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The Journal of Nutrition
Nutrient Physiology, Metabolism, and Nutrient-Nutrient Interactions

Dietary Actinidin from Kiwifruit (*Actinidia deliciosa* cv. Hayward) Increases Gastric Digestion and the Gastric Emptying Rate of Several Dietary Proteins in Growing Rats[1,2]

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Abstract

Dietary actinidin influences the extent to which some dietary proteins are digested in the stomach, and it is hypothesized that the latter modulation will in turn affect their gastric emptying rate (GE). In this study, the effect of dietary actinidin on GE and gastric digestion of 6 dietary protein sources was determined in growing rats. Each dietary protein source [beef muscle, gelatin, gluten, soy protein isolate (SPI), whey protein isolate, and zein] was included in 2 semisynthetic diets as the sole nitrogen source. For each protein source, 1 of the 2 diets contained actinidin [76.5 U/g dry matter (DM)] in the form of ground freeze-dried green kiwifruit (*Actinidia deliciosa* cv. Hayward), whereas the other diet contained freeze-dried gold kiwifruit (*Actinidia chinensis* cv. Hort16A), which is devoid of actinidin (3.4 U/g DM). For both diets, dietary kiwifruit represented 20% of the diet on a DM basis. The real-time GE was determined in rats gavaged with a single dose of the diets using magnetic resonance spectroscopy over 150 min ($n = 8$ per diet). Gastric protein digestion was determined based on the free amino groups in the stomach chyme collected from rats fed the diets ($n = 8$ per diet) that were later killed. GE differed across the protein sources [e.g., the half gastric emptying time ($T_{½}$) ranged from 157 min for gluten to 266 min for zein] ($P < 0.05$). Dietary actinidin increased the gastric digestion of beef muscle (0.6-fold), gluten (3.2-fold), and SPI (0.6-fold) and increased the GE of the diets containing beef muscle (43% $T_{½}$) and zein (23% $T_{½}$; $P < 0.05$). There was an inverse correlation between gastric protein digestion and DM retained in the stomach ($r = -0.67$; $P < 0.05$). In conclusion, dietary actinidin increased gastric protein digestion and accelerated the GE for several dietary protein sources. GE may be influenced by gastric protein digestion, and dietary actinidin can be used to modulate GE and protein digestion in the stomach of some dietary protein sources but not others. J. Nutr. 144: 440–446, 2014.

Introduction

Previous in vitro (1) and in vivo (2,3) studies have provided evidence that supports the hypothesis that the presence of dietary actinidin (a cysteine-protease present in green kiwifruit, *Actinidia deliciosa* cv. Hayward) increases the rate and extent of the gastric digestion of some individual proteins in protein sources, such as soy protein isolate (SPI)[6], beef muscle, gelatin, and gluten. However, in contrast, the gastric digestion of some individual proteins, such as some of the proteins in whey protein isolate (WPI) and zein, was unaffected by dietary actinidin. Interestingly, it has been suggested that the gastric emptying rate (GE) can be influenced by the rate at which proteins are digested in the stomach (4–7). It is possible then that dietary actinidin may affect the GE via its modulation of gastric protein digestion.

There is a dearth of published literature describing the relation between the rate and extent of gastric digestion and GE for foods that vary in the type of protein present and also the role that dietary actinidin may have on the GE. Given that the GE can affect the overall rate of digestion and absorption of nutrients (4,8,9), exploring the possibility that dietary actinidin can affect GE via an altered gastric digestion of proteins may be an important undertaking for individuals interested in increasing the rate of amino acid delivery to promote muscle protein synthesis (e.g., athletes and the elderly) (10,11). The present study may also be important for individuals experiencing feelings of fullness after consuming high-protein diets or possibly those with impaired gastric and small intestinal digestion. Recently, a magnetic resonance spectroscopy (MRS) technique that permits the measurement of GE

[1] Supported by Zespri International.
[2] Author disclosures: C. A. Montoya, J. P. Hindmarsh, L. Gonzalez, M. J. Boland, P. J. Moughan, and S. M. Rutherfurd, no conflicts of interest.
[6] Abbreviations used: DM, dry matter; GE, gastric emptying rate; MRS, magnetic resonance spectroscopy; SPI, soy protein isolate; $T_{½}$, half gastric emptying time; WPI, whey protein isolate.
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Manuscript received September 26, 2013. Initial review completed October 24, 2013. Revision accepted December 4, 2013.
First published online January 15, 2014; doi:10.3945/jn.113.185744.

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in real time in growing rats has been optimized and validated (12). This technique provides a straightforward and non-invasive approach for measuring GE and permits a more robust statistical analysis, based on repeated measures across time, when compared with the traditional slaughter method (12).

The study reported here aimed to extend the previous work of Rutherfurd et al. (2) by exploring the effect of dietary actinidin (in the form of ground freeze-dried green kiwifruit) on the GE (determined based on an MRS technique) of diets containing different protein sources and on gastric protein digestion. The study used the growing rat as an animal model for the adult human.

Materials and Methods

Sample preparation

Fresh green kiwifruit (*Actinidia deliciosa* cv. Hayward), which is rich in actinidin activity [284 U/g dry matter (DM)], and gold kiwifruit (*Actinidia chinensis* cv. Hort16A), which is almost devoid of actinidin activity (3,13), were provided by Zespri International. The preparation of the kiwifruit before inclusion in the diets is detailed in the study by Rutherfurd et al. (2). The protein sources (beef muscle protein, gelatin, wheat gluten, SPI, WPI, and zein) used in the study were also the same as those described by Rutherfurd et al. (2). Despite the small differences in the chemical composition between green and gold kiwifruit, gold kiwifruit is a suitable control for examining the impact of dietary actinidin on gastric digestion and GE studies (3). Therefore, in the present study, a diet containing gold kiwifruit was used as a control for each protein source.

Dietary treatments

Six diets, each containing 1 of the 6 different protein sources and each containing dietary actinidin from the addition of finely ground freeze-dried green kiwifruit (treatments), and 6 diets that were comparable but did not have dietary actinidin (gold replaced green kiwifruit, controls) were formulated to have a similar chemical composition [144 ± 2.7 g (mean ± SE) crude protein/kg DM] and to meet the nutrient requirements of the growing rat, with the exception of protein (14). A protein-free diet was also formulated to allow the determination of endogenous gastric protein flows. The diets are described fully by Rutherfurd et al. (2). Briefly, the diets were composed of (per kilogram of final diet): 50 g of soybean oil, 50 g of cellulose, 100 g of sucrose, 100 g of mineral–vitamin premix [as given by Balan et al. (15)], 3 g of titanium dioxide (an indigestible marker), 122–155 g of protein source (137, 122, 155, 143, 133, and 137 g for beef muscle, gelatin, gluten, SPI, WPI, and zein, respectively), 200 g of ground freeze-dried kiwifruit (either green kiwifruit for the diets containing actinidin or gold kiwifruit for the diets not containing actinidin), and wheat starch (342–374 g). In addition, 14 mg of purified actinidin containing 2154 U/g DM (New Zealand Pharmaceuticals) was added per gram of diet to all of the green kiwifruit–containing diets to compensate for the determined amount of actinidin activity lost during the freeze-drying step (284 U/g DM was present in the fresh green kiwifruit pulp). For the GE determination, positive control diets containing gold kiwifruit and supplemented with purified actinidin to provide similar actinidin activity as that present in the comparable green kiwifruit-based diet were also prepared for 2 of the protein sources (beef muscle and SPI). The latter protein sources were chosen as candidates for the positive control diets based on the reported positive effect of actinidin on the gastric digestion of their individual proteins (2,3). The amount of kiwifruit present in the diet was the equivalent of consuming 2 kiwifruit with an average-size meal for an adult human (2,3).

Experimental design

Ethics approval for the animal trial (applications 08/85 and 10/33) was obtained from the Animal Ethics Committee of Massey University.

GE. Twenty-four Sprague-Dawley male rats (285 ± 6.9 g bodyweight) consumed a proprietary unpurified rat diet formulated to meet the rat's nutritional requirements ad libitum (14). The MRS technique is non-invasive and therefore permits the same rats to be used for >1 treatment. However, the rats must be used when they are between 275 and 315 g bodyweight (12), which constitutes a period of ~2 wk. Given that the rats should have a 2-d stand-down period between scan days, there was insufficient time to allow all rats to undergo MRS analysis with all the experimental diets (i.e., 12 treatments plus 2 positive control diets). Consequently, the dietary treatments were randomly allocated to rats such that there were 8 rats per treatment, and each rat received 4–5 dietary treatments. Before each MRS test day, the rats were deprived of food and water was withdrawn for a 14-h period. On the test day, the rats were gavaged with 2 mL of a mixture containing 1 of the test or control diets described above (i.e., 1 replicate of each treatment was tested) as well as $AlCl_3$-$6H_2O$ and Milli-Q water acidified to pH 2 (7:1:9, wt:wt:v) (12). The rats were then scanned every 25 min up to 150 min in a Bruker AMX 200 horizontal bore instrument as described previously (12).

Gastric total protein hydrolysis. One hundred four Sprague-Dawley male rats (197 ± 2.9 g bodyweight) were housed individually and were randomly allocated to the 12 diets (n = 8 per treatment), as described previously (2). Briefly, the rats were adapted to the feeding regimen (frequent feeding) for 13 d with basal diets. The rats were then fed with the experimental diets before being killed as fully described by Rutherfurd et al. (2). Freeze-dried gastric chyme samples were used to determine the effect of dietary actinidin on gastric total protein digestion based on the content of free amino groups in the chyme. The free amino group content in the chyme samples and the total amino group content in the diets (determined after acid hydrolysis in 6 mol/L HCl for 24 h at 100°C in an oxygen-free environment) were determined using the *o*-phthaldialdehyde method as described by Montoya et al. (3) and based on the method described by Church et al. (16).

Actinidin activity analyses

Each experimental diet was mixed with 0.05 mol/L phosphate buffer (pH 6; 4°C) containing 0.4 mol/L sodium metabisulfite (~0.5 g/mL) to avoid enzyme oxidation. The mixture was centrifuged at 13,000 × g for 30 min at 4°C, and the supernatants were collected to measure actinidin activity as described previously (3). Actinidin activity (micromoles per liter of *p*-nitrophenol per minute per gram of DM) is expressed throughout the text as units per gram DM.

Calculations and statistical analyses

The gastric emptying profile was derived based on the determined relative Al concentration in the stomach as a function of time. The relative Al concentration was calculated as follows:

$$\text{Relative Al concentration}\,(\%) = \text{Al-MRS peak area}_{(\text{Time}i)}/\text{Al-MRS peak area}_{(\text{Time}0)} \times 100,$$

where time 0 is the time of the initial MRS scan taken immediately after gavaging, and time i is the time of the subsequent MRS scans.

The gastric digestion of total protein based on the free amino group content of gastric chyme was calculated as follows:

$$\text{True gastric total protein digestion}\,(\%) = \left[\left(\text{NH}_{2\,\text{Chyme}} \times \text{TiO}_{2\,\text{Diet}}/\text{TiO}_{2\,\text{Chyme}}\right) - \text{NH}_{2\,\text{EF}}\right]/\text{Total NH}_{2\,\text{Diet}} \times 100,$$

where Total $NH_{2\ Diet}$ is the free amino groups (grams per kilograms DM) in the diet determined after acid hydrolysis. $NH_{2\ Chyme}$ is the free amino groups (grams per kilograms DM) in the chyme. The units for the TiO_2 content were grams per kilograms DM. The NH_{2EF} (grams per kilograms DM intake) was the flow of gastric endogenous amino groups, which was calculated as follows from the stomach chyme collected from the rats receiving the protein-free diet (units are grams per kilograms):

$$\text{Endogenous NH}_2\ \text{flow}\,(\text{g/kg DM intake}) = \text{NH}_{2\,\text{Chyme}} \times \text{TiO}_{2\text{Diet}}/\text{TiO}_{2\text{Chyme}}.$$

The number of replications (n = 8) required to detect a statistical difference between treatments for each experiment was estimated, with a power >80% at a 2-tail 5% significance level, based on estimates of variance and means found in previous studies (3,12). All statistical

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analyses were performed using SAS statistical software (17). The effect of protein source or the effect of actinidin on the relative Al retention in the stomach over time for each protein source was tested after fitting the power exponential model reported by Elashoff et al. (18):

$$\text{Relative Al content remaining}_{\text{Time}} = \alpha_0 \exp-(\kappa \times \text{Time})^{\beta},$$

where α_0 is the relative Al content at time 0 (i.e., 100%), κ is the slope of the curve, and β is an index for the shape of the curve. A repeated-measures analysis was conducted using each rat as the experimental unit to estimate the parameters (κ and β) using the nonlinear mixed-effects model PROC NLMIXED of SAS (19). The above analysis was chosen after comparison with a linear repeated-measures model (20) based on the Akaike's and Bayesian's information criteria. The assumption of homoscedasticity was not fulfilled (a greater variation was observed for the data obtained at the shorter postprandial times). Therefore, a cubic transformation of the raw data was performed before conducting the statistical analysis.

The nonlinear mixed-effects model was also used to conduct a comparison of the parameters (κ and β) between treatments using the dummy variable technique (19). The $T_{½}$ (the time at which half the $AlCl_3$ present at time 0 had exited the stomach) was calculated from the estimated parameters (κ and β) using the "Estimate" statement of SAS and the equation proposed by Odunsi et al. (21):

$$T_{1/2} = (1/\kappa) \times [\text{Log}(1/0.5)]^{(1/\beta)}.$$

$T_{½}$ values were compared between individual treatments using Student's t test (22).

The interaction between protein source and actinidin activity with respect to gastric digestion of total protein (22) was tested using the PROC MIXED model of SAS. A significant interaction ($P < 0.001$) was observed between the protein source and actinidin activity for gastric digestion, and, consequently, the effect of actinidin on the gastric digestion of total protein was tested for each protein source individually using Student's t test (PROC TTEST of SAS). To examine the effect of protein source on the gastric digestion of total protein (diets without actinidin) and to test for differences in the food intake on day 14 across dietary treatments, a completely randomized analysis was performed using the PROC MIXED model of SAS. The model diagnostics (e.g., normal distribution, equal variance across treatments) of each variable were tested combining the PROC UNIVARIATE and the ODS GRAPHICS options of SAS. When the F value of the analysis of variance was significant ($P < 0.05$) for the PROC MIXED analysis, the means for each protein source (without actinidin) were compared using Tukey's test. Correlation analyses were performed between the gastric digestion of protein and the DM retained in the stomach or $T_{½}$ using the PROC CORR option of SAS.

Results

The rats for both gastric protein digestion and GE studies appeared healthy during the study, with the exception of a rat that died in the gastric protein digestion study for unknown reasons. For the rat study examining gastric protein digestion, the food intake on the final day was not different across any of the dietary treatments [voluntary feed intake was 1.6 ± 0.7 g/meal (mean ± SD); $P > 0.05$]. The actinidin activity of the experimental diets is shown in **Table 1**. As expected, the actinidin activity was much higher in the green kiwifruit–containing diets compared with the gold kiwifruit–containing diets (71–89 vs. 2–5 U/g DM). The low amount of actinidin activity in the gold kiwifruit–containing diets reflects the low degree of activity of this enzyme in gold kiwifruit.

The goodness of fit of the nonlinear models was based on the residuals (e.g., normal distribution), the plot of the observed and fitted values (**Fig. 1**), and the proportion of the variance explained by the model (e.g., when the effect of actinidin was tested, r^2 = 0.96, 0.94, 0.88, 0.84, 0.92, and 0.81 for beef muscle, gelatin, gluten, SPI, WPI, and zein, respectively).

TABLE 1 Actinidin activity in experimental diets containing either freeze-dried green kiwifruit or freeze-dried gold kiwifruit[1]

	Green kiwifruit diet (with actinidin)	Gold kiwifruit diet (no actinidin)
	U/g DM	*U/g DM*
Beef muscle	71.6 ± 4.73	4.0 ± 0.22
Gelatin	74.5 ± 3.01	3.1 ± 0.74
Gluten	73.1 ± 2.91	4.5 ± 0.49
Soy protein isolate	70.7 ± 2.04	3.7 ± 1.09
Whey protein isolate	80.2 ± 2.99	2.0 ± 0.66
Zein	88.7 ± 3.72	3.2 ± 0.23

[1] Values are means ± SEMs; n = 3. DM, dry matter.

Effect of protein source on stomach emptying rate and gastric total protein digestion. Protein source had a significant effect on the parameters (β and κ) estimated for the GE model ($P < 0.05$; **Table 2**). The β parameter (shape of the curve) was significantly higher for zein when compared with the other protein sources (1.7 vs. <1.3; $P < 0.05$). The κ parameter (slope of the curve) for gluten was higher than that estimated for beef muscle protein, gelatin, and zein (12.4 vs. 6.9, 7.5, and 6.1, respectively). In addition, the κ parameter for SPI was higher than that for beef muscle and zein ($P < 0.05$). This difference in the κ value observed across protein sources was reflected by the $T_{½}$ values in which zein, beef muscle, and gelatin had higher mean $T_{½}$ values (245–266 min) than gluten (157 min).

The flow of endogenous free amino groups (0.6 ± 0.08 g/kg DM intake) determined with the protein-free diet was used to correct apparent gastric digestion of total protein values to true gastric digestion of total protein values for the 6 protein sources (Table 2). Protein source had a significant effect on gastric total protein digestion ($P < 0.001$). The digestion of total protein in WPI was greater than that observed for other protein sources, and the digestion of beef muscle was greater than that observed for gluten, gelatin, or zein ($P < 0.001$).

Effect of dietary actinidin on stomach emptying rate and gastric total protein digestion. The gastric emptying profiles

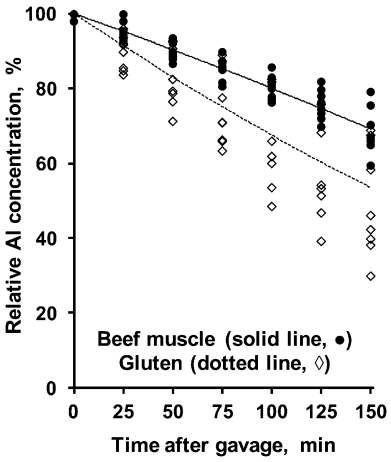

FIGURE 1 Observed values and fitted nonlinear model for the relative Al concentration in the stomachs of growing rats gavaged with beef muscle and gluten diets containing gold kiwifruit and determined by Al magnetic resonance spectroscopy. The fitted model was obtained after back transformation of the predicted values of gastric retention calculated from the β and κ parameters presented in Table 2.

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TABLE 2 Gastric diet retention parameters and gastric digestion of total protein for diets containing different protein sources and with either green kiwifruit (with actinidin) or gold kiwifruit (no actinidin) determined in growing rats[1]

Protein source	Gastric retention parameters			Gastric digestion
	β	κ	$T_{½}$	
		$\times 10^3$	*min*	%
No actinidin[2]				
Beef muscle	1.21 ± 0.139^{b}	6.9 ± 0.46^{c}	107 ± 4.9^{a} (260)	9.2 ± 0.62^{b}
Gelatin	1.25 ± 0.101^{b}	7.5 ± 0.85^{b,c}	100 ± 11.4^{a,b} (245)	5.4 ± 0.29^{c,d}
Gluten	1.19 ± 0.098^{b}	12.4 ± 1.92^{a}	59 ± 10.4^{c} (157)	4.4 ± 0.21^{d}
Soy protein isolate	1.33 ± 0.101^{b}	9.2 ± 0.78^{a,b}	82 ± 6.7^{b,c} (189)	7.7 ± 0.46^{b,c}
Whey protein isolate	1.08 ± 0.131^{b}	8.9 ± 1.78^{a,b,c}	80 ± 14.4^{b,c} (182)	12.1 ± 1.18^{a}
Zein	1.73 ± 0.114^{a}	6.1 ± 0.56^{c}	134 ± 11.8^{a} (266)	5.1 ± 1.41^{c,d}
With actinidin				
Beef muscle	1.12 ± 0.156	11.8 ± 2.60*	61 ± 13.1* (144)	14.9 ± 2.91*
Gelatin	1.08 ± 0.113	7.5 ± 1.13	95 ± 12.7 (228)	6.6 ± 0.66
Gluten	1.16 ± 0.120	12.7 ± 0.89	58 ± 4.4 (151)	18.3 ± 0.44*
Soy protein isolate	1.29 ± 0.101	10.7 ± 1.23	70 ± 8.0 (159)	12.3 ± 0.68*
Whey protein isolate	1.05 ± 0.082	10.4 ± 1.67	68 ± 10.7 (185)	13.3 ± 1.79
Zein	1.15 ± 0.170*	7.0 ± 0.97	103 ± 11.1 (249)	5.4 ± 1.52

[1] Values are means ± SEMs; $n = 8$. The gastric emptying parameters were obtained after cubic transformation of the raw data. The values in parentheses represent $T_{½}$ obtained after back transformation of the predicted values of gastric retention calculated from the β and κ parameters. The gastric digestion values represent the degree of total protein hydrolysis (free amino groups) corrected for endogenous free amino groups for the diets across the first 5 h of the postprandial period. Protein source ($P < 0.001$), actinidin activity ($P < 0.001$), and the interaction between protein source and actinidin activity ($P < 0.001$) had significant effects on gastric digestion. *Different from the no actinidin group ($P < 0.05$). $T_{½}$, half gastric emptying time.
[2] Values in a given column without a common letter (a > b > c) differ ($P < 0.05$).

for the 6 diets (different protein sources), either containing (freeze-dried green kiwifruit) or not containing (freeze-dried gold kiwifruit) actinidin, during the first 150 min after gavage are shown in **Figure 2**. The parameters (β, κ, and $T_{½}$) of the GE model (based on the relative Al content remaining in the stomach) are presented in Table 2. The parameter κ was significantly ($P < 0.05$) higher when actinidin was present in the diet for beef muscle protein and the parameter β for zein was lower compared with when actinidin was not present in the diet. The predicted $T_{½}$ was significantly lower for the beef muscle protein diet when actinidin was present in the diet. In contrast, the presence of dietary actinidin did not significantly influence β and κ parameters for the GE model or the predicted $T_{½}$ for the diets containing gelatin, gluten, SPI, and WPI ($P > 0.05$).

Dietary actinidin activity had a significant effect on the gastric digestion of total protein for beef muscle, gluten, and SPI ($P < 0.05$; Table 2). For the latter protein sources, the extent of gastric digestion was greater in the presence of dietary actinidin. In contrast, the presence of dietary actinidin did not influence the extent of gastric digestion for the diets containing gelatin, WPI, and zein ($P > 0.05$).

To determine the relation between the gastric digestion of total protein and GE, a correlation analysis was done using the 12 diets (6 protein sources × 2 levels of dietary actinidin), comparing gastric total protein digestion with both mean gastric retention of DM and the predicted $T_{½}$. There was a significant negative correlation between gastric digestion of total protein and the DM retained in the stomach after 60 min from gavaging ($r = -0.67$, $P < 0.05$, $n = 12$), as well as between gastric digestion of total protein and the predicted $T_{½}$ ($r = -0.65$, $P < 0.05$, $n = 12$) (**Fig. 3**).

Discussion

The aim of the present study was to evaluate the effect of dietary actinidin on the GE of a range of dietary protein sources in the growing rat and to investigate the relation between GE and the extent of gastric protein digestion. To do this, the gastric digestion of total dietary protein was quantified based on the analysis of free amino groups in the chyme. Furthermore, GE was characterized using a recently validated Al-MRS technique that is based on the amount of Al (GE marker) present in the stomach (MRS only detects Al in an acidic environment) at given times after a single meal (12). Montoya et al. (12) reported a significant correlation (r = 0.81–0.95) between the GE of Al, determined using MRS, and the GE of DM and nitrogen determined using the conventional slaughter technique. Consequently, it was assumed in the present study that the GE of Al reflected the GE of both DM and protein for the experimental diets. It should be noted that chyme likely contains proteins at various stages of digestion, but the protein and peptide composition of the chyme collected in the present study should have been representative of chyme across the first 5.5 h of the postprandial period. The fact that 2 different feeding regimens were used to measure protein digestion (frequent feeding regimen) and GE (single oral gavaged dose), respectively, is noted. In rats, the ileal nitrogen flow and nitrogen digestibility of diets containing different proteins sources was not influenced by different feeding regimens (frequent feeding regimen, ad libitum feeding, and meal feeding) (23). In the present study, it is assumed that the different feeding regimens will have no impact on the relation between gastric digestion and GE. It is also of note that heavier rats were required to determine GE compared with those used to determine gastric digestion of protein, but it would not be expected that bodyweight would affect the extent of gastric protein digestion over this bodyweight range. In addition, a protein-free diet was used to determine the flow of endogenous free amino groups in the stomach. Previous studies have shown that the flow of endogenous protein at the terminal ileum is lower for a protein-free diet than for a diet containing dietary peptides (24). It is possible that the endogenous free

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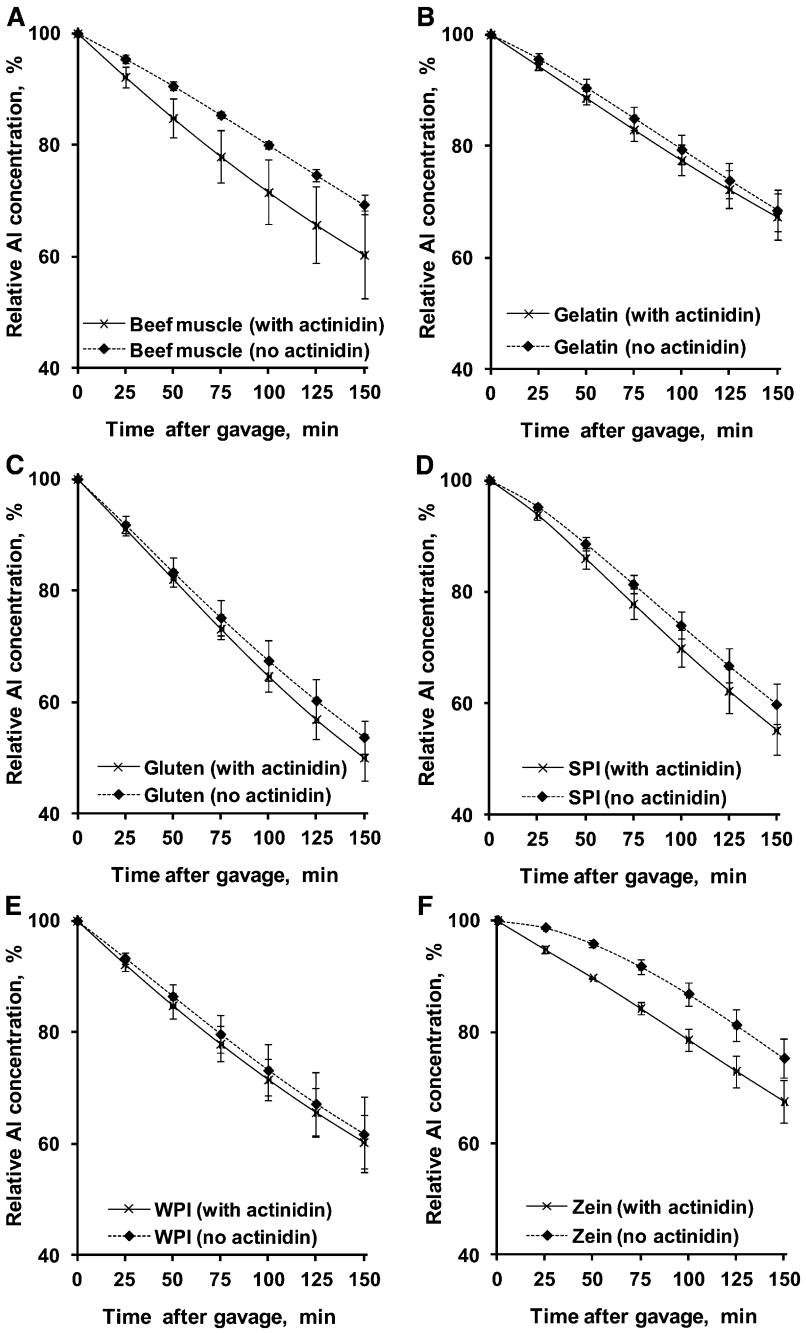

FIGURE 2 Relative AI concentration in the stomachs of growing rats gavaged with beef muscle (*A*), gelatin (*B*), gluten (*C*), soy protein isolate (*D*), whey protein isolate (*E*), and zein (*F*) diets containing either green kiwifruit (with actinidin) or gold kiwifruit (no actinidin) and determined by AI magnetic resonance spectroscopy. The values were obtained after back transformation (i.e., cubic root) of the predicted values of gastric retention calculated from the parameters (Table 2) estimated for the gastric emptying model (18). Values are means ± SEMs; n = 8. SPI, soy protein isolate; WPI, whey protein isolate.

amino acids in the stomach similarly also would be lower and that the true gastric digestion presented here for the different protein sources may have been overestimated. Nevertheless, the protein-free diet may be considered to be the best approach, because the free amino groups of a diet containing dietary peptides may be confounded with those from the endogenous proteins. Keeping the latter caveats in mind, the impact of dietary actinidin on gastric protein digestion is discussed below.

From the presently reported gastric protein digestion results, it can be concluded that dietary actinidin increased the extent of protein digestion in the stomach for diets containing beef muscle (0.6-fold), gluten (3.2-fold), and SPI (0.6-fold), but not for diets containing gelatin, WPI, or zein. Why the digestion of some dietary proteins is affected by the presence of actinidin in the diet whereas for others there was no effect may be attributable to differences in the structure (primary, secondary, or tertiary, or a combination) (25) of the individual proteins comprising each dietary protein source.

Recently, a study undertaken with pigs examined the effect of dietary actinidin (in the form of fresh green kiwifruit pulp) on the kinetics of gastric digestion of individual proteins in a beef muscle protein–containing diet during the first 7 h after meal ingestion. SDS-PAGE was used to estimate the amounts of individual proteins in both the diets and gastric chyme (3). Dietary actinidin increased the gastric digestion of the high-molecular–weight proteins (e.g., myosin-heavy chain, β-actinin, α-actinin, desmin, actin, tropomyosin–β-chain, and tropomyosin–α-chain) but not that of the low-molecular–weight proteins (<32 kDa as troponin I) (3). The latter result suggests that the extent and rate of digestion is protein-specific even within a single protein source. In the context of the presently reported study, the observed effect of actinidin across different protein sources is

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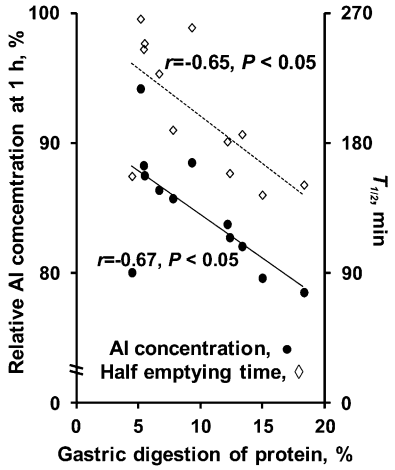

FIGURE 3 Linear correlations between the gastric digestion of protein and the relative Al concentration at 1 h in the stomach and the $T_{½}$. Values are means; n = 8. $T_{½}$, half gastric emptying time.

likely to be the result of the summation of the actinidin effect across the individual proteins within each protein source.

The hypothesis that the actinidin present in the diet, rather than some other dietary component, was responsible for the observed effect on the GE was supported when purified actinidin was incorporated in the beef muscle protein and SPI diets containing gold kiwifruit to give the same actinidin activity (67.5 and 69.2 U/g DM of actinidin activity, respectively) as the green kiwifruit diet (71.6 and 70.7 U/g DM of actinidin activity, respectively). The diets containing gold kiwifruit supplemented with purified actinidin had $T_{½}$ values that were not different from the equivalent diets containing green kiwifruit (198 ± 14 and 144 ± 31 min, respectively, for beef muscle protein and 142 ± 17 and 159 ± 18 min, respectively, for SPI; $P > 0.05$).

In the present study, the presence of actinidin in the diets increased the GE for diets containing beef muscle and zein, but it did not increase the GE of diets containing gelatin, gluten, SPI, and WPI. The positive effect of actinidin on the GE of beef muscle protein has also been observed in pigs (3), whereby 3 h postprandially, 59% of the consumed DM and 62% of the consumed nitrogen had exited the stomach when actinidin was present in the diet (in the form of fresh green kiwifruit pulp) compared with 50 and 54% for DM and nitrogen, respectively (when actinidin was not present in the diet).

Previous studies (4–6,8,9,26) have demonstrated that different dietary protein sources (e.g., casein, SPI, and WPI) are able to modulate the rate at which chyme exits the stomach in both animals and humans. Moreover, in some of these studies, it was hypothesized that the rate of protein digestion in the stomach may have been partially responsible for this modulation. However, no published studies have attempted to investigate the relation between the extent of gastric digestion of protein and GE. A significant negative relation between the extent of gastric total protein digestion and the DM remaining in the stomach was observed in the presently reported study ($r = -0.67$, n = 12) when all dietary treatments were examined together, providing direct evidence that GE is, at least in part, dependent on the gastric digestion of proteins. When the gluten and the zein treatments without actinidin were removed from the latter analysis "outliers," the correlation improved markedly ($r = -0.93$, $P < 0.001$, n = 10). The gluten and zein (without actinidin) treatments did not appear to follow the relation between GE and gastric total protein digestion observed for the other dietary treatments. It is perhaps not unexpected that the GE of protein is influenced to some degree by protein digestion in the stomach and that the latter relation is stronger for some proteins and not others. To illustrate the point, casein clots in the stomach and empties more slowly, whereas whey protein is soluble in the stomach and empties more quickly (5,8,9). The casein macrostructure must first be broken down via digestion before it can empty from the stomach, whereas the soluble whey proteins can empty from the stomach in their intact form.

Factors other than gastric protein digestion have also been reported to affect GE, and these include diet composition (e.g., fiber, fat, and protein) (27–29), the physical properties of the food (e.g., particle size, viscosity) (30,31), the rate of release of nutrients into the duodenum (6,7,32), and gut hormone secretion (e.g., cholecystokinin, gastrin, secretin) (6,33,34). Moreover, it is likely that, in addition to any direct effect on GE, some of the factors given above may influence GE via modulation of gastric protein digestion itself. For example, reducing the particle size may lead to a more rapid GE directly and also lead to a more rapid GE via a more rapid digestion of the smaller particles.

The presently reported results support the hypothesis that dietary actinidin increases the extent of gastric digestion of some, but not all, dietary proteins, and these findings are consistent with other in vitro (1) and in vivo (2,3) studies. In general, proteins that are able to reach the small intestine more rapidly are more readily available for subsequent small intestinal digestion, absorption, and therefore protein metabolism (4,8,9,35). In the context of the presently reported study, based on the GE at 1 h, the protein content of each diet, and the gastric digestion of protein, it is estimated that the amount of digestible protein released into the small intestine for the beef muscle, gelatin, gluten, SPI, WPI, and zein without actinidin (1.4, 0.9, 1.3, 1.5, 2.7, and 0.5 g/kg DM intake, respectively) was much lower than that for the same protein sources with actinidin (4.3, 1.5, 5.7, 3.0, 3.3, and 1.0 g/kg DM intake, respectively). Consequently, it is likely that dietary actinidin will lead to a more rapid amino acid absorption, which may be of interest to patients with gastric and small intestinal digestion problems or for individuals interested in increasing the rate of dietary amino acid delivery to cells for improving muscle protein mass, strength, and function (e.g., athletes or the elderly) via an increase in muscle protein synthesis (10,11). Additional studies focusing on the effect of actinidin on the rate of protein digestion and absorption in the small intestine and on muscle protein synthesis may be warranted.

In conclusion, the presence of dietary actinidin in the form of ground freeze-dried green kiwifruit in semisynthetic diets increased the extent of gastric digestion of some dietary protein sources (beef muscle protein, gluten, and SPI) but not others. Additionally, dietary actinidin increased the GE for some proteins (beef muscle protein and zein). A significant negative correlation between extent of gastric digestion of protein and GE was observed ($r = -0.67$; $P < 0.05$), suggesting that gastric protein digestion may be 1 of the factors that influences the GE for protein-containing diets.

Acknowledgments

The authors thank Trent Olson and Stuart Saigeman for their support in the animal trial, Estelle Bignaux for the amino-group determination, and Zespri International for supplying the kiwifruit. S.M.R., P.J.M., M.J.B., and C.A.M. contributed equally to the design of the experiment. Additionally, C.A.M. did the laboratory work and the statistical analysis and prepared the first draft of the paper that was revised by all the other authors. J.P.H. and L.G. did the laboratory work and the

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analysis of the magnetic resonance spectroscopy data. All authors read and approved the final manuscript.

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