• BASIC RESEARCH •

Tri-iodothyronine supplement protects gut barrier in septic rats

Zhi-Li Yang, Lian-Yue Yang, Geng-Wen Huang, He-Li Liu

Zhi-Li Yang, Lian-Yue Yang, Geng-Wen Huang, He-Li Liu,
Department of Surgery, Xiangya Hospital, Central South University,
Changsha 410008, Hunan Provice, China
Supported by the Scientific Research Fund of Chinese Ministry of
Health, No.98-1-112
Correspondence to: Lian-Yue Yang, MD, Department of Surgery,
Xiangya Hospital, Central South University, Changsha 410008,
Hunan Provice, China. damoyzl@163.net
Telephone: +86-731-4327326
Received: 2002-06-17   **Accepted:** 2002-07-19

Abstract

AIM: To investigate the role of tri-iodothyronine supplement
in protecting gut barrier in septic rats.

METHODS: Twenty-two rats were randomized into three
groups: sham group (n=6), sepsis group (n=8), and sepsis
plus tri-iodothyronine (T_3) group (n=8). Septic rat model
was established through cecal ligation and puncture (CLP).
After 5 h, sham and sepsis groups received saline, and the
remaining group received T_3 intraperitoneally. Twenty-one
hrs After CLP, intestinal permeability and serum free T_3 and
T_4 were measured with fluorescence spectrophotometer and
by radioimmunoassay, respectively. Intestinal ultrastructure
and histologic morphology were observed under transmission
electron microscopy (TEM) and light microscopy, respectively.

RESULTS: After 21 h, septic symptoms and signs in sepsis
plus T_3 group were milder than those in sepsis group. Serum
FT_3 or FT_4 concentration in sepsis group was lower than that
in sham group (1.59±0.20, 3.41±2.14 pmol/L vs 3.44±1.40,
9.53±3.39 pmol/L, P<0.05), and FT_3 concentration in sepsis
plus T_3 group (3.40±1.65 pmol/L, P<0.05) was corrected.
Portal concentration of fluorescein isothiocyanate-dextran
(FITC-D) in sepsis group (2.51±0.56 mg/L) was higher than
that in sham group (1.22±0.21 mg/L) (P<0.01), and in sepsis
plus T_3 group (1.68±0.38 mg/L) it was decreased significantly
(P<0.01). TEM and light microscopy showed that T_3
supplement preserved well ultrastructure and morphology
of intetinal mucosa in septic rats.

CONCLUSION: Tri-iodothyronine supplement protects gut
barrier in septic rats.

INTRODUCTION

In recent years, laboratory and clinical researches have strongly
shown that the gastrointestinal tract plays a pivotal role in the
occurrence of sepsis and multiple organ dysfunction syndrome
(MODS)[1-4]. It is believed that gut barrier failure is a "trigger
point" in sepsis and MODS, and some substances including
intestinal trefoil factor[5], glucagons-like peptide 2[6-8] and
glutamine[9,10] may protect gut barrier, but we are still facing

either poor efficacy in clinical application or the challenge of
translating the findings from the bench to the bedside.
Therefore, it has become a breakthrough that may prevent
MODS occurrence, improve septic prognosis and further seek
for protective substances of gut barrier.

The "euthyroid sick syndrome, ESS" is defined as a
decreased concentration of plasma tri-iodothyronine (T_3) with
normal or low thyroxine (T_4), but serum thyroid-stimulating
hormone concentration is normal[11,12]. This syndrome is seen
in states in which there are significant insults to the host
including surgery, starvation, myocardial infarction,
hypothermia and sepsis. In the study of ESS, it was reported
that T_3 supplement may protect some organ functions[13], such
as pulmonary function during sepsis[14], and donor myocardial
function after transplantation[15]. Recently, in sepsis, T_3
augmentation was shown by Chapital *et al* to increase the
circulating antithrombin III levels, which is a critical material
to prevent disseminated intravascular coagulation(DIC)[16]. An
earlier study revealed that hypothroid hormone resulted in
atrophy of intestinal epithelial cells, decreased mucosal DNA
and protein contents, shortened the villi height, decreased the
cryt depth and rates of utilization of glucose and glutamine,
and consequently impaired the gut barrier[17]. Based on these
studies, we hypothesized that T_3 supplement may protect gut
barrier in sepsis. This study was to investigate the relationship
between T_3 and gut barrier in septic rats.

MATERIALS AND METHODS

Animals
Twenty-two adult male specific pathogen free (SPF) Sprague-
Dawley rats weighing from 250 to 350 g were utilized in this
investigation. The animals were purchased from Department
of Animal Laboratory of Xiangya Medical College. Rats used
in the present study were cared in accordance with the directory
of Central South University Animal Care Unit, and the
guidelines of the National Institutes of Health on welfare of
laboratory animals.

Animal model
Rats were randomly divided into three groups: sham group
(n=6), sepsis group (n=8), and sepsis plus T_3 group (n=8).
Sepsis was induced by cecal ligation and puncture (CLP) as
described by Wichterman *et al*[18]. Under 3 % pentobarbital
natrium anesthesia, a laparotomy was performed (the size of
the incision was 2.0 cm), and the cecum was ligated just distally
to the ileocecal valve to avoid any intestinal obstruction and
was punctured across the intestine once with an 18 gauge
needle. Punctured holes were placed 1-0 silk thread in case
they were blocked up. The cecum was then returned to the
peritoneal cavity and the abdomen was closed in two layers.
Laparotomy in sham group was performed and the cecum was
manipulated, but neither ligated nor punctured. All animals
were resuscitated subcutaneously with 50 ml/kg body weight
of normal saline at the completion of surgery. After 5 h, sepsis
plus T_3 group were injected intraperitoneally with 1.5 ml/kg
body weight of T_3 (0.01 g/L, Sigma), and sham group and
sepsis group received 1.5ml/kg body weight of normal saline.
All animals were anesthetized 21hrs post-CLP once more with

two- thirds of original dose, their intestinal permeability and serum free T_3 and T_4 were measured, intestinal ultrastructure and histologic morphology were observed.

Intestinal permeability measurement

Intestinal permeability was determined essentially according to the method described by Chen et al[19]. A 20 cm segment of the ileum was dissected beginning at 3 cm ileum proximal to ileocecal valve with well protected superior mesenteric vessels. The bilateral end of the isolated ileum was clamped with rubber bands to prevent the leakage of   fluorescein isothiocyanate-dextran(FITC-D). One ml of 0.1M phosphate buffer saline (PBS, pH7.2) containing 25 mg of FITC-D (MW4400, Sigma) was injected into the lumen. After 30 min, a blood sample (100 μL) was taken by a puncture of the portal vein and immediately diluted with 1.9 ml of 50 Mm Tris(pH10.3) containing 150 mM chloride natrium. The diluted plasma was centrifuged at 3 000×g, 4 ℃ for 7 min. The supernatant was analyzed for FITC-D concentration with a Hitachi fluorescence spectrophotometer (F-2000) at an excitation wavelength of 480 nm and an emission wavelength of 520 nm. Standard curves for calculating the FITC-D concentration in the samples were obtained by diluting various amounts of FITC-D in a pool of rat plasma, then diluted and centrifuged in the same manner as the samples before measurement.

Transmission electron microscopy (TEM)

Segments of intestine were removed, small rings were cut from the intestine, and immediately fixed in 2.5 % glutaraldehyde for at least 24 hours, and counter-fixed in 2 % osmium tetroxide prepared in 0.1M phosphate buffer, pH7.4 for one hour. They were then dehydrated in graded series of ethanol and propylene oxide and embedded in Epon 812, Sections were cut, collected on copper grids, counter stained with uranyl acetate and lead citrate, and examined under a Hitachi H-600 electron microscope.

Histological assessment

A longitudinal 1 cm segment of intestine was removed and rinsed in normal saline, fixed in 10 % neutral formalin, and processed by conventional methods. The tissue was embedded in paraffin wax. Tissue sections (5 μm) were stained with haematoxylin and eosin. Intestinal morphologic characteristics were evaluated under light microscopy.

Assay of serum free T_3 and T_4 (FT_3, FT_4)

After 21hrs, 3 ml blood samples were collected from inferior vena cava, centrifuged at 1 500×g for 15 min. The supernatants were frozen at -20 ℃ for later FT_3 and FT_4 assay by radioimmunoassay (RIA).

Statistical analysis

Data were expressed as $\bar{x}\pm s$ and compared using one-way analysis of variance (ANOVA). The statistical analyses were made using the Statistical Package for the Social Science (SPSS10.0) software. Differences were considered as significant when the probability was less than 0.05.

RESULTS

All rats of sepsis group exhibited symptoms and signs of sepsis, including lethargy, piloerection, decreased grooming, and diarrhea. Above symptoms and signs of sepsis plus T_3 group were milder, and sham group were normal.

Serum FT_3 levels in sepsis group were decreased significantly compared with tri-iodothyronine treated rats at 21 hrs, sepsis plus T_3 rats had normal or slightly decreased FT_3 levels after CLP 21 hrs compared with sham operated rats,

and FT_4 levels in sepsis group were also much lower than those in sham operated rats (Table 1).

Table 1 Serum FT_3 and FT_4 concentrations measured by RIA ($\bar{x}\pm s$)

Groups	n	FT$_3$ (pmol/L)	FT$_4$ (pmol/L)
Sham	6	3.44±1.40[a]	9.53±3.39[a]
Sepsis	8	1.59±0.20	3.41±2.14
Sepsis plus T$_3$	8	3.40±1.65[a]	6.37±4.45

[a]$P<0.05$ vs sepsis group.

Rats receiving normal saline after CLP showed a significant increase in intestinal permeability in comparison with sham group ($P<0.01$), rats with administered T_3 after CLP showed a significant decrease in intestinal permeability in comparison with the sepsis plus normal saline group ($P<0.01$) (Figure 1).
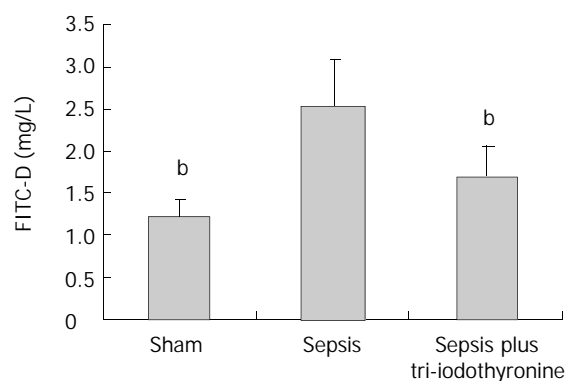

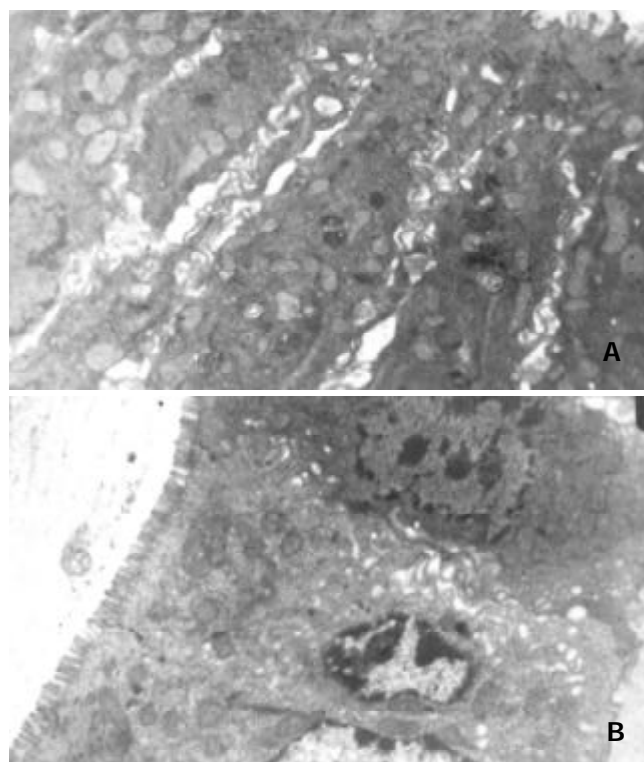**Figure 1** Effect of tri-iodothyronine on gut permeability. [b]$P<0.01$ vs sepsis group.

Figure 2 Transmission electron micrography of intestinal mucosal cells. (A) In sepsis group intestinal epithelial cells. (B) In sepsis plus T_3 group intestinal epithelial cells ×6 000.

In sepsis group, the ultrastructure of intestinal epithelial cells showed that the microvilli were sharply reduced and deformed, and loss was patchy. The edema of the villi cells was more pronounced with the mitochondrias dropsy and vacuolar change, gaps of enterocytes were sharply widened, junctional complex among enterocytes were shortened and widened (Figure 2 A). In contrast, ultrastructure of sepsis plus T_3 group showed that the microvilli were dense and regular with a jagged and interlocking pattern among enterocytes and the mitochondrias were clear (Figure 2 B).

Under photomicrography, septic rats showed severe edema and sloughing of the villous tips compared with sham animals (Figure 3A and B). Rats with administered T_3 after CLP showed relatively normal villous tips without sloughing (Figure 3 C).
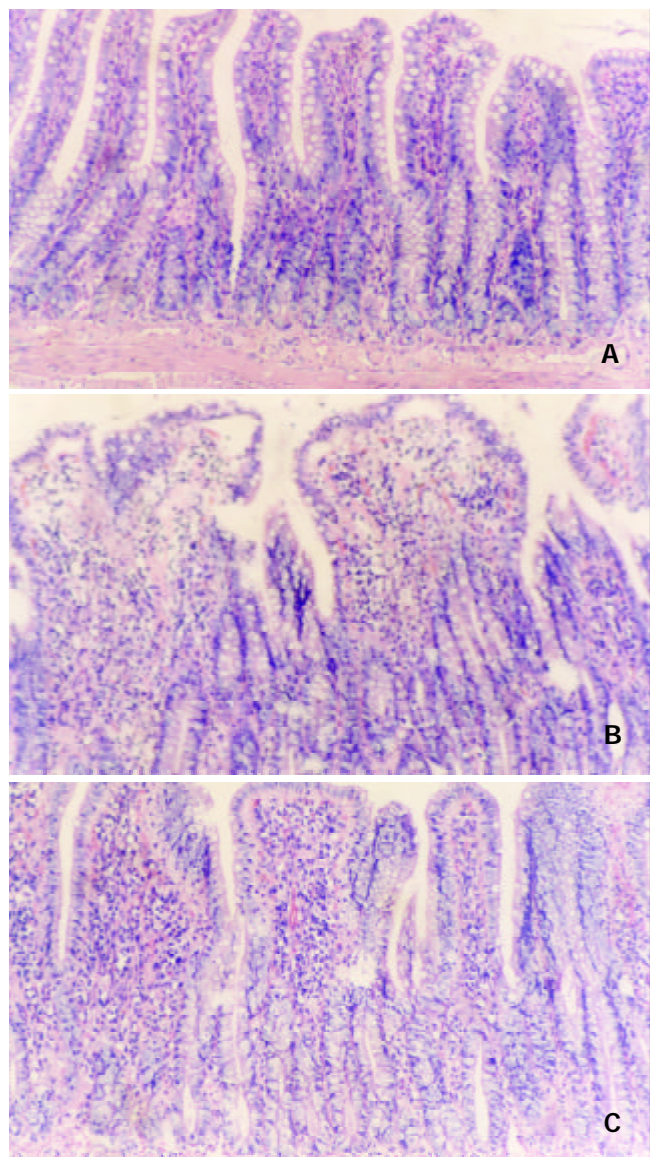

Figure 3 In sham rats, intestinal mucosal architecture is well preserved (A). Sepsis induced significantly edema of villous tips and sloughing of villous enterocytes (B).The tri-iodothyronine prevented the intestinal damages induced by sepsis (C). HE,×100.

DISCUSSION

For more than 3 decades, it has been known that the euthyroid sick syndrome exists probably in any severe illness, including starvation[20], pulmonary tuberculosis[21], sepsis[22,23], surgery[24,25], myocardial infarction[26,27], bypass[28] and bone marrow transplantation[29]. The syndrome is also called low T_3 syndrome or nonthyroidal illness, characterized by low serum T_3 levels, and serum free T_4 levels are commonly below normal, but may be normal or above normal. It is likely that mechanism of thyroid hormone suppression in these illness is multifactoral and may differ in different groups of patients, as for low serum T_3 levels in this syndrome, one important cause is a decreased generation of T_3 by type I iodothyronine deiodinase in the liver and a reduced degradation of r T_3[30]. Subsequently, Nagaya *et al* demonstrated that in severe illness activated NF-κB could inhibit T_3-dependent induction of type I 5' - deiodinase mRNA and enzyme activity[31]. In addition, the degree of low T_3 in circulation has been shown to correlate with the severity of the underlying disorders and the prognosis[32]. Some authors believe that this is an abnormal state with decreased production rather than increased degradation, others content that this may be the body's adaptation to stress protecting the body against exaggerated catabolism. Hitherto, some T_3 supplementing studies suggested that the former might be more reasonable than the latter[14-16,35].

In this study, we used cecum ligation and puncture (CLP) to establish classic animal model of sepsis. During the whole investigation, our septic models were coincident with the previous studies[33,34]. An acute decrease in circulating levels of free T_3 and T_4 was seen after 21hrs CLP, T_3 supplement prevented the decrease in serum free T_3 concentration with sepsis. ESS was seen in our septic model. Moley *et al* showed in their study that absence of thyroid hormone abolished the hyperdynamic phase of sepsis, increased susceptibility to sepsis, and significantly increased the mortality in sepsis, and thyroxine replacement following thyroidectomy prevented the increased mortality from sepsis[35]. In our research, T_3 supplement showed that septic symptoms and signs of the rats were abated to a certain extent. Thus, our data has confirmed that T_3 replacement in septic rats with ESS may be beneficial to the general condition of the patients.

The progression from sepsis to severe sepsis (sepsis with dysfunction of one organ) to multiple organ dysfunction syndrome and then to septic death requires escalation of treatment[36]. During the course of the progression, gut barrier disruption is believed to be the "motor" of "irreversible" shock and multiple system organ failure[37]. The critical cause is that translocation of bacteria and endotoxins contribute to the infection and injury of the body. Increased intestinal mucosal permeability is considered to be a quantitative index of injury or dysfunction of the intestinal mucosa barrier. The molecular probe FITC-D used in this study is considered to penetrate through a paracellular route toward portal vein via the tight junctions according to its size. Gut permeability indicated by FITC-D was coincident with the pathologic changes of injured gut in inflammation[38]. Assay of TITC-D does not depend on systemic circulation and renal function compared with other probe molecule, and so application of FITC-D assay can indicate more exactly the gut permeability in this experimental model. Our data showed that T_3 administration significantly decreased gut permeability in septic rats. Photomicrography and TEM showed that T_3 supplement well preserved the ultrastructure and mophology of intestinal mucosa. Therefore, these data indicate that T_3 augmentation can protect from the structural and functional damages of gut barrier in septic rats. The proximate molecular mechanism by which T_3 regulates gut barrier in sepsis is not known. It is possible that T_3 administration is associated with protective substances synthesis of intestinal epithelial cells. Smith *et al* reported that administration of T_3 could induce expression of heme oxygenase-1 (HO-1) and stimulate activity of HO-1 in liver of thyroidectomized rats[39]. HO-1 is a stress-associated protein whose expression is stimulated by hypoxia, and increases adaptive response of cells to hypoxia. Hypoxia inducible factor-1 (HIF-1) mediates transcriptional activation of HO-1 gene in

response to hypoxia[40]. Our study showed that T_3 supplement increased expression of HIF-1α in intestinal epithelial cells of septic rats (unpublished data). Thus, promoting adaptive response of cells to hypoxia may be one of approaches to improve gut barrier in sepsis by T_3.

In conclusion, thyroid hormone is one of the critical hormones in mammals and plays an indispensable role in development as well as in lipid, protein, and carbohydrate metabolism and energy generation. Our results demonstrate that tri-iodothyronine, active form of thyroid hormone, can protect gut barrier in septic rats. Obviously, biologic functions of thyroid hormone are expanded, and moreover, it may supply a novel method to protect from the injury of gut barrier in critic illness. It is of important theoretical significance and practical value to further investigate its protective mechanism.

ACKNOWLEDGEMENT

REFERENCES

1   **Moore FA**. Role of gastrointestinal tract in the postinjury multiple organ failure. *Am J Surg* 1999; **178**: 449-453
2   **Deitch EA**. Multiple organ failure: pathophysiology and potential future therapy. *Ann Surg* 1992; **216**: 117-134
3   **Wu CT**, Li ZL, Xiong DX. Relationship between enteric microecologic dysbiosis and bacterial translocation in acute necrotizing pancreatitis. *World J Gastroenterol* 1998; **4**: 242-245
4   **Yi JH**, Ni RY, Luo DO, Li SL. Intestinal flora translocation and overgrowth in upper gastrointestinal tract induced by hepatic failure. *World J Gastroenterol* 1999; **5**: 327-329
5   **Wong WM**, Poulsom R, Wright NA. Trefoil peptides. *Gut* 1999; **44**: 890-895
6   **Kouris GJ**, Liu Q, Rossi H, Djuricin G, Gattuso P, Nathan C, Weinstein RA, Prinz RA. The effect of glucagons-like peptide 2 on intestinal permeability and bacterial translocation in acute necrotizing pancreatitis. *Am J Surg* 2001; **181**: 571-575
7   **Wang FJ**, Zhao Y, Wang P, Wang SL, Liu J, Zhou X. Effects of glucagons-like peptide-2 on intestinal barrier function in severely burned rats. *Shijie Huaren Xiaohua Zazhi* 2002; **10**: 796-799
8   **Benjamin MA**, Mckay DM, Yang PC, Cameron H, Perdue MH. Glucagon-like peptide-2 enhances intestinal epithelial barrier function of both transcellular and paracellular pathways in the mouse. *Gut* 2000; **47**: 112-119
9   **Wischmeyer PE**, Kahana M, Wolfson R, Ren H, Mush MM, Chang EB. Glutamine reduces cytokine release, organ damage, and mortality in a rat model of endotoxemia. *Shock* 2001; **16**: 398-402
10   **Zhou X**, Li YX, Li N, Li JS. Effect of bowel rehabilitative therapy on structural adaptation of remnant small intestine: animal experiment. *World J Gastroenterol* 2001; **7**: 66-73
11   **Utiger RD**. Altered thyroid function in nonthyroidal illness and surgery:to treat or not to treat? *N Engl J Med* 1995; **333**: 1562-1563
12   **Yang LY**, Huang GW. Advance and development ofsick syndrome of normal thyroid in surgical sever pstients. *Zhongguo Shiyong Waike Zazhi* 2000; **20**: 114-115
13   **Liu JH**, Yang LY. Advance and development of research of protective mechanism of organ function by thyroxine. *Zhongguo Shiyong Waike Zazhi* 2001; **21**: 372-374
14   **Dulchavsky SA**, Bailey J. Triiodothyronine treatment maintains surfactant synthesis during sepsis. *Surgery* 1992; **112**: 475-479
15   **Jeevanandam V**, Todd B, Regillo T, Hellman S, Eldridge C, Mcclurken J. Reversal of donor myocardial dysfunction by tri-iodothyronine replacement therapy. *J Heart Lung Transplant* 1994; **13**: 681-687
16   **Chapital AD**, Hendrick SR, Lloyd L, Pieper D. The effects of tri-iodothyronine augmentation on antithrombin III levels in sepsis. *Am Surg* 2001; **67**: 253-255
17   **Ardawi MSM**, Jalalah SM, Effects of hypothyroidism on glucose and glutamine metabolism by the gut of the rat. *Clin Science* 1991; **81**: 347-355
18   **Wichterman KA**, Chaudry IH, Baue AE. Studies of peripheral glucose uptake during sepsis. *Arch Surg* 1979; **114**: 740-743
19   **Chen LW**, Hsu CM, Cha MC, Chen JS, Chen SC. Changes in gut mucosal nitric oxide synthase(NOS) activity after thermal injury and its relation with barrier failure. *Shock* 1999; **11**: 104-110
20   **Hennemann G**, Docter R, Krenning EP. Causes and effects of the low T_3 syndrome during caloric deprivation and non-thyroidal illness: an overview. *Acta Med Kaust* 1988; **15**: 42-45
21   **Chow CC**, Mak TW, Chan CH, Cockram CS. Euthyroid syndrome in pulmonary tuberculosis before and after treatment. *Ann Clin Biochem* 1995; **32**: 385-391
22   **Phillips RH**, Valente WA, Caplan ES, Connor TB, Wiswell JG. Circulating thyroid hormone changes in acute trauma:prognostic implications for clinical outcome. *J Trauma* 1984; **24**: 116-119
23   **Huang GW**, Yang LY, Yang JQ. Role of thyroid hormone in the protection of intestinal mucosal barrier of septic rats. *Zhonghua Shiyan Waike Zazhi* 2000; **17**: 531-532
24   **Cheren HJ**, Nellen HH, Barabejski FG, Chong MBA, Lifshitz GA. Thyroid function and abdorminal surgery.A longitudinal study. *Arch Med Res* 1992; **23**: 143-147
25   **Yang LY**, Liu HL. Application of thyroxine in surgery. *Zhongguo Shiyong Waike Zazhi* 2001; **21**: 328-329
26   **Vardarli I**, Schmidt R, Wdowinski JM, Teuber J, Schwedes U, Usadel KH. The hypothalamo-hypophyseal thyroid axis,plasma protein concentrations and the hypophyseogonadal axis in low T_3 syndrome following acute myocardial infarct. *Klin Wochenschrift* 1987; **65**: 129-133
27   **Eber B**, Schumacher M, Langsteger W, Zweiker R, Fruhwald FM, Pokan R, Gasser R, Eber O, Klein W. Changes in thyroid hormone parameters after acute myocardial infarction. *Cardiology* 1995; **86**: 152-156
28   **Holland FW**, Brown PS, Weintraub BD, Clark RE. Cardiopulmonary by pass and thyroid function: a "euthyroid sick syndrome". *Ann Thorac Surg* 1991; **52**: 46-50
29   **Vexiau P**, Perez-Castiglioni P, Socie G, Devergie A, Toubert ME, Aracting S, Gluckman E. The "euthyroid sick syndrome": incidence, risk factors and prognostic value soon after allogeneic bone marrow transplantation. *Br J Hematol* 1993; **85**: 778-782
30   **DE Groot LJ**. Dangerous dogmas in medicine: the nonthyroidal illness syndrome. *J Clin Endocrinal Metab* 1999; **84**: 151-164
31   **Nagaya T**, Fujieda M, Otsuka G,Yang JP, Okamoto T, Seo H. A potential role of activated NF-κB in the pathogensis of euthyroid sick syndrome. *J Clin Invest* 2000; **106**: 393-402
32   **Yang LY**, Hu M, Huang YS. The relation of critic septic shock and peripheric metabolism malfunction of thyroxine. *Zhonghua Shiyan Waike Zazhi* 1990; **7**: 134-135
33   **Shieh P**, Zhou M, Ornan DA, Chaudry IH,Wang P. Upregulation of inducible nitric oxide synthase and nitric oxide occurs later than the onset of the hyperdynamic response during sepsis. *Shock* 2000; **13**: 325-329
34   **Wang P**, Chaudry IH. Mechanism of hepatocellular dysfunction during hyperdynamic sepsis. *Am J Physiol* 1996; **270**: R927-938
35   **Moley JF**, Ohkawa M, Chaudry IH, Clemens MG, Baue AE. Hypothyroidism abolishes the hyperdynamic phase and increases susceptibility to sepsis. *J Surg Res* 1984; **36**: 265-273
36   **Tabrizi AR**, Zehnbauer BA, Freeman BD, Buchman TG. Genetic markers in sepsis. *J Am Coll Surg* 2001; **192**: 106-117
37   **Antonsson JB**, Fiddian-Green RG. The role of the gut in shock and multiple system organ failure. *Eur J Surg* 1991; **157**: 3-12
38   **Travis S**, Menzies I. Intestinal permeability :functional assessment and significance. *Clin Sci* 1992; **82**: 471-488
39   **Smith TJ**, Drummand GS, Kourides IA, Kappas A. Thyriod horomone regulation of heme oxidation in the liver. *Proc Natl Acad Sci USA* 1982; **79**: 7537-7541
40   **Lee PJ**, Jiang BH, Chin BY, Iyer NV, Alam J, Semenza GL, Choi AMK. Hypoxia-inducible factor-1 mediates transcriptional activation of the heme oxygenase-1 gene in response to hypoxia. *J Bio Chem* 1997; **272**: 5375-5381