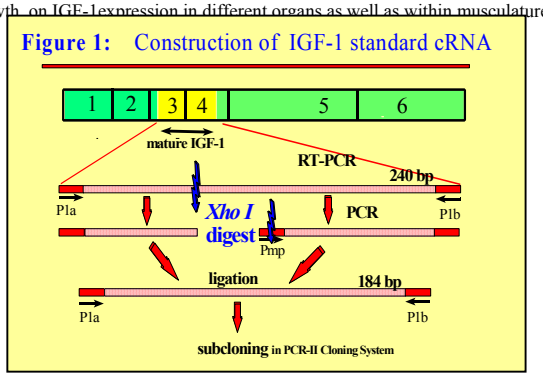
# Muscle and organ specific regulation of IGF-1 mRNA expression in growing steers: Quantification with an internally standardized competitive RT-PCR

M. Pfaffl, F. Schwarz, H.H.D. Meyer and H. Sauerwein,
TU München-Weihenstephan, Germany

## Introduction:

During postnatal growth, IGF-1 acts both as a mitogen as well as a stimulator of protein synthesis and of glucose utilization in tissues. IGF-1 is considered to mediate the anabolic actions of growth hormone in skeletal tissues. The observation of IGF-1 mRNA expression and peptide synthesis in many tissues led to the concept that IGF-1 does not act exclusively in an endocrine, but also in auto/paracrine manner. To elucidate the functional role of local IGF-1 expression for allometric growth phenomena, we aimed to investigate the tissue specific regulation of IGF-1 mRNA expression in various bovine muscles and organs. For a quantification of IGF-1 mRNA with the sensitivity required for tissues in which IGF-1 mRNA is of particular low abundance, e.g. in skeletal muscle, we designed, developed and validated an internally standardized RT-PCR assay. The competitive RT-PCR presented herein allows for a reliable and precise quantification of IGF-1 mRNA. It was first applied to investigate the effects of different feeding regimen, which lead to different growth rates including catch-up growth, on IGF-1expression in different organs as well as within musculature.

**Figure 1:** Construction of IGF-1 standard cRNA



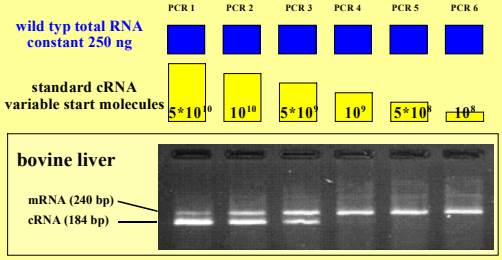

## Methodology:

The competitive standard IGF-1 cRNA (184 bp) was designed to have identical properties as the native IGF-1 mRNA in terms of the RT-PCR reaction , but to be distinguishable from the PCR product generated from the native sequence (240 bp). We therefore used RT-PCR to generate a 56 base deletion within the IGF-1 DNA target as illustrated in Figure 1. All primers used were derived from the bovine IGF-1 sequence (FOTSIS et al., 1990).

To obtain the IGF-1 mRNA concentration present in tissue RNA samples, series of 250 ng tissue RNA were spiked with different dilutions of competitor IGF-1 cRNA ($1.6x10^8$ to $16.0x10^{10}$ start molecules; Figure 2). Parallel amplification efficiencies (E) could be demonstrated for the two co-amplified RT-PCR products ($E_{IGF\text{-}1\ cRNA}$ = 66%; $r^2$ = 0.996; $E_{IGF\text{-}1\ mRNA}$ = 65%, $r^2$ = 0.954). Quantification of both products was done by HPLC anion exchange chromatography and UV ($OD_{260nm}$) detection. From the respective peak integrals, the amount of native IGF-1 mRNA start molecules present in the tissue RNA sample was then calculated as described by SIEBERT & LARRIK (1992).

The assay has a detection limit of 1600 IGF-1 cRNA molecules, an assay variation of 7.4% (n=5) and assay linearity (R=0.997) was given between 140 ng to 840 ng total-RNA input into competitive IGF-1 RT-PCR.

**Figure 2:** Competitive IGF-1 RT-PCR




## Experimental design:

26 Simmental steers were randomly allocated to three different feeding groups. Up to 220 kg of live weight all steers were kept under the same conditions, they were all castrated at 4 to 5 months of age. With 220 kg, 18 animals were transferred onto pasture feeding, the remaining 8 animals were fed as it is usual in common fattening systems (**I**). Having reached 500 kg live weight, the group kept on pasture was partitioned: 9 animals were further kept as before (**P**), the other 9 steers were transferred to the more intensive feeding regimen (**C**) to include the aspects of compensatory growth. The nutrient intakes as well as the growth rates and carcass composition of the different groups during the last fattening period (500 to 580 kg) is summarized in table 1. Animals were slaughtered at 570±12.5 kg and samples were collected from liver, heart, kidney and 4 muscles: m. splenius (SP), m. soleus (SO), m. cutaneus truncii (CT) and m. semispinalis capitis (SC), which were selected in order to represent maximal differences in fiber composition as well as in growth impetus.

**Table 1:** Nutrient intakes, growth rates and carcass composition

| | ME (MJ/d) | raw protein (g) | ADG (g/d) | lean meat[1] (%) | fat[1] (%) |
|---|---|---|---|---|---|
| I | 95.7 | 1174 | 1081 ∀86 ab[2] | 67.1 ∀1.8a | 12.3 ∀2.4a |
| C | 125.9 | 1400 | 1520 ∀252 b | 67.3 ∀1.2b | 11.5 ∀1.3b |
| P | 111.8 | 2535 | 866 ∀107 a | 61.2 ∀1.7a | 21.3 ∀2.0a |

[1] carcass composition was calculated according to Heindl et al., 1995;
[2] different letters indicate differences between feeding groups (p # 0.05);

## Results and Discussion:

The different growth velocities led to significant differences in IGF-1 mRNA expression rates in all organs and muscles (table 2).

Highest IGF-1 expression rates were observed in liver, followed by kidney and heart and were lowest in the different skeletal muscles. 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. When comparing the IGF-1 expression rates between the different treatment groups, a non-uniform reaction of the various tissues was apparent. In liver there was no difference between **P** and **I**, but in C undergoing compensatory growth, IGF-1 expression was reduced. The same was true for SP. In contrast, the lowest renal IGF-1 expression was observed in group **I**, which had average growth rates. In heart this group had the highest IGF-1 expression rates.

These differences were not attributable to alterations in DNA/RNA ratio. The results indicate that IGF-1 mRNA expression is regulated tissue specifically not only in different organs but also within skeletal muscles.

**Table 2:** IGF-1 mRNA expression in various bovine tissues

| | SP | SO | CT | SC | liver | heart | kidney |
|---|---|---|---|---|---|---|---|
| I | 7.9[1] (1.3)[2] a[3] | 8.4 (2.1) | 2.5 (1.0) | 4.8 (0.6) | 2872 (643) a | 32.3 (4.4) a | 11.1 (1.6) a |
| C | 1.6 (0.5) b | 13.9 (2.6) | 5.2 (1.9) | 6.9 (1.2) | 480 (110) b | 16.6 (3.8) b | 28.3 (3.1) b |
| P | 8.7 (2.2) a | 13.5 (2.8) | 1.3 (0.3) | 4.1 (0.6) | 2236 (171) a | 19.9 (3.9) b | 57.2 (19.4) b |

[1] x $10^{10}$ IGF-1 mRNA molecules/g tissue = mean;
[2] SEM are in parenthesis;
[3] different superscripts indicate differences between feedings (p<0.05);

**References:**

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