CHAPTER FOUR

The effect of fibers on coagulation of casein-based enteral nutrition in an artificial gastric digestion model

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ABSTRACT

Background A serious complication seen in critically ill patients is the solidification of enteral nutrition causing gastrointestinal obstruction. It has been suggested that enteral nutrition enriched with insoluble fibers may increase the risk of this complication.

Objective To evaluate the effect of soluble and insoluble dietary fibers on the coagulation of a casein-based enteral nutrition in an artificial gastric digestion model.

Methods A 100 % casein-based enteral nutrition was enriched with increasing concentrations of soluble fibers (acacia fiber, oligofructose and inulin) and insoluble fibers (soy polysaccharide, resistant starch and alpha cellulose). After digestion in a artificial gastric model, the chyme was poured over sequentially placed sieves, separating the coagulate into size fractions of larger than 2 mm, between 1 and 2 mm, and between 0.25 and 1 mm. Of these fractions we measured wet weight, dry weight and protein content. A significant effect on the fraction larger than 2 mm was considered to be clinically relevant.

Results Addition of high concentrations soy polysaccharide and resistant starch to a casein-based enteral nutrition, did not alter the wet weight, whereas dry weight and protein content of the coagulate was significantly reduced.

Conclusions When high concentrations of soy polysaccharide and resistant starch are added to a 100 % casein-based enteral nutrition, the coagulate consist of more water and less proteins, which may lead to an increased protein digestion and absorption in a clinical setting. The suggestion that insoluble fibers increase the risk of gastrointestinal obstruction in critically ill patients is not supported by these data.

INTRODUCTION

Fibers are added to enteral nutrition (EN) to improve gastrointestinal (GI) tolerance (e.g. prevention of diarrhea and constipation), for glycaemic and lipid control and for their pre-biotic effect (1). In patients who are not critically ill, or who require long-term EN, the use of a mixture of insoluble, bulking and soluble, fermentable fibers would appear to be the best approach (2). Based on case reports (level 4 evidence), international guidelines state that insoluble fibers should be avoided in all critically ill patients, as they may increase the risk of bowel obstruction by the solidification of EN (3-5). GI obstruction by the solidification is a serious complication observed in critically ill patients (6). It is increasingly acknowledged that the coagulation of EN can be influenced by the composition of EN. Van den Braak *et al*. recently studied the effect of individual proteins on the coagulating properties of EN after simulated gastric digestion (7). The current study focuses on the effect of fibers on the coagulation of casein-based EN.

Given that different types of fibers have diverse biological effects, the composition of fiber-enriched EN has evolved towards blends of soluble and insoluble fibers (8). Dietary fibers can be classified according to their solubility in water as either soluble or non-soluble. After the discovery of the biological effect of fermentation by colonic microbiota in humans, fibers were additionally classified as being fermentable and non-fermentable (9). In general, soluble fibers are easily fermentable, whereas insoluble fibers are less easily fermentable. However, few exceptions exist. Insoluble soy polysaccharides and resistant starch are for example fermentable. In addition, some fibers have been shown to have a prebiotic effect, as these are selectively metabolized by distinct gut bacteria. For example the specific oligosaccharides like gos and fos. The prebiotic effect has also been reported to promote GI health (10).

Proteins and polysaccharides are important in the structure and stabilization of EN. Protein-polysaccharide interactions have been studied intensively, both experimentally and theoretically (11-15). Various interaction mechanisms can operate. Strong attractive interactions lead to aggregation, phase separation and gelation. Repulsion, or absence of attraction, may result in thermodynamic incompatibility and phase separation. Additionally, casein proteins in solution can be brought to conditions where the proteins coagulate and form lumps. When polysaccharides are incorporated in the lumps they may weaken or harden the lumps. This situation, in case of insoluble fibers, resembles systems in reported studies were active- or passive filler particles are imbedded in gels. Both the rheological and fracture behaviors are influenced (16). All mentioned effects are strongly depending on the type and concentrations of proteins and fibers. There is still a lack in knowledge if fibers in EN can generate physico-chemical effects which are of biological relevance.

Coagulation of casein protein is a physiological process which changes the structure of the casein micelles is such a way that it cloths. Casein predominant EN tends to coagulate in the stomach, due to the precipitation of EN in an acidic environment (17). In the stomach, the pH of EN is lowered from about 7 to about 2. Casein coagulates at its isoelectric point of pH 4.6 (18). Coagulation of EN in the GI tract could potentially cause serious complications in critically ill patients in those with altered gastrointestinal motility and function.

The aim of this study is to outline the coagulating properties of casein predominant EN and

the interaction between fibers and casein complexes. The ***in vivo*** evaluation of such effects has proven to be challenging. Therefore we developed an artificial gastric model that closely resembles human gastric digestion. We used this model to investigate the influence of different amounts of soluble and insoluble dietary fibers on the coagulating properties of a 100 % casein-based EN. To optimize the nutritional care of critically ill patients with an altered digestive capacity, and to overcome complications, it is essential to understand GI kinetics of casein predominant EN enriched with fibers.

METHODS

Enteral nutrition and fibers

To examine the interaction and the coagulating properties in an artificial gastric digestion model, a standard 100 % casein-based EN (Nutrison Standard®, Nutricia N.V., Zoetermeer, the Netherlands) (Table 1) was used in combination with different types of dietary fibers. We used the soluble fibers acacia fiber (Acacia Gum Arabic, Willy Benecke GmbH, Hamburg, Germany), oligofructose and inulin (Raftilose P95 and Raftiline ST, Orafti-Active Food International, Tienen, Belgium). The insoluble fibers used were soy polysaccharide (Fibrim 2000 IP, Solae, St. Louis, Missouri, USA), resistant starch (Novelose 330, National Starch and Chemical GmbH, Hamburg, Germany) and alpha cellulose (Vitacel L 600-20, Rettenmaier & Söhne GmbH + CO. KG, Rosenberg, Germany). Oligofructose, inulin, acacia fiber and soy polysaccharide are fermentable fibers in the study. The non fermentable fibers selected were resistant starch and alpha cellulose.

Stock solutions were made of all fibers separately, with increasing concentrations to a maximum of 200 g/L of the soluble fibers. It was not technically possible to make 200 g/L concentrated stock solutions of soy polysaccharide, resistant starch, and alpha cellulose, therefore, stocks of lower concentration were prepared. These stocks were mixed and heat-treated. Concentrations of fibers added to the EN are listed in Table 2. Concentrations of 16-20 g/L are extremely high; in general concentrations in EN vary from 1-5 g/L. We added the fibers or water in increasing concentrations to the casein-based EN, thus maintaining the level of other nutrients, and simulated gastric digestion.

TABLE 1. *Composition of Nutrison Standard® (Nutricia N.V., Zoetermeer, the Netherlands).*

Nutrition information	100ml
Energy *kcal (kJ)*	100 (420)
Protein equivalent (16 %E) *g*	4.0
Casein (sodium-caseinate) *g*	4.0
Nitrogen *g*	0.6
Carbohydrate (49%E) *g*	12.3
As lactose *g*	0.02
Dextrine maltose *g*	11.9
Other *g*	0.4
Fat (35%E) *g*	3.9
as Saturates *g*	0.4

Nutrition information	100ml
Monounsaturated *g*	2.3
Polyunsaturates *g*	1.2
Linoleic acid *g*	0.9
α-Linolenic acid *g*	0.2
Fibre *g*	0
Minerals	
Sodium *mg*	100
Potassium *mg*	150
Chloride *mg*	125
Calcium *mg*	80
Phosphorus *mg*	72
Magnesium *mg*	23
Vitamins	
Vitamin A *µg-RE*	82
Carotenoids *mg*	0.2
Vitamin D *µg*	0.7
Vitamin E *mg-α-TE*	1.3
Vitamin K *µg*	5.3
Vitamin C *mg*	10
Thiamin *mg*	0.15
Riboflavin *mg*	0.16
Niacin *mg-NE*	1.8
Vitamin B6 *mg*	0.17
Vitamin B12 *µg*	0.21
Folic acid *µg*	27
Pantothenic acid *mg*	0.53
Biotin *µg*	4.0
Choline *mg*	37
Trace elements	
Iron *mg*	1.6
Zinc *mg*	1.2
Manganese *µg*	330
Copper *µg*	180
Iodine *µg*	13
Molybdenum *µg*	10
Selenium *µg*	5.7
Chromium *µg*	6.7
Fluoride *µg*	100

TABLE 2. *Concentrations of fibers added to the enteral nutrition.*

Fibers	Concentrations added to enteral nutrition[a]						
Soluble fibers							
Acacia fiber	0	0.625	1.25	2.5	5	10	20
Oligofructose	0	0.625	1.25	2.5	5	10	20
Inulin	0	0.625	1.25	2.5	5	10	20
Insoluble fibers							
Soy polysaccharide	0	0.625	1.25	2.5	5	-	-
Resistant starch	0	0.625	1.25	2.5	5	-	-
Alpha cellulose	0	0.5	1	2	4	8	16

a) All values are expressed in g/L

Artificial gastric digestion model

Gastric conditions were simulated using a setup that consists of eight parallel computer controlled bioreactor systems. Each reactor was equipped with a pH electrode and four dosing-lines (Multi Fermentor fed-batch; DASGIP AG, Juelich, Germany). The EN and digestive juices were mixed by an impellor (50 rpm). The reactors were placed in a 37°C water bath and filled with 450ml of EN and 50ml of fibers solution. Fifty ml of saliva (50 mM NaCl, 15 mM KCl, 1 mM $CaCl_2.H_2O$, 7 mM $NaHCO_3$, 0.036 % (w/v) α-amylase (*Aspergillus oryzae*, sigma A6211); pH 6.3) and 102ml of gastric juice (50 mM NaCl, 15 mM KCl, 1 mM $CaCl_2.H_2O$, 15 mM $NaHCO_3$, 0.014 % (w/v) pepsin (porcine stomach, sigma P7012), 0.019 % (w/v) lipase (*Rhizopus oryzae*, DF 15K Amano Pharmaceutical Co, Ltd Nagoya); pH 4.0) was added. The saliva was added with a flow rate of 30 ml/h. Gastric juice was added in the first two minutes with a flow rate of 300 ml/h, to simulate the gastric juice already present in the stomach at the time of ingestion, and subsequently 98 minutes with a flow rate of 55 ml/h. Gastric digestion was simulated for 100 minutes in which the pH was lowered following a set curve from a pH of 6.6 at start to a final pH of 2 by adding 1 M HCl while continuously mixing. If necessary, acidification was automatically corrected by the addition of 1 M $NaHCO_3$. The volume ratio of the EN and digestive juices, and enzyme concentration of gastric juice and saliva were used as described previously in the application of a dynamic model of the gastro-intestinal tract (19, 20). Previous studies using this artificial gastric digestion model show reproducible values with small error bars, therefore experiments were performed at least in duplicate ($n \geq 2$) to obtain a minimum of two independent observations of coagulates.

Determination of coagulation by wet and dry weight fractions

After 100 minutes of simulated gastric digestion, the content of the reactors was poured over three sequentially placed analytical sieves with mesh widths of 2 mm, 1 mm, and 0.25 mm (Retsch, VWR, Amsterdam, Netherlands). Particles were separated according to their particle diameter in four fractions: larger than 2 mm, between 1 and 2 mm, between 0.25 and 1 mm and smaller than 0.25 mm. A significant effect on the fraction larger than 2 mm is considered to be clinically relevant, because chyme particles larger than 2 mm are not able to pass the pyloric gatekeeper (21). Particles smaller than 2 mm are not clinically relevant, because

when the pyloric sphincter relaxes fluid and chyme particles, less than 1-2 mm pass into the duodenum.

The wet weight of the individual coagulate fractions was determined by weighing the sieves. The dry matter content of each fraction was determined after its collection from the sieve, according to a method described by Mojonnier (22). In short, the samples of the different fractions were heated on a thermostatically controlled heating plate (140 ± 10°C) to evaporate the water and subsequently placed in a vacuum oven (102 ± 2°C, 600 ± 50 mm Hg, 10 minutes). The residue constituted the absolute dry weight of the sample.

Protein content

The protein content was determined by combusting the sample to gas and ash and measuring the nitrogen content in the gas (% nitrogen / 100 g sample). The nitrogen combined gasses that are produced in this process are reduced to pure nitrogen by two separate gas filters. The nitrogen conversion factor used to obtain protein content was 6.25. This technique is based on a method first described by Dumas (23).

Statistics

Statistical analyses were performed using SPSS 15.0.1 software (SPSS Benelux, Gorinchem, The Netherlands). Analysis of variances was used for the analysis of the slopes of the regression lines with different types of fibers. We can predict the dependant variable with the following equation:

$$a * x + b = c$$

where 'a' represents the slope of the line in [L], 'x' the concentration of fibers added to the EN in [g/L], 'b' the baseline value with 0 g/L fibers added to the EN in [g], and 'c' is the predicted variable in [g] (e.g. wet weight, dry weight or protein content of the wet weight of the different sieves).

The slope of the line (also known as a regression coefficient) is considered significantly different from a horizontal line (slope 0 L) at $P < 0.05$.

RESULTS

Wet weight of digested EN with soluble fibers

All soluble fibers did not affect the wet weight of the coagulation fraction of particles larger than 2 mm. The addition of increasing concentrations of acacia fiber and oligofructose to EN resulted in a significant increase of the wet weight of the particles between 1 and 2 mm ($P < 0.05$) (Table 3). Increasing concentrations of inulin added to EN did not result in a significantly different wet weight of the coagulate fractions (Table 3).

Wet weight of digested EN with insoluble fibers

The addition of increasing concentrations of soy polysaccharide and resistant starch to EN had no effect on the coagulation fractions of particle larger than 2 mm. To elucidate, results of soy

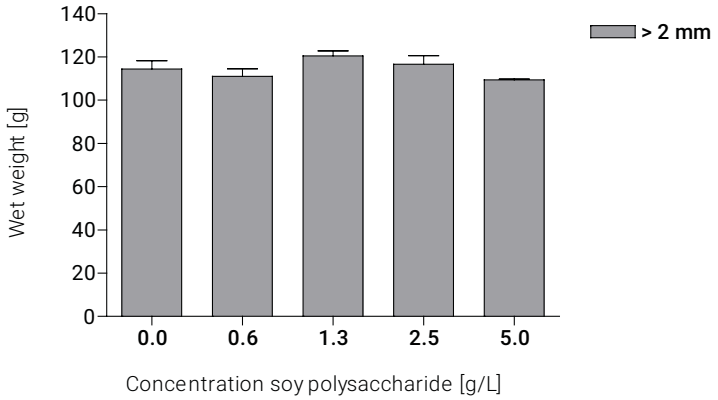
polysaccharide are shown in Figure 1. Soy polysaccharide and resistant starch significantly increased the wet weight of the particles between 1 and 2mm and particles between 0.25 and 1 mm (P < 0.05) (Table 3). Increasing concentrations of alpha cellulose added to EN resulted in a significant increase of the wet weight of the particles larger than 2 mm (P <0.05) and particles between 1 and 2 mm (P < 0.05) (Table 3).

TABLE 3. *Increase in wet weight of the coagulate.*

	Slopes of regression lines at different particle sizes[a]		
Fibers	**> 2 mm**	< 2 and > 1 mm	< 1 and > 0.25 mm
Soluble fibers			
Acacia fiber	**-0.04**	0.45*	-0.15
Oligofructose	**-0.25**	0.25*	0.15
Inulin	**-0.31**	0.06	0.30
Insoluble fibers			
Soy polysaccharide	**-0.72**	5.26*	5.10*
Resistant starch	**-0.46**	2.56*	7.67*
Alpha cellulose	**0.90***	0.92*	0.75

a) All values are expressed in L. The data were analysed by ANOVA. * P<0.05, significantly different from zero. Grey area is considered to be clinically relevant.

FIGURE 1. *Wet weight of digested enteral nutrition with increasing concentrations of soy polysaccharide (slope -0.72 L, ns).*



Dry weight of digested EN with soluble fibers

All soluble fibers did not affect the dry weight of the coagulation fraction of particles larger than 2 mm. The addition of increasing concentrations of acacia fiber and oligofructose to EN resulted in a significant increase of the dry weight of the particles between 1 and 2 mm (P < 0.05) (Table 4). Increasing concentrations of inulin added to EN resulted in no significant difference of the dry weight of the coagulate fractions (Table 4).

Dry weight of digested EN with insoluble fibers

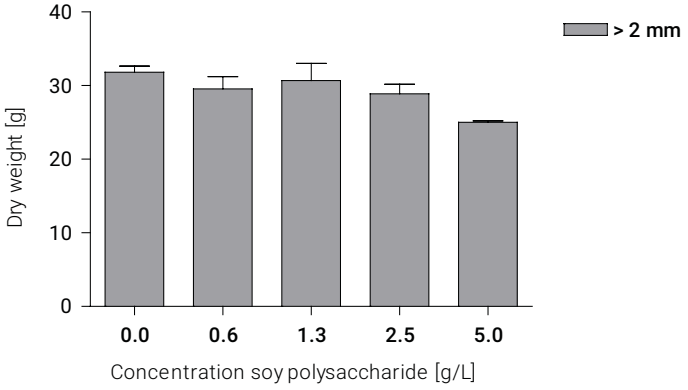
Adding increasing concentrations of soy polysaccharide and resistant starch to EN resulted in a significant decrease of the dry weight of the particles larger than 2 mm ($P < 0.05$), a significant increase of the particles between 1 and 2 mm ($P < 0.05$), and a significant increase of the particles between 0.25 and 1 mm ($P < 0.05$) (Table 4). To elucidate, results of soy polysaccharide are shown in Figure 2. Increasing concentrations of alpha cellulose added to EN resulted in a significant increase of the dry weight of the particles between 1 and 2 mm ($P < 0.05$), and particles between 0.25 and 1 mm ($P < 0.05$) (Table 4).

TABLE 4. *Increase in dry weight of the coagulate.*

	Slopes of regression lines at different particle sizes[a]		
Fibers	**> 2 mm**	< 2 and > 1 mm	< 1 and > 0.25 mm
Soluble fibers			
Acacia fiber	**0.01**	0.13*	0.03
Oligofructose	**-0.04**	0.06*	0.10
Inulin	**-0.04**	0.03	0.01
Insoluble fibers			
Soy polysaccharide	**-1.18***	1.13*	0.93*
Resistant starch	**-0.85***	0.63*	1.47*
Alpha cellulose	**-0.00**	0.21*	0.18*

a) All values are expressed in L. The data were analysed by ANOVA. * $P<0.05$, significantly different from zero. Grey area is considered to be clinically relevant.

FIGURE 2. *Dry weight of digested enteral nutrition with increasing concentrations of soy polysaccharide (slope -1.18 L, P < 0.05).*



Protein content of the wet weight of digested EN with soluble fibers

All soluble fibers did not affect the protein content of the coagulation fraction of particles larger than 2 mm. Addition of increasing concentrations of acacia fiber and oligofructose to EN resulted in a significant increase of the protein content of the wet weight of the particles

between 1 and 2 mm (P < 0.05) (Table 5). Increasing concentrations of inulin added to EN resulted in no significant difference of the protein content of the wet weight of the coagulate fractions (Table 5).

Protein content of the wet weight of digested EN with insoluble fibers

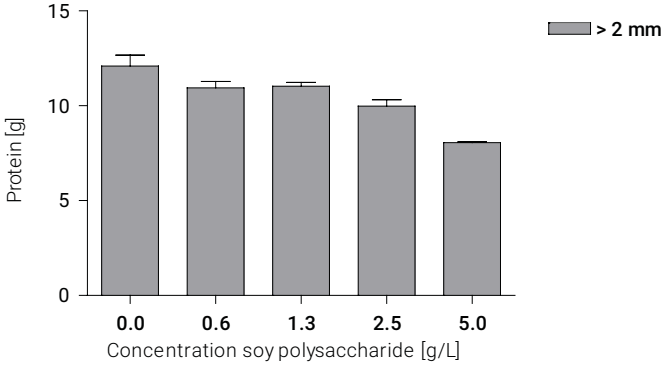
Increasing concentrations of soy polysaccharide and resistant starch added to EN resulted in a significant decrease of the protein content of the wet weight of the particles larger than 2 mm (P < 0.05), a significant increase of the particles between 1 and 2 mm (P < 0.05), and a significant increase of the particles between 0.25 and 1 mm (P < 0.05) (Table 5). To elucidate, results of soy polysaccharide are shown in Figure 3. Increasing concentrations of alpha cellulose added to EN resulted in a significant decrease of the protein content of the wet weight of the particles larger than 2 mm (P < 0.05) and a significant increase of the particles between 1 and 2 mm (P < 0.05) (Table 5).

TABLE 5. *Increase of protein content of the wet weight of the coagulate.*

	Slopes of regression lines at different particle sizes[a]		
Fibers	**> 2 mm**	< 2 and > 1 mm	< 1 and > 0.25 mm
Soluble fibers			
Acacia fiber	**-0.03**	0.04*	0.01
Oligofructose	**-0.04**	0.02*	0.01
Inulin	**-0.04**	0.01	0.01
Insoluble fibers			
Soy polysaccharide	**-0.70***	0.34*	0.19*
Resistant starch	**-0.42***	0.12*	0.21*
Alpha cellulose	**-0.13***	0.04*	0.03

a) All values are expressed in L. The data were analysed by ANOVA. * P<0.05, significantly different from zero. Grey area is considered to be clinically relevant.

FIGURE 3. *Protein content of the wet weight of digested enteral nutrition with increasing concentrations of soy polysaccharide (slope -0.70 L, P < 0.05).*



DISCUSSION

Use of EN is a well established method to provide adequate amounts of calories and proteins to minimize catabolism, diminish the suppression of immune competence, and decrease septic complications in critically ill patients (24). Fibers are added to EN as these provide a variety of health benefits, including a reduction of diarrhea and constipation, promotion of short chain fatty acid production, maintenance of healthy gut microbiota, and enhanced immune function (3). It has been suggested that insoluble fibers may be the cause of GI obstruction by solidification of EN in critically ill patients. Solidification of EN in the GI tract may lead to serious complications (6). It is worthy to note that the reported cases of bowel obstruction by coagulated EN concerned critically ill patients, which may indicate that this patient population is more at risk of developing this complication. Suggested causes of solidification of EN are the coagulation of casein in the GI tract, and as mentioned above the presence of insoluble fibers (3). Therefore, we simulated gastric digestion in an artificial gastric model with a casein-based EN enriched with soluble and insoluble fiber. We chose a casein-based EN because casein tends to coagulate in the stomach, due to its precipitation an acidic environment.

This study shows that the coagulating properties of a casein-based EN are modulated by the addition of fibers. Many processes are involved, e.g. solubility, water-holding capacity, viscosity, gelation, coagulation, adhesion, emulsifying, and foaming. We are interested in the coagulation of EN and the clinical impact of particles larger than 2 millimeters. Soluble fibers (e.g. acacia fiber, oligofructose and inulin), even in much higher concentrations than present in EN, did not affect the coagulate. Whereas high concentrations of soy polysaccharide and resistant starch added to a 100 % casein-based EN, made the coagulate consist of more water and less protein, making the coagulate more liquid. There is a substantial difference between the gastric emptying of liquids and solids (25). Solids have to be broken down to particles of less than two millimetres prior to their emptying from the stomach. Food particles that are larger than two millimetres are usually emptied in the interdigestive interval from the stomach during phase III of the migrating motor complex (MMC) (26, 27). Disorders of GI motility are, however, frequent among critically ill patients and MMC phase III has even been found to be absent in mechanically ventilated patients (28-31). Solids are thus retained longer in the stomach, which results in an increased gastric emptying time compared to liquids. A coagulate which is more liquid will therefore accelerate gastric emptying, resulting in a faster uptake of nutrients in the small intestine (32). The advantage of an EN, with a higher content of insoluble fibers, is that it is more easily digested in the small intestine, especially in patients having a decreased capacity of their pancreas. For example, patients who undergo major upper GI surgery, e.g. Whipple procedure, may have a disturbed intraluminal pH due to the lack of acid secretion which could contribute to the coagulation of EN. In addition, the remaining part of the pancreas after surgery may produce too little or no enzymes to digest the coagulate. Thus, a higher water content of the coagulate may also increase its interaction with digestive enzymes, improving the digestion and absorption process (e.g. hydrolysis of proteins) in the small intestine (33, 34).

The current study is the first showing the ***in vitro*** gastric digestion of soluble and insoluble fibers in EN. Although our study shows that fibers can modulate the coagulation of a casein-

based EN, we must emphasize that the concentrations used in this study are extensively exceeding concentrations added to EN. We are of the opinion that the concentrations of fibers used in EN administered to patients will not generate physic-chemical effects which are of biological relevance. Furthermore, we conclude from this study and previous studies that not fibers but casein protein is the major contributor to the coagulation of EN. These data strengthen our hypothesis that soluble and insoluble fibers do not influence the coagulation of EN in such a way that it may increase the risk of gastrointestinal obstruction in critically ill patients.

CONCLUSIONS

The current study provides information on the coagulation of 100 % casein-based EN enriched with soluble and insoluble fibers. We have demonstrated that in an artificial gastric digestion model high concentrations of the insoluble fibers soy polysaccharide and resistant starch modulate the coagulate in such a way, that it consist of more water and less protein, which may possibly accelerate gastric emptying and improve digestion and absorption. The suggestion that insoluble fibers increase the risk of gastrointestinal obstruction in critically ill patients is not supported by these data. This information, in combination with the known health benefits of fibers, must be taken into account when choosing an EN for critically ill patients at high risk for coagulation.

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