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CORTICOTROPIN RELEASING FACTOR (CRF) AND LEPTIN CONTRIBUTIONS
TO ENERGY BALANCE IN GENETICALLY OBESE (lep^{ob}/lep^{ob}) MICE:
POSSIBLE INVOLVEMENT OF CRF IN THE LEPTIN EFFECTS

BY

Bo Nancy Yu

A Thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfilment of the Requirements for the Degree of

Doctor of Philosophy

Department of Psychology
University of Manitoba
Winnipeg, Manitoba

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**A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University
of Manitoba in partial fulfillment of the requirements of the degree
of
Doctor of Philosophy**

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Abstract

Both corticotropin releasing factor (CRF), a central neuropeptide and initiating factor for adrenocorticotropic hormone and corticosterone, and leptin, the product of the OB gene, which is absent in genetically obese lep^{ob}/lep^{ob} mice, have similar effects on energy balance. Both CRF and leptin decrease food intake and body weight and increase thermogenesis. Both CRF and leptin receptors have been located mainly in the hypothalamus. Because of their shared anti-obesity effects and recent evidence that leptin coordinates central neuropeptide involvement in energy balance, three experiments were designed to investigate the possible involvement of CRF receptors in the central function of leptin in the genetic obesity of lep^{ob}/lep^{ob} mice: Study I investigated the effects of leptin in mediating food intake, body weight, and thermogenesis of lep^{ob}/lep^{ob} mice and the possible involvement of CRF receptors in leptin's function. Leptin (1.0 µg/µl/mouse) or vehicle [physiological saline (1 µl)] was given intracerebroventricularly (ICV) 30 min after pretreatment of either vehicle or α-helical CRF [(10 µg/µl/mouse), a CRF antagonist] to genetically obese (n=36) and lean (n=25) mice. Food intake, water intake, and body weight were monitored at 2, 4, and 23 hr, and core body temperature (T_c) was recorded every 20-30 min for 4 hr and at 23 hr after drug treatments. Results indicated that leptin decreased cumulative food intake at 23 hr in lep^{ob}/lep^{ob} mice only. Leptin suppressed weight gain in both obese and lean mice, but that occurred earlier in obese than in lean mice and tended to be greater in obese mice. Leptin elevated T_c in both obese and lean mice across 23 hr. α-helical CRF did not completely block leptin's effects on cumulative food intake and T_c in both obese and lean mice. However, it partially blocked

leptin's effect on body weight gain in obese mice but not in lean mice. Study II examined CRF's effects in mediating food intake, body weight, and thermogenesis of lep^{ob}/lep^{ob} mice. CRF (1.0 µg/µl/mouse) or vehicle was given ICV to obese (n=31) and lean (n=22) mice following the same pretreatments as in Study I. The same dependent variables were also measured. In contrast to leptin's effects in Study I, CRF had no significant effect on cumulative food intake, body weight change, or Tc in obese or lean mice. Study III tested the possible involvement of CRF receptors in leptin's effect on oxygen consumption of lep^{ob}/lep^{ob} mice. The lep^{ob}/lep^{ob} mice (N=19) were assigned to 1 of 3 treatment groups: vehicle--leptin, α-helical CRF--vehicle, α-helical CRF--leptin. Leptin and α-helical CRF doses were the same as in Studies I and II. Oxygen consumption was recorded via indirect calorimetry every 5-10 min for 1 hr after drug treatments. Leptin did not affect oxygen consumption in obese mice within 1 hr, but tended to stimulate oxygen consumption at 60 min after injection. Taken together, these data provide increasing support for the effect of leptin on energy balance in lep^{ob}/lep^{ob} mice. CRF receptors that are blocked by α-helical CRF did not appear to be involved in the anorexic effect of leptin on lep^{ob}/lep^{ob} mice. Nonetheless, CRF receptors may be involved, at least partially, in leptin's effects on energy balance, if the roles of the two different CRF receptor subtypes (CRF-R1 and CRF-R2) are considered in future research.

Corticotropin Releasing Factor (CRF) and Leptin Contributions to Energy Balance in Genetically Obese (lep^{ob}/lep^{ob}) Mice:

Possible Involvement of CRF in the Leptin Effects

Obesity is a major concern for human health. Studies on genetic obesity in mice have been carried out for almost 50 years and produced a variety of results. However, the physiological mechanisms of energy balance in obesity are still obscure. In 1994, Zhang et al. cloned the ob gene and identified its gene product, leptin. This new finding revolutionized obesity research, rekindled interest in animal models of genetic obesity, and opened a floodgate in obesity research with more than 1000 research articles on leptin since 1995.

Leptin is mainly secreted from white fat cells or white adipocytes (Zhang et al., 1994). It suppresses energy intake, decreases body weight and body fat percentage, and increases metabolism (e.g., Campfield, Smith, Guisez, Devos, & Burn, 1995; Halaas et al., 1995; Pellemounter et al., 1995). The neuropeptide, corticotropin releasing factor (CRF), has similar effects on energy balance (e.g., Arase, Shargill, & Bray, 1989a, 1989b). Both CRF (as reviewed by Rothwell, 1989) and leptin (Chen et al., 1996; Mercer et al., 1996) receptors are mainly expressed and function in the hypothalamus, particularly in the paraventricular nucleus (PVN). Thereby, a question emerges: Is CRF involved in the function of leptin on energy balance in genetic obesity?

The neuroendocrinological defects of genetically obese animal models indicate the involvement of central CRF in the development and maintenance of obesity. One accepted animal model for obesity is the Bar Harbor genetically obese mouse (lep^{ob}/lep^{ob}) (Storlien, 1984). lep^{ob}/lep^{ob} mice display many behavioral and neuroendocrinological characteristics,

such as overeating (hyperphagia), gross adiposity, less activity (hypoactivity), lowered core temperature (hypothermia), impaired fertility, reduced metabolic rate of brown adipose tissue (BAT), decreased muscle mass, high circulating glucose (hyperglycemia), high serum insulin (hyperinsulinemia), and elevated plasma corticosterone (hyperglucocorticoidemia) (Bray & York, 1979; Friedman & Leibel, 1992).

Neuroendocrine defects contribute to these abnormalities. Hypergluco-corticoidemia may be a major determinant for obesity, because removing either adrenal glands (adrenalectomy [ADX], Arase et al., 1989a, 1989b; Feldkircher, 1993) or pituitary glands (hypophysectomy [HYPX], Rothwell & Stock, 1985) reverses these behavioral and neuroendocrinological defects in genetically obese fa/fa rats and lep^{ob}/lep^{ob} mice. In addition, glucocorticoid replacement can block the effects of ADX (e.g., Saito & Bray, 1984) and HYPX (e.g., Holt, Rothwell, Stock, & York, 1988). Either ADX or HYPX blocks the negative feedback of corticosterone on central CRF and stimulates the activity of central CRF (Swanson & Simmons, 1989). Therefore, central CRF may play an important role in the development and maintenance of obesity.

CRF stimulates sympathetic activity and increases metabolism (Brown, 1986; Brown & Fisher, 1985; Brown et al., 1982), and suppresses caloric intake (Dunn & Berridge, 1990; Rothwell, 1990) in rats. Plotsky, Thiruvikraman, Watts, & Hauger (1992) reported that hypophyseal-portal levels of plasma CRF of genetically obese (fa/fa) rats are lower than those of lean rats. Bestetti et al. (1990) reported structurally changed hypothalamic CRF neurons in fa/fa rats. These structural changes may be responsible for Moore and Routh's (1988) finding of reduced CRF level in hypothalamic nuclei in fa/fa rats. How central CRF affects energy balance and the development and maintenance of

obesity in lep^{ob}/lep^{ob} mice has not been systematically investigated.

The central functions of CRF may be related to leptin. CRF, which are associated with the regulation of energy balance (Rothwell, 1989), and leptin receptors (e.g., Chen et al., 1996; Mercer et al., 1996) are mainly expressed in the hypothalamus. The hypothalamic PVN is a major area of CRF (Krahn, Gosnell, Levine, & Morley, 1988) and neuropeptide Y ([NPY], Billington, Briggs, Grace, & Levine, 1991; Stanley, 1993) to regulate energy balance. Therefore, the hypothalamus, specifically the PVN, may be a common central location for CRF, leptin, and NPY. However, what relationship exists between CRF and leptin in energy regulation in genetically lep^{ob}/lep^{ob} mice is unknown. Given (a) that corticosterone is elevated in obesity, (b) that ADX reverses obesity, (c) that CRF, acting in the CNS, has similar effects on energy regulation as leptin, and (d) that both hypothalamic CRF and leptin are implicated in the regulation of energy balance, the present study investigated the contributions of central CRF and leptin to the feeding behavior and thermogenesis of genetically obese mice.

Genetically Obese Mouse (lep^{ob}/lep^{ob})

Animal models of genetic obesity have been used extensively in an attempt to understand the regulation of body weight. The impairments of neuroendocrinological regulation in obese animal models may provide insights into the development and maintenance of obesity in humans. These models are produced by defects in genes that have been cloned recently (Bultman, Michaud, & Woychik, 1992; Chen et al., 1996; Kleyn et al., 1996; Naggert et al., 1995; Tartaglia et al., 1995; Zhang et al., 1994). Table 1 summarizes the genetic characteristics and related syndromes of these genetically obese rodents (as reviewed by Bray, 1997; Campfield, Smith, & Burn, 1996; Friedman & Leibel,

1992; Houseknecht, Baile, Matteri, & Spurlock, 1998; Johnson, Greenwood, Horwitz, & Stern, 1991; Spiegelman & Flier, 1997; Weigle & Kuijper, 1997). The genetically obese ($\text{lep}^{\text{ob}}/\text{lep}^{\text{ob}}$) mouse is the most popular of these animal models in obesity research. The discovery of leptin, which is the ob gene product and which the mutated gene in $\text{lep}^{\text{ob}}/\text{lep}^{\text{ob}}$ mice does not produce (Zhang et al., 1994), has provided an exciting clue for the treatment of obesity. Although some research (Considine, Sinha, et al., 1996; Maffei, Halaas, et al., 1995) reported that human obesity is associated with high circulating leptin levels, Montague et al. (1997) reported two human obesity cases in which patients were found with very low plasma leptin concentration, increased hunger and severely high body weight. Therefore, investigation of leptin's function on ingestive behavior and metabolism in genetically obese ($\text{lep}^{\text{ob}}/\text{lep}^{\text{ob}}$) mice, which do not produce functional leptin, may generate meaningful information for the treatment of some forms of human obesity.

The adiposity of the Bar Harbor genetically obese mouse is inherited as an autosomal recessive mutation (gene symbol $\text{lep}^{\text{ob}}/\text{lep}^{\text{ob}}$) on Chromosome 6, Linkage Group XI (Ingalls, Dickie, & Snell, 1950). This single gene mutation results in no functional leptin being produced, massive obesity, and Type II diabetes, which mimics human obesity (Coleman, 1982). Recently, Zhang et al. (1994) cloned the mouse ob gene and the relevant human homologue, which localizes to Chromosome 7q31.3 (Geffroy et al., 1995; Green et al., 1995). Hyperphagia can only partly explain the obesity in these mice, because pair-feeding obese mice with the amount of food eaten by lean mice does not prevent the development of obesity (Alonso & Maren, 1955; Hollifield & Parson, 1958). Thurlby and Trayhurn (1979) and Smith and Romsos (1985) indicated that $\text{lep}^{\text{ob}}/\text{lep}^{\text{ob}}$ mice use dietary energy more efficiently than do their lean litter mates,

Table 1
Genetic Models of Obese Rodents and Related Syndromes

Genetic Characteristics and Syndromes	Mice					Rats
	Obese	Diabetes	Yellow	Fatty	Tubby	Zucker
Symbol	lep ^{ob} /lep ^{ob}	db/db	A ^y	fat	tub	fa/fa
Gene mutation/ Chromosome	6	4	2	8	7	5
Inheritance	Recessive	Recessive	Dominant	Recessive	Recessive	Recessive
Gene product	Leptin	Leptin receptor	Agouti protein (Asp)	Carboxypeptidase E	TUB protein (phosphatase ?)	Leptin receptor
Gene defect	Stop codon 105, unfunctional leptin	Splicing defect, unfunctional leptin receptor	Agouti protein over-produced	Insert phosphate not cleaved	Insert pro-peptide not cleaved	Splicing defect, unfunctional leptin receptor
Hyperphagia	Moderate	Moderate	Mild	n/a	n/a	Moderate
Hyperglycemia	Severe	Severe	Variable	Absent	n/a	Absent
Hyperinsulinemia	Moderate	Transient	Mild	Moderate	Moderate	Moderate
Insulin resistance	Severe	Severe	Mild	Absent	Mild	Moderate
Hyperglucocorticoidemia	Severe	Severe	Severe	Moderate	Moderate	Severe
Hypothermia	Moderate	Moderate	Mild	n/a	n/a	Mild
Impaired fertility	Infertile	Infertile	Impaired	Impaired	Impaired	Infertile
Obesity	Severe/early	Severe/early	Mild/adult	Moderate/adult	Moderate/adult	Severe/early

with reduced energy expenditure for thermogenesis. In addition, BAT, a major site of heat production in rodents (Foster & Frydman, 1978) is usually hypertrophied but has less protein content (Freedman, Horwitz, & Stern, 1986) and defective SNS activation in genetically obese mice (Knehans & Romsos, 1982) and rats (Levin, Triscari, & Sullivan, 1980, 1982, 1983). Therefore, hypothermia and decreased oxygen consumption can provide a partial explanation for the increased energy efficiency of obese mice.

Hypothermia and decreased oxygen consumption occur at an early age. Obese mouse pups have lower rectal temperatures than lean littermates as early as 6 days old (Wilson, Currie, & Gilson, 1991) and lower oxygen consumption by 5 days postpartum (Boissonneault, Hornshuh, Simons, Romsos, & Leveille, 1976; Van der Kroon, Van Vroonhoven, & Douglas, 1977). Trayhurn and James (1978) have found that a 2.1-2.5 °C difference in core temperature exists between adult obese and lean mice housed at laboratory temperatures between 10-25 °C. Wilson and Sinha (1985) suggested that this hypothermia may reflect the absence of the opportunity for behavioral thermoregulation and a genetic defect in thermogenesis, since obese mice choose warmer environments than lean mice and in so doing raise their body temperatures closer to those of lean mice.

These biobehavioral abnormalities may originate from a variety of endocrine abnormalities. Margules, Moisset, Lewis, Shibuya, and Pert (1978) reported elevated concentrations of the plasma and pituitary opioid peptide, β -endorphin, in the lep^{ob}/lep^{ob} mouse. Because β -endorphin injected into the ventromedial hypothalamus ([VMH], Grandison & Guidotti, 1977) of satiated rats stimulated food intake, Margules et al. (1978) proposed that chronically high β -endorphin levels in the obese mouse are related to its hyperphagia. They also found that a small systemic dose of naloxone, an opiate antagonist,

selectively blocked hyperphagia in obese mice and rats after 20-hr food deprivation. This low dose of naloxone, however, did not produce similar food suppressing effects in lean mice as it did in obese mice. Gilson and Wilson (1989) supported and extended Margules et al.'s (1978) observations by showing that the effects of naloxone on food intake in obese mice were primarily central in origin. Subsequently, Khawaja, Chattopadhyay, and Green (1991) found that β -endorphin levels were 2-5 times greater in the VMH and dorsomedial hypothalamus of obese mice than leans. In short, opioid peptides play an important role in the regulation of food intake in obese rodents, although more recent interpretations suggest they may mediate the reinforcing properties of food rather than nutrient balance (Gosnell & Levine, 1996).

In addition to opioids, norepinephrine ([NE]; Lorden & Oltmans, 1977) and 5-hydroxytryptamine ([5-HT]; Garthwaite, Martinson, Tseng, Hagan, & Menahan, 1980) may contribute to the development of obesity in lep^{ob}/lep^{ob} mice. The hypothalamic NE level is higher in obese mice, especially in medial hypothalamic nuclei, including the VMH and the PVN (Oltmans, 1983) than in lean mice. The higher NE level may contribute to the hyperphagia of the obese mouse, because central injections of NE receptor agonists (especially those acting at α_2 - NE receptors) not only increased food intake in many species (as reviewed by Leibowitz, 1986), but also induced a stronger stimulating effect on food intake (especially of carbohydrate) in obese mice in comparison to lean controls (Currie & Wilson, 1991; 1992a). The level of brain serotonin is also increased in obese mice (Garthwaite et al., 1980). Likewise, central injection of 5-HT reduced food intake in obese and lean mice in a dose-related manner. Intakes of lean mice, however, were suppressed more than those of obese mice (Currie & Wilson, 1992b). This research

suggests that in obese mice elevated levels of central serotonin may cause tolerance to serotonin's suppressive effects on food intake. Therefore, higher levels of central NE and serotonin may work together to increase energy intake in obese mice.

The lep^{ob}/lep^{ob} mouse also exhibits a number of endocrine abnormalities, such as altered insulin and pituitary hormone levels. Dubuc (1977) found that the lep^{ob}/lep^{ob} mouse had high serum insulin and low glucose (hypoglycemia) during Postnatal Days (PD) 17-21. The lep^{ob}/lep^{ob} mouse's hypoglycemia is transient, however, changing to hyperglycemia by PD 21 due to insulin resistance, while serum insulin levels continue to increase. In addition to hyperinsulinemia, lep^{ob}/lep^{ob} mice also have markedly higher circulating levels of corticosterone by PD17 (Dubuc, 1977; Naeser, 1974). This hyperadrenocorticism persists throughout the life span of lep^{ob}/lep^{ob} mice (Saito & Bray, 1984). Edwardson and Hough (1975) also found 14-fold higher levels of pituitary adrenocorticotrophic hormone (ACTH), a stimulus for corticosterone production from the adrenal gland, in adult lep^{ob}/lep^{ob} mice than in lean controls.

High pituitary ACTH and plasma corticosterone levels contribute to many biobehavioral characteristics of obesity, including hyperphagia (e.g., Feldkircher, 1993) and lower thermogenesis (e.g., Rothwell & Stock, 1985). Specifically, Feldkircher (1993) confirmed that lowering corticosterone levels by ADX ameliorates body weight gains and daily food intake in lep^{ob}/lep^{ob} mice but not in lean mice. Replacement of cortisone in ADX lep^{ob}/lep^{ob} mice selectively restores their greater body weight gain and food intake (Shimomura, Bray, & Lee, 1987). Rothwell and Stock (1985) suppressed ACTH secretion by HYPX in rats and found decreased body weight gain and food intake and increased thermogenesis. Chronic administration of ACTH restored adrenal weight, plasma

corticosterone levels, energy intake, and energetic efficiency to normal in HYPX rats. Collectively, the hyperadrenocortism in lep^{ob}/lep^{ob} mice represents the hyperfunctional hypothalamo-pituitary-adrenal (HPA) axis in genetic obesity. Both ADX and HYPX diminish the negative feedback from peripheral corticosterone to central hypothalamic CRF by decreasing circulating corticosterone levels. This diminished negative feedback effect to the central nervous system may be the reason for the anti-obesity effect of ADX and HYPX in genetic obesity. Figure 1 summarizes the hyperfunctional HPA axis in lep^{ob}/lep^{ob} mice and the ameliorating effects of ADX and HYPX on activity.

Impaired functional feedback effect from corticosterone to hypothalamic CRF neurons has been implicated in obesity's development. Pepin, Pothier, and Barden (1992) produced a transgenic mouse strain in which the Type II glucocorticoid receptors were partially knocked out (i.e., the abundance of receptors mainly responsible for corticosterone's negative feedback on the HPA and central CRF production was decreased). Decreasing Type II glucocorticoid receptors resulted in impaired negative feedback from peripheral corticosterone to central CRF neurons, which are the beginning of the HPA axis. These mice developed hyperadrenocortism and also became obese. The possible impaired Type II receptor function by partially knocking out gene expression does not mean that the Type II receptors have no function at all. The Type II receptor may be nonfunctional at the HPA axis site, but hypersensitive in other central sites. Hence, the central CRF activity is extensively inhibited, but the HPA axis is not. As a result, the thermoregulatory effects of CRF are abolished by the over-sensitive negative feedback effects of glucocorticoid to central CRF. This impaired negative feedback system also appears in lep^{ob}/lep^{ob} mice. For instance, lep^{ob}/lep^{ob} mice have high corticosterone levels

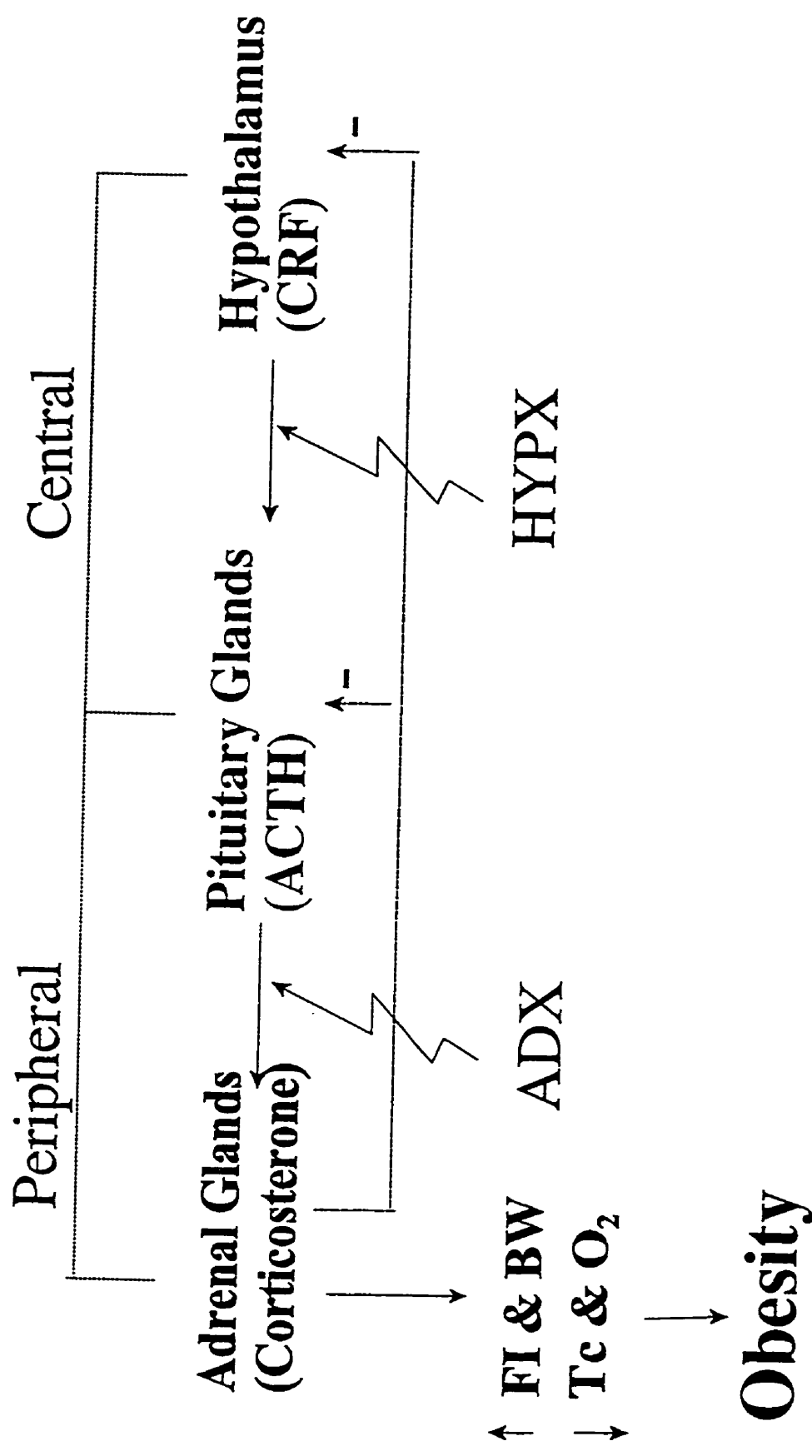
for most of their lives. On the other hand, other CRF functions, such as stimulating SNS activity, were inhibited in lep^{ob}/lep^{ob} mice.

In summary, the lep^{ob}/lep^{ob} mouse has been the most intensively studied animal model of human genetic obesity. The discovery that this mutant does not produce leptin, however, encourages a re-evaluation of the role that several neurohormonal factors are thought to play in determining this mouse's biobehavioral phenotypes. In addition to the lep^{ob}/lep^{ob} mouse's heightened sensitivity to manipulation of intake-stimulating noradrenergic and opioid peptidergic receptors and diminished sensitivity to manipulation of intake-suppressing serotonergic receptors, many studies suggest that hyperadrenocorticism may be one of the critical reasons for efficient energy expenditure (or lower thermogenesis for less energy output) and higher caloric intake in this and other obese models. Hyperadrenocorticism may be due to regulation problems at any (or all) of several points in the HPA axis (see Figure 1). Hence, enhanced body weight and food intake may be related to any of these points. CRF is one of the possible candidates for this impaired feedback regulation resulting in hyperadrenocorticism. CRF stimulates the secretion of ACTH, which initiates corticosterone production (Vale, Speiss, Rivier, & Rivier, 1981; Vale et al., 1983) and stimulates food intake and energy efficiency. On the other hand, CRF has antiobesity effects by decreasing food intake and stimulating thermogenesis (e.g., Arase et al., 1989a, 1989b; Plotsky et al., 1992). Therefore, the dual functions of CRF may be involved in the development and maintenance of obesity and may, in turn, be influenced by the absence of leptin in this model.

Figure Caption

Figure 1. Hyperfunctional HPA axis in genetic obesity. The hyperadrenocorticism plays an important role in stimulating food intake, increasing body weight, and suppressing metabolism (e.g., decreasing core temperature and oxygen consumption). Consequently, obesity develops. ADX and HYPX can abolish hyperadrenocorticism and reverse the obesity. BW = body weight; FI = food intake; Tc = core temperature; O₂ = oxygen consumption.

Hyperfunctional HPA Axis in Genetic Obesity



The Involvement of CRF in Obesity

CRF, a 41 amino acid peptide produced in the hypothalamus, initially stimulates the production of ACTH and other proopiomelanocortin (POMC)-derived peptides from the anterior pituitary (Emeric-Sauval, 1986; Gillies & Grossman, 1985; Vale et al., 1981; 1983). Centrally administered CRF also produces behaviors normally exhibited during conditions of high stress in rats (e.g., Britton, Koob, Rivier, & Vale, 1982). Further, high-affinity CRF binding sites have been identified in the brain areas that are involved in the regulation of the autonomic nervous system, particularly the sympathetic nervous system (SNS) (e.g., De Souza et al., 1985), which is activated during stress. The PVN CRF neurons project to the locus coeruleus (LC) (e.g., Swanson, Sawchenko, Rivier, & Vale, 1983; Valentino, Page, Van Bockstaele, & Aston-Jones, 1992) and autonomic preganglionic centers in the brain stem (Steffens, Scheurink, Luiten, & Bohus, 1988). LC contains the largest number of noradrenergic cell bodies within the CNS and stimulates the ANS (Foote, Bloom, & Aston-Jones, 1983). Therefore, the stress-related CRF function follows a particular pattern: (a) stressors activate PVN CRF neurons; (b) CRF activates the LC and the autonomic preganglionic centers in the brainstem; (c) the activated LC releases catecholamine, which triggers the SNS; (d) the activated autonomic preganglionic systems trigger the SNS; (e) simultaneously, CRF stimulates the HPA axis, which releases glucocorticoid; and (f) the increasing level of glucocorticoid inhibits CRF production by binding with glucocorticoid receptors (Miesfeld et al., 1984), and thereby, down-regulates the stress response. Overall, the major physiological functions of CRF include increasing plasma catecholamines, heart rate, blood pressure, metabolic rate, and locomotor activity; decreasing gastric acid secretion and sexual activity; inhibiting baroreceptor reflex

sensitivity; and producing hyperglycemia (as reviewed by Rothwell, 1990). Clearly, the function of central CRF to stress includes not only stimulating the release of ACTH and other POMC-derived peptides from the anterior pituitary, but also regulating energy intake and thermogenesis by activating the SNS, such as increasing metabolism and decreasing energy intake (see Figure 2). The stimulative effects on metabolism and suppressive effects on energy intake suggest that the CRF-SNS axis may have anti-obesity effects (see Figure 2).

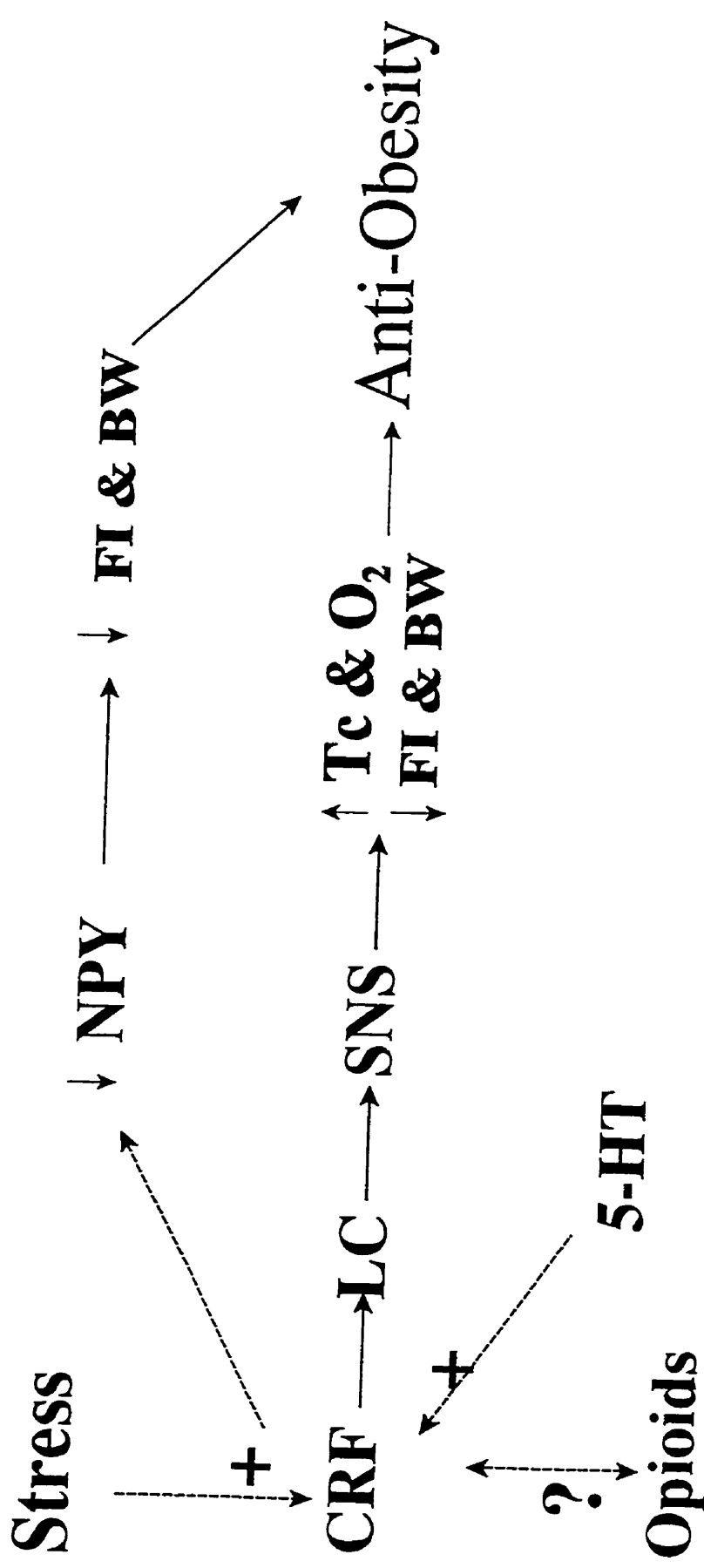
CRF and thermogenesis. In 1990, Rothwell compiled the available literature on the studies of CRF into a major review of the central effects of CRF on metabolism and energy balance. (The following section uses her organization and citations extensively and updates some areas.) Thermogenesis or heat production is defined as “additional adaptive or regulatory increases in metabolic rate associated with excess food consumption (diet-induced thermogenesis [DIT]), adaptation to cold or arousal from hibernation (nonshivering thermogenesis [NST]), or responses to disease, injury, and stress” (Rothwell, 1994, p.1). The main effector for NST and DIT in rodents is brown adipose tissue([BAT], Foster & Frydman, 1978; Girardier, 1983; Landsberg & Young, 1983). The activity of BAT depends on the SNS, which has stimulative input to BAT directly (Landsberg & Young, 1983; Rothwell & Stock, 1984). Thermogenesis, therefore, serves not only to maintain body temperature, but also to spend energy via heat production in BAT. If the regulation of thermogenesis is defective, the energy balance is impaired. Therefore, the defective regulation of thermogenesis is closely related to the development of obesity (as reviewed by Himms-Hagen, 1990).

Brown et al. (1982) initially found that CRF activates the SNS and metabolism.

Figure Caption

Figure 2. Central CRF stimulates locus coeruleus (LC) and the sympathetic nervous system (SNS). The activated SNS augments metabolism and suppresses energy intake, as a result, body weight is lessened. Thus, the CRF-SNS axis appears to have an anti-obesity effect. CRF is also related to other neuropeptides, such as NPY, opioids, and 5-HT.

Anti-Obesity Effect of CRF-LC-SNS Axis



Blatteis et al. (1989) found that endogenous brain CRF directly mediates thermogenic responses to 5-HT, certain cytokines and prostaglandins, and peripheral tissue or brain injury. Centrally injected CRF rapidly and dose-dependently stimulates sympathetically regulated thermogenesis in BAT (Rothwell, 1990), effects that could be dissociated from its effects on pituitary function. For example, LeFeuvre, Rothwell, and Stock (1987) and Rothwell and Stock (1985) reported that in rats HYPX decreases body weight much more than that of sham-operated controls, despite both groups having identical energy intake. HYPX also increased BAT activity. HYPX surgically abolishes the production and circulation of ACTH and corticosterone and blocks the negative feedback effects of corticosterone to central CRF. Therefore, the above HYPX effects on thermogenesis may be related to decreases in ACTH and corticosterone, or to blocked negative feedback of corticosterone to CRF, or both. However, LeFeuvre et al. (1987) found that centrally injected ACTH in rats failed to reverse the effects of HYPX to the extent that corticosterone did. Their study suggested that the activation of BAT following HYPX could be due to the blocking of negative feedback from corticosterone to central CRF and an increase in CRF. LeFeuvre et al. (1987) also injected CRF into the 3rd ventricle or the PVN of rats and found that CRF augmented BAT activity, physical activity, and arousal, which were followed by increases in rectal temperature. These results demonstrate that central CRF stimulates thermogenesis by BAT.

Are other neuromodulators involved in the effects of CRF on thermogenesis?

Rothwell, Hardwick, LeFeuvre, Crosby, and White (1991) have confirmed that CRF-induced thermogenesis appears to include the synthesis of POMC products within the CNS in rats, because the opioid antagonists naloxone or monoclonal antibody to γ -

melanocortin stimulating hormone (γ MSH) inhibited the effects of CRF on thermogenesis. Consistently, central injection of β -endorphin or γ MSH mimicked the CRF effects. The effect of CRF on thermogenesis may also mediate the effects of 5-HT. 5-HT stimulates the synthesis and release of CRF from the hypothalamus in vivo and in vitro (Calogero et al., 1989; Gibbs & Vale, 1983). The CRF receptor antagonist (CRF antibody) prevented the thermogenic and anorectic responses of 5-HT (LeFeuvre, Aisenthal, & Rothwell, 1991). The effects of 5-HT on energy intake and metabolism are probably, at least partially, the result of central CRF. In short, working with other neuropeptides together, CRF appears to play an integrating role in thermogenesis regulation.

CRF regulates food intake. Besides thermogenesis, CRF receptors in the hypothalamus regulate energy balance through altering food intake. Many studies have shown that central CRF inhibits food intake in rats (Rothwell, 1989, 1990). CRF suppresses spontaneous intake as well as hyperphagia caused by starvation and by the administration of insulin, muscimol, norepinephrine, and dynorphin (Levine, Rogers, Kneip, Grace, & Morley, 1983; Rothwell, 1990). The inhibitory effect of CRF on food intake may be linked to the PVN. Krahm et al. (1988) showed that injection of CRF into the PVN, but not into other brain areas, suppressed food intake in rats.

Several neuropeptides and neurotransmitters, which affect caloric intake through their action in PVN, interact; form a complex neural network to control energy intake and obesity (Leibowitz, 1986; Morley, 1989); and also moderate CRF production, secretion, or action (Blundell, 1989; Calogero, Gallucci, Chrousos, & Gold, 1988a, 1988b; Calogero et al., 1989; Leibowitz, Roland, Hor, & Squillari, 1984). Leibowitz and her colleagues (1984) and Leibowitz (1986) suggested that norepinephrine (NE) stimulates food intake

by a PVN α_2 -noradrenergic mechanism, which is enhanced by corticosterone.

Anatomically, central neuron pathways, which contain catecholamine, also connect directly to CRF neurons in PVN (Cunningham, Bohn, & Sawchenko, 1990; Cunningham & Sawchenko, 1988). Moreover, Calogero et al. (1988b) showed that α_2 -noradrenergic pathways inhibit CRF release. Hence, the intake-stimulating effects of either NE or an α_2 agonist may result from decreased CRF function via direct inhibition of CRF's release through either a PVN- α_2 mechanism or negative feedback on CRF secretion from circulating corticosterone. When lep^{ob}/lep^{ob} mice, which have naturally elevated corticosterone, received ICV NE or α_2 agonists, their intake (particularly of carbohydrate) increased above that of their pre-injection days and by a greater percentage than their lean controls (Currie & Wilson, 1992). This finding is consistent with both Leibowitz's data in rats and decreased CRF function in these mice. Central administration of 5-HT and serotonergic agonists potently inhibit food intake in both lep^{ob}/lep^{ob} and lean mice (Currie & Wilson, 1992). 5-HT also stimulates CRF production in vitro (Calogero et al., 1989; Oliver et al., 1990). Therefore, 5-HT's effects on energy intake may be related to its effect on CRF production in the hypothalamus. Taken together, CRF may be involved in mediating the effects of NE, α_2 agonists, and 5-HT on food intake.

CRF and genetic obesity. The anti-obesity effects of ADX and HYPX indicate that glucocorticoids participate in the development of obesity. This indication is supported by observations that ADX also normalizes many characteristics in the lep^{ob}/lep^{ob} mice, the fa/fa rats, and in rodents with experimental induction of obesity. For example, Solomon and Mayer (1973) reported that ADX suppressed body weight gain and plasma glucose levels and improved insulin resistance and the glucose response to food deprivation in

lep^{ob}/lep^{ob} mice compared with sham-operated controls. ADX also reduced plasma insulin levels in lep^{ob}/lep^{ob} mice (Yukimura & Bray, 1978). ADX has no or little effect on lean mice (e.g., Solomon et al., 1977; Smith & Romsos, 1985). In addition, Saito and Bray (1984) showed that ADX increased tail length, brain weight, spleen weight, and muscle weight in lep^{ob}/lep^{ob} mice. Those ADX effects could not be duplicated in pair-fed or lean mice.

As in lep^{ob}/lep^{ob} mice, ADX decreased body weight and food intake, suppressed body weight gain, and increased postprandial oxygen consumption and BAT activity (indexed by increased percentage of protein content, mitochondrial protein, specific GDP binding and total GDP binding) in Zucker fa/fa rats compared with lean or sham-operated controls (e.g., Marchington, Rothwell, Stock, & York, 1983). Furthermore, treatment with cortisone (Saito & Bray, 1984), deoxycorticosterone, or corticosterone (Yukimura, Bray, & Wolfson, 1978), or hydrocortisone (Freedman et al., 1986) can reverse the anti-obesity effects of ADX. Rothwell and Stock (1984) indicated that surgical denervation of the interscapular BAT depot could prevent the response of BAT to ADX. Taken together, these results indicate that ADX enhances thermogenesis by increasing the activity of the SNS; therefore, ADX normalizes most of the suppressed activity of SNS associated with genetic obesity. ADX has anti-obesity effects, which decrease energy intake and enhance thermogenesis, in genetically obese rodents.

The fact that the adrenal glands are functionally linked to the HPA axis would suggest that the anti-obesity effects of ADX might also occur with HYPX. Consistent with this expectation, Holt et al. (1988) demonstrated that HYPX produced a corticosterone reversible reduction in body weight and food intake and elevation in DIT

and BAT activity in the Zucker fa/fa rats, relative to pair-fed and sham-operated controls. HYPX also successfully decreased body weight of lep^{ob}/lep^{ob} mice to normal lean size two weeks after the operation (Herbai, 1970). Apparently, ADX and HYPX have similar anti-obesity effects in genetically obese rodents. Both ADX and HYPX remove or abolish the overproduction of glucocorticoids in genetically obese rodents. Glucocorticoids are final hormone products of the HPA axis and have a significant negative feedback effect, via glucocorticoid (type II) receptors, on central CRF (Meaney, Aitken, Viau, Sharma, & Sarrieau, 1989), which regulates the function of the HPA axis. Therefore, ADX and HYPX prevent the negative feedback loop of glucocorticoids to central CRF neurons. Is central CRF involved in the anti-obesity effects of ADX or HYPX?

There is evidence for such an involvement. Hardwick, Linton, and Rothwell (1989) showed that RU486, a glucocorticoid receptor antagonist, inhibited body weight gain and corticosterone action and acutely increased BAT activity in rats. However, pretreatment with a CRF antagonist prevented these responses. This indicates that RU486 first blocked the negative feedback of glucocorticoids to the CNS, then increased the production of CRF. Increased central CRF in turn stimulated BAT activity and suppressed body weight gain. Moreover, Walker and Romsos (1992) suggested the involvement of CRF in effects of ADX on lep^{ob}/lep^{ob} mice. They centrally injected CRF (5 µg) and found suppressed insulin levels and increased free fatty acids (i.e., stimulating fat cell metabolism) in lep^{ob}/lep^{ob} mice compared to vehicle controls. Central CRF also prevented the reverse effects of dexamethasone (DEX) to ADX on lep^{ob}/lep^{ob} mice. Namely, CRF reduced insulin and enhanced free fatty acid in ADX-DEX lep^{ob}/lep^{ob} mice. Consistently, the CRF antagonist, α -helical CRF (10 µg), hinders ADX effects (particularly on insulin secretion)

in lep^{ob}/lep^{ob} mice. Although the effects of CRF and CRF antagonist on BAT activity (thermogenesis) were not achieved at statistically significant levels, they may be related to the low dosage of CRF and CRF antagonist, the acute injection strategy of the study, or both. Accordingly, ADX and HYPX may abolish the obesity in genetically obese rodents by removing the negative feedback effects of glucocorticoid on central CRF and by increasing CRF production in the brain.

ADX and HYPX studies have suggested the dysfunction of central CRF in genetically obese rodents. Drescher, Chen, and Romsos (1994) have found that ICV CRF (2.1, 21, 105, and 210 pmol) dose-dependently suppressed food intake and decreased oxygen consumption. Higher doses of CRF were needed to reduce food intake in lean mice than in obese mice. This result suggested that lep^{ob}/lep^{ob} mice are more sensitive to exogenous CRF than their lean littermates. In fact, the inhibitory effect of chronic central infusion of CRF on obesity suggested that some impairment of the synthesis, or release of CRF, or both, may be related to impairments in appetite control and DIT of genetically obese rodents (Arase, York, Shimazu, Shargill, & Bray, 1988). Rohner-Jeanrenaud and Jeanrenaud (1991) found ICV administration of CRF for 7 days arrested the extra weight gain of Zucker fa/fa rats compared to pair-fed controls. CRF also reduced basal hyperinsulinemia, hepatic glycogen level, and epididymal fat pad weight, as well as elevating BAT weight and activity. Their study suggests that central administration of CRF can attenuate obesity in fa/fa rats and thereby prevents obesity in this animal model. Plotsky et al. (1992) found that initial CRF levels secreted into the hypophysial-portal circulation were lower in fa/fa rats than in lean rats and that stress (lowered blood pressure by nitroprusside injection) significantly increased CRF secretion to the hypophysial-portal

circulation in lean rats but not in fa/fa rats. That is, fa/fa rats have lower portal CRF levels in both a basal and a nitroprusside-induced stress situation. Moreover, exogenous CRF stimulated ACTH and corticosterone production in both fa/fa and lean rats. But the increased ACTH and corticosterone levels were restored to initial levels significantly more slowly in fa/fa rats than in leans, though the response patterns of ACTH and corticosterone to CRF challenge were similar in both. The results suggest that pituitary CRF receptors of obese rats are sensitive to exogenous CRF stimulation, and this sensitivity to CRF challenge may be related to either the low portal CRF tone, or continuous negative feedback effects from higher corticosterone concentration, or both. Plotsky et al. (1992) pharmacologically adrenalectomized fa/fa and lean rats and consistently found markedly enhanced portal CRF in fa/fa rats, but not in lean rats. In other words, ADX reversed the lower hypophysial-portal contents of CRF in obese rats. Plotsky et al. suggested that obese fa/fa rats display suppressed hypothalamic CRF release into the hypophysial-portal circulation, and that this is due to the abnormal glucocorticoid negative feedback effect on the hypothalamic CRF system of fa/fa rats.

Moore and Routh (1988) further showed a reduced CRF content of individual hypothalamic nuclei in fa/fa mutants. Bestetti et al. (1990) did a structural, immuno-cytochemical, and morphometrical study on the changes in the HPA axis of fa/fa rats. They found that most hypothalamic nuclei were structurally altered and the adrenal cortex was hypertrophic in fa/fa rats, thereby, indicating that central CRF in obese rats may not function normally. Recently, Timofeeva, Richard, and Huang (1996) have shown a lower expression of the CRF_{2α} receptor transcript in the VMH of fa/fa rats compared to lean rats. Thus, fa/fa rats may have a low density of CRF receptors, which suggests that the

decreased CRF effects in the hypothalamus may play a role in the development of obesity.

In summary, CRF is involved in the modulation of energy balance and defective thermogenesis and food intake of genetically obese rats, and the impaired synthesis, release, or function of CRF may contribute to the development of genetic obesity in rodents. The mechanism of CRF's defective regulation of energy balance in genetic obesity remains unknown. CRF regulates the activities of both the HPA axis and CRF - SNS axis. These two axes balance each other and maintain homeostasis. However, they are unbalanced in genetic obesity. Specifically, the CRF-HPA axis appears to be over active, while the CRF - SNS axis is underactive. Consequently, obesity develops (see Figure 3). The dysfunctional (or impaired) central CRF-SNS-HPA systems, particularly, the central regulation of CRF on thermogenesis and energy intake, may play a key role in the abnormal regulation of energy balance in genetic obesity.

Leptin and Obesity

The hypothesis that increased adipose tissue mass has negative feedback effects on the CNS (Davis, Gallagher, & Ladove, 1967; Hervey, 1959; Kennedy, 1953; Weigle, 1994) may give an insight to the defective function of CRF in genetic obesity. The recently cloned OB gene and its protein product (Zhang et al., 1994), leptin, have been strongly implicated in energy balance and obesity (Campfield et al., 1995; Halaas et al., 1995; Pelleymounter et al., 1995). Leptin may be the searched for adipose negative feedback signal (satiety factor) to the CNS. Leptin produces similar effects on thermogenesis and caloric intake as CRF. However, little research has investigated the relationship of leptin and CRF. Due to the direct effects of CRF on SNS and BAT activity, the primary inhibitory effects of leptin on obesity may be related to the function of CRF to

Figure Caption

Figure 3. The unbalanced CRF-HPA axis and CRF - SNS axis in genetic obesity. The hyperfunctional CRF - HPA axis induces hyperadrenocortism. The hyperadrenocortism promotes energy intake and lessens energy expenditure. On the other hand, the suppressed CRF - SNS axis decreases the activity of SNS. Thus, the anti-obesity effect of the CRF - SNS axis is ameliorated. The result of this unbalanced CRF-HPA axis and CRF - SNS axis is the development of obesity.

The diagram illustrates the role of CRF in the pathogenesis of obesity. It shows the following components and interactions:

- Stress** and an unknown factor (**?**) stimulate **CRF** (indicated by solid arrows with a **+** sign).
- CRF** stimulates the **HPA** axis (solid arrow) and the **SNS** (solid arrow).
- The **HPA** axis is inhibited by **ADX** and **HYPX** (indicated by lightning bolts).
- CRF** inhibits **NPY** (dashed arrow with a **-** sign).
- NPY** inhibits the **SNS** (dashed arrow with a **-** sign).
- The **SNS** promotes **Anti-Obesity** (solid arrow).
- The **HPA** axis promotes **Obesity** (solid arrow).
- The unknown factor (**?**) also promotes **Anti-Obesity** (solid arrow).
- The diagram also shows that **CRF** has a direct effect on the **SNS** (solid arrow).
- The diagram also shows that **CRF** has a direct effect on the **SNS** (solid arrow).
- The diagram also shows that **CRF** has a direct effect on the **SNS** (solid arrow).

Outcomes and associated changes:

- Obesity** is associated with $\uparrow$ FI & BW and $\downarrow$ Tc & O₂.
- Anti-Obesity** is associated with $\downarrow$ FI & BW and $\uparrow$ Tc & O₂.

modulate thermogenesis and energy intake. Particularly, leptin, as a satiety signal produced by adipocytes, may stimulate or trigger the suppressed hypothalamic CRF activity to rebalance the unbalanced CRF-HPA axis and the CRF-SNS axis. Consequently, leptin alters the negative feedback effects of the stored energy (i.e., constitutes the negative feedback loop from adipose tissue to hypothalamus). Therefore, leptin may link the periphery to the CNS by triggering CRF activity.

The search for a satiety factor. The presence of a satiety signal was first suggested in the circulation of parabiotic rats, which were surgically joined and shared blood circulation. Hervey (1959) showed that the hypothalamic lesion in one rat of a parabiotic pair produced hyperphagia and weight gain in the rat with the hypothalamic lesion, but hypophagia and obvious weight loss in the rat with the intact hypothalamus. His results indicated that some blood-borne signal from the hypothalamic-lesioned obese rat was transported to the partner with the intact hypothalamus, and this signal influenced food intake and body weight.

Several other parabiotic studies in rats (Harris & Martin, 1989; Parameswaran, Steffens, Hervey, & DeRuiter, 1977) also supported the presence of a circulating factor associated with fat cell size in satiety and body weight regulation (Keesey & Powley, 1986). Coleman and Hummel (1969) joined adult diabetic obese mice (db/db) with nondiabetic lean mice of the same sex in a parabiotic study. They found that lean controls of each parabiotic pair died of apparent starvation within 50 days after gradually losing weight and becoming hypoglycemic. Coleman (1973) found that parabiotic coupling of either genetically obese (lep^{ob}/lep^{ob}) mice with lean mice or with genetically diabetic mice (db/db) did not induce similar results in lean mice and diabetic mice: lep^{ob}/lep^{ob} mice ate less

and gained less weight. Accordingly, Coleman suggested that lean mice and diabetic mice produce some type of humoral substance, which regulates food intake and body weight. However, this humoral factor does not induce the same effects in diabetic mice, possibly due to defective satiety center. Lep^{ob}/lep^{ob} mice do not produce this type of body weight regulatory factor. Nevertheless, what this humoral factor is, where it is produced, and in which central neural area it works were unknown until Zhang et al. (1994) cloned the ob gene. Zhang et al. (1994) demonstrated that the lep^{ob}/lep^{ob} cDNA is “expressed specifically in fat cells”, and its gene product, leptin, “has 167 amino acids and the characteristics of a secreted protein” (Rink, 1994, p. 406). Harris (1997) and Harris, Zhou, Weigle, & Kuijper (1997) further confirmed that leptin is the signal produced from fat tissue, circulated in the blood, and exchanged between parabiosed mice to regulate energy balance.

Leptin and energy balance. Leptin is believed to function as an afferent satiety signal in a feedback loop that influences energy balance controlled by the brain (Zhang et al., 1994). Halaas et al. (1995) have also revealed that leptin is produced in lean mice and db/db mice, but not in lep^{ob}/lep^{ob} mice, whose OB mRNA does not produce functional leptin (called a nonsense mutation at codon 105). They found the circulating leptin level in db/db mice is much higher than in lean mice. Many studies (e.g., Lonnqvist, Arner, Nordfors, & Schalling, 1995; Ma et al., 1996; Maffei, Fei, et al., 1995; Masuzaki et al., 1995) have confirmed that leptin is also produced by white adipose tissue in humans and that the production of leptin is positively correlated to adiposity. Trayhurn, Thomas, Duncan, and Rayner (1995) have found the expression of the OB gene (OB mRNA) in several white adipose tissue depots of mice: The highest OB mRNA occurs in the epididymal and perirenal fat pads, and the lowest in the subcutaneous fat pads.

Furthermore, nutritional status influences OB mRNA levels (Frederich et al., 1995; MacDougald, Hwang, Fan, & Lane, 1995; Saladin et al., 1995; Trayhurn et al., 1995). That is, food deprivation reduces OB mRNA, and refeeding restores it. The leptin levels fall to undetectable levels when rodents are starved. Furthermore, Saladin et al. (1995) have shown that leptin secretion varies diurnally, increasing during the night after rats started eating. Food intake changes this diurnal variation. For instance, when rats were food-deprived overnight, not only was OB mRNA reduced, but cyclicity of OB mRNA was also prevented. Evidence indicates that leptin acts as an index of energy expenditure by signaling the CNS of the size of fat mass.

Leptin exerts major effects on energy balance. First, it suppresses caloric intake in rodents. Halaas et al. (1995) reported that daily IP injections of either mouse or human recombinant leptin decreased food intake and body weight of $\text{lep}^{\text{ob}}/\text{lep}^{\text{ob}}$ mice by 30% and of lean mice by 12% after 2 weeks of treatment. Similarly, Rentsch, Levens, and Chiesi (1995) demonstrated that a single intravenous injection of leptin reduced food intake in 24-hr fasted mice. Baker, Cullen, Karbon, and Pelleymounter (1996) and Pelleymounter et al. (1995) confirmed the chronic anti-obesity effects of leptin in $\text{lep}^{\text{ob}}/\text{lep}^{\text{ob}}$ mice. They injected (IP) $\text{lep}^{\text{ob}}/\text{lep}^{\text{ob}}$ and lean mice daily for 28 days with recombinant leptin. The results showed that leptin lowered food intake, body weight, percentage body fat, and serum levels of glucose and insulin selectively in the $\text{lep}^{\text{ob}}/\text{lep}^{\text{ob}}$ mice. These studies provided direct evidence that leptin serves as a satiety factor.

Second, leptin stimulates thermogenesis and thereby increases energy expenditure. Pelleymounter et al. (1995) reported that leptin (10 mg/kg, IP) also increased oxygen consumption, body temperature, and total activity in $\text{lep}^{\text{ob}}/\text{lep}^{\text{ob}}$ mice but not in lean

controls. The normalizing effects of leptin on these metabolic and behavioral variables of $\text{lep}^{\text{ob}}/\text{lep}^{\text{ob}}$ mice were within the level of their lean controls. Apparently, leptin plays a key role in the regulation of body weight, metabolism, and adiposity in mice, particularly in $\text{lep}^{\text{ob}}/\text{lep}^{\text{ob}}$ mice. Leptin seems to be not only an appetite suppressor, but also a metabolic stimulator.

Third, leptin acts directly on neuronal networks, which control food intake and energy balance. Campfield et al. (1995) first showed that a single ICV injection of leptin (1 $\mu\text{g}/\text{mouse}$) into the lateral ventricle stopped $\text{lep}^{\text{ob}}/\text{lep}^{\text{ob}}$ mice from eating after the first 30 min and most mice from eating during the remaining 6.5 hr of the experiment. This effect of central leptin was greater and lasted longer than the peripheral injection. Hwa, Ghibaudi, Compton, Fawzi, and Strader (1996) found that leptin (0.1 and 1 $\mu\text{g}/\text{mouse}$ single ICV) decreased food intake and body weight, and increased oxygen consumption, respiratory quotient, and percentage of energy derived from carbohydrate versus fat oxidation, in a 22-hr period in $\text{lep}^{\text{ob}}/\text{lep}^{\text{ob}}$ mice. All those findings suggest that leptin acts on energy homeostasis in the CNS.

In addition, leptin's modulation of white adipose tissue mass might be associated with signaling the SNS to increase thermogenesis and energy expenditure in BAT. Collins and Surwit (1996) have shown that treatment with CL316,243 (a β_3 -adrenergic receptor agonist that stimulates thermogenesis) decreases body weight and adipose tissue mass of high fat-feeding mice (a model of diet-induced obesity) and reduces the leptin mRNA level to the control level. Thus, β_3 -adrenergic receptor agonist's lowering of leptin production suggested that ob gene expression (leptin) works as a sensor of adipose tissue hypertrophy and that leptin's effects may be associated with SNS activity. Probably, leptin stimulates

sympathetic outflow and activates BAT, thereby regulating white adipose tissue mass indirectly. Collins and Surwit also reported that a single leptin injection (IP) selectively augmented NE turnover to interscapular BAT, but did not significantly influence NE turnover in retroperitoneal white adipose tissue in *lep^{ob}/lep^{ob}* mice; even though food intake did not change within the 2-hr period after leptin treatment. This result suggests leptin stimulates the SNS and then influences BAT thermogenesis. This specific effect of leptin on BAT may be an important mechanism by which leptin regulates body composition and metabolism.

The central mechanism of leptin. How leptin functions in the brain is a key question in the investigation of the central mechanism of its anti-obesity effects. Leptin receptors have been found in different isoforms and expressed in many tissues including adipocytes, ovary, testes (Chen et al., 1996; Cioffi et al., 1996; Lee et al., 1996), and choroid plexus (Lynn, Cao, Considine, Hyde, & Caro, 1996). However leptin receptors (OB-R) are also expressed in the hypothalamus of humans (Considine, Considine, Williams, Hyde, & Caro, 1996); mice (Baskin et al., 1996; Chen et al., 1996; Cioffi et al., 1996; Lee et al., 1996; Mercer et al., 1996); and rats (Cheung et al., 1996; Zamorano, Mahesh, Chorch, DeSevilla, & Brann, 1996). Mercer et al. (1996) adopted an *in situ* hybridization method to investigate the expression of the OB-R gene in mouse hypothalamus and other brain regions. They found OB-R mRNA was strongly expressed in the hypothalamus (arcuate, ventromedial, paraventricular, and ventral premammillary nuclei) and choroid plexus. OB-R in the hypothalamus may play a key role in the central regulation of energy balance.

Increasing evidence supports the hypothesis that leptin works in the hypothalamus as a hormonal negative feedback signal from adipose tissues to modify food intake and

energy expenditure. Direct evidence for the close relationship of leptin and hypothalamus is that leptin production is influenced by lesions of the hypothalamus, which augment both food intake and leptin mRNA levels in adipose tissues of rodents (Funahashi et al., 1995; Maffei, Fei, et al., 1995). Also, when the hypothalamus is lesioned, these receptors are presumably reduced in number or eliminated. For instance, Baker et al. (1996) lesioned the VMH in both lean and obese mice with goldthioglucose (GTG) before continuously systemically infusing them with leptin for 7-14 days. Although leptin decreased food intake and body weight of sham-operated lean and obese mice, it failed to affect either variable in GTG-lesioned mice. Dawson, Millard, Liu, Eppler, and Pelleymounter (1996) lesioned the arcuate nucleus of the hypothalamus via neonatal administration of monosodium glutamate (MSG) before chronic injection of leptin in rats. Leptin also failed to influence food intake and body weight gain in MSG-treated rats. Moreover, the activation effects of leptin have been indicated specifically in the hypothalamus of lep^{ob}/lep^{ob} and wild-type mice but not db/db mice, which lack an isoform of the leptin receptor (Chen et al., 1996; Lee et al., 1996). Vaisse et al. (1996) showed that leptin dose-dependently activated signal transducers and activators of transcription (STAT) proteins in the hypothalamus of obese and lean mice. In addition, Woods and Stock (1996) found that the PVN is another hypothalamic area that shows obvious and substantial neuronal activity in lep^{ob}/lep^{ob} mice after 3-hr leptin treatment. The PVN has been particularly prominent as a focus for the action of neuropeptide Y (NPY), CRF, and the monoamine neurotransmitters affecting energy homeostasis.

Are leptin's effects on energy homeostasis related to other neuromodulators?

Several studies have suggested that leptin may modulate the production or release of other

neuropeptides (e.g., NPY and CRF) in the hypothalamus (Schwartz, Seeley, Campfield, Burn, & Baskin, 1996; Seeley et al., 1996; Stephens et al., 1995). Stephens et al. (1995) first observed that the number of neurons expressing NPY mRNA in the arcuate nucleus was significantly reduced after a 30-day treatment of leptin (subcutaneous) in lep^{ob}/lep^{ob} mice; whereas, it had no effect on the NPY mRNA levels in db/db mice. Schwartz and Baskin et al. (1996) confirmed this result by IP injections of recombinant mouse leptin in lep^{ob}/lep^{ob} and db/db mice. This reduction of NPY mRNA in the hypothalamic arcuate nucleus was consistent with the inhibitory effects of leptin on food intake and its stimulatory effects on metabolism. Pair-feeding of obese mice to the intake of leptin-treated obese mice induced the same reduction of body weight, but did not decrease NPY mRNA levels in the arcuate nucleus. Consequently, the decreased NPY mRNA levels after leptin treatment were independent of the leptin weight-reducing effects. NPY is produced from NPY neurons in the arcuate nucleus, which project to the hypothalamic PVN. In the PVN, NPY release prompts caloric intake and suppresses energy expenditure (Billington et al., 1991; Stanley, 1993). Both lep^{ob}/lep^{ob} and db/db mice have elevated hypothalamic NPY gene expression (Chua et al., 1991; Wilding et al., 1993); thus, the development of obesity and related defective metabolism in these animal models could be, at least partly, due to increased NPY synthesis and release along the arcuate - PVN pathway. One possible mechanism by which leptin regulates energy balance is by inhibiting NPY synthesis and release in the hypothalamus (Stephens et al., 1995); or NPY interacts with leptin, or the function of NPY on energy balance is related to the leptin-dependent central neural system (Smith, Campfield, Moschero, Bailon, & Burn, 1996). As previously mentioned, central infusion of CRF for 7 days decreased the high NPY mRNA levels in the hypothalamus of fa/fa rats (Bchini-Hooft-van-Huijsduijnen, Rohner-Jeanrenaud, & Jeanrenaud, 1993).

Therefore, both leptin and CRF can suppress NPY production in the hypothalamus.

The hypothalamus is also an important central area for CRF production (as reviewed by Rothwell, 1989). CRF regulates HPA axis activity (Emeric-Sauval, 1986; Gillies & Grossman, 1985; Vale et al., 1981; Vale et al., 1983), and, as a potential neurotransmitter, stimulates the locus coeruleus and activates the CNS (e.g., Swanson et al., 1983; Valentino et al., 1992; Schulz & Lehnert, 1996). CRF itself has significant effects on food intake and metabolism (Dunn & Berridge, 1990; Emeric-Sauval, 1986; Rothwell, 1990). That is, CRF suppresses food intake and stimulates thermogenesis, just like leptin. However, the relationship of leptin and CRF in the hypothalamus is obscure. Central administration of leptin increases CRF mRNA levels in the PVN of lean rats (Schwartz, Seeley, et al., 1996; Seeley et al., 1996), although Schwartz, Seeley, et al. and Seeley et al. have failed to show any change of the CRF mRNA levels in the hypothalamic PVN of lep^{ob}/lep^{ob} mice after chronic IP administration of leptin. However, it is still too early to conclude that the effects of leptin on energy balance are not associated with the actions of central CRF in lep^{ob}/lep^{ob} mice. The amount of IP leptin reaching the relevant hypothalamic structures might be small. Evidently, central administration of leptin has greater and quicker effects on food intake and metabolism than peripheral administration does (Campfield et al., 1995; Stephens et al., 1995). These greater effects of ICV leptin may be related to the effects of NPY, or CRF, or both.

Statement of Research Problem

From the above discussion, both CRF and leptin play pivotal roles in the development and maintenance of obesity. The integrative actions of CRF are important in energy balance regulation where CRF modifies both components of energy balance -- food intake and thermogenesis. The dysfunction of CRF (impaired synthesis, release, or function

of CRF) is strongly implicated in the development and maintenance of genetic obesity in rodents. Most CRF and obesity studies are done with genetically obese Zucker rats. Few studies have focused on CRF and obesity in genetically obese mice. To date only two studies (Drescher et al., 1994; Walker & Romsos, 1992) have investigated the direct effects of central administration of CRF on genetic obesity in obese mice. The results of these studies partially confirmed CRF's anti-obesity effects. However, Drescher et al.'s (1994) results were controversial. That is, CRF dose-dependently suppressed food intake but decreased oxygen consumption in lep^{ob}/lep^{ob} mice. They offered a species-specific explanation for the different effects of CRF on mice and rats previously reported. However, their acute, nonstereotaxic ICV drug administration provides problems for a clear interpretation of their data. Other procedural choices add to the interpretation difficulties. Their mice were food deprived for a 12-hr period, which could interfere with endogenous corticosterone rhythms. They did not adapt their mice to either food intake assessments or oxygen consumption measurements. They anesthetized their mice with ether, a major sympathetic nervous system stimulant, before acute central injections. Finally, Drescher et al. used female mice, but Walker and Romsos used male mice, with no consideration of possible gender differences in endogenous corticosterone cycles. These studies require systematic replication, which avoids some procedural pitfalls, to identify the central effects of CRF on food intake, body weight, oxygen consumption, and core body temperature in lep^{ob}/lep^{ob} mice.

The negative feedback effects of glucocorticoids on central CRF can partly explain the suppressed regulatory effects of CRF on energy balance, namely, decreasing energy intake and stimulating energy expenditure. However, the manner in which peripherally stored energy signals the CNS (e.g., probably triggering CRF) is still not clear. Leptin, as a

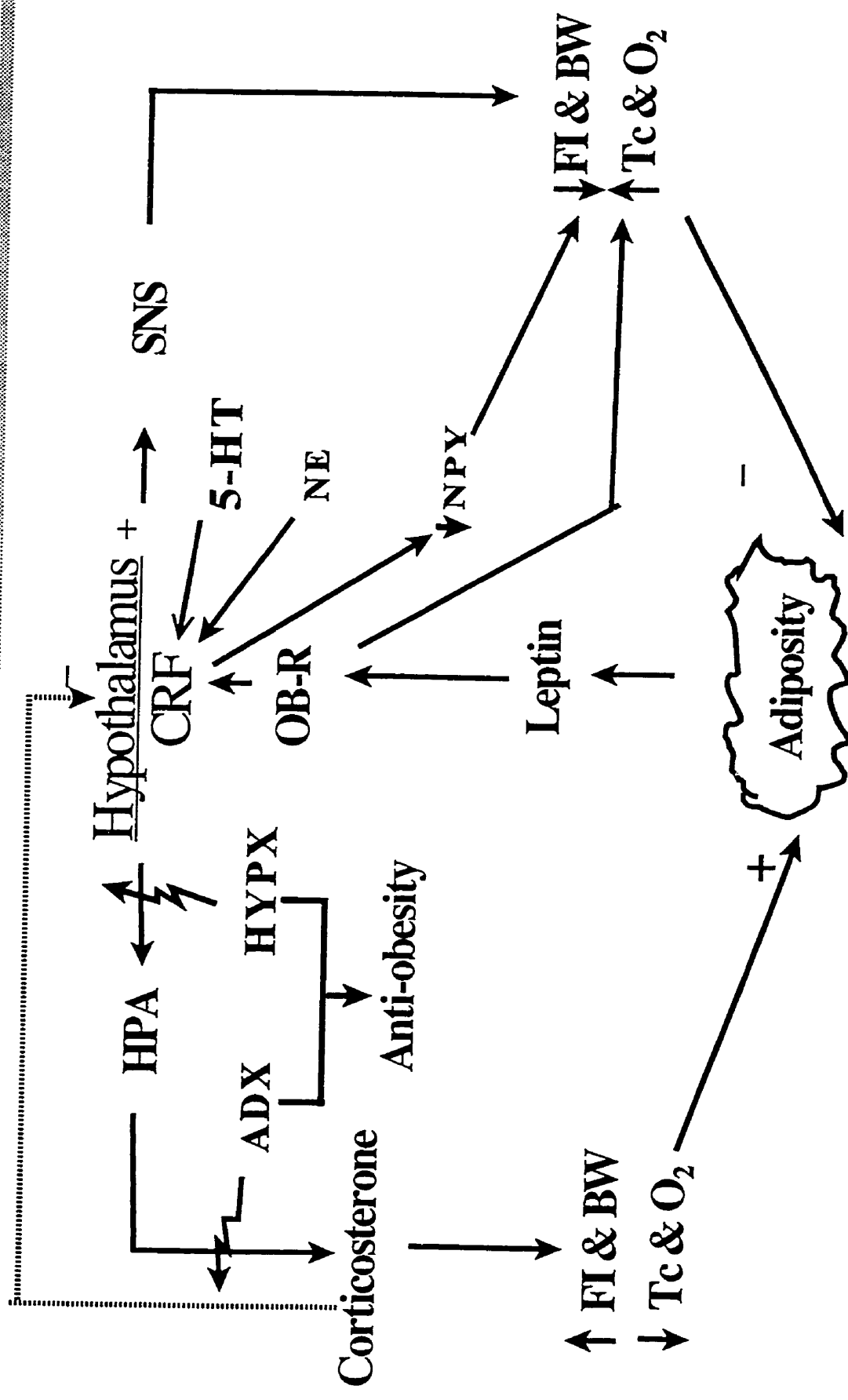
circulating hormone, regulates food intake and thermogenesis in lean and obese rodents. The central mechanism of leptin modulating food intake and metabolism is advancing the understanding of development and treatment of obesity. Leptin, a potential peripheral signal of stored energy, stimulates the CNS, then inhibits the development of obesity. How does leptin function in the CNS: by independent leptin-specific receptors, or by triggering other neuropeptides, or both? Because leptin and CRF have similar effects on the regulation of energy balance, it is reasonable to ask the question: Is CRF involved in the central function of leptin in genetic obesity (see Figure 4)? Central injection of leptin elevated CRF mRNA levels in the PVN of normal rats (Schwartz, Seeley, et al., 1996), but leptin had no effect on CRF mRNA levels in fa/fa rats (Seeley et al., 1996). The fact that obese rats (fa/fa) have a gene coding mutation in the leptin receptor may be the reason for no effect of leptin on CRF mRNA levels. A few studies have investigated the relationship between CRF and leptin in genetic obesity, and limited information has been provided from these studies. Therefore, this study focused on the effects of CRF and leptin on food intake, body weight, and thermogenesis in genetically obese (lep^{ob}/lep^{ob}) mice. In addition, the possible involvement of CRF in the central effects of leptin in obese mice was studied.

Based on these data, the hypotheses of the study follow. (1) If functional leptin receptors are in the hypothalamus, then central administrations of leptin could suppress food intake and body weight, augment thermogenesis in lep^{ob}/lep^{ob} mice, and, thereby, confirm the recent findings from other laboratories (Campfield et al., 1995; Pelleymounter et al., 1995). (2) The anti-obesity effects of CRF and leptin may implicate the same central network. If they do, and if central CRF is involved in producing the effects of leptin, then antagonizing central CRF should block leptin's effects on energy intake and expenditure. That is, leptin should produce smaller effects on intake, body weight, and energy

Figure Caption

Figure 4. Schematic illustration of unbalanced CRF-HPA axis and CRF-SNS axis and the possible involvement of CRF in the central function of leptin.

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expenditure if preceded by an injection of a CRF antagonist. (3) If CRF has anti-obesity effects on fa/fa rats, and fa/fa rats and lep^{ob}/lep^{ob} mice share some commonalities in their pathology, then CRF should also decrease food intake and body weight and increase thermogenesis (as measured by core body temperature or oxygen consumption) in lep^{ob}/lep^{ob} mice.

Overview of Design

To investigate the effect of central CRF and leptin and the involvement of CRF in the effects of leptin in mediating thermogenesis and food intake of lep^{ob}/lep^{ob} mice, three studies were conducted. Study I investigated the blocking effects of a CRF antagonist on the effects of centrally administered leptin on energy balance. Study II addressed the involvement of CRF in the food intake, body weight, and body temperature of lep^{ob}/lep^{ob} mice. Study III further explored the contribution of central CRF and leptin on thermogenesis (oxygen consumption) in lep^{ob}/lep^{ob} mice. The dose of CRF antagonist, α -helical CRF, which was used in the study of Walker and Romsos (1992) was used in Study I, II, and III. The dose of leptin, which was used in the study of Campfield et al. (1995), was employed in Study I and III. The CRF dose used in the study of Drescher et al. (1994), in which CRF induced a significant effect on cumulative food intake in lep^{ob}/lep^{ob} mice, was employed in Study II.

In Study I, the two pretreatments (vehicle and CRF antagonist) and two drug treatments (vehicle and leptin) produced the following four groups in each phenotype (obese and lean): (a) 1 μ l/mouse vehicle (physiological saline) — 1 μ l/mouse vehicle; (b) 1 μ l/mouse vehicle — 1.0 μ g/ μ l/mouse of leptin; (c) 10.0 μ g/ μ l/mouse of α -helical CRF — 1 μ l/mouse vehicle; (d) 10.0 μ g/ μ l/mouse of α -helical CRF — 1.0 μ g/ μ l/mouse of leptin (see Table 2). Both obese and lean mice were assigned to one of the four groups in a

systematic order of their body weights to counterbalance the differences in initial body weights within each phenotype.

Table 2

Overview of Design with Sample Size Completed Testing: Study I -- Leptin

Phenotype	Drug Treatment	Pretreatment	
		Vehicle	α -helical CRF
Obese	Vehicle	7	9
	Leptin	8	8
Lean	Vehicle	6	6
	Leptin	7	6

In Study II, there were two pretreatments (vehicle and CRF antagonist) and two drug treatments (vehicle and CRF), yielding four treatments for each phenotype: (a) 1 μ l vehicle (physiological saline) — 1 μ l vehicle; (b) 1 μ l vehicle — 1 μ g/ μ l/mouse of CRF (200 pmole); (c) 10.0 μ g/ μ l/mouse of α -helical CRF — 1 μ l vehicle; and (d) 10.0 μ g/ μ l/mouse of α -helical CRF — 1 μ g/ μ l/mouse of CRF (see Table 3). Mice from Study I were reused in Study II after at least 3 days rest. Some additional mice were added into the experiments to replace the mice with dislodged cannula. Obese and lean mice were given one of the four drug administrations in a systematic order of their body weights to counterbalance the differences in initial body weights within each phenotype.

In Study III, three drug treatment groups were used to determine the involvement of CRF in leptin's effect on oxygen consumption in lep^{ob}/lep^{ob} mice: (a) 10.0 μ g/ μ l/mouse of α -helical CRF — 1 μ l vehicle; (b) 1 μ l vehicle — 1 μ g/ μ l/mouse of leptin; (c) 10.0 μ g/ μ l/mouse of α -helical CRF — 1 μ g/ μ l/mouse of leptin (see Table 4). Each animal received only one treatment once.

Table 3

Overview of Design with Sample Size Completed Testing: Study II -- CRF

Phenotype	Drug Treatment	Pretreatment	
		Vehicle	α -helical CRF
Obese	Vehicle	7	9
	CRF	7	8
Lean	Vehicle	6	6
	CRF	4	6

Table 4

Overview of Design with Sample Size Completed Testing:Study III -- Oxygen Consumption

Vehicle-Leptin	α -helical CRF-Vehicle	α -helical CRF-Leptin
6	6	7

In Studies I and II, food intake and body weight were recorded 2, 4, and 23 hr after the two ICV administrations. Telemetered core body temperature was monitored every 20-30 min for 4 hr and at 23 hr after central administration. It was expected that (a) both ICV leptin and CRF would decrease food intake and body weight and increase body temperature in obese mice, and (b) α -helical CRF would completely, or partially, block the effects of leptin.

Central administration of leptin stimulated oxygen consumption at 3 hr (Mistry, Swick, & Romsos, 1997) and 22 hr (Hwa et al., 1996). However, neither Mistry et al. (1997) nor Hwa et al. (1996) reported the effect of leptin on oxygen consumption within 1 hr after central injection. Therefore, in Study III, oxygen consumption was monitored every 5 min for 1 hr after each treatment. It was anticipated that (a) leptin would stimulate oxygen consumption in obese mice compared with the α -helical CRF - vehicle

group, and (b) α -helical CRF would prevent the effects of leptin on oxygen consumption compared with the vehicle - leptin group.

The independent variables for these three studies were phenotype (obese, $\text{lep}^{\text{ob}}/\text{lep}^{\text{ob}}$ and lean, +/?), pretreatment (vehicle or CRF antagonist), drug treatment (vehicle, CRF, or leptin), and time (2, 4, 23 hr for food intake and body weight; every 20 - 30 min for 4 hr, and 23 hr for core temperature; every 5 - 10 min for 1 hr for oxygen consumption). The dependent variables were food intake (g), body weight (g), core body temperature ($^{\circ}\text{C}$), and oxygen consumption (ml/hr/g). All the dependent variables were measured repeatedly. As a result, mixed between- and within-subjects experimental designs were produced. Taken together, Study I and Study II gave rise to a 2 X 2 X 2 X 3 (Phenotype X Pretreatment X Drug X Time) design for food intake and body weight, and a 2 X 2 X 2 X 12 (Phenotype X Pretreatment X Drug X Time) design for body temperature; and Study III lead to a 3 X 12 (Drug X Time) mixed factorial design with repeated measurements.

Method

Animals

Genetically obese (Mus musculus, C57BL/6J, lep^{ob}/lep^{ob}, N = 63) and lean (Mus musculus, C57BL/6J, +/?, N = 48) mice (4 - 5 weeks old) were purchased from The Jackson Laboratory, Bar Harbor, ME, USA. Mice were individually housed in clean polypropylene nesting cages (approximately 28 X 17 X 12 cm) with ample wood chip bedding, Nestlets™ cotton-fibre pads for hygienic nest construction, and a cardboard paper tube for additional gnawing and play opportunities. Food (Prolab animal diet, which consists of 51% carbohydrate, 22% protein, 11% moisture, 6% ash, 5% fat, and 5% fibre, provides a metabolizable energy of 3.1 Kcal/g, PMI Feed Inc., St. Louis, MO) and water were continuously available. The colony room was maintained at $22 \pm 2^{\circ}\text{C}$ with a reversed 12-hr day-night cycle (lights on at 2000 hr) and 40-60% relative humidity. All animals were acclimatized to this day-night cycle for at least 4 weeks. Mice were weighed, gently handled for 2 min per day for at least 2 weeks before surgery, and given 3 - 4 weeks housing facility adjustment. The average body weight during the experiment was 53.5 - 54.9 g for obese mice and 27.4 - 28.6 g for lean mice, respectively.

Surgical Procedures

At 10 - 11 weeks of age, stereotaxical implantation of a unilateral 22-ga. cannula (Plastics One Inc., Roanoke, VA, USA) was done under pentobarbital anesthesia (5.0 mg/100g body weight). Once mice were anesthetized, aseptic surgery was done in two phases. Phase 1 included the chronic implantation of an indwelling thermister into the abdominal cavity. The abdominal area was shaved and cleaned by successive swabbings of 70% ethanol and sterile physiological saline. Under aseptic conditions, a longitudinal

incision on the midline at the anterior end of the abdomen was made, just long enough to allow insertion of a cold-sterilized telemetered AM transmitter (Mini-Mitter, Inc., Sunriver, OR, Models V-M, XM-FH). After the paraffin-insulated transmitter was put into the cavity, the incision of abdominal muscle was sutured (OO silk) and the incision of abdominal skin was closed with stainless steel wound clips (7.5 mm, Michel, Germany).

Phase 2 involved the unilateral implantation of a chronically indwelling ICV cannula and followed procedures described in Currie (1991). A 15-mm length of 22-ga. stainless steel guide cannula was stereotaxically implanted into the right ventricle with the following standard stereotaxic procedures. A Kopf stereotaxic instrument was fitted with a non-traumatic adaptor for mice constructed after a design described in Slotnick (1972). The interaural scalp hair of each mouse was clipped (Hair Clipper, Oster, No. 80), and the shaved scalp was swabbed successively with 70% ethanol and sterile saline. Because the midline suture is usually not closed in mice, the scalp was incised off-midline to expose the skull from the nasal bone to the occiput. Then, the scalp and the underlying fascia were retracted so that the bregmoidal and lambdoidal cranial sutures were clearly visible. Bregma and lambda were adjusted to the same dorsal-ventral readings by leveling the skull. The guide cannula tip was placed at the suitable point of anterior-posterior and lateral coordinates, before the location of entry was marked. The appropriate coordinates were 0.7 mm posterior to bregma, 1.5 mm lateral to the midline, and 2.0 mm ventral to the dorsal surface of the brain (Franklin & Paxinos, 1997). A medium dental burr was applied to drill through the skull in order to reveal the dura. Three additional holes were drilled in each of the remaining quadrants of the skull for securing stainless steel jeweler's screws, 2.0 mm in length. To place each screw into the skull, curved forceps and a small screw

driver were used. The screws were secured into the skull to a depth of 0.4 mm to prevent surgical trauma and cortical damage. A 23-ga. sterile needle was used to pierce the dura before cannula insertion. The guide cannula and the injection cannula were inserted into the lateral ventricle together. When the cannula was correctly implanted, sterile physiological saline flowed down the attached tubing, which connected to the injector cannula, into the ventricle. Dental acrylic cement (Jet Acrylic, Lang Dental Mfg. Co., Inc., Chicago, IL) was applied to the clean and dry skull and over the screws in a thin layer at the beginning, then followed by layers of acrylic cement to completely secure the cannula assembly. The incision was closed via OO silk suture. The injection cannula was removed and replaced by a stainless-steel stylet to cover each cannula.

Immediately following surgery, a local analgesic (2% Xylocaine hydrochloride, Astra Pharmaceuticals, Canada Ltd., Mississauga, Ontario) and an antibacterial cream (0.2% Furacin, Austin, Division of Vetoquinol Canada Inc., Joliette, Quebec) were topically applied to the sites of both abdominal and cranial incisions, and a broad spectrum antibiotic (Ethacilin, Rogar STB, 45000 units) was injected intramuscularly. Mice were weighed and housed individually in their original cages with cotton pads under their bodies. Mice had at least 6 days to recover from the surgery, while they continued to be handled every day postsurgically for 2 min. The handling procedure initially included (a) removing and reinserting the inner stylet from the cannula; (b) handling the mouse's body; and (c) weighing the mouse. However, step (a) was discontinued to minimize dislodging the cannula assembly before the completion of the injections and to, thereby, reduce the total number of mice in the studies. During the recovery period, slightly powdered food, as well as standard lab chow, and water were available continuously to all animals. Body weights,

temperatures, and food and water intakes were monitored to ensure recovery from surgery.

Apparatus

Food intake and body weight measurements. Mice were weighed to the nearest 0.01 g on a digital balance (Mettler PB300) and then placed in clean cages with a piece of paper towel under a small amount of cotton bedding pads and a preweighed quantity of fresh food (about 3 pellets) in a metal lid container with an internal diameter of 6.0 cm. The container was placed in a bigger metal container with an internal diameter of 8.5 cm and a height of 5.0 cm. Food intakes were measured by a digital balance (Mettler PM480) to the nearest 0.001 g.

Telemetric monitoring of core temperature. A 1.5g paraffin-coated AM transmitter was implanted into the peritoneal cavity of each mouse. The transmitters emit an AM pulse rate, which is linearly proportional to the animal's core temperature. Before the implantation, each transmitter was calibrated by establishing its pulse rate, while it was immersed in a temperature-controlled water bath (Model B-1, Lauda-Thermostat, Germany) at temperatures ranging from 30-40°C. Because the temperature sensor of the water bath was 15 cm away from the location of the transmitters, the water temperature at the transmitter location was about 1.5 - 2.5 °C lower than the temperature sensor point. Therefore, the recorded core temperatures were about 1.5 - 2.5 higher than the true body temperature of experimental animals. The telemetry pulses of high frequency transmitters were captured by an AM receiver (Data Quest III, Model R1010) and then passed through an electronic circuit, which amplifies and records the pulses on an IBM personal computer. The telemetry pulse of low frequency transmitters were monitored by an AM radio and counted by the experimenter.

Metabolic thermal testing. The metabolic chamber was located in a cabinet in which the temperature was kept at 23°C. A schematic representation of the apparatus is illustrated in Figure 5. The metabolic chamber is a 15-cm long glass bottle with an internal diameter of 8.5 cm (i.e., a volume of 900 cc). The rubber stopper (4.3 X 4.3 X 2.4 cm) provides an air-tight seal for the metabolic chamber and contains air inlet and outlet ports and an exit for temperature probes. Dry air passes through the chamber at a rate of 200 - 400 ml/min. Expired gases are dried by passage through Drierite (CaCO₃) and analyzed for oxygen content by a Beckman OM-11 Oxygen Analyzer. The gas analyzers are calibrated weekly and daily. The calculation of oxygen consumption (VO₂) is according to the following formula:

$$VO_2 = \frac{(V_{ATP}/60) * (P_b/760) * [273/(273+T^{\circ}C)] * [(20.94 - OM11/100)]}{\text{Body weight (g)}} \text{ (ml/hr/g)},$$

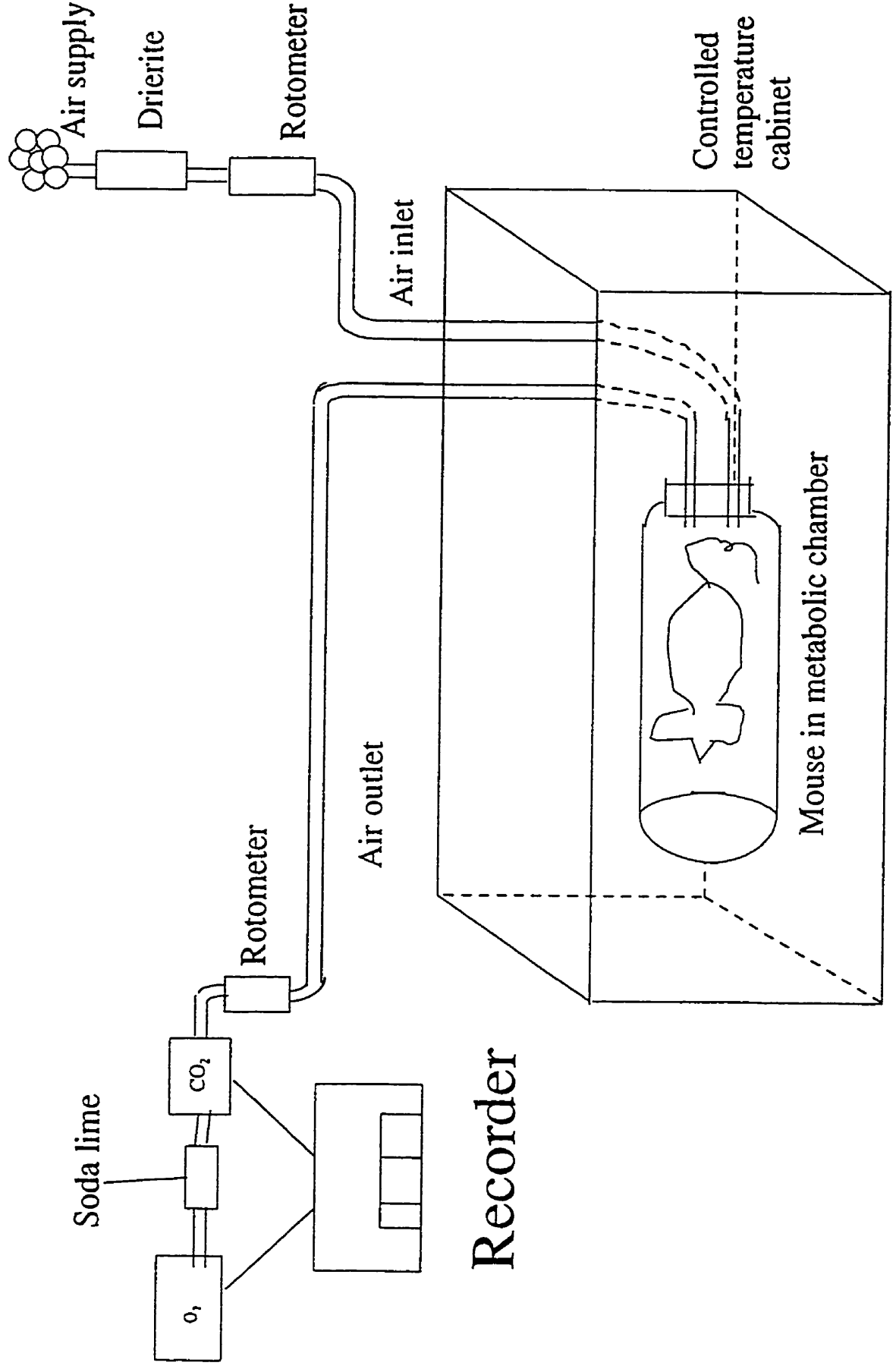
where V_{ATP} is the rate of air flow through the metabolic chamber in ml/hr; P_b is the barometric pressure in mm Hg; and OM11 is the percentage of oxygen consumption as expressed by the Beckman medical gas analyzer (OM11).

Chemicals

The injection vehicle is 0.9% (0.15 M) sterile physiological saline. Synthetic rat/human CRF₍₁₋₄₁₎ (Sigma-Aldrich, Mississauga, Ontario) was dissolved in physiological saline 1 hr before ICV administration and kept on ice. CRF was administered at a dose of 1.0 µg/µl/mouse. CRF antagonist, α-helical CRF₉₋₄₁ (Sigma-Aldrich, Mississauga, Ontario) was prepared as follows: 0.5 mg of α-helical CRF₉₋₄₁ was dissolved into 50 µl of physiological saline. Leptin (R&D Systems, Inc., Minneapolis, MN) was dissolved in

Figure Caption

Figure 5. Schematic representation of the apparatus used for metabolic testing in mice.



sterile physiological saline at a dose of 1.0 $\mu\text{g}/\mu\text{l}$ /mouse and stored in aliquots at -80°C .

Leptin was taken out of the freezer and defrosted at 1 hr before central injection.

Procedure

For the duration of the experiment mice were housed in wire-covered polypropylene cages with a piece of paper towel under three pieces of cotton bedding pads. After a 6-day postoperative recovery period, 2 more days of handling and basal data collection were carried out. Mice were weighed to the nearest 0.01 g on a digital balance and then placed in clean cages with a piece of paper towel under a small amount of cotton bedding pads and with preweighed fresh food in a metal lid container, which was placed in a bigger metal container to avoid food chips spilling out. When food intake was measured, the food spillage on the paper towel and feces in the food container were carefully picked up. A preweighed quantity of fresh food (about 3 pellets) was provided at the beginning of each test. Before central injection, mice were weighed, and weights within phenotype rank-ordered from highest to lowest. Mice within phenotypes were then assigned to one of the four drug administration groups (Study I & II) or of three drug injection groups (Study III) systematically according to their body weights to counterbalance the differences in initial body weights within each phenotype. Mice received two ICV injections, one for pretreatment and the other for drug treatment, right before lights off. The latency between the two injections was 30 min. Basal food intake and body weight were recorded before lights off, and after dark at 2 hr, 4 hr, and 23 hr for 2 days. Core temperature (T_c) was measured every 20-30 min for 4 hr (Studies I and II) and oxygen consumption was monitored at every 5 min for 1 hr (Study III with basal oxygen consumption data collected 2 days before the drug test).

In Studies I and II, mice were placed back into their own cages with preweighed fresh food after ICV injections. Food intake and body weight were monitored at 2 hr, 4 hr, and 23 hr. Body temperature was recorded according to the AM pulse rate of transmitters, which were captured by either an AM-FM radio or an AM receiver (Data Quest III, Model R1010), at every 20-30 min for 4 hr. In Study III, mice were placed in an oxygen testing chamber individually to measure the oxygen consumption, which was monitored by a Beckman OM-11 Oxygen Analyzer, at every 5 min for 1 hr. After all the experiments, mice were over-dosed with pentobarbital (1cc, IP, 5.0 mg/100 g mouse weight). After each mouse succumbed, injection of 10 μ l of India ink into the cannula was applied to verify the placement of ICV cannula at the right lateral ventricle. Particles of ink in the cerebral ventricles were revealed by postmortem histological examination.

Study I -- Leptin. The acute effects of leptin and the interaction of leptin and α -helical CRF on food intake, body weight, and body temperature in lep^{ob}/lep^{ob} and $+/?$ mice.

Thirty-five obese mice and 35 lean mice were employed in Study I and exposed to one of the four drug treatments. Three obese and 10 lean mice were excluded from the experiments and data analysis because their cannulae became dislodged during the central injection or their body weight did not recover to the pre-operation body weight. After basal data collection, obese ($N = 32$) and lean ($N = 25$) mice were assigned to one of the four ICV drug administrations, with a systematic order of their body weights to counterbalance the differences in initial body weights within each phenotype. The four drug treatments are (a) ICV 1 μ l vehicle (i.e., physiological saline, same for all vehicles) — 1 μ l vehicle, (b) 1 μ l vehicle — 1.0 μ g/ μ l/mouse of leptin, (c) 10.0 μ g/ μ l/mouse of α -helical CRF — 1 μ l vehicle, and (d) 10.0 μ g/ μ l/mouse of α -helical CRF — 1.0 μ g/ μ l/mouse of leptin. Each

ICV injection took 20 s. After each drug treatment, food intake and body weight were monitored at 2, 4, and 23 hr. Core temperatures were measured at every 20 - 30 min for 4 hr.

Study II -- CRF. The acute effects of CRF and the interaction of CRF and α -helical CRF on food intake, body weight, and Tc in lep^{ob}/lep^{ob} and $+/?$ mice. Thirty-five obese and lean mice, most of them from Study I, were used in Study II. Mice from Study I rested for at least 3 days. Five obese and 13 lean mice were excluded from the experiment because their cannulae were dislodged during the central injection. Both obese ($N = 31$) and lean ($N = 22$) mice were assigned to one of the four drug administration groups systematically according to their rank-ordered body weights to counterbalance the differences in initial body weights within each phenotype. The four drug administration groups are: (a) 1 μ l vehicle (physiological saline) — 1 μ l vehicle, (b) 1 μ l vehicle — 1 μ g/ μ l/mouse of CRF, (c) 10.0 μ g/ μ l/mouse of α -helical CRF — 1 μ l vehicle, (d) 10.0 μ g/ μ l/mouse of α -helical CRF — 1 μ g/ μ l/mouse of CRF. As in Study I, each ICV drug administration was given right before lights off, and food intake, body weight, and Tc were monitored the same as in Study I. This study would reveal the effects of central CRF on the energy balance of obese and lean mice. This study partially and systematically replicates the investigation of Drescher et al. (1994), but avoids some of their procedural pitfalls.

Study III -- Oxygen consumption. The effects of leptin and CRF antagonist on oxygen consumption in lep^{ob}/lep^{ob} mice. Twenty-four obese mice were used in Study III. Five mice were excluded from experiments due to unsuccessful central administration (cannulae dislodged). Mice ($N = 19$) were placed individually into the metabolism testing chamber for 1 hr for 3 consecutive days to adapt to the testing environment. On the third

adaptation day, basal oxygen consumption was recorded at every 5 min for 1 hr. There were three levels of drug treatments: (a) 10.0 $\mu\text{g}/\mu\text{l}$ /mouse of α -helical CRF — 1 μl vehicle, (b) 1 μl vehicle — 1 $\mu\text{g}/\mu\text{l}$ /mouse of leptin, (c) 10.0 $\mu\text{g}/\mu\text{l}$ /mouse of α -helical CRF — 1 $\mu\text{g}/\mu\text{l}$ /mouse of leptin. Mice were randomly assigned into one of these three groups according to systematic order of their body weights to counterbalance the differences in initial body weights within each phenotype. After the drug administration, Oxygen data were collected every 5 min for 1 hr.

Statistical Analysis

Both adjusted degrees of freedom univariate analysis of variance (ANOVA) and the multivariate analysis of variance (MANOVA) for repeated measurements are robust in balanced split-designs (Keselman & Keselman, 1988; Looney & Stanley, 1989). If sample sizes are unequal, the robustness of MANOVA depends on “the homogeneity of covariance matrices across the levels of the between-subjects grouping factor(s)” (Keselman, Carriere, & Lix, 1993, p. 306). Only SPSS MANOVA procedure provides the Box’s M test for homogeneity of variance-covariance matrices (SPSS Inc., 1997). Tabachnick and Fidell (1996) stated, “If sample sizes are equal, robustness of significance tests is expected. Disregard the outcome of Box’s M test, a notoriously sensitive test of homogeneity of variance-covariance matrices. However, if sample sizes are unequal and Box’s M test is significant at $p < 0.001$, then robustness is not guaranteed” (p. 382). All the dependent variables were checked for the homogeneity of variance-covariance across levels of grouping variables. Overall, most homogeneity tests (14 out of 16 tests) were satisfied at $p < 0.001$ level (see Appendix A). Moreover, the sample sizes of the current study are only slightly unequal. Therefore, MANOVA was employed as a statistical analysis method for

the three studies.

The statistical analysis approach was generally a repeated measures MANOVA with Phenotype (obese and lean), Pretreatment (Vehicle and α -helical CRF), and Drug (CRF and Leptin) as between-subjects variables, and Time (2, 4, and 24 hr) for food intake and body weight measurements, or Time (every 20-30 min for 4 hr and at 23 hr) for body temperature measurements, or Time (10, 20, 30, 40, 50, 60 min) for O_2 measurements, as within-subjects repeated measures variables. Significant effects were probed with t test and overall level of significance was set according to Bonferroni procedure.

General linear regression analyses were also utilized to summarize the contributions of pretreatment and drug treatment on body weight change. The regression analysis was not used as a major statistical analysis but as a further summation of the factors involved in the body weight change. Because general linear regression analysis is sensitive to the normality, linearity, and homoscedasticity assumptions, the "PROC UNIVARIATE" procedure and residuals scatterplots from SAS were used to check the above assumptions. Only a few dependent variables, (a) body weight change at 23 hr of Vehicle - Vehicle group and α -helical CRF - Vehicle group of the total 8 groups for Study I (Leptin Study), (b) body weight change at 23 hr of Vehicle - Vehicle group, and (c) body weight change at 2 hr of α -helical CRF - CRF group of the total 8 groups for Study II (CRF Study), violated the normality assumption. The data for these dependent variables also violated homoscedasticity assumptions. Accordingly, the regression analysis results for these variables should be interpreted with caution. All statistical analyses were performed with SAS statistical software (SAS Institute, 1990).

Results

Cumulative Food and Water Intake

Food and water intake in a free-feeding pattern were monitored right after the dark cycle started. Cumulative food and water intake at 2, 4, and 23 hr on pre-injection day (baseline day) and vehicle - vehicle injection day (adaptation) are shown in Table 5. Cumulative food intake, $F(2,63) = 300.74$, $p < .001$, and water intake, $F(2,59) = 41.131$, $p < .001$, were gradually increased over time in both obese and lean mice. Obese mice were hyperphagic over 23 hr (Time X Phenotype), $F(2,63) = 4.773$, $p < .05$. There were no significant differences in cumulative water intake between obese and lean mice. However, obese mice drank less water at 4 hr than lean mice did, $F(1,63) = 4.21$, $p < .05$.

To take into account the differences in initial body weight, the percentage cumulative food intake was calculated. As a percentage of initial body weight, percentage cumulative food intake was enhanced over time, Time effect, $F(2,63) = 344.367$, $p < .001$; so was percentage cumulative water intake, Time effect, $F(2,59) = 50.338$, $p < .001$ (see Table 5). Due to the fact that obese mice are heavier than lean mice, the percentage of food intake, Time X Phenotype, $F(2,63) = 15.249$, $p < .001$, and the percentage of water intake, Time X Phenotype, $F(2,59) = 9.394$, $p < .001$, of obese mice were less than those of lean mice. No significant differences in percentage food or water intake were found between baseline data and vehicle injection day data. Therefore, central administration alone did not induce a significant stress to animals regarding food and water intake.

Study I: Leptin and its interaction with α -helical CRF. As depicted in Figure 6, central administration of leptin decreased cumulative food intake, Time X Drug Treatment, $F(2,48) = 18.711$, $p < .001$, in both obese and lean mice. Animals with leptin

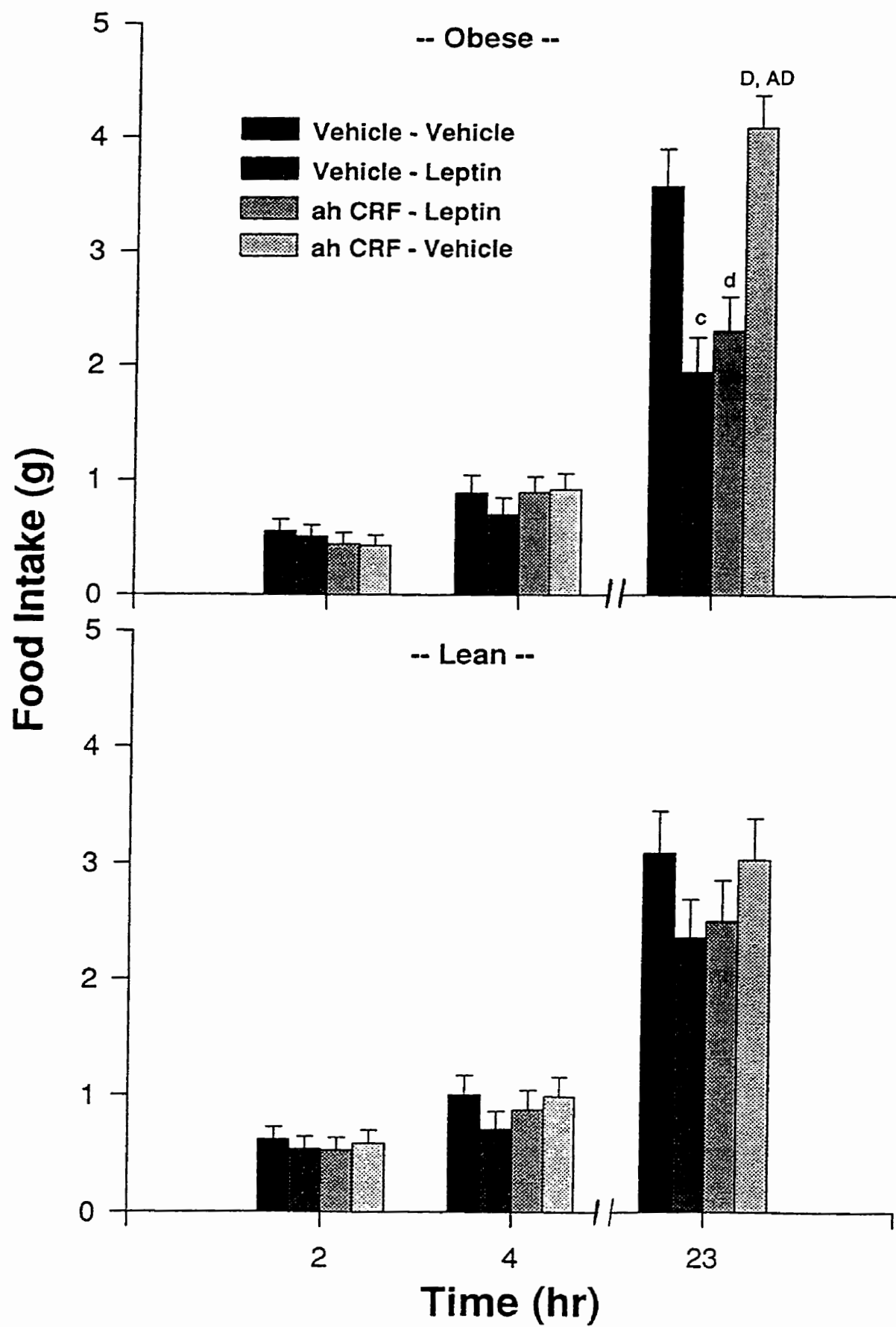
Table 5
Mean ($\pm$ SE) Cumulative Food and Water Intake and Body Weight Change of Obese and Lean Mice at Pre- and Vehicle Injection Day

Phenotype	Day	Time after dark			
		2 hr	4 hr	23 hr	
Obese					
Food intake (g)	Pre-injection	0.50 (0.06)	1.02 (0.07)	4.14 (0.17) ^b	
	Vehicle Injection	0.55 (0.13)	0.89 (0.13)	3.58 (0.34) ^a	
Food Intake (%)	Pre-injection	0.96 (0.17) ^{c, A}	1.93 (0.19) ^{c, C}	7.76 (0.45) ^{c, C}	
	Vehicle Injection	1.03 (0.35) ^{b, A}	1.63 (0.38) ^{c, C}	6.53 (0.92) ^{b, B}	
Water Intake (cc)	Pre-injection	1.29 (0.25)	2.52 (0.31) ^a	8.32 (1.33)	
	Vehicle Injection	1.67 (0.53)	2.33 (0.68) ^a	6.25 (2.88)	
Water Intake (%)	Pre-injection	2.44 (0.68) ^c	4.74 (0.87) ^{c, A}	15.77 (2.70) ^{a, A}	
	Vehicle Injection	3.04 (1.46) ^{c, A}	4.25 (1.87) ^{c, C}	11.39 (5.84)	
Body Weight Gain (g)	Pre-injection	0.13 (0.12)	0.12 (0.13)	0.12 (0.13)	
	Vehicle Injection	0.64 (0.21)	0.73 (0.24)	0.73 (0.24)	
Body Weight Gain (%)	Pre-injection	0.28 (0.34)	0.25 (0.43)	0.24 (0.53)	
	Vehicle Injection	1.19 (0.62)	1.34 (0.78)	1.12 (0.97)	
Lean					
Food Intake (g)	Pre-injection	0.57 (0.07)	0.98 (0.07)	3.36 (0.18)	
	Vehicle Injection	0.62 (0.14)	1.01 (0.14)	3.09 (0.37)	
Food Intake (%)	Pre-injection	2.01 (0.18)	3.46 (0.20)	11.88 (0.48)	
	Vehicle Injection	2.21 (0.38)	3.57 (0.41)	10.99 (0.99)	
Water Intake (cc)	Pre-injection	1.76 (0.27)	3.20 (0.34)	7.09 (1.44)	
	Vehicle Injection	2.33 (0.53)	3.83 (0.68)	7.75 (2.88)	
Water Intake (%)	Pre-injection	6.28 (0.73)	11.47 (0.94)	25.31 (2.92)	
	Vehicle Injection	8.30 (1.46)	13.65 (1.87)	27.54 (5.84)	
Body Weight Gain (g)	Pre-injection	0.31 (0.12)	0.19 (0.13)	0.19 (0.13)	
	Vehicle Injection	0.60 (0.23)	0.58 (0.26)	0.58 (0.26)	
Body Weight Gain (%)	Pre-injection	1.14 (.34)	0.71 (0.43)	0.11 (0.53)	
	Vehicle Injection	2.16 (0.67)	2.11 (0.85)	3.01 (1.04)	

^a $p < .05$, ^b $p < .01$, ^c $p < .001$ versus food intake of the same treatment in lean mice; ^A $p < .05$, ^B $p < .01$, ^C $p < .001$ versus Vehicle Injection data of lean mice.

Figure Caption

Figure 6. Effect of central injection of leptin on cumulative food intake in genetically obese and lean mice. Leptin decreased food intake at 23 hr in both obese and lean mice. CRF antagonist (α -helical CRF, 10 μ g/ μ l) did not block leptin's effect on food intake. Bars represent mean cumulative food intake; vertical lines depict standard errors of the means. (^c $p < .01$, ^d $p < .001$ compared to vehicle - vehicle group; ^D $p < .001$ compared to vehicle - leptin group; ^{AD} $p < .001$ compared to α -helical CRF - leptin group.)



as the drug treatment consumed less [Mean ($\bar{M}$) = 2.275 g] than animals with vehicle as the drug treatment consumed ($\bar{M}$ = 3.448 g) at 23 hr. Particularly, leptin suppressed cumulative food intake more in obese ($\bar{M}$ = 2.119 vs. $\bar{M}$ = 3.832) than in lean mice ($\bar{M}$ = 2.431 vs. $\bar{M}$ = 3.063), Time X Phenotype X Drug treatment, $F(2, 48) = 7.194$, $p < .01$. Neither the two-way interaction of pretreatment and drug treatment nor the 3-way interaction of phenotype, pretreatment, and drug treatment was found. That is, the CRF antagonist, α -helical CRF, did not block the effect of leptin on cumulative food intake.

Analysis of the effect of leptin on cumulative food intake, as a percentage of initial body weight, provided a similar picture as leptin on absolute cumulative food intake (see Figure 7). Because the average body weight of obese mice is much heavier than that of lean mice, the cumulative food intake of obese mice as a percentage of initial body weight was lower than that of lean mice, Time X Phenotype, $F(2, 48) = 36.865$, $p < .001$. Irrespective of pretreatment, leptin suppressed the percentage food intake successfully at 23 hr, Time X Drug Treatment, $F(2, 48) = 23.062$, $p < .001$. Also, leptin seemed to suppress food intake more in obese than in leans, Time X Phenotype X Drug Treatment, $F(2, 48) = 3.002$, $p = .06$. α -helical CRF alone did not affect percentage food intake in obese and lean mice. However, leptin with pretreatment of α -helical CRF did not successfully decrease cumulative food intake in lean mice at 23 hr, although it decreased the percentage food intake in obese mice (multiple comparison least square means, $ps < .05$).

Although obese mice consumed less water than lean mice did, Time X Phenotype, $F(1, 41) = 7.66$, $p < .05$, over the 23-hr period, leptin decreased cumulative water intake (see Figure 8) and percentage water intake in both obese and lean mice, Time X Drug Treatment, $F(2, 46) = 9.498$, $p < .001$, and $F(2, 46) = 6.321$, $p < .01$, respectively.

Figure Caption

Figure 7. Mean percentage cumulative food intake ($\pm$ SE) at 2, 4, and 23 hr after central administration of leptin in genetically obese and lean mice. Leptin elicited a significant decrease in percentage cumulative food intake (to initial body weight) at 23 hr post-injection, particularly in obese mice, although percentage food intake was also decreased in lean mice. α -helical CRF did not block leptin's effect on percentage food intake. (^b $p < .05$, ^c $p < .01$ compared to vehicle - vehicle group; ^c $p < .01$, ^D $p < .001$ compared to vehicle - leptin group; ^{AD} $p < .01$ compared to α helical CRF - leptin group.)

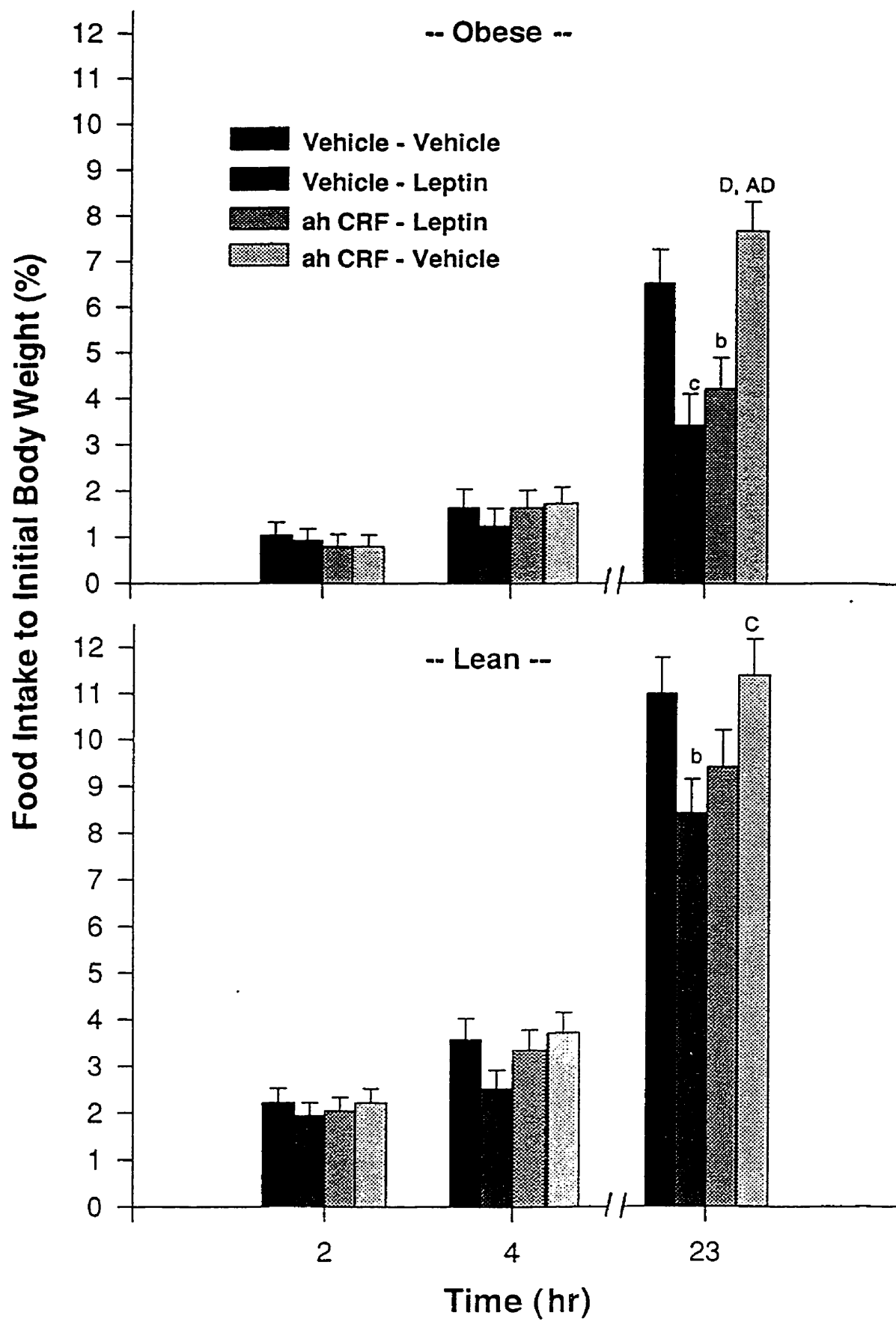
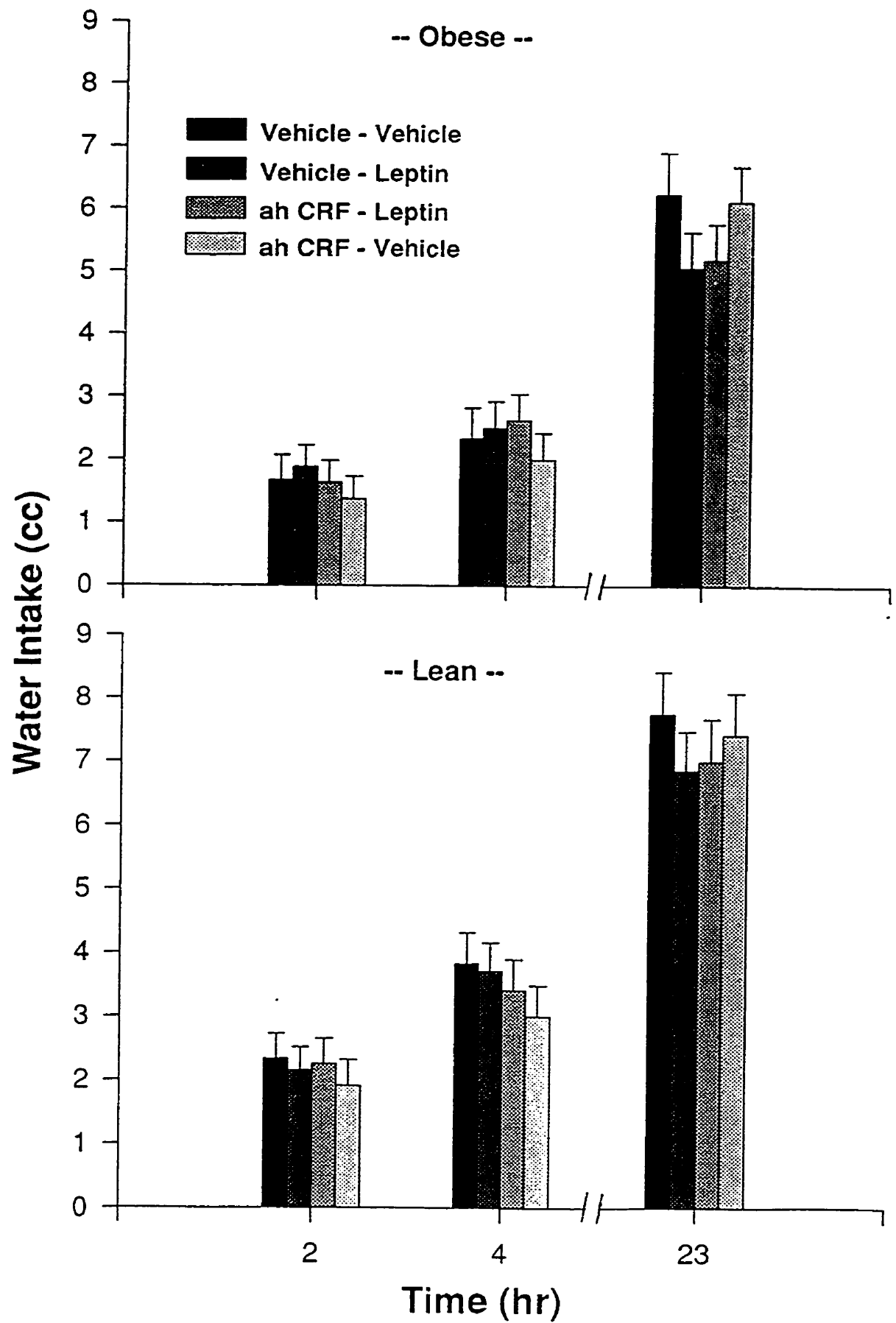


Figure Caption

Figure 8. Mean cumulative water intake ($\pm$ SE) at 2, 4, and 23 hr after ICV injection of leptin in genetically obese and lean mice. Leptin decreased water intake at 23 hr in obese mice. This effect was not blocked by α -helical CRF, and α -helical CRF alone had no effect on water intake in both obese and lean mice.



α -helical CRF alone did not influence water intake, and also did not block leptin's effect on water intake in obese and lean mice. Overall, obese mice drank less water than lean mice did, Phenotype, $F(1, 47) = 11.23$, $p < .01$.

Study II: CRF and its interaction with α -helical CRF. In contrast to leptin's effect on food and water intake, intraventricular administration of CRF had no effect on food intake or on percentage food intake in either obese or lean mice (see Tables 6 and 7). Obese mice consumed more food over a 23-hr free-feeding period, Time X Phenotype, $F(2, 44) = 11.193$, $p < .001$, than lean mice did. As shown in Figure 6, α -helical CRF alone did not affect food intake in obese and lean mice. CRF also had no effect on either cumulative water intake or percentage water intake in obese and lean mice (see Tables 6 and 7).

Mean Cumulative Body Weight Change

Overall, there was no difference in body weight gain between obese and lean mice in either pre-injection day or vehicle injection day (see Table 5). However, both obese and lean mice gained more body weight at the vehicle injection day, $F(1, 55) = 6.72$, $p < .05$, than at the pre-injection day across 23 hr (see Table 5). The same statistical result is found in analyzing body weight at each measurement time, as a percentage of initial body weight. Animals gained more weight on the vehicle injection day compared to baseline data (pre-injection day), $F(1, 55) = 5.11$, $p < .05$. Particularly, lean mice gained a larger percentage of their body weight on the vehicle injection day (multiple comparison least square means, $p < .05$).

Study I: Leptin and its interaction with α -helical CRF. Leptin reduced body weight gain in both obese and lean mice, Time X Drug Treatment, $F(2, 48) = 16.378$,

Table 6
Effects of CRF on Food and Water Intake, Body Weight Change in Obese and Lean Mice

Phenotype		Pretreatment	Drug Treatment	Time after ICV			
				2 hr	4 hr	23 hr	
Obese	Food intake (g)	Vehicle	Vehicle	0.55(0.11)	0.89(0.13)	3.58(0.30)	
		Vehicle	CRF	0.45(0.11)	1.00(0.13)	3.73(0.30)	
		α -helical CRF	Vehicle	0.43(0.09)	0.92(0.12)	4.09(0.26) _A	
		α -helical CRF	CRF	0.56(0.10)	0.98(0.12)	4.18(0.28)	
	Water Intake (cc)	Vehicle	Vehicle	1.67(0.47)	2.33(0.55)	6.25(0.81)	
		Vehicle	CRF	1.08(0.47)	2.83(0.55)	5.42(0.81)	
		α -helical CRF	Vehicle	1.38(0.41)	2.00(0.48)	6.13(0.70)	
		α -helical CRF	CRF	1.43(0.43)	2.86(0.51)	7.50(0.75)	
	Body Weight (g)	Vehicle	Vehicle	0.64(0.22)	0.73(0.23)	0.61(0.27)	
		Vehicle	CRF	0.15(0.22)	0.49(0.22)	0.52(0.27)	
		α -helical CRF	Vehicle	-0.00(0.20) _A	0.31(0.20)	0.91(0.24)	
		α -helical CRF	CRF	0.14(0.21) ^A	0.31(0.21)	0.80(0.25)	
	Lean	Food Intake (g)	Vehicle	Vehicle	0.62(0.11)	1.01(0.14)	3.09(0.32)
			Vehicle	CRF	0.67(0.14)	1.05(0.17)	3.42(0.40)
			α -helical CRF	Vehicle	0.59(0.11)	0.99(0.14)	3.03(0.32)
			α -helical CRF	CRF	0.80(0.11)	1.19(0.14)	3.52(0.32)
Water Intake (cc)		Vehicle	Vehicle	2.33(0.47)	3.83(0.55)	7.75(0.81)	
		Vehicle	CRF	1.75(0.58)	3.50(0.68)	7.38(0.99)	
		α -helical CRF	Vehicle	1.92(0.47)	3.00(0.55)	7.42(0.81)	
		α -helical CRF	CRF	2.25(0.47)	3.75(0.55)	7.75(0.81)	
Body Weight (g)		Vehicle	Vehicle	0.60(0.24)	0.58(0.25)	0.83(0.29)	
		Vehicle	CRF	0.53(0.03)	0.54(0.30)	0.74(0.36)	
		α -helical CRF	Vehicle	0.69(0.24)	0.56(0.25)	0.70(0.29)	
		α -helical CRF	CRF	0.84(0.24)	1.00(0.25)	1.18(0.29)	

^A $p < .05$ versus in lean mice; ^{*} $p < .05$ versus vehicle - vehicle of same phenotype.

Table 7

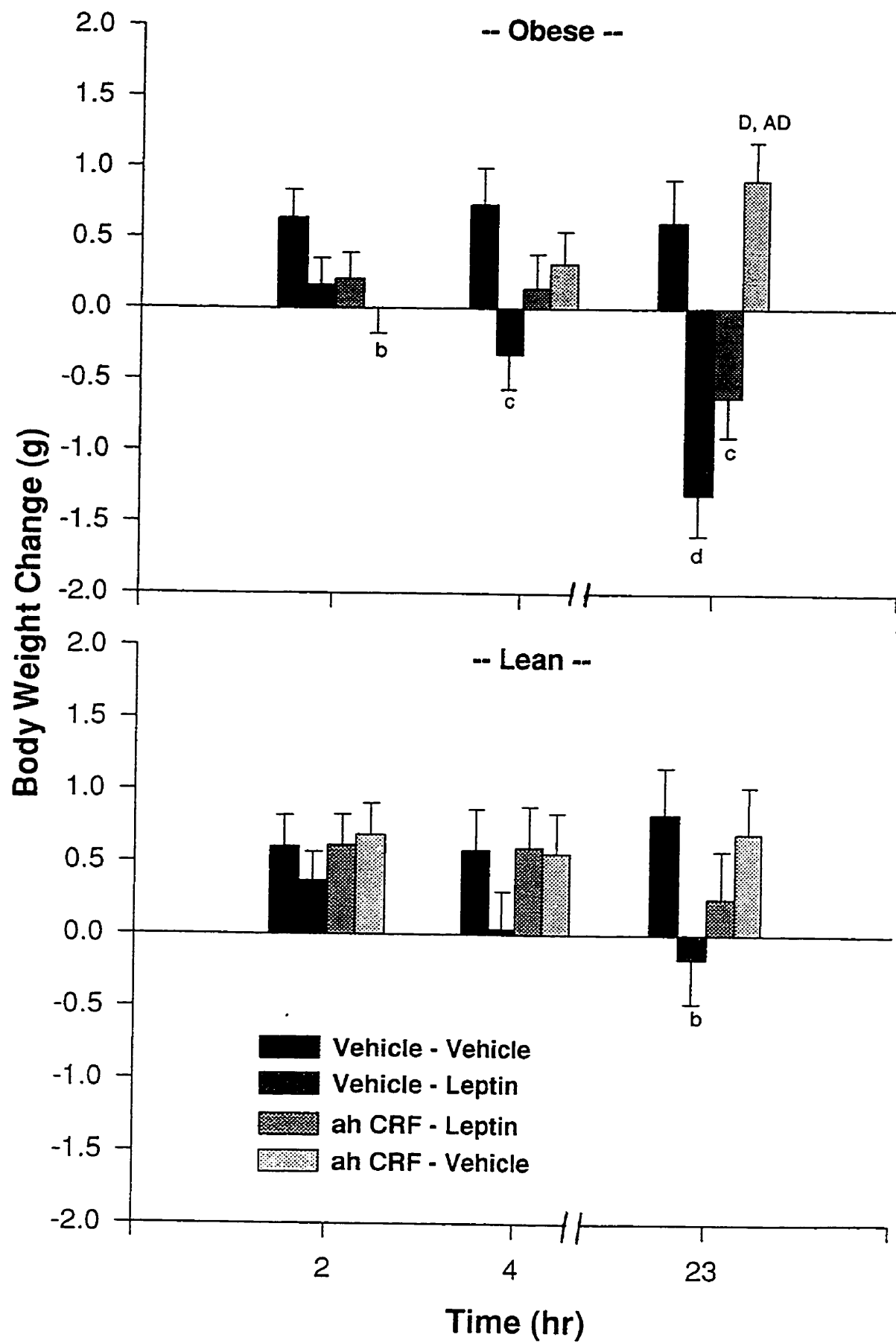
Effects of CRF on Percentage Food and Water Intake, Body Weight Change in Obese and Lean Mice

Phenotype	Pretreatment	Drug Treatment	Time after ICV		
			2 hr	4 hr	23 hr
Obese					
Food intake	Vehicle	Vehicle	1.03(0.27) ^A	1.63(.32) ^A	6.53(0.71) ^A
	Vehicle	CRF	0.86(0.27) ^A	1.88(.32) ^A	6.99(0.71) ^A
	α -helical CRF	Vehicle	0.80(0.24) ^A	1.73(.28) ^A	7.68(0.62) ^A
	α -helical CRF	CRF	1.08(0.26) ^A	1.88(.3) ^A	8.02(0.66) ^A
Water Intake	Vehicle	Vehicle	3.04(1.44) ^A	4.25(1.74) ^B	11.39(2.17) ^B
	Vehicle	CRF	2.04(1.44)	5.29(1.74) ^B	10.06(2.17) ^B
	α -helical CRF	Vehicle	2.60(1.24) ^A	3.75(1.51) ^B	11.65(1.88) ^B
	α -helical CRF	CRF	2.81(1.33) ^B	5.51(1.61) ^B	14.35(2.01) ^B
Body Weight	Vehicle	Vehicle	1.19(0.59)	1.34(0.65)	1.12(0.73)
	Vehicle	CRF	0.31(0.59)	0.94(0.65)	1.02(0.73)
	α -helical CRF	Vehicle	-0.03(0.52) ^A	0.56(0.57)	1.71(0.64)
	α -helical CRF	CRF	0.29(0.55) ^A	0.60(0.60) ^A	1.53(0.68) ^A
Lean					
Food Intake	Vehicle	Vehicle	2.21(0.29)	3.57(0.34)	10.99(0.76)
	Vehicle	CRF	2.43(0.36)	3.81(0.42)	12.42(0.94)
	α -helical CRF	Vehicle	2.20(0.29)	3.71(0.34)	11.4(0.76)
	α -helical CRF	CRF	3.02(0.29) ^a	4.48(0.34) ^a	13.17(0.76) ^a
Water Intake	Vehicle	Vehicle	8.30(1.44)	13.65(1.74)	27.53(2.17)
	Vehicle	CRF	6.43(1.76)	12.79(2.13)	26.81(2.66)
	α -helical CRF	Vehicle	7.20(1.44)	11.18(1.74)	27.96(2.17)
	α -helical CRF	CRF	8.62(1.44)	14.00(1.74)	28.98(2.17)
Body Weight	Vehicle	Vehicle	2.16(0.64)	2.11(0.07)	3.01(0.79)
	Vehicle	CRF	1.85(0.78)	1.97(0.85)	2.61(0.96)
	α -helical CRF	Vehicle	2.56(0.64)	2.13(0.07)	2.76(0.79)
	α -helical CRF	CRF	3.24(0.64)	3.83(0.07) ^a	4.47(0.79)

^A $p < .05$, ^B $p < .01$, versus in lean mice; ^a $p < .05$, ^b $p < .01$, versus vehicle - vehicle of same phenotype; ^{BB} $p < .10$, versus α -helical CRF-vehicle of same phenotype; ^{***} $p < .05$ versus Vehicle-CRF of the same phenotype.

Figure Caption

Figure 9. Effect of intraventricular injection of leptin on mean body weight change ($\pm$ SE) at 2, 4, and 23 hr postinjection in genetically obese and lean mice. Leptin suppressed body weight gain earlier and stronger in obese mice than in lean mice. α -helical CRF (α hCRF) did not block leptin's suppressive effect on body weight gain. Bars represent mean cumulative body weight change; vertical lines depict standard errors of the means. (^b $p < .05$, ^c $p < .01$, ^d $p < .001$ compared to vehicle - vehicle group; ^D $p < .001$ compared to vehicle - leptin group; ^{AD} $p < .001$ compared to α - helical CRF - leptin group.)



$p < .001$. Particularly, leptin suppressed body weight gain earlier and stronger in obese mice than in lean mice, Time X Phenotype X Drug Treatment, $F(2, 48) = 3.656$, $p < .05$ (see Figure 9). α -helical CRF alone did not influence body weight change in obese and lean mice. The pretreatment with α -helical CRF did not block the suppressive effect of leptin on body weight gain. However, there was no significant difference on body weight changes between the α -helical CRF - leptin group and the vehicle - vehicle group at 4 hr in obese mice and at 23 hr in lean mice. These results hinted that leptin did not successfully suppress the body weight gain in animals pretreated with α -helical CRF, although leptin alone resulted in a significant suppressive effect on body weight gain.

As a percentage of initial body weight, weight change was also decreased by leptin, Time X Drug Treatment, $F(2, 48) = 14.55$, $p < .001$ (see Figure 10). Irrespective of pretreatment or drug treatment, obese mice gained a smaller percentage of their initial body weights than lean mice did, Phenotype, $F(1, 49) = 12.20$, $p < .001$, due to the incomparable absolute weight gains against their heavier initial body weights. As described for absolute body weight change, α -helical CRF alone had no effect on percentage body weight change and also did not block leptin's effect on body weight change in obese mice.

Study II: CRF and its interaction with α -helical CRF. Overall, CRF did not reveal any suppressive effect on absolute body weight gain, although CRF tended to decrease body weight gain in obese mice (Figure 11). As depicted in Figure 11, CRF alone or with α -helical CRF showed a tendency to suppress body weight gain at 2 hr and 4 hr in obese mice, but not in lean mice. Re-analyzing the effect of CRF on body weight change, as a percentage of initial body weight (Table 7), did not reveal a suppressing effect of CRF on

Figure Caption

Figure 10. Mean percentage cumulative body weight change ($\pm$ SE) at 2, 4, and 23 hr postinjection of leptin in genetically obese and lean mice. Leptin significantly decreased percentage body weight gain in both obese and lean mice. α -helical CRF (α -h CRF) did not block leptin's effect on percentage body weight. (^b $p < .05$, ^c $p < .01$, ^d $p < .001$ compared to Vehicle - Vehicle group; ^D $p < .001$ compared to vehicle - leptin group; ^{AD} $p < .01$ compared to α -helical CRF - leptin group.)

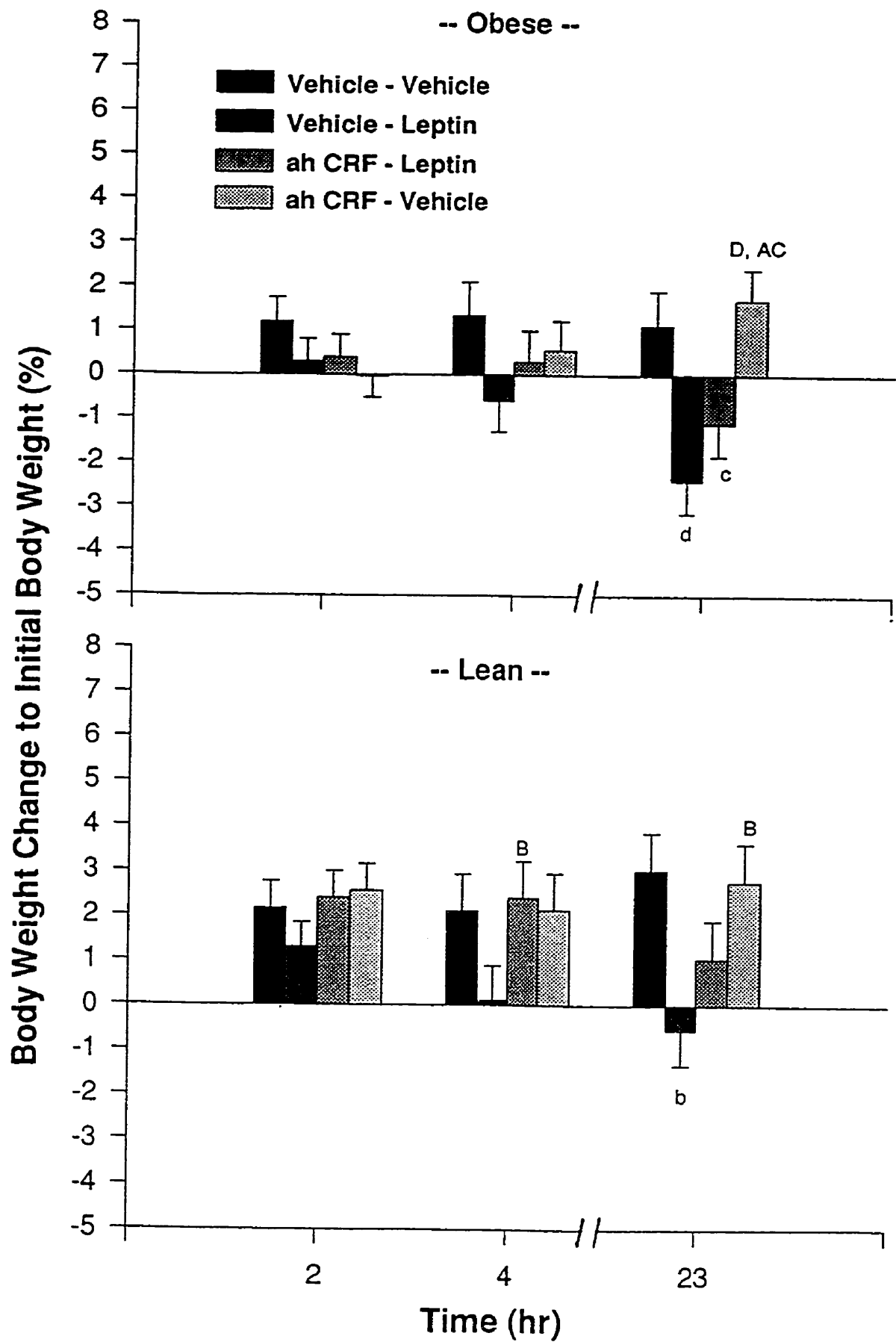
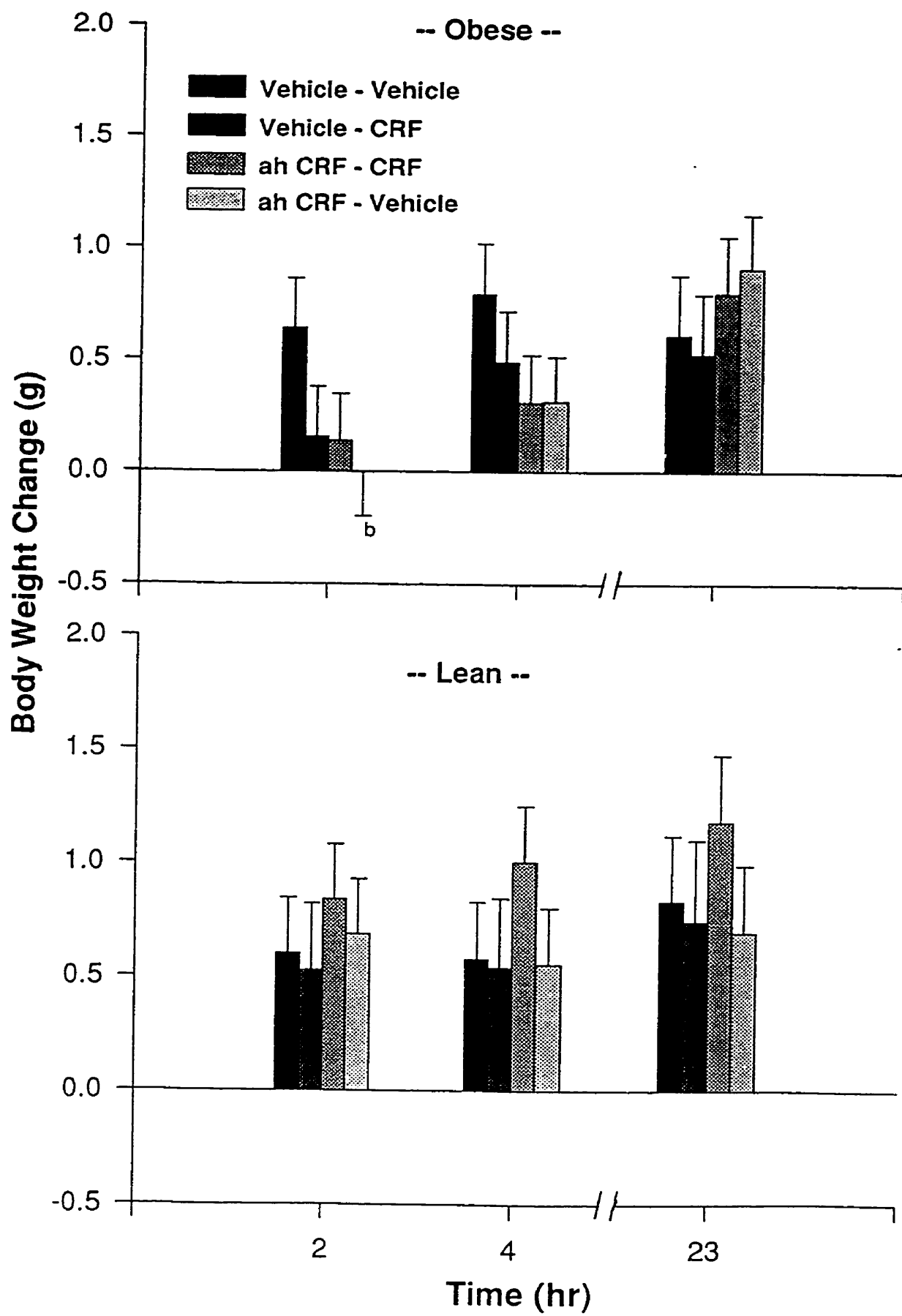


Figure Caption

Figure 11. Effect of intraventricular injection of CRF on mean body weight change ($\pm$ SE) at 2, 4, and 23 hr postinjection in genetically obese and lean mice. CRF did not successfully suppress body weight gain in either obese or lean mice. α -helical CRF (α -h CRF) alone abolished body weight gain only in obese mice at 2 hr. Bars represent mean cumulative body weight change; vertical lines depict standard errors of the means. (^b $p < .05$ compared to vehicle - vehicle group.)



body weight gain. However, obese mice with exogenous CRF, or α -helical CRF, or both, did not gain much body weight after the first 4 hr compared with lean mice ($-.03\% - .31\%$ vs. $1.85\% - 3.24\%$ at 2 hr, or $.56\% - .94\%$ vs. $1.97\% - 3.83\%$ at 4 hr), or compared with the vehicle-vehicle group ($-.03\% - .31\%$ vs. 1.19% at 2 hr, or $.56\% - .94\%$ vs. 1.34% at 4 hr).

Core Temperature and Percentage Temperature Change

As illustrated in Figure 12 and Figure 13, mouse's core temperatures followed the normal diurnal fluctuations, that is, higher temperatures in the dark period and lower temperatures in the light period, Time effect, $F(11, 26) = 49.37$, $p < .001$ (Figure 12, Study I -- Leptin), or $F(11, 16) = 12.36$, $p < .001$ (Figure 13, Study II -- CRF). As usual, obese mice had lower core temperatures than lean mice had over the 23-hr period, Phenotype, $F(1, 36) = 70.45$, $p < .001$ (Figure 12), or $F(1, 26) = 60.58$, $p < .001$ (Figure 13).

Study I: Leptin and its interaction with α -helical CRF. Leptin elevated core temperature significantly in both obese and lean mice, Time X Drug Treatment, $F(11, 26) = 2.96$, $p < .05$, across 23 hr (Figure 12). However, multiple comparisons of least square means revealed that leptin's enhancing effect on core temperature only occurred in obese mice ($ps < .01 - .10$) at 2.5 hr to 4 hr after central drug administration, but not in lean mice. α -helical CRF did not block leptin's enhancing effect on core temperature in either obese or lean mice.

To take into account the differences in initial core temperature, the percentage core temperature changes were calculated and analyzed. As a percentage of initial core temperature, temperature of obese mice changed more than that of lean mice across 23 hr, Time X Phenotype, $F(10, 27) = 3.76$, $p < .01$. Similar to the pattern in core temperature,

Figure Caption

Figure 12. Effect of leptin on core temperature at 30 min, 1 hr, 1 hr 20 min, 1 hr 40 min, 2 hr, 2 hr 20 min, 2 hr 40 min, 3 hr, 3 hr 30 min, 4 hr, and 23 hr after ICV injection in genetically obese and lean mice. Leptin increased core temperature in both obese and lean mice. CRF antagonist (α -h CRF, 10 μ g/ μ l) did not block leptin's enhancing effect on core temperature. (^b $p < .05$ compared to vehicle - vehicle group; ^B $p < .05$ compared to α -helical CRF - vehicle group.)

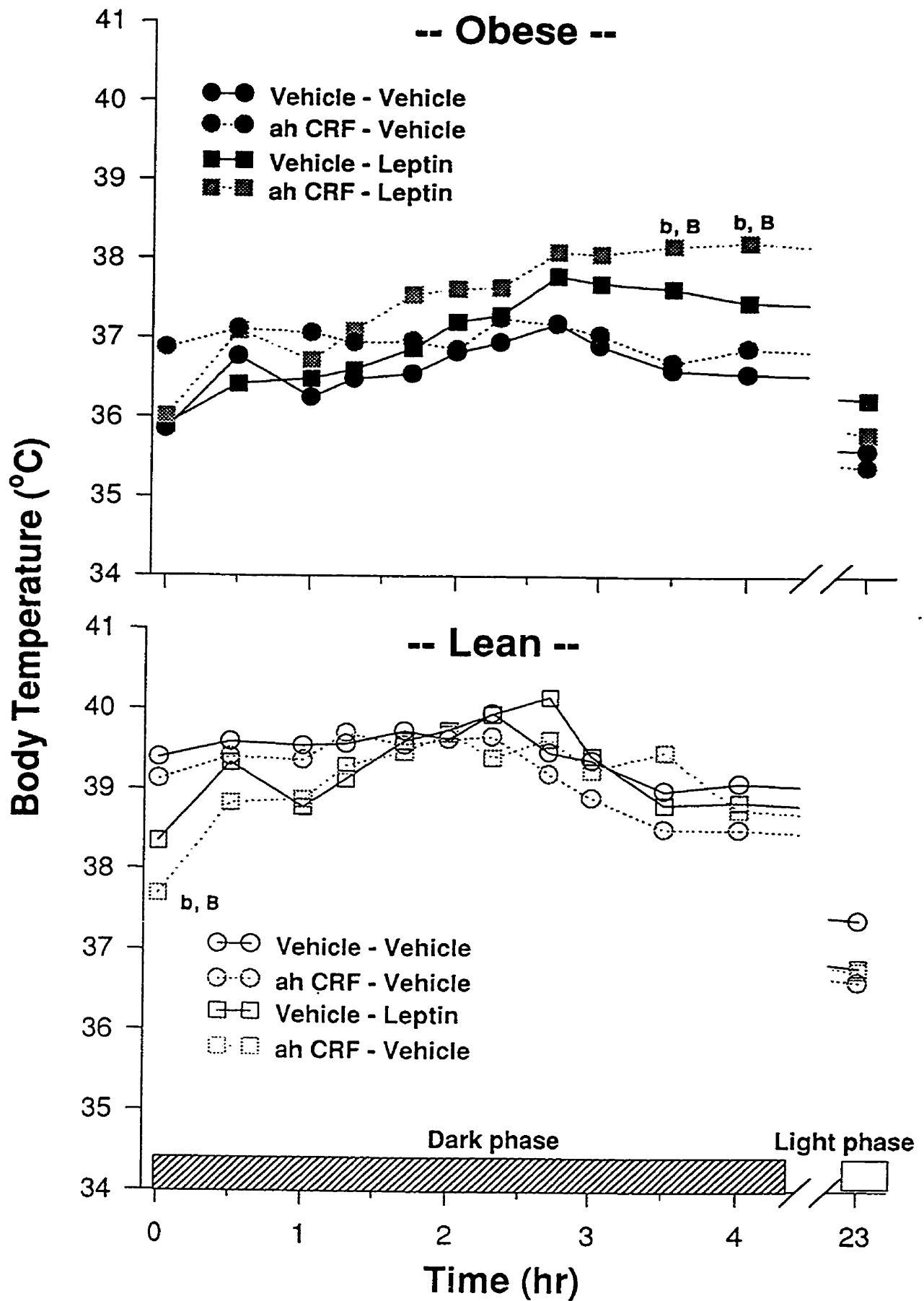


Figure Caption

Figure 13. Effect of central CRF on core temperature at 30 min, 1 hr, 1 hr 20 min, 1 hr 40 min, 2 hr, 2 hr 20 min, 2 hr 40 min, 3 hr, 3 hr 30 min, 4 hr, and 23 hr after injection in genetically obese and lean mice. CRF did not affect core temperature in either obese or lean mice. α -helical CRF (10 μ g/ μ l) alone or with CRF also had no effect on core temperature. (^b $p < .05$ compared to vehicle - vehicle group; ^B $p < .05$ compared to α -helical CRF - vehicle group.)

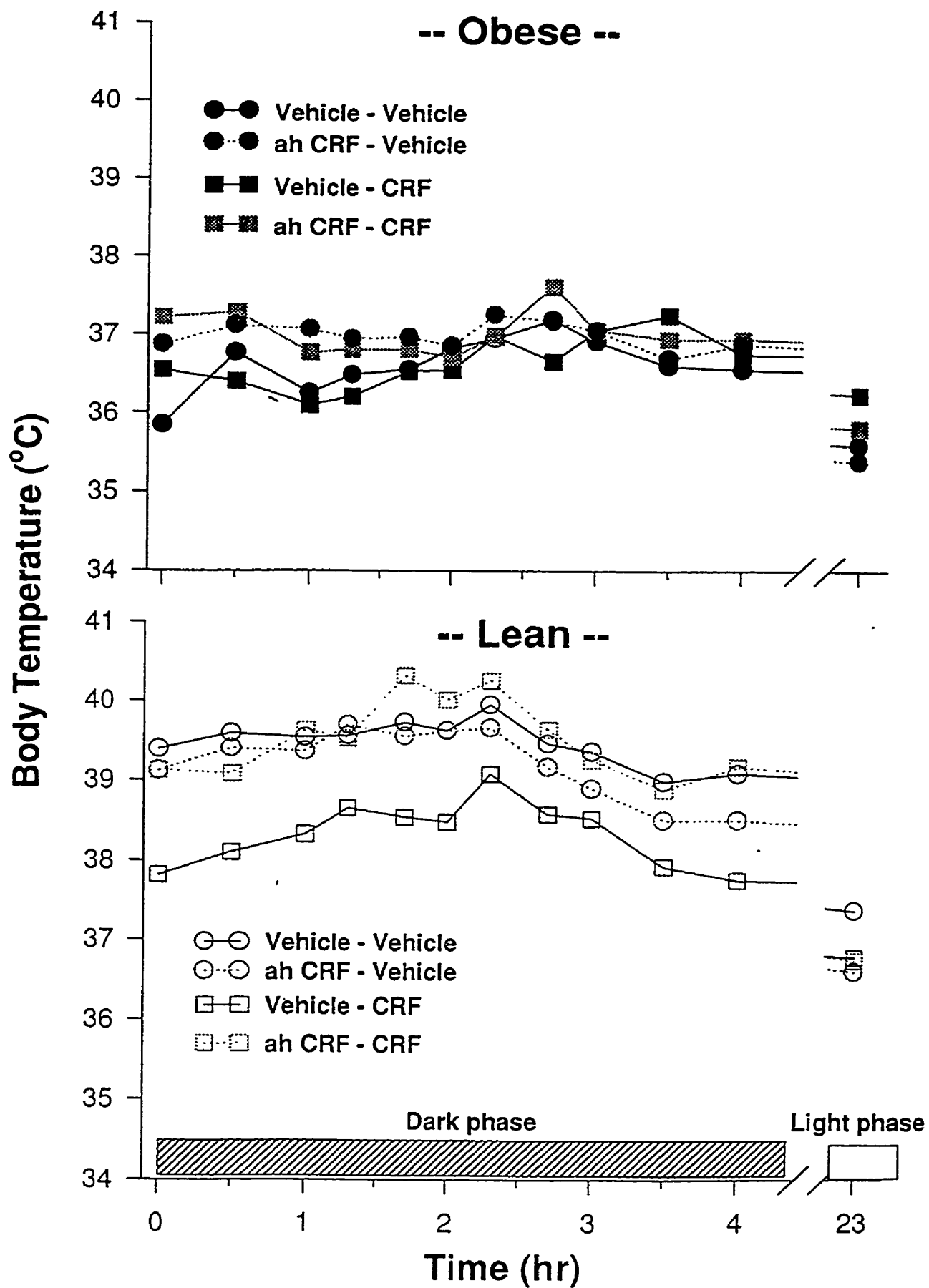


Figure Caption

Figure 14. Mean percentage core temperature change at 30 min, 1 hr, 1 hr 20 min, 1 hr 40 min, 2 hr, 2 hr 20 min, 2 hr 40 min, 3 hr, 3 hr 30 min, 4 hr, and 23 hr after ICV leptin in genetically obese and lean mice. Leptin induced a significant increase in percentage core temperature change across 23 hr, but earlier in lean mice than in obese mice. α -helical CRF (10 μ g/ μ l) did not block leptin's enhancing effect on percentage core temperature. (^b $p < .05$, ^c $p < .01$ compared to vehicle - vehicle group; ^B $p < .05$, ^C $p < .01$ compared to α helical CRF - vehicle group.)

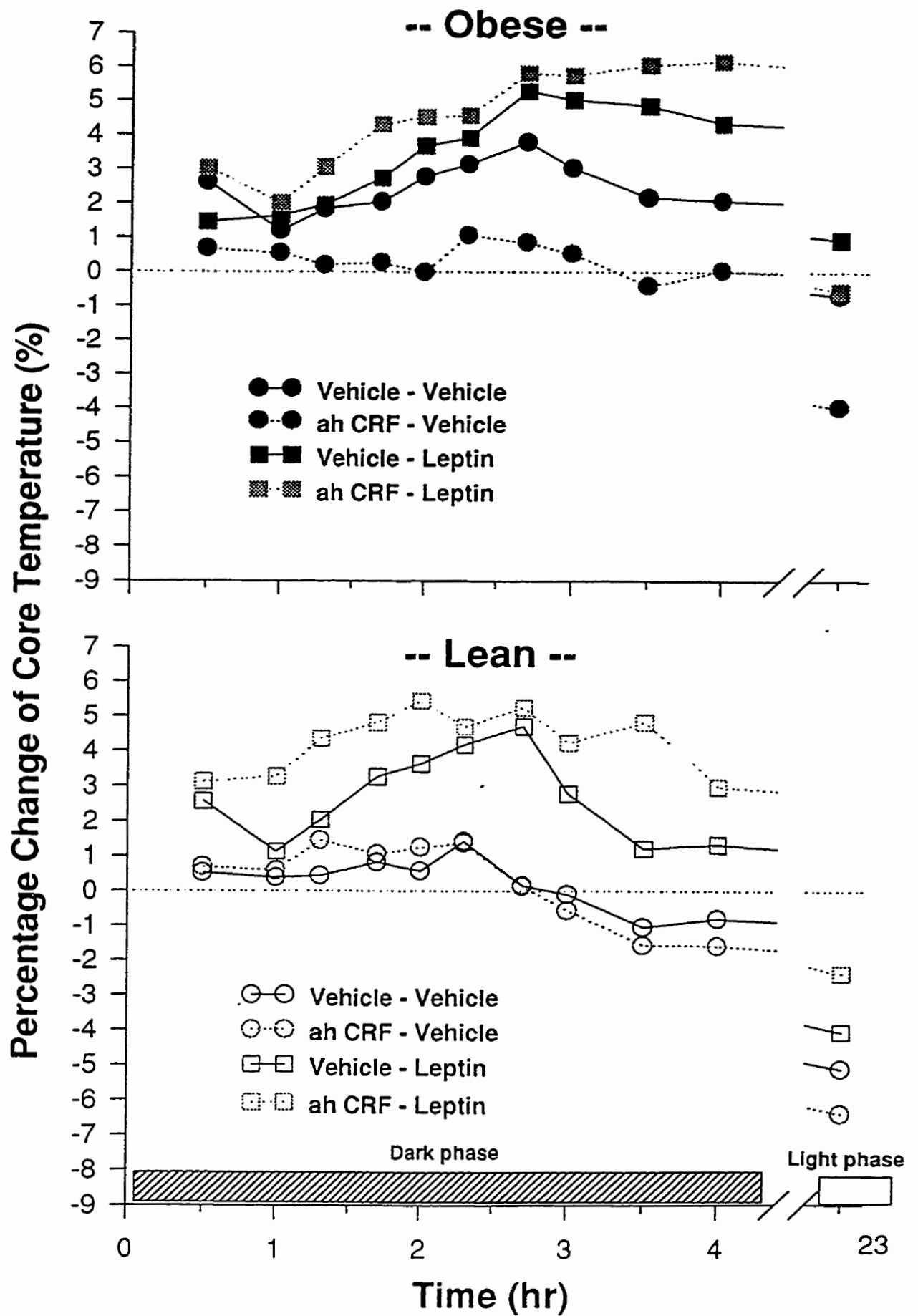
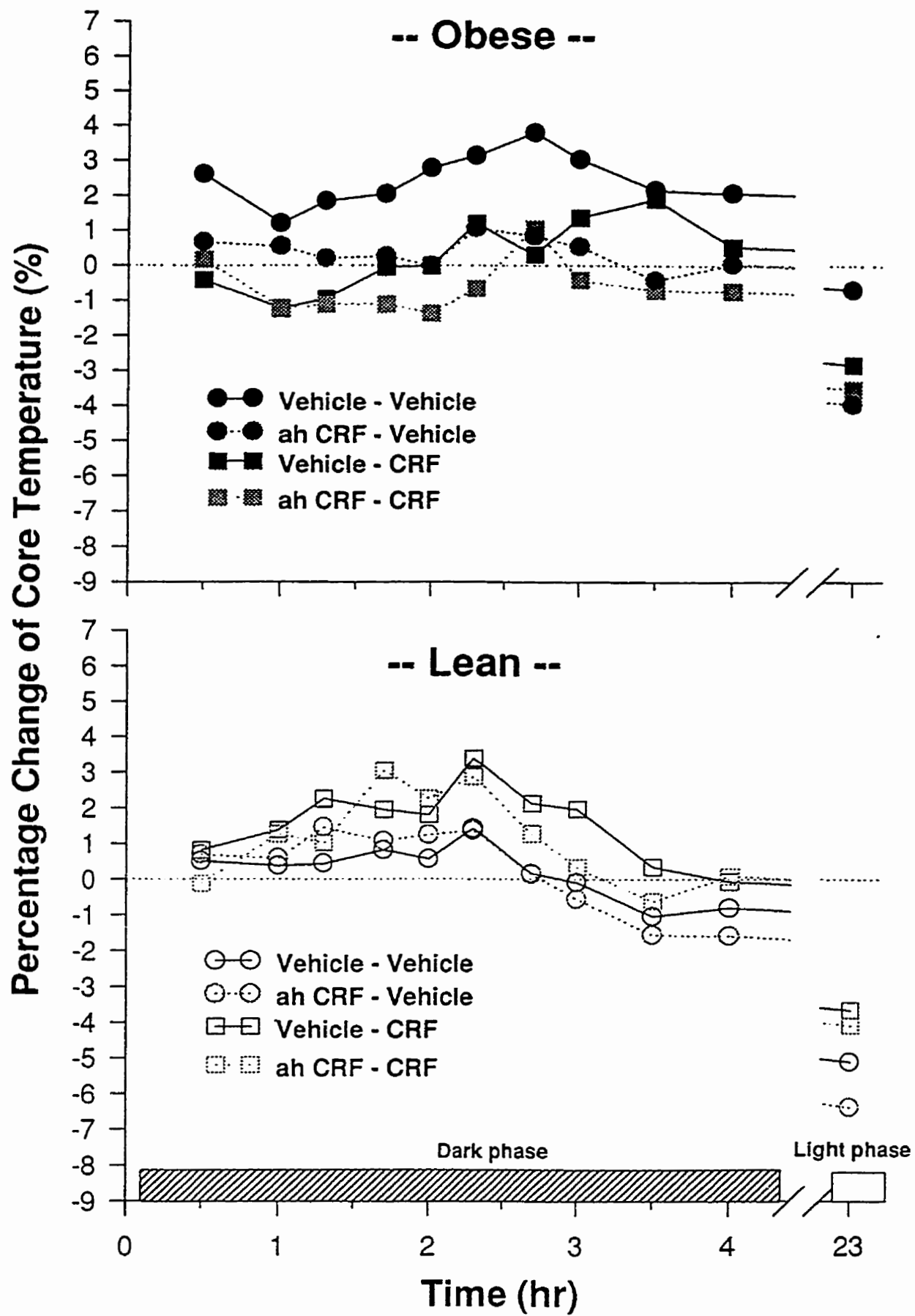


Figure Caption

Figure 15. Mean percentage core temperature change at 30 min, 1 hr, 1 hr 20 min, 1 hr 40 min, 2 hr, 2 hr 20 min, 2 hr 40 min, 3 hr, 3 hr 30 min, 4 hr, and 23 hr after ICV CRF in genetically obese and lean mice. CRF did not show any significant effect on percentage core temperature change across 23 hr. α -helical CRF (10 μ g/ μ l) alone or with CRF also did not influence percentage core temperature change.



leptin augmented the percentage core temperature change over 23 hr, Time X Drug Treatment, $F(10, 27) = 2.88$, $p < .05$, in both obese and lean mice (see Figure 14). Particularly, leptin increased the percentage core temperature change earlier in lean mice (about 1.5 hr after injection) than in obese mice (at least 2.5 hr after injection). α -helical CRF also did not block leptin's enhancing effect on core temperature change. Multiple comparison of least square means did not reveal any significant difference between the effects of α -helical CRF and vehicle on core temperature change, although obese mice, with α -helical CRF as pretreatment and vehicle as drug treatment, seemed to have the lowest core temperature changes across 23 hr.

Study II: CRF and its interaction with α -helical CRF. As depicted in Figure 13 and Figure 15, CRF did not have any significant effect on either absolute core temperature or percentage core temperature change in either obese or lean mice.

Factors Involved in the General Linear Regression Model of Absolute Body Weight Change

Study I: Leptin. General linear regression analysis revealed that leptin treatment played an important role in the absolute body weight change only in obese mice, but not in lean mice (Table 8). The effect of leptin on body weight change of obese mice is negative and significant at 4 hr, $t(1, 22) = -2.20$, $p = .04$. Food intake was a major factor contributing to body weight gain at 2 and 4 hr in obese mice, $t(1, 22) = 4.93$, $p = .0001$ and $t(1, 22) = 3.44$, $p = .003$, and lean mice, $t(1, 20) = 2.71$, $p = .02$ and $t(1, 20) = 3.21$, $p = .006$, respectively; however, at 23 hr food intake affected body weight change only in obese mice, $t(1, 22) = 5.08$, $p = .0001$. Core body temperature change did not contribute to the linear regression model of body weight change within 23 hr in obese mice.

However, core temperature change appeared to have positive impact on the regression model of body weight change in lean mice at 23 hr, $t(1, 20) = 2.52$, $p = .02$. Across 23 hr, lean mice's body weights were maintained at a stable level. Except for the core temperature change, no other investigated factors played a role in the regression models of body weight change at 23 hr. The R^2 of all these regression models of body weight change at 2, 4, and 23 hr were greater than .55, which means 55% of the variability of body weight change models had been explained by these factors. Pretreatment with α -helical CRF did not result in any significant effect on body weight change in all the regression models. Water intake also did not influence body weight change in either obese or lean mice.

Table 8

Factors Involved in General Linear Regression Models of Body Weight Change:

Leptin Study

	Change at 2 hr		Change at 4 hr		Change at 23 hr	
	Obese	Lean	Obese	Lean	Obese	Lean
Constant	-0.69 ($p = .06$)	NS	-1.06 ($p = .04$)	-1.00 ($p = .02$)	-2.78 ($p = .001$)	-0.51
Food Intake	1.85 ($p = .0001$)	0.98 ($p = .02$)	1.12 ($p < .003$)	1.30 ($p < .005$)	0.74 ($p = .0001$)	0.54 ($p = .07$)
Water Intake	-0.06	0.09	0.17	0.02	0.09	0.01
Pretreatment	0.04	0.28	-0.03	0.26	0.12	0.15
Leptin Treatment	-0.12	-0.05	-0.66 ($p = .04$)	-0.00	-0.26	-0.59 ($p = .06$)
Core Temperature	0.12	0.03	0.15	0.1	0.04	0.32 ($p = .02$)
R^2	0.61	0.57	0.55	0.6	0.78	0.59

Study II: CRF. As shown in Table 9, general linear regression analysis revealed that only food intake played a role in the regression model of body weight change. Food intake was a major factor involved in the body weight gain at 2, 4 hr in obese mice, $t(1, 17) = 3.18$, $p = .008$ and $t(1, 17) = 2.05$, $p = .06$, and in lean mice, $t(1, 15) = 4.04$, $p = .002$ and $t(1, 15) = 2.67$, $p = .02$. Core temperature change had a negative impact on body weight change only at 2 hr in lean mice, $t(1, 15) = -3.44$, $p = .006$. CRF did not show significant involvement in the regression model of body weight change at 2, 4, and 23 hr in either obese or lean mice. Water intake was also not significantly involved in the regression model of body weight change in obese and lean mice.

Table 9.

Factors Involved in General Linear Regression Models of Body Weight Change:
CRF Study

	Change at 2 hr		Change at 4 hr		Change at 23 hr	
	Obese	Lean	Obese	Lean	Obese	Lean
Constant	-0.36	-0.49 ($p = .08$)	-0.24	-1.03 ($p = .08$)	-0.82	0.85
Food Intake	1.68 ($p = .0079$)	1.36 ($p = .002$)	0.66 ($p = .06$)	1.30 ($p = .02$)	0.37	0.67
Water Intake	-0.11	0.09	0.11	0.04	0.05	-0.17
Pretreatment	-0.27	0.34	-0.33 ($p = .10$)	0.04	-0.12	0.26
CRF Treatment	-0.04	0.15	-0.26	0.22	-0.24	-0.27
Core Temperature	0.09	-0.37 ($p = .006$)	0.05	-0.08	0.03	0.53 ($p = .07$)
R^2	0.57	0.75	0.61	0.55	0.4	0.47

Study III: Oxygen Consumption

Oxygen consumption in obese mice seemed to be gradually suppressed across the first 60 min after central injection in the oxygen consumption testing chamber. As depicted in Table 10, there was no significant difference among the three treatment groups (α -helical CRF - Vehicle, Vehicle - Leptin, and α -helical CRF - Leptin).

As depicted in Figure 16, leptin did not affect the change of oxygen consumption, as a percentage of baseline oxygen consumption, in obese mice within 60 min after injection. However, at 60 min, the percentage change of oxygen consumption of the vehicle - leptin group ($\bar{M}$ = -4.58%) and the α -helical CRF - leptin group ($\bar{M}$ = 3.03%) was greater than that of the α -helical CRF - vehicle group ($\bar{M}$ = -30.03%) , Time X Treatment, $F(5, 13) = 4.133, p < .05$.

Table 10

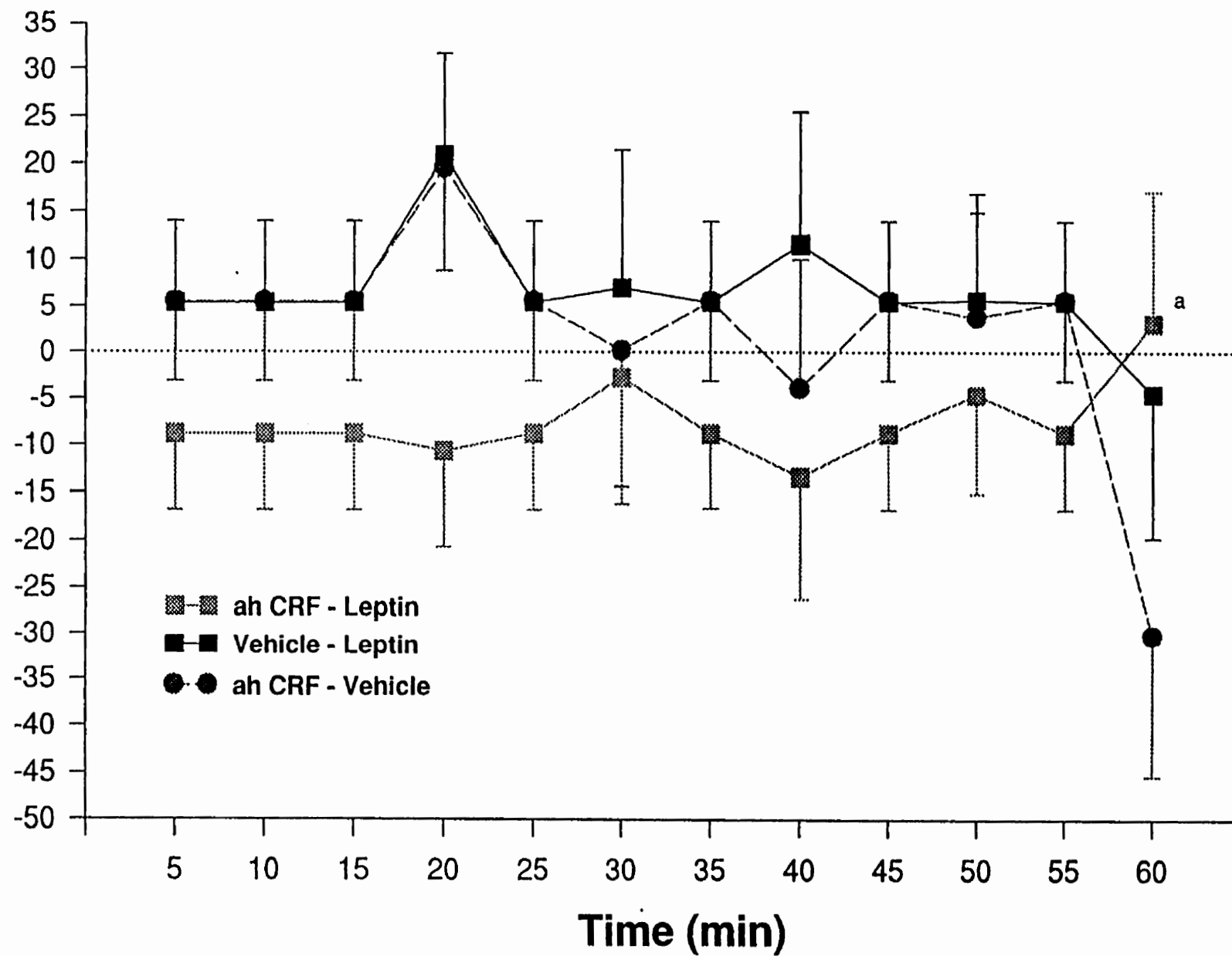
Effects of Leptin and α -helical CRF on Oxygen Consumption (ml/hr/g body weight) within 1 hr After ICV Injection on Genetically Obese Mice

Drug Treatment	Time					
	10 min	20 min	30 min	40 min	50 min	60min
Vehicle -- Leptin	2.33 (0.22)	2.41 (0.25)	2.20 (0.32)	2.25 (0.27)	2.01 (0.29)	2.02 (0.37)
α -helical CRF -- Leptin	2.55 (0.21)	2.53 (0.23)	2.47 (0.29)	2.28 (0.25)	2.42 (0.27)	2.59 (0.34)
α -helical CRF -- Vehicle	2.68 (0.12)	2.61 (0.25)	2.57 (0.32)	2.37 (0.27)	2.48 (0.29)	2.02 (0.37)

Figure Caption

Figure 16. Mean change ($\pm$ SE) in oxygen consumption, as a percentage of baseline oxygen consumption, of obese mice at 60 min after central administration. There was no significant difference among the three groups (α -helical CRF - vehicle, vehicle - leptin, and α -helical CRF - leptin). At 60 min, the percentage change in oxygen consumption of leptin-treated mice did not differ from vehicle as drug treatment. However, the oxygen consumption of the α -helical CRF - leptin group was about 30% greater than the α -helical CRF - vehicle group. (^a $p < .05$ compared to α -helical CRF - vehicle group.)

Percentage Change of O₂ from Baseline



Discussion

Effects of Leptin on Energy Intake, Thermogenesis, and Body Weight

The results of the current studies confirmed the effect of leptin on energy intake and expenditure in genetically obese (lep^{ob}/lep^{ob}) and lean mice. Central administration of leptin (1 μ g) decreased 23-hr cumulative food and water intake, increased core temperature, and suppressed body weight gain in both obese and lean mice, with a stronger effect on obese mice. Most recent studies on leptin's effect monitored food intake, oxygen consumption, and body weight (e.g., Campfield et al., 1995; Hwa et al., 1996; Mistry et al., 1997). Only a few studies have measured rectal temperature to investigate the metabolic effects of peripheral infusion of leptin in obese mice (Harris et al., 1998; Pelleymounter et al., 1995). Study I further demonstrated that centrally administered leptin can stimulate core temperature in obese and lean mice at 1.5 - 4 hr. These results confirmed the hypothesis that leptin, as a circulating signal from adipose tissue, works in the CNS and regulates ingestive behavior and metabolism.

Leptin decreased food intake more in obese mice than in lean mice. This result is consistent with other results of chronic intraperitoneal injections of leptin (Halaas et al., 1995). The stronger effects of leptin in obese mice suggested that the leptin receptor of this mutant is more sensitive to leptin's stimulation. Campfield et al. (1995) reported that central injection of leptin (1 μ g) reduced food intake at 30 min after central injection, but this decrease was statistically significant only at 7 hr. The present study (I) showed that the decrease in food and water intake by leptin occurred at 23 hr after central injection in both obese and lean mice. Pelleymounter et al. (1995) demonstrated that intraperitoneal administration of leptin decreased 24-hr water intake only in obese mice. However, the

current Study I showed that central injection of leptin decreased cumulative water intake at 23 hr in both obese and lean mice. This pattern is different from leptin's effects on food intake and body weight gain, which indicate a stronger and earlier effect in obese mice. The inhibitory effect of leptin on water intake may be a consequence of lowered food intake induced by leptin, rather than a specific effect of leptin. At this point, it is not known whether leptin's effects on food and water intake are specific or whether leptin's effects on one (e.g., food intake) affects the other (e.g., water intake).

The stimulating effect of leptin on metabolism in lep^{ob}/lep^{ob} mice has been reported in several studies (e.g., Hwa et al., 1996; Mistry et al., 1997; Pelleymounter et al., 1995). The current Study I further showed that central injection of leptin enhanced core temperature with a tendency of stronger effects in obese mice over a 23-hr period. The core temperature of obese mice with leptin treatment stayed higher than that of obese mice without leptin treatment at 23 hr. This result is consistent with the observation that leptin increases fat metabolism (Hwa et al., 1996) and increases sympathetic outflow in brown adipose tissue in obese mice (Collins & Surwit, 1996). Both of these effectors contribute to core body temperature. Besides increasing body temperature, a single ICV injection of leptin (1 µg) also augmented oxygen consumption at 3 hr in both obese and lean mice (Mistry et al., 1997) and cumulative oxygen consumption at 22 hr (Hwa et al., 1996) in lep^{ob}/lep^{ob} mice. The current Study III failed to show a significant effect of leptin on changing oxygen consumption during the first 60 min after central administration of leptin in obese mice. However, when baseline oxygen consumption differences between groups were normalized to percentage change from baseline, the current Study III found that oxygen consumption of lep^{ob}/lep^{ob} mice treated with leptin was greater than vehicle-treated

controls. It indicated a tendency towards a stimulating effect of leptin on oxygen consumption in lep^{ob}/lep^{ob} mice. Therefore, leptin's stimulating effect on oxygen consumption may not occur within 60 min but at sometime between 60 min and 3-4 hr (Mistry et al., 1997) after central administration. Taken together with Study I's finding that leptin did not affect food intake and core temperature until 4 hr and significantly suppressed food intake at 23 hr after central injection, these data suggest that leptin may stimulate metabolism and suppress energy intake over the same time frame. Until now there has been no direct evidence demonstrating that leptin increases metabolism and suppresses energy intake through different pathways. The stimulative effect of leptin on oxygen consumption may be a consequence of leptin's effects on food intake and body temperature.

As body weight is the result of overall energy regulation, leptin (Study I) blocked body weight gain earlier and stronger in lep^{ob}/lep^{ob} mice than in lean mice. Particularly, leptin successfully blocked body weight gain at 4 hr and even significantly reduced body weight at 23 hr in lep^{ob}/lep^{ob} mice. This effect was not seen in lean mice at the same time after ICV leptin. In contrast, leptin only blunted body weight gain at 23 hr in lean mice. These results further indicate that lep^{ob}/lep^{ob} mice are more sensitive to leptin than lean mice. As body weight reflects both intake and metabolism, the blocking effect of leptin on body weight gain in lep^{ob}/lep^{ob} mice is likely the direct result of leptin's stronger effects on their food intake and metabolism. The stronger effect of leptin on body weight in lep^{ob}/lep^{ob} mice confirms that these obese mice are more sensitive to leptin than lean mice.

The leptin-induced decrease in food intake can only partially explain the weight loss after leptin's infusion. For example, Hwa et al. (1996) reported that leptin stimulated

22-hr oxygen consumption, but pair-feeding, in which obese mice received the same amount of food as leptin-treated obese mice, did not induce the same effect in $\text{lep}^{\text{ob}}/\text{lep}^{\text{ob}}$ mice. The same pair-feeding also did not reduce body weight and adipose tissue size to the same degree as leptin did in $\text{lep}^{\text{ob}}/\text{lep}^{\text{ob}}$ mice (Levin, Nelson, Gurney, Vandlen, & De Sauvage, 1996). Moreover, leptin reduces fat tissues in rats, but the body weight and fat losses of rats did not recover even several weeks after the termination of leptin treatment (Chen et al., 1996). Therefore, due to (a) leptin's robust inhibitory effect on food intake, (b) leptin's stimulative effect on metabolism, (c) the sensitivity of $\text{lep}^{\text{ob}}/\text{lep}^{\text{ob}}$ mice to leptin, and (d) possible interaction or co-localizing with other neural transmitters, such as CRF and NPY, leptin blocked body weight gain earlier and decreased body weight more in obese mice than in lean mice.

Are CRF Receptors Involved in Leptin's Function?

Since this thesis was undertaken, several studies have suggested that CRF or CRF receptors may be involved in the central function of leptin in $\text{lep}^{\text{ob}}/\text{lep}^{\text{ob}}$ mice (Arvaniti, Huang, Picard, Bachelard, & Richard, 1998; Huang, Rivest, & Richard, 1998), in rats (Huang, Rivest, Picard, Deshaies, & Richard, 1998; Uehera, Shimizu, Ohtan, Sato, & Mori, 1998); and in humans (Wand & Schumann, 1998). For instance, Uehera et al. (1998) reported that α -helical CRF blocked the anorectic effects of leptin in normal rats. However, pretreatment of α -helical CRF failed to completely block leptin's effect on food intake, water intake, and core temperature in either obese or lean mice in the current Study I. Nonetheless, Study I demonstrated that α -helical CRF partially blocked leptin's effect on body weight gain. Particularly, leptin alone significantly decreased body weight gain at 4 hr in $\text{lep}^{\text{ob}}/\text{lep}^{\text{ob}}$ mice, but leptin did not suppress body weight gain at 4 hr when

combined with α -helical CRF. Moreover, leptin with α -helical CRF pretreatment also did not decrease body weight as much as it did with vehicle pretreatment at 23 hr in lep^{ob}/lep^{ob} mice. These results suggest that CRF receptors cannot be excluded from leptin's function regarding the overall effects of leptin on body weight regulation. Furthermore, the results that α -helical CRF partially blocked leptin's effect on body weight but did not block leptin's effect on food intake suggested that different central pathways may be involved in the function of leptin in lep^{ob}/lep^{ob} mice. The investigations on the complex relationship of leptin and NPY, CART (cocaine- and amphetamine-regulated transcript), neurotensin, CRF, POMC, and other neuropeptides may help to understand how those central pathways interact with leptin. For example, Agouti-related protein, an endogenous melanocortin antagonist and a stimulator of feeding, blocks leptin's anorectic effect on food intake (Seeley et al., 1997). Sahu (1998) administered leptin centrally for 3 days to rats and found decreased hypothalamic mRNA levels of galanin, melanin concentrating hormone, POMC, and NPY. All of these peptides have been implicated as intake stimulators (Inui, 1999). In addition, CART, a satiety factor, almost does not exist in the hypothalamic arcuate nucleus of lep^{ob}/lep^{ob} mice, which also have no functional leptin (Kristensen et al., 1998). Therefore, leptin may trigger different neural networks to induce anorectic effects and to stimulate metabolism in lep^{ob}/lep^{ob} mice.

Central CRF or CRF-like peptide has been suggested as one of the possible neural transmitters/modulators involved in leptin's function. Leptin decreased CRF production in the hypothalamus (Heiman et al., 1997; Huang, Rivest, & Richard, 1998; Raber, Chen, Mucke, & Feng, 1997) and also prevented the secretion of glucocorticoids from the adrenal gland (Huang, Rivest, & Richard, 1998) in lep^{ob}/lep^{ob} mice. Moreover, both

corticosterone and leptin dose-dependently suppressed CRF mRNA expression in the PVN of lep^{ob}/lep^{ob} mice (Arvaniti et al., 1998). Those findings suggested that leptin suppresses the hyperfunctional CRF-HPA axis in lep^{ob}/lep^{ob} mice by preventing the production of CRF in the PVN and the secretion of glucocorticoids from the adrenal gland. Consequently, leptin down-regulates CRF-HPA axis activity. Does leptin also regulate CRF-SNS axis activity? If it does, how are CRF or CRF receptors involved in this specific function? The different CRF receptors may provide some answers to this question.

CRF receptors, Type 1 (CRF-R1) and Type 2 (CRF-R2), appear to be distributed in different central and peripheral areas and to function differently. These two subtypes of CRF receptors exist not only in the brain but also in peripheral tissues; for instance, CRF-R1 mainly exists in anterior pituitary cells and regulates CRF's effect on ACTH and corticosterone production (Lovenberg, Chalmers, Liu, & De Souza, 1995). Antalarmin, a CRF-R1 antagonist, significantly reduced plasma ACTH and corticosterone levels, but had no effect on plasma leptin or leptin mRNA levels and body weight (Bornstein et al., 1998). Therefore, CRF-R1 is related to the CRF-HPA axis activity. Based on the findings that (a) leptin reduced CRF mRNA production (Arvaniti et al., 1998; Huang, Rivest, Picard, et al., 1998), (b) antalarmin did not affect leptin's mRNA expression, and (c) the major function of CRF-R1 is related to CRF-HPA axis, leptin down-regulates the CRF-HPA axis activity by reducing the production of CRF and consequently reduces the production of corticosterone. Conversely, CRF, at least via CRF-R1, does not affect leptin's function on the CRF-HPA axis.

Only sparse CRF-R1 is expressed in the hypothalamus and thalamus (Chalmers,

Lovenberg, & De Souza, 1995; Kresse et al., 1998; Potter et al., 1994; Owens, Mulchahey, Kasckow, Plotsky, & Nemeroff, 1995), while CRF-R2 is expressed extensively in the brain (Lovenberg et al., 1995), especially in the lateral septal nucleus, ventromedial hypothalamic nucleus, olfactory bulb, amygdala, and the choroid plexus (Chalmers et al., 1995). CRF-R2 is strongly implicated in the regulation of stress responses and eating behavior (Turnbull & Rivier, 1997). For example, urocortin, a CRF-like neuropeptide, mainly stimulates CRF-R2 receptors (Vaughan et al., 1995) and suppresses food intake in either overnight fasted rats (Smagin, Howell, Ryan, De Souza, & Harris, 1998) or 24-hr food deprived rats (Spina et al., 1996). The anorectic effect of urocortin was not the result of anxiety since urocortin did not induce anxiogenic-like behavior in rats (Spina et al., 1996). In short, CRF-R2 (and urocortin) may be directly involved in the appetite-suppressing effect of CRF and urocortin and may play an important role in the regulation of energy homeostasis.

The different roles of CRF-R1 and CRF-R2 in CRF actions suggest a dual function of CRF (see Figure 17). CRF via CRF-R1 regulates the HPA axis activity (Bornstein et al., 1998; Kresse et al., 1998). For example, Bornstein et al. (1998) found that peripheral chronic administration of antalarmin, a CRF-R1 antagonist, had no significant effect on body weight and leptin mRNA level, but significantly suppressed adrenal gland function in rats. In contrast, CRF and CRF-like peptide (urocortin) via CRF-R2 suppressed food intake and increased thermogenesis (Smagin et al., 1998; Spina et al., 1996), which are related to the function of the CRF-SNS axis. Further, chronic central infusion of leptin decreased CRF mRNA levels and CRF-R1 mRNA, but increased CRF-R2 mRNA expression in rat hypothalamus (Huang, Rivest, Picard, et al., 1998). This effect implies a

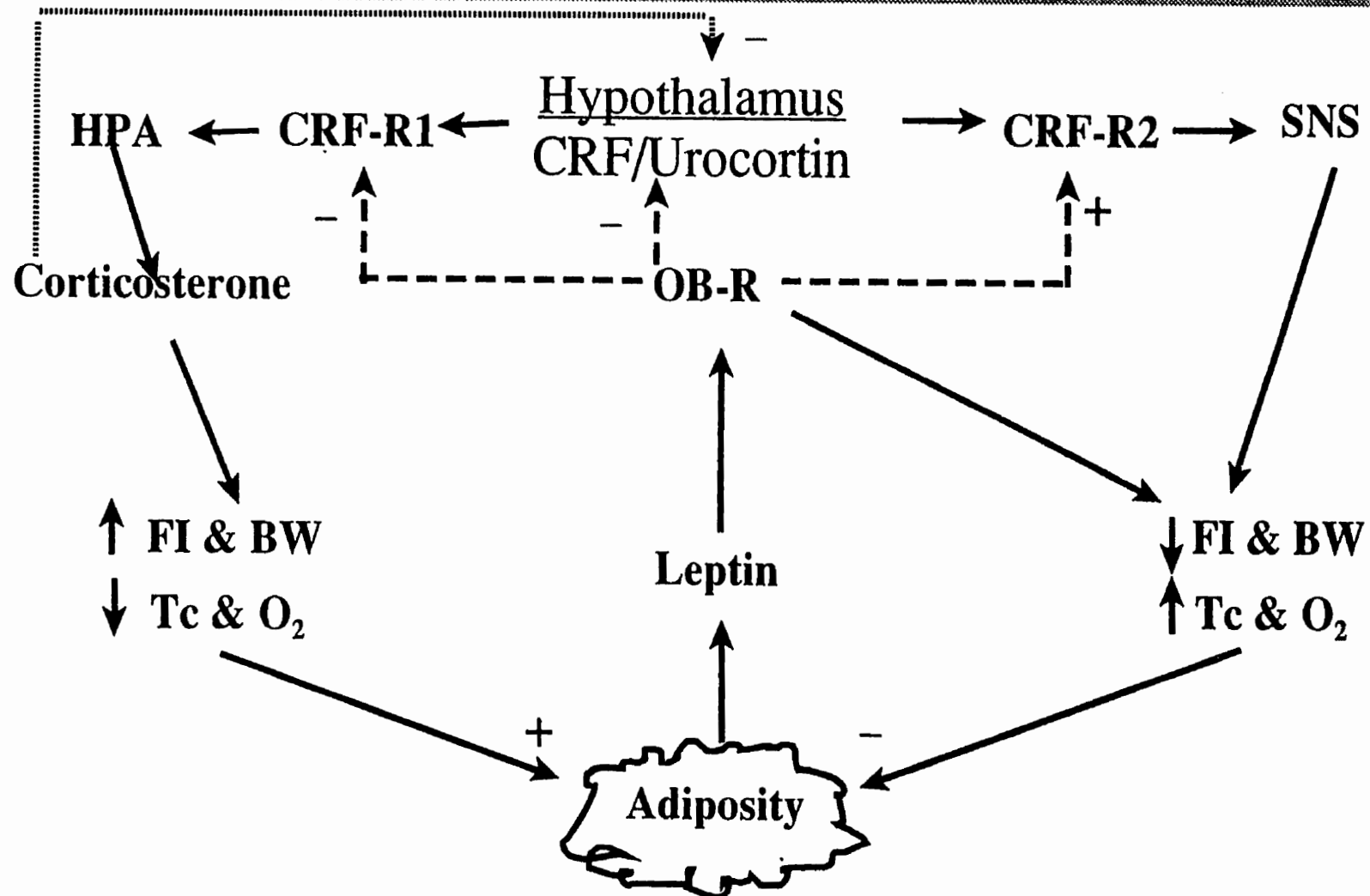
differential effect of leptin on the hypothalamic CRF-HPA axis and CRF-SNS axis activity (see Figure 17). That is, on one hand, leptin may reduce corticosterone production by decreasing CRF production. On the other hand, leptin increases CRF-R2 mRNA in the hypothalamus. The enhanced CRF-R2 availability may augment the opportunities of stimulating effects from either CRF, or urocortin, or both, to the CRF-SNS axis (see Figure 17). Consequently, thermogenesis or metabolism is increased. Simply blocking CRF receptors, without specifying CRF-R1 or CRF-R2, may not clearly demonstrate the differential effect of leptin on the two axes. It also cannot exclude the other neural transmitters and receptors involved in leptin's effects, such as NPY, POMC, urocortin, and CRF-binding proteins.

In summary, leptin significantly decreased food intake and suppressed body weight gain more in obese mice than in lean mice. Leptin also stimulated metabolism by increasing core temperature and oxygen consumption. The central mechanism of leptin's function still needs to be clarified. Leptin may lower the CRF-HPA axis activity by suppressing CRF production in the PVN, and leptin may stimulate the CRF-SNS axis activity by increasing CRF-R2 availability. However, more evidence is needed to determine that CRF receptors are involved in the effect of leptin on energy balance. Particularly, studies investigating the interactions of CRF receptors, with specific subtypes such as CRF-R1 and CRF-R2, or CRF binding protein, and leptin may enlighten this area.

Figure Caption

Figure 17. Schematic illustration of the dual function of CRF and the relationship of CRF with leptin on energy balance regulation.

The dual function of CRF and the relationship of CRF with leptin on energy balance regulation



Effects of Central Administration of CRF on Energy Intake, Thermogenesis, and Body Weight

In contrast to leptin's effects, central injection of CRF (1 µg/µl) (Study II) did not influence food intake, water intake, core temperature, and body weight gain. These results were not consistent with other reports related to CRF effects on energy regulation in either genetically obese (*fa/fa*) rats (e.g., Arase et al., 1989a, 1989b), nonobese rats (as reviewed by Rothwell, 1990), or genetically obese (*lep^{ob}/lep^{ob}*) mice (Drescher et al., 1994). The dose of CRF in the current study was adopted from Drescher et al.'s (1994) study, in which ICV injection of 1 µg (200 pmol) CRF inhibited food intake in 12-hr food-deprived obese and lean mice. In the current study, animals were housed in home cages with food and water ad lib. Central drug injection was given 30 min before the light cycle ended. The difference between 12-hr food deprivation and a natural ingestive pattern (dark-onset feeding) may explain the different results between the current Study II and Drescher et al.'s (1994) study. Therefore, CRF affected intake in a deprivation feeding paradigm, but not in a dark-onset feeding paradigm. In addition, Drescher et al. (1994) employed ether anesthetization and acute, nonstereotaxic ICV drug administration. All these procedures (12-hr food deprivation, ether anesthetization, and acute, nonstereotaxic ICV injection) are stressors and may stimulate central CRF production. Exogenous CRF with the stress-increased endogenous CRF may inhibit ingestive behavior and stimulate metabolism. The current Study II employed chronic implantation of ICV cannula and handling every day before and after the surgery. These procedures mitigated the stress induced by central injection and manipulation in the experiment and, presumably, avoided the extra production of endogenous CRF. Other studies, in which exogenous CRF

successfully decreased food intake, employed 5 μ g CRF (Arase et al., 1989a) in Zucker fa/fa rats or in nonobese rats (Levine et al., 1983). Despite the size difference of rat and mouse brains, 1 μ g CRF may be a relatively low dose to induce a significant effect on food intake.

Numerous studies have shown that intracerebral CRF has anti-obesity effects in rats (e.g., Arase et al., 1989; Britton et al., 1982; Hardwick et al., 1989; Rothwell, 1990). Rivest and Richard (1990) have suggested that central CRF negatively regulates energy balance by affecting all aspects of energy intake and expenditure. However, the current Study II demonstrated that a relatively low dose of exogenous CRF (1 μ g) has no effect on food intake and thermogenesis in lep^{ob}/lep^{ob} mice. The particular function of central CRF may explain these results. That is, CRF, as a coordinator, plays a dual role in energy balance (see Figure 17). On the CRF-HPA axis, CRF stimulates the production of glucocorticoids by increasing ACTH secretion (e.g., Vale et al., 1981; 1983). The high level of glucocorticoids causes obesity by inducing hyperphagia (e.g., Feldkircher, 1993) and lowering thermogenesis (e.g., Rothwell & Stock, 1985). On the CRF-SNS axis, CRF stimulates SNS activity and enhances brown adipose tissue activity (e.g., De Souza et al., 1985; Swanson et al., 1983). As a result, food intake is inhibited and thermogenesis is stimulated. Consequently, body weight is reduced. The peripheral glucocorticoids also have negative feedback effects on the CNS. However, based on the high level of glucocorticoids and low SNS activity in lep^{ob}/lep^{ob} mice, the negative feedback from corticosterone to hypothalamic CRF seems not to down-regulate the corticosterone production, but to down-regulate the SNS activity. Their high levels of plasma corticosterone represent the hyperactivity of the CRF-HPA axis in lep^{ob}/lep^{ob} mice. Their

lower body temperature and hypoactivity indicate suppressed CRF-SNS axis activity. Exogenous CRF in the hypothalamus may act through both axes, via CRF-R1 on CRF-HPA axis and CRF-R2 on the CRF-SNS axis, to regulate energy intake and expenditure. Because lep^{ob}/lep^{ob} mice do not produce functional leptin and leptin increases CRF-R2 mRNA production (Huang, Rivest, & Richard, 1998), lep^{ob}/lep^{ob} mice may have fewer CRF-R2 receptors. Fewer CRF-R2 receptors can directly lower the activity of CRF-SNS axis. Due to the lower activity of the CRF-SNS axis in genetic obesity, a single ICV administration of CRF (1 µg) may not be able to stimulate the CRF-SNS axis activity in lep^{ob}/lep^{ob} mice. Therefore, the inhibitory effect of CRF on food intake and stimulatory effect on thermogenesis could not be revealed in the current study.

Additionally, studies (Moreau, Kilpatrick, & Jenck, 1997; Smagin et al., 1998; Spina et al., 1996; Spina, Merlo-Pich, Rivier, Vale, & Koob, 1998) of the central function of urocortin, a recently identified mammalian CRF-like neuropeptide (Vaughan et al., 1995), suggest that urocortin may have more potential than CRF to stimulate CRF-SNS axis activity and to alter energy intake and thermogenesis. Central administration of urocortin dose-dependently decreased cumulative food intake in food deprived or nondeprived rats (Spina et al., 1996; Spina et al., 1998). This food intake-suppressing effect of urocortin was more potent than that of CRF (Spina et al., 1996; Spina et al., 1998). Urocortin is also stronger than CRF at activating CRF-R2 receptors (Vaughan et al., 1995), the receptor subtype through which CRF decreases food intake (Smagin et al., 1998). When Smagin et al. (1998) chronically infused antisense oligonucleotides to CRF-R2 receptors, which are mainly localized in VMH, PVN, and lateral hypothalamus (Grigoriadis, Lovenberg, Chalmers, Liaw, & De Souza, 1996), urocortin induced an

attenuated anorexia in rats. In short, urocortin, as a member of the CRF family, plays a more potent role than CRF in regulation of the CRF-SNS axis activity. Therefore, a relatively low dose of CRF (Study II), acting at both CRF-R1 and CRF-R2 sites, did not induce a significant effect on suppressing food intake and stimulating metabolism in mice as higher dosages of CRF or urocortin did in previous research in rats.

Conclusions

Due to the mutation of the ob gene, no functional leptin is produced in lep^{ob}/lep^{ob} mice (Zhang et al., 1994). Leptin, as a signal from adipose tissue, works in the CNS via leptin receptors (OB-R) (e.g., Chen et al., 1996). Therefore, the lack of functional leptin results in no negative feedback from adipose tissue to the CNS in lep^{ob}/lep^{ob} mice. Moreover, because OB-R of lep^{ob}/lep^{ob} mice is more sensitive to leptin, lep^{ob}/lep^{ob} mice showed a more striking response to leptin than lean mice did. On the other hand, the high levels of circulating corticosterone (Dubuc, 1977; Naeser, 1974) seem to be unable to inhibit central CRF activity in lep^{ob}/lep^{ob} mice. The reason for this nonfunctional negative feedback to CRF is still not clear. One possible suggestion is that glucocorticoid receptors, especially the Type II receptor, may not function properly in lep^{ob}/lep^{ob} mice. The absence of functional leptin results in no activation of OB-R in lep^{ob}/lep^{ob} mice. Because both OB-R (e.g., Chen et al., 1996; Cioffi et al., 1996; Mercer et al., 1996) and Type II receptors (e.g., Miesfeld et al., 1984; Pepin et al., 1992) are located in the hypothalamus, no activation of OB-R in the hypothalamus may affect the Type II receptors' function in lep^{ob}/lep^{ob} mice. Thus, the negative feedback from corticosterone may not be functional, even though the corticosterone level is extremely high in lep^{ob}/lep^{ob} mice. Whether the nonfunctional Type II receptor in the HPA axis is related to the lack of leptin production,

or the sensitive OB-R, or both, is still unknown. Furthermore, chronic infusion of leptin not only blunts the increase in hypothalamic CRF-R1 mRNA levels, induced by exercise or food deprivation, but also enhances hypothalamic CRF-R2 mRNA expression in rats (Huang, Rivest, Picard, et al., 1998). Therefore, the absence of activation of OB-R may also influence the activity of CRF-R2.

The current studies confirmed the anorectic effect of leptin and revealed the stimulative effect of leptin on metabolism (core temperature) in lep^{ob}/lep^{ob} mice. Leptin also stimulates oxygen consumption (e.g., Hwa et al., 1996; Mistry et al., 1997), but the current Study III showed that this effect did not happen within 60 min after ICV injection. Furthermore, the current research also suggested that (a) CRF receptors are partially involved in the central function of leptin, and (b) a low dose of CRF, without specifically agonizing either CRF-R1 or CRF-R2, could not stimulate metabolism or suppress food intake in lep^{ob}/lep^{ob} mice. Therefore, future studies on (a) the relationship of leptin and urocortin (which works mainly on CRF-R2), (b) the central function of leptin and glucocorticoid receptors (Type II), (c) the special roles of CRF-R1 and CRF-R2 in the function of leptin, and (d) leptin central pathways related to the dual function of CRF in the CRF-HPA axis and the CRF-SNS axis may enlighten understanding of the central regulation of energy homeostasis and eventually provide more information for the treatment of obesity.

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Footnotes

1. The genetically obese gene symbol lep^{ob}/lep^{ob} was displayed as ob/ob before Zhang et al. (1994) cloned the ob gene and identified its gene product, leptin.

Appendix A

Summary Tables of Homogeneity Test

Table A1.

Homogeneity of Variance Tests of Body Weight, Food Intake, and Water Intake as
Dependent Variables: Study I – Leptin

Dependent Variable	Univariate	Box's M - Test	
	Bartlett-Box F Test		
BW* Change at 2 hr	$F(7, 2312) = 1.36$ $p = 0.22$	$M = 91.69$	$F(42, 3321) = 1.71$ $p = 0.003$
BW Change at 4 hr	$F(7, 2312) = 2.27$ $p = 0.03$		
BW Change at 23 hr	$F(7, 2312) = 1.05$ $p = 0.40$		
Food Intake at 2 hr	$F(7, 2312) = 0.72$ $p = 0.65$	$M = 82.45$	$F(42, 3321) = 1.54$ $p = 0.02$
Food Intake at 4 hr	$F(7, 2312) = 0.61$ $p = 0.75$		
Food Intake at 23 hr	$F(7, 2312) = 2.56$ $p = 0.01$		
Water Intake at 2 hr	$F(7, 2157) = 1.55$ $p = 0.15$	$M = 46.23$	$F(42, 3149) = 0.85$ $p = 0.73$
Water Intake at 4 hr	$F(7, 2157) = 0.65$ $p = 0.71$		
Water Intake at 23 hr	$F(7, 2157) = 0.92$ $p = 0.49$		

Note: BW = Body Weight.

Table A2.

Homogeneity of Variance Tests of Percentage Body Weight Change, Food Intake, and Water Intake to Initial Body Weight: Study I – Leptin

Dependent Variable	Univariate Bartlett-Box F Test	Box's M - Test	
Percent BW *	$F(7, 2312) = 2.84$	$M = 110.13$	$F(42, 3321) = 2.06$
Change at 2 hr	$p = 0.006$		$p = 0.000$
Percent BW	$F(7, 2312) = 4.50$		
Change at 4 hr	$p = 0.000$		
Percent BW	$F(7, 2312) = 0.73$		
Change at 23 hr	$p = 0.65$		
Percent Food	$F(7, 2312) = 2.15$	$M = 77.69$	$F(42, 3321) = 1.45$
Intake at 2 hr	$p = 0.04$		$p = 0.030$
Percent Food	$F(7, 2312) = 2.38$		
Intake at 4 hr	$p = 0.02$		
Percent Food	$F(7, 2312) = 1.65$		
Intake at 23 hr	$p = 0.12$		
Percent Water	$F(7, 2157) = 4.92$	$M = 98.16$	$F(42, 3321) = 1.82$
Intake at 2 hr	$p = 0.000$		$p = 0.001$
Percent Water	$F(7, 2157) = 3.63$		
Intake at 4 hr	$p = 0.001$		
Percent Water	$F(7, 2157) = 3.84$		
Intake at 23 hr	$p = 0.000$		

Note: BW = body weight.

Table A3.

Homogeneity of Variance Tests of Core Temperature as Dependent Variables: Study I – Leptin

Dependent Variable	Univariate	Box's M - Test	
	Bartlett-Box F Test		
Temperature at 1 hr	$F(7,1263) = 3.79$ $p = 0.000$	$M = 160.89$	$F(60, 1314) = 1.45$ $p = .02$
Temperature at 2 hr	$F(7,1263) = 2.08$ $p = 0.04$		
Temperature at 3 hr	$F(7,1263) = 2.11$ $p = 0.04$		
Temperature at 4 hr	$F(7,1263) = 1.36$ $p = 0.22$		
Temperature at 23 hr	$F(7,1263) = 0.35$ $p = 0.93$		

Table A4.

Homogeneity of Variance Tests of Percentage of Core Temperature to Initial Core Temperature as Dependent Variables: Study I – Leptin

Dependent Variable	Univariate	Box's M - Test
	Bartlett-Box F Test	
Percentage Temperature	$F(7, 1263) = 0.86$	$M = 130.68$ $F(60, 1314) = 1.18$
at 1 hr	$p = 0.54$	$p = .17$
Percentage Temperature	$F(7, 1263) = 0.46$	
at 2 hr	$p = 0.86$	
Percentage Temperature	$F(7, 1263) = 0.50$	
at 3 hr	$p = 0.83$	
Percentage Temperature	$F(7, 1263) = 1.33$	
at 4 hr	$p = 0.23$	
Percentage Temperature	$F(7, 1263) = 0.68$	
at 23 hr	$p = 0.69$	

Table A5.

Homogeneity of Variance Tests of Body Weight, Food Intake, and Water Intake as
Dependent Variables: Study II – CRF

Dependent Variable	Univariate	Box's M - Test	
	Bartlett-Box F Test		
BW* Change at 2 hr	$F(7,1735) = 1.15$ $p = 0.33$	$M = 73.66$	$F(42, 2005) = 1.31$ $p = 0.091$
BW Change at 4 hr	$F(7,1735) = 1.12$ $p = 0.35$		
BW Change at 23 hr	$F(7,1735) = 0.51$ $p = 0.83$		
Food Intake at 2 hr	$F(7,1735) = 1.39$ $p = 0.21$	$M = 72.97$	$F(42, 2205) = 1.29$ $p = 0.099$
Food Intake at 4 hr	$F(7,1735) = 1.86$ $p = 0.07$		
Food Intake at 23 hr	$F(7,1735) = 1.68$ $p = 0.11$		
Water Intake at 2 hr	$F(7,1735) = 1.29$ $p = 0.25$	$M = 91.67$	$F(42, 1941) = 1.59$ $p = 0.01$
Water Intake at 4 hr	$F(7,1735) = 1.42$ $p = 0.19$		
Water Intake at 23 hr	$F(7,1735) = 2.37$ $p = 0.02$		

Note: BW = Body Weight.

Table A6.

Homogeneity of Variance Tests of Percentage Body Weight Change, Food Intake, and Water Intake to Initial Body Weight: Study II – CRF

Dependent Variable	Univariate Bartlett-Box F Test	Box's M - Test	
Percent BW *	$F(7,1725) = 2.14$	$M = 89.95$	$F(42, 2205) = 1.60$
Change at 2 hr	$p = 0.04$		$p = 0.009$
Percent BW	$F(7,1725) = 2.78$		
Change at 4 hr	$p = 0.007$		
Percent BW	$F(7,1725) = 1.55$		
Change at 23 hr	$p = 0.15$		
Percent Food	$F(7,1725) = 1.71$	$M = 55.51$	$F(42, 2205) = 0.98$
Intake at 2 hr	$p = 0.10$		$p = 0.50$
Percent Food	$F(7,1725) = 1.40$		
Intake at 4 hr	$p = 0.20$		
Percent Food	$F(7,1725) = 1.30$		
Intake at 23 hr	$p = 0.25$		
Percent Water	$F(7,1725) = 3.08$	$M = 129.86$	$F(42, 1941) = 2.25$
Intake at 2 hr	$p = 0.003$		$p = 0.000$
Percent Water	$F(7,1725) = 3.49$		
Intake at 4 hr	$p = 0.001$		
Percent Water	$F(7,1725) = 2.59$		
Intake at 23 hr	$p = 0.01$		

Note: BW = body weight.

Table A7.

Homogeneity of Variance Tests of Core Temperature as Dependent Variables:Study II – CRF

Dependent Variable	Univariate	Box's M - Test	
	Bartlett-Box F Test		
Temperature at 1 hr	$F(7,581) = 2.54$ $p = 0.01$	$M = 43.82$	$F(15, 420) = .997$ $p = .46$
Temperature at 2 hr	$F(7,581) = 2.26$ $p = 0.03$		
Temperature at 3 hr	$F(7,581) = 1.15$ $p = 0.33$		
Temperature at 4 hr	$F(7,581) = 0.85$ $p = 0.54$		
Temperature at 23 hr	$F(7,581) = 0.53$ $p = 0.81$		

Table A8.

Homogeneity of Variance Tests of Percentage of Core Temperature to Initial Core Temperature as Dependent Variables: Study II – CRF

Dependent Variable	Univariate	Box's M - Test	
	Bartlett-Box F Test		
Percentage Temperature	$\underline{F} (7,581) = 2.40$	$\underline{M} = 33.22$	$\underline{F} (15, 420) = .951$
at 1 hr	$\underline{p} = 0.02$		$\underline{p} = .51$
Percentage Temperature	$\underline{F} (7,581) = 0.51$		
at 2 hr	$\underline{p} = 0.83$		
Percentage Temperature	$\underline{F} (7,581) = 0.61$		
at 3 hr	$\underline{p} = 0.74$		
Percentage Temperature	$\underline{F} (7,581) = 1.20$		
at 4 hr	$\underline{p} = 0.30$		
Percentage Temperature	$\underline{F} (7,581) = 1.15$		
at 23 hr	$\underline{p} = 0.33$		

Appendix B

List of Abbreviations

ACTH:	Adrenocorticotrophic hormone
ADX:	Adrenalectomy
ANS:	Autonomic nervous system
BAT:	Brown adipose tissue
BW:	Body weight
CRF:	Corticotropin releasing factor
CRF-R1	CRF receptor Type 1
CRF-R2	CRF receptor Type 2
DEX:	Dexamethasone
FI:	Food intake
5-HT:	5-hydroxytryptamine
HPA:	Hypothalamus pituitary adrenal
HYPX:	Hypophysectomy
ICV:	Intracerebroventricular
LC:	Locus coeruleus
NE:	Norepinephrine
NPY:	Neuropeptide Y
<u>ob</u> :	Obese gene
O ₂	Oxygen consumption
POMC:	Proopiomelanocortin
PVN:	Paraventricular nucleus
SNS:	Sympathetic nervous system
T _c :	Core body temperature
VMH:	Ventromedial hypothalamus