

1 **CNS CONTROLS OF ADIPOSE TISSUE APOPTOSIS**

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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

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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 α), 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 α 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 α 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 α , 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 μ 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 μ g) 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 μ g/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 μ g/d CART only reduced food
188 intake. In contrast, rats receiving 15 μ g/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 μ 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 α -melanocortin stimulating hormone (α 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 β 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 β 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 β -
257 adrenergic receptors, particularly β 3 receptors (Collins and Surwit, 2001). Sympathetic
258 denervation of WAT increased fat cell number (Youngstrom and Bartness, 1998), whereas
259 stimulation of β 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 β 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 β 2-agonists increased bone
269 formation (Kellenberger et al., 1998), and β -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 β 1- and β 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 β -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 β -adrenergic receptors decreases bone formation (Takeda et al.,
276 2002) and mice lacking only β 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 β -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.

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544

545 **Figure Captions**

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

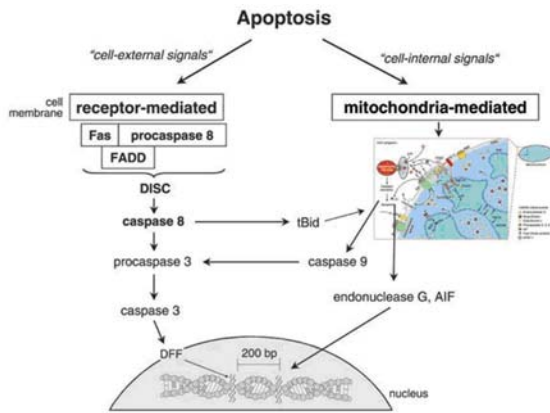
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549 Figure 2. Male Sprague Dawley rats (N=24) with chronic cannulas directed towards the
550 VMH were injected twice daily with synthetic cerebrospinal fluid (sCSF, control), 0.05 µg/
551 injection or 0.25 µg/injection rat recombinant leptin for four 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.

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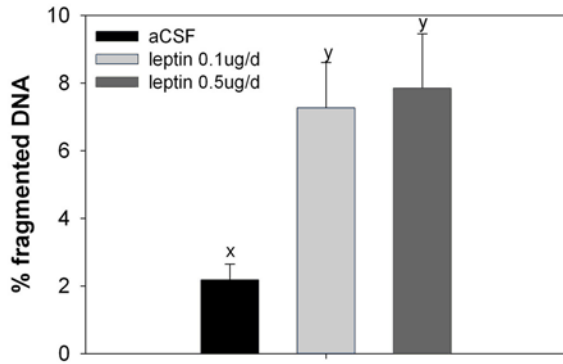
555 Figure 3. A. White adipose tissue weight of rats pre-injected ICV once a day for 4 days with
556 either artificial cerebrospinal fluid (aCSF, 5 µl) or SHU9119 (SHU, 1 nmol/5 µl) followed
557 one hour later with either aCSF (5 µl), leptin (10 µg/5 µl) or MTII (0.1 nmol/5 µl) ICV.
558 Food was removed 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)



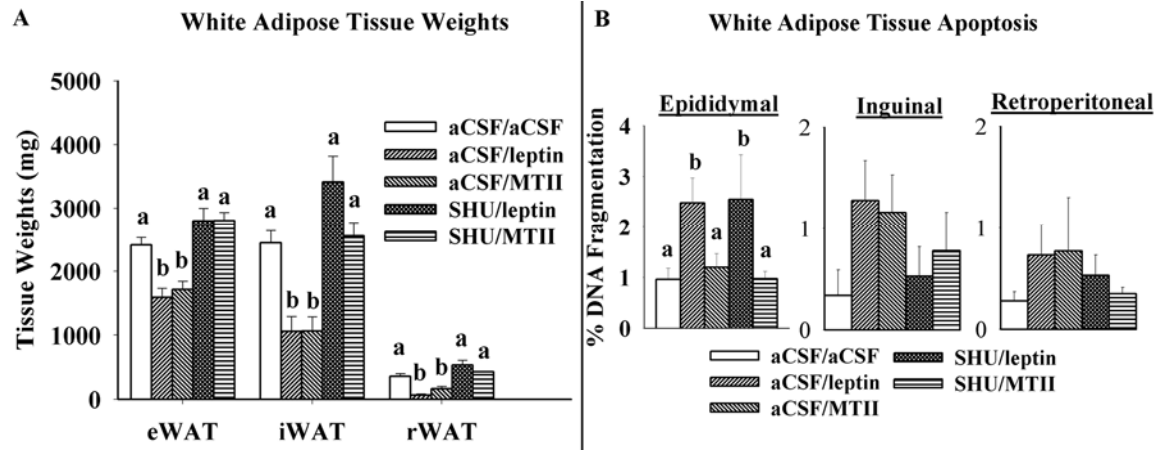
569

570 Figure 1



571

572 Figure 2



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574 Figure 3

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