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Research Article

Oxidative stress due to anesthesia and surgical trauma: Importance of early enteral nutrition

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Anesthesia and surgical trauma are considered major oxidative and nitrosative stress effectors resulting in the development of SIRS. In this study we evaluated the usefulness of early enteral nutrition after surgical trauma. Sixty male Wistar rats were subjected to midline laparotomy and feeding-gastrostomy. Twenty of these rats served as controls after recovering from the operation stress. The remaining rats received, through gastrostomy, enteral nutrition or placebo-feeding for 24 h. Oxidative stress markers and CC chemokine production were evaluated in rat serum and liver tissue. The operation itself was found to increase nitric oxide (NO) and malondialdehyde (MDA) and to decrease superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), as well as liver tissue energy charge (EC) in relation to controls. The rats receiving enteral feeding exhibited statistically significantly lower levels of NO and MDA, and higher levels of SOD, GSH-Px, and liver EC, in relation to placebo feeding rats. The operation significantly increased the chemokines monocyte chemoattractant protein (MCP)-1 and regulated upon activation, normal T-cell expressed, and secreted (RANTES) in rat serum, while enteral nutrition caused a further significant increase in chemokine levels in serum. mRNA chemokine expression in liver was increased in a similar pattern. These findings indicate that early enteral feeding might play an important role after surgery ameliorating oxidative stress, affecting positively the hepatic EC and regulating, *via* chemokine production, cell trafficking, and healing process.

Keywords: Anesthesia / Chemokines / Enteral nutrition / Oxidative stress / Surgical trauma

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1 Introduction

Anesthesia and surgery of any kind, but especially that of the abdominal viscera, are well recognized as oxidative stress inducing manipulations. Oxidative stress – described as an imbalance between the free radical production and the antioxidant defense – has an important role in the development and manifestation of systemic inflammatory response syndrome (SIRS), becoming an increasingly common cause of morbidity and mortality should the patient need hospitalization in the intensive care unit (ICU) [1, 2]. Besides free radical production, the stimuli of anesthesia and major surgery themselves, although less intense than in the case of infection or sepsis, also lead to mobilization of fuel stores and gluconeogenesis by the release of catecholomines, cortisol, glucagons, as well as by insulin resistance. In a similar manner, both surgery and anesthesia are responsible for immunological disturbances that are characterized by cell-mediated immunity, production of inflammatory mediators, and cell recruitment which is orchestrated mainly from an increased chemokine production [3–5].

Based on these data, we acknowledge today the beneficial effect of augmentation of distinct defense mechanisms,

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Abbreviations: ATP, adenosine triphosphate; **GSH-Px,** glutathione peroxidase; **ICU,** intensive care unit; **MCP-1,** monocyte chemoattractant protein-1; **MDA,** malondialdehyde; **NO,** nitric oxide; **RANTES,** regulated upon activation, normal T-cell expressed, and secreted; **SIRS,** systemic inflammatory response syndrome; **SOD,** superoxide dismutase

while other defense mechanisms must be suppressed simultaneously. To date, the best tool available is nutrition. Additionally, it is well accepted that the concept of a "therapeutic window" is essential in every "acute condition"; there appears to be an optimal time soon after the induction of oxidative stress and systemic inflammatory response, during which macro and micronutritional supplementation may still have a "preventive effect", although the response may also well depended on the previous nutritional and antioxidant status [6, 7]. Thus, according to the latest meta-analyses of currently available trial results, the primary aim should be to initiate enteral nutrition as early as possible [8–10].

Given the conflict between the benefits [11–14] and potential risk [15, 16] of early enteral nutrition, this study was conducted to evaluate the usefulness of early enteral nutrition in rats subjected to gastrointestinal tract surgery plus a major surgical trauma. The principal endpoint was the assessment of modulation of oxidative stress and acute phase response markers in the enteral feeding group.

2 Materials and methods

2.1 Materials

TRIzolR was obtained from Life Technologies (Paisley, UK). DNase was obtained from Boehringer-Mannheim (Mannheim, Germany). Oligo(dT) 12–18 primer, Superscript II, reverse transcription (RT) buffers, and deoxyribonucleotide triphosphates (dNTPs) were purchased from Gibco BRL (Life Technologies). RNasin was from Promega (Southampton, UK). PCR buffers, dNTPs, and expand polymerase were purchased from Roche Molecular Biochemicals (Lewes, Sussex, UK).

2.2 Animals

Sixty male Wistar rats weighing 250–300 g were housed together at room temperature with a 12 h light–dark cycle and were given free access to tap water and standard pellet rat food until 24 h before the time of operation. The experimental protocol was approved by the Governmental Animal Protection Committee and adhered to the European Community Guiding Principles for the care and use of animals.

2.3 Animal preparation

After food but not water was withheld for 24 h, each rat was operated on to perform a feeding gastrostomy. Under ketamine intramuscular anesthesia (50 mg/kg BW) a midline laparotomy was performed and a 1.0 mm silicon rubber tube (Dow Corning, Midland, MI, USA) was inserted aseptically through a fundal gastrotomy, advanced to the antrum and anchored to the stomach wall with a purse-string suture. The proximal end of the catheter was tunnelled subcutaneously, exteriorized at the midscapular region, and attached to a swivel-spring apparatus, which allowed unrestricted movement of the animals. The laparotomy was closed by means of a continuous suture, after a volume of 5 mL of normal saline 0.5% was infused into the peritoneal cavity for the initial fluid requirements. The rats were then placed in individual wire-bottomed cages to recover.

2.4 Experimental design

Anesthesia, laparotomy, and the performance of gastrotomy in relation to the soft tissue trauma for tunneling the catheter toward the midscapula area are considered a major stress. Upon recovery from anesthesia the rats were randomly divided into 3 groups: 20 of them were allocated to receive tap water and standard pellet rat food ad libidum. These rats were left free in their cage for a 10 days period for recovery from the anesthesia/operation-induced stress and served as controls (group 1: control). Twenty rats were allocated to receive, through gastrostomy, placebo-feeding (group 2: major trauma + placebo feeding), while the remaining twenty rats received enteral nutrition (group 3: major trauma + enteral feeding). Both regimens were given through the gastrostomy tube by continuous pump controlled infusion in a rate of 2 mL/h – under sterile conditions – immediately after the rats recovered from anesthesia and for a 24 h period, as previously published [7]. The rats of groups 2 and 3 were then sacrificed, having first been subjected to blood and liver sampling, 10 days later the same done with the controls (group 1).

2.5 Enteral nutrition

The enteral nutrition regimen used was the commercially available, mixed-nutrient, liquid formula Fresubin-HP Energy, purchased from Fresenius-Kabi, Hellas, while the placebo-treated rats were infused with an equal volume of normal saline solution 0.9%. Fresubin-HP energy contains 1.5 kcal/mL, with 20% of the kilocalories as proteins, 35% as lipids, and 45% carbohydrates. The volume of 2 mL/h was decided according to the literature, representing the hourly energy requirements of a small animal under stress (25 kcal × BW × 1.6 = kcal/day) [17].

2.6 Blood sampling

Five millilitres of blood was extracted by abdominal aorta puncture from all experimental animals, and was then mixed with 3.8% buffered sodium citrate, pH 7.4, in glass tubes in a ratio of 9:1, and after centrifugation at 2800 × *g* for 5 min, the serum was kept at –70°C, until use. Serum samples were used for the determination of Nitric Oxide (NO) synthesis as an index of free radical production, the activity of endogenous antioxidant enzymes superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px).

2.7 Liver sampling

The left lateral and medium liver lobes were excised from all animals. Specimens were divided into two equal parts and immediately frozen in liquid nitrogen for further analysis of products of oxidative damage to lipids malondialdehyde (MDA), as well as for determination of energy charge (EC) levels, and chemokine mRNA expression.

2.8 Assay of nitrite/nitrate

Serum samples were assayed for nitrite/nitrate (NOx) concentrations – as an index of an *in vivo* NO synthesis – using an autoanalyzer (TCI–NOX 1000, Tokyo Kasei Kogyo, Tokyo Japan) as follows: the samples were mixed with the carrier solution (0.07% EDTA and 0.3% NH_4Cl) and passed through a copperized cadmium reduction column to reduce nitrate to nitrite and reacted with a Griess reagent. Absorbance of a purple azo dye at 210 nm was measured using a flow-through visible spectrophotometer (Visible Detectors –3250, Tokyo Kasei Kogyo). Standards of sodium nitrite and nitrate, ranging from 2 to 10 μm, were analyzed daily to check column efficiency. The data are expressed in micromolar values, and the LOD of NOx was 0.5 M (99% confidence limit).

2.9 Assay of superoxide dismutase

SOD activity was measured with Ransod kits (Randox Laboratories, Crumlin, UK). This method employed xanthine and xanthine oxidase to generate superoxide radicals, which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) to form a red formazan dye. The SOD activity is then measured by the degree of inhibition of this reaction. All diluted sample rates were converted into percentages of the sample diluent rate, and subtracted from 100% to give a percentage of inhibition. The activity was measured at 37°C on a spectrophotometer, and absorbance was monitored at 505 nm for 3 min. The unit of activity is defined as the amount of enzyme that inhibits the rate of the formazan dye formation by 50%. SOD units were obtained from standard curve using percentage inhibition of the samples (SOD U/mL). Standards were prepared by diluting a commercial SOD preparation in order to obtain a standard curve.

2.10 Assay of glutathione peroxidase

GSH-Px activity was measured with Ransel kits (Randox Laboratories, Crumlin, UK) at 37°C on a spectrophotometer at 340 nm for 3 min. This assay required cumene hydroperoxide as a substrate. Before analysis the samples were diluted 40-fold to a hemolysate by adding Drabkin's reagent (double strength) to inhibit the peroxide activity of the hemoglobin. The final concentrations of reagents in the assay were those recommended by the manufacturer. The activity of GSH-Px was expressed in units/mL.

2.11 Assays of malondialdehyde

The extent of liver damage attributable to free radical production was assessed indirectly by measuring the MDA level, an intermediate product of lipid peroxidation. Liver specimens were weighed, minced, and homogenized in 0.02 M sodium phosphate buffer pH 7.4 (1:10 w/v), using a smooth glass with a Teflon pestle hand homogenizer. The supernatant of the homogenate, after centrifugation at $2800 \times g$ for 5 min, was withdrawn and analyzed with a technique described elsewhere [18]. Briefly, 1 mL of 17.5% trichloric acid and 1 mL of 0.6% thiobarbituric acid pH 2 were added to 1 mL of the homogenate. This mixture was placed in a boiling waterbath for 15 min and then allowed to cool. A 1 mL aliquot of 70% trichloric acid was added and the mixture allowed to incubate for 20 min. The sample was then centrifuged for 15 min at 2000 rpm and the optical density of the supernatant evaluated spectrophotometrically at 534 nm against a reagent blank. The amount of MDA was expressed in nanomoles *per* milligram of protein, with the protein content determined by the method of Lowry *et al.* [19].

2.12 Energy charge estimation

Hepatic tissue samples obtained for EC determination were immediately frozen (within 30 s) in liquid nitrogen and stored at –125°C to prevent adenosine triphosphate (ATP) degradation until analysis. Adenine-nucleotides -ATP, ADP, and AMP- hepatic tissue concentration, expressed in μmol/g of wet tissue weight, were determined by means of RP HPLC. Consequently, the tissue EC, which expresses liver functional adequacy [20], was estimated according to the formula proposed by Atkinson: (1/2 ADP + ATP)/(AMP + ADP + ATP) and expressed as raw numbers ± SD [21].

2.13 Chemokine ELISAs

Rat sera were collected as described above for chemokine assessment. The CC chemokines, monocyte chemoattractant protein (MCP)-1, and regulated upon activation, normal T-cell expressed, and secreted (RANTES) were measured with commercially available ELISA assay according to the manufacturer's instructions (Biosource, Nivelles, Belgium).

2.14 Reverse transcription-PCR (RT-PCR)

Total RNA was extracted from liver tissue homogenized on ice with a glass homogenizer into TRIzol and treated with

Table 1. Primer Sequences used for the RT-PCR studies

Gene	Primers	Product size (bp)
MCP-1	Sense: CCTGTTGTTCACAGTTGCTGCC Antisense: TCTACAGAAGTGCTTGAGGTGGTTG	369
RANTES	Sense: CGTGAAGGAGTATTTTTACACCAGC Antisense: CTTGAACCCACTTCTTCTCTGGG	110
18 S	Sense: GAGGTGAAATTCTTGGACCGG Antisense: CGAACCTCCGACTTTCGTTCT	93

DNAse as described by the manufacturers. RT-PCR were performed as previously described [22]. Briefly, 1 μg mRNA was denatured at 70(C for 10 min in the presence of 5 μM oligo (dT) 12–18 primer. It was then reverse transcribed in a 10 μL volume with Superscript II, 1 × RT buffer, 1 mM deoxyribonucletide triphosphates (dNTPs), 5 mM DDT, and 2.5 U/μL RNAsin at 42°C for 60 min. cDNAs (1 μL) were PCR amplified in a 25 μL reaction, containing: 1 × PCR buffer and 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 mM sense and antisense primers, and 0.4 U high fidelity expand polymerase. The oligonucleotide sequence and product size for specific primer pairs used are shown in Table 1. The conditions for amplification were: 5 min 94°C, 30 cycles of 30 s 94°C, 30 s annealing temperature, 30 s 72°C, followed by an extension for 7 min at 72°C. PCR products were resolved by electrophoresis on 2% agarose gels and visualized by ethidium bromide staining. In order to control for genomic contamination an identical parallel PCR reaction (RT-negative) was performed for each sample containing starting material which had not been reverse transcribed. Each set of primers was tested with at least three different RNA samples treated independently. The integrated density of the bands was calculated by digital image analysis (Scion image). The ratio of the integrated density of each gene divided by that of house keeping gene [18S] was used to quantify the results.

2.15 Statistical analysis

Data were expressed as means ± SD of the mean. Statistical analyses were performed by using the one-way repeated measures analysis of variance (ANOVA) for multiple comparisons between groups. Statistical significance was set at the p level of 0.05 for all tests.

3 Results

3.1 Nitrite/nitrate (NOx)

A basal production of NOx (31.98 ± 4.07 μmol/L) was found in control animals of group 1. The anesthesia/operation-induced stress was found to significantly increase NOx (89.61 ± 15.03 μmol, $p < 0.01$) in group 2 – placebo treated – rats, in relation to group 1, while group 3 – enteral feeding – rats exhibited a significantly lower NOx production (49.56 ± 8.78 μmol/L), in relation to group 2, indicating that feeding significantly decrease NOx production (Fig. 1).

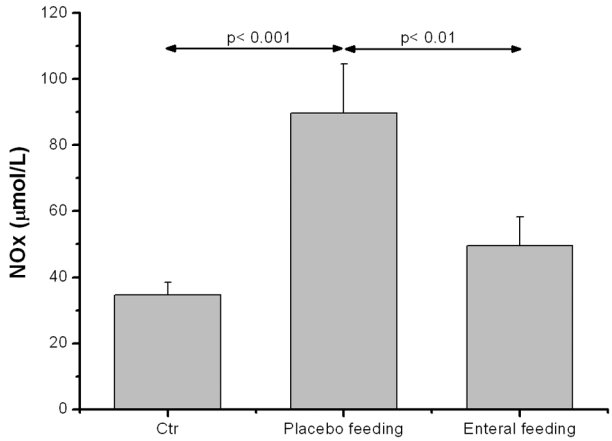

Figure 1. Basal production of nitrite/nitrate (NOx) in rat serum. Each column represents mean ± SD in μmol/L.

3.2 Superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px)

In control animals (group 1), the levels of endogenous antioxidant enzymes SOD and GSH-Px were found to be in high levels, taken as being the basal production. Group 2 rats were, as expected, exhibited a highly significant decrease in SOD and GSH-Px levels in relation to group 1. Enterally fed rats of group 3 showed a similar elimination trend of SOD and GSH-Px, but the difference between groups 2 and 3 was statistically significant, meaning that enteral feeding appears to preserve antioxidant defenses (Figs. 2 and 3).

3.3 Malondialdehyde (MDA)

MDA, a breakdown product of the most important chain reactions leading to oxidation of PUFAs, and therefore, a reliable marker of oxidative stress-mediated lipid peroxida-

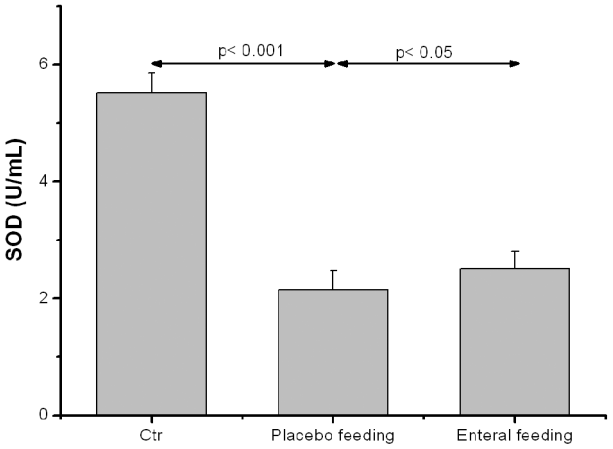

Figure 2. Endogenous production of SOD in rat serum, measured with Ransod kit. Each column represents mean ± SD in units/mL.

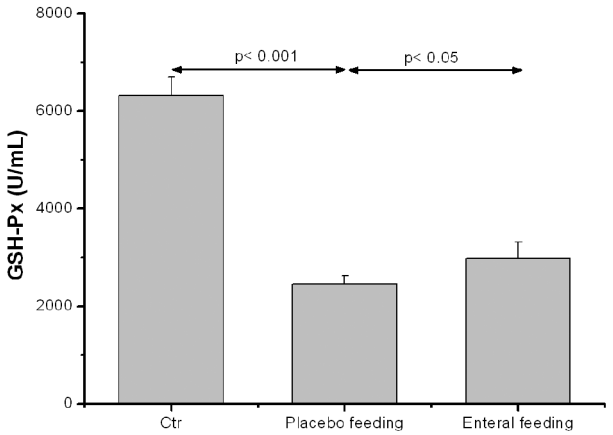

Figure 3. Endogenous production of GSH-Px in rat serum, measured by Ransel kit. The activity was expressed as mean ± SD in units/mL.

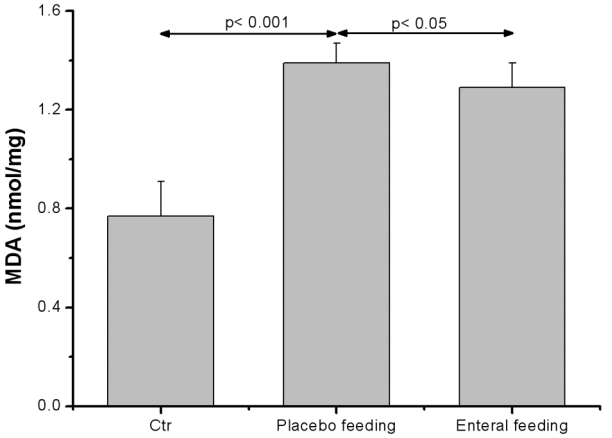

Figure 4. MDA production in rat liver tissue. Each column represents the amount of MDA as mean ± SD, expressed in nmol/mg of tissue protein.

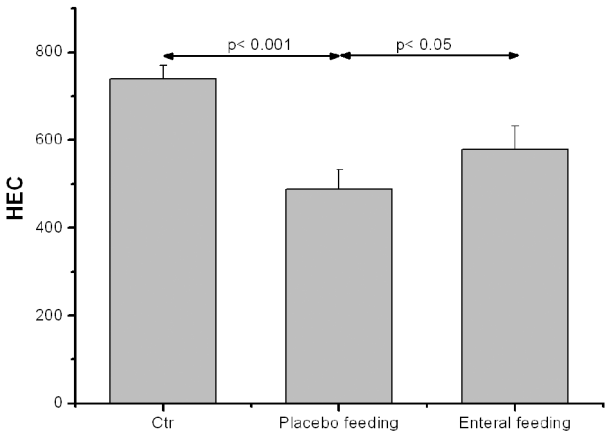

Figure 5. Hepatic EC, which represses the functional adequacy of rat liver. EC was estimated according to the formula proposed by Atkinson [21] and expressed as raw numbers. Each column represents mean ± SD in absolute values.

tion reveals, similarly to NOx products, a tremendous increase in the anesthesia/operation-induced stress in rats of group 2, which were placebo feeding. A significant decrease was prominent in group 3, which were subjected to enteral feeding, in relation to group 2. In both groups MDA levels were increased in relation to the baseline value of controls of group 1 (Fig. 4).

3.4 Hepatic energy charge

EC of liver tissue was found to be highly affected in group 2 rats subjected to operational stress without feeding support. In contrast, in group 3, however, enteral feeding seems to significantly re-establish hepatic EC and thus the functional adequacy of the liver, its level exhibiting a tendency to reach the normal value, 1, or that of group 1 (Fig. 5).

3.5 Chemokine levels in serum and chemokine mRNA expression in liver

ELISA measurement demonstrated a basal amount of circulating MCP-1 (23.22 ± 11.68 pg/mL, $n = 10$) and RANTES (36.66 ± 15.82 pg/mL, $n = 10$) in the serum from the control group (Figs. 6a and b). The operation (Placebo feeding Group) caused a significant ($p < 0.01$) elevation in the circulating levels of both MCP-1 (182.33 ± 124.83 pg/mL, $n = 10$) and RANTES (249.44 ± 158.36 pg/mL, $n = 10$). This elevation was significantly higher ($p < 0.001$) for MCP-1 (878.77 ± 142.73 pg/mL, $n = 10$) and RANTES (515.22 ± 39 pg/mL, $n = 10$) in the animals that received enteral feeding (group 3) compared to the group fed with placebo (Figs. 6a and b). Using RT-PCR, we examined the mRNA expression of both MCP-1 and RANTES in liver homogenates. Liver tissue from the animals which under-

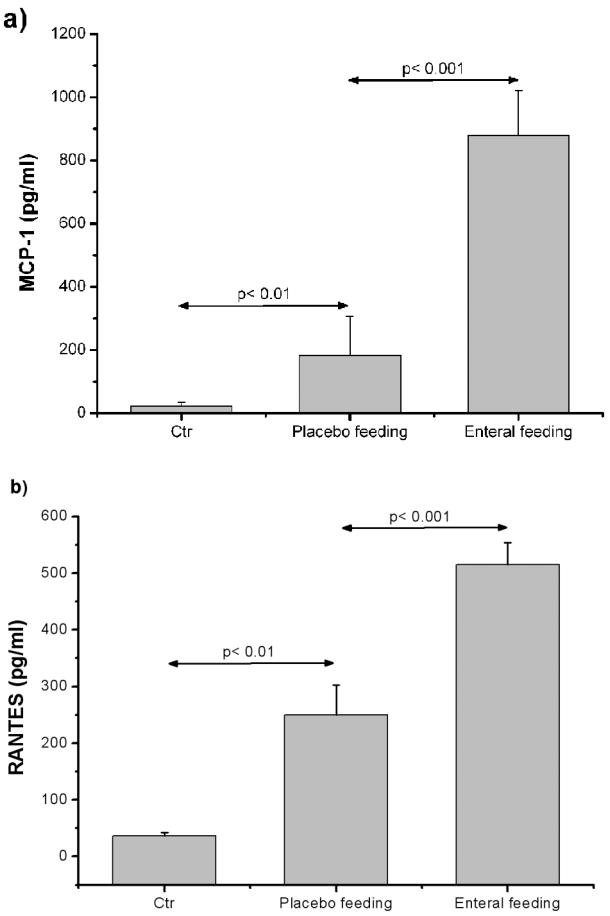

Figure 6. Circulating levels of (a) MCP-1 and (b) RANTES were measured by ELISA in serum from rats. Each column represents mean ± SD of chemokine levels in the groups.

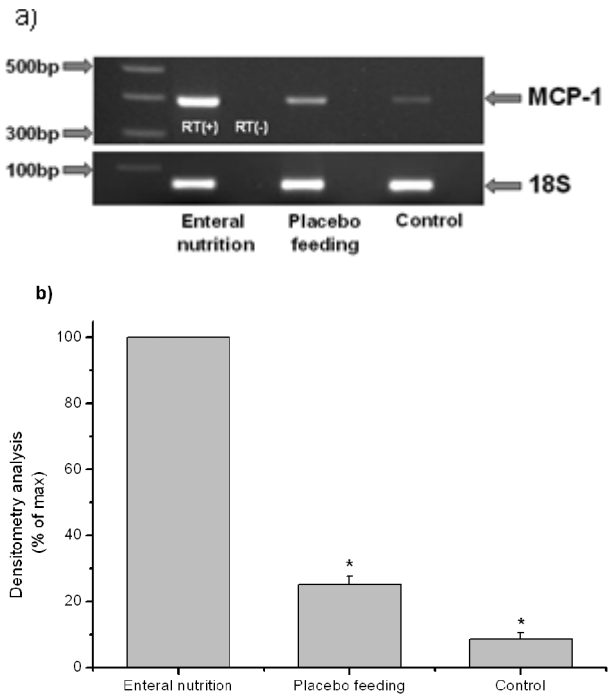

Figure 7. MCP-1 mRNA expression in rat liver tissue. (a) Total RNA was extracted from liver homogenates and RT-PCR for both MCP-1 and 18S was performed. Representative PCR blots of mRNA expression for MCP-1 and 18S. In order to control for genomic contamination each sample has a reverse transcriptase negative control (RT-). The gel shows fluorescence of ethidium bromide stained PCR products resolved by electrophoresis. (b) Densitometry analysis of PCR product of MCP-1 mRNA expression measured as a percentage of maximum MCP-1 mRNA expression after enteral nutrition. *$p <$ 0.001. Representative of three experiments.

went a gastrostomy and were fed with placebo was found to express MCP-1 mRNA transcripts (Figs. 7a and b), while enteral feeding was found to induce a stronger MCP-1 mRNA expression (Figs. 7a and b), in a pattern similar to the pattern of circulating serum levels of MCP-1 (Fig. 6a). RT-PCR for RANTES mRNA expression demonstrated similar findings (data not shown).

4 Discussion

Conventional management of individuals subjected to gastrointestinal surgery includes the institution of a nil-by-mouth regimen, until gastric motility recovers. However, it is now becoming clear that luminal nutrition has a central role in the maintenance of the normal gut function, by improving motility, accelerating splanchnic blood flow and preventing bacterial permeability, leading thus to a reduction in infective complications [9–12, 23].

In the present study, oxidative stress and chemokine production were multifactorial in origin; the main impacts were from the surgical trauma of both laparotomy and soft tissue (subcutaneous blunt manipulation in order the gastrostomy catheter to be exteriorized at the midscapular region), less from anesthesia and little, if any, from ischemia-reperfusion events due to visceral organs manipulation. Our results – with oxidative stress, chemokine production, and hepatic tissue EC – being highly affected after surgery, support the initial hypothesis. In a similar manner, recent studies on patients subjected to cardiosurgical operations, reported that surgical trauma alone, and not the cardiopulmonary by-pass procedure, caused the rise in chemokines and proinflammatory mediators at an early stage of the operation [5, 24–26]. To test the hypothesis that liver is a major source of circulating CC chemokines in various conditions, mainly *via* the chemokine secretion by Kupffer cells [27–30], we examined MCP-1 and RANTES mRNA expression in liver homogenates. Samples from the animals which had undergone operation and were fed with placebo (group 2) were found to express increased mRNA transcripts for both MCP-1 and RANTES compared to the controls. This data suggest that the liver might be the main

source of the CC chemokines in the blood of the rat following an operation.

Although it is difficult to assess the impact of anesthesia alone the group 1 rats, those which were allowed to recover from the operation for 10 days, were used as an indicator of anesthesia-induced stress. In that group, the values of all the above-mentioned parameters were no where near the values found in other groups: NO production as an index of oxidative stress was found to be significantly low; SOD and GSH-Px levels, as indexes of endogenous antioxidants were very high; CC chemokine circulating in blood and chemokine mRNA expression in liver tissues were also significantly low. Similarly, hepatic tissue MDA, as a reliable marker of lipid peroxidation, was significantly low and EC reflecting functional adequacy of the liver was found to be minimally affected from the normal value of 1. In support of our findings, Bravo-Cuéllar *et al.* [31] in a clinical study comparing plasma levels of proinflammatory cytokines, C-reactive protein, and lipoperoxides in patients submitted to laparoscopic cholecystectomy under general or regional anesthesia reported all parameters increased 24 h after surgery in both groups, except plasma levels of IL-1β in the regional anesthesia group and Jedynak *et al.* [5] did not find any effect of anesthesia on serum concentrations of MCP-1 and RANTES in patients during aortic surgery.

In our experimental study, a significant loss of oxidative balance was observed in the rats which received placebo feeding, a fact evidenced by the significant expenses of the antioxidant enzymes SOD and GSH-Px and increased levels of NO as well as of the oxidative damage marker MDA. In contrast, the enteral feeding rats (group 3), were found to have higher levels of antioxidant enzymes and lower production of free radical NO and MDA. Patients being in SIRS – whatever the cause of the initial disease – are indeed characterized by increased free radical production along with depressed circulating levels of nearly all antioxidants. The cause of these low levels are multifactorial: SIRS itself leads to redistribution of micronutrients from the circulating compartment to tissues and organs involved in protein synthesis [32], but acute losses through biological fluids, dilution due to resuscitation fluids, and insufficient intakes contribute heavily too [6].

Measuring CC chemokines in the serum of rats, we found that the enteral nutrition (group 3) resulted in a further increase in the concentration of circulating MCP-1 and RANTES compared to the animals which underwent operation and were fed with placebo (group 2). RT-PCR revealed a similar increase of the MCP-1 and RANTES mRNA expression in liver homogenates, suggesting that the elevation of circulating CC chemokines might be of hepatic origin [27–30]. Both CC chemokines MCP-1 and RANTES have been reported increased after surgery and trauma [5, 26, 33–35]. These CC chemokines serve as chemotactic and stimulating factors, modulating the host immune response to infection by direct effects on differentiating T-cells and indirect effects on antigen-presenting cells and playing a role in angiogenesis and the healing process [35–39]. These immunoregulatory properties of MCP-1 and RANTES might influence the development of immunosuppression and complications following surgery in humans or to improve the healing process, but their beneficial or harmful role has not been yet evaluated [40]. In our study, enteral feeding was found to significantly enhance the operation-induced production of both MCP-1 and RANTES. Another study have shown that decreased enteral stimulation results in decreased levels of chemokines [41]. If this effect of enteral nutrition is a result of increased nutrition uptake or an "immunoregulatory" effect of the diet, it is beyond the scope of the present work and further studies are necessary to clarify this data.

The most important source of antioxidants is provided by nutrition and in the present study the enteric formula used contains adequate amounts of vitamins and trace elements, as already mentioned. The trace elements Cu, Se, Mn, and Zn are essential components of the endogenous enzymatic defenses, thus nutritional support that exceeds recommended daily allowance is required for restoration [42]. Current investigations in enteral nutrition have focused on the ability to modulate the metabolic response *via* specially formulated enteral diets containing individual nutrients such as arginine, glutamine, antioxidants, and *n*-3 fatty acids in an effort to alter eicosanoid synthesis, cytokine production, and immune function during critical illness [2]. Additionally, the oxidation of fatty acids to acetyl coenzyme A is a central energy-yielding pathway in animals. Acetyl coenzyme A produced from the fatty acids may be completely oxidized in the citric acid cycle, resulting in further energy conservation [43]. Recently, Stadler *et al.* [44] reconfirmed that in rats exposed to *ex vivo* prolonged perfusion or normothermic ischemia-reperfusion injury over the past decades, the livers of fasting animals are much more sensitive than the livers of fed animals. Despite the fact that animal data, whatever the species, cannot be applied directly to humans, these findings suggest that antioxidants can limit extension of oxidative damage, when administered after the insult, but that the preventive effect is the most important.

In the present study, abundant glycogen was available in the livers of the feeding rats (group 3) because high concentrations of glucose and lipids were given, early postoperatively, *via* the enteral route. Dramatic changes to adenine nucleotides and liver EC were prominent in placebo treated animals *versus* those that received enteral nutrition. This significant difference in hepatic tissue EC between the two groups clearly indicates the beneficial effect of nutrition for alleviation of liver oxidative injury. Tang *et al.* [45] speculate that the abundant ATP in hepatocytes with high glycogen content plays a key role in stabilizing cell membrane and maintaining the function of organelles. This also is considered as the major reason why hepatocellular glycogen

can inhibit the production of oxygen-free radicals and the output of oxygen-free radicals is decreased during the course of a stressful stimulus.

As already known, fat has an energy value a little more than twice that of carbohydrates and is therefore an excellent source of energy. In an *ex vivo* rat liver model fat emulsion was found to have a better protective effect than glucose in anoxia-reoxygenation injury [46], while in an *in vivo* entotoxaemia model Kazamias *et al.* [23] demonstrated increasing splanchnic blood flow, increasing gut, and liver microcirculation and oxygenation, as well as increasing hepatic energy stores after animals were treated with enteral nutrition. These findings of increased liver microperfusion may well give an additional explanation for both the preserved EC and the reduced lipid peroxidation production found in our study – through MDA assessment in enteral feeding rats – underlining once more the antioxidant effect of nutrition.

Nowadays, there is strong evidence that avoidance of immune alterations associated with prolonged fasting, reduces the metabolic response to surgery, and improves outcome [47]. Previous experimental work demonstrates that lack of enteral stimulation, due to parenteral nutrition or long fasting, results in decreased production of IgA levels at intestinal and respiratory mucosal surfaces, and reduces lamina propria and Peyer's patch T and B lymphocyte mass, intestinal Th-2 type IgA-stimulating cytokines, and intestinal p*I*gR levels [48–50]. Parenteral nutrition was found to reduce significantly p*I*gR levels in the small intestine that is implicated in the deterioration of antibody-mediated mucosal immunity [51]. These mechanisms result in diminished luminal IgA levels by reducing IgA production or reducing transport of IgA due to reduced p*I*gR. In addition, lack of enteral nutrition was found to influence the degree of endothelial activation [52–54] and intravenous total parenteral nutrition significantly increased E-selectin expression in the small intestine of mice that might increase inflammatory response through enhanced gut E-selectin levels after LPS challenge [55]. Thus, the recognition of the importance of gut integrity and the link between enteral feeding and maintenance of the intestinal barrier opens new fields in the nutritional support of severely ill patients [12, 56, 57].

The results of the present experimental work on a rat model of oxidative stress induced by anesthesia and major surgical trauma show that early institution of a mixed-nutrient enteral feeding formula significantly ameliorates the oxidative damage observed in placebo treated animals. As the acute phase response is immediate, any attempt to modulate it by any treatment strategy must begin as early as possible; thus enteral feeding must provided early, at least before liver energy stores have been exhausted, leading to the amplification of detrimental events.

The authors have declared no conflict of interest.

5 References

[1] Mishra, V., Oxidative stress and role of antioxidant supplementation in critical illness, *Clin. Lab.* 2007, *53*, 199 – 209.

[2] Heyland, D. K., Dhaliwal, R., Day, A. G., Muscedere, J. *et al*., REducing Deaths due to OXidative Stress (The REDOXS Study): Rationale and study design for a randomized trial of glutamine and antioxidant supplementation in critically-ill patients, *Proc. Nutr. Soc.* 2006, *65*, 250 – 263.

[3] Faist, E., Schinkel, C., Zimmer, S., Update on the mechanisms of immune suppression of injury and immune modulation, *World J. Surg.* 1996, *20*, 454 – 459.

[4] Helmy, S. A., Wahby, M. A., El Nawaway, M., The effect of anaesthesia and surgery on plasma cytokine production, *Anaesthesia* 1999, *54*, 733 – 738.

[5] Jedynak, M., Siemiatkowski, A., Gacko, M., Mroczko, B. *et al*., Serum concentrations of MCP-1 and RANTES in patients during aortic surgery: The relationship with ischemia-reperfusion, *Arch. Immunol. Ther. Exp. (Warsz.)* 2004, *52*, 201 – 207.

[6] Berger, M. M., Can oxidative damage be treated nutritionally? *Clin. Nutr.* 2005, *24*, 172 – 183.

[7] Kolios, G., Kotzampassi, K., Manousou, P., Paramythiotis, D. *et al*., Enteral nutrition affects nitric oxide production in peripheral blood and liver after a postoperative lipopolysaccharide-induced endotoxemia in rats, *Nutrition* 2007, *23*, 575 – 581.

[8] Peter, J. V., Moran, J. L., Phillips-Hughes, J., A metaanalysis of treatment outcomes of early enteral versus early parenteral nutrition in hospitalized patients, *Crit. Care Med.* 2005, *33*, 213 – 220.

[9] Kreymann, K. G., Berger, M. M., Deutz, N. E., Hiesmayr, M. *et al*., ESPEN guidelines on enteral nutrition: Intensive care, *Clin. Nutr.* 2006, *25*, 210 – 223.

[10] Weimann, A., Omega-3 fatty acids-supplemented parenteral nutrition in the critically ill: Another step forward on the "great journey"? *Crit. Care Med.* 2006, *34*, 1253 – 1255.

[11] Kudsk, K. A., Gut mucosal nutritional support – enteral nutrition as primary therapy after multiple system trauma, *Gut* 1994, *35*, S52 – S54.

[12] Braga, M., Gianotti, L., Gentilini, O., Liotta, S. *et al*., Feeding the gut early after digestive surgery: Results of a nine-year experience, *Clin. Nutr.* 2002, *21*, 59 – 65.

[13] Kehlet, H., Wilmore, D. W., Multimodal strategies to improve surgical outcome, *Am. J. Surg.* 2002, *183*, 630 – 641.

[14] Brodner, G., Van Aken, H., Hertle, L., Fobker, M. *et al*., Multimodal perioperative management – combining thoracic epidural analgesia, forced mobilization, and oral nutrition – reduces hormonal and metabolic stress and improves convalescence after major urologic surgery. *Anesth. Analg.* 2001, *92*, 1594 – 1600.

[15] Heslin, M. J., Latkany, L., Leung, D., Brooks, A. D. *et al*., A prospective, randomized trial of early enteral feeding after resection of upper gastrointestinal malignancy, *Ann. Surg.* 1997, *226*, 567 – 577.

[16] Watters, J. M., Kirkpatrick, S. M., Norris, S. B., Shamji, F. M. *et al*., Immediate postoperative enteral feeding results in impaired respiratory mechanics and decreased mobility, *Ann. Surg.* 1997, *226*, 369 – 377.

[17] Bessey, P. Q., Nutritional support in critical illness, in: E. A. Deitch (Ed.), *Multiple Organ Failure. Pathophysiology and Basic Concepts of Therapy*, Thieme, New York 1990, pp. 126 – 149.

[18] Walker, P. D., Shah, S. V., Evidence suggesting a role for hydroxyl radical in gentamicin-induced acute renal failure in rats, *J. Clin. Invest.* 1988, *81*, 334 – 341.

[19] Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J., Protein measurement with the Folin phenol reagent, *J. Biol. Chem.* 1951, *193*, 265 – 275.

[20] Townsend, M. C., Hampton, W. W., Haybron, D. M., Schirmer, W. J. *et al*., Effective organ blood flow and bioenergy status in murine peritonitis, *Surgery* 1986, *100*, 205 – 213.

[21] Atkinson, D. E., The energy charge of the adenylate pool as a regulatory parameter. Interaction with feedback modifiers, *Biochemistry* 1968, *7*, 4030 – 4034.

[22] Linehan, J. D., Kolios, G., Valatas, V., Robertson, D. A. *et al*., Effect of corticosteroids on nitric oxide production in inflammatory bowel disease: Are leukocytes the site of action? *Am. J. Physiol. Gastrointest. Liver Physiol.* 2005, *288*, G261 – G267.

[23] Kazamias, P., Kotzampassi, K., Koufogiannis, D., Eleftheriadis, E., Influence of enteral nutrition-induced splanchnic hyperemia on the septic origin of splanchnic ischemia, *World J. Surg.* 1998, *22*, 6 – 11.

[24] Berg, K., Haaverstad, R., Astudillo, R., Bjorngaard, M. *et al*., Oxidative stress during coronary artery bypass operations: Importance of surgical trauma and drug treatment, *Scand. Cardiovasc. J.* 2006, *40*, 291 – 297.

[25] Prondzinsky, R., Knupfer, A., Loppnow, H., Redling, F. *et al*., Surgical trauma affects the proinflammatory status after cardiac surgery to a higher degree than cardiopulmonary bypass, *J. Thorac. Cardiovasc. Surg.* 2005, *129*, 760 – 766.

[26] Lotan, D., Zilberman, D., Dagan, O., Keller, N. *et al*., β-Chemokine secretion patterns in relation to clinical course and outcome in children after cardiopulmonary bypass: Continuing the search to abrogate systemic inflammatory response. *Ann. Thorac. Surg.* 2001, *71*, 233 – 237.

[27] Bukara, M., Bautista, A. P., Acute alcohol intoxication and gadolinium chloride attenuate endotoxin-induced release of CC chemokines in the rat. *Alcohol* 2000, *20*, 193 – 203.

[28] Valatas, V., Kolios, G., Manousou, P., Notas, G. *et al*., Octreotide regulates CC but not CXC LPS-induced chemokine secretion in rat Kupffer cells. *Br. J. Pharmacol.* 2004, *141*, 477 – 487.

[29] Fisher, J. E., Burger, P. C., Perlman, E. J., Dickman, P. S. *et al*., The frozen section yesterday and today: Pediatric solid tumors – crucial issues. *Pediatr. Dev. Pathol.* 2001, *4*, 252 – 266.

[30] Yamauchi, K., Akbar, S. M., Horiike, N., Michitaka, K. *et al*., Increased serum levels of macrophage inflammatory protein-3alpha in chronic viral hepatitis: Prognostic importance of macrophage inflammatory protein-3alpha during interferon therapy in chronic hepatitis C, *J. Viral. Hepat.* 2002, *9*, 213 – 220.

[31] Bravo-Cuellar, A., Romero-Ramos, J. E., Hernandez-Flores, G., Romo-Perez, F. D. *et al*., Comparison of two types of anesthesia on plasma levels of inflammatory markers, *Cir. Cir.* 2007, *75*, 99 – 105.

[32] Agay, D., Anderson, R. A., Sandre, C., Bryden, N. A. *et al*., Alterations of antioxidant trace elements (Zn, Se, Cu) and related metallo-enzymes in plasma and tissues following burn injury in rats, *Burns* 2005, *31*, 366 – 371.

[33] Brodsky, J. A., Brody, F. J., Endlich, B., Armstrong, D. A. *et al*., MCP-1 is highly expressed in peritoneum following midline laparotomy with peritoneal abrasion in a murine model, *Surg. Endosc.* 2002, *16*, 1079 – 1082.

[34] Pillai, R. G., Beutelspacher, S. C., Larkin, F. D., George, A. J., Upregulation of chemokine expression in murine cornea due to mechanical trauma or endotoxin, *Br. J. Ophthalmol.* 2008, *92*, 259 – 264.

[35] Wagatsuma, A., Endogenous expression of angiogenesis-related factors in response to muscle injury, *Mol. Cell. Biochem.* 2007, *298*, 151 – 159.

[36] Ward, S. G., Westwick, L., Chemokines: Understanding their role in T-lymphocyte biology, *Biochem. J.* 1998, *333*, 457 – 470.

[37] Low, Q. E., Drugea, I. A., Duffner, L. A., Quinn, D. G. *et al*., Wound healing in MIP-1alpha(−/−) and MCP-1(−/−) mice, *Am. J. Pathol.* 2001, *159*, 457 – 463.

[38] Dewald, O., Zymek, P., Winkelmann, K., Koerting, A. *et al*., CCL2/monocyte chemoattractant protein-1 regulates inflammatory responses critical to healing myocardial infarcts, *Circ. Res.* 2005, *96*, 881 – 889.

[39] Fivenson, D. P., Faria, D. T., Nickoloff, B. J., Poverini, P. J. *et al*., Chemokine and inflammatory cytokine changes during chronic wound healing, *Wound. Repair Regen.* 1997, *5*, 310 – 322.

[40] Takahashi, H., Tsuda, Y., Kobayashi, M., Herndon, D. N. *et al*., CCL2 as a trigger of manifestations of compensatory anti-inflammatory response syndrome in mice with severe systemic inflammatory response syndrome, *J. Leukoc. Biol.* 2006, *79*, 789 – 796.

[41] Hermsen, J. L., Gomez, F. E., Maeshima, Y., Sano, Y. *et al*., Decreased enteral stimulation alters mucosal immune chemokines, *JPEN J. Parenter. Enteral Nutr.* 2008, *32*, 36 – 44.

[42] Gasperino, J., Kvetan, V., Acute lung injury and nutritional support, *Crit. Care Med.* 2006, *34*, 1265 – 1267.

[43] Nelson, D. L., Cox, M. M., Oxidation of fatty acids, in: A. L. Lehninger (Ed.), *Lehninger Principles of Biochemistry*, Worth Publishers, Inc., New York 2000, pp. 598 – 622.

[44] Stadler, M., Nuyens, V., Seidel, L., Albert, A. *et al*., Effect of nutritional status on oxidative stress in an ex vivo perfused rat liver, *Anesthesiology* 2005, *103*, 978 – 986.

[45] Tang, L., Tian, F., Tao, W., Cui, J., Hepatocellular glycogen in alleviation of liver ischemia-reperfusion injury during partial hepatectomy, *World J. Surg.* 2007, *31*, 2039 – 2043.

[46] Stadler, M., Nuyens, V., Boogaerts, J. G., Intralipid minimizes hepatocytes injury after anoxia-reoxygenation in an ex vivo rat liver model, *Nutrition* 2007, *23*, 53 – 61.

[47] Sigalet, D. L., Mackenzie, S. L., Hameed, S. M., Enteral nutrition and mucosal immunity: Implications for feeding strategies in surgery and trauma, *Can. J. Surg.* 2004, *47*, 109 – 116.

[48] Li, J., Kudsk, K. A., Gocinski, B., Dent, D. *et al*., Effects of parenteral and enteral nutrition on gut-associated lymphoid tissue, *J. Trauma* 1995, *39*, 44 – 51.

[49] Wu, Y., Kudsk, K. A., DeWitt, R. C., Tolley, E. A. *et al*., Route and type of nutrition influence IgA-mediating intestinal cytokines, *Ann. Surg.* 1999, *229*, 662 – 667.

[50] Sano, Y., Gomez, F. E., Kang, W., Lan, J. *et al*., Intestinal polymeric immunoglobulin receptor is affected by type and route of nutrition, *JPEN J. Parenter. Enteral Nutr.* 2007, *31*, 351 – 356.

[51] Sano, Y., Gomez, F. E., Hermsen, J. L., Kang, W. *et al*., Parenteral nutrition induces organ specific alterations in polymeric immunoglobulin receptor levels, *J. Surg. Res.* 2008, *149*, 236 – 242.

[52] Fukatsu, K., Lundberg, A. H., Hanna, M. K., Wu, Y. *et al*., Route of nutrition influences intercellular adhesion molecule-1 expression and neutrophil accumulation in intestine, *Arch. Surg.* 1999, *134*, 1055 – 1060.

[53] Fukatsu, K., Lundberg, A. H., Kudsk, K. A., Hanna, M. K. *et al*., Modulation of organ ICAM-1 expression during IV-TPN with glutamine and bombesin, *Shock* 2001, *15*, 24 – 28.

[54] Fukatsu, K., Lundberg, A. H., Hanna, M. K., Wu, Y. *et al*., Increased expression of intestinal P-selectin and pulmonary E-selectin during intravenous total parenteral nutrition. *Arch. Surg.* 2000, *135*, 1177 – 1182.

[55] Fukatsu, K., Zarzaur, B. L., Johnson, C. D., Lundberg, A. H. *et al*., Lack of enteral feeding increases expression of E-selectin after LPS challenge, *J. Surg. Res.* 2001, *97*, 41 – 48.

[56] Silk, D. B., Green, C. J., Perioperative nutrition: Parenteral versus enteral, *Curr. Opin. Clin. Nutr. Metab. Care* 1998, *1*, 21 – 27.

[57] Sakurai, Y., Masui, T., Yoshida, I., Tonomura, S. *et al*., Randomized clinical trial of the effects of perioperative use of immune-enhancing enteral formula on metabolic and immunological status in patients undergoing esophagectomy, *World J. Surg.* 2007, *31*, 2150 – 2157.

