Body Growth and Plasma Concentrations of Metabolites and Metabolic Hormones during the Pubertal Period in Female Shiba Goats

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Abstract. It has been shown in various species that the onset of puberty is closely associated with body growth and nutritional state rather than age. The present study was conducted to determine the timing of puberty and to clarify body growth and metabolic changes around the pubertal period in female Shiba goats. Blood samples were collected between 10 to 38 weeks of age from 12 female goats, and plasma concentrations of progesterone, metabolites (glucose, nonesterified fatty acid, ketone body and acetic acid) and metabolic hormones (insulin and insulin-like growth factor-I (IGF-I)) were analyzed. Physical parameters (body weight, withers height and body length) were also measured at the blood sampling. The week when plasma progesterone concentrations first exceeded 1.0 ng/ml was designated as the onset of puberty. The results showed that the average age of the onset of puberty was 27.0 ± 0.9 (mean ± SEM) weeks in female Shiba goats. When the goats reached puberty, the average values of body weight and goat body mass index ((body weight (kg)/withers height (cm)/body length (cm)) × 10³) were 12.2 ± 0.5 kg and 5.7 ± 0.2, respectively. No particular change associated with puberty was apparent for plasma concentrations of the metabolites examined. Plasma insulin concentrations were maintained at lower levels until the onset of puberty, and then they began to gradually increase. Plasma IGF-I concentrations began to gradually increase 1 to 4 weeks before the onset of puberty and this increase continued throughout the peripubertal period. These results imply that IGF-I acts as a peripheral nutritional signal to trigger the onset of puberty in Shiba goats.

Key words: Puberty, Body growth, Metabolites, IGF-I, Shiba goat


The onset of puberty is the result of a series of complex developmental events that occur within the reproductive endocrine axis. Just before the onset, there is a high frequency rhythm of gonadotropin-releasing hormone (GnRH) secretion and a sustained rise in basal luteinizing hormone (LH) secretion as the frequency of LH pulses increases, and the follicular phase begins, leading to the first LH surge and ovulation [1]. It has also been shown in heifers that the responsiveness of the pituitary gland to GnRH [2] and the action of estrogen on LH secretion [3] also change just before puberty. Numerous studies have demonstrated a close association between body growth, nutritional status and the timing of puberty onset. In cattle [4, 5], sheep [6, 7] and rodents [8, 9], energy deficiency retards growth and delays the onset of puberty. Several lines of evidence from earlier investigations...
suggested that critical body weight [10] or critical body composition [11, 12] somehow initiates puberty in mammals. More recently, Suttie et al. [13] indicated that body weight and body fatness are a consequence, or correlate, of metabolic changes occurring before and around the onset of puberty, and proposed that blood-borne substances, which may be metabolites, hormones or a combination, influence the reproductive system and initiate puberty. However, the identities of the molecular messengers serving this metabolic signaling function remain unknown.

The Shiba goat (Capra hircus) is a Japanese miniature goat, and its inbred strain has been established for experimental use [14]. This species is thought to be a suitable animal model for investigating the mechanisms of puberty in ruminants, because details on the basic endocrine functions underlying reproduction have been clarified through a variety of intensive studies [15–18]. Further, because the Shiba goat is a non-seasonal breeder under natural daylight, experiments can be conducted without taking into account photoperiod, which is the other important signal for the onset of puberty in seasonal breeders such as sheep [1]. However, as far as we know, neither the precise timing of, nor metabolic changes around, puberty have been clearly demonstrated.

The objectives of the present study, therefore, were 1) to determine the timing of puberty; 2) to determine body growth at the onset of puberty; and 3) to clarify metabolic changes around the pubertal period, in female Shiba goats. The timing of puberty was determined based on profiles of plasma progesterone concentrations. Metabolic changes were assessed by measuring plasma concentrations of metabolites such as glucose, nonesterified fatty acid (NEFA), ketone body and acetic acid, all of which can be utilized as energy fuels in ruminants. Because circulating concentrations of insulin [19, 20] and insulin-like growth factor-I (IGF-I) [21–24] have been shown to be closely associated with nutritional status, plasma concentrations of both hormones were also measured as representatives of metabolic hormones.

**Materials and Methods**

**Animals**

Twelve female Shiba goats, born between March and June in 2001 at a closed colony of the National Institute of Livestock and Grassland Science (Tsukuba, Japan), were used. Newborn goats were raised with their mothers until weaning at 8 weeks of age. Additionally, cow milk was supplementary fed to all newborns to guarantee normal growth. The amount of supplemental milk was gradually increased, with 1.2 liters of milk fed just before the weaning. As newborns grew, they also started to eat dry hay and formula feed, composed of 16% crude protein, 2.5% crude fat, 6% crude fiber, 7% ash, 0.6% Ca, and 0.4% P. After weaning, they were randomly housed in two pens adjacent to each other (6 goats each), and 6 kg of formula feed and an adequate amount of dry hay were given to each pen everyday. Water was always freely available.

All experimental procedures were approved by the Committee on the Care and Use of Experimental Animals at the National Institute of Agrobiological Sciences.

**Experimental procedures**

Blood samples were collected by venipuncture between 1300 h and 1400 h at 10, 15 and 20 weeks of age, and then twice a week between 21 and 38 weeks of age. They were centrifuged at 3,000 rpm for 15 min at 4 C and plasma samples were stored at –30 C until assayed. At the time of blood sampling, body weight, withers height and body length were also measured.

**Assays**

Plasma progesterone concentrations were quantified in duplicate using an enzyme immunoassay (EIA) as previously described [25]. Two hundred microliters of plasma sample was extracted twice with 2 ml of diethyl ether in a glass tube. The extract was washed with 200 µl of methanol, and the resulting residue was dissolved in 400 µl of assay buffer (0.04 M phosphate-buffered saline, pH 7.2, containing 0.1% (w/v) bovine serum albumin) and used for the assay. The reaction of the EIA mixture was performed in a 96-well microtiter plate and the absorbance of each well was measured at 450 nm with a Benchmark Microplate Reader (Bio-Rad, Hercules, CA, USA). The least detectable level of progesterone was 0.196 ng/ml. The intra- and inter-assay coefficients of variation (CVs) were 3.2% at 6.0 ng/ml and 9.2% at 6.2 ng/ml, respectively.

Plasma IGF-I concentrations were measured in
duplicate by radioimmunoassay (RIA) using NIDDK-anti IGF-I rabbit serum (UBK 478). The assay was performed as previously described [26]. The least detectable level of IGF-I was 0.1 ng/ml. The intra- and inter-assay CVs were 5.4% at 16.4 ng/ml and 7.6% at 13.7 ng/ml, respectively.

Plasma insulin concentrations were measured in a single determination with a RIA kit (Insulin Eiken RIA kit, Eiken Chemical Co., Ltd., Tokyo, Japan). The assay procedure was in accordance with the protocol for the kit, except that the volumes of samples and all reagents were reduced to half of those recommended. The least detectable level of insulin was 5 \( \mu U \)/ml. The intra- and inter-assay CVs were 2.7% at 7.28 \( \mu U \)/ml and 6.7% at 7.15 \( \mu U \)/ml, respectively.

Plasma glucose concentrations were determined in duplicate using a Glucose B-test Wako kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan). The assay was performed as described in the manual of the kit, except that the volumes of samples and reagents were 2 \( \mu l \) and 200 \( \mu l \), respectively. The reaction of the assay mixture was performed in a microtiter plate, and the absorbance of each well was measured at 505 nm with the plate reader. The intra- and inter-assay CVs were 0.9% at 86.0 mg/dl and 5.3% at 85.0 mg/dl, respectively.

NEFA concentrations were measured in duplicate according to the method described by Johnson and Peters [27] using a NEFA C-Test Wako kit (Wako Pure Chemical Industries, Ltd.) with a slight modification. In brief, 5 \( \mu l \) of sample and 70 \( \mu l \) of color reagent A were placed in a 96-well microtiter plate, and incubated for 40 min at 37 C. Then, 160 \( \mu l \) of color reagent B was added and further incubated for 20 min at 37 C. The least detectable level of NEFA was 0.125 mEq/l. The absorbance of each well was measured at 550 nm with the plate reader. The intra- and inter-assay CVs were 2.8% at 0.25 mEq/l and 4.3% at 0.21 mEq/l, respectively.

Plasma ketone body concentrations were measured in duplicate with a D-\( \beta \)-hydroxybutyrate (\( \beta \)-HBA) kit (Sigma Diagnostics Co., St Louis, MO, USA). The assay was carried out following the protocol of the kit except that the volumes of \( \beta \)-HBA reagent, \( \beta \)-hydroxybutyrate dehydrogenase solution and plasma samples were 300 \( \mu l \), 5 \( \mu l \) and 5 \( \mu l \), respectively. The reaction of the assay mixture was performed in a microtiter plate, and the absorbance of each well was measured at 340 nm with the plate reader. The intra- and inter-assay CVs were 1.4% at 6.2 mg/dl and 6.3% at 6.4 mg/dl, respectively.

Plasma acetic acid concentrations were determined in duplicate with an F-kit (F. Hoffmann-La Roche Ltd., Basel, Switzerland). A mixture of 50 \( \mu l \) of each plasma sample or standard, 70 \( \mu l \) of distilled water, 100 \( \mu l \) of solution 1 and 50 \( \mu l \) of solution 2 diluted with distilled water (1:2.5) was put in a 0.6-ml Eppendorf tube. After centrifugation at 15,000 rpm for 10 min at 25 C, 100 \( \mu l \) of the supernatant was placed in a 96-well microtiter plate. Then, 50 \( \mu l \) of solution 3 diluted with distilled water (1:50) was added, and the reaction mixture was incubated for 3 min. After adding 50 \( \mu l \) of solution 4 diluted with distilled water (1:25), the mixture was further incubated for 15 min at room temperature. The absorbance of each well was measured at 340 nm with the plate reader. The least detectable level of acetic acid was 0.625 mg/dl. The intra- and inter-assay CVs were 1.4% at 3.27 mg/dl and 7.3% at 3.48 mg/dl, respectively.

Data analysis

Goat body mass index (gBMI) was calculated according to a previous definition [18] as follows:

\[
gBMI = \frac{\text{body weight (kg)} / \text{withers height (cm)} / \text{body length (cm)}}{10^3}
\]

Data were presented as mean ± SEM. Data were statistically analyzed using the repeated measures of ANOVA (StatView, SAS Institute Inc., Cary, NC, USA) followed by the Scheffe test for post-hoc comparisons. \( P \) values less than 0.05 were considered significant.

Results

In the present study, the onset of puberty was defined as the occurrence of first ovulation, which was estimated based on a rise in the plasma progesterone concentration to more than 1.0 ng/ml [7, 28]. A representative profile of time course change in plasma progesterone concentrations is shown in Fig. 1. In this example, plasma progesterone concentration was maintained at an undetectable level until 30 weeks of age. At 31 weeks of age, the concentration first exceeded 1.0 ng/ml, and then fluctuated with a regular 3-week cycle. The onset of puberty in this goat was,
therefore, determined at 31 weeks of age. In 11 out of 12 goats, the onset of puberty was identified to occur at an average age of 27.0 ± 0.9 weeks. In at least 5 goats, first estrous behavior was observed at the second ovulation (Fig. 1). In one goat (#307), no sign of puberty was identified by 38 weeks of age. Therefore, the values for this goat were excluded from the data analysis.

Body weight, withers height, body length and gBMI were aligned in terms of the timing of puberty (week 0, the onset of puberty) in each goat, and their time course changes are shown in Fig. 2. The average values and the ranges are summarized in Table 1. As can be seen from Fig. 2, although all physical parameters increased linearly as the animals grew, no particular change associated with puberty was apparent.

Time course changes in plasma concentrations of metabolites in each individual are shown in Fig. 3. Plasma glucose concentrations fluctuated around 80 mg/dl with a relatively large variation, and there was no obvious change associated with puberty. Plasma concentrations of acetic acids and ketone body fluctuated within a range of 2–6 mg/dl and 5–15 mg/dl, respectively, throughout the observation period. Almost all values for plasma NEFA concentrations were below the detection limit of 0.125 mEq/l (data not shown).

Time course changes in plasma concentrations of insulin and IGF-I in each individual are shown in Fig. 4A, and average values at weeks –5, 0 and 5 are shown in Fig. 4B. Plasma insulin concentrations were maintained at lower levels until the onset of puberty, and then they began to gradually increase. Plasma IGF-I concentrations did not markedly change until 5 weeks before the onset of puberty. However, they began to gradually increase between weeks –4 and –1 and this increase continued throughout the onset of puberty.

**Discussion**

It was believed that puberty occurs at around 7 months of age in female Shiba goats, and this was confirmed by the present study in which the timing of puberty was determined to be 27.0 ± 0.9 weeks of age based on profiles of plasma progesterone concentrations. In mammals, sufficient body growth is required for the onset of puberty. To assess body growth, gBMI as well as BW were employed in this study, because Tanaka et al. [18] suggested that in Shiba goats gBMI reflects energy status and well correlates with changes in pulsatile LH secretion induced by short-term energy deficiency. At the onset of puberty, mean BW and gBMI were 12.2 ± 0.5 kg (range, 9.9–14.2 kg) and 5.7 ± 0.2 (range, 4.9–6.5), respectively. Because there were slight variations in both parameters, we were unable to conclude whether gBMI is a better index than BW for assessing body growth around puberty. Nevertheless, they can be used as simple indices to predict the timing of puberty in female Shiba goats.

It has been suggested that sufficient body growth is a consequence of metabolic changes occurring before and around the onset of puberty [13]. Therefore, it is plausible to think that these changes serve as peripheral signals to initiate puberty. To examine this hypothesis, plasma concentrations of several metabolites were measured. Plasma concentrations of the metabolites examined in this study did not show any particular change associated with puberty. These results suggest that plasma metabolite concentrations per se are not a significant trigger for the onset of puberty in Shiba goats. However, Foster and Bucholtz [29] reported...
that LH secretion is suppressed by the central administration of a glucose inhibitor in developing sheep, and suggested that glucose availability has a critical role in the onset of puberty. Therefore, the sensitivity of central sensors that detect the availability of particular circulating metabolites may change before the onset of puberty. Alternatively, it may be that other metabolites such as propionic acid and amino acids are also involved and the total amount of energy available rather than the amount of a specific metabolite available is important for the onset of puberty, as suggested by Schillo et al. [5].

Insulin is considered to be one of the metabolic signals linking nutritional status with pulsatile GnRH/LH secretion in ruminants, because

Fig. 2. Changes in body weight, withers height, body length and gBMI during the pubertal period. Individual data for 11 goats were plotted based on the timing of puberty in each goat (week 0).

Table 1. Age and physical parameters at puberty in female Shiba goats (n=11)

<table>
<thead>
<tr>
<th>Age</th>
<th>Body weight (weeks)</th>
<th>Withers height (kg)</th>
<th>Body length (cm)</th>
<th>gBMI (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mean</td>
<td>27.0 ± 0.9</td>
<td>12.2 ± 0.5</td>
<td>43.8 ± 0.5</td>
<td>49.0 ± 0.6</td>
</tr>
<tr>
<td>range</td>
<td>25.3 – 33.0</td>
<td>9.9 – 14.2</td>
<td>41.0 – 46.0</td>
<td>46.0 – 51.0</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SEM.
circulating insulin concentrations are positively correlated with nutritional status [19, 20] and insulin acts centrally to stimulate pulsatile LH
release [30]. Steiner [31] indicated the involvement of this hormone in the onset of puberty in primates. Indeed, serum insulin concentrations increase before puberty in humans [32]. However, such actions of insulin may not be involved in the onset of puberty in Shiba goats, because plasma insulin concentrations were maintained at a relatively low level before puberty. This speculation is supported by the demonstration in developing sheep that insulin infusion did not improve the delay of puberty induced by food restriction [13]. Interestingly, plasma insulin concentrations started to rise after the onset of puberty in all goats. A similar profile of serum insulin concentrations during the peripubertal period was reported in cattle [33]. Therefore, the demand for insulin in the circulation may begin to progressively increase once sexual maturation occurs in ruminants.

In contrast with insulin secretion, plasma IGF-I concentrations increased during the onset of puberty in Shiba goats, as shown in a variety of species such as rodents [34, 35], ruminants [22, 36], and primates [37, 38] including humans [37–40]. IGF-I concentrations in the circulation are closely associated with nutritional status in both monogastric animals [21] and ruminants [22–24]. It has also been demonstrated that this hormone is involved in the control of GnRH/LH release. For example, IGF-I stimulates GnRH release from rat hypothalamic explants [41] and LH release from rat pituitary [42]. Furthermore, Hiney et al. [43] reported that the intraventricular administration of small amounts of IGF-I was capable of stimulating LH release from both juvenile and peripubertal rats. These lines of evidence, together with physiological profiles of plasma IGF-I concentrations during puberty presented by us and others [22, 34–40], suggest that IGF-I informs the central nervous system of the nutritional status and triggers the onset of puberty.

Leptin also has been suggested to play an important role in the transduction of the peripheral nutritional status [44] and thereby in the initiation of puberty [45]. We tried to measure plasma leptin concentrations in growing Shiba goats using a commercial leptin RIA kit, which is based on human leptin. Although this RIA kit has been used to detect plasma leptin concentrations in a number of species including domestic animals, we found it was not suitable for Shiba goats (unpublished data). To understand whether leptin is involved in the initiation of puberty in Shiba goats, the establishment of a leptin assay specific for this species is necessary.

In conclusion, the present study showed that female Shiba goats reached puberty at 27.0 ± 0.9 weeks of age with a BW of 12.2 ± 0.5 kg and gBMI of 5.7 ± 0.2. Whereas plasma concentrations of glucose, NEFA, acetic acids, ketones and insulin did not show any particular change related to the onset of puberty, plasma IGF-I concentrations started to increase around 5 weeks before the onset of puberty. The data implies a role for IGF-I as a metabolic signal in the initiation of puberty in ruminants.

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