Endocrine Diseases in Donkeys

Subjects: Veterinary Sciences

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Hyperlipemia, defined as abnormally high blood triglyceride concentrations, is the most common metabolic disease in donkeys. In contrast, metabolic syndrome, insulin dysregulation (ID) and pituitary pars intermedia dysfunction (PPID) are the most common endocrine disorders in this species. Although diagnostic approaches for these conditions are similar to horses, donkey-specific protocols must be used.

Keywords: asinine ; Cushing ; hyperinsulinemia ; hyperlipemia

1. Introduction

Donkey metabolic syndrome (DMS) and pituitary pars intermedia dysfunction (PPID) are the two most common endocrine disorders diagnosed in donkeys. Other less frequent diseases are thyroid gland abnormalities and disturbances of calcium and phosphorus secondary to parathyroid gland dysfunction and systemic inflammation (e.g., sepsis, colitis, intestinal strangulation, pleuropneumonia, etc.).

2. Pituitary Pars Intermedia Dysfunction (PPID)

2.1. Introduction

PPID, formerly named Cushing's disease (due to similarities with canine and human Cushing's disease), is a common disorder in old donkeys.

PPID keynotes

- Minimal epidemiological data available;
- Pathogenesis assumed to be like in horses;
- Beware of hypertrichosis and calm behavior;
- Resting ACTH in healthy donkeys is higher than in horses;
- Caution with seasonally adjusted ACTH ranges;
- TRH-stimulation test recommended for diagnosis;
- Pergolide dosing like in horses;
- Measure ACTH at 2–3 months and then 6–12 months.

2.2. Epidemiology

Although epidemiological data are scarce, it can be assumed that the prevalence of PPID is higher in donkeys than in horses and ponies due to their longer life expectancy. Based on data from horses, donkeys older than 15 years of age have a higher risk [1]. A breed predisposition has been documented for horses, with Arabian horses and ponies showing higher prevalence [2], but this remains unknown in donkeys. Sex does not seem to have an effect on the development of PPID or severity. A geographical (temperate and climate) effect has been described in horses [3], likely linked to daylight hours, but the same has yet to be elucidated in donkeys.

2.3. Pathophysiology

There is no reason to believe that the pathophysiology of PPID in donkeys is different to that in horses and ponies [4]. PPID is a neurodegenerative disease (which explains why age is a predisposing factor), where the dopaminergic inhibition

of the pars intermedia melanocytes from hypothalamic neurons is lost. This leads to an increase in the synthesis and subsequent cleavage of proopiomelanocortin (POMC), releasing different peptides (ACTH, α- and β-melanocyte-stimulating hormone [MSH], β-endorphin [β-END], corticotropin-like intermediate lobe [CLIP], etc.) into the bloodstream which are in part responsible for the clinical signs observed in PPID animals.

2.4. Clinical Signs

These are similar to those described for horses and ponies [1], including hypertrichosis, predisposition to infections and parasitism, polydipsia–polyuria, muscle wasting, pendulous abdomen, fat redistribution, recurrent laminitis, insulin dysregulation, reproductive abnormalities, lethargy, orthopedic problems, etc. Due to the calmed temperament of donkeys and the long hair coat of some breeds, PPID signs can go unnoticed for a long time.

2.5. Diagnosis

Diagnosis is based on resting plasma ACTH concentrations. It is important to mention that healthy donkeys have higher resting plasma ACTH concentrations than horses [5][6], and thus, clinicians must be cautious when using equine guidelines [7], which could result in misdiagnosis and false positives depending on the season. Similar to horses, a seasonal effect has been also described in donkeys, with higher concentrations in autumn [8], increasing from July to November. Moreover, ACTH concentrations in horses and ponies are different between radioimmunoassay (RIA: Millipore, Burlington, MA, USA), chemiluminescent immunoassay (CLIA: Immulite, Siemens, Munich, Germany), electrochemiluminescence immunoassay (ECLIA: Cobas e, Roche diagnostic, Rotkreuz, Switzerland) and immunofluorescent immunoassay (IFIA: AIA 360, Tosoh Bioscience, San Francisco, CA, USA) [9]. Thus, donkey-specific cut-off values adjusted to the season must be established for each analyzer. Meanwhile, the following ranges established using Immulite 1000 can be used: spring, 12.5 pg/mL; summer, 53.2 pg/mL; fall, 77.2 pg/mL; winter, 12.8 pg/mL [8]. It is noteworthy that these studies were carried out in the Northern Hemisphere, and ideally, they should be generated for the Southern Hemisphere (month modification), since ACTH concentrations seem to be influenced by a circadian rhythm associated with daylight hours.

Sample processing is crucial and can result in false negative results if samples are not collected in EDTA tubes and not centrifugated/separated/frozen rapidly or the shipment is not performed under refrigerated conditions.

Other peptides released by the pituitary pars intermedia such as α-MSH or β-END are also increased in PPID horses [10]. Alpha-MSH is also influenced by season (higher in autumn months), sample collection and TRH stimulation, but is not affected by stress, transportation, exercise or pain [10]. These peptides have not been evaluated in donkeys.

In donkeys with clinical signs consistent with PPID but resting ACTH concentrations within normal or non-diagnostic ranges, dynamic testing is recommended. Equine protocols including the TRH stimulation and dexamethasone suppression (DST) tests have been evaluated in donkeys, with the former giving more reliable results [11]. A lower protirelin (synthetic TRH) dose (0.5 mg/IV) is used in small and miniature donkeys. In countries where protirelin or chemical-grade TRH are not available, DST can be used, but it is more likely to give equivocal results, mainly in early stages of the disease. Other dynamic tests are no longer recommended.

2.6. Treatment

Treatment is similar to that in horses [1]. Pergolide (a dopaminergic agonist type 2) is the preferred pharmacologic agent that has been shown to be effective in donkeys with PPID. Recently, the pharmacokinetics and pharmacodynamics of pergolide were described in donkeys, with faster metabolism and clearance compared to horses [12]. Donkeys have good absorption of pergolide, but also display a cumulative pattern, suggesting that current doses used in horses are appropriate (Table 6). Like horses, some donkeys may fail to respond to pergolide. In these animals, increasing the dose or a combination with cyproheptadine (Table 6) should be considered. There is no pharmacologic information regarding cyproheptadine in donkeys. If anorexia develops, the daily dose should be split between morning and afternoon, reduced, or stopped and resumed 4–5 days later with lower doses and increased progressively. Based on its cumulative kinetics, after days of treatment, perhaps treatment every other day could be a potential option. Cabergoline (another dopamine receptor agonist) has been evaluated and occasionally used in horses refractory to pergolide, but there is no information regarding its use in donkeys.

Since PPID is a chronic, progressive and irreversible disease, life-long treatment is imperative to provide constant dopaminergic melanotrope inhibition. In most donkeys, clinical signs improve with pergolide treatment, including behavior and physical condition (lethargy, haircoat, weight gain and lameness). Follow-up ACTH measurements 3 and 6 months after treatment initiation, and then annually, is recommended.

2.7. Prognosis

Prognosis in donkeys is considered good with proper pharmacologic treatment and regular ACTH measurements. Additional geriatric husbandry care is needed, with regular deworming, vaccinations, dental care and farriery.

3. Donkey/Asinine Metabolic Syndrome (DMS/AMS)

3.1. Introduction

DMS is the most common endocrine disorder in donkeys, where obesity (regional adiposity) and insulin dysregulation (ID) are the main clinical features.

DMS keynotes

- High energy efficiency;
- Scarce epidemiological data available;
- Pathogenesis assumed to be like in horses;
- Functional entero-insular axis;
- Obesity, ID and HAL are common signs;
- Minimal information on resting insulin cut-off and ranges;
- Glucose curves are right-shifted;
- OST is recommended, but the protocol is not adapted to be donkey-specific;
- Weight loss is the main treatment, but equine diets are not recommended;
- Common drugs lack PK/PD studies.

3.2. Epidemiology

No donkey-specific epidemiological data are available, but animals of any age, gender and breed can suffer from DMS [13]. Although this syndrome is associated with overfeeding and obesity and is more frequent in developed countries, it also occurs in developing countries with high-quality pastures. The role of vitamin D deficiency (vitamin D_2 or D_3) in the pathogenesis of ID in donkeys has not been evaluated [14].

3.3. Pathophysiology

The mechanisms involved in the pathogenesis of DMS are similar to those in horses [15], although specific studies have not been carried out in donkeys. Briefly, hypertrophy of adipocytes (obesity) leads to the release of proinflammatory cytokines by macrophages residing in the adipose tissue (adipose tissue inflammation). These cytokines decrease glucose uptake by insulin-responsive tissues (peripheral insulin resistance) and stimulate pancreatic β-cells to release insulin. Hyperinsulinemia is also exacerbated by reduced hepatic insulin clearance. Carbohydrate-rich diets (grain; pastures) can also decrease insulin sensitivity. In horses and ponies, hyperinsulinemia alters the behavior of lamellar cells [16][17], resulting in hyperinsulinemia-associated laminitis (HAL) which has been linked to proliferation and epidermal lamellar cell dysfunction due to insulin-like growth factor 1 (IGF-1) receptor activation [18]. Other mechanisms including the direct effect of cytokines on lamellae tissue, reduced perfusion (by endothelin-1), impaired glucose metabolism by lamellar epithelial cells, and the direct effect of weight on the hoof likely contribute.

It was recently shown that donkeys have a functional entero-insular axis (EIA) [19]. Carbohydrate challenges stimulated a rapid release of glucose-dependent insulinotropic polypeptide (GIP) and active glucagon-like peptide-1 (aGLP-1) in healthy donkeys. An exaggerated EIA response has been proposed to contribute to ID in horses [20]. However, this remains to be determined in donkeys.

3.4. Clinical Signs

Obesity (regional adiposity) and recurrent endocrinopathic laminitis are the main clinical signs [1]. Importantly, laminitis can also be observed in lean donkeys. Infertility and altered lipid metabolism (hyperlipemia) can be also observed [15]. In

contrast, the clinical relevance of hypertension (important in human metabolic syndrome) remains to be documented in donkeys. In laminitis-prone ponies on summer pastures and in horses with metabolic syndrome after insulin infusion, it has been observed that there is an increase in blood pressure [21][22].

3.5. Diagnosis

- *Insulin dysregulation*

The principles for ID diagnosis in horses apply to donkeys [23]. In addition to physical evaluation, measurements of resting serum/plasma insulin concentrations are crucial in ID diagnosis. Donkeys have similar baseline insulin concentrations to horses [5][24][25], with values less than 20 µIU/mL considered normal. Donkey-specific cut-off values for ID diagnosis have not been established, and donkeys with basal not-fasted insulin concentrations higher than 20 µIU/mL need further investigation for ID diagnosis. Insulin concentrations can differ between methods [26]; beware that donkey-specific cut-off values have not been established for each technique/analyzer. A web application to convert insulin concentrations between analyzers has been developed [27].

It is not recommended to measure resting insulin concentrations in fasted donkeys, and thus, providing some hay or straw overnight is suggested. Resting insulin concentration after feeding is good indicator for laminitis risk development in ponies [28]. However, this has not been assessed in donkeys.

In donkeys with clinical signs compatible with DMS but resting serum insulin concentration in the normal (<20 µIU/mL) or non-diagnostic range (20–50 µIU/mL) according to horse guidelines [29], dynamic testing is recommended. Dynamic tests have been characterized in donkeys [19][30]. Glucose disposal is right-shifted in donkeys, suggesting lower intestinal absorption (oral challenges), delayed EIA response, reduced cellular glucose uptake (hepatic or peripheral tissues) or decreased renal clearance. Further studies are needed to elucidate the mechanisms involved.

The oral sugar test (OST) and oral glucose test (OGTT) are dynamic tests recommended for horses [29]; however, no data on their reliability for ID diagnosis in donkeys are available. The intravenous glucose tolerance test (IVGTT) was more reliable than the combined glucose–insulin test (CGIT) for ID donkeys [31]. Previous studies on the OST and OGT in healthy donkeys showed that glucose disposal is right-shifted (similar to CGIT and IVGTT), and peak glucose was lower compared to that in horses [19]. This indicates that sample timing and cut-off values should be modified, but guidelines for horses are still used in donkeys. For the OST, at least 0.45 mL/kg of corn syrup (Karo® Light corn syrup) is recommended for donkeys by the authors. An in-feed OST or the use of other commercial glucose mixtures have not been evaluated in this species.

Blood biomarkers (cytokines, amino acids and extracellular microvesicles) as well as metabogenomic and lipidomic profiles and their role in the pathogenesis of EMS/ID are being evaluated in horses [32][33]. No data are available for donkeys. The fecal microbiome has been suggested to contribute to ID/EMS in horses [34][35], and while the fecal microbiome has been described in donkeys, an association with DMS remains to be investigated.

- *Obesity*

Obesity is diagnosed based on physical evaluation and using a donkey-specific BCS (>6 out of 9) and neck score (NS, >2 out of 4) [36][37]. Ultrasonography can be useful to assess intra-abdominal and subcutaneous fat [38]. Hepatic enzyme activity can be increased in some obese ID donkeys. This can be explained by hepatic lipidosis inducing a decreased hepatic insulin clearance, or vice versa, an impaired glucose hepatic intake inducing hyperlipemia and hepatic lipidosis. It is important to mention that healthy donkeys have higher GGT activity (up to 80 IU/L) compared to horses [39]. Obese donkeys tend to have higher insulin concentrations and lower insulin sensitivity compared to lean ones [25]. Hyperinsulinemia was recently linked to non-alcoholic fatty liver disease in horses [40].

- *Laminitis*

The diagnosis of laminitis in donkeys is similar to that in horses. However, it is important to use donkey-specific radiographic measurements [41][42].

3.6. Treatment

The management of DMS in donkeys is based on reducing body weight, promoting insulin sensitivity and managing laminitis.

- *Obesity*

Weight loss is the cornerstone of DMS management [15]. A progressive diet modification with caloric restriction is crucial for an appropriate weight loss program without risk of hyperlipemia development [43]. The principles of diet management are similar for donkeys and horses. Ideally, diets should have less than 10% of non-structural carbohydrates (NSC), daily dry matter forage consumption should be between 1 and 1.5% of body weight, reduce the number of hours at pasture or consider a grazing muzzle (in some animals, this could increase the risk of hyperlipemia), avoid grazing when plants are growing fast or accumulating sugars, avoid treats (apples, carrots, mints, etc.) and promote physical activity considering orthopedic problems (walker or hand walking, turnout in a pen, etc.). Ideally, hay should be evaluated for nutritional composition and energy content. In addition, soaking the hay for 30–120 min or using a hay steamer to reduce NSC prior to feeding are valid options. Commercial diets to manage obesity in horses are not appropriate for donkeys due to their caloric content and high energy efficiency. Studies on the pharmacology of sodium levothyroxine (Thyro-L®) in donkeys have not been conducted , but equine doses are used to increase metabolic rate and promote insulin sensitivity in obese donkeys with laminitis (or other orthopedic problems) or those that are refractory to weight loss measures [15]. Nutraceuticals, such as resveratrol, that have antioxidant properties and increase insulin sensitivity in other species are being used in horses [44], ponies and donkeys, but research information on their use in donkeys is lacking.

- *Insulin dysregulation*

Weight loss can be sufficient to correct ID in most cases. When ID appears in lean donkeys or when weight loss is insufficient, pharmacological treatment should be considered. There are no studies on the pharmacology of metformin in donkeys and current dosing protocols have been extrapolated from horses. In donkeys that become anorectic, reducing the dose for a few days and resuming with a lower dose or frequency should be considered. Other drugs used in horses with ID, including sodium–glucose transporter type 2 (SGLT-2) inhibitors, incretin analogs or dipeptidyl peptidase-4 inhibitors (DPP-4), have not been evaluated in donkeys; however, they are being used by some clinicians. It is important to note that SGLT-2 inhibitors increase triglyceride concentrations and occasionally liver enzymes. Therefore, before starting treatment, it is important to measure triglyceride concentrations and repeat the measurement few days later to adjust dosing. Other side effects in humans include urinary tract infections secondary to glycosuria, but it has not been yet reported in horses or donkeys.

- *Laminitis*

Equine protocols for laminitis treatment apply to donkeys (analgesia, NSAIDs, acetaminophen, gabapentin, etc.), taking into consideration hoof anatomical differences [42]. An anti-IGF-1 receptor antibody has been evaluated with satisfactory results in horses with induced HAL [45]. Whether this novel therapeutic alternative could be used in laminitic donkeys is still unknown.

3.7. Prognosis

In donkeys where ID is addressed only with weight loss and they have mild laminitis (no sinking or rotation), the prognosis is usually good, but in donkeys with severe laminitis, insufficient weight loss and no response to metformin administration, the prognosis is poor.

4. Other Endocrine Disturbances

4.1. Thyroid Gland Diseases

Donkeys have higher plasma thyroid hormone concentrations (fT3, tT3, rT3, fT4 and tT4) than horses, with younger donkeys showing higher values compared to older ones [46]. No effect of gender has been observed. Drugs such as phenylbutazone or dexamethasone decrease thyroid hormone concentrations in horses, but their effect has not been evaluated in donkeys. Nonetheless, their effects should be taken into consideration prior to thyroid hormone measurements to avoid a misdiagnosis of hypothyroidism [13]. Dynamic tests (TRH stimulation test, TSH stimulation test and T3 suppression test) to assess thyroid function have not been evaluated in donkeys. Thyroid gland adenomas or neoplasia prevalence seem to be lower in donkeys than in horses (authors opinion).

4.2. Calcium–Phosphorus Homeostasis Disorders

Nutritional secondary hyperparathyroidism can be seen in donkeys consuming diets rich in phosphorus, poor in calcium or containing oxalate-rich plants [47]. Donkeys have higher ionized calcium and calcitriol, but lower parathyroid hormone (PTH) concentrations than horses [15]. Serum PTH concentrations in donkeys are variable.

Donkeys may develop hypercalcemia, hypophosphatemia and normal-to-low PTH concentrations due to tumors producing PTH-related protein (PTHrP). Healthy animals have very low or undetectable concentrations. This is important to be

differentiated from chronic renal failure, where there is also hypercalcemia and hypophosphatemia. In the case of chronic renal failure, creatinine concentrations are often elevated, which is not usually the case in paraneoplastic syndrome in which the neoplasia does not involve the kidneys. Similar to PTHrP, some malignancies may release other endocrine factors (endocrine tumors) with various consequences (paraneoplastic syndrome), but the prevalence is very low [48].

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