Available online at www.sciencedirect.com

SciVerse ScienceDirect

Journal of Nutritional Biochemistry 22 (2011) 1105–1111

Journal of Nutritional Biochemistry

RESEARCH ARTICLES

Chylomicron formation and glucagon-like peptide 1 receptor are involved in activation of the nutritional anti-inflammatory pathway☆,☆☆

Tim Lubbers[a], Jacco J. de Haan[a], M'hamed Hadfoune[a], Lennart Zabeau[b], Jan Tavernier[b], Yiren Zhang[c], David Grundy[c], Jan Willem M. Greve[d], Wim A. Buurman[a,*]

[a]Department of Surgery, NUTRIM School for Nutrition, Toxicology and Metabolism, Maastricht University Medical Centre+, P.O. Box 616, 6200 MD Maastricht, The Netherlands
[b]Flanders Interuniversity Institute for Biotechnology, Department of Medical Protein Research, Ghent University, 8500 Ghent, Belgium
[c]Department of Biomedical Science, University of Sheffield, S10 2TN Sheffield, UK
[d]Department of Surgery, Atrium Medical Centre, 6419 PC Heerlen, The Netherlands

Received 9 June 2010; received in revised form 12 September 2010; accepted 17 September 2010

Abstract

Enteral administration of lipid-enriched nutrition effectively attenuates inflammation *via* a cholecystokinin (CCK)-mediated vagovagal anti-inflammatory reflex. Cholecystokinin release and subsequent activation of the vagus are dependent on chylomicron formation and associated with release of additional gut peptides. The current study investigates the intestinal processes underlying activation of the CCK-mediated vagal anti-inflammatory pathway by lipid-enriched nutrition. Rats and mice were subjected to hemorrhagic shock (HS) or endotoxemia, respectively. Prior to the experimental procedures, animals were fasted or fed lipid-enriched nutrition. Pluronic L-81 (L-81) was added to the feeding to investigate involvement of chylomicron formation in activation of mesenteric afferent fibers and the immune-modulating potential of lipid-enriched nutrition. Ob/Ob mice and selective receptor antagonists were used to study the role of leptin, glucagon-like peptide 1 and peptide YY in activation of the nutritional reflex. Electrophysiological analysis of mesenteric afferents in mice revealed that lipid-enriched nutrition-mediated neural activation was abrogated by L-81 ($P<.05$). L-81 blunted the beneficial effects of lipid-enriched nutrition on systemic inflammation and intestinal integrity in both species (all parameters, $P<.01$). Ob/Ob mice required a higher dose of nutrition compared with wild-type mice to attenuate plasma levels of TNF-α and ileum-lipid binding protein, a marker for enterocyte damage (both $P<.01$), suggesting a higher stimulation threshold in leptin-deficient mice. Administration of a glucagon-like peptide 1-receptor antagonist, but not leptin or peptide YY antagonists, suppressed the effects of lipid-enriched nutrition. These data indicate that chylomicron formation is essential and activation of the glucagon-like peptide 1-receptor is involved in activation of the nutritional anti-inflammatory pathway by lipid-enriched nutrition.

Keywords: Pluronic L-81; Mesenteric afferents; Nutritional anti-inflammatory pathway; leptin; Peptide YY; Glucagon-like peptide 1 receptor

1. Introduction

Ingestion of nutrients triggers a multitude of regulatory functions in the digestive tract to maintain metabolic homeostasis [1,2]. Nutrient sensing and intestinal feedback require release of neuropeptides from entero-endocrine cells and activation of neural pathways. The vagus nerve in particular plays a prominent role in regulation of food intake and digestive capacities of the gastrointestinal tract *via* the so-called gut–brain axis [3,4].

Recently, our group described a novel feature of the gut–brain axis. Enteral administration of lipid-enriched nutrition attenuated local and systemic inflammation and prevented tissue damage *via* the vagus nerve [5–7]. The luminal presence of lipid-enriched nutrition triggers the brain *via* cholecystokinin (CCK)-mediated activation of CCK-1 receptors on afferent vagal fibers [8]. In turn, release of cytokines is inhibited through activation of nicotinic receptors on inflammatory cells *via* efferent vagal fibers [7].

CCK release following food intake is an important component in activation of the nutritional anti-inflammatory reflex [8]. However, little is known about the mechanisms that result in release of CCK and subsequent activation of afferent vagal fibers. Release of CCK from enterocrine-I cells is dependent on the intestinal processing of lipids, resulting in formation of chylomicrons [9,10]. In line, chylomicrons have been shown to inhibit gastric emptying *via* a CCK-1 receptor-mediated duodenal afferent pathway [9,10]. In addition to CCK, glucagon-like peptide 1 (GLP-1) and protein YY (PYY), and the adipokine leptin are involved in meal-induced activation of afferent vagal fibers [1,11] and inhibit food intake in conjunction with CCK [12–14]. The current study aimed to reveal the intestinal processes triggered by lipid-enriched

☆ Funding: This work was financially supported by Danone Research Centre for Specialised Nutrition, Wageningen, The Netherlands, and AGIKO-stipendium 920-03-522 (to T. Lubbers) from the Netherlands Organisation for Health Research and Development.
☆☆ Disclosure: None of the authors have any conflict of interest.
* Corresponding author. Tel.: +31 43 3881499; fax: +31 43 3884154.
E-mail address: w.buurman@maastrichtuniversity.nl (W.A. Buurman).

0955-2863/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.jnutbio.2010.09.006

nutrition that result in activation of the CCK-mediated nutritional anti-inflammatory reflex.

2. Materials and methods

2.1. Animals and experimental groups

Male Sprague-Dawley rats, weighing 300–350 g, and C57bl6 mice and obese Ob/Ob mice, both 10–12 weeks old, were purchased from Charles River Laboratories (Maastricht, The Netherlands) and housed under controlled conditions of temperature and humidity. Prior to the experiments, the animals were fed standard rodent chow *ad libitum* and had free access to water. The experimental protocols were approved by the Animal Ethics Committee of the Maastricht University Medical Centre+.

In rats, a non-lethal hemorrhagic shock (HS) model was used, as previously described [5]. In short, rats were anesthetized with isoflurane and the femoral artery was cannulated. At time of shock, 2.1 ml of blood per 100 g body weight was withdrawn. In all experiments, rats were fasted overnight (18 h) or fed lipid-enriched nutrition by oral gavage at −18 h (3 ml), −2 h (0.75 ml) and −45 min (0.75 ml) prior to HS (Fig. 1).

In mice, systemic inflammation was induced by intraperitoneal injection of 2 mg/kg lipopolysaccharide (LPS derived from *Escherichia coli* 055;B5, Sigma, St. Louis, MO, USA) in sterile phosphate-buffered saline (PBS; pH 7.4). Prior to endotoxemia, C57bl6 and Ob/Ob mice were fasted or fed lipid-enriched nutrition by oral gavage at −18 h (0.3 ml), −2 h (0.2 ml) and −45 min (0.2 ml). Next to this dose, a separate group of Ob/Ob mice received a higher dose (−18 h: 0.4 ml; −2 h: 0.3 ml; and −45 min: 0.3 ml). The amount of nutrition administered to C57/bl6 and Ob/Ob mice was based on daily energy expenditure (DEE). The energy provided by the standard dose approximates 7% DEE in both strains, while the high dose supplied 10% DEE [15]. All animals were killed at 90 min following either HS or LPS administration (Fig. 1).

The liquid lipid-rich diet contained 50.4 energy percent (en%) fat (30 en% are phospholipids), 8.7 en% protein and 40.9 en% carbohydrates. The lipid source was lecithin. Omega 3 and omega 6 fatty acids constituted less than 5 wt% (<5 g/100 ml). Proteins were derived from lean milk powder, containing 80% casein and 20% whey protein. The carbohydrate source was a mixture of sucrose and maltodextrins (Glucidex 19DE).

2.2. Prevention of chylomicron formation

The formation of chylomicrons was prevented by adding Pluronic L-81 (6 mg/ml; kindly provided by BASF, Brussels, Belgium) to the lipid-enriched nutrition. L-81 is a hydrophobic surfactant that inhibits the formation of chylomicrons, without affecting digestion, uptake or reesterification of absorbed lipid. Pluronic L-62D (BASF; comparable to Pluronic L-63), which is chemically similar to L-81, but does not prevent chylomicron formation, served as control [9].

2.3. Quantification of plasma triglycerides

The concentration of circulating triglycerides was measured in arterial plasma of rats collected at time of shock (t=0) and venous plasma of mice at time of sacrifice (t=90 min) using a triglyceride determination kit (Sigma) following the manufacturer's instructions (Fig. 1).

2.4. Receptor antagonists

Lipid-enriched nutrition-fed rats were treated with antagonists to the Y2-receptor (BIIE 0246 formate; 2 mg/kg in PBS) and GLP-1 receptor (exendin-3; 500 μg/kg in 30% polyethylene glycol saline solution; both Tocris Bioscience, Ellisville, MO, USA) 30 min prior to shock to investigate the involvement of PYY and GLP-1 release, respectively, in activation of the nutritional anti-inflammatory pathway.

2.5. Leptin receptor-specific nanobody

Mice were injected intraperitoneally with a blocking nanobody directed against the leptin receptor on two consecutive days prior to endotoxemia to delineate involvement of leptin in the anti-inflammatory potential of lipid-rich nutrition. The leptin receptor-specific nanobody was generated by immunization of lamas with the extracellular part of the mouse leptin receptor. It was genetically fused to a nanobody directed against mouse serum albumin to prolong the half-life *in vivo*. This bi-specific nanobody was produced in *E. coli* and purified up to 95% purity. LPS contamination was less than 0.1 EU/mg protein as measured using the amoebocyte lysate in combination with a chromogenic substrate (Cambrex, New Jersey, NY, USA). This nanobody acts as a potent leptin receptor antagonist both *in vitro* and *in vivo* (Zabeau et al., manuscript in preparation).

2.6. Cytokine and ileum-lipid binding protein analysis

IL-6 and TNF-α concentrations in arterial blood were measured using standard ELISAs for rat IL-6 and rat and mouse TNF-α (all BD Biosciences, Franklin Lakes, NJ, USA). Murine ileum-lipid binding protein (I-LBP) was determined in plasma using a specific ELISA (Hycult Biotech, Uden, The Netherlands).

2.7. Intestinal permeability assay and bacterial translocation

Intestinal permeability for macromolecules was assessed by measuring translocation of the 44-kD enzyme horseradish peroxidase (HRP) by the everted gut sac method [8]. Bacterial translocation (BT) to distant organs was determined as previously described [7,8]. In short, mesenteric lymph nodes, spleen and a segment of liver were collected aseptically. Tissue fragments were homogenized and transferred to agar plates (Columbia III blood agar base supplemented with 5% vol/vol sheep blood; BBL, Franklin Lakes, NJ, USA) and Chocolate PolyviteX agar (BioMérieux, Marcy l'Etoile, France). After 48 h of incubation, colonies were counted, determined using conventional techniques, adjusted to tissue weight and expressed as number of CFU per gram of tissue.

2.8. Mesenteric afferent discharge

Mice were killed by cervical dislocation in accordance with the UK Animals Scientific Procedures Act (1986). Intestinal tissue was prepared for nerve recording as previously described [15]. In short, proximal jejunal segments (2–3 cm) were dissected so that a non-bifurcating mesenteric bundle could be identified. The isolated segments were placed in oxygenated Krebs solution at 34°C. A single nerve bundle was drawn into a suction electrode for afferent recording. The jejunum was cannulated at each end and intraluminal pressure was recorded *via* a pressure recorder. The lumen was perfused with saline at 0.2 ml/min except during distension, when the outlet tap was closed allowing pressure to rise up to 55 mmHg and released by opening the tap.

Following a 60-min stabilization period, intestinal segments were distended at 15-min intervals and mean afferent firing rate (spikes per second) was displayed as peristimulus rate histogram. Once reproducible responses were obtained, the effect of nutrient was tested by switching luminal perfusion to lipid-enriched nutrition alone or to lipid-enriched nutrition with 3% L-81 (both 1 ml) with free drainage. The nutrient remaining within the lumen was trapped for 15 min following closure of the outlet port with termination of the continuous perfusion of saline. One period of distension was achieved by perfusion with saline, which also served to flush out luminal contents when the outlet tap was opened. Saline perfusion and repeat distensions at 15 min continued until the response had recovered to baseline.

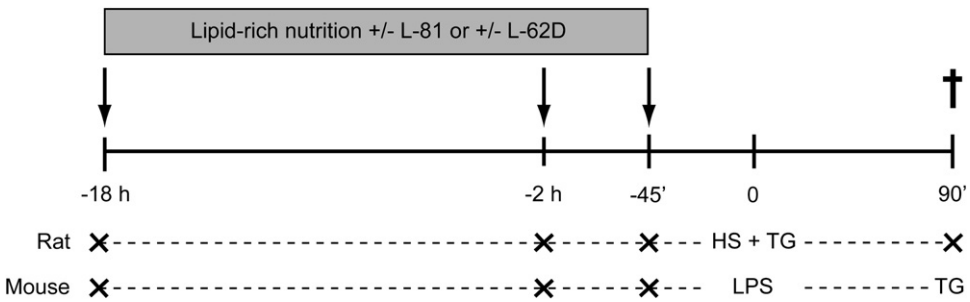

Fig. 1. Experimental protocol. All animals were deprived of food 18 h prior to HS or LPS administration. A liquid lipid-enriched diet was administered at three time points (−18 h, −2 h, −45 min) in the fed groups. L-81 or L-62D was added to the lipid-enriched nutrient to investigate involvement of chylomicron formation in activation of the nutritional anti-inflammatory reflex. In rats, 30–40% of the circulating volume was withdrawn at t=0 to induce HS. In the withdrawn blood, plasma triglyceride (TG) levels were determined. Mice received an intraperitoneal bolus of LPS (2 mg/kg) at t=0. All animals were killed at 90 min, at which time the plasma TG levels in mice were analyzed.

2.9. Statistical analysis

All experimental groups consisted of eight animals, unless otherwise indicated. A Mann–Whitney *U* test was used for between-group comparisons. Whole nerve afferent discharge was calculated from the number of spikes crossing a preset threshold and expressed as spikes per second. Baseline discharge was calculated as the mean firing in the 1-min period preceding distension. Discharge during distension was expressed as increase above baseline, calculated as the mean firing frequency in 5-s periods at each level of distending pressure. Low-threshold nerve afferent discharge was expressed as the difference between the baseline discharge and discharge at the 20 mmHg of the distending pressure. Data are expressed as mean±S.E.M. Data were compared statistically using repeated measure ANOVA with Dunnett post-test analysis. Prism 5.02 for Windows (GraphPad Software, Inc., San Diego, CA, USA) was used for computations. Differences were considered statistically significant at $P<.05$.

3. Results

3.1. Pluronic L-81 decreases plasma triglyceride levels after ingestion of lipid-enriched nutrition

First, we verified the effectiveness of L-81 to prevent chylomicron formation in rats and mice by measuring postprandial plasma concentrations of triglycerides. Ingestion of lipid-enriched nutrition resulted in increased circulating triglycerides in rats and mice compared with fasted animals (both $P<.01$; Fig. 2A and B). Addition of L-81 to the lipid-enriched nutrition reduced the amount of plasma triglycerides in both species compared with lipid-enriched nutrition plus control Pluronic L-62D (both $P<.01$), indicating that chylomicron formation is successfully prevented by L-81 in both species.

3.2. The inhibitory effect of lipid-enriched nutrition on systemic inflammation and loss of gut barrier is abrogated by Pluronic L-81

To investigate the role of chylomicron formation in activation of the anti-inflammatory pathway of lipid-enriched nutrition, we subjected rats to HS. Hemorrhagic shock resulted in markedly elevated plasma levels of TNF-α and IL-6, as well as increased ileal permeability and BT. These shock-induced changes were significantly attenuated by lipid-enriched nutrition, conform previous findings (Fig. 3) [7]. Prevention of chylomicron formation using Pluronic L-81 reduced the effect of lipid-enriched nutrition on plasma levels of TNF-α and IL-6 compared with vehicle (both $P<.01$; Fig. 3A and B). Moreover, L-81 reversed the protective effect of lipid-enriched nutrition on ileal leakage of HRP and BT compared with vehicle (both $P<.05$; Fig. 3C and D). These findings indicate that formation of chylomicrons is vital to activate the nutritional anti-inflammatory pathway in rats.

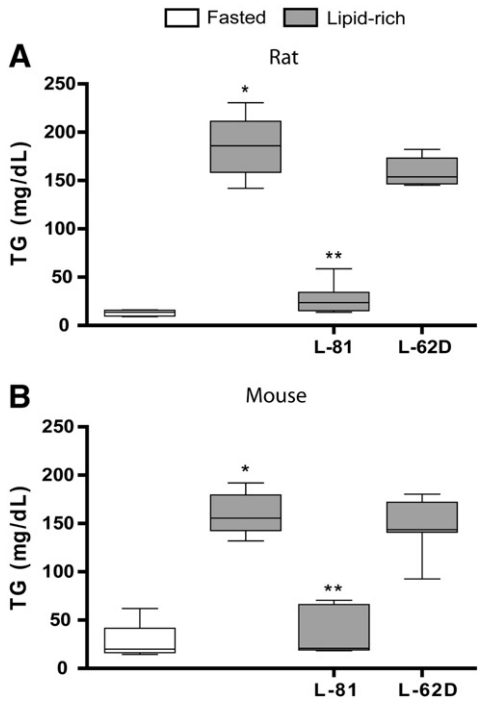

Fig. 2. L-81 prevents lipid-enriched nutrition-induced rise in plasma triglycerides. Ingestion of lipid-enriched nutrition increases plasma levels of triglycerides. Supplementation of the nutrient with L-81 reduces the amount of circulating triglycerides compared with control (Pluronic L-62D) in both rats (A) and mice (B). Data are represented as median, range and interquartile range. *$P<.05$ compared with fasted, **$P<.05$ compared with L-62D.

3.3. Prevention of chylomicron formation inhibits activation of mesenteric afferents and anti-inflammatory potential of lipid-enriched nutrition in mice

In order to investigate the role of chylomicron formation in activation of the autonomic nervous system by lipid-enriched nutrition, we determined mesenteric afferent discharge in response to lipid-enriched nutrition. First, we verified that L-81 inhibited the anti-inflammatory actions of lipid-enriched nutrition in mice similar to rats. Administration of lipid-enriched nutrition prior to LPS challenge attenuated plasma levels of TNF-α and I-LBP compared with fasted controls (both $P<.01$; Fig. 4A and B). L-81 abrogated the effect of lipid-enriched nutrition on systemic inflammation and enterocyte damage compared with vehicle (both $P<.01$), indicating that chylomicron formation also plays a crucial role in mice.

Next, we investigated firing of jejunal mechanosensitive afferents in response to lipid-enriched nutrition. The intrajejunal presence of lipid-enriched nutrition (treatment) enhanced vagal afferent discharge in response to luminal distension compared to the discharge before treatment and after treatment (both $P<.05$ from 8 to 55 mmHg; Fig. 5A). Addition of L-81 suppressed the increase in afferent discharge associated with lipid-enriched nutrition (Fig. 5B). Since previous studies demonstrated that low-threshold afferents preferentially project *via* vagal pathways [16], we specifically quantified the increase in afferent firing rate over the pressure range from 0 to 20 mmHg. Lipid-enriched nutrition enhanced afferent discharge to distension compared with the discharge before treatment and after treatment ($P<.001$; Fig. 5C). In the presence of L-81, the increase in afferent discharge triggered by lipid-enriched nutrition was prevented (Fig. 5D). Furthermore, L-81 reduced the discharge compared with the increase of lipid-enriched nutrition alone (treatment group in 5C; $P<.05$), indicating that chylomicron formation is an essential step in the lipid-enriched nutrition-mediated activation of vagal afferents.

3.4. Leptin is not involved in the nutritional anti-inflammatory pathway

Leptin is known to activate afferent vagal fibers and reduce food intake in combination with CCK [17]. Here, we investigated the involvement of leptin in activation of the anti-inflammatory reflex by lipid-enriched nutrition in Ob/Ob mice. Administration of the standard dose of lipid-enriched nutrition did not affect the endotoxin-induced systemic inflammatory response and enterocyte damage in Ob/Ob mice (Fig. 6A), indicating a potential role for leptin in activation of the nutritional anti-inflammatory pathway. However, providing a more potent stimulus *via* administration of a higher dose of lipid-enriched nutrition resulted in attenuated plasma levels of TNF-α ($P<.01$) and I-LBP ($P<.01$; Fig. 6B).

To further elucidate the role of leptin, we blocked the leptin receptor in wild-type mice using blocking nanobodies directed against the leptin receptor. Pretreatment of wild-type mice with nanobodies did not affect the anti-inflammatory potential of lipid-

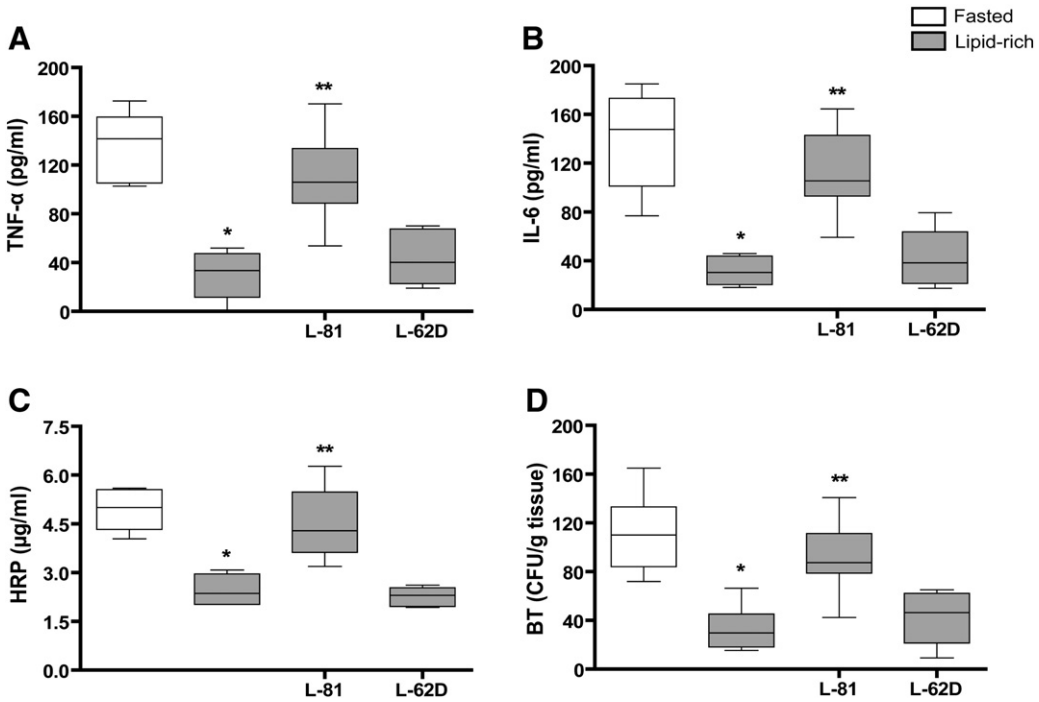

Fig. 3. Lipid-enriched nutrition-induced chylomicron formation is critical to reduce shock-induced inflammation and loss of intestinal integrity in rats. Hemorrhagic shock results in systemic inflammation and loss of intestinal integrity. Pretreatment with lipid-enriched nutrition attenuates systemic plasma levels of TNF-α (A) and IL-6 (B). Furthermore, lipid-enriched nutrition reduced ileal leakage of HRP (C) and BT (D). Addition of L-81 to lipid-enriched nutrition reduced the inhibitory effect of lipid-enriched nutrition on systemic inflammation and loss of intestinal integrity, while L-62D did not affect these parameters. Data are represented as median, range and interquartile range. *P<.01 compared with fasted, **P<.01 compared with lipid-enriched nutrition plus L-62D.

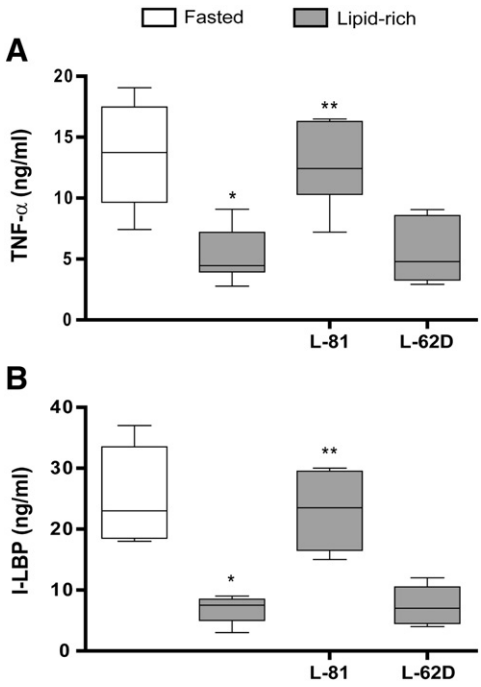

Fig. 4. Prevention of chylomicron formation blunts the anti-inflammatory and gut-protective potential of lipid-enriched nutrition in endotoxemic mice. Intraperitoneal administration of endotoxin results in marked systemic levels of TNF-α and I-LBP. The systemic inflammation and enterocyte damage were effectively reduced by lipid-enriched nutrition. L-81 abrogates the inhibitory effect of lipid-enriched nutrition on plasma levels of TNF-α and I-LBP, while supplementation with L-62D did not affect the anti-inflammatory response. Data are represented as median, range and interquartile range. *P<.05 compared with fasted, **P<.05 compared with L-62D.

enriched nutrition compared with vehicle treatment (Fig. 6C), indicating that activation of the nutritional anti-inflammatory pathway is independent of leptin receptors.

3.5. GLP-1 receptor antagonists reduce the immuno-modulatory properties of lipid-enriched nutrition

The intestinal peptides GLP-1 and PYY inhibit food intake in response to luminal fat and their release has been related to CCK release [14,18]. In rats, pretreatment with the GLP-1 receptor antagonist exendin-3 suppressed the effects of lipid-enriched nutrition on TNF-α, IL-6, leakage of HRP and BT to a limited extent (all parameters, P<.05; Fig. 7A and B). These findings suggest that activation of the GLP-1 receptor by lipid-enriched nutrition is involved in the vagal anti-inflammatory reflex. In contrast, administration of the selective antagonist to the PYY receptor failed to reduce the inhibitory actions of lipid-enriched nutrition on HS-induced plasma levels of TNF-α [64 (31 to 87) pg/ml vs. vehicle: 62 (38 to 95) pg/ml] and IL-6 [23 (17 to 53) pg/ml vs. vehicle: 30 (16 to 53) pg/ml]. Furthermore, loss of intestinal permeability [HRP: 2.4 (2.0 to 4.2) μg/ml vs. vehicle: 3.2 (1.9 to 3.8) μg/ml] and BT [38 (14 to 42) CFU/g tissue vs. vehicle: 33 (37 to 42) CFU/g tissue] were not affected, indicating that activation of the PYY receptor is not involved in the nutritional anti-inflammatory reflex.

4. Discussion

The current study provides insight into the mechanisms that occur at the level of the intestine, resulting in activation of the CCK-mediated nutritional anti-inflammatory reflex. First, we demonstrate that formation of chylomicrons induced by absorption of lipid-enriched nutrition plays a vital role in activation of the autonomic nervous system and the anti-inflammatory pathway in both rats and

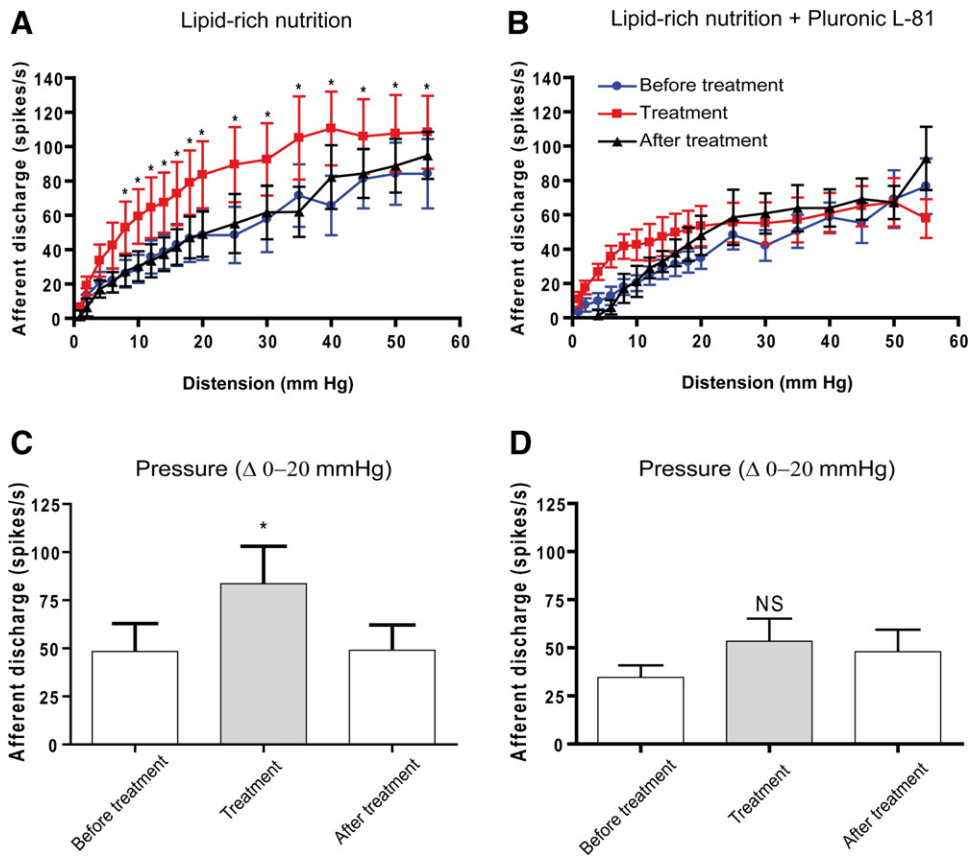

Fig. 5. Activation of mesenteric afferent fibers is dependent on chylomicron formation. Lipid-enriched nutrition enhances the afferent discharge of mesenteric afferents to distension (A). Prevention of chylomicron formation with L-81 abrogates the increased firing of mesenteric afferents by lipid-enriched nutrition (B). Lipid-enriched nutrition augmented mesenteric afferent discharge in a low-threshold experiment (C), whereas L-81 prevented this activation (D). Data represented as mean±S.E.M. *P<.05 compared with control, n=6.

mice. Second, we show that the intestinal peptide GLP-1, in addition to CCK, is involved in the immune-modulating properties of lipid-enriched nutrition.

Absorption of luminal nutrients exposes the host to antigenic components, which are able to activate the immune system [19,20]. Postprandial chylomicron formation and subsequent systemic dissemination have been shown to lead to an inflammatory response, due to their high affinity for dietary antigens, such as endotoxin [20,21]. Additionally, prevention of chylomicron formation attenuates LPS release from enterocytes and avoids postprandial endotoxemia [22]. Interestingly, we reveal that assembly and secretion of chylomicrons are important steps in the activation of the nutritional anti-inflammatory vagovagal reflex. The formation of chylomicrons, induced by ingestion of long-chain fatty acids and phospholipids, has been shown to activate the autonomic nervous system in a CCK-dependent manner [10,23,24]. Using a preparation of murine jejunum, we ascertained that lipid-enriched nutrition enhanced mesenteric afferent discharge to distension. Co-administration of L-81 reduced mesenteric afferent firing in the low-threshold range, providing evidence that activation of afferent vagal fibers depends on chylomicron formation [16]. It is noteworthy that this effect was observed within 15 min of exposure to L-81, suggesting that chylomicron assimilation rapidly leads to afferent activation.

As established in previous studies [5–7,15], lipid-enriched nutrition reduced inflammation and preserved intestinal integrity. Prevention of chylomicron formation reduced the effects of lipid-enriched nutrition on systemic inflammation and intestinal integrity in both HS rats and endotoxemic mice. These findings indicate that intestinal uptake and intracellular processing of lipids are important steps in initiation of the nutritional anti-inflammatory reflex in rodents. Previously, our group demonstrated that bile duct ligation (BDL) did not affect the anti-inflammatory potential of lipid-enriched nutrition, suggesting that uptake of lipids is not essential [25]. However, BDL does not completely obstruct lipid uptake. Specifically, the uptake of linoleic acid, which is a strong inducer of chylomicron formation and potent CCK secretagogue, remains largely unaffected [26–28]. Since CCK release and activation of vagal afferents are dependent on absorption of lipids and chylomicron formation [23,29], it is likely that the remaining lipid uptake in BDL rats results in sufficient chylomicron formation and CCK release to activate the anti-inflammatory pathway. Taken together, our data demonstrate that the intracellular processing of lipids plays a dominant role in activation of the anti-inflammatory reflex, since L-81 blocks assembly and secretion of chylomicrons, but does not affect lipid uptake [30]. Moreover, these data implicate that the nutritional compositions, aimed at modulating the immune response, should be rich in long-chain fatty acids and phospholipids. As reviewed by Calder [31] in detail, dietary supplementation with specific fatty acids, such as long-chain n-3 polyunsaturated fatty acids, results in profound immune-modulating effects by influencing metabolic processes, such as eicosanoid production and other as of yet unidentified mechanisms. In addition, our data indicate that enteral nutrition enriched with lipids directly activates an anti-inflammatory reflex.

Previous findings from our group demonstrated that the immunomodulatory effects of lipid-enriched nutrition are dependent on CCK receptors, suggesting that the pathway is largely CCK driven [6,7]. However, this does not exclude a role for other intestinal peptides. CCK has been termed “gatekeeper of the afferent vagus,” and nutrient-induced release of several intestinal peptides depends on

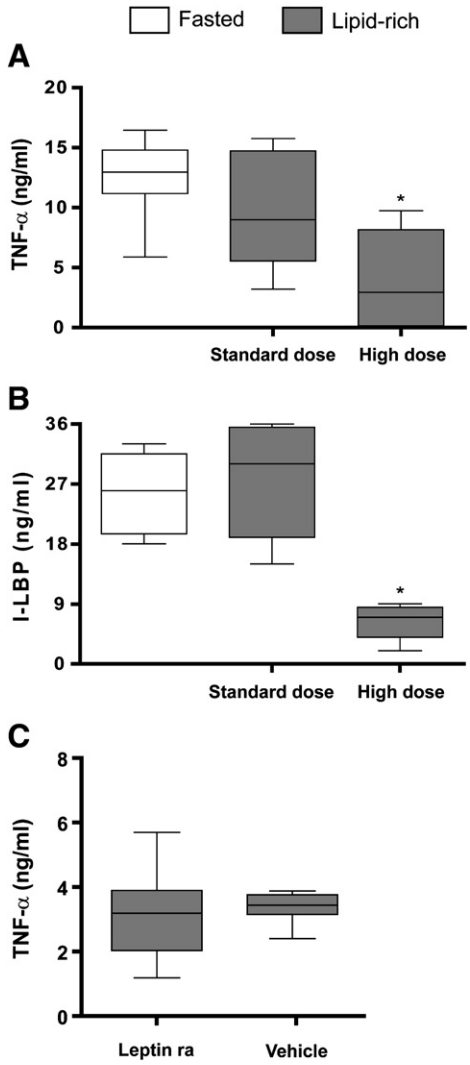

Fig. 6. Leptin lacks involvement in the nutritional anti-inflammatory reflex. Endotoxemia results in marked systemic inflammation and enterocyte damage in Ob/Ob mice. Administration of lipid-enriched nutrition did not reduce plasma levels of TNF-α and I-LBP (A). Increasing the dose of lipid-enriched nutrition from 0.9 kcal (standard dose) to 1.3 kcal (high dose) attenuated plasma levels of TNF-α and I-LBP (B). The anti-inflammatory potential of lipid-enriched nutrition was unaffected in wild-type mice treated with leptin receptor blocking nanobodies (C). Data are represented as median, range and interquartile range. *P<.01 compared with fasted.

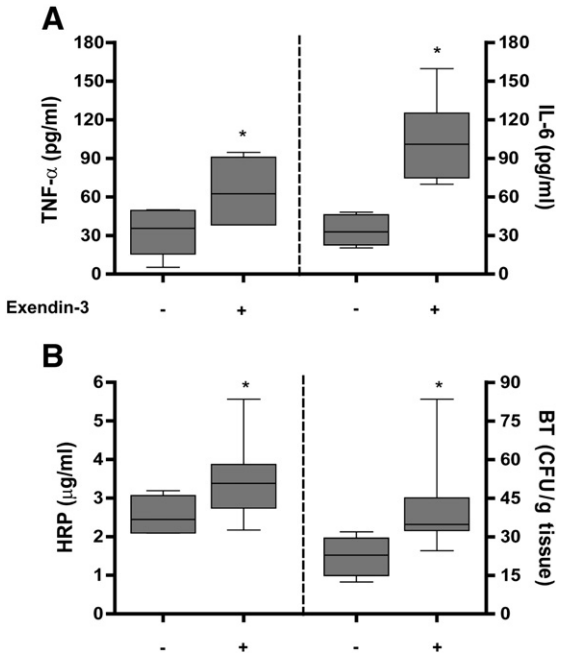

Fig. 7. GLP-1 receptor is involved in the immuno-modulatory effects of lipid-enriched nutrition. Pretreatment with the GLP-1 receptor antagonist exendin-3 reduced the effect of lipid-enriched nutrition on shock-induced systemic inflammation (A) and intestinal integrity (B). Data are represented as median, range and interquartile range. *P<.01 compared with vehicle.

activation of CCK-1 receptors [11, 14]. Leptin, GLP-1 and PYY are released in response to ingestion of dietary fat and function in combination with CCK to regulate satiety *via* afferent vagal fibers [14,18,32,33].

Involvement of leptin in the immune response was described more than a decade ago [34–36]. In the current study, lipid-enriched nutrition failed to reduce systemic inflammation and enterocyte damage in Ob/Ob mice. Increasing the nutrient dose, which enhances the anti-inflammatory potential [15], effectively reduced TNF-α and I-LBP plasma levels, suggesting that Ob/Ob mice have a higher stimulation threshold and implying a co-stimulatory role for leptin. In line, leptin has been described to ameliorate the anti-inflammatory and satiation potential of CCK [12,37]. However, a co-stimulatory role of leptin could not be established, since pharmacologic inhibition of leptin receptors in wild-type mice did not influence the effects of lipid-enriched nutrition. Considering that a higher dose of nutrition was needed in Ob/Ob mice, our data might indicate that the vagus nerve is less sensitive to lipid-enriched nutrition. This is supported by the fact that overfeeding, which is typical for leptin-deficient mice, desensitizes the afferent vagal pathway, resulting in defective intestinal lipid signaling [38,39]. To unravel these findings, future studies are needed to evaluate the effect of long-term high-fat intake on activation of the nutritional pathway.

There is little evidence thus far that GLP-1 and PYY are involved in regulation of the inflammatory response [40, 41]. However, a role for both peptides in nutrient-dependent activation of vagal afferents has been clearly established [42]. Moreover, release of GLP-1 and PYY is dependent on activation of CCK-1 receptors [14,18]. Here, we demonstrate that activation of GLP-1 receptors by lipid-enriched nutrition is involved in activation of the anti-inflammatory reflex. Administration of a GLP-1 receptor antagonist suppressed the anti-inflammatory potential of lipid-enriched nutrition, but did not abolish the effect. A role for the PYY receptor could not be demonstrated, as pretreatment with the Y2-receptor antagonist did not affect activation of the nutritional anti-inflammatory pathway. These findings are supported by Raybould et al. [43,44], who demonstrated that PYY-neutralizing antibodies do not affect lipid-induced gastric emptying, which is known to be regulated by vagal afferents. Taken together, the current findings suggest that activation of the GLP-1 receptor is involved in nutritional activation of the CCK-mediated anti-inflammatory vagovagal reflex.

In surgical and critically ill patients, nutritional support is vital to meet metabolic demand and prevent immunodeficiency [36,45]. Enteral administration of nutrients is preferred in these patient groups, since enteral nutrition reduces morbidity and length of hospital stay compared with parenteral nutrition [45,46]. The current study demonstrates that enteral nutrition not only delivers essential nutrients, but also triggers a potent intestinal immune feedback system. We believe that this endogenous anti-inflammatory mechanism evolved to counteract exposure of the interior milieu to antigenic substances, such as endotoxin, resulting in postprandial inflammation and potentially aggravating chronic inflammatory conditions [20,21]. Development of specific nutritional compositions that effectively

stimulate the vagovagal anti-inflammatory reflex, and well-timed administration could result in promising interventions to control inflammation and reduce organ damage during non-physiologic inflammatory conditions, such as surgical interventions and sepsis.

References

[1] Cummings DE, Overduin J. Gastrointestinal regulation of food intake. J Clin Invest 2007;117:13–23.
[2] Lam TK. Neuronal regulation of homeostasis by nutrient sensing. Nat Med 2010;16:392–5.
[3] Raybould HE, Glatzle J, Freeman SL, et al. Detection of macronutrients in the intestinal wall. Auton Neurosci 2006;125:28–33.
[4] Dockray GJ. Luminal sensing in the gut: an overview. J Physiol Pharmacol 2003;54 (Suppl 4):9–17.
[5] de Haan JJ, Lubbers T, Hadfoune M, et al. Postshock intervention with high-lipid enteral nutrition reduces inflammation and tissue damage. Ann Surg 2008;248: 842–8.
[6] Lubbers T, Luyer MD, de Haan JJ, et al. Lipid-rich enteral nutrition reduces postoperative ileus in rats via activation of cholecystokinin-receptors. Ann Surg 2009;249:481–7.
[7] Luyer MD, Greve JW, Hadfoune M, et al. Nutritional stimulation of cholecystokinin receptors inhibits inflammation via the vagus nerve. J Exp Med 2005;202:1023–9.
[8] Lubbers T, de Haan JJ, Hadfoune M, et al. Cholecystokinin/cholecystokinin-1 receptor-mediated peripheral activation of the afferent vagus by enteral nutrients attenuates inflammation in rats. Annals of Surgery 2010;252:376–82.
[9] Glatzle J, Kalogeris TJ, Zittel TT, et al. Chylomicron components mediate intestinal lipid-induced inhibition of gastric motor function. Am J Physiol Gastrointest Liver Physiol 2002;282:G86–91.
[10] Glatzle J, Wang Y, Adelson DW, et al. Chylomicron components activate duodenal vagal afferents via a cholecystokinin A receptor-mediated pathway to inhibit gastric motor function in the rat. J Physiol 2003;550:657–64.
[11] Dockray GJ. Cholecystokinin and gut–brain signalling. Regul Pept 2009;155:6–10.
[12] Barrachina MD, Martinez V, Wang L, et al. Synergistic interaction between leptin and cholecystokinin to reduce short-term food intake in lean mice. Proc Natl Acad Sci U S A 1997;94:10455–60.
[13] Lin HC, Chey WY, Zhao X. Release of distal gut peptide YY (PYY) by fat in proximal gut depends on CCK. Peptides 2000;21:1561–3.
[14] Beglinger S, Drewe J, Schirra J, et al. Role of fat hydrolysis in regulating glucagon-like peptide-1 secretion. J Clin Endocrinol Metab 2010;95:879–86.
[15] Lubbers T, de Haan JJ, Hadfoune M, et al. Lipid-enriched enteral nutrition controls the inflammatory response in murine gram-negative sepsis. Crit Care Med 2010;38:1996–2002.
[16] Booth CE, Kirkup AJ, Hicks GA, et al. Somatostatin sst(2) receptor-mediated inhibition of mesenteric afferent nerves of the jejunum in the anesthetized rat. Gastroenterology 2001;121:358–69.
[17] Peters JH, Simasko SM, Ritter RC. Modulation of vagal afferent excitation and reduction of food intake by leptin and cholecystokinin. Physiol Behav 2006;89: 477–85.
[18] Degen L, Drewe J, Piccoli F, et al. Effect of CCK-1 receptor blockade on ghrelin and PYY secretion in men. Am J Physiol Regul Integr Comp Physiol 2007;292:R1391–9.
[19] Aljada A, Mohanty P, Ghanim H, et al. Increase in intranuclear nuclear factor kappaB and decrease in inhibitor kappaB in mononuclear cells after a mixed meal: evidence for a proinflammatory effect. Am J Clin Nutr 2004;79:682–90.
[20] Erridge C, Attina T, Spickett CM, et al. A high-fat meal induces low-grade endotoxemia: evidence of a novel mechanism of postprandial inflammation. Am J Clin Nutr 2007;86:1286–92.
[21] Laugerette F, Vors C, Geloen A, et al. Emulsified lipids increase endotoxemia: possible role in early postprandial low-grade inflammation. J Nutr Biochem 2011;22:53–9.
[22] Ghoshal S, Witta J, Zhong J, et al. Chylomicrons promote intestinal absorption of lipopolysaccharides. J Lipid Res 2009;50:90–7.
[23] Lo CM, Ma L, Zhang DM, et al. The mechanism of the induction of brain c-fos expression by lipid absorption. Am J Physiol Regul Integr Comp Physiol 2007;292: R268–73.
[24] Tso P, Balint JA. Formation and transport of chylomicrons by enterocytes to the lymphatics. Am J Physiol 1986;250:G715–26.
[25] Luyer MD, Buurman WA, Hadfoune M, et al. High-fat enteral nutrition reduces endotoxin, tumor necrosis factor-alpha and gut permeability in bile duct-ligated rats subjected to hemorrhagic shock. J Hepatol 2004;41:377–83.
[26] Demarne Y, Corring T, Pihet A, et al. Fat absorption in germ-free and conventional rats artificially deprived of bile secretion. Gut 1982;23:49–57.
[27] Hand KV, Bruen CM, O'Halloran F, et al. Acute and chronic effects of dietary fatty acids on cholecystokinin expression, storage and secretion in enteroendocrine STC-1 cells. Mol Nutr Food Res 2010;54:S93–S103.
[28] Even P, Mariotti F, Hermier D. Postprandial effects of a lipid-rich meal in the rat are modulated by the degree of unsaturation of ^{18}C fatty acids. Metabolism 2010;59: 231–40.
[29] Raybould HE, Meyer JH, Tabrizi Y, et al. Inhibition of gastric emptying in response to intestinal lipid is dependent on chylomicron formation. Am J Physiol 1998;274: R1834–8.
[30] Tso P, Balint JA, Bishop MB, et al. Acute inhibition of intestinal lipid transport by Pluronic L-81 in the rat. Am J Physiol 1981;241:G487–97.
[31] Calder PC. n-3 Polyunsaturated fatty acids, inflammation, and inflammatory diseases. Am J Clin Nutr 2006;83:1505S–19S.
[32] Bado A, Levasseur S, Attoub S, et al. The stomach is a source of leptin. Nature 1998;394:790–3.
[33] D'Alessio D. Intestinal hormones and regulation of satiety: the case for CCK, GLP-1, PYY, and Apo A-IV. JPEN J Parenter Enteral Nutr 2008;32:567–8.
[34] Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. Nature 1998;395:763–70.
[35] Sarraf P, Frederich RC, Turner EM, et al. Multiple cytokines and acute inflammation raise mouse leptin levels: potential role in inflammatory anorexia. J Exp Med 1997;185:171–5.
[36] Matarese G, Moschos S, Mantzoros CS. Leptin in immunology. J Immunol 2005;174:3137–42.
[37] Bozkurt A, Cakir B, Ercan F, et al. Anti-inflammatory effects of leptin and cholecystokinin on acetic acid-induced colitis in rats: role of capsaicin-sensitive vagal afferent fibers. Regul Pept 2003;116:109–18.
[38] Wang PY, Caspi L, Lam CK, et al. Upper intestinal lipids trigger a gut–brain–liver axis to regulate glucose production. Nature 2008;452:1012–6.
[39] Cheung GW, Kokorovic A, Lam CK, et al. Intestinal cholecystokinin controls glucose production through a neuronal network. Cell Metab 2009;10:99–109.
[40] Ishii Y, Asai S, Kohno T, et al. Recovery of liver function in two-third partial hepatectomized rats evaluated by L-[$^{1-13}$C]phenylalanine breath test. Surgery 2002;132:849–56.
[41] Robinson K, Vona-Davis L, Riggs D, et al. Peptide YY attenuates STAT1 and STAT3 activation induced by TNF-alpha in acinar cell line AR42J. J Am Coll Surg 2006;202:788–96.
[42] Berthoud HR. The vagus nerve, food intake and obesity. Regul Pept 2008;149: 15–25.
[43] Holzer HH, Turkelson CM, Solomon TE, et al. Intestinal lipid inhibits gastric emptying via CCK and a vagal capsaicin-sensitive afferent pathway in rats. Am J Physiol 1994;267:G625–9.
[44] Raybould HE, Tabrizi Y, Meyer JH, et al. PYY immunoneutralization does not alter lipid-induced inhibition of gastric emptying in rats. Regul Pept 1999;79: 125–30.
[45] Kreymann KG, Berger MM, Deutz NE, et al. ESPEN Guidelines on enteral nutrition: intensive care. Clin Nutr 2006;25:210–23.
[46] Elke G, Schadler D, Engel C, et al. Current practice in nutritional support and its association with mortality in septic patients—results from a national, prospective, multicenter study. Crit Care Med 2008;36:1762–7.