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Original article

Insulin-like growth factor I during growth in bulls

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Summary — In 10 bulls, changes in blood plasma concentrations of insulin-like growth factor I (IGF_1) were studied during rearing and during the ensuing growth period. IGF_1 continuously increased from 32 μg/l at the age of 15 d to 194 μg/l at the age of 307 d together with body weight. However, IGF_1 was not related to daily rate of gain, which remained fairly constant during the growth period. An age-dependent increase was also observed for blood levels of insulin, thyroxine and triiodothyronine. The data suggest that insulin and thyroid hormones may be causally related to the age-dependent increase in IGF_1 levels.

growth — bulls — hormones — IGF_1

Résumé — IGF_1 pendant la croissance chez les taurillons. *Dix taurillons en croissance ont été utilisés pour étudier les variations des taux sanguins de IGF_1 (insulin-like growth factor I) au cours du sevrage et pendant la période d'engraissement. IGF_1 augmente progressivement avec l'âge de 32 μg/l à 15 jours jusqu'à 194 μg/l à 307 jours, et avec le poids corporel, mais pas avec le gain moyen quotidien. L'insuline et les hormones thyroïdiennes augmentent pendant la croissance. Ces résultats suggèrent que les concentrations d'insuline et d'hormones thyroïdiennes peuvent contribuer, au moins en partie, à l'augmentation de l'IGF_1 avec l'âge.*

croissance — taureaux — hormones — IGF_1

Introduction

Growth and body composition are regulated by various hormones. Growth hormone (GH) and insulin-like growth factor I (IGF_1) are of primary importance (Spencer, 1985). Exogenous GH stimulates growth of the skeleton, of muscle tissue, of the intestinal tract and of other tissues, but usually reduces fat deposition (Hart and Johnsson, 1986; Fabry *et al.*, 1987). However, experience with various farm animals demonstrates that blood levels of GH are only weakly, often not or even inversely related to growth rates and body size (Althen and Gerrits, 1976; Blum *et al.*, 1985b; Breier *et al.*, 1986; Hart and Johnsson, 1986).

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Effects of GH on the skeleton are mostly mediated by IGF_1 (Froesch *et al.*, 1986), but not exclusively (Isaksson *et al.*, 1982). On the other hand, IGF_1 effects on fat and muscle deposition are relatively small (Froesch *et al.*, 1986). In humans, blood concentrations of IGF_1 increase from birth up to puberty (Zapf *et al.*, 1981) and decrease again during adult life (Clemmons and Van Wyk, 1984). In some cases IGF_1 levels were associated with the final size of animals (Eigenmann *et al.*, 1984a, b; Buonomo *et al.*, 1987). Production of IGF_1, which primarily although not exclusively takes place in the liver, is enhanced by GH, insulin and thyroid hormones, besides other factors (Clemmons and Van Wyk, 1984; Spencer, 1985). In cattle IGF_1 is modified by growth hormone and by energy intake (Elsasser *et al.*, 1986; Ronge *et al.*, 1988; Ronge and Blum, unpublished observations). The administration of GH to heifers caused an increase in weight gain, associated with increased blood plasma levels of IGF_1 (Lemal *et al.*, 1988).

The objective of the present investigation was to study changes of IGF_1 in adequately fed bulls during an entire growth period. Blood metabolites, immunoreactive insulin (IRI), thyroxine (T_4) and triiodothyronine (T_3) were measured to describe the framework of changes in metabolism and the endocrine environment of IGF_1.

Materials and methods

Materials, feeding and experimental design

Ten bull calves (Red Holstein x Simmental) were available at the age of 15 d. They were held at the Federal Research Station for Animal Production, Grangeneuve, CH-1725 Posieux.

Calves were kept for 7 wk in groups until they weighed 150 kg (rearing period). During this time they received milk and increasing amounts of hay and corn silage, starting with the 2nd wk of the experiment.

The growth trial started when the animals reached 150 kg around the age of 120 d. They received grass silage *ad lib* as basic ration (BR) and concentrates (C; 1.5 kg fresh matter, corresponding to 1.3 kg dry matter) once daily between 09.00-10.00 h. Concentrates consisted of barley (10%), corn (81%), molasses (4%), fat (2%), vitamins and minerals (3%). During the growth trial animals were individually fed and feed intake was measured daily. Water was provided *ad lib*. Net energy (NE) and protein intake were calculated based on net energy of growth (NEV) and absorbable protein of the gut (AP), respectively (Schneeberger and Landis, 1984).

Blood was taken between 11.00-14.00 h from the jugular vein by puncture, using heparinized vacutainers (Becton-Dickinson, CH-4142 Münchenstein), during rearing at the age of 15 and 35 d, and then once monthly from 150 kg up to the end of the growth trial.

Laboratory methods

Feed analyses were performed by standard procedures (Weende procedure) at the Federal Research Station for Animal Production, Grangeneuve.

Glucose, protein, albumin, blood urea-nitrogen (BUN), IRI, T_4 and T_3 were measured as previously described (Blum *et al.*, 1985a).

Concentrations of IGF_1 were determined in triplicate by radioimmunoassay according to Zapf *et al.* (1981) with some modifications. IGF_1 (preparations 1/3 and 1/4) used for standards was kindly provided by Prof. Dr. R.E. Humbel, Zürich. For production of antiserum (in a rabbit) and for iodination (by the chloramin T method) we used recombinant human IGF_1 ($rIGF_1$, Mü 14 Fr 25-32 TOP). $rIGF_1$ was obtained from Prof. Dr. J. Nüesch and Dr. K. Scheibli, Ciba-Geigy A.G., Basle. All samples were pretreated with acid/ethanol to separate IGF_1 from its binding protein(s). The samples were neutralized with NH_4HCO_3, lyophilized and reconstituted in the assay buffer before further use. After incubation for 24 h with antibody and another 24 h with tracer, antibody-bound and free IGF_1 were separated after addition of 1% bovine gamma-globulin and 25% polyethy-

lene-glycol by centrifugation. Half-maximal binding was 1.0 ng/tube. The sensitivity was below 0.1 ng/tube (< 6.5 ng/ml). Recovery was 91 ± 5 and 111 ± 9%, respectively, for 10 and 20 ng $rIGF_1$ (10 µl) added to 1 ml bovine plasma. ^{125}I-labelled $rIGF_1$ added to bovine plasma was recovered with 104 ± 1%. Compared with separation of IGF_1 bound to plasma protein(s) by chromatography using a large column (30 x 2 cm, vol 92.5 ml) and acidified buffer, recovery by acid/ethanol extraction was increased by 27 ± 5%. Diluted sera from cattle in different physiological states paralleled the standard curve. Heparin in plasma did not modify the results. The intra- and inter-assay coefficients of variation were below 10%.

Statistical analysis

Values are expressed as means ± SEM. Significances of differences during the growth period compared to the period of rearing were evaluated with the Wilcoxon signed rank test (MSI Widas, Dr. Wälti AG, Buchs).

Results

Feed, energy and protein intake, changes of body weight and weight gain

Dry matter intake of basic ration, NE and AP increased in absolute terms (not shown), but decreased throughout the growth trial if related to body weight (Table I). Intake of concentrates was kept constant. Body weight continuously increased. Daily gain increased for the first 2—3 months and then remained relatively constant until the end of the study.

Metabolites

As shown in Table II, glucose levels did not change significantly during the whole experimental period. BUN became significantly increased after the rearing period

Table I. Dry-matter intake, related to body weight of basic ration (BR), concentrates (C), net energy for growth (NE), absorbable protein of the gut (AP), changes of body weight (BW) and daily weight gain during the growth trial.

Age (d)	*126*	*158*	*189*	*210*	*245*	*280*	*307*
BR-intake (kg/BW•d^{-1})	17.6 ± 0.2	18.3 ± 0.2	17.2 ± 0.1	17.4 ± 0.2	16.5 ± 0.2	16.0 ± 0.2	14.5 ± 0.1
C-intake (g/BW•d^{-1})	7.3 ± 0.0	6.1 ± 0.0	5.1 ± 0.0	4.5 ± 0.0	4.0 ± 0.0	3.5 ± 0.0	3.1 ± 0.0
NE-intake (kJ/BW•d^{-1})	199 ± 1.5	189 ± 1.2	172 ± 0.9	167 ± 1.1	162 ± 1.3	154 ± 1.5	138 ± 1.0
AP-intake (g/BW•d^{-1})	2.42 ± 0.02	2.31 ± 0.01	2.07 ± 0.01	2.01 ± 0.01	2.09 ± 0.02	2.01 ± 0.02	1.79 ± 0.01
Body weight (kg)	177 ± 5	212 ± 6	254 ± 6	291 ± 7	328 ± 7	372 ± 8	416 ± 9
Weight gain (kg/d)	1.08 ± 0.02	1.46 ± 0.02	1.54 ± 0.01	1.33 ± 0.01	1.44 ± 0.02	1.44 ± 0.02	1.52 ± 0.02

Values are means ± SE.
Animals before d 120 were held in groups, then were fed and held individually.

Table II. Blood plasma concentrations of glucose, blood urea nitrogen (BUN), albumin, protein, insulin (IRI), triiodothyronin (T_3) and thyroxine (T_4) during the period of rearing and during the growth trial.

Age (d)	*15*	*35*	*126*	*158*	*189*	*210*	*245*	*280*	*307*
Glucose (mmol/l)	5.31 ± 0.09	5.02 ± 0.06	4.59 ± 0.03	5.00 ± 0.03	4.82 ± 0.05	5.21 ± 0.03	4.97 ± 0.07	5.41 ± 0.05	5.28 ± 0.03
BUN (mmol/l)	5.91 ± 0.11	5.13 ± 0.08	6.33* ± 0.07	6.96* ± 0.08	5.98* ± 0.04	6.43* ± 0.05	8.29* ± 0.09	6.03 ± 0.09	5.74 ± 0.05
Albumin (g/l)	43.0 ± 0.54	40.8 ± 0.37	38.7* ± 0.23	38.6* ± 0.28	38.9* ± 0.28	39.0 ± 0.20	39.7 ± 0.21	41.6 ± 0.23	43.1 ± 0.23
Protein (g/l)	57.7 ± 0.27	63.2 ± 0.77	61.3 ± 0.22	59.2 ± 0.31	61.3 ± 0.31	61.2 ± 0.27	61.3 ± 0.29	62.2 ± 0.28	62.8 ± 0.24
IRI (µg/l)	0.49 ± 0.04	1.43 ± 0.15	0.63 ± 0.04	0.99 ± 0.04	1.71 ± 0.12	1.37 ± 0.06	1.66 ± 0.08	1.27 ± 0.07	2.27* ± 0.09
T_3 (nmol/l)	3.40 ± 0.13	3.04 ± 0.08	3.33 ± 0.08	3.52 ± 0.07	3.84* ± 0.06	3.64 ± 0.07	4.22* ± 0.10	3.41 ± 0.08	4.64* ± 0.05
T_4 (nmol/l)	93 ± 3	88 ± 1	96 ± 2	108* ± 2	118* ± 2	126* ± 1	134* ± 2	120* ± 3	146* ± 2

Values are means ± SE.
* Significantly different from mean levels during the period of rearing (d 15 and d 35); $P < 0.05$; no symbol, $P > 0.05$.

($P < 0.05$). Albumin marginally and transiently decreased ($P < 0.05$). Protein did not change significantly.

Hormones

Concentrations of IGF_1 continuously increased over the entire growth trial above those measured during the period of rearing ($P < 0.05$; Fig. 1). Concentrations of IRI slightly increased during the growth trial, but due to considerable variations the increase was only significant for the last value. Levels of T_3 and T_4 slightly increased over the entire growth trial and were significantly higher for the last months than during the period of rearing (Table II).

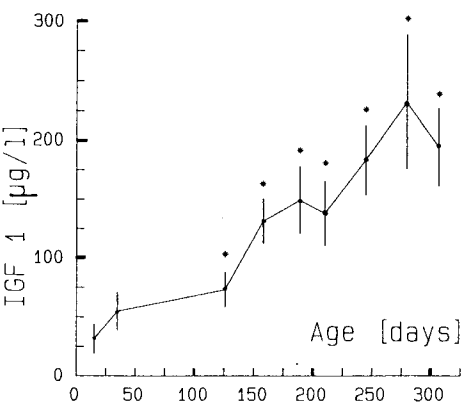

Fig. 1. Blood plasma concentrations (means ± SE) of insulin-like growth factor I during the period of rearing (d 15 and d 35) and during the growth trial in 10 bulls. Significance of difference from mean levels during the period of rearing : * $P < 0.05$; no symbol, $P > 0.05$.

Discussion

Concentrate intake was kept constant, as planned. Intake of absolute amounts of basic ration, NE and AP increased during the growth trial, whereas related to body weight, parameters of feed intake decreased. Towards the end of the trial absolute dry matter intake and consequently also NE- and AP-intake obviously reached an upper level. With an average daily gain during the growth trial of 1429 g nearly the maximal genetic growth potential was attained for the animals studied (Danuser and Lehmann, 1983).

Metabolites were measured to serve as indicators of shifts in energy and protein metabolism. Concentrations of glucose, albumin and protein were relatively constant and in the range found earlier (Blum *et al.*, 1985b). The small decrease in albumin concentrations during the growth trial relative to the period of rearing was of minor concern. The increase in BUN levels after the animals had reached 150 kg was probably the result of increased protein intake as the animals were switched from a ration consisting of hay and corn silage to one consisting of grass silage and concentrates.

We have shown that IGF_1 levels in cattle barely change during a 24-h period, despite wide variations in feed intake and in the presence of considerable diurnal alterations in metabolism and in other endocrine systems (Ronge *et al.*, 1988). In contrast to many other hormones, the IGF_1 status can therefore rather accurately be described by measuring single daily blood samples, as done in this study. Circulating IGF_1 continuously increased from the rearing period until the end of the growth trial which is in accordance with Schams *et al.* (1988). Values at the end of the study were markedly higher than in *et al.*, 1988). On the other hand, they were still markedly lower than IGF_1 concentrations (302 ± 16 µg/l) measured in 10 3-yr old bulls, weighing 930 kg and used for artificial insemination (Ronge and Blum, unpublished observations). Results of growing bulls and those of nearly mature AI bulls can only be compared with caution because of differences in feeding, environment and genetics. Nevertheless, data of the growth trial demonstrate an age-dependent rise in IGF_1, associated with body mass. Eigenmann *et al.* (1984a) and Buonomo *et al.* (1987) have already shown a positive relationship of IGF_1 with body weight in dogs and in pigs with a genetically different growth and size potential and suggested that IGF_1 may be one of the factors determining final body mass within a given species or breed. Exogenous IGF_1 enhances daily gain in hypophysectomized rats, demonstrating that its presence is necessary for normal growth (Schoenle *et al.*, 1982). IGF_1 was obviously not correlated with daily weight gain in our study, in accordance with Schams *et al.* (1988). It remains to be demonstrated that IGF_1 administration stimulates growth rate in farm animals.

Concentrations of IGF_1 in growing steers are influenced by the level of feed and protein intake (Breier *et al.*, 1986; Elsasser *et al.*, 1986, 1987) and we have demonstrated a positive correlation of IGF_1 levels with energy balances in heifers and lactating dairy cows (Ronge *et al.*, 1988; Ronge and Blum, unpublished observations). Although protein intake was increased during the growth period compared to the period of rearing, this cannot explain the continuous rise in IGF_1 after the animals had reached 150 kg because protein intake, on a body weight basis, was decreasing. The same held for energy intake.

The levels of IRI and thyroid hormones were in the normal range for growing bulls

and steers (Blum *et al.,* 1985b; Verde and Trenkle, 1987). In the present study concentrations of IRI, T_3 and T_4 all increased during the growth trial, as did IGF_1. IRI, T_3 and T_4 are known to enhance IGF_1 production (Clemmons and Van Vyk, 1984; Spencer, 1985), suggesting that they may have been responsible for the rise of IGF_1, at least in part. It has already been shown that there exists a positive correlation with blood testosterone and IGF_1 levels in bulls, whereas GH levels do not correlate with IGF_1 levels (Schams *et al.,* 1988). Accordingly, we could not demonstrate an association between GH and IGF_1 levels in the present study (data not shown). Additional and specifically designed studies are needed to elucidate the exact role of these hormones and of additional endocrine factors for regulation of IGF_1 production in growing cattle.

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