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Role of social interaction, exercise, diet, and age on developing and untreated diabetes in cynomolgus monkeys

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ABSTRACT

Type 2 diabetes mellitus is the most common form of diabetes that occurs in both human and nonhuman primates. Although spontaneously diabetic nonhuman primates are used extensively in diabetic related research and are a proven valuable tool for the study of the natural history of diabetes, little is known about the key factors that can cause this metabolic disorder and the preventative measures that could be employed to minimize the consequences of diabetes. Using a model of developing and untreated diabetes, this study describes the effects of housing arrangement (socially group- versus individually single-housed), exercise, diet, age, and sex on fasting plasma glucose, key lipids associated with diabetes, and bodyweight in two large cohorts of nonhuman primates. Key findings include exercise/housing arrangement's contribution to significant differences in bodyweight, levels of fasting plasma glucose, total cholesterol, and high- and low-density lipoproteins. Age also had profound effects on glucose, triglyceride and high-density lipoproteins, particularly in single-caged animals. Moreover, females had higher fasting glucose, total cholesterol and triglyceride levels than male counterparts within the same housing situations. These factors may be critical to identifying preventive measures that could eventually be used to minimize obesity and diabetes in humans.

1. Introduction

Prevention of type 2 diabetes mellitus (T2DM) is a major public health challenge because it accounts for 90–95% of the cases of diabetes, and with the proliferation of obesity and the aging of the population, T2DM is expected to become more common and widespread in many developed and developing countries (Strachan, 2011). Additionally, T2DM is prevalent more in individuals aged 40 years and older; yet, the incidence of T2DM in younger populations shows an increasing trend as the rate of obesity and physical inactivity in children continues to climb (Secretariat, 2009). Sedentary lifestyles and high-fat diets are behavioral factors conducive to the high prevalence of T2DM; meanwhile, the disease can be prevented by physical and behavioral interventions (Murea et al., 2012). For example, several clinical studies on randomized cohorts show that lifestyle interventions, including dietary modification, weight loss, and exercise in overweight adults with impaired glucose tolerance, can reduce up to 58% of the incidence of diabetes and development of metabolic syndrome (Knowler et al., 2002; Orchard et al., 2005; Tuomilehto et al., 2001). Nevertheless, these studies also found that some overweight adults with impaired glucose tolerance progressed to T2DM within three years despite a rigorous lifestyle modification. The result led to a series of questions as to why some individuals are susceptible to T2DM while others are not, what other existing risk factors could be associated with the development of diabetes especially in older individuals with pre-diabetic conditions, and how could better methods be implemented to prevent the development of metabolic disorders particularly in a younger population.

As in humans, the highest risk of developing T2DM occurs in obese nonhuman primates (NHPs) particularly in "Old-World" macaques like cynomolgus monkeys with hyperinsulinemia, impaired glucose tolerance, and impaired fasting glucose (IFPG). In general, these at-risk NHPs also have increased triglycerides and decreased high density lipoprotein cholesterol (HDL-C) concentrations, typically seen in humans with metabolic syndrome (Bauer et al., 2011). Therefore, with larger colonies of NHPs like cynomolgus monkeys established in our facilities, we have been able to examine the role housing arrangement, physical activity, diet, age, and sex play in obesity and in the development and progression to T2DM. A long line of studies has well documented that

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socially housed NHPs demonstrate a well-being that is superior to that of their singly-housed counterparts. For example, pair-housed rhesus macaques display more affiliative interactions, physical activity, play, and exploration than single-housed individuals and less abnormal, stereotyped, and self- injurious behavior (Baker et al., 2012; Eaton et al., 1994; Genuth et al., 2003; Schapiro et al., 1996; Wagenknecht et al., 2003). Finally, the similar pathogenetic characteristics and accompanying risk factors observed in both human and NHPs as they develop diabetes make the NHPs unique models for the study of environmental factors (e.g., social interactions and stress) that affect the development of T2DM.

Therefore, two large cohorts of NHPs were used to describe how diet and housing arrangement (group versus single housing; limited versus free exercise) are linked with the development of metabolic disorder and dyslipidemia in primates. The results generated from this comprehensive account of NHP populations with developing or untreated diabetes will provide critical information needed to further elucidate the underpinnings of age, social interaction, diet, and exercise in regulating metabolism while allowing us to develop better preventative strategies and treatments in humans.

2. Material and methods

2.1. Animals

A total 962 cynomolgus monkeys (*Macaca fascicularis*) with ages ranging from 8.5 to 26.2 years old (253 males and 709 females) were used in this study and were divided into two cohorts based on housing arrangement. All procedures used in the study were in compliance with guidelines established by the Institutional Animal Care and Use Committee (IACUC) of Wincon TheraCells Biotechnologies Co., Ltd. (Wincon) in Nanning, Guangxi, China.

In Cohort I, blood samples were collected from 862 cynomolgus monkeys which were randomly selected from a large primate colony at Grandforest Primate Breeding Co. Ltd., (Guangxi, China). Before each sampling session, informed consent from the vendor was obtained. The animals were housed outdoors in 15 m × 24 m open pens with eight separate covered feeding stations to facilitate food and supplement accessibility for all animals. The facility provided feeding areas and space to independently move/exercise. There were about 100 animals including 20 male and 80 females in each housing facility. The average year-around temperature was 21.5 °C (between 9.6 and 33.46 °C) and average humidity was 79%. Animals were fed in-house-prepared, grain-based pellets of a formulation that contained protein (18.8%, W/W), fat (4.9%), cholesterol (<0.0026%), and 8.5% fiber with the remaining amount as carbohydrates (53.5%) and minerals at 150 g/monkey/day along with water available ad libitum. In addition, animals were supplemented with fresh fruits and vegetables.

In Cohort II, 100 cynomolgus monkeys were initially selected from the same primate colony and purchased by Wincon at least 3 years before entering the study. The animals were regularly and individually housed in a custom-made primate cage (single cage dimension: width 0.65 m, depth 0.8 m, height 0.83 m) in the primate facility of Wincon, which is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International. Animals were maintained on a 12-h light/12-h dark cycle, and the room temperature was maintained between 18 and 29 °C and humidity between 30 and 70%. Animals were also fed an in-house-prepared, grain-based pellet (49.2% carbohydrates, 23.7% protein) but with higher levels of fat (6.1%) and cholesterol (0.016%). Water was available ad libitum. Similar to Cohort I, the diets were supplemented with fresh fruits and vegetables on a daily basis. Meanwhile, toys and a mirror were supplied to all animals. In addition, animals used in this cohort had scheduled play in a large cage equipped with enrichment apparatuses such as ladders and tree branches for 4 h at least twice a week. Furthermore, music, TV programs and movies randomly played in all animals' rooms during the lights-on period.

2.2. Blood collection and analysis

For animals in Cohort I at the time of blood collection, animals were transferred from a group-housed cage to a single-housing cage and left to acclimate for at least 48 h, then transferred back to the regular housing facility a day after the blood collection. For both cohorts, after 14-h of fasting, animals were anesthetized with 10 mg/kg ketamine and weighed after complete sedation. For each animal, 4 ml (ml) of blood was obtained from the femoral vein either in the left or right leg with a 20-gauge syringe, and transferred to a tube containing EDTA. The blood was immediately centrifuged at 3000 rpm for 15 min, and the plasma was stored at −80 °C and analyzed within 48 h of collection.

Fasting plasma concentration of glucose (FPG), total cholesterol (T-Cho), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were measured using a Hitachi 7600 Biochemical Analyzer (Hitachi, Tokyo, Japan). All assay kits were manufactured by Shanghai Zhicheng Biological Technology (Shanghai, China). All protocols used in the study were standard ones followed for human clinical laboratories including: GOD-PAP method for FPG concentration; CHOD-PAP method for total cholesterol; GPO-PAP method for triglyceride and direct clearance method for HDL- and LDL-C.

2.3. Statistical analysis

Statistical analyses were conducted using SPSS 24 (IBM, NY) and charts were plotted using Prism 6 GraphPad Software (San Diego, CA). All data shown in text and tables were presented as mean ± SD and in charts as mean ± SEM. Main effects and correlations were analyzed using multivariate analysis followed by posttest *t*-test, frequency distribution and linear regression corrected for multiple comparisons (Bonferroni correction). A P value < 0.05 was considered statistically significant for all analyses.

3. Results

3.1. Age and bodyweight

As shown in Fig. 1, the mean age of all animals used in the study was 15.74 ± 2.57 years old and differences between outdoor group-housed (15.74 ± 2.527) and indoor single-housed (15.79 ± 4.4) was not significant ($P = 0.903$) (Fig. 1A). By contrast, the mean body weight between the two cohorts was significant ($P < 0.0001$, Fig. 1B) i.e. animals living in single cages (6.60 ± 2.21 Kg) were, on average, 21.3% heavier than group-housed animals (5.24 ± 1.89 Kg). Also listed in Table 1 (2nd row), the body weights tended to decline with age in animals with group-housed conditions ($r^2 = 0.43$; $P < 0.0001$) while no such age-associated changes in body weight were seen in single-housed animals ($r^2 = 0.008$; $P = 0.37$). On average, males were significantly heavier than females observed in both cohorts (Fig. 1C). Based on published data, the average bodyweight for normal adult cynomolgus monkeys range from 3.860 to 11.350 Kg (Mean = 6.418 Kg) for males and 3.000 to 6.100 Kg (Mean = 4.490 Kg) for females (Andrade et al., 2004). According to this criteria, the most overweight animals were single-housed males (50%) followed by group-housed males (32.4%), single-housed females (23%) and finally, group-housed females (18.3%) (Fig. 1 D&E).

3.2. Fasting plasma glucose

According to the American Diabetes Association (ADA), normal plasma glucose level for humans is identified as <4.4 mmol/L, IFPG as 4.4–6.99 mmol/L, and diabetic as >6.99 mmol/L (Genuth et al., 2003). According to Wagner et al. (2001), ADA guidelines could be used to