FAT OR FIT

Correlates, consequences and control of overweight in group-housed primates

Dian Zijlmans

Fat or fit:
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Dian Gerdina Maria Zijlmans

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Promotors
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Prof. dr. J.A.M. Langermans

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“The only way to do great work is to love what you do”

Steve Jobs

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Chapter 1

General introduction

Dian G. M. Zijlmans

Introduction

Similar to humans, non-human primates (hereafter: primates) can develop overweight and obesity. This is defined as abnormal or excessive accumulation of adipose tissue in the body, which is caused by an imbalance between energy expenditure and energy intake (Chen et al. 2002). Energy expenditure concerns body processes such as basal metabolism and thermoregulation, but physical activity also expends energy (Landsberg et al. 2009). This should be matched with energy intake derived from food to maintain an equilibrium in the energy balance. An imbalance can lead to two outcomes: weight loss or weight gain. When energy intake is larger than energy expenditure, the remaining energy is being stored in the body, mainly as fat, which leads to weight gain and can result in overweight or even obesity (Chen et al. 2002). In general, obesity is considered a more severe condition than overweight, with a greater extent of body fat accumulation in obese individuals compared to overweight individuals. As such, we use the term overweight throughout this thesis to include both overweight and obesity.

Overweight has been identified in many primate species, ranging from common marmosets (*Callithrix jacchus*) to baboons (*Papio* spp.) and from orangutans (*Pongo* spp.) to squirrel monkeys (*Saimiri sciureus*) (Ausman et al. 1981; Altmann et al. 1993; Zihlman & McFarland 2000; Cocks 2007; Kavanagh et al. 2007; Videan et al. 2007; Tardif et al. 2009). Overweight is also found in several macaque species, e.g. crested black macaques (*Macaca nigra*: Howard 1972), pigtail macaques (*M. nemestrina*: Walike et al. 1977), bonnet macaques (*M. radiata*: Rosenblum & Smiley 1980), rhesus macaques (*M. mulatta*: Kemnitz & Francken 1986), long-tailed macaques (*M. fascicularis*: Chen et al. 2002) and Japanese macaques (*M. fuscata*: Takahashi et al. 2006). Thus, overweight is a condition that occurs in many primate species.

Wild versus captivity

Although primates can develop overweight, it does not naturally occur in the wild. Yet, a large proportion of captive primates develop overweight during adulthood (West & York 1998). Also, underweight is rare in captivity (Sterck et al. 2019), while wild primates can become slender or even underweight in periods of low food quality and availability (Hanuman langurs: Koenig et al. 1997). The prevalence of overweight primates in captivity has been reported to range between 10 and 50% of individuals in a population, depending on age, sex, species and housing condition (Crumley & Raleigh 1995; Hamada et al. 2003; Kavanagh et al. 2007; Yue et al. 2016, 2017). More specific, there is a difference in the amount of body fat between wild and captive primates. Body fat accounts for 2.1% of body weight in wild toque macaques (*Macaca sinica*: Dittus 2013) and 1.9% of body weight in wild baboons (Altmann et al. 1993). In contrast, body fat percentages up to 50% have been reported in captive rhesus macaques and Japanese macaques (Jen et al. 1984; Hamada et al. 2003). Life in captivity can thus be considered an “obesifying” lifestyle compared to the wild, which likely relates to differences in the type and availability of food, affecting energy intake, and the amount of space, affecting energy expenditure.

In the wild, the abundance, distribution and quality of food is seasonally variable (van Schaik 1986; Dasilva 1992). Wild primates therefore spend most of their day, namely up to 80%, searching for food (van Schaik et al. 1983; Altmann & Muruthi 1988; Kurup & Kumar 1993; Menon & Poirier 1996). The diet of wild primates can be categorized according to their main food source, being either frugivorous (fruit-eating), folivorous (leaf-eating) or insectivorous (insect-eating). Yet, most primates consume a varied diet of several different food items. Besides the availability and type of food, energy intake and behaviour around feeding are influenced by the distribution of food. When food is restricted to a limited area, interindividual distances are smaller and competitive interactions increase compared to when food is widespread (Japanese macaques: Furuichi 1983; Saito 1996). High-ranking individuals can monopolize the restricted feeding sites, while low-ranking ones are often displaced/chased away or avoid these locations. These low-ranking individuals may compensate by travelling more to find food, foraging away from the main group or switching to lower-quality food items (van Schaik & van Noordwijk 1988; Soumah & Yokota 1991; Saito 1996; Tsuji & Takatsuki 2012). Wild primates thus adjust their feeding behaviour according to their dominance rank. As a result, food intake does not differ between high-ranking and low-ranking individuals when food is abundant (long-tailed macaques: van Noordwijk & van Schaik 1987). In contrast, food intake is generally lower in low-ranking individuals compared to high-ranking individuals when food is scarce, resulting in energy shortage or even mortality (Japanese macaques: Tsuji & Takatsuki 2012).

Although overweight is absent in wild primate populations feeding on a natural diet, wild primates do have the potential to become overweight. This can be inferred from studies on provisioned primate populations, where food availability is artificially increased beyond the natural supply (Asquith 1989). Provisioning can take place either through presence of crops, garbage, or direct feeding by humans (Altmann & Muruthi 1988). In free-ranging rhesus macaques on Cayo Santiago, spontaneous overweight occurs in 7% of the adult population (Schwartz et al. 1993). Furthermore, body fat levels of semi-provisioned baboons that fed on a garbage dump were 23.2% compared to 1.9% in completely wild-feeding animals (Altmann et al. 1993). The garbage-feeding animals also reduced their physical activity, spending 20% of the time feeding and 50% resting, compared to respectively 60% and 10% in wild-feeding baboons (Altmann & Muruthi 1988). Similarly, provisioning reduced the time spent feeding and foraging by (more than) half in wild olive baboons (*Papio anubis*: Fortham-Quick 1984), vervet monkeys (*Cercopithecus aethiops*: Lee et al. 1986) and rhesus macaques (Malik 1986). Primates therefore seem to have a propensity to increase body fat levels and reduce physical activity in an environment where food is abundant and easily accessible.

In captivity, primates are typically fed a nutritionally balanced diet consisting of commercial monkey chow pellets supplemented with fruit, vegetables and other food items. However, the nutritional composition of the diet in captivity usually does not reflect the natural diet. Captive diets are generally lower in fiber and higher in protein and easily digestible carbohydrates, such as sugar, compared to wild diets (Cabana et al. 2018a). These high-quality diets in captivity are easily accessible and often provided *ad libitum* all year round (Bauer et al. 2010). Moreover, captive primates typically live in enclosures with less space than they occupy in the wild. As a result, captive primates do not have to search for food and spend considerably more time resting than wild primates (crested black macaques: Melfi & Feistner 2002). Captive primates generally spend half of their day resting and only 20% foraging and feeding (rhesus macaques: Beisner & Isbell 2008; Japanese macaques: Jaman & Huffman 2008). Thus, food in captivity has different nutritional values, is more abundant and easier to obtain compared to the wild, while the need and possibility for physical activity is limited. This results in an inherent risk to become overweight in captivity.

Captive primates thus experience low energetic demands and high food availability, which leads to a higher risk of overweight compared to the wild.

Overweight in captivity: past versus present

Overweight in captive primates can occur in different settings. In the past, studies using primates as a model for human obesity often induced obesity with high-fat diets or hypothalamic lesions (Hamilton et al. 1972; Hansen 1979; West & York 1988). Yet, many captive primates also develop overweight spontaneously during middle-age (Ausman et al. 1981; Kemnitz & Francken 1986; Ramsey et al. 2000a). In these studies, primates were mainly housed solitary in small cages and fed *ad libitum*. This type of housing impedes complex social interactions, which are considered a primate behavioural need (Schapiro et al. 2014). Moreover, the opportunity for physical activity is limited, resulting in a sedentary lifestyle and subsequent metabolic dysfunctions (Yue et al. 2017). As a result, overweight is relatively common in solitary-housed primates, i.e. they have higher body weights, fasting glucose and lipid levels compared to group-housed primates (long-tailed macaques: Yue et al. 2017). Although weight gain in solitary-housed primates is a prevalent problem, it can be prevented relatively easy with caloric restriction (Ingram et al. 1990; Colman et al. 1999a; Cefalu et al. 2004; Pifferi et al. 2019). Reducing the provided calories will shift the energy balance towards an equilibrium between energy intake and energy expenditure, resulting in weight stabilization, or higher energy expenditure than energy intake, resulting in weight loss. In solitary-housed male rhesus macaques, daily caloric intake to maintain a stable body weight during adulthood was 60% of the caloric intake of *ad libitum* fed monkeys (Hansen et al. 1995). Subsequently, *ad libitum* fed monkeys had significantly higher body weights (18.0 ± 1.5 kg vs. 11.0 ± 0.5 kg) and body fat levels (33.6 ± 4.0% vs. 21.3 ± 3.3%) compared to calorie restricted monkeys (Hansen et al. 1995).

While solitary-housing is still found, nowadays there is more emphasis on animal welfare, which is also translated into regulations concerning the housing and husbandry of captive primates. Positive animal welfare means that an animal can adequately adapt to its environment and thereby is able to reach a state which the animal perceives as positive (Ohl & van der Staay 2012). An important indicator of animal welfare is the expression of species-specific natural behaviour (Olsson & Westlund 2007). Most primates are highly social animals and have the behavioural need to engage in complex social interactions (Schapiro et al. 2014). Primates are therefore ideally housed in naturalistic social groups, i.e. groups that mimic important components of their wild social system. Next to the social aspect, there are also regulations concerning cage size, cage furniture and enrichment (McCann et al. 2007; Jennings et al. 2009; Directive 2010/63/EU). Captive primates perform more natural behaviour in large enclosures that simulate their natural habitat (Clarke et al. 1982; O'Neill et al. 1991; Novak et al. 1994; Ross et al. 2011). Group enclosures are relatively spacious and naturalistic, which provides more room and opportunity for physically active behaviour compared to solitary housing. Furthermore, food is provided to the group as a whole and must be shared among group members. However, energy intake and energy expenditure of individuals are difficult to control in group-housing. Indeed, food is often not equally divided among group members and especially high-ranking ones may seize the opportunity to take more food than others (olive baboons: Barton & Whiten 1993). This may have consequences for the relative adiposity levels, i.e. the amount of body fat relative to body weight, of individuals in the group (cf. Benn 1971). Some individuals will become overweight, while others stay lean. Thus, group housing makes it more difficult to prevent and treat overweight compared to solitary-housing (Bauer et al. 2011).

Altogether, group-housing provides social benefits relative to solitary housing, but it also affects an individual's energy intake and energy expenditure and thereby the risk to become overweight.

Animal welfare and research implications

Besides the expression of species-specific natural behaviour, health is also an important aspect of animal welfare. Overweight has empirically documented adverse effects on primate welfare, as overweight primates (1) are at risk for several health problems and (2) they likely experience compromised behavioural wellbeing. Like humans, overweight primates have a higher risk of developing health problems such as diabetes and cardiovascular disease (Kemnitz & Francken 1986; Shively & Clarkson 1987; Hansen & Bodkin 1993; Wagner et al. 2006). Diabetes develops in overweight individuals as the excessive fat tissue releases substances that lead to insulin resistance (Kahn et al. 2006). Insulin removes excess glucose from the blood, thereby keeping blood glucose levels constant. However, blood glucose levels increase when cells do not respond adequately to insulin, resulting in diabetes. An impaired glycemic response can be measured in blood as increased glucose levels lead to the formation of glycated proteins. The monitoring of these glycated proteins, e.g. fructosamine and glycated hemoglobin (HbA1c), facilitates the diagnosis of diabetes in both humans and primates

(Cefalu et al. 1993; McTighe et al. 2011; Williams-Fritze et al. 2011; Zemlin et al. 2011). A second health problem associated with overweight in primates and humans is cardiovascular disease. Overweight leads to changes in lipid metabolism, such as increased cholesterol, low-density lipoproteins (LDL) and triglyceride levels, and decreased high-density lipoproteins (HDL) concentrations (Hamilton et al. 1972; Wagner et al. 2006). These alterations in blood lipid concentrations contribute to atherosclerosis, i.e. narrowing of the arteries due to plaque formation, which increases the risk of heart attacks or strokes (Cole et al. 2020). In captive chimpanzees (*Pan troglodytes*), overweight results in elevated blood pressure (i.e. hypertension), a risk factor for cardiovascular disease (Ely et al. 2013), and cardiovascular disease accounts for ~35% of reported deaths (Varki et al. 2009).

Next to being at risk for certain health problems, overweight primates likely experience compromised behavioural wellbeing as they perform less species-specific natural behaviour (Bauer et al. 2012). Overweight primates rest more and perform less active behaviour compared to normal-weight primates (Wolden-Hanson et al. 1993; Bauer et al. 2012). This may be due to the physical discomfort they experience from the excessive amounts of body fat (Bauer et al. 2012). Alternatively, overweight animals may lack the motivation to be physically active, thereby sustaining their condition. In solitary-housed rhesus macaques, activity levels were related to weight gain with the most active monkeys gaining less weight compared to the least active monkeys (Sullivan et al. 2006). Low activity levels may thus be a causal factor in captive primates becoming overweight. These lines of evidence indicate that overweight is disadvantageous for both an individual's health and its natural behaviour.

Compromised animal welfare due to overweight in primates can also have implications for the scientific validity of experimental studies. Primates, especially macaques, are regularly used species in biomedical research. Spontaneously obese primates are excellent models to study human obesity, as they demonstrate similar physiological changes and disease risk as humans (Hansen 1979; Shively & Clarkson 1987; Comuzzie et al. 2003; Wagner et al. 2006; Kleinert et al. 2018). However, obese primates are also used in studies unrelated to obesity, which require the use of healthy animal models (Bauer et al. 2011). Here, the condition may be a confounding factor and bias experimental outcomes, thereby jeopardizing the scientific validity of study results (Martin et al. 2010; Bauer et al. 2011). For example, one study found that overweight led to slower breathing and a lower chest volume in long-tailed macaques (Young et al. 2003). Increased body fat levels thus result in reduced pulmonary function, implying that obesity may have serious adverse effects on lung function studies. Other inadvertent effects of overweight on research outcomes may also be present. Thus, besides overweight being a welfare problem to the animal itself, using these animals has possible confounding effects on the outcome of biomedical studies.

Measuring overweight

Due to the potential negative effects on animal welfare, overweight in captive primates should be closely monitored. Although many studies investigated body composition and relative adiposity in captive primates, a “gold standard” on how to measure and quantify overweight is lacking. Methods and criteria to measure overweight differ between studies and methods can be roughly divided in external and internal measurements.

External measurements include visual assessment, body condition scoring and anthropometry. Visual assessments use obesity rating scales in which observers determine the rating based on their judgement of the overall relative adiposity level of an individual (Berman & Schwartz 1988). Berman & Schwartz (1988) developed the method in free-ranging rhesus macaques and used a 7-point rating scale. These obesity rating scales are not very specific as they cannot distinguish between individuals with relatively similar relative adiposity levels, e.g. wild toque macaques feeding on a natural diet all score at or below 2, while even lean individuals differ in their relative adiposity level (Dittus & Gunathilake 2015). Besides, the method is subjective and prone to estimation errors, so adequate testing and validation are needed before use (Berman & Schwartz 1988). However, this method is most animal welfare-friendly because animals can stay in their home enclosure without being disturbed.

Body condition scoring (BCS) is a semiquantitative method to assess body fat and muscle tissue using palpation of key anatomic regions, such as the hips, spine, pelvis, thorax and abdomen (Clingerman & Summers 2005). The method uses a scale from 1.0 to 5.0 (compromised of both whole and half units) in which 1.0 indicates an emaciated condition, 3.0 represents optimal body condition and 5.0 indicates severe obesity (Clingerman & Summers 2005). Many primate facilities use BCS, which can be performed by veterinarians or veterinary technicians and is easily incorporated in routine health checks (Bauer et al. 2010). The method has been validated in rhesus macaques (Summers et al. 2012) and chimpanzees (Reamer et al. 2020), but has been used in many other species, e.g. common marmosets (Kramer et al. 2015), vervet monkeys (*Chlorocebus sabaeus*: Köster et al. 2016) and hamadryas baboons (*Papio hamadryas*: Cabana et al. 2018b). Like visual assessment, the method is subjective and requires adequate training before use. Furthermore, animals must be immobilized for raters to perform the scoring. Finally, the system is most consistent in adult and subadult individuals, but less so in juveniles and infants (Clingerman & Summers 2012).

A third method to estimate body fat levels is anthropometry, which refers to the systematic collection of body measures. Anthropometric measurements include body weight, height, which are used to calculate for example the Body Mass Index (BMI), body circumferences and skinfold thickness. Anthropometric measurements are a widely used and accepted method to estimate body fat levels in primates (Jen et al. 1984; Walker et al. 1984; Altmann et al. 1993; Bodkin et al. 1993; Kemnitz et al. 1993; Colman et al. 1999ab; Videan et al. 2007; Garcia et al. 2010). They are relatively easy to obtain during regular health checks and therefore feasible in large primate colonies.

Although body weight is often used to estimate body fat levels in primates, these estimates are not necessarily accurate because of confounding factors such as age, sex, height, muscle mass and bone density (Brozek & Henschel 1961; Walker et al. 1984). In humans, overweight is measured using BMI, which is a specific weight-for-height index (WHI) that scales body weight to the second power of height (coded in this thesis as WHI2.0; Keys et al. 1972). BMI has also been used in primates (Jen et al. 1984; Colman et al. 1999b; Raman et al. 2005; Videan et al. 2007). However, some people argue that the power of height is not always two and should be determined for each population, as populations may differ in body build (Benn 1971; Heymsfield et al. 1972; Lee et al. 1982). A population specific WHI likely provides a more direct measure of relative adiposity than body weight alone. Nevertheless, WHIs are still weight-dependent, so they do not differentiate between fat or muscle tissue, resulting in both heavily muscled and fat primates having a high WHI (Colman et al. 1999b).

Internal measurements have the power to separate body fat from other tissue and thus provide more information on body composition and the regional distribution of body fat. These techniques include tritiated water dilution (Walike et al. 1977; Walker et al. 1984; Kemnitz & Francken 1986; Altmann et al. 1993; Bodkin et al. 1993), dual energy X-ray absorptiometry (DXA: Colman et al. 1999ab; Hamada et al. 2003; Raman et al. 2005; Summers et al. 2012), magnetic resonance imaging (MRI: Tardif et al. 2009) and computed tomography (CT: Laber-Laird et al. 1991; Jayo et al. 1993; Cefalu et al. 1997; Wallace et al. 1999). These internal measurements are more labor intensive, expensive and time consuming compared to external measurements. Furthermore, they require different types of radiation, while exposure to radiation should be used only when it has high benefits and should be kept as low as reasonably achievable (the ALARA principle: Harvey 2008). Therefore, these methods are less practical and unsuitable for monitoring overweight in large primate colonies.

Next to the measuring methods, studies differ in the definition of their overweight criterion. Some studies consider their primates overweight when body weight and/or body fat percentage exceed a certain threshold (e.g. Hamilton et al. 1972; Bodkin et al. 1996; Cocks 2007; Ely et al. 2012; Yue et al. 2017). Other studies have relied on statistics for defining overweight, e.g. using percentiles (e.g. Hamada et al. 2003; Tardif et al. 2009) or standard deviations from the mean (e.g. Kemnitz et al. 1989; Schwartz et al. 1993). As a result, thresholds for defining overweight differ between studies, while these should ideally be based on negative health outcomes, such as hyperinsulinemia or hypertension (e.g. Raman et al. 2005). However, there are few studies that examine the relation between overweight and potential adverse health effects in captive group-living primates. The alternative approach to use statistics in defining overweight has the disadvantage that it depends on the population's descriptive statistics (such as mean, median and range), so when all individuals are relatively heavy, only few individuals will be considered overweight by definition and vice versa (Reamer et al. 2020). Thus, most studies agree that overweight in primates exists, yet they differ in their overweight criterion, impeding a fair comparison between studies.

Research setting

The research described in this thesis took place in the macaque colony of the Biomedical Primate Research Centre (BPRC) in Rijswijk, the Netherlands. The BPRC houses two species of macaques: rhesus macaques (*Macaca mulatta*) and long-tailed macaques (*M. fascicularis*). The two species are both housed in one of three housing conditions: (1) large naturalistic groups; (2) small same-sex groups; or (3) same-sex pairs in the (pre)experimental facility. Overweight prevalence in the large naturalistic groups was 23% for rhesus macaques and 17% for long-tailed macaques (Sterck et al. 2019). On the other hand, overweight is generally absent in the small same-sex groups, as these groups mainly consist of young subadult males that typically are not overweight (BPRC database). The research presented in this thesis therefore revolves around the macaques in the large naturalistic groups, while one chapter also involves female rhesus macaques in pair-housing.

Many research facilities across the world house their primates non-naturalistically, i.e. group composition, demography and migration processes do not resemble wild conditions. In contrast, the BPRC breeding colony houses macaques in large naturalistic groups that are formed and managed by adhering to natural group dynamics. Social groups at BPRC typically house 15–40 individuals, consisting of several adult females with their (grand-)offspring and one unrelated adult male. Similar to the wild, females are philopatric, stay in the natal group throughout their life and reside with relatives, while males are removed from the natal group when they reach sexual maturity to prevent inbreeding (Wrangham 1980; Mittermeier & Wilson 2013; Rowe & Myers 2016; Rox et al. 2021). This results in groups with several multigenerational matrilines. A matriline contains individuals that are all related to each other via one common female ancestor, and having these matrilines contributes to maintaining socially stable groups (rhesus macaques: Beisner et al. 2011; McCowan et al. 2018). Thus, the BPRC breeding colony provides a unique population where natural group composition and migration patterns are closely mimicked, which promotes the expression of natural species-specific behaviour and social interactions. Although this type of naturalistic group-housing enhances captive primate welfare, it also has some disadvantages such as a portion of animals becoming overweight.

Thesis aim and overview

This thesis aims to gain a better understanding of the correlates, consequences and control of overweight in group-housed primates. Therefore, we studied spontaneous overweight in relation to behaviour, diet, health and welfare in rhesus macaques and long-tailed macaques housed in naturalistic social groups at the BPRC in Rijswijk, the Netherlands. This research is relevant for two main reasons: (1) overweight has adverse effects on animal welfare; and (2) using overweight primates may reduce scientific validity of biomedical studies. The thesis addresses the topic mainly from an applied point of view, thereby aiming to improve animal welfare, and focusses on three central questions:

1. How to measure relative adiposity and quantify overweight in group-housed primates?
2. What are risk factors for group-housed primates becoming overweight?
3. Which husbandry strategies are suitable overweight management strategies in group-housed primates?

The first part of this thesis examines the question how to measure relative adiposity and quantify overweight in group-housed primates. Current measures of relative adiposity and boundaries for overweight and underweight are based on solitary-housed rhesus macaques living in small cages (Raman et al. 2005). **Chapter 2** explores the best relative adiposity measure for our group-housed macaque colony, with the premise that it can be easily collected during health checks. We used anthropometric data of group-housed rhesus macaques and long-tailed macaques to calculate species-specific weight-for-height indices (WHIs). For long-tailed macaques, similar anthropometric data on founder and wild individuals were available for comparison. We determine the best measure of relative adiposity and propose boundaries for overweight and underweight to investigate their incidence in the BPRC breeding colony. This new measure and corresponding boundaries are relatively objective, precise and allow for a fair comparison between animals and populations.

Body weight is an important welfare indicator, and it is included in the animal health program of most research facilities housing primates. In general, collecting body weight measurements requires animals to be sedated or trained, which can be stressful and time-consuming. A possible solution may be to let animals step onto a scale voluntarily. **Chapter 3** reports the participation, reliability and time investment of voluntary weighing in group-housed rhesus macaques. We established which individuals were more likely to participate and whether body weight from voluntary weighing matched body weight during sedation. Furthermore, recommendations for practical use are provided. The findings of this study can have a broad application, as voluntary weighing can be useful to monitor body weight in other species and other institutions, such as zoos. Moreover, combining body weight data from voluntary weighing with data on an individual's height, which is stable in full-grown adults, can be used to measure overweight (WHI; Chapter 2).

The second part of this thesis focuses on risk factors for developing overweight (question 2). Risk factors may differ or be influenced by the type of housing system. Macaques are a widely used species in biomedical studies, where they are usually pair-housed in a controlled environment. This differs substantially from the optimal housing conditions for macaques, i.e. group-housing in naturalistic enclosures. Housing conditions can have long-lasting effects on stress and body fat levels. Some individuals are likely more sensitive to these changes than others and selection procedures may be refined when these individuals can be identified. **Chapter 4** describes the effect of a change in housing condition on two (potential) biomarkers for long-term stress in female rhesus macaques. Hair cortisol concentrations and body fat levels, measured with both anthropometry and computed tomography (CT), were compared between group- and pair-housing. Since CT provides more information on the regional distribution of adipose tissue, we also used this to corroborate the species-specific WHI proposed in Chapter 2.

Although macaques in the BPRC breeding colony are housed under similar conditions, not every animal becomes overweight. Some individuals are relatively heavy, while others stay lean. Individual differences in behaviour related to energy intake, energy expenditure and dominance rank may lead to variation in relative adiposity. **Chapter 5** addresses the question whether behaviour predicts weight gain during adulthood in group-housed adult female rhesus macaques. Relative adiposity was quantified during annual health checks, while behavioural data on food intake, activity patterns and dominance rank were collected at baseline. We check which factors were related to relative adiposity at baseline, and whether behaviour predicted the change in relative adiposity over time. Identifying risk factors for becoming overweight may yield opportunities to improve animal husbandry and reduce weight gain, thereby increasing animal welfare.

The final part of this thesis addresses the third question and focusses on potential husbandry strategies to prevent and reduce overweight in group-housed primates. Diet likely plays an important role in captive primates becoming overweight and developing other health problems. Diets in captivity are generally lower in fiber and higher in sugar compared to wild diets (Cabana et al. 2018a). Overweight and overweight-related health problems, e.g. diabetes and cardiovascular disease, may be reduced when captive diets more closely mimic the natural diet. We implemented a minor dietary change, i.e. replacing the supplementary provisioning of bread by grains and vegetables, that reduced the glycemic index and increased fiber content, thereby adjusting the diet towards a more natural composition. **Chapter 6** provides a retrospective evaluation of this minor dietary change in adult female long-tailed macaques. Relative adiposity and biochemical parameters related to the risk of diabetes and cardiovascular disease were compared before and after the dietary change.

Next to adjusting the composition of the diet, reducing the provided amount of food likely improves overweight-related health parameters. Caloric restriction is a nutritional intervention that reduces caloric intake without malnutrition (Pifferi et al. 2019). Caloric restriction has been used in solitary- and pair-housed primates, but it is unknown whether it may be suitable for group-housed primates. Caloric restriction in group-housed primates provides a potential welfare risk, especially in multigenerational breeding groups, as food is often not equally divided among group members. In **Chapter 7**, we aim to determine the suitability of caloric restriction as an overweight management strategy in captive group-living primates. Therefore, we investigate whether mild (20%) caloric restriction affects adult overweight and blood chemistry, immature growth, veterinary interventions, and reproduction in ten long-tailed macaque breeding groups.

Primate facilities have become increasingly interested in potential husbandry strategies to reduce overweight in group-housed primates, though not many studies report on this. Some strategies require animals to be separated from their social group, either permanently or temporarily, which likely disrupts the social dynamics and has potential consequences for group stability and animal welfare. Moreover, many strategies require a lot of time investment from staff. **Chapter 8** evaluates four overweight management strategies that allow animals to stay in their social group and require minimal to no personnel time investment, namely (1) providing food in puzzles, (2) ideal body weight feeding, (3) caloric restriction and (4) a fruit-free diet. We use a combined approach to assess the effect of these four overweight management strategies on physiological and behavioural wellbeing in captive rhesus macaque groups. Anthropometry, biochemical parameters and hair loss were used as health and welfare indicators, while behavioural observations provided information on food intake, activity patterns, aggression and stress-related behaviour.

The main findings and conclusions from this thesis are summarized and discussed in **Chapter 9**. First, different overweight measures are compared regarding their suitability for group-housed primates and an optimal measure and boundaries for overweight in group-housed macaques are proposed. Variation in relative adiposity among group-housed primates and how this differs from the literature on solitary-housed primates is discussed. Second, the question whether overweight has negative health consequences in captive group-living primates is addressed. Third, risk factors for overweight in group-housed primates are discussed. Finally, potential overweight management strategies in group-housed primates and tools to prevent and reduce overweight are indicated. Overall, group-housing highly improves primate welfare, but also leads to an inherent risk of some animals becoming overweight. This thesis emphasizes the importance of measuring and monitoring overweight in group-housed primates and shows that critical evaluation of husbandry practices provides valuable insights to improve animal welfare.

Chapter 2

Determining overweight and underweight with a new weight-for-height index in captive group-housed macaques

Elisabeth H. M. Sterck*, Dian G. M. Zijlmans*,
Han de Vries, Lisette M. van den Berg,
Carel P. van Schaik, Jan A. M. Langermans

* Shared first authors

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Abstract

Housing primates in naturalistic groups provides social benefits relative to solitary housing. However, food intake may vary across individuals, possibly resulting in overweight and underweight individuals. Information on relative adiposity (the amount of fat tissue relative to body weight) is needed to monitor overweight and underweight of group-housed individuals. However, the upper and lower relative adiposity boundaries are currently only known for macaques living solitarily in small cages. We determined the best measure of relative adiposity and explored the boundaries of overweight and underweight to investigate their incidence in group-housed adult male and female rhesus macaques and long-tailed macaques living in spacious enclosures at the Biomedical Primate Research Centre (BPRC), the Netherlands. During yearly health checks different relative adiposity measures were obtained. For long-tailed macaques, comparable data on founder and wild animals were also available. Weight-for-height indices (WHI) with height to the power of 3.0 (WHI3.0) for rhesus macaques and 2.7 (WHI2.7) for long-tailed macaques were optimally independent of height and were highly correlated with other relative adiposity measures. The boundary for overweight was similar in group-housed and solitary-housed macaques. A lower boundary for underweight, based on 2% body fat similar to wild primates, gave a better estimate for underweight in group-housed macaques. We propose that for captive group-housed rhesus macaques relative adiposity should range between 42 and 67 (WHI3.0) and for long-tailed macaques between 39 and 62 (WHI2.7). The majority of group-housed macaques in this facility have a normal relative adiposity, a considerable proportion (17–23%) is overweight, and a few (0–3%) are underweight.

Introduction

Group housing of captive primates has beneficial social effects, but also changes other aspects of their life and environment. Group enclosures are more spacious compared with solitary housing situations and individuals have the ability to walk more and thus cover larger distances. Consequently, animals can become more muscular and thus relatively heavy. Moreover, food is typically provided for the whole group and some individuals (especially dominants) may seize the opportunity to take more food than others. All these changes can affect relative adiposity, that is the amount of fat tissue relative to body weight (cf. Benn 1971). A healthy relative adiposity is not signified by a single value but comprises a range within which animals (or humans) are considered to have a healthy weight (Raman et al. 2005). Individuals above the upper boundary are considered overweight, whereas those below the lower boundary are considered underweight. Both being overweight or underweight have empirically documented adverse effects on the health and welfare of an individual (Kemnitz & Francken 1986; Shively & Clarkson 1987; Scarlett & Donoghue 1998). However, there is no agreed-upon measure of relative adiposity and boundaries of over- and underweight for group-housed macaques. Current measures of relative adiposity and subsequent boundaries are based on solitary-housed macaques living in small cages (e.g. Raman et al. 2005) and these may be different for group-housed macaques.

The first challenge is how to measure relative adiposity. A valid measure fulfills two criteria: (a) its distribution should be independent of height; and (b) it should be highly correlated with other measures of relative adiposity (Benn 1971). Relative adiposity is often measured with weight-for-height indices (WHI) that scale body weight to a power of height (Benn 1971). A specific WHI measure, the body mass index (BMI), is well-known in humans. BMI is calculated by dividing body weight by the square of the height and can be coded WHI2.0 (indicating that the power of height is 2.0; Keys et al. 1972). BMI is generally independent of height in adult humans, but there is discussion whether the power of height is always two (Heymsfield et al. 2007). The Ponderal index is a WHI measure where body weight is normalized with the third power of height ($\text{weight/height}^{3.0}$; Rohrer 1921). As using an inappropriate WHI can produce misleading results about the relative adiposity, some authors state that the power of height is population specific; this is incorporated in the Benn index ($\text{weight/height}^{\beta}$; Benn 1971; Lee et al. 1982). The β can be population specific when populations differ in body build and ideally should be calculated for each population separately.

Additional measures of relative adiposity, besides WHI, concern body circumferences and skinfold thicknesses (Walker et al. 1984; Kemnitz & Francken 1986; Kemnitz et al. 1989; Bodkin et al. 1993; Colman et al. 1999b; Hamada et al. 2003). Relative adiposity has also been measured by body condition scoring (BCS). BCS uses palpation of key anatomic features such as hips, spine, pelvis, thorax, and abdomen and can be easily incorporated into routine health checks (Clingerman & Summers 2005). This measure is used in a wide variety of animal species, including horses, cats, dogs, sheep, mice, and cattle (Wildman et al. 1982; Carroll & Huntington 1988; Thompson & Meyer 1994; Ullman-Culleré & Foltz 1999; German et al. 2006). BCS has been validated in rhesus macaques (Summers et al. 2012).

The second challenge is to determine the boundaries of over- and underweight for group-housed macaques. In humans, the relationship between body fat percentage and BMI is background-specific due to variation in trunk-to-leg length, slenderness, and/or muscularity (Deurenberg et al. 2002). As a consequence, different BMI boundaries have to be considered (Deurenberg et al. 2002) and this may also apply to different primate populations. Raman et al. (2005) determined BMI boundaries for male and female rhesus macaques based on fat reserves in relatively old and solitary-housed animals living in small cages. The upper boundary was based on the insulin sensitivity index, which yielded an upper boundary of 23% body fat for males and 18% for females. The lower boundary was based on the body fat percentage below which individual health could quickly deteriorate, which yielded 9% body fat for males and 8% for females but included a large safety margin. Alternatively, the lower boundary can be based on the fat percentage of wild primates, for example, 1.9% in baboons and 2.1% in toque macaques (Altmann et al. 1993; Dittus 2013). The latter boundary (ca 2%) may be more appropriate for group-housed macaques living in relatively large enclosures, as they are more similar to wild than solitary-housed animals. In addition, boundaries can be based on deviation from the mean (Schwartz et al. 1993). Finally, the BCS also has boundary values for overweight (>3.5) and underweight (<2.5; Clingerman & Summers 2005; Summers et al. 2012).

The goal of the present study was to determine the best measure of relative adiposity and explore the boundaries of overweight and underweight to investigate their incidence in captive group-housed adult rhesus macaques (*Macaca mulatta*) and long-tailed macaques (*M. fascicularis*) living in spacious enclosures. For the long-tailed macaques comparable data on founder and wild animals were available. We determined species-specific WHI measures independent of height and correlated these with other measures of relative adiposity. We also used several methods to determine the upper and lower boundaries of relative adiposity and derived the appropriate boundaries for group-housed macaques.

Materials and methods

Subjects and housing current BPRC population

The subjects of this study were 300 adult rhesus macaques and 105 adult long-tailed macaques housed in social groups at the BPRC in Rijswijk, the Netherlands. Females older than 6 years of age and males older than 8 years of age were defined as adults, as they are skeletally mature (Schwartz et al. 1993; see Figure A1). Only nonpregnant females were included in the data set, because pregnant females are known to have higher body weight, BMI and abdominal circumferences than nonpregnant females in free-ranging rhesus macaques (Schwartz & Kemnitz 1992). Pregnancy was detected during the yearly health check or was determined afterwards as the 6 months preceding an infant's birth.

Four female rhesus macaques older than 25 years of age were excluded from the analyses, because elderly macaques may experience aging, just like humans, in which changes in physiology and metabolism are accompanied by changes in body size and weight (Ramsey et al. 2000a; Table A1). In the end, 273 female and 23 male rhesus macaques were included in the analyses, whereas the long-tailed macaque sample contained 92 females and 13 males housed at the BPRC. All monkeys were captive-born.

Social groups at the BPRC typically consist of 15–40 individuals and encompass several matrilines, that is females with descendants, and one non-natal adult male. Husbandry is aimed at mimicking natural demographic processes. Each social group had access to enriched (Vernes & Louwerse 2010) indoor (±72 m^2 and 2.85 m high) and outdoor (±260 m^2 and 3.1 m high) enclosures. The monkeys were fed monkey chow (Ssniff©) on a daily basis, complemented with fruit, vegetables, or bread. The amount of food was predetermined per group and linked to the summed needs of all individuals. Water was available *ad libitum*.

Subjects and housing other long-tailed macaque populations

In addition to the BPRC population, 24 founder female and 11 founder male long-tailed macaques were subjected to anthropometric measurements at the Utrecht University from 1987 to 1989. These founders were part of the population from which the current BPRC long-tailed macaques descended. Housing and feeding were comparable with the current BPRC conditions.

Data from the wild were available for nine female and six male long-tailed macaques from the Ketambe Research Station, Gunung Leuser National Park, Indonesia in 1989. The wild long-tailed macaques concerned individuals from the H-group and were all healthy (van Noordwijk & van Schaik 1999).

Anthropometric measurements current BPRC population

Relative adiposity levels of BPRC individuals were estimated by taking several anthropometric measures (Table A2; cf. Garcia et al. 2010) when the animals were sedated during their yearly health check. The yearly health checks are a veterinary management procedure at the BPRC colony. No invasive research or experimental procedures requiring ethics approval according to the European Directive 2010/63 and the Dutch law were performed. Therefore, no approval by the BPRC animal ethics committee was required. This study is consistent with the ASP Principles for the Ethical Treatment of Non-human Primates.

The measurements took place between 9.00 and 14.00 h from November 4, 2014 until May 10, 2017. Anthropometric measures concerned body weight, height, abdominal circumference, and skinfold thickness at four sites. All measurements were performed on the animal’s right side of the body. The measurements were performed in three subsequent years. Within any given year, one person was responsible for performing all the measurements. Whenever an individual was measured in more than one year, the average value was used for the analyses.

Body weight was measured with a standard scale and was expressed in kilograms accurate to one decimal. Height was measured as the crown-rump length, that is the distance from the highest point on the head to the bottom of the monkey. Height was measured using a SECA 210 measuring mat for human infants (Seca, Hamburg, Germany). The monkey was in a supine position and height was determined to the nearest 0.1 cm. Weight and height were used to calculate BMI and WHI measures. Abdominal circumference was measured at the height of the umbilicus with a tape measure to the nearest 0.1 cm with the animal in the lateral recumbent position (Colman et al. 1999b). Skinfold thickness was measured three times to the nearest 0.1 mm with a Baseline Pro skinfold caliper at four different sites, namely abdominal (at the height of the umbilicus), subscapular (1 cm below the inferior angle of the scapula), supra-iliac and at the triceps. A total skinfold thickness score was calculated by summing the average skinfold thicknesses at the four sites.

Furthermore, all macaques were subjected to BCS (Summers et al. 2012), which was performed by experienced veterinarians. In rhesus macaques, the BCS scale ranges from 1.0 to 5.0 comprising both whole and half units, in which the midrange (3.0) represents optimal body condition. Lower values (<2.5) represent emaciated to lean conditions and higher values (>3.5) indicate excessive body fat (Clingerman & Summers 2005). This BCS system was also applied to the long-tailed macaques.

Anthropometric measurements on founder and wild long-tailed macaques

Anthropometric measurements on the founder and wild long-tailed macaques were performed by CPvS (Table A2). Measurements on the founder long-tailed macaques were performed every few months between December 1987 and April 1989, with some slight deviations from the measurements in 2014–2017. Data from six body weight measurements and five height measurements were averaged to yield mean values for every individual. Data were not corrected for pregnancies. Similar to the current data, the body weight was measured with a standard scale and was expressed in kilograms accurate to one decimal. Differently from the current data, the height was measured by seating the animal and measuring the distance from its bottom to its head. This measure is similar to our crown-rump length. The abdominal circumference and skinfold thickness at four sites were based on two data points and measured similar to the current data. Skinfold thickness was calculated from the average of two measures.

Anthropometric measurements performed on the wild long-tailed macaques were the same as for the founder population. These measurements were performed once (February 1989).

Defining overweight and underweight

Five different methods were used to determine whether individuals were overweight or underweight: (a) BMI (=WHI2.0); (b) WHI; (c) abdominal circumference; (d) standard deviation (SD) from the mean WHI; and (e) BCS. WHI and SD from the mean WHI were calculated for this study, whereas the other boundaries were based on literature. First, the upper and lower BMI boundaries in solitary-housed rhesus macaques are between 32 and 44 kg/m^2 for males and between 27 and 35 kg/m^2 for females (Raman et al. 2005). We also applied these boundaries to the long-tailed macaques, as for females and males, respectively, the BMI did not differ significantly between the species (females: Mann–Whitney U test, $U = 10745.5$, $n = 362$, $p = 0.102$; males: Mann–Whitney U test, $U = 118$, $n = 36$, $p = 0.312$). Second, the upper and lower boundaries of the WHI3.0 and WHI2.7 we obtained (see *Results*) were calculated on the basis of BMI boundaries (Raman et al. 2005; see Appendix for data conversion). Likewise, we used the 2% body fat similar to wild primates (Altmann et al. 1993; Dittus 2013) for a female of average height to determine the lower boundary. Third, we used Raman et al.'s (2005) lower and upper boundaries for solitary-housed rhesus macaques for abdominal circumference: 40 and 54 cm for adult males and 35 and 44 cm for adult females, respectively. These were also applied to long-tailed macaques. Fourth, overweight and underweight were determined as being more than two standard deviations above and below the mean WHI (Schwartz et al. 1993). The fifth method used was BCS, which defines underweight as BCS < 2.5 and overweight as BCS > 3.5 (Summers et al. 2012).

Statistical analyses

The WHI measure that was least correlated with height was determined by calculating the appropriate power β in the formula WHI=weight/height$^{\beta}$ as determined using a custom program in R Core Team (2015). This program searches for the β that yields the regression coefficient closest to 0 for the regression line of WHI regressed on height. The value of β was determined separately for female rhesus macaques and female long-tailed macaques. The 95% confidence limits of each β were determined by means of the nonparametric bias-corrected accelerated (BCa) method using the function "bcanon" from the package "bootstrap" in R version 3.2.3 (2015; Efron & Tibshirani 1993).

The different anthropometric measurements of each species, sex and population were correlated using Spearman correlations. Sex differences in rhesus macaques were tested using independent samples t tests or Mann–Whitney U tests, depending on whether the data were normally distributed. In the long-tailed macaques, separate analyses were conducted for population and sex differences, because the differences in variance did not allow a combined (i.e. two-way ANOVA) analysis. Differences between the populations were determined with a one-way ANOVA or Kruskal–Wallis H test, depending on whether the data were normally distributed. Sex differences were tested in the same way as in the rhesus macaques. Normal distribution of the data was tested using the Shapiro–Wilk test. Statistical analyses were performed in IBM SPSS Statistics version 22 and the significance level used in this study was $\alpha = 0.05$. All statistical tests were two-tailed.

Results

Determining macaque WHI

The ideal WHI should be independent of height. WHI was calculated for females of each macaque species, as most data points were available on adult females of the current BPRC population and the sample size of adult males was small. BMI was significantly correlated with height in female rhesus macaques (Spearman correlation, $r = 0.276$, $n = 273$, $p < 0.0005$; Figure A2). Although only a trend, a positive slope was also found for female long-tailed macaques (Pearson correlation, $r = 0.201$, $n = 89$, $p = 0.059$; Figure A2). The WHI that was least correlated with height was determined. The correlation for rhesus macaque females was found to be closest to zero at a WHI with height to the power of 2.96 (rounded to 3.0; 95% confidence interval [CI] = [2.53, 3.39]) and for long-tailed macaque females 2.73 (rounded to 2.7; 95% CI = [1.76, 3.55]; Figures 1,2: black circles and black regression lines).

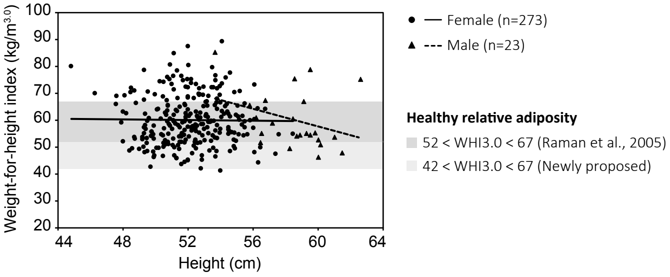

Figure 1. WHI3.0 plotted against height for adult female (black circles) and male (black triangles) rhesus macaques currently housed at the BPRC. WHI3.0 was optimally independent of height in females (black solid line), whereas there was a nonsignificant negative relationship between WHI3.0 and height in males (black dashed line). The dark gray bar represents the proposed relative adiposity boundaries by Raman et al. (2005), which correspond to 52 < WHI3.0 < 67. The light gray bar indicates the new lower boundary based on 2% body fat, similar to wild primates.

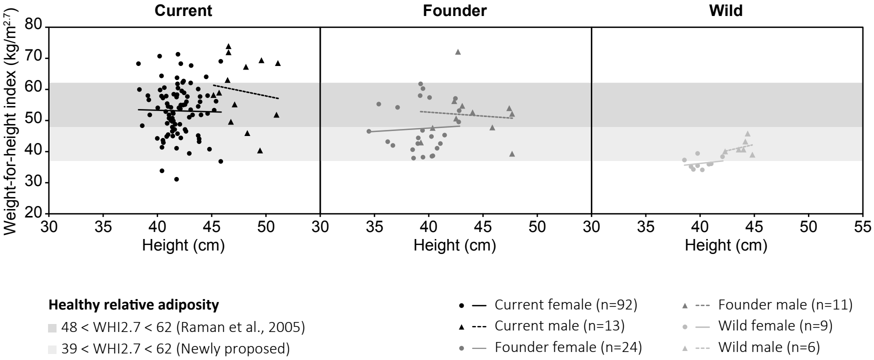

Figure 2. WHI2.7 plotted against height for different adult long-tailed macaque samples. First panel: Current BPRC females (black circles) and current BPRC males (black triangles); second panel: founder females (dark gray circles) and founder males (dark gray triangles); and third panel: wild females (light gray circles) and wild males (light gray triangles). The dark gray bar represents the proposed relative adiposity boundaries by Raman et al. (2005), which correspond to 48 < WHI2.7 < 62. The light gray bar indicates the new lower boundary based on 2% body fat, similar to wild primates. WHI2.7 values of different sex-population groups were generally independent of height.

The other relative adiposity measures, that is abdominal circumference, skinfold thickness, and BCS, were all highly correlated with BMI, WHI3.0 (rhesus macaques; Figure 3a; Table A3) and WHI2.7 (long-tailed macaques; Figure 3b; Table A4). Given that the WHI3.0 and WHI2.7 were independent of height (which BMI is not) and were highly correlated with other relative adiposity measures, we propose that these are better estimates of relative adiposity than the BMI (WHI2.0) for these macaques.

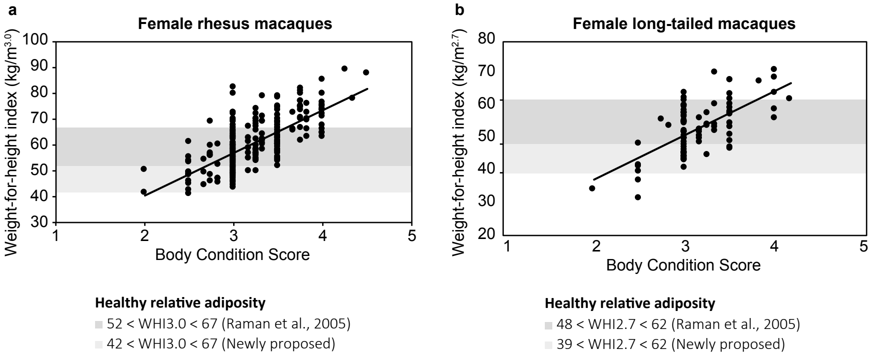

Figure 3. Relationship between body condition score and WHI3.0 (**a**; rhesus macaque females) and WHI2.7 (**b**; long-tailed macaque females) at the BPRC. The dark gray bar represents the proposed relative adiposity boundaries by Raman et al. (2005), which are 52 < WHI3.0 < 67 in rhesus macaques and 48 < WHI2.7 < 62 in long-tailed macaques. The light gray bar indicates the new lower boundary based on 2% body fat levels in the wild, which corresponds to WHI3.0 = 42 (rhesus macaques) and WHI2.7 = 39 (long-tailed macaques). The y-axes are differently scaled.

Relative adiposity in males and other populations

The general applicability of WHI3.0 (rhesus) and WHI2.7 (long-tailed macaques) was tested per species to see whether these measures also apply to adult males (both species) and to different population samples (long-tailed macaques).

Sex differences in rhesus macaques

In male rhesus macaques, there was an almost significant negative relationship between height and WHI3.0 (Spearman correlation, r = −0.380, n = 23, p = 0.074; Figure 1), whereas WHI3.0 was highly correlated with other relative adiposity measures (Table A3). Next, we compared female and male characteristics (Table A7, statistics). Male rhesus macaques had a significantly higher body weight and height than females. Abdominal circumference was significantly higher in males, while females had higher subscapular skinfold thickness. Abdominal skinfold thickness, supra-iliac skinfold thickness, triceps skinfold thickness, total skinfold thickness and BCS did not differ between the sexes. Male rhesus macaques had significantly higher BMI compared with females, whereas the sexes did not differ in WHI3.0.

Correlations of adiposity measures in males and different long-tailed macaque populations

In the current male long-tailed macaque population, WHI2.7 did not depend significantly on height (Pearson correlation, r = −0.132, n = 13, p = 0.668; Figure 2) and was highly correlated with other relative adiposity measures (Table A4). Similarly, WHI2.7 did not depend significantly on height (Figure 2) in founder females (Spearman correlation, r = 0.051, n = 24, p = 0.813), founder males (Pearson correlation, r = −0.090, n = 11, p = 0.793), wild females (Pearson correlation, r = 0.231, n = 9, p = 0.550) and wild males (Pearson correlation, r = 0.273, n = 6, p = 0.600). Moreover, WHI2.7 was highly correlated with other relative adiposity measures in the founder population (Table A5). In the wild population, only a few relative adiposity measures were significantly correlated with WHI2.7 (Table A6).

Population and sex differences in long-tailed macaques

For each sex, we examined variation in various body measures among the current, founder and wild populations (Figure 2; Table A7, statistics). In both sexes, body weight, height, abdominal circumference, subscapular skinfold thickness, supra-iliac skinfold thickness, triceps skinfold thickness, and total skinfold thickness were higher for the current population compared with the founder and wild population. Furthermore, female BMI and WHI2.7 were higher in the founder population compared with the wild population. Abdominal skinfold thickness, BMI and WHI2.7 of males were significantly higher in the current than wild population, but did not differ from the founder population.

Sex differences were tested for the different populations separately (Table A7, statistics). The sexes did not differ significantly in abdominal circumference in all three populations. Males had higher body weight, height, and BMI than females in all three populations. Males had significantly higher WHI2.7 compared with females in the current and wild population, but not the founder population. Skinfold thicknesses were higher for males from the wild population than females. Similarly, current BPRC male triceps thickness and the founder male total skinfold thickness was significantly higher than for females, yet other male and female skinfold thicknesses did not differ in the current and founder populations. BCS did not differ significantly between the sexes in the current BPRC population.

Estimating boundaries of overweight and underweight with the new WHI measures

The new relative adiposity measures, WHI3.0 for rhesus macaques and WHI2.7 for long-tailed macaques, can be applied to both females and males and, for long-tailed macaques, to different populations. We, therefore, calculated boundaries of overweight and underweight on the basis of these measures. This resulted in WHI boundaries (cf. Raman et al. 2005 for solitary-housed macaques), with a lower boundary based on 8–9% body fat, for rhesus macaques of $52 < \text{WHI3.0} < 67$ and for long-tailed macaques $48 < \text{WHI2.7} < 62$. The lower WHI boundary based on 2% body fat, similar to wild primates, was 42 for rhesus macaques (WHI3.0) and was 39 for long- tailed macaques (WHI2.7).

Estimating the incidence of overweight and underweight

The incidence of overweight and underweight in the current BPRC population was determined employing five different methods (Table 1). Depending on the method, estimates of overweight percentages in the population varied between 4.1% and 31.8% in rhesus macaques and between 2.0% and 24.8% in long-tailed macaques. Methods 1, 2, and 5 provided intermediate estimates of the percentages of overweight

Table 1. Incidence of overweight and underweight in the BPRC breeding colony per species-sex group assessed with different methods.

	Incidence of overweight					**Incidence of underweight**					
	M1: BMI boundaries	**M2: WHI boundaries** [a]	**M3: AC boundaries**	**M4: mean WHI ± 2 SD**	**M5: BCS**	**M1: BMI boundaries**	**M2: WHI boundaries with 8% body fat** [a,b]	**M2: WHI boundary with 2% body fat** [c]	**M3: AC boundaries**	**M4: mean WHI ± 2 SD**	**M5: BCS**
Criterion	♂: BMI > 42 ♀: BMI > 35	RM: WHI3.0 > 67 LTM: WHI2.7 > 62	♂: AC > 54 ♀: AC > 44	♂♀: WHI > mean + 2 SD	♂♀: BCS > 3.5	♂: BMI < 32 ♀: BMI < 27	RM: WHI3.0 < 52 LTM: WHI2.7 < 48	RM: WHI3.0 < 42 LTM: WHI2.7 < 39	♂: AC < 40 ♀: AC < 35	♂♀: WHI < mean – 2 SD	♂♀: BCS < 2.5
Rhesus macaques											
Males (♂)	4 / 23 17.4%	6 / 23 26.1%	4 / 23 17.4%	1 / 23 4.3%	5 / 23 21.7%	8 / 23 34.7%	5 / 23 21.7%	0 / 23 0%	6 / 23 26.1%	0 / 23 0%	2 / 23 8.7%
Females (♀)	60 / 273 22.0%	62 / 273 22.7%	89 / 269 33.1%	11 / 273 4.0%	40 / 273 14.7%	59 / 273 21.6%	56 / 273 20.5%	1 / 273 0.4%	53 / 269 19.7%	1 / 273 0.4%	2 / 273 0.7%
Total	64 / 296 **21.6%**	68 / 296 **23.0%**	93 / 292 **31.8%**	12 / 296 **4.1%**	45 / 296 **15.2%**	67 / 296 **22.6%**	61 / 296 **20.6%**	1 / 296 **0.3%**	59 / 292 **20.2%**	1 / 296 **0.3%**	4 / 296 **1.4%**
Long-tailed macaques											
Males (♂)	4 / 13 30.8%	6 / 13 46.2%	1 / 12 8.3%	0 / 13 0%	3 / 13 23.1%	3 / 13 23.1%	2 / 13 15.4%	0 / 13 0%	5 / 12 41.7 %	0 / 13 0%	0 / 13 0%
Females (♀)	13 / 88 14.8%	11/ 88 12.5%	24 / 89 27.0%	2 / 88 2.2%	6 / 92 6.5%	24 / 88 27.3%	23 / 88 26.1%	3 / 88 3.4%	12 / 89 13.5%	3 / 88 3.4%	1 / 92 1.1%
Total	17 / 101 **16.8%**	17 / 101 **16.8%**	25 / 101 **24.8%**	2 / 101 **2.0%**	9 / 105 **8.6%**	27 / 101 **26.7%**	25 / 101 **24.8%**	3 / 101 **3.0%**	17 / 101 **16.8%**	3 / 101 **3.0%**	1 / 105 **1.0%**

Notes: The boundaries were based on the literature. Method 1 (M1): BMI boundaries (Raman et al. 2005); Method 2 (M2): WHI boundaries per species; Method 3 (M3): abdominal circumference boundaries (Raman et al. 2005); Method 4 (M4): based on two standard deviations from the mean WHI per species (Schwartz et al. 1993); Method 5 (M5): based on BCS (Clingerman & Summers 2005).

Abbreviations: AC, abdominal circumference; BCS, body condition score; BMI, body mass index; BPRC, Biomedical Primate Research Centre; SD, standard deviation; WHI, weight-for-height index

[a] The WHI measures of rhesus macaque males and females did not differ significantly (Table A7). The WHI measures of long-tailed macaque males were higher than of females (Table A7), but we could not determine a boundary. Therefore, we calculated the WHI per species and used the same (female) boundary for both sexes.

[b] Based on Raman et al. (2005)

[c] Based on body fat of wild primates (Altmann et al. 1993; Dittus 2013)

in both species. Method 3 provided a high proportion, whereas Method 4 provided a low proportion of overweight.

The incidence of underweight varied between 0.3% and 22.6% in rhesus macaques and between 1.0% and 26.7% in long-tailed macaques. Methods 1, 2 (based on 8% body fat) and 3 resulted in a large proportion of underweight individuals. Methods 2 (based on 2% body fat), 4 and 5 provided relatively low proportions of underweight.

Discussion

We determined the best measure of relative adiposity and explored the boundaries of overweight and underweight in captive group-housed rhesus and long-tailed macaques. The WHI with height to the power of 3.0 (rhesus macaques) and 2.7 (long-tailed macaques) depended least on height and were highly correlated with other relative adiposity measures. Therefore, we considered these WHI measures better than the BMI (i.e. WHI2.0). The percentages of overweight and underweight individuals were estimated with five different methods, based on upper and lower boundaries derived from the literature. These showed large differences in their outcomes. The upper WHI boundary based on solitary-housed macaques (cf. Raman et al. 2005) gave an intermediate incidence of overweight and may apply to group-housed macaques. In contrast, the lower boundary proposed for solitary-housed macaques resulted in a large percentage of underweight individuals. A lower boundary based on 2% body fat of wild primates yielded few underweight individuals and may constitute a better estimate of the incidence of underweight in group-housed macaques.

The best measure of WHI in captive group-housed macaques

A WHI can be used to measure relative adiposity, but the power of height may be population specific (Benn 1971). In the BPRC population BMI was positively correlated with height in both female rhesus and female long-tailed macaques. Therefore, BMI is not the best measure of relative adiposity in these macaques. The WHI that correlated least with height was determined. This differed between the two macaque species: it was WHI3.0 (or the Ponderal index, weight/height^3) for rhesus macaques and WHI2.7 (weight/height$^{2.7}$) for long-tailed macaques. Both WHIs were highly correlated with other adiposity measures, that is abdominal circumference, skinfold thicknesses and BCS. Altogether, WHI3.0 for rhesus and WHI2.7 for long-tailed macaques fit the two criteria for a valid measure of relative adiposity.

In rhesus macaques, sex differences in BMI indicated that males had a significantly higher relative adiposity than females, whereas no such difference was found for WHI3.0. However, males were also taller, and the correlation of BMI with height may have been responsible for this outcome. Indeed, most other adiposity measures did not show a sex difference. Similarly, in the founder long-tailed macaques WHI2.7 did not show a sex difference, whereas BMI did. This indicates that using a WHI that depends on height can result in spurious outcomes. However, WHI2.7 and BMI of the current BPRC and wild long-tailed macaques indicated that males had a higher relative adiposity than females. This suggests a population-specific sex difference in WHI2.7 in long-tailed macaques.

As for both macaque species the “traditional” BMI measure was not the best way to measure relative adiposity, this may also apply to other macaque species. The two study species differed in the power of height that gave the best estimate: for rhesus macaques WHI3.0 and for long-tailed macaques WHI2.7. The difference between the species may be related to the more terrestrial habits of rhesus macaques and the mostly arboreal habits of long-tailed macaques that have resulted in relatively robust rhesus and slender long-tailed macaques (Rodman 1979; Cant 1988). Alternatively, the differences may be due to body size. However, the two species do overlap in height. Moreover, the CIs include a large range of power estimates, especially in long-tailed macaques due to the smaller sample size. Ideally, the power of WHI should be determined for each species separately, yet this requires large sample sizes. When this cannot be calculated, we suggest that, depending on the robustness of the species and their terrestrial or arboreal lifestyle, the rhesus or long-tailed macaque WHI measure should be used.

WHI3.0 in rhesus macaques

Although the WHI3.0 was determined for female rhesus macaques, males also had measures within the female range. Similarly, the WHI3.0 and most other relative adiposity measures did not differ systematically between the sexes, suggesting that these measures did not depend on sex. The suggestion that WHI3.0 is also the best measure for males seems contradicted by the almost significant negative relationship between male height and WHI3.0 (see also Figure 1). However, the number of males was relatively low ($n = 23$) and this outcome hinged on one exceptionally short and stocky individual. When excluding this nonrepresentative male, a weak relationship between male height and WHI3.0 was found (Spearman correlation, $r =-0.296$, $n = 22$, $p = 0.182$). Currently, WHI3.0 seems a good measure of relative adiposity in both full-grown female and male rhesus macaques. Future research should aim to estimate the power of height in WHI for rhesus macaque males based on a larger data set.

WHI2.7 in long-tailed macaques

For female long-tailed macaques, the WHI estimates of the current BPRC population could be compared with other samples, namely the founders of the current BPRC population and wild long-tailed macaques. In all populations and in both sexes, WHI2.7 was independent of height. Moreover, WHI2.7 was correlated with other adiposity measures. Therefore, WHI2.7 seems a measure of relative adiposity applicable to all measured populations of long-tailed macaques.

The founder and the wild individuals were smaller, lighter and had a lower WHI2.7 than the current BPRC animals, whereas founder and wild individuals were similar in many of the adiposity measures. The animals in the current population being taller than the founder and wild animals suggest that they may obtain maximum length in captive conditions with optimal food and few diseases, or that captive management unintentionally selected for taller animals. We cannot distinguish between these two options. The comparison of the WHI2.7 and other relative adiposity measures of the current BPRC population with the founders and wild macaques suggests that the current BPRC population is relatively heavy.

Male long-tailed macaques of the current BPRC population were compared with the females. Males were larger, heavier and had a higher WHI2.7 than females. They had a higher triceps skinfold thickness than females as well, but the other relative adiposity measures did not differ between the sexes. This may have several explanations. First, the male long-tailed macaques were from a different genetic origin than the females, to prevent inbreeding. These populations may have a different relative adiposity. Second, the higher male WHI2.7 may reflect a relatively high muscle mass. As most relative adiposity measures of current BPRC males and females did not differ, this suggests that males may indeed be more muscular. We did not find a similar effect in the other populations. In the founder population, females and males were similar in most relative adiposity measures, whereas in the wild population males had higher adiposity than females. This may either indicate a real difference between the populations or can be due to the small sample sizes.

The incidence of overweight and underweight

We determined the incidence of overweight and underweight in the current BPRC populations based on five different methods. The different measures showed highly variable outcomes.

Method 1 (Raman et al. 2005) determined BMI boundaries for male and female rhesus macaques based on fat reserves in relatively old and solitary-housed animals living in small cages. The estimates for the percentage of overweight individuals were intermediate between the other measures, yet the estimates for underweight were high. This may have two explanations. First, many of the studied animals may be underweight (see below). Second, the boundary for underweight may be set at a relatively high value. Indeed, Raman et al. (2005) based the lower boundary on 8–9% body fat and included a large safety margin (3%). Therefore, the lower boundary for solitary-housed rhesus macaques may not represent the correct reference values for group-housed macaques that have more opportunities to move around in their enclosures.

The calculations for the WHI boundaries in Method 2 were based on the BMI measures of Raman et al. (2005) and give a similar pattern in their estimations of overweight and underweight. Like for Method 1, the lower boundary of WHI based on 8–9% body fat resulted in a large percentage of underweight individuals. Alternatively, when considering a lower boundary based on 2% body fat in wild primates (Altmann et al. 1993; Dittus 2013), the percentage of underweight individuals becomes similar to the (low) estimates of two other measures (rhesus macaque females: 0.3%; long-tailed macaque females: 3.0%). Based on 2% body fat the lower boundary for underweight is WHI3.0 = 42 for rhesus macaques and WHI2.7 = 39 for long-tailed macaques.

Method 3 was based on the boundaries of abdominal circumference for solitary-housed male and female rhesus macaques (Raman et al. 2005). This resulted in many overweight and many underweight individuals for both rhesus and long-tailed macaques. This method was not in line with the overall pattern and probably overestimates problematic weights.

Method 4 (Schwartz et al. 1993) is based on the population average in WHI and its variation. This method gives the lowest proportion of overweight and underweight individuals in both species, although more individuals are overweight than underweight. Some individuals had weights above the normal variation, arguing that overweight does exist in both species. In addition, only three female long-tailed macaques and one female rhesus macaque have a value below the normal variation, indicating that underweight is rare in this population. However, a weakness of this method is that it depends on the population average: when all individuals are relatively heavy, relatively few individuals will be considered overweight and vice versa. The boundary for underweight yields WHI3.0 = 42 for rhesus monkeys and WHI2.7 = 37 for long-tailed macaques.

Method 5 measuring the BCS (Clingerman & Summers 2005) is based on expert evaluation of body fat and muscle tissue and uses palpation of key anatomic features. Similar to Methods 1 and 2, this method resulted in intermediate percentages of overweight, whereas the percentage of underweight individuals was very low. Therefore, this method suggests that overweight is found in these macaques, but that underweight is rare. When the optimal body condition (BCS = 3) was used as a reference to create WHI boundaries, rhesus macaques have an optimal relative adiposity range between 44 < WHI3.0 < 82 and long-tailed macaques between 41 < WHI2.7 < 64.

Proposed WHI boundaries for overweight and underweight

Based on estimates of the five methods, we propose WHI boundaries for group-housed macaques that live in relatively large enclosures with inside and outside compartments. For overweight, we propose to follow the intermediate values from Method 2 (based on Method 1) to determine the WHI boundary. This results for rhesus macaques in WHI3.0 = 67 and for long-tailed macaques in WHI2.7 = 62. This is the same boundary as proposed previously by Raman et al. (2005) for solitary-housed macaques. They based their upper boundary on health considerations. Whether this also applies to group-housed macaques remains to be established.

For underweight, we propose to follow Methods 4 and 5 and the boundary of Method 2 when using 2% body fat (Altmann et al. 1993; Dittus 2013). This leads to very few underweight individuals in the current BPRC population (rhesus macaques: 0.3%; long-tailed macaques: 3.0%) and fits the observation that females with a low WHI give birth to offspring at a normal rate (non-published data). In addition, individuals with a low WHI were typically considered "normal" (i.e. BCS = 3) with the BCS method. This also complies with the impression that individuals with a low WHI are similar in build to reproducing wild long-tailed macaques (EHMS personal observation; cf. Altmann et al. 1993; Dittus 2013). Actually, most females of the wild population had an even lower WHI2.7. This suggests that the lower boundary is not stricter than living in the wild. Higher boundaries would consider “normally” slender individuals underweight. In addition, a relatively low weight in macaques may improve longevity and not necessarily be unhealthy (Mattison et al. 2017). For rhesus macaques, the estimates of Methods 2 and 4 are similar and propose a WHI3.0 = 42 (Figure 3a). For long-tailed macaques, Method 2 results in WHI2.7 = 39, whereas Method 4 results in WHI2.7 = 37, we propose to use the more conservative WHI2.7 = 39 as the lower boundary (Figure 3b).

The BCS (Method 5) was the only method that yielded both an intermediate incidence of overweight and the proposed proportion of underweight individuals. The BCS correlated with all measures indicating adiposity as well. However, BCS also correlated positively with height, which is undesirable. In addition, the experts who determined BCS seemed to vary in how they applied the BCS system, some being more conservative than others (unpublished data). As a result, animals with an optimal BCS (BCS = 3) vary greatly in WHI (Figure 3) and some were even considered overweight based on WHI measures. In contrast, some individuals considered overweight based on their BCS (BCS > 3.5), had a normal relative adiposity when based on WHI measures. Therefore, the two methods do not agree. We propose to use the WHI estimates as it is relatively objective and precise, identifying individuals near the higher or lower boundary of the normal WHI range. Moreover, monitoring of individual-specific relative adiposity between different measuring moments can be more precise.

In conclusion, relative adiposity in macaques is best measured for rhesus macaques with WHI3.0 (weight/height^3) and for long-tailed macaques WHI2.7 (weight/height$^{2.7}$), as these WHI measures are independent of height and are highly correlated with other relative adiposity measures. We propose that a healthy relative adiposity in captive group-housed rhesus macaques ranges between 42 < WHI3.0 < 67 and in long-tailed macaques between 39 < WHI2.7 < 62. The lower boundary is based on fat percentages similar to wild primates, whereas the upper WHI boundary complies with a previously proposed boundary for overweight in solitary-housed rhesus macaques (i.e. Raman et al. 2005). The more objective identification of over- and underweight via appropriate WHI measures may aid in more focussed clinical and husbandry decisions for macaques. The use of the established upper boundary and this new lower boundary results in an acceptable weight for the majority of the group-housed macaques in spacious enclosures with very few underweight animals and a considerable proportion of overweight animals. Further research into health parameters in group-housed individuals with high and low WHI values is still required.

Appendix

Age – height relationship

We checked the premise from the literature that females older than six years of age can be defined as skeletally mature in the BPRC population (Schwartz et al. 1993). Surprisingly, adult female rhesus macaque age was positively correlated with height (Spearman correlation, $r = 0.261$, $n = 273$, $p < 0.0005$; Figure A1). Additional calculations with different age cut-off points indicated that with a cut-off point of seven years the correlation was still significantly positive (Spearman correlation, $r = 0.162$, $n = 210$, $p = 0.019$), but this disappeared when the sample encompassed only females above the age of eight (Spearman correlation, $r = 0.067$, $n = 172$, $p = 0.385$). Linear regression showed that the relationship between age and height in adult female rhesus macaques was height (cm) = 51.461 + 0.068 × age (years). This means that adult rhesus females grow 0.068 cm per year and 0.68 cm in ten years, which we considered negligible relative to the range in height (about 45-58 cm) and ignored in the further analyses.

There was no significant correlation between age and height in adult female long-tailed macaques (Spearman correlation, $r = -0.114$, $n = 88$, $p = 0.292$).

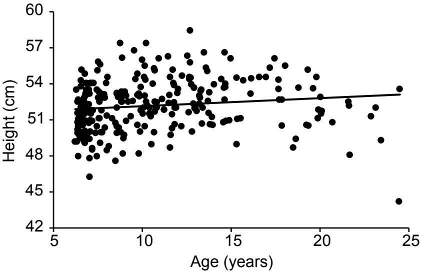

Figure A1. Height plotted against age for adult female rhesus macaques. The line shows a slight, but significant positive correlation between age and height. This relationship can be expressed as height (cm) = 51.461 + 0.068 × age (years).

Converting BMI in WHI-values

According to Raman et al. 2005, BMI of solitary housed female macaques should be between 27 and 35 kg/m^2. The average height of female rhesus macaques at the BPRC population is 52.19 cm, while female long-tailed macaques are on average 44.31 cm tall. See table for data conversion.

	BMI = kg/m^2	**Average height**	**Average weight (kg = BMI*m^2)**	**WHI (kg/m$^\beta$)**
Lower boundary rhesus ♀	27	52.19 cm = 0.5219 m	$27*0.5219^2 = 27*0.272 =$ 7.35 kg	$7.35 / 0.5219^3 = 51.73 =$ 52 kg/m^3
Upper boundary rhesus ♀	35	52.19 cm = 0.5219 m	$35*0.5219^2 = 35*0.272 =$ 9.53 kg	$9.53 / 0.5219^3 = 67.06 =$ 67 kg/m^3
Lower boundary long-tailed ♀	27	44.31 cm = 0.4431 m	$27*0.4431^2 = 27*0.1963 =$ 5.30 kg	$5.30 / 0.4431^{2.7} = 47.73 =$ 48 kg/m$^{2.7}$
Upper boundary long-tailed ♀	35	44.31 cm = 0.4431 m	$35*0.4431^2 = 35*0.1963 =$ 6.87 kg	$6.87 / 0.4431^{2.7} = 61.88 =$ 62 kg/m$^{2.7}$

Table A1. The age, body weight, height and WHI3.0 of four elderly female rhesus macaques

Name	Age (years)	Weight (kg)	Height (cm)	WHI3.0 (kg/m^3)
Bertha	34.5	5.1	46.2	51.72
Wizco	29.7	5.7	47.4	53.46
Alarm	26.9	6.9	47.8	63.18
Mol	25.7	8.1	49.3	67.60

Table A2. Descriptive statistics of the studied populations (mean ± SE)

	Current female ♀ rhesus macaques	Current male ♂ rhesus macaques	Current female ♀ long-tailed macaques	Current male ♂ long-tailed macaques	Founding female ♀ long-tailed macaques	Founding male ♂ long-tailed macaques	Wild female ♀ long-tailed macaques	Wild male ♂ long-tailed macaques
Sample size	n = 273	n = 23	n = 92	n = 13	n = 24	n = 11	n = 9	n = 6
Age (years)	10.73 ± 0.26	13.21 ± 0.68	12.25 ± 4.35	12.07 ± 0.54	10.88 ± 0.86	10.40 ± 0.74	10.53 ± 1.17	11.09 ± 1.69
Weight (kg)	8.58 ± 0.10	12.03 ± 0.46	5.91 ± 1.19	9.86 ± 0.58	4.38 ± 0.22	6.65 ± 0.46	3.51 ± 0.13	5.29 ± 0.21
Length (cm)	52.19 ± 0.13	58.60 ± 0.43	44.31 ± 1.94	51.38 ± 0.64	41.22 ± 0.53	46.66 ± 1.06	42.11 ± 0.43	46.63 ± 0.43
$WHI^{2.0}$ (BMI)	31.33 ± 0.30	35.04 ± 1.23	30.05 ± 4.69	37.24 ± 1.84	25.50 ± 0.89	30.28 ± 1.51	19.74 ± 0.39	24.29 ± 0.66
$WHI^{2.7}$	NA	NA	53.14 ± 8.14	59.41 ± 2.93	47.42 ± 1.57	51.69 ± 2.56	36.16 ± 0.60	41.43 ± 1.01
$WHI^{3.0}$ (Ponderal)	60.05 ± 0.56	59.90 ± 2.20	NA	NA	NA	NA	NA	NA
AC (cm)	41.19 ± 0.40	45.50 ± 1.62	40.60 ± 5.57	44.59 ± 1.88	25.85 ± 0.68	26.33 ± 0.90	26.53 ± 1.03	26.71 ± 1.02
AST (mm)	6.46 ± 0.24	9.95 ± 1.62	6.55 ± 3.86	8.25 ± 1.76	2.54 ± 0.13	3.21 ± 0.51	1.58 ± 0.07	2.42 ± 0.21
SBST (mm)	5.37 ± 0.12	4.67 ± 0.40	5.85 ± 1.81	7.23 ± 0.72	3.83 ± 0.17	4.25 ± 0.40	2.50 ± 0.06	3.52 ± 0.20
SIST (mm)	6.10 ± 0.17	6.61 ± 0.83	7.86 ± 2.78	9.23 ± 1.10	4.09 ± 0.26	4.75 ± 0.66	2.37 ± 0.06	3.68 ± 0.21
TRST (mm)	2.25 ± 0.03	2.31 ± 0.09	2.12 ± 0.35	2.66 ± 0.20	1.16 ± 0.02	1.35 ± 0.11	1.06 ± 0.04	1.42 ± 0.06
TOST (mm)	20.15 ± 0.50	23.56 ± 2.79	22.27 ± 7.16	27.37 ± 3.57	10.46 ± 0.47	13.57 ± 1.59	7.50 ± 0.21	11.03 ± 0.63
BCS	3.19 ± 0.02	3.12 ± 0.12	3.15 ± 0.38	3.25 ± 0.12	NA	NA	NA	NA

Abbreviations: WHI, weight-for-height index; AC, abdominal circumference; AST, abdominal skinfold thickness; SBST, subscapular skinfold thickness; SIST, supra-iliac skinfold thickness; TRST, triceps skinfold thickness; TOST, total skinfold thickness; BCS, body condition score

Table A3. Spearman correlations for body measurements indicating relative adiposity in adult male (n=23) and female (n=273) rhesus macaques that currently live in the BPRC breeding colony †. Grey-marked areas represent non-significant results.

Males Females	Height	Weight	WHI$^{2.0}$ (BMI)	WHI$^{3.0}$ (Ponderal)	Abdominal circumference	Abdominal skinfold	Subscapular skinfold	Supra-iliac skinfold	Triceps skinfold	Total skinfold	Body Condition Score
Height		r=0.245 n=23 p=0.260	r=-0.171 n=23 p=0.435	r=-0.380 n=23 p=0.074	r=0.077 n=23 p=0.727	r=-0.022 n=23 p=0.922	r=-0.047 n=23 p=0.830	r=0.124 n=23 p=0.574	r=-0.275 n=23 p=0.204	r=0.009 n=23 p=0.967	r=-0.207 n=23 p=0.334
Weight	r=0.634 n=273 p<0.0005		r=0.858 n=23 p<0.0005	r=0.739 n=23 p<0.0005	r=0.833 n=23 p<0.0005	r=0.659 n=23 p=0.001	r=0.699 n=23 p<0.0005	r=0.796 n=23 p<0.0005	r=0.520 n=23 p=0.011	r=0.775 n=23 p<0.0005	r=0.514 n=23 p=0.012
WHI$^{2.0}$ (BMI)	r=0.276 n=273 p<0.0005	r=0.902 n=273 p<0.0005		r=0.956 n=23 p<0.0005	r=0.842 n=23 p<0.0005	r=0.771 n=23 p<0.0005	r=0.791 n=23 p<0.0005	r=0.792 n=23 p<0.0005	r=0.686 n=23 p<0.0005	r=0.845 n=23 p<0.0005	r=0.695 n=23 p<0.0005
WHI$^{3.0}$ (Ponderal)	r=0.039 n=273 p=0.522	r=0.766 n=273 p<0.0005	r=0.965 n=273 p<0.0005		r=0.745 n=23 p<0.0005	r=0.699 n=23 p<0.0005	r=0.737 n=23 p<0.0005	r=0.724 n=23 p<0.0005	r=0.652 n=23 p=0.001	r=0.775 n=23 p<0.0005	r=0.739 n=23 p<0.0005
Abdominal circumference	r=0.416 n=269 p<0.0005	r=0.885 n=269 p<0.0005	r=0.890 n=269 p<0.0005	r=0.812 n=269 p<0.0005		r=0.887 n=23 p<0.0005	r=0.743 n=23 p<0.0005	r=0.777 n=23 p<0.0005	r=0.600 n=23 p=0.002	r=0.884 n=23 p<0.0005	r=0.667 n=23 p=0.001
Abdominal skinfold	r=0.184 n=271 p=0.002	r=0.599 n=271 p<0.0005	r=0.668 n=271 p<0.0005	r=0.656 n=271 p<0.0005	r=0.638 n=268 p<0.0005		r=0.822 n=23 p<0.0005	r=0.842 n=23 p<0.0005	r=0.615 n=23 p=0.002	r=0.958 n=23 p<0.0005	r=0.723 n=23 p<0.0005
Subscapular skinfold	r=0.224 n=271 p<0.0005	r=0.662 n=271 p<0.0005	r=0.730 n=271 p<0.0005	r=0.711 n=271 p<0.0005	r=0.669 n=268 p<0.0005	r=0.714 n=271 p<0.0005		r=0.833 n=23 p<0.0005	r=0.687 n=23 p<0.0005	r=0.890 n=23 p<0.0005	r=0.734 n=23 p<0.0005
Suprailiac skinfold	r=0.180 n=271 p=0.003	r=0.627 n=271 p<0.0005	r=0.697 n=271 p<0.0005	r=0.683 n=271 p<0.0005	r=0.638 n=268 p<0.0005	r=0.764 n=271 p<0.0005	r=0.817 n=271 p<0.0005		r=0.700 n=23 p<0.0005	r=0.944 n=23 p<0.0005	r=0.708 n=23 p<0.0005
Triceps skinfold	r=0.222 n=270 p<0.0005	r=0.560 n=270 p<0.0005	r=0.588 n=270 p<0.0005	r=0.556 n=270 p<0.0005	r=0.551 n=267 p<0.0005	r=0.544 n=270 p<0.0005	r=0.591 n=270 p<0.0005	r=0.549 n=270 p<0.0005		r=0.696 n=23 p<0.0005	r=0.498 n=23 p=0.016
Total skinfold	r=0.205 n=270 n=0.001	r=0.675 n=270 p<0.0005	r=0.749 n=270 p<0.0005	r=0.732 n=270 p<0.0005	r=0.693 n=267 p<0.0005	r=0.929 n=270 p<0.0005	r=0.875 n=270 p<0.0005	r=0.914 n=270 p<0.0005	r=0.637 n=270 p<0.0005		r=0.767 n=23 p<0.0005
Body Condition Score	r=0.317 p=273 p<0.0005	r=0.710 n=274 p<0.0005	r=0.716 n=273 p<0.0005	r=0.659 n=273 p<0.0005	r=0.669 n=269 p<0.0005	r=0.609 n=271 p<0.0005	r=0.541 n=271 p<0.0005	r=0.635 n=271 p<0.0005	r=0.460 n=270 p<0.0005	r=0.657 n=270 p<0.0005	

† Correlations for males appear in the upper-right triangle and those for females appear in the lower-left triangle. Numbers represent the Spearman correlation coefficient (r), sample size (n) and level of significance (p-value).

Table A4. Spearman correlations for body measurements indicating relative adiposity in adult male (n=13) and female (n=92) long-tailed macaques that currently live in the BPRC breeding colony [†]. Grey-marked areas represent non-significant results.

Males Females	Height	Weight	WHI$^{2.0}$ (BMI)	WHI$^{2.7}$	Abdominal circumference	Abdominal skinfold	Subscapular skinfold	Supra-iliac skinfold	Triceps skinfold	Total skinfolds	Body Condition Score
Height		r=0.327 n=13 p=0.275	r=-0.063 n=13 p=0.837	r=-0.157 n=13 p=0.609	r=0.448 n=12 p=0.144	r=-0.056 n=12 p=0.863	r=0.186 n=12 p=0.564	r=0.172 n=12 p=0.594	r=0.224 n=12 p=0.484	r=-0.053 n=12 p=0.871	r=-0.060 n=13 p=0.846
Weight	r=0.607 n=88 p<0.0005		r=0.879 n=13 p<0.0005	r=0.835 n=13 p<0.0005	r=0.895 n=12 p<0.0005	r=0.657 n=12 p=0.020	r=0.818 n=12 p=0.001	r=0.881 n=12 p<0.0005	r=0.727 n=12 p=0.007	r=0.734 n=12 p=0.007	r=0.830 n=13 p<0.0005
WHI$^{2.0}$ (BMI)	r=0.223 n=88 p=0.037	r=0.887 n=88 p<0.0005		r=0.978 n=13 p<0.0005	r=0.811 n=12 p=0.001	r=0.734 n=12 p=0.007	r=0.860 n=12 p<0.0005	r=0.895 n=12 p<0.0005	r=0.727 n=12 p=0.007	r=0.839 n=12 p=0.001	r=0.898 n=13 p<0.0005
WHI$^{2.7}$	r=-0.041 n=88 p=0.706	r=0.782 n=88 p<0.0005	r=0.976 n=88 p<0.0005		r=0.769 n=12 p=0.003	r=0.804 n=12 p=0.002	r=0.846 n=12 p=0.001	r=0.923 n=12 p<0.0005	r=0.685 n=12 p=0.014	r=0.881 n=12 p<0.0005	r=0.944 n=13 p<0.0005
Abdominal circumference	r=0.363 n=84 p=0.001	r=0.835 n=87 p<0.0005	r=0.846 n=84 p<0.0005	r=0.808 n=84 p<0.0005		r=0.741 n=12 p=0.006	r=0.881 n=12 p<0.0005	r=0.888 n=12 p<0.0005	r=0.790 n=12 p=0.002	r=0.811 n=12 p=0.001	r=0.785 n=12 p=0.002
Abdominal skinfold	r=0.383 n=85 p<0.0005	r=0.546 n=87 p<0.0005	r=0.511 n=85 p<0.0005	r=0.464 n=85 p<0.0005	r=0.614 n=84 p<0.0005		r=0.874 n=12 p<0.0005	r=0.839 n=12 p=0.001	r=0.818 n=12 p=0.001	r=0.958 n=12 p<0.0005	r=0.864 n=12 p<0.0005
Subscapular skinfold	r=0.201 n=86 p=0.064	r=0.550 n=88 p<0.0005	r=0.574 n=86 p<0.0005	r=0.540 n=86 p<0.0005	r=0.633 n=85 p<0.0005	r=0.552 n=87 p<0.0005		r=0.916 n=12 p<0.0005	r=0.867 n=12 p<0.0005	r=0.944 n=12 p<0.0005	r=0.853 n=12 p<0.0005
Supra-iliac skinfold	r=0.347 n=85 p=0.001	r=0.572 n=87 p<0.0005	r=0.517 n=85 p<0.0005	r=0.457 n=85 p<0.0005	r=0.641 n=84 p<0.0005	r=0.465 n=87 p<0.0005	r=0.711 n=87 p<0.0005		r=0.762 n=12 p=0.004	r=0.923 n=12 p<0.0005	r=0.961 n=12 p<0.0005
Triceps skinfold	r=0.175 n=86 p=0.108	r=0.493 n=88 p<0.0005	r=0.557 n=86 p<0.0005	r=0.519 n=86 p<0.0005	r=0.600 n=85 p<0.0005	r=0.410 n=87 p<0.0005	r=0.632 n=88 p<0.0005	r=0.602 n=87 p<0.0005		r=0.825 n=12 p=0.001	r=0.728 n=12 p=0.007
Total skinfolds	r=0.359 n=85 p=0.001	r=0.650 n=87 p<0.0005	r=0.624 n=85 p<0.0005	r=0.570 n=85 p<0.0005	r=0.734 n=84 p<0.0005	r=0.824 n=87 p<0.0005	r=0.847 n=87 p<0.0005	r=0.822 n=87 p<0.0005	r=0.624 n=87 p<0.0005		r=0.914 n=12 p<0.0005
Body Condition Score	r=0.226 n=88 p=0.034	r=0.595 n=92 p<0.0005	r=0.585 n=88 p<0.0005	r=0.544 n=88 p<0.0005	r=0.539 n=87 p<0.0005	r=0.473 n=87 p<0.0005	r=0.488 n=88 p<0.0005	r=0.459 n=87 p<0.0005	r=0.414 n=88 p<0.0005	r=0.558 n=87 p<0.0005	

[†] Correlations for males appear in the upper-right triangle and those for females appear in the lower-left triangle. Numbers represent the Spearman correlation coefficient (r), sample size (n) and level of significance (p-value).

Table A5. Spearman correlations for body measurements indicating relative adiposity in adult male (n=11) and female (n=24) long-tailed macaques that founded the current BPRC breeding population [†]. Grey-marked areas represent non-significant results.

Males Females	Height	Weight	WHI$^{2.0}$ (BMI)	WHI$^{2.7}$	Abdominal circumference	Abdominal skinfold	Subscapular skinfold	Supra-iliac skinfold	Triceps skinfold	Total skinfolds
Height		r=0.728 n=11 p=0.011	r=0.323 n=11 p=0.332	r=-0.023 n=11 p=0.947	r=0.556 n=11 p=0.076	r=-0.354 n=11 p=0.286	r=0.173 n=11 p=0.611	r=0.105 n=11 p=0.759	r=0.077 n=11 p=0.821	r=-0.073 n=11 p=0.831
Weight	r=0.747 n=24 p<0.0005		r=0.829 n=11 p=0.002	r=0.533 n=11 p=0.091	r=0.911 n=11 p<0.0005	r=0.032 n=11 p=0.926	r=0.560 n=11 p=0.073	r=0.621 n=11 p=0.041	r=0.601 n=11 p=0.050	r=0.432 n=11 p=0.185
WHI$^{2.0}$ (BMI)	r=0.297 n=24 p=0.158	r=0.837 n=24 p<0.0005		r=0.882 n=11 p<0.0005	r=0.918 n=11 p<0.0005	r=0.337 n=11 p=0.311	r=0.755 n=11 p=0.007	r=0.902 n=11 p<0.0005	r=0.836 n=11 p=0.001	r=0.729 n=11 p=0.011
WHI$^{2.7}$	r=0.051 n=24 p=0.813	r=0.661 n=24 p<0.0005	r=0.952 n=24 p<0.0005		r=0.718 n=11 p=0.013	r=0.638 n=11 p=0.035	r=0.827 n=11 p=0.002	r=0.907 n=11 p<0.0005	r=0.909 n=11 p<0.0005	r=0.861 n=11 p=0.001
Abdominal circumference	r=0.323 n=24 p=0.124	r=0.757 n=24 p<0.0005	r=0.829 n=24 p<0.0005	r=0.763 n=24 p<0.0005		r=0.223 n=11 p=0.509	r=0.682 n=11 p=0.021	r=0.806 n=11 p=0.003	r=0.691 n=11 p=0.019	r=0.588 n=11 p=0.057
Abdominal skinfold	r=0.096 n=24 p=0.656	r=0.542 n=24 p=0.006	r=0.743 n=24 p<0.0005	r=0.769 n=24 p<0.0005	r=0.441 n=24 p=0.031		r=0.743 n=11 p=0.009	r=0.614 n=11 p=0.044	r=0.415 n=11 p=0.205	r=0.849 n=11 p=0.001
Subscapular skinfold	r=0.335 n=24 p=0.109	r=0.736 n=24 p<0.0005	r=0.818 n=24 p<0.0005	r=0.749 n=24 p<0.0005	r=0.726 n=24 p<0.0005	r=0.484 n=24 p=0.017		r=0.834 n=11 p=0.001	r=0.691 n=11 p=0.019	r=0.879 n=11 p<0.0005
Supra-iliac skinfold	r=0.142 n=24 p=0.507	r=0.631 n=24 p=0.001	r=0.822 n=24 p<0.0005	r=0.838 n=24 p<0.0005	r=0.788 n=24 p<0.0005	r=0.548 n=24 p=0.006	r=0.823 n=24 p<0.0005		r=0.720 n=11 p=0.013	r=0.900 n=11 p<0.0005
Triceps skinfold	r=0.078 n=24 p=0.717	r=0.355 n=24 p=0.089	r=0.408 n=24 p=0.048	r=0.413 n=24 p=0.045	r=0.133 n=24 p=0.535	r=0.454 n=24 p=0.026	r=0.239 n=24 p=0.261	r=0.270 n=24 p=0.201		r=0.651 n=11 p=0.030
Total skinfolds	r=0.234 n=24 p=0.270	r=0.724 n=24 p<0.0005	r=0.884 n=24 p<0.0005	r=0.871 n=24 p<0.0005	r=0.766 n=24 p<0.0005	r=0639 n=24 p=0.001	r=0.939 n=24 p<0.0005	r=0.930 n=24 p<0.0005	r=0.300 n=24 p=0.155	

[†] Correlations for males appear in the upper-right triangle and those for females appear in the lower-left triangle. Numbers represent the Spearman correlation coefficient (r), sample size (n) and level of significance (p-value).

Table A6. Spearman correlations for body measurements indicating relative adiposity in wild adult male (n=6) and female (n=9) long-tailed macaques [†]. Grey-marked areas represent non-significant results.

Males Females	**Height**	**Weight**	**WHI$^{2.0}$ (BMI)**	**WHI$^{2.7}$**	**Abdominal circumference**	**Abdominal skinfold**	**Subscapular skinfold**	**Supra-iliac skinfold**	**Triceps skinfold**	**Total skinfolds**
Height		r=0.829 n=6 p=0.042	r=0.429 n=6 p=0.397	r=0.143 n=6 p=0.787	r=0.257 n=6 p=0.623	r=0.290 n=6 p=0.577	r=0.696 n=6 p=0.125	r=0.464 n=6 p=0.354	r=-0.319 n=6 p=0.538	r=0.314 n=6 p=0.544
Weight	r=0.837 n=9 p=0.005		r=0.829 n=6 p=0.042	r=0.657 n=6 p=0.156	r=0.314 n=6 p=0.544	r=0.667 n=6 p=0.148	r=0.928 n=6 p=0.008	r=0.812 n=6 p=0.050	r=-0.116 r=6 p=0.827	r=0.714 n=6 p=0.111
WHI$^{2.0}$ (BMI)	r=0.569 n=9 p=0.110	r=0.900 n=9 p=0.001		r=0.943 n=6 p=0.005	r=0.543 n=6 p=0.266	r=0.754 n=6 p=0.084	r=0.783 n=6 p=0.066	r=0.812 n=6 p=0.050	r=-0.029 n=6 p=0.957	r=0.771 n=6 p=0.072
WHI$^{2.7}$	r=0.226 n=9 p=0.559	r=0.683 n=9 p=0.042	r=0.917 n=9 p=0.001		r=0.429 n=6 p=0.397	r=0.812 n=6 p=0.050	r=0.696 n=6 p=0.125	r=0.812 n=6 p=0.050	r=0.203 n=6 p=0.700	r=0.829 n=6 p=0.042
Abdominal circumference	r=0.414 n=9 p=0.269	r=0.714 n=9 p=0.031	r=0.672 n=9 p=0.047	r=0.672 n=9 p=0.047		r=0.319 n=6 p=0.538	r=0.232 n=6 p=0.658	r=0.261 n=6 p=0.618	r=-0.116 n=6 p=0.827	r=0.200 n=6 p=0.704
Abdominal skinfold	r=0.697 n=9 p=0.037	r=0.485 n=9 p=0.186	r=0.323 n=9 p=0.396	r=0.034 n=9 p=0.931	r=-0.056 n=9 p=0.887		r=0.868 n=6 p=0.025	r=0.971 n=6 p=0.001	r=0.603 n=6 p=0.205	r=0.986 n=6 p<0.0005
Subscapular skinfold	r=0.722 n=9 p=0.028	r=0.462 n=9 p=0.210	r=0.269 n=9 p=0.484	r=-0.017 n=9 p=0.966	r=-0.042 n=9 p=0.914	r=0.837 n=9 p=0.005		r=0.956 n=6 p=0.003	r=0.250 n=6 p=0.633	r=0.899 n=6 p=0.015
Supra-iliac skinfold	r=0.672 n=9 p=0.047	r=0.816 n=9 p=0.007	r=0.869 n=9 p=0.002	r=0.727 n=9 p=0.026	r=0.376 n=9 p=0.319	r=0.557 n=9 p=0.119	r=0.452 n=9 p=0.222		r=0.441 n=6 p=0.381	r=0.986 n=6 p<0.0005
Triceps skinfold	r=0.518 n=9 p=0.153	r=0.507 n=9 p=0.153	r=0.324 n=9 p=0.396	r=0.175 n=9 p=0.653	r=0.384 n=9 p=0.308	r=0.692 n=9 p=0.039	r=0.556 n=9 p=0.120	r=0.307 n=9 p=0.422		r=0.551 n=6 p=0.257
Total skinfolds	r=0.731 n=9 p=0.025	r=0.577 n=9 p=0.104	r=0.410 n=9 p=0.273	r=0.142 n=9 p=0.715	r=0.114 n=9 p=0.770	r=0.975 n=9 p<0.0005	r=0.861 n=9 p=0.003	r=0.579 n=9 p=0.103	r=0.808 n=9 p=0.008	

[†] Correlations for males appear in the upper-right triangle and those for females appear in the lower-left triangle. Numbers represent the Spearman correlation coefficient (r), sample size (n) and level of significance (p-value).

Table A7. Results of the statistical tests in which the effect of sex (male or female) and origin of the population (wild, founder or current) on different long-tailed macaque body measures were tested. Population differences were tested with one-way ANOVAs or Kruskal-Wallis H-tests, for males and females separately. Sex differences were tested with independent samples t-tests or Mann-Whitney U-test, for the different populations separately. Grey-marked areas represent non-significant results.

	Rhesus macaques	Long-tailed macaques				
Measure	**Sex [†] – current population (n=296)**	**Population origin [‡] – data of females (n=125)**	**Population origin [‡] – data of males (n=30)**	**Sex [†] – current population (n=105)**	**Sex [†] – founder population (n=35)**	**Sex [†] – wild population (n=15)**
Body weight	U=485.5, n=296, p<0.0005 M > F	H(2)=41.891, p<0.0005 Cu > Fo ~ Wi (p<0.0005; p<0.0005; p=0.306)	H(2)=18.418, p<0.0005 Cu > Fo ~ Wi (p=0.009; p<0.0005; p=0.396)	t=6.712, n=105, p<0.0005 M > F	t=5.088, n=35, p<0.0005 M > F	t=7.779, n=15, p<0.0005 M > F
Height	t=13.884, n=296, p<0.0005 M > F	F(2,120)=23.790, p<0.0005 Cu > Fo ~ Wi (p<0.0005; p=0.008; p=0.798)	F(2,120)=13.541, p=0.001 Cu > Fo ~ Wi (p=0.005; p=0.009; p=1.000)	t=11.960, n=101, p<0.0005 M > F	t=5.142, n=35, p<0.0005 M > F	t=7.088, n=15, p<0.0005 M > F
Body mass index	U=2022, n=296, p=0.005 M > F	H(2)=35.302, p<0.0005 Cu > Fo > Wi (p<0.0005; p=0.001; p=0.042)	H(2)=15.952, p<0.0005 Cu ~ Fo; Cu > Wi; Fo ~ Wi (p=0.065; p<0.0005; p=0.160)	t=3.767, n=101, p=0.002 M > F	U=59, n=35, p=0.008 M > F	t=6.376, n=15, p<0.0005 M > F
Weight-for-height index	U=2958, n=296, p=0.645 M ~ F	H(2)=29.869, p<0.0005 Cu > Fo > Wi (p=0.017; p<0.0005; p=0.011)	H(2)=12.284, p=0.002 Cu ~ Fo; Cu > Wi; Fo ~ Wi (p=0.393; p=0.001; p=0.087)	t=2.489, n=101, p=0.014 M > F	U=95, n=35, p=0.198 M ~ F	t=4.776, n=15, p<0.0005 M > F
Abdominal circumference	U=2171, n=292, p=0.018	H(2)=67.688, p<0.0005 Cu > Fo ~ Wi (p<0.0005; p<0.0005; p=1.000)	H(2)=20.442, p<0.0005 Cu > Fo ~ Wi (p=0.003; p<0.0005; p=1.000)	U=375.5, n=99, p=0.116 M ~ F	t=0.409, n=35, p=0.685 M ~ F	t=0.119, n=15, p=0.907 M ~ F
Abdominal skinfold thickness	U=2411.5, n=292, p=0.079 M ~ F	H(2)=58.631, p<0.0005 Cu > Fo ~ Wi (p<0.0005; p<0.0005; p=0.272)	H(2)=7.951, p=0.019 Cu ~ Fo; Cu > Wi; Fo ~ Wi (p=0.108; p=0.031; p=1.000)	U=482, n=99, p=0.668 M ~ F	U=97, n=35, p=0.224 M ~ F	t=4.371, n=15, p=0.001 M > F
Subscapular skinfold thickness	U=2123, n=293, p=0.012 M < F	H(2)=47.212, p<0.0005 Cu > Fo ~ Wi (p<0.0005; p<0.0005; p=0.112)	H(2)=16.204, p<0.0005 Cu > Fo ~ Wi (p=0.014; p = 0.001; p=0.520)	U=367.5, n=100, p=0.089 M ~ F	U=101, n=35, p=0.283 M ~ F	t=4.787, n=15, p=0.003 M > F
Supra-iliac skinfold thickness	U=3088.5, n=293, p=0.966 M ~ F	H(2)=56.373, p<0.0005 Cu > Fo ~ Wi (p<0.0005; p<0.0005; p=0.209)	H(2)=14.722, p=0.001 Cu > Fo ~ Wi (p=0.006; p=0.003; p=1.000)	U=416, n=99, p=0.256 M ~ F	U=107.5, n=35, p=0.390 M ~ F	U=0, n=15, p<0.0005 M > F
Triceps skinfold thickness	U=2804.5, n=292, p=0.457 M ~ F	H(2)=71.613, p<0.0005 Cu > Fo ~ Wi (p<0.0005; p<0.0005; p=1.000)	H(2)=18.233, p<0.0005 Cu > Fo ~ Wi (p=0.009; p<0.0005; p=1.000)	U=250.5, n=100, p=0.003 M > F	t=1.716, n=35, p=0.115 M ~ F	t=4.935, n=15, p<0.0005 M > F
Total skinfold thickness	U=2901.5, n=292, p=0.621 M ~ F	H(2)=66.320, p<0.0005 Cu > Fo ~ Wi (p<0.0005; p<0.0005; p=0.535)	H(2)=16.039, p<0.0005 Cu > Fo ~ Wi (p=0.005; p=0.001; p=1.000)	U=411, n=99, p=0.234 M ~ F	U=71.5, n=35, p=0.030 M > F	t=6.214, n=15, p<0.0005 M > F
Body Condition Score	U=2598, n=296, p=0.148 M ~ F	NA	NA	U=535.5, n=105, p=0.523 M ~ F	NA	NA

[†] M = male, F = female [‡] Cu = current population, Fo = founder population, Wi = Wild population, the p-values between brackets show outcomes of the post-hoc comparisons with Bonferroni correction of Cu vs. Fo, Cu vs. Wi and Fo vs. Wi, respectively.

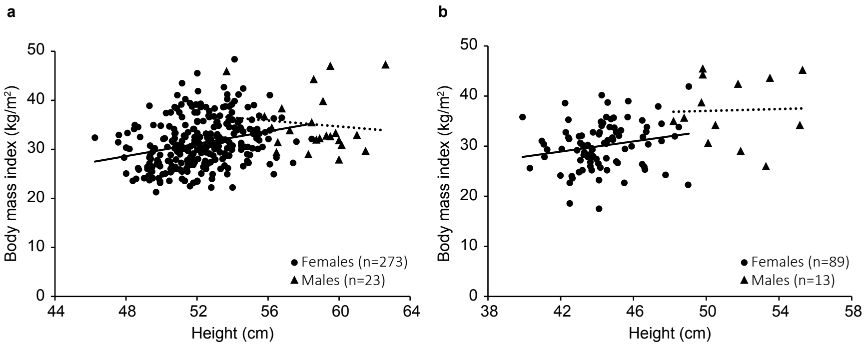

Figure A2. BMI plotted against height for adult rhesus macaques (**a**) and long-tailed macaques (**b**) currently living at the BPRC breeding colony. Dots represent females, while triangles represent males.

Chapter 3

The utility of voluntary weighing in captive group-living rhesus macaques

Dian G. M. Zijlmans, Marit K. Vernes,
Elisabeth H. M. Sterck*, Jan A. M. Langermans*

* Joint senior authors

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Abstract

Body weight is an important health and welfare indicator for captive non-human primates (NHPs). Body weight can be measured during routine handling procedures, which cause stress. Alternatively, animals can be trained to step onto a scale, but training success varies greatly between individuals. Being able to weigh animals regularly without having to handle or train them is thus desirable for monitoring animal health and welfare. This study investigates the utility, i.e. the participation, reliability and time investment, of voluntary weighing in captive NHPs living in large social groups. Subjects of the study were 92 rhesus macaques (*Macaca mulatta*) housed in four social groups at the Biomedical Primate Research Centre in Rijswijk, the Netherlands. A scale was placed in their home enclosure during several sessions. Individuals were unwilling to step onto an unbaited scale. When likeable food items were used to attract individuals to the scale, 68% of them stepped onto the scale. Age and dominance rank did not affect stepping onto the scale, whereas exploratory tendency and social group did. The level of agreement between body weight by voluntary weighing and body weight measured during sedation was very high. These results show that the majority of rhesus macaques in social groups can be weighed voluntarily and that voluntary weighing is reliable. When optimising and further developing the method, voluntary weighing can form a valuable tool in the captive management of NHPs.

Introduction

Body weight is an important health and welfare indicator for captive non-human primates (NHPs) (Terranova & Coffman 1997). Hence, every research facility housing NHPs includes body weight measurements in their animal management programme (Bauer et al. 2010). Body weight can quickly change when animals become sick, i.e. macaques that suffer disease may lose up to 30% of their body weight in 17 days (Bronson et al. 1982). Furthermore, stress can lead to changes in body weight (Schapiro et al. 2012). The detection of these body weight changes, which may signal underlying welfare problems, requires regular body weight measurements (Jennings et al. 2009).

Body weight can be measured during routine handling procedures, such as when animals are relocated or during health checks. This usually means the animals have to be caught and sedated prior to being weighed, which causes stress to both animals and staff (Balcombe et al. 2004; Suleman et al. 2004). Moreover, anaesthesia can have several side-effects (Horne 2001). Since stress and sedation of animals should be avoided as much as possible, this method does not allow frequent weighing. Colony animals (i.e. animals not involved in experimental studies) are therefore weighed only once or a few times per year (Bauer et al. 2010). A possible solution allowing more frequent weighing may be to let animals step onto a scale voluntarily.

At present, positive reinforcement training is often used to enable animals to co-operate voluntarily in various husbandry and veterinary procedures (Desmond & Laule

1994; Prescott et al. 2005). Common marmosets (*Callithrix jacchus*) and cotton-top tamarins (*Saguinis oedipus*) are relatively quickly and easily trained for weighing, while being in their family group (Sánchez et al. 1999; Layne & Power 2003; McKinley et al. 2003). This may be more challenging with other NHPs, like macaques. While macaques are housed at many facilities, only one study reports training group-housed macaques for weighing (Reinhardt 1990). However, this study did not train weighing within the social group. Instead, the macaques were trained to be individually captured in a transport box and weighed in a separate room away from their group members.

Macaques may be more difficult to train in their social group, because they live in large groups with strict dominance hierarchies. Individuals vary greatly in their response to training (Coleman et al. 2005) and training success can be influenced by individual characteristics such as age, dominance rank and personality. In general, younger (Lacreuse et al. 2005), higher-ranking (Drea & Wallen 1999; Kemp et al. 2017) and more exploratory individuals (Coleman et al. 2005; Wergård et al. 2016) are trained more easily. As a result, training all individuals in a social group is time-consuming and it may not be possible to train all group members. For example, station-training of group-housed rhesus macaques (*Macaca mulatta*) — average group size, six individuals — took, on average, 3 h per individual and 26/30 individuals were trained successfully (Schapiro et al. 2003). In larger groups, the success rate may even be lower and the time investment higher.

These disadvantages of training may be prevented by voluntary weighing, i.e. the animal itself decides to step onto a scale and prior training is not required. Voluntary weighing is not a new concept, as it has previously been used in studies on (semi-) wild ungulates, birds and NHPs. It has been applied to Alpine ibex (*Capra ibex*), bighorn sheep (*Ovis canadensis*), mountain goats (*Oreamnos americanus*), king penguins (*Aptenodytes patagonicus*) and dark-eyed juncos (*Junco hyemalis*) (Le Maho et al. 1993; Vézina et al. 2001; Bassano et al. 2003; Gendreau et al. 2005). Wild toque macaques (*Macaca sinica*) and free-ranging Japanese (*Macaca fuscata*) and rhesus macaques have also been weighed successfully using baited scales (Hazama 1964; Mori 1979; Dittus 1998; Zhang et al. 2016). Wild immature yellow baboons (*Papio cynocephalus*) were even weighed without feeding or baiting the scale (Altmann & Alberts 1987). Therefore, voluntary weighing may be applicable to captive, group-living NHPs.

This study aims to determine whether voluntary weighing can be a valuable tool to monitor health and welfare of captive NHPs living in large social groups. We therefore investigated the utility, i.e. participation, reliability and time investment, of voluntary weighing in captive rhesus macaques living in naturalistic groups. We determined participation in four groups. The reliability of the method was tested by comparing body weight measured during voluntary weighing to body weight measured during sedation. We also checked whether the same individual characteristics that often affect training success can predict which individuals are more likely to participate in voluntary weighing. In addition, the time investment required for voluntary weighing in large social groups was addressed.

Materials and methods

Study animals and housing

Study animals consisted of 92 rhesus macaques of Indian origin living in four socially stable groups at the Biomedical Primate Research Centre (BPRC) in Rijswijk, the Netherlands. The groups were established for the purpose of breeding between 1996 and 2002 but were not active breeding groups at the time of this study, i.e. they did not contain an adult male. The groups contained one or several matrilines and were formed by adhering to natural group dynamics (females stay in their natal group, while males three years and older are removed to prevent inbreeding). Table 1 shows the characteristics of each study group and data collection per group. Each group had free access to large interior (72 m^2; 2.85 m high) and exterior (250 m^2; 3.1 m high) enclosures. The interior enclosure contained sawdust bedding, which was replaced regularly while the exterior facility had sand bedding and natural plant growth was possible. The enclosures were enriched with a large variety of items, such as climbing structures, fire hoses, car tyres, slides and a swimming pool (Vernes & Louwerse 2010). Animals were fed daily with monkey chow (Ssniff, Soest, Germany) in the morning and vegetables, fruit or a grain mixture in the afternoon. Drinking water was available *ad libitum* via automatic water dispensers.

Table 1. Group characteristics and data collection in the study groups.

Group	Size	Gender	Matrilines (n)	Mean ± SE (min–max) age (years)	Date scale sessions	Date health check
Chloor	n = 18	18F, 0M	1	7.4 ± 0.9 (3.3–15.4)	Dec 2019 + Jan 2020	28th Oct 2019
Bertha	n = 27	27F, 0M	3	9.2 ± 1.1 (2.7–21.4)	Feb 2020	7th Feb 2020
Grey	n = 24	23F, 1M	1	7.4 ± 0.9 (3.0–17.1)	July 2020	17th July 2020
Marieke	n = 23	22F, 1M	1	7.9 ± 1.1 (2.5–18.9)	Feb 2021	9th Nov 2020

Measuring body weight during sedation

Body weight of individuals in the BPRC breeding colony is measured once per year during the annual health check. After an overnight fast, the animals were sedated with an intramuscular injection of 10 mg/kg ketamine (100 mg/mL; Ketamine 10%, Alfasan, Woerden, the Netherlands) in combination with 0.05 mg/kg medetomidine (1 mg/mL; Sedastart, AST Farma, Oudewater, the Netherlands), which was reversed after the health check with 0.25 mg/kg atipamezole (5 mg/mL; Sedastop, AST Farma, Oudewater, the Netherlands). Body weight was measured to the nearest 0.1 kg during the health check and hereafter will be referred to as sedated body weight (SBW).

Voluntary weighing

The scale used for voluntary weighing, a Kern EOB 60K-2LF (Kern, Balingen, Germany), is a wireless scale with a remote display, which was 'monkey-proof' as it did not contain any wires, screws or buttons. This scale is especially suitable for weighing animals, because the scale calculates an average value to obtain a stable body weight. Since the display automatically shuts down after 3 min of inactivity, observers had to be present to manually restart the display. Additional weight was added to the bottom of the scale to ensure that it could not be lifted or moved. During voluntary weighing, the remote display was kept outside of the enclosure, while the scale was placed inside the home enclosure. The scale was placed on the ground or in a corridor, which was located approximately 1 m above the ground (Figure 1). Before placing the scale in the enclosure, the sawdust bedding was swept aside if necessary. The observers sat a minimum of 2 m away from the scale.

During almost 11 h of try-out sessions with an unbaited scale in the Chloor-group, only one female stepped onto the scale. From this, we concluded that the individuals were not motivated enough to step onto an unbaited scale. Thereafter, small amounts of likeable food items (e.g. apple syrup, jam, lemonade, raisins and ice cubes filled with fruit) were applied onto and close to the scale to attract individuals. Most of the food items were applied prior to each session, while lemonade was also re-applied during the sessions with a syringe. In the other groups, the scale was baited from the first day it was made available.

Data were collected from each group during several sessions, which took place between December 2019 and February 2021 and varied in duration between 1.5 and 3.5 h per session. Individuals were habituated to the presence of one or two observers, who replenished some of the food items when they were consumed. The frequency of

Figure 1. Location of the scale on the ground within the interior of one of the macaque home enclosures. Only part of the enclosure is visible.

stepping onto the scale, frequency of weighing and body weights were scored for each individual. Stepping onto the scale was recorded only when the individual's body was located entirely on the scale, including all four limbs. The mean body weight by voluntary weighing was calculated per individual and will be referred to as voluntary body weight (VBW).

Two cameras (JVC Everio GZ-R15BE, JVC, Yokohama, Japan) filmed the display and scale throughout all the sessions. When body weight or stepping events were missed during live observations, videos were checked to enable completion of the data. Not all stepping events resulted in body weight measurements. For example, when an animal walked over the scale too fast, it was not possible to obtain a reliable body weight. Also, individual weighing was not possible when multiple individuals sat on the scale together. As a result, body weight was not measured for one female from the Grey-group, even though she did step onto the scale. Eventually, 897 body weight measurements were recorded from the 1345 stepping onto the scale events. After a session, the scale would occasionally be left in the enclosure to facilitate habituation but was always removed at the end of each day.

Dominance rank

All occurrence observations took place in all groups to score submissive behaviour, concerning bared teeth, making room, giving ground and escape (Table A1). Submissive behaviour was used to calculate the dominance hierarchies with MatMan in R (Netto et al. 1993). Submissive behaviour was observed 1516 times in the Chloor-group, 2042 times in the Bertha-group, 2749 times in the Grey-group and 2535 times in the Marieke-group. Dominance hierarchies were significantly linear and highly consistent in all groups (Chloor-group: $h' = 1$; $p < 0.005$, DCI = 0.977, 0% unknown relationships; Bertha-group: $h' = 0.924$; $p < 0.005$, DCI = 0.988, 5% unknown relationships; Grey-group: $h' = 0.982$; $p < 0.005$, DCI = 0.968, 1% unknown relationships; Marieke-group: $h' = 0.895$; $p < 0.005$, DCI = 0.992, 9% unknown relationships). Dominance rank was re-scaled by dividing absolute dominance rank by group size to correct for the fact that groups varied in size.

Exploratory tendency

Exploration is a personality trait that is often measured using novel object tests (Carter et al. 2012; Massen et al. 2013). Exploratory tendency was measured in all groups by exposing individuals to a novel feeding method, concerning a new construction for distributing the monkey chow. Individuals were divided into three exploration categories based on their latency to first approach and exploratory behaviour towards the novel feeding method. The first category contained individuals that immediately started using the novel feeding method, i.e. within the first 5 min (high exploratory tendency; n = 35). The second category of individuals did this within 30 min (medium exploratory tendency; n = 31), while individuals from the third category took more time to explore the novel feeding method (low exploratory tendency; n = 26).

Data analysis

Besides participation per group, latency and stepping rate of participants were used to measure the efficiency of voluntary weighing. Latency was the time it took an individual, after the scale was made available, to step onto it for the first time. Stepping rate per individual was calculated by dividing the total number of times an individual stepped onto the scale by the total amount of time the scale was available to the group. Since the data contained too many zeroes to comply with the assumptions for linear regression, we used the binary occurrence of stepping onto the scale (1 = yes, 0 = no) rather than the stepping rate for the analysis. A binary logistic regression model was used to test whether age, dominance rank, exploratory tendency or group ID could predict which individuals were more likely to participate in voluntary weighing.

Reliability of voluntary weighing was tested by checking the level of agreement between VBW and SBW with an intra-class correlation coefficient (ICC). Spearman correlations were used for all other correlations. Some individuals had VBWs available in both the morning (before 1200 h) and the afternoon (after 1200 h) of the same day. A paired samples t-test was used to test whether VBW differed between time of day. We checked whether reliability of voluntary weighing could be improved by testing which factors influenced body weight deviation, which was calculated as VBW minus SBW. A linear regression model with age, dominance rank, number of body weight measurements and group ID as predictor variables was used to test this.

Two adult females were excluded from the analyses on reliability as they were recovering from illness and showed increased body weight after veterinary treatment. Descriptive statistics in the results are reported as mean ± SE. Data were analysed statistically with IBM SPSS Statistics version 26. The significance level was $\alpha = 0.05$ and all tests were two-tailed.

Ethical statement

Since the annual health check is a veterinary management procedure, this study did not perform any invasive or experimental procedures that would require ethics approval according to European Directive 2010/63 or Dutch law. This study complied with guidelines for the ethical use of animals in applied ethology studies (Sherwin et al. 2003) and was approved by BPRC’s Animal Welfare Body (IvD 017A).

Results

Participation and time investment

This study found that 63 out of 92 (68%) rhesus macaques participated in voluntary weighing when the scale was baited. It took individuals, on average, 6 h and 50 min ± 46 min to step onto the scale for the first time. The mean stepping rate of participants was 2.0 ± 0.3 times per h. In total, 1345 events of stepping onto the scale were recorded within a total time of almost 62 h. The efficiency of the voluntary weighing method was highly variable per group (Table 2).

Table 2. Mean ± SE (minimum–maximum) of the efficiency of voluntary weighing per social group, measured as participation per group and stepping rate and latency per participant.

Group	Participation	Total stepping events (n)	Total observation time	Stepping rate of participants	Latency of participants
Chloor	n = 13, 72%	258	12 h, 15 min	1.6 ± 0.6 (0.2–6.6)	7 h, 01 min ± 1 h, 12 min (0 h, 27 min–11 h, 45 min)
Bertha	n = 18, 67%	419	20 h	1.2 ± 0.3 (0.1–5.3)	8 h, 53 min ± 1 h, 21 min (0 h, 27 min–18 h, 37 min)
Grey	n = 9, 38%	113	22 h, 30 min	0.6 ± 0.1 (0.0–2.4)	15 h, 02 min ± 2 h, 33 min (1 h, 52 min–21 h, 49 min)
Marieke	n = 23, 100%	555	7 h, 10 min	3.3 ± 0.5 (0.3–9.7)	1 h, 54 min ± 0 h, 20 min (0 h, 44 min–6 h, 20 min)

Participation in voluntary weighing occurred independent of age (logistic regression, $\chi^2 = 0.911$, $n = 92$; $p = 0.340$) and dominance rank (logistic regression, $\chi^2 = 0.330$, $n = 92$; $p = 0.566$). The likelihood of an individual stepping onto the scale significantly differed between exploration categories (logistic regression, $\chi^2 = 11.098$, $n = 92$; $p = 0.004$) and between groups (logistic regression, $\chi^2 = 7.902$, $n = 92$; $p = 0.048$). Individuals with a low exploratory tendency were less likely to step onto the scale compared to individuals with high and medium exploratory tendencies (Figure 2a). Additionally, individuals from the Grey-group were less likely and individuals from the Marieke-group more likely to step onto the scale compared to individuals from the other groups (Figure 2b).

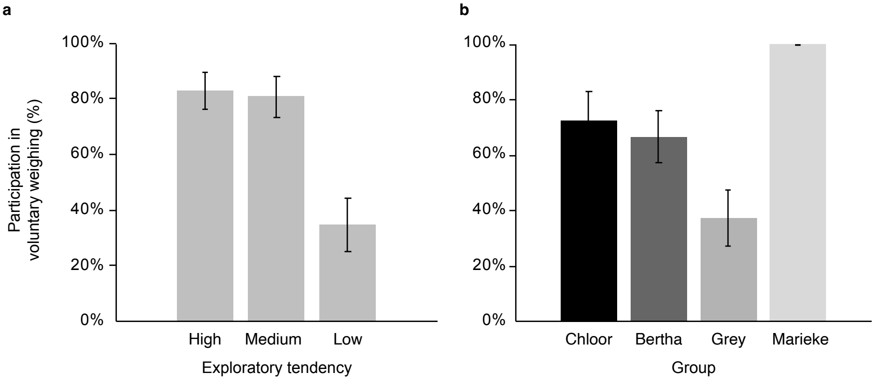

Figure 2. The effects of exploratory tendency (**a**) and social group (**b**) on the participation in voluntary weighing.

Reliability of voluntary weighing

Sedated body weight (SBW) varied between 3.45 and 14.4 kg and was highly correlated to age (Spearman correlation, r = 0.780, n = 92; p < 0.005). Voluntary body weight (VBW) ranged from 4.15 to 14.3 kg and was, on average, 0.44 ± 0.07 kg (6.9 ± 1.0%) higher than SBW. All individuals from the Chloor-group had higher VBW than SBW, while 12 out of 18 individuals from the Bertha-group, six out of eight individuals from the Grey-group and 20 out of 22 individuals from the Marieke-group had a higher VBW than SBW. The level of agreement between VBW and SBW was very high with ICC = 0.966 (F(59,59) = 97.60; p < 0.005, 95% CI [0.835; 0.987]; Figure 3). VBW was significantly higher in the afternoon compared to the morning (paired samples t-test, t = –5.191, n = 13; p < 0.005). VBW increased, on average, 0.145 kg (range 0–0.4 kg) from morning to afternoon, which corresponds to a relative increase of 1.7% (range 0–4.3%).

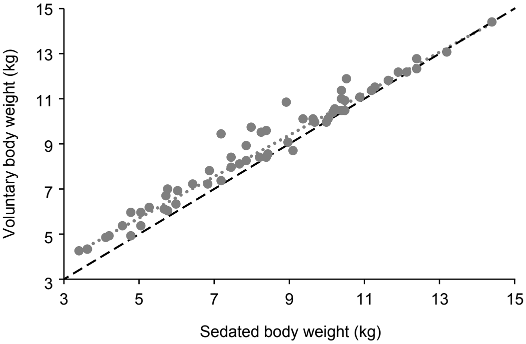

Figure 3. The relationship between voluntary body weight (VBW) and sedated body weight (SBW). The dashed line represents perfect agreement.

Body weight deviation (i.e. VBW minus SBW) was independent of dominance rank (GLM, F(1,50) = 0.757; p = 0.388) and the number of body weight measurements (GLM, F(1,50) = 0.908; p = 0.345). Body weight deviation was higher in younger individuals (GLM, F(1,50) = 11.793; p < 0.005) and differed per group (GLM, F(3,50) = 18.059; p < 0.005). Body weight deviation was significantly higher in the Chloor- and Marieke-group compared to the Bertha- and Grey-group (Table 3). There was also a significant interaction effect between age and group in the model (GLM, F(3,50) = 7.331; p < 0.005), which implies that the relationship between age and body weight deviation differed per group. There was no correlation present between age and body weight deviation in the Bertha-group (Spearman correlation, r = –0.083, n = 18; p = 0.744), Grey-group (Spearman correlation, r = –0.347, n = 8; p = 0.399) or Marieke-group (Spearman correlation, r = –0.357, n = 22; p = 0.103). In contrast, there was a significant negative correlation between age and body weight deviation in the Chloor-group (Spearman correlation, r = –0.676, n = 12; p = 0.016). Younger individuals thus had higher body weight deviation compared to older individuals in the Chloor-group, but not in the other groups (Figure 4).

Table 3. Mean ± SE (minimun–maximum) of body weight deviation and body weight deviation relative to sedated body weight per social group.

Group	Size	Body weight deviation (kg)	Relative body weight deviation (%)
Chloor	n = 12	0.92 ± 0.20 (0.10–2.18)	12.1 ± 2.6 (0.9–30.3)
Bertha	n = 18	0.07 ± 0.07 (-0.57–0.87)	1.0 ± 0.7 (-6.2–8.3)
Grey	n = 8	0.10 ± 0.05 (-0.10–0.30)	1.5 ± 0.8 (-0.7–4.4)
Marieke	n = 22	0.61 ± 0.02 (-0.05–1.23)	10.9 ± 1.5 (-0.4–22.8)

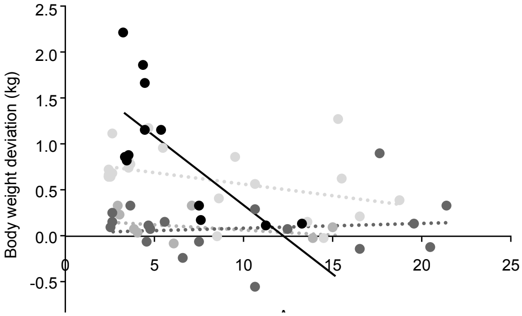

Figure 4. Body weight deviation (i.e. VBW minus SBW) plotted against age per group. A solid line indicates a significant relationship, while dotted lines represent non-significant relationships.

Long-term participation in voluntary weighing

Voluntary weighing was repeated in the Grey-group three months after the initial scale sessions. The nine individuals that participated during the initial scale sessions were again weighed voluntarily within two sessions of 3 h each. Furthermore, two additional individuals stepped onto the scale within these 6 h.

Discussion

This study aimed to determine whether voluntary weighing can be a valuable tool to monitor health and welfare of captive NHPs living in large social groups. We therefore assessed the utility, i.e. participation, reliability and time investment, of voluntary weighing in captive group-housed rhesus macaques. Although individuals were not willing to step onto an unbaited scale, 63 out of 92 individuals (68%) were weighed voluntarily when likeable food items were applied to the scale. When one group was tested again after three months, the same individuals (and two additional ones) still stepped onto the scale within a much shorter time-period than before. Furthermore, voluntary weighing resulted in reliable body weight measurements.

Participation in voluntary weighing

The overall level of participation in voluntary weighing was 68%. Previous studies found participation rates between 21 and 71% in free-ranging rhesus macaques (seven groups; Zhang et al. 2016), while body weights of more than 90% of the individuals were obtained in free-ranging Japanese macaques (one troop; Mori 1979). Approximately 85% of immature yellow baboons in the wild were measured after presenting the scale two or three times a week for almost a year (two groups; Altmann & Alberts 1987). Although participation was somewhat lower in our study on captive rhesus macaques, the wild studies took a much longer period for weighing individuals, namely several weeks to months. Participation rates may thus be higher when extending the weighing period. Another way to increase participation rates may be to use more scales simultaneously, which provides more opportunities to step onto a scale.

To the authors' knowledge, there have been no studies reporting on the participation and success rate of training macaques in large social groups. Station- and target-training are very successful (87–97%) when training macaques in pairs and small groups (2–9 individuals per group; Schapiro et al. 2003; Fernström et al. 2009; Kemp et al. 2017). In our breeding colony, which houses macaques in large naturalistic groups, participation in oral syringe training was, on average, 47% (range 24–66%) per session. Although 87% of the individuals participated at least once, only 65% (range 30–95%) were trained successfully (n = 14 groups, 9–27 individuals per group; unpublished data). Besides, training success depends very much on dominance rank as high-ranking individuals often monopolise training (Schapiro et al. 2003), biasing the sample. In contrast, we found that voluntary weighing occurred independently of dominance rank. Therefore, voluntary weighing may yield data on a similar number of individuals as scale training, but likely measures partly different individuals.

Predicting participation

Individual characteristics, such as age, dominance rank and exploratory tendency, may determine who steps onto the scale and so it may be possible to predict which individuals are more likely to participate in voluntary weighing. An age effect with younger individuals being more likely to step onto the scale was expected, but not found. Although the highest stepping rates were indeed measured for young individuals, there were several juveniles that did not step onto the scale at all. As a result, individuals of different ages were equally likely to participate in voluntary weighing. This contrasts with studies indicating that younger individuals are usually more exploratory and attracted towards novelty (rhesus macaques: Insel et al. 2008; vervet monkeys [*Cercopithecus aethiops*]: McGuire et al. 1994).

Since there was only one scale in the enclosure during the sessions and high-ranking individuals usually have priority of access to restricted resources (Boccia et al. 1988; Brennan & Anderson 1988), high-ranking individuals were expected to monopolise the scale. However, no association between dominance rank and stepping onto the scale was found. This is in contrast with wild toque macaques, where low-ranking individuals were under-represented at the baited weighing scale as a result of competition by high-ranking individuals (Dittus 1998). Initially, the novelty of the scale may have decreased competition, thereby providing an opportunity for low-ranking individuals to step onto the scale. In addition, the relatively long time-period the scale was available — namely at least 90 min per session — may have reduced monopolisation of the baited scale.

Exploratory tendency was measured by exposing individuals to a novel feeding method and, as expected, more exploratory individuals were more likely to step onto the scale. When individuals have similar behavioural responses in different contexts, personality traits may be involved, since personality is defined as individual differences in behaviour that are consistent over time and across situations (Réale et al. 2007; Freeman & Gosling 2010). The reaction towards the novel feeding method and stepping onto the scale both seem to reflect an individual's exploratory tendency and may therefore be considered a personality trait. Thus, exploratory tendency measured by novel object tests can be used to predict which individuals are more likely to participate in voluntary weighing.

Finally, the likelihood to step onto the scale differed per group. Participation was significantly lower in the Grey-group and higher in the Marieke-group compared to the other groups. The efficiency of voluntary weighing thus differs per group. Inter-group variation in the ratio of measured individuals was also apparent in free-ranging rhesus macaques (Zhang et al. 2016).

Reliability of voluntary weighing

The level of agreement between voluntary (VBW) and sedated body weight (SBW) was high, indicating voluntary weighing results in reliable body weights. This complies with previous studies that also tested the reliability of voluntary weighing (Vézina et al. 2001; Bassano et al. 2003). VBW was, on average, 0.44 kg higher compared to SBW, but there were considerable differences in body weight deviation between the four groups. Body weight deviation was higher in the Chloor- and Marieke-group compared to the Bertha- and Grey-group. These findings can be explained by two limitations in the study design, both of which are related to the timing of the measurements.

First, the time of day may have confounded the data, as individuals were heavier in the afternoon compared to the morning. Some individuals even increased VBW by 0.4 kg, which amounts to 3–4% of their body weight, from morning to afternoon. Body weight of immature yellow baboons was 6% higher in the evening, after a day of feeding, compared to the morning (Altmann & Alberts 1987). In free-ranging Japanese macaques, a maximum daily increase in body weight of 0.3 kg was found, which corresponds to a relative increase of 3–4% (Kurita 1999). This study concluded that the increase in body weight resulted from food intake during the day and this should be considered when collecting body weight data (Kurita 1999). As SBW was always measured in the morning when the animals were fasted, whereas VBW was measured during the day while the monkeys had access to food, this may have confounded the data. Since we were unaware of this confounding effect at the beginning of the study, most sessions in the Chloor-group were performed in the afternoon, which provides a partial explanation for the relatively large body weight deviation in this group.

Secondly, the difference between SBW and VBW may be due to natural body growth. SBW was measured two to three months prior to the scale sessions in the Chloor- and Marieke-group. There was thus a considerable time-period between the two measurements in these groups. At six years of age, female rhesus macaques reach skeletal maturation and body weight increase flattens (Schwartz & Kemnitz 1992). Since many individuals were yet to reach skeletal maturation, they were still growing, and their body weight still increased. Accordingly, VBW was higher than SBW in the Chloor- and Marieke-group, especially in the younger individuals, due to the time-period between the measurements. In contrast, VBW and SBW were collected around the same time in the Bertha- and Grey-group, so growth of juveniles was not a problem there. Thus, higher VBW relative to SBW does not represent false body weights, but most likely reflects individuals' real body weights that have increased as a result of food intake and/or natural body growth over time.

Time investment in voluntary weighing

The current study took a considerable amount of time. Setting up the scale and cameras, baiting the scale, observing and coding the videos (if necessary) took approximately 100 h in total. On average, 1 h and 35 min of time investment was thus necessary to weigh one individual. Time investment in voluntary weighing can be reduced by incorporating automated systems for identification and weighing in the home enclosure of NHPs, which are already available for livestock and birds (Turner et al. 1984; Peiper et al. 1993; Vézina et al. 2001). These automated systems enable regular weighing of animals without human intervention. This may have the additional advantage of increasing participation in voluntary weighing, as human presence can impact animal behaviour, even when animals are properly habituated (cf. Caine 1990). We therefore conclude that optimising and further developing the method will greatly enhance the utility of voluntary weighing as a means of monitoring health and welfare of captive, group-housed NHPs.

Recommendations for practical use

Based on the results of this study, we have several recommendations for the use of voluntary weighing in other institutions and/or other species of NHPs. Firstly, make sure that the monkeys cannot break the scale or injure themselves when exploring it. Use a 'monkey-proof' scale (i.e. no wires, screws, buttons, etc.) and make sure that the monkeys cannot move it or lift it (either by weighing down the scale or fixing it to a surface). Second, apply small amounts of likeable, preferably sticky, food items to the scale and its immediate surroundings to attract individuals. Note that food preferences can differ per group and species. Third, perform voluntary weighing in the morning when individuals are yet to eat. This limits the effect of individual variation in food intake on body weight.

Animal welfare implications

Voluntary weighing is an animal welfare-friendly method of obtaining regular body weight measurements from NHPs. Regular body weight measurements allow the quick detection of body weight changes, which may signal underlying welfare issues. Voluntary weighing does not require animals to be caught and/or sedated, which minimises stress and prevents side-effects from anaesthesia. Furthermore, the animals are free to choose to participate or not.

Conclusion

Voluntary weighing is a reliable method of weighing captive socially housed NHPs. This study shows that the majority of individuals (68%) step onto the scale, but voluntary weighing is still relatively time-consuming, and efficiency of the method varies per group. When optimising and further developing the method, voluntary weighing can form a valuable tool in the captive management of NHPs.

Appendix

Table A1. Ethogram of submissive behaviour (Utrecht University 2019).

Behaviour	Description
Bared teeth (BT)	Individual lifts its eyebrows and scalp, while the ears are positioned against the head. Individual pulls its lips up, displaying teeth and gum.
Make room (MR)	Individual makes a small avoiding movement away from another individual that approaches. The individual does not move over a large distance and may only move the upper body.
Give ground (GG)	Individual creates a distance between itself and another individual that approaches, by moving away at moderate speed.
Escape (ES)	Individual flees away from the interaction partner at high speed.

Chapter 4

Effect of housing conditions on cortisol and body fat levels in female rhesus macaques

Dian G. M. Zijlmans, Lisette Meijer, Marit K. Vernes, Jacqueline A. M. Wubben, Linda Hofman, Annet L. Louwerse, Elisabeth H. M. Sterck, Jan A. M. Langermans, Marieke A. Stammes

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Abstract

Macaques are among the most commonly used non-human primates in biomedical research. They are highly social animals, yet biomedical studies often require group-living animals to be pair-housed in a controlled environment. A change in environment causes only short-term stress in adapting individuals, while non-adapting animals may experience long-term stress that can adversely affect study results. Individuals likely differ in their ability to adapt depending on individual characteristics. Changes in cortisol and body fat levels may reflect these different individual responses. Here, we investigate the long-term effect of a change from group- to pair-housing on cortisol and body fat levels in 32 female rhesus macaques, exploring whether age, dominance rank, original cortisol, and body fat levels are related to long-term stress in pair-housing. Hair samples were analyzed for cortisol levels, while anthropometric measurements and computed tomography were performed to quantify body fat. Monkeys served as their own control with a 7.5-month period between the measurements. Cortisol levels increased, while average body fat levels did not differ when individuals were moved from group- to pair-housing. Cortisol and body fat levels were not significantly correlated. Changes in cortisol were independent of age and dominance rank, whereas individual variation in body fat alterations was related to the group-housed body fat level and dominance rank. Although this study did not identify individual characteristics related to long-term stress in pair-housing, the individual variation confirms that some individuals are more resilient to change than others and provides possibilities for future refinement studies.

Introduction

Macaques are among the most commonly used non-human primates (NHPs) in biomedical research due to their high level of similarity to humans (Pieters 2007; Estes et al. 2018). The regulations around the use of NHPs as animal models are strict. All studies need to comply with the principles of the 3Rs: replacement, reduction, and refinement (Tannenbaum et al. 2015), and welfare of the animals must be ensured as well as possible. Since the expression of natural behaviour is a commonly used indicator of animal welfare, housing conditions should provide possibilities to perform species-specific natural behaviour (Olsson & Westlund 2007; Cassidy et al. 2020). Macaques are highly social animals and have the behavioural need to engage in complex social interactions. Furthermore, the physical environment has to meet certain requirements concerning cage size, cage furniture, and enrichment (National Research Council 1998; McCann et al. 2007; Jennings et al. 2009; Cassidy et al. 2020). Large enclosures with natural substrate and environmental enrichment lead to more natural and less stereotypic behaviour (Clarke et al. 1982; O’Neill et al. 1991; Novak et al 1994; Ross et al. 2011). Optimal housing conditions for macaques thus consist of naturalistic group-housing in large enclosures that mimic their natural habitat. However, these conditions are generally not feasible in biomedical research, since this requires a more controlled environment (Snowdon 1994; Schapiro et al. 2012; Hannibal et al. 2017). As a result, NHPs in biomedical studies are usually pair-housed in smaller cages in an indoor facility (Hau & Schapiro 2007; Hannibal et al. 2017).

When animals are selected for experiments, they usually move from their larger groups to pair-housing in this controlled environment. The effect of relocations on primate physiology and behaviour have been extensively studied. Relocations have been reported to cause stress, i.e. they lead to alterations in body weight, a suppressed immune response, increased cortisol levels, increased heartrate, and behavioural changes (Clarke et al. 1988, 1994; Cohen et al. 1992; Gust et al. 1994; Fernström et al. 2008; Schapiro et al. 2012). These changes are often temporary, consistent with short-term stress, which implies that the individuals are able to adapt to their new situation (Cheney & Seyfarth 2009). Unfortunately, some animals remain stressed for an extended period and experience long-term stress, as their physiological parameters and behaviour do not return to baseline levels (Honess et al. 2004; Davenport et al. 2008; Schapiro et al. 2012). This long-term stress indicates that these animals have difficulty to adapt to their new environment and, subsequently, this may adversely affect study results. These differences between individuals in their ability to adapt suggest that some individuals are more resilient than others, and this may depend on individual characteristics, such as age and dominance rank (Cohen et al. 1997; Bethea et al. 2008; Cheney & Seyfarth 2009; Goncharova & Oganyan 2018).

Measuring the biomarkers mentioned above for stress can be divided as invasive and non-invasive. Invasive measurements, which require blood sampling or telemetry, will likely have its impact on stress too. Non-invasive biomarkers include hair cortisol concentrations (HCCs) and body weight, which is monitored anyway to follow course of growth, development, and basic health. Long-term stress can be reliably measured in HCCs, as stressful events can be found back in hair samples for at least 14 weeks (Davenport et al. 2006). During stress, the hypothalamic-pituitary-adrenal axis produces glucocorticoids such as cortisol. These have been associated with a suppression of the growth hormone system to metabolize fat (Shively et al. 2009; Bauer et al. 2011). Stress-induced changes in fat metabolism may increase body fat, especially in the abdominal region (Jayo et al. 1993; Rhyu et al. 2010). Evidence for increased body fat due to stress was found in several studies: social stress from subordination resulted in a high abdominal fat deposition in female long-tailed macaques (*Macaca fascicularis*; Shively et al. 2009). Similarly, the incidence of obesity increased when group composition was altered, inducing social stress, in male vervet monkeys (*Cercopithecus aethiops*; Crumley & Raleigh 1995; Bauer et al. 2011). Thus, changes in HCCs and body fat levels may both serve as biomarkers for long-term stress and signal individual differences in adaptive ability.

The current study aims to identify individual characteristics associated with long-term stress resulting from change in housing conditions when individuals enter an experiment, thereby providing possibilities to refine selection procedures. This contributes to optimizing animal welfare of NHPs in biomedical studies, thereby increasing scientific validity of experimental results (Hannibal et al. 2017). We therefore investigate the long-term effect of a change from group- to pair-housing on HCCs and body fat levels of captive female rhesus macaques (*Macaca mulatta*). As this study is based on an opportunistic data collection from another study, solely females are incorporated, and we only investigated the change in housing conditions from group- to pair-housing. Individuals served as their own control as they moved from group-housing encompassing indoor and outdoor enclosures to pair-housing in smaller indoor enclosures with a 7.5-month adaptation period. Hair samples were analyzed for HCCs, while anthropometric measurements and computed tomography (CT) were performed to quantify body fat. HCCs and body fat levels are expected to be higher in pair-housing compared to group-housing; and to be correlated.

Materials and methods

Animals and ethics

For this study, 32 female rhesus macaques housed at the Biomedical Primate Research Centre (BPRC) in Rijswijk, the Netherlands, were examined. BPRC is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC International). All procedures performed in this study, as well as housing and husbandry, were in accordance with the European Directive 2010/63 and Dutch law. Ethical approval for this separate study was not required as the data were obtained as part of a tuberculosis study (animal license AVD5020020172645). As a result, animals were not randomly selected, as they had to fulfill several study criteria, including age, sex, and body composition. Animals were aged between 4 and 9 years old (mean = 6.3, SE = 0.2) and weighed between 5.4 and 10.1 kg (mean = 7.7, SE = 0.2) at the start of the study. An exclusion criterion was applied on maximum body weight for the animals to fit in the PET–CT. The tuberculosis study consisted of an adaptation and vaccination phase of 7.5 months after which the infectious challenge took place. Our study was performed during this initial 7.5-month period.

Housing and husbandry

The initial housing conditions for 27 females consisted of group-housing, while five females had already been pair-housed for a longer period (at least nine months). This last group of animals was also relocated from one building to another but remained pair-housed in a similar enclosure. They served as a control group to test whether the changes in HCCs and body fat levels were related to the housing condition and not due to the relocation itself. In both housing conditions, the same light/dark cycles were applied with a 12/12 light/dark cycle from 7 to 19 h. Both conditions also had access to natural light, by the outside enclosure, or by the windows in the stable.

Group-housing

Group-housed individuals came from nine different groups, which were formed by adhering to natural group dynamics. Each group consisted of 15–40 individuals from several multigenerational matrilines and had free 24 h access to enriched indoor (72 m^2 and 2.85 m high) and outdoor (250 m^2 and 3.1 m high) enclosures. Both indoor and outdoor enclosures contained several compartments and visual barriers. The inside enclosure contained sawdust bedding, while the outside enclosure had a sand bedding with natural plant growth. The enclosures were equipped with several climbing structures, beams, perches, fire hoses, car tires, slides, and a swimming pool to stimulate natural behaviour (Vernes & Louwerse 2010). Drinking water was available *ad libitum* via automatic water dispensers. The monkeys were fed monkey chow (Ssniff, Soest, Germany) daily in the morning, complemented with fresh fruit, vegetables, or a grain mixture in the afternoon.

Pair-housing

After moving to the experimental facility, all individuals were pair-housed in four inside rooms with six enclosures per room. Most individuals of a pair were maternally related to each other (sisters, cousins, or nieces). One pair was not related but came from the same breeding group. Two pairs were formed by introducing unfamiliar females to each other: one formerly group-housed female was coupled to a formerly pair-housed female and two other formerly pair-housed females also formed a new pair. Ten pairs had access to a single enclosure, while six pairs had access to a double enclosure. A single enclosure is 2 m high with a surface of 2 m^2. The enclosures were split in three height levels and contained visual barriers, perches, and fire hoses. Water was available *ad libitum* and monkeys were fed monkey chow daily around noon. Fresh fruit was provided in the morning and vegetables in the afternoon. Food enrichment was offered daily and additional non-food enrichment (toys) varied weekly according to a rotation schedule.

Relocation

All females were relocated from their initial housing condition to pair-housing on two consecutive days. On the day of the relocation, animals were anesthetized with an intramuscular injection of ketamine (ketamine hydrochloride, Ketamine 10%; Alfasan, Woerden, the Netherlands, 100 mg/mL, 10 mg/kg) in combination with the $\alpha 2$-adrenoceptor agonist medetomidine (medetomidine hydrochloride, Sedastart; AST Farma, Oudewater, the Netherlands, 1 mg/mL, 0.05 mg/kg), which was reversed afterwards with atipamezole (atipamezole hydrochloride, Sedastop; AST Farma, Oudewater, the Netherlands, 5 mg/mL, 0.25 mg/kg). Hair was shaved from the posterior vertex region of the neck to analyze hair cortisol concentrations (HCCs). Anthropometric measurements and computed tomographies (CTs) of the abdominal area were performed to quantify body fat. These three procedures were repeated in exactly the same manner after 7.5 months in pair-housing.

Procedures

Hair cortisol analysis

Hair was shaved from the posterior vertex region of the neck, packed in aluminum foil, and stored in a freezer at −20 °C. Hair samples were collected in the three months preceding relocation and at the day of the relocation, representing HCCs in group-housing. Hair samples were collected again during a required management procedure after roughly six months in pair-housing and after 7.5 months in pair-housing. This resulted in two hair samples per housing condition for each animal.

HCCs were measured from the samples as described by Davenport et al. 2006. In short, the hair was washed with isopropanol twice and allowed to dry for five days before being ground to powder in a beadbeater. Approximately 50 mg of the powder was incubated for 24 h in 1 mL methanol to extract the cortisol, followed by centrifugation. The supernatant was transferred into another tube and left to dry on a heating block for approximately 5.5 h. The dried cortisol extract was dissolved in 400 µL phosphate buffer and analyzed with an enzyme immunoassay kit (Salimetrics, State College, PA, USA) according to the manufacturer's instructions. HCCs were corrected for powder weight by calculating the pg cortisol/mg hair. All samples were analyzed twice with an average coefficient of variation of 3.8%.

Since HCCs in the two samples of the same housing condition were highly correlated (group-housing: Spearman correlation, $r = 0.825$, $n = 31$, $p < 0.0005$; pair-housing: Spearman correlation, $r = 0.809$, $n = 32$, $p < 0.0005$), the average value for each housing condition was used in further analyses.

Anthropometric measurements

Anthropometric measurements were performed following a standard procedure (Sterck et al. 2019). Briefly, body weight was measured with a standard scale, crown-rump length (height) was measured with a measuring mat for human infants (Seca, Hamburg, Germany), and abdominal circumference was measured with a measuring tape. A Baseline Pro skinfold caliper was used to measure abdominal skinfold thicknesses at the height of the umbilicus. All measurements were performed three times and always by the same person (DGMZ). The average values of the three measurements were used in further analyses. A species-specific weight-for-height index for rhesus macaques, known as WHI3.0, was calculated as body weight (kg) divided by the third power of height (m) (Sterck et al. 2019). We will refer to this as WHI.

CTs

To obtain CTs, the monkeys were positioned head first supine in a LFER 150 PET-CT (Mediso, Budapest, Hungary). A single cone beam scan of 360 projections was performed at the lumbar vertebra level at 80 kVp, 980 mA, and a scan time of 50 ms for each projection. For analysis, only the area between the third (L3) and the fifth (L5) lumbar vertebra was included. Three observers analyzed the scans together in

VivoQuant 4.5rc4 (InviCRO, Boston, MA, USA). Fat was measured with a density range, because fat is a solid though flexible type of tissue, the shape and density of which are defined by the surrounding tissue.

Since abdominal fat deposition was found to have a significant impact on the well-being and more important the risk of developing diseases, we aimed to investigate both the subcutaneous fat and the abdominal fat found around the organs in the abdominal area (Bauer et al. 2011). This cannot be captured in a single value and therefore different Hounsfield unit (HU) ranges were used for abdominal (−170 HU to −90/−60/−30/0 HU) and subcutaneous fat tissue (−170 HU to 0/30/60/90/120 HU). One HU range for each fat tissue type did not suffice, as there was individual variation in the density of fat tissue between monkeys with more or less fat. The HU range included as much fat tissue as possible without covering any other tissue, such as muscles, intestines, or kidneys.

Next, abdominal fat tissue, subcutaneous fat tissue, and the body volume of the monkey were defined for each scan in mm^3 (Figure 1). Abdominal and subcutaneous body fat percentages were calculated by dividing the amount of fat tissue by the body volume. When the monkeys had too little body fat to differentiate on CT, body fat percentage was set at 0% (Dittus 2013). Total body fat percentage (TBF) was measured as the sum of abdominal and subcutaneous body fat percentages. All scans were randomized and re-analyzed by two of the observers separately three months later (LM, MAS). The level of agreement between the ratings was moderate ($\kappa = 0.555$) for abdominal body fat, while there was substantial agreement ($\kappa = 0.666$) for subcutaneous body fat (Landis & Koch 1977). The median value of the assessments was used for further analyses.

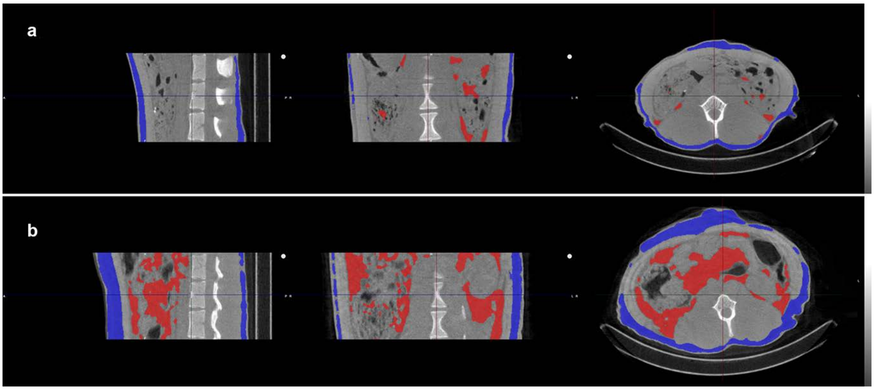

Figure 1. Representative CT sections of two female rhesus macaques with regions of interest defining the abdominal body fat (red) and subcutaneous body fat (blue). The three panels represent one animal visualized in sagittal, coronal, and transversal direction in which the center of the cross-hairs represents the same point in the three directions. The respective macaques had body weights of 6.45 kg (a) and 7.43 kg (b). (**a**) A lean individual with a WHI of 53.5 kg/m^3 and a TBF of 10.1%, while (**b**) represents an individual with a relatively high amount of body fat (WHI = 62.6 kg/m^3, TBF = 32.7%).

Dominance rank

Experienced ethologists and colony managers regularly recorded dominance rank in the colony and were able to categorize the females as being low-ranking (n = 16) or high-ranking (n = 11) in their social group before relocation. Dominance rank was evaluated again when the females were relocated and pair-housed as being low-ranking (n = 13) or high-ranking (n = 14) based on video analyses of agonistic behaviour between the individuals of a pair. This approach resulted in 86% agreement with the subjective evaluation from the animal trainer (MKV).

Data analyses

Data analyses were performed in IBM SPSS Statistics v26. Anthropometric measures and body fat percentages from the CTs were highly correlated under both housing conditions ($r > 0.784$, $n = 64$, $p < 0.0005$; Table A1; Figure A1) and overall provided similar results (Table A2). We therefore only report statistical outcomes for WHI and TBF. HCCs and body fat levels were compared between group- and pair-housing with a paired samples t-test or Wilcoxon signed ranks test, depending on whether the data were normally distributed or not. Normal distribution of the data was visually checked using boxplots and histograms, as well as tested with the Shapiro-Wilk test. Pearson or Spearman correlations were used to test the relationship between HCCs and body fat levels. The delta values for HCC, WHI, and TBF were calculated by subtracting the values in pair-housing from group-housing (the baseline value).

Multiple linear regression analyses were used to check which individual factors, i.e. age and dominance rank, affected HCC and body fat levels of females that moved from group- to pair-housing in both housing conditions and also the changes in HCC and body fat. Normality and homoscedasticity of the residuals were visually checked for each model. These assumptions were violated in the models for pair-housed HCC and changes in HCC when including all individuals. The data contained one major outlier (pair-housed HCC = 150.8 pg/mg, delta HCC = 130.3 pg/mg), which was the only group-housed individual that was coupled to an unfamiliar female in pair-housing. We considered this a valid reason to exclude this individual from the cortisol analyses. After this individual was excluded, the assumptions of normality and homoscedasticity of the residuals were fulfilled. There was no collinearity between the variables in the models as the variance inflation factor was equal to or lower than 1.26 for all variables.

Initial housing condition could not be included in the models on the delta values due to collinearity. To check whether changes in HCC and body fat were related to the housing condition and not the relocation, an independent samples t-test or a Mann-Whitney U-test was performed on the delta values. Other potential confounding factors, i.e. number of sedations, number of reported injuries, exposure to humans, cage size (double or single enclosure), and relatedness between the individuals of a pair, were tested but did not significantly influence the outcome measures (Table A3). The outcomes were considered statistically significant at $\alpha = 0.05$ and all tests were two-tailed.

Results

Group-housing

HCCs in group-housing were on average 24.0 pg/mg with little variation between individuals (SE: 1.2, range: 12.0–34.3 pg/mg). HCCs were independent of age (F(1,24) = 1.336, p = 0.259) and dominance rank (F(1,24) = 1.254, p = 0.274). TBF of group-housed individuals was on average 21.1% and there was considerable variation between individuals (SE: 2.8, range: 0–45.3%; Table A4). TBF and WHI were independent of dominance rank (F(1,24) = 0.041, p = 0.841; F(1,24) = 0.162, p = 0.691). Age had no significant effect on WHI (F(1,24) = 2.030, p = 0.167), but older individuals did have a higher TBF compared to younger macaques (F(1,24) = 6.145, p = 0.021). There was no significant correlation between HCC and WHI (Pearson correlation, r = −0.253, n = 27, p = 0.203) or between HCC and TBF (Pearson correlation, r = −0.272, n = 27, p = 0.171; Figure 2a) in group-housing.

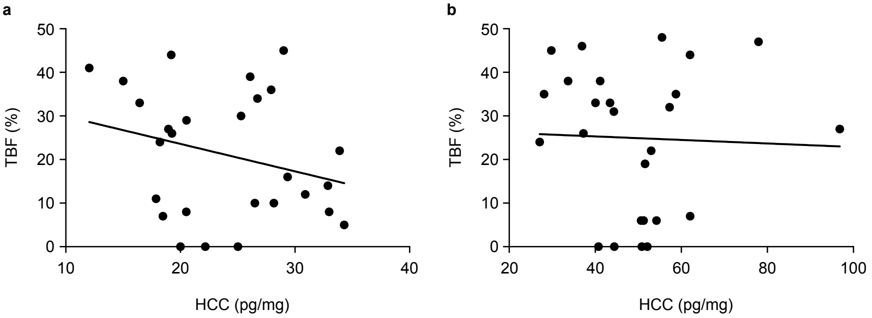

Figure 2. Total body fat percentage (TBF) in the abdominal area plotted against the hair cortisol concentration (HCC) of female rhesus macaques in group-housing (**a**) and pair-housing (**b**).

Pair-housing

Pair-housed individuals had average HCCs of 49.3 pg/mg and variation between individuals was higher compared to the variation in group-housing (SE: 3.0, range: 27.1–96.8 pg/mg). Dominance rank (F(1,23) = 0.961, p = 0.337) and age (F(1,23) = 0.000, p = 0.996) had no significant effect on HCCs in pair-housing. TBF was on average 24.8% and the variation between individuals was roughly similar compared to group-housing (SE: 3.1, range 0–48.3%; Table A4). TBF and WHI were independent of age (F(1,24) = 2.441, p = 0.131; F(1,24) = 1.493, p = 0.234) and dominance rank (F(1,24) = 0.000, p = 0.985; F(1,24) = 0.097, p = 0.758) in pair-housing. There was no significant correlation between HCC and WHI (Spearman correlation, r = −0.210, n = 26, p = 0.304) or between HCC and TBF (Spearman correlation, r = −0.053, n = 26, p = 0.798; Figure 2b) in pair-housing.

Comparison between housing conditions

HCCs increased for all individuals, except one when individuals were moved from group- to pair-housing (Figure 3a). HCCs significantly increased with an average of 112% (SE: 12, range: −4–247%; Wilcoxon signed ranks test, Z = −4.432, n = 26, $p < 0.0005$). Delta HCC was highly variable (SE: 2.7, range: −1.08–68.9 pg/mg) and independent of age (F(1,21) = 0.389, p = 0.540), group-housed HCC (F(1,21) = 0.378, p = 0.545), group-housed dominance rank (F(1,21) = 2.669, p = 0.117), and pair-housed dominance rank (F(1,21) = 1.826, p = 0.191). Delta HCC was not significantly related to delta WHI (Pearson correlation, r = −0.083, n = 26, p = 0.688) or delta TBF (Pearson correlation, r = −0.046, n = 26, p = 0.824). Delta HCC significantly differed between formerly group-housed (25.1 ± 2.7 pg/mg) and formerly pair-housed females (−5.7 ± 7.7 pg/mg; independent samples t-test, t = 4.400, n = 31, $p < 0.0005$), which confirms that the increase in HCC resulted from changing from group- to pair-housing and not from the relocation.

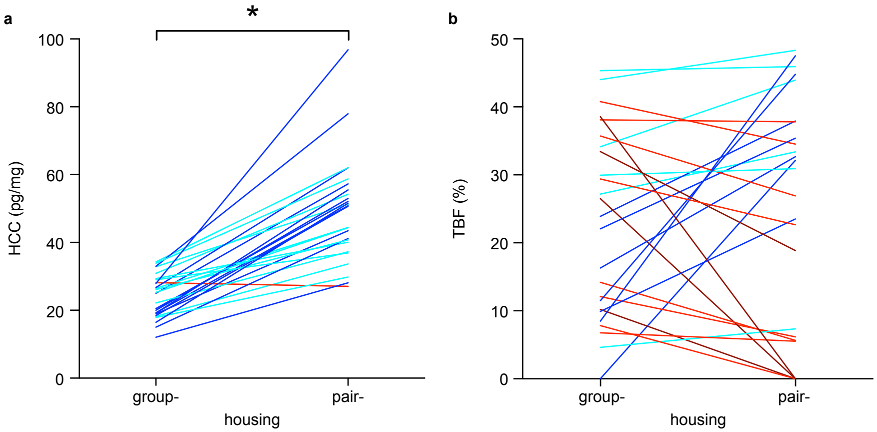

Figure 3. The effect of group- and pair-housing on hair cortisol concentrations (HCC; (**a**)) and total body fat percentage (TBF) in the abdominal region (**b**) of female rhesus macaques. Each line represents an individual. Dark blue lines indicate large relative increases, while light blue lines represent small relative increases. Similarly, dark red lines represent large decreases and normal red lines represent relatively small decreases. * $p < 0.05$.

WHI and TBF did not significantly change when individuals moved from group- to pair-housing (paired samples t-test, t = 1.490, n = 27, p = 0.148; Wilcoxon signed ranks test, Z = −0.937, n = 27, p = 0.349). However, there was large individual variation in body fat alterations (Table A2; Figure 3b). Eight individuals lost more than 10% of their original body weight, while five individuals gained more than 10% of their original body weight. WHI increased in 13 individuals, while TBF increased in 15 individuals. For TBF, the average increase was 15.6% (SE: 3.2, range 0.6–39.0%) and the average decrease was 11.3% (SE: 3.1, range 0.3–38.5%).

Delta WHI was independent of age (F(1,22) = 0.367, p = 0.551) and pair-housed dominance rank (F(1,22) = 0.440, p = 0.514). WHI decreased in individuals with a high group-housed WHI, while WHI generally increased in individuals with a low group-housed WHI (F(1,22) = 4.487, p = 0.046). There was also a trend for individuals with a high rank in their social group to decrease WHI in pair-housing, while low-ranking individuals increased their WHI after the relocation (F(1,22) = 3.409, p = 0.078). A similar pattern was found for TBF. Neither age (F(1,22) = 0.955, p = 0.339) nor pair-housed dominance rank (F(1,22) = 0.010, p = 0.923) influenced alterations in TBF. TBF increased in individuals with low group-housed TBF (F(1,22) = 9.373, p = 0.006; Figure 4a) and dominance rank (F(1,22) = 4.282, p = 0.050; Figure 4b), while TBF decreased in individuals with high group-housed TBF and dominance rank. Formerly group-housed and formerly pair-housed individuals did not significantly differ in delta WHI (independent samples t-test, t = −1.040, n = 32, p = 0.307) or delta TBF (Mann-Whitney U-test, U = 62, n = 32, p = 0.775).

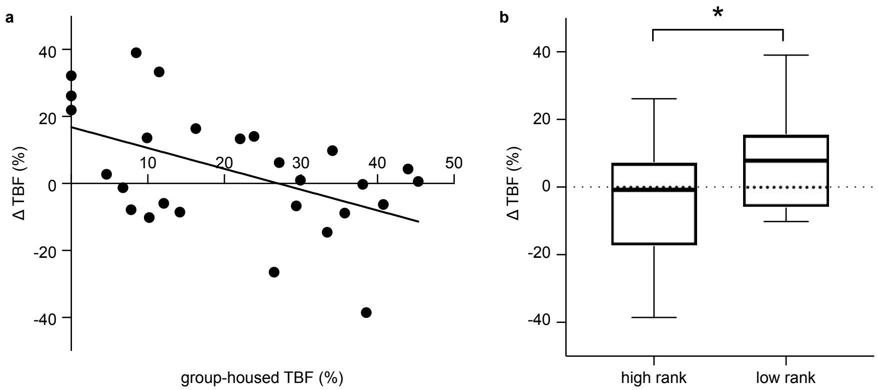

Figure 4. The effect of group-housed total body fat percentage (**a**) and group-housed dominance rank (**b**) on change in total body fat percentage (ΔTBF). * $p < 0.05$.

Discussion

This study investigated the effect of moving female rhesus macaques from group- to pair-housing on hair cortisol concentrations (HCCs) and body fat levels and aimed to identify individual characteristics associated with long-term stress in pair-housing. When individuals moved from group- to pair-housing, HCCs increased for all individuals, except one, while average weight-for-height index (WHI) and total body fat percentage (TBF) did not change. Delta HCC was independent of age, dominance rank, and group-housed HCC, while alterations in body fat levels were related to the group-housed body fat level and dominance rank. However, HCCs and body fat levels were not significantly correlated.

This study used HCCs as a biomarker for long-term stress. Hair sampling is minimally invasive and provides a reliable method for the measurement of long-term cortisol (Davenport et al. 2006; Heimburge et al. 2019). Nevertheless, several points need to be considered when using hair samples, e.g. location on the body, amount of sunlight, repeated sampling, and season (Behringer & Deschner 2017; Heimburge et al. 2019). To minimize the effect of these possible confounders, we standardized the location of the hair sample at the posterior vertex region of the neck and collected all samples at the same time points.

In the current study, HCCs increased from 24.0 pg/mg in group-housing to 49.3 pg/mg in pair-housing. These values should be placed in perspective to other publications. Semi-free ranging adult female rhesus macaques have mean HCCs of 44.0 pg/mg, while individually housed male rhesus macaques have mean HCCs of 110.3 pg/mg (Davenport et al. 2006; Wooddell et al. 2017). HCCs of group-housed male and female rhesus macaques are population density-dependent: young adults (5–9 year) in a low-density population have mean HCCs of 37.7 pg/mg, while HCCs are on average 77.7 pg/mg in the high-density populations (Dettmer et al. 2014). Housing conditions thus have a strong effect on HCCs and HCCs are generally higher in smaller and more crowded environments. Early life social experience in naturalistic groups may have resulted in monkeys being less responsive to stressful environments later in life in our study (Stevens et al. 2009). In addition, HCCs in pair-housing were likely lower as we mostly selected maternally related pairs instead of forming new pairs with unrelated animals. HCCs in this study were thus relatively low compared to the other studies, even in pair-housing. However, due to the fact that HCCs in pair-housing were in general higher, it may not be possible to measure alterations in the same way between group-housed vs. pair-housed animals and the pair-housed control animals.

Still, the changes in HCC were highly variable, indicating that some individuals experienced less long-term stress and were indeed more resilient than others. We expected higher responsiveness to stress in monkeys with higher age, low dominance rank, and lower baseline HCCs. Yet, delta HCC was independent of these factors in our models. This is in contrast with reports that older animals and animals with lower baseline stress levels are generally more responsive to stress (Cheney & Seyfarth 2009; Goncharova & Oganyan 2018). In addition, low-ranking long-tailed macaques are more vulnerable to a viral infection than high-ranking monkeys (Cohen et al. 1997). Since stress is known to suppress the immune function, this suggests that low-ranking animals are less resilient to stress compared to high-ranking individuals, but this was not found in our study. Based on changes in HCCs, we thus did not find individual characteristics associated with long-term stress when individuals enter an experiment.

Although social subordinates often have higher cortisol levels compared to dominants in NHPs (Chamove & Bowman 1978; Sapolsky 1995; Shively 1998; Gust et al. 1993), we found that HCCs were independent of dominance rank in both housing conditions. Control and predictability are commonly mentioned as important factors herein (Abbott et al. 2003; Cheney & Seyfarth 2009). A meta-analysis by Abbott et al. (2003) showed that higher cortisol levels by subordinates are predicted by two factors: high stressor rates and low opportunities for social support (Abbott et al. 2003). The groups included in the present study contain several matrilines, in which females from the same matriline provide social support to each other. Besides, the enclosures in group- and pair-housing contain visual barriers and hiding places, which enables low-ranking females to avoid aggression by dominants, thereby having more control over their social interactions (Cheney & Seyfarth 2009). The lack of a correlation with dominance rank and the relatively low HCCs compared to other studies imply that both our housing conditions (group- and pair-housing) provide sufficient opportunities, at least for females, to cope with potential stressors.

Although HCCs were expected to correlate with body fat levels, this was not found. This contradicts with previous studies suggesting that long-term stress increases fat deposition in the abdominal area. These studies induced social stress by altering group composition, while in our study individuals were mostly paired with familiar individuals in a new environment. Only one formerly group-housed female was paired to an unfamiliar female and she had an extremely high pair-housed HCC. This suggests that a different social environment (i.e. cage mates) may be more stressful than a change in physical environment (i.e. enclosure). This may explain the lack of correlation between HCCs and body fat levels. In addition, the increase in HCC may have been too little or the time span too small to affect body fat levels in our study. Nevertheless, body fat levels did not reflect long-term stress in our study animals. Our results imply that changes in body fat levels may not always be suitable as biomarker for long-term stress. Nevertheless, they are still useful as indicators of health and welfare (Jennings et al. 2009).

To determine body fat levels, both anthropometric measurements and CT analyses were used, a combination of external and internal measurements. Anthropometric measurements are a widely used and accepted method to estimate body fat levels in NHPs (Walker et al. 1984; Altmann et al. 1993; Bodkin et al. 1993; Kemnitz et al. 1993; Colman et al. 1999b; Garcia et al. 2010). The use of CT to determine the percentage of body fat present around the abdomen is less common, though has been used before in both humans and NHPs (Laber-Laird et al. 1991; Seidell et al. 1990; Jayo et al. 1993; Cefalu et al 1997; Wallace et al. 1999; Yoshizumi et al. 1999). The advantage of CT is that it provides information on the regional distribution of body fat to complement and validate the anthropometric measurements (van der Kooy 1993). Although the two approaches yielded highly correlated measures, TBF and WHI produced slightly different results. TBF increased in 15 individuals, while WHI increased in only 13 individuals. However, WHI is based on body weight and height, which compromises more than body fat alone. Since smaller enclosures in pair-housing provide less space for physical activity, individuals may have lost some body weight due to loss of muscle tissue after the relocation. In addition, control over access to food may be different in pair-housing compared to group-housing. An increase in body fat counteracted by a decrease in muscle tissue may therefore result in a smaller increase, or even a decrease, in WHI compared to the TBF measured by CT.

Overall, body fat levels did not differ between housing conditions. This contrasts with the literature, where individually housed long-tailed macaques had higher body fat levels compared to group-housed monkeys (Yue et al. 2017). Yet, there was considerable individual variation in body fat change between females. The change of housing conditions affected body fat alterations in a specific way depending on group-housed body fat level and group-housed dominance rank: lean and low-ranking individuals mainly gained body fat, while heavy and high-ranking individuals generally lost body fat. Monkeys with low group-housed body fat levels and dominance rank may have relatively low food intake in group-housing, while food is more equally divided in pair-housing. As a result, lean and low-ranking individuals may easily gain body fat, while heavy and high-ranking individuals may lose body fat in pair-housing.

Although high dominance rank has been associated with higher body fat and weight in other NHP studies (Small 1981; Whitten 1983), rank had no effect on body fat levels in this study. Our selection criteria may have introduced some bias here, as obese individuals were excluded from the study. Since group-housed dominance rank did affect the changes in body fat levels, group-housed dominance rank may be considered in animal selection when it is important that individuals do not gain/lose body fat. Low-ranking individuals should be preferred when animals should not lose body fat, while high-ranking individuals should be selected when the research requires animals not to gain much body fat. Based on the results of this study, this recommendation can be applied to females; however, it may be different for males.

Conclusion

In conclusion, HCCs significantly increased when female rhesus macaques were moved from group to pair-housing, while average body fat levels did not differ. Changes in HCCs were independent of age, dominance rank, and group-housed HCC. There was large individual variation in body fat alterations, which was related to the group-housed body fat levels and dominance rank. However, there was no significant correlation between HCCs and body fat levels. This study did therefore not find individual characteristics related to long-term stress in pair-housing. However, the individual variation confirms that some individuals are more resilient than others and provides possibilities for future refinement studies.

Appendix

Table A1. Spearman correlations between anthropometric measures and body fat percentages, measured by CT. n = 64, p < 0.0005. $^{\#}$Pearson correlation

	WHI	AC	AST	ABF	SBF	TBF
BW	r=0.857$^{\#}$	r=0.945$^{\#}$	r=0.847$^{\#}$	r=0.859	r=0.838	r=0.894
WHI	X	r=0.911$^{\#}$	r=0.861$^{\#}$	r=0.784	r=0.855	r=0.855
AC	X	X	r=0.905$^{\#}$	r=0.893	r=0.847	r=0.930
ASF	X	X	X	r=0.846	r=0.878	r=0.892
ABF	X	X	X	X	r=0.795	r=0.977
SBF	X	X	X	X	X	r=0.822

Abbreviations: BW = body weight (kg), WHI = weight-for-height index (kg/m^{3}), AC = abdominal circumference (cm), AST = abdominal skinfold thickness (mm), ABF = abdominal body fat (%), SBF = subcutaneous body fat (%), TBF = total body fat (%).

Table A2. Outcome of the regression models for body fat change, as measured by all anthropometric and CT measures. Factors included in the models are age, group-housed dominance rank, pair-housed dominance rank and the baseline value. n = 27. * 0.05 < p < 0.10, ** p < 0.05.

	Age	Group-housed dominance rank	Pair-housed dominance rank	Baseline value
BW	F=0.965, p=0.337	F=1.636, p=0.214	F=0.219, p=0.644	F=10.261, p=0.004**
WHI	F=0.367, p=0.551	F=3.409, p=0.078*	F=0.440, p=0.514	F=4.487, p=0.046**
AC	F=0.535, p=0.472	F=4.048, p=0.057*	F=0.106, p=0.748	F=8.764, p=0.007**
ASF	F=1.296, p=0.267	F=3.526, p=0.074*	F=0.044, p=0.836	F=9.229, p=0.006**
ABF	F=1.697, p=0.206	F=4.594, p=0.043**	F=0.005, p=0.946	F=10.936, p=0.003**
SBF	F=0.019, p=0.892	F=2.433, p=0.133	F=0.144, p=0.708	F=4.033, p=0.057*
TBF	F=0.955, p=0.339	F=4.282, p=0.050*	F=0.010, p=0.923	F=9.373, p=0.006**

Abbreviations: BW = body weight (kg), WHI = weight-for-height index (kg/m^{3}), AC = abdominal circumference (cm), AST = abdominal skinfold thickness (mm), ABF = abdominal body fat (%), SBF = subcutaneous body fat (%), TBF = total body fat (%).

Table A3. Checking the effect of potential confounding factors on the study's major outcome measures with one-way ANOVA (F-value) and independent samples t-tests (t-value).

	Delta WHI (n = 27)	**Delta TBF (n = 27)**	**Delta HCC (n = 26)**
Number of procedures (0/5/8)	F=0.397, p=0.677	F=0.862, p=0.435	F=0.365, p=0.698
Number of injuries (0/1/2/3)	F=0.132, p=0.940	F=0.288, p=0.833	F=1.801, p=0.176
Exposure to humans (high vs. low)	t=0.570, p=0.575	t=0.365, p=0.719	t=-0.122, p=0.912
Cage size (single vs. double)	t=0.547, p=0.590	t=0.484, p=0.633	t=1.867, p=0.074
Related pair (yes/no)	t=-0.181, p=0.858	t=-0.369, p=0.715	t=-0.950, p=0.351
Trainability (high vs. low)	t=-0.801, p=0.431	t=-1.988, p=0.058	t=0.573, p=0.572

Table A4. Descriptive statistics on body fat measures in group-housing, pair-housing and body fat change. n = 27. Mean ± SE (minimum–maximum) are reported.

Measure	**Group-housing**	**Pair-housing**	**Change**
BW	7.9 ± 0.2 (5.9–10.1)	7.7 ± 0.2 (5.6–9.6)	-0.2 ± 0.2 (-2.5–1.7)
WHI	58.3 ± 1.4 (43.9–74.7)	56.3 ± 1.6 (41.1–71.7)	-2.0 ± 1.4 (-18.1–10.1)
AC	35.3 ± 1.0 (26.2–43.7)	35.0 ± 1.0 (26.9–44.4)	-0.3 ± 1.0 (-11.2–8.9)
ASF	9.9 ± 0.8 (3.0–17.9)	9.7 ± 1.0 (1.9–20.3)	-0.2 ± 1.0 (-12.7–9.5)
ABF	11.9 ± 1.9 (0–32.2)	14.8 ± 2.2 (0–40.5)	3.0 ± 2.4 (-26.6–35.1)
SBF	9.3 ± 1.1 (0–17.1)	10.0 ± 1.2 (0–20.7)	0.7 ± 1.2 (-13.9–11.5)
TBF	21.1 ± 2.8 (0–45.3)	24.8 ± 3.1 (0–48.3)	3.7 ± 3.4 (-38.5–39.0)

Abbreviations: BW = body weight (kg), WHI = weight-for-height index (kg/m^3), AC = abdominal circumference (cm), AST = abdominal skinfold thickness (mm), ABF = abdominal body fat (%), SBF = subcutaneous body fat (%), TBF = total body fat (%).

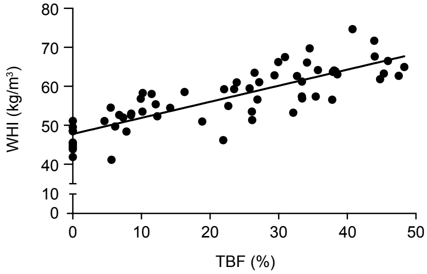

Figure A1. Weight–for–height index (WHI) plotted against total body fat percentage (TBF) in the abdominal region of female rhesus macaques in group– and pair–housing. n = 64.

Chapter 5

Does behaviour predict weight gain during adulthood in captive group-living rhesus macaques?

**Dian G. M. Zijlmans, Lisette M. van den Berg,
Jan A. M. Langermans, Elisabeth H. M. Sterck**

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Abstract

Many non-human primates (NHPs) in captivity become overweight. Individual variation in adiposity may result from differences in behaviour related to energy intake, energy expenditure and dominance rank. Understanding how behaviour contributes to becoming overweight may provide opportunities to improve housing and husbandry of captive group-living NHPs. This study therefore investigated whether behaviour predicts weight gain during adulthood in captive female rhesus macaques (*Macaca mulatta*) housed in three social groups at the Biomedical Primate Research Centre in Rijswijk, the Netherlands. Relative adiposity was quantified with a species-specific weight-for-height index (WHI) during three consecutive annual health checks. Behavioural data were collected at baseline on food intake, activity budgets and dominance rank. We examined which factors were associated with WHI at baseline and whether behaviour predicted the subsequent change in WHI over time, while controlling for individual characteristics. At baseline, a high WHI was associated with low activity levels, but not related to food intake, age or dominance rank. WHI did not differ from baseline after one year but had significantly increased after the second year. This increase in WHI was independent of food intake and activity budgets, while higher-ranking females had a higher increase in WHI compared to lower-ranking monkeys. This suggests that captive NHPs with a high dominance rank are more prone to becoming overweight, whereas differences in activity budgets are merely a consequence and not a cause. Thus, dominance rank, but not behaviour, likely predicts weight gain during adulthood in captive group-living NHPs.

Introduction

Non-human primates (NHPs) in captivity are susceptible to becoming overweight, characterized by excessive amounts of body fat resulting from an imbalance between energy intake and expenditure (Kemnitz 1984; Chen et al. 2002). Although overweight NHPs do not naturally occur in the wild (Altmann et al. 1993; Dittus 2013), a considerable proportion of captive NHPs become overweight during their life (West & York 1998; Sterck et al. 2019). The welfare of these animals may be compromised as being overweight is associated with a higher risk of several health problems, such as diabetes and cardiovascular disease (Kemnitz & Francken 1986; Shively & Clarkson 1987). Besides, overweight NHPs may be restricted in performing natural, species-specific behaviour due to their size and condition (Bauer et al. 2011, 2012). The prevalence of overweight individuals in captive group-living NHPs has been reported to range between 10 and 30% (Crumley & Raleigh 1995; Hamada et al. 2003; Yue et al. 2016, 2017; Sterck et al. 2019). This high prevalence in captivity may be caused by differences in energy intake and/or energy expenditure compared to the wild, since captive housing conditions differ from wild conditions in the type and availability of food and the amount of space.

Energy intake and expenditure of wild primates depend on the distribution, seasonality and abundance of food, as well as habitat quality (van Schaik 1986; Dasilva 1992; Murray et al. 2006). In the wild, food availability is seasonally variable and food is typically widespread, while in captivity high-quality and nutritionally balanced diets are easily accessible and often provided *ad libitum* all year round (Bauer et al. 2010). As a result, food may be more abundant and easier to obtain for captive than wild NHPs. In addition, activity budgets generally differ between wild and captive NHPs, with more resting and less foraging and moving in captivity (Melfi & Feistner 2002). Wild NHPs spend up to 60% of their waking hours foraging and feeding and 10–30% resting (Altmann & Muruthi 1988; Kurup & Kumar 1993). In contrast, captive NHPs generally spend less than 20% of their waking hours foraging and feeding and may spend up to 50% resting (Beisner & Isbell 2008; Jaman & Huffman 2008). Captive enclosures are also restricted in space, which may further reduce activity. Thus, captive NHPs are likely more at risk for becoming overweight due to limited motivation for physical activity associated with foraging in an environment where food is abundant (Bauer et al. 2011).

Captive NHPs living in the same group experience the same housing conditions, yet not every individual in a group becomes overweight (Sterck et al. 2019). Some group members become relatively heavy, while others stay lean. Middle-aged NHPs are most likely to be overweight (Schwartz & Kemnitz 1992; Ramsey et al. 2000a). Variation in relative adiposity is likely related to individual differences in energy intake and expenditure. In squirrel monkeys (*Saimiri sciureus*) housed under similar conditions, overweight individuals had significantly higher caloric intake compared to normal weight individuals (Ausman et al. 1981). Furthermore, overweight individuals were more likely to be inactive in group-housed long-tailed (*Macaca fascicularis*) and rhesus macaques (*Macaca mulatta*) (Wolden-Hanson et al. 1993; Bauer et al. 2012). Behaviour related to energy intake and expenditure may potentially play an important role in captive NHPs becoming overweight.

In addition, dominance rank may be related to relative adiposity in captive group-living NHPs. Dominant individuals typically have priority of access and can monopolize preferred resources such as food (Whitten 1983; Boccia et al. 1988; Barton 1993; Overduin-de Vries et al. 2020). High-ranking individuals generally have greater access to food and are interrupted less during feeding (Smuts & Nicolson 1989; Deutsch & Lee 1991; Altmann & Alberts 2005). Dominant individuals were also more likely to be engaged in eating behaviour in group-housed long-tailed macaques (Bauer et al. 2012). In contrast, low-ranking individuals cannot monopolize food. As a result, high-ranking individuals may have a higher body weight and body fat levels compared to low-ranking monkeys (Small 1981; Schwartz et al. 1993; Michopoulos & Wilson 2011).

Identifying risk factors for becoming overweight in captive group-living NHPs may yield opportunities to improve animal husbandry and reduce weight gain during adulthood, thereby increasing animal welfare. This study therefore investigates whether behaviour related to energy intake and expenditure predicts weight gain during adulthood in captive group-living rhesus macaques. Relative adiposity was quantified with a species-specific weight-for-height index (WHI) during three consecutive annual health checks (2015–2017), while behavioural data on food intake, activity budgets and dominance rank were collected at baseline (i.e. 2015). Our study encompasses a two-year period during adulthood, which is a relatively short period in relation to the average lifespan for captive rhesus macaques of approximately 25 years (Chiou et al. 2020). Linear mixed models were used to determine which factors were associated with relative adiposity at baseline, and whether behaviour predicted the change in WHI after one and two years, while controlling for individual characteristics.

Materials and methods

Animals and housing

Subjects of this study were 31 sexually mature (i.e. 3 years and older) non-pregnant female rhesus macaques of Indian origin housed in three social groups at the Biomedical Primate Research Centre (BPRC) in Rijswijk, the Netherlands. The animals were on average 8.0 ± 0.8 (range: 3.5–18.8) years old and weighed 8.2 ± 0.4 (range: 4.8–12.5) kg at the start of the study. The groups contained 20–40 individuals per group that were managed by adhering to natural group dynamics. The groups consisted of several multigenerational matrilines, encompassing adult females with their offspring, and one group contained an adult breeding male temporarily. We refer to the three groups by using the names of the dominant females: Macaroni, Avocado and Calimero. The number of subjects per group was 12 (Macaroni), 12 (Avocado) and 7 (Calimero). These groups were selected for this study as they contained both normal- and overweight individuals. The groups also contained two elderly females (> 25 years old), but these females were not included in the study as aging likely affected their behaviour, physiology and body measures (Veenema et al. 1997; Ramsey et al. 2000a).

Each social group had access to an enriched indoor (± 72 m^2 and 2.85 m high) and outdoor (± 250 m^2 and 3.1 m high) enclosure. The indoor enclosure contained sawdust bedding, while the outdoor enclosure had a sand bedding with natural plant growth. The enclosures were divided into several compartments and contained several climbing structures, fire hoses, beams, sitting platforms and an outside swimming pool (Vernes & Louwerse 2010). In the morning, the macaques were provided with monkey chow (Ssniff, Soest, Germany). The amount of monkey chow fed to each group consisted of the sum of all individual's energetic requirements, which was based on their basal metabolic rate and depended on their age, sex and body weight (National Research Council 2003). Rhesus macaques can store food in their cheek pouches and retrieve this at a later moment. Monkey chow was provided in several (i.e. four to seven) feeding trays to prevent monopolization. In addition, fresh fruit, vegetables, bread or a grain mixture were presented in the afternoon. Fruit and vegetables included apple, banana, bell pepper, cabbage, cauliflower, chicory, cucumber, endive, leek, lettuce, maize, orange, pear, sweet potato and tomato. Most of these items were handed to the animals by a caretaker. Water was available *ad libitum*.

Measuring relative adiposity

Relative adiposity was measured using a species-specific weight-for-height index (WHI), which was calculated as body weight (kg) divided by the third power of height (m) (Sterck et al. 2019). WHI is independent of height and highly correlated with other relative adiposity measures, e.g. abdominal circumference and skinfold thicknesses (Sterck et al. 2019). Body weight and height were measured following a standard procedure during three consecutive annual health checks (2015–2017).

Although measured by different people, height of skeletally matured females (i.e. females ≥ 6 years old; Schwartz & Kemnitz 1992) was highly correlated between the three health checks ($r_p > 0.856$, $p < 0.0005$), indicating high inter-observer reliability. Baseline WHI was measured in 2015, while the WHI measured in 2016 and 2017 was used to determine the change in relative adiposity after one and two years. The change in relative adiposity was calculated by subtracting the WHIs in 2016 and 2017 from the baseline WHI in 2015. WHI ideally ranges between 42 and 67 kg/m^3, so individuals with a WHI exceeding the upper boundary were considered to be overweight (Sterck et al. 2019).

Behavioural observations

All behavioural observations were performed at baseline in 2015. Food intake was observed using video recordings. First, behaviour around the feeding trays with monkey chow was recorded between 8.30 h and 16.00 h with two video cameras (JVC Everio GZ-R15BE, JVC, Yokohama, Japan) for two days per group with roughly one month between the two recordings. At the end of each recording, the monkeys had consumed all the food in the feeding trays, and leftover monkey chow pieces on the ground outside of the enclosure were counted. The number of monkey chow pieces consumed per individual was counted from the videos. A piece was considered eaten when an individual put this into its mouth or when an individual disappeared out of view with the piece(s) in hand. A hand full counted as four pieces. The error factor on total monkey chow intake, i.e. relative difference between the observed and expected total number of monkey chow pieces, was on average 10% (range: 0–27%). The expected number of monkey chow pieces was calculated by dividing the total weight of the chow provided to the group by the average weight of one chow piece. Second, the afternoon feeding of apples and bread, handed to the animals by a caretaker, was observed by video recordings. These items were chosen, because bread was fed three times a week and apples were the most commonly fed type of fruit during the study period. Apple and bread feedings were both filmed five times per group and always in the presence of the same caretaker. Apple and bread feedings were performed opportunistically whenever apples or bread were fed, and the principal caretaker of the building was present to perform the feeding.

Food intake of individual monkeys is generally consistent over time (Sullivan et al. 2006). However, monkey chow intake per individual did not correlate between the two days in the groups (Macaroni group; $r_p = 0.231$, $n = 12$, $p = 0.469$; Avocado group: $r_p = 0.121$, $n = 12$, $p = 0.707$; Calimero group $r_p = 0.593$, $n = 7$, $p = 0.161$). In contrast, both individual apple and bread intake correlated well between the five sessions of each item in all three groups, so intake of these food items was consistent over time (Appendix A). The average monkey chow intake of the two days and the average apple and bread intake of the five sessions per individual were used in further analyses. The average amount of monkey chow, apples and bread per individual was converted into a measure of energy intake in kcal/day using the average weight per piece and information regarding the energy content of each food item (i.e. 3.28 kcal/gram monkey chow, 0.61 kcal/gram apple and 1.98 kcal/gram bread). Total daily food intake per individual was calculated by proportionally adding mean apple (4times/7days) and bread (3times/7days) intake per day to the mean monkey chow intake per day. Total food intake relative to body weight per individual was calculated as the total food intake divided by body weight.

Activity budgets were observed by focal animal sampling during 15-minute periods for a total of three hours per animal (Altmann 1974). Data were collected by DGMZ using The Observer XT version 11 (Noldus Information Technology, Wageningen, the Netherlands) according to a predetermined balanced randomized observation schedule. Animals had free access to both indoor and outdoor enclosures during the observations, which took place between 9.00 h and 16.00 h. Focal animals were scored continuously for the duration of activity-related behaviour (see ethogram: Appendix B). Behavioural data were categorized in resting, moving, foraging, grooming and other behaviour. Each category was expressed as a percentage of time from the total observation time per individual. Moving combined all behaviour in which the body was not stationary, i.e. walking, running, climbing, aggressive behaviour and playing, while resting consisted of lying, sitting and standing. Foraging is defined as "searching for and investigating (potential) food items such as sifting through bedding", which is considered a searching activity and not related to food intake.

Finally, all occurrence sampling was used to score submissive behaviour during the live observations and video analyses (Altmann 1974). Submissive behaviour concerned bared teeth, give ground and make room (Appendix B). These behaviours were used to construct a dominance hierarchy for each group using MatMan (Netto et al. 1993). All three dominance hierarchies were significantly linear and highly consistent (Macaroni: $h' = 0.88$, DCI = 0.99, $p < 0.0005$; Avocado: $h' = 0.95$, DCI = 0.99, $p < 0.0005$, Calimero: $h' = 0.79$, DCI = 0.99, $p < 0.0005$). For the data analyses, absolute dominance rank was rescaled to a relative measure (ranging from zero to one) to correct for the different number of subjects per group. The higher the dominance rank, the closer the score is to zero. Dominance rank of all three groups remained stable over the study period (2015–2017).

Data analyses

Behaviour related to energy intake and expenditure, i.e. food intake and activity budgets, at baseline were analyzed first. Spearman correlations were used to determine whether the intake of different food items (i.e. monkey chow, apples and bread) and the percentage of time spent on several behavioural categories (i.e. moving, resting, foraging and grooming) were correlated. Linear mixed models were used to test whether age (in years) and dominance rank were related to the variation in food intake and activity-related behaviour. The parametric paired samples t-test was used to compare WHI at baseline with WHI after one year, because both were normally distributed. The non-parametric Wilcoxon signed ranks test was used to compare baseline WHI with WHI after two years, as WHI after two years was not normally distributed. The Shapiro-Wilk test was used to test normal distribution.

Linear mixed models were used to check whether behaviour and individual characteristics were related to baseline WHI. The behavioural categories, age and dominance rank were included in the models as covariates, while the social group in which the individual was housed was included as a random factor. Two separate models were run, as there was high collinearity between resting and the other behavioural categories, i.e. moving, foraging and grooming. In the results section, we report the outcomes for the models including moving, foraging and grooming. The models with only resting provided similar outcomes (Appendix C). Finally, we examined which variables were predictive of the change in WHI after one and two years. The behavioural categories, age, dominance rank and baseline WHI were included as covariates, while social group was included as a random factor. Three individuals were omitted from this final analysis as one individual developed chronic diarrhea and two animals passed away during the two-year study period. The models were visually checked for normal distribution and homoscedasticity of the residuals and the variance inflation factor did not exceed 3.62. Descriptive statistics in the results are reported as mean ± SE.

Statistical analyses and figure editing were performed in SPSS Statistics version 28 and R studio version 1.2.5 using the packages *car*, *lmerTest*, *jtools* and *ggplot2*. All tests were two-tailed and the level of significance was set at $\alpha = 0.05$, but trends ($0.05 < p < 0.10$) are reported as well.

Ethical statement

BPRC is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC International). Ethical review and approval for this study were waived as the annual health checks are a routine veterinary procedure related to the regular health management of the colony (cf. Balansard et al. 2019). This study performed no experimental procedures that would require ethical approval according to the European Directive 2010/63 or the Dutch law. All applicable international, national, and institutional guidelines and laws for the care and ethical use of animals were followed.

Results

Energy intake

Total food intake was derived from an individual's daily consumption of monkey chow, apples and bread. Monkey chow intake was not correlated with apple ($r_s = -0.016$, $n = 31$, $p = 0.933$) or bread intake ($r_s = 0.030$, $n = 31$, $p = 0.871$). In contrast, there was a highly significant positive correlation between apple and bread intake ($r_s = 0.893$, $n = 31$, $p < 0.0005$). Monkey chow intake was independent of age ($F(1,26) = 1.796$, $p = 0.192$) and dominance rank ($F(1,26) = 0.899$, $p = 0.352$). Apple intake was independent of dominance rank ($F(1,26) = 0.877$, $p = 0.358$), while older individuals tended to have a higher apple intake than younger monkeys ($F(1,26) = 3.378$, $p = 0.078$). Bread intake was not related to age ($F(1,26) = 2.505$, $p = 0.126$) or dominance rank ($F(1,26) = 0.076$, $p = 0.785$).

Total food intake, combining the three measured food items, was on average 679 ± 31 kcal/day. There was a 2.6-fold difference in the number of calories consumed between individuals with the highest and lowest total food intake (range: 394–1028 kcal/day). Total food intake was independent of dominance rank ($F(1,26) = 0.530$, $p = 0.473$), while older individuals had higher total food intake compared to younger individuals ($F(1,26) = 4.310$, $p = 0.048$). Total food intake relative to body weight was on average 87 ± 4 kcal/kg. There was also considerable variation in food intake per kilogram body weight between monkeys (range: 44–146 kcal/kg). Total food intake relative to body weight was higher in younger individuals compared to older monkeys ($F(1,26) = 5.411$, $p = 0.028$), while dominance rank ($F(1,26) = 0.413$, $p = 0.526$) had no significant influence.

Energy expenditure

Energy expenditure was estimated using activity budgets. Females spent on average 9.3 ± 0.9% of the time moving, 54.6 ± 2.1% resting, 11.5 ± 1.2% foraging, 21.6 ± 1.5% grooming and 3.0 ± 0.4% on other behaviour. The most and least active monkeys differed more than tenfold in their percentage of time spent moving (range: 1.8–19.2%), while resting differed only 2.1-fold (range: 39.3–82.2%). Time spent resting was negatively correlated with time spent moving ($r_s = -0.776$, $n = 31$, $p < 0.0005$) and foraging ($r_s = -0.642$, $n = 31$, $p < 0.0005$), but not related to grooming ($r_s = -0.154$, $n = 31$, $p = 0.407$). Moving and foraging were positively correlated ($r_s = 0.736$, $n = 31$, $p < 0.0005$), while moving was not related to grooming ($r_s = -0.269$, $n = 31$, $p = 0.144$). There was a significant negative association between foraging and grooming ($r_s = -0.427$, $n = 31$, $p = 0.016$).

Time spent moving and foraging were independent of dominance rank ($F(1,26) = 1.748$, $p = 0.198$; $F(1,26) = 2.659$, $p = 0.115$), while younger individuals moved and foraged more than older monkeys ($F(1,26) = 16.013$, $p < 0.0005$; $F(1,26) = 8.678$, p = 0.007). Similarly, resting was not related to dominance rank (F(1,26) = 0.778, p = 0.386), but older animals spent more time resting compared to younger individuals (F(1,26) = 15.152, p < 0.0005). Time spent grooming was independent of age (F(1,26) = 0.106, p = 0.924) and dominance rank (F(1,26) = 0.395, p = 0.535).

Baseline and change in relative adiposity

At baseline, mean WHI was 61.1 ± 1.4 kg/m^3 and 9 of 31 individuals (29%) exceeded the upper boundary for a healthy relative adiposity and were considered to be overweight. Baseline WHI was independent of age (F(1,24) = 0.144, p = 0.708), dominance rank (F(1,24) = 0.124, p = 0.728), total food intake (F(1,24) = 0.004, p = 0.953) and grooming (F(1,24) = 0.029, p = 0.866). Baseline WHI was negatively correlated with the time spent moving (F(1,24) = 6.415, p = 0.018) and tended to be negatively correlated with time spent foraging (F(1,24) = 3.557, p = 0.071), meaning individuals with low activity levels had a higher relative adiposity (Figure 1ab). Similarly, resting was significantly related to baseline WHI with more resting in relatively heavy individuals (Appendix C; Figure 1c).

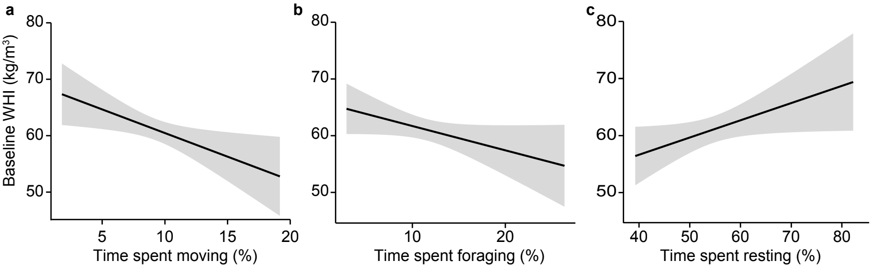

Figure 1. Females that spent less time moving (**a**), less time foraging (**b**) and more time resting (**c**) had a higher baseline weight-for-height index (baseline WHI; mean + 95 % confidence interval).

The baseline data were employed to determine whether behaviour, age or dominance rank affected the change in WHI after one and two years. One year after the baseline period, mean WHI was 62.4 ± 1.7 kg/m^3, which is roughly comparable to the baseline WHI (t = −1.673, n = 31, p = 0.105), and 10 out of 31 individuals (32%) were overweight (Figure 2a). Change in WHI after one year was independent of age (F(1,23) = 0.057, p = 0.814), dominance rank (F(1,23) = 1.019, p = 0.324), baseline WHI (F(1,23) = 0.260, p = 0.616), food intake (F(1,23) < 0.0005, p = 0.989), moving (F(1,23) = 0.229, p = 0.637), foraging (F(1,23) = 0.054, p = 0.819) and grooming (F(1,23) = 1.314, p = 0.265). When only resting was used in the model instead of moving, foraging and grooming, this resulted in similar non-significant outcomes (Appendix C).

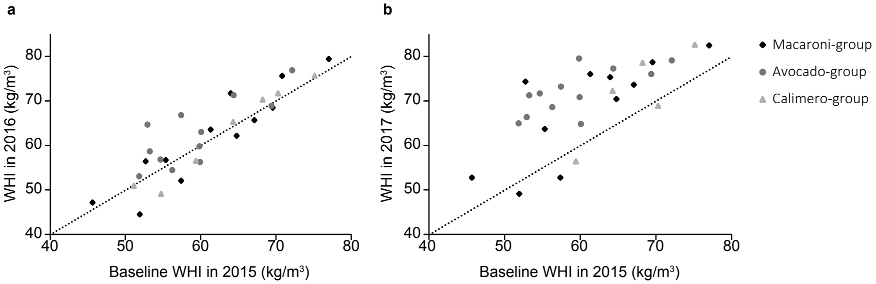

Figure 2. Baseline WHI in 2015 plotted against WHI in 2016 (**a**) and 2017 (**b**) per group. The black dotted line separates the individuals that increased (top-left) or decreased (bottom-right) their relative adiposity compared to baseline.

Two years after the baseline period, mean WHI was 70.4 ± 1.7 kg/m^3, which is significantly higher compared to the baseline WHI ($Z = -4.463$, $n = 28$, $p < 0.0005$; Figure 2b). WHI increased in 86% of the animals and more than two-third (20 of 28 individuals, 71%) of the individuals was overweight in 2017. Change in WHI after two years was independent of age ($F(1,20) = 2.869$, $p = 0.107$), baseline WHI ($F(1,20) = 0.936$, $p = 0.346$), food intake ($F(1,20) = 1.356$, $p = 0.258$), moving ($F(1,20) = 0.557$, $p = 0.465$), foraging ($F(1,20) = 0.479$, $p=0.497$) and grooming ($F(1,20) = 0.017$, $p = 0.897$). However, change in WHI was significantly related to dominance rank, as high-ranking individuals had a higher increase in WHI compared to low-ranking monkeys ($F(1,20) = 11.481$, $p = 0.003$; Figure 3). A similar result was obtained when only resting was used instead of moving, foraging and grooming (Appendix C).

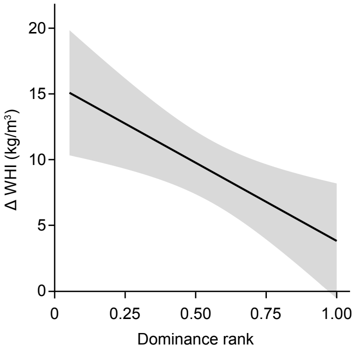

Figure 3. Dominance rank plotted against the change in weight-for-height index after the second year (Δ WHI; mean + 95 % confidence interval). A higher dominance rank is indicated by a score closer to zero.

Discussion

This study aimed to identify risk factors for becoming overweight in captive group-living NHPs. First, we checked whether behaviour related to energy intake and expenditure was associated with relative adiposity, measured using a weight-for-height index (WHI), at baseline. Second, we determined whether behaviour at baseline predicted the subsequent change in relative adiposity, while controlling for individual characteristics. A high WHI was associated with low activity levels at baseline, but was not related to food intake, age or dominance rank. WHI did not differ from baseline after one year but had significantly increased after the second year. This increase in WHI was independent of food intake and activity budgets, while higher-ranking females had a higher increase in WHI compared to lower-ranking monkeys.

Energy intake

This study used behavioural observations to quantify energy intake, yet accurate measurements of food intake are notoriously difficult to obtain in a group setting (Chivers 1998; Wilson et al. 2008). Observations have previously been used to quantify food intake in individually housed NHPs (e.g. Hannah & Hansen 1990; Wolden-Hanson et al. 1993; Hansen et al. 1995). In these studies, the error factor is usually less than 5% (Hansen et al. 1995). Our average error factor on the monkey chow pellets was 10% (range: 0–27%) and the observed number of pellets was always lower than the expected number of pellets, which likely resulted in an underestimation of the actual food intake. Similarly, human observers underestimate food intake compared to an automated feeding system by 12.9% (Wilson et al. 2008). These automated feeders release one pellet upon activation by a microchip in the wrist, which is then recorded by a computer (Wilson et al. 2008). Besides, individuals could take a piece of food, put it in their cheek pouches and disappear out of view from the camera. So, whether they actually ate the food could not be observed. More accurate ways to quantify energy intake of group-housed NHPs are needed.

Furthermore, apple and bread intake may have been influenced by the feeding and scoring method. Apples and bread were handed to the animals by a caretaker, and were thus monopolizable, while monkey chow was distributed over multiple feeding trays. Apple and bread intake were highly correlated, while intake of monkey chow was not correlated with the intake of apples or bread. Apple and bread intake were independent of dominance rank though. The intake of these food items is likely more related to personality and the quality of the animal-keeper relationship. In stump-tailed macaques (*Macaca arctoides*), individuals with a “friendly” personality are more likely to approach and accept food offered by caretakers (Waitt et al. 2002). In our study, the intake of apples and bread was unequally distributed, with some individuals receiving several pieces while others received none. However, individuals that did not accept food from the caretaker still managed to obtain some food as they scavenged the ground for leftovers or stole pieces from other animals (personal observation). As a result, our method likely overestimated apple and bread intake for some individuals, while underestimating it for others. Thus, also this estimate of food intake may be less accurate than the observations suggest.

Although the estimated energy intake based on monkey chow, apples and bread should be interpretated with caution, some interesting results were found. Total food intake was highly variable between individuals, ranging between 394 and 1028 kcal/day. Such individual differences may be due to different energetic requirements based on individual characteristics, such as age. For example, young animals generally require more energy to enable growth (Draper 1966). Indeed, in our study, food intake relative to body weight was higher in younger individuals compared to older monkeys. Thus, the relatively high energetic demands of younger individuals were reflected in their food intake.

Dominance rank is a second possible factor to affect food intake as dominant individuals typically have priority of access to food (Whitten 1983; Boccia et al. 1988; Barton 1993). However, none of the food intake measures were related to dominance rank, implying that low-ranking individuals were able to somehow compensate for this. In the wild, low-ranking individuals travel more to find food, forage away from the main group or compensate by switching to other, lower-quality food items (van Schaik & van Noordwijk 1988; Soumah & Yokota 1991; Saito 1996; Tsuji & Takatsuki 2012). In captivity, low-ranking females store food in their cheek pouches and then move away from the group to eat it (Brennan & Anderson 1988; Deutsch & Lee 1991). NHPs thus adjust their foraging and eating behaviour according to their dominance rank (Soumah & Yokota 1991; Bauer et al. 2012). As a result, food intake probably does not differ systematically between high- and low-ranking individuals (cf. van Noordwijk & van Schaik 1987; Michopoulos et al. 2012).

All in all, some measures of food intake were related to age, but food intake was independent of dominance rank. The method of feeding and observations may have affected the accuracy of our food intake measures and more accurate ways to measure energy intake in group-housed NHPs are needed to verify our results.

Energy expenditure

Similar to energy intake, energy expenditure may vary depending on individual characteristics. There was a tenfold difference in time spent moving between the most and least active monkeys, whereas this difference is eightfold in individually housed female rhesus macaques (Sullivan et al. 2006). Younger animals spent more time moving and foraging and less time resting compared to older monkeys, which complies with previous studies (Veenema et al. 1997; Ramsey et al. 2000a; Melfi & Feistner 2002; Jaman & Huffman 2008). Furthermore, dominance rank had no effect on the time spent moving, foraging, grooming or resting. Similarly, in group-housed long-tailed macaques dominance rank has no effect on the level of moving or resting (Bauer et al. 2012). Activity budgets of captive group-housed female rhesus macaques thus vary depending on age, but not dominance rank.

The enclosure environment plays an important role in shaping activity budgets. In captive Sulawesi crested macaques (*Macaca nigra*), resting increases and moving and feeding behaviour decrease with smaller enclosure sizes (Melfi & Feistner 2002). Larger enclosures with natural substrate and vegetation increase foraging behaviour and lead to activity budgets that are more similar to activity budgets of wild NHPs (Beisner & Isbell 2008; Jaman & Huffman 2008). In our study, the macaques had 24 h access to relatively spacious indoor and outdoor enclosures with environmental enrichment. However, space and foraging opportunities were still restricted, which likely affected their activity budgets. Animals spent on average 9.3% of the time moving, 54.6% resting, 11.5% foraging and 21.6% grooming. Captive group-living Japanese (*Macaca fuscata*) and rhesus macaques on a similar diet, but with larger outside enclosures (500–6400 m^2), also spend more time resting compared to other activities (Beisner & Isbell 2008; Jaman & Huffman 2008). However, their activity budgets are comprised of 10–15% moving, 35–50% resting, 15–30% foraging and feeding and 10–15% grooming (Beisner & Isbell 2008; Jaman & Huffman 2008). In our study, resting and grooming were thus higher, while moving and foraging were lower. Differences in housing conditions likely explain why activity budgets differ between captive studies.

Baseline and change in relative adiposity

This study aimed to identify risk factors for becoming overweight in captive group-living NHPs by testing whether behaviour was related to the baseline adiposity and to change in relative adiposity, while controlling for individual characteristics. Baseline WHI was independent of food intake, age and dominance rank, while a higher baseline WHI was associated with less moving, less foraging and more resting. Less active monkeys were thus more likely to be overweight, which complies with previous studies (Wolden-Hanson et al. 1993; Bauer et al. 2012). Similar to our findings, food intake was not related to body weight in individually housed rhesus macaques (Hannah & Hansen 1990; Wolden-Hanson et al. 1993). In contrast, high-ranking female NHPs generally have higher body fat levels compared to low-ranking females (Small 1981; Schwartz et al. 1993). Altogether, our results largely replicate previous findings from cross-sectional studies, but our aim was to identify risk factors instead of studying associations.

We therefore determined whether age, dominance rank, WHI and behaviour at baseline predicted the change in WHI. WHI did not differ from baseline after one year, but significantly increased after the second year. This increase in WHI was independent of behaviour related to energy intake and expenditure, i.e. food intake and activity budgets, at baseline. Food intake neither correlates with weight gain in individually housed female rhesus macaques (Sullivan et al. 2006). In contrast, total caloric intake is significantly correlated with weight gain when feeding high-fat diets in group-housed female rhesus macaques (Wilson et al. 2008). When monkeys are maintained on a standard laboratory (low-fat) diet, body weight does not change (Wilson et al. 2008). Even though food intake was not predictive of change in WHI and commercial monkey chow was provided to the monkeys in our study, relative adiposity increased during the study period in the majority (86%) of individuals. This indicates that the animals consumed more energy than required. The standard feeding routine at BPRC, i.e. providing 100% of the required energy in monkey chow and providing other food items in addition, may have led to a higher prevalence of overweight individuals and this should therefore be critically evaluated.

The time spent moving, foraging and resting were significantly correlated with baseline WHI, but it did not predict the change in WHI. This suggests that the time spent on these activities is merely a consequence and not a cause of captive NHPs being overweight. Yet, activity is a risk factor of weight gain in individually housed female rhesus macaques maintained on a high-fat diet: more active individuals gain less body weight compared to less active monkeys (Sullivan et al. 2006). These contradicting findings may result from differences in housing and diet and call for more studies on risk factors of weight gain in captive group-living NHPs. Nevertheless, dominance rank was a significant predictor of change in WHI: higher-ranking individuals had a higher increase in WHI than lower-ranking monkeys. Similarly, juvenile rhesus macaques with a high dominance rank gain more body weight between one and three years of age compared to low-ranking juveniles (Bastian et al. 2003). Thus, dominance rank likely predicts weight gain during adulthood in captive group-living NHPs.

A potential mechanism behind this relationship between dominance rank and weight gain may lie in the role of physiology and circulating hormones in the deposition of fat tissue. Glucocorticoids, such as cortisol, are produced by the hypothalamic-pituitary-adrenal axis during stress and have been linked to alterations in fat deposition (Shively et al. 2009). Several studies found that dominance rank was related to cortisol levels with low-ranking female NHPs generally having higher levels compared to high-ranking females (Cavigelli & Caruso 2015). These findings indicate a possible relationship between dominance rank of an individual and glucocorticoid-mediated accumulation of adipose tissue. Contrasting with this proposal, hair cortisol concentrations did not differ between high- and low-ranking female rhesus macaques from the BPRC colony, suggesting that cortisol levels did not play a role in the weight gain during our study (Zijlmans et al. 2021a). However, further studies are needed to assess the role of hormones in weight gain in group-housed NHPs.

Conclusion

This study aimed to identify risk factors for becoming overweight in captive group-living rhesus macaques. Although it seems plausible that the provisioning of food and housing conditions in captivity both contribute to the problem (Bauer et al. 2012), this study found no evidence for a link between behaviour related to energy intake and expenditure and weight gain. More accurate ways to measure energy intake in group-housed NHPs are needed to verify this. Thus, not individual differences in behaviour, but dominance rank seems to predict weight gain during adulthood in captive group-living NHPs.

Appendix

Appendix A: Correlation matrices for intake of apples (white) and bread (grey) per group

Table A1. Spearman correlations between apple intake and bread intake during the five different feeding sessions in the Macaroni group (n=12)*

	Session 1	**Session 2**	**Session 3**	**Session 4**	**Session 5**
Session 1		$r_s = 0.719$ $p = 0.008$	$r_s = 0.849$ $p < 0.0005$	$r_s = 0.830$ $p < 0.0005$	$r_s = 0.675$ $p = 0.016$
Session 2	$r_s = 0.502$ $p = 0.096$		$r_s = 0.731$ $p = 0.007$	$r_s = 0.811$ $p = 0.001$	$r_s = 0.852$ $p < 0.0005$
Session 3	$r_s = 0.762$ $p = 0.004$	$r_s = 0.520$ $p = 0.083$		$r_s = 0.805$ $p = 0.002$	$r_s = 0.792$ $p = 0.002$
Session 4	$r_s = 0.861$ $p < 0.0005$	$r_s = 0.529$ $p = 0.077$	$r_s = 0.714$ $p = 0.009$		$r_s = 0.804$ $p = 0.002$
Session 5	$r_s = 0.874$ $p < 0.0005$	$r_s = 0.495$ $p = 0.102$	$r_s = 0.529$ $p = 0.077$	$r_s = 0.810$ $p = 0.001$	

Table A2. Spearman correlations between apple intake and bread intake during the five different feeding sessions in the Avocado group (n=12)*

	Session 1	**Session 2**	**Session 3**	**Session 4**	**Session 5**
Session 1		$r_s = 0.615$ $p = 0.033$	$r_s = 0.714$ $p = 0.009$	$r_s = 0.751$ $p = 0.005$	$r_s = 0.657$ $p = 0.020$
Session 2	$r_s = 0.890$ $p < 0.0005$		$r = 0.731$ $p = 0.007$	$r_s = 0.866$ $p < 0.0005$	$r_s = 0.776$ $p = 0.003$
Session 3	$r_s = 0.633$ $p = 0.027$	$r_s = 0.632$ $p = 0.027$		$r_s = 0.858$ $p < 0.0005$	$r_s = 0.794$ $p = 0.002$
Session 4	$r_s = 0.820$ $p = 0.001$	$r_s = 0.945$ $p < 0.0005$	$r_s = 0.754$ $p = 0.005$		$r_s = 0.897$ $p< 0.0005$
Session 5	$r_s = 0.936$ $p < 0.0005$	$r_s = 0.845$ $p = 0.001$	$r_s = 0.646$ $p = 0.023$	$r_s = 0.764$ $p = 0.004$	

Table A3. Spearman (r_s) and Pearson (r_p) correlations between apple intake and bread intake during the five different feeding sessions in the Calimero group (n=7)*

	Session 1	**Session 2**	**Session 3**	**Session 4**	**Session 5**
Session 1		$r_p = 0.934$ $p = 0.002$	$r_p = 0.621$ $p = 0.136$	$r_p = 0.935$ $p = 0.002$	$r_p = 0.798$ $p = 0.032$
Session 2	$r_s = 0.532$ $p = 0.219$		$r_p = 0.583$ $p = 0.169$	$r_p = 0.833$ $p = 0.020$	$r_p = 0.806$ $p = 0.029$
Session 3	$r_s = 0.132$ $p = 0.778$	$r_s = 0.310$ $p = 0.499$		$r_p = 0.681$ $p = 0.092$	$r_p = 0.543$ $p = 0.208$
Session 4	$r_s = 0.031$ $p = 0.947$	$r_s = 0.155$ $p = 0.739$	$r_s = 0.835$ $p = 0.019$		$r_p = 0.557$ $p = 0.194$
Session 5	$r_s = 0.120$ $p = 0.797$	$r_s = 0.057$ $p = 0.904$	$r_s = 0.365$ $p = 0.421$	$r_s =-0.010$ $p = 0.984$	

* Correlations for the apple intake appear in the upper-right triangle (white area) and those for the bread intake appear in the lower-left triangle (grey marked area). Numbers represent the correlation coefficient (r) and level of significance (p-value).

Appendix B: Ethogram of all scored activity-related and submissive behaviour

Category	Behaviour	Description
Resting	Lying	Individual is lying still in one place
	Sitting	Individual's but cheeks touch a horizontal object so that there is no locomotion
	Standing	Individual remains stationary on its two legs (bipedal) or on all fours (quadrapedal)
Moving	Walking	Individual repetitively places one limb before the other to make a horizontal movement at moderate speed. Distinguished from running primarily by speed.
	Climbing	Individual uses arms and legs to make a vertical movement on a steep surface such as a wall or fence
	Running	Same as walking but at higher speed
	Aggression	Aggressive behaviour that may be energetically costly, e.g. lunge, slap, bite, push and pull
	Playing	All types of interactions between two or more individuals using the relaxed open mouth play face (in which the mouth is half wide open and teeth are covered by the lips). The interaction can be touch, pull, pug, push, hit, wrestle, chase and bite.
Foraging	Foraging	Searching for and investigating (potential) food items such as sifting through bedding.
Grooming	Grooming	Individual uses its hands and fingers to go through its own or another individual's fur to remove foreign objects. This includes taking care of the skin, teeth and fur.
Other	Feeding	Ingestion of food/water from the feeding trays/water dispensers
	Object exploration	Exploring or manipulating a non-food object (e.g. plastic bottle) with the hands or mouth
	Hanging	Individual grasps with all four limbs onto a vertical surface and remains stationary
Submissive behaviour	Bared teeth	Individual lifts its eyebrows and forehead, while the ears are positioned against the head and the lips are drawn back, thus displaying teeth and gum.
	Make room	Individual makes a small avoiding movement away from another individual that approaches, within three seconds after the approach. The actor remains in approximately the same spot and may make room by only moving the upper body.
	Give ground	Individual creates a distance between itself and another individual that comes closer than one meter or within three seconds after the individual arrived, by moving away from it at moderate speed.

Appendix C: Results from linear regression models on baseline WHI and change in WHI after one and two years when resting was used instead of moving, foraging and grooming. * p<0.05.

	Baseline WHI	Change in WHI (1st year)	Change in WHI (2nd year)
Age	F(1,26)=2.445, p=0.130	F(1,25)=0.207, p=0.653	F(1,22)=2.685, p=0.116
Dominance rank	F(1,26)=0.265, p=0.611	F(1,25)=1.406, p=0.248	F(1,22)=12.444, p=0.002*
Total food intake	F(1,26)=0.025, p=0.875	F(1,25)=0.006, p=0.940	F(1,22)=1.223, p=0.281
Resting	F(1,26)=4.362, p=0.047*	F(1,25)=1.878, p=0.184	F(1,22)=0.012, p=0.914
Baseline WHI	NA	F(1,25)=1.100, p=0.305	F(1,22)=1.010, p=0.327

Chapter 6

Retrospective evaluation of a minor dietary change in non-diabetic group-housed long-tailed macaques (*Macaca fascicularis*)

Dian G. M. Zijlmans, Annemiek Maaskant,
Elisabeth H. M. Sterck, Jan A. M. Langermans

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Abstract

Macaques in captivity are prone to becoming overweight and obese, which may cause several health problems. A diet that mimics the natural diet of macaques may prevent these problems and improve animal welfare. Adjusting captive diets towards a more natural composition may include increasing fiber content and lowering the glycemic index, i.e. reducing the impact on blood glucose levels. Such a dietary change was implemented in our long-tailed macaque (*Macaca fascicularis*) breeding colony. The basic diet of monkey chow pellets remained the same, while the supplementary provisioning of bread was replaced by grains and vegetables. This study is a retrospective evaluation, based on electronic health records, that investigated whether this minor dietary change had a beneficial effect on relative adiposity and overweight-related health parameters in 44 non-diabetic, group-housed, female long-tailed macaques. Relative adiposity was measured with a weight-for-height index and blood samples were collected during yearly health checks. Glycemic response and lipid metabolism were evaluated using several biochemical parameters. Relative adiposity and overweight status did not differ after dietary change. Yet, relatively heavy individuals generally lost body weight, while relatively lean individuals gained body weight, leading to a more balanced body weight dynamic. Dietary change did not affect HbA1c and triglyceride levels, while fructosamine and cholesterol levels were significantly reduced. Thus, the minor dietary change had no significant effect on overweight status, but some biochemical parameters related to the risk of diabetes and cardiovascular disease were positively affected. This study emphasizes the importance of evaluating husbandry changes and that critically reviewing husbandry practices can provide valuable insights to improve animal health and welfare.

Introduction

Macaques in captivity are susceptible to becoming overweight and obese. Similar to humans, this can cause several health problems, such as type 2 diabetes mellitus (T2DM) and cardiovascular disease (Kemnitz & Francken 1986; Shively & Clarkson 1987). Diet likely plays an important role in becoming overweight and the related health problems (Hansen & Bodkin 1986). A diet that mimics the natural diet of macaques may prevent these problems. Wild macaques mainly eat wild fruits, supplemented with seeds, flowers, leaves, buds, bark and small animals, e.g. insects (Yeager 1996; Hill 1997; Hanya 2004; Sarker et al. 2008). This natural diet is high in fiber and low in fat (Riley et al. 2013; Kassim et al. 2017), resulting in little to no overweight in wild macaques (Dittus 2013; Sterck et al. 2019). In contrast, diets in captivity tend to be low in fiber and high in easily digestible carbohydrates, such as sugar (Cabana et al. 2018a). Accordingly, 10–15% of captive macaques develop obesity during their life (West & York 1998).

Adjusting the diet towards a more natural composition may decrease overweight-related health problems and improve animal welfare in captivity. For example, increasing fiber and decreasing sugar content in the diet led to a reversal of prediabetes and more natural behaviour in great apes (Cabana et al. 2018a). Fruits, vegetables, and grains generally contain a high amount of fiber and have a low glycemic index (GI ≤ 55) (Ludwig 2002; Atkinson et al. 2008; Dias 2019). Other food items, such as bread, have a high GI (GI ≥ 70) as they contain carbohydrates that are quickly digested and metabolized (Atkinson et al. 2008; Augustin et al. 2015). This leads to postprandial hyperglycemia, i.e. a high increase in blood glucose after consumption, which has been proposed to increase the risk of T2DM and cardiovascular disease in humans (Ludwig 2002).

Various biochemical parameters can be used to assess the risk of developing overweight-related health problems such as T2DM and cardiovascular disease. As animals are progressing towards T2DM, the glycemic response becomes impaired and blood glucose levels increase (Wagner et al. 2006). As a result, glycated proteins are formed, e.g. fructosamine and glycated hemoglobin (HbA1c), both accurate biomarkers to measure the intermediate and long-term glycemic response, respectively (Cefalu et al. 1993; Williams-Fritze et al. 2011). In addition, obese macaques experience changes in markers for lipid metabolism, e.g. increased total cholesterol and triglyceride concentrations, which are risk factors for the development of both T2DM and cardiovascular disease (Hamilton et al. 1972; Wagner et al. 2006). These four biochemical parameters provide information regarding health risks and can thus be useful in the diagnosis and management of overweight-related health problems in macaques (Williams-Fritze et al. 2011).

In an attempt to reduce overweight incidence and overweight-related health problems, a dietary change was implemented in our long-tailed macaque (*Macaca fascicularis*) breeding colony. The supplementary provisioning of bread was replaced by grains and vegetables, while the basic diet of monkey chow pellets remained the same. Although wild long-tailed macaques mainly eat fruits, it would not be appropriate to feed similar amounts of fruit in captivity. Cultivated fruits have a different nutritional composition, i.e. less protein and fiber and more sugar, compared to wild fruits (Kaumanns et al. 2006; Cabana et al. 2018a). Since the nutritional composition of cultivated vegetables is more like wild fruits, more vegetables than fruits were provided. The dietary change led to an increased fiber content and a lower GI. The implementation of this dietary change had little impact on daily husbandry practices, i.e. feeding times and routines remained unchanged.

This study is a retrospective evaluation that investigated whether this minor dietary change had a beneficial effect on relative adiposity and overweight-related health parameters in non-diabetic, group-housed, female long-tailed macaques. The evaluation was based on data retrieved from electronic health records. Relative adiposity and biochemical parameters were measured during annual health checks before and after dietary change. Overweight status was determined with a species-specific weight-for-height index, which represents relative adiposity levels of long-tailed macaques (Sterck et al. 2019). Biochemical parameters related to glycemic response, i.e. fructosamine and HbA1c, and lipid metabolism, i.e. cholesterol and triglyceride, were compared to evaluate the effect of dietary change on the risk of T2DM and cardiovascular disease.

Materials and methods

Subjects and housing

Subjects of this study were 44 full-grown adult female long-tailed macaques from the breeding colony of the Biomedical Primate Research Centre (BPRC), an AAALAC accredited facility, in Rijswijk, the Netherlands. The animals were aged between 6 and 22 (10.7 ± 0.61) years old and weighed between 3.4 and 9.15 (5.5 ± 0.20) kg at the time of initial data collection. Pregnant and (pre)diabetic individuals were excluded to prevent the possibility of pregnancy or disease progression interfering with our outcome parameters. All females lived with their offspring and typically with one adult breeding male in multi-generational groups (N = 9 groups). The groups were formed by adhering to natural group dynamics, i.e. females are philopatric, while males are removed from their natal group at puberty. The amount of data on adult males was therefore insufficient to include them in the data analyses.

Individuals had access to enriched indoor (±72 m^2 and 2.85 m high) and outdoor (±250 m^2 and 3.1 m high) compartments. The inside enclosure contained sawdust bedding, while the outside enclosure had a sand bedding where natural plant growth was possible. Environmental enrichment consisted of several climbing structures, beams, fire hoses, car tires, sitting platforms, and a swimming pool to stimulate natural behaviour (Vernes & Louwerse 2010). Drinking water was freely available throughout the day via automatic water dispensers.

Diet and dietary change

The basic diet of the macaques consisted of monkey chow pellets (Ssniff, Soest, Germany) that were daily fed in the morning. The amount of monkey chow per individual was calculated based on the basal metabolic rate and depended on their age, sex, and body weight (National Research Council 2003). Adult females were calculated to require on average 90 g of monkey chow per day. In addition, one slice of wheat bread (~30 g, three times a week), 120 g of fruit/vegetables (three times a week), or 15 g of a grain mixture (once a week) were provided per individual in the afternoon (Table 1). Since the sum of all individuals' needs was provided to the group and the distribution of food among group members could not be controlled, actual food intake likely varied per individual. Food enrichment was provided occasionally but its contribution to daily nutritional intake was carefully controlled and considered negligible.

Table 1. Nutritional values of food items in the daily diet of female long-tailed macaques at BPRC based on the (average) amount fed per animal. Information was obtained from the food item's manufacturer or The Dutch Food Composition Database (NEVO, online version 2019/6.0; Table A1).

Food item	Amount (gram)	Energy (kcal)	Protein (gram)	Carbo-hydrates (gram)	Fiber (gram)	Fat (gram)	Glycemic index
Monkey chow	90	295	22.7	32.5	3.78	3.87	NA #
Fruit/vegetables	120	33	1.48	4.97	2.40	0.31	Low
Maize silage	120	71	3.12	17.6	7.01	1.38	Low
Grain mixture	15	50	1.66	10.7	0.47	0.38	Medium
Wheat bread	30	59	2.20	11.8	1.34	0.40	High

NA = not available.

A dietary change took place in June 2019 to reduce the diet's glycemic index and enhance fiber content. The supplementary provisioning of wheat bread in the afternoon (three times a week) was replaced by maize silage, grain mixture, and vegetables. As a result, the ratio of fruit to vegetables changed from approximately 1:3 before dietary change to 1:5 after dietary change. The 10 most commonly fed fruit/vegetables were banana, bell pepper, cabbage, chicory, Chinese cabbage, cucumber, endive, leek, lettuce, and tomato (Table A1). The dietary change led to a 15.4% increase in fiber content in the average daily diet, while the amount of energy, protein, carbohydrates, and fat remained approximately the same (Table 2). Besides an increase in fiber content, the removal of bread led to a lower overall GI after dietary change.

Table 2. Average daily intake of energy, protein, carbohydrates, fiber, and fat before and after dietary change in adult female long-tailed macaques.

Nutritional component	Before dietary change	After dietary change	Relative change (%)
Energy (kcal)	342	339	-1.0%
Protein (gram)	24.5	24.4	-0.2%
Carbohydrates (gram)	41.2	40.9	-0.7%
Fiber (gram)	5.45	6.29	+15.4%
Fat (gram)	4.23	4.35	+3.0%

Data collection

This retrospective evaluation was based on data retrieved from BPRC's electronic health records, which included data from annual health checks. These health checks are a routine veterinary procedure related to the regular health management of the colony (Balansard et al. 2018). No additional procedures were performed, and all procedures complied with regulations in the European Directive 2010/63 and the Dutch law. The health checks prior to dietary change took place in spring 2018. Since the dietary change took place in June 2019, data from the health checks in autumn 2020 were used for testing the effect of the dietary change. Subjects served as their own control to exclude possible confounding factors, e.g. dominance rank, genetics, etc.

Prior to the health checks, individuals were fasted overnight, while water was freely available throughout the night. At the assessment in spring 2018 (before dietary change), individuals were sedated with an intramuscular injection of ketamine (10 mg/kg, Ketamine 10%; Alfasan, Woerden, the Netherlands). There was a subsequent change in the routine anesthesia protocol for the benefit of the animals. In autumn 2020 (after dietary change), monkeys were thus sedated with a combination of ketamine (10 mg/kg, Ketamine 10%; Alfasan, Woerden, the Netherlands) and medetomidine (0.05 mg/kg, Sedastart; AST Farma, Oudewater, the Netherlands) IM, which was reversed after the procedures with atipamezole (0.25 mg/kg, Sedastop; AST Farma, Oudewater, the Netherlands) administration IM. Medetomidine induces muscle relaxation and results in mild hyperglycemia (Nishimura et al. 1994; Greene 1999; Kanda & Hikasa 2008).

As part of the health check, body weight and height were determined, as described earlier (Sterck et al. 2019). Briefly, a standard scale was used to measure body weight to the nearest 0.1 kg. Height was measured as crown–rump length by placing the monkeys on their back on a measuring mat (SECA, Hamburg, Germany). Height was measured to the nearest 0.1 cm. Body weight and height were used to calculate a species-specific weight-for-height index (hereafter referred to as WHI). WHI was calculated as weight (in kilograms) divided by height (in meters) to the power of 2.7 (WHI2.7 in Sterck et al. 2019). This measure of relative adiposity was preferred over solely using body weight as the latter does not take into account individual variation in height. Although all females were full-grown and skeletally matured, height was highly variable (range: 40.1–47.7 cm). We determined overweight status and individuals were considered overweight when their WHI exceeded the upper boundary of 62 kg/m$^{2.7}$ (Sterck et al. 2019).

Furthermore, blood samples were collected for complete blood count and blood chemistry. The samples were analyzed for fructosamine (umol/L), HbA1c (%), total cholesterol (mmol/L) and triglyceride (mmol/L) levels using a Cobas Integra 400 plus (Roche Diagnostics, Rotkreuz, Switzerland). Blood samples were collected from the vena femoralis into EDTA and serum tubes (Vacuette, Greiner Bio-One international GmbH, Alphen aan den Rijn, the Netherlands), left for 30 min and centrifuged at 3000 rpm for 10 min. Afterwards, the remaining serum was transferred to polypropylene tubes and stored below −20 °C.

All biochemical parameters, except for triglyceride after dietary change, were analyzed on the same day of the sample collection. Triglyceride levels after dietary change were analyzed roughly five months after sample collection. A correction was applied to the data as triglyceride levels in serum are only stable up until three months when stored at −20 °C (Finley & Tietz 1996). The correction was based on the regression equation between triglyceride levels after five months and deviation from the actual value from thirty samples for which the original triglyceride values were available (Appendix B).

Data analyses

Statistical testing was performed in IBM SPSS Statistics version 26. The effect of dietary change on body weight, WHI, and biochemical parameters was tested with a paired samples t-test or Wilcoxon signed ranks test. Normal distribution of the data was checked with the Shapiro–Wilk test. Pearson and Spearman correlations were used to test the association between age and WHI and associations between the different biochemical parameters. Linear regression analyses were used to evaluate whether age and WHI affected delta WHI, fructosamine, HbA1c, cholesterol, and triglyceride levels. Normality and homoscedasticity of the residuals were visually checked. Delta WHI was calculated as WHI after dietary change (in 2020) minus WHI before dietary change (in 2018). Finally, Fisher's exact test was used to compare the proportion of overweight individuals before and after dietary change. Descriptive statistics in the results are reported as mean ± SE. The level of significance was $\alpha = 0.05$ and all tests were two-tailed.

Results

Relative adiposity and overweight status

Mean body weight was 5.5 ± 0.20 kg before dietary change and 5.4 ± 0.19 kg after dietary change, which is not a statistically significant difference (paired samples t-test, $t = 0.959$, $n = 44$, $p = 0.343$). Similarly, WHI did not differ after dietary change (paired samples t-test, $t = 0.991$, $n = 44$, $p = 0.327$; Figure 1). WHI was independent of age in our study population both before (Spearman correlation, $r = 0.180$, $n = 44$, $p = 0.242$) and after dietary change (Spearman correlation, $r = 0.053$, $n = 44$, $p = 0.731$).

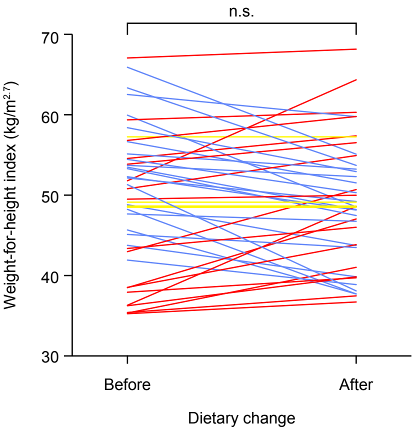

Figure 1. Effect of dietary change on weight-for-height index (WHI) in adult female long-tailed macaques. Each line represents an individual (n = 44). Blue lines represent a decrease, while red lines indicate an increase and yellow lines represent no change. n.s.: $p \geq 0.05$.

Delta WHI was independent of age (F(1,41) = 0.689, p = 0.411), but was significantly associated with baseline WHI (F(1,41) = 11.731, p = 0.001). Delta WHI was significantly higher in individuals with a low baseline WHI, implying that WHI increased in relatively lean individuals, while WHI decreased in relatively heavy individuals after dietary change (Figure 2).

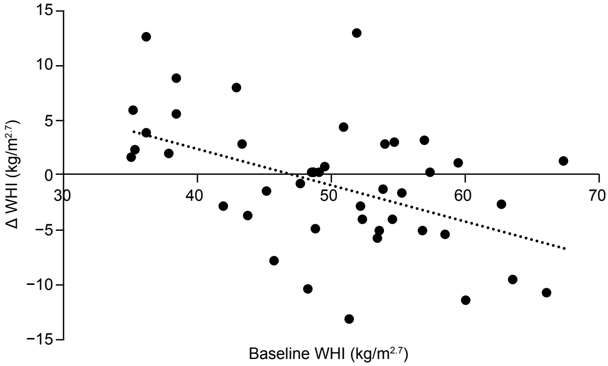

Figure 2. Delta weight-for-height index (Δ WHI) plotted against the baseline WHI in adult female long-tailed macaques that were subjected to a minor dietary change.

The year before dietary change, four individuals (9.1%) had WHIs above the upper boundary for overweight, while only two individuals (4.5%) were overweight after dietary change. Overweight status did not significantly differ before or after dietary change though (Fisher's exact test, p = 0.676).

Biochemical parameters

Table 3 shows descriptive statistics on fructosamine, HbA1c, cholesterol, and triglyceride levels before and after dietary change. Correlations between the different biochemical parameters were weak or absent (Table A2).

Table 3. Descriptive statistics on biochemical parameters related to glycemic response and lipid metabolism before and after dietary change. n = 44. Mean ± SE (minimum–maximum) are reported. * $p < 0.05$.

	Before dietary change	After dietary change
Fructosamine (umol/L)	187 ± 2.3 (153–220)	171 ± 2.5 (134–213) *
HbA1c (%)	4.25 ± 0.04 (3.66–4.95)	4.29 ± 0.04 (3.48–4.90)
Cholesterol (mmol/L)	3.10 ± 0.08 (1.95–4.52)	2.84 ± 0.10 (1.62–4.16) *
Triglyceride (mmol/L)	1.02 ± 0.11 (0.24–3.68)	1.05 ± 0.09 (0.33–3.51)

Glycemic response

Fructosamine levels were independent of age and WHI both before (F(1,41) = 0.053, p = 0.820; F(1,41) = 1.520, p = 0.225) and after dietary change (F(1,41) = 1.698, p = 0.200; F(1,41) = 0.026, p = 0.873). After the dietary change, fructosamine levels were significantly reduced (paired samples t-test, t = 7.060, n = 44, $p < 0.0005$; Figure 3a).

WHI had no significant influence on HbA1c levels before (F(1,41) = 0.057, p = 0.812) or after dietary change (F(1,41) = 1.214, p = 0.277). Age did not affect HbA1c levels before dietary change (F(1,41) = 0.680, p = 0.414), yet age was positively associated with HbA1c levels after dietary change (F(1,41) = 7.261, p = 0.010), i.e. older individuals had higher HbA1c levels. The regression equation indicated that HbA1c values increased 0.028% per year of age ($R^2 = 0.174$). Dietary change had no significant effect on HbA1c levels (paired samples t-test, t = −0.759, n = 44, p = 0.452; Figure 3b).

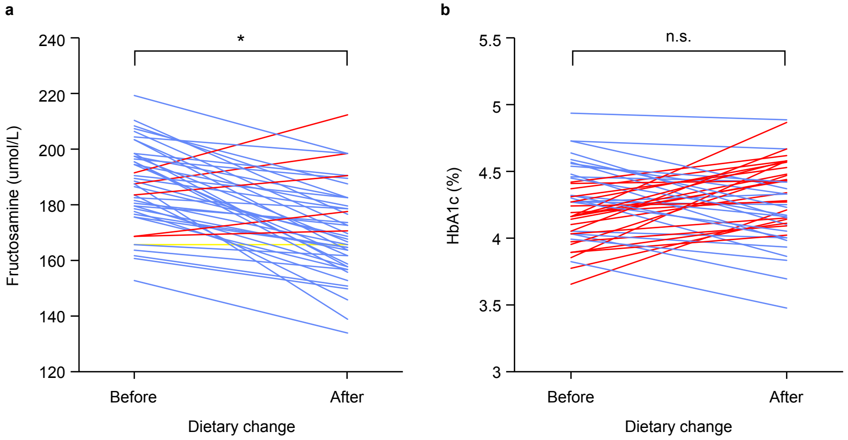

Figure 3. Effect of dietary change on fructosamine (**a**) and HbA1c levels (**b**) in adult female long-tailed macaques. Each line represents an individual (n = 44). Blue lines represent a decrease, while red lines indicate an increase and yellow lines represent no change. * $p < 0.05$, n.s.: $p \geq 0.05$.

Lipid metabolism

Cholesterol levels were independent of age and WHI before (F(1,41) = 0.164, p = 0.687; F(1,41) = 0.257, p = 0.615) and after dietary change (F(1,41) = 0.082, p = 0.775; F(1,41) = 2.567, p = 0.117). Cholesterol levels were significantly reduced after dietary change (paired samples t-test, t = 3.971, n = 44, $p < 0.0005$; Figure 4a).

Triglyceride levels were independent of WHI before (F(1,41) = 0.793, p = 0.378) and after dietary change (F(1,41) = 3.353, p = 0.074), while triglyceride levels significantly increased with age before (F(1,41) = 7.146, p = 0.011) and after dietary change (F(1,41)

= 5.491, p = 0.024). The regression equations showed that triglyceride levels increased with every additional year of age with 0.072 mmol/L before dietary change ($R^2 = 0.177$) and 0.051 mmol/L after dietary change ($R^2 = 0.181$). Triglyceride levels were not significantly different after dietary change (Wilcoxon signed ranks test, Z = −0.604, n = 44, p = 0.546; Figure 4b).

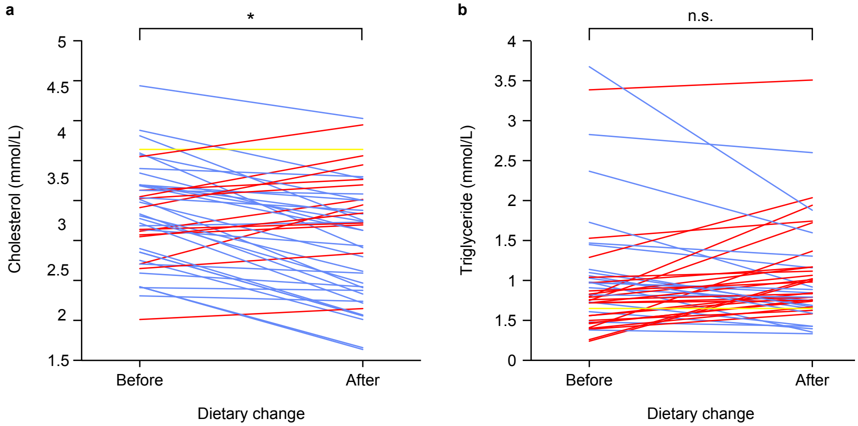

Figure 4. Effect of dietary change on cholesterol (**a**) and triglyceride levels (**b**) in adult female long-tailed macaques. Each line represents an individual (n = 44). Blue lines represent a decrease, while red lines indicate an increase and yellow lines represent no change. * $p < 0.05$, n.s.: $p \geq 0.05$.

Discussion

This study evaluated, based on electronic health records, the effect of a minor dietary change on relative adiposity and overweight-related health parameters in non-diabetic, group-housed, female long-tailed macaques. Relative adiposity and biochemical parameters related to glycemic response and lipid metabolism were compared before and after the supplementary provisioning of bread was replaced by grains and vegetables. Relative adiposity and overweight status did not differ after dietary change. Yet, relatively heavy individuals generally lost body weight, while relatively lean individuals gained body weight, leading to a more balanced body weight dynamic. Dietary change had no effect on HbA1c and triglyceride levels, while fructosamine and cholesterol levels were significantly reduced. Thus, the minor dietary change had no significant effect on overweight status but had a positive effect on some biochemical parameters related to the risk of T2DM and cardiovascular disease.

Relative adiposity and overweight status

Body weight, WHI, and overweight status did not differ after dietary change. Based on the increased fiber content, a reduction in relative adiposity was expected. Fiber intake increases satiety and decreases the feeling of hunger after a meal, which results in reduced energy intake, even when food is available *ad libitum* (Howarth et al. 2001). The importance of fiber in the diet of captive primates is increasingly being recognized and the provisioning of browse (e.g. willow twigs), which is high in fiber, is therefore often recommended and becoming more popular in zoos and other institutions (Nijboer & Dierenfeld 1996; Campbell et al. 2001). A reduction in body weight after transitioning to a high-fiber diet was found in a vervet monkey (*Chlorocebus aethiops sabaeus*) breeding colony (Fairbanks et al. 2010), but not in our study. However, the relative increase in fiber content was almost tenfold higher in the vervet monkey study (140%) compared to our study (15.4%). Higher fiber contents may be needed for relative adiposity to decrease overall.

Nevertheless, dietary change had a differential effect on relative adiposity of individual animals, depending on their baseline value. Relatively lean individuals gained body weight, while relatively heavy animals generally lost body weight after dietary change. This finding may be explained by an unexpected secondary effect of the dietary change. Although food intake was not measured in this study, this finding suggests that dietary change resulted in a different distribution of food among group members. Wheat bread was easy to monopolize, resulting in some individuals obtaining several slices, while others obtained none (personal observation, cf. Wilson et al. 2008; Overduin-de Vries et al. 2020). In contrast, grains, maize silage, and leafy vegetables were likely divided more equally, as these items were spread through and/or in front of the cages. The more equal distribution of these food items may have led to relatively lean individuals obtaining more food than before, thus gaining body weight, while relatively heavy individuals obtained less food, thereby losing body weight. Even though relative adiposity did not decrease overall, dietary change had a differential effect on individual animals resulting in a more balanced body weight dynamic.

Relative adiposity was not related to any of the biochemical parameters in this study, while other studies found several associations between being overweight and indicators of glycemic response and lipid metabolism in macaques. Cholesterol and triglyceride levels are generally higher in obese male and female rhesus macaques (*Macaca mulatta*) compared to their non-obese counterparts (Hamilton et al. 1972; Kemnitz & Francken 1986; Kemnitz et al. 1989). Body weight is also positively correlated with triglyceride and glucose levels in adult female long-tailed macaques (Chen et al. 2002). These studies often included highly obese subjects with body fat accounting for up to 61% of their body weight (Kemnitz & Francken 1986). This body fat percentage would equal a body weight of roughly 12.65 kg (Raman et al. 2005), while the heaviest monkey in our study initially weighed 9.15 kg. Accordingly, no effect of body weight on cholesterol, triglyceride, or glucose levels is found in long-tailed macaques with relatively low body weights (Schuurman & Smith 2004). Thus, the relatively low overweight prevalence and little variation in relative adiposity between individuals in our study may explain the lack of significant associations between WHI and biochemical parameters.

The absence of these relationships implies that this long-tailed macaque population is generally healthy regarding overweight-related health parameters. All biochemical parameters, i.e. fructosamine, HbA1c, cholesterol, and triglyceride levels, also fit well within previously reported ranges for this species (Yoshida et al. 1986; Cefalu et al. 1993; Schuurman & Smith 2004; Koga et al. 2005; Wagner et al. 2006; Marigliano et al. 2011; Xie et al. 2013; Fernandes 2018). However, this does not mean that overweight-related health problems do not occur in our breeding colony.

Glycemic response

Since a high-GI food (bread) was replaced with low/medium-GI foods (grains and vegetables), the glycemic response was expected to improve, thereby reducing the risk of T2DM. Glycemic response was measured using fructosamine and HbA1c levels, which produced different results regarding the effect of dietary change. Fructosamine levels decreased, while HbA1c levels showed no significant difference after dietary change. These results may be explained by the difference in sensitivity of albumin and hemoglobin to bind to glucose. Fructosamine is formed when plasma glucose binds to albumin, while HbA1c results from glycation of hemoglobin (Williams-Fritze et al. 2011). Albumin has been suggested as being more sensitive to postprandial glycemic variation compared to hemoglobin and therefore larger alterations in blood glucose would be needed to affect HbA1c levels similar to fructosamine levels (Day et al. 1980; Ryle et al. 1990). As a result, varying fiber and glucose intake in humans does not affect HbA1c, but significantly influenced fructosamine levels (Ryle et al. 1990). Similarly, fructosamine levels differ between long-tailed macaques fed a standard or high-fat diet, while no difference is found in HbA1c (Cefalu et al. 1993). These findings are consistent with the outcome of our study. Although no significant effect on HbA1c levels was found, the decrease in fructosamine suggests that the dietary change had a positive impact on glycemic response. As the decrease in fructosamine was observed across the study population, this was likely a primary effect of dietary change and independent of the potentially new food distribution.

A third possible biochemical parameter to quantify glycemic response is plasma glucose concentration. Although plasma glucose levels were measured, a fair comparison was not possible as medetomidine is known to affect glucose levels and this was added to the anesthesia protocol (Nishimura et al. 1994; Kanda & Hikasa 2008). Moreover, plasma glucose levels provide information about instant glucose levels, while fructosamine levels reflect blood glucose levels from the past two to three weeks and HbA1c represents the previous two to three months (Williams-Fritze et al. 2011). Fructosamine and HbA1c are thus more suitable parameters to detect long-term changes in glycemic response as they reflect glucose levels over a longer period.

Nevertheless, there was no significant association between fructosamine and HbA1c levels. Cefalu et al. (1993) found that fructosamine and HbA1c are significantly correlated ($r = 0.61$) in a long-tailed macaque population, which included both diabetic and non-diabetic monkeys. Fructosamine and HbA1c also correlate well in diabetic humans ($0.55 < r < 0.88$; Cockram et al. 1990; Braatvedt et al. 1997; Narbonne et al. 2001; Cohen et al. 2003), but no correlation has been found in non-diabetic humans ($r = 0.01$; Davidson & Schriger 2010). Our study included only non-diabetic individuals, which might explain the lack of correlation between fructosamine and HbA1c.

Furthermore, HbA1c levels increased with age after dietary change, but this was not found before dietary change. HbA1c levels are also positively associated with age in non-diabetic humans (Yang et al. 1997; Davidson & Schriger 2010; Roth et al. 2016), but not in other studies with macaques and squirrel monkeys (*Saimiri* species; Cutler et al. 1992; Fernandes 2018). Since higher age is a risk factor for the development of T2DM in both humans and primates (Wagner et al. 2006; Jones et al. 2014), the link between age and HbA1c and their relation to T2DM in primates may need further investigation.

Lipid metabolism

Multiple studies show that an increased fiber intake has a positive effect on lipid metabolism, i.e. leads to reduced total cholesterol, LDL and triglyceride levels, in humans and rats (Nishina & Freedland 1990; Hannon et al. 2019). Especially water-soluble fibers seem to have this cholesterol-lowering effect in humans (Haskell et al. 1992). Therefore, it was expected that the serum cholesterol and triglyceride levels would decrease after the dietary change. In line with this expectation, cholesterol levels decreased after dietary change. In contrast, triglyceride levels showed no significant difference after dietary change.

In the present study, higher triglyceride levels were found in older individuals compared to younger monkeys both before and after the dietary change. Similar age-effects have been reported in other studies with both long-tailed macaques and rhesus macaques (Yoshida et al. 1986; Smucny et al. 2001; Xie et al. 2013; Yu et al. 2019). Possibly, no significant effect of dietary change on triglyceride levels was found because the effect of dietary change was counteracted by an age-effect. Furthermore, triglyceride levels after dietary change had to be corrected due to the period between blood sample collection and analysis. This correction may have introduced some bias, thereby reducing reliability of the triglyceride data.

Altogether, the minor dietary change had a beneficial effect on at least one of the two biochemical parameters related to cardiovascular disease.

Conclusion

This study evaluated in retrospect the effect of a minor dietary change on relative adiposity and overweight-related health parameters in non-diabetic, group-housed, female long-tailed macaques. The basic diet of monkey chow pellets remained the same, while the supplementary provisioning of bread was replaced by grains and vegetables. Although this minor dietary change had no significant effect on overweight status, dietary change had a differential effect on individual animals resulting in a more balanced body weight dynamic. Also, some biochemical parameters related to the risk of diabetes and cardiovascular disease were positively affected. These results emphasize the importance of evaluating husbandry changes and shows that critically reviewing husbandry practices can provide valuable insights to improve animal health and welfare.

Appendix

Appendix A

Table A1. Nutritional values of the 10 most commonly fed fruit and vegetables at BPRC (in alphabetical order) based on the amount fed per adult: 120 g. Source: The Dutch Food Composition Database (NEVO, online version 2019/6.0).

Fruit/vegetable	Energy (kcal)	Protein (gram)	Carbohydrates (gram)	Fiber (gram)	Fat (gram)
Banana	110	1.32	24.0	2.28	0.36
Bell pepper	30	0.84	4.68	3.00	0.24
Cabbage	43	2.88	4.92	4.92	0.24
Chicory	23	1.56	2.88	1.44	0.24
Chinese cabbage	20	1.20	2.40	3.00	0.00
Cucumber	16	0.84	1.56	0.72	0.48
Endive	19	1.80	1.20	2.04	0.36
Leek	34	1.80	4.20	3.60	0.24
Lettuce	16	1.68	0.36	1.44	0.48
Tomato	24	0.84	3.48	1.56	0.48

Table A2. Correlations between different biochemical parameters in adult female long-tailed macaques (n = 44). The type of correlation, correlation coefficient (r), and significance (p) are reported[1].

	Fructosamine (umol/L)	HbA1c (%)	Cholesterol (mmol/L)	Triglyceride (mmol/L)
Fructosamine (umol/L)	X	Pearson, r=0.025, p=0.873	Pearson, r=0.229, p=0.135	Spearman, r=0.205, p=0.182
HbA1c (%)	Pearson, r=0.233, p=0.127	X	Pearson, r=-0.370, p=0.014	Spearman, r=0.071, p=0.646
Cholesterol (mmol/L)	Pearson, r=-0.223, p=0.145	Pearson, r=0.035, p=0.822	X	Spearman, r=-0.010, p=0.950
Triglyceride (mmol/L)	Spearman, r=0.212, p=0.167	Spearman, r=0.220, p=0.151	Spearman, r=-0.012, p=0.940	X

[1] The numbers in the lower left triangle represent correlations before dietary change, while the upper right triangle contains the correlations after dietary change.

Appendix B

Triglyceride samples were analyzed roughly five months after blood collection, while triglyceride is only stable for three months when stored at −20 °C (Finley & Tietz 1996). To check the reliability of the triglyceride levels analyzed after five months, we used 30 samples for which the original triglyceride values (i.e. analyzed on the same day as blood collection) were available. These samples were re-analyzed after being stored at −20 °C for five months. We then calculated the deviation between triglyceride levels analyzed on the same day as blood collection and triglyceride levels analyzed five months after blood collection. All values after five months appeared to be equal to or higher than the original value. The deviation depended on the actual triglyceride level with deviation being higher in samples with lower triglyceride levels (Figure A1). The data resulted in the following regression equation: $y = -0.046x + 0.1481$, with y being the deviation and x being the triglyceride concentration after five months. This equation was used to correct the data.

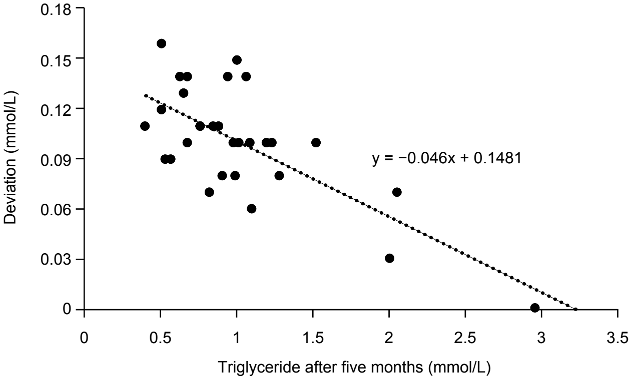

Figure A1. Deviation in triglyceride levels plotted against the triglyceride levels when analyzed after being stored at −20 °C for five months (n = 30).

Chapter 7

Overweight management through mild caloric restriction in multigenerational long-tailed macaque breeding groups

Dian G. M. Zijlmans, Annemiek Maaskant,
Annet L. Louwerse, Elisabeth H. M. Sterck,
Jan A. M. Langermans

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Abstract

Caloric restriction (CR) is an effective method to reduce overweight in captive non-human primates (NHPs). CR has been applied to individually- and pair-housed NHPs, but whether applying CR can be effective and safe in group-housed NHPs has not yet been assessed. This study investigates the effect of mild (20%) CR on adult overweight and biochemical parameters, immature growth, veterinary consultations, and reproductive success in multigenerational long-tailed macaque (*Macaca fascicularis*) breeding groups. Data were derived from anthropometric measurements and blood samples during yearly health checks, complemented with retrospective data on veterinary consultations and reproductive success. Adult body measures decreased after CR, with heavier individuals and females losing more weight compared to leaner individuals and males. CR lowered cholesterol levels in adults but had no overall effect on other biochemical parameters. Yet, biochemical parameters of individuals with high baseline values were reduced more compared to individuals with low baseline values. Immature growth, veterinary consultations and reproductive success were not influenced by CR. Thus, CR targeted the right individuals, i.e. overweight adults, and had no adverse effects on the variables examined in this study. This implies that mild CR can be a valuable overweight management strategy in group-housed NHPs.

Introduction

Non-human primates (NHPs) are susceptible to developing overweight in captivity, even when maintained on a nutritionally balanced diet (West & York 1998). Many primate facilities feed their social groups *ad libitum* or provide a predetermined amount of food that is available throughout the day (Bauer et al. 2010). This way of feeding has likely contributed to the increasing overweight prevalence among group-housed NHPs. In research facilities, overweight prevalence of group-housed NHPs roughly varies between 10 and 30% of the individuals (Crumley & Raleigh 1995; Hamada et al. 2003; Yue et al. 2016, 2017; Sterck et al. 2019). This overweight prevalence may continue to rise, as little research and information are available on overweight prevention and treatment in group-housed NHPs (Bauer et al. 2011). Primate facilities should remedy overweight, as overweight can cause similar health problems in NHPs as in humans, e.g. type 2 diabetes mellitus and cardiovascular disease (Kemnitz & Francken 1986; Shively & Clarkson 1987; Hansen & Bodkin 1993).

An effective method to prevent overweight and overweight-related health problems in NHPs is caloric restriction (CR), i.e. a nutritional intervention that reduces caloric intake without malnutrition (Hansen & Bodkin 1993; Pifferi et al. 2019). Beneficial effects of CR on health- and lifespan have been found in several NHP species, among others in long-tailed macaques (*Macaca fascicularis*: Cefalu et al. 2004), rhesus macaques (*Macaca mulatta*: Hansen & Bodkin 1993; Colman et al. 1999a), squirrel monkeys (*Saimiri sciureus*: Ingram et al. 1990) and grey mouse lemurs (*Microcebus *murinus*: Pifferi et al. 2018). The animals in these studies were housed individually or in pairs due to the experimental need to control and quantify food intake. One study performed CR in group-housed NHPs and found that baboon mothers (*Papio* spp.) subjected to CR during pregnancy weighed less than *ad libitum* fed control mothers (Rodriguez et al. 2012). However, these baboons were trained to feed in individual cages (Schlabritz-Loutsevitch et al. 2004), impeding social interactions around food intake. Although CR seems to be a promising method to prevent and decrease overweight without separating individuals from their social group, its use has not yet been validated for NHPs housed and fed in social groups.

Some researchers argue that CR may not be appropriate or feasible in social groups (Bauer et al. 2011), because food is often not equally divided among group members (e.g. Barton & Whiten 1993; Wilson et al. 2008). This is especially important in multigenerational breeding groups, because immatures need enough energy to enable growth. CR reduces the number of calories and therefore has the potential side-effect to restrict immature growth. Growth rates of immature yellow baboons (*Papio cynocephalus*) are lower in completely wild-feeding than in partially food-enhanced (i.e. 4.9 g/day vs. 8.7 g/day) social groups (Altmann & Alberts 2005). Offspring growth was also slower in grey mouse lemur infants of calorie restricted mothers compared to infants reared by *ad libitum* fed mothers (Canale et al. 2012). Thus, CR may slow immature growth.

CR may also negatively affect other, especially low-ranking, individuals, as CR likely increases feeding competition. High-ranking individuals typically have priority of access to preferred resources such as food (Boccia et al. 1988; Barton & Whiten 1993; Overduin-de Vries et al. 2020). They can fulfil their energetic requirements during CR by monopolizing food, while low-ranking individuals cannot. In wild Japanese macaques (*Macaca fuscata*), metabolizable energy intake was lower in low-ranking females compared to high-ranking females in times of food scarcity, resulting in energy shortage and three lower-ranking females dying (Tsuji & Takatsuki 2012). Besides, several studies have shown that aggressive behaviour of NHPs increases when the availability and distribution of food are restricted (Belzung & Anderson 1986; Boccia et al. 1988). CR may therefore result in more veterinary consultations as competition can potentially lead to severe weight loss and increased number of injuries or even mortality.

Besides maintaining stable groups and maximizing the animals' health and welfare, another important aspect of managing NHP breeding colonies is to produce offspring. Reproduction is energetically costly and reproductive success is sensitive to a limited food supply (Wade & Schneider 1992). After periods of drought and extreme heat, when food becomes scarce, wild female yellow baboons were less likely to cycle, conceive and give birth to living infants (Beehner et al. 2006). Similarly, in wild Japanese macaques, only one (high-ranking) female produced offspring after a food-scarce season, while twelve females did so when there was no food scarcity (Tsuji & Takatsuki 2012). In wild long-tailed macaques, increased food supply during highly productive mast years resulted in higher birth rates and earlier conception (van Schaik & van Noordwijk 1985). In captive studies, CR inhibited ovulation in rhesus macaques (Lujan et al. 2006), and even induced pregnancy loss in common marmosets (*Callithrix jacchus*: Tardif et al. 2004). Thus, CR may lead to reduced reproductive success, which would be an undesirable outcome.

To the authors' knowledge, it is currently unknown whether applying CR can be effective and safe in group-housed NHPs. CR should ideally target overweight adults, while having no adverse effects on other group members. We investigate the effect of mild (20%) CR on adult overweight and biochemical parameters, immature growth, veterinary consultations, and reproductive success in multigenerational long-tailed macaque breeding groups, thereby aiming to determine the suitability of CR as an overweight management strategy in group-housed NHPs. Anthropometric measurements and blood samples were collected during annual health checks. Adult overweight and immature growth were derived from body weight and height measurements. Blood samples were analyzed for several biochemical parameters related to lipid metabolism and glycemic response. Veterinary consultations and reproductive success were derived in retrospect from the year-round electronic health records.

Materials and methods

Animals and housing

Ten multigenerational long-tailed macaque (*Macaca fascicularis*) breeding groups from the Biomedical Primate Research Centre (BPRC) in Rijswijk, the Netherlands, were examined for this study. The breeding colony housed approximately 200 animals (10–40 animals per group) throughout the study period, ranging between 0 and 24 years of age. Natural group dynamics were adhered to when the groups were formed, i.e. females are philopatric and males leave the natal group at puberty. The multigenerational groups consisted of adult females from several matrilines with their (mature) offspring and one (or two) unrelated breeding male(s). Breeding males were almost continuously present, except for some short periods between the removal of an old breeding male and the introduction of a new one. Not every individual in the breeding colony was included in all statistical analyses due to varying reasons (see *Data analyses*).

The breeding groups were housed in enriched enclosures with free access to both indoor (±72 m^2 and 2.85 m high) and outdoor (±250 m^2 and 3.1 m high) compartments. The indoor compartments contained sawdust, while the outdoor compartments had a sand floor where natural plant growth was possible. The enclosures contained several climbing structures, fire hoses, car tires, beams, sitting platforms and an outside swimming pool (Vernes & Louwerse 2010). Water was available throughout the day from automated water dispensers. Experienced ethologists and colony managers regularly observed dominance interactions in the groups and assigned rank categories to all females based on their knowledge and experience with the groups. Females were categorized as being high-ranking (H), middle-ranking (M) or low-ranking (L) within their social group. Roughly one-third of each group was assigned to each category, while taking into consideration the matrilineal structures. Adult breeding males were always considered high-ranking.

Diet and caloric restriction

The daily diet of the macaques started with monkey chow (Primates/NHP–pellets, Ssniff, Soest, Germany) in the morning. The amount of monkey chow was calculated using the basal metabolic rate (BMR), which depended on the age, sex and body weight of each individual (National Research Council 2003). The monkey chow provided all the daily required energy per individual and was provided in multiple feeding trays per group (i.e. typically six trays per group, but at least one tray per five animals with a minimum of two trays) to prevent monopolization. In addition, fresh fruit, vegetables, bread or a grain mixture were presented in the afternoon. For fruit and vegetables, 150 g was prepared for each individual aged four years and older, and 100 g for individuals between one and three years old. One slice of bread and 20 g of the grain mixture were counted per animal. Food enrichment and treats were provided occasionally. The daily diet was accessible for all group members. In this situation, overfeeding likely occurred as the monkey chow provided all required energy per individual and other items were provided in addition.

Due to the relatively high prevalence of overweight in the breeding colony (i.e. 22%), the veterinary team decided to initiate mild CR in August 2017, reducing the provided number of calories by 20% (Table 1). The amount of monkey chow based on the BMR was multiplied by 0.8 to obtain the new amount per individual. Since the feeding trays regularly contained left-over pellets the following morning before CR (Stalknecht 2016), we consider 20% to be a reasonable reduction, while still providing enough food to meet energetic requirements and prevent malnutrition. Additionally, the amount of fruit and vegetables was reduced from 100/150 g to 80/120 g per individual and the grain mixture from 20 g to 15 g per individual. The number of bread slices remained equal after CR.

Table 1. The amount of food per individual in the diet of the long-tailed macaques at the Biomedical Primate Research Centre (BPRC) before and after mild caloric restriction (CR) was initiated in August 2017. The variation reflects variation in the amount of food for individuals of different ages and body weights.

Food item	Before CR	After CR
Monkey chow	141 ± 36 g (range 79–294)	115 ± 37 g (range 65–265)
Fruit and vegetables	100/150 g	80/120 g
Bread	1 slice (~30 g)	1 slice (~30 g)
Grain mixture	20 g	15 g

Data collection

Health checks

Anthropometric measurements and blood samples were collected once per year during annual health checks. These health checks are a routine veterinary procedure related to the regular health management of the colony (cf. Balansard et al. 2018). Individuals were fasted overnight and sedated with an intramuscular injection of ketamine (10 mg/kg, Ketamine 10%; Alfasan, Woerden, the Netherlands) prior to the health check. Individuals younger than eight months old were not sedated and were left with their mother during the entire procedure (if possible). The health checks before CR took place in November 2016 or spring 2017, while the health checks after CR took place in spring 2018.

Anthropometric measurements of full-grown adults and immatures were collected as previously described (Sterck et al. 2019). Body weight was measured with a standard scale. Height was measured as crown-rump length with the animal in a supine position using a measuring mat for human infants (SECA, Hamburg, Germany). Abdominal circumference was measured at the level of the umbilicus with the animal in a supine position using a flexible tape measure. Body weight was measured for all individuals, whereas height and abdominal circumference could not always be accurately measured, e.g. when individuals woke up from their sedation. Body weight, height and abdominal circumference were measured to one decimal point. Overweight status of adults was based on a species-specific weight-for-height index, known as WHI2.7 (Sterck et al. 2019), hereafter referred to as WHI. This was calculated as weight in kilograms divided by height in meters to the power of 2.7. WHI of group-housed long-tailed macaques ideally ranges between 39 and 62 kg/m$^{2.7}$ (Sterck et al. 2019). Individuals with a WHI below the lower boundary were considered underweight, while exceeding the upper WHI boundary resulted in overweight.

Blood samples of full-grown adults were collected from the vena femoralis and analyzed for total cholesterol (mmol/L), triglyceride (mmol/L), glucose (mmol/L), fructosamine (umol/L) and glycated hemoglobin (HbA1c; %) levels using a Cobas Integra 400 plus (Roche Diagnostics, Rotkreuz, Switzerland). One female had no blood sample available after CR, so she was not included in the analyses on biochemical parameters. Due to technical issues, the HbA1c values determined in spring 2017 were not comparable to the other values, reducing the sample size for this analysis.

Health and reproductive records

Animal caretakers monitored the animals twice daily (during the feeding rounds) and reported births and abnormalities in a digital database, which contained the health records of all animals that live at the BPRC. Cases of severe weight loss that required veterinary consultation were obtained in retrospect from the database. The proportion of individuals with severe weight loss before and after CR was calculated as the number of individuals with severe weight loss per year divided by the total size of the breeding colony. Furthermore, we calculated the proportion of individuals with injuries that required veterinary consultation, one year before and after CR. Injuries were included when the integumentary system of the animal was affected, e.g. lacerations, and a veterinarian consult was required. The necessity of a consultation is determined according to a standardized protocol. Finally, we examined cases of mortality when the integumentary system of the animal was affected after a suspected or observed conflict. However, the number of mortality cases was too limited for statistical analyses.

Reproductive success was measured using the proportion of pregnant females and the proportion of successful pregnancies in the year before and after CR. The number of females that conceived was calculated backwards from the number of births based on an average pregnancy length of 164 days (Honjo et al. 1978). The proportion of pregnant females per year was calculated as the number of females that conceived divided by the total number of fertile females, i.e. females that were sexually mature (>3 years old) and not on contraceptives. Pregnancy outcome was considered successful when the offspring was born alive. The proportion of successful pregnancies before and after CR were calculated by dividing the number of infants born alive by the total number of pregnancies per year.

Data analyses

The effectiveness of CR was tested by comparing anthropometric measurements and biochemical parameters of full-grown adults (females > 6 years old, males > 8 years old; Schwartz & Kemnitz 1992) before and after CR. To exclude possible confounding factors, adults were used as their own control in a paired design. Some adult males were added or removed from the breeding colony during the study period to prevent inbreeding, so there was limited paired data for adult males. Females that were pregnant during one or both health checks (n = 19) were excluded as pregnancy is known to affect body fat levels (Schwartz & Kemnitz 1992). Six (pre-)diabetic individuals were excluded to prevent possible confounding effects of disease progression on our outcome measures. Eventually, 41 adult females and five adult males aged between 6 and 22 years old were included in the pairwise comparisons.

The effectiveness of CR on body measures and biochemical parameters of full-grown adults was tested with a paired samples t-test or Wilcoxon signed ranks test. A Shapiro–Wilk test was used to test whether the data were normally distributed. A linear regression analysis checked which adult individuals were affected most by CR. The effect of CR, i.e. delta of a measure, was calculated by subtracting the value before CR (in 2016/2017) from the value after CR (in 2018). We tested whether an individual's age, sex and baseline value were related to delta WHI, cholesterol, triglyceride, glucose, fructosamine and HbA1c. Breeding group was included as a random factor in all models, and normality and homoscedasticity of the residuals were visually checked. Whether female dominance influenced delta WHI and overweight prevalence before and after CR was tested with a one-way ANOVA and Fisher's exact tests, respectively.

In addition, we checked whether CR had adverse effects on immature growth, veterinary consultations, and reproductive success. Immature growth was derived from cross-sectional body weight and height data of 111 immatures aged between eight months and four years old. Some immatures contributed to the dataset twice (n = 58), while others were included only once (n = 53), depending on their age. Body weight of three-year old females was excluded when pregnant (n = 6). Linear regression analyses checked whether age, sex and CR affected immature body weight and height. Interaction effects were included in the model when they had a significant impact on the outcome measure, and breeding group was included as a random factor.

Finally, data from the health and reproductive records were examined. Whether the number of individuals with severe weight loss relative to the total breeding colony size (n = 196 before CR, n = 211 after CR) differed before or after CR was tested with Fisher's exact test. A chi-square test was used to test the proportion of individuals with injuries that required veterinary consultation before and after CR. The effect of CR on female reproduction was tested by comparing the proportion of pregnant females and the proportion of successful pregnancies before and after CR. Only fertile females were included in these analyses (n = 74 before CR, n = 87 after CR). Chi-square tests and a Fisher's exact test were used to examine the effects of CR and female dominance on likelihood of conception and pregnancy outcome.

Data analyses were performed in SPSS version 28 and R studio version 1.2.5. All tests were two-tailed and a value of $p < 0.05$ was considered significant, but trends ($0.05 \leq p < 0.10$) are also reported. Descriptive statistics are presented in the results as mean ± SE.

Animal ethics

Ethical approval for this study was waived by BPRC's Animal Welfare Body (Instantie voor Dierenwelzijn). The research involved less than minimal discomfort to the animals used in this manuscript according to the definition of an animal experiment as defined by the European Directive 2010/63/EU. All procedures performed in this study complied with the applicable international, national and institutional guidelines and regulations.

Results

Adult overweight

Body weight of full-grown adults significantly decreased from 6.7 ± 0.2 kg before CR to 6.3 ± 0.2 kg after CR (Wilcoxon signed ranks test, $Z = -3.533$, $n = 46$, $p < 0.0005$). Similarly, abdominal circumference (paired samples t-test, $t = 5.960$, $n = 38$, $p < 0.0005$) and WHI (paired samples t-test, $t = 4.498$, $n = 46$, $p < 0.0005$) decreased after CR (Table 2). Delta WHI was independent of age ($F(1,33) = 2.304$, $p = 0.139$), but significantly related to baseline WHI ($F(1,33) = 6.488$, $p = 0.016$) and sex ($F(1,33) = 5.201$, $p = 0.029$). WHI decreased more in individuals with a higher baseline WHI (Figure 1a). Furthermore, WHI significantly decreased in females, but not in males (Figure 1b). Delta WHI did not differ between females from different rank categories (one-way ANOVA, $F(2,38) = 1.927$, $p = 0.160$).

Table 2. Mean ± SE (minimum–maximum) body measures and biochemical parameters of full-grown adult long-tailed macaques before and after mild caloric restriction (CR) started. * $p < 0.05$.

	n	Before CR	After CR
Body measures			
Body weight (kg)	46	6.7 ± 0.2 (4.25–11.5)	6.3 ± 0.2 (3.7–12.0) *
Abdominal circumference (cm)	38	43.2 ± 0.9 (29.4–56.9)	39.4 ± 0.9 (25.0–51.5) *
WHI (kg/m$^{2.7}$)	46	57.2 ± 1.2 (40.5–78.4)	53.8 ± 1.0 (35.5–68.9) *
Biochemical parameters			
Cholesterol (mmol/L)	45	3.16 ± 0.11 (1.69–4.93)	2.96 ± 0.11 (1.53–4.74) *
Triglyceride (mmol/L)	45	1.46 ± 0.13 (0.35–4.67)	1.33 ± 0.14 (0.26–3.91)
Glucose (mmol/L)	45	3.56 ± 0.20 (1.67–7.61)	3.48 ± 0.24 (1.56–11.7)
Fructosamine (umol/L)	45	189 ± 3.5 (140–250)	187 ± 2.8 (153–244)
HbA1c (%)	12	4.37 ± 0.14 (3.81–5.59)	4.18 ± 0.07 (3.86–4.67)

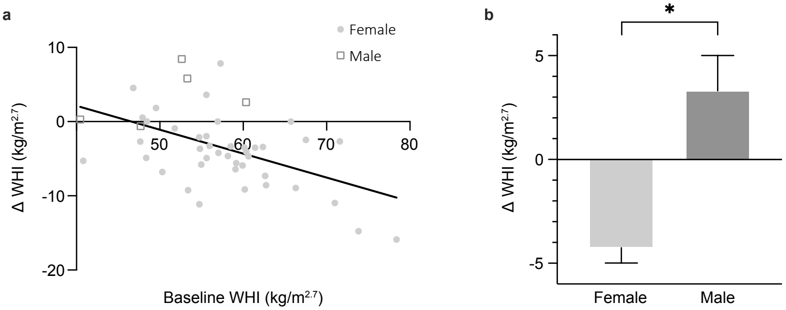

Figure 1. Effect of baseline weight-for-height index (WHI) (**a**) and sex (**b**) on delta WHI in full-grown adult male and female long-tailed macaques. Note that the y-axes are not equally scaled. Circles represent females; squares represent males. Error bars represent the standard error. * $p < 0.05$.

Overall, ten individuals (22%; rank category 4H, 3M, 3L) met the overweight criterium before CR, and six individuals (13%; 2H, 4M, 0L) did so after CR. Female dominance did not affect overweight prevalence before CR (Fisher’s exact test, p = 0.587) or after CR (Fisher’s exact test, p = 0.174). When data were split by sex, overweight prevalence in females went from ten individuals (24%) before CR to five individuals (12%) after CR. In contrast, no adult male was overweight before CR, while one male developed overweight after CR. Underweight was absent from the breeding colony before CR, whereas one high-ranking female (2%) met the underweight criterion after CR.

Biochemical parameters of adults

Cholesterol levels significantly decreased after CR (paired samples t-test, t = 3.650, n = 45, p = 0.001; Table 2). Although triglyceride levels went from 1.46 ± 0.13 mmol/L before CR to 1.33 ± 0.14 mmol/L after CR, this change was not statistically significant (Wilcoxon signed ranks test, Z = −1.236, n = 45, p = 0.216; Table 2). When triglyceride data were split by sex, there was a trend for a significant reduction in triglyceride levels in females (Wilcoxon signed ranks test, Z = −1.754, n = 40, p = 0.079), but not in males (paired sample t-test, t = −1.294, n = 5, p = 0.265). Delta cholesterol and delta triglyceride were independent of age (F(1,32) = 0.059, p = 0.809; F(1,32) = 1.048, p = 0.314) and sex (F(1,32) = 0.401, p = 0.531; F(1,32) = 0.654, p = 0.425), but there was a trend for a negative association between the delta and baseline values (F(1,32) = 4.125, p = 0.051; F(1,32) = 3.377, p = 0.075; Figure 2a,b). Thus, individuals with higher baseline values tended to have a higher decrease in cholesterol and triglyceride after CR.

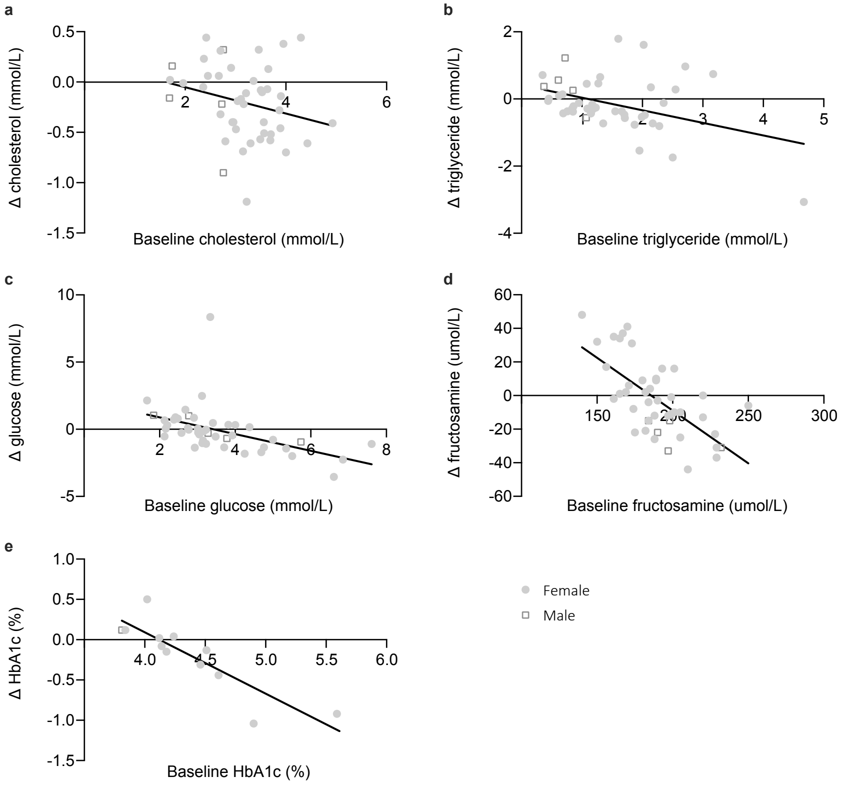

Figure 2. Effect of baseline value on delta cholesterol (**a**), delta triglyceride (**b**), delta glucose (**c**), delta fructosamine (**d**), and delta HbA1c (**e**) in full-grown adult male and female long-tailed macaques. Circles represent females; squares represent males.

CR had no significant influence on glucose (Wilcoxon signed ranks test, Z = −1.230, n = 45, p = 0.219), fructosamine (paired samples t-test, t = 0.684, n = 45, p = 0.498) and HbA1c levels (paired samples t-test, t = 1.494, n = 12, p = 0.163; Table 2). Delta glucose was independent of age (F(1,32) = 0.045, p = 0.833) and sex (F(1,32) = 0.132, p = 0.719), while individuals with higher baseline glucose levels had a higher glucose reduction after CR (F(1,32) = 15.115, p < 0.0005; Figure 2c). Similarly, delta fructosamine and delta HbA1c were inversely related to the baseline values (F(1,32) = 15.856, p < 0.0005); F(1,7) = 11.930, p < 0.0005; Figure 2d,e), but not related to age (F(1,32) = 0.390, p = 0.536; (F(1,7) = 0.007, p=0.937). Delta HbA1c was also independent of sex (F(1,7) = 0.422, p = 0.537), while males had higher delta fructosamine compared to females (F(1,32) = 8.452, p = 0.007). When data were split by sex, fructosamine levels decreased after CR in males (paired samples t-test, t = 6.063, n = 5, p = 0.004), but did not differ in females (paired samples t-test, t = -0.100, n = 40, p = 0.921). Thus, individuals with higher baseline values had a higher decrease in glucose, fructosamine and HbA1c after CR.

Immature growth

Body weight of immatures was independent of sex (F(1,150) = 0.915, p = 0.340) and CR (F(1,150) = 1.463, p = 0.228; Figure 3a), while body weight was positively associated with age (F(1,150) = 656.152, p < 0.0005). Body weight increased on average with 0.99 kg per year. There was also a significant interaction effect between age and sex in the model (F(1,150), 6.740, p = 0.010). This means that the relationship between age and body weight differed per sex. The regression equations showed that male body weight = 0.55 + 1.11 × age, while female body weight = 0.78 + 0.88 × age (Figure A1).

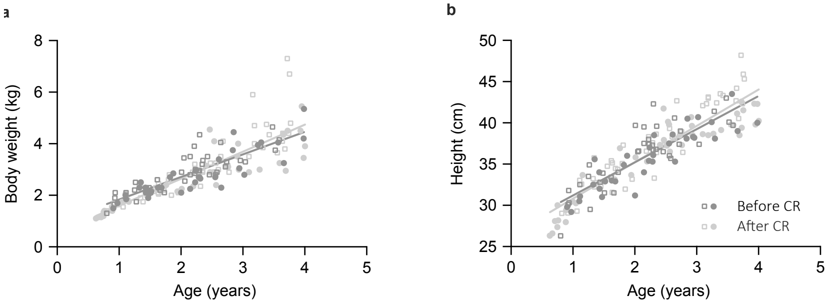

Figure 3. Growth patterns of immature male and female long-tailed macaques based on body weight (**a**) and height (**b**) before and after caloric restriction (CR). Circles represent females; squares represent males

Similarly, height of immatures was independent of sex (F(1,148) = 0.418, p = 0.519) and CR (F(1,148) = 0.270, p = 0.604; Figure 3b), but increased with age (F(1,148) = 768.054, p < 0.0005). On average, height increased with 4.2 cm per year. There was again a significant interaction effect between age and sex (F(1,148) = 4.133, p = 0.044).

The regression equations showed that male height = 26.27 + 4.73 × age, while female height = 27.1 + 3.85 × age (Figure A1). Thus, the age–sex interaction effects show that male immatures have a higher increase in body weight and height per year compared to female immatures, meaning males grow faster than females, while CR had no significant effect on immature growth.

Health records

Severe weight loss that required veterinary consultation occurred in three individuals (1.5%) before CR and seven individuals (3.3%) after CR. The proportion of individuals with severe weight loss did not differ significantly before or after CR (Fisher's exact test, $p = 0.341$). Besides, five of these individuals had a confirmed diagnosis of diabetes mellitus, while five other cases of severe weight loss were linked to chronic or intermittent diarrhea (Table A1). Thus, the severe weight loss of these individuals was likely caused by underlying medical conditions, rather than by CR.

Six individuals (3.1%) were seen by the veterinarian for injuries prior to CR and six individuals (2.8%) after CR. The nature and location of most injuries were suggestive to be inflicted by other group members. The proportion of individuals with injuries that required veterinary consultation did not differ after CR (chi-square test, $\chi^2 = 0.017$, $p = 0.897$). In addition, mortality due to suspected or observed conflict occurred twice in the year before CR and once in the year after CR. This suggests that CR had no effect on injuries or mortality due to suspected or observed conflicts.

Reproductive success

Finally, we checked whether CR influenced female reproduction in the breeding colony. In the year prior to CR, 37 out of 74 (50%) fertile females conceived, while 44 out of 87 (51%) fertile females became pregnant in the year after CR (Figure 4a). The proportion of females that became pregnant did not significantly change after CR (chi-square test, $\chi^2 = 0.005$, $p = 0.942$). Female dominance had no effect on the likelihood to conceive both before CR (chi-square test, $\chi^2 = 0.881$, $p = 0.644$) and after CR (chi-square test, $\chi^2 = 1.191$, $p = 0.551$). Thirty out of 33 (91%) pregnancies resulted in viable offspring before CR, while 42 out of 45 (93%) pregnancies were successful after CR. The proportion of successful pregnancies did not significantly differ before or after CR (Fisher's exact test, $p = 0.694$; Figure 4b). Thus, CR did not affect female reproduction.

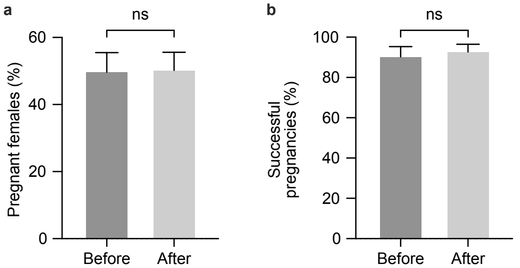

Figure 4. Proportion of pregnant females (**a**) and successful pregnancies (**b**) in a long-tailed macaque breeding colony before and after caloric restriction (CR). Error bars represent the standard error. ns: $p \geq 0.05$.

Discussion

This study aimed to determine whether applying caloric restriction (CR) can be effective and safe in NHPs housed and fed in social groups. CR should ideally target overweight adults, while having no adverse effects on other group members. We investigated the effect of mild (20%) CR on adult overweight and biochemical parameters, immature growth, veterinary consultations, and reproductive success in multigenerational long-tailed macaque breeding groups. Overall, CR significantly reduced adult body measures and it had more effect on females and heavier individuals compared to males and leaner individuals. CR reduced cholesterol levels but did not affect overall triglyceride, glucose, fructosamine and HbA1c levels. Yet, biochemical parameters of individuals with high baseline values were reduced more compared to individuals with low baseline values. Immature growth, veterinary consultations and reproductive success remained equal after CR. Thus, mild CR can be considered effective and safe.

Adult overweight and biochemical parameters

Overweight prevalence of full-grown adult male and female long-tailed macaques at BPRC combined was 22% before CR and 13% after CR. The WHI, used to evaluate overweight status, significantly decreased after CR. This complies with previous studies in individually- and pair-housed NHPs and indicates that CR is effective in reducing body weight. We also checked which adult individuals were affected most by CR. Delta WHI was independent of age and female dominance, but significantly related to baseline WHI and sex. Heavier individuals lost more weight compared to leaner individuals, which implies that CR targets the right individuals. Furthermore, WHI of females generally decreased, while four out of five males gained weight after CR. In contrast to our group-housed animals, who experience food competition, body weight reduction after CR was more pronounced in males compared to females in individually-housed rhesus macaques (Mattison et al. 2005). In the BPRC breeding colony, females likely experience more competition than males as groups contain multiple adult females and generally only one adult male, who is always top-ranking. This likely explains the sex-effect in our study. Alternatively, the small sample size for adult males (n = 5) may have caused this effect by chance.

CR reduced cholesterol levels of adults, but it had no overall effect on other biochemical parameters in our study. Yet, individuals with high baseline values had a higher reduction in their biochemical parameters after CR, i.e. were affected more by CR, compared to individuals with low baseline values. This again implies that CR targets the right individuals. This is partially consistent with findings from previous studies. Calorically restricted rhesus macaques had significantly lower cholesterol and triglyceride levels compared to *ad libitum* fed controls (Verdery et al. 1997; Lane et al. 1999). Biochemical parameters related to glycemic response, i.e. glucose, fructosamine and HbA1c, did not differ between CR- and control-fed long-tailed macaques (Cefalu et al. 2004). Although a reduction in fasting glucose was not apparent immediately in rhesus macaques (Cutler et al. 1992; Kemnitz et al. 1993), glucose levels were significantly reduced after several years (Kemnitz et al. 1994; Lane et al. 1995). These previous studies generally evaluate the effect of 30–40% CR over several years, while we studied the effect of 20% CR after several months. This lower intensity and shorter duration may explain why we found no overall reduction in triglyceride and glucose levels after CR. Beneficial effects of CR on biochemical parameters may become more pronounced over time.

Thus, CR effectively reduced body weight measures and cholesterol levels of group-housed adult long-tailed macaques and had most effect on individuals with high baseline values for WHI and biochemical parameters.

Immature growth

The suitability of CR in NHP breeding groups depends on whether CR is safe, which means there should be no adverse effects of CR on group members. Therefore, we first checked whether CR influenced immature body weight and height. Body weight and height increased with age but did not differ between the sexes. In free-ranging rhesus macaques, sex differences in body weight and height are first apparent in the age group 4–5 years old (Schwartz & Kemnitz 1992). Sexual dimorphism occurs at a slightly younger age in rhesus and long-tailed macaques in captivity, i.e. between 3 and 4 years old (Andrade et al. 2004). This complies with our findings as our analyses included individuals between 8 months and 4 years old. Although sex itself had no effect, there was a significant interaction effect between age and sex in the models. The age-related increase in body weight and height differed per sex, and regression equations showed that males grew faster, both in weight and height, than females. Higher growth rates in males compared to females are also found in captive Japanese macaques, captive rhesus macaques and wild yellow baboons (Hamada et al. 1999; Altmann & Alberts 2005; Haertel et al. 2018).

Despite the reduced amount of food, CR had no adverse effect on immature growth. Decreased growth rates could have occurred as immatures are generally less efficient in competitive foraging than adults (van Schaik 1989). However, many primates show high levels of feeding tolerance towards infants and juveniles (Rapaport & Brown 2008; von Rohr et al. 2010). Juveniles stealing food from other group members is even being tolerated in captive white-naped mangabeys (*Cercocebus lunulatus*: Waasdorp et al. 2021). This high tolerance may enable immatures to obtain enough food and energy for growth, and likely explains why no effect of CR on immature growth was found.

Health records and reproductive success

Health records were consulted in retrospect to obtain data on veterinary consultations and female reproduction. First, underweight and cases of severe weight loss were examined. Based on the ideal WHI range, underweight did not occur before CR, while one high-ranking female (2%) met the underweight criterion after CR. This was not considered a reason for concern, as this percentage fits within the normal variation (Sterck et al. 2019). Moreover, this female had similar reproductive output before and after CR and did not deviate in the number of births from other females. The proportion of individuals with severe weight loss did not differ before or after CR. Moreover, all cases were related to underlying medical conditions that are unlikely to be related to CR, i.e. diabetes or diarrhea. Actually, CR even prevents or delays the onset of type 2 diabetes mellitus and impaired glucose tolerance in captive male rhesus macaques (Hansen & Bodkin 1993). In humans, diarrhea occurred equally often in participants on mild CR compared to participants on an *ad libitum* diet (Romashkan et al. 2016). Thus, CR did not affect cases of severe weight loss as these were related to medical conditions that are likely not caused by CR.

Second, injuries and mortality due to suspected or observed conflicts were examined, as restricted resources may lead to increased levels of aggression. The relatively low number of injuries and mortality in this study imply that the breeding groups are socially stable in general. Although the cases of mortality were too limited for statistical testing, the proportion of individuals with injuries that required veterinary consultation did not differ before or after CR. This may be due to the provisioning of chow in multiple feeding trays per group, as this prevents monopolization and likely also aggression. Neither did our animal caretakers report higher levels of aggressive behaviour after CR (personal communication). These results suggest that CR had no effect on aggression. Similarly, aggressive behaviour did not change after a one-week 25% food reduction in a rhesus macaque group (Southwick 1967). Behavioural observations on aggression were not performed in this study but are needed to confirm these results.

Third, the number of pregnancies and pregnancy outcomes were used to measure reproductive success. Females were equally likely to conceive and pregnancy outcomes did not differ before or after CR. Similar to our findings, female mouse lemurs housed in same-sex groups exposed to 40% CR for eight months became pregnant at similar rates and had an equal number of surviving offspring at weaning as control females (Canale et al. 2012). Chronic CR also did not affect reproductive cycling and hormone concentrations in pair-housed female rhesus macaques (Lane et al. 2001). Furthermore, the likelihood of conception was independent of female dominance before and after CR. In free-ranging Japanese macaques on Koshima island, birth rates were equal between females from different rank categories when artificial food supplies were abundant (Watanabe et al. 1992). However, when artificial feeding was severely reduced, the most dominant females gave birth more frequently than the others (Watanabe et al. 1992). Such a rank-effect was not found in our study, implying that CR equally influenced females from different rank categories. Altogether, this indicates that CR did not alter the reproductive success of the breeding colony.

Future research

Although the number of calories fed were restricted by 20%, the fed amount was probably still enough to meet all individuals' energetic requirements, i.e. malnutrition did not occur. Immature growth, veterinary consultations and female reproduction were unaffected by CR and females from different rank categories were equally likely to conceive. This indicates that we may have been overfeeding the colony before CR, while after CR caloric intake was closer to 'optimal'. Similarly, a review on CR in NHPs concludes that the caloric intake of control animals is probably above 'optimal', i.e. they are generally overfed, compared to CR animals (Pifferi & Aujard 2019). Using these animals in biomedical studies may bias experimental outcomes (Martin et al. 2010), so further investigation is needed to determine 'optimal' caloric intake for captive group-living NHPs.

This study provides the first evidence that mild CR may be a useful overweight management tool in captive group-living NHPs. However, several factors were not included in this study and should still be evaluated in group-housed NHPs, e.g. effects of CR on thermoregulation, stress response and behaviour. Some studies found physiological effects of CR. For example, core body temperature was ~0.5 °C lower in male rhesus macaques subjected to 30% CR compared to age-matched controls (Lane et al. 1996). Besides, limited resources may elicit a stress response. In grey mouse lemurs, urinary cortisol concentrations increased after a two-week 60% CR (Noiret et al. 2020). Yet, more long-term studies found no evidence for a stress-related increase in cortisol levels in CR compared with control NHPs (Ramsey et al. 2000b; Downs et al. 2008; Canale & Henry 2010; Dal-Pan et al. 2011). Whether CR triggers a stress response likely varies depending on its intensity and duration (Noiret et al. 2020). This and other effects of CR should be further studied in group-housed NHPs, as previous studies are limited to individually- and pair-housed NHPs.

Conclusion

Mild caloric restriction may be a valuable overweight management strategy in group-housed non-human primates as caloric restriction targeted the right individuals and had no adverse effects on the variables examined in this study.

Appendix

Table A1. Health record examination of individuals with severe weight loss one year before and after caloric restriction (CR).

Case	Period	Age/sex/rank	Medical condition
1	Before CR	12, M, high	Diabetes mellitus and diarrhea
2	Before CR	20, F, low	Diabetes mellitus
3	Before CR	12, F, high	Diarrhea
4	After CR	17, F, middle	Diabetes mellitus
5	After CR	13, F, high	Diarrhea
6	After CR	20, F, middle	Diabetes mellitus
7	After CR	22, F, low	Diarrhea
8	After CR	11, F, middle	Diarrhea
9	After CR	19, F, middle	Diabetes mellitus
10	After CR	24, F, low	Diarrhea

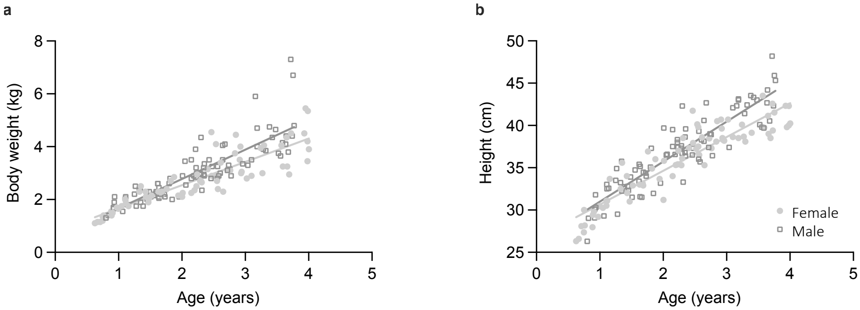

Figure A1. Growth patterns of immature male and female long-tailed macaques based on body weight (**a**) and height (**b**). The dark grey line implies that the growth rate of immature males is higher compared to females.

Chapter 8

Evaluating overweight management strategies in captive rhesus macaque groups: a multidisciplinary approach

Dian G. M. Zijlmans, Rosanne Vreugdenhil,
Annet L. Louwerse, Jan A. M. Langermans[*],
Elisabeth H. M. Sterck[*]

* Joint senior authors

Abstract

Overweight is a well-known problem in captive group-living primates. Overweight primates have a higher risk of several health problems and perform less species-specific natural behaviour suggesting decreased welfare. Primate facilities are therefore interested in potential husbandry strategies to reduce overweight, though not many studies report on this. Our study uses a multidisciplinary approach to assess the effect of four overweight management strategies on relative adiposity, physiological and behavioural wellbeing of group-housed rhesus macaques (*Macaca mulatta*), namely: (1) providing food in puzzles, (2) ideal body weight feeding, (3) 20% caloric restriction and (4) a fruit-free diet. Anthropometric measures, biochemical parameters and hair loss (i.e. alopecia) were used as health indicators, while behavioural observations provided information on food intake, activity budgets, aggression and self-directed behaviour. Food puzzles decreased feeding rate but had no effect on overall activity budgets. Food puzzles and ideal body weight feeding did not reduce overweight, while body weight even increased with the standard feeding routine. Caloric restriction increased feeding rate and reduced overweight, but it also resulted in problematic weight loss in four low-ranking individuals and more alopecia. Finally, a fruit-free diet did not affect relative adiposity, but resulted in lower glucose levels, more natural activity budgets and less self-directed behaviour. In conclusion, food puzzles provide a form of enrichment, but are not effective in reducing overweight. Caloric restriction reduced overweight, yet compromised physiological wellbeing of some individuals and is therefore not generally applicable as an overweight management strategy, whereas implementation of ideal body weight feeding and a fruit-free diet is recommended to enhance health and welfare in group-housed rhesus macaques.

Introduction

Overweight in captive group-living primates is a common phenomenon, which results from an imbalance between energy derived from food and energy expended by physical activity (Kemnitz 1984; Chen et al. 2002). Like humans, being overweight is associated with several health problems in primates, such as cardiovascular disease and diabetes (Kemnitz & Francken 1986; Shively & Clarkson 1987; Hansen & Bodkin 1993). Overweight primates are generally more inactive and spend less time performing species-specific natural behaviour, indicating their welfare may be compromised (Bauer et al. 2012). Standard feeding routines in primate facilities likely contribute to the overweight problem. In general, food provided to captive primates is easily accessible and mainly consists of commercially available monkey chow pellets, supplemented with fresh fruit and/or vegetables (Bauer et al. 2010). The pellets are often available throughout the day as they are fed *ad libitum* or in a predetermined amount that depends on the number of individuals in a group and their body weight (Bauer et al. 2010). This standard diet has an inherent risk to result in some individuals becoming overweight. Primate facilities should aim to prevent and reduce overweight to enhance the physiological and behavioural wellbeing of their primates. Finding appropriate overweight management strategies for captive group-living primates, such as dietary and husbandry adjustments, is important, yet not many studies report on this.

A weight management plan combining dietary and husbandry adjustments can lead to significant weight reduction, physiological and behavioural improvements in group-housed primates. For example, a combined dietary and husbandry adjustment was highly successful in highly obese chimpanzees (*Pan troglodytes*: Lambeth et al. 2011). These chimpanzees had to climb and support their own body weight to obtain food from an elevated device. Simultaneously, the provided number of calories from pellets was reduced and the amount of fiber in the diet was increased. As a result, individuals lost 12 to 30% of their body weight, blood glucose levels improved and respiratory problems during anesthesia disappeared. Other chimpanzees experienced a switch from a diet consisting of *ad libitum* group-fed pellets and little greens to individually-fed pellets and more leafy vegetables (Clay et al. 2022). After three years, this had resulted in 14 out of 15 overweight individuals improving their body condition score and 67% not being overweight anymore. Furthermore, individuals spent more time feeding, resulting in more natural activity budgets, while abnormal, stress-related and agonistic behaviour remained equal. Although the above-mentioned strategies have proven highly effective, they also come with a cost. Both strategies included separating individuals from their social group, either permanently or temporarily, which disrupts the social dynamics and has potential consequences for group stability and animal welfare. Furthermore, both strategies required a lot of personnel time investment.

Our study focuses on four overweight management strategies that allow individuals to stay in their social group and require minimal or no extra time investment of staff, namely: (1) providing food in puzzles, (2) ideal body weight feeding, (3) 20% caloric restriction and (4) a fruit-free diet. The first husbandry adjustment we examined concerned providing all pellets in food puzzles. Food puzzles are feeding devices that typically increase the time and effort required to obtain food and are often used to decrease stereotypic behaviour in primates (Gottlieb et al. 2011; Coleman et al. 2012). When modified feeding devices were placed on different heights of the cage in group-housed chimpanzees, time spent feeding increased and overweight individuals lost body weight, while other, normal-weight, individuals remained similar in their body weight (Bridges et al. 2013). Younger primates are generally more curious and perform more object manipulation (McGuire et al. 1994; Byrne & Suomi 1996; Bergman & Kitchen 2009), so we expect them to spend more time using the food puzzles compared to adults. This may lead to a different division of food, increasing food intake in younger individuals and decreasing food intake in adults. Since overweight is most common in adult macaques (i.e. 10–17 years old; Ramsey et al. 2000), food puzzles are predicted to reduce overweight and increase foraging time at the same time.

The second overweight management strategy we termed “ideal body weight feeding”, which calculates the amount of food per group based on each monkey’s ideal body weight instead of their actual body weight. This alternative method provides an amount of food to each group that matches the sum of all individuals' required amount of energy based on their optimal basal metabolic rate (BMR). BMR is the amount of energy an animal requires for basic life-sustaining body functions and is calculated based on an individual's body weight (National Research Council 2003). Instead of using an individual's current body weight to calculate BMR, we use the individual's ideal body weight depending on their height (see *Material and methods*). This method is expected to better fit each group's energetic requirements, i.e. providing less food when groups contain many heavy individuals, thereby reducing overweight.

Third, a more drastic approach is to decrease the number of calories provided to all animals, known as caloric restriction (CR). CR is highly effective in reducing overweight and associated health problems in solitary- and pair-housed primates (Ingram et al. 1990; Hansen & Bodkin 1993; Kemnitz et al. 1993). Besides, adult rhesus macaques and grey mouse lemurs (*Microcebus murinus*) on 30% long-term CR were more active compared to *ad libitum*-fed controls (Weed et al. 1997; Dal-Pan et al. 2011). Yet, this method may be inappropriate and/or difficult to implement in group-housed primates (Bauer et al. 2011). Still, we showed in a previous study on long-tailed macaque (*Macaca fascicularis*) breeding groups that mild (20%) CR specifically targeted overweight individuals and had no adverse effects on immature growth, reproduction and veterinary consultations (Zijlmans et al. 2022a). However, potential effects of CR on physiology and behaviour were not examined, while restricted food availability likely increases competition and may therefore lead to increased aggression, stress or malnutrition. Increasing evidence suggests a link between stress and hair loss (i.e. alopecia) in primates (Steinmetz et al. 2006; Novak et al. 2016). Furthermore, nutritional shortages can cause alopecia in primates, e.g. protein deficiency in western lowland gorillas (*Gorilla gorilla*: Mundy et al. 1998), zinc deficiency in moustached tamarins (*Saguinus mystax*: Chadwick et al. 1979), and folacin deficiency in squirrel monkeys (*Saimiri sciureus*: Rasmussen et al. 1979).

Finally, there seems to be ample evidence that fruit-free diets enhance physiological and behavioural wellbeing of captive primates. Although fruit is typically part of the natural diet for macaques, cultivated fruits have a different nutritional composition from wild fruits and are therefore less appropriate to feed in captivity (Kaumanns et al. 2006; Cabana et al. 2018a). Removing all fruits (as well as bread, eggs and seeds) from the diet improved dental health and caused some overweight individuals to lose weight in several primate species at a zoo (Plowman 2013). Similarly, increasing fiber content and decreasing the glycemic index of the diet resulted in more balanced body weights and improved biochemical parameters related to glucose response and lipid metabolism in group-housed long-tailed macaques (Zijlmans et al. 2021b). Likewise, a low-sugar, high-fiber diet led to more natural behaviour, less abnormal behaviour and decreased glucose levels in captive great apes (Cabana et al. 2018a) and improved coat condition in Hamadryas baboons (*Papio hamadryas*: Cabana et al. 2018b). Moreover, a fruit-free diet reduced aggression and self-directed behaviour in captive lemurs (Britt et al. 2015). Similar beneficial physiological and behavioural effects of a fruit-free diet are therefore expected in our study.

To assess the suitability of these four overweight management strategies, our study combines several indicators of physiological and behavioural wellbeing in captive group-living rhesus macaques (*Macaca mulatta*). The study groups contained, next to mature individuals with and without overweight, many individuals that were not yet skeletally matured (i.e. they were still growing). Therefore, overweight management strategies should target overweight individuals, while not hampering growing and normal-weight individuals. In addition, there should be no adverse effects on physiological and behavioural wellbeing of the entire group, e.g. increased aggression or stress. Anthropometric measurements and standard blood chemistry were performed during annual health checks, which are part of the regular health management of the colony. Next to anthropometric measurements during the annual health checks, overweight was monitored regularly during the study using non-invasive methods, i.e. visual obesity scoring (adapted from Berman & Schwartz 1988) and voluntary weighing (Zijlmans et al. 2021c). Photos of hair coat condition were taken during some of the health checks, and these were included in the study post-hoc. Finally, behavioural observations provided information on changes in food intake, activity budgets, aggression and self-directed behaviour.

Material and methods

Subjects and housing

Subjects of this study were 91 female rhesus macaques (*Macaca mulatta*) housed in groups at the Biomedical Primate Research Centre in Rijswijk, the Netherlands. The animals lived in five multigenerational groups, which we will refer to using the names of the matriarchs: Grey, Liby, Marieke, Bertha and Iffy. The animals were aged between 1.7 and 20.5 (7.8 ± 0.5) years old and weighed between 3.1 and 14.4 (8.3 ± 0.3) kg at the start of the study (Table 1). The groups contained ten to thirty individuals per group and were managed by mimicking natural migration processes, i.e. females are philopatric and males leave the group around puberty. Therefore, groups consisted of adult females from several matrilines with their offspring. None of the groups contained an adult breeding male, so there were no pregnant or lactating females. The groups contained in total five juvenile males at the start of this study, but these males were excluded because they were removed from their natal group during the study. The groups also contained three adult females with health problems, but these were excluded because of internal diseases, i.e. chronic diarrhea and endometriosis. Each group had 24/7 access to indoor (72 m^2 and 2.85 m high) and outdoor (250 m^2 and 3.1 m high) enclosures. The enclosures contained climbing structures, slides, car tires, platforms, beams and an outside swimming pool (Vernes & Louwerse 2010). Water was continuously available via automated water dispensers.

The daily diet of the macaques consisted of monkey chow (Ssniff, Soest, Germany) in the morning, complemented in the afternoon with fruit (2x per week), vegetables (2x per week), maize silage (1x per week) or a grain mixture (2x per week). In general, the monkey chow was provided between 7.30 h and 9.00 h and the afternoon feeding took place between 15.00 h and 16.30 h. Monkey chow was the main component of the diet, providing 100% of the required energy per individual. At the start of the study, the standard amount of monkey chow per individual was based on an individual's basal metabolic rate (BMR) and depended on their age and body weight (National Research Council 2003; Table 2). The sum of all individual's standard amounts of monkey chow was provided to the entire group. The monkey chow was provided in multiple feeding trays per group (i.e. typically six trays, but at least one tray per five animals with a minimum of two trays). The amount of fruit, vegetables and maize silage was 150 g for individuals over four years old and 100 g for individuals from one to four years old (Table 2). For the grain mixture, 20 g was counted per animal irrespective of age. Food enrichment and treats were provided occasionally but were considered to have a negligible effect on daily energy intake.

Table 1. Group characteristics of the study groups. Mean ± SE (minimum–maximum) age and body weight at the start of the study are reported, as well as dietary and husbandry adjustments per study phase.

Group	Size	Age (years)	Body weight (kg)	Strategies phase 1	Strategies phase 2
Grey	n=20	7.8 ± 0.9 (3.0–17.1)	9.3 ± 0.5 (5.1–14.4)	Food puzzles + IBWF	CR
Liby	n=11	7.6 ± 1.4 (2.6–16.6)	9.2 ± 0.7 (4.8–13.4)	IBWF	CR
Marieke	n=21	7.9 ± 1.2 (2.3–18.7)	7.2 ± 0.6 (3.5–12.2)	Food puzzles	CR + fruit-free
Bertha	n=26	8.3 ± 1.1 (1.7–20.5)	7.9 ± 0.5 (3.1–12.0)	Control	NA
Iffy	n=13	6.9 ± 0.9 (3.4–13.6)	8.4 ± 0.3 (6.4–10.3)	NA	CR + fruit-free

Abbreviations: IBWF, ideal body weight feeding; CR, caloric restriction; NA, not applicable.

Table 2. The average amount of food that was provided daily per individual during standard feeding, phase 1 and phase 2. The variation reflects individual differences in energetic requirements based on age and body weight.

Food item	Standard feeding	Phase 1: standard or IBWF	Phase 2: CR
Monkey chow – standard	203 ± 7 (114–330) g	203 ± 7 (114–330) g	167 ± 7 (97–264) g
Monkey chow – IBWF	208 ± 9 (143–351) g	180 ± 9 (138–314) g	144 ± 7 (110–251) g
Fruit, vegetables, maize silage	100/150 g	100/150 g	80/120 g
Grain mixture	20 g	20 g	16 g

Abbreviations: IBWF, ideal body weight feeding; CR, caloric restriction.

Study design

The study was comprised of two phases. During the first phase of the study, the effect of food puzzles and ideal body weight feeding on physiological and behavioural wellbeing was investigated, while during the second phase of the study the effect of CR and a fruit-free diet was examined. The start and end points of each phase were marked by the annual health checks, during which most of the data were collected.

Four study groups were involved in the first phase of the study, which lasted approximately one year (range 355–367 days). These groups were selected for the study as they contained both normal-weight and overweight individuals (i.e. each group contained at least four overweight individuals). Each group was randomly assigned to a condition according to a two-by-two factorial design. Food puzzles were provided to the Grey- and Marieke-group, while ideal body weight feeding (see below) was applied to the Grey- and Liby-group (Table 1). The Bertha-group served as a control group in which the standard feeding method was maintained. Food puzzles consisted of 500 mL Gibco medium bottles with holes, each 18 millimeters in diameter, drilled into them and placed in 20 L jerrycans attached to the wired mesh (Figure 1). Monkeys had to shake the bottles to obtain chow and all monkey chow was provided in the food puzzles. There were multiple jerrycans per group (i.e. at least six) to prevent monopolization and each jerrycan contained one or two bottles. The number of holes in the bottles was high (i.e. five to seven) to familiarize the monkeys with the new feeding method during the first week. Hereafter, the initial bottles with many holes were replaced by bottles with fewer (i.e. two to four) holes.

Figure 1. Food puzzle design. Gibco medium bottles (**a**) were drilled with 18-millimeter holes (**b**) and placed in jerrycans attached to the wired mesh in the indoor enclosure (**c**), based on a design by S. Dumay.

Ideal body weight feeding means that individuals are fed monkey chow based on their ideal body weight instead of their actual body weight. Ideal body weight feeding thus allots more monkey chow to individuals with a low relative body weight, while it allots less monkey chow to individuals with a high relative body weight. The ideal body weight was determined using a species-specific weight-for-height index for group-housed rhesus macaques. WHI3.0, hereafter referred to as WHI, was calculated as body weight in kilograms divided by the third power of the height in meters (Sterck et al. 2019). Ideal body weight was the body weight that matched the individual's height to yield an optimal WHI of 52 kg/m^3, which roughly equals 8% body fat (Raman et al. 2005) and was calculated by multiplying the third power of an individual's height by 52. As many subjects were relatively heavy at the start of the study, ideal body weight feeding resulted in a lower amount of monkey chow per group than standard feeding. On average, ideal body weight feeding reduced the amount of monkey chow compared to the standard feeding method by 13% ± 2% in the Grey-group and 15% ± 2% in the Liby-group (Table 2).

The second phase of the study involved again four groups and lasted around 200 days (range 189–206 days). The Bertha-group was replaced by the Iffy-group for management reasons. All four groups were subjected to 20% CR by reducing the amount of food provided to them at the end of phase 1. The amount of all food items was multiplied by 0.8 to obtain the new amount per individual (Table 2). In addition, fruit was completely removed from the diet by replacing fruit with vegetables, on average, two times per week in the Marieke- and Iffy-group, i.e. the fruit-free groups (Table 1). Fruit was still provided, on average two times per week, to the Grey- and Liby-group, i.e. the fruit-provided groups. Food puzzles were maintained during the second study phase in the Grey- and Marieke-group.

Health checks

Anthropometric measurements were performed, and standard blood chemistry values were determined during the macaques' annual health checks (Table A2). These health checks are a routine veterinary management procedure related to the health monitoring program of the colony (Balansard et al. 2019). Individuals were fasted overnight and sedated in their home enclosure with a combination of ketamine (10 mg/kg, Ketamine 10%; Alfasan, Woerden, the Netherlands) and medetomidine (0.05 mg/kg, Sedastart; AST Farma, Oudewater, the Netherlands). After the procedures, the sedation was reversed with atipamezole (0.25 mg/kg, Sedastop; AST Farma, Oudewater, the Netherlands) and individuals were returned to their home enclosure.

Body weight was measured using a standard scale. Height was measured as crown-rump length with the animals in a supine position. A measuring mat for human infants (SECA, Hamburg, Germany) was adapted for primates by making a hole for the tail. Body weight and height were measured to one decimal point. Height was measured three times independently and averaged. All anthropometric measurements were performed by the same person (DGMZ). Body weight and height were used to calculate a species-specific weight-for-height index (WHI) as body weight in kilograms divided by the third power of height in meters (Sterck et al. 2019). WHI is a measure of relative adiposity, i.e. the amount of body fat relative to body weight, that is optimally independent of height and highly correlated with other relative adiposity measures, such as abdominal circumference and skinfold thicknesses (Sterck et al. 2019). WHI of captive group-living rhesus macaques ideally ranges between 42 and 67 kg/m^3, so individuals exceeding the upper boundary of 67 kg/m^3 were defined as being overweight.

Blood samples were collected from the vena femoralis and analyzed for standard blood chemistry values using a Cobas Integra 400 plus (Roche Diagnostics, Rotkreuz, Switzerland). For the purpose of this study, we focused on biochemical parameters related to overweight and health in primates, i.e. total cholesterol and glucose levels.

We noticed that hair loss increased during the second study phase, while hair pulling was not observed, and decided to include alopecia scores in the study post-hoc. As a result, photos of hair coat condition were not available for all groups throughout the study. Photos were available for all groups at baseline, for the Grey- and Bertha-group after phase 1 and for the Liby-, Marieke- and Iffy-group after phase 2. During the health checks, photos were taken of the animals' coats while they were lying on their belly with the limbs adjacent to the body (in a so-called "frog" position). The photos were scored for the severity of hair loss by two observers independently (DGMZ and RV). Scores ranged from 0 to 5 with increasing severity of hair loss: 0 being no alopecia and 5 being completely bald (Honess et al. 2005). The observers had no previous experience with scoring alopecia, so two half hour training sessions were needed to get familiar with the scoring system. Also, the observers conjointly selected several reference photos for every score to facilitate the actual scoring. There was substantial agreement between the two observers' alopecia scores (Cohen's kappa, $\kappa = 0.767$, $p < 0.0005$; Landis & Koch 1977). Whenever the two observers disagreed in their score, a third observer with experience in alopecia scoring (ALL) was consulted to determine the final score. All observers were blind to the identity of the individual and the condition.

Behavioural observations

Due to practical issues and time limitations, behavioural observations were conducted in two groups per study phase. Behavioural observations took place in the Grey- and Marieke-group during the first phase and the Marieke- and Liby-group during the second phase. Behavioural observations focused on several aspects, namely food intake, activity budgets, aggression, self-directed behaviour and submission. Behavioural data were collected roughly two months before and two months after the start of each phase. Animals had free access to both the indoor and outdoor enclosure during the observations. The observations took place between 8.30 h and 16.00 h in the indoor enclosure, as this is the location where food is provided.

Food intake was mainly measured on a group-level, as food intake per individual is notoriously difficult to quantify in a group-setting (Chivers 1998; Wilson et al. 2008; Zijlmans et al. 2022b). The amount of monkey chow was weighed in the morning when provided to the animals, and the remaining amount was weighed again at a random time during the day (between 8.25 h and 16.30 h). The difference in chow weight between the two time points reflected the consumed amount of chow up until that moment. A daily feeding rate was then calculated as the consumed amount of chow divided by the time difference and expressed as grams per hour. In addition, scan sampling was used to score cheek pouch fullness on an individual level. Cheek pouch fullness had four levels: empty (0), some (1), half (2) and full (3). These scores were converted to numbers (0–3) and averaged to obtain a mean cheek pouch score per animal per study phase.

Focal animal sampling and scan sampling were used to construct activity budgets per individual (Altmann 1974). Prior to and during the first phase, focal animals were observed in 15-minute periods for a total of 2–3 hours per animal per phase using The Observer XT version 11 (Noldus, Wageningen, the Netherlands). Focal animals were scored continuously on the duration of activity-related behaviour and their presence at the feeding trays (see ethogram: Appendix A). Prior to and during the second phase, activity-related behaviour was scored for the entire group using scan sampling with 10-minute intervals to increase the efficiency of data collection. All observations were balanced between the morning and afternoon. Activity-related behaviour was divided in several behavioural categories for data analyses, namely resting, moving, foraging, feeding, grooming and other behaviour. The activities were expressed as a percentage of time from the total observation time per individual (for focal animal sampling) or a percentage of scans from the total number of scans per individual (for scan sampling).

Aggression, self-directed behaviour and submission were observed by focal and all occurrence sampling (Altmann 1974). Aggression and self-directed behaviour were calculated per individual by dividing the total number of occurrences by the total time an individual was observed. Submissive behaviour included bared teeth, give ground, make room and escape (see ethogram: Appendix A). Dominance hierarchies were constructed per group based on submissive behaviour using MatMan in R (Netto et al. 1993). The dominance hierarchies of all groups were significantly linear and highly consistent (Grey-group: $h' = 0.982$; $p < 0.0005$, DCI = 0.968; Liby-group: $h' = 1$, $p < 0.0005$, DCI = 1; Marieke-group: $h' = 0.97$, $p < 0.0005$, DCI = 0.991; Bertha-group: $h' = 0.924$, $p < 0.0005$, DCI = 0.988; Iffy-group: $h' = 0.728$, $p < 0.0005$, DCI = 0.877). Absolute dominance rank of an individual was divided by the number of individuals in the group to correct for different group sizes. This resulted in values between zero and one in which a score closer to zero indicates a higher dominance rank.

Overweight monitoring

Next to the anthropometric measurements during the annual health checks, overweight was monitored regularly during the study using two non-invasive methods, i.e. visual obesity scoring (VOS; adapted from Berman & Schwartz 1988) and voluntary weighing (Zijlmans et al. 2021c). During VOS, each individual's appearance was rated on a scale from 1 to 4 (including both whole and half units) based on several criteria, e.g. girth, excessive skin, protruding bones, etc. A score of 1 represents underweight, 2 indicates optimal body condition, while scores 3 and 4 represent overweight and obesity respectively. Photos of reference animals were taken to facilitate scoring, which had to take place whenever an individual was either walking or standing. A pilot study at BPRC showed that visual obesity scores were highly correlated with WHIs in female rhesus macaques (Zijlmans et al. 2019). VOS was used to monitor overweight of all individuals in the groups throughout the study. VOS was performed by two observers (DGMZ and RV) with high inter-observer reliability (Cohen's kappa, $\kappa = 0.872$, $p < 0.0005$).

Voluntary weighing, i.e. animals stepping onto a scale in their home enclosure voluntarily without prior training, is a reliable and non-invasive method to obtain regular body weight measurements of captive group-living primates (Zijlmans et al. 2021c). During voluntary weighing sessions, a monkey-proof scale was placed in the home enclosure and sticky food items (e.g. apple syrup, jam and lemonade) were used to attract individuals to the scale. Although participation differed between social groups (Zijlmans et al. 2021c), many individuals stepped onto the scale voluntarily and their body weight could thus be monitored throughout the study. An observer (DGMZ or RV) was present during all sessions to note down the body weights from the display. The sessions were also videotaped to complete the data in case certain stepping events were missed during live observations. Voluntary weighing took place quarterly throughout the study and monthly during the first quarter of the second phase. The body weights obtained during voluntary weighing combined with the heights collected during the health checks allowed us to monitor WHIs during the study (Figure A1). Height is stable in skeletally matured individuals (i.e. females > 6 years old; Schwartz & Kemnitz 1992), while linear growth between consecutive annual health checks was assumed for females that were not yet skeletally matured.

Data analyses

Data analyses were executed with SPSS Statistics version 28. Paired comparisons were performed with a paired samples t-test or Wilcoxon signed ranks test, depending on the distribution of the data. We calculated delta values for WHI and indicators of physiological wellbeing, i.e. cholesterol, glucose and alopecia score, by subtracting the values before and after a husbandry change. We used general linear models with group as fixed factor and age, dominance rank and baseline value as covariates to examine whether the change in WHI, cholesterol, glucose and alopecia score varied between groups with different overweight management strategies. Whenever group had a significant effect on the delta value, we used Tukey's HSD post-hoc test to see which groups differed. The variance inflation factor of the models did not exceed 2.35. We also checked for interaction effects between group and other variables (i.e. age, dominance rank or baseline value). Pearson or Spearman correlations were used to examine the strength and significance of these correlations per group. McNemar's test was used to test whether the proportion of overweight individuals differed before and after each phase. Independent samples t-tests or Mann-Whitney U-tests per group were used to compare daily feeding rates before and after a husbandry change. One female was healthy for most part of the study but developed severe diarrhea at the end of the study and, for that reason, she was euthanized at the final health check. She was included in most analyses except for analyses including data on WHI and indicators of physiological wellbeing collected during the final health check.

Ethical statement

The Biomedical Primate Research Centre (BPRC) is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC International). All procedures, housing and husbandry during the study were in accordance with the European Directive 2010/63 and the Dutch law. The study was approved by BPRC's Animal Welfare Body (IvD 017A).

Results

First study phase: the effect of food puzzles and ideal body weight feeding

At the start of the study, mean WHI was 61.1 ± 1.1 kg/m^3 and 19 out of 78 individuals (24%) were categorized as being overweight. Baseline WHI was independent of dominance rank (F(1,72) = 0.223, p = 0.638), but older individuals had a higher WHI than younger individuals (F(1,72) = 42.454, p < 0.0005). Baseline WHI was also related to the social group (F(3,72) = 4.768, p = 0.004), yet post-hoc testing showed no significant differences in the pairwise comparisons (all p > 0.05). Contrary to what was expected, mean WHI (61.1 ± 1.1 kg/m^3 vs. 65.8 ± 1.1 kg/m^3; Z = -6.373, n = 78, p < 0.0005), visual obesity scores (2.36 ± 0.07 vs. 2.62 ± 0.07; Z = -5.343, n = 78, p < 0.0005) and overweight prevalence (24% vs. 42%; p = 0.001) significantly increased from baseline after phase 1. WHI increased on average by 8.3 ± 0.9% with a maximum increase of 34% in one year time.

The relative effects of the different overweight management strategies in phase 1 were determined. Delta WHI was independent of an individual's age, baseline WHI and dominance rank, but significantly differed between groups (Table A3). Post-hoc testing revealed that delta WHI was significantly higher in the Marieke- and Bertha-group compared to the Grey-group (p < 0.0005, p = 0.001), and higher in the Marieke-group compared to the Liby-group (p = 0.008; Figure 2a). WHI significantly increased in the Liby- (t = -2.389, n = 11, p = 0.038), Marieke- (Z = -4.015, n = 21, p < 0.0005) and Bertha-group

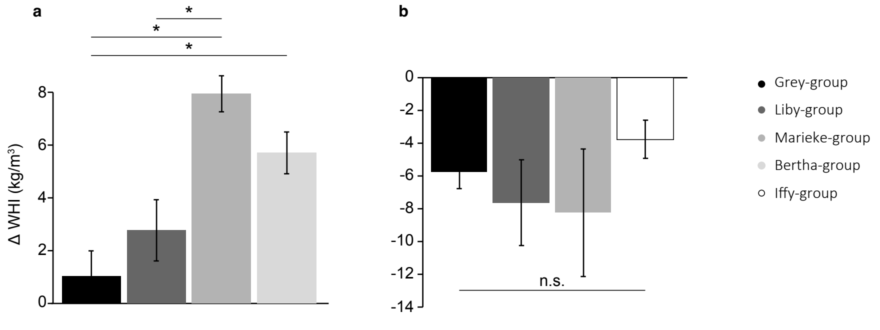

Figure 2. Delta weight-for-height index (Δ WHI) per group after the first phase (**a**) and second phase (**b**) of the study. Note that the y-axes are not equally scaled. * p<0.05.

(t = -7.156, n = 26, p < 0.0005), but not the Grey-group (t = -1.093, n = 20, p = 0.288). Thus, groups with the standard feeding method gained more weight compared to groups with the ideal body weight feeding method, while food puzzles did not affect weight gain.

Cholesterol levels significantly increased from 3.22 ± 0.06 mmol/L at baseline to 3.33 ± 0.05 mmol/L after phase 1 (Z = -2.523, n = 78, p = 0.012). Delta cholesterol was independent of age, dominance rank, baseline cholesterol and group (Table A3). Similarly, glucose levels significantly increased from 5.30 ± 0.24 mmol/L at baseline to 6.30 ± 0.17 mmol/L at the end of phase 1 (Z = -3.584, n = 78, p < 0.0005). Delta glucose was related to the baseline value, but independent of age, dominance rank and group (Table A3). Thus, cholesterol and glucose levels increased after phase 1, but the increase did not differ between groups with different overweight management strategies.

Alopecia scores in the Grey- and Bertha-group after phase 1 did not significantly differ from baseline (Z = -0.962, n = 44, p = 0.336). Delta alopecia score was independent of age and dominance rank, while individuals with a higher baseline alopecia score had a lower increase compared to individuals with a lower baseline alopecia score (Table A3). Furthermore, delta alopecia scores differed between the groups, as they were higher in the Grey-group compared to the Bertha-group (Table A3), suggesting that the husbandry change combining food puzzles with ideal body weight feeding increased alopecia scores.

Behavioural observations before and during the first study phase were performed in the Grey-group with food puzzles and ideal body weight feeding, and Marieke-group with food puzzles and standard feeding. Daily feeding rate significantly decreased in both groups when the food puzzles were present (Grey-group: t = 7.019, n = 34, p < 0.0005; Marieke-group: t = 6.284, n = 26, p < 0.0005). The percentage of time spent at the feeding trays increased with food puzzles in the Marieke-group, but remained similar in the Grey-group (Table A4). Cheek pouch fullness was significantly lower in the Grey-group (0.15 ± 0.03) compared to baseline (0.32 ± 0.04), but did not change in the Marieke-group. Time spent resting, moving, foraging, feeding or grooming did not differ when food puzzles were present (Table A4). Aggressive behaviour remained equal in both groups, while self-directed behaviour decreased in both groups (Table A4). In summary, ideal body weight feeding had no measurable effect on behaviour, except for a decrease in cheek pouch fullness. Food puzzles decreased feeding rate and self-directed behaviour, but did not affect overall activity budgets or aggression.

Second study phase: the effect of caloric restriction and a fruit-free diet

For the second phase of the study, the Bertha-group was replaced by the Iffy-group. The husbandry changes in phase 2 had a significant impact on relative adiposity levels, as mean WHI (64.8 ± 1.1 kg/m^3 vs. 58.4 ± 1.1 kg/m^3; Z = -6.601, n = 64, p < 0.0005) and visual obesity scores (2.53 ± 0.07 vs. 2.20 ± 0.06; Z = -5.344, n = 64, p < 0.0005) decreased from the start to the end of phase 2. Similarly, the prevalence of overweight individuals was significantly reduced by the end of phase 2 (33% vs. 16%; p = 0.003).

WHI decreased on average by 9.7 ± 1.0%, but there were large individual differences. In some individuals in the Liby- and Marieke-group, large weight loss occurred with relative decreases in WHI of up to 34% in 28 weeks. Thus, 20% CR was highly effective in reducing relative adiposity.

Delta WHI was independent of age and baseline WHI, but significantly related to dominance rank and group (Table A3), yet post-hoc testing revealed no significant differences in the pairwise comparisons of groups (all $p > 0.05$; Figure 2b). Lower-ranking individuals experienced a higher reduction in WHI compared to higher-ranking individuals. Furthermore, there were two significant interaction effects in the model, namely between group and rank, and between group and baseline WHI (Table A3). The relation between dominance rank and delta WHI was significant in the fruit-provided Liby-group ($r_s = -0.770$, $n = 10$, $p = 0.009$) and fruit-free Marieke-group ($r_s = -0.617$, $n = 21$, $p = 0.003$), but non-significant in the fruit-provided Grey-group ($r_s = -0.337$, $n = 20$, $p = 0.146$) and fruit-free Iffy-group ($r_s = -0.451$, $n = 13$, $p = 0.122$; Figure 3a). In addition, individuals with a higher baseline WHI lost more weight compared to individuals with a lower baseline WHI in the fruit-provided Grey-group ($r_p = -0.831$, $n = 20$, $p < 0.0005$), but not the other groups (Liby-group: $r_p = 0.156$, $n = 10$, $p = 0.667$; Marieke-group: $r_p = -0.388$, $n = 21$, $p = 0.083$; Iffy-group: $r_p = 0.121$, $n = 13$, $p = 0.695$; Figure 3b). Thus, the effect of baseline WHI and dominance rank on delta WHI differed per group, but this was not related to whether the groups had a fruit-free diet or not.

Cholesterol levels did not change from before to after phase 2 ($Z = -0.147$, $n = 64$, $p = 0.883$). Delta cholesterol was independent of age and dominance rank, but related to baseline cholesterol level and group (Table A3). Yet, post-hoc testing revealed no significant differences between the groups (all $p > 0.05$). Glucose levels significantly decreased from 6.17 ± 0.16 mmol/L before phase 2 to 5.23 ± 0.26 mmol/L at the end of phase 2 ($t = 2.980$, $n = 64$, $p = 0.004$). Delta glucose was independent of age and dominance rank, but there were significant interaction effects between group and age and group and rank, implying that the relation between these variables and delta

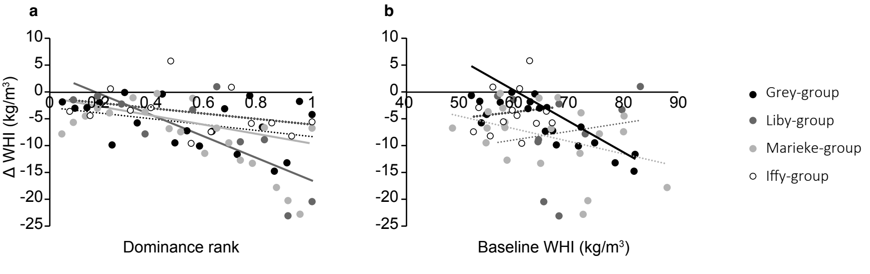

Figure 3. The effect of dominance rank (**a**) and baseline WHI (**b**) on delta weight-for-height index (Δ WHI) after the second phase differs between the four study groups. A higher dominance rank is indicated by a score closer to zero. Solid lines indicate a significant relationship, while dotted lines represent non-significant relationships.

glucose varied per group. Furthermore, delta glucose was significantly related to baseline glucose and group (Table A3). Post-hoc testing showed that glucose levels were reduced more in the fruit-free Iffy-group compared to the fruit-provided Grey-group ($p = 0.028$) and tended to be reduced more in the fruit-free Marieke-group compared to the fruit-provided Grey-group ($p = 0.071$), but not the fruit-provided Liby-group ($p = 0.953$, $p = 1.000$).

Alopecia scores after phase 2 were available for the Liby-, Marieke- and Iffy-group. Alopecia scores significantly increased from 0.66 ± 0.14 at baseline to 2.05 ± 0.17 at the end of phase 2 ($Z = -5.107$, $n = 44$, $p < 0.0005$). Delta alopecia score was again independent of age and dominance rank, but significantly affected by baseline alopecia score and group (Table A3). Individuals with low baseline alopecia scores had a higher increase compared to individuals with a high baseline alopecia score. Mean delta alopecia scores were higher in the fruit-provided Liby-group and fruit-free Marieke-group compared to the fruit-free Iffy-group ($p = 0.027$, $p = 0.004$). Change in alopecia was thus not related to whether groups had fruit included in their diet or not.

Behavioural observations before and during the second study phase were performed in the Liby-group, where food supply was reduced by 20%, and Marieke-group, where fruit in the diet was replaced by vegetables in addition to the 20% CR. Daily feeding rate significantly increased in both groups after CR was initiated (Liby-group: $t = -7.184$, $n = 15$, $p < 0.0005$; Marieke-group: $U = 30.00$, $n = 24$, $p = 0.015$). Food intake became more restricted in time as monkey chow was no longer available in the afternoon. The percentage of time spent at the feeding trays and cheek pouch fullness significantly decreased in the fruit-free Marieke-group, but remained equal in the fruit-provided Liby-group (Table A4). Activity budgets remained similar in the fruit-provided Liby-group, while resting and feeding decreased and foraging increased in the fruit-free Marieke-group (Table A4). Aggressive behaviour was similar before and after CR was initiated, while self-directed behaviour decreased in the fruit-free Marieke-group, but not the fruit-provided Liby-group (Table A4). Thus, CR resulted in higher feeding rates and food availablility being restricted to the morning hours. CR combined with a fruit-free diet led to less self-directed behaviour and more natural activity budgets, i.e. less resting and more foraging, while aggressive behaviour was not affected.

Discussion

The effect of four overweight management strategies on relative adiposity, physiological and behavioural wellbeing of captive rhesus macaque groups was examined. The study groups contained, next to mature individuals with overweight, many individuals that were not yet skeletally matured and several adults that were not overweight. Therefore, we could establish whether overweight management strategies indeed targeted overweight individuals, while not hampering growing and normal-weight individuals or having other adverse effects. The first study phase investigated food puzzles and ideal body weight feeding, while 20% caloric restriction (CR) and a fruit-free diet were the focus of the second study phase. Food puzzles resulted in lower feeding rates and a food intake more spread over the day, yet overall activity budgets did not differ. Ideal body weight feeding resulted in less weight gain compared to the standard feeding routine based on actual body weight. CR was highly effective in reducing overweight and did not affect aggression, but it also resulted in large weight loss in some low-ranking individuals in two groups and more alopecia. The fruit-free diet had no effect on relative adiposity, but resulted in lower glucose levels, more natural behaviour and less self-directed behaviour. These results have several implications for husbandry and overweight management of group-housed rhesus macaques.

Food puzzles

First and foremost, we analysed whether our overweight management strategies actually reduced overweight, starting with the food puzzles. Food puzzles are feeding devices that typically increase the time and effort required to obtain food (Gottlieb et al. 2011; Coleman et al. 2012). We expected younger individuals to be more interested in the food puzzles compared to adults, thereby leading to a different division of food. We predicted relative adiposity to increase in younger individuals and decrease in older individuals. However, WHI, visual obesity scores and overweight prevalence did not decrease after the food puzzles were employed and change in WHI was independent of age. Although interest in objects, e.g. toys, decreased with age in Barbary macaques (*Macaca sylvanus*), interest was modulated by the presence of food (Almeling et al. 2016). Individuals lost interest in objects without food in early adulthood, while interest in objects with food remained high until monkeys were 24 years old (Almeling et al. 2016). Similarly, age had no effect on interest in food puzzles in a large sample of rhesus macaques (Bliss-Moreau & Baxter 2019). The food puzzles thus did not have the expected age-effect nor did they result in the desired weight reduction.

Cholesterol and glucose levels both increased after phase 1, but the increase did not differ between groups with different overweight management strategies. Presence of food puzzles therefore did not change cholesterol or glucose levels relative to absence of food puzzles. Nevertheless, food puzzles may have had an adverse effect on coat condition (see next paragraph). Furthermore, behavioural observations on food intake, activity budgets, aggression and self-directed behaviour showed that food puzzles had a positive effect on behavioural wellbeing. Daily feeding rate decreased in both groups when the food puzzles were present, while time spent at the feeding tray increased in the Marieke-group, but not the Grey-group. This difference likely results from a different effect of food puzzles on feeding behaviour in the two groups. The food puzzles still contained more than half of the monkey chow at the end of the observations (around 16.00h) in the Grey-group, while food puzzles were almost empty at that time in the Marieke-group (personal observation RV). Still, food puzzles were empty the following morning in both groups, implying that the food puzzles shifted feeding behaviour towards the late afternoon and evening, when the observer was no longer present, in the Grey-group. Although it generally took monkeys more time to obtain the chow and food intake was more spread over the day, overall activity budgets remained similar. Furthermore, food puzzles did not change aggressive behaviour and decreased self-directed behaviour. This complies with the conventional view that food puzzles have a positive effect on primate welfare and are nice enrichment devices (Brent & Eichberg 1991; Novak et al. 1998; Gottlieb et al. 2011).

All in all, food puzzles had no effect on physiological wellbeing and did not reduce overweight, but had a positive effect on behavioural wellbeing. Food puzzles thus provide a nice form of enrichment.

Ideal body weight feeding

Although WHI, visual obesity scores and overweight prevalence increased during the first study phase, body weight increase was significantly lower in groups with ideal body weight feeding compared to groups with the standard feeding routine. The standard feeding routine calculates a basal metabolic rate (BMR) based on actual body weight, which is suitable in groups with normal-weight individuals, but will lead to overfeeding when groups contain many overweight individuals. Ideal body weight feeding, which calculates a BMR based on ideal body weight, therefore better fits the energetic requirements of rhesus macaque groups. Ideal body weight feeding led to an average reduction in the amount of monkey chow of 14% ± 2%. Still, ideal body weight feeding resulted in significant weight gain in the Liby-group, but not the Grey-group. Although ideal body weight feeding did not reduce overweight, weight gain was lower with ideal body weight feeding compared to standard feeding. Implementation of ideal body weight feeding instead of the standard feeding routine is therefore recommended, at least in non-breeding groups. Further studies are needed to determine whether this also applies to breeding groups with pregnant and/or lactating females, which have higher metabolic demands to enable fetal development and nursing (Portman 1970; O'Brien & Kinnaird 1997).

Ideal body weight feeding did not alter cholesterol and glucose levels compared to standard feeding, as the change in cholesterol and glucose levels did not differ between groups. However, it may have had an effect on coat condition. Alopecia scores were only available for the Grey-group with both food puzzles and ideal body weight feeding, and the Bertha-group with no food puzzles and the standard feeding routine. Alopecia scores did not differ in the Bertha-group, but slightly increased in the Grey-group. However, the range of alopecia scores did not change and the difference was small (Table A2), so it may not be clinically relevant. Furthermore, we were not able to determine whether this increase was due to the food puzzles or due to ideal body weight feeding as alopecia scores were not available in the other groups. Finally, ideal body weight feeding had no measurable effect on behaviour, except for a decrease in cheek pouch fullness. Cheek pouch fullness decreased in the Grey-group with ideal body weight feeding, but not the Marieke-group with the standard feeding method.

In conclusion, ideal body weight feeding did not reduce overweight and did not affect physiological or behavioural wellbeing. Yet, ideal body weight feeding should be preferred over the standard feeding routine as it limits weight gain during adulthood, thereby likely preventing or at least delaying the onset of overweight.

Caloric restriction

Four groups were subjected to 20% CR during the second study phase. At the end of this phase, WHI, visual obesity scores and overweight prevalence had decreased in all four groups. Weight loss did not differ between the groups. In the Grey- and Liby-group, the 20% caloric restriction was added up to the effect of ideal body weight feeding (Table 2). Therefore, a more severe reduction in relative adiposity was expected in these groups, but this was not found. Age and baseline WHI had no overall effect on change in WHI, while low-ranking individuals lost more weight than high-ranking individuals. This implies that high-ranking individuals obtained more food than low-ranking ones, even though food was divided in multiple feeding trays to prevent monopolization. Similarly, wild chimpanzees in Gombe National Park with a high dominance rank tended to keep more stable body weights over time, demonstrating a better position in contest competition (Pusey et al. 2005). This rank-effect is undesirable when it leads to energy shortage and undernutrition in low-ranking animals, such as in wild Japanese macaques (*Macaca fuscata*) during periods of food scarcity (Tsuji & Takatsuki 2012). However, the rank-effect was not equally strong in all four groups.

The study groups significantly differed in how dominance rank and baseline WHI were related to change in WHI, as indicated by the interaction effects in the model. Ideally, there should be a negative correlation between delta WHI and baseline WHI, implying that individuals with a high baseline WHI lost more weight than individuals with a low baseline WHI. While this relation was found in the Grey-group, it was absent in the other three groups. Furthermore, there was a strong negative correlation between dominance rank and delta WHI in the Liby- and Marieke-group, but not the Grey- and Iffy-group. This also led to large weight loss in four low-ranking individuals from the Liby- and Marieke-group, with relative decreases in WHI up to 34% in 28 weeks. In overweight humans, a medically safe rate to lose weight is ~1% of the initial body weight per week (National Heart Lung and Blood Institute 1998), which translates to ~24.5% weight loss during our study period. This safe rate to lose weight was exceeded by the four low-ranking individuals, implying their weight loss was problematic.

Although CR had the desired effect in the Grey-group, CR did not have that effect in the three other groups, suggesting that the suitability of CR depends on the group. These group differences were not related to our overweight management strategies, but may result from inter-group variation in dominance style and social tolerance. In Barbary macaques, groups differed in dominance style as measured by hierarchy steepness and the proportion of agonistic interactions against the hierarchy (Amici et al. 2021). Similarly, chimpanzee groups can have different social styles, even when they live under comparable conditions (Cronin et al. 2014; DeTroy et al. 2021). This implies that not only species can differ in social style, but such differences are likely also present in groups from the same species, with some groups being more despotic than others. CR is probably not appropriate as overweight management strategy in highly despotic rhesus macaque groups, but may be suitable in more tolerant groups. This hypothesis is supported by our previous finding that mild CR had no adverse effects and there was no rank-effect on weight loss in multigenerational long-tailed macaque breeding groups (Zijlmans et al. 2022a), as long-tailed macaques are considered more tolerant than rhesus macaques (Thierry 2007).

Based on previous research in solitary-housed rhesus macaques, CR was expected to improve overweight related biochemical parameters, i.e. cholesterol and glucose levels (Lane et al. 1995, 1999; Verdery et al. 1997). However, cholesterol levels in our study did not improve after CR. This contrasts with our previous finding that mild CR improved cholesterol levels in adult long-tailed macaques housed in the BPRC breeding colony (Zijlmans et al. 2022a). Glucose levels significantly decreased, but the reduction was mainly present in groups on a fruit-free diet, but not in the groups with fruit still present in the diet. Therefore, the change in glucose levels was likely a result of the fruit-free diet and not due to CR. Similarly, glucose levels of adults, as well as fructosamine and Hba1c levels, did not change by mild CR in BPRC's long-tailed macaque colony (Zijlmans et al. 2022a). Since effects of CR can depend on its duration (cf. Nakamura et al. 2016), the 28-week period of CR in this study may have been too short to find significant beneficial effects of CR on biochemical parameters.

Because an increase in hair loss during the second study phase was seen, alopecia scores were included in the study post-hoc. Alopecia is a relatively common phenomenon in captive primates that can be caused by multiple factors, e.g. seasonal variation, aging, nutrition, hormones, genetics, immunologic and psychological factors (Novak & Meyer 2009). Although its etiology is not very well understood, alopecia in captive primates has been linked to age, sex, season, pregnancy and housing conditions (Steinmetz et al. 2006; Kroeker et al. 2014; Lutz et al. 2016, 2019). In our study, alopecia increased in all groups after CR was initiated and delta alopecia did not differ between groups with and without fruit in the diet. It was thus clear that coat condition deteriorated due to the 20% CR. Further research is needed to determine the mechanism behind the increased hair loss after CR. CR could have led to elevated stress levels, nutritional deficiencies or a combination of factors that resulted in more alopecia. Previous studies found that rhesus macaques with high alopecia scores have higher hair cortisol concentrations compared to monkeys without alopecia, but the strength of this relationship differs between facilites (Novak et al. 2014, 2016; Lutz et al. 2016). It would therefore be interesting to correlate alopecia scores with hair cortisol concentrations to see whether hair loss in our study was related to increased stress levels.

CR in the current form is not generally applicable as an overweight management strategy in rhesus macaque groups as it led to problematic weight loss in four low-ranking individuals in two out of four groups and more alopecia. These problems may be prevented by introducing CR more gradually (e.g. starting with 10% CR), and closely monitoring the effects before further reducing the food supply. For example, food supply was reduced by 10% per month over a period of three months to reach the desired 30% restriction in solitary-housed rhesus macaques (Colman et al. 2009). Besides, increasing the number of feeding trays during CR may reduce the effect of dominance rank on weight loss. The social dynamics make it difficult to reduce overweight effectively and safely in despotic groups with both normal-weight and overweight individuals. Therefore, primate facilties should prioritize overweight prevention rather than overweight reduction. Implementation of another standard feeding routine, such as ideal body weight feeding, and regular evaluation of diets is essential to really tackle the overweight problem in group-housing.

Thus, CR is highly effective in reducing overweight, but the suitability of CR as an overweight management strategy differs per group in a despotic primate species like the rhesus macaque.

Fruit-free diet

In addition to the 20% CR, fruit was replaced by vegetables in the Marieke- and Iffy-group. Change in WHI did not differ between groups, indicating that a fruit-free diet had no significant impact on relative adiposity in addition to CR. Dietary adjustments are known to affect biochemical parameters related to glucose response and lipid metabolism (e.g. Zijlmans et al. 2021b). In this study, glucose levels tended to improve in the groups with the fruit-free diet, which corresponds with previous studies (Cabana et al. 2018a). Furthermore, dietary adjustments reduced alopecia and improved coat condition in common marmosets (*Callithrix jacchus*: Ghebremeskel et al. 1991) and Hamadryas baboons (Cabana et al. 2018b). Therefore, the fruit-free diet was expected to reduce alopecia. However, alopecia scores increased during the second study phase in all groups, even in groups on a fruit-free diet. Any positive effect of a fruit-free diet on coat condition was likely nullified by the detrimental effects of CR. Again, it would be interesting to add cortisol concentrations to the study as cortisol concentrations of zoo-housed spider (*Ateles* spp.) and wooly monkeys (*Lagothrix* spp.) were higher in zoos that provided the highest amount of fruit (Ange-van Heugten et al. 2009). This suggests that an unnatural diet is a potential stressor for captive primates and cortisol concentrations should decrease after fruit is removed from the diet.

Besides impacting physiological wellbeing, dietary adjustments can also improve behavioural wellbeing of captive primates. In our study, the combination of CR with a fruit-free diet led to more natural activity budgets, i.e. less resting and more foraging, and reduced self-directed behaviour. Similarly, the removal of fruit from the diet of captive pygmy slow lorises (*Nycticebus pygmaeus*) diversified their activity budgets and reduced abnormal behaviour (Cabana & Plowman 2014). Also, regurgitation and reingestion in captive gorillas and self-directed behaviour in captive lemurs decreased by removing fruit from the diet (Lukas 1999; Britt et al. 2015). Our study thus complies with previous studies and is thereby adding more evidence to the idea that fruit-free diets enhance captive primate welfare.

In summary, a fruit-free diet did not reduce overweight, but resulted in lower glucose levels, more natural activity budgets and less self-directed behaviour.

Conclusion

This study evaluated the effect of four overweight management strategies on the physiological and behavioural wellbeing of rhesus macaque groups. Food puzzles had positive effects on behavioural wellbeing but did not reduce overweight. Ideal body weight feeding is recommended as it limits weight gain during adulthood. Caloric restriction (CR) had the desired effect in one group, but compromised physiological wellbeing of some low-ranking individuals in two other groups. In the current form, CR is therefore not generally applicable as an overweight management strategy in rhesus macaque groups. Finally, a fruit-free diet did not affect overweight, but resulted in physiological and behavioural improvements, and is therefore recommended. Thus, different overweight management strategies have diverse effects on group-housed primates and combining these strategies in a careful manner may prevent and reduce overweight while simultaneously improving physiological and behavioural wellbeing.

Appendix

Table A1. Ethogram of all scored activity-related and submissive behaviour.

Category	Behaviour	Description
Location	At tray	Animal is within one arm's length of the feeding tray
	Not at tray	Animal is more than one arm's length away from the feeding tray
Resting	Lying	Individual is lying still in one place
	Sitting	Individual's but cheeks touch a horizontal object so that there is no locomotion
	Standing	Individual remains stationary on its two legs (bipedal) or on all fours (quadrapedal)
Moving	Walking	Individual repetitively places one limb before the other to make a horizontal movement at moderate speed. Distinguished from running primarily by speed.
	Climbing	Individual uses arms and legs to make a vertical movement on a steep surface such as a wall or fence
	Running	Same as walking but at higher speed
	Aggression	Aggressive behaviour that may be energetically costly, e.g. lunge, slap, bite, push and pull
	Playing	All types of interactions between two or more individuals using the relaxed open mouth play face (in which the mouth is half wide open and teeth are covered by the lips). The interaction can be touch, pull, pug, push, hit, wrestle, chase and bite.
Foraging	Foraging	Searching for and investigating (potential) food items such as sifting through bedding.
Feeding	Feeding	Ingestion of food/water from the feeding trays/water dispensers
Grooming	Grooming	Individual uses its hands and fingers to go through its own or another individual's fur to remove foreign objects. This includes taking care of the skin, teeth and fur.
Other	Object exploration	Exploring or manipulating a non-food object (e.g. plastic bottle) with the hands or mouth
	Hanging	Individual grasps with all four limbs onto a vertical surface and remains stationary
Submissive behaviour	Bared teeth	Individual lifts its eyebrows and forehead, while the ears are positioned against the head and the lips are drawn back, thus displaying teeth and gum.
	Make room	Individual makes a small avoiding movement away from another individual that approaches, within three seconds after the approach. The actor remains in approximately the same spot and may make room by only moving the upper body.
	Give ground	Individual creates a distance between itself and another individual that comes closer than one meter or within three seconds after the individual arrived, by moving away from it at moderate speed.
	Escape	Individual flees away from the interaction partner at high speed.

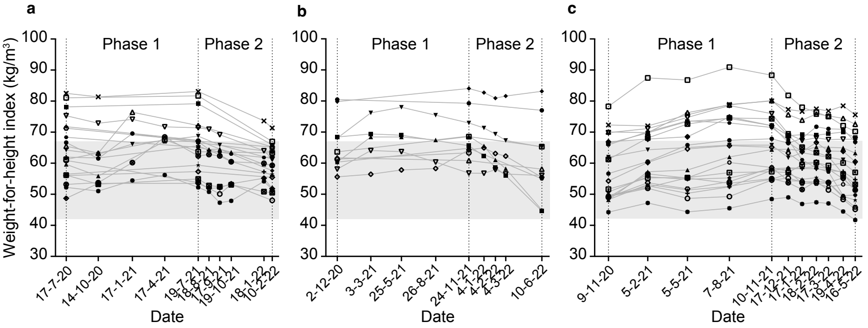

Figure A1. Weight-for-height index (WHI) of individuals in the Grey-group (**a**), Liby-group (**b**) and Marieke-group (**c**) throughout the study. Dots represent time points where WHI was measured, either during the health checks (dashed lines) or during voluntary weighing.

Table A2. Descriptive statistics of WHI and indicators of physiological wellbeing per group collected during annual health checks at baseline, after phase 1 and after phase 2. Mean ± SE (minimum–maximum) are reported.

Group	Study phase	Grey-group (n=20)	Liby-group (n=11)	Marieke-group (n=21)	Bertha-group (n=26)	Iffy-group (n=13)
WHI (kg/m³)	Baseline	64.6 ± 2.2 (48.7–82.5)	65.3 ± 2.5 (55.6–80.5)	57.8 ± 2.2 (44.2–78.3)	59.3 ± 1.7 (48.2–78.5)	NA
	Phase 1	65.6 ± 2.0 (52.2–83.1)	68.0 ± 2.4 (56.8–84.0)	65.8 ± 2.3 (48.4–88.3)	65.1 ± 1.9 (51.0–84.6)	59.7 ± 1.3 (52.3–66.8)
	Phase 2	59.8 ± 1.3 (48.0–70.5)	60.6 ± 4.0 (44.8–84.1)	57.5 ± 2.1 (41.7–75.6)	NA	55.9 ± 1.8 (45.0–68.5)
Cholesterol (mmol/L)	Baseline	3.10 ± 0.08 (2.60–3.69)	3.62 ± 0.18 (2.79–5.15)	2.86 ± 0.09 (2.08–3.61)	3.45 ± 0.07 (2.79–4.19)	NA
	Phase 1	3.36 ± 0.10 (2.48–4.20)	3.48 ± 0.20 (2.59–4.62)	3.11 ± 0.09 (2.28–3.71)	3.43 ± 0.08 (2.55–4.35)	3.64 ± 0.09 (3.21–4.36)
	Phase 2	3.24 ± 0.09 (2.65–3.94)	3.76 ± 0.17 (3.13–4.80)	3.09 ± 0.09 (2.35–3.83)	NA	3.71 ± 0.11 (2.94–4.66)
Glucose (mmol/L)	Baseline	7.53 ± 0.46 (4.17–12.2)	6.13 ± 0.36 (3.98–8.10)	4.89 ± 0.29 (2.53–7.60)	3.56 ± 0.16 (1.95–5.59)	NA
	Phase 1	5.89 ± 0.38 (3.46–9.89)	6.57 ± 0.22 (5.22–7.62)	6.23 ± 0.26 (3.49–8.49)	6.56 ± 0.37 (3.36–10.1)	6.08 ± 0.27 (4.45–7.51)
	Phase 2	6.35 ± 0.18 (4.94–7.72)	5.27 ± 0.68 (2.81–9.62)	4.84 ± 0.59 (1.27–9.57)	NA	4.12 ± 0.43 (0.61–5.58)
Alopecia score	Baseline	0.40 ± 0.13 (0–2)	0.73 ± 0.24 (0–2)	0.76 ± 0.25 (0–3)	0.69 ± 0.19 (0–3)	0.46 ± 0.14 (0–1)
	Phase 1	0.85 ± 0.15 (0–2)	NA	NA	0.54 ± 0.16 (0–2)	NA
	Phase 2	NA	2.27 ± 0.30 (1–4)	2.52 ± 0.22 (1–5)	NA	1.00 ± 0.16 (0–2)

Table A3. Results of the general linear models on the change in weight-for-height index (WHI), cholesterol, glucose and alopecia score, in four rhesus macaque groups per study phase. * p<0.05.

	Age	**Dominance rank**	**Baseline value**	**Group**	**Interaction effects**
Phase 1					
WHI (kg/m^3)	F(1,71)= 0.111, p=0.740	F(1,71)= 3.380, p=0.070	F(1,71)= 0.980, p=0.325	F(3,71)= 9.535, p<0.0005*	NA
Cholesterol (mmol/L)	(1,71)= 1.543, p=0.218	F(1,71)= 0.490, p=0.486	F(1,71)= 3.579, p=0.063	F(3,71)= 2.515, p=0.065	NA
Glucose (mmol/L)	F(1,71)= 0.354, p=0.554	F(1,71)= 0.201, p=0.656	F(1,71)= 36.901, p<0.0005*	F(3,71)= 1.746, p=0.165	NA
Alopecia score	F(1,39)= 0.358, p=0.553	F(1,39)= 2.457, p=0.125	F(1,39)= 15.369, p<0.0005*	F(1,39)= 6.443, p=0.015*	NA
Phase 2					
WHI (kg/m^3)	F(1,51)= 0.711, p=0.403	F(1,51)= 41.458, p<0.0005*	F(1,51)= 0.388, p=0.536	F(2,51)= 5.424, p=0.007*	Group:rank (F(3,51)=5.888, p=0.002); group:baseline (F(3,51)=5.218, p=0.003)
Cholesterol (mmol/L)	F(1,57)= 0.485, p=0.489	F(1,57)= 2.798, p=0.100	F(1,57)= 11.612, p=0.001*	F(3,57)= 4.640, p=0.006*	NA
Glucose (mmol/L)	F(1,51)= 0.935, p=0.338	F(1,51) =0.263, p=0.610	F(1,51)= 40.854, p<0.0005*	F(3,51)= 2.827, p=0.048*	Group:age (F(3,51)=6.676, p<0.0005); group:rank (F(3,51)=2.877, p=0.045)
Alopecia score	F(1,38)= 0.144, p=0.706	F(1,38) =0.979, p=0.329	F(1,38)= 8.437, p=0.006*	F(2,38)= 11.654, p<0.0005*	NA

Table A4. Results of the pairwise comparisons on indicators of behavioural wellbeing, i.e. food intake measures, activity budgets, aggression and self-directed behaviour, in two rhesus macaque groups per study phase. * p<0.05.

	Phase 1	**Phase 1**	**Phase 2**	**Phase 2**
	Grey-group (n=20)	**Marieke-group (n=21)**	**Liby-group (n=11)**	**Marieke-group (n=21)**
Food intake				
Time at tray	Z=-0.224, p=0.823	Z=-2.972, p=0.003*	t=1.539, p=0.155	Z=-3.458, p<0.0005*
Cheek pouch fullness	t=3.601, p=0.002*	t=-1.297, p=0.209	Z=-0.711, p=0.477	Z=-4.015, p<0.0005*
Activity budgets				
Resting	t=-0.079, p=0.937	t=0.733, p=0.472	t=1.927, p=0.083	t=4.714, p<0.0005*
Moving	Z=-0.411, p=0.681	Z=-0.122, p=0.903	t=1.463, p=0.174	t=1.146, p=0.265
Foraging	Z=-1.008, p=0.313	Z=-0.330, p=0.741	t=-1.378, p=0.198	t=-4.071, p<0.0005*
Feeding	Z=0.000, p=1	Z=-1.130, p=0.259	Z=-0.533, p=0.594	t=3.544, p=0.002*
Grooming	Z=-0.075, p=0.940	t=-1.535, p=0.140	t=-2.159, p=0.056	t=-1.731, p=0.099
Other behaviour	Z=-1.157, p=0.247	Z=-1.651, p=0.099	Z=-1.600, p=0.110	Z=-1.860, p=0.063
Aggression	Z=0.000, p=1	Z=-0.392, p=0.695	t=1.185, p=0.263	Z=-0.365, p=0.715
Self-directed behaviour	Z=-3.360, p<0.0005*	t=2.421, p=0.025*	t=2.111, p=0.061	Z=-3.736, p<0.0005*

Chapter 9

Primate overweight: general discussion and future prospects

Dian G. M. Zijlmans

Background and aims

Like humans, non-human primates (hereafter: primates) can become overweight or even obese. Overweight is a condition of excessive body fat, resulting from an imbalance between energy intake and energy expenditure (Kemnitz 1984; Chen et al. 2002). Living conditions have a great influence on both energy intake and energy expenditure, affecting their balance and subsequently the risk to develop overweight. Wild primates feeding on a natural diet do not become overweight, while captive primates are susceptible to gaining excessive amounts of body fat (West & York 1998). This likely relates to differences in food availability and energetic demands between the wild and captivity. In the wild, food quality and availability are uncertain and seasonally variable, so wild primates spend most of their day looking for food (van Schaik 1986; Altmann & Muruthi 1988; Dasilva 1992; Kurup & Kumar 1993; Menon & Poirier 1996). In contrast, captive primates are fed high-quality and nutritionally balanced diets throughout the year (Bauer et al. 2010). Food is abundant and easily accessible in captivity, limiting the need for physical activity and leading to a more sedentary lifestyle. As a result, overweight does not naturally occur in wild primates (Altmann et al. 1993; Dittus 2013), whereas a considerable portion of captive primates develop overweight during their life (Crumley & Raleigh 1995; Hamada et al. 2003; Yue et al. 2016, 2017; Sterck et al. 2019).

In the past, captive primates were often solitary-housed in small cages with little space for physical activity. These primates have been used in studies on human obesity and herein overweight was sometimes experimentally induced using high-caloric, high-fat diets. Luckily, there is more emphasis on primate welfare nowadays. Positive animal welfare means that an animal can adequately adapt to its environment and is thereby able to reach a state which the animal perceives as positive (Ohl & van der Staay 2012). An important indicator of animal welfare is the expression of species-specific natural behaviour (Olsson & Westlund 2007). Primates are highly social animals with a behavioural need to engage in complex social interactions and should preferably be housed in naturalistic social groups. Group-housing provides social benefits compared to solitary-housing, but it likely also affects an individual's balance between energy intake and energy expenditure. Group enclosures are relatively spacious and naturalistic, which provides more opportunity for species-specific natural behaviour such as grooming, running and climbing. Also, food is typically provided to the whole group and must be shared among group members. Some group members, especially high-ranking ones, may seize the opportunity to take more food than others. While group members experience the same housing conditions, this can lead to variation in relative adiposity, i.e. the amount of body fat relative to body weight, so some individuals might become overweight while others stay lean. Altogether, group-housing enhances the psychological wellbeing of primates, but it also provides an inherent risk of some individuals becoming overweight.

Being overweight has empirically documented adverse effects on primate welfare. First, overweight primates have a higher risk of certain health problems, like diabetes and cardiovascular disease (Kemnitz & Francken 1986; Shively & Clarkson 1987). Second, overweight primates perform less species-specific natural behaviour compared to normal weight primates (Bauer et al. 2012). Research into spontaneous (i.e. non-induced) overweight in group-housed primates is thus essential to optimize husbandry and enhance primate welfare. Therefore, relative adiposity should be measured objectively and overweight regularly monitored. Primate facilities can estimate relative adiposity and monitor overweight using several methods, which can be divided in external measurements (i.e. visual scoring, body condition scoring and anthropometry) and internal measurements (i.e. tritiated water dilution, DXA, MRI and CT). However, there is no “golden standard” on how overweight is measured or defined in group-housed primates. An appropriate measure and corresponding boundaries may depend on the setting in which they are employed. Furthermore, few studies examine potential husbandry strategies to prevent and reduce overweight in group-housed primates. To find effective overweight management strategies, we first need to identify risk factors, i.e. determine which factors are related to weight gain.

This thesis aimed to gain a better understanding of the correlates, consequences and control of overweight in group-housed primates. Therefore, we studied spontaneous overweight in relation to behaviour, diet, health and welfare in captive group-living rhesus macaques (*Macaca mulatta*) and long-tailed macaques (*M. fascicularis*) housed in naturalistic social groups at the Biomedical Primate Research Centre (BPRC) in Rijswijk, the Netherlands. This research is important for two main reasons: (1) overweight has adverse effects on primate welfare; and (2) using overweight primates might compromise the scientific validity of biomedical studies. We focused on three central questions concerning measuring overweight (Chapter 2-3), risk factors for overweight (Chapter 4-5) and overweight management strategies (Chapter 6-8) in group-housed primates. Altogether, answering these questions can contribute to improving husbandry and enhancing animal welfare of group-housed primates.

Measuring overweight in group-housed primates

Multiple methods, both invasive and non-invasive, have been used to measure relative adiposity and quantify overweight in captive primates. Here, we explore what measures are most suitable to determine overweight in captive group-living primates. Invasive methods require animals to be captured, immobilized and sedated, while non-invasive methods do not. Sedation should be minimized as it can lead to stress, injuries and side-effects from anesthesia (Horne 2001; Balcombe et al 2004; Suleman et al 2004). For that reason, group-housed primates are generally only sedated for routine handling procedures, such as during relocations or health examinations. Invasive methods that were used in our studies include body condition scoring (BCS: Chapter 2), computed tomography (CT: Chapter 4) and anthropometry (Chapter 2, Chapter 4-8), while non-invasive methods include visual obesity scoring (Chapter 8) and voluntary weighing (Chapter 3, Chapter 8). Note that all invasive measurements were performed during other required (veterinary) management procedures, when monkeys were already being sedated for that purpose. Furthermore, abdominal CTs were performed in combination with thorax CTs for a tuberculosis study. Therefore, no monkeys were sedated only to measure their relative adiposity. The employed methods have different advantages and drawbacks, thereby affecting their suitability for measuring overweight in group-housed primates.

Regarding the invasive methods, anthropometry should be preferred over BCS and CT for measuring overweight in group-housed primates. BCS was quickly discharged as measuring method to quantify overweight in BPRC's macaque colony, as our veterinarians varied in how they applied the BCS system, some being more conservative than others. This resulted in a wide range of body weights associated with the optimal BCS (BCS = 3) and some individuals being misclassified, i.e. individuals being classified as having a healthy weight while they were actually overweight and vice versa. When animals are already being sedated, anthropometry is more objective and precise than BCS and should thus be preferred. This does not mean that BCS cannot be a valuable tool for primate facilities as it can still provide a general picture of the average condition in a primate colony. Nevertheless, using BCS on an individual level with multiple raters should be considered with caution. Alternatively, CT provides more information on the regional distribution of body fat compared to anthropometry and BCS. CTs are valuable when a study requires differentiation between different tissue types, e.g. intra-abdominal and subcutaneous body fat. However, this amount of detail is not needed for overweight monitoring in group-housed primates. Moreover, CT uses radiation and is more expensive, labor intensive and time-consuming compared to the other methods. Thus, CTs are less practical in large primate colonies, and so anthropometry is the preferred method to measure overweight in group-housed primates.

Collecting anthropometric measurements such as body weight, height, abdominal circumference and skinfold thicknesses during routine health examinations is cheap, easy and quick. Based on body weight and height, species-specific weight-for-height indices (WHIs) can be calculated. In humans, the best known WHI is the body mass index (BMI), yet BMI was positively correlated with height in female macaques at BPRC and thereby violates an important prerequisite for a valid WHI. Therefore, we propose to measure overweight in group-housed primates using species-specific WHIs: WHI3.0 in rhesus macaques and WHI2.7 in long-tailed macaques (Chapter 2). These WHIs were optimally independent of height and were highly correlated with other measures of relative adiposity, i.e. abdominal circumference and skinfold thicknesses. Abdominal circumference provides only a regional estimate and skinfold thicknesses were highly observer-dependent, even after ample training. These measures are therefore less appropriate overweight measures. Furthermore, total body fat percentage in the abdominal area, measured by CT, was used to corroborate the species-specific WHI in female rhesus macaques. Total body fat percentage and WHI were highly corrected, indicating that WHI nicely reflects internal body fat (Chapter 4). Thus, relative adiposity of group-housed primates is ideally measured using species-specific WHIs.

A non-invasive method to collect WHIs, which does not require animals to be sedated, would allow more frequent measurements and better overweight monitoring. Luckily, methods to collect body measures in a more animal welfare-friendly way are becoming increasingly available and more advanced. The development of these methods mainly originates from studies in the wild, where collecting data on physical traits is even more challenging compared to captivity (Rothman et al. 2008). Previous studies measured body weight in wild primates by letting animals themselves decide whether to use a (baited) scale or not, which we refer to as voluntary weighing (Chapter 3). This method was effective in wild chimpanzees (*Pan troglodytes*: Pusey et al. 2005), yellow baboons (*Papio cynocephalus*: Altmann & Alberts 1987), bearded capuchin monkeys (*Sapajus libidinosus*: Fragaszy et al. 2010), toque macaques (*Macaca sinica*: Dittus 1998), Japanese macaques (*M. fuscata*: Mori 1979) and rhesus macaques (Zhang et al. 2016). A pilot study was performed to measure body weight using voluntary weighing in captive group-living rhesus macaques at BPRC (Chapter 3). Like the wild studies, the method showed promising results in captivity, as 68% of the study animals participated and the method yielded reliable body weights. In the meantime, voluntary weighing has been applied in six groups with a participation rate of 74% (unpublished data). Still, voluntary weighing is relatively time-consuming and should be further developed to become more efficient, e.g. incorporating automated systems for identification and weighing in the primates’ home enclosures. These systems enable automatic body weight monitoring without human presence, thereby increasing efficiency and saving time, and are already available for livestock and birds (Turner et al. 1984; Peiper et al. 1993; Vézina et al. 2001; Dickinson et al. 2013). Voluntary weighing thus allows more frequent body weight measurements and quicker detection of changes in body weight, which may signal underlying welfare problems (Jennings et al. 2009). This becomes especially valuable for overweight monitoring when body weight data can be combined with height data. In full-grown animals, height will no longer change after reaching skeletal maturation, so WHI can be calculated repeatedly once their height is known.

Besides using anthropometry during sedation, height of the animals can be collected non-invasively using photogrammetry. Photogrammetry refers to measuring body dimensions with photographs and has been used in several free-ranging and wild primates, e.g. chimpanzees (Sandel et al. 2022), gorillas (*Gorilla gorilla*: Breuer et al. 2007), geladas (*Theropithecus gelada*: Lu et al. 2016), red colobus monkeys (*Procolobus rufomitratus*: Rothman et al. 2008), mantled howling monkeys (*Alouatta palliata*: Barrickman et al. 2015), Japanese macaques (Kurita et al. 2012), rhesus macaques and long-tailed macaques (Jadejeroen et al. 2014). Different techniques have been developed, which vary in how the scale of the object is determined. Some studies use a scaling bar or object of known size (e.g. Kurita et al. 2012), while others calculate the size of the object by the ratio of the focal length of the lens and the distance between the camera and the object (e.g. Breuer et al. 2007). Finally, the parallel laser method uses two laser beams with a fixed distance from each other as a scale, as the lasers are projected onto the animal when taking the photo (e.g. Rothman et al. 2008; Barrickman et al. 2015; Lu et al. 2016; Sandel et al. 2022). Although non-invasive height measurements were not used in this thesis, as height was measured during annual health checks, photogrammetry can likely also be applied to captive group-living primates. Combining voluntary weighing and photogrammetery provides a non-invasive method to collect WHIs and allows better overweight monitoring of group-housed primates.

The species-specific WHIs proposed in Chapter 2 were used to assess the variation in relative adiposity of captive group-living macaques housed in relatively spacious indoor and outdoor enclosures. The incidence of overweight and underweight in the BPRC breeding colony was determined using previously proposed relative adiposity boundaries from relatively old and solitary-housed rhesus macaques living in small cages (Raman et al. 2005). The overweight criterion or upper boundary was based on the insulin sensitivity index, which yielded an upper boundary of 23% body fat in males and 18% body fat in females (Raman et al. 2005). We adopted the same upper boundary, as it was based on health parameters that were not included in our study. This led to a considerable portion of overweight individuals (i.e. 17–23%) in the BPRC breeding colony. Employing the same lower boundary from Raman et al. (2005), based on 8–9% body fat, resulted in a high portion of underweight individuals (i.e. 21–25%), which does not comply with our impression that these were actually lean animals that resembled body proportions of wild macaques. Similarly, in group-housed Japanese macaques, many adults (i.e. 23–34%) were regarded as lean when the minimum limit of DXE measurement, i.e. 4% body fat, was used as a criterion (Hamada et al. 2003). Thus, the underweight criterion for solitary-housed macaques does not correspond to the lower boundary for group-housed macaques with more opportunities for social interactions and locomotion.

Alternatively, a lower boundary based on the body fat percentage of wild primates, i.e. 1.9% in baboons (Altmann et al. 1993) and 2.1% in toque macaques (Dittus 2013), is recommended (Chapter 2). This resulted in underweight being limited to a few individuals (i.e. 0–3%) and complies with the impression that individuals with a WHI close to the lower boundary are similar in build to reproducing wild primates. A higher value for the lower boundary would consider normally slender individuals as being underweight, while individuals with a low WHI were typically scored as having an optimal body condition score (i.e. BCS = 3) and these females gave birth at a normal rate (unpublished data). Furthermore, data of the wild long-tailed macaque population show that our lower boundary is not stricter than living in the wild, as most females from the wild population had a WHI below the lower boundary. Being lean might even be beneficial, as it improves longevity and delays the onset of age-related diseases in rhesus macaques (Mattison et al. 2017). Altogether, relative adiposity should ideally range between 42 and 67 (WHI3.0) in rhesus macaques and between 39 and 62

(WHI2.7) in long-tailed macaques, resulting in most group-housed macaques at the BPRC breeding colony having a healthy relative adiposity.

In summary, relative adiposity is ideally measured using species-specific WHIs and non-invasive methods to calculate WHIs enable better overweight monitoring in captive group-living primates. Furthermore, group-housed primates living in relatively spacious enclosures are more similar to wild than solitary-housed individuals regarding the underweight criterion.

Health consequences of overweight in group-housed primates

Measuring relative adiposity and monitoring overweight in group-housed primates is important to guard primate health. Health consequences of overweight are well-studied in solitary-housed primates, but it remains uncertain to what extent overweight-related health problems adversely affect primates in a more naturalistic, group-housed setting. Various blood parameters can be used to assess overweight-related health problems such as diabetes and cardiovascular disease. In this thesis we used glucose, fructosamine, glycated hemoglobin (HbA1c), total cholesterol and triglyceride levels as biomarkers for overweight-related health problems as these were included in the standard blood chemistry. Many previous studies found associations between overweight and these blood parameters, so they are often used in the diagnosis and management of overweight-related health problems in captive primates. For example, body weight is positively correlated with triglyceride and glucose levels in adult female long-tailed macaques (Chen et al. 2002). Furthermore, cholesterol and triglyceride levels are generally higher in obese rhesus macaques compared to their non-obese counterparts (Hamilton et al. 1972; Kemnitz & Francken 1986; Kemnitz et al. 1989). These studies mainly included solitary-housed animals with body fat percentages up to 61%, and obesity was often experimentally induced (Kemnitz & Francken 1986). When solitary-housed long-tailed macaques are given the opportunity to exercise, total cholesterol does not differ between monkeys in the exercise and non-exercise group (Kramsch et al. 1981). However, “good” cholesterol (HDL) increased and “bad” cholesterol (LDL) and triglyceride levels decrease, suggesting that moderate exercise has a protective role against heart disease in primates (Kramsch et al. 1981). The literature thus provides ample evidence that overweight has adverse health consequences in solitary-housed primates with little physical activity and/or overweight being experimentally induced.

Similar physiological changes and associated health problems can be expected in overweight primates living in groups. Macaques in the BPRC population live in naturalistic social groups with ample space for locomotion and they exhibit spontaneous onset of overweight during adulthood. Surprisingly, no relation between relative adiposity and any of the blood parameters was found in adult female long-tailed macaques (Chapter 6). This implies that the BPRC long-tailed macaque population may experience relatively few overweight-related health problems. Similarly, obese group-housed Japanese macaques (i.e. > 22% body fat) did not develop overweight-related health problems as serum levels of adiponectin, insulin, glucose, fructosamine and HbA1c did not differ between obese and non-obese monkeys (Takahashi et al. 2006). Furthermore, group-housed bonnet macaques (*Macaca radiata*) in the heaviest tertile had higher insulin and triglyceride levels compared to the rest of the colony, but did not differ in glucose, total cholesterol, HDL or LDL concentrations (Kaufman et al. 2005). Adverse health effects related to overweight may be more pronounced in solitary-housed primates compared to group-housed primates. Indeed, diabetic long-tailed macaques in solitary-housing had higher plasma glucose levels compared to diabetic individuals in group-housing (Yue et al. 2017). Thus, increased opportunities for social interactions and locomotion in group-housing may have a protective role against overweight-related health problems.

When health consequences related to overweight differ between solitary-housing and group-housing, this can have implications for our overweight criterion. The WHI boundaries presented in this thesis provide a tool to monitor overweight and underweight in group-housed macaques, but we have already shown that the underweight criterion in solitary-housing did not match the lower boundary in group-housing. Likewise, the upper boundary for group-housed primates may differ from the upper boundary of solitary-housed primates. The upper boundary should ideally be based on adverse health effects associated with overweight. Besides the biomarkers used in this thesis, HDL, LDL, insulin, leptin and adiponectin could be interesting candidates for developing a physiological overweight criterion (Takahashi et al. 2006; Power et al. 2013; Dennis et al. 2019). For example, the ratio of leptin to adiponectin has been proposed as an index to identify overweight in long-tailed macaques (Chen et al. 2003). Thus, the findings in this thesis indicate that health consequences of overweight may differ between housing conditions. We therefore call for more studies on health risks associated with spontaneous overweight in group-housing to allow a more objective identification of overweight in group-housed primates.

Risk factors for overweight in group-housed primates

To find effective overweight management strategies for group-housed primates, identifying risk factors for overweight and determining which factors are related to weight gain is important. Several potential risk factors were studied in this thesis, namely aging, housing condition, stress, dominance rank and behaviour. Age is a major risk factor for overweight and overweight-related health problems, both in humans and primates (Schwartz & Kemnitz 1992; Baumgartner et al. 1995). Significant body fat deposition in free-ranging rhesus macaques occurs after skeletal maturation is complete and linear growth has stopped (Schwartz & Kemnitz 1992). Body fat deposition and the associated risk to develop metabolic disorders are greatest between 10 and 17 years of age in captive rhesus macaques (Ramsey et al. 2000a).

Similarly, positive correlations between age and relative adiposity were found in rhesus macaques at BPRC (Chapter 4, Chapter 8).

Risk factors for developing overweight may differ between housing conditions. The type of housing itself may also be a risk factor, as overweight is generally more common in solitary-housing compared to group-housing (Kemnitz 1984). Body weight was 21.3% higher in solitary-housed long-tailed macaques compared to their group-housed counterparts (Yue et al. 2017). Similar differences in relative adiposity were expected between group- and pair-housing at BPRC, as the indoor cages in pair-housing provide less space for physical activity compared to the larger indoor and outdoor enclosures in group-housing. However, we found no overall effect of the housing condition on relative adiposity, yet there was individual variation in body fat alterations when female rhesus macaques moved from group- to pair-housing (Chapter 4). This individual variation in body fat alterations was related to the baseline adiposity level and dominance rank in group-housing: lean and low-ranking individuals mainly gained body fat, while heavy and high-ranking individuals generally lost body fat. We expected these individual changes in body fat to be mediated by glucocorticoids, i.e. stress hormones, since social stress increases abdominal fat deposition in long-tailed macaques (Shively et al. 2009). However, hair cortisol concentrations were not related to body fat levels, thereby not supporting the hypothesis of glucocorticoid-mediated alterations in body fat (Chapter 4). Still, housing condition was related to stress levels with higher hair cortisol concentrations in pair-housing compared to group-housing (Chapter 4). Similarly, fecal glucocorticoids were higher among female Japanese macaque in indoor single cages compared to females in outdoor social groups (Takeshita et al. 2014). Thus, housing condition affects stress levels, but neither of them seem to be significant risk factors for developing overweight in group-housed primates.

The social environment, i.e. an animal's position in the group relative to other group members, may have more effect on relative adiposity than an animal's physical environment. Various chapters in this thesis show that dominance rank is an important determinant of changes in relative adiposity. When female rhesus macaques moved from group- to pair-housing, dominance rank in group-housing predicted which individuals were gaining and losing body fat. Females that were low-ranking in their social group gained body fat in pair-housing, while individuals that were high-ranking in their social group lost body fat in pair-housing (Chapter 4). In contrast, high-ranking female rhesus macaques gain more weight over time compared to low-ranking ones when they reside in group-housing (Chapter 5). Dominance rank not only affected weight gain, but also influenced weight loss after caloric restriction (see next section). Although dominance rank had no effect on weight loss after caloric restriction in adult female long-tailed macaques (Chapter 7), low-ranking females lost more weight after caloric restriction than high-ranking females in two of the four rhesus macaque groups (Chapter 8). The effect of dominance rank on changes in relative adiposity thus differs between the species. This may be due to variation in the level of despotism and social tolerance, because long-tailed macaques are considered more tolerant than rhesus macaques (Thierry 2007). To test this hypothesis, it would be interesting to study the relation between dominance rank and relative adiposity in other macaque species, especially more egalitarian ones.

The exact mechanism behind the effect of dominance rank on relative adiposity is still unclear, but it likely relates to high-ranking individuals having higher energy intake and/or lower energy expenditure than low-ranking individuals. High-ranking primates generally have priority of access to resources and can monopolize these resources, thereby allowing them to obtain food at a low energetic cost (Whitten 1983; Boccia et al. 1988; Overduin-de Vries et al. 2020). As a result, high-ranking primates have greater access to food, are interrupted less during feeding and spend more time eating compared to low-ranking ones (Smuts & Nicolson 1989; Deutsch & Lee 1991; Bauer et al. 2012). However, dominance rank was not related to any measure of food intake or activity budgets in female rhesus macaques at BPRC (Chapter 5). Although behaviour related to energy expenditure, i.e. an individual's activity budget, was related to relative adiposity at baseline, it did not predict weight gain during adulthood. Therefore, behaviour related to energy expenditure is more likely a consequence than a risk factor of being overweight. Food intake was unrelated to weight gain in solitary-housed female rhesus macaques maintained on a high-fat diet (Sullivan et al. 2006). Similarly, no evidence was found for a link between food intake and weight gain in our study. However, food intake of group-housed primates is notoriously difficult to quantify on an individual level (Chivers 1998; Wilson et al. 2008). Still, total caloric intake correlated with weight gain when feeding high-fat diets in group-housed female rhesus macaques (Wilson et al. 2008). Studies thus provide inconclusive results on the role of energy intake in overweight development in group-housed primates. More accurate ways to measure energy intake in group-housed primates are needed as it seems plausible that the provisioning of food in captivity contributes to the overweight problem.

All in all, we found no evidence for type of housing or stress levels being significant risk factors for developing overweight in group-housed primates. Age and dominance rank are important determinants of relative adiposity, but the rank-effect effect may differ between housing conditions and species. Low physical activity is more likely a result of being overweight, while high energy intake may be a risk factor, but food intake is hard to quantify in a group setting.

Overweight management strategies in group-housed primates

Overweight management strategies combining dietary and husbandry changes can lead to significant weight loss, physiological and behavioural improvements in group-housed primates. Many primate facilities probably perform dietary and husbandry changes on a trial-and-error basis, the outcome of which is rarely reported. However, it is important to objectively measure and report (e.g. as short communication or case study) the effects of dietary and husbandry changes on body measures, physiology and behaviour to allow a systematic evaluation of effective and safe overweight management strategies in group-housed primates. Since behaviour related to energy expenditure was likely more a consequence than a risk factor of overweight in group-housed primates (Chapter 5), our overweight management strategies mainly aimed at reducing energy intake rather than increasing energy expenditure. The provisioning of food (Chapter 8), the type of food (Chapter 6, Chapter 8) and the amount of food (Chapter 7-8) were adapted to examine their effect on overweight and overweight-related health problems.

First, food in captivity is often provided in feeding trays and is easily accessible. We adapted the way in which monkey chow was provided by putting all monkey chow in food puzzles. Food puzzles are feeding devices that typically increase the time and effort required to obtain food (Gottlieb et al. 2011; Coleman et al. 2012). Food puzzles can be manufactured relatively easy and cheap with left over materials. In our case, Gibco medium bottles from the lab and jerrycans were utilized for the food puzzle design. The effort individuals must put in to obtain food may prevent them from eating out of boredom. As younger primates are generally more curious and perform more object manipulation (McGuire et al. 1994; Byrne & Suomi 1996; Bergman & Kitchen 2009), we expected the food puzzles to increase food intake in younger individuals and decrease food intake in adults, thereby reducing overweight. Nevertheless, food puzzles did not have the expected age-effect nor did they result in the desired weight reduction (Chapter 8). Our food puzzles may have been too little energy-demanding, as individuals could sit and reach the food puzzles easily from inside their home cage. In group-housed chimpanzees, food puzzles were placed on different heights of the cage, forcing animals to climb and support their own body weight while feeding (Bridges et al. 2013). As a result, overweight individuals lost weight while normal-weight individuals remained similar in body weight. Although no effect of food puzzles on relative adiposity was found, food puzzles decreased feeding rate and self-directed behaviour while aggression remained similar (Chapter 8). This supports the general notion that food puzzles positively affect primate welfare and are nice enrichment devices (Brent & Eichberg 1991; Novak et al. 1998; Gottlieb et al. 2011).

Second, the nutritional composition of the diet likely plays an important role in captive primates becoming overweight and developing overweight-related health problems. As these issues do not occur in wild primates feeding on a natural diet, a diet that mimics the nutritional composition of wild diets may prevent overweight and overweight-related health problems. Captive diets generally contain less fiber and more fat and easily digestible carbohydrates, such as sugar, compared to wild diets (Cabana et al. 2018a). Accordingly, two adjustments in the type of food were investigated in this thesis, namely removing wheat bread and fruits from the diet. Wheat bread was fed several times a week at BPRC until June 2019. Wheat bread has a high glycemic index, indicating the carbohydrates are easily digested and metabolized (Atkinson et al. 2008; Augustin et al. 2015). This leads to a high increase in blood glucose after consumption, which has been linked to increased risk of diabetes and cardiovascular disease in humans (Ludwig 2002). Replacing bread by grains and vegetables resulted in more balanced body weights and positively affected fructosamine and triglyceride in adult female long-tailed macaques (Chapter 6). Fruit is currently (i.e. December 2022) still part of the standard diet of rhesus macaques at BPRC (2x per week). Although many wild primates are frugivores, meaning fruit is their main food source, fruits cultivated for human consumption have a different nutritional composition from wild fruits and are therefore less appropriate to feed in captivity (Kaumanns et al. 2006). The nutritional value of cultivated vegetables may be more similar to wild fruits. While replacing fruits with vegetables had no effect on relative adiposity of female rhesus macaques at BPRC, our results indicated that glucose levels were improved (Chapter 8). Similarly, blood glucose levels of captive great apes improved with a low-sugar, high-fiber diet (Cabana et al. 2018a). Besides beneficial effects on health parameters, providing more natural diets in captivity has the additional benefit of improving behavioural wellbeing. For example, removing milk and pellets from the diet of captive gorillas reduced undesirable behaviour, such as regurgitation and reingestion, and increased foraging behaviour (Lukas et al. 1999; Less 2012). Similarly, switching to a more fiber-rich pellet reduced regurgitation and reingestion in zoo-housed chimpanzees (Mulder et al. 2016). A fruit-free diet diversified the activity budgets and reduced abnormal behaviour of captive pygmy slow lorises (*Nycticebus pygmaeus*), and reduced aggression and self-directed behaviour in captive lemur species (Cabana & Plowman 2014; Britt et al. 2015). Finally, a fruit-free diet led to more natural activity budgets and less self-directed behaviour in rhesus macaques at BPRC (Chapter 8). Thus, a natural diet enhances primate welfare, as it likely improves both physiological and behavioural wellbeing.

Third, the amount of food provided in captivity likely contributes to the overweight problem. The standard feeding routine of many primate facilities is to feed pellets *ad libitum* or in a predetermined amount depending on the number of animals in the group and their body weight, so that food is available throughout the day (Bauer et al. 2010). In this thesis, two ways to adjust caloric intake in group-housed macaques were investigated, namely ideal body weight feeding and caloric restriction. Ideal body weight feeding refers to calculating the amount of food per individual based on its ideal body weight, as opposed to the standard feeding method that uses the individual's actual body weight. We considered a body fat percentage of 8% to represent an ideal body weight and calculated the amount of food accordingly. Ideal body weight feeding results in less food in groups with many overweight individuals, while it increases food supply in groups with many lean individuals. It was therefore predicted to better fit each group's energetic requirements than standard feeding. Ideal body weight feeding did not reduce overweight and did not affect physiological and behavioural wellbeing in rhesus macaque groups, while relative adiposity increased with the standard feeding routine (Chapter 8). Thus, ideal body weight feeding may prevent, or at least delay, the onset of overweight and should be preferred over the standard feeding routine as it limits weight gain during adulthood.

Finally, we studied the effect of caloric restriction on both macaque species at BPRC. Caloric restriction means reducing the amount of food by a certain percentage (e.g. 10%, 20% or 30%) irrespective of an individual's body weight. Caloric restriction is a highly effective strategy to prevent and reduce overweight in solitary- and pair-housed primates (Ingram et al. 1990; Hansen & Bodkin 1993; Kemnitz et al. 1993). Some researchers argue that caloric restriction may not be appropriate in group-housing as food is often not equally divided among group members (Bauer et al. 2011). It could potentially lead to malnutrition when food is not equally divided among group members, but also stress and increased aggression. Therefore, the effectivity and safety of mild (20%) caloric restriction was examined in ten long-tailed macaque groups and four rhesus macaque groups. Mild caloric restriction effectively reduced overweight, independent of dominance rank, and improved cholesterol levels in adult long-tailed macaques (Chapter 7). Furthermore, mild caloric restriction had no adverse effects on infant growth, reproduction or veterinary consultations in the breeding groups (Chapter 7). Although the effects of caloric restriction on stress response and behaviour were not examined in this study, our data on the long-tailed macaques suggest that caloric restriction can potentially be a valuable overweight management strategy in group-housed primates.

To further look into the effects of caloric restriction on physiological and behavioural wellbeing, 20% caloric restriction was studied in four rhesus macaque groups. Similar to the long-tailed macaques, caloric restriction significantly decreased relative adiposity in rhesus macaques (Chapter 8). Although caloric restriction had the desired effect in one group, caloric restriction had adverse effects on two other groups. Hair loss (or alopecia), a potential indicator of stress, increased after caloric restriction and low-ranking females lost more weight than high-ranking females. Four low-ranking individuals from two groups even experienced problematic weight loss, i.e. weight loss of >1% per week (National Heart Lung and Blood Institute 1998). One possible explanation for these group differences may be inter-group variation in dominance style and social tolerance. Different groups from the same species can differ in dominance style, even when living under comparable conditions (Cronin et al. 2014; Amici et al. 2021; DeTroy et al. 2021). This implies that not only species (cf. Thierry 2007), but also groups from the same species can differ in dominance style, with some groups being more despotic than others. When groups contain both normal-weight and overweight individuals, the social dynamics might complicate safe overweight reduction in highly despotic groups. Primate facilities should therefore focus on overweight prevention rather than overweight reduction. This requires regular evaluation of the diet and overweight status in group-housed primates. When using caloric restriction to reduce overweight, caloric restriction should be introduced gradually and groups should be closely monitored throughout to prevent adverse effects. Thus, caloric restriction is highly effective in reducing overweight, but its suitability as an overweight management strategy differs per group.

Concluding remarks

Overall, group-housing highly improves primate welfare, but also leads to an inherent risk of some individuals becoming overweight. This thesis emphasizes the importance of monitoring overweight in group-housed primates and provides non-invasive measurements as a tool to do this. Overweight management strategies in group-housed primates are rather successful, but can sometimes result in undesirable outcomes. Overweight prevention should therefore be favored over overweight reduction, and this can be achieved by providing diets that mimic the natural diets of wild primates with regard to provisioning, composition and amount of food. Thus, having an overweight management program is recommended for all facilities housing primates as it contributes to improving primate welfare.

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Nederlandse samenvatting

Overgewicht komt niet alleen voor bij mensen, maar ook bij apen. Er is sprake van overgewicht als er te veel vetweefsel aanwezig is in het lichaam. Dit ontstaat wanneer de energie-inname via voedsel groter is dan het energieverbruik. Hoewel apen in het wild normaal niet dik worden, worden apen in gevangenschap dit vaak wel. Dit komt waarschijnlijk doordat energie-inname en energieverbruik verschillen tussen apen in het wild en apen in gevangenschap. Voedselkwaliteit en-beschikbaarheid zijn moeilijk te voorspellen in het wild, waardoor apen in het wild met een natuurlijk dieet een groot deel van de dag bezig zijn met voedsel zoeken. Wanneer er te weinig voedsel te vinden is, kan dit leiden tot ondergewicht of zelfs sterfte. Ook moeten apen in het wild vaak grote afstanden lopen om eten te vinden en moeten ze soms klimmen om bij het eten te komen. Daarentegen wordt er genoeg eten met kwalitatief hoge voedingswaardes gevoerd aan apen in gevangenschap en is dit eten meestal makkelijk verkrijgbaar. Apen in gevangenschap hoeven zelf niet op zoek naar voedsel en kunnen het makkelijk opeten, zonder daar veel moeite voor te doen. Apen in gevangenschap hebben dus meer voedselzekerheid en zijn daardoor minder actief dan apen in het wild.

Vroeger werden apen in gevangenschap vaak in hun eentje gehuisvest in kleine kooien met weinig bewegingsruimte en een afgemeten hoeveelheid eten. Er is veel onderzoek gedaan naar overgewicht bij deze apen, waarbij de apen soms expres dik werden gemaakt met een ongezond dieet. Gelukkig is er vandaag de dag meer oog voor huisvesting en dierenwelzijn. Apen zijn zeer sociale dieren en moeten bij voorkeur samenleven met andere apen in groepen met grote binnen- en buitenverblijven. Ze hebben dan meer mogelijkheden voor natuurlijk gedrag zoals vlooien en klimmen. Verder wordt het voedsel aangeboden aan de hele groep en moeten de apen dit onderling verdelen. Sommige apen, vooral apen die hoog in de rangorde staan, zullen proberen meer voedsel te krijgen dan anderen. Hierdoor kan binnen een groep de ene aap dik worden, terwijl anderen op een gezond gewicht blijven. Kortom, apen huisvesten in sociale groepen is beter voor dierenwelzijn, maar heeft tegelijkertijd ook gevolgen voor de balans tussen energie-inname en energieverbruik, waardoor sommige apen te dik kunnen worden.

Overgewicht kan nadelig zijn voor het welzijn van apen. Apen met overgewicht zijn namelijk minder actief en vertonen minder natuurlijk gedrag dan apen met een gezond gewicht. Daarnaast hebben apen met ernstig overgewicht, net als mensen, meer kans op diabetes en hart- en vaatziekten. Onderzoek naar overgewicht bij apen gehuisvest in groepen is dus belangrijk om dierenwelzijn te waarborgen. Er zijn verschillende manieren om overgewicht in apen te meten. Bij mensen wordt de Body Mass Index (BMI) vaak gebruikt, maar hoe zit dat bij apen? Een universele manier om te bepalen of een dier ondergewicht of overgewicht heeft bestaat nog niet voor groepslevende apen. Naast het meten en monitoren van overgewicht, moeten er ook oplossingen worden gevonden om overgewicht bij apen in groepen te verminderen. Daarvoor moet eerst bestudeerd worden welke factoren een rol spelen bij het ontstaan van overgewicht, dus wat zijn risicofactoren voor overgewicht en welke apen hebben meer kans om dik te worden.

Dit proefschrift heeft als doel om meer inzicht te krijgen in de oorzaken en gevolgen van, en oplossingen voor overgewicht bij apen gehuisvest in groepen. Daarvoor hebben we overgewicht bestudeerd in twee soorten makaken, te weten: resusapen (*Macaca mulatta*) en Java-apen (*M. fascicularis*), welke leven in sociale groepen bij het Biomedical Primate Research Centre (BPRC) in Rijswijk, Nederland. De apen leven hier in natuurlijke groepen waarbij de samenstelling en het beheer van de groepen zoveel mogelijk overeenkomt met de situatie in het wild. Vrouwen blijven hun hele leven in hun geboortegroep, terwijl mannen de groep verlaten rond hun puberteit. De groepen bestaan dus voornamelijk uit volwassen vrouwen met hun (klein-)kinderen en vaak een volwassen (fok)man. Van de volwassen vrouwen heeft ~20% overgewicht en van de mannen ~30%. De BPRC-kolonie biedt dus een unieke populatie om onderzoek te doen naar overgewicht in relatie tot gedrag, dieet, gezondheid en welzijn. De hoofdstukken in dit proefschrift zijn met name gericht op drie aspecten, namelijk het meten van overgewicht (Hoofdstuk 2-3), risicofactoren voor overgewicht (Hoofdstuk 4-5) en het verminderen van overgewicht (Hoofdstuk 6-8) bij apen gehuisvest in groepen.

Het meten van overgewicht

Om te beginnen wordt in **Hoofdstuk 2** een universele maat en definitie voor overgewicht bij apen gehuisvest in groepen voorgesteld. De huidige definitie van onder- en overgewicht is namelijk afgeleid uit onderzoek naar apen die in hun eentje in kleine kooien gehuisvest zijn. Een goede maat voor overgewicht is onafhankelijk van lengte en hangt samen met andere maten voor vetzucht. Deze maat moet ook makkelijk te verzamelen zijn tijdens de jaarlijkse gezondheidscontroles, omdat de apen dan kort verdoofd zijn. De oplossing werd gevonden in een soortspecifieke gewicht/lengte index (weight-for-height index), waarvan BMI bij mensen een voorbeeld is. Bij makaken wordt lengte echter niet gekwadrateerd (verheven tot de tweede macht) zoals bij BMI, maar verheven tot de macht 3 in resusapen en tot de macht 2.7 in Java-apen. Door de lichaamsmaten van Java-apen in gevangenschap te vergelijken met data van Java-apen uit het wild, kon bepaald worden dat de grens voor ondergewicht bij apen gehuisvest in groepen 2% lichaamsvet is, vergelijkbaar met apen in het wild. Dat slanke vrouwtjes makkelijk kinderen krijgen en grootbrengen bevestigt eveneens deze ondergrens. De grens voor overgewicht is gelijk gehouden aan het vetpercentage van apen die in hun eentje gehuisvest zijn, omdat dit idealiter gekoppeld moet zijn aan negatieve gezondheidseffecten. Met deze nieuwe maat en definities van onder- en overgewicht zijn 23% van de resusapen en 17% van de Java-apen bij BPRC te dik, terwijl ondergewicht zelden (0–3%) voorkomt. De meeste makaken in groepen bij BPRC hebben dus een gezond gewicht.

In **Hoofdstuk 3** wordt onderzocht of het gewicht van de apen betrouwbaar kan worden gemeten zonder verdoving. Gewicht is namelijk een belangrijke indicator van dierenwelzijn. Vaak moet een aap verdoofd of getraind zijn om gewicht te kunnen meten, wat zowel stressvol als tijdrovend kan zijn. Door een weegschaal met plakkerig voedsel (zoals jam, appelstroop of limonade) in het binnenverblijf te plaatsen, hoopten we dat de apen uit zichzelf op de weegschaal zouden gaan staan. Dit noemen we vrijwillig wegen. Op deze manier kon het gewicht van de meerderheid (68%) van de resusapen gemeten worden. Welke apen zich vrijwillig lieten wegen werd niet bepaald door leeftijd of plaats in de rangorde, maar wel door hoe onderzoekend (exploratief) de apen waren. Apen die meer onderzoekend zijn, stonden vaker op de weegschaal dan apen die meer terughoudend zijn. Ook waren sommige groepen meer geneigd deel te nemen dan andere groepen. Verder kwam het gewicht gemeten tijdens het vrijwillig wegen goed overeen met het gewicht gemeten terwijl de dieren onder verdoving waren, dus is de methode betrouwbaar. Hoewel vrijwillig wegen nog redelijk tijdrovend is, kan het dus een belangrijk hulpmiddel zijn bij het monitoren van overgewicht bij apen gehuisvest in groepen.

Risicofactoren voor overgewicht

Risicofactoren voor overgewicht kunnen verschillen tussen verschillende soorten huisvesting. Makaken worden vaak gebruikt voor biomedisch onderzoek naar voor de mens levensbedreigende ziektes. Hier worden ze gehuisvest in duo’s in een gecontroleerde omgeving. Dit verschilt behoorlijk van de optimale huisvesting in sociale groepen met grote, natuurlijke verblijven. Huisvesting kan lange-termijn gevolgen hebben op stressniveaus en vetpercentages van apen, waardoor de dieren mogelijk minder geschikt zijn voor deelname aan biomedisch onderzoek. **Hoofdstuk 4** beschrijft het effect van een verandering van groep- naar duo-huisvesting op stressniveaus en vetpercentages van vrouwelijke resusapen. Het stresshormoon cortisol is bepaald in haarmonsters van de apen, terwijl de gewicht/lengte index (Hoofdstuk 2) in combinatie met CT-scans van de buik gebruikt zijn om vetpercentage te meten. Hoewel cortisol hoger was in duo’s dan in groepen, was er geen verschil in vetpercentage tussen de twee soorten huisvesting. De verandering in vetpercentage hing samen met de plaats in de rangorde in de groep: vetpercentage nam toe bij vrouwen laag in de rangorde en nam af bij vrouwen die hoog in de rangorde stonden. Cortisol hing echter niet samen met vetpercentage, waardoor stress en type huisvesting geen belangrijke risicofactoren lijken te zijn voor overgewicht bij apen gehuisvest in groepen.

Apen in dezelfde groep kunnen verschillen in de mate van overgewicht: sommige dieren worden dik, terwijl anderen op een gezond gewicht blijven. Dit kan veroorzaakt worden door verschillen in energie-inname, verschillen in energieverbruik en plaats in de rangorde. Wanneer voorspeld kan worden welke dieren dik worden, kunnen mogelijk ook oplossingen worden bedacht om overgewicht te voorkómen en verminderen. De onderzoeksvraag van **Hoofdstuk 5** luidt dan ook: is gedrag voorspellend voor gewichtstoename in volwassen vrouwelijke resusapen? Energie-inname is geschat door de hoeveelheid apenbrokken, brood en fruit die de apen opeten te observeren met videocamera’s. Daarnaast is met gedragsobservaties bepaald hoe de apen hun tijd besteden, dus hoeveel procent van de tijd ze rusten, bewegen, vlooien, eten zoeken en ander gedrag vertonen. Dikke dieren waren minder actief, maar gewichtstoename hing niet samen met de mate van beweging en hoeveel de apen aten. Daarentegen werd gewichtstoename wel voorspeld door de plaats van een vrouw in de rangorde: vrouwen hoog in de rangorde kwamen meer aan dan vrouwen laag in de rangorde. Kortom, niet gedrag maar plaats in de rangorde lijkt een risicofactor voor overgewicht bij apen gehuisvest in groepen.

Verminderen van overgewicht

De samenstelling, hoeveelheid en wijze van aanbieden van het dieet speelt waarschijnlijk een belangrijke rol in de ontwikkeling van overgewicht bij apen in gevangenschap. Diëten in gevangenschap bevatten doorgaans minder vezels, meer vet en meer suikers dan het dieet van apen in het wild. Overgewicht en gerelateerde gezondheidsproblemen, zoals diabetes en hart- en vaatziekten, kunnen mogelijk worden verminderd door een meer natuurlijk dieet aan te bieden. **Hoofdstuk 6** bevat een evaluatie van een kleine dieetverandering in volwassen vrouwelijke Java-apen, namelijk het vervangen van brood door graan en groente. Hierdoor kreeg het dieet een meer natuurlijke samenstelling. Na de dieetverandering was het gewicht van de apen gezonder en waren bepaalde bloedwaardes gerelateerd aan diabetes en hart- en vaatziekten verbeterd. Dus, relatief kleine aanpassingen in het dieet kunnen al een positieve bijdrage hebben op het welzijn van apen in gevangenschap.

Behalve de samenstelling van het dieet, kan ook de hoeveelheid voedsel worden aangepast om zo overgewicht te verminderen. Voervermindering is zeer succesvol gebleken bij apen die in hun eentje of in duo's gehuisvest zijn, maar het is nog onbekend of dit ook veilig kan worden gebruikt bij apen in groepen. Aangezien voedsel meestal niet gelijk verdeeld is onder groepsleden, zit er een risico aan het verminderen van de hoeveelheid voedsel. Te weinig voedsel kan leiden tot ondervoeding, stress en agressie. Het doel van het onderzoek beschreven in **Hoofdstuk 7** was om te kijken of voervermindering geschikt is voor apen gehuisvest in groepen. Het effect van 20% voervermindering op overgewicht en bloedwaardes van volwassen apen, groei van jonge apen, voortplanting en het aantal dierenartsbezoeken werd bestudeerd in tien groepen Java-apen. Na de 20% voervermindering is overgewicht afgenomen en zijn cholesterolwaardes in het bloed verbeterd. Verder werden er geen negatieve effecten gevonden op groei van jonge apen, voortplanting en het aantal dierenartsbezoeken. Kortom, de uitkomsten bij Java-apen geven aan dat voervermindering een geschikte oplossing zou kunnen zijn om overgewicht bij apen gehuisvest in groepen te verminderen.

In **Hoofdstuk 8** worden vier mogelijke oplossingen voor het verminderen van overgewicht in groepen resusapen bekeken, namelijk (1) brokken aanbieden in voerpuzzels, (2) voeren op ideaal gewicht, (3) 20% voervermindering en (4) een fruitloos dieet. Gegevens over vetzucht, gezondheid en gedrag zijn gebruikt om een afweging te kunnen maken over de geschiktheid van deze mogelijke oplossingen. Het aanbieden van apenbrokken in voerpuzzels had geen invloed op de mate van overgewicht, maar had wel positieve effecten op gedrag. Voeren op basis van ideaal gewicht in plaats van het daadwerkelijke gewicht van een aap zorgt dat er minder apenbrokken worden toebedeeld aan dikke apen en meer aan dunne apen. Hierdoor past de hoeveelheid voedsel die wordt aangeboden aan een groep beter bij de energiebehoefte van de apen in die groep. Er was inderdaad minder gewichtstoename met voeren op ideaal gewicht. Voervermindering was zeer effectief in het verminderen van overgewicht, maar had in de helft van de groepen ook nadelige effecten, vooral bij apen die laag in de rangorde staan. Een fruitloos dieet had geen effect op overgewicht, maar verlaagde glucosewaardes in het bloed en zorgde voor meer natuurlijk gedrag. Kortom, voerpuzzels zijn leuk als verrijking om de apen bezig te houden, maar dragen niet bij aan het verminderen van overgewicht. Voeren op ideaal gewicht en een fruitloos dieet worden aanbevolen, terwijl voervermindering in de hier gebruikte vorm niet geschikt is voor alle groepen resusapen.

Tot slot worden de belangrijkste bevindingen samengevat en tegen elkaar uitgezet in **Hoofdstuk 9**, met het doel te evalueren hoe in groepslevende apen het best alle dieren een gezond gewicht kunnen behouden of krijgen. Het voorkómen van overgewicht is makkelijker dan het verminderen, omdat rangorde een grote invloed heeft op vetzucht bij apen in groepen. Daarom zou het dieet van apen in gevangenschap zoveel mogelijk moeten lijken op het dieet van apen in het wild qua samenstelling, hoeveelheid en wijze van aanbieden. Zelfs kleine veranderingen in voederpraktijken kunnen al een gunstig effect hebben op het dierenwelzijn. Al met al toont dit proefschrift aan dat het meten en monitoren van overgewicht belangrijk is en dat kritisch kijken naar zaken als huisvesting en dieet kan bijdragen aan het verbeteren van het welzijn van apen gehuisvest in groepen. Alle instellingen die apen huisvesten wordt dan ook aanbevolen hier actief mee bezig te zijn.

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Curriculum vitae

Dian Gerdina Maria Zijlmans was born on the 15th of March 1993 in Tilburg, the Netherlands. In 2011, she obtained her VWO diploma (Beatrix College, Tilburg) and started her bachelor's in Biology at Utrecht University. During the bachelor, she greatly enjoyed the courses on animal behaviour and did her research project and thesis on animal group-living. Hereafter, she enrolled in the Behavioural Ecology track of the Environmental Biology master program at Utrecht University, where she focused her curriculum on the behaviour and welfare of animals living under human care. Dian performed her major research project at the Biomedical Primate Research Centre (BPRC), where she studied factors related to overweight in group-housed macaques. Next, she investigated social relationships and behaviour of African wild dogs in three Dutch zoos. Finally, she wrote a literature thesis on the phenomenon of adaptation to captivity and its implications for zoo animal management.

After she graduated in January 2017, Dian worked as a research and education assistant for the Animal Behaviour and Cognition (formerly Animal Ecology) group at Utrecht University. During this time, she assisted in courses in the behavioural biology curriculum, supervised students, created video content of primate behaviour for new teaching material and gained experience in animal training. She was also allowed to keep working on her major research project and published her first article on the subject in 2019. In the same year, she started her PhD at BPRC's Animal Science Department further investigating correlates, consequences and control of overweight in captive group-living macaques, which resulted in this thesis.

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