Effects of Dipeptides Administered to a Perfused Area of the Skin in Angora Goats[1,2]

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ABSTRACT: The effect of dipeptide infusion on mohair growth of Angora goats was investigated using a skin perfusion technique. Six Angora wethers (average BW 32 ± 2 kg) were implanted bilaterally with silicon catheters into the superficial branches of the deep circumflex iliac artery and to the deep circumflex iliac vein. For the first 14 d of the experiment, animals received infusions into the deep circumflex iliac arteries of either a mixture of Met-Leu and Lys-Leu (one side) or saline (other side). Infusion rates of amino acids were .72 mg/h Met-Leu and .72 mg/h Lys-Leu. The area of skin supplied by the deep circumflex iliac artery was approximately 300 cm^2. An area of 150 cm^2 within the perfused region was used to determine mohair growth. Two weeks after the cessation of infusions, perfused areas were shorn, and greasy and clean mohair production, staple length, and diameter were determined. Greasy and clean mohair production from the perfused region were increased by dipeptide infusion compared to the side infused with saline (1.91 vs 1.66 g, $P < .05$ and 1.56 vs 1.31 g, $P < .04$, respectively). No significant changes were observed in mohair diameter; however, staple length tended to increase as a result of dipeptide infusion (18.0 vs 16.1, $P < .1$). Decreased concentrations of Met, Cys, Lys, Phe, Val, Ileu, Leu, and Arg were observed in the venous blood taken from the deep circumflex iliac vein on the side infused with the amino acid mixture compared with blood taken from the saline side ($P < .05$). There were no treatment differences in triiodothyronine, thyroxine, or insulin concentrations in venous blood taken from the deep circumflex iliac vein. Direct skin infusion with dipeptide may have resulted in mobilization of amino acids for increased protein synthesis, or the infused dipeptides may have acted as growth promoters stimulating skin amino acid uptake and protein synthesis.

Key Words: Skin, Perfusion, Goats, Dipeptides, Methionine, Lysine

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Introduction

Dietary proteins are sources of amino acids (**AA**) that are required for maintenance and growth. Free AA in the blood are believed to be the sole source of these important nutrients and it is believed that only free AA can be used for protein synthesis (Bender, 1984). Besides free AA, many small peptides have been identified in the circulation. There is evidence of peptide clearance from the blood (Druml et al., 1991). Peptides have been shown to support serum protein concentrations and nitrogen retention equivalent to that of intravenous free AA (Adibi et al., 1993). Intravenous small peptides (dipeptides and tripeptides) have shown great capability as mechanisms for the provision of AA that may be difficult to deliver via nutrient infusions (Radmacher et al., 1993), such as AA that are relatively unstable or poorly soluble in aqueous solutions (Christensen, 1995).

Several studies with Angora goats have demonstrated increased fiber diameter and faster growth as the level of protein in the diet was increased (Hart et al., 1993; Sahlu et al., 1993). In a recent study, Puchala et al. (1995) showed that increasing the supply of methionine, leucine, and lysine to the skin increased mohair growth. Certain tissues may utilize small peptides; however, this is difficult to determine by conducting production experiments. Direct infusion of nutrients to the defined area of the skin allows for the quantification of metabolites and permits estimation of the minimum amounts of limiting nutrients that can be utilized by the skin (Hoey and Hopkins,

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Table 1. Composition of experimental diet

Item	% (DM basis)
Ingredient	
Alfalfa	7.0
Ground corn	19.0
Oats	25.0
Solvent-extracted soybean meal	9.0
Bermudagrass hay	37.8
Trace mineral salts[a]	1.0
Calcium carbonate	1.0
Vitamin ADE[b]	.2
Nutrients[c]	
CP	12.6
NDF	54.2
ADF	22.4
Ash	7.5

[a]Containing (in percentages) NaCl, 94 to 95; Mn > .2; ferrous Fe, > .16; ferric Fe, > .14; Cu, > .033; Zn, > .10; I, > .007; Co, > .005.
[b]Each gram contained 2,200 IU of vitamin A, 2,200 IU of vitamin D, and .2 IU of vitamin E.
[c]Average from four samples.

1983; Pierzynowski et al., 1994). Such a technique also allows identification of specific AA utilization by the skin.

The objective of this study was to examine the effect of infusing small amounts of a mixture of dipeptide (Met-Leu and Lys-Leu) to a specific area of skin on mohair growth and blood metabolites.

Materials and Methods

Animals

Six Angora wethers (average BW 32 ± 2 kg) were implanted bilaterally with silicon catheters in the superficial branches of the deep circumflex iliac artery and the deep circumflex iliac vein as described by Pierzynowski et al. (1994). The area supplied by the artery was identified using methylene blue (Sigma, St. Louis, MO) infusion and an area (10 × 15 cm) was marked by tattoo in the middle of the perfused region. Goats recovered from the surgery within 5 to 6 h and were put into individual cages and continued to receive their diet (Table 1). For the experimental period, intake was restricted to 80% of that consumed during the last month before the experiment. Goats were fed once daily at 0800 and had free access to water. Goats were handled humanely at all times according to the guidelines of the Institutional Animal Care and Use Committee of Langston University as described by the Animal Welfare Act of 1966.

Experimental Design

Goats were shorn before the 56-d experimental procedure. The tattooed region was shorn using a small clipper with surgical blade no. 40 (Oster, Milwaukee, WI). For the first 14 d of the experiment, goats were infused either with a mixture of dipeptide or saline into the deep circumflex iliac arteries. The site of infusion was randomly chosen. For daily infusions, 75 mg of Met-Leu and 75 mg of Lys-Leu were dissolved in 250 mL of saline, and the pH was adjusted to 7.4 using NaOH. Solutions were infused with 60-mL syringe pumps (Harvard Apparatus, South Natick, MA). Infusion rates were 2.4 mL/h for the saline and dipeptide mixtures. The infusion provided .72 mg/h of both dipeptide mixtures. On d 5, 9, and 14 of dipeptide infusion, blood was collected at 0800 from the deep circumflex iliac veins. Two weeks after infusions were stopped, tattooed regions were shorn and mohair collected for analysis of yield and fiber diameter. Tattooed regions of all goats were shorn again after another 4 wk and mohair was analyzed.

Blood Collection

Blood samples were taken from treated (dipeptide infusion) and control (saline infusion) catheterized deep circumflex ilium veins. Blood was collected into three 7-mL tubes containing K_3 EDTA for hormone assay, potassium oxalate-sodium fluoride for metabolites, or sodium heparin for AA analysis (Becton Dickinson, Vacutainer systems, Rutherford, NJ). The tubes were immediately chilled in an ice bath, transported to the laboratory, and centrifuged at 1,500 × *g* at 4°C for 20 min. Plasma aliquots were stored at –20°C until analysis.

Analyses

Plasma hormones were analyzed using commercially available kits from ICN Biomedicals, Inc. (Costa Mesa, CA): insulin (Kit #RINI9302), cortisol (Kit #FI9303), total triiodothyronine (T_3; Kit #T3I9302) and total thyroxine (T_4; Kit #T4I9302). The intraassay coefficients of variation averaged 5.7% for insulin, 5.7% for cortisol, 3.6% for T_3, and 4.9% for T_4. Analyses for the specific hormones were carried out in one assay. Amino acid analyses were performed using AminoQuant 1090 (Hewlett-Packard, San Fernando, CA) utilizing precolumn derivatization with *o*-phthalaldehyde and 9-fluorenylmethyl-chloroformate and UV detection (Hewlett Packard, San Fernando, CA). Plasma (1 mL) with .1 mL added internal standard (norvaline and sarcosine) was deproteinized with .9 mL Seraprep (Pickering, Mountain View, CA). Infused dipeptides were analyzed using the same system (AminoQuant), but gradient and wavelength (215 nm) were modified to achieve the best resolution. Plasma glucose concentrations were analyzed colorimetrically using a kit (catalog no. 315 Sigma Diagnostic, St. Louis, MO). Plasma urea N was determined as described by Chaney and Marbach (1962). Staple length and grease and clean mohair yields were determined according to ASTM Standards

Table 2. Mohair measurements after 14 days of dipeptide or saline infusion and 14 days of regrowth[a]

Mohair	Infusion			
	Dipeptide	Saline	SEM[b]	$P <$
Greasy mohair, g	1.91	1.66	.16	.051
Clean mohair, g	1.56	1.31	.07	.040
Length, mm	18.00	16.06	.53	.095
Diameter, μm	32.89	32.66	.94	.868

[a]n = 6 goats.
[b]SEM = standard error of the mean.

(ASTM, 1988). Fiber diameter was determined using the Peyer FDA 200 System (Wallerau, Switzerland).

Mohair data were analyzed as a one-way structural covariance model using SAS (1985), and mohair data from the control period (no infusion) served as a covariate. Blood data were analyzed as a split plot in time using SAS (1985). The main plot was treatment and was tested with the error term of animal within treatment. The sub plot was time of sampling, and the interaction of time of sampling × treatment was tested with the residual error.

Results

The skin perfusion model developed in our laboratory (Pierzynowski et al., 1994) allowed us to successfully infuse dipeptides or saline for 14 d into Angora goats. There were no problems in obtaining blood samples from the deep circumflex iliac veins, and feed intake (mean = 800 g/d) was not reduced by infusion. Dipeptide infusion increased greasy (1.91 vs 1.66 g; $P < .05$) and clean (1.56 vs 1.31 g; $P < .04$) mohair production in the tattooed area compared to the area infused with saline (Table 2). There were no significant changes in mohair diameter (mean = 32.8 μm); however, infusion of dipeptide tended to increase mohair length (18.0 vs 16.0 mm; $P < .1$).

Infusion of the dipeptide mixture decreased ($P < .05$) the concentrations of Met, Cys, Lys, Phe, Val, Ileu, Leu, and Arg in plasma from the deep circumflex iliac veins (Table 3). Overall, the plasma total concentration of essential AA was affected by dipeptide infusion (719.3 vs 863.0 μM for saline infusion, $P < .04$). There was no change in the plasma concentration of nonessential AA. Infused dipeptides were not detected in the blood taken from the deep circumflex iliac veins. There were no differences in plasma urea N or glucose due to the infusions (Table 4). Insulin, T_3, and T_4 were also not affected by the AA infusion.

Discussion

The nutritional and metabolic significance of peptide absorption is not fully understood, especially in ruminants. Muscle, liver, kidney, and other tissues have been shown to have, or are suspected to have, the ability to utilize peptides as a source of AA to meet cellular demands (Backwell et al., 1994, Hubl et al., 1994). The extent to which intact peptides may be absorbed into the blood is controversial. Peptide absorption seems to be an important physiological process in ruminants and may constitute the primary source of absorbed AA (Webb et al., 1993).

In this study, we investigated whether peptides containing the limiting AA for mohair growth could influence skin metabolism and mohair growth. Methionine and lysine are reported to be the most limiting AA for wool and mohair growth (Reis et al., 1990; Sahlu and Fernandez, 1992; Puchala et al., 1995). Leucine was also included in the mixture because it is known to serve as an energy-yielding substrate, is an important regulator of protein turnover, and modifies the uptake of tyrosine, phenylalanine and tryptophan. Leucine stimulates protein synthesis, and its metabolite, α-oxoisocaproate, is responsible for inhibiting protein catabolism (Bender, 1984).

Relatively small amounts of peptides were used in this study because supplementation was directed toward skin metabolism. An amount of .72 mg/h of each peptide was used to ensure that supplementation of one side with peptide would not appreciably influence the other side infused with saline. The skin perfusion model and local peptide infusions allowed us to separate skin metabolism from total body metabolism and to use the contralateral side of an

Table 3. Blood amino acid levels in the superficial branches of the deep circumflex iliac vein during amino acid or saline infusion[a]

Amino acids	Infusion			
	Dipeptide	Saline	SEM[b]	$P <$
Essential	μM			
Met	15.59	23.18	1.49	.03
Cys	44.23	55.31	2.84	.05
Lys	80.84	94.64	4.18	.02
Phe	58.35	65.37	2.71	.03
Val	124.50	141.62	7.16	.05
Ileu	65.22	73.39	4.16	.03
Leu	121.74	135.95	6.17	.04
Thr	81.91	88.55	5.92	.35
Arg	123.21	188.12	8.26	.01
Total	719.29	862.96	22.66	.04
Nonessential				
Glu	141.20	152.11	4.58	.52
Ser	59.91	53.67	3.21	.56
Gly	1,083.18	1,159.68	60.90	.58
Ala	191.01	205.80	9.96	.23
His	53.12	57.32	4.69	.59
Tyr	84.26	90.22	5.44	.29
Total	1,613.32	1,708.46	42.27	.21

[a]n = 6 goats.
[b]SEM = standard error of the mean.

Table 4. Blood glucose, urea N, and hormone levels in the superficial branches of the deep circumflex iliac vein during dipeptide or saline infusion[a]

Item	Infusion			
	Dipeptide	Saline	SEM[b]	$P <$
Glucose, mg/dL	55.62	59.24	4.04	.66
Urea N, mg/dL	5.65	6.02	.52	.89
Insulin, μIU/mL	26.58	22.26	2.12	.13
Triiodothyronine, ng/dL	182.27	181.64	9.49	.92
Thyroxine, μg/dL	5.24	4.83	.23	.77

[a]n = 6 goats.
[b]SEM = standard error of the mean.

animal as a control (Pierzynowski et al., 1994; Puchala et al., 1995, 1996).

It is not clear whether the increase in mohair production with dipeptide infusion was due to a direct stimulating role of peptides on skin metabolism and protein synthesis or to utilization of AA supplied by the infused peptides. There are several reports indicating that small peptides can be found in the circulation (Gardner, 1988; Savoie et al., 1988, Caspary, 1992). Gardner published several reports of intact peptide absorption in rats (Gardner, 1982, 1988). He estimated that about 30% of the AA appearing in the secretion at the serosal surface were as peptides. Species differences may be important when absorption of small peptides is considered. McCormick and Webb (1982) observed very high concentrations of peptide AA in the plasma of calves that accounted for about 70 to 80% of the total AA appearing in the blood. Small peptides are also absorbed and digested by hydrolase present in the brush border and cytosol of the intestinal mucosa (Alpers, 1986; Tobey and Heizer, 1986).

Several studies with humans have demonstrated that intravenous dipeptides are cleared rapidly from plasma, and this is an indication of accessibility of infused peptides as sources of AA (Furst and Stehle, 1993, Vazquez et al., 1993). The short half-lives (approximately 3 min) of Ala-Cys, Gly-Cys, and Ala-Gln (Albers et al., 1988; Furst et al., 1989) suggest that they are catalyzed by soluble and(or) plasma membrane-bound peptidases. However, longer half-lives of Ala-Tyr (12.3 min) and Gly-Tyr (101.7 min) in humans were observed by Druml et al. (1991). This may indicate that some peptidases may not be present in plasma and some peptides must be degraded in such organs as liver or kidney. Hubl et al. (1994) suggested that the kidney is the most important organ for the clearance of dipeptides and the release of amino acid residues into the circulation.

There is no information available on whether peptidases are present in the skin, and it is difficult to speculate on the mode of action of peptides used in this experiment. There are three possible reasons for the increased mohair production due to the infusion of peptides. The most likely reason is that the peptides were hydrolyzed in the blood and(or) skin tissue, and constituent AA were used for protein synthesis (mohair growth). The other possible explanations are that peptides acted as local growth promoters and indirectly affected mohair growth or that the peptides were used directly for the synthesis of mohair protein.

In an experiment of similar design, Puchala et al. (1995) infused .36 mg/h Met, .36 mg/h Lys and .72 mg/h Leu into the superficial branches of the deep circumflex iliac artery of Angora goats and observed that clean mohair production was increased by AA infusion compared to the side infused with saline (3.13 vs 2.70 g, $P < .07$). Numerical values are less for the present experiment (probably due to seasonal differences in mohair growth); however, the percentage increase in mohair production was similar in both experiments (17%). Because the observed increase in mohair production was similar it is possible that the constituent AA derived from infusion of peptides were used to increase mohair production. In this case, it must be assumed that hydrolysis of infused peptides was rapid and utilization of resulting AA was as effective as during AA infusion.

In this experiment, increased mohair length was observed as a result of dipeptide infusions (18.0 vs 16.1; $P < .1$) but this was not observed during local AA infusion to the skin (Puchala et al., 1995). This may suggest a different mode of utilization of the infused peptides. Peptides have been recognized as vital biological effectors such as hormones and neurotransmitters (Duta, 1989). Therefore, it may be possible that the infused peptides may have an indirect stimulatory action on mohair growth.

In this study, the infused peptides were not detected in the blood taken from the superficial branches of the deep circumflex iliac vein. This suggests that the peptides were either hydrolyzed by enzymes present within red blood cells or taken up rapidly by the skin. In a recent study, Backwell et al. (1994) demonstrated the in vivo utilization of AA of peptide origin by lactating dairy goats. In their study the extent of utilization was not different for Gly-[^{13}C]Phe and Gly-[^{13}C]Leu. The authors concluded that the mechanism by which [^{13}C]Phe and [^{13}C]Leu were incorporated into milk protein is not clear, but that it may involve peptide hydrolysis by either mammary cell surface or red blood cell hydrolases followed by uptake of liberated AA by the mammary gland.

Increased mohair production as a result of dipeptide infusion required increased energy utilization; however, glucose concentration was not affected by the treatment. It seems that a source of energy other than glucose was used for increased mohair protein synthesis. Energy metabolism in the skin may be unusual, with only 25% of glucose metabolized by hair follicles entering the tricarboxylic acid cycle (Harris et al., 1989). Acetate may account for about 50% of the substrate utilized by the tricarboxylic cycle in wool

follicles, and that may explain the lack of differences in plasma glucose concentration.

In this experiment, we observed a decrease in essential AA concentrations in the blood taken from the superficial branches of the deep circumflex iliac vein on the dipeptide-treated side, probably due to increased utilization for mohair growth. Only some AA were affected in the experiment using a mixture of AA (Puchala et al., 1995), and this also suggests a different action of peptides and free AA. Pan et al. (1996) demonstrated that Met- containing peptides can be used as methionine sources for protein accretion. The authors cultured myogenic and mammary epithelial cells with different Met-containing peptides and observed that many of them were superior to free methionine in supporting protein accretion. Those results also suggested indirect support of protein synthesis by peptides.

Implications

Supply of small peptides (Met-Leu and Lys-Leu) to the skin increased mohair production and mohair length. Infused peptides were not detected in blood taken from the superficial branches of the deep circumflex iliac vein, and this suggests that the peptides were utilized by the skin. Altered amino acid concentrations in venous blood from the dipeptide-perfused side indicated that supplementation enhanced amino acid uptake for protein synthesis, but mechanisms for enhanced mohair production are not clear. Further research is needed to investigate the biochemical mechanism of used peptides in skin and effect of other small peptides on skin metabolism.

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