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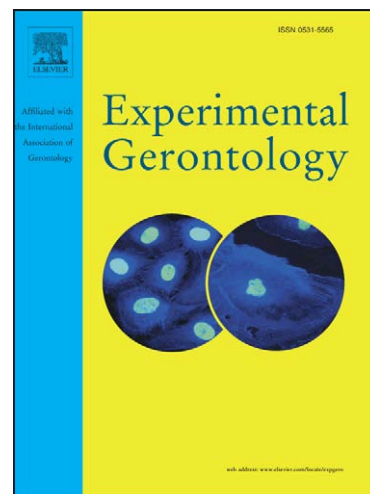
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**Changes in the neuroendocrine control of energy homeostasis by adiposity signals during aging**

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## SUMMARY

Energy balance in mammals is modulated by peripheral signals that inform the brain about the magnitude of fat stores, the amount of food in the gastrointestinal tract, and the level of nutrients such as glucose in the circulation. Among these, insulin and leptin are considered adiposity signals involved in the long-term maintenance of fat stores. Here we review the mechanisms of action of leptin and insulin in the hypothalamus and how these mechanisms are altered during aging in rat models. Aged rats are characterized by increased fat mass, central leptin and insulin resistance, and hyperleptinemia. Leptin resistance is manifested by its failure to inhibit food intake, deplete fat stores, down regulate its own expression in adipose tissue, and increase energy expenditure. Moreover, leptin and insulin signalling are decreased in hypothalamus from aged rats. Calorie restriction and fasting studies provide controversial data on the cause-effect interrelationship between increased adiposity and development of central leptin resistance. Although in the absence of obesity leptin resistance seems to be a characteristic of aged animals, adiposity could either reinforce it or cause an early onset of this resistance. More studies are necessary to clarify the role of the hypothalamus in the development of age-associated obesity and insulin resistance.

## 1. Introduction

Neuroendocrine regulation of energy homeostasis involves cross-talk between the periphery and the CNS. Several peptidic factors, mainly originating in the periphery, interact with specific brain targets such as hypothalamic nuclei or transmit their signal through the vagus nerve and sympathetic fibers, which activate neurons in another brain area, the nucleus of the tractus solitarius (NTS). Thus, hypothalamus and NTS seem to play a key role integrating peripheral signals and generating homeostatic responses, transmitted by the autonomic nervous system which regulate food intake and energy expenditure (Badman and Flier, 2005; Takahashi, 2003).

Peripheral signals regulating energy homeostasis can be classified into one of the three following types: 1) long-term energy balance signals, such as leptin and insulin which act as adiposity signals indicating the amount of fat stored in the organism (Schwartz et al., 2000; Spiegelman and Flier, 2001); 2) hunger and satiety signals, such as ghrelin, glucagon-like peptide 1 (GLP-1) or cholecystokinin (CCK) among others, which regulate the short-term eating behavior (Badman and Flier, 2005; Stanley et al., 2005); and 3) nutrient signals, such as glucose or fatty acids, which reflect the whole-body nutrient status and can modulate appetite, body weight and liver metabolism (Hu et al., 2003; Obici et al., 2003). The first and the last types of signals act mainly through activation of hypothalamic neurons whereas hunger and satiety signals target additionally to the NTS and other brainstem areas. In addition to these signals, other factors currently under study, such as adiponectin or resistin, seem to have an effect on energy balance acting either centrally on hypothalamus, or peripherally modulating energy metabolism of several tissues. Moreover, it should be

pointed out that most of the hormonal factors mentioned above, in addition to their central targets, can modulate peripheral tissues via endocrine and paracrine action.

In this review we examine the role of the long-term modulators of energy balance, leptin and insulin, with special emphasis in the changes that occur during aging in their respective effects on food intake and energy expenditure.

## **2. Long-term energy balance signals**

### *2.1.- Lipostatic theory: leptin and insulin as adiposity signals*

The original lipostatic hypothesis of Kennedy aimed to understand how the brain can monitor the status of fat stores in the organism and proposed the existence of a humoral factor circulating in proportion to the magnitude of these stores. This factor would interact with specific areas in the CNS involved in the regulation of energy homeostasis, closing a classical feed-back loop. Insulin was the first factor so far described that met the criteria to be considered as an “adiposity signal”: its fasting circulating concentration is proportional to the amount of body fat, it is transported through the blood brain barrier, insulin receptors are expressed in key hypothalamic areas, and central insulin administration reduces food intake and body weight (Woods and Seeley, 2001). Moreover, mice with brain-specific disruption of the insulin receptor gene become more obese than wild-type mice when fed a high-fat diet, and mice lacking the protein tyrosine phosphatase-1B which present increased insulin signalling are resistant to diet-induced obesity (Woods and Seeley, 2001). Furthermore, selective decrease of insulin receptor in arcuate nucleus using intracerebroventricular (i.c.v.) administration of insulin receptor antisense

oligodeoxynucleotide leads to hyperphagia and increased fat mass in rats (Obici et al., 2002). Neuronal insulin control of fat storage has also been found in animals as *C. elegans* (Wolkow et al., 2000) and *D. melanogaster* (Bohni et al., 1999) suggesting that it is a conserved function throughout the evolution.

In 1994 the protein leptin arising from the adipose tissue was identified as a second adiposity signal involved in the control of energy balance. Plasma leptin concentration is shown to be proportional to the amount of fat stores, with the exception of *ob/ob* mice lacking a functional leptin gene (Seeley and Woods, 2003). A saturable transport of leptin across the blood brain barrier has also been reported, and leptin receptors (Ob-Rb) have been identified in several hypothalamic areas as well as in neurons in the NTS (Seeley and Woods, 2003). Administration of leptin to rodents decreases food intake and body weight, and increases energy expenditure. As expected, the obesity syndrome in *ob/ob* mice is corrected by leptin administration. On the other hand, *db/db* mice and Zucker Diabetic Fatty rats which lack a functional leptin receptor show severe obesity (Ahima and Flier, 2000; Spiegelman and Flier, 2001). Together these data give experimental support to the existence of an adipose-brain axis in the regulation of energy balance as proposed originally by the lipostatic theory.

The current model of control of adipose tissue mass postulates that both, leptin and insulin, regulate the activity of two type of neurons situated in the arcuate nucleus that express leptin and insulin receptors. One type synthesizes neuropeptide Y (NPY) and agouti-related peptide (AgRP), and the other synthesizes cocaine and amphetamine related transcript (CART) and pro-

opiomelanocortin (POMC), a precursor of the anorectic factor  $\alpha$  melanocyte-stimulating hormone ( $\alpha$ MSH). Both, leptin and insulin, stimulate the expression of POMC and the secretion of  $\alpha$ MSH, and inhibit the NPY/AgRP neurons. The inhibition by leptin and insulin of the synthesis of orexigenic NPY is thought to contribute to the anorectic effects of these adiposity signals. On the other hand, AgRP is known to function as an antagonist of  $\alpha$ MSH-4 receptors (MC4R) and its decrease by insulin and leptin leads to a greater anorectic effect of  $\alpha$ MSH (Schwartz et al., 2000; Spiegelman and Flier, 2001). NPY/AgRP and POMC/CART neurons project to second order neurons in ventromedial (VMH) and dorsomedial (DMH) hypothalamus as well as in the paraventricular nucleus (PVN) and lateral hypothalamic area (LHA). PVN includes neurons producing anorectic factors such as TRH, CRH and oxytocin which inhibit food intake and stimulate the autonomic function, and these neurons are innervated by NPY/AgRP and POMC/CART neurons from the arcuate nucleus. On the other hand, orexigenic factors such as MCH and orexins A and B are produced by neurons situated in LHA. Many other factors have been reported so far that influence energy homeostasis and a complex series of neuronal interrelationships between several brain areas is emerging which has been described elsewhere and whose detailed analysis is beyond the scope of this review (Hillebrand et al., 2002; Schwartz et al., 2000; Spiegelman and Flier, 2001; Stanley et al., 2005).

## *2.2.-Leptin and insulin signal transduction*

Although cellular and physiological effects of leptin and insulin on the hypothalamus are similar, they use different signal transduction pathways. Leptin receptor Ob-Rb is a member of the type 1 cytokine receptor family that, after



binding of leptin, recruits the Janus-activated kinase-2 (JAK2), a cytosolic tyrosine kinase. JAK2 phosphorylates specific intracellular tyrosine residues of leptin receptor which bind in turn the signal transducer and activator of transcription-3 (STAT3). This transcription factor is phosphorylated by JAK2 leading to its activation, dimerization and translocation to the nucleus where it modulates gene transcription. JAK2 also phosphorylates other tyrosine residue of the leptin receptor building a binding site for the SH2 domain-containing tyrosine phosphatase (SHP). The recruitment of SHP results in its tyrosine phosphorylation and the subsequent recruitment of GRB2, the first step in the p21ras-extracellular-signal regulated (ERK) signalling pathway. Leptin signalling also promotes the transphosphorylation of JAK2 and this phosphorylated kinase presumably binds to insulin receptor substrate-2 (IRS-2) and activates the PI 3-kinase (PI3K) pathway (Bates and Myers, 2003).

Insulin binds to the heterotetrameric insulin receptor activating its tyrosine kinase activity, that phosphorylates several tyrosine residues in the receptor  $\beta$  subunit. This leads to the tyrosine phosphorylation of a family of proteins (IRSs) which interact with the p85 $\alpha$  regulatory subunit of PI3K, activating the p110 catalytic subunit which in turn phosphorylates phosphatidylinositol-(4,5)-bisphosphate to phosphatidylinositol-(3,4,5)-trisphosphate. This phospholipid mediates most of insulin effects activating a cascade of protein kinases including PDK1, PKB and GSK3 (Saltiel and Pessin, 2002).

Activation by insulin and leptin of the PI3K pathway constitutes a case of “cross-talk” that could explain the similarities of their physiological effects on energy homeostasis. Electrophysiological studies revealed that leptin and insulin

modulate the membrane potential and firing rates of hypothalamic neurons by a signaling mechanism involving PI3K. On the other hand, the inhibition by i.c.v. administrated insulin of food intake has been shown to require PI3K signaling and the same was observed for the anorectic effect of leptin (Niswender et al., 2003).

Nevertheless, more recent studies have demonstrated that leptin, in contrast to insulin, elicits only a transient stimulation of PI 3-kinase that does not result in the stimulation of PKB. Thus, modulation of neuropeptide expression in POMC and AgRP/NPY neurons is elicited through different pathways by the two effectors (Plum et al., 2006). In addition, regulation of neuronal electrical activity via PI 3-kinase and stimulation of ATP-sensitive potassium channels also show differences for leptin and insulin. Thus, in POMC neurons leptin stimulates and insulin inhibits electrical activity. This differential action could partially explain the severe obese phenotype observed in db/db mice if compared to the subtle body weight increase characteristic of IR-knockout mice (Plum et al., 2006).

### **3.- Effect of aging on physiological leptin action**

Changes in leptin action with aging have been mainly investigated in rat models. Common features of humans and rat models of aging are the increase in body weight, the accretion of fat mass and the development of hyperleptinemia. This last characteristic is also observed in most obese animals and humans and could be the consequence of central leptin resistance. Thus, it can be postulated that aging is associated with the failure of leptin to regulate fat stores that increase up to the beginning of the late senescent phase. Fat mass accretion should be the consequence of a positive energy balance due to

either increased food intake or decreased energy expenditure or both, and these changes should reflect alterations in leptin and/or insulin action at their central targets.

### *3.1.- Leptin action on food intake during aging*

Absolute daily food intake remains approximately constant with aging in male F344xBN (Li et al., 1997), Wistar (Fernández-Galaz et al., 2001), and BN rats (Wolden-Hanson, 2006). Food intake can be expressed as relative to body mass and this would lead to the conclusion that aging is associated with anorexia that could be explained by the elevated serum leptin levels (Wolden-Hanson, 2006). Nevertheless, considering the organism as a whole, it seems more appropriated to evaluate the absolute food intake as an index of overall caloric intake that would be regulated by circulating adiposity signals among other factors. This interpretation would conversely suggest the presence of leptin resistance which is responsible for the maintenance of food intake along aging despite marked increases in circulating leptin levels (Fernández-Galaz et al., 2001; Li et al., 1997). Aging-associated anorexia has been observed in studied rat models but only in an advanced senescent phase (Wolden-Hanson, 2006).

Leptin resistance in aged rats has been tested by direct infusion of leptin, either peripherally (Gabriely et al., 2002; Scarpace et al., 2000) or i.c.v. (Fernández-Galaz et al., 2002; Shek and Scarpace, 2000). In both cases a clear anorectic effect of leptin was observed in young (3 months) but not in 24-month old rats. Using two different doses of i.c.v. leptin we demonstrated that aged rats respond to elevated doses of leptin (10 µg/day) decreasing food intake and

body weight similarly to young rats but are unresponsive to doses of leptin (0.2  $\mu\text{g/day}$ ) that elicit a significant reduction of food intake and a maximal decrease of body weight in young rats (Fernández-Galaz et al., 2002). These data suggest that aged Wistar rats show lower central sensitivity to leptin with normal maximal response. Nevertheless, the physiological central levels of leptin are likely lower than those reached by i.c.v. leptin infusion, even in hyperleptinemic old rats, and this allows postulating an attenuated central effect of leptin in aged rats.

Using in situ hybridization in arcuate sections from Brown Norway rats a progressive decline with aging in NPY and Agrp expression was observed (Wolden-Hanson, 2006), in spite of increasing serum leptin levels. In addition, an increase in CART and no changes in POMC expression were reported (Wolden-Hanson, 2006). Interestingly, i.c.v. injection of AgRP elicits an increase over 7 days in food intake similar in young and old rats, suggesting that leptin resistance could be localized at the level of first-order neurons while the rest of melanocortin system remains functional (Wolden-Hanson, 2006). Using the in situ hybridization and RT-PCR techniques we have also observed a decrease in NPY expression in arcuate of 24-month old Wistar rats, whereas a lower amount of NPY protein was found in immunohistochemistry studies both in arcuate and in periventricular hypothalamic area (Fernández-Agulló et al., 2006). On the other hand, studies on NPY expression in whole hypothalamus of F344xBN rats by RT-PCR also demonstrated a slight decrease in aged animals and no changes after peripheral (Scarpace et al., 2000) or central leptin infusion

(Shek and Scarpace, 2000) whereas a significant effect was observed in young animals.

Fasting is a robust inducer of appetite that is associated with significant increases of hypothalamic NPY and AgRP and decreases of POMC and CART expression. Studies in fasted rats (Li et al., 1998; Wolden-Hanson, 2006) revealed that aged animals show blunted NPY and AgRP expression in response to fasting suggesting that they have impaired orexigenic capacity. Interestingly, a lower effect of fasting on decreasing anorexigenic POMC and CART expression was also reported. Fasting also induces a decrease in circulating leptin to levels slightly higher than in 3-months old rats. Thus, it could be postulated that the leptin concentration is still high enough to preclude the recovery of full leptin sensitivity in aged rats. In fact, at moderately high serum leptin concentrations the correlation between leptin and hypothalamic NPY mRNA is rather weak (Li et al., 1998). Nevertheless, despite this attenuated response to fasting, aged rats increase food intake and gradually recover their original body weight after 15-20 days. In contrast, young animals return to their original body weight in 5-6 days. In Wistar rats we designed a three month calorie restriction protocol that results in a 15% reduction in body weight, lowers serum leptin to levels higher than in young rats, and decreases visceral adiposity up to values similar to those observed in young animals (Escrivá et al., 2007; Fernández-Galaz et al., 2002). After calorie restriction, 24-months old Wistar rats show a marked increase of NPY mRNA in arcuate and increased levels of NPY protein in arcuate and periventricular areas (Fernández-Agulló et al., 2006). These animals increase food intake more than double after the end of the restriction protocol and i.c.v. infusion of a leptin dose, which is ineffective

in ad libitum fed old rats, markedly suppresses the drive to eat in these animals and the subsequent increase in body weight. These data indicate that this caloric restriction protocol reverts the central leptin resistance characteristic of aged rats (Fernández-Galaz et al., 2002). Gabriely et al. (2002) studied the anorectic effect of leptin in aged Sprague-Dawley rats that underwent calorie restriction throughout their lives maintaining low fat mass and serum leptin levels. Interestingly, these animals displayed a blunted effect of i.v. infused leptin in decreasing food intake, suggesting that leptin resistance is intrinsic to aging and not consequence of progressive development of obesity.

### *3.2.- Leptin action on fat stores during aging*

Leptin depletes fat stores in rodents and humans. However, in aged animals fat mass increases in spite of elevated leptin (Escrivá et al., 2007; Scarpace et al., 2000), suggesting that the effect of leptin is impaired. It has been demonstrated that leptin induces enzymes and transcription factors involved in fatty acid oxidation in adipose tissue which acts not only as storage of fat, but also as protector of nonadipose tissues from fat overloading (Wang et al., 2001). We have recently reported that central leptin also promotes a lipid flux between adipose tissue and liver, stimulating fat tissue lipolysis and inhibiting glyceroneogenesis and re-esterification of fatty acids which are delivered to the liver (Gallardo et al., 2007). By inducing liver fatty acid oxidation and inhibiting re-esterification and synthesis of VLDL, leptin seems to have a global effect of decreasing fat reserves in the organism. Moreover, central infused leptin has been shown to impair insulin action in adipose tissue, contributing to favor triglyceride hydrolysis (Pérez et al., 2004). Studies performed with lean ZDF rats have revealed that 18-month old animals are insensitive to adenovirus-

induced hyperleptinemia showing a lower stimulation of fatty acid oxidation enzymes expression than in young rats, despite similar levels of circulating leptin. Moreover, leptin induces the pre-adipocyte marker Pref-1 in young rats, but not in old animals (Wang et al., 2001). Preliminary data from our group suggest that the above mentioned central effects of leptin in adipo-hepatic lipid flux are also blunted in aged Wistar rats.

Leptin also down-regulates its own expression in adipose tissue. Elevated leptin levels could be due to the increase in fat depots together with the absence of a leptin effect on leptin expression. In Sprague-Dawley rats under high-fat diet adipose tissue leptin mRNA increases in presence of hyperleptinemia (Wang et al., 1999). Nevertheless, the combination of diet-induced hyperleptinemia and infusion of adenovirus containing the leptin cDNA produces a further increase in serum leptin that results in a significant 20% reduction in adipose tissue leptin mRNA, likely consequence of a direct effect on adipose tissue non mediated by the CNS (Wang et al., 1999). In F344xBN aged rats which serum leptin levels similar to those of diet-induced obese rats, elevated leptin expression has been reported to occur in some, but not all, visceral fat depots (Li et al., 1997, 1998). In contrast, our own data in aged Wistar rats show a significant decrease in leptin mRNA levels in retroperitoneal fat from 24-months old rats if compared to 3- and 8-months old animals, whereas in epididymal fat the decrease does not reach statistical significance (unpublished observations). Together with differences in rat strain, these discrepancies could be due to a slightly higher leptinemia in the aged Wistar rat (Escrivá et al., 2007) that could bring about direct effects on adipose tissue independent of central action (Wang et al., 1999). It should also be pointed out that either fasting for 48 h (Li et al., 1998) or

calorie restriction for three months (our own unpublished data) cause a decrease of leptin mRNA in fat depots in aged rats indicating that leptin gene expression remains sensitive to changes in caloric intake. Interestingly, rats maintained under food restriction throughout their lives show a lower effect of i.v. infused leptin on adipose tissue leptin expression than young animals (Gabriely et al., 2002). Moreover the ability of leptin to decrease visceral fat mass in these animals is blunted and similar to that observed in aged-obese rats (Gabriely et al., 2002; Scarpace et al., 2000).

### *3.3.- Effect of leptin on energy expenditure during aging*

Leptin stimulation of energy expenditure is associated with elevation of UCP1 and thermogenesis in brown adipose tissue, and increased resting metabolic rate, mediated by a higher sympathetic outflow. Human aging is accompanied by lower thermogenesis, resting metabolic rate and physical activity, resulting in an state of positive energy balance that could be responsible for increased adiposity (Seals and Bell, 2004). Both in F344xBN and F344 rats no changes were observed in UCP1 expression with aging (Li et al., 1997). Nevertheless, oxygen consumption was decreased (Li et al., 1997). Moreover, in response to peripheral leptin no increment in oxygen consumption was observed in aged rats (Scarpace et al., 2000). Young lean rats respond to central infusion of leptin with increases in UCP1 expression and this effect is maintained during chronic leptin infusion. In contrast, in 18 months old rats with mild adiposity, chronic exposure to high central leptin results in a marked failure of leptin to induce UCP1 expression and oxygen consumption. These data suggest that leptin, in combination with mild adiposity and/or age induces leptin resistance in regard to



its effects on sympathetic outflow stimulation. A current hypothesis postulates that during aging, progressively elevated levels of leptin result in an activation of sympathetic nervous system (SNS) that brings about its desensitization, thus at advanced ages leptin fails to increment energy expenditure (Seals and Bell, 2004).

#### **4.- Effect of aging on hypothalamic leptin signaling**

Both, in F344xBN and Wistar rats, it has been demonstrated that 24-month old animals show a lower presence of Ob-Rb leptin receptors in the hypothalamus (Fernández-Galaz et al., 2001, 2002; Scarpace et al., 2001). In Wistar rats this decrease is not observed in 8-month old rats, despite the fact that these animals show already elevated leptin levels and an adiposity index similar to that observed in 24-month old rats. Scarpace et al. (Scarpace et al., 2001) have studied the dose-response stimulation of STAT3 phosphorylation and the P-STAT3 transcription factor binding activity in hypothalamus after i.v. and i.c.v. injections of leptin. When leptin was administrated i.c.v. they found a decrease in aged rats of the maximal stimulation of STAT3 phosphorylation without changes in the dose of leptin required for half-maximal stimulation. These differences are not accounted for by a decrease in STAT3 protein expression in aged animals. The P-STAT3 transcription factor binding activity was also lower in aged rats (Scarpace et al., 2001). They concluded that leptin resistance might be due in part to an impairment of leptin transport in the hypothalamus, and to decreased leptin signalling, likely as a consequence of decreased amounts of Ob-Rb leptin receptors.

As mentioned above, our own data in Wistar rats (Fernández-Galaz et al., 2002) indicate however, that centrally administrated leptin during 7 days is able to reduce food intake and body weight in aged animals to similar levels as in young rats when a dose of 10  $\mu$ g/day is used, suggesting a lower sensitivity to leptin but similar maximal response in old animals. This discrepancy, apart from differences in the rat model, could rely on the methodological approaches used in each case: an in vitro assay after acute injections of leptin (Scarpace et al., 2001) versus in vivo responses under lower amounts of leptin chronically infused (Fernández-Galaz et al., 2002). Calorie restriction for three months improves central leptin action and this is associated with a marked increase in Ob-Rb mRNA and protein in the hypothalamus (Fernández-Galaz et al., 2002). Beyond the decrease in leptin receptor expression, leptin resistance could be due to the impairment of leptin signalling. In this sense, the expression of SOCS-3, the suppressor of cytokine signalling 3, which has been demonstrated to inhibit leptin and insulin signalling, is increased in the hypothalamus from aged Wistar rats (Peralta et al., 2002). Moreover, calorie restriction for three months brings about a 30% decrease in SOCS-3 expression in the hypothalamus (Peralta et al., 2002) in agreement with its effect on the recovery of central leptin action in aged rats (Fernández-Galaz et al., 2002). A similar increase in hypothalamic SOCS-3 expression has been reported in 18-month old lean wild-type Zucker diabetic fatty rats (Wang et al., 2001). Thus, these data suggest that the increase in SOCS-3, together with the decrease in the expression of Ob-Rb could be responsible for the decreased leptin response observed in aged rats.

## 5. - Effect of aging on central insulin action and signaling

To the best of our knowledge we have reported the unique study on aging-associated changes in central insulin action (García-San Frutos et al., 2007). Using two different doses of centrally infused insulin for 7 days we demonstrated that it causes a dose-dependent decrease in daily food intake and body weight, and these effects were blunted in 8- and 24-month old rats. The fact that the beginning of the attenuation of central insulin response concurs with the major increase in adiposity index (Escrivá et al., 2007) after sexual maturity suggests a role for adiposity in the deterioration of central insulin response. This is supported by the data from food-restricted animals, which show improved central insulin responsiveness in terms of changes in body weight and food intake, associated with a decreased adiposity (García-San Frutos et al., 2007). An impaired central insulin response has also been described in obese rats (Carvalheira et al., 2003). The study of the insulin signal transduction pathway in hypothalamus showed an impairment of the PI3K branch of insulin signaling in aged rats as indicated by the decreased stimulation by insulin of Akt, GSK3 $\beta$ , p70S6K and Foxo1 phosphorylation (García-San Frutos et al., 2007). This would likely have an effect on catabolic and anabolic neuropeptides expression and neuronal electrical activity leading to a decreased anorectic action (Plum et al., 2006). The impairment in insulin signaling also occurs in earlier steps in the cascade such as phosphorylation of IR and IRS-2. This might in turn be explained by the increased serine phosphorylation of both proteins. Interestingly, in food-restricted old rats serine phosphorylation of IR and IRS-2 is lower and an overall increase of insulin signaling is shown. These data seem to contrast with those obtained in

peripheral tissues, where calorie restriction improves insulin action in 8-, but not in 24-month old animals (Escrivá et al., 2007). It is likely that peripheral tissues are more sensitive to prolonged increases in adiposity than the hypothalamus. Another factor possibly responsible for hypothalamic insulin resistance in 24-month old rats is the increase in SOCS-3 expression, a protein that is known to inhibit not only leptin, but also insulin signaling, and whose expression increases in aged Wistar rats and decreases after calorie restriction (Peralta et al., 2002).

Hypothalamic and peripheral insulin resistance develop with aging in the absence of fasting hyperinsulinemia. Nevertheless, at the age of 8 months when central insulin resistance is detected, Wistar rats show elevated serum leptin levels, and a percent of visceral fat similar to that observed in older rats. Hence leptin or another factor associated with increased adiposity may play a role in the impairment of central insulin signalling with aging. Although leptin acts as an insulin sensitizer in peripheral tissues, in adipocytes we have shown that it impairs insulin signalling (Pérez et al., 2004). The study of possible interactions between leptin and insulin signalling in hypothalamus would shed more light on this issue.

## **6.- Summary and perspective**

Leptin, together with insulin, seems to serve as sensor of available fuels and by interacting with its hypothalamic targets modulates changes in eating behaviour and sympathetic outflow that decrease food intake and increase energy expenditure. The available data in rodents and humans indicate that during aging the serum leptin concentration increases, leptin resistance develops, and

there is a progressive increment of fat mass and visceral adiposity. A major unanswered question refers to the time course of these two alterations with aging and their cause-effect relationship. On one hand it can be postulated that fat mass accretion leads to increased circulating leptin and the down-regulation of leptin signalling in the hypothalamus, having as consequence a further expansion of adipose tissue and the worsening of leptin resistance. On the other hand it is possible that progressive impairment of central leptin signalling is a characteristic of the aging process itself and obesity is simply a consequence of this resistance.

Studies on F344xBN and Wistar rats suggest that increased adiposity is an early change in the aging process necessary for the development of leptin resistance. Furthermore, aged rats under three months of food restriction, with adiposity levels similar to those of young animals and moderately elevated serum leptin, show a normal anorectic response to central leptin suggesting that adiposity plays a key role in the development of leptin resistance (Fernández-Galaz et al., 2002). In contrast, studies in aged Sprague –Dawley rats subjected to calorie restriction throughout their lives, that maintained leptin and fat mass levels similar to those of young mates, demonstrated an age-associated decrease in the anorectic response to i.v. administrated leptin. These studies suggest that leptin resistance develops with aging independent of the presence of adiposity. According to the evolutionary theory of aging this postreproduction phenotype should be evolutionarily neutral. Thus, adiposity would be the consequence of this age-associated leptin resistance that leads to other complications such as insulin resistance, hypertension and decreased life-span (Gabriely et al., 2002). More studies will be needed to clarify this point.

Concerning future research several aspects should be considered. Adiponectin, another adipokine, has recently emerged as a new player in the energy homeostasis control that stimulates energy expenditure at the central level. Even though plasma adiponectin concentration does not change with aging (Escrivá et al., 2007) a possible alteration of its central action in aged animals should be investigated. Additionally, proinflammatory cytokines such as TNF- $\alpha$  are known to promote insulin resistance, a phenomenon clearly associated with aging. On the other hand, it seems clear that a relationship between aging and/or age-associated adiposity could be related with a sub-acute state of inflammation. Thus, the possible role of cytokines in the development of central leptin and insulin resistance would deserve more attention. Finally, the relationship between the mechanisms involved in long-term and short-term control of energy homeostasis currently under study shows an enormous complexity and the contribution of its alteration to the defective control of energy balance with aging remains to be explored.

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