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Abstract

Adipose tissue is not only required for energy storage but is an essential endocrine organ with a central role in the pathology of obesity. The understanding of its role, both in human and equine medicine, is continually evolving. With obesity being an ever-growing problem in the equine population, gaining owner compliance is critical when implementing management plans. The aim of this review is to encourage the inclusion of the concept of adiposity in discussions with horse owners on obesity and metabolic syndrome. In doing this, we hope to improve clients understanding and therefore maximise the impact of diagnostic tests, monitoring tools and management.

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Summary

Adipose tissue is not only required for energy storage but is an essential endocrine organ with a central role in the pathology of obesity. The understanding of its role, both in human and equine medicine, is continually evolving. With obesity being an ever-growing problem in the equine population, gaining owner compliance is critical when implementing management plans. The aim of this review is to encourage the inclusion of the concept of adiposity in discussions with horse owners on obesity and metabolic syndrome. In doing this, we hope to improve clients understanding and therefore maximise the impact of diagnostic tests, monitoring tools and management.

Introduction

Adipose tissue is a multifaceted organ, essential for health. While its role in lipid storage is well established, understanding the importance of adipose tissue as an endocrine organ, with a central role in the pathology of

obesity, is evolving. Helping clients to understand the systemic impacts of excess adipose tissue can maximise the impact of diagnostic tests, monitoring tools, management and owner compliance.

Adipose as a storage organ

The amount of adipose tissue varies hugely between individual horses and is affected by factors such as age, sex and breed (Wallis and Raffan, 2020). Estimation of body fat content by deuterium oxide dilution found adipose to account for 2.7-35.6% of total bodyweight in horses (Dugdale et al., 2012). There are two distinct types of adipose: white adipose tissue and brown adipose tissue. The white adipose tissue predominates and is specialised for energy storage. The brown adipose, named due to its colouration from the huge concentration of mitochondria, has a role in thermoregulation and, as a consequence, is abundant in new-borns and animals in hibernation (Kiranmayi and Bhargav, 2019). Most adult mammals have very low quantities of brown adipose and indeed, this form has not been described at all in the adult horse. There is great interest in brown adipose activation as a novel therapeutic target in human obesity (Liu et al., 2022); further research into brown adipose in the horse may therefore be of interest.

White adipose tissue is primarily an energy storage organ and is composed of adipocytes, in which lipid accumulates, as well as connective tissue, immune cells and blood vessels. Adipose can store almost 100 times more megajoules of energy than muscle and this resource allows mammals to cope with changes in energy availability over time. Humans with congenital lipoatrophy (Berardinelli-Seip syndrome), who have a functional failure of their adipose, rapidly develop severe insulin resistance and hepatic lipidosis, leading to liver failure and cardiovascular disease (Garg, 2004). This demonstrates that adipose an essential component of energy homeostasis and should not be viewed as always detrimental to health.

Adipocytes are formed from mesenchymal stem cells in a process called adipogenesis; a complex multistep process which includes the formation of pre-adipocytes from undifferentiated stem cells and the formation of mature adipocytes from these preadipocytes by accumulation of lipid. Equine adipose mesenchymal stem cells (MSCs) are well understood due to their regular use in the developments of treatments of musculoskeletal disorders (stem cell therapy) which has evolved due to their relative ease of acquisition and robust response to *in vitro* manipulation (Marycz et al., 2016). The *in vitro* characteristics of equine MSCs are very similar to those of humans, including the pre-adipocyte response to insulin and glucocorticoid stimulation which induces lipid accumulation and differentiation to mature adipocytes (Bukowska et al., 2021).

Adipocytes store fat as triglyceride (triacylglycerol) in one large lipid droplet per cell. Triglycerides from dietary fat are absorbed from the gut and transported to the adipose in the form of chylomicrons. Adipose, as well as liver, can also synthesise triglycerides from excess carbohydrates, a process known as de novo lipogenesis (DNL) (Ameer et al., 2014). The starting compound for DNL varies greatly between species, with horses using acetate, unlike humans who primarily use glucose or pigs who can use either (Suagee et al., 2010). Adipose tissue, as opposed to the liver, is the primary site of DNL in horses in contrast to humans (Suagee et al., 2010; Adolph et al., 2019), which may go some way to explain why fatty liver is less common in horses than other species. Aberrant DNL is associated with insulin resistance and cardiovascular risk in humans and is a potential link between excess carbohydrate intake and these conditions (Ameer et al., 2014), however very little is known about changes in DNL in equine disease.

In the face of increased calorie intake, adipose can expand by hyperplasia (increased adipocyte numbers derived from preadipocytes) or by hypertrophy (increase in individual cell size by lipid accumulation). The capacity for hyperplasia varies between adipose depots, as discussed later. When required, the stored triglycerides are broken down into glycerol and free fatty acids by lipolysis. These fatty acids are then available to mitochondria for respiration. The ability and speed at which adipose tissue breaks down stored triglycerides is one of the main distinguishing features between adipose depots.

Adipose as an endocrine organ

In addition to its primary role in storage, adipose tissue is the largest endocrine organ in the body, producing and responding to hormonal signals and critical in the cross-talk between metabolic organs which govern

energy homeostasis. Adipocytes secrete bioactive peptides (adipokines and adipocytokines) which can act locally (autocrine/paracrine) or systemically. Adipose also possesses a complex receptor profile which allows it to respond to endocrine and nervous input. Finally, adipocytes are able to exert fine control over endocrine signalling through their enzyme machinery, important in the metabolism of hormones, particularly steroid hormones. Our understanding of the equine adipocyte endocrine profile is more limited than that of humans or rodents but some data exist.

Adipokines

Leptin is a critical regulator of energy storage through appetite/satiety control: when energy stores are adequate, leptin signals to the hypothalamus to reduce appetite drive, thus reducing food intake. As in other species, leptin is secreted from equine adipocytes in proportion to body fat mass. Indeed, animals with increased body fat (without disease) have higher plasma and adipose tissue expression of leptin (Staub et al., 2019; Buff et al., 2002).

Adiponectin is produced almost exclusively by adipocytes (Fang & Judd, 2011) and it acts primarily on muscle and liver to increase insulin sensitivity and reduce inflammation. In most species, including horses, there is an inverse relationship between fat mass and plasma adiponectin (Kearns et al., 2006).

Resistin is also an adipose-specific protein, whose transcription is induced during differentiation of adipocytes. In rodents, it has been shown to decrease gluconeogenesis in the liver (Banerjee et al., 2004). Numerous human studies have failed to demonstrate a reliable association between resistin levels, obesity and/or insulin dysregulation. In contrast, resistin may be a marker of inflammation (Banerjee and Lazar, 2003), consistent with findings in horses (Fuentes-Romero et al., 2021).

Adipocytokines

Adipocytes, stromovascular and inflammatory cells all contribute to adipocytokines secretion. Cytokines released include IL-6, TNFα, MCP1, CCL2 and IL1β. The actions of the adipocytokines are diverse and are not limited to just inflammatory effects such as chemoattraction and immune cell activation. For example, TNFα suppresses free fatty acid and glucose uptake into adipocytes in humans (Ruan et al., 2002). Little is known about the actions of these cytokines in equine adipose but expression of several, including *TNΦα* , *IΛ1β* , IL-6 and *CCL2* , has been shown (Reynolds et al., 2019; Basinska et al., 2015). The analysis of these cytokines may also be a potential diagnostic target in equine metabolic syndrome.

Steroid hormones

Adipose is crucial for the action and metabolism of steroid hormones, particularly glucocorticoids and sex steroids. Furthermore, glucocorticoids are essential in differentiation of mature adipocytes. Adipose tissue in humans and horses contains high levels of glucocorticoid receptor (GR) and also the metabolizing enzyme 11β-hydroxysteroid dehydrogenase type 1 (HSD1,) which metabolises inactive cortisone into active cortisol and thus potentiates activation of GR (Morgan et al., 2018). Horses predominantly metabolise their cortisol through the carbonyl reductase 1 pathway which is very active in adipose tissue and produces a metabolite which impacts glucose homeostasis (Morgan et al., 2017). Less is known about sex steroid metabolism and receptors in equine adipose tissue, though sexually dimorphic patterns of adipose distribution are noted (such as the large nuchal crest adipose depots in stallions) suggesting androgens may impact adipose deposition.

Insulin

Adipocytes are exquisitely sensitive to the effects of insulin. Binding of insulin to its receptor initiates the movement of glucose transporter GLUT4 to the plasma membrane to allow uptake of glucose by the adipocyte. Adipocyte GLUT4 trafficking not only keeps substrate available for DNL but also seems to mediate the cross-talk of adipose with liver and muscle to maintain systemic glucose homeostasis (Abel et al., 2001). GLUT4 trafficking is evident in equine adipocytes but there is a suggestion that more complex and additional insulin signalling pathways may also contribute (Warnken et al., 2017). Insulin also supresses

lipolysis in equine and human adipocytes (Warnken et al., 2017; Duncan et al., 2007) and this acts to regulate the availability of free fatty acids and glycerol to the liver for gluconeogenesis.

Figure 1 Diagram showing some of the key functions of adipose tissue. Created with BioRender.com.

Not all adipose is created equal

The anatomical location of adipose tissue appears to impart several important characteristics, most likely due to the slightly different role adipose will play in each different site. The simplest distinction is that of subcutaneous adipose tissue (SAT) which lies immediately underneath the skin and visceral (VAT) which surrounds organs. In addition to these two major categories, adipose tissue within the bone marrow, around the heart (epicardial) and blood vessels and in/around skeletal muscle have all been found to have distinct phenotypes in humans and rodents. Very little is known about these tissue specific depots in the horse and so this review will focus on what is known about SAT and VAT.

Subcutaneous versus visceral adipose tissue

The distinction between SAT and VAT is important because of the independent association of VAT with increased metabolic risk. People who carry their weight around their femorogluteal region (SAT) which is the predominant pattern seen in women, are relatively protected from metabolic risk in obesity (Booth et al., 2014). In contrast, those who predominantly lay down visceral adipose are at increased risk (Kwon et al., 2017). The reason for this different impact on metabolic health is not clear, but several features of SAT and VAT have been implicated.

- SAT has a greater capacity for expansion by hyperplasia, which results in more abundant but smaller and "healthier" adipocytes. VAT however favours hypertrophy for expansion which results in large and less healthy adipocytes, which can more readily induce lipolysis and release fatty acids into the circulation or directly to the liver via the portal circulation (Bergman et al., 2006). A similar pattern of adipocyte size is found in horses, with peri-renal and retroperitoneal adipocytes having a significantly larger cross-sectional area than nuchal adipose (Bruynsteen et al., 2013).
- In humans, VAT has a more inflammatory phenotype, compared with SAT. This is the case both in 'normal' individuals and those in the face of persistent calorie excess (Ibrahim, 2010). In horses there was increased mRNA levels of inflammatory cytokines in visceral depots compared with the nuchal depot (Bruynsteen et al., 2013).
- Leptin secretion is greater from SAT relative to VAT in humans and horses (Bruynsteen et al., 2013). In humans, adiponectin expression is higher in SAT than VAT (Fain et al., 2004) but the opposite is found in horses (Bruynsteen et al., 2013). There is also some evidence in humans and horses (Warnken et al., 2017) that SAT is more insulin sensitive than VAT, potentially contributing to "safer" storage of lipids.

This distinction between SAT and VAT is relevant in horses because clinical measures of adipose tissues (body condition scoring, weigh tapes) almost exclusively measure SAT. It is important to recognise this as a limitation and remember that visceral adipose may be more important in predicting disease risk. Body condition score is only strongly correlated with total body fat as determined by deuterium oxide dilution (eTBF%) (Dugdale et al., 2011) in lean or non-obese horses. As BCS increases, the predictive ability of BCS reduces significantly (Dugdale et al., 2012). In addition, reliance on BCS may lead us to miss horses with body fat carried almost exclusively around the viscera, a state referred to as TOFIs in human medicine: Thin on the Outside Fat on the Inside (Thomas et al., 2012). Whilst we cannot use the MRI techniques employed in human medicine, it is worth considering abdominal ultrasound in horses if a TOFI phenotype is suspected.

Adipose tissue in obesity

Obesity is defined by the World Health Organisation (WHO) as 'abnormal or excessive fat accumulation that may impair health.' In horses we have yet to define this parameter, so our definition of obesity is based

solely on measures of subcutaneous fat. We do know that in obese horses, as defined by BCS, adipose can account for up to 35% of body mass (Dugdale et al, 2012).

Obese adipose tissue, particularly VAT, is markedly dysfunctional in humans and directly related to the development of obesity associated morbidities including insulin resistance and cardiovascular risk (Santillana et al., 2023). In horses, dysfunction of obese adipose tissue is also clear. Reynolds *et al* (2019) found marked hypertrophy of VAT adipocytes, a hallmark of dysfunctional adipose in humans. Fibrosis, a key feature of human dysregulated adipose, was not found in the adipose of these horses but has been reported by others (Basinska et al., 2015), this discrepancy perhaps due to the chronicity of disease in the animals studied. Dysfunctional adipose has also been shown to have increased expression of leptin (Reynolds et al., 2019). There is conflicting data regarding adipocytokine expression in obese adipose of horses, as there is in the human literature. Whilst Burns*et al* (2010) showed no change in TNFα and IL1β in obese adipose (Burns et al., 2010), several groups have since shown increases associated with obesity and insulin dysregulation in VAT (Reynolds et al., 2019; Jayathilake et al., 2022) and SAT (Basinska et al., 2015). Basinska *et al* also reported macrophage infiltration. It should be noted that the populations studied are invariably diverse in terms of disease state. In humans, obese adipose expansion by hypertrophy fails to stimulate angiogenesis which is driven by increased cell number (hyperplasia), therefore the blood supply eventually becomes limited and hypoxia occurs which contributes to inflammation (Hammarstedt et al., 2018). Although this has not yet been investigated, the same process may occur in the horse.

A key question in understanding how obesity results in insulin resistance, is whether obese adipose tissue is itself insulin resistant. There is currently not a clear answer to this question in humans as it appears to be the case in some individuals but not in others. This alludes to the increasing acceptance that many subtypes of insulin resistance syndromes exist (Imi et al., 2023). Very little is known about insulin sensitivity of obese adipose in horses. A crude measure of insulin signalling components in adipose showed no differences between lean horses and those with equine metabolic syndrome (Reynolds et al., 2019); however, insulin signalling is dynamic and relies on an altered phosphorylation state, so much more work is required in this area.

Figure Diagram showing some of the known and proposed effects of unhealthy adipose tissue seen in overweight/obese patients. Created with BioRender.com

The next question is whether dysfunctional obese adipose tissue contributes to whole body insulin dysregulation. The answer is invariably yes in human and rodent models, although there is still debate over the temporal nature of this relationship (Blüher, 2016; Kahn and Flier, 2000; Kahn et al., 2006). In general, it is thought that factors released in greater or lesser quantities from obese adipose perturb insulin signalling, whether that is a function of the insulin receptor itself or the downstream signalling cascade, particularly in liver and muscle but also in adipose. These downstream factors include adipocytokines (Blüher, 2016), leptin and adiponectin (Yadav et al., 2013). Overloaded adipocytes, especially in VAT, can release free fatty acids into the portal circulation and overwhelm hepatic gluconeogenesis thus impacting insulin sensitivity (Longo et al., 2019). If adipocytes have reached capacity for safe storage then lipid will be deposited ectopically such as in muscle and in liver which is detrimental to insulin sensitivity in those tissues (Longo et al., 2019).

Conclusion

The aim of this review was to demonstrate our growing understanding of the complexities of adipose tissue and its essential role in the morbidity of equine obesity. It is vital that knowledge gleaned from this work should be translated into practical benefits in terms of equine welfare; helping owners and veterinary surgeons to mitigate the impact of obesity on the domestic equine population. The authors have found it is useful to include discussion of "unhealthy" adipose when communicating with owners about obesity. In doing this, the owner should become aware that the horse is not just fat, but this fat is producing factors which negatively impact most other organs in the body.

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