GROWTH PERFORMANCE AND PLASMA INSULIN-LIKE GROWTH FACTOR I CONCENTRATIONS IN SHEEP SELECTED FOR HIGH WEANING WEIGHT

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ABSTRACT

A study was undertaken to determine the effect of selection for high weaning weight on concentrations of plasma insulin-like growth factor I (IGF-I) in sheep and to evaluate the usefulness of measuring IGF-I as an aid in identification of genotypes with a higher growth potential. Lambs from two lines selected for high 120-d weight (HW and DH) and an unselected control (C) were weighed and blood samples collected monthly from birth to weaning (4 mo of age). A clear differentiation in size occurred after 1 mo of age between lines, between sexes, and between singles and twins. At weaning, selected lines were 3.8 and 5.0 kg heavier than controls. Plasma IGF-I concentrations were 1.5 to 2 times higher (P < .001) in males than in females after 1 mo of age. There were no significant differences in IGF-I concentration between lines or types of birth. However, line DH and single lambs on average had higher concentrations of IGF-I. Within sex and type of birth correlations between IGF-I concentrations at 0, 1, 2, 3, and 4 mo and 4-mo BW ranged from −.16 to .49 in the three lines, and most were not significant. Coefficients of variation for IGF-I concentrations (36 to 50%) were two to three times higher than those for BW (11 to 15%). Due to the high variability of IGF-I measurements, the low correlations between IGF-I concentration and BW, and the small differences in IGF-I between control and selected lines, measurement of plasma IGF-I is unlikely to be an effective aid to selection for growth rate in sheep.

Key Words: Sheep, IGF-I, Somatomedin, Growth Factors, Growth, Selection


Introduction

Body weight is an important selection criterion in meat-producing animals. Few long-term selection experiments for this characteristic that would allow study of physiological factors affecting growth and differentiation among lines have been done in sheep (See review by McGuirk et al., 1986).

A long-term selection experiment for high 120-d weaning weight in Targhee sheep was initiated in 1960 and has been continued for 10 generations under range conditions (Lasslo et al., 1985a,b). Two selected lines have differentiated significantly in weaning weight from a randomly selected control line. Postweaning growth rate of ram lambs from selected lines is also superior to that of controls (Brown et al., 1987).

These lines provide an excellent model to study underlying physiological factors associated with genetic differentiation for growth in animals.

Studies of the mechanisms by which growth hormone (GH) stimulates somatic cell growth have led to the proposition that GH induces growth factors, such as insulin-like growth factor I (IGF-I), which mediate many of the growth-promoting actions of GH (Hall and Sara, 1983).
The objectives of this study were 1) to determine the effect of selection for growth on circulating concentrations of plasma IGF-I in sheep and 2) to evaluate the usefulness of measuring IGF-I in plasma as an aid in genetic selection for growth at an early age in meat animals.

Materials and Methods

Experimental Animals. The animals in this study include all the lambs produced in 1987 from two lines selected for high 120-d weaning weight (HW and DH) and a randomly selected control (C). A detailed description of the lines was given by Lasslo et al. (1985a,b). The animals were maintained under range conditions at the University of California Hopland Field Station. Birth of the lambs was synchronized to occur within a 7-d period in December 1986 and January 1987. All the animals were weaned at 4 mo of age (none of the animals reached puberty before weaning).

Ewes with singles and ewes with twins were reared on different pastures; the twin group was assigned to the pastures with better estimated feed quality. This led to fewer differences in mean weight between singles and twins than expected, based on previous years' results (Lasslo et al., 1985a). However, the comparisons between lines should be unbiased, because ewes from all lines, within each type of birth group, were run together.

Approximately 4 h after birth, the lambs were weighed and a blood sample was collected from the jugular vein in 5-ml vacutainer tubes with EDTA (K$_3$) as an anticoagulant. The animals were weighed and blood samples collected each month until weaning at 4 mo of age. The blood samples were kept in ice, centrifuged, and the plasma stored at -20°C until it was assayed for IGF-I.

Insulin-like Growth Factor I Assay. Plasma concentration of IGF-I was determined by RIA using reagents and protocol supplied by L. E. Underwood and J. I. Van Wyk through the National Hormone and Pituitary Program. The tracer used in the RIA was $[^{125}I]$human somatomedin-C/IGF-I, and antiserum UB805C was used at a dilution of 1:10,000. The effects of binding proteins that interfere with the RIA were eliminated by acid extraction of the plasma and immediate dilution before addition to the assay tubes (Underwood et al., 1982). For acid extraction, plasma samples were incubated with equal volumes of .1 M glycine-glycine HCl buffer, pH 3.2, at 37°C for 8 h in 2-ml stoppered polypropylene tubes. After incubation, extracted plasma samples were neutralized and diluted 1:10 with assay buffer (.03 M sodium phosphate, .05% Tween-20, .01 M EDTA, .02% sodium azide, and .2% protamine sulfate, pH 7.5). Plasma samples were further diluted 1:20 with assay buffer and 50 µl was used as assay volume.

Assay standards were prepared using recombinant human IGF-I as pure standard. Recombinant IGF-I has been shown to be suitable for use as standard in heterologous assays for human (Baxter et al., 1987) and bovine plasma (Elsasser et al., 1988). An acid-extracted plasma pool from 5-mo-old rams was used as assay standard; this standard had an activity of 95 ng of recombinant IGF-I/ml. All the plasma samples were assayed with the same batch of labeled IGF-I in a 3-wk period in 15 assays of 240 tubes each. Between-assay variation was found to be 13.6%, and within-assay variation was 9%. The lower limit of detectable IGF-I was 15.6 pg/ml.

In the earlier validation of the RIA by Underwood et al. (1982), serum was acid-extracted for 48 h to eliminate the interference effects of binding proteins. In the course of this study it was determined that an 8-h extraction was sufficient to eliminate such effect. This was validated based on the parallelism of dose-response curves from 8-h acid-treated sheep plasma, human plasma, and recombinant human IGF-I (Figure 1). Recovery of recombinant IGF-I was 97% for 3.8, 7.5, 15, 30, and 60 pg added to assay tubes containing a standard quantity of acid-extracted and diluted plasma.

Statistical Analyses. The data on lamb weights and IGF-I concentrations in plasma at each measured time were analyzed by least squares analysis of variance using the GLM procedure of SAS (1986). Main effects consisted of line (C, HW, DH), sex, and type of birth (single or twin). There were no interactions ($P > .05$) between main effects for any of the dependent variables measured. Pearson correlations between 4-mo weight of lambs and monthly (0, 1, 2, 3, and 4) concentrations of plasma IGF-I were calculated for each line.

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3IGF-I lot No. 404, Amgen, Thousand Oaks, CA.
Figure 1. Dose-response displacement curves of acid-extracted sheep (♦) and human plasma (●), and of human recombinant insulin-like growth factor I (1.95 pg/ml) (▲) in the insulin-like growth factor I RIA. B/Bo represents the ratio of bound to unbound hormone.

Results and Discussion

Monthly measurements from birth to weaning (4 mo of age) of plasma IGF-I concentrations and body weight of lambs classified by line (C, control; HW and DH, selected), sex, and type of birth (singles and twins) are shown in Figure 2. A clear differentiation in size occurred after 1 mo of age between lines, between sexes, and between singles and twins. With the exception of male singles (the least numerous subclass in each line) at 2 mo of age, the lambs from selected lines were consistently larger than control-line animals. At weaning, selected lines were, on average, 3.8 to 5.0 kg heavier than controls (Table 1). The weight dimorphism between sexes (Table 1) was paralleled by the IGF-I concentrations in plasma (Table 2); males were larger ($P < .001$) and had higher concentrations of IGF-I ($P < .001$) in the three lines. There was no clear differentiation in IGF-I concentration between the selected and control lines, although line DH, on average, had higher concentrations of IGF-I in both sexes. There were no significant differences in IGF-I concentrations between singles and twins, although on average singles had higher concentrations of IGF-I than twins (Figure 2), paralleling the higher weight of single lambs.

Males lambs had IGF-I concentrations 1.5 to 2 times higher than females. This magnitude of sex dimorphism has not been reported in other animal species. In adolescent children, girls tend to have slightly higher IGF-I concentrations than boys, which is consistent with their more rapid maturation (Harris et al., 1985).

Correlations between IGF-I concentrations in plasma at 0, 1, 2, 3, and 4 mo of age and 4-mo BW (Table 3) ranged from −.16 to .49 in the three lines. Correlations calculated without accounting for differences between sexes and type of birth were consistently larger (.17 to .61). However, the larger correlations would be expected because males had higher weights and IGF-I concentrations than females. Type of birth would have a similar effect on these correlations.

A great deal of attention has been directed to determining the significance of IGF-I in growth and development and the utility of this measurement in humans (Rosenfeld et al., 1985). Furlanetto et al. (1977) reported that growth hormone-deficient children had low concentrations of somatomedin-C/IGF-I, and individuals with acromegaly had elevated concentrations. From this initial report, numerous publications have appeared describing the clinical relevance of IGF-I concentrations in the evaluation of disease states of short stature, growth hormone excess, and nutritional status (Underwood, 1985). A few earlier human studies reported some positive correlations between IGF-I concentrations and growth rates (Gourmelen et al., 1986; Rappaport et al., 1987), but there is no consistent evidence in the literature, even in a recent IGF-I review (Daughaday and Rotwein, 1989), that a positive correlation exists between IGF-I and growth rate in normal individuals.

Genotype effects on plasma concentrations of IGF-I have been demonstrated. Dwarf hypopituitary human individuals (Zapf et al., 1981), Snell mice (D’Ercole and Underwood, 1980), and dogs (Eigenmann et al., 1984) deficient in GH have low concentrations of plasma IGF-I. Blair et al. (1989) have reported that seven generations of divergent selection in mice for concentrations of IGF-I resulted in size differentiation between two lines, although the realized heritability was low, .15 ± .12.

In sheep, Gluckman et al. (1983) observed that IGF-I concentrations had a positive association with birth weight ($r = .43$) and birth length ($r = .28$). Olsen et al. (1981) reported a positive association between serum somatomedin-like activity and relative weight gain in lambs. Blair et al. (1990) have reported
Figure 2. Means and standard errors for plasma insulin-like growth factor I (IGF-I) levels and weight of lambs by monthly period classified by line, sex, and type of birth. Line C (shaded bars), line HW (crosshatched bars) and line DH (solid bars).


### TABLE 1. LEAST SQUARES MEANS ± SE FOR WEIGHT OF LAMBS (kg) BY LINE, SEX, AND TYPE OF BIRTH

<table>
<thead>
<tr>
<th>Age, mo</th>
<th>Line</th>
<th>Sex</th>
<th>Type of birth</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>HW</td>
<td>DH</td>
<td>Male</td>
</tr>
<tr>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.5 ± .1</td>
<td>4.7 ± .2</td>
<td>4.9 ± .2</td>
<td>4.8 ± .2</td>
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<tr>
<td></td>
<td>(38)</td>
<td>(32)</td>
<td>(25)</td>
<td>(47)</td>
</tr>
<tr>
<td>1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.4 ± .3</td>
<td>11.9 ± .4</td>
<td>12.5 ± .4</td>
<td>12.5 ± .3</td>
</tr>
<tr>
<td></td>
<td>(38)</td>
<td>(32)</td>
<td>(25)</td>
<td>(47)</td>
</tr>
<tr>
<td>2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>17.8 ± .4</td>
<td>19.4 ± .6</td>
<td>19.7 ± .5</td>
<td>19.9 ± .5</td>
</tr>
<tr>
<td></td>
<td>(38)</td>
<td>(32)</td>
<td>(24)</td>
<td>(46)</td>
</tr>
<tr>
<td>3&lt;sup&gt;d&lt;/sup&gt;</td>
<td>23.6 ± .6</td>
<td>26.1 ± .7</td>
<td>26.7 ± .7</td>
<td>26.7 ± .6</td>
</tr>
<tr>
<td></td>
<td>(33)</td>
<td>(31)</td>
<td>(24)</td>
<td>(44)</td>
</tr>
<tr>
<td>4&lt;sup&gt;e&lt;/sup&gt;</td>
<td>29.6 ± .6</td>
<td>33.4 ± .8</td>
<td>34.6 ± .8</td>
<td>34.7 ± .6</td>
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<tr>
<td></td>
<td>(38)</td>
<td>(31)</td>
<td>(24)</td>
<td>(46)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Effect due to type of birth (P < .001).
<sup>b</sup>Effects due to line (P < .05), sex (P < .001), and type of birth (P < .001).
<sup>c</sup>Effect due to sex (P < .001).
<sup>d</sup>Effects due to line (P < .001), sex (P < .001), and type of birth (P < .001).
<sup>e</sup>Effects due to line (P < .05) and sex (P < .001).
<sup>f</sup>Numbers in parentheses are observations contributing to each mean.
<sup>g</sup>Coefficient of variation (square root of residual variance × 100 divided by the overall weighted mean).

A 20 ng/ml difference between lines after one generation of male selection for high and low concentration of IGF-I in Romney sheep. However, no correlated changes in live weight at 12, 16, and 26 wk were observed between the lines.

From the point of view of evaluating the usefulness of a physiological measurement such as IGF-I concentration at an early age as an aid to increase the accuracy of genetic selection for growth in meat animals, it is essential that a moderately high correlation exist consistently between the two variables. The sample of animals that we have analyzed does not provide evidence of such an association. The CV of IGF-I concentration (Table 2)

### TABLE 2. LEAST SQUARES MEANS ± SE FOR INSULIN-LIKE GROWTH FACTOR I CONCENTRATIONS IN PLASMA (ng/ml) BY LINE, SEX, AND TYPE OF BIRTH

<table>
<thead>
<tr>
<th>Age, mo</th>
<th>Line</th>
<th>Sex</th>
<th>Type of birth</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>HW</td>
<td>DH</td>
<td>Male</td>
</tr>
<tr>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>196 ± 14</td>
<td>196 ± 15</td>
<td>223 ± 22</td>
<td>204 ± 16</td>
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<tr>
<td></td>
<td>(27)</td>
<td>(26)</td>
<td>(15)</td>
<td>(33)</td>
</tr>
<tr>
<td>1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>220 ± 17</td>
<td>209 ± 23</td>
<td>267 ± 22</td>
<td>284 ± 18</td>
</tr>
<tr>
<td></td>
<td>(36)</td>
<td>(27)</td>
<td>(22)</td>
<td>(43)</td>
</tr>
<tr>
<td>2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>190 ± 16</td>
<td>162 ± 21</td>
<td>188 ± 21</td>
<td>245 ± 17</td>
</tr>
<tr>
<td></td>
<td>(34)</td>
<td>(28)</td>
<td>(23)</td>
<td>(43)</td>
</tr>
<tr>
<td>3&lt;sup&gt;d&lt;/sup&gt;</td>
<td>171 ± 17</td>
<td>217 ± 21</td>
<td>215 ± 20</td>
<td>278 ± 17</td>
</tr>
<tr>
<td></td>
<td>(32)</td>
<td>(31)</td>
<td>(23)</td>
<td>(44)</td>
</tr>
<tr>
<td>4&lt;sup&gt;e&lt;/sup&gt;</td>
<td>206 ± 15</td>
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<td>245 ± 18</td>
<td>284 ± 15</td>
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<td></td>
<td>(32)</td>
<td>(27)</td>
<td>(23)</td>
<td>(41)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Effect due to type of birth (P < .001).
<sup>b</sup>Effects due to sex (P < .001) and type of birth (P < .001).
<sup>c</sup>Effect due to sex (P < .001).
<sup>d</sup>Effect due to sex (P < .001).
<sup>e</sup>Effect due to sex (P < .001).
<sup>f</sup>Numbers in parentheses are observations contributing to each mean.
<sup>g</sup>Coefficient of variation (square root of residual variance × 100 divided by the overall weighted mean).
was high, 36 to 50%. These values were two to three times the CV for BW (Table 1), which argues against measurement of IGF-I concentration in plasma as a useful aid in identifying animals with high genetic potential for growth.

**Implications**

The generally positive correlation between insulin-like growth factor I concentrations and growth rates at early ages indicates that insulin-like growth factor I plays a role in growth in sheep, as in other species. However, the rather low values of this correlation and the small differences in insulin-like growth factor I concentrations between the selected and unsel ected lines suggest that measurement of plasma insulin-like growth factor I is not likely to be a very effective aid to selection for growth rate in this species.

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