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Oral metformin for type-2 diabetes mellitus treatment in a black-tufted marmoset (*Callithrix penicillata*)[1]

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ABSTRACT.- Duz J., Surita L.E., Machado L., Costa P.M.D., Machado B.S., Valle S.F., Alievi M.M. & Pöppl A.G. 2024. **Oral metformin for type-2 diabetes mellitus treatment in a black-tufted marmoset (*Callithrix penicillata*).** *Pesquisa Veterinária Brasileira 44:e07359, 2024.* Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves 9090, Porto Alegre, RS 91540-000, Brazil. E-mail: gomespoppl@hotmail.com

Type-2 diabetes mellitus (T2DM) is characterized by defects in insulin secretion and combined peripheral resistance to the hormone. Several non-human primates (NHP) species develop T2DM, mainly captive animals with reduced physical activity and incorrect feeding. This case report describes the T2DM treatment of a black-eared marmoset (*Callithrix penicillata*) by diet reformulation and metformin oral administration. An adult female was diagnosed with T2DM after hyperglycemia and high serum fructosamine associated with glycosuria and obesity. Metformin hydrochloride (125mg/animal, orally, q24h) associated with feeding intervention was started. After 26 days, a significant reduction in weight, glycemia, and serum fructosamine could be observed, showing satisfactory results for the adopted therapy. Metformin is considered a safe drug for T2DM treatment due to its low hypoglycemia risk. The new diet consisted of sweet potato, squash, and varied fruits offered twice daily. In addition, thawed-mice newborns, egg whites, and small portions of pelleted primate food. In the present report, metformin use, associated with a low glycemic index diet, was effective in treating this particular marmoset and may present a potential for T2DM treatment in other NHPs.

INDEX TERMS: Non-human primates, callitrichid, insulin resistance, oral hypoglycemic drug, metformin, type-2 diabetes mellitus, black-tufted marmoset, *Callithrix penicillata*.

RESUMO.- [**Tratamento de diabetes mellitus tipo 2 em um sagui-de-orelha-preta (*Callithrix penicillata*).**] Diabetes mellitus tipo 2 (DM2) caracteriza-se por uma combinação de defeitos na secreção de insulina e resistência periférica ao hormônio. Diversas espécies de primatas não humanos (PNH) desenvolvem DM2, sobretudo animais cativos com atividade física reduzida e alimentados incorretamente. Este trabalho descreve o tratamento de DM2 em sagui-de-tufo-preto (*Callithrix penicillata*), através da reformulação da dieta e administração oral de metformina. Uma fêmea adulta foi diagnosticada com DM2 após apresentar hiperglicemia e frutosaminemia elevadas associadas à glicosúria e à obesidade. Iniciou-se o uso do cloridrato de metformina (125mg/animal, VO, SID) associado ao controle de consumo alimentar com ajustes da dieta. Após 26 dias pode-se observar redução significativa de peso, adequação da glicemia e frutosaminemia, constatando resultado satisfatório da terapêutica adotada. A metformina é considerada um medicamento seguro para o tratamento de DM2, devido ao baixo risco de hipoglicemia. A base da nova dieta era batata-doce, abóbora e frutas variadas oferecidas

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duas vezes ao dia. Além disso, camundongos recém-nascidos descongelados, clara de ovo e pequenas porções de ração primata peletizada. No presente relato, a metformina associada a uma dieta com baixo índice glicêmico, foi eficaz para tratamento de DM2 podendo apresentar potencial terapêutico de DM2 em outros PNH.

TERMOS DE INDEXAÇÃO: Primatas não humanos, calitriquídeos, resistência insulínica, hipoglicemiante oral, diabetes mellitus tipo 2, sagui-de-orelha-preta, *Callithrix penicillata*.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic syndrome characterized by persistent hyperglycemia resulting from a deficiency in insulin action and/or secretion (American Diabetes Association 2014). In humans, Type-2 diabetes mellitus (T2DM) is the most common type of DM and is mainly characterized by insulin resistance, β-cell dysfunction, and elevated hepatic glucose output (Zhou et al. 2018). The T2DM etiology is diverse and includes genetic predisposition, obesity, sedentarism, abdominal fat storage, and dysbiosis, among other factors (American Diabetes Association 2014). In non-human primates (NHP), T2DM is often associated with obesity in captive animals (Araújo et al. 2000, Ruivo 2017). However, symptoms are rarely observed, and the diagnosis is often random (Ruivo 2017, Baker & de la Garza 2022). Also, NHPs are sometimes considered models for T2DM studies (Wagner et al. 2006).

The black-tufted marmoset (*Callithrix penicillata*) is a Brazilian native neotropical primate species (Ruivo 2017). Captive animals that are overfed or incorrectly fed, along with reduced physical activity, have a high chance of becoming obese. Studies have shown that callitrichids kept in captivity had higher body mass than their wild counterparts, a fact considered a consequence of differences in diet and physical activity (Filomeno Encarnación & Heymann 1998, Araújo et al. 2000). With obesity, these NHP are prone to develop skeletal abnormalities, heart disease, diabetes, and cancer, all of which can affect animal’s well-being and longevity (Bray 2004).

Metformin (1,1-dimethyl biguanide) is among the most utilized drugs for T2DM treatment in humans. It consists of an oral biguanide class antihyperglycemic drug, and it is considered one of the safest drugs for T2DM treatment because of its intrinsic minor risk of hypoglycemia when compared to other oral hypoglycemic medications (Araújo et al. 2000, Viollet et al. 2012). This drug has high efficiency in reducing fasting and postprandial blood glucose and improving glucose metabolism (Nasri & Rafieian-Kopaei 2014). Metformin has an antihyperglycemic action through hepatic gluconeogenesis inhibition and glucagon action opposition. Also, it improves hepatic and peripheral insulin sensitivity (Zhou et al. 2018).

This study aimed to describe the T2DM treatment in a black-eared marmoset (*C. penicillata*) through metformin administration associated with dietary reformulation.

MATERIALS AND METHODS

Animal Ethics. The procedures herein reported agree with the ethical standard of care for NHPs. The owners signed an agreement consent to publish data related to the case at hospital´s admission.

Case report. A four-year-old female black-tufted marmoset (*Callithrix penicillata*) kept as a pet was taken to a veterinary teaching hospital due to hemorrhagic gastroenteritis. During anamnesis, signs such as prostration, anorexia, emesis, and hematochezia were reported. The animal’s diet consisted of homemade food *ad libitum* (human suckling babies’ formula, fruits with sugar, seasoned cooked chicken, vegetables, and snacks such as mealworm, *petit Suisse* cheese, and bread). Over physical examination, the animal was alert, about 8% dehydrated, weighing 640g, with a rectal temperature of 103.1F (39.5°C), and had hypocolored mucosa. The patient was admitted to the hospital to undergo complementary exams, which included blood count, serum biochemistry, abdominal ultrasound, radiography, coproparasitological exam, and urinalysis while gastroenteritis treatment was being performed.

Abdominal ultrasound showed an enlarged liver with multiple irregular margins and hyperechoic areas throughout the parenchyma; also, a full gallbladder with biliary sludge. Radiographic examination showed no abnormalities but hepatomegaly. The serum biochemistry panel showed marked hyperglycemia (512mg/dL), high serum fructosamine (656µmol/L), and glycosuria (4+) in the urinalysis, confirming a diabetic state. Due to the patient’s obesity, T2DM and hepatic steatosis diagnosis were assumed. Table 1 shows the patient’s main clinical and laboratory data over time.

During hospitalization, food intake adjustments and dietary reformulation were performed, so the diet was based on nutritional demands (Crissey et al. 1998). The new diet consisted of sweet potato, squash, and varied fruits (apple, pear, guava, kaki, kiwi, and grapes) offered twice daily. In addition, thawed-mice newborns, egg whites, and small portions of pelleted primate food were used as protein sources, adding up to approximately 150g per day. In addition, treatment for gastroenteritis based on antibiotic therapy (metronidazole 15mg/kg, PO, q12h and amoxicillin with clavulanic acid 25mg/kg, PO, q12h), antiemetics, and fluid therapy was instituted, to which the patient had a good clinical response. Besides monitoring blood glucose and body mass, metformin hydrochloride (125mg/animal, PO, q24h) in the form of macerated tablets within the food in the day’s first meal was introduced eight days after hospital admission.

RESULTS

The patient remained hospitalized for 26 days. Over this period, complementary laboratory tests were repeated on day 21, five days before the patient’s discharge from

Table 1. Laboratory and clinical parameters of a black-tufted marmoset (*Callithrix penicillata*) with type 2 diabetes mellitus (T2DM)

Parameter	Day 1	Day 21	Day 46	Reference*
Weight (g)	640	505	520	±340
Hematocrit (%)	17	29	30	45-48
Glycemia (mg/dL)	512**	142***	220***	124-220
Fructosamine (µmol/L)	656	213	207	148-275
Albumin (g/L)	38	44	43	44-58
ALP (UI/L)	221	163	160	34-88
ALT (UI/L)	0	5	14	38-72
AST (UI/L)	6	75	88	106-196

Day 1 = Admittance and first blood work, Day 21 = evaluation performed 21 days after treatment onset, Day 46 = clinical review 20 days after hospital discharge under domiciliary care; * Fortman et al. (2002), Viollet et al. (2012), Strike & Feltrer (2017), ** Fasting, *** postprandial; ALP = alkaline phosphatase, ALT = alanine aminotransferase, AST = aspartate aminotransferase.

the hospital (Table 1), while body mass score control and glycemic results were more closely monitored. The patient lost 140g and achieved good glycemic control (Fig.1), as well as a relevant reduction in the body condition score from 4.5/5 to 3/5 (Clingerman & Summers 2005) was obtained (Fig.2-3). Despite the glycemic variability documented over the days at the hospital, the mean glycemia after metformin introduction on day 8 (181.4±41.6, range = 90-326mg/dL) was significantly smaller (*P*=0.007; Student's t-test) than the mean registered from days 1 to 8 (473.3±39.4, range = 410-512mg/dL). Despite the discrete increase in serum albumin, the reduction in serum fructosamine results confirms a better glycemic status of the marmoset after three weeks of hospitalization. A discrete fluctuation in liver enzymes' activity with a reduction in ALP activity without reaching the reference interval, increases in the activities of ALT and AST below the reference interval, and improved hematocrit was also documented on day 21, allowing hospital discharge five days later. The initial domiciliary treatment consists of metformin hydrochloride (125mg/animal, PO, q24h), the continuation of the adequate balanced diet prescribed for the species, vitamins, and mineral supplementation. Also, periodic clinical rechecks were requested. After 25 days at home (Table 1, day 46), a clinical recheck with blood sampling occurred, and observed parameters were considered stable. A metformin dose reduction for 62.5mg/animal, PO, q24h was then suggested, and a new recheck was programmed; however, the owners refused to return, leading to follow-up loss.

DISCUSSION

The present study refers to a patient who presented the main clinical characteristics of primates with DM (Ruivo 2017). Husbandry mistakes with animals kept under human care and behavioral and feeding adaptation failures are common compared to free wild animals. In this case, it is common that inadequate intake of sugars and fibers occurs, which predisposes to obesity and associated metabolic disease onset. Such factors justify an overweight value two-fold greater than the average weight for healthy female specimens of *Callithrix penicillata* (±340g) (Viollet et al. 2012). For this reason, diet adjustment aimed to reduce the offer of simple carbohydrates and promote a diet with lower sugar levels associated with higher fiber and protein content.

T2DM is a chronic metabolic condition in which, despite the pancreas being capable of producing insulin, there is peripheral resistance to the hormone's actions. This condition is often reported in obese and senile females, and, in addition to obesity, other risk factors are related, such as physical inactivity, hormones associated with stress, female reproductive hormones, and the use of progestogens (Araújo et al. 2000). Feeding history associated to obesity, hyperglycemia, and glycosuria strongly suggests DM2 diagnosis in this particular case (Baker & de la Garza 2022). Even though the renal threshold that determines glycosuria in callitrichids is unknown, this parameter was useful to determine the diagnosis and discard stress-related hyperglycemia. Humans' threshold is around 198mg/dL (American Diabetes Association 2014), and in NHP, this value might be similar. Serum fructosamine level increase was also a main data as it suggests chronic hyperglycemia occurrence, discarding restraint-hyperglycemia and associated glycosuria. In addition, serum fructosamine monitoring was useful for evaluating treatment response (Sako et al. 2009). Moreover, the patient's satisfactory and safe response to metformin and nutritional management argues in favor of a DM2 diagnosis.

Metformin has antihyperglycemic action by inhibiting hepatic gluconeogenesis and opposing the actions of glucagon. In a complementary way, it improves the sensitivity of hepatic and peripheral tissue to insulin, reducing insulin resistance. Therefore, the decision to use metformin was based on the safety of continuing domiciliary treatment compared to injectable insulin. Despite some gastrointestinal side-effects described in people (Vallianou et al. 2019), the drug seemed well-tolerated by the blacked-tufted marmoset. Minor fluctuations in liver enzymes were considered without clinical significance. These parameters were documented to be highly variable according to different conditions in *Callithrix jacchus* (Kramer et al. 2022) and also can be influenced by physical restrain and inhalant anesthetics in *C. penicillata* (Costa et al. 2023). Since the animal needed to be sedated with isoflurane inhalation for the blood sampling on days 1, 21, and 46, some impact of this procedure can be expected in the analyzed parameters. However, no hepatotoxicity was suggested by these parameter changes during this treatment period.

Metformin is considered one of the safest drugs for treating T2DM, as it has less risk of causing hypoglycemia when compared to other groups of hypoglycemic agents, such as second-generation sulfonylureas (Viollet et al. 2012). Despite the nutritional modifications after hospital admission, glycemic results only showed a clear reduction tendency after metformin introduction. The fluctuations documented over days 3 to 8 are attributable to glycemic variability (Suh & Kim 2015) due to factors such as environmental or physical restrain stress, insulin-antagonistic hormones, increased gluconeogenesis, and variable glycemic index of the meals. The initial dose of 125mg/animal/day had previously been reported in callitrichids (Strike & Feltrer 2017). It was associated with serum fructosamine and glycemia reductions, allowing further dose adjustment to 62.5mg/day. However, adjustments in dose, frequency, and

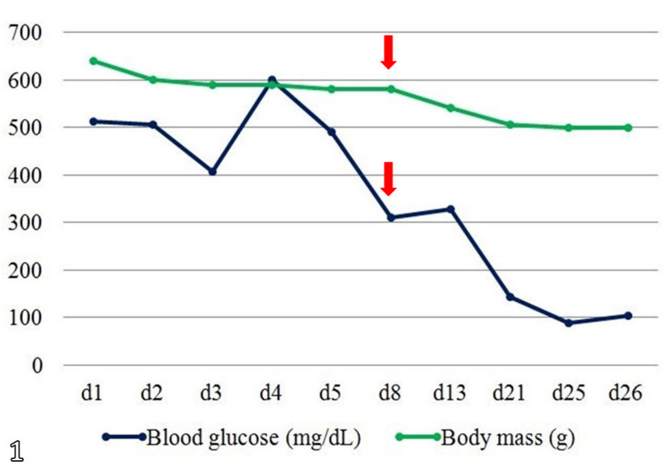

Fig.1. Colored lines show blood glucose levels (mg/dL, blue line) and body weight (g, green line) evolution over the period hospitalized, represented in days on the horizontal bar. On day one, diet reformulation started, while the onset of metformin treatment was on day eight (red arrow). 2023.

association with other hypoglycemic drugs might be necessary for some specimens. This fact demonstrates that treatment needs to be adapted for the individual and adjusted regularly, as it is for human T2DM (DeFronzo 1999). In this report, the initial dose resulted in a satisfactory response verified through glycemia and serum fructosamine reduction within the reference values for the species. The need for continuous medication is questionable, as environmental enrichment helps reduce sedentary behavior, and dietary adjustments can effectively re-establish a eutrophic body condition, thus reducing insulin resistance and the need for hypoglycemic drugs.

Another point of discussion is the fact that the initial presentation with hemorrhagic gastroenteritis, prostration, and anemia, not associated with the presence of gastrointestinal parasites, documentable intestinal mass or foreign body, pancreatitis, or other conditions in the complementary exams performed, motivated the use of systemic antibiotics. The rectal temperature near the upper limit was also considered a clue due to the relationship between body temperature and hematological parameters in this species (Pereira & Barros 2016) and the unfeasibility of performing a complete blood count due to volume sample limitation. The gut microbiome has a strong relationship with insulin resistance and diabetes in people. Eventual dysbiosis at diagnosis could have presented a role in the glycemic status of the marmoset, and the impact of metformin and the antibiotics prescribed in the gut microbiome could have had a role in the reported glycemic response (Vallianou et al. 2019).

Also, the case herein reported argues in favor of the need for awareness against NPHs and other captive animals' obesity. Before developing overt diabetes, NHPs have a period of obesity-associated insulin resistance that is initially met with compensatory insulin secretion. When a relative or absolute deficiency in pancreatic insulin production occurs, fasting glucose concentrations increase and diabetic signs become apparent (Wagner et al. 2006, Baker & de la Garza 2022). In this way, promoting responsible guard of captive animals regarding maintenance of adequate nutritional and environmental requirements to keep eutrophic condition is mandatory.

Fig.2-3. Black eared marmoset (Callithrix penicillata). Comparative images of the patient's body condition score on the hospitalization day (D1=4.5/5) and on the day of hospital release (D26=3/5) after dietary intervention and metformin (125mg/animal, PO, q24h) treatment. 2023.

CONCLUSION

The use of the oral antihyperglycemic metformin associated with a low sugar level diet was efficient in treating type-2 diabetes mellitus (T2DM) in *Callithrix penicillata,* which may be helpful to treat T2DM in other non-human primates (NHP) as well. Also, this study reinforces the need for adequate nutritional and environmental care when NHPs are kept as pets.

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Conflict of interest statement.- The authors declare that there are no conflicts of interest.

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