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

(Requests for offprints should be addressed to A Sohlström who is now at Department of Physiology and Pharmacology, Karolinska Institute, S-171 77 Stockholm, Sweden)

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.

Journal of Endocrinology (1998) **157**, 107–114

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 $\pm$ 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.

Figure 1 Average food intake, expressed as g/day and g/kg body weight/day, in *ad libitum*-fed and food-restricted guinea pigs throughout the experiment.

Figure 2 Average body weight of *ad libitum*-fed and food-restricted guinea pigs throughout the experiment.

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, ultra-filtered 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 IGF-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

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 ± s.d.

	Fed	Fasted	Level of significance (P)	Ad libitum fed	Food restricted	Level of significance (P)
Albumin (g/dl)	2.85 ± 0.47	3.07 ± 0.32	ns	3.48 ± 0.26	2.83 ± 0.21	<0.001
Urea (mmol/l)	N.A.	N.A.	—	9.20 ± 1.14	13.56 ± 2.23	<0.001
Glucose (mmol/l)	7.59 ± 1.05	8.53 ± 1.36	ns	11.70 ± 3.74	11.11 ± 2.89	ns
Lactate (mmol/l)	1.52 ± 0.56	1.01 ± 0.14	ns	8.09 ± 3.86	10.91 ± 3.59	<0.05
Cholesterol (mmol/l)	0.54 ± 0.12	0.89 ± 0.20	<0.01	0.67 ± 0.19	0.46 ± 0.15	<0.001
Triglycerides (mmol/l)	N.A.	N.A.	—	0.62 ± 0.14	0.45 ± 0.09	<0.001
Free fatty acids (meq/l)	0.57 ± 0.14	2.26 ± 0.48	<0.001	0.50 ± 0.26	0.54 ± 0.15	ns
IGF-I (ng/ml)	489 ± 128	427 ± 76	<0.07	343 ± 107	172 ± 121	<0.001
IGF-II (ng/ml)	1638 ± 161	1512 ± 236	ns	1743 ± 294	677 ± 264	<0.001
IGFBP-1 (% of control)	170 ± 23	193 ± 40	ns	180 ± 118	80 ± 56	<0.001
IGFBP-2 (% of control)	59 ± 10	64 ± 15	ns	68 ± 45	117 ± 48	<0.01
IGFBP-3 (% of control)	333 ± 36	356 ± 96	ns	228 ± 69	72 ± 43	<0.001
IGFBP-4 (% of control)	162 ± 29	178 ± 45	ns	178 ± 93	101 ± 56	<0.01

N.A., not analysed; ns, not significant.

were calculated by comparison to ¹⁴C-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 ¹²⁵I-IGF-I or ¹²⁵I-IGF-II as described by Hossenlopp *et al.* (1986) and exposed to X-ray film at − 80 °C for one week (IGFBP-3) or two weeks (IGFBP-1, -2, -4). The relative amount of IGFBP was assessed by densitometry (Image Quant, version 3.22, Computing Densitometer, Model 300A, Molecular Dynamics, Sunnyvale, CA, USA). Each band was expressed as a percentage of the control sample.

Metabolite analysis The concentrations of plasma metabolites (glucose, lactate, albumin, free fatty acids, cholesterol in both experiments and triglycerides and urea in experiment II) in individual animals were analysed enzymatically, in duplicate, using a COBAS MIRA automated analyser (Roche Diagnostic Systems). The interassay coefficients of variation, assessed by repeated analysis of the same sample, were 1.4 to 7.8% for the different metabolites.

Statistics

Data were analysed using BMDP Statistical Software (Los Angeles, CA, USA). In experiment I, differences between fed and fasted samples were analysed by paired *t*-test. In experiment II, the values were analysed by one-way ANOVA. Levene’s test for equal variances was performed and if significant, differences between *ad libitum*-fed and food-restricted groups were assessed by Kruskal-Wallis one-way analysis of variance. Associations between IGFs and IGFBPs on the one hand, and body weight, weight gain and food conversion efficiency on the other, were

analysed by linear regression analysis in *ad libitum*-fed and 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, urea and lactate 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 ~46 kDa band (IGFBP-3), a ~30 kDa band (IGFBP-2), a ~28 kDa band (including IGFBP-1, but possibly also other IGFBPs) and a ~24 kDa band (IGFBP-4). 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.

Figure 3 Western ligand blot of IGFBP in pooled plasma of (a) fed (lane 1) and fasted (lane 2) guinea pigs and (b) *ad libitum*-fed (lane 1) and chronically food-restricted (lane 2) guinea pigs. The molecular masses (kDa) of the IGFBPs are indicated on the left.

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

Table 2 Organ size as a percentage of body weight for *ad libitum*-fed and chronically food-restricted guinea pigs in experiment II. Values are means $\pm$ S.D.

	<i>Ad libitum</i> fed	Food restricted	Level of significance (<i>P</i>)
Adrenals	0.052 $\pm$ 0.007	0.064 $\pm$ 0.009	<0.001
Kidneys	0.700 $\pm$ 0.077	0.758 $\pm$ 0.072	<0.05
Spleen	0.190 $\pm$ 0.029	0.138 $\pm$ 0.029	<0.001
Pancreas	0.268 $\pm$ 0.066	0.322 $\pm$ 0.045	<0.01
Liver	4.709 $\pm$ 0.448	4.039 $\pm$ 0.385	<0.001
Gastrointestinal tract	4.567 $\pm$ 0.639	6.455 $\pm$ 0.797	<0.001
Uterus	0.154 $\pm$ 0.034	0.158 $\pm$ 0.052	ns
Retroperitoneal fat	1.008 $\pm$ 0.106	0.059 $\pm$ 0.069	<0.001
Interscapular fat	1.754 $\pm$ 0.178	0.310 $\pm$ 0.197	<0.001
Parametrial fat	0.629 $\pm$ 0.104	0.075 $\pm$ 0.059	<0.001
Heart	0.364 $\pm$ 0.038	0.334 $\pm$ 0.040	<0.05
Lung	0.599 $\pm$ 0.168	0.643 $\pm$ 0.094	ns
Thymus	0.071 $\pm$ 0.008	0.034 $\pm$ 0.013	<0.001
Thyroids	0.012 $\pm$ 0.002	0.013 $\pm$ 0.003	ns
M. Biceps	0.118 $\pm$ 0.007	0.134 $\pm$ 0.014	<0.01
M. Soleus	0.047 $\pm$ 0.004	0.061 $\pm$ 0.008	<0.001
M. Gastrocnemius	0.512 $\pm$ 0.036	0.547 $\pm$ 0.065	ns
Brain	0.569 $\pm$ 0.030	0.845 $\pm$ 0.099	<0.001

ns, not significant.

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

	IGF-I		IGF-II		IGFBP-1		IGFBP-2		IGFBP-3		IGFBP-4	
	<i>r</i>	<i>P</i> value	<i>r</i>	<i>P</i> value	<i>r</i>	<i>P</i> value	<i>r</i>	<i>P</i> value	<i>r</i>	<i>P</i> value	<i>r</i>	<i>P</i> value
<i>Ad libitum</i>-fed animals												
Body weight	0.196	ns	0.358	ns	−0.210	ns	−0.435	ns	−0.215	ns	−0.205	ns
Weight gain	0.369	ns	0.350	ns	−0.562	<0.05	−0.639	<0.01	−0.307	ns	−0.097	ns
Food conversion efficiency	0.370	ns	0.361	ns	−0.707	<0.01	−0.708	<0.01	−0.259	ns	0.042	ns
Food-restricted animals												
Body weight	0.723	<0.001	0.776	<0.001	0.718	<0.001	−0.272	ns	0.695	<0.001	0.583	<0.01
Weight gain	0.747	<0.001	0.872	<0.001	0.637	<0.01	−0.383	ns	0.812	<0.001	0.626	<0.01
Food conversion efficiency	0.716	<0.001	0.874	<0.001	0.605	<0.01	−0.401	ns	0.816	<0.001	0.607	<0.01

ns, not significant.

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 levels of IGF-II have previously been observed in severely malnourished children (Soliman *et al.* 1986). Taken together 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 *et al.* 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 -II are related to growth and food efficiency in food-restricted, but not in *ad libitum*-fed animals, indicate that the involvement of these

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, Peterkofsky *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 (Peterkofsky *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 & Peterkofsky 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.* 1990, Davies *et al.* 1991). IGFBP-3 is then degraded into smaller fragments, resulting in decreased affinity for IGF-I. We tested (data not shown) for the presence of protease activity by incubating serum from the food-restricted group with serum from the *ad libitum*-fed group which was then subjected to a Western ligand blot, according to Gargosky *et al.* (1992). No reduction in the intensity of the band corresponding to IGFBP-3 could be found and it was therefore concluded that with this method, no protease activity could be identified in plasma from the food-restricted guinea pigs. This is in agreement with a study of chronically protein-restricted rats, where no protease activity could be identified in this way (Oster *et al.* 1995).

Chronic food restriction decreased growth, and thus decreased body weight. However, different organs/tissues were not affected to the same extent. The results indicate that 'key organs' such as brain and gastrointestinal tract, but also muscles, adrenals and pancreas, were relatively unaffected by the food restriction, while the sizes of fat depots, liver and lymphatic tissues such as thymus and spleen, were considerably reduced. The large decrease in

fat depots in the body found in the food-restricted group in this study is different from previous published results of malnourished rats (Sohlström *et al.* 1994). In that study (Sohlström *et al.* 1994) a similar design of chronic food restriction was used, but the food-restricted rats were fed 70% of the absolute intake of the *ad libitum*-fed rats. That study showed that the food-restricted rats, although they were significantly smaller than the *ad libitum*-fed rats, had the same concentration of body fat. This suggests species differences in the response of adipose tissue and other tissues to chronic malnutrition. When rats are subjected to fasting or food restriction their plasma levels of GH are drastically reduced (Oster *et al.* 1995). However, in guinea pigs, as in humans, malnutrition is associated with increased circulating levels of GH (Fairhall *et al.* 1990). As GH is known to stimulate lipolysis, an increased GH secretion during malnutrition would enhance mobilization of body fat in the guinea pig and human while decreased GH levels in malnourished rats would have the opposite effect. Alternatively, the different response may simply reflect the fact that the period of food restriction and its severity that guinea pigs were subjected to in the current study were 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 help and technical assistance and Professor Kerstin Hall for constructive discussions. This study was supported by Johan and Henning Throne-Holst's Foundation, the Swedish Medical Research Council and CRC for Tissue Growth and Repair, Australia and the Australian Research Council.

References

- Bell GI, Stempien 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.
- Cohick WS & Clemmons DR 1993 The insulin-like growth factors. *Annual Review of Physiology* **55** 131–153.
- Conlon MA, Tomas FM, Owens PC, Wallace JC, Howarth GS & Ballard FJ 1995a Long R³ insulin-like growth factor-I (IGF-I) infusion stimulates organ growth but reduces plasma IGF-I, IGF-II and IGF binding protein concentrations in the guinea pig. *Journal of Endocrinology* **146** 247–253.

- Conlon MA, Francis GL, Tomas FM, Wallace JC, Howarth GS & Ballard FJ 1995b Continuous 14-day infusion of IGF-II increases the growth of normal female rats, but exhibits a lower potency than IGF-I. *Journal of Endocrinology* **144** 91–98.
- Daughaday WH, Yanow CE & Kapadia M 1986 Insulin-like growth factors-I and -II in maternal and fetal guinea pig serum. *Endocrinology* **119** 490–494.
- Davenport ML, Svoboda ME, Koerber KL, Van Wyk JJ, Clemmons DR & Underwood LE 1988 Serum concentrations of insulin-like growth factor-II are not changed by short term fasting and refeeding. *Journal of Clinical Endocrinology and Metabolism* **67** 1231–1236.
- Davies SC, Wass JAH, Ross RJM, Cotterill AM, Buchanan CR, Coulson VJ & Holly JMP 1991 The induction of a specific protease for insulin-like growth factor-binding protein-3 in the circulation during severe illness. *Journal of Endocrinology* **130** 469–473.
- Donovan SM, Oh Y, Pham H & Rosenfeld RG 1989 Ontogeny of serum insulin-like growth factor binding proteins in the rat. *Endocrinology* **125** 2621–2627.
- Fairhall KM, Gabriellson BG & Robinson ICAF 1990 Effect of food withdrawal and insulin on growth hormone secretion in the guinea pig. *Endocrinology* **127** 716–723.
- Gabriellson B, Fairhall KM & Robinson ICAF 1990 Growth hormone secretion in the guinea pig. *Journal of Endocrinology* **124** 371–380.
- Gargosky SE, Moyse KJ, Owens JA, Wallace JC, Robinson JS & Owens PC 1990 Circulating levels of insulin-like growth factors increase and molecular forms of their serum binding proteins change with human pregnancy. *Biochemical and Biophysical Research Communications* **170** 1157–1163.
- Gargosky SE, Pham HM, Wilson KF, Liu F, Giudice LC & Rosenfeld RG 1992 Measurement and characterization of insulin-like growth factor binding protein-3 in human biological fluids: discrepancies between radioimmunoassay and ligand blotting. *Endocrinology* **131** 3051–3060.
- Gilbert M, Sparks JW, Girard SJ & Battaglia FC 1985 Effects of fasting on glucose turnover rate and metabolite levels in conscious pregnant guinea pigs. *Biology of the Neonate* **48** 90–99.
- Giudice LC, Farrell EM, Pham H, Lamson G & Rosenfeld RG 1990 Insulin-like growth factor binding proteins in maternal serum throughout gestation and in the puerperium: effects of a pregnancy-associated serum protease activity. *Journal of Clinical Endocrinology and Metabolism* **71** 806–816.
- Gosiewska A & Peterkofsky B 1995 Insulin-like growth factor (IGF) binding proteins and their mRNAs in connective tissues of fasted guinea pigs. *Endocrine* **3** 889–897.
- Gosiewska A, Wilson S, Kwon D & Peterkofsky B 1994 Evidence for an *in vivo* role of insulin-like growth factor binding protein-1 and -2 as inhibitors of collagen gene expression in vitamin C-deficient and fasted guinea pigs. *Endocrinology* **134** 1329–1339.
- Hossenlopp P, Seurin D, Segovia-Quinson B, Hardouin S & Binoux M 1986 Analysis of serum insulin-like growth factor binding proteins using Western blotting: use of the method for titration of the binding proteins and competitive binding studies. *Analytical Biochemistry* **154** 138–143.
- Jones JI & Clemmons DR 1995 Insulin-like growth factors and their binding proteins: biological actions. *Endocrine Reviews* **16** 3–34.
- Kerr DE, Laarveld B & Manns JG 1990 Effects of passive immunization of growing guinea pigs with an insulin-like growth factor-I monoclonal antibody. *Journal of Endocrinology* **124** 403–415.
- Ketelslegers J-M, Maiter D, Maes M, Underwood LE & Thissen J-P 1995 Nutritional regulation of insulin-like growth factor-I. *Metabolism* **44** (Suppl 4) 50–57.
- Khalifa MH 1986 Concentrations of carbohydrates and lipids of guinea pigs after five days starvation. *Comparative Biochemistry and Physiology* **85A** 225–227.
- Levinovitz A, Norstedt G, van den Berg S, Robinson ICAF & Ekström T 1992 Isolation of an insulin-like growth factor-II cDNA from guinea pig liver: expression and developmental regulation. *Molecular and Cellular Endocrinology* **89** 105–110.
- Merimee TJ, Zapf J & Froesch ER 1982 Insulin-like growth factors in the fed and fasted states. *Journal of Clinical Endocrinology and Metabolism* **55** 999–1002.
- Murphy LJ, Seneviratne C, Moreira P & Reid RE 1991 Enhanced expression of insulin-like growth factor-binding protein-1 in the fasted rat: the effects of insulin and growth hormone administration. *Endocrinology* **218** 689–696.
- Oster MH, Fielder PJ, Levin N & Cronin MJ 1995 Adaptation of the growth hormone and insulin-like growth factor-I axis to chronic and severe calorie or protein malnutrition. *Journal of Clinical Investigation* **95** 2258–2265.
- Owens PC, Johnson RJ, Campbell RG & Ballard FJ 1990 Growth hormone increases insulin-like growth factor-I (IGF-I) and decreases IGF-II in plasma of growing pigs. *Journal of Endocrinology* **124** 269–275.
- Owens PC, Conlon MA, Campbell RG, Johnson RJ, King R & Ballard FJ 1991 Developmental changes in growth hormone, insulin-like growth factors (IGF-I and IGF-II) and IGF binding proteins in plasma of young growing pigs. *Journal of Endocrinology* **128** 439–447.
- Peterkofsky B, Palka J, Wilson S, Takeda K & Shah V 1991 Elevated activity of low molecular weight insulin-like growth factor binding proteins in sera of vitamin C-deficient and fasted guinea pigs. *Endocrinology* **128** 1769–1779.
- Sohlström A, Kabir N, Sadurskis A & Forsum E 1994 Body composition and fat distribution in the first 2 weeks of gestation in *ad lib*-fed and energy-restricted rats. *British Journal of Nutrition* **71** 317–333.
- Soliman AT, El Hadi ABD, Aref MK, Hintz RL, Rosenfeld RG & Rogol AD 1986 Serum insulin-like growth factors-I and -II concentrations and growth hormone and insulin responses to arginine infusion in children with protein-energy malnutrition before and after nutritional rehabilitation. *Pediatric Research* **20** 1122–1130.
- Thissen J-P, Ketelslegers J-M & Underwood LE 1994a Nutritional regulation of the insulin-like growth factors. *Endocrine Reviews* **15** 80–101.
- Thissen J-P, Pucilowska JB & Underwood LE 1994b Differential regulation of insulin-like growth factor-I (IGF-I) and IGF binding protein-1 messenger ribonucleic acids by amino acid availability and growth hormone in rat hepatocyte primary culture. *Endocrinology* **134** 1570–1576.
- Zangger I, Zapf J & Froesch ER 1987 Insulin-like growth factor-I and -II in 14 animal species and man as determined by three radioligand assays and two bioassays. *Acta Endocrinologica* **114** 107–112.

Received 11 April 1997

Revised manuscript received 6 October 1997

Accepted 13 November 1997