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Title: The manner of the inflammation-boosting effect caused by acute hyperglycemia secondary to overfeeding and the effects of insulin therapy in a rat model of sepsis

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Running title: Inflammation-boosting effect of acute hyperglycemia and the effects of insulin therapy

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Abstract

Background: The aim of the study was to investigate both the inflammation-boosting effect and the metabolic stress induced by acute hyperglycemia secondary to overfeeding with excessive glucose infusion, and the effects of insulin therapy on those events in a rat model of sepsis.

Materials and Methods: Sprague-Dawley rats underwent cecal ligation and puncture (CLP) or a sham operation. Pre-established continuous intravenous glucose infusion was initiated immediately after surgery. First, rats with CLP-inducing sepsis were divided into three groups on the basis of the target blood glucose (BG): high glucose (HG) group (overfed: >300 mg/dL), moderate glucose (MG) group (moderate hyperglycemia: 200 to 300 mg/dL), and no glucose (NG) group (100 to 150 mg/dL). The Sham group received the same glucose infusion as HG. Blood glucose and plasma interleukin (IL)-6 levels were monitored over time. All rats were sacrificed 9 hours after surgery to evaluate lung histology and measure hepatic total glutathione and malondialdehyde contents. Based on the results, the high glucose and insulin (HI) group was added to septic groups as a model of insulin therapy, in which insulin with the same high glucose dose as HG was administered to maintain moderate hyperglycemia.

Results: BG in all groups remained in the pre-established target range throughout the experiment. Plasma IL-6 in all septic groups increased in a time-dependent manner, while that in the Sham group with moderate hyperglycemia hardly increased. Nine hours after CLP, plasma IL-6 in HG rose to 7407.5 ± 1987.3 pg/ml, which was three times higher than the other septic groups. There was no significant difference among MG, NG and HI, in which BG remained constant at <300 mg/dL. HG showed the worst consequences of lung injury and oxidative stress in the liver, which were completely stable in HI.

Conclusion: Acute severe hyperglycemia in critical illness might excessively boost the existing systemic inflammatory response in a threshold-based manner. Insulin therapy

under overfeeding could strongly inhibit such a boosting effect and oxidative stress in the liver.

Key Words: sepsis; hyperglycemia; inflammation; oxidative stress; metabolic stress; insulin

Introduction

Since the Leuven studies, glycemic control during critical illness has been considered essential and effective in improving prognosis [1–3]. Sepsis is a representative critical illness, and many therapeutic guidelines, as typified by the Surviving Sepsis Campaign, stress the importance of glycemic control as a treatment for the causes of sepsis [4]. There is growing evidence of the deleterious effects of acute hyperglycemia, one of which is the promotion of the inflammatory cascade [5, 6]. Previous studies have shown that acute hyperglycemia is associated with rapidly increasing concentrations of circulating cytokines such as interleukin (IL)-6 in both non-stressed and critically ill patients [5–8]. Likewise, in vivo studies in animals have shown that acute hyperglycemia enhances cytokine production and the oxidative response within hours under both non-stressed conditions and stressful conditions induced by endotoxin [9, 10]; however, these studies offer few suggestions regarding the extent to which acute hyperglycemia during critical illness amplifies the existing systemic inflammatory response. Thus, there has been no investigation in either the clinical setting or in vivo experiments to answer this important question. These considerations prompted us to clarify three key questions as follows. The first is whether acute hyperglycemia actually promotes excessive systemic inflammation in severe infection. If so, further detailed questions arise: (1) By how much is the systematic inflammatory response boosted?; (2) How much time is required before the onset of this phenomenon?; (3) What is the manner of the systemic inflammatory response boost, i.e. proportionate to blood glucose levels or threshold-based? The second key question is whether insulin therapy can inhibit such an inflammation-boosting effect caused by acute hyperglycemia. The third key question is whether insulin therapy also improves metabolic stress associated with acute hyperglycemia induced by overfeeding, such as oxidative stress [9, 11]. To examine these issues, we designed a rat model of acute hyperglycemia obtained by adjusting intravenous glucose loading under septic conditions induced by cecal ligation

and puncture (CLP). CLP is widely used as a standard model to induce sepsis in laboratory animals [12, 13]. This rat model is suitable to test the deleterious effects of acute hyperglycemia mimicking the common clinical condition of overfeeding with excessive glucose infusion under septic conditions in clinical settings.

Materials and Methods

Animals

Thirty-five male, seven-week-old Sprague-Dawley rats (Nippon Clea, Tokyo, Japan), weighing approximately 300 g, were used in the experiment. The animals were maintained at 21 °C under 12 h light/dark cycles and allowed free access to water and standard chow for 3 to 5 days. The experimental protocols were carried out in a humane manner after receiving approval from the Institutional Animal Experiment Committee of the University of Tsukuba and in accordance with the Regulations for Animal Experiments of our university and the Fundamental Guidelines for Proper Conduct of Animal Experiments and Related Activities in Academic Research Institutions under the Jurisdiction of the Ministry of Education, Culture, Sports, Science, and Technology.

Operative procedure

Prior to surgery, all rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (Somnopentyl; Kyoritsu Seiyaku Co., Tokyo, Japan) at a dose of 40 mg/kg body weight. Under aseptic conditions, the following was performed: (1) Left carotid artery catheterization. The arterial catheter was a polyethylene tube of 0.58 mm inner diameter and 0.965 mm outer diameter (Becton, Dickinson and Co., Franklin Lakes, NJ, USA). (2) Right jugular vein catheterization in a central venous position. The tip of the central venous catheter was a silicone tube of 0.5 mm inner diameter and 1.0 mm outer diameter (Fuji Systems Co., Tokyo, Japan), and the rest of the catheter was a plastic tube of the same diameter (Imamura Co., Chiba, Japan). The distal ends of each catheter were tunneled subcutaneously and exited in the cephalad portion of the

interscapular area. The catheters were fixed to the skin using a harness attached to a swivel assembly. (3) After catheterization, CLP was performed. Following a 3 cm midline incision, the cecum was exposed and ligated with a 3-0 silk suture below the ileocecal valve. The cecum was then punctured through both sides with an 18 gauge needle. After a small amount of feces had extruded from the punctured site, the cecum was placed back into the peritoneum. Sham-operated animals received catheterization and simple laparotomy, but not CLP. The abdominal wall and skin were closed with 2-0 synthetic absorbable sutures (VICRYL; Ethicon, Tokyo, Japan). To ensure technical uniformity, all procedures including CLP were performed by only one surgeon (lead author of this article). Following these procedures, the rats were maintained in individual metabolic cages and were not allowed access to food or water. Immediately after surgery, an arterial catheter was infused with normal saline containing 0.05 U/ml of heparin at a rate of 0.1ml/h using infusion pumps (SP-115; JMS Co., Ltd., Tokyo, Japan) for catheter maintenance.

First experimental design

Rats were divided into two main groups: Groups under septic conditions induced by CLP and the Sham group without sepsis. Moreover, the groups under septic conditions were divided into three groups on the basis of the target blood glucose (BG) levels: the high glucose (HG) group (n = 7), in which intravenous infusion of HG at approximately 40 mg/kg body weight/min, equivalent to 228 kcal/kg body weight/day, led to severe hyperglycemia (blood glucose >300 mg/dL); moderate glucose (MG) group (n = 7), in which intravenous moderate glucose infusion of approximately 25 mg/kg body weight/min, equivalent to 148 kcal/kg body weight/day, led to moderate hyperglycemia (200 to 300 mg/dL); and no glucose (NG) group (n = 7), in which normal saline was administered as a non-glucose solution allowed glycemic control (BG <150 mg/dL), recommended by the second edition (2008) of the Surviving Sepsis Campaign guidelines (100 to 150 mg/dL) [14]. The Sham group (n = 7) received the same

intravenous glucose infusion as the HG group (40 mg/kg body weight/min), leading to the development of moderate hyperglycemia (200 to 300 mg/dL). The amount of glucose infusion to achieve each targeted blood glucose level was determined by pilot studies (data not shown). Immediately after surgery, continuous intravenous administration of each solution using infusion pumps (SP-115; JMS Co., Ltd.) was initiated uniformly at a rate of 9 ml/kg body weight/h (approximately 2.7 ml/h) via the central venous catheter. In the HG, MG and Sham groups, each solution was adjusted by mixing two different glucose-added electrolytic solutions (Hicaliq NC-L and Hicaliq NC-H; TERUMO Co., Tokyo, Japan) to ensure a predetermined amount of glucose infusion (Table 1).

Just before surgery (CLP or sham operation), and 3 and 6 hours after surgery, 0.4 ml blood samples were obtained, respectively, via the arterial catheter to determine blood glucose and plasma inflammatory cytokine levels. Nine hours after surgery, all rats were sacrificed to obtain tissue and blood samples in order to analyze lung histology, oxidative stress markers and clinical chemistry markers. The reason why the experimental period was designed for nine hours was because pilot studies revealed that the HG group had a mortality of over 40 percent after 12 hours (data not shown).

Second experimental design

The results of the first experiment indicated that plasma IL-6 did not increase depending on the increase in BG levels, but was likely to rise sharply when BG exceeded a certain threshold level, which may be equivalent to 300 mg/dl. To confirm this possibility, the high glucose and insulin (HI) group (n = 7) was added to the groups under septic conditions as a model of insulin therapy, in which recombinant human insulin (6 IU/kg/h; Eli Lilly Japan, Kobe, Japan) with the same intravenous high glucose infusion as the HG group (Table 1) was administered by constant intravenous infusion so as not to exceed the upper limit of moderate hyperglycemia (300 mg/dL) at 9 hours after CLP. According to this experimental design, all examination items were evaluated

in the HI group.

Analytical Procedures

Glucose monitoring

Blood samples (10 μl) from the arterial catheter were analyzed using a blood glucose meter (FreeStyle; NIPRO, Osaka, Japan) at each measurement time point.

Cytokine monitoring

We measured blood IL-6 levels to estimate the degree of systemic inflammatory response because blood IL-6 levels accurately reflect the activation of cytokine cascades and are well correlated with the severity of sepsis [15, 16]. Blood samples (0.4 ml) from the arterial catheter at each measurement time point were anticoagulated with ethylenediaminetetraacetic acid (EDTA) and centrifuged to obtain plasma. Plasma levels of IL-6 were determined using an enzyme linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, MN, USA).

Blood biochemistry analysis

Blood samples (4 ml) were collected at the end of the study. Serum samples were analyzed with FUJI DRY-CHEM 3500 (FUJIFILM Co., Tokyo, Japan) to measure aspartate amino transferase (AST), alanine transaminase (ALT), blood urea nitrogen (BUN) and creatinine (CRE) levels, which roughly represent liver and renal functions.

Oxidative stress analysis

We analyzed the total glutathione (GSH) and malondialdehyde (MDA) content in the liver as an oxidative stress marker [11, 17]. The depletion of GSH, regarded as an anti-oxidant molecule, and increment of the MDA, regarded as a lipid peroxidative end product, are consequential findings of oxidative stress enhancement [18–20]. Liver tissues were collected at the end of the study, immediately frozen with liquid nitrogen, and kept at -80 °C until analysis. The GSH and MDA contents in the liver were determined using a colorimetric determination kit (Japan Institute for the Control of Aging, Nikken SEIL Co., Shizuoka, Japan).

Histopathological Analysis

Right lungs were obtained at the end of the study. Lung tissue specimens were fixed with 10% phosphate-buffered formaldehyde and then embedded in paraffin. Histopathological sections (4 μm) were stained with hematoxylin-eosin. Two pathologists blind to treatment assignment evaluated the extent of lung injury according to Murakami's technique [21]. Briefly, 24 areas of lung parenchyma were graded on a scale of 0–4 (0, absent and appears normal; 1, light; 2, moderate; 3, strong; 4, intense) for congestion, edema, inflammation, and hemorrhage.

Statistics

All data are presented as the mean ± standard error of the mean. For comparison of more than two groups, statistical analyses were performed with one-way factorial analysis of variance (ANOVA). When ANOVA was found to be significant at the 95% confidence level, multiple comparisons between groups were made by the Tukey-Kramer test. For the histological study, the Kruskal-Wallis test was performed. Comparisons between the HG and HI groups were made by the two-sided Mann-Whitney's U test. $P < 0.05$ was considered significant.

Results

Data of blood biochemical examinations (Table 2)

At the end of this experiment, some markers in the septic groups showed a higher value than those of the Sham group. There were no significant differences in serum levels of AST, ALT, BUN and CRE among the HG, MG and NG groups ($P = 0.54$, 0.63, 0.16, 0.15, respectively). Serum levels of BUN in the HG group were higher than those in the HI group ($P < 0.01$). As a result, no liver or kidney failure occurred during the experimental period.

Blood glucose levels (Figure 1)

Just before surgery (CLP or sham operation), there was no significant difference

among any of the groups. In the groups under septic conditions without insulin treatment (HG, MG and NG groups), BG was likely to increase depending on the amount of glucose infusion. In the HG group, BG remained markedly raised (severe hyperglycemia: >300 mg/dL) throughout the experiment, and reached 463.3 ± 48.8 mg/dL 9 hours after CLP. The HI group, receiving the same high glucose infusion as the HG group with insulin, showed moderate hyperglycemia (200 to 300 mg/dL) from 6 to 9 hours after CLP. Thus, BG in all groups remained in the pre-established target range throughout the experiment.

Plasma interleukin-6 levels (Figure 2)

Just before surgery, there were no significant differences among any of the groups. Plasma IL-6 in the septic groups (HG, MG, NG and HI groups) increased with time. The HG group with severe hyperglycemia (BG >300 mg/dL) showed a sudden rise in plasma IL-6 at 3 hours after CLP, and plasma IL-6 eventually rose to 7407.5 ± 1987.3 pg/ml, which was more than three times the values measured in the other septic groups and significantly higher ($P < 0.01$). On the other hand, plasma IL-6 in the Sham group with moderate hyperglycemia peaked 3 hours after the sham operation, and then exhibited a declining trend, which resulted in 49.1 ± 26.3 pg/ml and showed no significant difference between the initial value and the last value after the start of the experiment ($P = 0.12$). In the HI group with insulin therapy, a sudden increment in plasma IL-6 was prevented in spite of administering the same glucose infusion as the HG group. There was no significant difference among MG, NG and HI groups, in which BG remained constant at <300 mg/dL ($P = 0.25$).

Histological examination (Figure 3, 4)

Typical histological appearances of lung tissues accompanying septic conditions are shown in Figure 3. In contrast to the Sham group (Fig. 3A), lung tissues in the septic groups demonstrated an increase in both interstitial edema and inflammatory cells (Fig. 3B, 3C, 3D, 3E). As an overview, the lung injury in the HG group appeared to be worse

than in the other septic groups. Figure 4 shows the quantitative result of the extent of lung injury in each group. Histological scores of edema and inflammation in the HG group were significantly higher than in MG NG, and Sham groups ($P < 0.01$). In the HI group, histological scores of edema and inflammation were significantly lower than in the HG group ($P < 0.01$).

Total glutathione contents in the liver (Figure 5)

Total GSH contents in the liver in the HG, MG, NG, HI and Sham groups were 1.26 ± 0.23, 1.64 ± 0.23, 3.17 ± 0.30, 2.24 ± 0.26 and 1.86 ± 0.38 mmol/mg tissue, respectively. Among the septic groups without insulin treatment, total GSH content in the liver showed a tendency to decrease inversely with the amount of glucose infusion, and the HG group had the lowest value. In the NG group, total GSH content in the liver was significantly higher than in the other groups ($P < 0.01$ versus HG and MG, $P = 0.02$ versus Sham). Hepatic total GSH contents in the HI group were significantly higher than in the HG group, in spite of receiving the same intravenous high glucose infusion ($P = 0.02$).

Malondialdehyde content in the liver (Figure 6)

MDA contents in the liver in the HG, MG, NG, HI and Sham groups were 46.7 ± 4.1, 40.2 ± 1.8, 35.2 ± 2.5, 39.3 ± 4.4 and 40.9 ± 3.0 mmol/mg tissue, respectively. The change patterns of hepatic MDA content were opposite those of hepatic total GSH content; however, the differences were not statistically significant ($P = 0.09$).

Discussion

In this study, we first demonstrated that acute hyperglycemia actually promotes excessive systemic inflammation in severe infection. In addition, the present study might provide new insight into two important issues; the manner of the inflammation-boosting effect caused by acute hyperglycemia, and the effects of insulin therapy on acute hyperglycemia under overfeeding.

As explained earlier, plasma IL-6 levels can be regarded as a useful index of systemic inflammatory response [15, 16]. BG in the NG group fluctuated between 100 to 150 mg/dL throughout this experiment, which is comparable to the target range recommended by Surviving Sepsis Campaign guidelines [14]; therefore, in the NG group without harmful hyperglycemia, the changes in plasma IL-6 levels were considered to be purely induced by septic conditions. Thus, using the change in plasma IL-6 in the NG group as the base allowed us to evaluate whether plasma IL-6 changes in the two other septic groups (HG and MG groups) were affected by factors other than septic conditions, such as harmful hyperglycemia. In the HG group, severe hyperglycemia (>300 mg/dL) led to a sharp rise in plasma IL-6 after six hours of sepsis, which then markedly increased by 1000 pg/ml, with the result being more than three times that of the NG group. On the other hand, in the MG group with moderate hyperglycemia, plasma IL-6 fluctuated in the same manner as in the NG group, and there was no significant difference between the two groups. In the Sham group with moderate hyperglycemia under non-septic conditions, there was a slight increase in plasma IL-6, which was less than one seventieth that of the HG group. As previously reported, in non-stressed subjects, the increment in circulating cytokine concentrations induced by acute severe hyperglycemia was very small [5]. Likewise, in the Sham group, which was not associated with septic conditions, the increment in plasma IL-6 was hardly promoted by moderate hyperglycemia. As might be expected, the presence of inflammation above a certain degree is a precondition when acute hyperglycemia strongly amplifies an inflammatory response. While HG and MG groups were associated with the same septic conditions, there was a major and significant difference in the fluctuations in plasma IL-6 between the two groups. Moreover, plasma IL-6 in the MG group fluctuated in the same manner as in the NG group. A notable point is that the HG group remained severely hyperglycemic, while the MG group remained moderately hyperglycemic. These results indicated that plasma IL-6 did not increase depending on

the increase in BG levels, but was likely to rise sharply when BG exceeded a certain threshold level, which may be equivalent to 300 mg/dl. This presumption could be validated by the evidence obtained from the HI group receiving the same high glucose dose as the HG group. In the HI group, insulin therapy could maintain moderate hyperglycemia and subsequently prevent a sudden increment in plasma IL-6, which allowed for maintenance of the same level as in both MG and NG groups. Here, we can provide an answer to our first key question as follows: acute severe hyperglycemia excessively boosts an existing systemic inflammatory response, such as sepsis, on a short-term basis (within half a day) and in a threshold-based manner. These experimental findings provide new insight into the deleterious effects of acute hyperglycemia in critical illness. The maintenance of BG below a certain level is imperative as a treatment measure to prevent excessive promotion of the inflammatory cascade in critical illness.

Another point to keep in mind is that the existing systemic inflammatory response enhanced by acute severe hyperglycemia caused organ damage on a short-term basis. The results of histological scores regarding lung injury were consistent with the degree of systemic inflammatory response evaluated by plasma IL-6 levels. In the HG group with a marked increase in plasma IL-6, the severity of lung injury showed the worst findings of interstitial edema and inflammatory cell infiltration in both the overview image and histological scoring. Meanwhile, in the HI group, such harmful events were completely avoided by insulin therapy, the underlying mechanism of which might be blood glucose-lowering action, mainly through stimulation of skeletal muscle glucose uptake [22, 23]. Here, we can provide an answer to the second key question as follows: insulin therapy could strongly inhibit the boosting effect on the existing systemic inflammatory response due to acute hyperglycemia, allowing for the prevention of a cytokine storm and organ damage.

The last key question is whether insulin therapy can improve metabolic stress

associated with acute hyperglycemia induced by overfeeding, namely, excessive glucose infusion. We used total GSH and MDA content in the liver to evaluate the state of oxidative stress. Not only chronic, but also acute hyperglycemia enhances oxidative stress under non-stress conditions [9, 11, 24]. Sepsis itself is also a powerful factor generating oxidative stress [18, 25]. The finding that glucose infusion increases oxidative stress depending on the applied dose is corroborated by the discovery that the total GSH content in the liver showed a tendency to decrease inversely with the amount of glucose infusion among the septic groups (HG, MG, and NG groups). Moreover, in the NG group receiving no glucose infusion, the hepatic total GSH content was maintained at a significantly higher level than in the Sham group. On the other hand, the change patterns of hepatic MDA content were opposite those of hepatic total GSH content because the increment of MDA reflected an increase in oxidative stress. Unlike in the case of GSH, the differences among all groups did not reach statistical significance (P = 0.09). This result might be likely to be due to the experimental period. The increment in hepatic MDA content should start to occur subsequent to the depletion of anti-oxidant molecules such as GSH, so the experimental period of 9 hours was too short for changes in hepatic MDA content to show a significant difference. From the above standpoint, administration of a large dose of glucose appears to have a stronger impact on oxidative stress in the liver than severe systemic inflammation such as sepsis, which means that glucose infusion promotes the enhancement of oxidative stress in the liver. In the HI group, hepatic total GSH content was significantly higher than in the HG group, in spite of administering the same intravenous high glucose infusion. Thus, insulin therapy exerts antioxidant effects in the liver; however, this benefit is achieved by enhancing glucose uptake into skeletal muscle cells instead of hepatocytes, which might put a lot of stress on muscle cells, e.g., increasing the production of reactive oxygen species and/or by-products [9].

In the present study, important limitations need to be addressed. We could analyze each

finding based on clear observations and provide unequivocal answers to three key questions posed in the introduction; however, the underlying mechanism of each observation remains to be fully elucidated. Further investigations are needed to clarify each mechanism.

Conclusions

First, the present study clarified the interaction between acute hyperglycemia under septic conditions and with an inflammatory response. Acute severe hyperglycemia might excessively boost the existing systemic inflammatory response on a short-term basis and in a threshold-based manner. Second, we examined the effects of insulin therapy for acute severe hyperglycemias secondary to overfeeding with excessive glucose infusion. Under overfed conditions, insulin therapy could strongly inhibit such a boosting effect on the existing systemic inflammatory response and oxidative stress in the liver.

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Figure Legends

Figure 1. Changes in blood glucose levels in each group after surgery.

Sham: 40 mg/kg/min glucose infusion after sham operation. HG: 40 mg/kg/min glucose infusion after cecal ligation and puncture (CLP). MG: 25 mg/kg/min glucose infusion after CLP. NG: normal saline infusion after CLP. HI: 40 mg/kg/min glucose and 6 IU/kg/h insulin infusion after CLP. Data are expressed as the mean ± SE. *P <0.05 versus MG, NG and Sham (one way measures ANOVA and Tukey-Kramer test). †P <0.05 versus HG (two-sided Mann-Whitney U test).

Figure 2. Changes in plasma interleukin -6 levels in each group after surgery.

Sham: 40 mg/kg/min glucose infusion after sham operation. HG: 40 mg/kg/min glucose infusion after cecal ligation and puncture (CLP). MG: 25 mg/kg/min glucose infusion after CLP. NG: normal saline infusion after CLP. HI: 40 mg/kg/min glucose and 6 IU/kg/h insulin infusion after CLP. Data are expressed as the mean ± SE. *P <0.05 versus MG, NG and Sham (one way measures ANOVA and Tukey-Kramer test). †P <0.05 versus HG (two-sided Mann-Whitney U test).

Figure 3. Typical histological appearance of lung tissue.

(A) Lung tissue obtained from rats in Sham group (40 mg/kg/min glucose infusion after sham operation). (B) Lung tissue obtained from rats in HG group (40 mg/kg/min glucose infusion after cecal ligation and puncture). (C) Lung tissue obtained from rats in MG group (25 mg/kg/min glucose infusion after cecal ligation and puncture method). (D) Lung tissue obtained from rats in NG group (normal saline infusion after cecal ligation and puncture method). (E) Lung tissue obtained from rats in HI group (40 mg/kg/min glucose and 6 IU/kg/h insulin infusion after cecal ligation and puncture). Sections of lung specimens were stained with hematoxylin and eosin at ×100 magnification.

Figure 4. Histological scores of lung tissue.

Sham: 40 mg/kg/min glucose infusion after sham operation. HG: 40 mg/kg/min glucose infusion after cecal ligation and puncture (CLP). MG: 25 mg/kg/min glucose infusion after CLP. NG: normal saline infusion after CLP. HI: 40 mg/kg/min glucose and 6 IU/kg/h insulin infusion after CLP. Data are expressed as the mean ± SE. ‡P <0.05 versus HG (Kruskal-Wallis test). †P <0.05 versus HG (two-sided Mann-Whitney U test).

Figure 5. Total glutathione contents in the liver in each group.

Sham: 40 mg/kg/min glucose infusion after sham operation. HG: 40 mg/kg/min glucose infusion after cecal ligation and puncture (CLP). MG: 25 mg/kg/min glucose infusion after CLP. NG: normal saline infusion after CLP. HI: 40 mg/kg/min glucose and 6 IU/kg/h insulin infusion after CLP. Data are expressed as the mean ± SE. §P <0.05 versus HG (one way measures ANOVA and Tukey-Kramer test). †P <0.05 versus HG (two-sided Mann-Whitney's U test).

Figure 6. Malondialdehyde contents in the liver in each group.

Sham: 40 mg/kg/min glucose infusion after sham operation. HG: 40 mg/kg/min glucose infusion after cecal ligation and puncture (CLP). MG: 25 mg/kg/min glucose infusion after CLP. NG: normal saline infusion after CLP. HI: 40 mg/kg/min glucose and 6 IU/kg/h insulin infusion after CLP. Data are expressed as the mean ± SE.

Table 1

Table 1 Composition of nutrient solution in five groups

	Sham	HG	MG	NG	HI
Glucose (g/l)	264	264	171	0	264
Sodium (mEq/l)	71.4	71.4	71.4	154	71.4
Potassium (mEq/l)	42.9	42.9	42.9	0	42.9
Insulin dose (IU/kg body weight/h)	0	0	0	0	6
Energy intake (kcal/kg body weight/day)	228	228	148	0	228

Sham: 40 mg/kg/min glucose infusion after sham operation. HG: 40 mg/kg/min glucose infusion after cecal ligation and puncture method (CLP). MG: 25 mg/kg/min glucose infusion after CLP. NG: normal saline infusion after CLP. HI: 40 mg/kg/min glucose and 6 IU/kg/h insulin infusion after CLP.

Table 2

Table 2 Blood biochemical examination data

	Sham	HG	MG	NG	HI	
AST	99.5 ± 7.0	124.0 ± 9.8	129.7 ± 11.6	113.5 ± 9.3	109.7 ± 6.3	N.S.
ALT	31.6 ± 2.6	49.6 ± 4.2 ¶	48.4 ± 4.3 ¶	44.3 ± 3.8	47.0 ± 3.3	
BUN	3.0 ± 0.3	9.5 ± 1.6 ¶	7.9 ± 1.1 ¶	11.6 ± 1.1 ¶	5.5 ± 1.0 †	
CRE	0.14 ± 0.02	0.29 ± 0.06 ¶	0.20 ± 0.02	0.19 ± 0.01	0.1 ± 0.0 †	

Sham: 40 mg/kg/min glucose infusion after sham operation. HG: 40 mg/kg/min glucose infusion after cecal ligation and puncture method (CLP). MG: 25 mg/kg/min glucose infusion after CLP. NG: normal saline infusion after CLP. HI: 40 mg/kg/min glucose and 6 IU/kg/h insulin infusion after CLP. AST: aspartate amino transferase, ALT: alanine transaminase, BUN: blood urea nitrogen, CRE: creatinine. Numbers for blood biochemical measurements are the mean ± standard error. N.S.: no significance, ¶: $P < 0.05$ versus Sham (one way measures ANOVA and Tukey-Kramer test), †: $P < 0.05$ versus HG (two-sided Mann-Whitney U test).

Figure 1

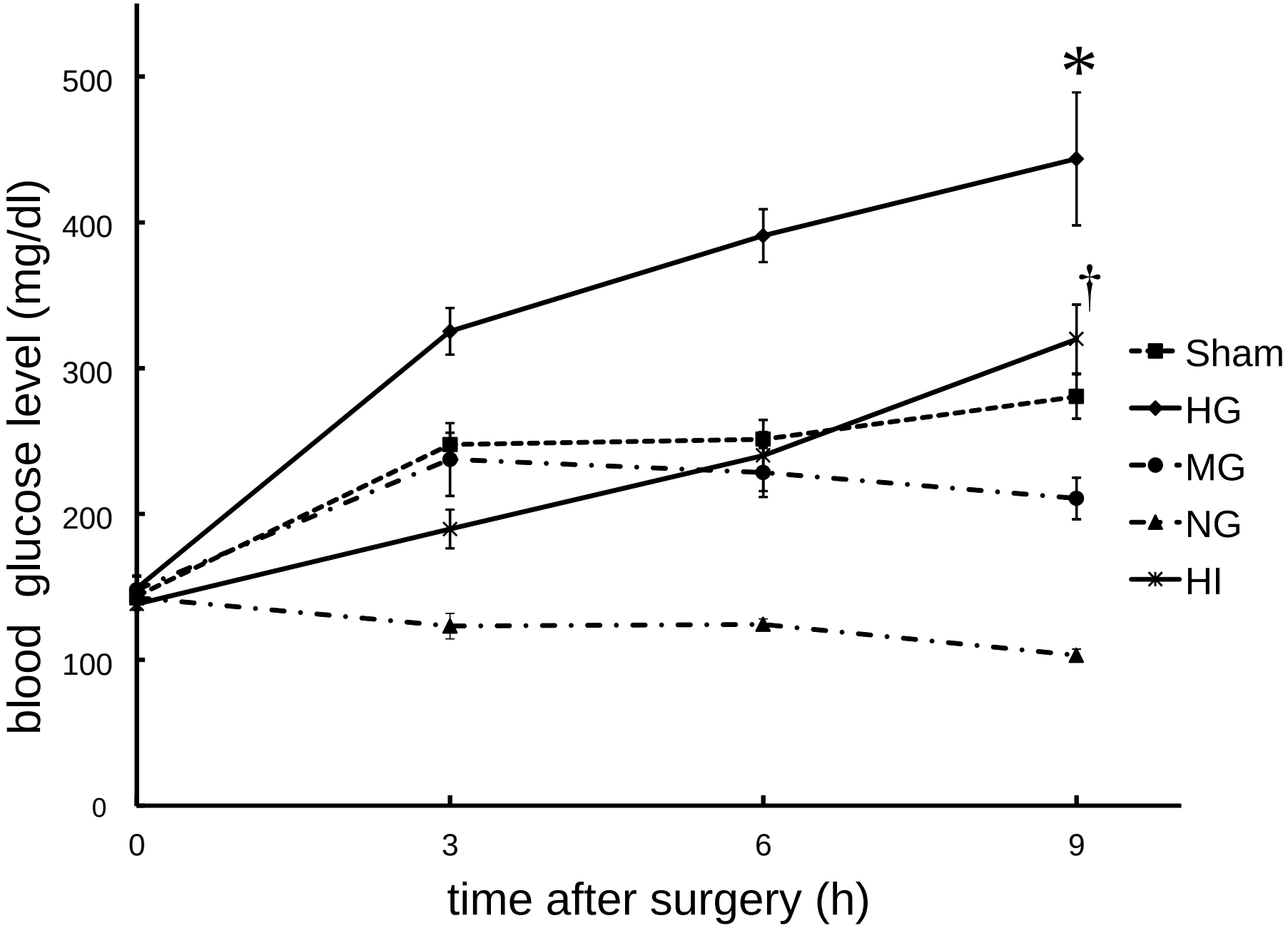

500
400
300
200
100
0
blood glucose level (mg/dl)
0
3
6
9
time after surgery (h)
*
†
Sham
HG
MG
NG
HI

Figure 2

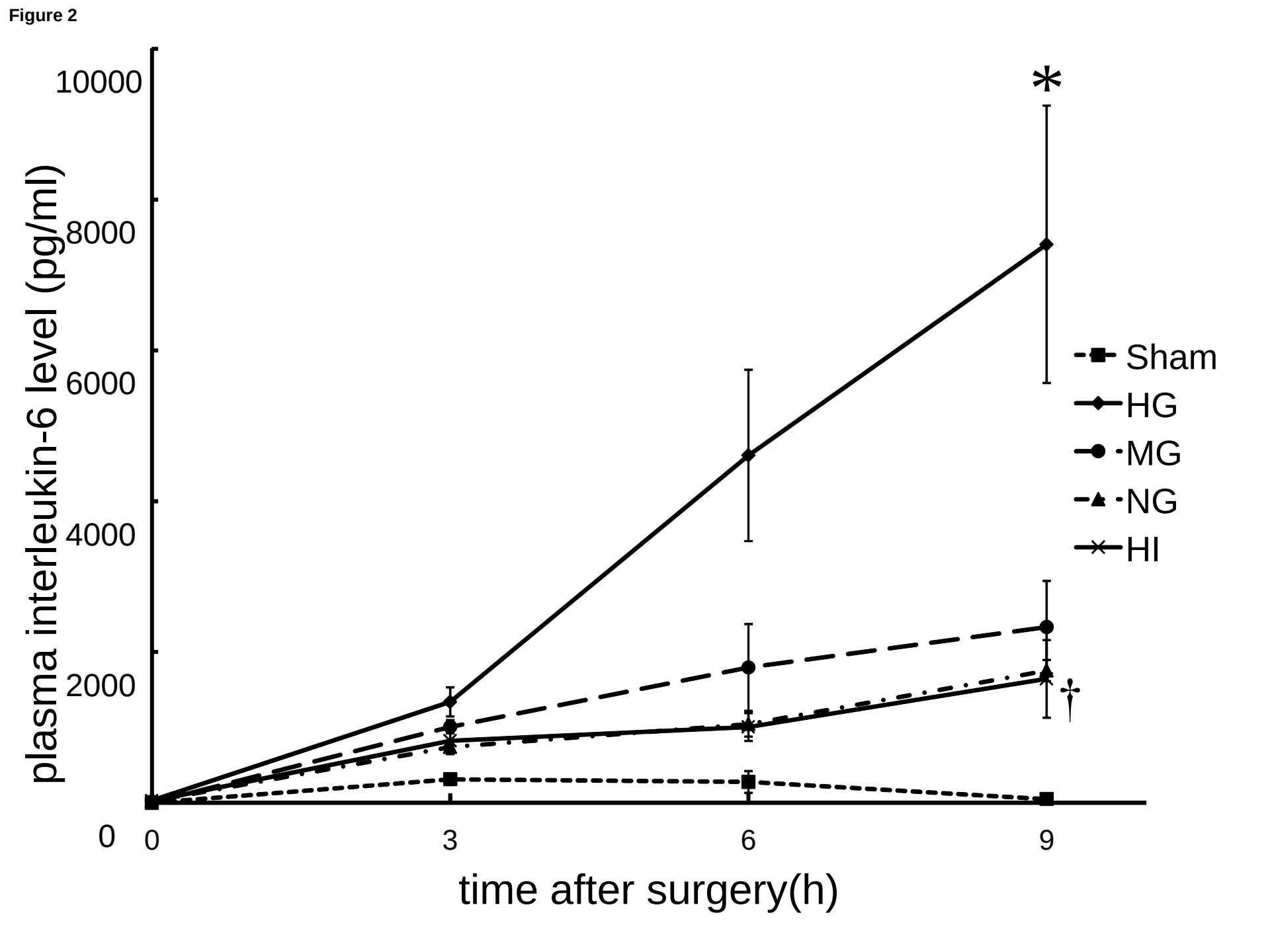

10000
8000
6000
4000
2000
0
plasma interleukin-6 level (pg/ml)
0
3
6
9
time after surgery(h)
*
†
Sham
HG
MG
NG
HI

Figure 3

Click here to download high resolution image

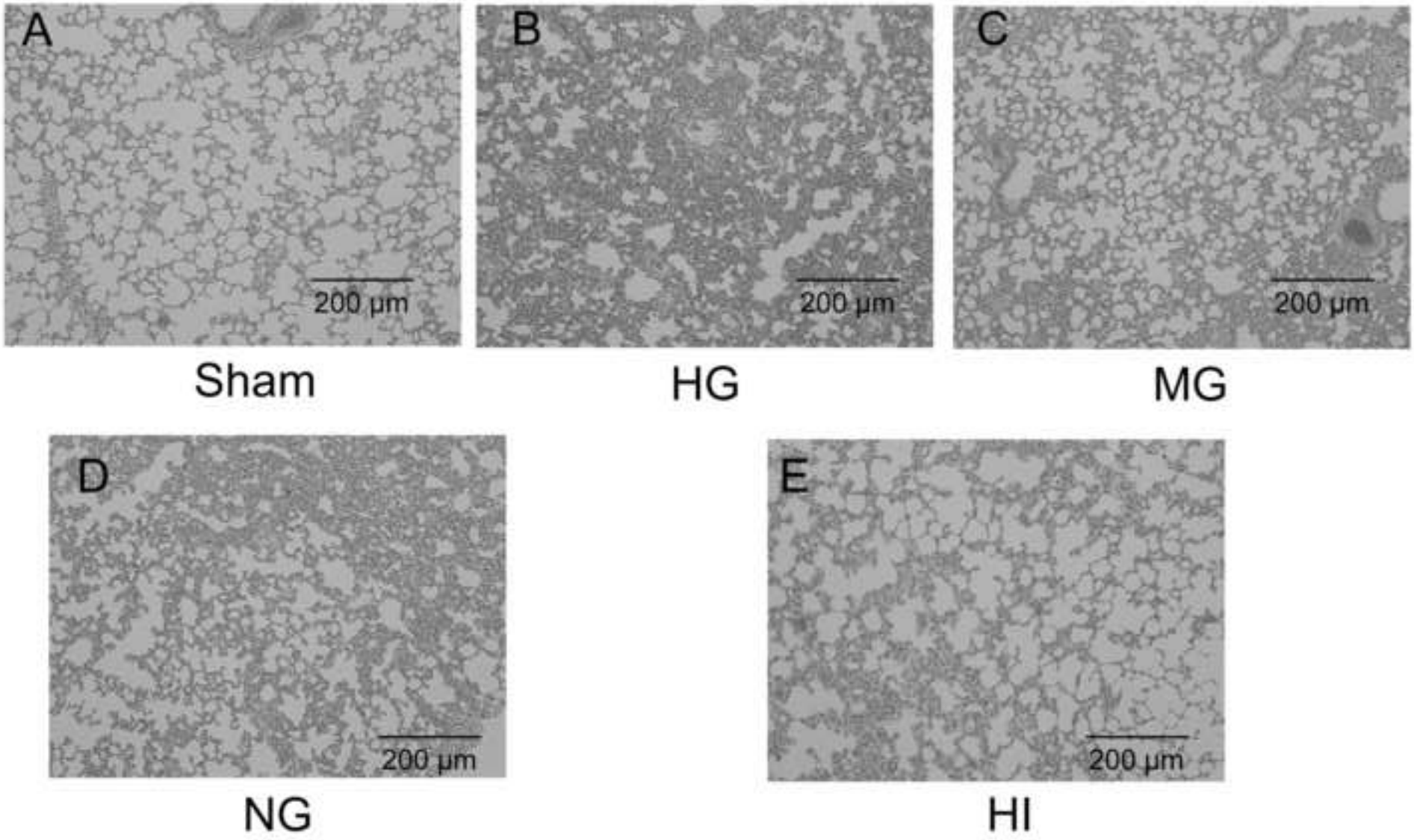


Figure 4

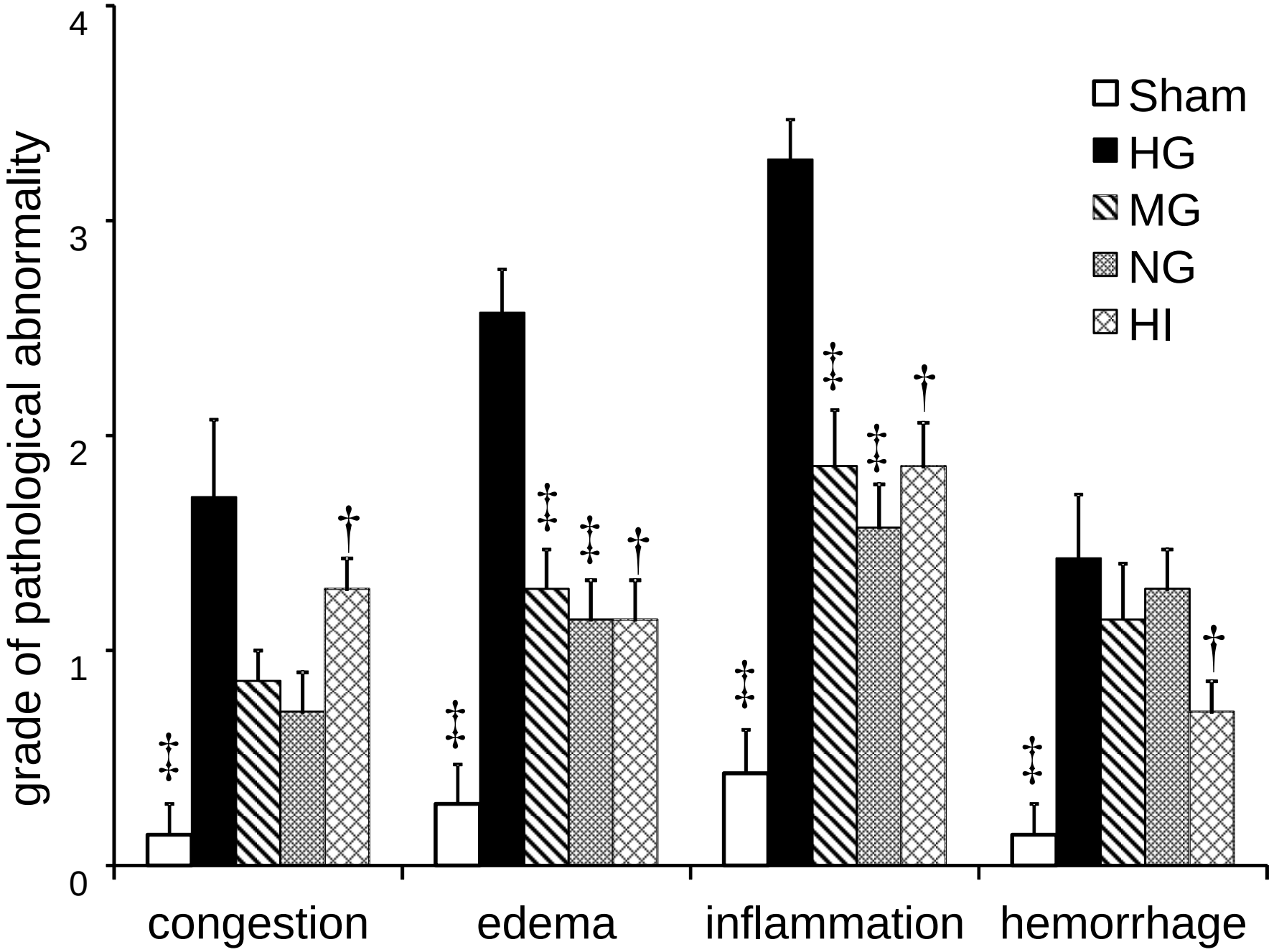

grade of pathological abnormality
4
3
2
1
0
Sham
HG
MG
NG
HI
congestion
edema
inflammation
hemorrhage

Figure 5

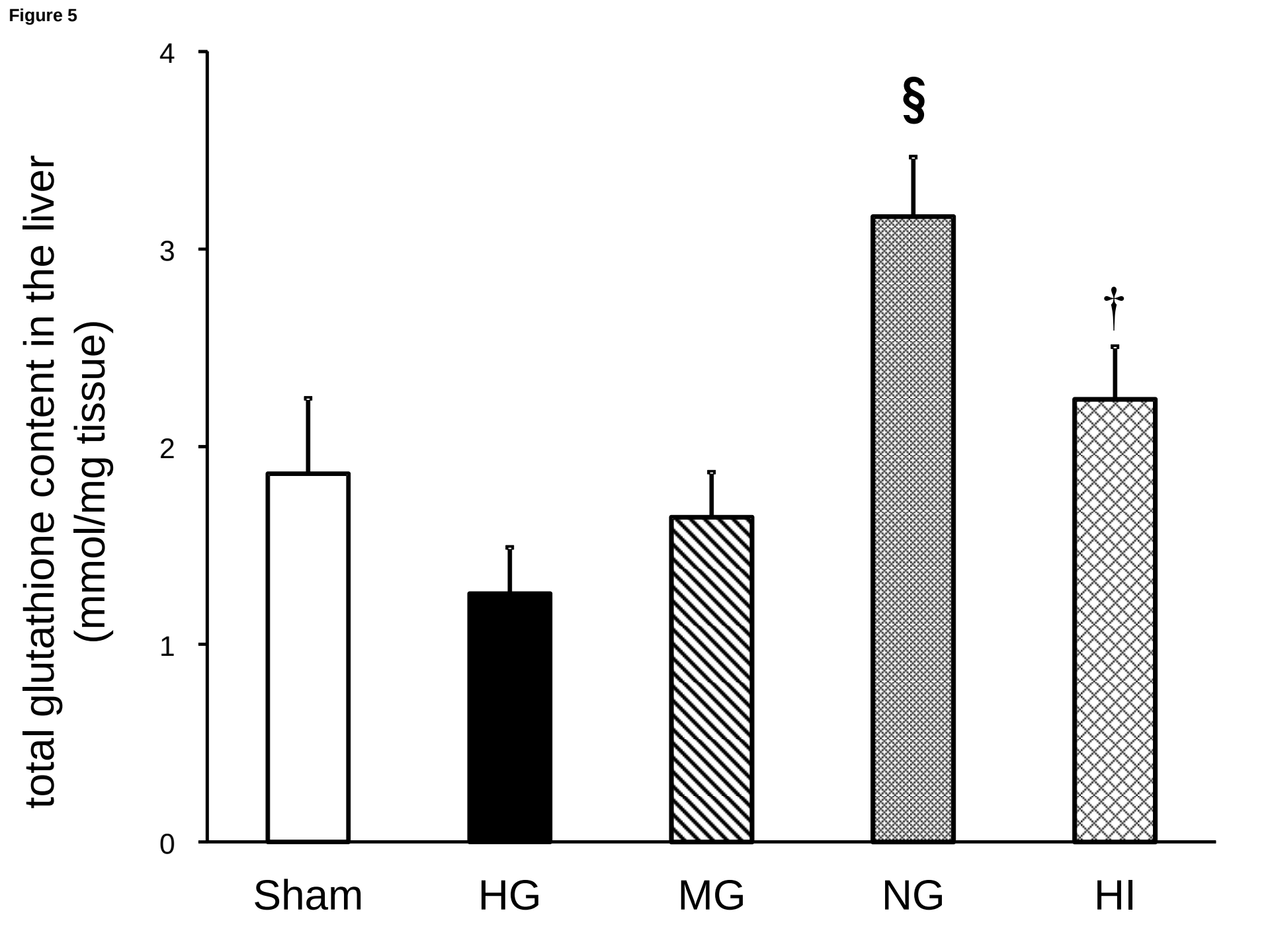
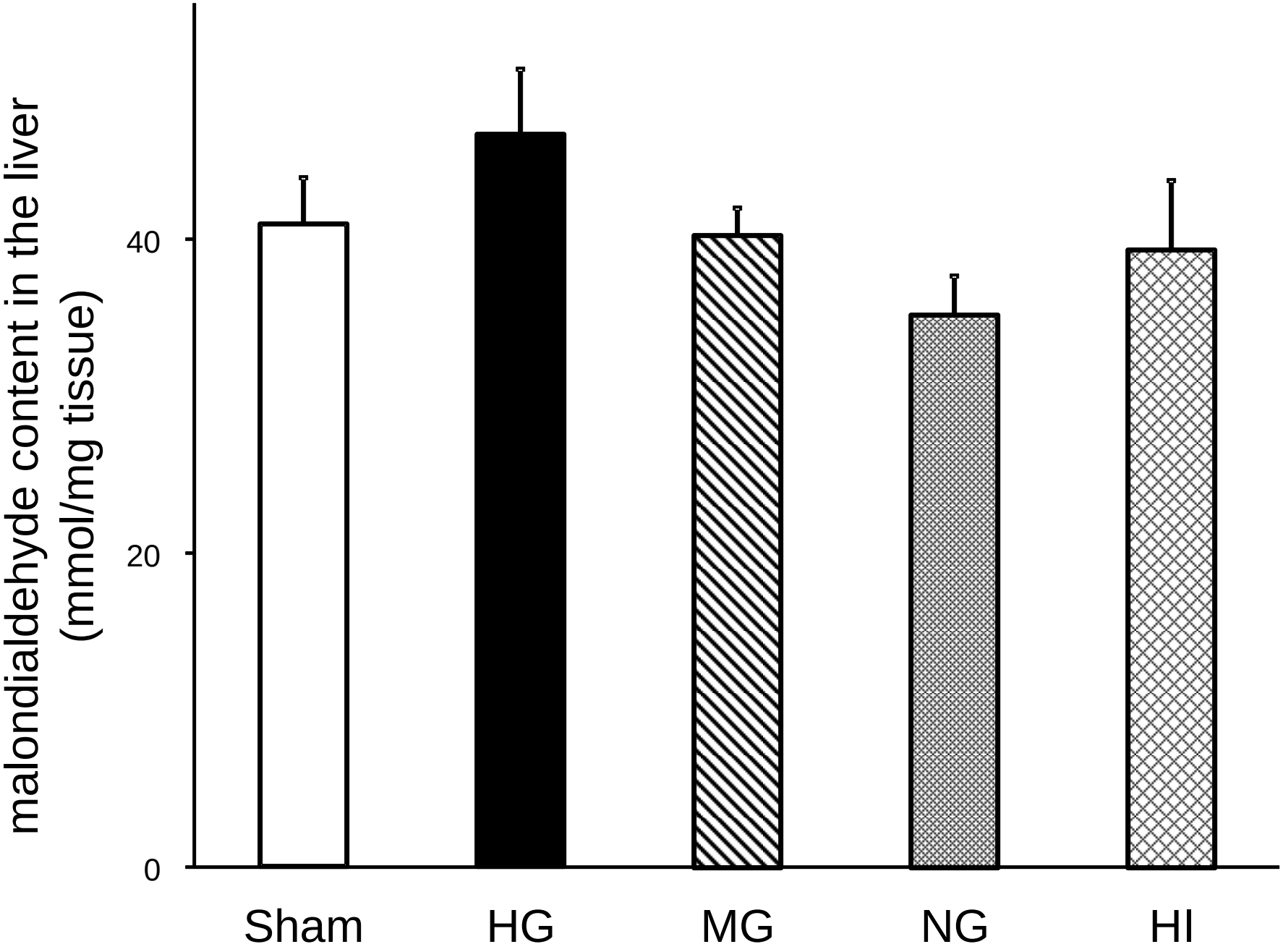

total glutathione content in the liver
(mmol/mg tissue)
4
3
2
1
0
§
†
Sham
HG
MG
NG
HI

Figure 6


malondialdehyde content in the liver
(mmol/mg tissue)
40
20
0
Sham
HG
MG
NG
HI