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Marginal Copper Deficiency Increases Liver Neutrophil Accumulation After Ischemia/Reperfusion in Rats

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Abstract

Copper deficiency can cause a host of major cardiovascular complications including an augmented inflammatory response through effects on both neutrophils and the microvascular endothelium. In the present study, we evaluated the effect of marginal copper deficiency on the neutrophilic response to hepatic ischemia/reperfusion injury, a condition that induces an inflammatory response. Male weanling Sprague–Dawley rats were fed purified diets which were either copper-adequate (6.3 mg/kg) or copper-marginal (1.62 mg/kg) for 4 weeks prior to undergoing 90 min of partial hepatic ischemia followed by 8 h of reperfusion. Liver injury was assessed by serum levels of alanine aminotransferase and by liver histology. Liver neutrophil accumulation was determined by tissue myeloperoxidase content. There was no significant difference in liver injury between copper-adequate and copper-marginal rats. However, liver neutrophil accumulation was significantly increased in copper-marginal rats. These findings were confirmed histologically. Liver expression of the adhesion molecule, intercellular adhesion molecule-1 (ICAM-1), was increased in copper-marginal rats compared to copper-adequate rats. The results suggest that neutrophil accumulation is increased through enhanced ICAM-1 expression in liver of copper-marginal rats after ischemia/reperfusion, but that this does not result in increased liver injury.

Keywords

Copper; Ischemia/reperfusion injury; Neutrophils; ICAM-1

Introduction

Copper (Cu) is an important micronutrient that is essential for the normal function of mammalian enzyme systems. Cu plays a pivotal role as a co-factor for several metalloenzymes including Cu/zinc (Zn) superoxide dismutase (SOD), cytochrome c oxidase, lysyl oxidase, tyrosinase, ceruloplasmin, hephaestin, dopamine β -hydroxylase, and peptidylglycine α -amidating mono-oxygenase [1]. Although distinct Cu deficiency is rare in the clinical setting, marginal Cu deficiency can be a risk factor of various pathological conditions, such as immunocompromised and acute respiratory distress syndrome [2]. Moreover, idiopathic Cu deficiency in some specific conditions, such as use of long-term parenteral nutrition or tube feedings [3-5], inappropriate Zn supplementation [6], and malabsorption following bariatric surgery (e.g., Roux-en-Y gastric bypass) for severe obesity [7,8], has recently been reported.

We previously demonstrated that pulmonary inflammatory responses were augmented in Cu-deficient animals [9]. Dietary Cu deficiency caused activation of neutrophils and endothelial cells as evidenced by F-actin polymerization [10,11] and increased neutrophil accumulation within the lung microcirculation through increased intercellular adhesion molecule-1 (ICAM-1) expression [9,12]. Cu deficiency reduces Cu/Zn SOD activity leading to accumulation of reactive oxygen species. The reduced activity of Cu/Zn SOD is representative of a general shift to a pro-oxidant environment in Cu-deficient animals that includes depression of other antioxidant enzymes [9,13]. Cu/Zn SOD has been shown to be greatly reduced in liver during dietary Cu depletion compared to that of other organs [14].

Liver ischemia/reperfusion (I/R) injury is a major cause of acute liver inflammation and associated with various clinical settings, such as hemorrhagic shock, liver resection, and transplantation [15]. It is widely accepted that there are two distinct phases of liver injury after I/R. The first phase of injury occurs during the initial hours after reperfusion and is related to the production of reactive oxygen species from Kupffer cells, leading to mild hepatocellular injury [16,17]. The late phase injury is initiated by inflammatory mediators released by activated Kupffer cells and hepatocytes. These mediators, including IL-12, TNF α , and IL-1, induce the expression of CXC chemokines and adhesion molecules which recruit activated neutrophils from the liver microcirculation to the parenchyma [18-23]. These neutrophils then directly injure hepatocytes and vascular endothelial cells through their release of oxidants and protease [16,24,25]. In the present study, we evaluated the effect of marginal Cu deficiency on the inflammatory injury response to hepatic I/R.

Materials and Methods

Animals and diet

This project was approved by the University of Louisville Animal Care and Use Committee and was in compliance with the National Institutes of Health guidelines. Male weanling Sprague–Dawley rats were purchased from Charles River Breeding Laboratories (Wilmington, MA, USA). On arrival, rats were housed individually in stainless steel cages in a temperature- and humidity-controlled room with a 12:12-h light: dark cycle. The rats were given free access to distilled water and to one of two diets for 4 weeks. The basal diet was a casein-sucrose-cornstarch-based diet (TD84469, Teklad Test Diets; Madison, WI, USA) containing all known essential vitamins and minerals except for Cu and iron. The Cu-adequate (CuA) diet consisted of the basal diet (940 g/kg of total diet) with safflower oil (50 g/kg) and a Cu-iron mineral mix that provided 0.22 g of ferric citrate (16% iron) and 24 mg of CuSO₄·H₂O/kg of diet. The Cu-marginal (CuM) diet was the same except for partial replacement of Cu with cornstarch in the mineral mix to provide 1.5 mg Cu/kg diet. Analysis of diets indicated average Cu concentrations of 6.30 and 1.62 mg of Cu/kg of diet

for CuA and CuM diets, respectively. After consuming their respective diets for 4 weeks, rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (65 mg/kg), and a midline laparotomy was performed. An atraumatic clip was used to interrupt blood supply to the left lateral and median lobes of the liver. After 90 min of partial hepatic ischemia, the clip was removed to initiate hepatic reperfusion. Sham surgery was performed on five rats from each diet treatment group. These rats underwent all surgical procedures except for the use of the atraumatic clip. Rats were sacrificed after 8 h of reperfusion, and blood and samples of the left lateral and median lobes were taken for analysis.

Myeloperoxidase assay

Liver myeloperoxidase (MPO) content was assessed by methods described elsewhere [26]. Briefly, liver tissue (100 mg) was homogenized in 2 ml of buffer A (3.4 mmol/L KH_2HPO_4 , 16 mmol/L Na_2HPO_4 , pH7.4). After centrifugation for 20 min at $10,000\times g$, the pellet was resuspended in 10 vol of buffer B (43.2 mmol/L KH_2HPO_4 , 6.5 mmol/L Na_2HPO_4 , 10 mmol/L EDTA, 0.5% hexadecyl-trimethylammonium, pH6.0) and sonicated for 10 s. After heating for 2 h at 60°C , the supernatant was reacted with 3,3',3,5'-tetramethylbenzidine, and the optical density was read at 655 nm.

Blood and tissue analysis

Blood was obtained by cardiac puncture. Serum was analyzed for alanine aminotransferase (ALT) by bioassay (Wiener Laboratories, Rosario, Argentina) as an index of hepatocellular injury. Liver tissues were fixed in 10% neutral-buffered formalin, processed, and then embedded in paraffin for light microscopy. Sections were stained with hematoxylin and eosin for histological examination. Liver and kidney Cu concentrations were determined with inductively coupled plasma emission spectrometry.

Western blot analyses

Liver samples were homogenized in lysis buffer (10 mM HEPES, pH7.9, 150 mM NaCl, 1 mM EDTA, 0.6% NP-40, 0.5 mM PMSF, 1 $\mu\text{g}/\text{ml}$ leupeptin, 1 $\mu\text{g}/\text{ml}$ aprotinin, 10 $\mu\text{g}/\text{ml}$ soybean trypsin inhibitor, 1 $\mu\text{g}/\text{ml}$ pepstatin). Samples were then sonicated and incubated for 30 min on ice. Cellular debris was removed by centrifugation at 10,000 rpm. Protein concentrations of each sample were determined. Samples containing equal amounts of protein in equal volumes of sample buffer were separated in a denaturing 10% polyacrylamide gel and transferred to a 0.1- μm pore nitrocellulose membrane. Nonspecific binding sites were blocked with Tris-buffered saline (TBS; 40 mM Tris, pH7.6, 300 mM NaCl) containing 5% non-fat dry milk for 1 h at room temperature. Membranes were then incubated with antibodies to ICAM-1 (R&D Systems, Minneapolis, MN, USA) in TBS with 0.1% Tween 20 (TBST). Membranes were washed and incubated with secondary antibodies conjugated to horseradish peroxidase. Immuno-reactive proteins were detected by enhanced chemiluminescence.

Statistical analysis

All data are expressed as mean $\pm$ standard error of the mean (SEM). Data were analyzed with a one-way analysis of variance with subsequent Student–Newman–Keuls test. Differences were considered significant when $P<0.05$.

Results

Establishment of copper deficiency

The degree of Cu deficiency was determined by measuring hepatic and renal Cu contents. Both liver and kidney Cu concentrations correlated with the respective dietary

concentrations after 4 weeks of feeding. The hepatic and renal Cu concentrations were significantly decreased in CuM rats compared to CuA rats (Table 1).

Effect of marginal copper deficiency on liver I/R injury

The degree of liver injury after I/R surgery was assessed by serum levels of ALT and by histological evaluation. There was no difference in serum ALT levels between CuA and CuM rats (Fig. 1). Histologically, CuA rats displayed the typical pattern of severe hepatocellular necrosis and destruction of liver architecture (Fig. 2). Injury in CuM rats was similar to that of CuA rats. However, there was evidence of increased neutrophil accumulation in the livers of CuM rats compared to CuA rats (Fig. 2).

Marginal copper deficiency increases liver neutrophil accumulation after I/R

To confirm the histological findings of increased neutrophil accumulation in livers of CuM rats, we processed liver tissue for analysis of MPO, a neutrophil-specific enzyme that is routinely used to quantitate the degree of neutrophil accumulation [26]. I/R caused a significant increase in tissue MPO content in CuA rats (Fig. 3). However, MPO content in CuM rats was significantly higher than that observed in CuA rats, confirming increased neutrophil accumulation (Fig. 3). To explore the potential mechanism for this effect, we examined the expression of the cellular adhesion molecule, ICAM-1, in liver tissues. ICAM-1 is an essential vascular cell adhesion molecule used by neutrophils for recruitment into the liver [27]. Western blot analysis demonstrated increased ICAM-1 expression in livers from CuM rats compared to CuA rats (Fig. 4).

Discussion

Marginal Cu diets have been used in animal studies to simulate the Cu content of some Western diets [28,29]. We have previously used the regimen of 1.5 mg Cu/kg diet for 4 weeks starting at weaning to study the cardiovascular effects of marginal Cu intake [30-32]. The effect of the marginal Cu diet on systemic Cu status in the current study (Table 1) is similar to our previous results with this diet. Previous work with this diet suggests that this concentration of Cu appears to be near the tipping point for several functional changes associated with inadequate dietary Cu. These changes include prolonged hemostasis [30], diminished vasoreactivity [31], and upregulation of the inflammatory protein COX-2 [32]. The diminished vasoreactivity is also seen in a long-term adult model of Cu deficiency [33] validating the use of the short-term weanling model. Based on these results, we used the 1.5-mg Cu/kg in the current study of hepatic inflammation.

We previously demonstrated that dietary Cu deficiency can lead to an augmented inflammatory response in the lung [9,12]. Dietary Cu deficiency had proinflammatory effects on both neutrophils and the microvascular endothelium that promoted neutrophil-endothelial interactions associated with enhanced expression of CD11b adhesion molecule [9,20]. The increase of neutrophil accumulation in the lungs of Cu-deficient rats is thought to be caused by loss of biomechanical deformability of neutrophils [10]. In liver I/R injury, neutrophils play a critical role in the inflammatory tissue injury [34]. We hypothesized, therefore, that the inflammatory response to liver I/R would be exaggerated by the proinflammatory effects of marginal Cu deficiency. Interestingly, acute liver injury was not different in CuM rats compared to CuA rat as indicated by serum ALT (Figs. 1 and 2). However, neutrophil accumulation was greatly increased in CuM rats (Figs. 2 and 3). We also showed that liver ICAM-1 protein expression was enhanced in CuM rats (Fig. 4). It has been shown that ICAM-1 plays an important role for neutrophil recruitment during the later phase of inflammation after ischemia reperfusion [27].

In an earlier study, we demonstrated that lipopolysaccharide (LPS) does not affect the liver ICAM-1 expression in rats fed a Cu-adequate or completely Cu-deficient (CuD) diet [12]. Similarly, there was no difference in liver MPO activity between CuA and CuD rats without LPS stimulation, but MPO activity was significantly higher in CuA rats compared to CuD rats after LPS stimulation [12]. This discrepancy between those findings and our current data seem to be related to the stimulus (I/R vs. LPS). The liver is known to be highly resistant to LPS as a protective mechanism against intestinal translocation. I/R on the other hand is a direct and significant hepatocellular insult.

In contrast to our expectation, there was no difference in the degree of liver injury measured by serum ALT levels. Normally, neutrophils are primary contributors to I/R injury of the liver [34] and once sequestered in the hepatic parenchyma, cause hepatocyte damage through the release of oxidants and proteases [25,35]. However, Cu-deficient neutrophils are known to be immature [36], and the function of neutrophils and macrophages is suppressed in marginally Cu-deficient rats [37,38]. Babu and Failla [37] showed that generation of superoxide anion (O_2^-) and microbicidal activity of neutrophils were suppressed in marginally as well as severely Cu-deficient rats. Despite the developmental and functional defects of Cu-deficient neutrophils, inadequate Cu nutriture does not impair neutrophil adhesion to the endothelium but actually promotes sequestration [9,12,36]. These phenomena may help explain why we observed more neutrophils in the livers of CuM rats, but not an enhanced liver injury.

Another explanation can be proposed in terms of host environment. Allen et al. showed that recovery of cardiac functions after I/R was improved in Cu-deficient rats rather than Cu-adequate rats [39]. Their interpretation of this observation was that adaptation to the Cu-deficient conditions might induce “preconditioned” status for the stress of I/R in the hearts of Cu-deficient rats.

It is well known that Cu deficiency results in anemia and suppression of Cu-dependent antioxidant enzymes. Anemia can induce adaptation to hypoxic condition and therefore resistance to ischemic damage. In addition, chronically suppressed antioxidant enzyme activity may enhance other antioxidant systems, which can reduce injury caused by oxidative stress during reperfusion [39]. Therefore, it could be speculated that some cytoprotective mechanisms which might have occurred in the hearts of the previous study were also induced in the liver and caused relatively less injury in spite of more neutrophil accumulation in CuM rats compare to CuA rats.

In conclusion, the present study demonstrates that marginal Cu deficiency significantly enhances neutrophil accumulation in liver, but not liver injury after I/R. Increased neutrophil accumulation is associated with enhanced ICAM-1 expression in the livers of CuM rats. Further investigation is needed to clarify the role of Cu and the influence of Cu deficiency on liver pathophysiology.

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References

1. Bertinato J, L'Abbe MR. Maintaining copper homeostasis: regulation of copper-trafficking proteins in response to copper deficiency or overload. *J Nutr Biochem*. 2004; 15:316–322. [PubMed: 15157936]
2. Sarricolea ML, Villa-Elizaga I, Lopez J. Respiratory distress syndrome in copper deficiency: an experimental model developed in rats. *Biol Neonate*. 1993; 63:14–25. [PubMed: 8443291]
3. Chen CC, Takeshima F, Miyazaki T, et al. Clinicopathological analysis of hematological disorders in tube-fed patients with copper deficiency. *Intern Med*. 2007; 46:839–844. [PubMed: 17575375]
4. Jayakumar S, Micallef-Eynaud PD, Lyon TD, et al. Acquired copper deficiency following prolonged jejunostomy feeds. *Ann Clin Biochem*. 2005; 42:227–231. [PubMed: 15949160]
5. Sriram K, O'Gara JA, Strunk JR, et al. Neutropenia due to copper deficiency in total parenteral nutrition. *JPEN J Parenter Enteral Nutr*. 1986; 10:530–532. [PubMed: 3093712]
6. Prodan CI, Holland NR, Wisdom PJ, et al. CNS demyelination associated with copper deficiency and hyperzinemia. *Neurology*. 2002; 59:1453–1456. [PubMed: 12427906]
7. Griffith DP, Liff DA, Ziegler TR, et al. Acquired copper deficiency: a potentially serious and preventable complication following gastric bypass surgery. *Obesity*. 2009; 17:827–831. [PubMed: 19148115]
8. Steinbrook R. Surgery for severe obesity. *N Engl J Med*. 2004; 350:1075–1079. [PubMed: 15014179]
9. Lentsch AB, Kato A, Saari JT, et al. Augmented metalloproteinase activity and acute lung injury in copper-deficient rats. *Am J Physiol Lung Cell Mol Physiol*. 2001; 281:L387–L393. [PubMed: 11435213]
10. Gordon SA, Lominadze D, Saari JT, et al. Impaired deformability of copper-deficient neutrophils. *Exp Biol Med*. 2005; 230:543–548.
11. Lominadze D, Saari JT, Percival SS, et al. Proinflammatory effects of copper deficiency on neutrophils and lung endothelial cells. *Immunol Cell Biol*. 2004; 82:231–238. [PubMed: 15186252]
12. Schuschke DA, Percival SS, Lominadze D, et al. Tissue-specific ICAM-1 expression and neutrophil transmigration in the copper-deficient rat. *Inflammation*. 2002; 26:297–303. [PubMed: 12546139]
13. Saari JT. Copper deficiency and cardiovascular disease: role of peroxidation, glycation, and nitration. *Can J Physiol Pharmacol*. 2000; 78:848–855. [PubMed: 11077985]
14. Paynter DI, Moir RJ, Underwood EJ. Changes in activity of the Cu-Zn superoxide dismutase enzyme in tissues of the rat with changes in dietary copper. *J Nutr*. 1979; 109:1570–1576. [PubMed: 225457]
15. Lentsch AB, Kato A, Yoshidome H, et al. Inflammatory mechanisms and therapeutic strategies for warm hepatic ischemia/reperfusion injury. *Hepatology*. 2000; 32:169–173. [PubMed: 10915720]
16. Jaeschke H, Farhood A. Neutrophil and Kupffer cell-induced oxidant stress and ischemiareperfusion injury in rat liver. *Am J Physiol*. 1991; 260:G355–G362. [PubMed: 2003603]
17. Jaeschke H. Reactive oxygen and ischemia/reperfusion injury of the liver. *Chem Biol Interact*. 1991; 79:115–136. [PubMed: 1884426]
18. Suzuki S, Toledo-Pereyra LH. Interleukin 1 and tumor necrosis factor production as the initial stimulants of liver ischemia and reperfusion injury. *J Surg Res*. 1994; 57:253–258. [PubMed: 7518017]
19. Wanner GA, Ertel W, Muller P, et al. Liver ischemia and reperfusion induces a systemic inflammatory response through Kupffer cell activation. *Shock*. 1996; 5:34–40. [PubMed: 8821101]
20. Lentsch AB, Yoshidome H, Kato A, et al. Requirement for interleukin-12 in the pathogenesis of warm hepatic ischemia/reperfusion injury in mice. *Hepatology*. 1999; 30:1448–1453. [PubMed: 10573524]
21. Colletti LM, Remick DG, Burtch GD, et al. Role of tumor necrosis factor- α in the pathophysiologic alterations after hepatic ischemia/reperfusion injury in the rat. *J Clin Invest*. 1990; 85:1936–1943. [PubMed: 2161433]

22. Clarke CN, Kuboki S, Tevar A, et al. CXC chemokines play a critical role in liver injury, recovery, and regeneration. *Am J Surg.* 2009; 198:415–419. [PubMed: 19716886]
23. Lentsch AB, Yoshidome H, Cheadle WG, et al. Chemokine involvement in hepatic ischemia/reperfusion injury in mice: roles for macrophage inflammatory protein-2 and KC. *Hepatology.* 1998; 27:1172–1177. [PubMed: 9537464]
24. Okaya T, Lentsch AB. Cytokine cascades and the hepatic inflammatory response to ischemia and reperfusion. *J Invest Surg.* 2003; 16:141–147. [PubMed: 12775430]
25. Jaeschke H. Mechanisms of liver injury. II. Mechanisms of neutrophil-induced liver cell injury during hepatic ischemia-reperfusion and other acute inflammatory conditions. *Am J Physiol Gastrointest Liver Physiol.* 2006; 290:G1083–G1088. [PubMed: 16687579]
26. Schierwagen C, Bylund-Fellenius AC, Lundberg C. Improved method for quantification of tissue PMN accumulation measured by myeloperoxidase activity. *J Pharmacol Meth.* 1990; 23:179–186.
27. Farhood A, McGuire GM, Manning AM, et al. Intercellular adhesion molecule 1 (ICAM-1) expression and its role in neutrophil-induced ischemia-reperfusion injury in rat liver. *J Leukoc Biol.* 1995; 57:368–374. [PubMed: 7884306]
28. Wildman RF, Hopkins R, Failla ML, et al. Marginal copper-restricted diets produce altered cardiac ultrastructure in the rat. *Proc Soc Exp Med.* 1995; 210:43–49.
29. Li Y, Wang L, Schuschke DA, et al. Marginal dietary copper restriction induces cardiomyopathy in rats. *J Nutr.* 2005; 135:2130–2136. [PubMed: 16140888]
30. Schuschke LA, Saari JT, Miller FN, et al. Hemostatic mechanisms in marginally copper-deficient rats. *J Lab Clin Med.* 1995; 125:748–753. [PubMed: 7769369]
31. Schuschke DA, Percival SS, Saari JT, et al. Relationship between dietary copper concentration and vasodilation in the microcirculation of rats. *Biofactors.* 1999; 10:321–327. [PubMed: 10619699]
32. Schuschke DA, Adeagbo ASO, Patibandla PK, et al. Cyclooxygenase-2 is upregulated in copper-deficient rats. *Inflammation.* 2009; 32:333–339. [PubMed: 19609832]
33. Falcone JC, Saari JT, Kang YJ, et al. Vasoreactivity in an adult rat model of marginal copper deficiency. *Nutr Res.* 2005; 25:177–186.
34. Jaeschke H, Farhood A, Smith CW. Neutrophils contribute to ischemia/reperfusion injury in rat liver in vivo. *FASEB J.* 1990; 4:3355–3359. [PubMed: 2253850]
35. Ramaiah SK, Jaeschke H. Role of neutrophils in the pathogenesis of acute inflammatory liver injury. *Toxicol Pathol.* 2007; 35:757–766. [PubMed: 17943649]
36. Karimbakas J, Langkamp-Henken B, Percival SS. Arrested maturation of granulocytes in copper deficient mice. *J Nutr.* 1998; 128:1855–1860. [PubMed: 9808634]
37. Babu U, Failla ML. Copper status and function of neutrophils are reversibly depressed in marginally and severely copper-deficient rats. *J Nutr.* 1990; 120:1700–1709. [PubMed: 2175782]
38. Babu U, Failla ML. Respiratory burst and candidacidal activity of peritoneal macrophages are impaired in copper-deficient rats. *J Nutr.* 1990; 120:1692–1699. [PubMed: 2175781]
39. Allen CB, Saari JT. Isolated hearts from copper-deficient rats exhibit improved postischemic contractile performance. *J Nutr.* 1993; 123:1794–1800. [PubMed: 8229293]

Fig. 1.

a Effects of copper deficiency on liver ischemia/reperfusion injury. Liver injury was measured by serum levels of alanine aminotransferase. Data are mean $\pm$ SEM with $n=5$ per group. Asterisk, $P<0.05$ compared to sham group

Fig. 2. Effect of copper (Cu) deficiency on liver histopathology. Representative pictures of CuA (*left*) and CuM (*right*). Both of the pictures showed similar degree of hepatocellular necrosis. However, more neutrophil accumulation was observed in CuM group compare to CuA group

Fig. 3.

Effects of copper (Cu) deficiency on neutrophil accumulation after liver ischemia/reperfusion injury. Neutrophil accumulation was determined by liver content of myeloperoxidase. Data are mean $\pm$ SEM with $n=5$ per group. *Single asterisk*, $P<0.05$ compared to sham group. *Double asterisk*, $P<0.05$ compared to CuA group

Fig. 4. Intercellular adhesion molecule-1 protein expression after ischemia/reperfusion injury was determined by Western blot

Table 1Indices of Copper (Cu) Status in Rats Fed Cu-adequate and Cu-marginal Diets ($n=5$ per group)

	Cu-adequate	Cu-marginal
Liver Cu ($\mu\text{g/g}$ dry wt.)	13.88 $\pm$ 0.64 a	9.08 $\pm$ 0.59 b
Kidney Cu ($\mu\text{g/g}$ dry wt.)	16.42 $\pm$ 1.44 a	13.89 $\pm$ 0.29 b

All values are mean SE. Values with different lowercase letters are significantly different ($P<0.05$)