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Dietary nitrogen and calcium modulate CYP27B1 expression in young goats

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ABSTRACT

In livestock, feeding a reduced nitrogen (N) diet is favored for economic and ecological reasons. Ruminants cope more easily with a reduced N diet than monogastric species. However, changes in mineral homeostasis such as a reduction in 1,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$) concentrations, calcium (Ca), and IGF1 levels were observed in goats kept on a reduced N diet. The decrease in 1,25-$(OH)_2D_3$ occurred even during a simultaneous reduction in dietary N and Ca, whereas a solitary Ca reduction stimulated 1,25-$(OH)_2D_3$ synthesis. The aim of the present study was to examine the effects of N- and/or Ca-reduced diets on the expression of 24-hydroxylase (CYP24A1), 1-alpha-hydroxylase (CYP27B1), vitamin D receptor (VDR), retinoid X receptor alpha (RXRα), IGF1 receptor (IGF1R), Klotho, and fibroblast growth factor receptor 1c (FGFR1c) in kidneys of young goats. Four groups were kept on a control diet, an N-reduced diet, a Ca-reduced diet or an N- and a Ca-reduced diet. Renal expression of CYP24A1 was not affected, whereas CYP27B1 expression was significantly diminished in the N-reduced diet fed goats ($P < 0.05$) and significantly elevated with the Ca reduction ($P < 0.001$). The VDR expression was not modified, whereas RXRα ($P < 0.05$) and Klotho expression ($P < 0.001$) were stimulated during Ca reduction. The IGF1R ($P < 0.05$) and FGFR1c ($P < 0.05$) expression were enhanced with the N reduction. From these data, it can be concluded that the downregulation of renal CYP27B1 expression observed with dietary N reduction is probably mediated by a complex interaction between the somatotropic axis and the Klotho/FGF signaling pathway in young goats.

1. Introduction

In ruminant nutrition, it is preferable to lower dietary nitrogen (N) content for economic and ecological reasons, that is, to decrease feed costs and to reduce the excretion of N into the environment. Owing to efficient ruminohepatic circulation of urea, ruminants are thought to cope more easily with a reduced N diet than monogastric species as long as adequate energy supply is maintained [1]. The reason for this ability to adapt to low-protein diets is the efficient use of microbial protein as the main protein source for the ruminant host. In times of N scarcity, urea transport across the caprine rumen epithelium is stimulated [2] to deliver more urea as an N source to ruminal microbes for microbial protein synthesis. However, changes in mineral homeostasis such as reduced blood calcium (Ca) concentrations, diminished intestinal Ca absorption, and decreased 1,25 dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$) levels were detected in young goats kept on a reduced N diet [3,4]. Interestingly, the observed decrease in 1,25-$(OH)_2D_3$ occurred during a simultaneous reduction in dietary N and Ca, whereas a solitary dietary Ca reduction stimulated 1,25-$(OH)_2D_3$ synthesis [4].

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In general, the synthesis of 1,25-$(OH)_2D_3$ from 25 hydroxyvitamin D_3 (25-OHD_3) is mainly catalyzed by the mitochondrial enzyme 1-alpha-hydroxylase (CYP27B1), whereas catabolism of 1,25-$(OH)_2D_3$ is initiated by mitochondrial 24-hydroxylase (CYP24A1) in the kidney [5–7]. To regulate circulating levels of 1,25-$(OH)_2D_3$ to maintain Ca and phosphorus homeostasis, the activities of CYP27B1 and CYP24A1 are modulated in a diametrically opposed manner in the kidney. During hypocalcemia, elevated parathyroid hormone (PTH) levels stimulate the expression and activity of CYP27B1 in proximal tubule cells and concomitantly decrease CYP24A1 action [8–10]. As a regulatory circuit, 1,25-$(OH)_2D_3$ itself decreases expression of CYP27B1 and increases CYP24A1. However, this mechanism can be modulated. The abundance of renal retinoid X receptor alpha (RXRα) and vitamin D receptor (VDR) that was altered by dietary Ca was demonstrated to be a crucial factor in regulating 1,25-$(OH)_2D_3$ concentrations in rats [11].

In addition, there are other regulatory factors such as IGF1 that stimulate the production of 1,25-$(OH)_2D_3$ in kidney cells in vitro independently of GH [10] by affecting the expression and activity of CYP27B1 [12]. A negative regulator of CYP27B1 expression is the fibroblast growth factor 23 (FGF23), which binds to a constitutive binary complex of Klotho and FGF receptor 1c (FGFR1c) in renal proximal tubular cells [13]. A concurrent decrease in IGF1 and 1,25-$(OH)_2D_3$ could be observed in rats fed a low-protein diet under isoenergetic conditions [14]. As IGF1 and 1,25-$(OH)_2D_3$ concentrations were also reduced during dietary N reduction in young goats [15], it was postulated that the expression of renal CYP27B1 or CYP24A1 was modified by dietary N in ruminants.

To further reveal the underlying mechanisms and potential interactions with reduced Ca intake, the impact of the respective feeding regimes (control, reduced N, reduced Ca, reduced N and Ca) on renal expression of CYP24A1, CYP27B1, RXRα, VDR, IGF1 receptor (IGF1R), Klotho, and FGFR1c were investigated in samples taken from an earlier experiment [4]. More information about further parameters changed under N and Ca variation are available in these articles [4,16].

2. Materials and methods

The protocol of the animal feeding and handling was approved by the Animal Welfare Commissioner of the University of Veterinary Medicine Hannover (Hannover, Germany) and was in line with the German Animal Welfare Law.

2.1. Animals: feeding and sampling

The samples for this molecular study originate from a previous experiment [4,16]. When working with a large animal model, it is important to keep the number of animals as low as possible for animal welfare-based reasons (3R principles).

Twenty-six male colored German goats aged 2 mo were randomly divided into 4 feeding groups comprising 6 to 7 animals per group. Dietary ingredients and exact conditions of feeding regimes were published recently [4]. Briefly, the animals received either a diet with an adequate N and Ca content (control group [N+/Ca+], 21% CP, 1% Ca), with reduced N content ([N−/Ca+], 8% CP, 1% Ca), with reduced Ca content ([N+/Ca−], 22% CP, 0.4% Ca), or with reduced N and Ca content ([N−/Ca−], 8% CP, 0.4% Ca). The diets were isoenergetic (12.5 MJ ME per kg dry matter). Feeding was carried out 3 times a day. Chopped wheat straw was offered at 25% of the concentrate weight. The goats had free access to water and were weighed weekly.

2.2. Blood and tissue sampling

At the end of the feeding period (after 6–8 wk), blood samples were taken shortly before slaughter, but always at the same time in the morning to avoid circadian effects. Samples were taken by puncturing the Vena jugularis with EDTA-coated syringes (Sarstedt, Nümbrecht, Germany). Plasma were separated by centrifugation (2000 *g* at room temperature for 15 min) and stored at −20°C until further analysis. One animal per day was sacrificed by exsanguination after captive bolt stunning in a group-alternating manner to avoid time effects.

The kidneys were removed within 5 min after death and tissue samples were taken from the cortex. Samples were rinsed with ice-cold saline (0.9% NaCl, w/v), immediately frozen in liquid N_2 and stored at −80°C until further preparation.

2.3. Biochemical analysis of GH concentrations

The concentration of GH in plasma was measured by an ELISA 8 wk after taking to avoid uncertainties about stability of the samples at the Clinic for Cattle, Endocrinology Laboratory, University of Veterinary Medicine, Hannover, Germany, as previously described [15].

2.4. Ribonucleic acid isolation, reverse transcription, and quantitative real-time PCR

The total RNA was isolated with the RNeasy Plus Mini Kit (Qiagen; Hilden, Germany), with genomic DNA eliminator spin columns in accordance with the manufacturer's protocol. The concentration of RNA was measured by UV absorbance (BioPhotometer plus, Eppendorf AG, Hamburg, Germany). The quality and the RNA integrity number (RIN) of the extracted RNA was evaluated using an RNA 6000 nanoassay for an Agilent 2100 Bioanalyzer (Agilent Technologies, Böblingen, Germany).

Using random hexamers, oligo-dT primers and TaqMan-Reverse Transcription Reagents (Applied Biosystems, Darmstadt, Germany), 200 ng of isolated RNA were reverse transcribed in accordance with the manufacturer's protocol. Negative controls of template and reaction mix without reverse transcriptase were included.

The primers used for production of recombinant DNA were derived either from caprine, ovine, or bovine sequences. Primers were designed to span exon-exon junctions. The FGFR1c primer pair was designed to amplify all splice variants of the gene. Therefore, the spanning of exon-exon junction could not be included. For VDR no information about exon-intron structure is available in the published sequence database GenBank (https://www.ncbi.