Effects of food restriction and pregnancy on the expression of insulin-like growth factors-I and -II in tissues from guinea pigs

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Abstract

The insulin-like growth factor (IGF) system is subjected to pregnancy-associated changes in the circulation and is suggested to be of importance for partitioning of nutrients between the mother and the foetus. Interestingly, maternal undernutrition alters the pregnancy-associated changes, with possible adverse consequences for the mother and the foetus. However, it is not known how malnutrition and pregnancy alter the expression of mRNA for IGFs locally in different tissues. The aims of this study were to investigate where IGF-I and IGF-II are expressed in guinea pigs and how this expression is altered during food restriction and pregnancy. *Ad libitum*-fed and food-restricted (fed 70% of the *ad libitum*-fed intake four weeks before pregnancy and throughout the study) guinea pigs were mated. On day 40 of pregnancy and on the corresponding day for virginal females the animals were killed. mRNA for IGF-I and IGF-II was analysed in various

organs/tissues by solution hybridisation. mRNA for IGF-I was expressed in high amounts in uterus, liver and adipose tissues. The expression was not affected by food restriction, but was increased in liver and adipose tissue and decreased in uterus by pregnancy. mRNA for IGF-II was expressed in high amounts in the placenta and liver. In the placenta the expression was decreased by food restriction. Pregnancy increased the levels of mRNA for IGF-II in the liver. Food-restricted dams had smaller foetuses and placentas. In conclusion, this study indicates an important role for the adipose tissue during gestation, not only as an energy store but also as an endocrine tissue expressing IGF-I. The decreased expression of IGF-II in the placenta due to food restriction is suggested to have adverse effects on placental structure and function.

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Introduction

In order to be able to provide the foetus and the newborn with sufficient energy and nutrients, the pregnant mother undergoes a number of physiological, metabolic and endocrine adaptations (Prentice *et al.* 1995). Changes in the insulin-like growth factor (IGF) system is one of these. The IGF system has been suggested to influence the substrate supply to the foetus, and hence its growth, by modulating maternal metabolism and placental function (Owens 1991). Such a role may be of particular significance when access to energy and nutrients is limited.

The IGF system includes the ligands IGF-I and -II, six characterised high affinity binding proteins (IGFBP-1 to -6) and three cell surface receptors (type 1 IGF receptor, type 2 IGF receptor and insulin receptor) (Jones & Clemmons 1995). IGF-I and -II are growth factors responsible for inducing proliferation, differentiation and metabolic changes in a variety of cell types (Jones & Clemmons 1995). IGF-I and -II are associated to a great extent with IGFBPs (Jones & Clemmons 1995), and are

able to affect the bioavailability and the action of IGFs (Clemmons 1998). Growth hormone (GH) (Butler & LeRoith 2001) and nutrition (Thissen *et al.* 1994) are the main regulators of the IGF system. While the polypeptides IGF-I and -II, the IGFBPs and the receptors are synthesised in many tissues (Cohick & Clemmons 1993, Jones & Clemmons 1995), the liver is thought to be the major contributor to the circulatory levels of IGFs (Cohick & Clemmons 1993, Butler & LeRoith 2001, Ohlsson *et al.* 2000).

During pregnancy, maternal plasma levels of IGF-I increase early in most species (Wilson *et al.* 1982, Gargosky *et al.* 1990, Donovan *et al.* 1991, Hills *et al.* 1996, Nason *et al.* 1996, Sohlström *et al.* 1998b). The abundance of circulatory IGF-II increases in some species (Nason *et al.* 1996), including humans (Wilson *et al.* 1982, Gargosky *et al.* 1990), but remains unchanged in others (Donovan *et al.* 1991, Sohlström *et al.* 1998b). On the one hand, results from rat studies indicate that maternal serum IGF-I targets the mother's own anabolism and substrate retention rather than the foetus (Gargosky *et al.* 1991,

Monaco & Donovan 1996). On the other hand, in humans (Hall *et al.* 1986, Holmes *et al.* 1998) and guinea pigs (Sohlström *et al.* 2001) maternal IGF-I seems to be associated with foetal and placental growth. Maternal plasma levels of IGF-II have been shown to promote the structural development of the placenta (Roberts *et al.* 2001b) and IGF-II produced by the trophoblasts has been suggested to be important for implantation and placentalization (Hamilton *et al.* 1998, Han *et al.* 1999, Rutanen 2000). However, maternal undernutrition alters these pregnancy-associated changes in the IGF system, with possible adverse consequences for the mother, the placenta and the foetus (Sohlström *et al.* 1998b, Roberts *et al.* 2001b).

Studies on the role of maternal IGFs during pregnancy have so far mainly been focused on plasma levels and the endocrine action of these peptides. Recently, it was shown that genetically modified mice with inactivated liver-derived IGF-I had dramatically decreased IGF-I levels in plasma without any impairment of postnatal growth, indicating that non-hepatic, autocrine/paracrine-acting IGF-I is one main determinant of postnatal growth (Sjögren *et al.* 1999, Ohlsson *et al.* 2000, Butler & LeRoith 2001). However, these mice had increased serum levels of GH, since the circulating IGF-I normally exerts negative feedback regulation on GH secretion (Sjögren *et al.* 1999). Thus, the tissue-specific expression of IGFs and their autocrine/paracrine actions are of great importance for growth and metabolism. It is possible that non-hepatic IGFs may also contribute to the circulating concentrations of IGFs.

In this study the expression of mRNA for IGF-I and -II was studied in various tissues in *ad libitum*-fed and food-restricted guinea pigs in midpregnancy and on the corresponding day for virginal females. Guinea pigs (Daughaday *et al.* 1986), like humans (Gargosky *et al.* 1990) but unlike rodents (Donovan *et al.* 1989), express substantial amounts of IGF-II in adult life and have a progressive increase in circulatory IGF-I during pregnancy (Sohlström *et al.* 1998b).

The aims of this study were to investigate where IGF-I and IGF-II are expressed in guinea pigs and how this expression is altered by food restriction and pregnancy.

Materials and Methods

Nulliparous female Dunkin-Hartley guinea pigs ($n=34$), 2–3 months old and weighing 475 ± 26 g at arrival, were obtained from Charles River Laboratories, Sulzfeld, Germany. The animals were housed individually in a room with a 12 h light:12 h darkness cycle and a temperature of 21 °C. They were fed with a guinea pig diet from Special Diet Services, Witham, Essex, UK. The animals had free access to water. One week after arrival and four

weeks before mating, guinea pigs were randomly divided into two groups, one that had feed available *ad libitum* and one that was subjected to food restriction. Food intake and body weight were measured three times a week. Food-restricted animals were given 70% of the average food intake during the previous 2 or 3 days of the *ad libitum*-fed animals throughout the study. During the four weeks before mating it was noticed however that the *ad libitum*-fed animals including those being mated later had a stable food intake. Thus, the food ration of the food-restricted dams was set to 21 g every day and for the food-restricted virginal animals this ration continued throughout the study. For the pregnant animals the food intake increased during pregnancy. After four weeks of nutritional control, half of the animals in each dietary group were mated. A female in oestrus was put with a male overnight, and the female was regarded as pregnant when a vaginal copulatory plug was found the following morning. This day was regarded as day 1 of gestation. The following four groups were investigated and their mean body weight in grams at day 1 of gestation and on the corresponding day for virginal animals are shown within parentheses: virginal/*ad libitum*-fed (692 ± 42), virginal/food-restricted (501 ± 16), pregnant/*ad libitum*-fed (580 ± 44) and pregnant/food-restricted (538 ± 36). Since pregnant animals also took part as controls in another study (Sohlström *et al.* 2001), osmotic mini-pumps (Alzet, Model 2002, Scanbur BK AB, Sollentuna, Sweden) filled with 240 µl 0.1 mol/l acetic acid were placed s.c. on their backs at day 20 of gestation after the animals were anaesthetised with injections of ketamine, 25 mg/kg i.m. (Pfizer) and xylocaine, 4 mg/kg s.c. (Bayer AB, Leverkusen, Germany), and locally with a s.c. injection of lidocaine (Astra Zeneca). The virginal guinea pigs were not subjected to this procedure. On day 40 of pregnancy or on the corresponding day in virginal females, the animals were sedated in CO₂ for 15 s and then killed by an intracardiac overdose of pentobarbital sodium 60 mg/kg (Astra Zeneca). Immediately after death, 5–10 ml blood were collected by cardiac puncture into chilled plastic tubes containing 10 IU/ml heparin (Lövens Läkemedel, Malmö, Sweden) and 500 IU/ml aprotinin (Bayer AB), and the animals were thereafter put on ice. The tubes were stored on ice until centrifugation. Plasma was harvested and frozen at –20 °C until analysis. The following tissues and organs were removed from the carcass and immediately weighed: individual fetuses and placentas, liver, spleen, muscle biceps, muscle triceps, and parametrial and interscapular adipose tissues. Tissues and organs were immediately snap frozen on dry ice and stored at –80 °C until analyses of mRNA. This protocol was approved by the Stockholm Ethical Committee for Experiments in Animals, Sweden. The animal care conformed to the European Convention for Protection of Vertebrates used for Experimental and Other Scientific Purposes published by the Council of Europe 1985 (no. 123), Strasbourg.

Blood analyses

IGF-I and -II were measured by radioimmunoassay (RIA) as previously reported for guinea pigs (Conlon *et al.* 1995, Sohlström *et al.* 1998a). Plasma was acidified to pH 2.5 and then fractionated by size exclusion high performance liquid chromatography at pH 2.5. Individual plasma samples were extracted with freon according to Owens *et al.* (1990) and then injected onto a Protein-Pak 125 exclusion column (Waters/Millipore, Lane Cove, NSW, Australia). Triplicate aliquots were neutralised with Tris-base and their IGF-I and IGF-II content measured by specific RIAs. Recombinant human IGF-I and IGF-II (GroPep Pty. Ltd, Adelaide, Australia) were used for preparation of radioligands and standards for their respective RIAs. In the IGF-I RIA, a rabbit anti-human (h) IGF-I (MAC 89/1), having a cross-reactivity with hIGF-II of <1%, was used at a final dilution of 1:60 000. In the IGF-II RIA, a mouse anti-rat IGF-II monoclonal antibody (IGF S1-F2) was used, with a cross-reactivity with hIGF-I of 2.5% at 1 ng/reaction tube and 100% to human IGF-II (Conlon *et al.* 1995). Guinea pig IGF-I (Bell *et al.* 1990) and IGF-II (Levinovitz *et al.* 1992) are identical to human IGF-I and -II. The intra- and inter-assay coefficients of variation were, respectively, 5.5% and 6.9% for IGF-I and 7.8% and 10.1% for IGF-II.

Tissue analyses

All maternal tissues were analysed and one placenta from each dam was randomly selected.

Total nucleic acid (TNA) preparation

TNA was prepared as described earlier (Durnam & Palmiter 1983). Tissues were homogenised in 4 ml SET buffer (1% (w/v) sodium dodecyl sulphate (SDS), 20 mM Tris-HCl (pH 7.5), and 10 mM EDTA) and digested with proteinase K (800 µg) for 45 min at 45 °C. TNA was extracted by phenol-chloroform, followed by precipitation by adding two volumes of ethanol. The concentrations of the nucleic acids in TNA that were sampled were measured spectrophotometrically.

Solution hybridisation

Quantification of mRNA for IGF-I and IGF-II was performed using RNA probes labelled with ³⁵S-UTP (Amersham International plc, Amersham, Bucks, UK) (Durnam & Palmiter 1983). cDNA for IGF-I and -II probes was cloned separately into expression vector pGEM-T (Promega, Madison, WI, USA), allowing *in vitro* synthesis (Melton *et al.* 1984), using SP6 and T7 RNA polymerases. cDNA for IGF-I and -II was kindly obtained from G Bell (Departments of Biochemistry and Molecular Biology and Medicine, University of Chicago, IL, USA)

and B Peterkofsky (Laboratory of Biochemistry, National Cancer Institute, Bethesda, MD, USA). Each vector was sequenced to assure significant alignment of mRNA for IGF-I and IGF-II in guinea pig and 100% homology was obtained. mRNA probes were hybridised for 16–18 h at 70 °C with samples of isolated TNA. Hybridised probes were protected from RNase digestion and precipitated by addition of trichloroacetic acid (6 M) and collected after filtration on glass fibre filters (Whatman GF/C, Maidstone, Kent, UK). The radioactivity on the filters was counted in a scintillation counter. Hybridisation signals from each sample were compared with a standard curve of known amounts of *in vitro* synthesised mRNA (Durnam & Palmiter 1983, Möller *et al.* 1991). Each sample was analysed in duplicate.

Statistical analyses

Results are presented as means ± standard deviation and were considered statistically significant when $P < 0.05$. The effects of food restriction *in utero* on the number of foetuses and on foetal and placental weight were assessed using Student's unpaired *t*-test. Differences between groups regarding expression of mRNA for IGF-I and -II in various tissues, plasma levels of IGF-I and -II, body weight, body weight gain, and tissue and organ weights were analysed by ANOVA with nutrition (*ad libitum*-fed versus food-restricted) and reproductive status (virginal versus pregnant) as components of variation. When interactions were found, planned comparisons were made to assess differences between (1) virginal/*ad libitum*-fed and virginal/food-restricted, (2) virginal/*ad libitum*-fed and pregnant/*ad libitum*-fed, (3) virginal/food-restricted and pregnant/food-restricted and (4) pregnant/*ad libitum*-fed and pregnant/food-restricted. Levene's test for equal variances was performed and found to be significant for the expression of mRNA for IGF-I in interscapular adipose tissue and uterus, and for the expression of mRNA for IGF-II in uterus. Differences between the groups in those cases were assessed by the Mann-Whitney U test.

Results

mRNA for IGF-I in tissues from virginal and pregnant dams

mRNA for IGF-I was expressed in all tissues being analysed from virginal females and the expression was most pronounced in the uterus, liver and parametrial and interscapular adipose tissues (Fig. 1). Food restriction had no effect on the expression of mRNA for IGF-I except in the spleen, where the relatively small expression of IGF-I mRNA was increased. In pregnant animals the expression of mRNA for IGF-I was approximately doubled in the liver, parametrial and interscapular adipose tissues as compared with virginal counterparts, regardless of nutritional

Figure 1 Expression of mRNA for IGF-I in various tissues and organs from virgin and pregnant guinea pigs. a, significant effect ($P < 0.05$) of nutrition; b, significant effect ($P < 0.05$) of pregnancy; nd, non detectable levels of mRNA for IGF-I.

status. However, in the uterus of the pregnant dams, the expression of mRNA for IGF-I was approximately half of that for the virgin dams. No mRNA for IGF-I was detected in the placenta.

mRNA for IGF-II in tissues from virgin and pregnant dams

mRNA for IGF-II was expressed in all tissues being analysed from virgin and pregnant females, except in the soleus muscle (Fig. 2), and the expression was highest in the placenta and liver. Food restriction decreased the expression of mRNA for IGF-II in the placenta to 76% of that for *ad libitum*-fed dams. However, in the parametrial adipose tissue the expression was slightly increased by food restriction. In pregnant animals the expression of mRNA for IGF-II was doubled in the liver and increased to a small

extent in the parametrial adipose tissue. Food restriction increased the expression of mRNA for IGF-II in the interscapular adipose tissue in virgin animals, but no such effect was seen in pregnant dams.

Circulatory IGF-I and -II

Food restriction decreased circulatory plasma levels of IGF-I in both virgin and pregnant animals as compared with their *ad libitum*-fed counterparts. Both *ad libitum*-fed and food-restricted pregnant dams had twice as high circulatory levels of IGF-I as their virgin counterparts (Table 1). The plasma levels of IGF-II were also decreased by food restriction in both virgin and pregnant animals as compared with their *ad libitum*-fed counterparts. Pregnant *ad libitum*-fed animals had lower plasma levels of IGF-II

Figure 2 Expression of mRNA for IGF-II in various tissues and organs from virgin and pregnant guinea pigs. a, significant effect ($P < 0.05$) of nutrition; b, significant effect ($P < 0.05$) of pregnancy; c, interaction between nutrition and pregnancy; nd, non detectable levels of mRNA for IGF-II.

Table 1 Number of dams, reproductive outcome, plasma levels of IGF-I and -II, body weight, tissue and organ weights in virginal and pregnant dams in the absence or the presence of food restriction. Values are means $\pm$ standard deviation

	Virginal		Pregnant	
	<i>Ad libitum</i> -fed	Food-restricted	<i>Ad libitum</i> -fed	Food-restricted
Number of dams	5	10	7	12
Number of foetuses/dam ^a	—	—	3.7 $\pm$ 0.5	2.7 $\pm$ 0.5
Foetal weight (g) ^a	—	—	9.81 $\pm$ 1.18	8.37 $\pm$ 1.05
Plasma IGF-I (ng/ml) ^{a,b}	312 $\pm$ 97	139 $\pm$ 81	612 $\pm$ 173	283 $\pm$ 143
Plasma IGF-II (ng/ml) ^{a,b,c,d,g}	1149 $\pm$ 256	500 $\pm$ 270	564 $\pm$ 377	355 $\pm$ 181
Body weight at arrival ^{c,d,e,f,g}	496 $\pm$ 15	462 $\pm$ 14	454 $\pm$ 10	489 $\pm$ 30
Body weight at day 1 of gestation ^{1,a,b,c,d,e,f,g}	692 $\pm$ 42	502 $\pm$ 16	580 $\pm$ 44	538 $\pm$ 36
Body weight at day 40 of gestation ^{1,a,b,c,d,e,f}	768 $\pm$ 48	575 $\pm$ 26	775 $\pm$ 45	644 $\pm$ 28
Body weight gain arrival–day 1 ^{2,a,b,c,d,f,g}	196 $\pm$ 42	40 $\pm$ 23	126 $\pm$ 40	50 $\pm$ 27
Bodyweight gain day 1–day 40 ^{3,a,b,c,e,f,g}	76 $\pm$ 22	73 $\pm$ 18	196 $\pm$ 26	106 $\pm$ 27
Parametrial adipose tissue (% of net body weight) ^{4,a,c,d,f}	0.69 $\pm$ 0.15	0.19 $\pm$ 0.07	0.60 $\pm$ 0.09	0.26 $\pm$ 0.11
Interscapular adipose tissue (% of net body weight) ^{4,a}	1.34 $\pm$ 0.08	0.76 $\pm$ 0.22	1.40 $\pm$ 0.22	0.65 $\pm$ 0.21
Liver (% of net body weight) ⁴	3.09 $\pm$ 0.23	3.10 $\pm$ 0.37	3.28 $\pm$ 0.35	2.98 $\pm$ 0.28
Muscle biceps (% of net body weight) ^{4,a,b,c,d,g}	0.05 $\pm$ 0.01	0.06 $\pm$ 0.01	0.06 $\pm$ 0.01	0.06 $\pm$ 0.01
Muscle soleus (% of net body weight) ^{4,a,c,d,g,e}	0.27 $\pm$ 0.03	0.36 $\pm$ 0.04	0.33 $\pm$ 0.02	0.33 $\pm$ 0.03
Spleen (% of net body weight) ^{4,b}	0.13 $\pm$ 0.01	0.12 $\pm$ 0.02	0.12 $\pm$ 0.01	0.10 $\pm$ 0.02
Placental weight (g) ^a	—	—	2.20 $\pm$ 0.18	1.96 $\pm$ 0.24

¹Body weight at day 1 and day 40 of gestation or on the corresponding day for virginal animals. ²Body weight at day 1 of gestation (or on the corresponding day for virginal animals) minus body weight at the day of arrival. ³Body weight at day 40 of gestation minus the weight on day 1 of gestation (or on the corresponding days for virginal animals). ⁴Net body weight is the maternal body weight at day 40 of gestation (or on the corresponding day for virginal animals) minus weight of placenta and foetuses.

^aSignificant effect of nutrition ($P < 0.05$) as assessed by ANOVA. ^bSignificant effect of pregnancy ($P < 0.05$) as assessed by ANOVA. ^cInteraction between nutrition and reproductive status ($P < 0.05$) as assessed by ANOVA. ^dSignificant difference between virginal/*ad libitum*-fed and virginal/food-restricted ($P < 0.05$) animals as assessed by planned comparison. ^eSignificant difference between virginal/food-restricted and pregnant/food-restricted ($P < 0.05$) animals as assessed by planned comparison. ^fSignificant difference between pregnant/*ad libitum*-fed and pregnant/food-restricted ($P < 0.05$) animals as assessed by planned comparison. ^gSignificant difference between virginal/*ad libitum*-fed and pregnant/*ad libitum*-fed ($P < 0.05$) animals as assessed by planned comparison.

than their virginal counterparts, while no such difference was observed in pregnant and virginal food-restricted dams.

Reproductive outcome, body weight and tissue and organ weights

Food restriction reduced litter size, foetal weight and placental weight (Table 1). Body weight gain was also affected by food restriction; however this was only evident during the first weeks. Thereafter, only the pregnant food-restricted dams gained less weight as compared with their *ad libitum*-fed counterparts (Table 1). Food-restricted animals had less body fat and more lean tissue relative to their net body weight compared with *ad libitum*-fed controls (Table 1). Furthermore, food-restricted pregnant dams tended to have more parametrial adipose tissue relative to net body weight than their virginal counterparts ($P = 0.092$).

Discussion

This study showed that in guinea pigs mRNA for IGF-I is expressed in the highest amounts in the uterus, liver and adipose tissues. The expression of IGF-I in these tissues

was not affected by food restriction, but it was significantly increased in the liver and adipose tissues and decreased in the uterus in pregnant animals. The results indicate a potential endocrine function for adipose tissue during gestation. mRNA for IGF-II was highly expressed in the liver and placenta. Food restriction decreased the expression of mRNA for IGF-II in the placenta, whereas pregnant animals had a higher expression in the liver. The decreased expression of IGF-II in the placenta in undernourished dams may have adverse effects on placental size, structure and function.

The animals in this study were studied on day 40 of pregnancy, which is about midgestation as the length of gestation in guinea pigs is around 70 days. Plasma levels of IGF-I increase progressively during the first half of pregnancy in guinea pigs, and although they do not decrease during the last trimester as in rodents, the increase is modest at the end of pregnancy (Sohlström *et al.* 1998b). Therefore, we assume that the expression of mRNA for IGFs was measured at a time during pregnancy when it was at the highest levels and also at a time when the major growth of the placenta was occurring (Saintonge & Rosso 1983). As shown previously in rats, chronic food restriction affects weight gain primarily at an early stage (Sohlström *et al.* 1994). The metabolism of the animal probably adapts to the decreased food intake by lowering the energy needs

thereby saving energy for growth. This is probably why food restriction is shown to affect weight gain during the time before mating, but not during the last 40 days of the study. The fact that food-restricted animals had less adiposity at day 40 of gestation or on the corresponding day for the virginal animals confirmed the fact that these animals were affected by the low food intake.

Our results showed that non-hepatic tissues, particularly adipose tissues in guinea pigs, expressed as high amounts of mRNA for IGF-I as the liver. This has previously been observed in pigs (Coleman *et al.* 1994), rats (Peter *et al.* 1993) and humans (Möller *et al.* 1991, Peter *et al.* 1993), but is in contrast to findings in rats, where the liver has been shown to be the major site of expression (Möller *et al.* 1991). Considering the size of adipose tissue as compared with the liver, this tissue may contribute substantially to the circulating pool of IGF-I. The uterus also expressed high levels of mRNA for IGF-I. Previous studies in pregnant rats (Correia-Da-Silva *et al.* 1999) and pigs (Sterle *et al.* 1998) also showed an expression of mRNA for IGF-I in uterus, although it was not compared with other tissues besides the placenta. Consistent with our results, low levels or no expression of mRNA for IGF-I have been detected in the placenta in guinea pigs (Han *et al.* 1999) and rats (Möller *et al.* 1991). The human placenta expresses mRNA for IGF-I, although the level of the expression is dependent on the trimester of pregnancy and the site of the placenta that is analysed (Han *et al.* 1996).

Food restriction did not affect the expression of mRNA for IGF-I in most of the tissues that were analysed, although the plasma levels of IGF-I were decreased. This is consistent with the finding of Monaco and Donovan (1996), but in contrast to Muaku *et al.* (1995) and Straus and Takemoto (1991) where food restriction during gestation lowered both the plasma levels and the expression of IGF-I in the liver in rats. However, in the latter two studies the type of food restriction was different, since a very low protein diet and an extreme energy restriction were used (Straus & Takemoto 1991, Muaku *et al.* 1995). Based on the findings of Monaco and Donovan (1996) and our results, we suggest that the transcription is not affected by a moderate general food restriction which primarily has low energy content and a mild protein restriction. Regulation of the plasma levels of IGF-I in food-restricted animals probably occurs post-transcriptionally, such as by altered mRNA stability, altered translation, or altered profile of the IGFBPs. Food-restricted guinea pig dams have previously been shown to have a more pronounced shift of IGF-I binding activity from higher (IGFBP-3) to lower (IGFBP-1 and IGFBP-2) molecular weight binding proteins as compared with *ad libitum*-fed controls (Sohlström *et al.* 1998b). Such a change would increase the clearance of plasma IGF-I (Bastian *et al.* 1993) and result in lower levels of plasma IGF. In pregnant guinea pigs, the profile of binding proteins is relatively stable until late in pregnancy

(Sohlström *et al.* 1998b). Furthermore, guinea pigs do not appear to have any protease activity against IGFBP-3 during pregnancy (Sohlström *et al.* 1998b).

In pregnant dams the expression of mRNA for IGF-I was doubled in the liver, which confirms findings of Grant *et al.* (P A Grant, K L Kind, C T Roberts, A Sohlström, J A Owens & P C Owens, personal communication). Interestingly, the expression of mRNA for IGF-I in adipose tissues and the plasma levels of IGF-I were also doubled compared with virginal counterparts, regardless of dietary group. This may indicate that adipose tissue, together with the liver, is responsible for the increased plasma levels of IGF-I seen during pregnancy. This has been suggested earlier, but was based on results from *in vitro* studies of human adipocytes (Wabitsch *et al.* 2000).

Our results showed that the placenta expressed even higher levels of mRNA for IGF-II than the liver. This is consistent with findings in humans (Möller *et al.* 1991) and pigs (Sterle *et al.* 1998). Interestingly, Sterle *et al.* (1998) suggested that in pigs, IGF-II synthesis by the placenta may exert a negative feedback on the IGF-I expression in the uterus. This may also be true for guinea pigs, as the levels of IGF-I expression in the uterus were markedly decreased in pregnant as compared with virginal animals in the present study. Another similarity to data presented by Sterle *et al.* (1998) is the expression of mRNA for IGF-II in the uterus. They state that studies of the pregnant uterus endometrium should be interpreted with caution, as there may be a placental contamination. However, in our study the uterus from virginal animals also expressed mRNA for IGF-II. Thus, the expression in the uterus in the present study is not thought to be due to placental contamination.

Food restriction did not affect the expression of mRNA for IGF-II in most tissues, with the exception of the placenta and the parametrial adipose depot. In agreement with our results, Leaman *et al.* (1990) showed that food deprivation did not affect the expression of mRNA for IGF-II in the muscle and liver of adult swine. The fact that the food-restricted animals in our study had lower plasma levels of IGF-II than the *ad libitum*-fed animals suggests that the expression of IGF-II is not regulated at the transcriptional level, but rather by post-transcriptional events, as is the case for IGF-I. In general, the circulatory levels of IGF-II are less affected by malnutrition than those of IGF-I. In studies where humans and guinea pigs have been subjected to short acute fasting, no reduction or minor reductions in IGF-II have been shown (Davenport *et al.* 1988, Sohlström *et al.* 1998a). However, chronic food restriction decreases IGF-II levels in plasma in guinea pigs (Sohlström *et al.* 1998a,b). Similarly, severely malnourished children (Soliman *et al.* 1986) and severely ill patients (Davies *et al.* 1991) have reduced IGF-II concentrations in plasma.

In contrast to most other tissues in the pregnant guinea pig, food restriction decreased the expression of mRNA for IGF-II in the placenta. This is of interest since

food-restricted pregnant dams also had reduced placental weights and numbers of fetuses. Previous studies have shown that the placental weight is not the only factor adversely affected by food restriction, but also the placental structure, resulting in an impaired capacity for placental substrate transfer (Roberts *et al.* 2001a). Interestingly, maternal plasma levels of IGF-II have been shown to be related to structural development of the placenta (Roberts *et al.* 2001b). Thus, the expression of mRNA for IGF-II in the placenta indicates an important effect on placental size, structure and function and hence foetal growth.

Pregnancy increased the expression of mRNA for IGF-II in the liver and in the parametrial adipose tissue in this study. Apart from the expression of mRNA for IGF-II in the placenta, which has been widely studied (Shen *et al.* 1986, Reynolds *et al.* 1997), few studies have investigated how pregnancy affects the expression of mRNA for IGF-II in non-reproductive tissues. The plasma levels of IGF-II were reduced in the pregnant dams as compared with the virgin animals in this study, which is in contrast to findings in a previous study performed in a similar animal model (Sohlström *et al.* 1998a). The reason for these opposing results is unknown, but may be explained, in part, by the fact that the studies were performed in different strains of guinea pigs.

In conclusion, this study has shown that in guinea pigs, adipose tissue expresses as high amounts of mRNA for IGF-I as the liver, and that this expression is doubled during pregnancy. This indicates an important role for the adipose tissue during gestation, not only as an energy store but also as an autocrine/paracrine as well as an endocrine tissue, both in *ad libitum*-fed and food-restricted animals. mRNA for IGF-II was expressed at the highest levels in the placenta and liver. The expression in the placenta was reduced by food restriction. It is suggested that the decreased expression of IGF-II in the placenta may have adverse effects on placental size, structure and function.

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