Seasonal variation in the body fat of Japanese macaques *Macaca fuscata*

Yuzuru Hamada1*, Seiji Hayakawa1, Juri Suzuki2, Kunio Watanabe3 and Satoshi Ohkura4

1 Section of Morphology, Primate Research Institute, Kyoto University, 41 Kanrin, Inuyama, Inuyama 848-8506, Japan
2 Center for Human Evolution Modeling Research, Primate Research Institute, Kyoto University, 41 Kanrin, Inuyama, Inuyama 848-8506, Japan
3 Laboratory of the conservation and management of Japanese macaques, Primate Research Institute, Kyoto University, 41 Kanrin, Inuyama, Inuyama 848-8506, Japan
4 Laboratory of neuro-endocrinology, National Institute of Agrobiological Sciences, Kan-non-dai 2-1-2, Tsukuba 305-8602, Japan

Abstract. Japanese macaques *Macaca fuscata* inhabit the Japanese archipelago where they experience markedly seasonal warm and cool temperate climates. Their reproduction and their nutritional status follow clearly seasonal patterns. Their status can be effectively evaluated based on their total body fat, but making such measurements has been difficult. In this study, the body fat of Japanese macaques was studied using Double Energy X-ray Absorptiometry (DXA). Adult males were found to have a median fat mass of 7% and adult females 9%. Using the 90th percentile as the criterion, we determined the levels of obesity in Japanese macaques as 17% in males and 27% in females. Fatness indicators (physique index and skinfold thickness) were found to correlate well with total body fat and fat mass percentage. Fat mass is estimated fairly well from these indicators. Fatness indicators change seasonally in artificially reared Japanese macaques.

Key words: DXA, fat, *Macaca fuscata*, nutritional status, seasonality.

The Japanese archipelago lie between latitudes 20°N and 45°N having warm monsoon to cool temperate climatic zones. Seasonality in climate is remarkable in terms of both temperature and precipitation variation. Japanese macaques *Macaca fuscata* are widely distributed in the islands of Honshu, Kyushu, and Shikoku, and also occur on neighboring smaller islands. They are medium sized mammals with an average body mass of ca. 12 kg in males and 8 kg in females (Hamada et al. 1996) that do not hibernate. In tune with the climate, they show a clear seasonality in reproduction, that is, they mate from late autumn to early winter, and bear young from spring to early summer (Kawai 1969). Japanese macaques suffer from food shortage in winter more or less depending on the local climatic condition (Wada 1979). Therefore, a nutritional reservoir is of vital importance for survival (Hamada 2002; Nakayama 2002), and the nutritional status is considered to show a seasonal fluctuation. Change in body mass in free-ranging macaques is reflected in their status (Hazama 1964; Mori 1979; Matsuoka 2000; Kurita et al. 2002) as it is in artificially reared macaques (Matsubayashi and Mochizuki 1982; Matsubayashi and Enomoto 1983). Macaques lose body mass from winter to spring and gain mass from summer to autumn. Other mammal species inhabiting the Japanese islands show a similar seasonal change in body mass (Shiraishi et al. 1996; Tsubota 1998). Quantitative evaluation of how much Japanese macaques depend on nutritional reserves in winter, however, has not yet been made.

Nutritional status can be inferred from body fat mass, because fat contains more than twice as much energy per unit weight as carbohydrate or protein, while also functioning as a heat insulator. Fat accumulation has been studied in various primates, including humans and other anthropoids (McFarland 1997), human infants (Kuzawa 1998; Pawlowski 1998), captive macaques (Pond and Mattacks 1987; Tanaka et al. 1995), captive African hominoids (McFarland and Zihlman 1997), and wild

*To whom correspondence should be addressed. E-mail: hamada@pri.kyoto-u.ac.jp*
orangutans (Knott 1997). Cheirogaleid lemurs, such as *Cheirogaleus medius* (Fietz and Ganzhorn 1999) and *Microcebus murinus* (Schmid 1999) are exceptional primates because they hibernate, relying on fat deposited in the tail. There have not been, however, any detailed studies of seasonal variation of the fat mass Japanese macaques. Although many research methods have been developed for studies of humans (e.g. weighing in water; measurement of displacement of air using rare gases; bio-impedance; and isotopes; see Roche et al. (1996)), many of them are difficult or impossible to apply to non-human primates. Skinfold thickness and physique index (Hamada et al. 1996) are the simplest indirect methods, and these fatness indicators have been applied to wild vervet monkeys (*Cercopithecus aethiops*, Crumley and Raleigh 1995). These methods have been criticised, however, because skinfold thickness, for example, does not always reflect the mass of visceral fat, while the physique index, relying on calculations of body mass and anterior trunk length, suffers from body mass variation not correlated with fat mass. Some researchers have evaluated the development of fat tissues in the omentum majus or around the visceral organs by observation using an endoscope (Nigi et al. 1995; Nigi and Morimitsu 1997; Morimitsu 1997). Koganezawa (1995) dissected macaques and weighed the fat around the kidneys and other visceral organs to obtain fatness indices. Hayama et al. (1998), who dissected macaques to measure whole body fat extracted not only visceral fat but also subcutaneous fat. The simple, indirect methods, such as physique index, are not precise, observation by endoscope requires invasive veterinary procedures, and dissection is tedious and not applicable to living macaques. Furthermore, total body fat mass, information on which is indispensable for the evaluation of nutritional status, can only be obtained by dissection.

Recently, Double Energy X-ray Absorptiometry (DXA) has become a popular method for the measurement of body composition. DXA also provides precise total body fat mass information quickly. Tanaka et al. (1995) have already used the DXA technique in their study of captive long-tailed macaques *Macaca fascicularis*. Although there have been numerous experimental studies of fat mass or obesity from the biomedical perspective (e.g. Colman et al. 1999b), there have been few studies of wild or free-ranging populations (Altmann et al. 1993). In the present study, fat mass data for Japanese macaques were obtained by DXA. The experimental animals were reared in out-door cages, where they were physically active. In this paper we present statistical evidence for relationships between fatness indicators and fat mass or fat mass percentage in order to evaluate the applicability of these indicators as parameters of nutritional status. We also present data on seasonal change of fat mass in Japanese macaques, and briefly discuss the function of fat in Japanese macaques.

### Materials and methods

#### Subjects

Japanese macaques reared at the Primate Research Institute, Kyoto University, were used in the research experiments while conforming to that institute’s rearing and experimental use of primates guidelines. These macaques were originally from Takahama in Fukui Prefecture, Arashiyama in Kyoto Prefecture, and Wakasa in Tottori Prefecture. Date of birth and health data were available for most subjects, however the ages of several old individuals were estimated. Individuals were reared either in group cages with outdoor enclosures or in outdoor enclosures. Thus they lived under conditions resembling those of natural populations in terms of temperature and physical activity. Although the same individuals were measured twice or more, all data was analyzed without considering the number of measurements made (see Table 1).

<table>
<thead>
<tr>
<th>Age</th>
<th>Female</th>
<th>Male</th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10</td>
<td>9</td>
<td>89</td>
<td>123</td>
</tr>
<tr>
<td>1</td>
<td>20</td>
<td>13</td>
<td>66</td>
<td>56</td>
</tr>
<tr>
<td>2</td>
<td>49</td>
<td>7</td>
<td>175</td>
<td>52</td>
</tr>
<tr>
<td>3</td>
<td>49</td>
<td>10</td>
<td>61</td>
<td>57</td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>10</td>
<td>51</td>
<td>52</td>
</tr>
<tr>
<td>5</td>
<td>24</td>
<td>21</td>
<td>44</td>
<td>55</td>
</tr>
<tr>
<td>6</td>
<td>39</td>
<td>42</td>
<td>45</td>
<td>38</td>
</tr>
<tr>
<td>7</td>
<td>10</td>
<td>5</td>
<td>38</td>
<td>29</td>
</tr>
<tr>
<td>8</td>
<td>7</td>
<td>3</td>
<td>37</td>
<td>25</td>
</tr>
<tr>
<td>9</td>
<td>7</td>
<td>1</td>
<td>27</td>
<td>11</td>
</tr>
<tr>
<td>≥10</td>
<td>23</td>
<td>15</td>
<td>89</td>
<td>41</td>
</tr>
<tr>
<td>≥15</td>
<td>20</td>
<td>16</td>
<td>65</td>
<td>31</td>
</tr>
<tr>
<td>≥20</td>
<td>37</td>
<td>15</td>
<td>82</td>
<td>20</td>
</tr>
<tr>
<td>Total</td>
<td>307</td>
<td>167</td>
<td>869</td>
<td>590</td>
</tr>
<tr>
<td>TOTAL</td>
<td>474</td>
<td>1459</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
We measured body mass (BM) in kg with a balance and anterior trunk length (ATL) in mm with an anthropometer. Somatometrical data is derived from a total of 1,459 macaques (869 females and 590 males). From BM and ATL, a physique index was calculated as follows (Hamada et al. 1996): \(1.05 \times \frac{BM}{ATL^{2.3}} \times 10^7\) for females, and \(1.01 \times \frac{BM}{ATL^{2.3}} \times 10^7\) for males.

Skinfold thickness
Skinfold thickness was measured, using standard skinfold callipers (Eiken-shi ki) to the nearest 0.1 mm, at three sites, the belly (at the level of the navel), subscapular and supra-iliac sites. The total skinfold thickness at those three sites was used for the numerical analysis.

DXA measurement
Total body fat mass was measured by DXA (Lunar Co. Ltd) using Total Body for Pediatrics software. DXA measurements were obtained from 474 subjects in total (307 females and 167 males). The percentage fat mass was calculated as [fat mass in kg]/[BW in kg] \(\times 100\). Numerical relationships between whole body fat mass or percentage fat mass and fatness indicators, such as physique index and skinfold thickness, were examined.

Numerical analyses
Age-related patterns were analyzed as follows: data were first grouped into age-classes; secondly the median, 10th and 90th percentiles were obtained for each age-class; and thirdly line graphs connecting medians, 10th percentiles and 90th percentiles, respectively, were smoothed using the Loess-smooth function (S-Plus 4, MathSoft, Co. Ltd.). Coefficients of correlation (r) were calculated between physique index and percentage fat mass, and between skinfold thickness and percentage fat mass. Linear regression equations were calculated either by the least-square method (LS) when the coefficient of correlation was high, or by the reduced major axis method (RMA).

Results
Age-related patterns of Physique index
At birth, the physique index is about 100 in females and 105 in males. It decreases rapidly during the first two years, to about 95 in females and to about 100 in males. The index then gradually increases, reaching a plateau (the adult stage) at 112 for females and 123 for males at about eight years of age (see Fig. 1). Thus the physique of Japanese macaques matures at approximately the same age as do other somatic systems (e.g. body dimensions, eruption of teeth, or reproductive maturation).

Line graphs of the 10th and 90th percentiles, showing the range of variation, lie parallel to the median. The degree of deviation is widest for the 90th percentile in
females, which appears to increase throughout life, until the oldest age class (24 years) (see Fig. 1). The criteria used to classify animals as either lean or obese, were the 10th and 90th percentiles in adults (8–20 years of age); 105 and 140 for males and 93 and 143 for females. Extraordinarily obese individuals (index >180) were found only among females.

Age-related patterns of Skinfold thickness

Skrinfeld thickness follows a different age-related pattern from that of physique index (Fig. 2). Skinfold thickness increases slowly in both sexes. From birth to about 6.5 years of age it is 5–7 mm, thereafter it increases rapidly attaining a plateau at the adult stage, at about eight years of age. Median thicknesses of adults (≥8 years) are about 11 mm in females and about 9 mm in males. Line graphs of 10th and 90th percentiles lie parallel to the median, and the graph of 90th percentile tends to deviate from the graph of median further than that of 10th percentile does. Skinfold thickness at the 10th percentile, is about seven mm in adults and does not change with age. The minimum thickness among adults, that is when no subcutaneous fat has been accumulated, is considered to be 7 mm. Among individuals 10–14 years old, the 90th percentile was greater than 20 mm for females and 15 mm for males. Thus females tend to accumulate more subcutaneous fat than males. Median and 10th percentile thicknesses of animals older than 20 were smaller than for those of younger adults, perhaps because the skin itself becomes thinner with age, though this requires further clarification in future research.

Based on the 10th and 90th percentiles of skinfold thickness, adult males (8–20 years of age) were considered lean when they measured 6.68 mm or less and obese if they measured 14.74 mm or more. The corresponding figures for females were 6.56 mm and 18.76 mm.

Whole body fat mass and percentage fat mass: DXA measurements

The minimum limits of DXA measurement is set at 4% of fat mass against body mass. Therefore, age-related changes in total body fat mass and percentage fat mass were not analyzed in the same way as described above (see Fig. 3). Individuals younger than five years of age had percentage fat masses of 8% or less. Among five year olds, a considerable number of individuals, especially females, had accumulated more than 10% body fat, but the majority of adolescents and younger adults (5–10 years old) had whole body fat masses of 5–8%. Among adults older than 10, the median and 90th percentiles were 7% and 17% for males, and 9% and 27% for females (see Fig. 3). Thus DXA indicates that there is considerable difference in fat accumulation between males and females and females have greater percentage fat mass than males, confirming result obtained from skinfold thickness measurements.

Obesity among adult Japanese macaques, as determined in this study by the 90th percentile values, was 17% for males and 27% for female. Eight females were regarded as obese, two of which had levels as high as 45% and 51%, whereas among males the highest fat
level was 27% (two individuals). Leanness was tentatively defined as 4% in both sexes by DXA (the minimum limit of DXA measurement), however, as by this criterion many adults were regarded as lean (22.6% of females and 34.2% of males), the criterion for defining lean must be re-evaluated.

The relationship between body mass and fat mass

Accumulation of fat begins when body mass exceeds 8 kg in females and 12 kg in males (see Fig. 4). Regression equations between BM (kg) and total body fat mass (g) were calculated with LS, and total body fat mass is expressed by the following functions: 

- For immature females with BM <8.0 kg: 
  \[ -11.32 + 40.710 \times BM \]  
  \( R^2 = 0.894, P < 0.01 \)

- For adult females with BM ≥8.0 kg: 
  \[ -5772.26 + 723.37 \times BM \]  
  \( R^2 = 0.885, P < 0.01 \)

- For immature males with BM <12.0 kg: 
  \[ 1.598 + 37.993 \times BM \]  
  \( R^2 = 0.975, P < 0.01 \)

- For adult males with BM ≥12.0 kg: 
  \[ -8571.83 + 718.87 \times BM \]  
  \( R^2 = 0.285, P < 0.01, \text{ calculated with RMA} \)

The relationship between physique index and percentage fat mass

The physique index was regressed with percentage fat mass using all data regardless of age with LS: 

- For immature females with BM <8.0 kg, and \( -5772.26 + 723.37 \times BM \) (\( R^2 = 0.885, P < 0.01 \)) for adult females with BM ≥8.0 kg; and 
- For immature males with BM <12.0 kg, and \( -8571.83 + 718.87 \times BM \) (\( R^2 = 0.285, P < 0.01, \text{ calculated with RMA} \)) for adult males with BM ≥12.0 kg.

\[ -25.76 + 0.310 \times \text{physique index} \]  
\( R^2 = 0.762, P < 1\% \) for
females, and $-14.39 + 0.180 \times \text{physique index}$ ($R^2 = 0.513, P < 1\%$) for males. For adults, functions were obtained from individuals older than six and with percentage fat mass greater than 4% as follows (Fig. 5): $-29.05 + 0.344 \times \text{physique index}$ ($R^2 = 0.777, P < 0.01$) for females and $-23.13 + 0.260 \times \text{physique index}$ ($R^2 = 0.556, P < 0.01$) for males.

The relationship between skinfold thickness and percentage fat mass

A relationship was sought between skinfold thickness and percentage fat mass (Fig. 6) for all data, and it was expressed by functions as follows: $-0.402 + 0.472 \times \text{skinfold thickness}$ ($R^2 = 0.748, P < 0.01$) for females, and $-1.765 + 0.545 \times \text{skinfold thickness}$ ($R^2 = 0.651, P < 0.01$) for males. Regressions are calculated for individuals of 6.0 years of age or older and with percentage fat mass greater than 4.0% (Fig. 6) as follows: $2.407 + 0.428 \times \text{skinfold thickness}$ ($R^2 = 0.671, P < 0.01$) for females, and $-2.596 + 0.628 \times \text{skinfold thickness}$ ($R^2 = 0.572, P < 0.01$) for males. The plots of obese individuals deviates greatly from the regression line, indicating that the fat accumulation pattern changes as fat increases. It appears that more fat may accumulate subcutaneously rather than in the deep body in relatively obese individuals. Therefore regression of second or third orders may fit better between skinfold thickness and percentage fat mass. It is also probable that individuals differ in their fat accumulation, with some bias towards subcutaneous fat deposition and some to deep body fat deposition.
Monthly physique index and skinfold thickness were analyzed using all adult data (≥8.0 years of age) combined. Among females, the maximum index of 123 was observed in August and September, and the minimum of 103 was found in February and March (Fig. 7). Females had largest skinfolds (15 mm) in September and smallest skinfolds (5 mm) in February, indicative of an annual gain and loss of about 0.54 kg of fat in adult females.

In males, the physique index fluctuates less remarkably with season and the higher index of about 123 was maintained from July to November. From November to April it decreased to the lowest index of 110, then increased again from April to July. Monthly skinfold thickness among males follows a typical sine curve with a slight deviation, fluctuating between the largest, ca 13 mm from October and November, and the smallest, ca 5 mm in May, indicative of an gain and loss of about 0.50 kg of fat (see Fig. 7).

**Discussion**

**Fat accumulation in Japanese macaques**

Until the development of DXA, the only means to precisely measure total body fat mass in non-human primates were the isotope method (Altmann et al. 1993), and dissection to extract as much fat as possible (McFarland and Zihlman 1997; Hayama et al. 1998). The development of DXA has allowed total body fat mass to be accurately measured in 10 minutes or less. Using DXA, the median percentage fat mass of captive Japanese macaques was found to be 7% for males and 9% for females, thus total fat mass of females amounts to about 700 g in females and 800 g in males.

This study has shown that fat mass or percentage fat mass can be estimated by such fatness indicators as body mass, physique index and skinfold thickness. Similar results have been reported by Colman et al. (1999a) who made statistical analyses of DXA and such fatness indicators as body mass, body mass index, body circumference, and abdominal skinfold thickness in rhesus macaques (*Macaca mulatta*). Although body mass and skinfold thickness appear to reflect fat mass well for immature individuals, the physique index does not, probably because growth in linear dimensions greatly affects the physique index.

The relationship between skinfold thickness and percentage fat mass is expressed fairly well by the linear regression. In females, however the relationship may be expressed better by 2nd or 3rd order regressions than by linear regression, because fat tends to be accumulated more heavily in subcutaneous tissues than in deep body tissues as total body fat mass increases. Koganezawa (1995) has shown that loss of fat mass in Japanese macaques occurs first subcutaneously, then from around the kidneys, and finally from the omentum majus, with increase in fat mass following the reverse pattern. He concluded that the subcutaneous fat mass is the better indicator of nutritional status for well nourished macaques and that fat in the omentum majus is a better indicator for those poorly nourished.
Age change and sex difference in fat in Japanese macaques

Skinfold thickness and percentage fat mass measured by DXA show similar age-related patterns, that is, thickness and percentage fat mass are both small and do not clearly increase or decrease from birth to adolescence (about 6 years of age). Reproductively immature macaques seldom have fat masses greater than 4%, presumably because they use their nutrition for growth without accumulating fat. Some adolescents (5–8 years of age), exhibiting signs of reproductive maturation, such as menarche or swelling of the sexual skin in females, or testicular development and descent into the scrotum in males, have fat masses of 5–10%. Some of the fatter adolescent females had already engaged in reproductive activity, and therefore, their higher fat mass were considered to be related to reproduction. Among males, individuals with higher percentage fat mass appear to be exceptional; most of them began to accumulate fat from their seventh autumn (about 6.5 years of age). After cessation of growth and after reproductive maturation, nutrition surplus to maintenance and physical activity is accumulated in the form of fat tissue.

Obesity and leanness in Japanese macaques

Obesity in adult Japanese macaques, as determined by the 90th percentile of adult subjects in the present study, is 17% in males and 27% in females. Leanness was tentatively defined as 4% body fat mass in the present study coincident with the lower limit of sensitivity of DXA. However, as a considerable number of macaques were found to have with fat masses of 4% or less, the criterion requires re-evaluation. Based on the physique index adult (8–20 years old) males were obese when over 140 and lean when less than 105, the corresponding figures for adult females were 143 and 93. Based on skinfold thickness, males over 14.7 mm were obese, and at less than 6.7 mm they were lean. Females over 18.8 mm were obese and at less than 6.6 mm they were lean. It is noteworthy that many of the obese Japanese macaques in this study were derived from one troop (Wakasa, 7 out of 8 obese individuals in female, and all of three obese individuals in male), suggesting that obesity in Japanese macaques may be genetically determined.

Norms and ranges of fatness obtained captive-reared animals may not necessarily be applicable to macaques reared differently or to wild macaques. We have, therefore, estimated percentage fat mass for wild Japanese macaques based on existing somatometrical data (Hamada et al. 1996). For troops living in cold and snowy areas, these were 12.56% for males in Shiga, and 17.62% for females in Shiga, while for Hakusan females that figure was 20.59%. For the Koshima troop, living in warm area, the corresponding figures were 3.73% for males and 3.87% for females. The estimated values were well within the range found in captive-reared macaques.

Several studies have been made on fatness in non-human catarrhines other than Japanese macaques indicating that the range in percentage fat of non-human catarrhines is between about 2% and about 50%. Altman et al. (1993) reported that baboons in troops relying on garbage for food have 23.2% fat mass whereas, wild baboons, which do not depend on garbage, have as low as 1.9%. They also showed that females tend to carry more fat than males. Pond and Mattacks (1987) reported a low fat percentage in captive-reared pig-tailed (Macaca nemestrina) and long-tailed macaques (M. fascicularis). Tanaka et al. (1995) regarded 35% as a criterion for obesity among captive-bred long-tailed macaques, based on division using the fat-free soft tissue mass as the denominator, its value being equivalent to about 25% using body mass as the denominator. McFarland and Zihlman (1997) reported captive African hominoids (Pan troglodytes, P. paniscus, and Gorilla g. gorilla), as having from as little as 2% to 40% or more body fat mass.

The criteria for assessing obesity differ between authors, perhaps reflecting the difficulty in providing a reasonable definition of obesity. In the present study, we have relied on statistics, that is, the 90th percentile. Perhaps the most reasonable criteria may be based on mortality, longevity, or reproductive success; determining such criteria is the aim of future research.

The fat mass of healthy humans lies within the range 20–30% of body mass, which is comparable to the levels of obesity in Japanese macaques determined in the present. Humans have been described as a primate species evolving to accumulate fat (Pond 1996; Kuzawa 1998). The higher average percentage fat mass in humans, however, may be the result of the modern lifestyle, as a recent study has shown that the fatness of children and adolescents increased from the 1960s to the 1990s (Thompson et al. 2002).

Seasonality in fat mass and percentage fat mass in Japanese macaques

Seasonality in fat mass and percentage fat mass was exhibited by captive-reared Japanese macaques (Fig. 7).
From our preliminary analyses of longitudinal studies these animals also showed seasonality in total body fat mass as measured by DXA. These results suggest that body fat accumulation and loss depends on a physiological mechanism based on a fixed circannian cyclicity.

Nutritional status relates to the feeding behavior of wild macaques (Koganezawa 1995). In addition to seasonal variation in temperature, phenological seasonality, which differs with locality, is also an important determinant in the seasonality of body mass and fat mass of Japanese macaques. This may be shown by the fact that wild macaques lose weight in summer (Matsuoka 2000; Kurita et al. 2002).

The functions of body fat, as a nutritional reserve, or in reproduction, will be discussed elsewhere.

Acknowledgments: The authors thank the staff of the section of Morphology and the Center for Human Evolution Modelling Research, Primate Research Institute, Kyoto University, for their support. We are indebted to Mr. Harumoto Gunji for his help with the DXA measurement. This research was financially supported by a Grant-in-Aid for COE Research 2001, a Grant-in-Aid for Specially Promoted Research (COE) 2002, and Grant-in-Aid Nos. 11833008 and 11304059.

References


Received 5 November 2002. Accepted 26 June 2003.