Continuous Administration of Enteral Lipid- and Protein-Rich Nutrition Limits Inflammation in a Human Endotoxemia Model

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Objective: An overzealous inflammatory response is an important cause of morbidity and mortality in surgical, trauma, and critically ill patients. Enteral administration of lipid-rich nutrition was previously shown to attenuate inflammation and reduce organ damage via a cholecystokinin-1 receptor–mediated vagovagal reflex in animal studies. The current preclinical study investigates the immunomodulatory potential of a custom-made enteral nutrition during systemic inflammation in man.

Design: Double-blind, randomized controlled trial.

Setting: Intensive care research unit.

Subjects: Male volunteers.

Interventions: After an overnight fast, 18 healthy male subjects received an IV bolus of Escherichia coli lipopolysaccharide (2 ng/kg). Subjects in the fasted group (n=6) were deprived of food throughout the study, while subjects in the intervention groups were fed either custom-made lipid- and protein-rich nutrition (n=6) or isocaloric control nutrition (n=6) via nasojejunal tube, starting 1 hour prior to lipopolysaccharide administration until 6 hours afterward.

Measurements and Main Results: Bolus lipopolysaccharide administration resulted in a marked inflammatory response. Continuous postpyloric administration of nutrition significantly increased plasma cholecystokinin levels throughout the lipopolysaccharide-induced inflammatory response. Lipid- and protein-rich nutrition attenuated circulating levels of the proinflammatory cytokines tumor necrosis factor-α and interleukin-6 and the interleukin-1 receptor antagonist compared with control nutrition (all p<0.05) and fasted subjects (all p<0.05). In additional, lipid- and protein-rich nutrition augmented the anti-inflammatory response, reflected by increased plasma levels of interleukin-10 compared with fasted subjects (p=0.0001).

Conclusions: The current preclinical study expands the immunomodulating effects of enteral nutrition as previously observed in rodents to man. Continuous administration of enteral nutrition resulted in a rapid anti-inflammatory effect. Furthermore, enrichment of the nutritional composition with lipid and protein was shown to enhance the anti-inflammatory potential. Therefore, continuous enteral administration of lipid- and protein-rich nutrition is a promising intervention to modulate the immune response in the early course of systemic inflammation in man. (Crit Care Med 2013; 41:1258–1265)

Key Words: anti-inflammatory reflex; endotoxin; enteral nutrition; enterocyte damage; systemic inflammation
novel treatment modalities which broadly affect the inflammatory response are warranted to reduce morbidity and mortality (5–7).

Previously, our group demonstrated in rodents that enteral administration of lipid-rich nutrition attenuates inflammation and reduces organ damage via a hard-wired pathway (8). The luminal presence of lipid-rich nutrition triggers a vagal reflex via peripheral cholecystokinin-1 receptors, which reduces local and systemic inflammation and decreases organ damage through activation of peripheral nicotinic acetylcholine receptors on inflammatory cells (8–11). Well-timed nutritional stimulation of this novel gut-brain-immune axis could be a promising intervention to prevent or even treat overzealous inflammation in the clinical setting, as lipid-rich nutrition was also shown to control the immune response and reduce organ damage when administered after the inflammatory trigger (9). Therefore, the aim of this preclinical study was to investigate the anti-inflammatory potential of a nutritional intervention, specifically designed to result in a marked and prolonged cholecystokinin release, in man. Based on observations that predominantly enteral lipids and proteins trigger cholecystokinin release (12), continuous postpyloric administration of a custom-made lipid- and protein-rich nutrition was compared with an isocaloric low-lipid and low-protein control nutrition and with fasted subjects. The effect of lipid- and protein-rich nutrition on acute inflammation was studied in a model of human endotoxia (13). Furthermore, the influence of lipid- and protein-rich nutrition on endotoxin-induced subclinical intestinal damage was investigated.

MATERIALS AND METHODS

Subjects

This study was registered at ClinicalTrials.gov (NCT01100996). After approval from the ethics committee of the University Medical Centre Nijmegen, 12 healthy male subjects gave written informed consent to participate in the experiments in accordance with the Declaration of Helsinki. Samples of fasted subjects (n = 6) were obtained from the placebo-group that participated in another double-blind lipopolysaccharide study performed using the same lot of endotoxin (NCT00513110). There were no differences in subject characteristics (Table 1). All subjects tested were negative for human immunodeficiency virus and hepatitis B. The subjects did not have any febrile illness or use any medications in the 2 weeks preceding the study. All subjects refrained from food and drinks 12 hours before admittance to the ICU on the experimental day.

Experimental Human Endotoxemia

A schematic overview of the experimental procedures is provided in Figure 1. On the experimental day, subjects were admitted to the ICU and prehydrated with 1.5 L glucose 2.5%/NaCl 0.45% after which they received an IV bolus of 2 ng/kg body weight U.S. reference Escherichia coli endotoxin (Escherichia coli O:113; Clinical Center Reference Endotoxin Lot EC-5, National Institute of Health, Bethesda, MD) within 1 min (14). The prehydration protocol was issued by the local ethics committee to reduce the risk of endotoxin-induced hypovolemia. Blood pressure was continuously monitored by an arterial pressure monitoring set. Body temperature was measured using an infrared tympanic thermometer (FirstTemp Genius; Sherwood Medical, Crawley/Sussex, UK). Heart rate was continuously monitored using a 3-lead electrocardiogram. Administration of endotoxin results in flu-like symptoms, such as headache, nausea, vomiting, shivering, and myalgia. Symptoms were rated using grades ranging from 0 (no symptoms) to 5 (most severe ever experienced), resulting in a cumulative sickness score (15). Blood was drawn from the arterial line before the start of postpyloric feeding and serially thereafter up to 24 hours after lipopolysaccharide administration (Fig. 1). Routine hematology parameters were determined using flow cytometry (Sysmex XE-2100; Goffin Meyvis, Etten-Leur, the Netherlands).

Postpyloric Feeding

On the experimental day, two groups received a nutritional intervention in a double-blind randomized fashion, while one group was fasted during the entire experiment (all groups n = 6). The nutritional intervention groups received continuous postpyloric infusion with a liquid-, lipid-, and protein-rich nutrition or an isocaloric control nutrition for 7 hours via self-advancing nasojejunal feeding tube (Tiger 2, Cook Medical, Bloomington, IN). No feeding was aspirated via nasogastric tube or regurgitated throughout the experiment. The rate of feeding for each subject was based on their total energy requirement (TER). TER was calculated by multiplying the

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Fasted</th>
<th>Enriched</th>
<th>Control</th>
<th>p-Value Between Groups</th>
</tr>
</thead>
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<tr>
<td>Age (yr)</td>
<td>24 ± 1</td>
<td>23 ± 1</td>
<td>25 ± 2</td>
<td>0.30</td>
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<tr>
<td>BMI (kg/cm²)</td>
<td>22.0 ± 0.7</td>
<td>23.0 ± 0.6</td>
<td>23.1 ± 0.9</td>
<td>0.60</td>
</tr>
<tr>
<td>TER (kcal)</td>
<td>2822 ± 44</td>
<td>2845 ± 79</td>
<td>3020 ± 137</td>
<td>0.22</td>
</tr>
<tr>
<td>Rate of infusion (kcal/min)</td>
<td>NA</td>
<td>2.0 ± 0.1</td>
<td>2.1 ± 0.1</td>
<td>0.31</td>
</tr>
</tbody>
</table>

BMI = body mass index; TER = total energy requirement; NA = not applicable. Data are represented as mean ± SEM of six subjects per group.
basal metabolic rate of each subject with their activity level using the Harris-Benedict equation (Table 1). All subjects exercised moderately, which equals an activity level of 1.55. The 24-hour energy requirement was administrated over a 7-hour time course to obtain sufficient cholecystokinin-plasma levels during the inflammatory phase (16).

Feeding Composition
The lipid- and protein-rich nutrition contained 44 energy percent (en%) fat, 25en% protein, and 31en% carbohydrates. The protein fraction consisted of intact casein, whey protein, and soy protein hydrolyzate. The control nutrition contained 20en% fat, 16en% protein, and 64en% carbohydrates. The lipid fraction of both feedings contained less than 5 weight percent omega-3 fatty acids. Both the lipid- and protein-rich and control nutrition provided 1 kcal/mL. The specific constituents of both feeding compositions are given in Supplemental Digital Content 1 (http://links.lww.com/CCM/A602).

Determination of Plasma Cholecystokinin, Cytokines, and Subclinical Intestinal Damage
During the experiment, EDTA-anticoagulated blood was collected from the arterial line and immediately centrifuged at 4°C to obtain plasma. Samples were stored at −80°C until batch-wise analysis. Tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-10, and IL-1 receptor antagonists (IL-1RAs) were measured using a multiplex Luminex Assay (Millipore, Billerica, MA). Intestinal-fatty acid–binding protein (i-FABP), a marker of intestinal epithelial cell damage, was determined in plasma using an in-house developed, validated, and standardized enzyme-linked immunosorbent assay (17, 18). Systemic cholecystokinin levels were determined in plasma using a cholecystokinin radioimmunoassay (Eurodiagnostica, Malmö, Sweden).

Statistical Analysis
Power analysis indicated that based on α = 0.05, a power of 80% (β = 0.2), an anticipated reduction of 40% in TNF-α levels between subject receiving enriched nutrition and fasted subjects and an SD of 20%, six subjects were needed per group. Data were normally distributed (calculated by the Kolmogorov-Smirnov test) and depicted as mean ± SEM. Repeated-measures two-way analysis of variance (ANOVA) was used to detect differences between groups for serial data. Differences in serial data within groups, subject characteristics between groups, and area under curve (AUC) of i-FABP between groups were analyzed by one-way ANOVA with Bonferroni post hoc test. Data were excluded from the analysis after being identified as a significant outlier using the Grubb’s test (extreme studentized deviate method). The TNF-α values of one subject in the lipid- and protein-rich nutrition group were removed from the analysis after being identified as a significant outlier. A p value of less than 0.05 was considered statistically significant. All statistical analyses were performed with Graphpad Prism 5 (Graphpad Software, La Jolla, CA).

RESULTS
Hematologic and Clinical Response
As summarized in Table 2, administration of endotoxin resulted in changes in hematologic and clinical parameters in the fasted and nutritional intervention groups. In all groups, mean arterial blood pressure decreased from 90 minutes after lipopolysaccharide administration onwards (p < 0.001), while a compensatory rise in heart rate was observed (p < 0.001). Also, endotoxemia resulted in a rise in core body temperature (p < 0.001) and white blood cell count (p < 0.001) in both the fasted and nutritional intervention groups. The lipopolysaccharide-induced changes in hemodynamic parameters, core temperature, and white blood cell count were not affected by enteral nutrition.

The sickness score of all subjects peaked at 90 minutes following lipopolysaccharide administration. Administration of lipid- and protein-rich or control nutrition did not affect the sickness score compared with fasted subjects (p = 0.43 and p = 0.28, respectively).

Enteral Feeding With Lipid- and Protein-Rich Nutrition Attenuates Proinflammatory and Augments Anti-Inflammatory Cytokines During Experimental Human Endotoxemia
IV administration of lipopolysaccharide resulted in a marked proinflammatory response. Treatment with lipid- and protein-rich nutrition significantly attenuated TNF-α levels compared...
with fasted ($p < 0.0001$) and control nutrition ($p < 0.05$; Fig. 2A). Lipid- and protein-rich nutrition lowered peak TNF-α levels with 40% ± 8% compared with fasted subjects and 29% ± 10% compared with control nutrition. The control nutrition demonstrated a trend towards lower TNF-α plasma levels compared with fasted subjects ($p = 0.06$). Lipid- and protein-rich nutrition also significantly reduced IL-6 plasma concentrations during the endotoxemia protocol compared with control nutrition ($p < 0.001$) and fasting ($p < 0.05$; Fig. 2B), while the control nutrition did not affect interleukin-6 compared with fasted. Administration of lipid- and protein-rich nutrition attenuated peak levels of IL-6 with 41% ± 9% compared with fasted subjects and 54% ± 7% compared with control nutrition.

IV injection of lipopolysaccharide is known to trigger a complex compensatory anti-inflammatory response. Lipid- and protein-rich nutrition decreased circulating levels of the specific IL-1RA throughout the experiment compared with control nutrition ($p < 0.0001$) and fasting ($p < 0.0001$; Fig. 2C). Peak levels of IL-1RA were 37% ± 8% lower in the lipid- and protein-rich nutrition group compared with fasted subjects and 25% ± 6% compared with control nutrition. The control nutrition did not affect IL-1RA levels compared with fasted.

Continuous postpyloric infusion of lipid- and protein-rich nutrition resulted in elevated plasma concentrations of IL-10 over time compared with fasting ($p < 0.0001$), while the control nutrition demonstrated a trend toward higher IL-10 levels ($p = 0.07$; Fig. 2D). Lipid- and protein-rich nutrition enhanced peak levels of interleukin-10 with 231% ± 19% compared with fasted subjects and 130% ± 12% with control nutrition.

### Endotoxin-Induced Enterocyte Damage

In all subjects, administration of lipopolysaccharide resulted in a gradual increase in i-FABP plasma levels until 4 hours postlipopolysaccharide, representing the occurrence of enterocyte damage (Fig. 3A). From 4 hours postlipopolysaccharide to 8 hours, levels of i-FABP in all groups returned to baseline. Fasted subjects and subjects receiving control nutrition displayed a more prominent increase in i-FABP levels during the experiment compared with control nutrition ($p < 0.001$) and fasting ($p < 0.0001$; Fig. 2D). Peak levels of IL-1RA were 37% ± 8% lower in the lipid- and protein-rich nutrition group compared with fasted subjects and 25% ± 6% compared with control nutrition. The control nutrition did not affect IL-1RA levels compared with fasted.

### Table 2: Hemodynamic Parameters, Blood Leukocyte Count, and Sickness Score During Human Endotoxemia

<table>
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<tr>
<th>Parameters</th>
<th>$T = 0$</th>
<th>$T = 1$</th>
<th>$T = 2$</th>
<th>$T = 3$</th>
<th>$T = 4$</th>
<th>$T = 8$</th>
<th>$T = 24$</th>
<th>$p$ Between Groups</th>
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<td>Mean arterial pressure (mm Hg)</td>
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<td></td>
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<td></td>
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<tr>
<td>Fasted</td>
<td>96 ± 3</td>
<td>94 ± 5</td>
<td>85 ± 5</td>
<td>81 ± 4</td>
<td>81 ± 3</td>
<td>88 ± 3</td>
<td>ND</td>
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<td>Enriched</td>
<td>104 ± 5</td>
<td>95 ± 4</td>
<td>91 ± 5</td>
<td>89 ± 3</td>
<td>83 ± 2</td>
<td>85 ± 1</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>100 ± 2</td>
<td>98 ± 3</td>
<td>94 ± 5</td>
<td>89 ± 3</td>
<td>82 ± 3</td>
<td>87 ± 2</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Fasted</td>
<td>68 ± 2</td>
<td>77 ± 4</td>
<td>78 ± 4</td>
<td>87 ± 3</td>
<td>85 ± 4</td>
<td>79 ± 2</td>
<td>ND</td>
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<tr>
<td>Enriched</td>
<td>63 ± 3</td>
<td>66 ± 3</td>
<td>75 ± 3</td>
<td>91 ± 2</td>
<td>82 ± 3</td>
<td>77 ± 4</td>
<td>ND</td>
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<tr>
<td>Control</td>
<td>68 ± 6</td>
<td>81 ± 5</td>
<td>84 ± 6</td>
<td>90 ± 5</td>
<td>90 ± 6</td>
<td>79 ± 5</td>
<td>ND</td>
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<tr>
<td>Temperature (°C)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Fasted</td>
<td>36.7 ± 0.2</td>
<td>37.0 ± 0.2</td>
<td>38.0 ± 0.3</td>
<td>38.5 ± 0.4</td>
<td>38.3 ± 0.3</td>
<td>37.5 ± 0.1</td>
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<td>0.72</td>
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<tr>
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<td>37.1 ± 0.2</td>
<td>37.7 ± 0.2</td>
<td>38.3 ± 0.3</td>
<td>38.1 ± 0.2</td>
<td>37.5 ± 0.1</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>36.7 ± 0.1</td>
<td>37.0 ± 0.1</td>
<td>38.0 ± 0.3</td>
<td>38.3 ± 0.2</td>
<td>38.3 ± 0.2</td>
<td>37.4 ± 0.2</td>
<td>ND</td>
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<tr>
<td>Leukocytes ($\times 10^9$/L)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Fasted</td>
<td>5.7 ± 1.2</td>
<td>3.1 ± 0.9</td>
<td>5.2 ± 0.7</td>
<td>ND</td>
<td>8.7 ± 0.2</td>
<td>11.7 ± 0.7</td>
<td>75 ± 0.9</td>
<td>0.81</td>
</tr>
<tr>
<td>Enriched</td>
<td>5.9 ± 0.6</td>
<td>2.7 ± 0.7</td>
<td>5.2 ± 0.4</td>
<td>ND</td>
<td>9.7 ± 0.8</td>
<td>12.2 ± 0.9</td>
<td>71 ± 0.5</td>
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<tr>
<td>Control</td>
<td>5.2 ± 0.4</td>
<td>3.1 ± 0.6</td>
<td>5.6 ± 0.6</td>
<td>ND</td>
<td>9.7 ± 0.9</td>
<td>13.2 ± 1.3</td>
<td>79 ± 0.6</td>
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</tr>
<tr>
<td>Sickness score</td>
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<td></td>
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<tr>
<td>Fasted</td>
<td>0.3 ± 0.3</td>
<td>1.3 ± 0.8</td>
<td>2.3 ± 0.6</td>
<td>1.8 ± 0.6</td>
<td>0.5 ± 0.3</td>
<td>0.5 ± 0.3</td>
<td>ND</td>
<td>0.12</td>
</tr>
<tr>
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<td>1.7 ± 1.3</td>
<td>4.0 ± 1.4</td>
<td>3.2 ± 1.4</td>
<td>1.3 ± 0.6</td>
<td>0.7 ± 0.5</td>
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<td></td>
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<tr>
<td>Control</td>
<td>0.3 ± 0.3</td>
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<td>1.7 ± 0.3</td>
<td>1.7 ± 0.4</td>
<td>0.8 ± 0.6</td>
<td>0.3 ± 0.2</td>
<td>ND</td>
<td></td>
</tr>
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</table>

- $T =$ time expressed in hours after lipopolysaccharide administration; ND = not determined.
- Data expressed as mean ± SEM. $p$-values are comparisons between groups over time and were determined by two-way repeated-measures analyses of variance.
the experiment compared with subjects fed with lipid- and protein-rich nutrition. Total i-FABP release (represented by AUC) tended to be lower for the lipid- and protein-rich nutrition group compared with control nutrition and fasted subject, although this did not reach statistical significance ($p = 0.39$; Fig. 3B).

**Enteral Nutrition Increases Plasma Cholecystokinin Levels During the Inflammatory Response**

To assess the effect of continuous duodenal infusion on cholecystokinin release, plasma cholecystokinin levels were measured on indicated time points (Fig. 1). In fasted subjects, plasma cholecystokinin levels were below detection level throughout the protocol. Cholecystokinin levels increased from nondetectable values ($< 0.3$ pmol/L) before administration of enteral nutrition ($T = -1$ hr) to $2.3 \pm 0.5$ pmol/L at 1 hour after onset of continuous administration of lipid- and protein-rich and to $2.2 \pm 0.6$ pmol/L for the control group ($T = 0$; Fig. 4) at which time the bolus of endotoxin was administered. Cholecystokinin plasma levels in the control dropped 4 hours after IV lipopolysaccharide injection ($0.7 \pm 0.2$ pmol/L; $p < 0.05$) compared with the levels at $T = 0$ hours, while the drop in the lipid- and protein-rich nutrition group was not significant ($1.1 \pm 0.3$ pmol/L; $p = 0.15$, respectively). Cholecystokinin levels dropped to nondetectable levels at 8 hours after cessation of the nutrient infusion. There were no significant differences in total plasma cholecystokinin release between lipid- and protein-rich and control nutrition (data not shown).

**DISCUSSION**

During the last decades, the catabolic state of surgical and critically ill patients increasingly gained interest (19, 20). The observed negative correlation between catabolism and clinical outcome resulted in more liberal nutritional regimes, such as reduced preoperative fasting and early administration of enteral nutrition (20). Implementation of these renewed nutritional support regimes reduced morbidity and length of hospital stay (21, 22). Although the exact mechanisms behind these beneficial effects are not well known, it is assumed that adequate nutritional support prevents immunodeficiency induced by caloric deficits (23). Enteral nutrients are known to also activate digestive and metabolic feedback responses (24, 25). Previously, our group described a novel pathway via which enteral nutrition is able to modulate the inflammatory response in rodents. Short-term administration of lipid-rich nutrition was shown to limit inflammation and reduce organ damage via cholecystokinin-1 receptor–mediated activation of the so-called cholinergic anti-inflammatory pathway in several experimental models (8, 9, 26–28). Herein, we present the first evidence that continuous postpyloric administration of enteral nutrition during the course of endotoxemia modulates inflammation in man.

Virtually every surgical, trauma, and ICU patient suffers from systemic inflammation. The complex interplay between pro- and anti-inflammatory mechanisms during such a systemic inflammatory response is still incompletely understood (29). Excess release of TNF-$\alpha$ is known to contribute to the development of systemic inflammatory response syndrome, organ damage, and mortality in sepsis (30). Furthermore, circulating levels of TNF-$\alpha$ and IL-6 are correlated with the severity of sepsis in patients (31). In line with these clinical observations, excessive release of proinflammatory mediators should be minimized in the early course of systemic inflammation to improve clinical outcome (32). This study demonstrates that lipid- and protein-rich nutrition limits systemic inflammation during human...
experimental endotoxemia by lowering circulating levels of TNF-α and IL-6. These findings are in line with rodent data from our group (8, 26). Furthermore, the intervention with lipid- and protein-rich nutrition resulted in decreased IL-1RA plasma levels. These data conform previous reports from others, demonstrating that TNF-α and IL-6 enhance IL-1RA release during endotoxemia, while inhibition of these cytokines using epinephrine or glucocorticoids lowers circulating IL-1RA (33–36). In parallel with these reports, our findings that lipid- and protein-rich nutrition not only decreases plasma levels of TNF-α and IL-6 but also lowers circulating IL-1RA reflect an overall reduced proinflammatory state. Interestingly, postpyloric administration of lipid- and protein-rich nutrition amplified the anti-inflammatory response to endotoxin as evidenced by a pronounced increase in circulating IL-10. Production of the cytokine IL-10 is considered part of the host-protective mechanism that counterbalances the proinflammatory response during acute infection and inflammation (35).

Furthermore, administration of interleukin-10 has been shown to reduce endotoxin-induced lethality in mice (37). Together, these data indicate that continuous administration of lipid- and protein-rich enteral nutrition is a physiological intervention to control acute systemic inflammation in man. These promising findings warrant future studies investigating the beneficial effect of lipid- and protein-enriched enteral nutrition on clinical endpoints in surgical and critically ill patients.

Intestinal epithelial cell damage often accompanies sepsis, trauma, and major surgery and is related to the degree of gastrointestinal hypoperfusion (17, 38, 39). In addition, intestinal compromise has been implicated in the development of inflammatory complications following injury (40). Here, IV administration of lipopolysaccharide resulted in increased i-FABP levels. i-FABP is a small cytosolic protein that is present in mature enterocytes and released into the circulation upon cell damage (17). The rise in plasma i-FABP levels was smaller in subjects treated with lipid- and protein-rich nutrition compared with control nutrition or fasted subjects, although this did not reach statistical significance. These data are supported by rodent studies demonstrating that lipid-rich nutrition preserves intestinal integrity in several models (26, 41). The small overall increase in i-FABP plasma levels is likely attributable to the relative low dose of lipopolysaccharide in combination with the prehydration protocol. It is to be expected that the prehydration protocol resulted in limited endotoxin-induced (splanchnic) hypoperfusion, thereby reducing enterocyte damage (17). Future studies are needed to investigate a gut-protective effect of lipid- and protein-rich nutrition in man.

In rodents, activation of the nutritional anti-inflammatory pathway was shown to be mediated via peripheral cholecystokinin-1 receptors (8). Intestinal release of cholecystokinin and subsequent activation of cholecystokinin receptors are predominantly triggered by the luminal presence of lipid and protein (12, 42), while termination of nutrient exposure results in a rapid drop of cholecystokinin levels (42). To this end, we continuously administered lipid- and protein-enriched nutrition to optimally stimulate peripheral cholecystokinin-1 receptors throughout the endotoxin-induced inflammatory response. Although the observed effects of enteral lipid- and protein-enriched nutrition on systemic inflammation were comparable with our rodent data, the human data do not support a clear role for cholecystokinin in the anti-inflammatory potential of lipid- and protein-enriched nutrition. The differences in anti-inflammatory potential between our nutritional compositions might be explained by the fact that circulating cholecystokinin levels do not reflect local intestinal concentrations and
subsequent activation of afferent vagal fibers in the gut (43). Furthermore, it can also be explained by species differences between rodent and man combined with involvement of other neuropeptides, as the luminal presence of nutrients induces release of several neuropeptides resulting in a complex interplay of signals (44). Therefore, it remains to be determined via which pathway enteral lipid- and protein-enriched nutrition modulates inflammation in man.

Together, this study reveals a novel application of enteral nutrition to modulate inflammation in man. In contrast to the immunomodulating effects transferred by prolonged ingestion of omega-3 fatty acids and glucose (45, 46), our intervention demonstrated a rapid anti-inflammatory effect, which is suggestive for a direct feedback mechanism. This is underlined by the fact that the anti-inflammatory effect is achieved by continuous infusion of small amounts of nutrients, while bolus administration of enteral nutrition, delivering nearly twice the amount of lipid 2 hours prior to the induction of endotoxia, did not affect the inflammatory response (47).

In conclusion, the current preclinical study demonstrates that 1) short-term continuous administration of enteral nutrition starting prior to the inflammatory trigger modulates the acute systemic inflammatory response in humans, and 2) enrichment of the nutritional composition with lipid and protein reinforces this anti-inflammatory potential. Taken together, our data implicate continuous administration of lipid- and protein-rich nutrition as a promising intervention to control an excessive inflammatory response in the clinical setting. Future studies are being performed to investigate the significance of the cholecystokinin-mediated anti-inflammatory response in man and to study the beneficial effects of enteral lipid- and protein-enriched nutrition on surgical and critically ill patients.

REFERENCES