

1 **CNS CONTROLS OF ADIPOSE TISSUE APOPTOSIS**

2 MARY ANNE DELLA-FERA\*, MARK W. HAMRICK§ AND CLIFTON A. BAILE\*,#

3 **SUMMARY**

4 Adipose tissue apoptosis is a novel approach that could be useful for treating both obesity  
5 and osteoporosis. Activation of adipocyte apoptosis via the CNS has been demonstrated,  
6 particularly following leptin treatment, but the neural pathways involved are only beginning  
7 to be defined. Although the sympathetic nervous system is the most likely transduction  
8 pathway from brain to adipocytes, either in fat pads or in bone marrow, the involvement of  
9 an intermediary humoral factor acting directly on adipocytes has not yet been ruled out. It is  
10 also possible that adipocyte apoptosis is triggered as a result of alteration of blood supply.  
11 Remodeling of tissues is usually accompanied by local changes in angiogenesis, although it  
12 can be difficult to determine which is the initiating process. Although there are many  
13 technical issues involved in studying adipocyte apoptosis in animals, a better understanding  
14 of the biochemical and anatomical pathways involved could lead to development of  
15 treatments resulting in the controlled removal of adipocytes in fat depots and bone marrow,  
16 and possibly in a site-specific manner.

17  
18 **BACKGROUND**

19 Increased adipose tissue mass is a common denominator in both obesity and osteoporosis.  
20 Obesity is characterized by increased fat storage in subcutaneous and visceral adipose depots  
21 resulting from an imbalance between energy intake and energy expenditure, whereas  
22 osteoporosis is associated with increased adipocyte production in bone marrow and is not  
23 necessarily associated with increased overall adiposity. In obesity a reduction of body fat is  
24 accompanied by amelioration of the pathophysiological effects. There are currently no  
25 therapies that specifically reduce bone marrow adipocyte populations. However, bone  
26 formation decreases with increasing proportion of marrow adipocytes (Verma et al., 2002);  
27 thus, it is likely that reversal or prevention of bone marrow adiposity will improve bone  
28 quality.

29 In the United States, the prevalence of overweight among adults increased by 61% from 1991  
30 to 2000; currently, more than half of all adults are considered overweight and approximately  
31 20% are extremely overweight or obese (Flegal et al., 1998). Obesity is not just a cosmetic

---

Departments of Animal & Dairy Science\* and Foods & Nutrition# University of Georgia  
Athens, GA 30602

§Department of Cellular Biology & Anatomy, Medical College of Georgia, Augusta, GA  
30912

32 problem—there is much evidence indicating that higher levels of body fat are associated with  
33 an increased risk for the development of numerous adverse health consequences (Visscher  
34 and Seidell, 2001). There is also a tremendous economic burden associated with the recent  
35 rise in prevalence of obesity. The economic costs of obesity are estimated to be ~7% of total  
36 health care costs in the United States (Colditz, 1999). In addition, approximately 10% of the  
37 total costs of loss of productivity due to sick leave and work disability are attributable to  
38 obesity-related diseases (Narbro et al., 1996). However, the remedies provided by the \$100  
39 billion/year diet industry have failed in providing long-term maintenance of weight loss for  
40 obese or overweight people (Wadden, 1993).

41 Approximately 10 million people in the U.S. are estimated to have osteoporosis, a disease  
42 that results in over 1.5 million bone fractures a year. It is now known that the accumulation  
43 of adipocytes in bone marrow is a major factor contributing to age-related bone loss. Women  
44 with osteoporosis have higher numbers of marrow adipocytes than women with healthy bone  
45 (Justesen et al., 2001; Kajkenova et al., 1997; Meunier et al., 1971), and bone formation rate  
46 is inversely correlated with adipocyte number in bone tissue biopsies from both men and  
47 women (Verma et al., 2002). Recent in vivo and in vitro studies provide important insights  
48 into why marrow adipogenesis is associated with bone loss. First, mesenchymal stem cells  
49 within bone marrow can differentiate to form adipocytes or osteoblasts. Conditions favoring  
50 adipocyte differentiation will therefore have adverse effects on bone formation because  
51 precursor cells are directed towards the adipocyte lineage rather than the osteoblast lineage  
52 (Akune et al., 2004; Jilka, 2002). Second, adipocytes secrete osteoclastogenic cytokines such  
53 as IL-6 (Fried et al., 1998), and adipocytes can inhibit osteoblast activity in culture (Maurin  
54 et al., 2000). Finally, adipocyte development and hypertrophy can compress intraosseous  
55 capillaries, which decreases blood supply within bone (Laroche, 2002). Experimental studies  
56 have shown that treatments that reduce bone marrow adipocyte number are associated with  
57 increased bone formation, thus suggesting a new approach to the treatment of osteoporosis  
58 (Nuttall and Gimble, 2004).

59 Weight loss reduces risk factors for and improves symptoms of obesity-related conditions  
60 (National\_Heart\_Lung\_and\_Blood\_Institute, 1998). Treatments for obesity have  
61 traditionally focused on drugs or behavioral strategies that restrict food intake, although  
62 surgical options such as gastric reduction are options for those who are morbidly obese.  
63 Surgical adipose tissue removal by liposuction is increasingly being used both as a treatment  
64 for obesity and for cosmetic body sculpturing. Recent studies have shown that removal of  
65 even a relatively small percentage of adipose tissue can lead to significant improvements in  
66 levels of vascular inflammatory markers and in insulin resistance (D'Andrea et al., 2005;  
67 Giugliano et al., 2004). Other findings indicate that a high percentage of patients maintain  
68 postoperative weights at least one year after liposuction (Commons et al., 2001). The cost  
69 and increased morbidity and mortality associated with this procedure severely limit its use as  
70 a treatment for obesity; however, these studies do suggest that stimulation of the endogenous  
71 removal of adipose tissue by apoptosis could be a valuable option for obesity treatment.  
72

## 73 **MOLECULAR MECHANISMS OF APOPTOSIS**

74 Apoptosis is a physiological form of cell suicide that is executed in a precise manner without  
75 generating inflammation. Apoptosis is necessary to eliminate excess cells during

76 development and to remove damaged and potentially dangerous cells (Alberts, 2002;  
77 Hengartner, 2000; Kaufmann and Hengartner, 2001). Disorders of apoptosis can result in  
78 either runaway cellular proliferation, as occurs in cancer, or excessive loss of cells, which  
79 occurs in certain immunodeficiency and neurodegenerative disorders.  
80 Apoptosis is characterized by loss of cellular contact with the surrounding matrix,  
81 cytoplasmic contraction, chromatin condensation and DNA fragmentation. Other changes  
82 that occur include externalization of the phosphatidylserine component of the phospholipid  
83 bilayer and formation of apoptotic bodies that are removed through endocytosis by  
84 macrophages and other cells. Although a number of stimuli appear to trigger the process of  
85 apoptosis, there are two major signaling pathways: the death receptor pathway and the  
86 mitochondrial pathway (Figure 1) (Gupta, 2001; Mayer and Oberbauer, 2003). In both  
87 pathways a series of molecular and biochemical steps leads to the activation of cysteine  
88 proteases, or caspases. This results in subsequent cleavage of a number of nuclear and  
89 cytoplasmic substrates, including those responsible for the maintenance of nuclear integrity,  
90 cell cycle progression and DNA repair, resulting in cell death (Alberts, 2002; Hengartner,  
91 2000; Kaufmann and Hengartner, 2001).  
92 The death receptor pathway involves cell membrane receptors that have an extracellular  
93 recognition domain and a cytoplasmic sequence, the death domain. Ligands for these  
94 receptors belong to the tumor necrosis factor gene family. Binding of a ligand to the  
95 extracellular domain leads to formation of an intracellular complex consisting of the death  
96 domain, other intracellular molecules and procaspase 8. This aggregation leads to activation  
97 of procaspase 8 to caspase 8, which triggers a proteolytic cascade ultimately resulting in  
98 formation of enzymes that degrade chromosomal DNA.  
99 The mitochondrial pathway of apoptosis is usually activated by internal stimuli, stress  
100 molecules (reactive oxygen species, reactive nitrogen species), chemotherapeutic agents or  
101 UV radiation (Mayer and Oberbauer, 2003). Under normal conditions, mitochondria  
102 maintain an electrochemical gradient between the inner matrix and the cytoplasm.  
103 Mitochondria contain two compartments—the matrix, surrounded by the inner mitochondrial  
104 membrane (IMM) and the intermembrane space, surrounded by the outer mitochondrial  
105 membrane (OMM).  
106 The intermembrane space contains several apoptosis-inducing factors, including cytochrome  
107 c, procaspases and AIF (apoptosis-inducing factor). The apoptotic mechanism is initiated as  
108 a result of increased permeability of the outer and/or inner mitochondrial membranes, which  
109 is controlled by a variety of members of the anti-apoptotic Bcl-2 family and pro-apoptotic  
110 proteins, such as Bax. Increased permeability of the outer mitochondrial membrane results in  
111 release of cytochrome C, which triggers the cascade of caspase activation (Page et al., 2004).  
112 The final stages of apoptosis are the same as those initiated by the death receptor pathway.  
113

## 114 **ADIPOCYTE APOPTOSIS**

115 It was once believed that the total number of adipocytes remained constant over one's  
116 lifetime; however, studies over the last 10 years have shown that the endogenous elimination  
117 of adipocytes through apoptosis occurs normally (Prins and O'Rahilly, 1997), and can also be  
118 associated with certain pathological conditions or induced by specific pharmacological  
119 agents. Adipocyte apoptosis has been detected in rats with streptozotocin-induced diabetes

120 (Geloan et al., 1989; Loftus et al., 1998), in humans with malignancy-associated weight loss  
121 (Prins et al., 1994), and in humans infected with HIV who are treated with protease inhibitors  
122 (Dowell et al., 2000; Lagathu et al., 2004; Lagathu et al., 2005). Recently, several models of  
123 inducible adipose tissue apoptosis in rodents have been described (Pajvani et al., 2005)  
124 (Felmer et al., 2002; Kolonin et al., 2004; Trujillo et al., 2005). These models have been  
125 useful both to study the process of apoptosis in adipose tissue and to demonstrate the  
126 beneficial effects of removal of adipocytes in obesity.

127 Certain natural compounds have also been shown to induce adipocyte apoptosis in vitro, and  
128 in some cases, in vivo as well. For example, epigallocatechin gallate (EGCG, a flavonoid in  
129 green tea), genistein (an isoflavonoid in soy), esculetin (a coumadin compound), ajoene  
130 (from garlic) and conjugated linoleic acid (CLA) all increased apoptosis of 3T3-L1  
131 adipocytes in vitro (Evans et al., 2000; Hargrave et al., 2004; Kim et al., 2005; Lin et al.,  
132 2005; Yang et al., 2005a; Yang et al., 2005b). Both CLA and genistein have also been  
133 shown to increase adipose tissue apoptosis in mice in vivo (Hargrave et al., 2002; Kim et al.,  
134 2005; Tsuboyama-Kasaoka et al., 2000).

135 Endogenous factors that may be involved in adipose tissue apoptosis, under either  
136 physiological or pathological conditions, have only begun to be explored, and much of this  
137 work has involved factors that exert their effects via the CNS, as discussed below. Tumor  
138 necrosis factor alpha (TNF $\alpha$ ), which is produced and secreted by adipocytes, was first shown  
139 by Prins et al to induce adipocyte apoptosis in vitro (Prins et al., 1997). Because TNF $\alpha$  is  
140 produced and secreted by adipocytes, it may act as a paracrine agent to control adipose tissue  
141 mass in part through apoptosis, but there is not yet sufficient information to determine  
142 whether TNF $\alpha$  acts physiologically to regulate apoptosis of adipocytes (Warne, 2003).  
143

## 144 **CENTRAL NERVOUS SYSTEM CONTROL OF ADIPOSE TISSUE APOPTOSIS**

### 145 *Leptin*

146 We have shown that the hormone leptin, secreted by adipose tissue, reduces fat mass in  
147 rodents not only by increasing lipolysis, but also by stimulating adipocyte apoptosis both in  
148 fat depots and in bone marrow (Della-Fera et al., 2001; Gullicksen et al., 2003; Hamrick et  
149 al., 2005a). Like TNF $\alpha$ , leptin is a cytokine produced and secreted by adipocytes, but our  
150 studies have shown that its effect on adipose tissue apoptosis is mediated by the CNS and not  
151 locally. As little as 0.1  $\mu$ g leptin/day administered into the ventromedial nucleus of the  
152 hypothalamus (VMH) for four days significantly increased adipose tissue apoptosis in rats  
153 (Della-Fera et al., 2005) (Figure 2).  
154

### 155 *Melanocortins*

156 We have recently begun investigating the downstream pathways involved in leptin-induced  
157 adipose tissue apoptosis. Because melanocortin receptors appear to mediate a number of  
158 leptin's effects, we carried out a study to determine the role of melanocortin receptors in  
159 adipose tissue apoptosis (Choi et al., 2003b). Rats with cannulas implanted in the lateral  
160 cerebral ventricles (LV) were injected ICV with either sCSF or the melanocortin receptor

161 blocker SHU9119 (1 nmol) followed one hour later by injection of either sCSF (control),  
162 leptin (10 µg) or MTII (0.1 nmol). Treatments were administered for 4 days and food intake  
163 and body weight were measured daily. Twenty four hr after the final injections, the rats were  
164 sacrificed and blood and adipose tissues were collected. Both MTII and leptin significantly  
165 decreased food intake and body weight. Leptin, but not MTII, significantly decreased serum  
166 insulin and leptin concentrations and increased serum free fatty acid concentrations. Both  
167 leptin and MTII also decreased epididymal white adipose tissue weight (eWAT), but only  
168 leptin increased adipose tissue apoptosis. Pretreatment of rats with SHU9119 blocked the  
169 effects of both MTII and leptin on food intake, body weight and adipose tissue weight and  
170 reversed the effects of leptin on serum leptin, insulin and free fatty acid concentrations, but  
171 SHU9119 pretreatment had no effect on leptin-induced adipose tissue apoptosis (**Figure 3**).  
172 The results of this study indicated that leptin-induced adipose tissue apoptosis is not  
173 mediated by downstream melanocortin receptors.  
174

### 175 ***CART***

176 CART is one of the most abundantly expressed mRNAs in the rat hypothalamus (Gautvik et  
177 al., 1996), and neuroanatomical studies have shown that CART mRNA is expressed within  
178 hypothalamic areas implicated in the CNS control of feeding behavior and metabolism,  
179 including the paraventricular (PVN), arcuate and dorsomedial nuclei (DMN), as well as the  
180 lateral hypothalamus (LH) (Dall Vechia et al., 2000; Koylu et al., 1998; Koylu et al., 1997).  
181 A number of studies indicate that CART peptides act centrally to inhibit feeding (Kristensen  
182 et al., 1998; Lambert et al., 1998; Larsen et al., 2000), and CART may be a downstream  
183 effector for specific leptin actions in some areas (Elias et al., 1998; Kristensen et al., 1998).  
184 We carried out a study to determine if CART administered icv produced effects similar to  
185 leptin on feeding behavior, body weight and adipose tissue. After 4 days of continuous  
186 administration, 9.6 µg/d CART decreased food intake and body weight but caused behavioral  
187 abnormalities and loss of muscle as well as fat. A dose of 2.4 µg/d CART only reduced food  
188 intake. In contrast, rats receiving 15 µg/d leptin had normal behavior, but they ate less and  
189 lost weight and body fat, but not muscle.

190 We had predicted that if CART acted centrally as a downstream mediator of leptin, then it  
191 would induce adipose tissue apoptosis. This hypothesis was based on evidence pointing to  
192 the possibility that leptin-induced adipose apoptosis is a result of increased sympathetic  
193 stimulation of adipose tissue (Gullicksen et al., 2003; Haynes et al., 1997; Page et al., 2004),  
194 and because leptin has been shown to activate CART-containing neurons in the  
195 hypothalamus that innervate preganglionic sympathetic neurons in the thoracic spinal cord  
196 (Elias et al., 1998). We found, however, that adipose tissue apoptosis was significantly  
197 increased only by leptin. Thus, it appears that CART does not act as a downstream mediator  
198 of leptin-induced adipose tissue apoptosis.  
199

### 200 ***Ciliary Neurotrophic Factor***

201 Ciliary neurotrophic factor (CNTF) is a pluripotent neurocytokine expressed by glial cells in  
202 peripheral nerves and in the central nervous system (Ip and Yancopoulos, 1996; Manthorpe

203 et al., 1993). However, unlike the prototypical cachectic cytokines, recent studies have  
204 shown that CNTF can induce weight loss without exhibiting the typical deleterious  
205 characteristics of these cytokines (Lambert et al., 2001; Xu et al., 1998). CNTF has been  
206 compared to leptin for its similar effects on food intake, weight loss and energy expenditure.  
207 Central administration of CNTF decreases food intake and body weight, and like leptin, after  
208 cessation of treatment, there is not an immediate rebound in weight gain (Gloaguen et al.,  
209 1997; Kalra et al., 1998; Lambert et al., 2001; Xu et al., 1998). Because leptin and CNTF  
210 have been shown to have similar actions on adipose tissue mass, body weight and food intake,  
211 we hypothesized that CNTF administered ICV would increase adipose tissue apoptosis.  
212 After 4 days of once daily ICV injections (5  $\mu$ g), both leptin and CNTF significantly  
213 increased apoptosis in epididymal and retroperitoneal adipose tissue in rats (Duff et al., 2004).  
214 It is of interest that the long term, but not short term, effect of centrally administered CNTF  
215 on body weight reduction can be eliminated by blocking its effect on neural cell proliferation  
216 (Kokoeva et al., 2005). Kokoeva et al also showed that in ob/ob mice CNTF treatment did  
217 not cause long term reduction of body weight, indicating that a leptin-sensitive component of  
218 the new cells produced in the hypothalamus after CNTF treatment is required for the long  
219 term effect of CNTF on body weight reduction. Thus, it would be of interest to determine  
220 whether ob/ob mice exhibit adipose tissue apoptosis in response to central CNTF treatment.  
221 Although the mechanisms involved in either leptin or CNTF-induced adipose tissue  
222 apoptosis are not yet known, both similarities and differences between these two peptides are  
223 beginning to suggest a likely CNS pathway. Two important CNS peptides that act as  
224 downstream effectors of leptin are  $\alpha$ -melanocortin stimulating hormone ( $\alpha$ MSH) and  
225 neuropeptide Y (NPY) (Inui, 1999). Leptin and CNTF both activate STAT-3 in areas of the  
226 hypothalamus involved in feeding behavior and body weight regulation (Lambert et al.,  
227 2001; Sleeman et al., 2000). However, CNTF causes weight loss in animal models that are  
228 resistant to the effects of leptin, including mice lacking leptin receptors (db/db), mice with  
229 diet-induced obesity (DIO) and mice with melanocortin-4 receptor deficiency (Gloaguen et  
230 al., 1997; Marsh et al., 1999). It is of interest to note that mice with DIO have enhanced  
231 sensitivity to the anorectic effects of melanocortins, suggesting that DIO may involve  
232 reduced melanocortin signaling (Hansen et al., 2005). Thus, the effect of CNTF on food  
233 intake and body weight appears to be mediated downstream or independently of  
234 melanocortin receptors.

235 In contrast, both leptin and CNTF have been shown to suppress NPY levels and their effects  
236 on food intake and body weight can be reversed by concurrent NPY administration (Jang et  
237 al., 2000; Kotz et al., 1998; Lambert et al., 2001; Yokosuka et al., 1998). Likewise, the lack  
238 of rebound eating after CNTF or leptin treatments are terminated have been suggested to be a  
239 result of the decrease in NPY levels, compared to the increase that occurs with food  
240 deprivation (Lambert et al., 2001). Because we found that melanocortin receptors are not  
241 involved in leptin-induced adipose tissue apoptosis, these findings suggest that NPY is a  
242 critical component of the downstream mechanism involved in adipose tissue apoptosis  
243 mediated by leptin: Both Gong et al (Gong et al., 2003) and Margareto et al (Margareto et al.,  
244 2000) have shown that inhibition of NPY receptors increases adipose tissue apoptosis in rats;  
245 thus, these findings suggest that adipose tissue apoptosis increased by icv injections of leptin  
246 or CNTF may be a result of suppression of NPY expression in the hypothalamus.

247

248 *Sympathetic Nervous System*

249 We have recently found that chronic oral administration of a  $\beta$ 2-adrenergic agonist resulted  
250 in increased adipose tissue apoptosis in mice (Page et al., 2004). Because leptin has been  
251 shown to increase sympathetic nervous system activity (Dunbar et al., 1997; Haynes et al.,  
252 1997; Tang-Christensen et al., 1999), while NPY suppresses sympathetic activity (van Dijk  
253 et al., 1994), it is possible that leptin-induced increased  $\beta$ 2-adrenergic receptor activation in  
254 specific fat depots could trigger adipocyte apoptosis.

255 There is extensive innervation of white adipose tissue (WAT) by the sympathetic nervous  
256 system (SNS) (Bartness and Bamshad, 1998), and adipocytes have been shown to express  $\beta$ -  
257 adrenergic receptors, particularly  $\beta$ 3 receptors (Collins and Surwit, 2001). Sympathetic  
258 denervation of WAT increased fat cell number (Youngstrom and Bartness, 1998), whereas  
259 stimulation of  $\beta$ 3-adrenergic receptors induced apoptosis through activation of a tyrosine  
260 kinase pathway (Ma and Huang, 2002). Indeed, treatment of estrogen-deficient rats with an  
261 agonist for the  $\beta$ 3-adrenergic receptor significantly decreased bone marrow adiposity in the  
262 spine (Kurabayashi et al., 2001).

263

264 **ROLE OF CNS IN BONE MARROW ADIPOSE APOPTOSIS**

265 Preliminary studies suggest that the sympathetic nervous system plays a significant role in  
266 the regulation of bone marrow adipocyte populations. Bone marrow is richly innervated with  
267 sympathetic nerve fibers, and neuronal signals appear to play a significant role in the  
268 regulation of bone mass. Kellenberger et al showed that  $\beta$ 2-agonists increased bone  
269 formation (Kellenberger et al., 1998), and  $\beta$ -agonists have been found to decrease bone loss  
270 with disuse and muscle atrophy (Martin et al., 2005; Zeman et al., 1991). Moreover, mice  
271 lacking both  $\beta$ 1- and  $\beta$ 2-adrenergic receptors have decreased cortical bone mass (Pierroz,  
272 2004), suggesting that beta-adrenergic signaling is necessary for the normal maintenance and  
273 accumulation of bone tissue. Signaling through  $\beta$ -adrenergic receptors can also inhibit the  
274 expression of adipogenic factors in vivo (Margareto et al., 2001). Other studies, however,  
275 suggest that stimulation of  $\beta$ -adrenergic receptors decreases bone formation (Takeda et al.,  
276 2002) and mice lacking only  $\beta$ 2-adrenergic receptors were shown to exhibit a high bone mass  
277 phenotype (Eleftheriou et al., 2005). Thus, at present, the role of beta-adrenergic signaling in  
278 regulating bone metabolism is unclear.

279 We have hypothesized that  $\beta$ -adrenergic signaling in bone marrow, activated centrally via  
280 leptin, not only induces bone marrow adipocyte apoptosis but also inhibits bone marrow  
281 adipogenesis.

282 Although Ducy et al have shown that leptin-deficient mice (*ob/ob*) and mice lacking the  
283 leptin receptor (*db/db*) have increased bone mineralization of the spine (Ducy et al., 2000),  
284 others have shown that *ob/ob* mice have lower total bone mass and reduced bone density in  
285 their femora compared to normal mice (Hamrick et al., 2005b; Steppan et al., 2000) and that  
286 *db/db* mice have reduced length, bone mineral density and bone mineral mass of their tibias  
287 (Lorentzon et al., 1986). Furthermore, the limb bones of *ob/ob* mice showed increased bone  
288 marrow adipogenesis (Hamrick et al., 2005b). Hamrick et al (Hamrick et al., 2005b) tested  
289 the hypothesis that leptin treatment would reduce adipocyte stores in bone marrow and would  
290 increase bone formation and bone mass in leptin-deficient *ob/ob* mice. Leptin (2.5 & 10

291  $\mu\text{g/d}$ ) was administered continuously by subcutaneously implanted osmotic pumps in female  
292 *ob/ob* and *OB/?* lean control mice for 14 d. Both doses of leptin decreased the number of  
293 marrow adipocytes by more than 20% ( $P<0.05$ ) compared to control-treated *ob/ob* mice. The  
294 decrease in adipocyte number with leptin treatment was accompanied by an increase in  
295 concentration of the apoptosis marker caspase-3 in bone marrow adipocytes and  
296 hematopoietic cells. Both doses also increased the bone-forming endosteal surface by more  
297 than 30% ( $P<0.05$ ) compared to control-treated *ob/ob* mice. Leptin treatment increased  
298 whole-body bone mineral content by more than 30% in the *ob/ob* mice receiving the highest  
299 leptin dose. These results demonstrated that leptin is an osteogenic factor that eliminates  
300 bone marrow adipocytes, increases bone formation, and increases bone mineral and density  
301 in leptin-deficient animals. More recently we showed that leptin injected directly into the  
302 VMH of rats significantly increased endosteal osteoblast surface area, reduced bone marrow  
303 adipocyte number by more than 50% and increased bone marrow caspase-3 levels ( $P<0.001$ )  
304 (Hamrick et al., 2005a). Thus, these data indicate that leptin regulates adipocyte apoptosis in  
305 bone marrow through a central, hypothalamic signaling pathway.

306

307

- 309 Akune, T. et al. 2004. Ppargamma insufficiency enhances osteogenesis through osteoblast  
310 formation from bone marrow progenitors. *J Clin Invest* 113: 846-855.
- 311 Alberts, B. 2002. Programmed cell death (apoptosis) *Molecular biology of the cell*. p 1010-  
312 1014. Garland Science, New York.
- 313 Bartness, T. J., and M. Bamshad. 1998. Innervation of mammalian white adipose tissue:  
314 Implications for the regulation of total body fat. *Am J Physiol* 275: R1399-1411.
- 315 Choi, Y. H. et al. 2003a. Melanocortin receptors mediate leptin effects on feeding and body  
316 weight but not adipose apoptosis. *Physiol Behav* 79: 795-801.
- 317 Choi, Y. H. et al. 2003b. Melanocortin receptors mediate leptin effects on feeding and body  
318 weight, but not adipose apoptosis. *Physiol Behav* 79: 795-801.
- 319 Colditz, G. A. 1999. Economic costs of obesity and inactivity. *Medicine & Science in Sports*  
320 *& Exercise* 31: S663-S667.
- 321 Collins, S., and R. S. Surwit. 2001. The beta-adrenergic receptors and the control of adipose  
322 tissue metabolism and thermogenesis. *Recent Prog Horm Res* 56: 309-328.
- 323 Commons, G. W., B. Halperin, and C. C. Chang. 2001. Large-volume liposuction: A review  
324 of 631 consecutive cases over 12 years. *Plast Reconstr Surg* 108: 1753-1763;  
325 discussion 1764-1757.
- 326 D'Andrea, F. et al. 2005. Changing the metabolic profile by large-volume liposuction: A  
327 clinical study conducted with 123 obese women. *Aesthetic Plast Surg*.
- 328 Dall Vechia, S., P. D. Lambert, P. C. Couceyro, M. J. Kuhar, and Y. Smith. 2000. Cart  
329 peptide immunoreactivity in the hypothalamus and pituitary in monkeys: Analysis of  
330 ultrastructural features and synaptic connections in the paraventricular nucleus. *J*  
331 *Comp Neurol* 416: 291-308.
- 332 Della-Fera, M. A. et al. 2005. Leptin injected into the ventromedial hypothalamus (vmh)  
333 reduces food intake (fi), body weight (bw) and bone marrow adiposity and increases  
334 apoptosis of adipose tissue and bone marrow. *Experimental Biology Annual Meeting*.
- 335 Della-Fera, M. A., H. Qian, and C. A. Baile. 2001. Adipocyte apoptosis in the regulation of  
336 body fat mass by leptin. *Diabetes Obes Metab* 3: 299-310.
- 337 Dowell, P., C. Flexner, P. O. Kwiterovich, and M. D. Lane. 2000. Suppression of  
338 preadipocyte differentiation and promotion of adipocyte death by hiv protease  
339 inhibitors. *J Biol Chem* 275: 41325-41332.
- 340 Ducy, P. et al. 2000. Leptin inhibits bone formation through a hypothalamic relay: A central  
341 control of bone mass. *Cell* 100: 197-207.
- 342 Duff, E. et al. 2004. Ciliary neurotrophic factor injected icv induces adipose tissue apoptosis  
343 in rats. *Apoptosis* 9: 629-634.
- 344 Dunbar, J. C., Y. Hu, and H. Lu. 1997. Intracerebroventricular leptin increases lumbar and  
345 renal sympathetic nerve activity and blood pressure in normal rats. *Diabetes* 46:  
346 2040-2043.
- 347 Eleftheriou, F. et al. 2005. Leptin regulation of bone resorption by the sympathetic nervous  
348 system and cart. *Nature* 434: 514-520.
- 349 Elias, C. F. et al. 1998. Leptin activates hypothalamic cart neurons projecting to the spinal  
350 cord. *Neuron* 21: 1375-1385.
- 351 Evans, M. et al. 2000. Conjugated linoleic acid suppresses triglyceride accumulation and  
352 induces apoptosis in 3t3-l1 preadipocytes. *Lipids* 35: 899-910.

353 Felmer, R., W. Cui, and A. J. Clark. 2002. Inducible ablation of adipocytes in adult  
354 transgenic mice expressing the e. Coli nitroreductase gene. *J Endocrinol* 175: 487-498.

355 Flegal, K. M., M. D. Carroll, R. J. Kuczmarski, and C. L. Johnson. 1998. Overweight and  
356 obesity in the united states: Prevalence and trends, 1960-1994. *International Journal*  
357 *of Obesity and Related Metabolic Disorders* 22: 39-47.

358 Fried, S. K., D. A. Bunkin, and A. S. Greenberg. 1998. Omental and subcutaneous adipose  
359 tissues of obese subjects release interleukin-6: Depot difference and regulation by  
360 glucocorticoid. *Journal of Clinical Endocrinology and Metabolism* 83: 847-850.

361 Gautvik, K. M. et al. 1996. Overview of the most prevalent hypothalamus-specific mRNAs, as  
362 identified by directional tag PCR subtraction. *Proc Natl Acad Sci U S A* 93: 8733-8738.

363 Geloan, A., P. E. Roy, and L. J. Bukowiecki. 1989. Regression of white adipose tissue in  
364 diabetic rats. *Am J Physiol* 257: E547-553.

365 Giugliano, G. et al. 2004. Effect of liposuction on insulin resistance and vascular  
366 inflammatory markers in obese women. *Br J Plast Surg* 57: 190-194.

367 Gloaguen, I. et al. 1997. Ciliary neurotrophic factor corrects obesity and diabetes associated  
368 with leptin deficiency and resistance. *Proc Natl Acad Sci U S A* 94: 6456-6461.

369 Gong, H. X. et al. 2003. Lipolysis and apoptosis of adipocytes induced by neuropeptide y-y5  
370 receptor antisense oligodeoxynucleotides in obese rats. *Acta Pharmacol Sin* 24: 569-  
371 575.

372 Gullicksen, P. S., M. A. Della-Fera, and C. A. Baile. 2003. Leptin-induced adipose apoptosis:  
373 Implications for body weight regulation. *Apoptosis* 8: 327-335.

374 Gupta, S. 2001. Molecular steps of death receptor and mitochondrial pathways of apoptosis.  
375 *Life Sci* 69: 2957-2964.

376 Hamrick, M., M. A. Della-Fera, D. L. Hartzell, Y.-H. Choi, and C. A. Baile. 2005a. Central  
377 control of bone marrow adipocyte populations by leptin. *J. Bone and Mineral*  
378 *Research* 20: S368.

379 Hamrick, M. W. et al. 2005b. Leptin treatment induces loss of bone marrow adipocytes and  
380 increases bone formation in leptin-deficient ob/ob mice. *J Bone Miner Res* 20: 994-  
381 1001.

382 Hansen, M. J., H. B. Schioth, and M. J. Morris. 2005. Feeding responses to a melanocortin  
383 agonist and antagonist in obesity induced by a palatable high-fat diet. *Brain Res* 1039:  
384 137-145.

385 Hargrave, K. M. et al. 2002. Adipose depletion and apoptosis induced by trans-10, cis-12  
386 conjugated linoleic acid in mice. *Obes Res* 10: 1284-1290.

387 Hargrave, K. M. et al. 2004. Influence of dietary conjugated linoleic acid and fat source on  
388 body fat and apoptosis in mice. *Obes Res* 12: 1435-1444.

389 Haynes, W. G., W. I. Sivitz, D. A. Morgan, S. A. Walsh, and A. L. Mark. 1997. Sympathetic  
390 and cardiorenal actions of leptin. *Hypertension* 30: 619-623.

391 Hengartner, M. O. 2000. The biochemistry of apoptosis. *Nature* 407: 770-776.

392 Inui, A. 1999. Feeding and body-weight regulation by hypothalamic neuropeptides--  
393 mediation of the actions of leptin. *Trends Neurosci* 22: 62-67.

394 Ip, N. Y., and G. D. Yancopoulos. 1996. *Annu. Rev. Neurosci.* 19: 491-515.

395 Jang, M., A. Mistry, A. G. Swick, and D. R. Romsos. 2000. Leptin rapidly inhibits  
396 hypothalamic neuropeptide y secretion and stimulates corticotropin-releasing  
397 hormone secretion in adrenalectomized mice. *J Nutr* 130: 2813-2820.

398 Jilka, R. L. 2002. Osteoblast progenitor fate and age-related bone loss. *J Musculoskelet*  
399 *Neuronal Interact* 2: 581-583.

400 Justesen, J. et al. 2001. Adipocyte tissue volume in bone marrow is increased with aging and  
401 in patients with osteoporosis. *Biogerontology* 2: 165-171.

402 Kajkenova, O. et al. 1997. Increased adipogenesis and myelopoiesis in the bone marrow of  
403 samp6, a murine model of defective osteoblastogenesis and low turnover osteopenia.  
404 *J Bone Miner Res* 12: 1772-1779.

405 Kalra, S. P. et al. 1998. Leptin and ciliary neurotropic factor (cntf) inhibit fasting-induced  
406 suppression of luteinizing hormone release in rats: Role of neuropeptide y. *Neurosci*  
407 *Lett* 240: 45-49.

408 Kaufmann, S. C., and M. O. Hengartner. 2001. Programmed cell death: Alive and well in the  
409 new millennium. *Trends in Cell Biology* 11: 526-534.

410 Kellenberger, S., K. Muller, H. Richener, and G. Bilbe. 1998. Formoterol and isoproterenol  
411 induce c-fos gene expression in osteoblast-like cells by activating beta2-adrenergic  
412 receptors. *Bone* 22: 471-478.

413 Kim, H.-K. et al. 2005. Genistein decreases food intake, body weight and fat pad weight and  
414 causes adipose tissue apoptosis in ovariectomized female mice. *J Nutr* in press.

415 Kokoeva, M. V., H. Yin, and J. S. Flier. 2005. Neurogenesis in the hypothalamus of adult  
416 mice: Potential role in energy balance. *Science* 310: 679-683.

417 Kolonin, M. G., P. K. Saha, L. Chan, R. Pasqualini, and W. Arap. 2004. Reversal of obesity  
418 by targeted ablation of adipose tissue. *Nat Med* 10: 625-632.

419 Kotz, C. M. et al. 1998. Neural site of leptin influence on neuropeptide y signaling pathways  
420 altering feeding and uncoupling protein. *Am J Physiol* 275: R478-484.

421 Koylu, E. O., P. R. Couceyro, P. D. Lambert, and M. J. Kuhar. 1998. Cocaine- and  
422 amphetamine-regulated transcript peptide immunohistochemical localization in the  
423 rat brain. *J Comp Neurol* 391: 115-132.

424 Koylu, E. O. et al. 1997. Immunohistochemical localization of novel cart peptides in rat  
425 hypothalamus, pituitary and adrenal gland. *J Neuroendocrinol* 9: 823-833.

426 Kristensen, P. et al. 1998. Hypothalamic cart is a new anorectic peptide regulated by leptin.  
427 *Nature* 393: 72-76.

428 Kurabayashi, T. et al. 2001. Effects of a beta 3 adrenergic receptor agonist on bone and bone  
429 marrow adipocytes in the tibia and lumbar spine of the ovariectomized rat. *Calcif*  
430 *Tissue Int* 68: 248-254.

431 Lagathu, C. et al. 2004. Antiretroviral drugs with adverse effects on adipocyte lipid  
432 metabolism and survival alter the expression and secretion of proinflammatory  
433 cytokines and adiponectin in vitro. *Antivir Ther* 9: 911-920.

434 Lagathu, C. et al. 2005. Hiv antiretroviral treatment alters adipokine expression and insulin  
435 sensitivity of adipose tissue in vitro and in vivo. *Biochimie* 87: 65-71.

436 Lambert, P. D. et al. 2001. Ciliary neurotrophic factor activates leptin-like pathways and  
437 reduces body fat, without cachexia or rebound weight gain, even in leptin-resistant  
438 obesity. *Proc Natl Acad Sci U S A* 98: 4652-4657.

439 Lambert, P. D. et al. 1998. Cart peptides in the central control of feeding and interactions  
440 with neuropeptide y. *Synapse* 29: 293-298.

441 Laroche, M. 2002. Intraosseous circulation from physiology to disease. *Joint Bone Spine* 69:  
442 262-269.

443 Larsen, P. J., N. Vrang, P. C. Petersen, and P. Kristensen. 2000. Chronic  
444 intracerebroventricular administration of recombinant cart(42-89) peptide inhibits and  
445 causes weight loss in lean and obese Zucker (fa/fa) rats. *Obes Res* 8: 590-596.

446 Lin, J., M. A. Della-Fera, and C. A. Baile. 2005. Green tea polyphenol epigallocatechin  
447 gallate inhibits adipogenesis and induces apoptosis in 3T3-L1 adipocytes. *Obes Res* 13:  
448 982-990.

449 Loftus, T. M., F. P. Kuhajda, and M. D. Lane. 1998. Insulin depletion leads to adipose-  
450 specific cell death in obese but not lean mice. *Proc Natl Acad Sci U S A* 95: 14168-  
451 14172.

452 Lorentzon, R., U. Alehagen, and L. Boquist. 1986. Osteopenia in mice with genetic diabetes.  
453 *Diabetes Res Clin Pract* 2: 157-163.

454 Ma, Y. C., and X. Y. Huang. 2002. Novel signaling pathway through the beta-adrenergic  
455 receptor. *Trends Cardiovasc Med* 12: 46-49.

456 Manthorpe, M., J. C. Louis, T. Hagg, and S. Varon. 1993. Ciliary neurotrophic factor. In: S.  
457 E. Loughlin and J. H. Fallon (eds.) *Neurotrophic factors*. p 443-473. Academic Press,  
458 San Diego.

459 Margareto, J. et al. 2000. A new npy-antagonist strongly stimulates apoptosis and lipolysis  
460 on white adipocytes in an obesity model. *Life Sci* 68: 99-107.

461 Margareto, J., E. Larrarte, A. Marti, and J. A. Martinez. 2001. Up-regulation of a  
462 thermogenesis-related gene (*ucp1*) and down-regulation of *pparg* and *ap2* genes  
463 in adipose tissue: Possible features of the antiobesity effects of a beta3-adrenergic  
464 agonist. *Biochem Pharmacol* 61: 1471-1478.

465 Marsh, D. J. et al. 1999. Response of melanocortin-4 receptor-deficient mice to anorectic and  
466 orexigenic peptides. *Nat Genet* 21: 119-122.

467 Martin, A. et al. 2005. Leptin modulates both resorption and formation while preventing  
468 disuse-induced bone loss in tail-suspended female rats. *Endocrinology* 146: 3652-  
469 3659.

470 Maurin, A. C. et al. 2000. Influence of mature adipocytes on osteoblast proliferation in  
471 human primary cocultures. *Bone* 26: 485-489.

472 Mayer, B., and R. Oberbauer. 2003. Mitochondrial regulation of apoptosis. *News Physiol Sci*  
473 18: 89-94.

474 Meunier, P., J. Aaron, C. Edouard, and G. Vignon. 1971. Osteoporosis and the replacement  
475 of cell populations of the marrow by adipose tissue. A quantitative study of 84 iliac  
476 bone biopsies. *Clin Orthop* 80: 147-154.

477 Narbro, K. et al. 1996. Economic consequences of sick-leave and early retirement in obese  
478 Swedish women. *International Journal of Obesity and Related Metabolic Disorders*  
479 20: 895-903.

480 National Heart Lung and Blood Institute. 1998. Clinical guidelines of the identification,  
481 evaluation, and treatment of overweight and obesity in adults: The evidence report,  
482 National Institutes of Health, Bethesda, MD.

483 Nuttall, M. E., and J. M. Gimble. 2004. Controlling the balance between osteoblastogenesis  
484 and adipogenesis and the consequent therapeutic implications. *Curr Opin Pharmacol*  
485 4: 290-294.

486 Page, K. A. et al. 2004. Beta-adrenergic receptor agonists increase apoptosis of adipose tissue  
487 in mice. *Domest Anim Endocrinol* 26: 23-31.

488 Pajvani, U. B. et al. 2005. Fat apoptosis through targeted activation of caspase 8: A new  
489 mouse model of inducible and reversible lipoatrophy. *Nat Med* 11: 797-803.

490 Pierroz, D. D. 2004. B1 $\beta$ 2-adrenergic receptor ko mice have decreased total body and  
491 cortical bone mass despite increased trabecular bone number. *J Bone Miner Res* 19: 1121.

492 Prins, J. B. et al. 1997. Tumor necrosis factor-alpha induces apoptosis of human adipose cells.  
493 *Diabetes* 46: 1939-1944.

494 Prins, J. B., and S. O'Rahilly. 1997. Regulation of adipose cell number in man. *Clin Sci*  
495 (Lond) 92: 3-11.

496 Prins, J. B., N. I. Walker, C. M. Winterford, and D. P. Cameron. 1994. Human adipocyte  
497 apoptosis occurs in malignancy. *Biochem Biophys Res Commun* 205: 625-630.

498 Sleeman, M. W., K. D. Anderson, P. D. Lambert, G. D. Yancopoulos, and S. J. Wiegand.  
499 2000. The ciliary neurotrophic factor and its receptor, cntfr alpha. *Pharm Acta Helv*  
500 74: 265-272.

501 Steppan, C. M., D. T. Crawford, K. L. Chidsey-Frink, H. Ke, and A. G. Swick. 2000. Leptin  
502 is a potent stimulator of bone growth in ob/ob mice. *Regul Pept* 92: 73-78.

503 Takeda, S. et al. 2002. Leptin regulates bone formation via the sympathetic nervous system.  
504 *Cell* 111: 305-317.

505 Tang-Christensen, M., P. J. Havel, R. R. Jacobs, P. J. Larsen, and J. Cameron. 1999. Central  
506 administration of leptin inhibits food intake and activates the sympathetic nervous  
507 system in rhesus macaques. *Journal of Clinical Endocrinology and Metabolism* 84:  
508 711-717.

509 Trujillo, M. E., U. B. Pajvani, and P. E. Scherer. 2005. Apoptosis through targeted activation  
510 of caspase8 ("Attac-mice"): Novel mouse models of inducible and reversible tissue  
511 ablation. *Cell Cycle* 4.

512 Tsuboyama-Kasaoka, N. et al. 2000. Conjugated linoleic acid supplementation reduces  
513 adipose tissue by apoptosis and develops lipodystrophy in mice. *Diabetes* 49: 1534-  
514 1542.

515 van Dijk, G., A. E. Bottone, J. H. Strubbe, and A. B. Steffens. 1994. Hormonal and metabolic  
516 effects of paraventricular hypothalamic administration of neuropeptide y during rest  
517 and feeding. *Brain Res* 660: 96-103.

518 Verma, S., J. H. Rajaratnam, J. Denton, J. A. Hoyland, and R. J. Byers. 2002. Adipocytic  
519 proportion of bone marrow is inversely related to bone formation in osteoporosis. *J*  
520 *Clin Pathol* 55: 693-698.

521 Visscher, T. L., and J. C. Seidell. 2001. The public health impact of obesity. *Annual Review*  
522 *of Public Health* 22: 355-375.

523 Wadden, T. A. 1993. Treatment of obesity by moderate and severe caloric restriction. Results  
524 of clinical research trials. *Annals of Internal Medicine* 119: 688-693.

525 Warne, J. P. 2003. Tumour necrosis factor alpha: A key regulator of adipose tissue mass. *J*  
526 *Endocrinol* 177: 351-355.

527 Xu, B. et al. 1998. Anorectic effects of the cytokine, ciliary neurotropic factor, are mediated  
528 by hypothalamic neuropeptide y: Comparison with leptin. *Endocrinology* 139: 466-  
529 473.

530 Yang, J.-Y. et al. 2005a. Esculetin induces apoptosis and inhibits adipogenesis in 3t3-l1 cells.  
531 *Obes Res* in press.

532 Yang, J. Y., M. A. Della-Fera, C. Nelson-Dooley, and C. A. Baile. 2005b. Molecular  
533 mechanisms of apoptosis induced by ajoene in 3t3-l1 adipocytes. *Obes Res* submitted.

534 Yokosuka, M., B. Xu, S. Pu, P. S. Kalra, and S. P. Kalra. 1998. Neural substrates for leptin  
535 and neuropeptide y (npy) interaction: Hypothalamic sites associated with inhibition of  
536 npy-induced food intake. *Physiol Behav* 64: 331-338.

537 Youngstrom, T. G., and T. J. Bartness. 1998. White adipose tissue sympathetic nervous  
538 system denervation increases fat pad mass and fat cell number. *Am J Physiol* 275:  
539 R1488-1493.

540 Zeman, R. J., A. Hirschman, M. L. Hirschman, G. Guo, and J. D. Etlinger. 1991. Clenbuterol,  
541 a beta 2-receptor agonist, reduces net bone loss in denervated hindlimbs. *Am J*  
542 *Physiol* 261: E285-289.

543

544

545 **Figure Captions**

546 Figure 1. Two principal pathways: the receptor and the mitochondria-mediated apoptosis  
547 (Mayer and Oberbauer, 2003).

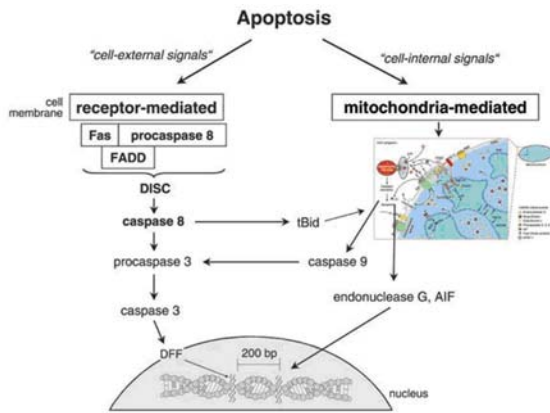
548

549 Figure 2. Male Sprague Dawley rats (N=24) with chronic cannulas directed towards the  
550 VMH were injected twice daily with synthetic cerebrospinal fluid (sCSF, control), 0.05 µg/  
551 injection or 0.25 µg/injection rat recombinant leptin for four consecutive days. Adipose  
552 tissue apoptosis (epididymal fat pad) was quantified as the percent of fragmented DNA.  
553 Data shown are means ± SEM. x,y: means without a common letter are different, p<0.01.

554

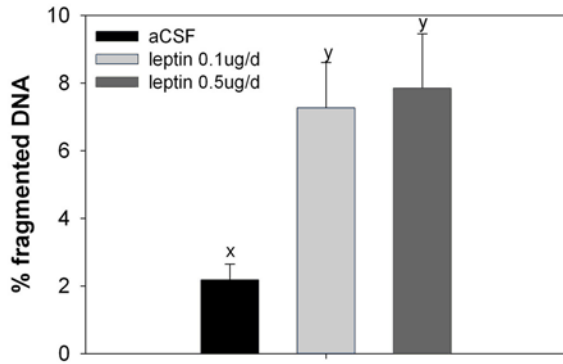
555 Figure 3. A. White adipose tissue weight of rats pre-injected ICV once a day for 4 days with  
556 either artificial cerebrospinal fluid (aCSF, 5 µl) or SHU9119 (SHU, 1 nmol/5 µl) followed  
557 one hour later with either aCSF (5 µl), leptin (10 µg/5 µl) or MTII (0.1 nmol/5 µl) ICV.  
558 Food was removed for 1 h between injections. Tissues were collected on day 5 between 24-  
559 28 h after the last injections. Epididymal white adipose tissue (eWAT); inguinal WAT  
560 (iWAT); retroperitoneal WAT (rWAT). a,b: Means with different letters are significantly  
561 different at P < 0.05. Data are means ± SEM (n = 8–10) (Choi et al., 2003a).

562 B. Fragmented-to-total DNA ratio (%) in fat tissues collected from rats pre-injected ICV  
563 once a day for 4 days with either artificial cerebrospinal fluid (aCSF, 5 µl) or SHU9119  
564 (SHU, 1 nmol/5 µl) followed one hour later with either aCSF (5 µl), leptin (10 µg/5 µl) or  
565 MTII (0.1 nmol/5 µl) ICV. Food was removed for 1 h between injections. Fresh tissues,  
566 taken on day 5 between 24-28 h after the last injections, were immediately analyzed for DNA.  
567 a,b: Means with different letters are significantly different at P < 0.05. Data are means ±  
568 SEM (n = 7–10). (Choi et al., 2003a)



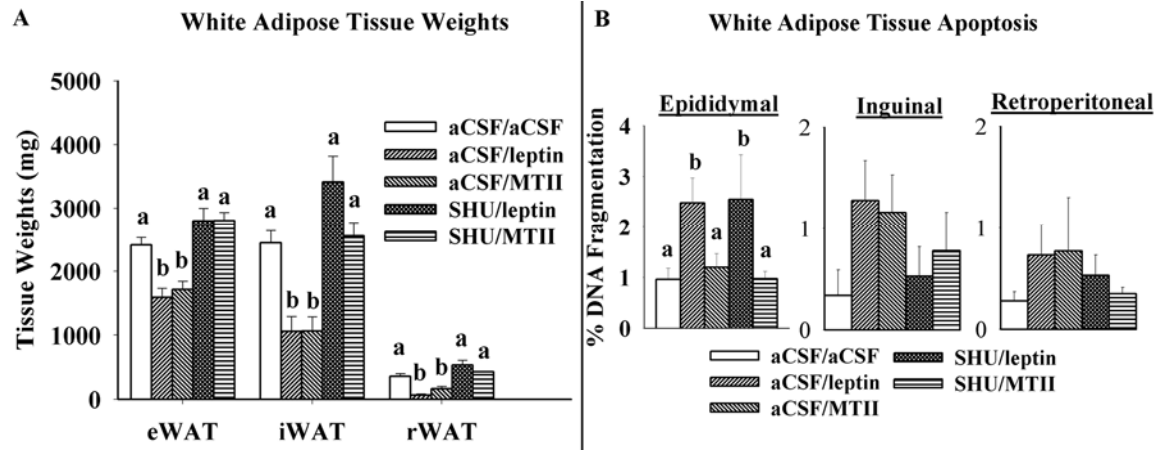
569

570 Figure 1



571

572 Figure 2



573

574 Figure 3

575