Effects of acute and chronic food restriction on the insulin-like growth factor axis in the guinea pig

A Sohlström, A Katsman¹, K L Kind², P A Grant, P C Owens, J S Robinson and J A Owens¹

Department of Obstetrics and Gynaecology, University of Adelaide, South Australia 5005, Australia, ¹Department of Physiology, University of Adelaide, South Australia 5005, Australia and ²Division of Human Nutrition, CSIRO, PO Box 10041, Gouger Street, Adelaide, South Australia 5000, Australia

Abstract

The effect of fasting (17–18 h) versus food restriction (70% for 80 ± 13 days) on the IGF–IGF binding protein (BP) axis in female guinea pigs was studied and related to body weight, weight gain and food conversion efficiency. Circulating IGF-I was reduced in the fasted (13%) and food-restricted (50%) animals. IGF-II was only decreased (61%) in the food-restricted group. There was no effect of fasting on IGFBP-1 to -4 while IGFBP-1, -3 and -4 were reduced by 56%, 60% and 44% respectively, and IGFBP-2 increased by 72%, in the food-restricted group. Food restriction reduced the relative sizes of fat depots, spleen, liver, thymus and heart, increased those of adrenals, kidneys, pancreas, gastrointestinal tract, M. Biceps, M. Soleus and brain while those of uterus, lungs, thyroids and M. Gastrocnemius were unchanged. IGFBP-1 and -2 were negatively correlated to weight gain and food conversion efficiency in the ad libitum-fed group, while IGF-I, -II, IGFBP-1, -3 and -4 were positively correlated to body weight, weight gain and food conversion efficiency in the food-restricted group. The results show that acute and chronic food restriction have different consequences for the IGF–IGFBP axis. Furthermore, IGF-II as well as IGF-I are implicated in the control of body weight, weight gain and food conversion efficiency under conditions of restricted nutrition. Finally, IGFBP-1 and -2 may have different roles during chronic undernutrition compared with unrestrained nutrition in adult life.


Introduction

Insulin-like growth factors (IGF)-I and -II are important polypeptide growth factors expressed in most tissues in the body but present in their highest concentrations in blood, largely in association with IGF-binding proteins (IGFBPs). The major source of circulating IGFs is supposed to be the liver, but other tissues may also contribute, particularly in larger animals (Ketelslegers et al. 1995). IGFs have metabolic, mitogenic and differentiating effects on a wide variety of cell types (Jones & Clemmons 1995). During fasting or chronic food restriction, the concentration of circulating IGF-I is reduced and correlates with growth rate in humans and rodents, indicating an important role for IGF-I in the regulation of growth (Ketelslegers et al. 1995). The expression of IGF-I is considered to be controlled by several hormones but mainly by growth hormone (GH). However, a limited availability of nutrients can impair IGF-I gene expression independent of hormonal status (Thissen et al. 1994a), a result which is further supported by studies showing that certain amino acids may have a direct effect on the transcription of the IGF-I gene (Thissen et al. 1994b).

Circulating levels of IGF-II are higher than IGF-I throughout life in most species studied except in rats and mice which express very small amounts of IGF-II after birth (Daughaday et al. 1986, Zangger et al. 1987, Donovan et al. 1989, Owens et al. 1991). IGF-II stimulates growth of numerous cell lines and tissues, but it has not been strongly linked to animal growth (Cohick & Clemmons 1993, Jones & Clemmons 1995). However, the effects of IGF-II have been mostly studied in rats and mice which do not normally express IGF-II postnatally other than in the central nervous system. Recently, Conlon et al. infused female guinea pigs (1995a) and rats (1995b) with IGF-II and were able to stimulate weight gain and food conversion efficiency in rats but not in guinea pigs. Plasma concentrations of IGF-II seem to be less affected by malnutrition than those of IGF-I. Studies in humans and guinea pigs where a short acute fasting has been applied, have shown minor or no reductions in IGF-II (Merimee et al. 1982, Davenport et al. 1988, Peterkofsky et al. 1991), while studies of severely malnourished children indicate that IGF-II concentrations can be reduced by long-term restricted nutrition (Soliman et al. 1986).
The plasma concentrations of the IGFBPs are also changed during fasting or reduced food intake. IGFBP-3 is generally not affected by a short-term fast, while chronic malnutrition decreases circulating IGFBP-3 (Thissen et al. 1994a). Serum concentrations of IGFBP-1 decrease after a meal, in association with increased insulin and glucose levels, and increase during periods of no food intake (Thissen et al. 1994a). The levels of IGFBP-2 increase with restricted food intake (Thissen et al. 1994a).

Increased knowledge of how nutrition controls growth and development, and the role of IGFs and their binding proteins in this process, is important in many areas, such as in the agricultural sector and in treatment of protein-energy malnutrition in children and of patients with catabolic disorders. Many studies in this field have used acute fasting or severe food restriction of animals, usually rodents. The changes that occur during a chronic, moderate food restriction may be different and more relevant to situations under which animals, patients and many children live. The purpose of this study was to investigate the effect of fasting versus a moderate chronic food restriction on the systemic IGF–IGFBP axis in the guinea pig, a species that expresses IGF-II postnatally but also has been shown to be less GH-dependent in its postnatal growth (Gabrielsson et al. 1990), suggesting that other factors may have growth regulating roles in this species. Furthermore, the effects of a long-term moderate food restriction on body weight, growth, body composition and food conversion efficiency were studied and related to any concomitant changes in the IGF axis.

Materials and Methods

Experimental design

Female guinea pigs (IMVS Coloured strain) were obtained from the Gilles Plains Animal Resource Centre (Gilles Plains, SA, Australia). The animals were housed in individual wire bottom cages in a room with a 12 h light:12 h darkness cycle and a temperature of 25 °C. They were fed a guinea pig and rabbit ration (Milling Industries Stockfeeds, May Terrace, Murray Bridge, SA, Australia), with an increased content of vitamin E (165 mg/kg). The guinea pigs had free access to tap water containing vitamin C (400 mg/l). Throughout the experiment food intake and body weight were monitored three times per week and the average daily food intake per kg body weight was calculated. The study was approved by the animal ethics committee of the University of Adelaide, Australia.

Experiment I Catheters were inserted into the right carotid artery in six guinea pigs (654 ± 45 g) under general anaesthesia induced by ketamine (70 mg/kg body weight) and xylazine (6 mg/kg body weight). The animals were allowed to recover from surgery for 9 ± 4 days. At this stage all animals had normal food intake and were gaining body weight. Blood was sampled between 1300 h and 1500 h without any prior food withdrawal (fed sample). Four (4 ± 2) days later the animals were fasted from 1700 h and blood was sampled the following day, between 1000 and 1100 h (fasted sample). Blood was stored on ice until centrifugation at 2500 r.p.m. at 4 °C for 10 min. The plasma was harvested and frozen at −20 °C until analysis.

Experiment II Guinea pigs were divided into two groups, one ad libitum-fed (n=14, body weight 470 ± 56 g) and one food-restricted (n=21, body weight 451±38 g) group. The animals were killed 74 to 100 days (mean ± s.d.: 80 ± 13) after arrival. In the ad libitum-fed group five animals remained after 80 days and three animals remained after 90 days. The corresponding number of animals in the food-restricted group was 13 and 3. The animals in the food-restricted group were given 70% of the average food intake/kg body weight of that of the ad libitum-fed group during the previous two or three days. For the food-restricted animals still participating in the study 85 days after arrival (n=7), the food ration was increased to 90% of the average food intake/kg body weight of the ad libitum-fed group. The reason for this is shown in Figs 1 and 2. In the ad libitum-fed group the food intake (g/day) was stable but the body weight increased resulting in a successive decrease in food intake/kg body weight. In the food-restricted group the body weight remained stable and therefore the food ration (g/day) tended to decrease with time. To keep the food ration of the food-restricted animals stable, the initial level of restriction (70%) was increased to 90% of the food intake/kg body weight of the ad libitum-fed group. The average food intake (g/day) in the food-restricted animals receiving 70% of the intake/kg body weight of the ad libitum-fed group was 19-4 ± 2-0 g, while that for the animals receiving 90% of the intake/kg body weight of the ad libitum-fed group after 85 days of restriction was 20-7 ± 2-1 g. There was no significant difference between these two groups of food-restricted animals with respect to any of the parameters studied. All food-restricted animals were therefore considered as one group. The food-restricted animals received on average 55% of the absolute intake of the ad libitum-fed animals.

The animals were killed by an intraperitoneal overdose of sodium pentobarbitone. To make the ad libitum-fed and food-restricted animals comparable with regard to time between food intake and sacrifice, food-restricted animals were killed in the afternoon, 3–4 h after they had received their daily food ration, while the ad libitum-fed animals were killed in the morning.

Immediately after killing 10 ml blood was collected by cardiac puncture. The blood was stored on ice until centrifugation at 2500 r.p.m. at 4 °C for 10 min. The plasma was harvested and immediately frozen and kept at −20 °C until analysis.

The weight of the following organs/tissues was recorded: adrenals, kidneys, spleen, pancreas, liver, gastrointestinal tract (consisting of cleaned stomach, small intestine, caecum and large bowel), retroperitoneal fat pad, interscapular fat pad, parametrial fat pad, heart, uterus, lungs, thymus, thyroids, M. Biceps brachii, M. Soleus, M. Gastrocnemius and brain.

**Analytical measurements**

**IGF-I and IGF-II RIA** IGF-I and -II were measured by RIA as previously reported for guinea pig (Conlon et al. 1995a). Briefly, plasma was first acidified to pH 2.5 to dissociate IGFs from IGFBPs, and then delipidated, ultrafiltered and fractionated by size exclusion high performance liquid chromatography at pH 2.5 (Carr et al. 1995). This procedure completely separated IGFBPs from IGFs in guinea pig plasma, as previously demonstrated with serum and plasma from pig (Owens et al. 1990), sheep (Carr et al. 1995) and human (Gargosky et al. 1990). All of the IGF-I and IGF-II in guinea pig plasma was recovered in the chromatography effluent eluting between 8.75 min and 10.75 min after injection of diluted, acidified guinea pig plasma. This effluent was routinely collected as a single 2 ml fraction for each plasma specimen. Triplicate aliquots were neutralised with Tris–base (Owens et al. 1990) and their IGF-I and IGF-II contents measured by specific RIAs. Recombinant human IGF-I and -II (GroPep Pty. Ltd, Adelaide, Australia) were used for preparation of radioligands and standards (Owens et al. 1990) for their respective RIAs (Carr et al. 1995, Conlon et al. 1995a). Guinea pig IGF-I (Bell et al. 1990) and IGF-II (Levinovitz et al. 1992) are identical to human IGF-I and -II. The recoveries of IGF-I and -II were >95%. The intra- and interassay coefficients of variation were, respectively, 5.5% and 6.9% for IGF-I and 7.8% and 10.1% for IGF-II.

**Western ligand blotting** The individual IGFBPs in plasma were characterised by Western ligand blotting. Samples (20 µl of a 5% dilution of plasma) were subjected to non-reducing discontinuous SDS-PAGE on a 4% stacking gel and a 10% polyacrylamide separating gel, 2 h at 20 mA followed by overnight at 8 mA. A sample containing a mix of plasma from ad libitum-fed pregnant, virginal and food-restricted virginal animals was run on each gel as a control. Molecular masses of IGFBP bands...
were calculated by comparison to $^{14}$C-labelled ‘rainbow’ molecular weight markers (Amersham International plc, Amersham, Bucks, UK). Separated proteins were transferred onto nitrocellulose filters at 250 mA for 3.5 h. Membranes were blocked and probed with $^{125}$I-IGF-I or $^{125}$I-IGF-II as described by Hossenlopp et al. (1986) and exposed to X-ray film at $-80 \, ^\circ$C for one week (IGFBP-3) or two weeks (IGFBP-1, -2, -4). The relative amount of IGFBPs was expressed as a percentage of the control sample. The blot shows a distinct band of abundant binding protein in all groups of animals, followed by the chronically food-restricted animals.

**Results**

In the fasted animals the plasma concentrations of free fatty acids and cholesterol were increased while those of albumin, glucose and lactate were unchanged (Table 1). In the chronically food-restricted animals the plasma concentrations of albumin, cholesterol and triglycerides were reduced, whereas lactate and urea were increased while those of glucose and free fatty acids were unchanged compared with the ad libitum-fed group. When the animals in experiment I were compared with the animals in experiment II, it was found that the latter group had significantly higher plasma concentrations of glucose and lactate.

Fasting reduced ($P<0.07$) the plasma concentration of IGF-I to 87% of the fed value, while that of IGF-II was unchanged (Table 1). Chronic food restriction, however, reduced IGF-I to 50% and IGF-II to 39% of the corresponding values for the ad libitum-fed group.

Figure 3 shows a representative Western ligand blot of the IGFBPs. The blot shows a distinct $28 \, \text{kDa}$ band (IGFBP-3), a $30 \, \text{kDa}$ band (IGFBP-2), a $28 \, \text{kDa}$ band (including IGFBP-1), and possibly also other IGFBPs. IGFBP-3 was the most abundant binding protein in all groups of animals, followed by IGFBP-2, -1 and -4. There was no effect of fasting on any of the binding proteins studied (Table 1). However, in the chronically food-restricted animals the abundance of IGFBP-1, -3 and -4 in plasma was reduced by 56%, 60% and 44% respectively, while that of IGFBP-2 was increased by 72% compared with the ad libitum-fed animals.

**Table 1** Plasma metabolites, circulating IGF-I, IGF-II and IGFBP-1, -2, -3 and -4 in fed and fasted (experiment I) and in ad libitum-fed and food-restricted (experiment II) female guinea pigs. Values are means $\pm$ S.D.

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Fed</th>
<th>Fasted</th>
<th>Level of significance ($P$)</th>
<th>Level of significance ($P$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin (g/dl)</td>
<td>2.85 $\pm$ 0.47</td>
<td>3.07 $\pm$ 0.32</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Urea (mmol/l)</td>
<td>N.A.</td>
<td>N.A.</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>7.59 $\pm$ 1.05</td>
<td>8.53 $\pm$ 1.36</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Lactate (mmol/l)</td>
<td>1.52 $\pm$ 0.56</td>
<td>1.01 $\pm$ 0.14</td>
<td>$&lt;0.01$</td>
<td>$&lt;0.01$</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>0.54 $\pm$ 0.12</td>
<td>0.89 $\pm$ 0.20</td>
<td>$&lt;0.01$</td>
<td>$&lt;0.01$</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>N.A.</td>
<td>N.A.</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Free fatty acids (meq/l)</td>
<td>0.57 $\pm$ 0.14</td>
<td>2.26 $\pm$ 0.48</td>
<td>$&lt;0.001$</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>IGF-I (ng/ml)</td>
<td>489 $\pm$ 128</td>
<td>427 $\pm$ 76</td>
<td>$&lt;0.07$</td>
<td>$&lt;0.07$</td>
</tr>
<tr>
<td>IGF-II (ng/ml)</td>
<td>1636 $\pm$ 161</td>
<td>1512 $\pm$ 236</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>IGFBP-1 (% of control)</td>
<td>170 $\pm$ 23</td>
<td>193 $\pm$ 40</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>IGFBP-2 (% of control)</td>
<td>59 $\pm$ 10</td>
<td>64 $\pm$ 15</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>IGFBP-3 (% of control)</td>
<td>333 $\pm$ 36</td>
<td>236 $\pm$ 96</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>IGFBP-4 (% of control)</td>
<td>162 $\pm$ 29</td>
<td>178 $\pm$ 45</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

N.A., not analysed; ns, not significant.
Chronic food restriction reduced body weight (Fig. 2), weight gain and food conversion efficiency (data not shown) compared with food available ad libitum. In the food-restricted group, the relative sizes of the spleen, liver, retroperitoneal fat, interscapular fat, parametrial fat, heart and thymus were reduced while those of the adrenals, kidneys, pancreas, gastrointestinal tract, M. Biceps, M. Soleus and brain were increased compared with those of the ad libitum-fed group (Table 2). There were no differences in the relative sizes of the uterus, lungs, thyroids and M. Gastrocnemius between the two groups of animals. The tissues which were most affected by chronic food restriction were the retroperitoneal fat, parametrial fat, interscapular fat, liver, thymus and spleen (Fig. 4). The least affected tissues were brain, gastrointestinal tract, M. Soleus, adrenals and pancreas.

In the ad libitum-fed group, IGFBP-1 and IGFBP-2 were negatively correlated to weight gain and food conversion efficiency, while no significant correlations were found between IGF-I or IGF-II and these parameters (Table 3). In the food-restricted group, IGF-I, IGF-II, IGFBP-1, IGFBP-3 and IGFBP-4 were all positively correlated to body weight, weight gain and food conversion efficiency, while no correlation was found between IGFBP-2 and these parameters.

**Discussion**

In this study, acute fasting was imposed for an average of 17–18 h, which is a relatively short period compared with other studies in rodents which have often used 24–72 h of fasting. However, as guinea pigs eat mainly at night it is likely that the fasted animals were without food for at least 24 h. The large increase in the plasma concentration of free fatty acids does indicate that these animals were metabolically affected by the fasting. The unchanged levels of glucose are in agreement with previously published studies of fasted guinea pigs (Gilbert et al. 1985, Khalifa 1986). The high glucose and lactate levels observed in plasma sampled from the animals killed by an overdose of pentobarbitone are possibly due to stress.

The results of this study show that the effects of acute versus chronic food restriction in guinea pigs on plasma...
metabolites and hence metabolism and on the IGF axis
differ substantially. In the current study, acute fasting
reduced circulating IGF-I, but not IGF-II, in agreement
with previous studies of fasted animals or humans showing
that IGF-I is affected by a short-term fast while IGF-II
remains unchanged (Thissen et al. 1994a). Chronic food
restriction further reduced the plasma concentrations of
IGF-I and to an even greater extent of IGF-II. Reduced
levelsofIGF-IIhavepreviouslybeenobservedinseverely
malnourished children (Soliman et al. 1986). Taken to-
gether these results show that in species which have

circulating IGF-II postnatally, long-term malnutrition
leads to a substantial decrease in circulating IGF-II.
Previous studies in guinea pigs have shown that passive
immunoneutralization of IGF-I fails to slow growth (Kerr
etal. 1990) and that chronic infusions of IGF-I and IGF-II
are unable to stimulate weight gain (Conlon et al. 1995a).
These findings suggest a less active role for the IGF axis in
growth regulation in the guinea pig. However, the results
of this study, showing that IGF-I and IGF-II are related to
growth and food efficiency in food-restricted, but not in ad
libitum-fed animals, indicate that the involvement of these

Figure 4 Organ/tissue sizes of the chronically food-restricted group, expressed as a
percentage of the corresponding value for the ad libitum-fed group in experiment II. The
horizontal line represents the total body weight of the food-restricted group as a
percentage of the corresponding value for the ad libitum-fed group. GI tract,
gastrointestinal tract.

Table 3 Correlations (r) between IGF-I, IGF-II and IGFBP-1 to -4 on the one hand, and body weight, weight gain and food conversion
efficiency on the other hand, in ad libitum-fed and chronically food-restricted virginal guinea pigs

<table>
<thead>
<tr>
<th></th>
<th>IGF-I</th>
<th>IGF-II</th>
<th>IGFBP-1</th>
<th>IGFBP-2</th>
<th>IGFBP-3</th>
<th>IGFBP-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ad libitum-fed animals</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight</td>
<td>0·196</td>
<td>0·358</td>
<td>-0·210</td>
<td>-0·435</td>
<td>-0·215</td>
<td>-0·205</td>
</tr>
<tr>
<td>Weight gain</td>
<td>0·369</td>
<td>0·350</td>
<td>-0·562</td>
<td>&lt;0·05</td>
<td>-0·639</td>
<td>&lt;0·01</td>
</tr>
<tr>
<td>Food conversion efficiency</td>
<td>0·370</td>
<td>0·361</td>
<td>-0·707</td>
<td>&lt;0·01</td>
<td>-0·708</td>
<td>&lt;0·01</td>
</tr>
<tr>
<td>Food-restricted animals</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight</td>
<td>0·723</td>
<td>&lt;0·001</td>
<td>0·776</td>
<td>&lt;0·001</td>
<td>0·718</td>
<td>&lt;0·001</td>
</tr>
<tr>
<td>Weight gain</td>
<td>0·747</td>
<td>&lt;0·001</td>
<td>0·872</td>
<td>&lt;0·001</td>
<td>0·637</td>
<td>&lt;0·01</td>
</tr>
<tr>
<td>Food conversion efficiency</td>
<td>0·716</td>
<td>&lt;0·001</td>
<td>0·874</td>
<td>&lt;0·001</td>
<td>0·605</td>
<td>&lt;0·01</td>
</tr>
</tbody>
</table>

ns, not significant.
growth factors may be dependent on the nutritional status of the animal.

In this study, the relative plasma concentration of a 28 kDa binding protein, assumed to be mostly IGFBP-1, was unchanged in the fasted guinea pigs, which is in disagreement with other studies of fasted animals in this and other species (Murphy et al. 1991, Peterkošky et al. 1991, Gosiewska et al. 1994). IGFBP-1 is assumed to have inhibitory effects on the anabolic actions of IGFs (Gosiewska et al. 1994) and, as expected, there was a negative correlation between this binding protein and weight gain in the ad libitum-fed group. Surprisingly, in the food-restricted group there was a positive correlation between IGFBP-1 and body weight, weight gain and food conversion efficiency. The results in this study indicate that changes in IGFBP-1 are dependent on the type, severity and/or duration of the food restriction and that IGFBP-1 may have different roles in ad libitum-fed and food-restricted animals.

There was no effect of fasting on the circulating levels of IGFBP-2, indicating that this binding protein requires a longer period of food restriction before being increased. This is also confirmed by the results showing that the chronically food-restricted guinea pigs had increased levels of IGFBP-2, and by other studies in guinea pigs showing increased levels of IGFBP-2 after 4 days of fasting (Peterkošky et al. 1991). The fact that IGFBP-2 was strongly and negatively correlated to weight gain and food conversion efficiency in the ad libitum-fed group supports the idea that this binding protein, along with IGFBP-1, can inhibit the anabolic actions of the IGFs (Gosiewska & Peterkošky 1995).

In studies of rats and humans, it has been reported that IGFBP-3 can be modified by a specific protease during pregnancy and catabolic disorders (Giudice et al. 1994). IGFBP-3 is assumed to have inhibitory effects on the anabolic actions of IGFs (Peterkošky et al. 1995). In our study, the relative plasma concentration of a specific protease was not detected in the food-restricted rats, but did occur in the ad libitum-fed rats. Surprisingly, in the food-restricted group the intensity of the band corresponding to IGFBP-3 was greater than those used in previous studies in rats.

In conclusion, this study has shown that the effects of acute fasting versus chronic food restriction in guinea pigs differ with regard to circulating metabolites, IGFs and IGFBPs. The results implicate IGF-I as well as IGF-II in the regulation of weight gain in undernourished animals and an inhibiting role for IGFBP-1 and IGFBP-2 on weight gain in ad libitum-fed animals.

Acknowledgements

We thank Jan Jaap Erwich, Jane Lang, Linda Mundy and Karina Irvine for helpful advice and the constructive discussions. This study was supported by the Swedish Medical Research Council and CRC for Tissue Growth and Repair. Australia and the Australian Research Council.

References

Bell GI, Stempień MM, Fong NM & Seino S 1990 Sequence of a cDNA encoding guinea pig IGF-I. Nucleic Acids Research 18 4275.

Carr JM, Owens JA, Grant PA, Walton PE, Owens PC & Wallace JC 1995 Circulating insulin-like growth factors (IGFs) and IGF-binding proteins (IGFBPs) and tissue mRNA levels of IGFBP-2 and IGFBP-4 in the ovine fetus. Journal of Endocrinology 145 545–557.


Received 11 April 1997
Revised manuscript received 6 October 1997
Accepted 13 November 1997