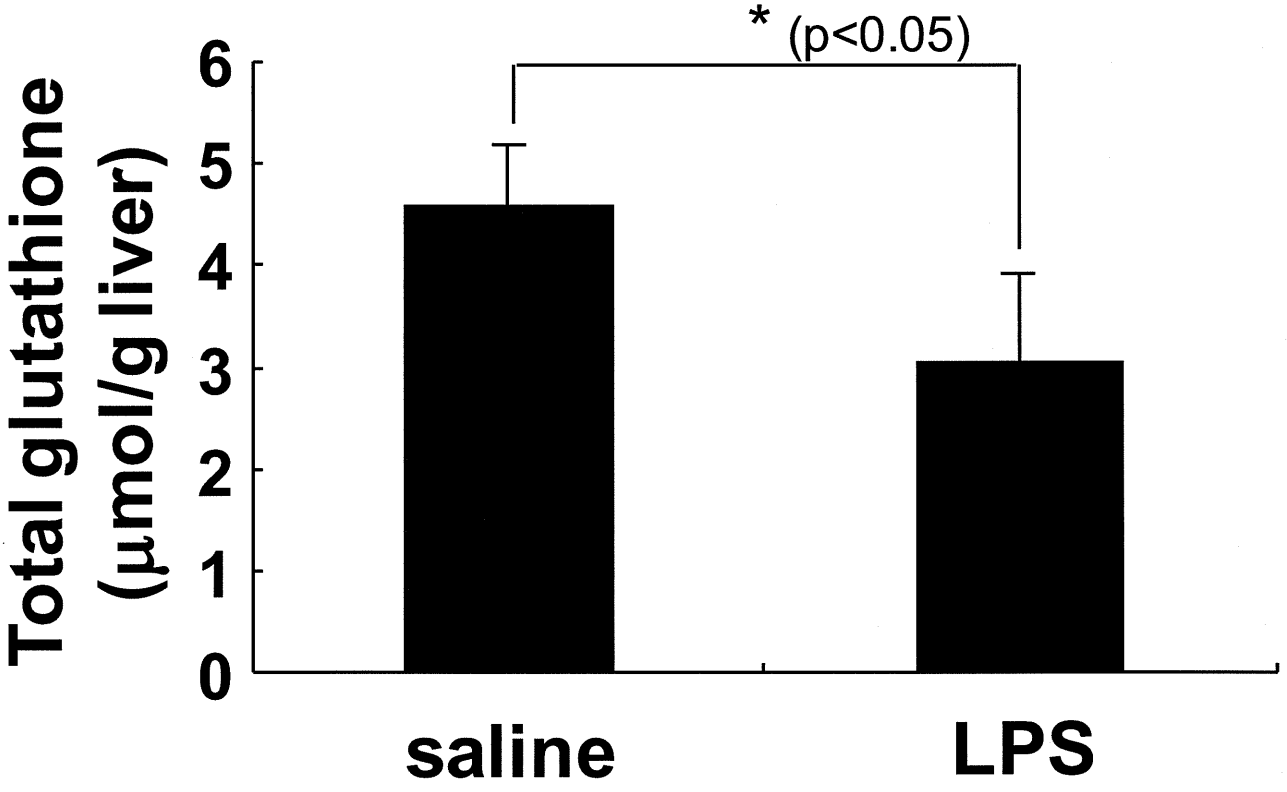
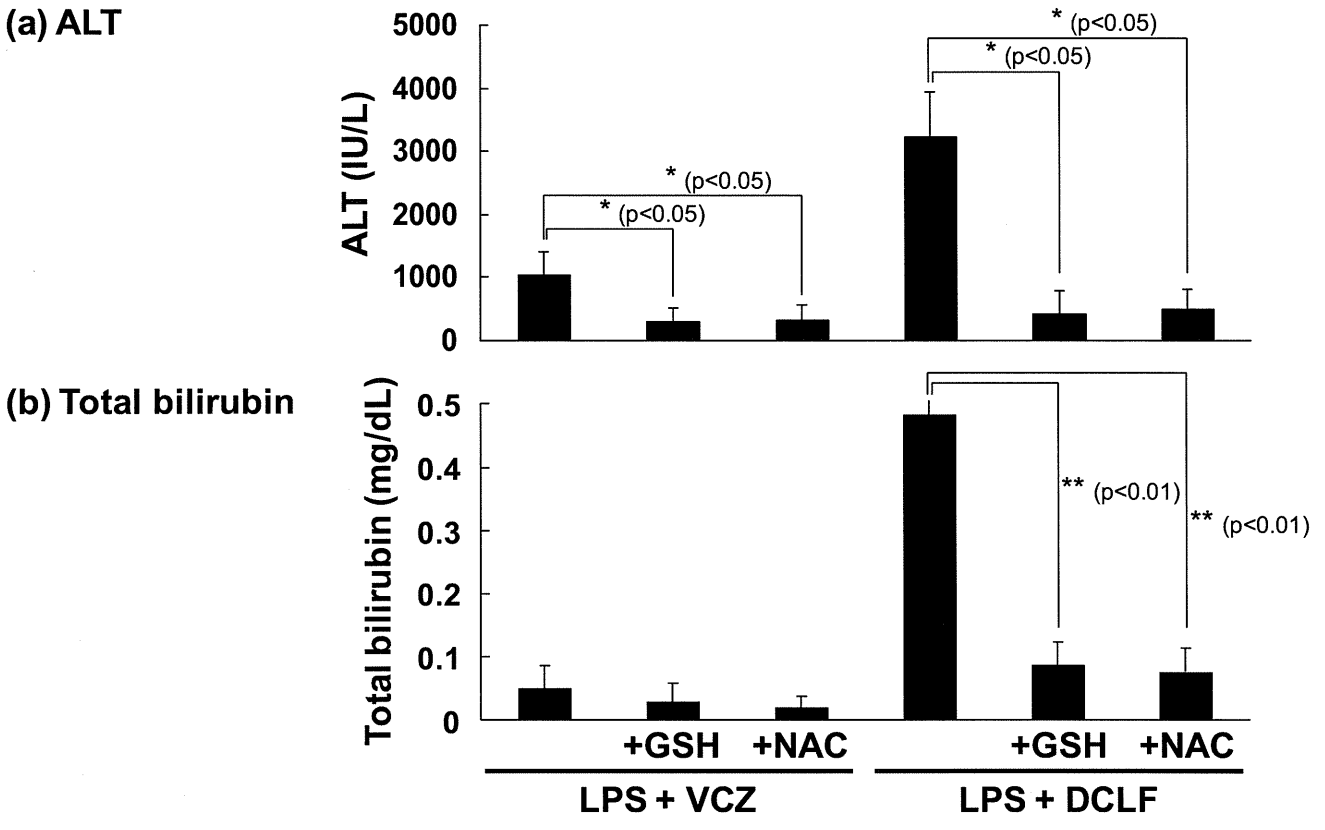
図 4　　LPS 処理による肝臓中グルタチオンの低下



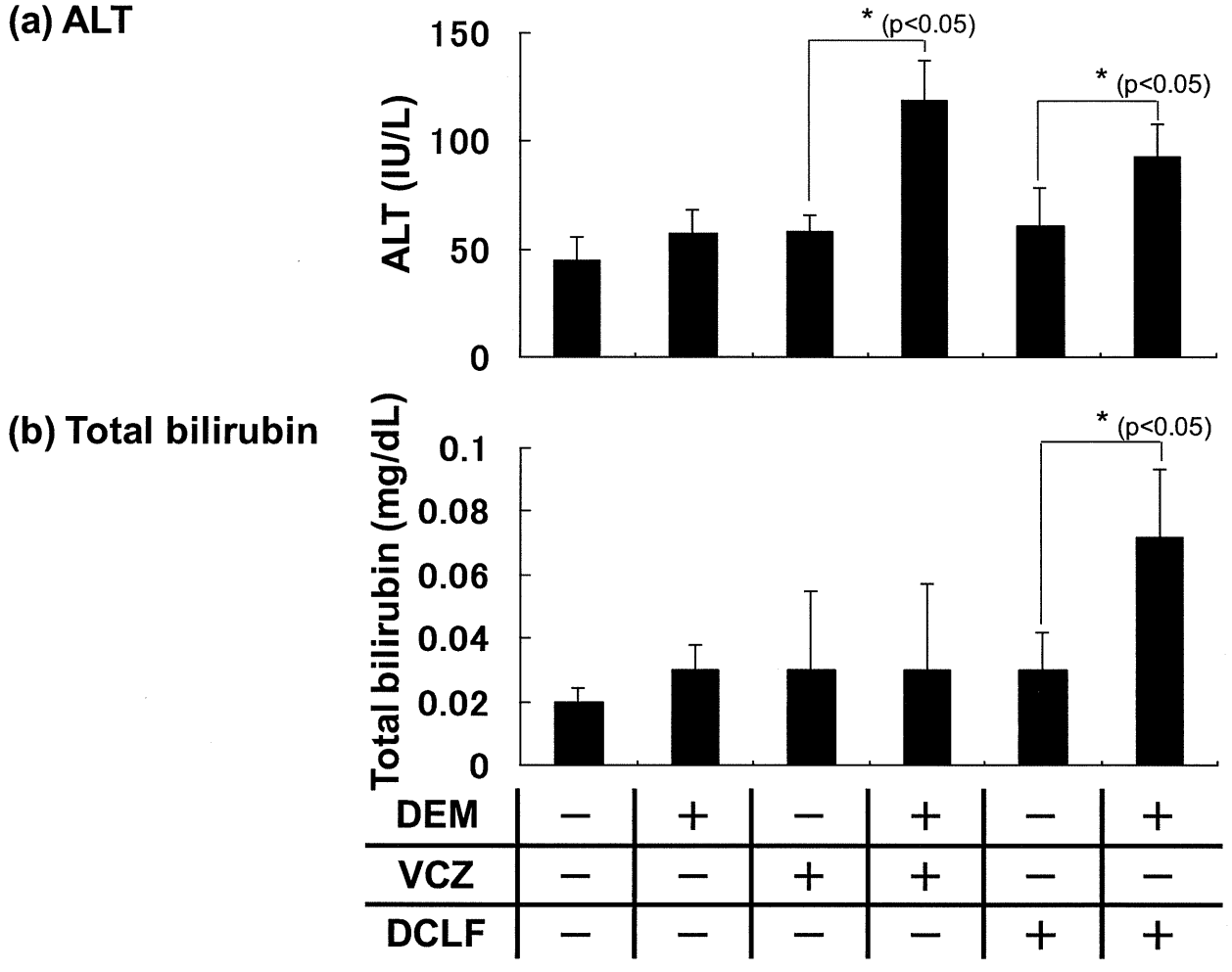
LPS(1mg/kg, i.p.)投与2時間後に肝臓を回収・調製し、Dithiobisnitrobenzoic acid (DTNB)を用いた酵素サイクル法によりグルタチオン濃度を測定。

図 5　GSH/NAC投与による肝障害の抑制



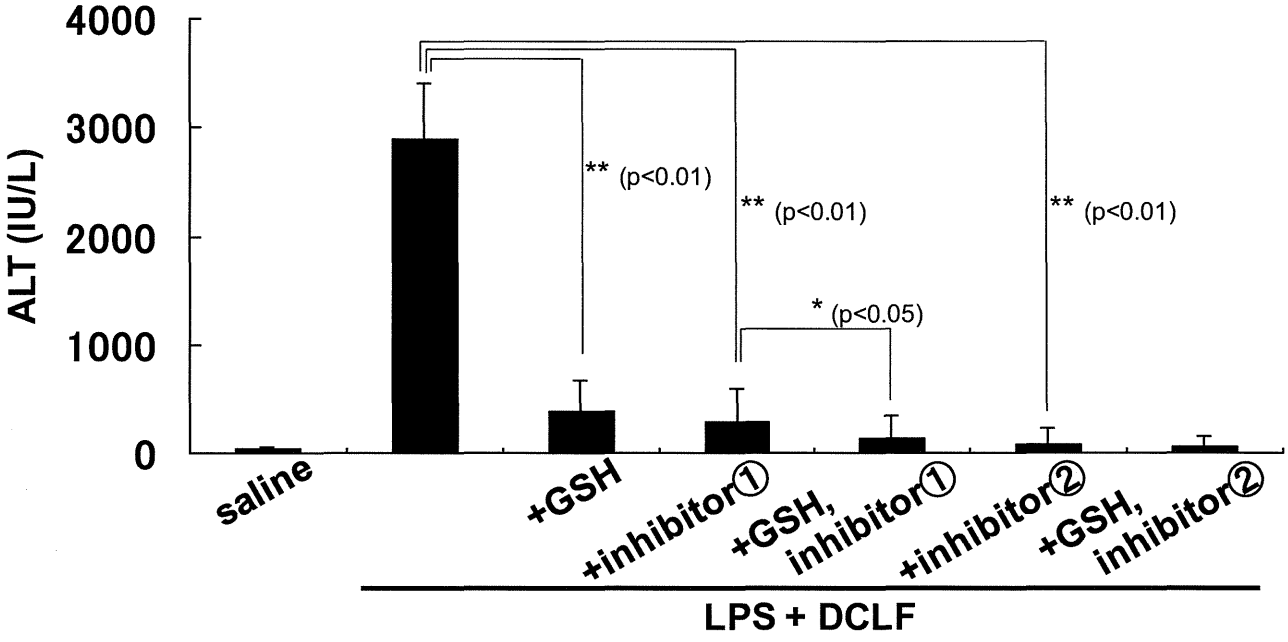
LPS投与1時間前より、GSH(100mg/kg, s.c.)もしくはNAC(100mg/kg, s.c.)を投与し、薬物投与12時間後に血清を回収し、(a)ALT、(b)総ビリルビンを測定。

図 6　　グルタチオン枯渇による肝障害の誘発



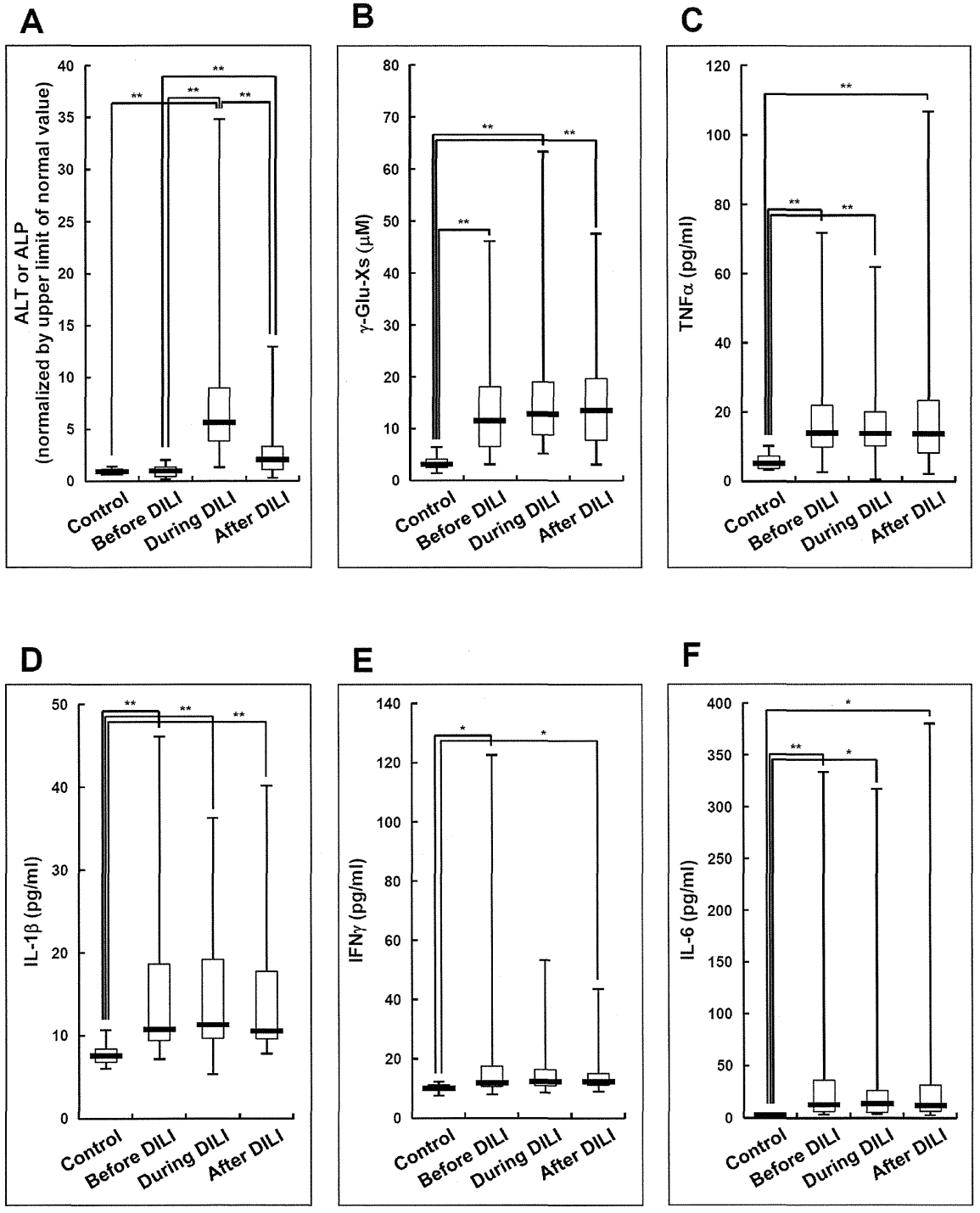
DEM(3mmol/kg, i.p.)投与2時間後にVCZ(30mg/kg, i.v.)もしくはDCLF(100mg/kg, i.v.)を投与。
薬物投与12時間後に血清を回収し、(a)ALT、(b)総ビリルビンを測定。

図７　　サイトカイン阻害による肝障害の抑制



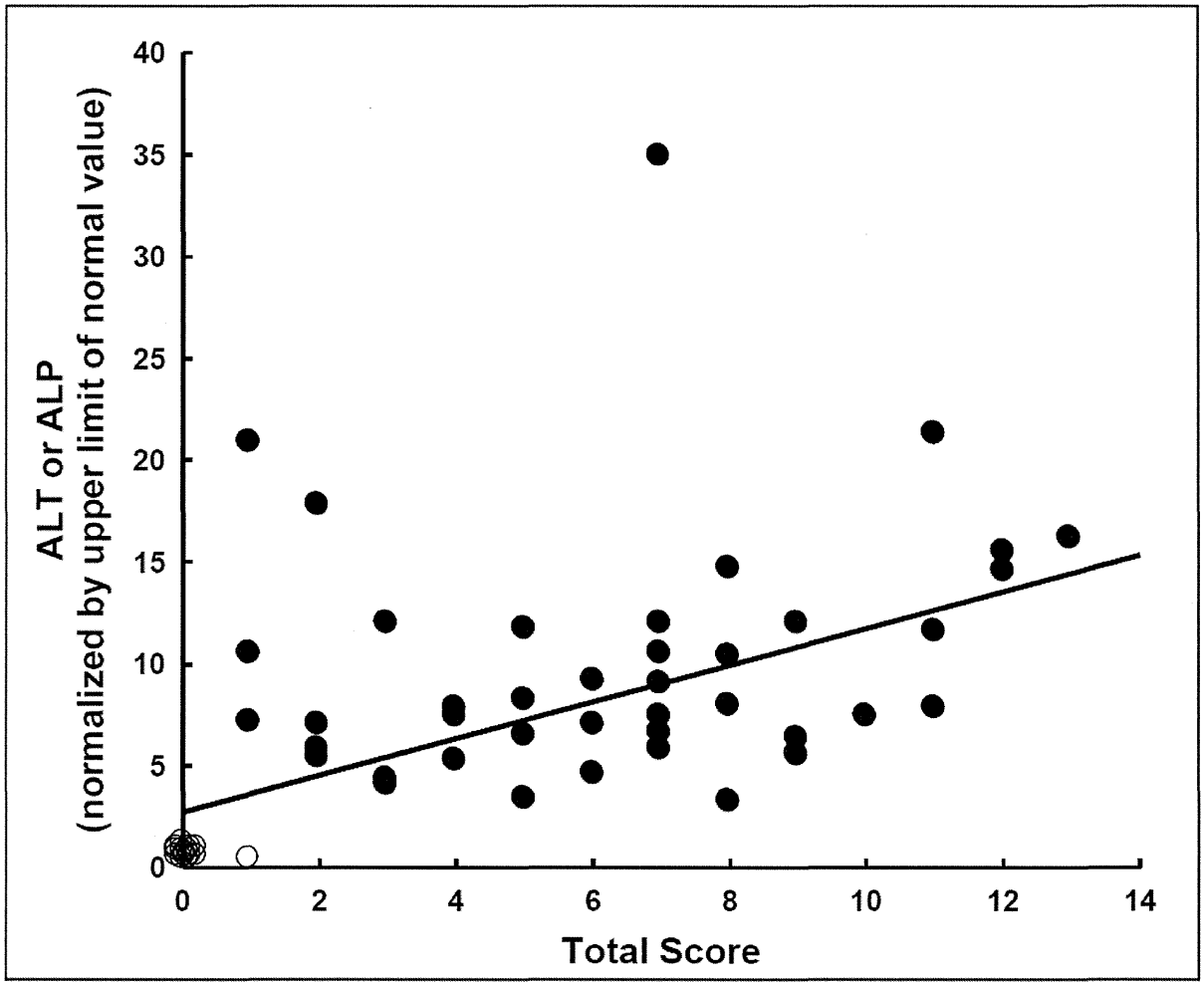
LPS投与1時間前より、GSH(100mg/kg, s.c.)もしくはサイトカインに対する阻害剤(inhibitor ①,②)を投与し、薬物投与12時間後に血清中ALTを測定。

図8



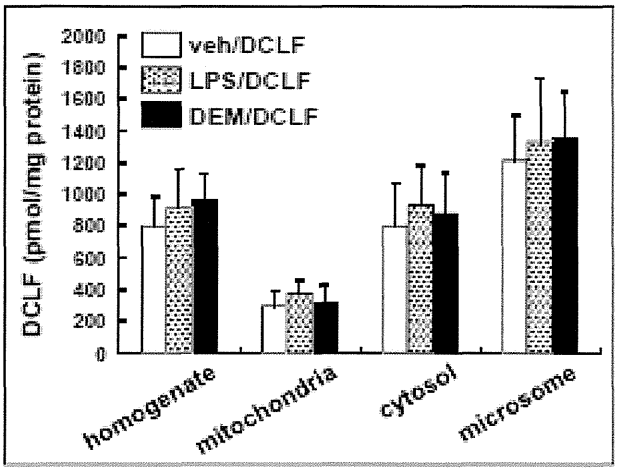
Serum concentrations of substances were determined in patients before the onset of DILI (Before DILI), during DILI (During DILI) and after the recovery of DILI (After DILI). Panel A shows the increase in the concentration of serum liver injury markers normalized by the upper limit of normal values. If ALT ≧ ALP or ALT < ALP, normalized values of ALT or ALP was selected, respectively. For control subjects, n=15 from 15 patients, whereas for Before DILI, During DILI and After DILI patients, n=281, n=278, and n=446, respectively, from 63 patients. Panel B shows the serum g-Glu-Xs concentrations. For control subjects, n=15 from 15 patients, whereas for Before DILI, During DILI and After DILI patients, n=116, n=140, and n=215, respectively, from 63 patients. Panel C shows the serum concentrations of TNFa. For control subjects, n=15 from 15 patients, whereas Before DILI, During DILI and After DILI patients, n=93, n=114, and n=81, respectively, from 63 patients. Panels D, E and F show the serum concentrations of IL-1b, IFNg and IL-6, respectively. In Panels D, E and F, the n for control subjects were 15 from 15 patients, whereas those for Before DILI, During DILI and After DILI patients were 90, 95 and 75, respectively, 63 patients). Each bar and box represents the median and the range from lower to upper median. $*p < 0.05$ and $**p < 0.01$, significantly different from the respective control groups.

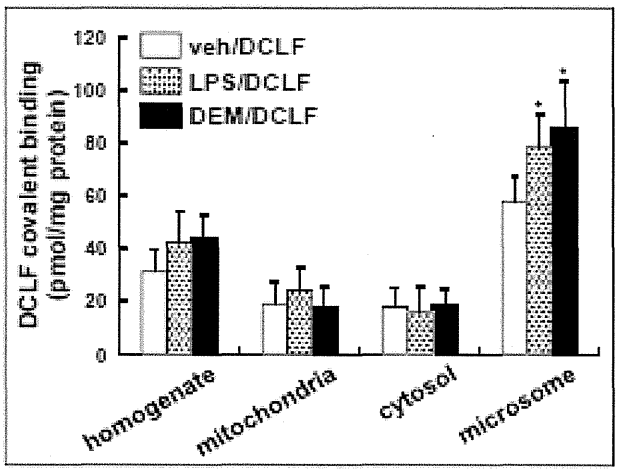
図9



Scoring analysis for DILI. Serum concentrations of g-Glu-Xs, TNFa, IL-1b, IFNg, IL-6 and CRP were normalized by that of control and scored. In each patient, the highest scores of each marker, which are observed within 10 days before the peak of liver injury, were summed and these values were plotted with the peak amount of normalized ALT/ALP. The open and closed circles represent the results from control (n = 15) and DILI (n =42) patients, respectively.

図10

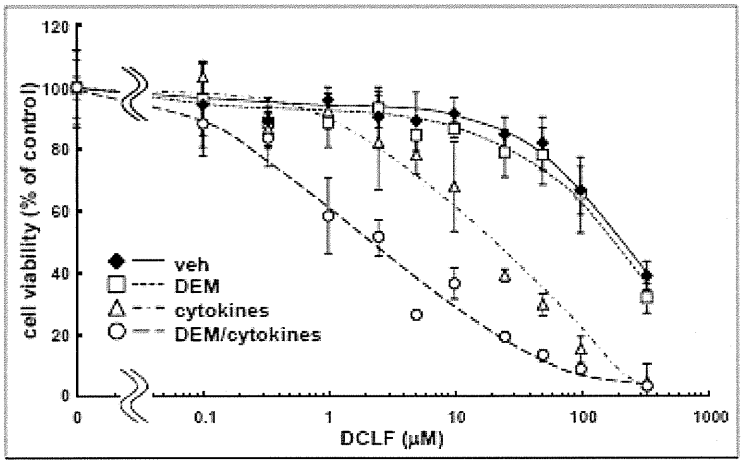




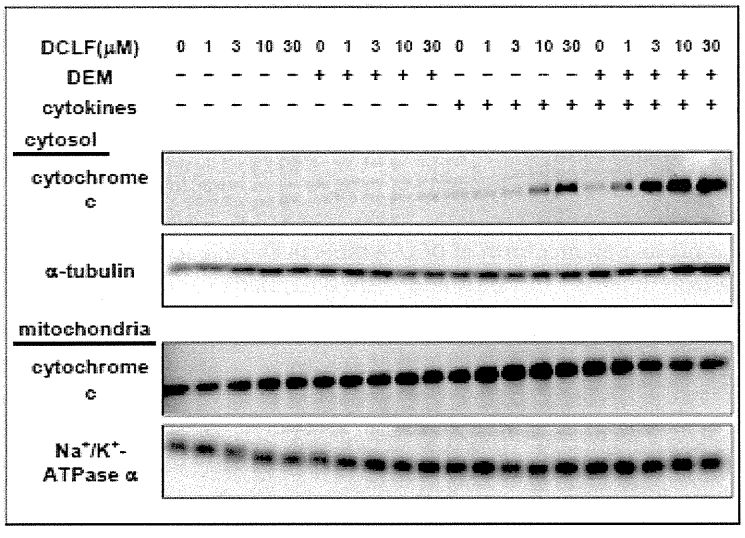
The total amount of DCLF and protein-adduct form of DCLF, respectively, in each fraction. Results are given as the mean ± SD of 3 independent experiments. *$p < 0.05$ and **$p < 0.01$, significantly different from the respective control group.

図11

A



B



Rat hepatocytes were cultured in the medium containing DEM, cytokines and DCLF at indicated concentrations for 24 h. (A) Cell viability was normalized by that in the absence of DCLF in each treatment. Closed diamond and open square, triangle and circle represent the results obtained in the presence of vehicle (veh), DEM, cytokines and both DEM and cytokines, respectively. Each approximation curve was drawn according to the least-squares analysis. Results are given as the mean ± SD of 6 independent determinations. (B) Cytochrome c release from mitochondria to cytosol was determined by Western blotting. After 24 h incubation, fractions of mitochondria and cytosol were prepared from hepatocytes to determine the amount of cytochrome c. As a control protein in each fraction, Na^+/K^+-ATPase a and a-tubulin were confirmed in mitochondria and cytosol fraction, respectively. Results are given as the mean ± SD of 4 independent determinations. $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$, significantly different from the control group.

図 12

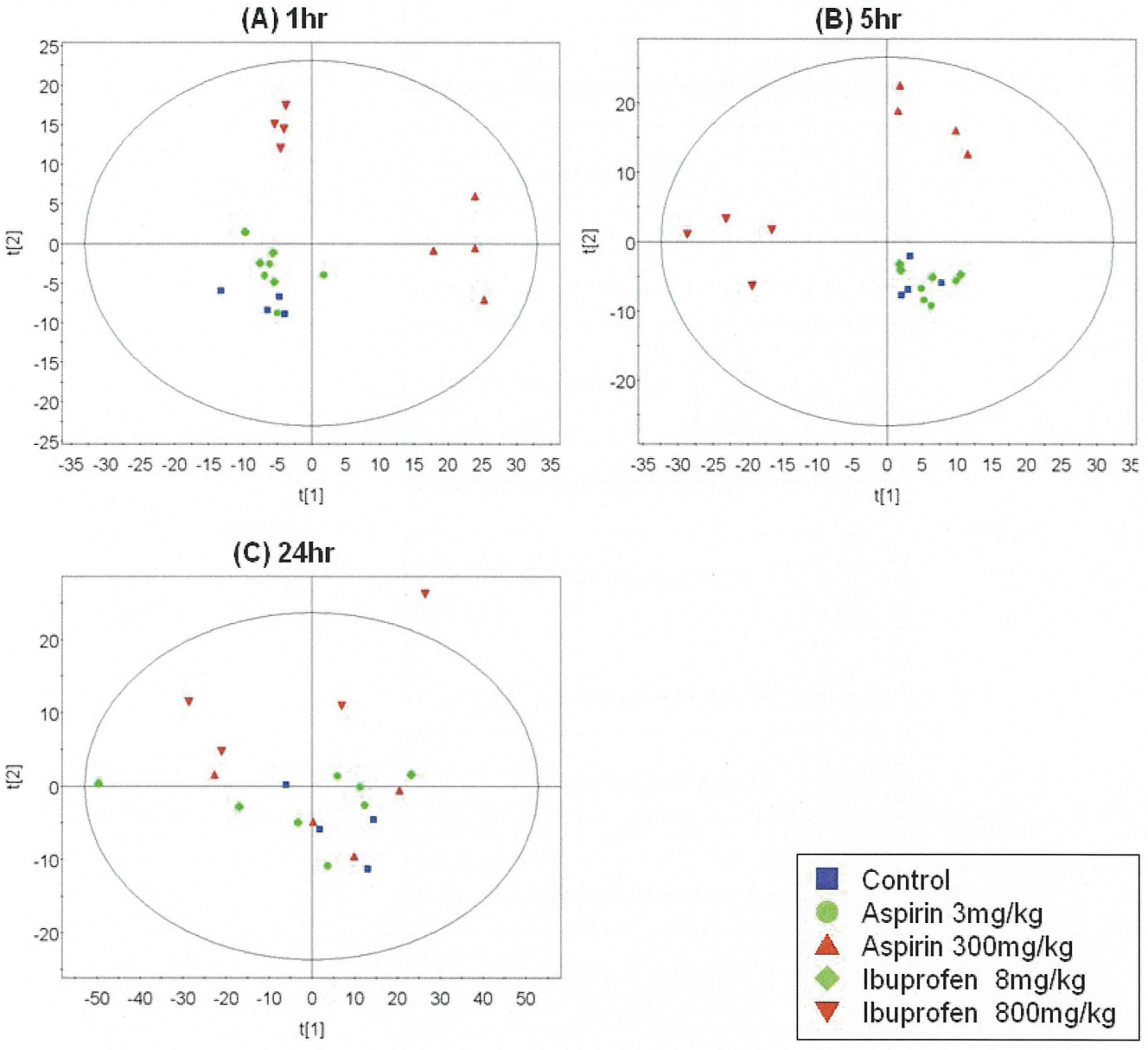
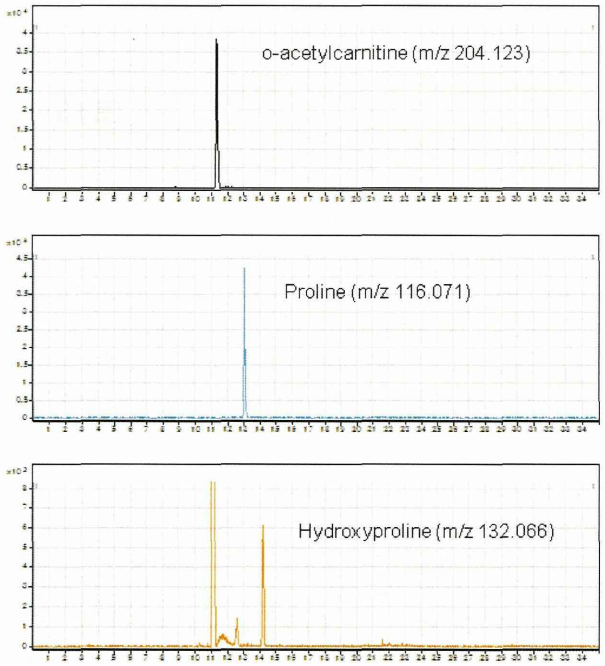
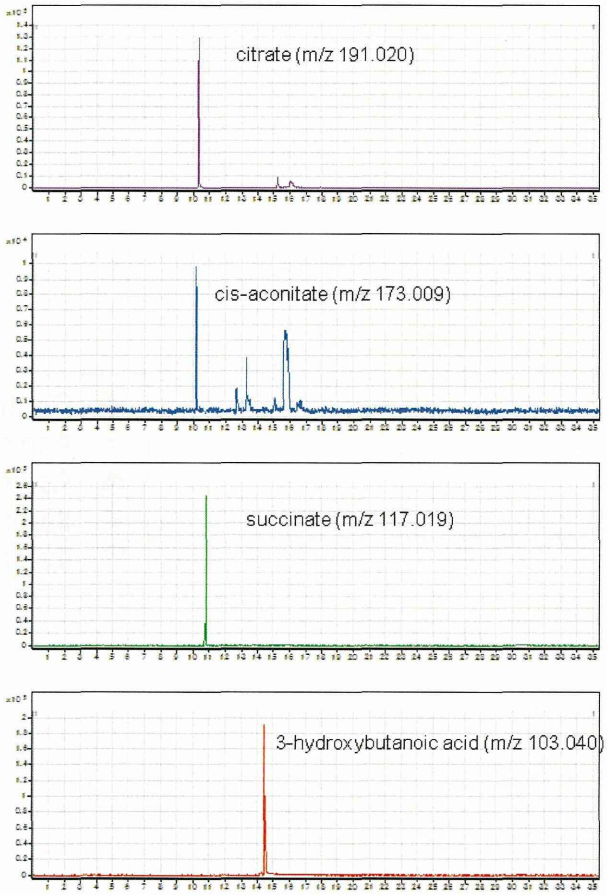

(A) 1hr
(B) 5hr
(C) 24hr
t[1]
t[2]
Control
Aspirin 3mg/kg
Aspirin 300mg/kg
Ibuprofen 8mg/kg
Ibuprofen 800mg/kg

図 13

(a)



(b)



Time (min)

図 14

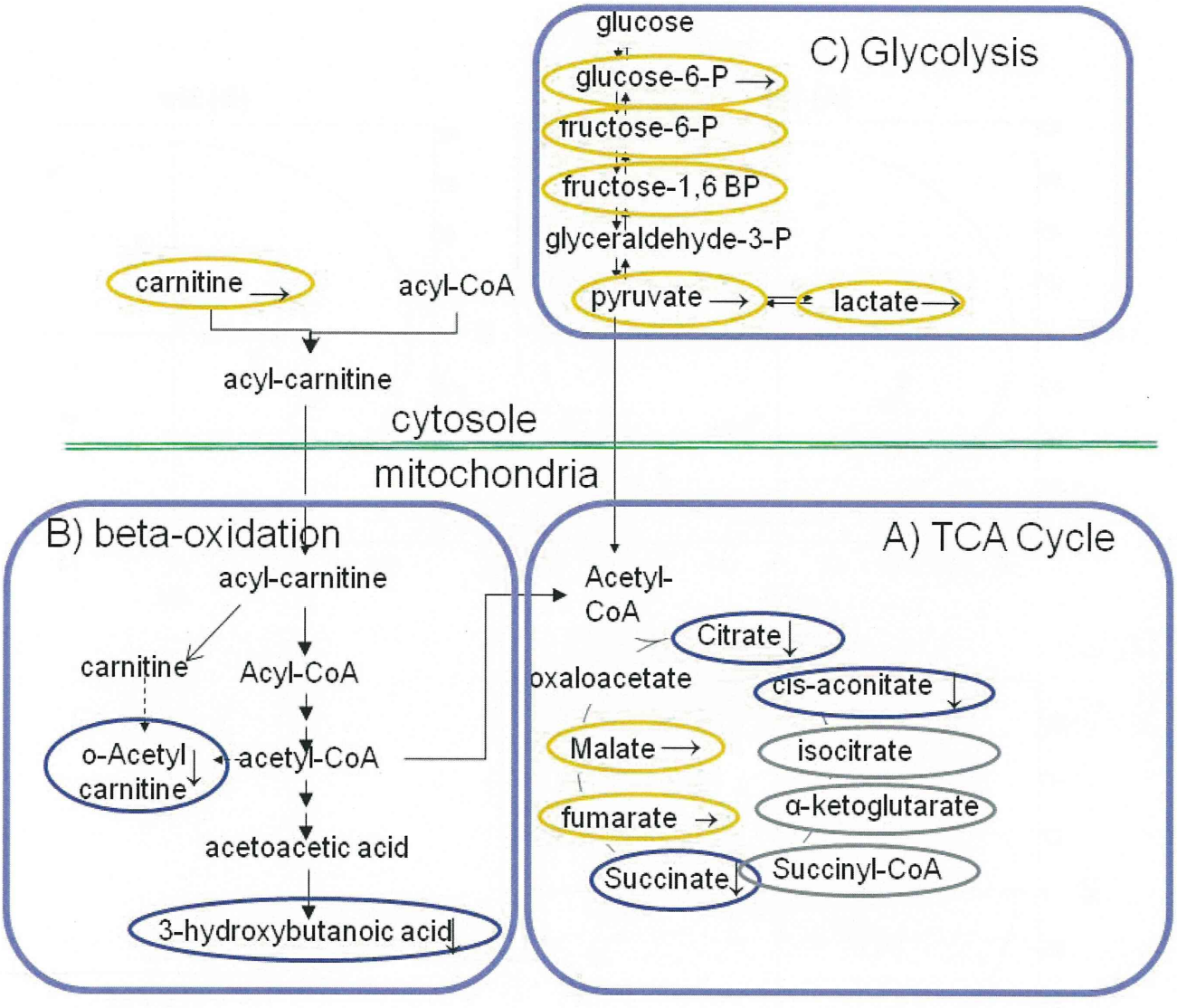

glucose
C) Glycolysis
glucose-6-P
fructose-6-P
fructose-1,6 BP
glyceraldehyde-3-P
pyruvate
lactate
carnitine
acyl-CoA
acyl-carnitine
cytosole
mitochondria
B) beta-oxidation
acyl-carnitine
carnitine
Acyl-CoA
o-Acetyl carnitine
acetyl-CoA
acetoacetic acid
3-hydroxybutanoic acid
A) TCA Cycle
Acetyl-CoA
Citrate
oxaloacetate
cis-aconitate
Malate
isocitrate
fumarate
α-ketoglutarate
Succinate
Succinyl-CoA

図 15

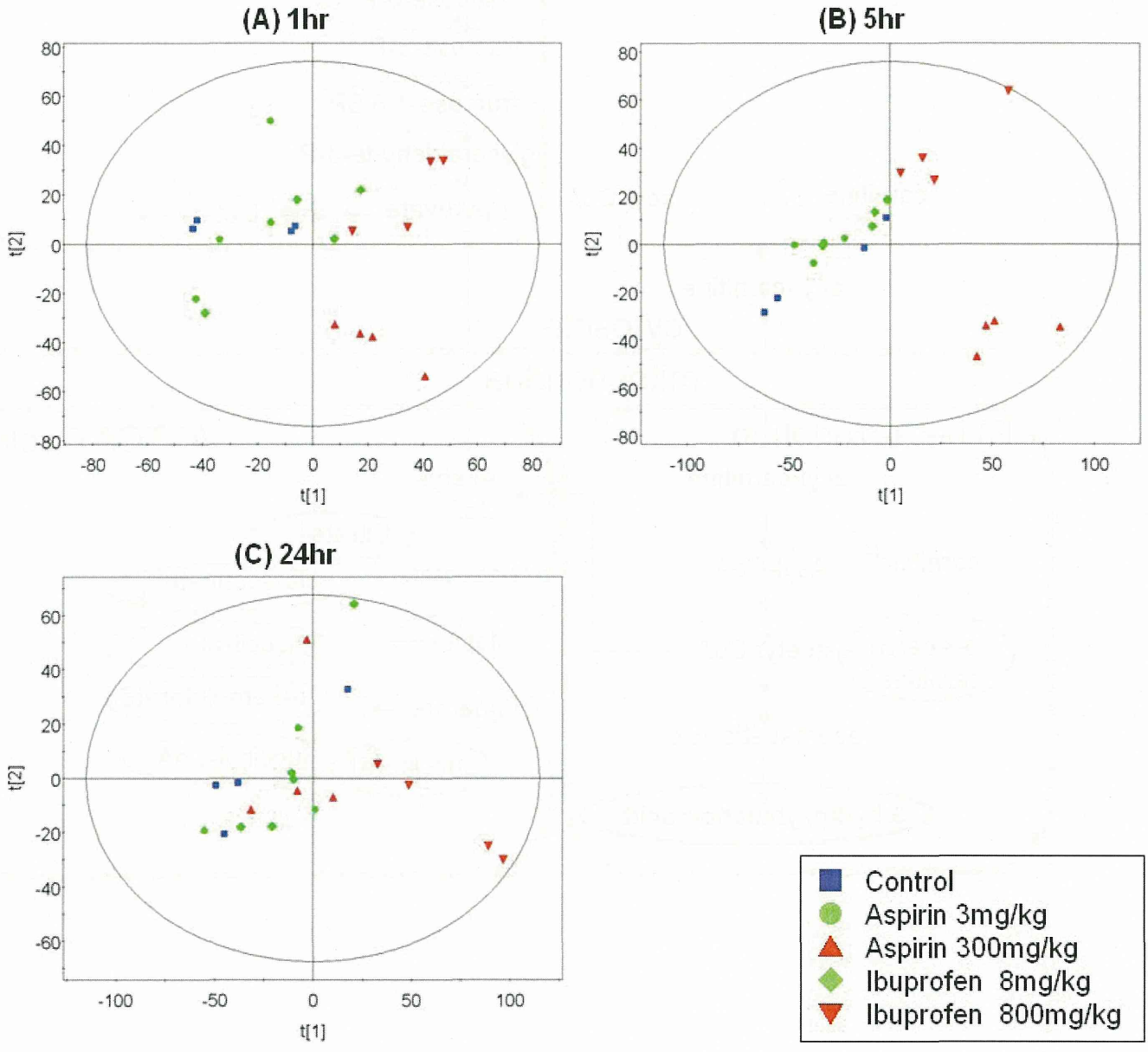

(A) 1hr
(B) 5hr
(C) 24hr
t[1]
t[2]
Control
Aspirin 3mg/kg
Aspirin 300mg/kg
Ibuprofen 8mg/kg
Ibuprofen 800mg/kg

図 16

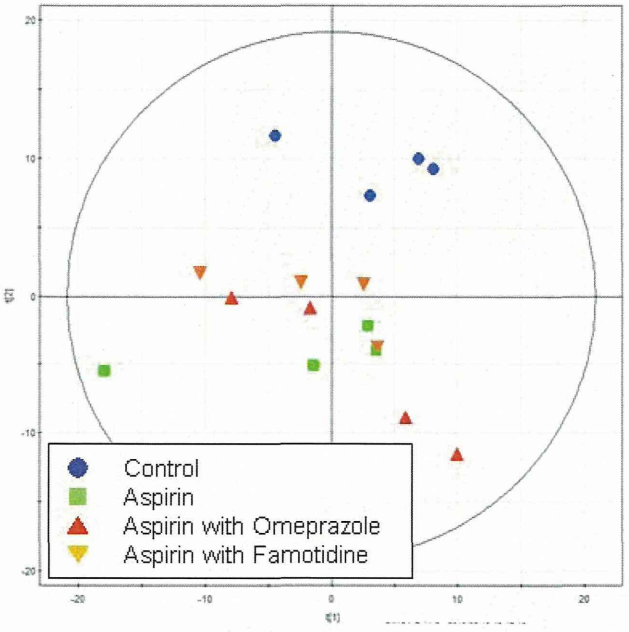
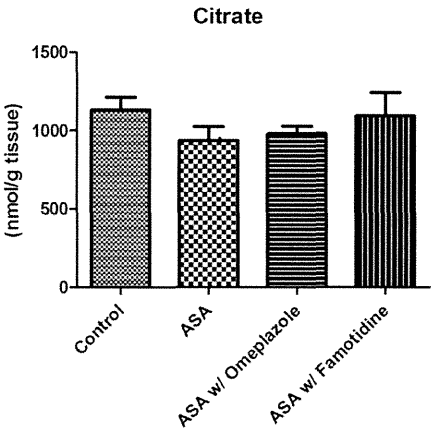
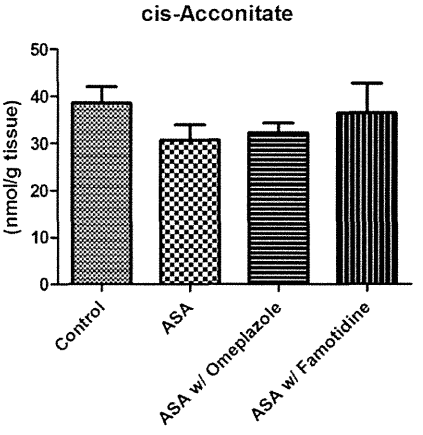

Control
Aspirin
Aspirin with Omeprazole
Aspirin with Famotidine

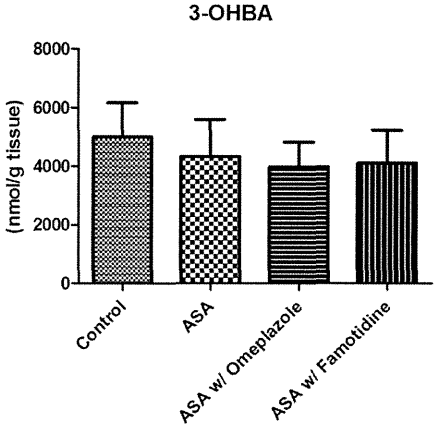
図 17

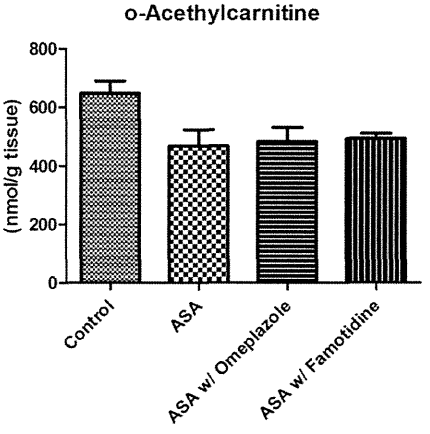
TCA Cycle



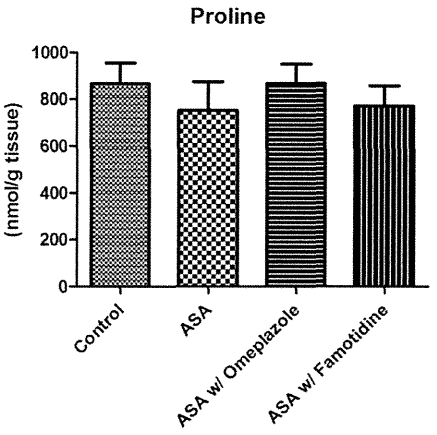


beta-Oxidization





Collagen metabolism



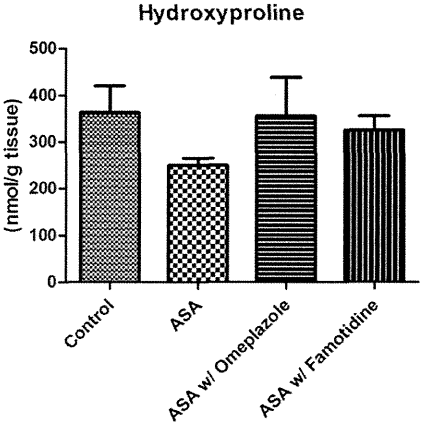


図 18

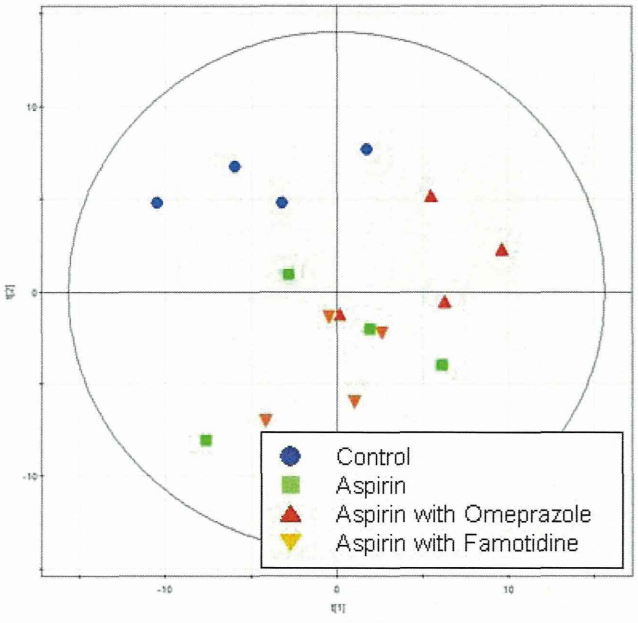
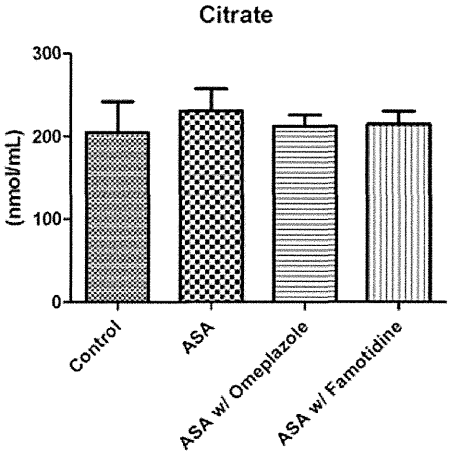
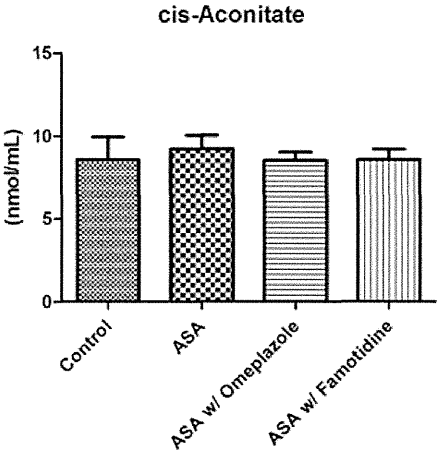

Control
Aspirin
Aspirin with Omeprazole
Aspirin with Famotidine

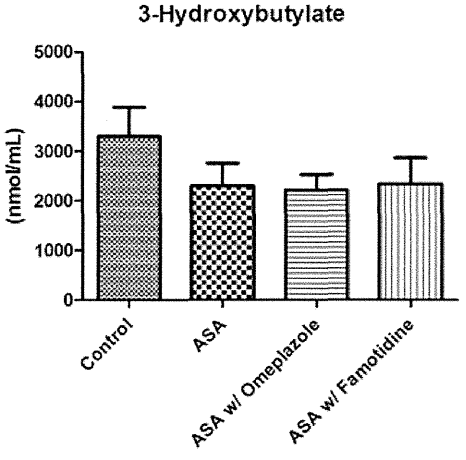
図 19

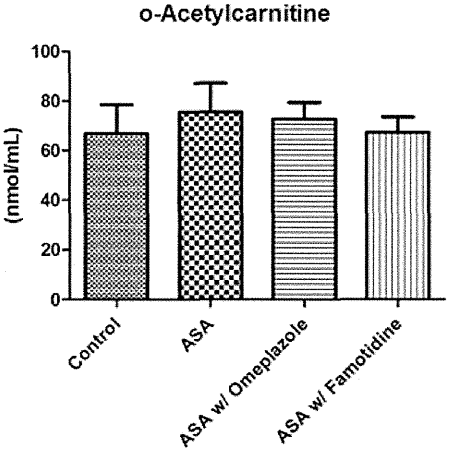
TCA Cycle



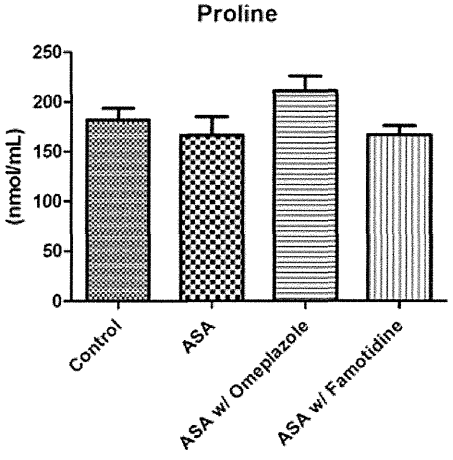


beta-Oxidization





Collagen metabolism



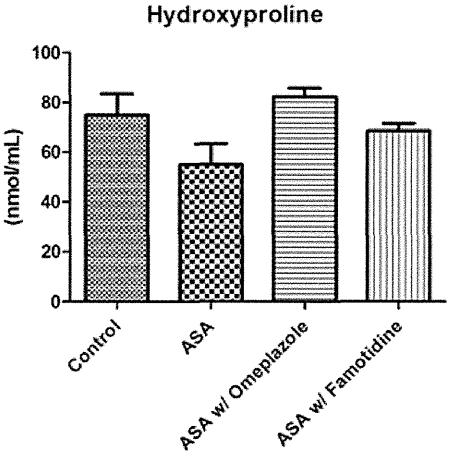


図 20

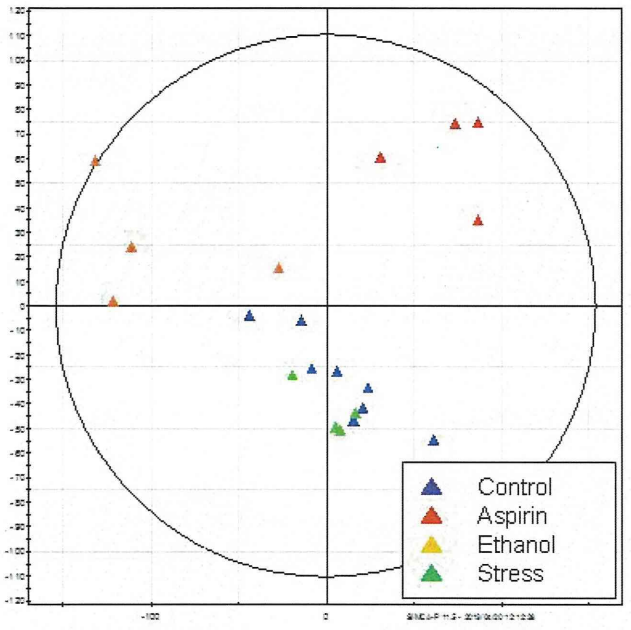


図 21

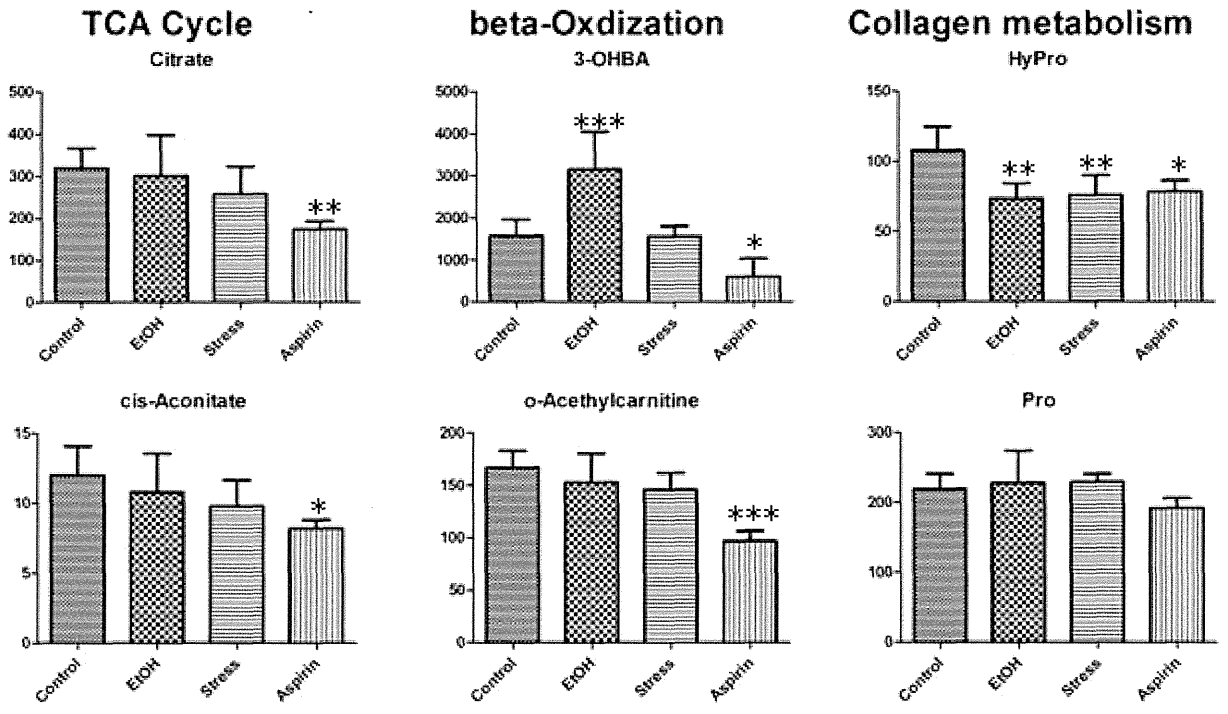
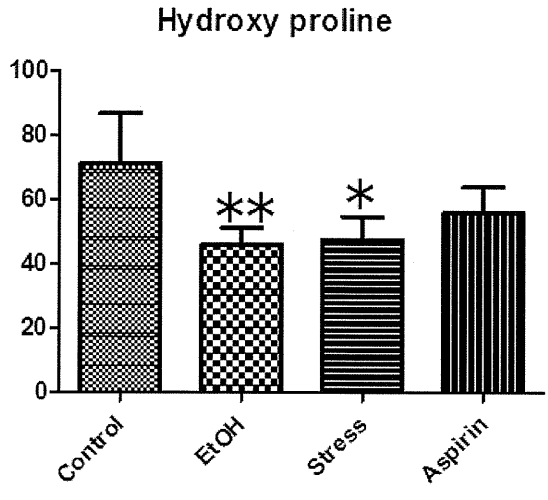

TCA Cycle
Citrate
500
400
300
200
100
0
**
Control
EtOH
Stress
Aspirin
beta-Oxdization
3-OHBA
5000
4000
3000
2000
1000
0
***
*
Collagen metabolism
HyPro
150
100
50
0
**
**
*
cis-Aconitate
15
10
5
0
*
o-Acethylcarnitine
200
150
100
50
0
***
Pro
300
200
100
0

図 22


Hydroxy proline
100
80
60
40
20
0
**
*
Control
EtOH
Stress
Aspirin

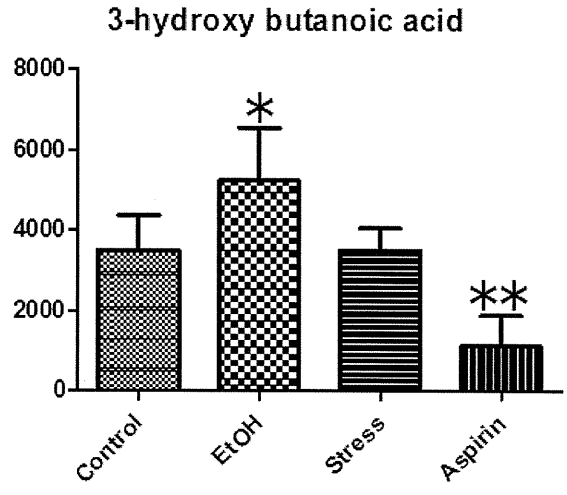

3-hydroxy butanoic acid
8000
6000
4000
2000
0
*
**
Control
EtOH
Stress
Aspirin

表 1　代謝物質標準液濃度

	各代謝物質濃度（μM）	内部標準物質(IS)濃度（μM）
陽イオン ALLSTD	20	200
陽イオン 112STD	20	200
陰イオン ALLSTD	20	200
陰イオン 112STD	50	200
陰イオン 追加依頼 3 物質 (アスピリン、サリチル酸、イブプロフェン)	50	200