



## Symposium on Biological Causes and Coping Strategies

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Additional information and declarations  
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## Obesity and thermogenic adipose tissue plasticity in dogs‡

### Abstract

Obesity in pet dogs is a growing concern in veterinary medicine in developed countries. Obesity is associated with the expansion of white fat, the main tissue that stores metabolic energy in mammals. Brown and beige adipose tissues, which express thermogenic uncoupling protein-1, are oppositely related to obesity due to their thermogenic activity and associated energy expenditure properties. Dogs possess high amounts of brown adipose tissue early after birth; however, its involution with aging causes adult dogs to possess minimal active brown fat. However, adult dogs retain a remarkable capacity to activate the browning of adipose tissue depots in response to sustained  $\beta$ -adrenergic stimulation or other inducers, such as cannabinoid receptor-1 inhibitors. Therefore, dogs retain the capacity of adipose tissue plasticity to acquire thermogenic properties, which should be considered when developing obesity prevention and/or treatment strategies for dogs.

**Keywords:** Dogs, obesity; Brown adipose tissue; Beige adipose tissue;  $\beta$ -adrenergic receptors.

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## Introduction

Obesity is a health disorder characterized by accumulation of fat due to a permanent positive energy balance where the energy input to the organism (food consumption and absorption of metabolites) is higher than the energy expenditure. This is a common disorder in human health known to increase the risk of diabetes, cardiovascular diseases, and even some types of cancer.

Obesity is also increasingly appearing in companion animals and has long been a concern in veterinary medicine.<sup>(1)</sup> Close to one-half of pet dogs in developed countries are considered to be overweight or obese.<sup>(2-6)</sup> Obese dogs show multiple comorbidities similar to those seen in human obesity such as insulin resistance and hyperlipidemia,<sup>(6, 7)</sup> along with undesirable behaviors.<sup>(8)</sup>

Certain canine breeds show distinct susceptibilities to obesity, suggesting that genetics can impact the propensity for obesity in dogs.<sup>(9, 10)</sup> Recent advances in the recognition of the plasticity of adipose tissues in relation to the energy storage-versus-energy balance status in mammals are expected to be relevant to obesity in dogs. This review summarizes the current view of thermogenic adipose plasticity in relation to obesity, and presents knowledge of this process in dogs.

## White and brown adipose tissues and obesity

Obesity is characterized by a massive expansion of white adipose tissue (WAT), which is the physiological site for energy storage in mammals. This energy-storing function of white adipocytes reflects their ability to store large amounts of fat and their capacity to be hypertrophied in obese individuals. In addition, WAT itself can enlarge due to adipocyte hyperplastic processes such as those seen in cases of massive obesity appearing early in development. The ectopic presence of fat in nonadipose tissues (liver, heart, and muscle) is a hallmark of the harmful systemic consequences of obesity.<sup>(11)</sup>

As observed in humans, adipose tissue hypertrophy in obese dogs correlates positively with plasma leptin concentrations<sup>(12)</sup> and negatively with plasma adiponectin concentrations.<sup>(13)</sup> Moreover, the WAT of obese dogs shows high expression of TNF- $\alpha$ , a well-known inflammatory marker in adipose tissue, concomitant with a repressed expression of the Glut4 glucose transporter and lipoprotein lipase,<sup>(13)</sup> which is expected to contribute to the metabolic dysregulation in obese canines.<sup>(6, 13, 14)</sup>

Studies have shown that the involvement of adipose tissues in obesity goes far beyond the role of WAT as a site of massive fat storage, adding complexity. In mammals, researchers long ago identified another type of adipose tissue distinct from WAT: brown adipose tissue (BAT). This tissue is a major site for both cold- and diet-induced nonshivering thermogenesis in mammals.

BAT contains brown adipocytes, a particular type of adipose cell characterized by the multilocular distribution of lipid droplets inside the cell and the presence of large amounts of highly oxidative mitochondria. Moreover, the mitochondria in brown adipocytes contain uncoupling protein-1 (UCP1). This protein, which is

uniquely present in this type of cells, uncouples mitochondrial respiration from oxidative phosphorylation and enables the mitochondria of brown adipocytes to use active oxidation of metabolic foodstuff to produce heat instead of ATP. Thus, UCP1 expression is both the key molecular event that gives BAT its thermogenic function and an unequivocal molecular marker of the “brown” identity of an adipose cell.<sup>(15)</sup>

Based largely on studies in rodent models (mice, rats), BAT activity has been shown to protect against obesity by contributing to energy expenditure.<sup>(16)</sup> Although the existence of BAT and the expression of UCP1 in humans was first recognized several decades ago, the traditional concept held that BAT is restricted mostly to the neonatal and early infancy periods in humans. More recently, however, positron emission tomography-based studies demonstrated that active BAT is present in adult humans,<sup>(17)</sup> and researchers identified an inverse relationship between BAT activity and obesity in adult human populations.<sup>(18)</sup>

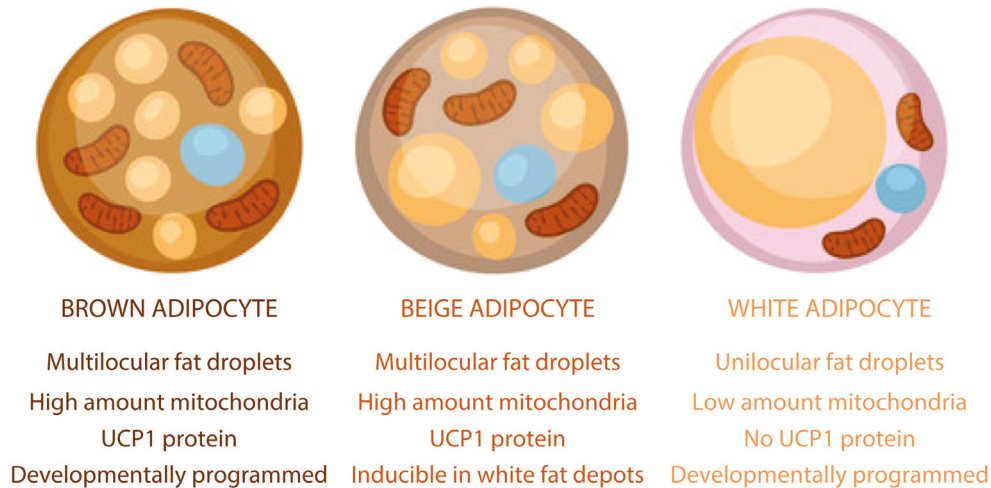
Although BAT studies have historically been performed in rodents and humans, it was assumed that BAT was present in all mammals to different degrees according to species and developmental stage. An exception was found early on in pigs. Apart from cell morphology in adipose depots, there is an evolutionary-based invalidation of the UCP1 gene in pigs, such that no functional UCP1 is present and therefore no BAT-mediated thermogenesis occurs.<sup>(19)</sup> Surprisingly, recent advances in the massive sequencing of genomes from multiple mammalian species have revealed that the UCP1 gene is invalidated in other mammals, such as elephants, horses, and some types of sea mammals which, accordingly, appear to lack thermogenically active BAT.<sup>(20)</sup>

### Adipose tissue plasticity: the browning process

In addition to WAT and BAT, recent years have witnessed growing recognition that adipose tissue can show thermogenic plasticity. In rodents subjected to environmental (e.g., thermal stress) or dietary stimuli, researchers observed not only BAT activation but also the appearance of brown adipocyte-like cells at anatomical locations that typically harbor WAT.<sup>(21)</sup> This process is called the “browning” or “beiging” of WAT.

Several studies have shown that the brown adipocytes that appear in WAT have a totally different cell lineage from that of “classic” brown adipocytes. While “classic” brown adipocytes originate during development from cellular precursors like those of muscle tissue, this is not the case for brown adipocytes that appear in WAT due to browning. To distinguish the latter cells from “classic” brown adipocytes, researchers call these cells “beige” or “brite” (from “brown-in-white”) adipocytes.<sup>(21, 22)</sup>

Beige adipocytes express the UCP1 protein and produce heat like classic brown adipocytes, although some studies suggest that they possess additional thermogenic mechanisms based on a futile creatine kinase cycle.<sup>(23, 24)</sup> Similar to classical brown adipocytes, UCP1 expression is a major marker gene of the beige-versus-white phenotype of adipose cells but high expression of other marker genes (CIDEA, Dio2, PGC-1 $\alpha$ ) is also a characteristic feature of brown and beige cells (Figure 1).<sup>(21)</sup>



**Figure 1.** The three types of adipocytes in mammals and their main features.

Some researchers proposed that beige cells arise from the differentiation of WAT-resident precursor cells; while others postulated that the white adipocytes themselves can “transdifferentiate” to beige adipocytes.<sup>(25, 26)</sup> In any case, the browning process became a focus of research interest in obesity based on the observation that strains of mice that have a low capacity to activate browning are especially prone to obesity.<sup>(27)</sup>

Mammalian species larger than small rodents, in which classical BAT depots whiten to WAT with age, maintain a remarkable capacity to exhibit browning of WAT in adulthood under certain conditions (Figure 1). In fact, BAT biopsies from adult humans were found to contain both “classic” brown and beige adipocytes.<sup>(28)</sup> This, together with the inducibility of beige adipocytes in response to environmental determinants, makes the browning process especially interesting as a potential tool for promoting thermogenic activity and protecting against obesity.

Activation of the sympathetic nervous system at adipose depots is a main mechanism for activating BAT and promoting WAT browning in response to the environmental temperature changes and dietary stimuli.<sup>(25)</sup> Additionally, these processes can be induced by non-sympathetic actors, such as circulating endocrine factors (e.g., fibroblast growth factor-21, irisin) and also dietary components ranging from vitamin A derivatives to capsinoids.<sup>(29, 30)</sup> Going forward, it will be critical to establish the molecular mechanisms responsible for eliciting BAT activation and browning to support the targeting of these of these processes in developing pharmacological and/or dietary strategies to promote energy expenditure and protect against obesity.

### Brown adipose tissue in dogs

Relatively few studies have examined BAT in dogs, indicating that it shows remarkable dependence on the stage of development. These studies, which were performed mostly in Beagle dogs, found that most of the adipose depots (lumbar, interscapular, perirenal, around the bladder) in neonatal dogs (1-2 days old) have

a BAT appearance, including a preponderance of cells with multilocular distribution of lipid droplets, abundant mitochondria with strong oxidative activity, and robust expression of UCP1 protein.<sup>(31, 32)</sup>

Functional assays in puppy dogs indicated that the perirenal BAT exhibits thermogenic activity.<sup>(33)</sup> In adult dogs (over half-a-year of age), however, the adipose depots were found to exhibit WAT appearance, with unilocular adipocytes, decreased mitochondrial oxidative capacity, and negligible UCP1 expression.<sup>(12,31)</sup> Molecular analysis of gonadal fat from adult dogs of different breeds and sizes did not detect substantial levels of UCP1 gene expression,<sup>(14)</sup> and UCP1 protein expression was not detected in five tested adipose depots (around kidney, around bladder, pericardial, omental and subcutaneous) of adult Beagle dogs.<sup>(34)</sup>

The ontogeny of BAT in dogs appears to be similar to that of other large mammals, including some livestock species (e.g., cows, lambs, goats).<sup>(35)</sup> BAT is highly relevant in the immediate postnatal period, possibly as an ontogeny-programmed event to ensure that the body temperature is maintained after the thermal stress associated with birth. Thereafter, if the animal lives in a temperate (not intensely cold) environment, BAT is replaced by WAT with age. Noteworthy, some case reports of hibernomas, rare benign tumors composed of brown adipocytes expressing UCP1, in adult dogs<sup>(36)</sup> may be interpreted as the persistence of cell lineages retaining susceptibility to acquire brown adipocyte features, even in a pathological context, in the adult dog.

### Adipose tissue plasticity and browning in dogs

Available data indicate that even when dogs practically exhibit no BAT in adulthood, their WAT maintains a remarkable capacity to undergo browning under relevant stimuli. Possibly because dogs are often used as standard research models in pharmacology, most data on adipose browning in dogs reflect the response to drug treatments rather than physiological stimuli related to environmental temperature or dietary components.

Several reports consistently showed that sympathomimetics (general  $\beta$ -adrenoceptor agonists, or agonists specific for  $\beta_3$  adrenergic receptor) have a strong capacity to induce WAT browning in dogs and subsequently reduce adiposity. Chronic administration of the  $\beta$ -agonist, LY79730, to adult dogs was reported to dramatically change the appearance of all adipose depots (except subcutaneous adipose tissue), via the massive development of brown-like (beige) cell clusters at former anatomical WAT depots <sup>(31,32)</sup>. This was reflected by the induction of molecular markers of the brown/beige phenotype, such as cytochrome c oxidase (indicative of mitochondrial oxidative capacity) and active UCP1 protein levels.

In humans and rodent models, specific activation of the  $\beta_3$  type of adrenergic receptors has been shown to be the most powerful in inducing BAT activation and WAT browning.<sup>(37, 38)</sup> Dogs possess significant levels of this adrenoceptor in their adipose tissue,<sup>(39, 40)</sup> and adipocytes from dogs show a robust lipolytic responsiveness to  $\beta_3$ -adrenoceptor agonists.<sup>(41)</sup>

**Table 1.** Summary of the reported effects of pharmacological treatments on the browning process of adipose tissue in dogs

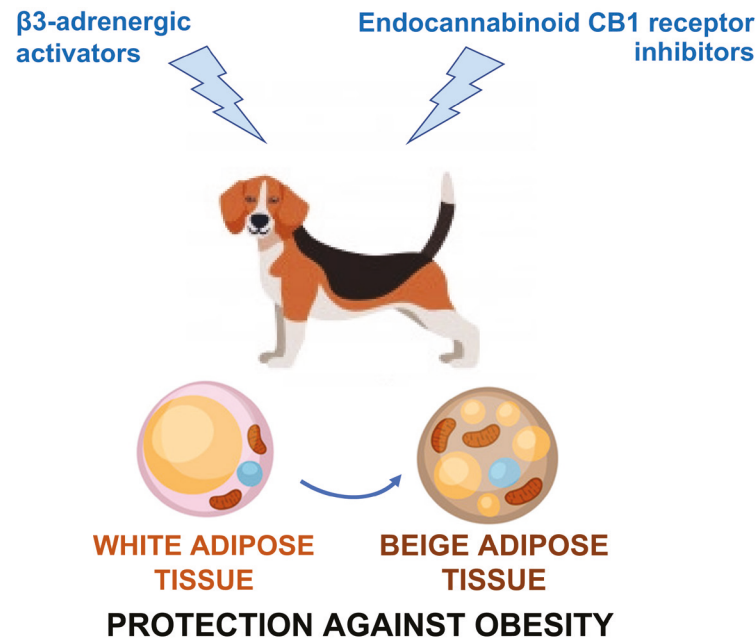
Drug (Company)	Action	Effects on adiposity and energy balance	Evidence of adipose browning	Adipose depots affected
LY 79730 <sup>(31-32)</sup> (ICI Pharmaceuticals)	$\beta$ -adrenergic agonist	Increased metabolic rate	Multilocular adipocytes UCP1 Mitochondria	Perirenal Peri-bladder
ICI D7114 <sup>(34)</sup> (ICI Pharmaceuticals)	$\beta$ 3-adrenergic agonist	Reduced body weight	Multilocular adipocytes UCP1 Mitochondria	Perirenal Peribladder Pericardial (Subcutaneous) (Omental)
CL316,243 <sup>(40)</sup> (American Cyanamid Corporation)	$\beta$ 3-adrenergic agonist	Reduced body weight Reduced adiposity	Multilocular adipocytes UCP1 Mitochondria	Perirenal Omental Subcutaneous
AJ-9677 <sup>(42-43)</sup> (Sumitomo-Dainihon Pharmaceutical)	$\beta$ 3-adrenergic agonist	Reduced body weight Reduced adiposity	Multilocular adipocytes UCP1	Subcutaneous
Rosiglitazone <sup>(44)</sup> (GlaxoSmithKline)	PPAR $\gamma$ -agonist	Not shown	Multilocular adipocytes Mitochondria	Perirenal
Rimonabant <sup>(50)</sup> (Sanofi-Aventis)	Cannabinoid receptor-1 antagonist	Reduced adiposity	Multilocular adipocytes Mitochondria Non-UCP1 beige marker genes	Visceral Subcutaneous

Several studies consistently found that chronic treatment of adult dogs with distinct  $\beta$ 3-adrenergic receptor agonist molecules had strong effects in promoting WAT browning (Table 1). Chronic treatment of dogs with  $\beta$ 3-adrenergic agonists led to the appearance of clusters of cells with the full features of beige adipocytes: multilocular distribution of intracellular lipid droplets, numerous mitochondria with densely packed cristae and high oxidative activity, and expression of the UCP1 transcript and UCP1 protein.<sup>(34, 40, 42, 43)</sup>

The perirenal, peri-bladder and pericardial adipose tissues showed more intense browning, whereas less intense browning was seen in the omental and subcutaneous fat depots. *Ex vivo* analysis showed that the cells appearing as a consequence of browning showed the characteristic thermogenic behavior of beige adipocytes.<sup>(43)</sup> In most of these studies, the browning of adipose tissue was accompanied by a reduction in adiposity<sup>(34, 40)</sup> and enhanced oxygen consumption,<sup>(32)</sup> especially when the dogs had been made obese previously.<sup>(42)</sup> Together, these data confirm that browning develops in adipose tissue in response to  $\beta$ -adrenergic stimulation in dogs. As in rodents and humans, this is expected to reflect the plasticity of adipose tissue to acquire BAT-like features in dogs under physiological stimuli known to involve  $\beta$ -adrenergic mediated signaling, such as cold environments.

A few nonadrenergic agents have also been proven to affect adipose tissue plasticity and promote browning in dogs. Treatment of dogs with rosiglitazone, an agonist of the PPAR $\gamma$  nuclear receptor, was found to modify adipocytes at perirenal adipose tissue to resemble beige cells, as evidenced by the appearance of





**Figure 2.** Pharmacological treatment of dogs with  $\beta_3$ -adrenergic receptors and endocannabinoid receptor-1 antagonists induces browning of adipose tissue in association with protection against obesity.

multilocular adipocytes and increased amounts of mitochondria.<sup>(44)</sup> However, no specific marker of the brown/beige phenotype (e.g., UCP1 expression) was tested. PPAR $\gamma$  is a master regulator of both brown and white adipogenesis and a promoter of adipocyte differentiation;<sup>(45)</sup> it remains unclear whether the altered features of adipose tissue from rosiglitazone-treated dogs reflects some extent of browning or an increased abundance of white adipocytes at early stages of differentiation.

Much more consistent is the evidence that rimonabant, an inhibitor of the type I endocannabinoid receptor (CB1R), promotes browning of adipose tissue in dogs. CB1R has been shown to be involved in feeding and energy expenditure, and the treatment of experimental rodent models, dogs, and humans with the CB1R antagonist rimonabant leads to weight loss.<sup>(46-48)</sup> In fact, rimonabant was used for several years in Europe to treat patients with obesity before it was withdrawn due to harmful psychiatric side effects.<sup>(49)</sup>

Adult dogs fed a high fat diet and treated with rimonabant exhibited decreased body weight despite unchanged food consumption and marked induction of browning-related genes (e.g., those encoding CIDEA, Dio2, mitochondrial components, non-UCP1-related beige thermogenic actors) in subcutaneous and visceral adipose depots,<sup>(50)</sup> and minor induction of UCP1 gene expression. The researchers proposed that there may be crosstalk between CB1R-mediated and  $\beta$ -adrenergic-mediated intracellular signaling in eliciting beige activation in adipose tissue of dogs.

In conclusion, although adult dogs appear to lack relevant amounts of active BAT under basal conditions, their WAT exhibit the capacity of browning of WAT under relevant stimuli (Figure 2). In principle, promoting this process in pet dogs is desirable because the active brown/beige adipose tissue protects against obesity. However, as in humans, the use of  $\beta$ -adrenergic activators to promote browning

and enhance energy expenditure to reduce adiposity probably infeasible in dogs due to the unwanted cardiovascular side effects of sympathomimetics.<sup>(51)</sup>

Other non-pharmacological strategies aimed at inducing browning could be investigated, such as lifestyle changes related to avoid an excessively warm environment and promote exercise (e.g. exercise may lead to BAT activation and WAT browning<sup>(52)</sup> and/or the use of functional foods enriched in potential inducers of brown/beige activity.

It is notable that nearly all studies on BAT activation and WAT browning have been performed in Beagle dogs. Distinct dog breeds differ largely in size, which is inversely related to the thermogenic effort required to maintain body temperature and the requirements of adipose thermogenic activity. Moreover, dog breeds may differ in their genetic predisposition for thermogenic adipose tissue plasticity.

On the other hand, the age of the dog is expected to influence the capacity of WAT for beige adipocyte-recruitment and activation, which may be related to reports on age-dependent differences in the propensity to obesity in dogs.<sup>(3)</sup> All these are factors that may lead to variable contribution of the brown and beige adipose tissues to the dog physiology and thereafter to the propensity to obesity. In any case, future research is needed to ascertain the specific mechanisms underlying adipose tissue plasticity and development in dogs in light of the expansion of obesity and associated comorbidities in pet dogs.



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## Conflicts of interest

Authors declare no conflicts of interest.

## Author contributions

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