Duodenal infusion of fatty acids differentially affects plasma glucagon-like peptide-1 and ghrelin concentrations in sheep

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ABSTRACT: The aim of this study was to investigate how intraduodenal infusions of fatty acids (FA) affect appetite-related gut peptides such as glucagon-like peptide-1 (GLP-1) and ghrelin in sheep. We hypothesized that these peptides can be highly reactive to unsaturated long-chain FA, because they are well known to decrease dry matter intake (DMI). Four ewes were fitted with a duodenal cannula and a jugular vein catheter for a 6-h duodenal infusion of the 9 FA (C8:0, C10:0, C12:0, C14:0, C16:0, C18:0, C18:1, C18:2, and C18:3) and water (control). The concentration of each FA was 1.6 g per metabolic body weight (BW), approximately corresponding to the amount of supplemented fat in a standard dairy cow diet. Each infusion was separated by at least 2 d. During the infusion period, blood samples were collected periodically to determine changes in plasma GLP-1, ghrelin, and metabolite concentrations. Duodenal infusions of

C18:1, C18:2, and C18:3 led to higher plasma GLP-1 ($P < 0.05$) and lower glucose ($P < 0.05$) than control. Plasma ghrelin concentrations were greater in C18:1 and C18:3 infusions than control ($P < 0.05$). Plasma ketone bodies were higher in C8:0 and C10:0 infusions ($P < 0.05$), but plasma triglyceride concentrations were lower in C8:0, C10:0, C12:0, and C16:0 infusions ($P < 0.05$) than control. Fatty acid infusions except for C18:3 led to higher plasma NEFA concentrations than control ($P < 0.05$). These results confirmed that the hypophagic effect of dietary unsaturated long-chain FA is mediated by GLP-1 (an anorexigenic effect) secretion. However, we also observed higher plasma ghrelin (an orexigenic effect) partially by unsaturated long-chain FA. Thus, the gut peptide secretions when ruminant animals ingest FA supplements would complexly affect satiety and further studies are needed to determine their each impact on DMI.

Key words: ghrelin, GLP-1, long-chain fatty acids, medium-chain fatty acids, ruminants

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INTRODUCTION

Lipid supplementation is a common strategy to improve domestic ruminant performance. Lipid additives increase the energy density in diets, but often decrease dry matter intake (DMI) and their hypophagic effect differs in fatty acids

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(FA) source, form, and type (Allen, 2000). Lipids also modulate the secretion of gut peptides regulating feed intake and energy metabolism. Thus, any hypophagic effects are probably the result of variation in FA characteristics (length and saturation) that influence gut peptide activity. Some key gut peptides include glucagon-like peptide-1 (GLP-1) and ghrelin. GLP-1, an anorexigenic hormone that enhances insulin secretion, is secreted by small intestine (Kreymann et al., 1990; Faulkner, 1991), and ghrelin, an orexigenic hormone, is mainly secreted by abomasum in ruminants (Hayashida et al., 2001). Feed ingestion increases plasma GLP-1 concentrations (Elsabagh et al., 2017), while plasma ghrelin concentrations exhibit a preprandial rise and a postprandial fall in sheep (Sugino et al., 2004). Secretions of these gut peptides are regulated by nutrients present in the gastrointestinal tract or circulating blood. Despite some research demonstrating that lipids can alter gut peptide concentrations, for instance elevating plasma GLP-1 concentrations (Relling et al., 2007; Relling and Reynolds, 2009), it has not been determined which specific FA stimulates gut-peptide secretion in ruminants. Furthermore, data on how FA affect ghrelin have been equivocal. In one study, consuming unsaturated long-chain fatty acids (LCFA) decreased plasma ghrelin concentration (Bradford et al., 2008), whereas previous research showed that plasma ghrelin concentration either increased (Fukumori et al., 2012b) or was unaffected (Relling et al., 2008). We hypothesized that GLP-1 and ghrelin respond to each individual FA in a different manner, which might contribute to the lipid supplement-induced DMI decreasence. Particularly, we hypothesized that these gut peptides might be highly reactive to unsaturated LCFA because the hypophagic effect of unsaturated LCFA is greater than that of other FA (Allen, 2000). Thus, here we conducted duodenal FA infusions to determine the secretory responses of GLP-1 and ghrelin in sheep.

MATERIALS AND METHODS

Animals and Treatments

Experiments were approved by the Animal Care and Use Committee of Utsunomiya University, Utsunomiya, Japan (A14-0014).

This study used 4 mature ewes (Suffolk × Corriedale crossbred; 47.4 ± 4.9 kg body weight [BW]) fitted with T-type duodenal cannulas posterior to the pylorus. Surgery was performed under

anesthesia at least 1 mo before the experiment. Throughout the experimental period, ewes were fed timothy and alfalfa hay equivalent to their metabolizable energy requirements (NRC, 2007) once daily at 0700 h. Fresh water was provided ad libitum throughout the experiment. Duodenal infusions of individual FA emulsions (C8:0, C10:0, C12:0, C14:0, C16:0, C18:0, C18:1, C18:2, and C18:3) and water (control) were conducted. Emulsions were prepared with free FA (Wako Pure Chemical Industries Ltd., Osaka, Japan) as described previously (Enjalbert et al., 1998), with modifications. First, 50 g of fatty acid was suspended in 1 g of xanthan gum (MP Biomedicals, LLC., Illkirch, France) mixed with water to form a gel. Next, 2.5 g of crude soy lecithin (Wako Pure Chemical Industries Ltd.) was added to the gel, and the total volume was adjusted to 450 g by water. The control infusion contained xanthan gum and lecithin, but no FA. The amount of FA infused was 1.6 g per metabolic BW, approximately corresponding to the amount of fat supplement generally fed to dairy cows. A 2-d interval separated each treatment to avoid carryover effects, and the infusion order was randomized by individual.

Sampling and Analysis

One day before the experiment, ewes were fitted with jugular vein catheters (Argyle 16G CV catheter kit; Nippon Sherwood Medical Industries Ltd., Tokyo, Japan) containing 200 U/mL heparinized saline. The treatment infusion began 3 h after feeding (1,000 h). Gastrostomy feeding tubes (Terumo, Tokyo, Japan) were connected to the ewes' cannulas for administering FA emulsions with a 50 mL syringe (Terumo) every 15 min for 6 h. Blood samples (5 mL) were taken at 0 (preinfusion), 1, 2, 3, 4, 4.5, 5, 5.5, and 6 h from experiment start and then immediately placed in tubes containing heparin (2 U/mL of blood: Wako Pure Chemical Industries Ltd.) and aprotinin (500 Kallikrein Inhibitor U/mL of blood: Sigma-Aldrich Inc., St Louis, MOUSA). This mixture was then centrifuged at $1,500 \times g$ and 4°C for 20 min. The resultant harvested plasma was stored at -20°C until needed for assays.

Plasma ghrelin and GLP-1 concentrations were determined using time-resolved fluoro immunoassays (2030 ARVO X4; PerkinElmer, Inc., Waltham, MA, USA), following published methods (Sugino et al., 2004; Fukumori et al., 2012a). Plasma triglyceride, NEFA, and total ketone bodies were determined using an automated biochemistry analyzer (Beckman Coulter, Inc., Tokyo, Japan),

while plasma glucose concentrations were measured with a glucose analyzer (Arkay, Kyoto, Japan).

Statistical Analysis

Data were expressed as least squares means of 4 ewes with SEM. Data were analyzed using the mixed models in JMP (version 13 for Windows, SAS Institute Inc., Cary, NC, USA). Treatment (infused FA or control), time (time after infusion), and the interaction were fixed effects, and sheep was random effect. If the treatment effect was significant, individual treatment means were compared using Tukey's test. Significant differences were set at $P < 0.05$.

RESULTS AND DISCUSSION

Plasma GLP-1 concentrations were higher than control during the infusions of C18:1, C18:2, and C18:3 ($P < 0.05$, Table 1). Figure 1 showed the time-dependent changes in plasma GLP-1 concentrations during the infusion period of control, C18:1, C18:2, and C18:3. There were significant rises of GLP-1 in C18:1 and C18:2 infusions against at the start of infusion. Our results are in line with several reports showing that feeding FA increased plasma GLP-1 concentrations (Bradford et al., 2008; Relling and Reynolds, 2007). For instance, plasma GLP-1 concentrations were found to be greater among cows fed diets high in monounsaturated and polyunsaturated LCFA than among those fed diets high in saturated LCFA (Relling and Reynolds, 2007). However, monounsaturated

and polyunsaturated LCFA did not differ in their effects on GLP-1 (Relling and Reynolds, 2007). We also determined plasma insulin concentrations in this study (data not shown), but did not show any similar changes with GLP-1. Previous studies have reported decreases to plasma insulin concentrations under a LCFA diet (in lactating cows: Relling and Reynolds, 2007; Fukumori et al., 2012b, 2013; in growing wethers: Relling et al., 2009). Thus, insulin response to lipid supplementation is generally not associated with GLP-1, although elevated GLP-1 potentially stimulates insulin secretion. On the other hand, plasma glucose concentrations were decreased by C18:1, C18:2, and C18:3 infusions ($P < 0.05$, Table 1), which might be related to GLP-1 secretion because GLP-1 had a glucose-lowering effect independently of insulin (Fukumori et al., 2012a).

We also observed differences in plasma ghrelin, where C18:1 and C18:3 infusions were greater than control (Table 1), although the time-dependent changes in plasma ghrelin concentrations were not clear in contrast to GLP-1 (Fig. 1). We had previously found that feeding cows calcium salts of LCFA derived from soybean and palm oil (rich in unsaturated FA) increased plasma ghrelin concentrations (Fukumori et al., 2012b). In contrast, other reports suggested that LCFA caused a decrease in ghrelin (Bradford et al., 2008) or caused no change at all (Relling et al., 2010; Fukumori et al., 2015). In the present study, we also found that the direct infusion of medium-chain fatty acids (MCFA: C8:0, C10:0, and C12:0) into the duodenum did not stimulate ghrelin secretion. The active form of ghrelin is acylated

Table 1. Plasma concentrations of gut peptides and metabolites during the duodenal infusions of 9 FA in sheep

Item	Intraduodenal infusion (treatment)										SEM	P values		
	Control	C8:0	C10:0	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3		Treatment	Time	Interaction
GLP-1 (ng/mL)	0.0127 ^c	0.0271 ^c	0.0521 ^{bc}	0.0322 ^c	0.0451 ^{bc}	0.0504 ^{bc}	0.0160 ^c	0.0823 ^{ab}	0.121 ^a	0.107 ^a	0.016	<0.001	<0.001	0.266
Ghrelin (ng/mL)	0.323 ^{cd}	0.230 ^d	0.303 ^{cd}	0.382 ^{bcd}	0.275 ^{cd}	0.329 ^{cd}	0.310 ^{cd}	0.550 ^a	0.416 ^{abc}	0.504 ^{ab}	0.127	<0.001	0.228	0.987
Ketone bodies (mmol/L)	0.342 ^c	1.17 ^a	0.794 ^b	0.468 ^c	0.423 ^c	0.311 ^c	0.362 ^c	0.299 ^c	0.320 ^c	0.330 ^c	0.061	<0.001	<0.001	<0.001
Glucose (mg/dL)	61.8 ^{abc}	54.4 ^{cdef}	54.5 ^{cdef}	65.6 ^a	57.3 ^{bcd}	60.6 ^{abcd}	62.4 ^{cdef}	52.3 ^{ef}	53.7 ^{def}	46.7 ^f	2.41	<0.001	0.017	1.000
NEFA (mEq/L)	0.154 ^d	0.291 ^{bc}	0.371 ^{ab}	0.435 ^a	0.363 ^{ab}	0.347 ^{abc}	0.284 ^{bc}	0.306 ^{bc}	0.301 ^{bc}	0.226 ^{cd}	0.066	<0.001	<0.001	0.880
Triglyceride (mg/dL)	12.4 ^a	7.27 ^b	6.95 ^b	6.96 ^b	10.3 ^{ab}	6.24 ^b	10.6 ^{ab}	10.4 ^{ab}	10.3 ^{ab}	10.0 ^{ab}	1.24	<0.001	<0.001	0.919

Values are presented as least squares means with SEM ($n = 4$).

Values carrying different superscripts (a, b, c, d, e, f) are significantly different among treatments ($P < 0.05$).

Figure 1. Plasma concentrations of glucagon-like peptide-1 (GLP-1) and ghrelin during the 6-h infusions of control (water), C18:1, C18:2, and C18:3. Data are expressed as the least squares means ($n = 4$). Open plots indicate significant differences with 0 h of each treatment ($P < 0.05$).

with MCFA; accordingly, MCFA consumption increased stomach ghrelin in rats (Nishi et al., 2005) and in dairy cows (Fukumori et al., 2013). However, whether directly infusing MCFA to the small intestine (where MCFA are absorbed) would increase plasma ghrelin concentration has not been unclear. Plasma ketone body concentrations were greater in C8:0 and C10:0 infusions than control ($P < 0.05$, Table 1) in this study, consistent with our previous study that supplemented bovine diets with MCFA (Fukumori et al., 2013). These results are likely due to the rapid absorption of MCFA into the portal vein and then to the liver, where they are metabolized into ketone bodies. In support of this pathway, they might be difficult to transport from the liver as MCFA because plasma triglyceride concentrations were lower in C8:0, C10, and C12:0 infusions than for control ($P < 0.05$, Table 1). Therefore, plasma ghrelin concentrations are probably related to peripheral circulating MCFA, and the 6-h infusion period in our study was too short to generate the conditions that stimulate ghrelin secretions. Our previous studies that examined the effects of feeding FA on gut-peptide secretions were evaluated by 2-wk feeding trial. Because we did not measure plasma FA profiles in this study, we were unable to discuss the relationship between FA profiles in the blood circulation and ghrelin. Future studies should aim to test whether intravenous FA infusions could trigger ghrelin secretion.

During the experimental periods, plasma NEFA concentrations were higher under FA infusions except for C18:3 than under control conditions ($P < 0.05$, Table 1). Similar increases in plasma NEFA were observed when cow diets were supplemented with fat (Relling et al., 2007; Fukumori et al., 2012b). However, we did not observe the relationship between NEFA and these gut peptides.

Among the available lipid supplement sources, calcium salts of palm oil FA decreased DMI, whereas hydrogenated FA did not affect DMI (Allen, 2000). The profile of FA reaching the duodenum is important to decide DMI (Drackley et al., 1992). Numerous studies recognized that FA have potent physiologic effects on endocrine signaling (Benson and Reynolds, 2001; Reeling and Reynolds, 2007, 2008; Bradford et al., 2008). Our present study is the first to show the effects of individual FA on GLP-1 and ghrelin secretion in ruminants. As a result, we demonstrated that C18:1, C18:2, and C18:3 increase plasma GLP-1 concentrations. On the other hand, C18:1 and C18:3 also increased plasma ghrelin concentrations. The results suggest that these gut peptide secretions by lipid supplementations would complexly modulate DMI in ruminants and further studies are required to elucidate their each impact on feed intake.

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LITERATURE CITED

- Allen, M. S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. *J. Dairy Sci.* 83:1598–1624. doi:10.3168/jds.S0022-0302(00)75030-2
- Benson, J. A., and C. K. Reynolds. 2001. Effects of abomasal infusion of long-chain fatty acids on splanchnic metabolism of pancreatic and gut hormones in lactating dairy cows. *J. Dairy Sci.* 84:1488–1500. doi:10.3168/jds.S0022-0302(01)70182-8
- Bradford, B. J., K. J. Harvatine, and M. S. Allen. 2008. Dietary unsaturated fatty acids increase plasma glucagon-like

- peptide-1 and cholecystokinin and may decrease premeal ghrelin in lactating dairy cows. *J. Dairy Sci.* 91:1443–1450. doi:10.3168/jds.2007-0670
- Drackley, J. K., T. H. Klusmeyer, A. M. Trusk, and J. H. Clark. 1992. Infusion of long-chain fatty acids varying in saturation and chain length into the abomasum of lactating dairy cows. *J. Dairy Sci.* 75:1517–1526. doi:10.3168/jds.S0022-0302(92)77908-9
- Elsabagh, M., Y. Inabu, T. Obitsu, and T. Sugino. 2017. Response of plasma glucagon-like peptide-2 to feeding pattern and intraruminal administration of volatile fatty acids in sheep. *Domest. Anim. Endocrinol.* 60:31–41. doi:10.1016/j.domaniend.2017.03.001
- Enjalbert, F., M. C. Nicot, C. Bayourthe, and R. Moncoulon. 1998. Duodenal infusions of palmitic, stearic or oleic acids differently affect mammary gland metabolism of fatty acids in lactating dairy cows. *J. Nutr.* 128:1525–1532.
- Faulkner, A. 1991. Sensitivity of serum insulin concentrations to glucagon-like peptide 1 (7-36) amide in sheep. *Biochem Soc Trans.* 19:311S.
- Fukumori, R., T. Mita, T. Sugino, T. Obitsu, and K. Taniguchi. 2012a. Plasma concentrations and effects of glucagon-like peptide-1 (7-36) amide in calves before and after weaning. *Domest. Anim. Endocrinol.* 43:299–306. doi:10.1016/j.domaniend.2012.05.003.
- Fukumori, R., T. Sugino, H. Shingu, N. Moriya, Y. Hasegawa, M. Kojima, K. Kangawa, T. Obitsu, S. Kushibiki, and K. Taniguchi. 2012b. Effects of calcium salts of long-chain fatty acids and rumen-protected methionine on plasma concentrations of ghrelin, glucagon-like peptide-1 (7 to 36) amide and pancreatic hormones in lactating cows. *Domest. Anim. Endocrinol.* 42:74–82. doi:10.1016/j.domaniend.2011.09.005
- Fukumori, R., T. Sugino, H. Shingu, N. Moriya, H. Kobayashi, Y. Hasegawa, M. Kojima, K. Kangawa, T. Obitsu, S. Kushibiki, et al. 2013. Ingestion of medium chain fatty acids by lactating dairy cows increases concentrations of plasma ghrelin. *Domest. Anim. Endocrinol.* 45:216–223. doi:10.1016/j.domaniend.2013.09.005
- Fukumori, R., T. Sugino, H. Shingu, N. Moriya, H. Kobayashi, K. Yamaji, M. El-Sabagh, Y. Hasegawa, M. Kojima, K. Kangawa, et al. 2015. Effects of fat-enriched diet and methionine on insulin sensitivity in lactating cows. *J. Anim. Sci.* 93:2778–2784. doi:10.2527/jas.2015-8868
- Hayashida, T., K. Murakami, K. Mogi, M. Nishihara, M. Nakazato, M. S. Mondal, Y. Horii, M. Kojima, K. Kangawa, and N. Murakami. 2001. Ghrelin in domestic animals: distribution in stomach and its possible role. *Domest. Anim. Endocrinol.* 21:17–24.
- Kreymann, B., G. Williams, M. A. Ghatei, and S. R. Bloom. 1990. Glucagon like peptide-1 7-36: A physiological incretin in man. *J. Endocrinol.* 126:169–173.
- Nishi, Y., H. Hiejima, H. Hosoda, H. Kaiya, K. Mori, Y. Fukue, T. Yanase, H. Nawata, K. Kangawa, and M. Kojima. 2005. Ingested medium-chain fatty acids are directly utilized for the acyl modification of ghrelin. *Endocrinology* 146:2255–2264. doi:10.1210/en.2004-0695
- NRC. 2007. Nutrient requirements of small ruminants: sheep, goats, and New World camelids. Natl. Acad. Press., Washington, DC.
- Relling, A. E., S. C. Loerch, and C. K. Reynolds. 2010. Plasma ghrelin and oxyntomodulin concentrations in lactating dairy cows receiving abomasal soybean oil, corn starch, and casein infusions. *Domest. Anim. Endocrinol.* 38:284–288. doi:10.1016/j.domaniend.2009.12.003
- Relling, A. E., J. L. Pate, C. K. Reynolds, and S. C. Loerch. 2010. Effect of feed restriction and supplemental dietary fat on gut peptide and hypothalamic neuropeptide messenger ribonucleic acid concentrations in growing wethers. *J. Anim. Sci.* 88:737–748. doi:10.2527/jas.2009-2316
- Relling, A. E., and C. K. Reynolds. 2007. Feeding rumen-inert fats differing in their degree of saturation decreases intake and increases plasma concentrations of gut peptides in lactating dairy cows. *J. Dairy Sci.* 90:1506–1515. doi:10.3168/jds.S0022-0302(07)71636-3
- Relling, A. E., and C. K. Reynolds. 2008. Abomasal infusion of casein, starch and soybean oil differentially affect plasma concentrations of gut peptides and feed intake in lactating dairy cows. *Domest. Anim. Endocrinol.* 35:35–45. doi:10.1016/j.domaniend.2008.01.005
- Relling, A. E., J. L. Pate, C. K. Reynolds, and S. C. Loerch. 2009. Effect of feed restriction and supplemental dietary fat on gut peptide and hypothalamic neuropeptide messenger ribonucleic acid concentrations in growing wethers. *J. Anim. Sci.* 88:737–748. doi:10.2527/jas.2009-2316
- Sugino, T., Y. Hasegawa, Y. Kurose, M. Kojima, K. Kangawa, and Y. Terashima. 2004. Effects of ghrelin on food intake and neuroendocrine function in sheep. *Anim. Reprod. Sci.* 82-83:183–194. doi:10.1016/j.anireprosci.2004.05.001