Differential expression of IGF-I and IGF-binding protein-1 and -2 in periportal and perivenous zones of rat liver

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Abstract

IGF-I has important roles in regulating growth and metabolism. Circulating IGF-I is bound to specific binding proteins (IGFBP-1 to -6), with hepatocytes containing IGF-I, IGFBP-1 and -2 mRNA. Although many hepatic proteins are regionally expressed in the liver acinus, no studies have reported zonation of IGF protein expression. In this study we investigated the pattern of hepatic mRNA for the IGF proteins, vs the previously reported periportal gradient of phosphoenolpyruvate carboxykinase (PEPCK) expression. *In situ* hybridisation was used to analyse IGF-I, IGFBP-1, -2 and PEPCK mRNA in female Sprague-Dawley rats fed diets containing low (6%), normal (21%) or high (35%) protein. We report for the first time that IGFBP-1 and -2 and IGF-I are differentially expressed in the liver acinus. In the normal- and high-protein groups, levels of IGFBP-1 mRNA were higher in the perivenous

region, i.e. the opposite gradient to PEPCK, with a higher gradient of IGFBP-1 expression in the high-protein group. In contrast, IGFBP-2 had a similar pattern to PEPCK, and a periportal gradient of IGF-I mRNA was also seen in the low-protein group. Using computerised image analysis, levels of IGFBP-1 and -2 mRNA were elevated 2- and 10-fold respectively, in the low- vs normal-protein groups. The level of IGF-I mRNA was reduced to 65% of normal, with circulating IGF-I levels at 30% and insulin levels 39% of normal. These results demonstrate that hepatocytes are a heterogeneous population with respect to regulation of IGF proteins, having specific expression patterns dependent on the position of the hepatocyte within the liver acinus.

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Introduction

Insulin-like growth factors I and II (IGF-I and -II) are circulating peptides with important roles in the regulation of growth and metabolism in the body (Jones & Clemmons 1995). Many of the effects of IGF-I are insulin-like, including hypoglycaemia, stimulation of glucose uptake and increased gene expression of glucose transporters (Zapf *et al.* 1986, Maher & Harrison 1990, Lewitt *et al.* 1993). These actions suggest that IGF-I, like insulin, plays a physiological role in the regulation of glucose metabolism in the body. In contrast to insulin, total levels of circulating IGF-I are not regulated acutely with respect to the fed and fasted states. Instead, circulating IGF-I is bound to a group of specific high-affinity binding proteins (IGFBPs), with the majority bound with IGFBP-3 in a complex of approximately 150 kDa, consisting of IGF-I, IGFBP-3 and an acid-labile subunit (Jones & Clemmons 1995). However, evidence does also exist for acute regulation of IGF-I activity, through the fraction of free IGF-I, which is less than 1% of the total circulating IGF-I (Frystyk *et al.* 1995). Free IGF-I has

been negatively correlated to levels of the lower molecular weight IGFBP-1 under some conditions (Frystyk *et al.* 1995), consistent with a role for the lower molecular weight IGFBPs in regulating the fraction of free IGF-I. In addition, the 31–40 kDa binary complexes that the lower molecular weight IGFBPs form with IGF-I may pass through the endothelial spaces (Bar *et al.* 1990), and thus be important in tissue targeting of IGF-I. Hepatocytes express mRNA for IGF-I, and IGFBP-1, -2 and -4, and the liver appears to be the primary source of circulating IGF-I and IGFBP-1 (Jones & Clemmons 1995).

One important factor, which has been ignored with respect to hepatic production of the IGF-related proteins, is that the expression of many hepatic proteins is dependent on their relative position within the liver acinus. While hepatocytes in different parts of the liver acinus appear similar under a light microscope, cells located in the periportal region have a functional capacity different from those surrounding the central vein, leading to 'metabolic zonation' of the liver (Jungermann & Kietzmann 1996). Functions of the liver which are zonally distributed include glucose metabolism, amino acid utilisation and

ammonia detoxification, bile formation, xenobiotic metabolism and formation of plasma proteins. These differences are related to gradients in concentrations of substrates and hormones, due to the passage of blood from the periportal to the perivenous zone, while variations also occur in nerve supply, extracellular matrix composition, and non-parenchymal cell populations (Jungermann & Kietzmann 1996). Although no zonation of IGF-I, IGFBP-1 or -2 expression has been reported, to our knowledge, some degree of zonation should be expected, at least for IGFBP-1, due to the many similarities between the regulation of the IGFBP-1 gene and the phosphoenolpyruvate carboxykinase (PEPCK) gene (Wynshaw-Boris *et al.* 1984, Magnuson *et al.* 1987, Lee *et al.* 1993). PEPCK is the rate-limiting enzyme of gluconeogenesis, and is expressed at higher levels in the periportal than the perivenous zone of the liver (Bartels *et al.* 1993). The aim of the present study was to determine if any gradient of expression of IGF-I, IGFBP-1 or -2 mRNA could be found in the liver acinus, using *in situ* hybridisation. In addition, varying protein diets were used to determine if any zonal pattern found was dependent on the overall level of mRNA expression, as a low-protein diet increases hepatic IGFBP-1 and -2 and reduces hepatic IGF-I mRNA levels (Lemozy *et al.* 1994).

Materials and Methods

Animals

Female Sprague–Dawley rats were purchased from B&K Universal, Sollentuna, Sweden. A total of 29 rats was used in the experiment, divided randomly into groups fed low (6%), normal (21%) or high (35%) protein diets *ad libitum* ($n=9$, 10 and 10 respectively). The body weights of the groups of animals at the beginning of the experiment were 159 ± 1 , 162 ± 1 and 163 ± 1 g (mean $\pm$ S.E.M.) for the low-, normal- and high-protein groups. The diets were calculated to contain: raw protein 6.1, 21.0 and 35.0%, and non-fat energy 67.9, 53.0 and 39.0% respectively, with 7.0% raw fat, 4.0% fibre, 5.0% ash, 10.0% water and 13.3 MJ/kg in each diet (AnalyCen Nordic, Special Diets, Lidköping, Sweden). The raw materials were casein, corn starch, sucrose, dextrose, cellulose powder, mineral premix and soy bean oil.

All rats were maintained on a 12 h day/night rhythm with free access to water. Forty-seven days following the start of the experiment the rats received an intraperitoneal injection of fluanisone and fentanyl (Hypnorm, 10 mg/ml and 0.2 mg/ml respectively), followed by exsanguination using cardiac puncture. Rats were killed in the period 1000 to 1730 h. For practical reasons the rats could not be killed simultaneously, but the groups were uniformly distributed to ensure that there was no biased effect of the time of day. The livers were immediately

removed, frozen on dry ice, and stored at -70°C until further processing. The experimental design was approved by the Animal Ethics Committee of the Karolinska Hospital.

In situ hybridisation

Oligonucleotide probes with sequences complementary to mRNAs encoding rat IGF-I (nucleotides 947–994) (Sandberg Nordqvist *et al.* 1996), rat IGFBP-1 (nucleotides 432–477) (Murphy *et al.* 1990), rat IGFBP-2 (nucleotides 609–654) (Sandberg Nordqvist *et al.* 1996), rat IGFBP-4 (nucleotides 488–534) (Sandberg Nordqvist *et al.* 1996), rat PEPCK (nucleotides 1206–1251) (Beale *et al.* 1985) and a rat IGFBP-2 sense probe (nucleotides 609–654) (Sandberg Nordqvist *et al.* 1996) were synthesised on a PAC-amidite and purified through NAP-25 columns at Pharmacia Upjohn, Uppsala, Sweden. The oligonucleotides were labelled at the 3'-end with $\alpha^{35}\text{S}$ -dATP (Du Medical Scandinavia, Sollentuna, Sweden) using terminal deoxynucleotidyl transferase (Amersham International, Amersham, Bucks, UK) in a buffer containing 10 mM cobalt chloride, 1 mM dithiothreitol (DTT), 300 mM Tris and 1.4 M potassium cacodylate (pH 7.2). Labelled probes were purified with Nensorb-20 columns (Du Medical Scandinavia), and DTT was added to a final concentration of 10 mM. The specific activities obtained were between 1 and 4×10^9 c.p.m./ μg oligonucleotide. Sections of 14 μm were cut on a cryostat (3000 Leica Instruments GmbH, Nussloch, Germany) at -15°C and thaw-mounted on to microscope glass slides (Probe On, Fisher Scientific, Pittsburgh, PA, USA). Animals from each group were represented in the same proportions on each slide, and each section analysed in triplicate. The tissues were hybridised according to published procedures (Schalling *et al.* 1991). Briefly, sections were air dried and covered with a hybridisation buffer containing 50% formamide, $4 \times$ SSC buffer, $1 \times$ Denhardt's solution (0.02% polyvinylpyrrolidone, 0.02% BSA and 0.02% Ficoll), 1% sarcosyl, 0.02 M phosphate buffer (pH 7.0), 10% dextran sulphate (Pharmacia Upjohn), 500 $\mu\text{g}/\text{ml}$ heat-denatured salmon sperm DNA (Sigma, St Louis, MO, USA), 200 mM DTT (Pharmacia Upjohn), and 1×10^7 c.p.m./ml of the labelled probe. The slides were placed in a perspex box humidified with 50% formamide and $4 \times$ SSC buffer ($1 \times$ SSC, 0.15 M NaCl, 0.015 M sodium citrate), and incubated for 16–18 h at 42°C . After hybridisation, sections were sequentially rinsed in four changes of $1 \times$ SSC buffer at 55°C for 60 min, rinsed in distilled water and then dehydrated in 60% and then 95% ethanol. Following air drying the sections were exposed to Hyperfilm beta-max X-ray film (Amersham International) for 1–7 days, and the films developed with Kodak LX 24 (Kodak, Rochester, NY, USA) for 2 min and fixed in Kodak AL 4 fixative for 5 min.

Table 1 Body weight increases, serum IGF-I and serum insulin levels in female Sprague–Dawley rats fed 6, 21 or 35% protein diets for 47 days (mean $\pm$ S.E.M.)

	6% protein (n=9)	21% protein (n=10)	35% protein (n=10)
Body weight increase (g)	$-8 \pm 1^*$	96 ± 2	106 ± 2
(%)	$-5.1 \pm 0.6^*$	59.3 ± 1.2	64.9 ± 1.2
Serum IGF-I (ng/ml)	$83 \pm 5^*$	262 ± 15	281 ± 21
Serum insulin (μ U/ml)	$12.5 \pm 2.0^*$	31.8 ± 3.5	36.9 ± 4.6

* $P < 0.05$ vs 21% protein group.

Analyses

Serum rat IGF-I was measured using RIA. Samples were acid-ethanol extracted and cryoprecipitated prior to the assay, and des(1–3)IGF-I used as a ligand to eliminate interaction of the IGFBPs (Bang *et al.* 1991). Immuno-reactive insulin was measured by RIA using guinea pig antibodies raised against porcine insulin, and 125 I-labelled porcine insulin as a tracer. Rat insulin (Novo, Copenhagen, Denmark) was used as a standard. Dextran-coated charcoal was used to separate bound from free insulin. The sensitivity of the assay was approximately 0.2μ U per tube, and intraassay coefficient of variation 5.1%.

Quantification of film autoradiographs was performed using a previously published micro-densitometry technique (Sandberg Nordqvist *et al.* 1996). Since the slides for each probe could not fit on the same film, the densitometry units for the normal-protein group were used as the reference in each film, and the levels in the low- and high-protein groups expressed as a percentage of this group. The overall level of expression of each protein in the liver was measured using areas covering approximately ten acini, using a relatively low magnification of the autoradiographs. Levels of mRNA in the periportal and perivenous regions were measured at higher magnification of the autoradiographs, similar to Fig. 2.

Statistics

Normally distributed data were compared by ANOVA, and significant differences were then compared using the method of Student–Newman–Keuls. Non-normally distributed data, or data with unequal variances, were tested using Kruskal–Wallis one-way ANOVA on ranks. Differences between the periportal and perivenous levels of mRNA were compared using paired *t*-tests. Linear regression was used to determine if a relationship was present between parameters. Statistical analyses were performed using SigmaStat for Windows (Jandel Scientific GmbH, Erkrath, Germany). Significance was designated as $P < 0.05$.

Results

In rats fed the low-protein diet, significant growth retardation occurred compared with the normal- and high-

protein fed groups (Table 1). The low-protein group lost approximately 5% of their original body weight during the experiment, while the normal- and high-protein groups gained approximately 60% of their original body weight during the same time period. These differences were reflected in the serum IGF-I concentrations, since serum IGF-I in the low-protein group was only 30% of the levels in the normal- and high-protein groups. A similar reduction in serum insulin levels in the low-protein group was found, with levels approximately 36% of the normal- and high-protein groups (Table 1). No significant difference in serum insulin or IGF-I levels occurred between the normal- and high-protein diets.

Feeding rats different protein diets altered the overall level of hepatic expression of IGF-I, IGFBP-1 and -2 mRNA. In animals fed the low-protein diet, the expression of IGF-I in the liver was reduced to approximately 65% of the group fed the normal-protein diet (Fig. 1a; $P < 0.05$). In contrast, IGFBP-1 mRNA levels were increased approximately 2-fold by the low-protein diet (Fig. 1b; $P < 0.05$), with an even greater increase in IGFBP-2 expression in this group of about 10-fold versus the normal-protein diet (Fig. 1c; $P < 0.05$). No significant differences in expression of IGF-I, or IGFBP-1 and -2 were found between the normal- and high-protein diets. Levels of IGFBP-2 mRNA in the normal- and high-protein groups were barely above background. IGFBP-4 was expressed at high levels in the liver, but this level was not affected by the protein content of the diet (Fig. 1d). There were also no significant differences in the level of PEPCK expression between the protein groups (Fig. 1e).

Examination of the autoradiographs demonstrated that in some of the animals there was higher expression of IGFBP-1 in the perivenous compared with the periportal region of the liver. This pattern is shown in Fig. 2a for a high-protein fed animal, and contrasts with the periportal expression of PEPCK mRNA from the same animal shown in Fig. 2b. In comparison, homogeneous levels of hepatic IGFBP-4 mRNA were present in the liver (Fig. 2c), and only background labelling was seen with the sense IGFBP-2 probe (Fig. 2d). To determine whether there was a significant difference in levels of mRNA in the periportal and perivenous zones, densitometry measurements of each region were performed. In Fig. 3a and b the

levels of expression of IGFBP-1 and PEPCK in the perivenous and periportal zones are shown. No significant difference in IGFBP-1 expression between the two zones was present in the low-protein group, but a 23% higher expression was found in the perivenous vs the periportal zone in the normal-protein group ($P<0.05$), with a greater difference of approximately 73% present in the high-protein group (Fig. 3a; $P<0.001$). Levels of PEPCK mRNA were significantly greater in the periportal vs the perivenous zones in all groups (Fig. 3b; $P<0.001$). The level of PEPCK mRNA in the periportal zone was 61, 201 and 198% higher than perivenous PEPCK mRNA levels in the low-, normal- and high-protein groups respectively.

As a further measure of the degree of zonation for each protein, the ratios between the levels of periportal and perivenous mRNA were calculated for each animal. The average ratios for IGFBP-1 mRNA in the perivenous/periportal zones were 1.10 ± 0.05 , 1.37 ± 0.21 and 1.85 ± 0.13 in the low-, normal- and high-protein groups respectively. The ratio of perivenous/periportal IGFBP-1 mRNA was higher in the high- compared with the low- and normal-protein groups ($P<0.05$). In contrast, ratios for PEPCK mRNA in the periportal/perivenous zones (i.e. the opposite gradient) were 2.02 ± 0.50 , 3.68 ± 0.56 and 3.48 ± 0.42 in the low-, normal- and high-protein groups respectively (mean $\pm$ s.e.m.). PEPCK had a higher periportal/perivenous mRNA ratio in both the normal- and high-protein groups vs the low-protein diet ($P<0.05$). In 7/10 of the rats in the high-protein group there was a 50% or greater level of IGFBP-1 mRNA in the perivenous vs the periportal region, while 0/9 and 3/10 rats in the low- and normal-protein groups respectively showed a 50% or greater difference. There appeared to be no relationship between the degree of zonation for IGFBP-1 and PEPCK mRNA (Fig. 4; $r=0.10$, $P=0.62$, $n=29$). In addition, no correlation due to the time of day at which the rats were killed was apparent for the ratio of IGFBP-1 or PEPCK mRNA between the periportal and perivenous zones ($r=-0.09$, $P=0.64$ for IGFBP-1 mRNA and $r=-0.30$, $P=0.11$ for PEPCK mRNA).

In contrast to the gradient found for IGFBP-1 expression, the pattern of IGFBP-2 mRNA in the low-protein group was similar to PEPCK mRNA (Fig. 5a and b). A high concentration of IGFBP-2 mRNA was present in the periportal region, with little or no IGFBP-2 expression in the area surrounding the central vein. The difference in the level of expression of IGFBP-2 in the low-protein

Figure 1 Expression of IGF-I (a), IGFBP-1 (b), IGFBP-2 (c), IGFBP-4 (d) and PEPCK (e) in female Sprague-Dawley rats fed low (6%, $n=9$), normal (21%, $n=10$) or high (35%, $n=10$) protein diets. *In situ* hybridisation was performed for each probe, and computerised image analysis of the autoradiographs used to determine relative expression. Data were converted to a percentage of the normal-protein group for comparisons. * $P<0.05$ vs normal-protein group.

Figure 2 Darkfield autoradiographs following *in situ* hybridisation of rat liver for IGFBP-1 (a), PEPCK (b) IGFBP-4 (c) or sense IGFBP-2 (d). The animal had consumed a high (35%) protein diet for 47 days prior to killing. Bright areas represent high mRNA expression, and dark areas relatively lower expression. Arrows are pointing to central veins of the liver.

group was significantly greater than for either IGFBP-1 or PEPCK, with a ratio of periportal to perivenous mRNA levels of 26.1 ± 4.9 (mean $\pm$ S.E.M.). Measurements could not be made in the two zones in the normal- and high-protein groups, as the levels of expression were barely above background.

A gradient of IGF-I mRNA levels was also apparent in some of the animals, with a significantly greater expression of IGF-I of almost 100% in the periportal vs the perivenous zone in the low-protein group (Fig. 3c; $P < 0.001$). This pattern is compared with the distribution of PEPCK mRNA in Fig. 6a and b. No difference in the level of IGF-I expression between the two zones was present in the normal-protein group, while in the high-protein group there was slightly higher IGF-I expression in the perivenous vs the periportal zone ($P < 0.05$). The levels of IGF-I mRNA expression in the periportal vs perivenous zone were also expressed as a ratio for each animal. This ratio was significantly greater in the low-protein vs the

normal- and high-protein groups (2.04 ± 0.17 for the low- vs 1.03 ± 0.02 and 0.92 ± 0.03 in the normal- and high-protein groups; mean $\pm$ S.E.M., $P < 0.05$).

Discussion

This study has demonstrated for the first time that in rat liver the levels of IGFBP-1, -2 and IGF-I mRNA can be heterogeneous across the liver acinus. The expression of IGFBP-2 was clearly periportal, as for PEPCK mRNA, and, in rats fed a low-protein diet, higher levels of IGF-I mRNA were also found in the periportal versus the perivenous area. In contrast, the gradients of expression of IGFBP-1 and PEPCK mRNA were opposing, demonstrating that although IGFBP-1 and PEPCK share many regulatory features (Wynshaw-Boris *et al.* 1984, Magnuson *et al.* 1987, Lee *et al.* 1993), significant differences in their regulation must also be present.

Figure 4 The relationship between the ratio of IGFBP-1 and PEPCK mRNA levels in the periportal and perivenous zones of rat liver. Rats were fed a low (6%, $n=9$, $\square$), normal (21%, $n=10$, $\circ$) or high (35%, $n=10$, $\bullet$) protein diet. ($r=0.10$, $P=0.62$, $n=29$).

Levels of IGFBP-1 mRNA were higher in the perivenous compared with the periportal zone of the liver in the normal- and high-protein groups. A number of possible factors can contribute to gradients in the levels of specific mRNA species within the liver. Probably the most important influence on hepatocyte function is the direction of blood flow within the acinus, from the terminal portal venule and hepatic arteriole, to the central vein (Jungermann & Kietzmann 1996). During the passage of blood through the sinusoids, concentration gradients for oxygen, hormones and substrates are produced, e.g. the oxygen tension falls from around 65 mmHg to 35 mmHg from the periportal to perivenous zones, and there is a 50% drop in insulin between meals and 15% decline after meals (Jungermann & Kietzmann 1996). There are also differences in biomatrix, innervation, and the populations of non-parenchymal liver cells between the liver zones (Reid *et al.* 1992, Jungermann & Kietzmann 1996). It may be speculated that part of the reason for a gradient in IGFBP-1 expression is due to changes in insulin levels across the liver acinus. Up to 50%

Figure 3 Expression of IGFBP-1 (a), PEPCK (b) and IGF-I (c) in the periportal (solid bars) and perivenous (open bars) zones of rat liver in animals fed a low (6%, $n=9$), normal (21%, $n=10$) or high (35%, $n=10$) protein diet. *In situ* hybridisation was performed for each probe, and computerised image analysis of the autoradiographs used to determine relative expression vs the normal-protein group in the periportal region. * $P<0.05$ and ** $P<0.001$ vs the periportal region.

Figure 5 Darkfield autoradiographs following *in situ* hybridisation of rat liver for IGFBP-2 (a) or PEPCK (b). The animals had consumed a low (6%) protein diet for 47 days prior to killing. Bright areas represent high mRNA expression, and dark areas relatively lower levels of expression. Arrows are pointing to central veins of the liver.

Figure 6 Darkfield autoradiographs following *in situ* hybridisation of rat liver for IGF-I (a) or PEPCK (b). Animals had consumed a low (6%) protein diet for 47 days prior to killing. Bright areas represent high mRNA expression, and dark areas relatively lower expression. Arrows are pointing to central veins.

of insulin is degraded from the periportal to perivenous zones (Jungermann & Kietzmann 1996), with a higher density of ^{125}I -insulin-binding sites in the periportal zone (Nakanishi *et al.* 1995). Higher insulin binding in the periportal zone could in turn lead to relatively reduced IGFBP-1 expression in this region. In addition, IGFBP-1 expression is increased by glucagon (Lee *et al.* 1993), and a greater number of immunoreactive glucagon receptors are present in the perivenous region of rat liver (Berthoud *et al.* 1992). As insulin is inhibitory and glucagon stimulatory for both PEPCK and IGFBP-1 expression (Magnuson *et al.* 1987, Lee *et al.* 1993), this does not, however, explain why the gradient of PEPCK mRNA in the liver acinus is in the opposite direction. Part of the reason why PEPCK is expressed at higher levels in the periportal zone may be that an oxygen-sensing pathway in the gene, mediated by a ferro-haeme protein, leads to 100% glucagon-dependent activation at 16% O_2 , but only 60% at 8% O_2 , levels similar to periportal and perivenous oxygen tensions respectively (Kietzmann *et al.* 1993). The possibility exists that this oxygen-sensing pathway is either lacking, or opposing, in the regulation of IGFBP-1. Additional data confirm that while IGFBP-1 and PEPCK mRNA share regulatory elements in their genes, they also

have opposing patterns of regulation. For example, IGFBP-1 expression is high in the foetus (Lee *et al.* 1993) while significant expression of PEPCK only commences postnatally (Lyonnet *et al.* 1988), and phorbol esters, agonists for protein kinase C, increase IGFBP-1 and decrease PEPCK expression (Unterman *et al.* 1992).

In animals fed the low-protein diet little zonation of IGFBP-1 was present. The gradient of IGFBP-1 mRNA may have been lessened in this group by increased IGFBP-1 expression, to maximal levels, in both regions of the acinus. It is interesting to note that the difference in PEPCK expression between the two zones was also reduced in the low-protein diet. The reason for the apparent increase in the degree of IGFBP-1 zonation in the high- compared with the normal- and low-protein groups is unknown. Serum insulin levels were not significantly different in the normal- and high-protein groups, and no significant difference in serum glucagon levels has been reported between rats fed normal and 90% protein diets (Wimmer *et al.* 1990). However, changes in other regulators of IGFBP-1 expression, such as growth hormone and corticosteroids (Lee *et al.* 1993), may be contributory factors, and further studies will be necessary to determine their role in the zonation of IGFBP-1 expression.

Although it was surprising to find opposing gradients of IGFBP-1 and PEPCK mRNA in the liver acinus, different regions of expression of these proteins also occur in the kidney. IGFBP-1 is expressed at the highest levels in the medullary thick ascending limb of Henle (Chin *et al.* 1992), while the proximal tubule is the site of higher PEPCK expression (Ross *et al.* 1986). It is even more interesting to note that these regions are homologous to the liver zones with respect to their glucose metabolism. The medullary thick ascending limb of Henle is the area with the highest rate of glucose-dependent glycolysis, but little or no gluconeogenesis (i.e. perivenous zone), while the proximal tubule is the site of gluconeogenesis in the kidney (i.e. periportal zone) (Ross *et al.* 1986).

No relationships were evident between the degrees of zonation for IGFBP-1 and PEPCK. This would suggest that differing factors are responsible for the gradients of IGFBP-1 and PEPCK in the liver acinus. There was also no relationship between the time of day at which the rats were killed and the gradient of IGFBP-1 or PEPCK mRNA in the liver acinus. It is possible that the differences in killing times in the present study (1000 to 1730 h) were insufficient to lead to clear changes, with the added factor that the rats were not fasted overnight, and there may have been some variability in the time of their last meal.

Regulation of IGFBP-2 expression in the liver is incompletely understood. Levels of IGFBP-2 mRNA in adult rat liver are increased following hypophysectomy, fasting, protein deficiency and diabetes (Ooi *et al.* 1990, Orłowski *et al.* 1990). In the present study, it appeared that IGFBP-2 had a more stable regional expression in the liver compared with IGFBP-1, as, provided the level of expression was high enough to be measurable, the level of IGFBP-2 expression was always significantly higher in the periportal versus the perivenous zone. It has been demonstrated that non-parenchymal cells influence IGFBP-2 secretion by hepatocytes, as insulin inhibits IGFBP-2 secretion in pure hepatocyte culture, but not when hepatocytes are grown in co-culture with Kupffer cells (Scharf *et al.* 1996). A greater number of Kupffer cells are found in the periportal than the perivenous region (Bouwens *et al.* 1992), and this could contribute to higher levels of IGFBP-2 mRNA in the periportal region.

The periportal gradient of IGF-I expression was only present in the rats fed the low-protein diet, in which the overall level of expression of IGF-I was reduced to approximately 65% of the normal group. The appearance of zonation in this group is likely to be due to the greater reduction in IGF-I expression in the perivenous compared with the periportal zone. IGF-I expression in the liver is positively regulated by insulin (Jones & Clemmons 1995), and levels of serum insulin were reduced by the low-protein diet. Growth hormone is the dominant regulator of

IGF-I expression (Jones & Clemmons 1995), and it may also have a role in hepatic zonation of IGF-I mRNA. The more subtle increase in IGF-I expression in the perivenous zone of the high-protein group will need to be confirmed in future experiments.

The reduction in hepatic IGF-I mRNA to 60% of normal levels is similar to a previous report with a 50% reduction in IGF-I mRNA in the liver of rats fed a 5% vs a 15% protein diet (Lemozy *et al.* 1994). However, the increase in hepatic IGFBP-1 mRNA levels of approximately 2-fold is less than the 5.7-fold increase reported by Lemozy and colleagues, which may be related to the slightly lower protein content in the diet used in that study (Lemozy *et al.* 1994). The 10-fold increase of hepatic IGFBP-2 mRNA in the low-protein group is higher than in two previous studies with increments of 3-fold (Lemozy *et al.* 1994) and 6.5-fold (Straus & Takemoto 1990). Since increases in hepatic IGFBP-2 mRNA of approximately 10-fold have been reported in rats fasted for 48 h (Ooi *et al.* 1990, Orłowski *et al.* 1990), the increase in hepatic IGFBP-2 levels in the low-protein group could have been partly caused by reduced food intake.

It was not possible to determine from the densitometry measurements whether mRNA levels were altered only in the hepatocytes, or also in non-parenchymal cells. IGF-I immunoreactivity is present in Kupffer cells, and hepatic endothelial cells (Andersson *et al.* 1986), while secretion of IGFBP-2 has been demonstrated in Kupffer cells (Scharf *et al.* 1996). However, the most populous cell in the liver is the hepatocyte, which accounts for about 60% of total cell number and 80% of cell volume (Blouin *et al.* 1977), and the changes seen are likely to be primarily due to changes in hepatocyte mRNA levels. In addition, in mice fed a low-protein diet, the number of Kupffer cells is decreased to only two-thirds of the number in normally fed mice (Honda *et al.* 1995), meaning that the Kupffer cells probably made an even smaller contribution to total cell number in the low-protein group.

Gradients in IGF-I, IGFBP-1 and -2 mRNA levels in the liver acinus may have important influences on the form of hepatic IGF-I which is released into the circulation. For example, IGF-I secreted by the periportal hepatocytes is likely to interact with IGFBP-2 before the more distally expressed IGFBP-1. Although the significance of the zones of expression of IGF-I, IGFBP-1 and -2 within the liver acinus is speculative, there is no doubt that the present study adds a new level of complexity to the regulation of these proteins in the liver.

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References

- Andersson I, Billig H, Fryklund L, Hansson H-A, Isaksson O, Isgaard J, Nilsson A, Rozell B, Skottner A & Stemme S 1986 Localization of IGF-I in adult rats. Immunohistochemical studies. *Acta Physiologica Scandinavica* **126** 311–312.
- Bang P, Eriksson U, Sara V, Wivall IL & Hall K 1991 Comparison of acid ethanol extraction and acid gel filtration prior to IGF-I and IGF-II radioimmunoassays: improvement of determinations in acid ethanol extracts by the use of truncated IGF-I as a radioligand. *Acta Endocrinologica* **124** 620–629.
- Bar RS, Clemmons DR, Boes M, Busby WH, Booth BA, Dake BL & Sandra A 1990 Transcapillary permeability and subendothelial distribution of endothelial and amniotic fluid insulin-like growth factor binding proteins in the rat heart. *Endocrinology* **127** 1078–1086.
- Bartels H, Freimann S & Jungermann K 1993 Predominant periportal expression of the phosphoenolpyruvate carboxykinase gene in liver of fed and fasted mice, hamsters and rats studied by *in situ* hybridisation. *Histochemistry* **99** 303–309.
- Beale EG, Chrapkiewicz NB, Scoble HA, Metz RJ, Quick DP, Noble RL, Donelson JE, Biemann K & Granner DK 1985 Rat hepatic cytosolic phosphoenolpyruvate carboxykinase (GTP): structures of the protein, messenger RNA, and gene. *Journal of Biological Chemistry* **260** 10748–10760.
- Berthoud VM, Iwanij V, Garcia AM & Saez JC 1992 Connexins and glucagon receptors during development of rat hepatic acinus. *American Journal of Physiology* **263** G650–G658.
- Blouin A, Bolender RP & Weibel ER 1977 Distribution of organelles and membranes between hepatocytes and nonhepatocytes in the rat liver parenchyma. A stereological study. *Journal of Cell Biology* **72** 441–455.
- Bouwens L, De Bleser P, Vanderkerken K, Geerts B & Wisse E 1992 Liver cell heterogeneity: functions of non-parenchymal cells. *Enzyme* **46** 155–168.
- Chin E, Zhou J & Bondy C 1992 Anatomical relationships in the patterns of insulin-like growth factor (IGF)-I, IGF binding protein-1, and IGF-receptor gene expression in the rat kidney. *Endocrinology* **130** 3237–3245.
- Frystyk J, Vestbo E, Skjærbaek C, Mogensen CE & Ørskov H 1995 Free insulin-like growth factors in human obesity. *Metabolism* **44** 37–44.
- Honda Y, Takahashi K, Naito M & Fujiyama S 1995 The role of macrophage colony-stimulating factor in the differentiation and proliferation of Kupffer cells in the liver of protein-deprived mice. *Laboratory Investigation* **72** 696–706.
- Jones JI & Clemmons DR 1995 Insulin-like growth factors and their binding proteins: biological actions. *Endocrine Reviews* **16** 3–34.
- Jungermann K & Kietzmann T 1996 Zonation of parenchymal and nonparenchymal metabolism in liver. *Annual Review of Nutrition* **16** 179–203.
- Kietzmann T, Schmidt H, Unthan-Fechner K, Probst I & Jungermann K 1993 A ferro-heme protein senses oxygen levels, which modulate the glucagon-dependent activation of the phosphoenolpyruvate carboxykinase gene in rat hepatocyte cultures. *Biochemical and Biophysical Research Communications* **195** 792–798.
- Lee PDK, Conover CA & Powell DR 1993 Regulation and function of insulin-like growth factor-binding protein-1. *Proceedings of the Society for Experimental Biology and Medicine* **204** 4–29.
- Lemozy S, Pucilowska JB & Underwood LE 1994 Reduction of insulin-like growth factor-I (IGF-I) in protein-restricted rats is associated with differential regulation of IGF-binding protein messenger ribonucleic acids in liver and kidney, and peptides in liver and serum. *Endocrinology* **135** 617–623.
- Lewitt MS, Saunders H, Cooney GJ & Baxter RC 1993 Effect of human insulin-like growth factor-binding protein-1 on the half-life and action of administered insulin-like growth factor-I in rats. *Journal of Endocrinology* **136** 253–260.
- Lyonnet S, Coupe C, Girard J, Kahn K & Munnich A 1988 *In vivo* regulation of glycolytic and gluconeogenic enzyme gene expression in newborn rat liver. *Journal of Clinical Investigation* **81** 1682–1689.
- Magnuson MA, Quinn PG & Granner DK 1987 Multihormonal regulation of phosphoenolpyruvate carboxylase-chloramphenicol acetyltransferase fusion genes. *Journal of Biological Chemistry* **262** 14917–14929.
- Maher F & Harrison LC 1990 Stabilization of glucose transporter mRNA by insulin/IGF-I and glucose deprivation. *Biochemical and Biophysical Research Communications* **171** 210–215.
- Murphy LJ, Seneviratne C, Ballejo G, Croze F & Kennedy TG 1990 Identification and characterization of a rat decidua insulin-like growth factor-binding protein complementary DNA. *Molecular Endocrinology* **4** 329–336.
- Nakanishi M, Watanabe M & Shimada M 1995 Heterogeneous distribution of ¹²⁵I-binding sites in the liver of fed and fasted mice. *Cellular and Molecular Biology* **41** 137–144.
- Ooi GT, Orlowski CC, Brown AL, Becker RE, Unterman TG & Rechler MM 1990 Different tissue distribution and hormonal regulation of messenger RNAs encoding rat insulin-like growth factor-binding proteins-1 and -2. *Molecular Endocrinology* **4** 321–328.
- Orlowski CC, Brown AL, Ooi GT, Yang YW-H, Tseng Ly-h & Rechler MM 1990 Tissue, developmental, and metabolic regulation of mRNA encoding a rat insulin-like growth factor binding protein (rIGFBP-2). *Endocrinology* **126** 644–652.
- Reid LM, Fiorino AD, Sigal SH, Brill S & Holst PA 1992 Extracellular matrix gradients in the space of Disse: relevance to liver biology. *Hepatology* **15** 1198–1203.
- Ross BD, Espinal J & Silva P 1986 Glucose metabolism in renal function. *Kidney International* **29** 54–67.
- Sandberg Nordqvist A-C, von Holst H, Holmin S, Sara VR, Bellander B-M & Schalling M 1996 Increase of insulin-like growth factor (IGF)-1, IGF binding protein-2 and -4 mRNAs following cerebral contusion. *Molecular Brain Research* **38** 285–293.
- Schalling M, Franco-Cereceda A, Hemsén A, Dagerlind Ö, Seroogy K, Persoon H, Hökfelt T & Lundberg JM 1991 Neuropeptide Y and catecholamine synthesizing enzymes and their mRNAs in rat sympathetic neurons and adrenal glands: studies on expression, synthesis and axonal transport after pharmacological and experimental manipulations using hybridisation techniques and radioimmunoassay. *Neuroscience* **41** 753–766.
- Scharf J-G, Ramadori G, Braulke T & Hartmann H 1996 Synthesis of insulin-like growth factor binding proteins and of the acid-labile subunit in primary cultures of rat hepatocytes, of Kupffer cells, and in cocultures: regulation by insulin, insulin-like growth factor, and growth hormone. *Hepatology* **23** 818–827.
- Straus DS & Takemoto CD 1990 Effect of dietary protein deprivation on insulin-like growth factor (IGF)-I and -II, IGF binding protein -2, and serum albumin gene expression in rat. *Endocrinology* **127** 1849–1860.
- Unterman TG, Lacson RG, Jentel JJ & Oehler DT 1992 Divergent effects of phorbol esters and insulin on insulin-like growth factor binding protein-1 (IGFBP-1) production and mRNA in rat H4IIE hepatoma cells. *Biochemical and Biophysical Research Communications* **182** 262–268.
- Wimmer M, Luttringer CH & Colombi M 1990 Changes in the acinar activity patterns of phosphoenolpyruvate carboxykinase in livers of male and female rats upon feeding a high protein and a high fat diet. *Histochemistry* **93** 257–262.

- Wynshaw-Boris A, Lugo TG, Short JM, Fournier REK & Hanson RW 1984 Identification of a cAMP regulatory region in the gene for rat cytosolic phosphoenolpyruvate carboxykinase (GTP). *Journal of Biological Chemistry* **259** 12161–12169.
- Zapf J, Hauri C, Waldvogel M & Froesch ER 1986 Acute metabolic effects and half-lives of intravenously administered insulin-like

growth factors I and II in normal and hypophysectomized rats. *Journal of Clinical Investigation* **77** 1768–1775

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