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## Substance P as a novel anti-obesity target

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### Abstract

**Background and Aims:** Substance P (SP) is an 11-amino acid peptide that belongs to the tachykinin family of peptides. SP acts in the brain and in the periphery as a neuropeptide, neurotransmitter and hormone affecting diverse physiological pathways, mainly via its high affinity neurokinin-1 receptor (NK-1R). Its presence in the hypothalamus and other areas of the brain that regulate feeding as well as in the stomach and small intestine prompted us to investigate its role on appetite control and energy balance.

**Methods:** CJ 012,255 (CJ), a SP antagonist which binds to NK-1R, was injected in lean, diet induced (DIO), and genetically obese mice *ob/ob* and its effects on body weight, adiposity and insulin sensitivity were investigated.

**Results:** CJ administration prevented weight gain and accumulation of fat after two weeks of high fat feeding, while similar CJ treatment in obese mice (following two months of high fat diet) resulted in weight loss, reduction in adiposity and improvement of insulin sensitivity, in part due to inhibition of food intake. The effects of SP in the control of energy balance are, at least in part, leptin independent, since CJ treatment was also effective in leptin deficient mice. Peripheral SP administration resulted in a mild, dose-dependent increase in food intake, evident 3 hrs post-SP injection.

**Conclusions:** SP per se acts as an orexigenic neuropeptide and promotes weight gain in mice via NK-1R coupling. We speculate that NK-1R antagonists, already tested in clinical trials for various diseases, may represent a potential target against obesity.

### Introduction

Obesity-related pathology has surpassed tobacco use as a cause of death in the United States<sup>1</sup> and has become an alarming public health problem worldwide. According to World Health

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Organization, more than 1 billion adults are overweight (body mass index (BMI) > 25) and among them 300 million are truly obese (BMI>30)<sup>2, 3</sup>. The National Institutes of Health reported that approximately 50% of the US population is overweight or obese, and 15 million obese patients require surgical intervention<sup>4</sup>, with similar percentages reported in Europe<sup>5</sup> and with numbers increasing during the last half decade, especially among children adolescents and men<sup>6</sup>. Alarmingly, this epidemic now affects about 25% of children in the developed world. Obesity represents a major risk factor for the development of non-insulin dependent diabetes (type II) and its complications such as the metabolic syndrome, coronary heart disease and peripheral neuropathy<sup>7</sup>. Obesity develops as a result of the disruption of the homeostasis between food intake and energy expenditure, and therefore factors affecting these processes are the focus of extensive research targeting the development of effective anti-obesity drugs, thus far, with only limited success<sup>8, 9</sup>.

Substance P (SP), an eleven amino acid peptide member of the tachykinin peptide family<sup>10</sup> is expressed in the central nervous system as well as in peripheral tissues, including the gastrointestinal and respiratory tracts, the urinary and immune systems, blood vessels and skin<sup>11-14</sup>. SP exerts its effects on neurogenic inflammation<sup>15, 16</sup>, intestinal motility<sup>17</sup>, mucosal permeability<sup>18</sup>, and epithelial ion transport and proliferation<sup>19, 20</sup> via its high affinity neurokinin-1 receptor (NK-1R). NK-1R is present in several cell types including neurons, epithelial cells and various types of immune cells<sup>21-23</sup> and its expression is up-regulated in numerous inflammatory conditions<sup>21, 24, 25</sup>. However, there is no evidence that SP is involved in the regulation of food intake and energy homeostasis.

SP binds to three G-protein coupled receptors, neurokinin (NK) 1,2, and 3, with NK-1R being the receptor with the highest affinity<sup>26</sup>. NK-1 mediates several physiological and pathophysiological responses<sup>27</sup> and pharmacological antagonists of NK-1R have been used so far for treating diverse conditions, such as mood disorders (depression, anxiety and stress), nausea associated with chemotherapy, rheumatoid arthritis, and inflammatory bowel disease<sup>28, 29</sup>. In the intestine SP produced by several cell types may circulate in the blood as a hormone or act locally in a paracrine fashion<sup>14</sup>. Recently, accumulating evidence points to an important role of gut derived peptides in obesity, by means of conveying meal-related signals to appetite regulating centers, mainly in the hypothalamus, and the nucleus tractus solitarius, and/or by directly influencing insulin production or sensitivity<sup>4</sup>.

CJ 012,255 (CJ, Pfizer) is a selective NK-1R antagonist<sup>30</sup> that has been used to reduce ileal pouch inflammation<sup>31</sup> and postoperative peritoneal adhesions formation in rats<sup>32</sup>. We recently reported that human preadipocytes bear functional NK-1R that signal to proinflammatory pathways<sup>33</sup>. This observation along with the notion that obesity represents a low-grade chronic sub-acute inflammation in the adipose tissue<sup>34</sup>, prompted us to evaluate the role of CJ 012,255 treatment in the development of obesity. The fact that SP is abundant in the stomach, duodenum, and jejunum, all important areas for digestion and nutrient uptake, as well as in hypothalamic areas implicated in feeding behavior, such as the arcuate and ventromedial nuclei, along with the presence of NK-1R in the hypothalamus<sup>35</sup> and adipose tissue<sup>33</sup>, led us also to investigate a potential role for SP in the regulation of energy balance. In the present study we describe the effect of SP administration and blockage of NK-1R in the feeding behavior and weight of the C57BL/6 models of diet induced obesity (DIO) and high fat diet (HF). We report that SP has an orexigenic effect in mice and that administration of the NK-1R pharmacologic inhibitor CJ 012,255 counteracts the increase in feeding, ameliorates the weight gain induced by feeding with high fat/high caloric content diets, and improves their ability to remove glucose from the blood and respond to insulin.

## Materials and Methods

### Animals and treatments

*High fat (HF) model.* Twelve-week-old male C57BL6 mice (Taconic) were housed individually and fed a high fat (HF) diet (45kcal % fat, 4.73 kcal/gm, Research Diets #D12451) for 5 days prior to CJ 12,255 (Pfizer) administration for acclimatization and during the study. Mice (two separate experiments, n=10 mice per group per cohort) received daily intraperitoneal (i.p.) injections of 300 µg CJ 12,255 in 200 µl of saline or saline alone for a week, 1 hr prior to initiation of the dark cycle. Mouse body weight and food intake was monitored daily at the time of injection. To investigate whether the CJ 12,255-mediated effects on body weight were attributed to caloric intake or increases in energy expenditure, we included a third group of mice (pair-fed) who had access to restricted food equal in grams to the food consumed by CJ 12,255-treated mice during the previous day. *Diet-induced obesity (DIO) model.* Eight-week-old male C57BL6 mice were placed in single cages and fed a HF diet for 12 weeks. Mice were then divided into the following groups (two separate experiments, n=8 mice per group) matched by weight: Group C (initial weight  $42.3 \pm 1.0$  grams): mice receiving saline injections. Group J (initial weight  $40.8 \pm 1.2$  grams): mice receiving a low dose of CJ 12,255 (150µg/mouse). Group JJ (initial weight  $40.8 \pm 1.0$  grams): mice receiving a high dose of CJ 12,255 (300µg/mouse). Group PF (initial weight  $40.8 \pm 0.8$  grams): mice pair-fed to those receiving the high dose of CJ 12,255. Mice were treated for a total of 18 days and their food consumption and weight were monitored daily 2 hrs prior to the dark cycle. *Leptin deficient (ob/ob) mice.* *Ob/ob* mice (two separate experiments, n=8 per group per cohort) (Jackson Laboratories), were housed separately and treated with 300 µg CJ 12,255 for 7 days followed by 600 µg CJ 12,255 for 10 additional days. Their food intake and body weight were monitored daily 2 hours prior to their dark cycle. The control group received mock i.p. injections of 200 µl saline.

### SP treatment

*Acute effect.* Two different groups of male C57BL6 mice (n=8 per group) were injected i.p. either with a low (36µg/mouse) or a high (72µg/mouse) dose of SP, one hour (1 hr) before the start of the dark cycle (0 h). Mice were monitored for food intake after 1, 2, 3, 14 and 22 hrs, and compared to saline-injected controls. *Chronic effect.* Male C57BL/6 mice (n=8 per group) were injected with 72 µg/mouse of SP or saline daily, 1 hr before the start of their dark cycle and their body weight and food intake monitored every 24 hrs.

### Glucose and Insulin tolerance tests (GTT and ITT)

Mice were fasted overnight (1700–0800) and tested for glucose and insulin tolerance at the initial and final days of each experiment. Dextrose and insulin (Humulin, Lilly) were injected i.p. (1 gram/kg and 2 Units/kg, respectively) and serum glucose was measured at 0, 15, 30, 120 and 240 min via tail blood collection. Glucose levels were measured using standard glucose test strips with an ACCU-CHECK Advantage meter (Roche, Nutley, NJ). The groups were selected before CJ 012,255 administration and matched for weight.

### Real time RT-PCR

RNA was isolated from tissues kept at  $-80^{\circ}\text{C}$  using the RNeasy kit followed by DNaseQ treatment (Qiagen). Fifty ng of RNA were subjected to RT-PCR amplification using TaqMan one step predeveloped assays and in a GeneAmp 5700 sequence detection system (Applied Biosystems). Results were normalized by TBP expression and expressed as arbitrary mRNA units.

## Dual-energy X-ray absorptiometry (DEXA)

Mice were scanned, using the Lunar PIXImus2 mouse densitometer (GE Medical Systems, Madison, WI) and total body fat and lean body mass were determined using the analysis program as described by the manufacturer.

## Serum analysis

Serum insulin and leptin were measured by EIA (Linco) and serum alanine aminotransferase by Ani Lytics (Gaithersburg, MD).

## Statistical Analysis

Results were analyzed by protected ANOVA factorial with Dunn/Bonferroni correction, ANOVA repeated measures and student's t-test using Statview (SAS) and expressed as mean  $\pm$ SE.

## Results

### Effects of the NK-1R antagonist CJ on High Fat (HF) diet- induced weight gain in lean mice

The drug was well tolerated with no apparent toxicity as evaluated by serum alanineaminotransferase and TNF $\alpha$  levels and gross pathology of all organs, except for a mild tissue reaction at the site of injection. However, CJ-treated mice showed an immediate hyperkinetic response that lasted 30 sec after the injection with no other apparent unusual behaviour relative to the controls. In contrast to vehicle-treated mice that gained on average 7% of their initial body weight, CJ-treated mice lost 4% of their weight within a week of treatment (Figure 1A,  $p < 0.001$  for weight gain between control and CJ-treated mice by ANOVA). Upon discontinuation of treatment, weight gain (Figure 3A) and food intake (Figure 3B) returned gradually to pre-treatment levels, indicating that CJ does not induce a persistent state of wasting. Weight loss was due to a reduction in fat, but not in lean mass, as assessed by epididymal fat pad weight (Figure 1B,  $1.6 \pm SE 0.1$  g in control mice vs  $1.2 \pm SE 0.1$  g in CJ-treated mice and  $1.3 \pm 0.1$  g in the PF mice,  $p < 0.05$ , ANOVA), and DEXA analysis (Figure 4). Weight loss was also associated with 50% lower serum leptin levels in these mice (Figure 1C,  $20.7 \pm SE 1.5$  vs  $47.1 \pm SE 2.5$  vs  $28.3 \pm SE 1.5$  ng/ml for CJ and vehicle-treated and pair-fed mice, respectively,  $p < 0.01$ , ANOVA). Interestingly, pair-fed mice had 25% higher leptin levels compared to CJ-treated mice ( $p < 0.05$ ), which may suggest a potential direct SP effect in the adipose tissue<sup>33</sup>.

The similar weight loss among CJ-treated and pair-fed mice suggested that CJ might affect appetite. Indeed, CJ administration resulted in 25% reduction in cumulative food intake in the high fat fed mice (Figure 1D,  $18.8 \pm SE 0.7$  g and  $13.4 \pm SE 0.3$  g for vehicle and CJ-treated mice respectively,  $p < 0.0001$ , paired T-test). Consistent with reduced food intake and lower adiposity, CJ-treated mice had also lower serum insulin levels (Fig. 1E,  $1.4 \pm SE 0.1$  vs  $1.1 \pm SE 0.1$  vs  $3.7 \pm SE 0.6$  ng/ml respectively,  $p < 0.001$ , ANOVA), and improved responses to glucose (Figure 2A) and insulin tolerance tests (Figure 2B), suggesting increased insulin sensitivity compared to control mice.

### Effects of Substance P on orexigenesis and weight gain

CJ treatment prior to the start of the dark cycle, when mice consume the majority of their food, resulted in reduction of food intake evident as early as 1 hr post injection (Figure 5A,  $2.3 \pm SE 0.2$  vs  $3.4 \pm SE 0.1$  g, for vehicle and CJ-treated mice, respectively,  $p < 0.0001$ , paired T-test). Likewise, during refeeding after an overnight fast, CJ-treated mice consumed 50% less food compared to vehicle-treated control mice (Figure 5E). Similar CJ-mediated anorectic effects were also evident when mice fed standard chow (data not shown). These results suggest an

orexigenic effect of peripheral SP. We next treated control mice with two different doses of SP (36 or 72  $\mu\text{g}/\text{mouse}$ ) i.p. and found that peripheral SP administration had a mild, dose-dependent increase in food intake (Fig. 5B,  $1.8 \pm \text{SE } 0.2$ ,  $2.1 \pm \text{SE } 0.2$  and  $2.4 \pm \text{SE } 0.2$  g for vehicle, SP-low and SP-high-treated mice, respectively,  $p < 0.05$ , ANOVA), evident 3 hrs post-injection. It has been reported that in rats, intravenous administration of SP induced acute (5–30 min) hypoinsulinemia, hyperglucagonemia and hyperglycemia<sup>36</sup>. We injected i.p. random-fed mice with SP (72  $\mu\text{g}/\text{mouse}$ ), in the absence of food, and found no differences in blood glucose levels up to 4 hrs post-treatment (Figure 5C).

Food intake is under the control of several hypothalamic neuropeptides, the orexigenic neuropeptide-tyrosine (NPY) and the anorexigenic pro-opiomelanocortin (POMC) among them<sup>4</sup>. We thus investigated whether SP administration had an effect on the hypothalamic expression of appetite regulating neuropeptides. Mice ( $n=16/\text{group}$ ) were injected i.p with SP (100  $\mu\text{g}/\text{mouse}$ ) immediately after the end of their feeding cycle and food was removed from their cages. After 3 hrs, the hypothalami were extracted and RNA was analyzed by real-time RT-PCR. We found a small increase in NPY mRNA expression and reduced POMC expression in two separate cohorts of mice (Figure 5D). Such a finding might be compatible with the food intake pattern in mice treated acutely with SP (Figure 5B).

### Effects of NK-1R antagonism on food intake and body weight in leptin deficient *ob/ob* mice

We next investigated whether the effects of CJ in food intake and body weight were leptin-mediated, using leptin deficient *ob/ob* mice. During a 10 day period, body weight was increased by 4% in vehicle-treated mice and decreased by 8% with CJ treatment (Figure 6A,  $-5.2 \pm \text{SE } 1.2$  vs  $3.1 \pm \text{SE } 0.5$  g change for CJ and vehicle-treated mice, respectively,  $p < 0.01$ , paired T-test). The effect of CJ on body weight reduction was dose-dependent and associated with 30% less adiposity (Figure 6B,  $2.1 \pm 0.2$  vs  $1.4 \pm 0.1$  g of epididymal fat in vehicle and CJ-treated mice, respectively,  $p < 0.05$ , ANOVA). Consistent with our results with the high fat fed lean mice (Figure 1D), CJ-treated *ob/ob* mice had reduced appetite as measured by their food consumption (Fig. 6C,  $63.9 \pm \text{SE } 1.3$  vs  $44.6 \pm \text{SE } 2.3$  g for vehicle and CJ-treated mice, respectively,  $p < 0.0001$ , ANOVA), which could explain, at least in part, the weight loss evident in these mice (Figure 6A). We also examined whether hypothalamic SP expression is under the control of leptin, as was observed with the appetite-controlling peptide NPY<sup>4</sup>. We found that fasting, which results in a significant drop of leptin levels, did not affect hypothalamic SP mRNA expression, while it increased NPY mRNA expression in the same animals (Figure 6D).

### Effects of CJ treatment on body weight and adiposity in Diet-Induced Obese (DIO) mice

Having established that NK-1R receptor antagonism prevents weight gain in lean mice on a high fat diet, we next examined whether CJ treatment could stimulate weight loss in mice with diet-induced obesity (DIO). We found that at the end of the treatment period mice treated with the higher dose of CJ lost 14.5% ( $p < 0.0001$ , ANOVA) and pair-fed mice lost 11.1% of their initial body weight (Figure 7A,  $42.2 \pm \text{SE } 1.0$  vs  $39.7 \pm \text{SE } 1.5$  vs  $35.0 \pm \text{SE } 1.9$  vs  $36.3 \pm \text{SE } 1.0$  g in C, J, JJ and PF, respectively). In contrast, control mice slightly increased their body weight (Figure 7A). Interestingly, CJ-mediated weight loss was also reflected in a 30% ( $p < 0.01$ , ANOVA) reduction in epididymal fat mass, whereas pair-fed mice did not lose any significant fat mass (Figure 7B,  $1.5 \pm \text{SE } 0.1$  vs  $1.3 \pm \text{SE } 0.1$  vs  $1.0 \pm \text{SE } 0.1$  vs  $1.5 \pm \text{SE } 0.1$  g in C, J, JJ and PF, respectively). Consistent with reduced adiposity, mice that received CJ had 50% lower serum leptin levels, in contrast to pair-fed mice (Figure 7C,  $8.6 \pm \text{SE } 1.8$  vs  $4.8 \pm \text{SE } 0.7$  vs  $4.2 \pm \text{SE } 1.2$  vs  $4.9 \pm \text{SE } 1.4$  ng/ml for C, J, JJ and PF respectively,  $p < 0.01$ , ANOVA). Such findings suggest that SP has direct effects on adipocytes. Indeed, our previous study demonstrated the presence of functional NK-1R in mouse adipose tissue and in human isolated preadipocytes<sup>33</sup>. Moreover, treatment of human preadipocytes with SP resulted in significant

upregulation of IL-8 expression, a potent proinflammatory cytokine that has been implicated in obesity and its complications. Our results also show that food consumption in CJ-treated obese mice was reduced by 30% (Figure 7D,  $36.1 \pm \text{SE } 2.7 \text{ g}$  vs  $48.9 \pm \text{SE } 2.0 \text{ g}$ , for CJ and vehicle-treated mice, respectively,  $p < 0.001$ , ANOVA), while random serum insulin levels were also substantially reduced (Figure 7E,  $41.3 \pm \text{SE } 0.8$  vs  $31.6 \pm \text{SE } 2.6$  vs  $19 \pm \text{SE } 4.4$  vs  $37.2 \pm \text{SE } 3.3 \text{ ng/ml}$  for C, J, JJ and PF respectively,  $p < 0.01$ , ANOVA). In glucose tolerance tests performed in the same mice just before and at the end of CJ treatments, mice receiving CJ had improved blood glucose clearance (Figure 7F). Taken together, these findings indicate increased insulin sensitivity in obese mice as a result of CJ administration.

### Effects of CJ on food and water intake

To exclude the possibility that the CJ effects on food intake and weight loss are due to illness or malaise, we examined whether CJ administration was associated with a taste aversion response. Our results show that while LiCl administration resulted in a significant decrease (Supplemental Figure 1,  $p < 0.0001$ ) in saccharine preference, even a high dose of CJ administration did not have any significant effect. Thus it does not appear that CJ reduces food intake as a result of malaise or illness. Furthermore, we have observed that CJ administration reduced acutely, water intake, like food intake, during the hours of activity by approximately 40%, when compared to saline-injected controls (data not shown,  $p < 0.0001$ ).

### Discussion

Very few cases of human obesity are caused by genetic factors<sup>37</sup> leaving the high in fat and calories western type of diet as the most important contributor to the development of obesity and type II diabetes<sup>2, 3</sup>. Here we show prevention of weight gain in lean mice fed a western type diet and induction of weight loss and improvement of insulin sensitivity in obese and lean mice treated with an NK-1R antagonist. Moreover, we present evidence indicating that NK-1R antagonist-mediated weight loss is at least in part due to decreased appetite. Further analysis of high fat diet fed or fasted mice treated with either CJ or SP did not reveal any differences in their activity or oxygen consumption as evaluated by a Comprehensive Laboratory Animal Monitoring System (CLAMS; Columbus Instruments, Columbus, OH) (data not shown). Although the mechanism(s) by which CJ mediates weight loss remains to be elucidated, the effects of CJ treatment on food intake and weight gain are, at least in part, leptin independent, since CJ was also effective in leptin deficient mice. Furthermore, fasting did not alter hypothalamic SP expression, in contrast to other appetite regulated hypothalamic neuropeptides.

Our results using an NK-1R antagonist indicate a role for SP in promoting appetite and weight gain. However, a previous study in rats described reduction in food intake 1hr after i.p. SP injection ( $150\text{-}250 \mu\text{g}$ )<sup>38</sup>. In another report, intracerebroventricular SP injection ( $20 \mu\text{g}$ ) in fasted and water deprived rats resulted in more than 90% decrease in food intake during refeeding<sup>39</sup>. Different animal species and experimental approaches may account for the discrepant results between our study and these previous studies<sup>38, 39</sup>. Furthermore, CJ-induced effects on weight gain as well as on obesity related pathologies may be due to the antagonist's potential signaling capabilities. Such is the case for other neuropeptide receptor antagonists such as Rimonabant, an antagonist of cannabinoid receptor type 1 (CB1)<sup>40</sup>.

It is also possible that the effects of SP and SP receptor antagonism described here are the result of an interaction(s) with other neuropeptides known to co-localize with SP<sup>41</sup>. Additional studies are required to identify the functional importance of these putative interactions in the effects of SP antagonism described in our study.

Previous studies have shown that NK-1R antagonism is ineffective on locomotive behavior when injected during the dark cycle in hamsters<sup>42</sup>. Based on this evidence, and since in our experiments mice were injected with CJ just before the initiation of the dark cycle, we do not expect that the NK-1R antagonist had any effects on locomotive behavior. It is also likely that the magnitude of SP effects of appetite described in Figure 5B may represent an underestimate due to the effects of the former in reducing locomotion<sup>43, 44</sup>.

Currently, there is no information as to whether the NK-1R used in our study crosses the blood-brain barrier, making it difficult to hypothesize the site of action of this antagonist. However, our results from the acute feeding studies in conjunction with the presence and proinflammatory effects of NK-1R in adipocytes<sup>39</sup>, suggest that the effects of CJ may be directed at both the central and/or peripheral levels.

The effects of CJ administration in total epididymal fat pad weight and total body weight may be due to alterations in the lipolytic potential of fat cells. Indeed, it has been reported that NPY (an orexigenic neuropeptide) decreases the capacity of 3T3-L1 cells to secrete free fatty acids, and additionally inhibits  $\alpha$ -MSH (appetite-reducing neuropeptide)-induced lipolysis in these cells<sup>45</sup>. Such a suggestion is also supported by our findings showing that mice in the pair-fed group, in contrast to CJ-injected mice, had similar epididymal fat pad weights as vehicle-injected animals (Fig. 7B). Thus, there is potentially a direct physiological SP effect on fat cells, which, as suggested by our results, is mediated by NK-1R. Consistent with this hypothesis, we have recently shown that functional NK-1R are expressed on the surface of human preadipocytes<sup>33</sup>.

Importantly, we found that the beneficial effects of NK-1R antagonism on body weight following high fat diet also lead to marked improvements in blood glucose levels, even before challenge (Figure 7F, right panel, 0 min), accompanied by reduced serum leptin and insulin levels (Figures 7C and 7E, respectively). Interestingly, starting serum glucose levels were almost identical for the CJ-treated mice (compared to the elevated serum glucose observed in vehicle-treated mice at the end of the experiment), both before and after treatment (Figure 7F). Thus NK-1R blockade might affect the development of type-2 diabetes<sup>2</sup>. Furthermore, the short time between CJ administration (within 15 days) and the improvements in serum glucose levels as well as its removal from the blood, may be an indication for involvement of SP either in a direct or indirect manner, given its pro-inflammatory capacity<sup>25</sup> in fat cell signaling pathways that regulate responses to insulin.

Taken together, the current findings reveal a previously unrecognized role for SP in appetite regulation and metabolism, in addition to the already established effects of this peptide in gastric motility and digestion<sup>46</sup>. The SP paradigm further supports the emerging concept of the gastrointestinal tract as an endocrine organ with important sensing and signaling roles towards total body energy homeostasis<sup>4</sup>. Most importantly, the effects of NK-1R blockade on appetite, body weight and adiposity point to a novel approach for treating obesity and insulin resistance. NK-1 or combined NK-1 and NK-3 receptor antagonists have already been tested in clinical trials for anxiety and depression, urinary incontinence, irritable bowel syndrome, and nausea associated with chemotherapy<sup>47</sup>.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Abbreviations

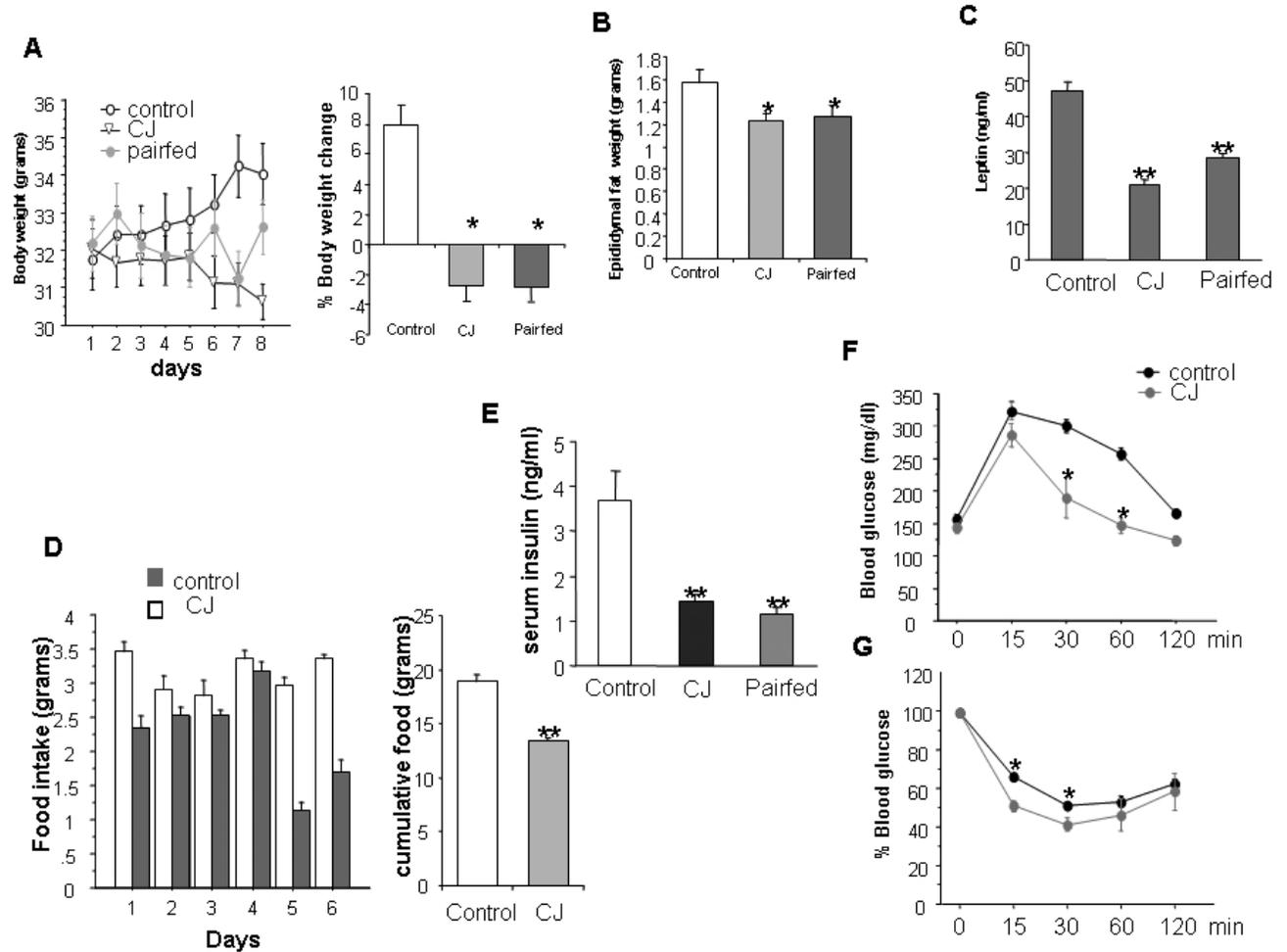
SP, substance P; NK-1R, neurokinin-1 receptor; DIO, diet induced obesity; GTT, glucose tolerance test; ITT, insulin tolerance test; TNF $\alpha$ , tumor necrosis factor  $\alpha$ ; NPY, neuropeptide-tyrosine; POMC, pro-opiomelanocortin; DEXA, Dual Energy X-ray Absortometry.

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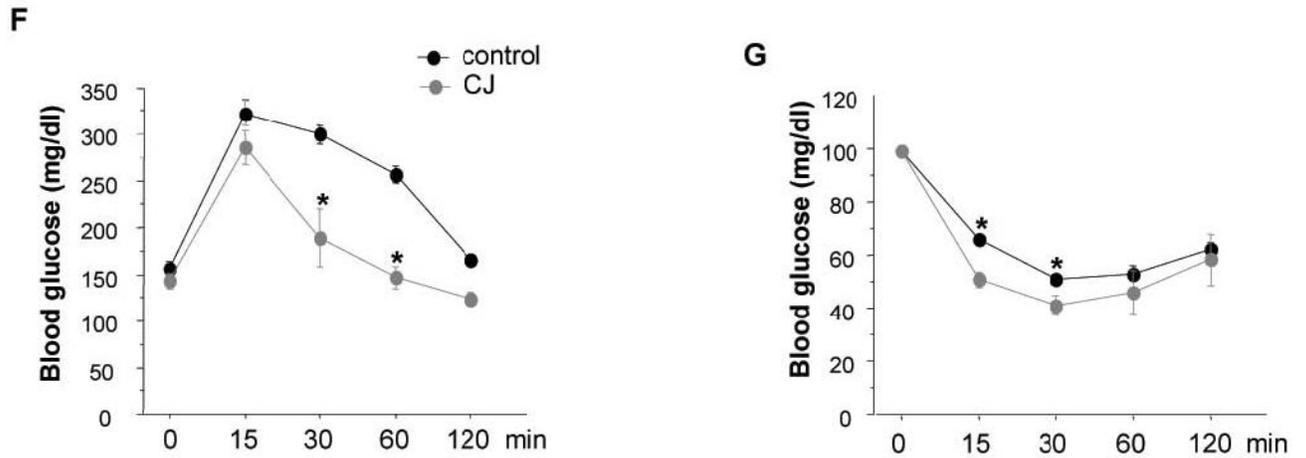
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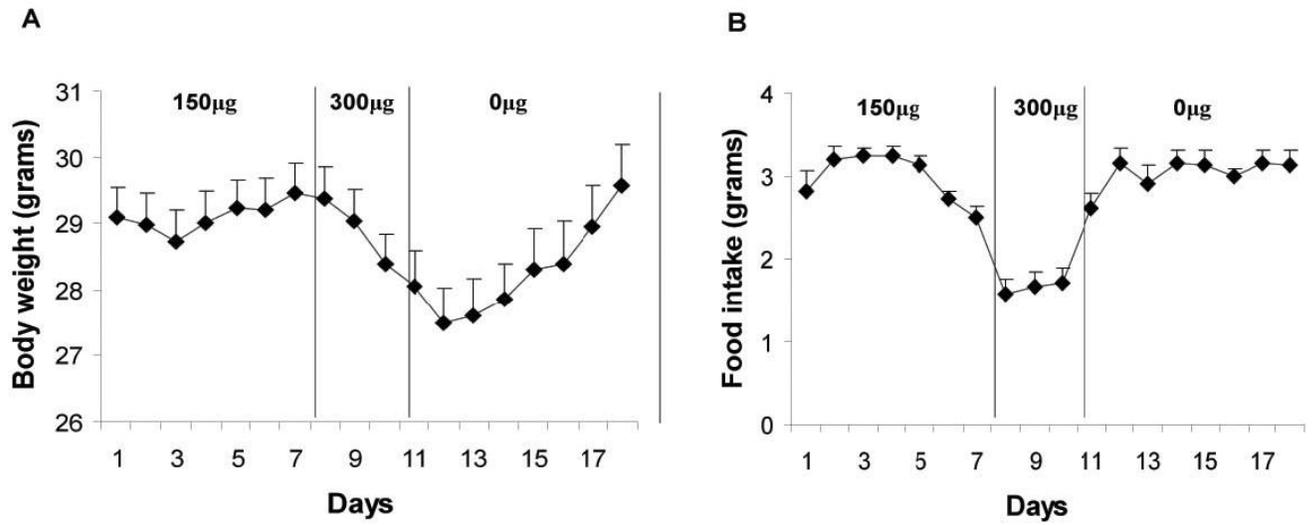


**Figure 1.** CJ, a substance P, neurokinin-1 receptor antagonist, reduces appetite, prevents weight gain, and improves insulin sensitivity in lean mice. (A) CJ (300  $\mu$ g)-treated mice gained significantly less weight on a high fat diet compared to control mice, (B) had reduced adiposity as evaluated by epididymal fat weight, and (C) reduced serum leptin levels. (D) Weight loss in CJ treated mice was associated with a significant reduction in food intake. (E) Consistent with lower adiposity and reduced food intake, CJ-treated mice had significantly lower serum insulin levels.



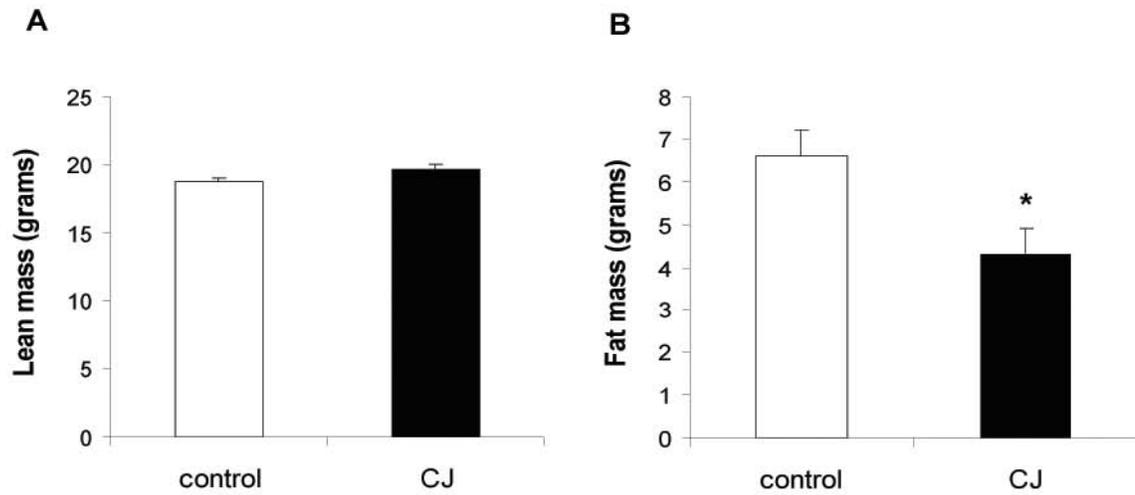
**Figure 2.**

CJ-treated mice showed significantly improved insulin sensitivity when (F) glucose and (G) insulin tolerance tests were performed. Results are the mean  $\pm$  SEM of 10 mice per group and are representative of two separate experiments. \*  $p < 0.05$ , and \*\*  $p < 0.01$  versus control treatment.



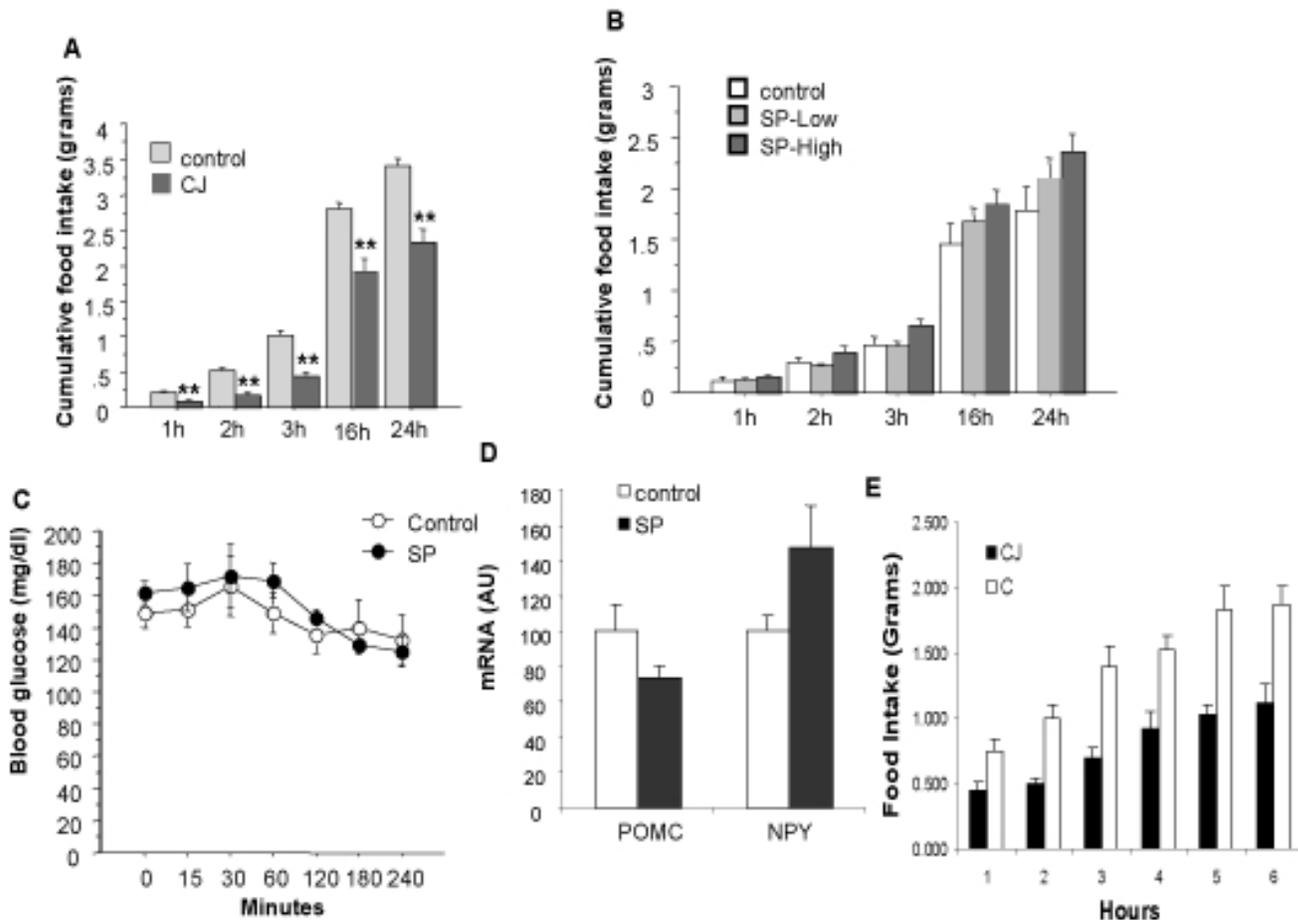
**Figure 3.**

CJ treated mice rapidly regain weight after treatment termination. (A) C57BL6 male mice (n=8/group) fed a high fat diet were injected daily ip with CJ according to the following scheme: 150µg of CJ for 7 days followed by 300µg for the next three days and 0µg for up to day 17. Body weight returned to normal values after discontinuation of treatment. (B) Similar results were obtained for food intake.



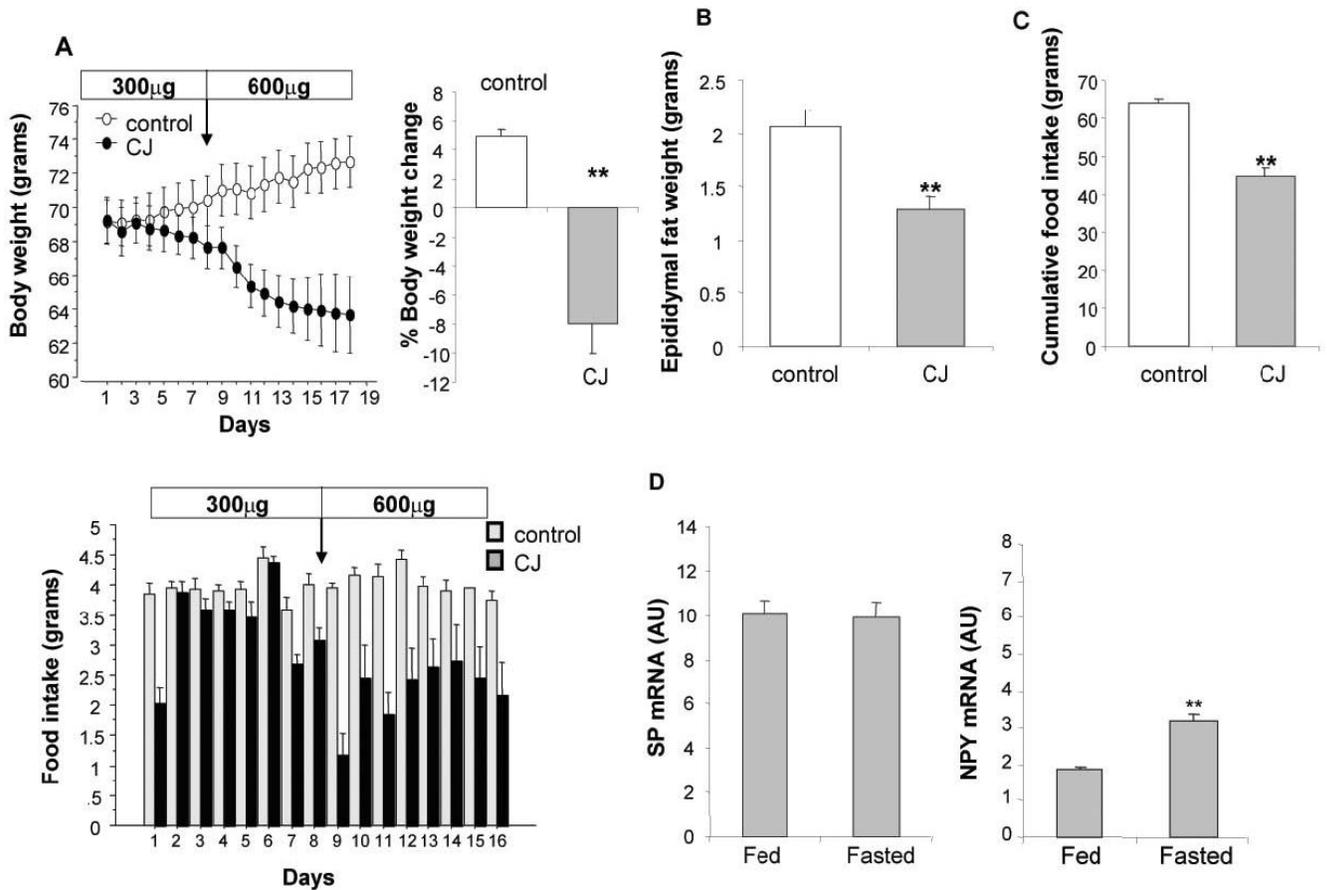
**Figure 4.**

Weight loss in CJ treated mice is not due to reduction of lean mass. (A) No changes in lean body mass were observed when C57BL6 male mice were fed a high fat diet and then treated with CJ (300  $\mu$ g) daily for 5 days. Body composition was evaluated by Dual Energy X-ray Absortometry (DEXA; Lunan PIXImus2, GE Medical Sciences). A) Lean mass of CJ and vehicle treated mice. (B) Fat mass decreased significantly after CJ treatment in the same mice (\* $p < 0.05$ )



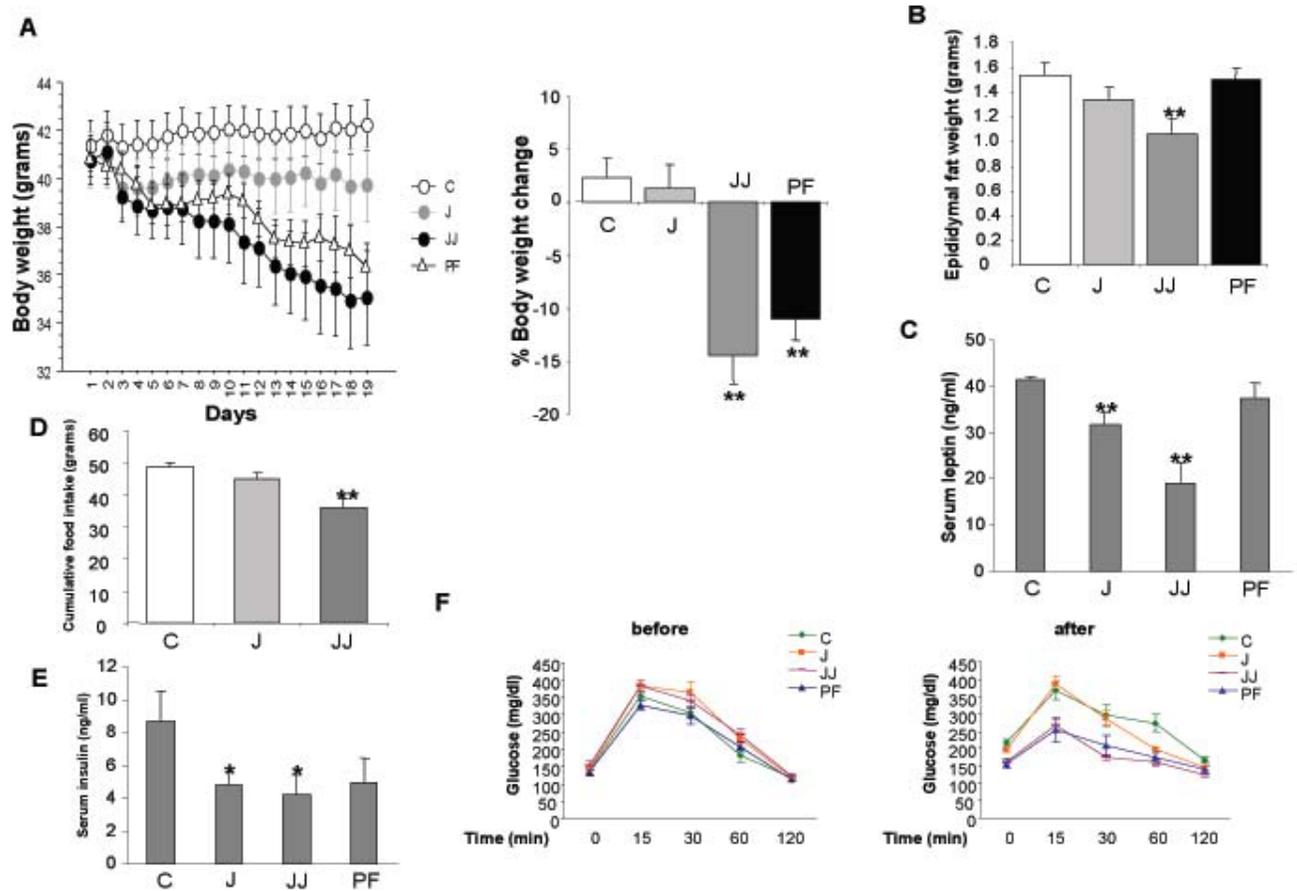
**Figure 5.**

CJ reduces while SP induces feeding after i.p. administration in C57BL/6 mice. (A and B) In an acute feeding paradigm, male C57BL6 mice were treated i.p. with CJ (300 µg) or with a low (36 µg) or a high (72 µg) dose of SP prior to the start of their feeding cycle (dark cycle) and food intake was monitored at intervals up to 24 hrs. As expected, CJ reduced food intake acutely, whereas SP dose-dependently induced feeding. (C) Intraperitoneal SP injection does not affect blood glucose levels in C57BL6 male mice (n=8/group) that were injected i.p. with SP (100 µg) at the start of their light cycle and food was removed from the cages. Glucose levels in tail vein blood were measured at various time points up to 4 hrs using a glucometer. (D) No effect was observed when mice were treated with SP (100µg) for 3 hrs at the start of the light cycle in the absence of food and mRNA expression of the appetite regulating neuropeptides POMC and NPY was measured by real-time RT-PCR. (E) CJ treatment inhibited refeeding in C57BL6 male mice (n=8/group) fasted overnight and treated ip with CJ (300 µg) 30min prior to refeeding. Food intake was monitored hourly for 6hrs. Results are mean ± SEM of 8 mice per group and are representative of two separate experiments. \*  $p < 0.05$ , and \*\*  $p < 0.01$



**Figure 6.**

CJ treatment reduces food intake and weight gain in leptin deficient mice. (A) There was a significant reduction in the weight of leptin deficient *ob/ob* mice treated with 300  $\mu$ g CJ for 7 days followed by 600  $\mu$ g CJ for 10 additional days. (B) The weight loss was associated with smaller epididymal fat pads, and thus reduced adiposity in the same animals. (C) Consistent with our previous observation mice with reduced weight and adiposity (A and B) exhibited decreased food intake. (D) Fasting does not affect hypothalamic SP mRNA expression. Male C57BL6 mice (n=6/group) were fasted overnight (16hrs) and hypothalamic mRNA expression of substance P and NPY were measured by real time RT-PCR. Results are the mean  $\pm$  SEM of 8 mice per group and are representative of two separate experiments. \*  $p < 0.05$ , and \*\*  $p < 0.01$  versus control treatment.



**Figure 7.** CJ treatment results in weight loss and increased energy expenditure in obese (DIO) mice. (A) Eight weeks old male C57BL6 mice were placed on a high fat/hypercaloric diet for 12 weeks and then treated i.p. with 150 µg (J) or 300 µg (JJ) of CJ or saline ( C: control and PF: pair-fed) for 18 days. CJ treatment resulted in a dose-dependent weight loss. (D) Weight loss after CJ treatment was associated with reduced food intake in the same mice. (B) Epididymal fat weight, a marker of total body adiposity, was also reduced in CJ-treated mice, even more than in pair-fed mice, indicating increased energy expenditure. (C and E) Consistent with lower adiposity, CJ-treated mice had significantly lower serum leptin (C) and insulin (E) levels. (F) Obese mice that received CJ also demonstrated improved insulin sensitivity as shown by more efficient glucose clearance in a glucose tolerance test. Results are the mean ± SEM of 8 mice per group and are representative of two separate experiments. \*  $p < 0.05$ , and \*\*  $p < 0.01$  versus control treatment.