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Quantification of insulin-like growth factor -1 (IGF-1) mRNA: Modulation of growth intensity by feeding results in inter- and intra-tissue-specific differences of IGF-1 mRNA expression in steers

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Summary: The effect of constant and compensating body growth velocities on IGF-1 mRNA expression was studied in various tissues of growing steers. Twentysix steers were allocated to three groups in which the average daily gains were kept either constantly high on intensive feeding, low on pasture feeding or were accelerated to compensatory growth after feed restriction. All animals were slaughtered at 570 ± 2.6 kg and samples were collected from liver, heart, kidney and from 4 different muscles (m. splenius, m. soleus, m. cutaneus truncii and m. semispinalis capitis), which were selected in order to include maximal differences in fibre composition as well as in growth impetus. IGF-1 mRNA was quantified by a validated internally standardised RT-PCR method. The amount of RNA extracted from the various tissues investigated was constant within each type of tissue and showed no differences between treatment groups. As indicated by a constant ratio between the amount of RNA extracted and the DNA concentrations, there was no effect of the feeding on total transcriptional activity. The order of IGF-1 mRNA abundance per g tissue was liver $\gg$ kidney $>$ heart $>$ skeletal muscle. The different feeding regimen resulted in significant differences of IGF-1 mRNA expression rates in all organs showing different patterns between organs. IGF-1 mRNA concentrations showed muscle specific differences and also divergent reactions in response to the differing growth rates. These results support that the liver is the main IGF-1 producing tissue; above that they indicate that skeletal muscle, in particular when taking its absolute mass into account, might considerably contribute to the IGF-1 levels in blood. Our findings demonstrate that IGF-1 mRNA expression is regulated tissue specifically not only between different organs but also within musculature.

Introduction

Changes in overall growth rate are not necessarily paralleled by respective changes in growth velocity of individual tissues and organs. Although the allometric growth of individual organs is a well known phenomenon, conclusive explanations are lacking so far. In skeletal muscle the occurrence of different growth rates of individual muscles or muscle groups is generally noticed and is, above that, of special interest for meat production. Focusing on muscle growth patterns, alterations in muscle growth velocity are commonly attributed to changes in function, to hereditary tendencies of serial development as well as to the influence of bone growth on muscle growth (Hammond, 1932; Bryden, 1969; Stewart, 1972; Berg and Butterfield, 1976). Besides these basic developmental changes affecting muscle growth, a variety of other factors such as sex, rate of growth, loss and gain of body weight or the deposition of fat within the musculature modifies the muscle growth patterns. The regulatory factors which possibly contribute to allometric growth are most probably located in the respective tissue itself since alterations in circulating concentrations of hormones or growth factors as well as blood supply cannot sufficiently explain why certain tissues grow faster or slower than the body in total. The levels of regulation coming into question for the mediation of differing growth rates are:

- the sensitivity of the tissue to growth regulating hormones, i.e. the density and the affinity of the respective receptors
- the local synthesis of growth factors acting in a paracrine or autocrine manner

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– the local synthesis of specific binding proteins which may increase or inhibit the action of the relevant growth factors.

In ruminants, differing receptor densities in various individual muscles which might be related to allometric growth have been reported for sex steroids (Sauerwein and Meyer, 1989), for glucocorticoids (Sauerwein et al., 1991) as well as for insulin and insulin like growth factor-1 (Boge et al., 1995). The latter growth factor is of particular interest since all the regulatory levels listed potentially apply for it. During postnatal muscle growth, IGF-1 has been demonstrated to stimulate protein synthesis and to improve glucose utilisation (Daughaday and Rotwein, 1989; Simmen, 1991; Dimitriadis et al., 1994). IGF-1 is considered to mediate the anabolic growth hormone (GH) actions in skeletal tissues; above that it is an important growth regulator in numerous tissues acting in an endocrine as well as in an autocrine and paracrine way, the latter ones being potentially independent of GH (Weimann and Kiess, 1990; Thissen et al., 1994). To elucidate the functional role of IGF-1 for allometric growth phenomena, we aimed to quantify IGF-1 mRNA expression in various individual muscles as well as in different organs from growing steers in which growth intensity was modulated by differing feeding regimen which also included the aspects of compensatory growth. For these low abundant tissues, e.g. muscles, a method is required which allows for a reliable quantification of IGF-1 mRNA. Considering these limitations, a validated competitive RT-PCR offers the most potent instrument to detect low-abundance mRNAs (Saiki et al., 1988; Pfaffl et al., same issue).

Material and methods

Animals, experimental design and tissue sampling

Twenty six male Simmental calves were castrated being 3 to 4 months old and were initially fed together until all animals had reached a life weight of 220 kg. The steers were then randomly allocated to two different feeding regimen: 9 steers (group I) were housed in pens and were fed a common fattening diet with maize silage ad libitum, 1 kg soybean meal and mineral mixture. The remaining steers were fed up to 420 kg live weight on restricted amounts of grass silage and hay. At 420 kg they were subdivided into two groups: one group (n = 9) was kept on pasture only (group P); the other group was initially kept identically; after having reached 500 kg live weight, 8 animals were transferred to the more intensive feeding regimen of group I in pens with maize silage ad libitum, 1 kg soybean meal and mineral mixture (group C) in order to include the aspects of compensatory growth in the study. Growth rates were recorded by biweekly weighing of the animals. All steers were slaughtered at the same live weight (570.6 ± 2.6 kg). Immediately after slaughter, samples were dissected from liver, heart and kidney and from different muscles, i.e. from m. splenius, m. soleus, m. cutaneus trunci and from m. semispinalis capitis. The selection of the muscles was done in order to include maximal differences in fibre type composition and growth impetus; it is based on data provided by Totland and Kryvi (1991) and Berg and Butterfield (1976). Aliquots of all tissue samples were frozen in liquid nitrogen and were then stored at −80 °C until further processing. Carcass and organ weights were recorded. In order to estimate carcass composition, the fat, muscle and bone content was quantified in brisket and flatribs; total carcass composition was then estimated from these data using the regressions by Heindl et al. (1995) with a certainty (R^2%) of 91 for lean meat, 97 for fat and 86 for bone. Protein, fat, water and ash content were measured in m. longissimus dorsi as described by Reimann et al. (1993).

IGF-1 mRNA quantification

RNA extraction, competitive IGF-1 RT-PCR with the appropriate internal IGF-1 standard cRNA and IGF-1 mRNA quantification with HPLC-UV was performed with the validated method as described (Pfaffl et al., same issue).

RNA/DNA ratio

To be able to distinguish differences in IGF-1 mRNA expression from more general differences in transcriptory efficiency, the DNA concentrations were determined in aliquots from the tissue homogenates used to extract total RNA. The amount of total RNA extracted from the tissue samples was constant and showed no significant differences between the treatment groups. The initial homogenates were diluted 1/200 (muscle) or 1/400 (organs) in TNE buffer (10 mM Tris, 100 mM NaCl and 1 mM EDTA) and stored at −20 °C until needed. Calf thymus DNA (Sigma Chemicals Co., St. Louis, Missouri, USA) was used as standard (range: 0−900 ng/mL). The assay was performed in C96 White Maxisorp Fluoro Nunc™ microtitration plates (Nunc GmbH, Wiesbaden, Germany) by co-incubation of 225 mL diluted tissue homogenate or DNA standard with 75 mL of 1 mg/mL Hoechst 33258 fluorescent dye (Sigma) for 15 min at 37 °C. Fluorescence was read in a Titertek Fluroscan II (Labsystems, Helsinki, Finland) with $590_{im.}/485_{em.}$ nm.

Statistical analyses

The data are presented as mean ± SEM. Statistical comparisons were performed by using the Sigma-Stat software (version 2.0 for Windows 95; Jandel Scientific

Table 1 Growth rates and carcass characteristics from three steer groups fed for differing growth rates

group	average daily gain (g)		carcass weight (kg)	carcass composition[1]			muscle/fat ratio
	up to 500 kg	500–570 kg		muscle (%)	fat (%)	bone (%)	
I	1023 ± 43 a A	1081 ± 86 a A	336.9 ± 4.1 a	61.2 ± 0.6 a	21.3 ± 0.7 a	12.9 ± 0.3 a	2.9 ± 0.1 a
P	718 ± 56 b A	866 ± 107 b A	317.5 ± 2.7 b	67.3 ± 0.5 b	11.5 ± 0.5 b	16.4 ± 0.4 b	5.7 ± 0.4 b
C	826 ± 62 b A	1520 ± 252 a B	320.9 ± 2.4 b	67.1 ± 0.6 b	12.3 ± 0.8 b	15.6 ± 0.4 b	5.9 ± 0.3 b

[1] As calculated according to Heindl et al. (1995) from brisket and flatrib composition; Different small letters indicate differences between feeding regimen groups ($p < 0.05$); Different capital letters indicate differences between weight groups ($p < 0.001$)

Table 2 Organ and organ fat weights in steers fed for differing growth rates

group	heart	kidneys	liver	kidney and pelvic fat
	(% of the individual carcass weights)			
I	0.61 ± 0.03 a	0.29 ± 0.01 a	2.00 ± 0.07 a	2.31 ± 0.17 a
P	0.75 ± 0.02 b	0.40 ± 0.01 b	2.60 ± 0.07 b	1.12 ± 0.09 b
C	0.72 ± 0.02 b	0.34 ± 0.01 c	2.44 ± 0.09 b	1.30 ± 0.05 b

Different letters indicate differences between feeding regimen groups ($p < 0.05$)

Software, San Rafael, CA, USA). After testing all data groups for normality by the Kolmogorow-Smirnov test, normally distributed groups were tested by One Way Analysis of Variance with the Student-Newman-Keuls test for pairwise comparisons. Comparisons of more than two non-parametric groups were done by the Kruskall-Wallis Analysis of Variance on Ranks followed by posthoc Dunn's Method with pairwise comparisons. Differences of $p < 0.05$ were regarded as significant.

Results

Growth rates and body composition

Until the begin of the last feeding period, i.e. until 500 kg of live weight, the steer group fed indoor had the highest average daily gains which were significantly higher than in the two groups fed on pasture only (Table 1). For the last feeding period (500 to 570 kg) in which the aspect of compensatory growth was included for one half of the previously pasture fed animals, the rates of gain remained constant for the indoor and the pasture fed animals, respectively, but were strong increased ($p < 0.001$) in group C undergoing compensation. As shown in Table 1, steers fed permanently at a high level (I) had higher carcass weights than those steers from groups P and C. With regard to carcass composition again the steers from group I were differing significantly from the other two groups with an increased carcass fat content together with a decreased portion of lean meat. The ratio lean meat to fat (*muscle/fat ratio*) was only half of the ratio seen in the other two groups which grew slower and accumulated less fat and more lean muscle. The weight of the different organs (Table 2) was relatively lower in the fast growing fattening steers whereas the organ fat pads from kidneys and pelvis were heavier when compared to the two other groups. The acceleration of growth velocity during the final fattening period in group C did not result in significant changes of body composition as compared to group P which remained on pasture feeding. When looking at the composition of an individual muscle, the m. longissimus dorsi (Table 3), a similar pattern was observed. Again, the fat content was highest in the constantly fast growing group I, accompanied by decreased protein accretion as compared to the other groups. Muscular composition was not influenced by compensation as demonstrated by the lack of differences between groups P and C.

IGF-1 mRNA expression in organs and individual muscles

The IGF-1 mRNA expression rates measured in the various tissues of the three steer groups are depicted in Fig. 1. The differences observed between tissues and between groups were also evident when relating the IGF-1 mRNA concentrations measured to the amount of total RNA analysed. The general transcriptional activity, i.e. the RNA/DNA ratio was relatively constant (0.1429 ± 0.0054) in all tissues investigated ($n = 182$) and showed no group specific differences. The highest IGF-1 expression rates were observed in liver being up to 500 times higher than in skeletal muscles. In kidney and heart the expression

Table 3 Composition of m. longissimus dorsi from steers fed for differing rates of gain

group	fat (%)	protein (%)	water (%)	ash (%)
I	4.53 ± 0.42 a	22.0 ± 0.13 a	72.5 ± 0.34 a	1.10 ± 0.006 a
P	1.31 ± 0.11 b	22.9 ± 0.08 b	74.8 ± 0.12 b	1.14 ± 0.004 b
C	1.53 ± 0.13 b	22.7 ± 0.07 b	74.8 ± 0.17 b	1.13 ± 0.006 b

Different letters indicate differences between feeding regimen groups ($p < 0.05$)

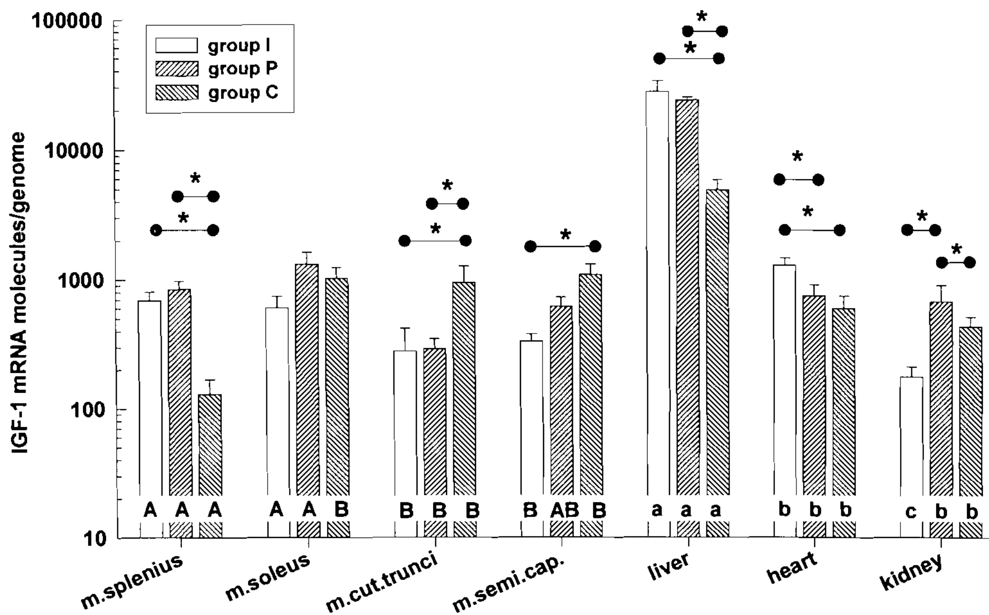

Fig. 1 IGF-1 mRNA concentrations (mean ± S.E.M.) in various tissues from steers growing with different velocities and slaughtered at a final live weight of 570 kg.; group P: pasture fed steers with constant average growth rates of 853 g/d, group C: initially pasture fed steers which were finally (500 to 570 kg) transferred to a more intensive indoor feeding regimen, then gaining 1.84 fold more weight per day than before (1520 g). Group I : constantly indoor fed steers with mean growth rates of 1053 g/day. Asterisks designate within tissue differences between groups ($p < 0.05$); Different capital letters written on the bars of the four different muscles designate differences between individual muscles and within group ($p < 0.05$). Different small letters written on the bars of the organs designate differences between individual organs and within group ($p < 0.05$)

was in between liver and muscle. Comparing the IGF-1 mRNA expression in the livers of the different groups, there was no difference between the constantly slow and the constantly fast growing groups P and I. In group C undergoing compensation significantly lower expression rates were observed. In heart and in kidney the pattern was divergent, showing the highest expression rates in heart muscle from group I, whereas equally lower IGF-1 mRNA concentrations were found in the relatively slower growing group P and the compensating group C. In contrast, the highest IGF-1 mRNA expression in kidney was observed in the pasture fed group, followed by the compensating group and the fast growing group I. In the different skeletal muscles investigated, there were differences between muscles as well as between feeding groups. For a more detailed comparison of the individual muscles, the mean differences between the particular muscle IGF-1 mRNA concentrations and the overall mean value of muscular IGF-1 mRNA (680 IGF-1 mRNA molecules/genome) are demonstrated in Fig. 2 together with the respective data available from the literature (Totland and Kryvi, 1991) on fibre type distribution. Comparing the IGF-1 expression within the groups, a consistent ranking of the four muscles investigated was not obvious. In groups I and P the concentrations in m. cutaneus trunci were lower than in the other three muscles which were not differing. In contrast, in group C the lowest concentrations were observed in m. splenius. Between group differences were noticed in all muscles investigated with the only exception of m. soleus. In m. splenius, the pattern of the IGF-1 mRNA concentrations closely resembled the one seen in liver. In m. cutaneus trunci as well as in m. semispinalis capitis the IGF-1 expression in the compensating group C was higher than in the constantly fast growing group I.

Discussion

The results demonstrate that IGF-1 mRNA expression in tissues is regulated divergently, both be-

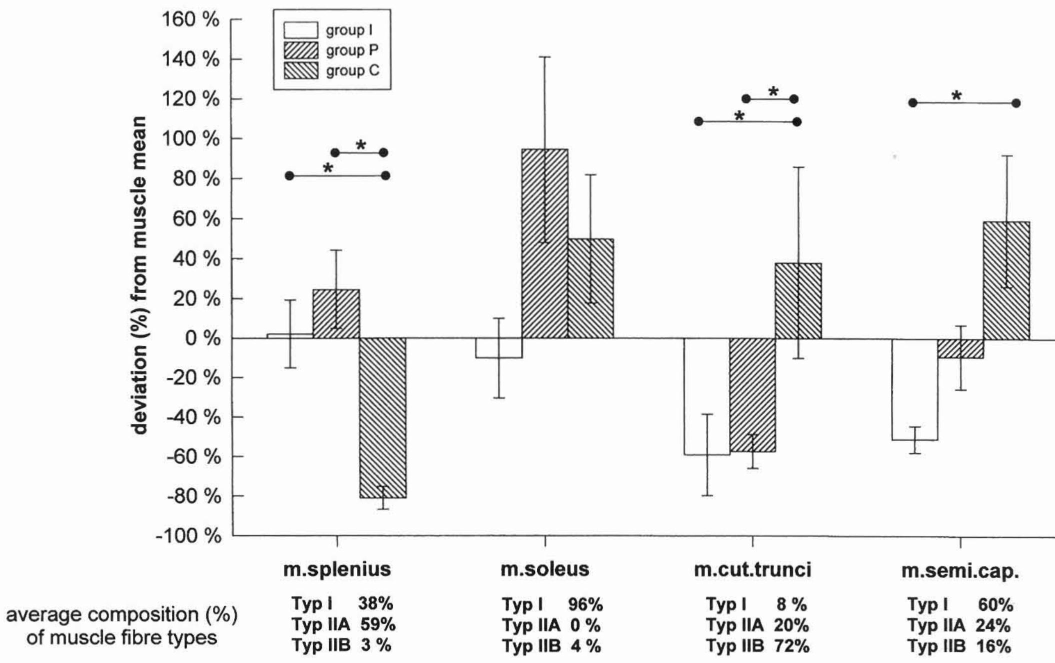

Figure 2 Mean ± S.E.M. deviation (%) of the average expression (= 680 IGF-1 mRNA molecules/genome) in the four shown muscles from steers growing with different velocities. Average composition of muscle fibre types were taken from Totland and Kryvi (1991). Asterisks designate within tissue differences between groups ($p < 0.05$)

tween different tissues as well as within skeletal muscle.

Comparing the expression niveaus measured in the different tissues, the importance of the liver as a major site of IGF-1 production in postnatal animals (Schwander et al., 1983) is confirmed. When taking the other organs investigated into consideration, their contribution to the total amount of IGF-1 produced by the body seems rather substantial: i.e. when projecting the amounts of IGF-1 mRNA on a per organ basis (based on the factors of RNA extracted per g tissue and the organ weights), heart and kidney IGF-1 mRNA production amounts to 0.5 and 1.3% of the one from liver in group I. In the constantly slower growing group P the relations were 0.5 and 8.7% for heart and for kidney, respectively. Skeletal muscle, being one of the bodies largest organs contributes 22 and 25% of liver IGF-1 mRNA synthesis in groups I and P. However, the question as to whether muscle and other organs do analogously translate the mRNA and secrete the IGF-1 protein into circulation and thus contribute to IGF-1 blood levels as the liver does remain open.

Tissue IGF-1 mRNA concentrations in continuously slow or fast growing steers

Liver IGF-1 expression was not related to growth velocity when comparing the constantly fed groups I and P. This finding is not in accordance with the common observation of IGF-1 response to nutrient intake, which has been demonstrated in fasted as well as in protein or energy restricted animals (Straus and Takemoto, 1990a, 1990b, 1991). Possibly the difference between the two different feeding regimen was not severe enough to induce alterations in IGF-1 liver mRNA abundance. As indicated by the daily gains in the pasture fed animals, the nutrient intake was well above the level required for maintenance. In heart and in kidney differences were observed between the constantly slow and fast growing steers, but only in heart a positive relationship to growth velocity was evident from the relatively higher IGF-1 mRNA concentrations in the fastly growing group I. In contrast, kidney IGF-1 mRNA levels were higher in slow than in fast growing animals. For heart and kidney an autocrine or paracrine IGF-1 action can be assumed since the presence of type 1 IGF receptors has been documented for those two tissues (Toyozaki et al., 1993; Chin and Bondy, 1992); the situation is different for liver in which no IGF-1 receptor mRNA is seen (Le Roith et al., 1995). In skeletal muscle the presence of IGF-1 receptors is markedly reduced during postnatal life as compared to fetal development (Alexandrides et al., 1989; Boge et al., 1995); this reduction is apparently related to the increased differentiation of myoblasts into myotubes (Rosenthal et al., 1991). The myogenic potential of the postnatal tissue is limited to relatively few satellite cells which remain responsive to proliferative signals; thus the mitogenic IGF-1 effects are most probably limited to those satellite cells and other non-myotube cells present in muscle i.e. connective tissue cells. However, IGF-1 effects on protein and carbohydrate metabolism of myotubes have been demonstrated, e.g. increased protein synthesis together with decreased protein degradation (Harper et al., 1987) as well as increased glucose transport (Duclos et al., 1993) and glucose

utilisation (Dimitriadis et al., 1992). Considering the relatively higher organ weights as well as the higher portion of muscle in carcass from steers growing constantly slow (group P), only in kidney the pattern of relative organ growth is paralleled by the relatively higher local IGF-1 mRNA expression rates. In heart, the highest organ weight is accompanied by comparing lowest IGF-1 mRNA concentrations. In these two tissues in which growth results from hyperplasia rather than from hypertrophy, no conclusive relationship of local IGF-1 synthesis and tissue growth seems to exist. However, the concomitant regulation of all other factors of the IGF system, i.e. the binding proteins and the receptor, needs to be considered to be able to judge the relative importance of the observed divergent regulation of local IGF-1 mRNA by growth velocity for organ growth. For skeletal muscle in which hypertrophy is the main growth determinant, the situation seems even more complex since our study demonstrates a divergent reaction of IGF-1 mRNA expression in different individual muscles. For animals restricted in dietary energy intake a reduction in muscle fibre size has been consistently reported (Goldspink and Ward 1979; Yambayamba and Pierce, 1991b; Klosowski et al., 1992 b; Ward and Stickland 1993; Picard et al., 1995). According to our study local IGF-1 mRNA levels are relatively higher in three of the four muscles investigated from group P than from group I thus indicating that IGF-1 expression might be negatively related to fibre size. Regarding the composition of muscle tissue as done exemplary for m. longissimus dorsi, relatively higher portions of fat were observed for group I. We suggest that local muscular IGF-1 might rather be effective to stimulate protein, water and ash accretion than to induce fat accumulation. This hypothesis would imply that IGF-1 expression is also decreased with increasing maturity of muscular tissue; a relation which is supported by reports about decreasing skeletal muscle IGF-1 mRNA expression in postnatal animals with increasing age (Dickson et al., 1991; Oldham et al., 1996). Comparing the IGF-1 expression in the four muscles investigated within group I, a relation to fibre type distribution might be assumed since the relatively lowest expression rates were observed in those muscles having a relatively high portion of type IIb (fast glycolytic). In general a positive correlation between dietary energy level and glycolytic fibre portion has been established (Moody et al., 1980; Johnston et al., 1981; Yambayamba and Price 1991a; Klosowski et al., 1992a). A tendency towards relatively higher IGF-1 mRNA expression rates in muscles from slow growing steers as compared to faster growing animals and thus a dependency from fibre type proportion might be speculated. However, in m. cutaneus trunci no such difference was observed, indicating that either fibre composition and/or IGF-1 expression were not sensitive to food supply. More detailed studies are necessary to sort out as to whether IGF-1 mRNA is indeed expressed in dependency of muscle type.

Carcass composition suggests a relatively higher growth impetus of lean tissue in general for groups P and C; data available for allometric growth rates of individual muscles indicate that m. semispinalis capitis and m. soleus belong to those muscles growing with a low or average impetus; in contrast m. cutaneus trunci and m. splenius are high impetus muscles (Berg and Butterfield, 1976). The IGF-1 mRNA concentrations measured herein showed no obvious relation to either growth velocity in general or allometric growth rate of the individual muscle. As mentioned above, the importance of local IGF-1 production for local growth phenomena depends on binding proteins as well as receptor density and function. Taking the muscular IGF-1 receptor densities measured in the same muscles but from female calves (Boge et al., 1995) into consideration, again no such relation could be established.

Aspects of compensatory growth and tissue IGF-1 mRNA concentrations

After accelerating the growth rates by shifting to a more intensive feeding regimen in group C, an elevation of liver IGF-1 expression might have been anticipated. Surprisingly the IGF-1 mRNA abundance amounted to only 20% of the concentrations observed in liver tissue from the steers in groups I and P; this difference is only marginally reduced when taking the relatively higher liver weights into consideration. In heart and kidney again no relation of IGF-1 mRNA concentrations to either total growth velocity or organ growth was obvious. Similarly reduced IGF-1 mRNA expression rates as in liver were observed in m. splenius from compensating animals. With regard to carcass composition no significant difference between the constantly slow growing group P and the compensating group C was evident. The increased growth rate in group C during the final finishing period thus seems attributable to a continuation of the pattern of gain composition observed during pasture feeding. This gain was nevertheless more effective and faster as indicated by the shorter time in which the compensating animals reached the final live weight (500−570 kg: 105 days in P; 70 days in C). These aspects of increased growth rates together with relatively lower portions of fat as compared to constantly fast growing animals explains the pronounced interest of applied research; however, the mechanisms underlying the phenomenon are not well understood. It remains open what the metabolic or endocrine signal is since a number of studies has demonstrated that after feeding animals at a more intensive level than previously, the adaptation of the hormonal

system occurs within a few days and thus seems not responsible for the effects on growth rates maintained beyond this relatively short time (Blum et al., 1979, 1985). Supported by recent data from runt pigs in which no relation between IGF-1 plasma concentrations and compensatory growth could be established (Ritacco et al., 1997), it seems as if the IGF-1 does either not play a major role for the mediation of compensatory growth or is even negatively related to this phenomenon. Hereby the regulation of the other components of the IGF-1 system needs to be included to be finally able to judge the functional relevance of the IGF system for postnatal muscle growth.

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