VASCO DA GAMA UNIVERSITARY SCHOOL

MASTER´S DEGREE

THE ROLE OF LEPTIN IN OBESITY AND DIABETES *MELLITUS* IN DOG

Patrícia Isabel Cunha Santos

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Abstract

Canine obesity is a serious breeding and health issue. Recent studies reported that 34-59% of dogs visiting veterinary are overweight and 5-20% are obese.

Recent studies have shown that during the development of obesity there is several alterations at the cellular and molecular levels that contribute to the secretion of local and systemic molecules, called adipokines. These molecules contribute to the existence of a chronic low grade inflammatory state, to angiogenesis, to the control of appetite and satiety and to the control of the glucose and lipid metabolism. Together, these alterations may explain the reduced life span of obese animals and the development of several pathologies.

One of main adipokines is leptin that plays a key role in the regulation of body weight, energy balance and feeding behaviour. Leptin is mainly regulated by adiposity, existing a positive correlation between the degree of adiposity and the plasma leptin concentration. In addition to adiposity, others factors regulate the leptin production such as insulin, glucocorticoids, endotoxin, and cytokines.

Due to the contribution of leptin to the metabolism of glucose and lipids, several studies suggested that it may to establish an association between obesity, insulin resistance and ultimately diabetes. In fact, in humans the occurrence of obesity, dyslipidaemia and hypertension has been long associated to the development of insulin resistance and diabetes. However, the association between canine obesity and diabetes is not well understood, and there is no consensus whether obesity induces diabetes.

Considering that obesity and diabetes reduce life span and the mechanisms underlying its development are not clearly understood, in this article we review the current knowledge on the association between obesity, diabetes and leptin.

Keywords: Obesity; Diabetes *mellitus*; Leptin; Insulin resistance.

Resumo

A obesidade canina é uma grave questão de bem-estar. Estudos recentes mostram que 34-59% dos cães que visitam o veterinário têm excesso de peso e 5-20% são considerados obesos.

Estudos recentes mostram que, durante o desenvolvimento da obesidade há várias alterações a nível celular e molecular que contribuem para a secreção de moléculas locais e sistémicas, chamadas adipocinas. Estas moléculas contribuem para a existência de um estado inflamatório crónico, para a angiogénese, para o controlo do apetite e saciedade e para o controlo do metabolismo da glucose e dos lípidos. Em conjunto, estas alterações podem explicar a redução do tempo de vida dos animais obesos e o desenvolvimento de várias patologias.

Uma das principais adipocinas é a leptina que desempenha um papel fundamental na regulação do peso corporal, balanço energético e comportamento alimentar. A leptina é regulada principalmente pela adiposidade, existindo uma correlação positiva entre o grau de adiposidade e a concentração de leptina no plasma. Além da adiposidade, outros factores regulam a produção de leptina, tais como a insulina, os glucocorticóides, endotoxinas e citoquinas.

Devido à contribuição da leptina para o metabolismo da glicose e dos lípidos, vários estudos sugerem que esta adipocina pode estabelecer uma associação entre obesidade, resistência à insulina e, finalmente, diabetes. De facto, nos humanos a ocorrência de obesidade, dislipidemia e hipertensão têm sido associados com o desenvolvimento de resistência à insulina e com a diabetes. No entanto, a associação entre a obesidade canina e diabetes não é bem compreendida, e não há consenso se a obesidade induz diabetes.

Considerando que a obesidade e a diabetes reduzem o tempo de vida e os mecanismos subjacentes ao seu desenvolvimento não são claramente compreendidos, neste artigo vamos rever o conhecimento atual sobre a associação entre obesidade, diabetes e leptina.

Palavras-chave: Obesidade; Diabetes *mellitus*; Leptina; Resistência à insulina.

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List of abbreviations

BCS	Body condition score
BMI	Body max índex
DM	Diabetes *mellitus*
IL-1	Interleukin-1
IL-1RA	Interleukin-1 receptor antagonist
IL-6	Interleukin-6
PI3K	Phosphatidylinositol-3-kinase
PPARɣ	Peroxisome-proliferator
TGFβ	Transforming growth factor β
TNFα	Tumor necrosis factor α

1. Introduction

Canine obesity is a growing problem that results from accumulation of body fat due to a mismatch between energy intake and energy expenditure. It is not possible to determine the exact prevalence of obesity due to the lack of an accurate method to measure adiposity but it is considered that it ranges from 5% to 20%, in several countries (Courcier *et al.*,2010; Switonski & Mankowska, 2013).

Obesity has negative effects on health, leading to reduced life expectancy and/or increased health problems such as dyslipidaemia, arthritis, intolerance to exercise, tracheal collapse, hyperadrenocorticism, hypoventilation, heat intolerance and increased risk to develop diabetes *mellitus* (DM) (German *et al.*, 2010; Zoran, 2010). Among dogs the relationship between obesity and DM is not clearly understood but it is generally accepted that omental fat induces insulin resistance and also that obesity contributes to hypertension and to the alteration of lipid profile, alterations that improve with weight loss (Tvarijonaviciute *et al.*, 2012; German *et al.*, 2010; German 2006).

Considering the increased prevalence of obesity and its negative consequences to the dog's quality of life it is important to establish preventive attitudes and to implement the adequate therapeutic measures. For that, it is important to understand the mechanisms underlying the development of obesity and the molecular and cellular alterations associated to the increased adiposity.

There are several factors associated to obesity such as age, sex, breed, neutering, endocrinopathies such as hypothyroidism and hyperadrenocorticism, feeding, sedentary lifestyle, and behavioural factors (Colliard *et al.*,2006; Courcier *et al.*, 2010; Diez *et al.*, 2006; German, 2006). Some of these factors could be avoid if the owners adopt healthy habits, but other factors are intrinsic to the dogs. In these last situations it is important to understand the characteristics of the adipose tissue.

Recent studies showed that adipose tissue produce several active molecules such as chemokines, pro-inflammatory mediators such as tumour necrosis factor alpha, interleukin-1 (IL-1), interleukin-1 receptor antagonist (IL-1Ra), interleukin-6 (IL-6) and

transforming growth factor β (TGFβ), and hormone-like molecules such as leptin (Kwon & Pessin 2013; Cammisotto & Bendayan 2007; Griffin & Guilak 2009; Balistreri *et al.* 2010; Tvarijonaviciute *et al.* 2012).

Leptin is a small non-glycosylated peptide of 146 amino acids and of 16 kDa. Once produced, leptin mainly reduces appetite and regulates the metabolism of carbohydrates and lipids (Iwase *et al.* 2000; Ishioka *et al.* 2005; Ishioka *et al.* 2007; Ishioka *et al.* 2002).

Recent studies pointed to a loss of the leptin function in obese dogs that may worsen the obesity. Since obesity is associated to the impaired glucose metabolism and to the development of insulin resistance it is possible that the impaired effect of leptin in obesity contributes to the worsening of insulin resistance and ultimately for the development of DM. However, the association between obesity, leptin and diabetes is unclear and consequently it is difficult to establish therapeutic targets in order to treat obesity and DM.

2. Canine Obesity

Canine obesity is a serious breeding and health issue that among other consequences is associated to a decrease in dogs life span, orthopaedic problems, development of DM, and to an increased risk to the development of tumours (German *et al.*, 2010). Recent studies reported that 34-59% of dogs visiting veterinary are overweight and 5-20% were considered obese (Switonski & Mankowska, 2013). Taking in consideration the incidence of obesity and the fact that this clinical condition has several health and wellness implications for pets and their owners (Corbee, 2012) it is important to understand the pathophysiology and the risk factors associated to it, in order to implement the adequate treatment and to establish preventive measure (Zoran, 2010).

Obesity occurs when for an extended period of time, animals have an excessive dietary intake or inadequate energy usage that induce a positive energy balance (Courcier *et al.*, 2010). This situation will induce an abnormal or excessive fat

accumulation and consequently the development of adiposity whose quantification will establish the degree of obesity. However, among companion animals it is difficult to establish an accurate protocol to quantify the adiposity and there is no consensus on the best method (Ishioka *et al.*, 2007).

Some authors have considered overweight when the dog's weight is 10 to 20% higher of the ideal weight and obesity when dog's weight is 20% higher of the ideal weight (Ishioka *et al.*, 2002; Rand *et al.*,2004). However, this criteria was dependent on the definition of "optimal body weight" for each breed which is not defined (German, 2006). Nonetheless, the body weight is still a valuable tool whilst dogs are on a diet since it allows a control of the weight loss. Others clinicians tried to applied the body mass index (BMI), generally used to diagnose obesity in humans, but due to the morphological and anatomical peculiarities of each breed, this index did not give objective data in small animal clinic (Ishioka *et al.*, 2002).

In order to overwhelm these difficulties, clinicians started to evaluate body condition using a score based on visual assessment and palpation of several parameters such as bony prominences evidence, muscle mass and presence of fat (Switonski & Mankowska, 2013). Three main body condition scoring (BCS) exist, all of which use similar visual and palpable characteristics, but differ by the number of categories within the scoring system (e.g., 5 points, 6 points, and 9 points) (German, 2006). The most widely accepted system is the 9 points BCS, since it was demonstrated that it correlates better with body fat mass determined by dual-energy X-ray absorptiometry (German, 2006). According to this nine points BCS, dogs classified with 1 are emaciated animals and dogs classified with 9 are considered grossly obese, table 1. Dogs classified with 4-5 points are considered as having an adequate body condition.

In spite of the efforts, the application of the 9 points BCS is still dependent on the experience and subjectivity of the clinician (Ishioka *et al.*, 2002). Most of the clinicians considered that dog has ideal weight when the ribs are easily palpable, waist is easily noted and abdominal tuck is evident.

The lack of a quantitative method for determining overweight and obesity complicates the analysis of the results obtained in epidemiological studies of canine obesity and the identification of risk factors.

Table 1. Body condition score based on 9 categories.

Body condition	**Score**	**Overall body appearance**
Too thin	1	Ribs, lumbar vertebrae, pelvic bones and all bony prominences evident from a distance. No discernible body fat. Obvious loss of muscle mass.
Too thin	2	Ribs, lumbar vertebrae and pelvic bones easily visible. No palpable fat. Some evidence of other bony prominence. Minimal loss of muscle mass.
Too thin	3	Ribs easily palpated and may be visible with no palpable fat. Tops of lumbar vertebrae visible. Pelvic bones becoming prominent. Obvious waist and abdominal tuck.
Ideal	4	Ribs easily palpable, with minimal fat covering. Waist easily noted, viewed from above. Abdominal tuck evident.
Ideal	5	Ribs palpable without excess fat covering. Waist observed behind ribs when viewed from above. Abdomen tucked up when viewed from side.
Too heavy	6	Ribs palpable with slight excess fat covering. Waist is discernible viewed from above but is not prominent. Abdominal tuck apparent.
Too heavy	7	Ribs palpable with difficulty; heavy fat cover. Noticeable fat deposits over lumbar area and base of tail. Waist absent or barely visible. Abdominal tuck may be present.
Too heavy	8	Ribs not palpable under very heavy fat cover, or palpable only with significant pressure. Heavy fat deposits over lumbar area and base of tail.

		Waist absent. No abdominal tuck. Obvious abdominal distention may be present.
Too heavy	9	Massive fat deposits over thorax, spine and base of tail. Waist and abdominal tuck absent. Fat deposits on neck and limbs. Obvious abdominal distention.

Obesity is usually associated to dietary factors and consequently to energy imbalance, too many calories consumed or too few calories burned. Nonetheless, it is accepted that obesity is multifactorial and is also dependent on factors such as age, breed, sex, gonadal status and hormonal influences, occurrence of endocrinopathies, sedentary lifestyle, and behavioural factors (Zoran, 2010).

It is considered that the incidence of obesity increases with the age of both the dog and the owner (Courcier *et al.*, 2010; Diez *et al.*, 2006). In a study performed by Courcier *et al.* in the UK, 39.9% of the obese dogs were between three and eight years old and 38.6% were nine years or older which is in agreement with other studies (Diez *et al.* 2006; Lund *et al.*2006; Colliard *et al.* 2006). The predisposition to obesity of older animals seems to be associated to the reduction in the metabolic rate and to a reduced physical activity (Zoran 2010; Lund *et al.*2006; Colliard *et al.* 2006).

Considering the breed predisposition, previous studies showed that certain breeds including Cairn Terriers, West Highland White Terriers, Scottish Terriers, Shetland Sheepdogs, Basset Hounds, Cavalier King Charles Spaniels, Dachshunds, Beagles, Cocker Spaniels, and Labrador Retrievers had an increased predisposition to develop obesity (Diez *et al.*, 2006; Edney & PM, 1986). Conversely, certain breeds, particularly Greyhounds and various Sheep Herding breeds appear to be resistant to the development of obesity (Zoran, 2010). However, recently in a study performed in the UK, Courcier *et al.* reported that it was not found a statistical association between breed and obesity (Courcier *et al.*, 2010). Moreover, Courcier *et al.* also suggested that the

previous association was due to the existence of confounders factors such as the environmental factors (Courcier *et al.*, 2010).

Another clear risk factor for obesity is gender and neutering. Previous studies reported that obesity is more prevalent in females than in males (Lund *et al.* 2006; Diez *et al.* 2006; Colliard *et al.* 2006; Courcier *et al.* 2010). In addition to gender, neutering is also considered a risk factor to the development of obesity, being the incidence of obesity higher in neutered dogs of both sexes (Colliard *et al.*, 2006; Courcier *et al.*, 2010; Diez *et al.*, 2006; German, 2006; Lund *et al.*, 2006). In a study performed across United States, 38% of all castrated male and spayed female adult dogs were overweight or obese. This problem is believed to be due to hormonal changes associated with neutering that change the feeding behaviour, contribute to reduction of the metabolic rate and to a reduction in physical activity. Recent studies also showed that the loss of sex hormones induce alterations in the brain centres that affect satiety, metabolism and the production of hormonal regulators of food intake such as leptin (Zoran, 2010).

In addition to these causes, there are others factors that may contribute to the development of obesity like the lifestyle, human-animal relationship and behavioural factors like anxiety, depression, failure to establish a normal feeding behaviour, and failure to develop control of satiety (German, 2006). However, the contribution of these factors to obesity is dependent on several variables (owner's age and lifestyle, existence of several animals at the same place, time that the animal spent alone) that limit the evaluation of its contribution *per si* for the development of obesity.

Independently of the subjacent cause of obesity, it is considered that obese dogs have a significant reduction in mean life span and require medication for chronic health problems sooner than non-obese dogs (Kealy *et al.*, 2002). In fact, the deposition of adiposity is associated to the occurrence of cardiovascular diseases, respiratory difficulties and osteoarticular problems (German *et al.*, 2010). The adiposity increases the circulating blood volume and the cardiac output in order to irrigate the excess of adipose tissue (Waltham, 1999).This increase in workload together with the fact that in

obese dogs there is also deposition of fatty in heart that will contribute to the reduction of cardiovascular reserves and to the development of congestive heart problems (Laflamme, 2012). In addition, the deposition of fat around airways of laryngeal and pharyngeal areas may exert pressure and lead to a narrowing of the airways, contributing to respiratory insufficiency and to exercise intolerance (Waltham, 1999). Obesity also increases biomechanical loading contributing to the development of osteoarthritis, which in turns leads to reduced mobility and to a reduction in energy expenditure worsening the obesity (Griffin & Guilak 2009; Laflamme 2012).

Although early studies indicate that the effect of obesity was mainly due to fat deposition in different organs, recent studies have shown that there were also several alterations at the cellular and molecular level (Laflamme,2012; Tvarijonaviciute *et al.* 2012). During the development of obesity, cells become overload with fatty acids that will contribute to hypertrophy and hyperplasia of adipocytes. This cellular stress induces the secretion of local and systemic molecules called adipokines, including more than 50 cytokines, chemokines, hormone-like factors such as leptin and adiponectin and pro-inflammatory mediators such as tumour necrosis factor alpha (TNFα), interleukin-1 (IL-1), interleukin-1 receptor antagonist (IL-1Ra), interleukin-6 (IL-6) and transforming growth factor β (TGFβ), figure 1 (Balistreri *et al.*, 2010; Cammisotto & Bendayan, 2007; Griffin & Guilak, 2009; Kwon & Pessin, 2013; Tvarijonaviciute *et al.*, 2012). Together, these mediators contribute to the existence of a chronic low grade inflammatory state, to angiogenesis, alter the control of appetite and satiety and control the glucose and lipid metabolism, which may explain the reduced life span of obese animals. (Laflamme, 2012; Kwon & Pessin 2013; Balistreri *et al.* 2010).

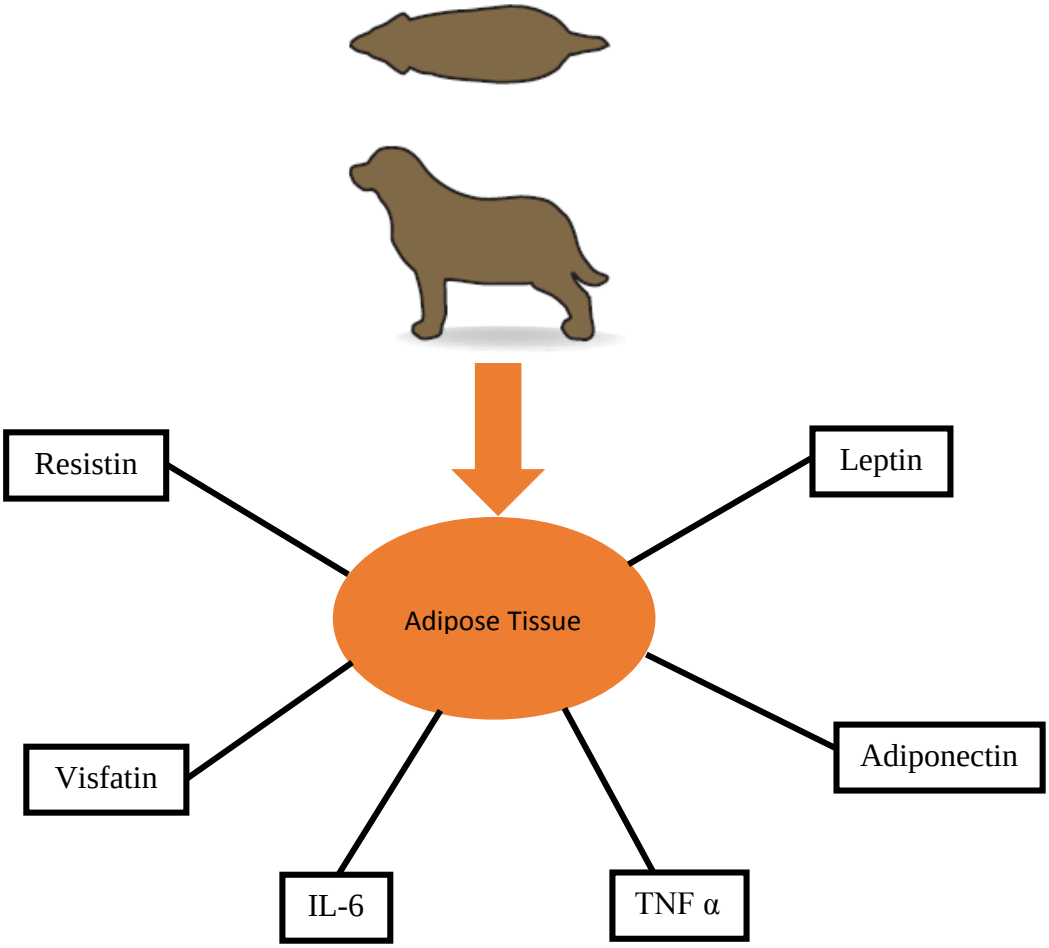

Figure 1: Adipose tissue and the production of adipokines.

3. Diabetes *mellitus*

Diabetes *mellitus* (DM) is one of the most frequently diagnosed endocrinopathies among dogs that is characterised by a chronic excess of blood glucose, resulting from defects in impaired insulin secretion, impaired insulin action, or both (Fall, 2009; Rand *et al.*, 2004).

In companion animals, like in humans, two types of DM are recognized.(Rand *et al.*, 2004). Among cats it is considered that 80-95% of diabetic present clinical characteristics and histological findings similar to the DM type 2 in humans. This type of diabetes is characterized by abnormal insulin secretion and peripheral insulin resistance,

being obesity and physical inactivity major risk factors for its development. (Fall, 2009; Hoenig, 2014; Rand *et al.*, 2004).

On the other hand, dogs usually suffer from DM that resembles human DM type 1 which is characterized by an impaired insulin production. The stimulation of beta cells by glucose, it is not accompanied by an increase in the insulin releasing, suggesting that there is a decrease of beta cells or that beta cells became unresponsive (Hoenig, 2002). One of the main causes of diabetes in dogs is chronic pancreatitis associated to auto-immune diseases that induces an extensive pancreatic damage and consequently an impaired insulin production. Another cause of DM among dogs is age which is associated to a decline in beta cell function. In fact, DM rarely occurs in dogs younger than one year of age but affects 70 % of dogs older than seven at the time of diagnosis (Hoenig, 2002). However, it is also important to consider that dogs may also develop a diabetes that resembles a human type 2 diabetes, associated to peripheral insulin resistance induced by others endocrinopathies such as Cushing disease and acromegaly, or by the long-term use of steroid drugs (Kahn *et al.*, 2006).

In addition to pancreatitis and age it is also considered that breed, and sex may also contribute to the development of diabetes among dogs. In fact, there are several breeds that present an increased risk to the development of diabetes such as Keeshonds, Pulis, Cairn Terriers, Miniature Pinschers, Poodles, Samoyeds, Australian Terriers, Schnauzers, Spitz, Fox Terriers, Bichon Frise, and Siberian Huskies. In females, it is considered that insulin resistance may accompany the heat cycle and gestational diabetes may occur during pregnancy (Zoran, 2010).

More recently, obesity has also been associated to the development of insulin resistance and also to DM. However, the association between canine obesity and diabetes is not well understood (Ellmerer *et al.* 2006; German 2006; German *et al.* 2010, Laflamme, 2012). Among humans, it is considered that central obesity may contribute to the development of glucose intolerance and posteriorly to the development of diabetes type 2. Nevertheless, in veterinary medicine the link between obesity and diabetes it is

not well established. In cats, it is known that insulin sensitivity decreases by about 30 % for each kilogram of body weight gain (German, 2006;Hoenig *et al.*,2007; Rand *et al.*, 2004;Laflamme,2012) and feline obesity is associated with up to an 4-fold increased risk for development of DM (Laflamme, 2012,Lund *et al.* 2006).

Among dogs, it is considered that omental fat induces insulin resistance and also that obesity contributes to hypertension and to the alteration of lipid profile, alterations that ameliorated by weight loss (German *et al.*, 2010; German, 2006; Tvarijonaviciute *et al.*, 2012). Nonetheless, the molecular mechanisms that correlate obesity with insulin resistance and diabetes are not well understood (Hoenig *et al.*, 2007)

According previous studies, there are two theories that may explain this correlation (Bergman *et al.*, 2007). One of the theories is based on the fact that visceral fat release non-esterified fatty acids that accumulate in the pancreatic beta cells inducing deterioration of β-cell function and reducing the production of insulin (Bergman *et al.*, 2007).

The other theory is based on the ability of adipocytes to produce adipokines such as IL-1, IL-6, TGFβ, leptin, resistin, visfatin and adiponectin that may induce an inflammatory state and reduce insulin sensitivity of liver and muscle cells, figure 1 and 2 (Kwon & Pessin 2013; Cammisotto & Bendayan 2007; Griffin & Guilak 2009; Balistreri *et al.* 2010; Tvarijonaviciute *et al.* 2012; German *et al.* 2010, Laflamme, 2012).

The insulin resistance seems to be associated with the inhibition of glucose oxidation by the non-esterified fatty acids. The impaired glucose metabolism could be explained by two different pathways that may occur simultaneously. On the one hand, the intracellular beta oxidation of fatty acids generates a great amount of acetil-coenzyme A that inhibits the phosphofructokinase and hexokinase activity and subsequently the use of glucose (Kahn *et al.*, 2006). On the other hand, the increase in the intracellular content of fatty acid metabolites such as diacylglycerol, fatty acyl-coenzyme A, and ceramides may activate a serine/threonine kinase cascade leading to serine/threonine phosphorylation of insulin receptor substrate and consequently to a

decreased in the activity of PI3K/AKT (Kahn *et al.*, 2006). The PI3K/AKT is the signalling pathway that facilitates glucose uptake in adipocytes and muscle cells by allowing the translocation of glucose transporters to the plasma membrane, figure 2 (Kahn *et al.*, 2006; Vargas *et al.*, 2004). Therefore, a reduction in the activation of this signalling pathway will induce impaired glucose uptake and will generate insulin resistance (Hoenig, 2002).

In addition to the production of adipokines and to the metabolic alterations, recent studies reported that obesity also alters the expression of several genes involved in glucose and lipid metabolism. One of these genes is the peroxisome-proliferator activated receptor γ (PPAR γ) which encoded the protein with the same name. PPAR γ seems to contribute to increase insulin sensitivity in muscle and adipose tissue due to its ability to regulate the transcription of other genes. However, Gayet *et al.* reported that in obese dogs there is a decrease in PPAR γ mRNA expression. Furthermore, since the target genes of PPAR γ encode proteins involved in the release and storage of fatty acids, such as leptin, and adiponectin and of proteins directly involved in glucose uptake such as the insulin-dependent glucose transporter GLUT4, several studies pointed that PPAR γ contributes to the insulin resistance developed in obese dogs, figure 2 (Gayet *et al.*,2007; Kintscher & Law, 2005; Leonardini *et al.*,2009)

Considering the hypothesis that obesity contributes to the development of insulin resistance and also that obesity is a serious problem among dogs, it is expecting that the percentage of dogs developing diabetes type 2 will increase in the next years. Therefore, it is important to implement preventive measures to avoid obesity and to investigate the role of adipokines such as leptin produced by adipose tissue.

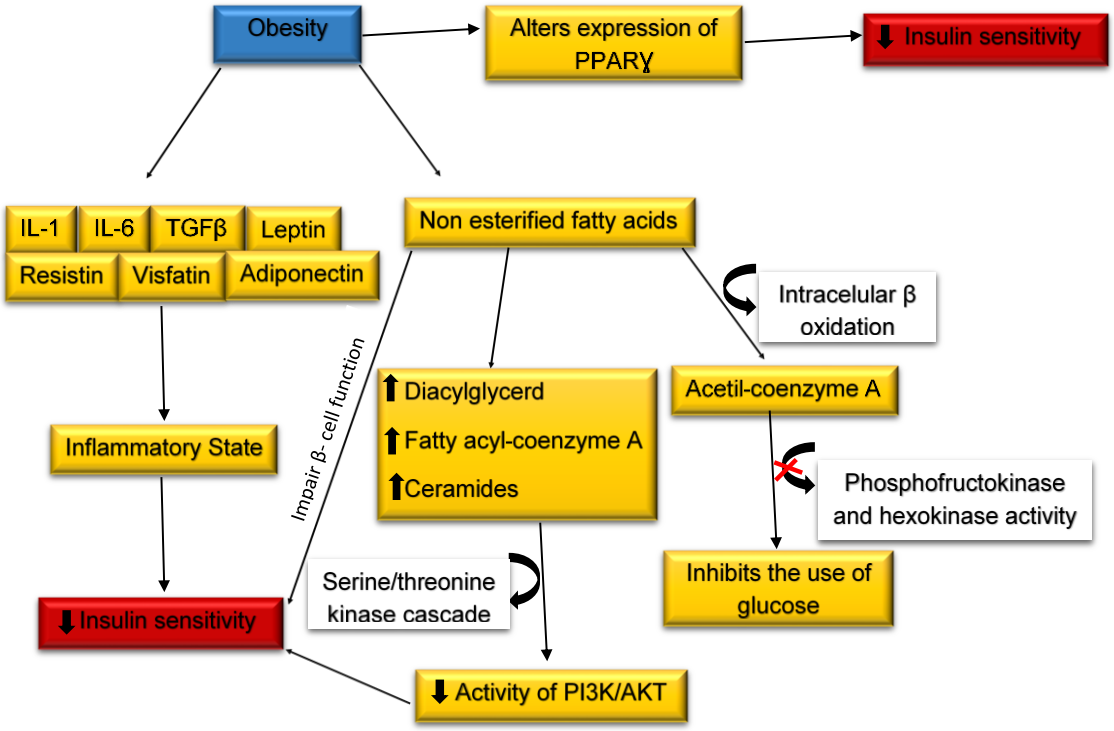

Figure 2. Molecular and cellular alterations associated to obesity

4. Correlation between obesity, diabetes and leptin

As previously referred adipose tissue produces adipokines that influence various biologic processes including energy balance, glucose and lipid metabolism, inflammation, immune function, haemostasis, vascular function and angiogenesis, figure 1 and 2 (Zoran 2010; German *et al.* 2010; Kwon & Pessin 2013; Cammisotto & Bendayan 2007; Griffin & Guilak 2009; Balistreri *et al.* 2010; Tvarijonaviciute *et al.* 2012; Laflamme, 2012).

One of the main adipokines is leptin, the *ob (obese)* gene product that plays a key role in the regulation of body weight, energy balance and feeding behaviour (Ishioka *et al.*, 2005, Ishioka *et al.*,2007; Ishioka *et al.*, 2002; Iwase *et al.*, 2000). In dogs, this adipokine is synthesized and secreted mainly by adipocytes but in humans and mice it is also produced by gastric epithelial cells, placenta, mammary gland and liver (Cammisotto & Bendayan, 2007; Zoran, 2010). Structurally, leptin is a small non-

glycosylated peptide of 146 amino acids and of 16 kDa discovered in 1994 by Zhang et al (Cammisotto & Bendayan, 2007; Zhang *et al.*, 1994).

The production of leptin is mainly regulated by adiposity, existing a positive correlation between degree of adiposity and plasma leptin concentration observable in several species, including dogs. In addition to adiposity, other factors also regulate the leptin production such as insulin, glucocorticoids, endotoxin, and cytokines (TNFα, IL1b, and IL-6) (Zoran, 2010).

According the feeding-fasting cycles and to the net effects of various neuroendocrine and nutritional factors such as glucose, insulin, glucocorticoids and catecholamines, serum leptin concentration shows diurnal changes. More specifically, plasma leptin rises immediately following food intake, shows a peak 5-8 hours afterwards and then slowly decreases to basal levels 19-23 hours after a meal. In contrast when dogs are fasted, plasma leptin levels gradually decrease to levels even lower than basal values and then return to baseline limits within 12 hours of re-feeding (Ishioka et al., 2005; Ricci & Bevilacqua, 2012).

In spite of the diurnal variations, Ishioka *et al.* demonstrated that among dogs the plasma leptin concentration was independent of age, sex and of reproductive status (neutered or intact) regardless they were obese or of optimal body condition (Ishioka *et al.*, 2007; Ishioka *et al.*, 2002).

Once produced leptin reduces appetite and regulates the cellular metabolism (stimulates lipolysis, inhibits lipogenesis, improves insulin sensitivity, increases glucose metabolism and stimulates fatty acid oxidation). In addition, recent studies reported that leptin also contributes to the proliferation of lymphocytes (particularly CD4+) and to the induction of Th1 response, cytokine production, phagocytosis, regulation of hypothalamic-pituitary-adrenal-axis, reproduction, angiogenesis, and oxidative stress (Balistreri *et al.*, 2010; Cammisotto & Bendayan, 2007; Nishii *et al.*, 2010).

The effects of leptin are initiated through interaction with its receptor. The leptin receptor present six isoforms, belongs to the family of the gp130 receptors and is closely

related to members of the IL-6 family of receptors. Five of the receptors are membrane bound and the sixth is a soluble form that binds leptin in circulation to enhance its half-life (Cammisotto & Bendayan, 2007). The highest numbers of receptors are expressed at the satiety centres of the hypothalamus, but they can be found widely distributed throughout the body, reflecting leptin's involvement in the regulation of several physiologic processes (Ricci & Bevilacqua, 2012).

Binding of leptin to its receptor in the hypothalamus activates a signalling pathway that reduces appetite, through the stimulation of anorexigenic neurons, suppression of orexigenic neurons, and suppression of the release of endocannabinoids, which are regulators of orexigenic neurons (Amitani et al.,2013; Marroquí et al., 2012). These properties of leptin were evidenced by several studies and explain the reason why leptin is one of the main adypokine implicated in obesity.

Previous studies performed in mice showed that mutation of the DNA sequence encoding leptin was associated with the development of hyperphagia and morbid obesity (Ricci & Bevilacqua, 2012) . Conversely leptin administration in obese mice resulted in reduction of food intake and weight loss (Ricci & Bevilacqua, 2012).

Furthermore, it was showed that after a fatty or high-energy meal dogs present a 2- to 3-fold increase in the leptin concentrations which was maintained for as long as 8 hours, which confirms that leptin is associated to fatty acids and glucose metabolism (Ishioka 2005). This increased in leptin concentration was also confirmed in obese dogs from either experimentally induced obesity or pet dogs with increased body condition scores. In contrast, reduction in fat mass was accompanied by a decrease in leptin concentration (Ricci & Bevilacqua, 2012).

These results pose a question, if leptin controls food intake, and the plasma concentration of leptin in obese animals is increased as compared to non-obese how to explain the increase in obesity. A possibility to explain this contradiction was the occurrence of a mutation on the leptin receptor, turning cells resistant to the plasma leptin. However, several studies performed in humans reported that the majority of obese

humans do not present mutations at the leptin receptor and as far as we know there is no report of leptin receptor mutations in dogs (Dincer *et al.*, 2005; Farooqi *et al.*, 2007; Zoran, 2010).

The other possibility is the development of leptin resistance as a result of the increased fat tissue. Leptin crosses the blood-brain barrier via a transport system that becomes saturated with the increased plasma concentration of leptin (Zoran 2010; Amitani *et al.* 2013, Marioqui). This hypothesis was evidenced in rodents studies where obesity has been associated with a state of leptin resistance mainly due to the inability of leptin to reach is target sites in the brain and/or to the reduction of the intracellular leptin receptor signalling cascade (Ricci & Bevilacqua, 2012). Although no studies have evaluated leptin resistance in dogs, it is fair to speculate that this condition also occurs in dogs (Ricci & Bevilacqua, 2012).

Considering these results, we may hypothesise that the impaired effect of leptin will contribute to the dysregulation of the appetite and of the lipid metabolism and therefore it will also contribute to the alteration of the glucose metabolism and to the development of insulin resistance. Moreover, recent studies performed in mice reported that leptin regulates not only glucose metabolism through its effects on body weight and food intake but also by independently pathway (Amitani *et al.*, 2013; Denroche *et al.*, 2012; Kwon & Pessin, 2013; Zoran, 2010).

These effects of leptin seem to be mediated centrally via activation of specific neuronal subpopulations in the hypothalamus and through the activation of phosphatidylinositol-3-kinase (PI3K) signalling pathway which is also the pathway associated to the insulin signal transduction. As leptin exerts its effect upstream of PI3K, by the modulation of the phosphorylation satus of insulin receptor substrate-1 (Amitani *et al.*, 2013; Denroche *et al.*, 2012; Koch *et al.*, 2010; Kwon & Pessin, 2013), it may override the need of insulin to regulate the glucose metabolism. Through this signalling pathway leptin has the ability to enhance hypothalamic sensitivity to insulin and to contribute to the homeostasis of glucose metabolism.

Therefore, in obesity, the reduced ability of leptin to transverse blood-brain barrier has two main consequences: impairs the control of food intake and reduces the response of hypothalamus to insulin, dysregulating the glucose homeostasis (Amitani *et al.*, 2013; Denroche *et al.*, 2012; Koch *et al.*, 2010; Kwon & Pessin, 2013; Zoran, 2010).

In addition to the effect of leptin at hypothalamus, it is also important to consider its effect at the pancreatic β-cells where leptin exerts two different actions: inhibits insulin synthesis and secretion from β-cells; and controls β-cell proliferation, apoptosis, and cell growth (Amitani *et al.*, 2013; Denroche *et al.*, 2012; Koch *et al.*, 2010; Kwon & Pessin, 2013; Marroquí *et al.*, 2012; Tomoaki, 2012; Zoran, 2010).

The effect on insulin occurs in two different ways: leptin suppresses the expression of pre proinsulin mRNA in pancreatic β-cells and inhibits the signalling pathways involved in the transport of glucose and in the increased intracellular calcium concentration, both essentials to the secretion of insulin (Amitani *et al.*, 2013; Marroquí *et al.*, 2012; Tomoaki, 2012).

This effect of leptin in pancreatic β-cells is part of a loop that controls the levels of plasmatic insulin and leptin (Amitani *et al.*, 2013; Marroquí *et al.*, 2012; Tomoaki, 2012). These observations were obtained using animal models of obesity and therefore additional data will be necessary to clarify the function of leptin in dogs. Nevertheless, these consideration highlight the possible role of leptin in the control of obesity, glucose homeostasis and insulin resistance a triangle that needs to be better investigated among dogs in order to treat and prevent metabolic disorders.

Among humans the first results regarding the plasma level of leptin in diabetic patients were controversial. Several studies reported that leptin levels were independently associated with DM after adjustment for BMI and total cholesterol (Bandaru & Shankar, 2011). However, more recently Chen *et al.* reported that higher leptin levels were found to be associated with elevated risk of type 2 DM in men but not in women (Chen *et al.*, 2014).

In dogs, Nishii et al reported that obese diabetic dogs had lower plasma leptin

concentration than the body condition score-matched normal dogs but re-inforced that in diabetes other factors than adiposity may contribute to this reduced level (Nishii *et al.*, 2010). In fact, considering that obesity also contributes to the damage of β-cells reducing the insulin production and considering that the synthesis and storage of fat requires insulin, it is possible to hypothesize that from a certain point of diabetes there will be a persistent hyperglycaemia and a progressive loss of body fat stores, similar to that observed in diabetes type 1, which will induce a reduction in the circulating levels of leptin (Morton & Schwartz, 2012).This hypothesis needs to be investigated in order to better understand the role of leptin in the pathophysiology of diabetes (Amitani *et al.*, 2013; Denroche *et al.*, 2012; Dincer *et al.*, 2005).

Independently on the controversy among the leptin plasma levels, studies performed in obese leptin deficient mice showed that the exogenous administration of leptin reduces obesity, restores insulin sensitivity and reverse hyperglycaemia which may indicate that leptin could be considered a potential therapeutic target (Amitani *et al.*, 2013; Denroche *et al.*, 2012; Dincer *et al.*, 2005; Koch *et al.*, 2010).

5. Conclusion

In dogs, leptin is one of the first parameters that increase during weight gain, unlike plasma cholesterol, triglycerides, glucose and insulin, which do not change in the initial phase. This observation turns leptin as a valuable tool in the follow up of obesity and related disorders (Ishioka *et al.*, 2007). Since there is a direct correlation between adiposity and plasma level of leptin its monitorization during obesity- control programs may ensure fat loss instead of muscle loss. In addition, leptin could also be a helpful parameter in research to evaluate the effect of newly developed anti-obesity drugs (Ishioka *et al.*, 2007; Ishioka *et al.*, 2002).

Regarding the leptin role in diabetes, further studies are needed to clarify its contribution to the pathophysiology of this metabolic disease and its potential as a therapeutic target.

6. Appendix

Classification of diabetes in dogs:

a. Insulin deficiency diabetes includes beta cell loss hypoplasia or abiotrophy, exocrine pancreatic disease (such as pancreatitis), or idiopathic causes.
b. Insulin resistant diabetes results from the insulin antagonistic effect of either endogenous or exogenous hormones, which oppose the effect of insulin and/or decrease insulin secretion such as sex steroids, glucocorticoids, and growth hormone, among others. (Hoenig, 2014)

However, some cases don't fit in this classification like gestational and dioestrous diabetes mellitus and for this reason a new classification system for canine diabetes mellitus was proposed. The following classes are proposed: juvenile, progesterone – related, secondary to pancreatic insult, endocrine tumours, iatrogenic, immune – mediated and idiopathic. (Fall, 2009)

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