

## Introduction

Model studies on zinc (Zn) deficiency are usually performed with fast growing rats. However the intensive anabolic situation produces severe interactions between Zn deficiency *per se* and the metabolism *in toto*. Respective results may thus not fully reflect the situation in adults. To overcome this methodological disadvantage, we have used an animal model to study Zn deficiency in adult non-growing rats (1).

Metabolic effects on key players of the somatotrophic axis were analyzed on **protein level**:

- Growth hormone (GH)
- Insulin-like growth factor-1 (IGF-1)

and on **mRNA level**:

- Growth hormone receptor (GHR)
- Insulin-like growth factor-1 (IGF-1)
- Insulin-like growth factor-1 receptor (IGF-1R)
- IGF binding protein-1 (IGF-BP1)
- IGF binding protein-2 (IGF-BP2)
- IGF binding protein-3 (IGF-BP3)

As metabolic markers **non-esterified fatty acids (NEFA)** and **glucose plasma levels** were measured enzymatically.

## Animal experiment

The rat tissues were retrieved from an animal model described earlier (1): 31 female, non-growing rats weighing 212 g were fed a purified, phytate-enriched diet at restricted amounts covering the energy requirement for maintenance (8.0 g per head and day). Dietary Zn remained either at its native level (2 µg/g, Zn deficiency) or was supplemented with ZnSO<sub>4</sub> at amounts covering the requirement of Zn (58 µg/g, control). 8 subgroups of animals (each n = 3) were submitted to Zn deficiency for 1, 2, 4, 7, 11, 16, 22, or 29 days and then euthanised. Baseline values were retrieved from animals fed the control diet and euthanised at day 0 (n = 3) and day 29 (n = 4) of the study. Liver was removed immediately after euthanising and total RNA was extracted.

## Real-time RT-PCR and quantification method

We developed and validated various quantitative RT-PCR assays and established them on a fully quantitative real-time platform (LightCycler, Roche Diagnostics, Mannheim, Germany). Herein a relative quantification was applied (2). The relative expression is based on the expression levels of a target gene versus a reference gene and adequate for the most purposes to investigate physiological changes in gene expression levels. Expression studies were done in real-time RT-PCR and each sample was normalised to the internal GAPDH expression (= *housekeeping gene*). Relative expression levels of Zn deficiency group and control group were compared with the corresponding control group, which were set to 1.0. The relative expression ratio (R) was calculated in real-time RT-PCR from the PCR efficiencies (E = 2) and the mean crossing point (CP) deviation (ΔCP) of the unknown sample group mean versus the control group mean (3).

$$R = \frac{(E_{\text{target gene}})^{\Delta CP_{\text{target gene}} (\text{mean control} - \text{mean sample})}}{(E_{\text{GAPDH}})^{\Delta CP_{\text{GAPDH}} (\text{mean control} - \text{mean sample})}}$$

## Radioimmuno- and enzyme-assays

Plasma GH and IGF-1 concentrations in rats were analyzed by radioimmunoassay, as described earlier (4, 5). NEFA and glucose plasma levels were measured enzymatically as described by the supplier (r-biopharm, Darmstadt, Germany).

**Table 1: Blood plasma concentrations of hormones and metabolic parameters during 29 days Zn depletion.**

day	control 0	time course of Zn deficiency								control 29	Con. vs. Def. <sup>1)</sup>	S. E.
		1	2	4	7	11	16	22	29			
IGF-I (ng/ml)	301	264	306	310	310	228	267	275	266	237	n.s.	43
GH (ng/ml)	27	41	27	20	41	38	30	30	39	40	n.s.	16
NEFA (ng/ml)	0.58	0.49	0.60	0.51	0.52	0.50	0.85	0.53	0.56	0.66	n.s.	0.14
Glucose (g/l)	0.22	0.25	0.28	0.29	0.27	0.23	0.25	0.26	0.24	0.24	n.s.	0.03

1) contrast between control (day 0 and 29) and Zn deficiency (day 1 to 29);  
2) n.s. = statistically not significant

## Results & Discussion

### Zn deficiency

Zn deficiency was evident from reduced plasma Zn concentrations, plasma alkaline phosphatase activity and severe mobilization of Zn from tissue stores (mainly skeleton), while feed intake and body weight remained unaffected (1).

### mRNA expression in liver

Expression levels of IGF-1, IGF-1R, GHR, and three binding proteins (IGF-BP 1-3) were detected and quantified by kinetic RT-PCR. Each factor exhibited a specific expression pattern in liver.

IGF-1 mRNA expression was dominant, followed by IGF-BP1, GAPDH, IGF-BP2, GHR and IGF-BP3. The IGF-1R mRNA expression was very low abundant.

GAPDH was used as reference and housekeeping gene in order to compare the quantified mRNA concentrations (GAPDH expression was set to 100% = 1e+0 at all treatment days). GAPDH expression showed no significant regulation under Zn deficiency in liver and a low variation of 3.8 % during treatment period.

### Relative changes in mRNA expression due to Zn deficiency

Figure 1 shows the regulative response of mRNA expression to Zn deficiency and the respective correlation coefficients and significance level (p-value).

IGF-BP2 mRNA expression declined slightly over 29 days Zn depletion (1.8-fold down-regulation, p<0.05).

All other transcripts remained unchanged over 29 d of Zn deficiency.

### Plasma protein levels

GH and IGF-1 plasma concentrations remained constant during Zn depletion (mean concentrations over 29 day Zn deficiency: 33±14 ng/ml and 275±46 ng/ml, respectively). Furthermore the concentrations of NEFA (mean concentrations: 0.58±0.16 ng/ml) and glucose (mean concentrations: 254±34 ng/ml) did not change with time.

## Conclusion

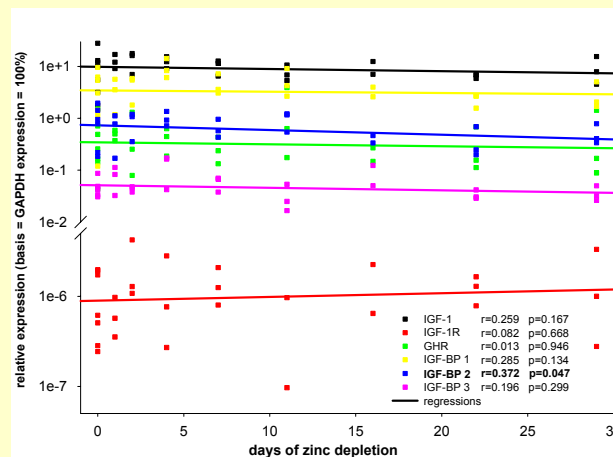
This study provides a comparative view of metabolic markers and gene expression regulation of key players of the somatotrophic axis in liver in a non-growing adult rat model.

The expression results indicate the existence of all investigated transcripts in rat liver, their different expression levels and their tissue specific regulation under Zn deficiency treatment.

Under the condition of unchanged nutrient intake and the absence of growth (and presumably of any other production performance), the Zn deficiency did not obviously affect directly the mRNA expression and protein levels on key players of the somatotrophic axis, except for IGF-BP2, as well as key metabolites.

## References

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**Figure 1: Relative mRNA expression of IGF, IGF-1R, GHR, IGF-BP1, IGF-BP2 and IGF-BP3 on the basis of GAPDH expression (= 100%).**