The effect of stanozolol on ^{15}nitrogen retention in the dog

Merle E. Olson, Douglas W. Morck, K. Bruce Quinn

Abstract

The objective of the study was to determine the influence of either oral or intramuscular administration of stanozolol on nitrogen retention in dogs by using a non-invasive ^{15}N-amino acid tracer technique. Ten healthy, intact, adult male sled dogs received either stanozolol tablets, 2 mg/dog PO, q12h, for 25 days (Group 1, $n = 5$) or an intramuscular injection of 25 mg of stanozolol on Days 7, 14, 21, and 28 (Group 2, $n = 5$). A ^{15}N amino acid (5.27 mmol) was infused intravenously into each dog on Day 0 (before stanozolol treatment) and on Day 31 (after stanozolol treatment). Urine was collected by catheterization from each animal 3 times daily for 3 consecutive days. The ^{15}N-urea enrichment in urine was determined by high-resolution mass spectrometry and the total amount of urea in the urine was determined. Both oral and injectable stanozolol resulted in significant ($P < 0.05$) increases in amino acid nitrogen retention compared to pretreatment values. Oral stanozolol increased nitrogen retention from 29.2 ± 8.2% to 50.3 ± 9.2%, while stanozolol injection increased nitrogen retention from 26.6 ± 9.9% to 67.0 ± 7.5%. The response to intramuscular administration was significantly greater than the response to the oral dosing regime. Stanozolol increases amino acid nitrogen retention in dogs, as has been previously observed in rats. This action of stanozolol may be beneficial in dogs under stress of surgical trauma and chronic disease.

Résumé

Cette étude avait comme objectif de déterminer l'influence de l'administration de stanozolol par voie orale (PO) ou intramusculaire (IM) sur la rétention d'azote chez les chiens à l'aide d'une méthode non-invasive utilisant un acide aminé marqué avec du ^{15}N. Dix chiens de traîneau mâles entiers en santé ont reçu du stanozolol, soit en tablettes à une dose de 2 mg/chien PO q12h pour 25 j (groupe 1, n = 5) ou en injection im à une dose de 25 mg aux jours 7, 14, 21 et 28 (groupe 2, n = 5). Un acide aminé marqué ^{15}N (5,27 mMol) fut infusé par voie intra-veineuse chez chaque chien au jour 0 (avant le traitement au stanozolol) et au jour 31 (après le traitement). De l'urine fut prélevée chez chaque animal par cathétérisation trois fois par jour (q8h) pour trois jours consécutifs. La présence d'urée-^{15}N dans l'urine fut évaluée par spectrométrie de masse à haute résolution, et la quantité totale d'urée dans l'urine fut déterminée. L'administration orale et parentérale de stanozolol entraîna une augmentation significative (P < 0,05) de la rétention d'azote provenant des acides aminés lorsque comparée aux valeurs observées avant le traitement. L'administration orale de stanozolol augmenta la rétention azotée de 29,2 ± 8,2 % à 50,3 ± 9,2 %, alors que par injection le stanozolol augmenta la rétention d'azote de 26,6 ± 9,9 % à 67,0 ± 7,5 %. La réponse à l'administration IM était significativement supérieure à la réponse au traitement oral. Tout comme chez le rat, le stanozolol a augmenté la rétention azotée chez le chien. Cette propriété du stanozolol pourrait être bénéfique chez des chiens soumis à un stress suite à un trauma chirurgical ou une maladie chronique.

(Traduit par docteur Serge Messier)

Protein synthesis (anabolism) and breakdown (catabolism) occur continuously in all active cells. Anabolism and catabolism are regulated by endogenous hormones and can be influenced by exogenous hormones. Endogenous anabolic hormones include growth hormone, insulin, androgens, and estrogens; glucocorticoids, thyroxin, and adrenaline are endogenous catabolic hormones (1,3). Anabolic steroidal hormones act to increase muscle mass and protein content and these hormones have been used in food-producing animals to increase weight gain, enhance feed conversion, and improve carcass quality (2,3). These drugs have been used to treat clinical conditions associated with muscle wasting, such as injury, surgical trauma, and certain debilitating infectious diseases of humans and animals (1,3). Anabolic steroids act directly on the muscle cells to increase protein synthesis or indirectly by inhibition of glucocorticoid-induced protein catabolism (4). In male rats, stanozolol has been shown to have minimal anabolic and catabolic effects. However, in female rats, it increased muscle protein synthesis, body growth rate, and skeletal muscle growth rate without stimulating degradation (4). Historically, stanozolol has been used in dogs for the treatment of muscle wasting disease and, recently, it has been evaluated for use in chronic renal disease in castrated male dogs (5). There is limited experimental information on the effect of the drug on

Animal Health Unit and Gastrointestinal Sciences, University of Calgary, 3330 Hospital Drive NW, Calgary, Alberta T2N 4N1 (Olson, Morck); The Upjohn Company, Animal Health Division, Orangeville, Ontario (Quinn).

Address correspondence and reprint requests to Dr. M.E. Olson, telephone: (403) 220-6836, fax: (403) 270-0954, e-mail: molson@ucalgary.ca

Supported by grants from the Natural Sciences and Engineering Research Council of Canada and the Upjohn Company.

Received November 26, 1999. Accepted June 29, 2000.

Table I. ^{15}N amino acid infusion solution

Amino acid	Purity	Molecular weight	Total weight (g)	Amino acid received (mg/dog)	Amino acid received (mmol/dog)
Glycine	99.8%	76.06	6.00	300.0	3.94
Alanine	99.2%	90.09	1.75	87.5	0.97
Glutamate	98%	148.13	0.50	25.0	0.17
Leucine	98%	132.17	0.50	25.0	0.19
Total			8.75	437.5	5.27

Table II. Effect of oral or injectable stanozolol on body weight and nitrogen balance

Group	Treatment	Body weight (kg)	Weight change (kg)	Urea (mmol/L)	Urine volume (mL)	^{15}N recovered (mmol)	^{15}N infused (mmol)	^{15}N retained (%)
PO	Before stanozolol (Day 0)	27.3 ± 5.2	0[a]	866 ± 66	316 ± 21	3.73 ± 0.43[a]	5.27 ± 0.0	29.2 ± 8.2[a]
PO	After stanozolol (Day 31)	28.2 ± 6.0	0.8 ± 0.8[a]	1068 ± 240	274 ± 49	2.62 ± 0.49[b]	5.27 ± 0.0	50.3 ± 9.2[b]
IM	Before stanozolol (Day 0)	28.6 ± 3.8	0[a]	1105 ± 177	266 ± 33	3.87 ± 0.52[a]	5.27 ± 0.0	26.6 ± 9.9[a]
IM	After stanozolol (Day 31)	29.7 ± 4.0	1.1 ± 1.1[a]	1099 ± 281	266 ± 42	1.74 ± 0.40[c]	5.27 ± 0.0	67.0 ± 7.5[c]

Data are expressed as mean values ± SEM

Values with different superscripts are significantly different ($P < 0.05$)

nitrogen retention in this species. The objective of this study was to determine the influence of both oral and intramuscular administration of the anabolic steroid, stanozolol, on body weight and nitrogen retention by using a non-invasive ^{15}N-amino acid tracer technique in intact male dogs (6,7).

Ten (10) healthy, intact, male sled dogs, 1 y to 2 y of age (Animal Resource Centre, University of Calgary, Calgary, Alberta), and weighing 20.5 kg to 35 kg (45 lbs to 77 lbs) were used in the investigation. Animals were housed individually in kennels (approximately 2 m × 3.25 m) and provided standard dog food (Nu-Way Dog Food; Unifeed, Calgary, Alberta), consisting of 22% crude protein, 8% crude fat, 4.5% crude fibre, 10% ash and 12.5% moisture, and water ad libitum throughout the study. Dogs received moderate exercise for 1 h daily. In this study, each dog served as its own control to addresses the objective, which was to determine the effects of an anabolic steroid on metabolic changes within an individual animal. Dogs were conditioned to the same experimental environment for 2 mo prior to the experiment to reduce the opportunity of temporal metabolic changes within each dog. Dogs were randomly allocated into experimental groups. Group 1 contained 5 dogs, each receiving daily oral (PO) stanozolol (Winstrol-V; 2 mg/tablet, The Upjohn Company, Kalamazoo, Michigan, USA). Group 2 contained 5 dogs, each receiving 4 intramuscular (IM) injections of stanozolol (Winstrol-V; 50 mg/mL, The Upjohn Company) during the experimental period. The body weight of each dog was determined on Day 0 and on Day 31.

Prior to treatment (Day 0) a catheter was inserted into the right cephalic vein of each dog. A urinary catheter was inserted and the bladder was emptied of urine. A solution of ^{15}N-labeled amino acids (Aldrich Chemical Company, Milwaukee, Wisconsin, USA) was infused into the venous blood of each dog in 10 mL of sterile saline (Table I). Urine was collected by catheterization from each animal 3 times daily (q8h) for 3 consecutive days. Total collected urine volumes were measured and the urine was stored at −70°C until the time of analyses.

On Day 7, the Group 1 dogs began the stanozolol tablets, PO, 2 mg/dog, q12h, for 25 d (until Day 33). Group 2 dogs received parenteral stanozolol at a dosage of 25 mg/dog via deep IM injection into the semimembranosus/semitendinosus muscles on each of Days 7, 14, 21, and 28. On Day 30, the urinary bladders of Group 1 and 2 animals were emptied, the cephalic veins were catheterized as described above, and ^{15}N-amino acid solutions were infused as described for Day 0. Urine was collected by catheterization q8h for 3 consecutive days. Urine volumes were recorded and the urine specimens were stored at −70°C until all samples could be analyzed.

Urine samples were freeze-dried and placed in a high resolution mass spectrometer (Kratos MS 80; Kratos Inc., Manchester, England). The ^{15}N enrichment was determined by analyzing the parent ion and the parent ion + 1 peak (60 and 61 mass ion peaks, urea and ^{15}N urea). The non-enriched peak was normalized to 100%, and all other peaks were referred to this peak. The peak at mass 61 was corrected for urea enrichment associated with natural abundance of ^{15}N (subtract 1.91%). The urea concentration in the urine was determined spectrophotometrically using an autoanalyzer (Kodak E250; Rochester, New York, USA).

Labeled nitrogen apparent retention was calculated before and after administration of anabolic steroid so that the influence of the anabolic steroid on nitrogen retention could be evaluated. One-way analysis of variance was used to compare labeled nitrogen

apparent retention between the 2 treatment groups and the nitrogen retention before and after administration of anabolic steroid. A 95% confidence interval was used in all comparisons.

Adverse reactions to IM stanozolol, PO stanozolol, or IV amino acid solution were not observed. The ^{15}N-urea enrichment could be detected in the urine only during the first 8 h after infusion of amino acids. Retention of ^{15}N associated with protein anabolism is summarized in Table II. Both the oral and injectable forms of stanozolol resulted in significant ($P < 0.05$) increases in amino acid nitrogen retention. Oral stanozolol increased labeled nitrogen retention from 29.2 ± 8.2% to 50.3 ± 9.2%, while IM stanozolol increased labeled nitrogen retention from 26.6 ± 9.9% to 67.0 ± 7.5%. The response to the injectable stanozolol was significantly greater than the response to oral dosing of the drug. Body weight did not significantly increase following administration of oral or injectable forms of stanozolol.

The single dose tracer technique has been employed previously in humans and in rats (6,7). We have demonstrated the procedure to be useful in evaluation on nitrogen and protein metabolism in dogs. It is non-invasive, therefore, animals do not need to be subjected to euthanasia or be anesthetized to collect muscle biopsy samples. The procedure does not require use of radioisotopes. One must consider that collecting urine specimens for labeled urea measurement does not account for labeled nitrogen loss through other urinary metabolites such as NH_3 and hippuric acid or the loss of labeled nitrogen in feces or perspiration. The data presented in this study demonstrate that measuring labeled urea can adequately be used to evaluate labeled nitrogen apparent retention in dogs.

Differences between the action of various anabolic steroids on muscle protein turnover have been observed in rats (4,6,8,9) and ruminants (10). Testosterone proprionate and trenbolone acetate increased both muscle protein anabolism and catabolism (6,9). In rats, stanozolol and nandrolol increase muscle protein synthesis and inhibit glucocorticoid-induced protein catabolism (4,8). From the information gained in this study, the effects of stanozolol in dogs may be similar to that in rats. The anabolic and anti-catabolic actions of stanozolol may be beneficial in dogs under certain conditions, such as stress of surgical trauma and chronic disease.

References

1. Ziegler TR, Leader LM, Jonas CR. Adjunctive therapies in nutritional support. Nutrition 1997;13:64S–72S.
2. Lamming GE. Future developments in the manipulation of growth in farm animals. Vet Rec 1987;120:495–499.
3. Barragry TB. Anabolic steroids. Irish Vet J 1974:28–33.
4. Bates PC, Chew LF, Millward DJ. Effects of the anabolic steroid stanozolol on growth and protein metabolism in the rat. J Endocrinol 1987;114:373–381.
5. Cowan LA, McLaughlin R, Toll PW, et al. Effect of stanozolol on body composition, nitrogen balance, and food consumption in castrated dogs with chronic renal failure. J Am Vet Med Assoc 1997;211:719–722.
6. Menschikowski M, Jung K, Junghans P, Petzke KJ, Albrecht V. The influence of a steroid hormone and of physical exercise on protein metabolism in rats. Exp Clin Endocrinol 1988;92:341–348.
7. Edwards RH, Dworzak F, Gerber RP, et al. Stanazolol in patients with muscular dystrophy increases muscle protein synthesis measured in vivo with stable isotopes. Brit J Clin Pharm 1984;19: 124–125.
8. Choo JJ, Emery PW, Rothwell NJ. Dose-dependent effects of an anabolic steroid, nandrolone phenylpropionate, on body composition, muscle protein metabolism in female rats. Ann Nutr Met 1991;35:141–147.
9. Vernon BG, Buttery PJ, Pearson JT. The effect of trenbolone acetate with time on the various responses of protein synthesis of the rat. Brit J Nutr 1984;52:515–521.
10. Sinnett-Smith PA, Dumelow NW, Buttery PJ. Effect of trenbolone acetate and zeranol on protein metabolism in male castrated and female lambs. Brit J Nutr 1983;50:225–234.