running wheel for 3 weeks post-weaning and injected icv with rabbit anti-IL-6 diluted with aCSF (Ex/Sed/anti-IL-6). All injections were given daily during the 2nd–3rd week of the experiment. (2  $\mu\text{L}$ , 2 hours following the onset of darkness). During the treatment period for the preliminary study, body weight was measured weekly. Following treatment, rats were sacrificed and four visceral (epididymal, retroperitoneal, perirenal, mesenteric) and one subcutaneous (inguinal) fat pad were removed and weighed. In addition, trunk blood was obtained and centrifuged to acquire plasma for analysis of glucose by an Analox glucometer. Throughout the treatment period for the IL-6 study, food intake and body weight were measured daily prior to injection. Following treatment, food intake and body weight were monitored on a weekly basis for 10 additional

weeks. Groups were compared by 1-way and repeated measures ANOVA depending upon experimental design. When significant intergroup differences were found post-hoc analysis was performed by Bonferroni's assessment. All statistical analyses were performed using Systat software.

## RESULTS

### Preliminary Study

Treatment of DIO rats with icv aCSF or rabbit-anti goat IgG for 2 weeks had no overall effect on body weight gain [ $F(1,3)=0.32$ ,  $P=0.81$ ], adiposity ( $P=.47$ ), or plasma glucose levels ( $P=1.0$ ).

### IL-6 Study

Following the first week of the experiment (after first week of experiment, post-surgery, prior to injections) both groups of exercising rats (Ex3wk/SED and Anti-IL-6) had lower body weight gain compared to the SED IL-6 rats (IL-6 > anti-IL-6,  $P=0.03$ , IL-6 > Ex3wk,  $P=0.03$ ), whereas all of the other groups had equal body weight gain. Treatment of DIO Sed rats with IL-6 or anti-IL-6 begun at 5 weeks of age had no overall effect on body weight gain. All rats had statistically equal weight gain following the 2 week treatment period [ $F(2,8)=2.29$ ,  $P=0.09$ ].

## CONCLUSION

In conclusion, our preliminary studies served as critical controls for the IL-6 and anti-IL-6 study. We showed that icv injection of aCSF or nonspecific IgG had no effect on body weight gain, adiposity, or glucose levels. During the treatment period, neither IL-6 nor anti-IL-6 had any effect on weight gain. It is possible that these treatments may have more long-lasting effects on body weight

gain and adiposity and thus long-term monitoring of body weight gain and food intake are still being conducted in the Levin lab. Furthermore, tail blood will be taken from these rats every 2 weeks for plasma leptin analysis by radioimmunoassay. This will allow us to have an indication of their adiposity at various time points throughout the remainder of the experiment. Terminally fat pad mass will be obtained as an overall measure of their adiposity to determine if these treatments had any effect on long-term adiposity. If prior icv treatment has no long-term effect on body weight gain then it can be concluded that IL-6 is not the key factor in the long term effects of post-weaning exercise on lowering body weight gain and adiposity. This will mean that some other exercise-induced signal must be responsible for this effect and further investigation of this factor will be necessary.

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