Histidine and carnosine alleviated hepatic steatosis in mice consumed high saturated fat diet

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running title: anti-lipogenic effects of carnosine

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Abstract

The effects of histidine, alanine and carnosine on activity and/or mRNA expression of lipogenic enzymes and sterol regulatory element-binding proteins (SREBPs) in liver and adipose tissue from high fat diet treated mice were examined. Histidine, alanine or carnosine, each agent at 1 g/l was added into drinking water for 8-wk supplement. Histidine or carnosine supplement increased hepatic levels of alanine, histidine and carnosine. High fat diet evoked lipogenesis via raising the activity and mRNA expression of glucose-6-phosphate dehydrogenase, malic enzyme, fatty acid synthase (FAS), 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, SREBP-1a, -1c and -2 in liver and adipose tissue ($P<0.05$), which consequently increased mice body weight, epididymal fat, and hepatic triglyceride and cholesterol contents ($P<0.05$). The intake of histidine or carnosine significantly diminished the activity and mRNA expression of malic enzyme, FAS, HMG-CoA reductase, SREBP-1c and SREBP-2, which led to lower body weight, epididymal fat, and hepatic triglyceride and cholesterol levels ($P<0.05$). Mice consumed high fat diet exhibited hyper-insulinemia, hyper-leptinemia, hypo-adiponectinemia and hypo-ghrelinemia. Histidine or carnosine treatments significantly improved insulin sensitivity and attenuated hyper-insulinemia ($P<0.05$). These results support that histidine and carnosine are effective agents for mitigating high fat diet induced hepatic steatosis.

Keywords: Carnosine; Histidine; Steatosis; SREBPs; Insulin

1. Introduction

Hepatic steatosis, hyperlipidemia and obesity due to lipid accumulation are risk factors associated with the prevalence of cardiovascular diseases and metabolic disorders (Daskalopoulou et al., 2004; Franks et al., 2010). Enhanced lipogenesis is a major contributor toward lipid accumulation. Thus, any agent with anti-lipogenic effect may improve lipid metabolism disorders, and alleviate steatosis and obesity.

Carnosine (beta-alanyl-l-histidne) is a dipeptide synthesized from the precursors L-histidine and β-alanine by carnosine synthetase, and present in brain, skeletal muscle and liver (Boldyrev and Severin, 1990). Several *in vivo* studies have reported that carnosine, histidine or alanine could provide anti-oxidative, anti-inflammatory and anti-glycative protection, and support that they are potent nutraceutical agents (Shimizu, 2004; Liu et al., 2008; Hipkiss, 2009). Our previous study observed that histidine or carnosine treatments dose-dependently reduced hepatic triglyceride and cholesterol contents under diabetic condition, and suggested that these compounds might possess anti-lipogenic activity (Lee et al., 2005). Hence, a further study was designed to explore whether these agents were able to attenuate lipogenesis in liver and adipose tissue, especially when high fat diet was supplied.

Malic enzyme, fatty acid synthase (FAS) and 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase are lipogenic enzymes, and involved in the biosynthesis of triglyceride and cholesterol in liver and adipose tissue. Sterol regulatory element-binding proteins (SREBPs) are important transcription factors responsible for fatty acid and cholesterol metabolism (Shimano et al., 1999), in which SREBP-1c is more effective in modulating the expression of genes involved in fatty acid synthesis, whereas SREBP-2 is more predominant in mediating genes associated with cholesterol synthesis

(Shimomura et al., 1997; Horton et al., 1998). If carnosine, histidine or alanine could down-regulate these lipogenic enzymes and SREPBs, they may decline lipogenesis and reduce triglyceride and cholesterol levels in tissues. In addition, hormones such as insulin, leptin and adiponectin also affect lipid metabolism. It is reported that food and food components such as fish oil, tea polyphenols and chlorogenic acid could improve obesity and metabolic disorders via regulating these hormones (Bose et al., 2008; Saraswathi et al., 2009; Cho et al., 2010). If carnosine, histidine or alanine could mediate these hormones, they may regulate lipid metabolism through endocrinal pathways.

The major purpose of this study was to investigate the effects of histidine, alanine and carnosine on activity and/or mRNA expression of lipogenic enzymes and SREBPs in liver and adipose tissue from high fat diet treated mice. The influence of these compounds upon the variation of insulin, leptin, adiponectin and ghrelin in circulation was also evaluated.

2. Materials and methods

2.1. Animals and diets

Male 3-week-old C57BL/6 mice were obtained from National Laboratory Animal Center (National Science Council, Taipei City, Taiwan). Mice were housed on a 12-h light-12-h dark schedule, and fed with water and mouse standard diet for one week acclimation. Mice were then divided into two groups, one continuously consumed control diet, and the other was switched to a high fat diet containing 60% calories as fat (Research Diet D12492; Research Diets, New Brunswick, NJ, USA), in which saturated fat and monounsaturated fat were 55% and 35% of total fat, respectively. Use of the

mice was reviewed and approved by both Chung Shan Medical University and China Medical University animal care committees.

2.2. Experimental design

Either control diet group or high fat diet group was further divided into four sub-groups, in which water or each of histidine, alanine and carnosine was supplied. Histidine (99%), alanine (99.5%) and carnosine (98%) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Each agent at 1 g/l was directly added into the drinking water. Body weight was measured every week. After 8 weeks, mice were killed with carbon dioxide. Liver and epididymal white adipose tissue from each mouse were collected and weighted. Then, 0.1 g liver tissue was homogenized in 2 ml phosphate buffered saline (pH 7.2) on ice, and the homogenate was collected. The protein concentration of homogenate was determined by the method of Lowry et al. (1951) using bovine serum albumin as a standard. In all experiments, sample was diluted to a final concentration of 1 mg protein /ml.

2.3.Hepatic alanine, histidine and carnosine measurement

The content of alanine, histidine and carnosine in liver was determined according to the method of Chan et al. (1994). Briefly, liver at 0.1 g was homogenized in 1 ml, 0.36 M perchloric acid and followed by centrifugation at 2000 xg for 10 min. The supernatant was filtrated through a 0.45 μM filter membrane, and then 50 μl filtrate was derivatized with 200 μl fluoaldehyde o-phthalaldehyde reagent (Pierce, Rockford, IL, USA). Alanine, histidine and carnosine concentrations in the derivatized extract were determined by a HPLC equipped with a 5 μm Hypersil ODS high-resolution column.

2.4. Blood analysis

Serum activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) was determined by using commercial assay kits (Randox Laboratories Ltd., Crumlin, UK). Commercial enzymatic colorimetric kits were used for the determination of plasma free fatty acids (Wako Chemicals GmbH, Neuss, Germany), triglyceride and cholesterol (Sigma Diagnostics, Madrid, Spain).

2.5. Hepatic triglyceride and cholesterol determination

The methods described in Miura et al. (2005) were used to measure hepatic triglyceride and cholesterol content. Briefly, 1 ml liver homogenate was mixed with 2.5 ml chloroform/methanol (2:1, v/v). The chloroform layer was collected and concentrated by a rotary evaporator. After mixing with 10% Triton X-100 in isopropanol, the sample was assayed by Wako Triglyceride E-Test and Total Cholesterol E-Test kits according to the manufacturer's instructions (Wako Pure Chemical, Osaka, Japan). Hepatic triglyceride and cholesterol levels were expressed as mg/g wet tissue.

2.6. Steatosis grade assay

Resected liver specimens were used to determine the grade of steatosis by a licensed pathologist. According to the grading system of Pekow et al. (2007), steatosis was assayed by a percentage of hepatocytes containing fat droplets, grade 0 = absent, grade 1 = 1-5%, grade 2 = 6-32%, grade 3 = 33-66%, and grade 4 = >66% of hepatocytes affected.

2.7. Fecal lipid analysis

Feces, at 0.5 g, were mixed with 3.5 ml deionized water. After sitting at 4 °C overnight, feces were homogenized by vortexing. The fecal lipid was extracted with methanol/chloroform (2:1, v/v) using a method described in Tsujita et al. (2006). The lipophilic layer was collected and dried under a nitrogen stream. Total lipids were

measured gravimetrically.

2.8. Assay for activity of glucose-6-phosphate dehydrogenase (G6PDH), malic enzyme, FAS and HMG-CoA reductase

G6PDH activity was assayed by a commercial kit (Sigma-Aldrich, Co., Ltd. St. Louis, MO, USA) and determined by using a plate-reader spectrophotometer and measuring the rate of absorbance increase at 340 nm due to the conversion of $NADP^+$ to NADPH. The activity of malic enzyme and FAS was measured according to the methods of Stelmanska et al. (2004) and Nepokroeff et al. (1975). Both were determined by spectrophotometric assays. HMG-CoA reductase activity was measured by a radiochemical method as described in Kita et al. (1980), in which [3-^{14}C]HMG-CoA was used as a substrate, and [3-^{14}C]mevalonone synthesized during the assay was isolated by anion-exchange column chromatography.

2.9. Measurement of plasma insulin, leptin, adiponectin and ghrelin

Plasma insulin, leptin and adiponectin levels were measured using Insulin Mouse Ultrasensitive ELISA kit (DRGInstruments GmbH, Marburg, Germany), Mouse Leptin Quantikine ELISA kit (R&D Systems, Minneapolis, MN, USA) and Rat/mouse Adiponectin ELISA kit (Phoenix Europe GmbH, Karlsruhe, Germany), respectively. Plasma immunoreactive ghrelin concentration was measured using a commercial radioimmunoassay kit (Phoenix Pharmaceuticals, Belmont, CA, USA).

2.10. Calculation for insulin resistance

Insulin resistance, determined as HOMA-IR, was calculated using the final blood glucose and insulin levels in food-deprived mice via the formula: [glucose (mmol/l) x insulin (mU/l)]/22.5.

2.11. Quantitative reverse transcription-polymerase chain reaction (RT-PCR) for mRNA

expression

Quantitative RT-PCR was used to examine the mRNA expression in liver and epididymal white adipose tissue. RNA was extracted using TRIizol reagent and further digested with DNase. Total RNA was isolated using the SV Total RNA Isolation kit (Promega, Madison, WI, USA) according to the manufacturer's protocol. Two μg of total RNA was used to generate cDNA. Reverse transcription was performed in a one-step protocol using the iScript cDNA Synthesis Kit (Bio-Rad Co., Hercules, CA, USA) according to the manufacturer's instructions. The primers for PCR were synthesized based on previously published primer sequences (Kim et al., 1999; Yu et al., 2005; Lin and Yin, 2008). Malic enzyme: forward, 5'-CCA CCA GCG CGG CTA CCT GCT GAC GCG GGA-3', reverse, 5'-CCT CTG ACT CGC CGG TGC CGC AGC CCG ATG-3'; FAS: forward, 5'-CAT GAC CTC GTG ATG AAC GTG T-3', reverse, 5'-CGG GTG AGG ACG TTT ACA AAG-3'; HMG-CoA reductase: forward, 5'-CCT GAC ACT GAA CTG AAG CG-3', reverse, 5'-TCT TTC CAG AAC ACA GCA CG-3'; SREBP-1a: forward, 5'-TAG TCC GAA GCC GGG TGG GCG CCG GCG CCAT-3', reverse, 5'-GAT GTC GTT CAA AAC CGC TGT GTG TCC AGT TC-3'; SREBP-1c: forward, 5'-ATC GGC GCG GAA GCT GTC GGG GTA GCG TC-3', reverse, 5'-ACT GTC TTG GTT GTT GAT GAG CTG GAG CAT-3'; SREBP-2: forward, 5'-CAT GGA CAC CCT CAC GGA GCT GGG CGA CGA-3', reverse, 5'-TGC ATC ATC CAA TAG AGG GCT TCC TGG CTC-3'; glyceraldehyde-3-phosphate dehydrogenase (GAPDH): forward, 5'-TGA TGA CAT CAA GAA GGT GGT GAA G-3', reverse, 5'-CCT TGG AGG CCA TGT AGG CCA T-3'. The target concentration was expressed relative to the concentration of a reference housekeeping gene, GAPDH. The cDNA was amplified

under the following reaction conditions: 94 ℃ for 1 min, 57 ℃ for 1 min, and 72 ℃ for 1 min. 28 cycles were performed for GAPDH, malic enzyme, FAS, HMG-CoA reductase and 32 cycles for SREBPs. The corresponding PCR products were analyzed by 1% (w/v) agarose gel electrophoresis and revealed with ethidium bromide. Quantitative analysis was performed with a BAS 2000 BIO-image analyzer (Fuji Photo Film Co., Tokyo, Japan). In this present study, mRNA level was calculated as percentage value of the control diet with water intake group.

2.12. Statistical analysis

The effect of each treatment was analyzed from 10 mice (n = 10) in each group. All data were expressed as mean ± standard deviation (S.D.). Statistical analysis was done using one-way analysis of variance (ANOVA), and post-hoc comparisons were carried out using Dunnet's t-test. *P* values <0.05 were considered as significant.

3. Results

Mice consumed high fat diet had significantly higher final body weight, liver weight, epididymal white adipose tissue, water intake and feed intake (Table 1, $P<0.05$); however, histidine or carnosine treatments significantly lowered mice final body weight, liver weight and epididymal white adipose tissue ($P<0.05$). As shown in Table 2, the supplement of three test agents significantly increased the hepatic concentrations of these compounds in mice with control diet ($P<0.05$). High fat diet alone decreased hepatic levels of three agents ($P<0.05$); however, the supplement of histidine or carnosine in high fat diet groups significantly elevated their concentrations in liver ($P<0.05$).

As shown in Table 3, high fat diet significantly increased plasma levels of ALT, AST, triglyceride and cholesterol ($P<0.05$); the intake of histidine or carnosine significantly

decreased these parameters in circulation ($P<0.05$). High fat diet also significantly increased hepatic triglyceride and cholesterol contents and fecal lipid, as well as caused 9 of 10 mice with steatosis at grades 3 and 4 (Table 4, $P<0.05$). Treatments from histidine or carnosine significantly decreased hepatic triglyceride and cholesterol levels ($P<0.05$), and only 2 of 10 mice exhibited steatosis at grades 3 and 4. All test compounds did not affect fecal lipid content ($P>0.05$).

The effects of three compounds on the activity of four lipogenic enzymes are presented in Table 5. Compared to control diet groups, high fat diet significantly enhanced the activity of these enzymes ($P<0.05$). However, the intake of histidine or carnosine significantly attenuated high fat diet induced elevation in malic enzyme, FAS and HMG-CoA reductase activities ($P<0.05$). Histidine or carnosine treatments failed to affect G6PDH activity ($P>0.05$). The mRNA expression of malic enzyme, FAS and HMG-CoA reductase in liver and epididymal white adipose tissue is shown in Figure 1. Compared to control diet groups, high fat diet significantly up-regulated mRNA expression of these enzymes, and the intake of histidine or carnosine significantly suppressed high fat diet induced mRNA expression of these enzymes ($P<0.05$). The mRNA expression of SREBP-1a, SREBP-1c and SREBP-2 in liver and adipose tissue is shown in Figure 2. High fat diet also significantly up-regulated mRNA expression of these SREBPs in two tissues ($P<0.05$). Histidine or carnosine treatments significantly suppressed high fat diet induced mRNA expression of SREBP-1c and SREBP-2 ($P<0.05$), but did not affect SREBP-1a expression of ($P>0.05$).

Plasma levels of insulin, leptin, adiponectin and ghrelin, and HOMA-IR were presented in Table 6. High fat diet increased insulin and leptin levels, decreased adiponectin and ghrelin levels, and elevated HOMA-IR ($P<0.05$). The treatments from

histidine or carnosine decreased insulin level and improved HOMA-IR (P<0.05); but did not affect leptin, adiponectin and ghrelin levels (P>0.05).

4. Discussion

The high fat diet we used for present study contained 55% saturated fat, which contributed to 33% of total energy. This high saturated fat diet effectively enhanced the activity and/or mRNA expression of four enzymes and three SREBPs responsible for triglyceride and cholesterol biosynthesis, which clearly explained the observed elevated lipogenesis in liver and epididymal white adipose tissue. Our present study further found that the intake of histidine or carnosine increased their levels in liver, and markedly attenuated high saturated fat diet caused weight gain, hyperlipidemia, hepatic steatosis and epididymal fat deposit. Since histidine or carnosine did not affect fecal lipid content, the lipid-lowering effect of these agents was not due to an increase in lipid excretion. These findings support that these two compounds were effective anti-lipogenic agents.

Both malic enzyme and fatty acid synthase involved in biosynthesis of fatty acids and triglycerides; and HMG-CoA reductase is a cholesterol synthesis associated enzyme. It has been indicated that the suppression on the activity and/or mRNA expression of these enzymes could diminish de novo lipogenesis, and lower fat accumulation in liver (Kang et al., 2006; Huong and Ide, 2008). Our present study found that histidine or carnosine treatments markedly declined the activity and mRNA expression of these three enzymes, which subsequently decreased triglyceride and cholesterol levels in plasma, liver and adipose tissue, and finally attenuated hepatic steatosis and lowered body weight.

Our results of steatosis grade also agreed that histidine and carnosine treatments alleviated high fat diet-induced fatty liver. In addition, SREBP-1c is responsible for modulating the expression of fatty acid synthase, whereas SREBP-2 could regulate HMG-CoA reductase (Horton et al., 1998; Lin and Yin, 2008). Our SREBPs results revealed that high fat diet enhanced mRNA expression of SREBP-1c and SREBP-2, which subsequently up-regulated the mRNA expression of their downstream factors, fatty acid synthase and HMG-CoA reductase, and finally promoted lipogenesis in liver and adipose tissue. Furthermore, we found that histidine or carnosine was able to suppress high fat diet evoked expression of SREBP-1c and SREBP-2 in both liver and adipose tissue. Thus, the less available SREBP-1c and SREBP-2 in turn diminished the expression of fatty acid synthase and HMG-CoA reductase, and lowered triglyceride and cholesterol augmentation in tissues. These findings indicated that histidine and carnosine could provide anti-steatotic and anti-obese protection via regulating certain upstream lipogenic factors.

On the other hand, we notified that histidine or carnosine treatments caused similar expression reduction (25-40%) in liver and adipose tissue for malic enzyme, fatty acid synthase and HMG-CoA reductase. However, the suppressive effects of these two compounds upon SREBP-1c and SREBP-2 expression in liver (30-45% reduction) was greater than in adipose tissue (20-33% reduction). These findings suggested that these agents were more effective in attenuating hepatic steatosis. Further study is necessary to examine whether higher doses of these two agents could enhance their anti-lipogenic effects in liver and other tissues.

As reported by others (Handjieva-Darlenska and Boyadjieva, 2009; Parra et al.,

2010) and our present study, high fat diet also altered circulating levels of several hormones. The results of our study revealed that histidine or carnosine treatments did not improve high fat diet caused hyper-leptinemia, hypo-adiponectinemia and hypo-ghrelinemia although these two compounds attenuated hyper-insulinemia. It seems that histidine and carnosine were not able to mediate these hormones. These findings implied that the improved hepatic steatosis and obesity in histidine or carnosine treated groups were not associated with these hormones. The alleviation of hyper-insulinemia from either histidine or carnosine might be simply due to these agents already decline lipogenesis and reduce lipid accumulation in liver and tissues, which in turn elevated insulin sensitivity and lowered the requirement for insulin. The reduction of HOMA-IR also agreed that these agents modified high fat diet induced insulin resistance.

In our present study, each mouse obtained 336-400 mg test compound during the treatment period. However, the deposit of histidine and carnosine in liver was 12 mg and 1.67 mg per 100 g tissue, respectively. Human studies reported that intact carnosine could be absorbed through oral administration, and alanine supplement could increase carnosine content in tissues like muscle (Gardner et al., 1991; Derave et al., 2007). Therefore, the metabolism and utilization of these compounds in human might be different from rodents. In our present study, carnosine exhibited greater effects than histidine in suppressing mRNA expression of HMG-CoA reductase, SREBP-1c and SREBP-2. Thus, carnosine supplement might provide greater anti-lipogenic protection. Muscle foods such as chicken and beef are the major food source of carnosine (Park et al., 2005). However, these muscle foods also contain considerable fat. Thus, it may not

be practical to increase muscle foods consumption in order to obtain carnosine.

In conclusion, high saturated fat diet caused hyperlipidemia, obesity and hepatic steatosis. The intake of histidine and carnosine markedly lowered lipid accumulation in circulation, liver and adipose tissue via diminishing the activity and/or mRNA expression of malic enzyme, fatty acid synthase, HMG-CoA reductase, SREBP-1c and SREBP-2. These compounds also improved insulin resistance and hyper-insulinemia. These results suggest that these two compounds could be considered as potent agents for attenuating high saturated fat diet induced hepatic steatosis.

Conflict of Interest Statement

The authors declare that there are no conflicts of interest.

Table 1

Body weight, water intake, feed intake, liver weight and epididymal white adipose tissue in mice treated with control diet or high fat diet with water, histidine, alanine or carnosine for 8 weeks. Values are mean ± S.D., n=10.

	Body weight g		Water intake ml/mouse/day	Feed intake g/mouse/day	Liver weight g	Epididymal white adipose tissue g
	Initial	Final				
control diet						
water alone	17.6±0.5[a]	25.3±1.3[a]	2.7±0.6[a]	2.5±0.4[a]	1.31±0.25[a]	0.29±0.10[a]
water+histidine	17.1±0.6[a]	24.6±1.1[a]	3.0±1.1[a]	2.3±0.7[a]	1.36±0.37[a]	0.38±0.11[a]
water+alanine	18.0±0.7[a]	25.1±1.4[a]	2.5±0.9[a]	2.8±0.4[a]	1.45±0.28[a]	0.33±0.08[a]
water+carnosine	17.4±0.4[a]	26.0±0.8[a]	2.6±1.2[a]	2.2±0.8[a]	1.42±0.32[a]	0.35±0.12[a]
high fat diet						
water alone	17.0±1.0[a]	38.6±2.4[c]	6.6±1.0[b]	5.2±0.9[b]	2.17±0.40[c]	1.56±0.23[c]
water+histidine	16.9±0.7[a]	32.5±1.6[b]	6.0±1.3[b]	4.7±0.7[b]	1.82±0.35[b]	1.07±0.17[b]
water+alanine	17.2±0.8[a]	37.0±2.0[c]	6.3±1.1[b]	5.3±1.5[b]	2.24±0.46[c]	1.48±0.27[c]
water+carnosine	17.4±0.9[a]	31.7±1.5[b]	5.9±1.2[b]	4.9±1.0[b]	1.79±0.30[b]	0.96±0.15[b]

[a-c]Means in a column without a common letter differ, $P<0.05$.

Table 2

Concentration (mg/100 g tissue) of alanine, histidine and carnosine in liver from mice treated with control diet or high fat diet with water, histidine, alanine or carnosine for 8 weeks. Values are mean ± S.D., n=10.

	alanine	histidine	carnosine
control diet			
water alone	14.71±2.06[c]	15.84±1.65[c]	2.11±0.36[c]
water+histidine	17.35±1.13[d]	19.27±2.15[d]	3.32±0.47[d]
water+alanine	18.21±1.50[d]	18.77±1.24[d]	3.29±0.23[d]
water+carnosine	17.79±1.64[d]	19.05±2.28[d]	5.13±0.39[e]
high fat diet			
water alone	9.34±1.39[a]	10.55±1.68[a]	0.46±0.10[a]
water+histidine	11.57±1.18[b]	12.57±1.71[b]	1.43±0.17[b]
water+alanine	10.65±1.26[b]	11.06±1.30[a]	0.81±0.22[a]
water+carnosine	11.08±1.31[b]	13.08±1.46[b]	1.67±0.29[b]

[a-e]Means in a column without a common letter differ, $P<0.05$.

Table 3

ALT, AST, triglyceride, cholesterol and free fatty acids in plasma from mice treated with control diet or high fat diet with water, histidine, alanine or carnosine for 8 weeks. Values are mean ± S.D., n=10.

	ALT	AST	Triglyceride	Cholesterol	Free fatty acids
	U/l	U/l	g/l	g/l	mmol/l
control diet					
water alone	56±6[a]	75±4[a]	2.41±0.38[a]	1.42±0.46[a]	0.76±0.08[a]
water+histidine	54±3[a]	70±5[a]	2.28±0.41[a]	1.51±0.35[a]	0.78±0.07[a]
water+alanine	57±5[a]	73±4[a]	2.37±0.37[a]	1.37±0.50[a]	0.75±0.05[a]
water+carnosine	52±4[a]	73±7[a]	2.50±0.29[a]	1.46±0.27[a]	0.79±0.08[a]
high fat diet					
water alone	104±13[b]	118±16[b]	5.61±0.60[c]	3.83±0.82[c]	0.84±0.06[a]
water+histidine	67±8[a]	81±10[a]	3.84±0.37[b]	2.98±0.65[b]	0.86±0.09[a]
water+alanine	93±12[b]	110±13[b]	5.25±0.52[c]	4.05±1.01[c]	0.85±0.07[a]
water+carnosine	65±6[a]	84±9[a]	3.46±0.49[b]	2.67±0.74[b]	0.82±0.08[a]

[a-c]Means in a column without a common letter differ, $P<0.05$.

Table 4

Hepatic triglyceride and cholesterol content, fecal lipid level and steatosis grade in mice treated with control diet or high fat diet with water, histidine, alanine or carnosine for 8 weeks. Values are mean ± S.D., n=10.

	Hepatic triglyceride mg/g wet tissue	Hepatic cholesterol mg/g wet tissue	Fecal lipid mg/g feces	Steatosis grade 0/1/2/3/4
control diet				
water alone	30.1±1.8[a]	3.0±0.6[a]	10.28±2.13[a]	10/0/0/0/0
water+histidine	28.4±1.3[a]	2.9±0.5[a]	12.24±3.76[a]	10/0/0/0/0
water+alanine	31.5±1.9[a]	3.1±0.7[a]	11.59±1.90[a]	10/0/0/0/0
water+carnosine	27.0±2.1[a]	2.6±0.4[a]	12.90±2.54[a]	10/0/0/0/0
high fat diet				
water alone	54.9±4.1[c]	7.8±1.0[c]	15.68±3.42[b]	0/0/1/5/4
water+histidine	41.7±3.3[b]	6.0±0.8[b]	14.37±4.07[b]	0/3/5/2/0
water+alanine	50.8±4.5[c]	7.5±1.1[c]	16.10±4.41[b]	0/1/2/4/3
water+carnosine	38.1±2.9[b]	5.7±0.7[b]	15.02±3.75[b]	0/4/5/1/0

[a-c]Means in a column without a common letter differ, $P<0.05$.

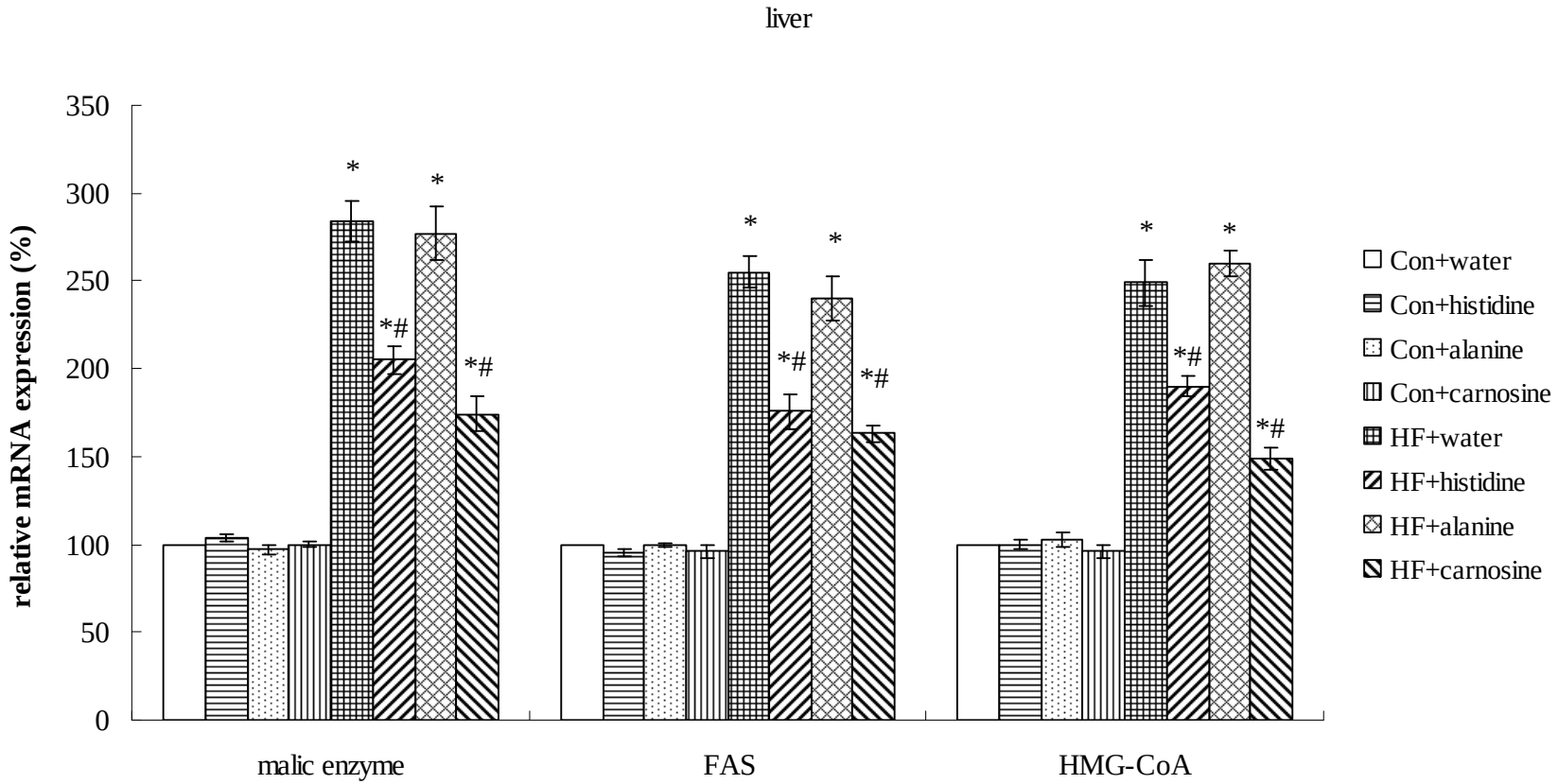
Table 5

Hepatic activity of glucose-6-phosphate dehydrogenase (G6PDH, nmol/min/mg protein), malic enzyme (nmol/min/mg protein), fatty acid synthase (FAS, nmol/min/mg protein) and 3-hydroxy-3-methylglutaryl coenzyme A (HMG–CoA) reductase (pmol/min/mg protein) in mice treated with control diet or high fat diet with water, histidine, alanine or carnosine for 8 weeks. Values are mean ± S.D., n=10.

	G6PDH nmol/min/mg protein	Malic enzyme mmol/min/mg protein	FAS nmol/min/mg protein	HMG-CoA reductase pmol/min/mg protein
control diet				
water alone	1.7±0.5[a]	3.1±0.6[a]	2.6±0.9[a]	20.5±1.4[a]
water+histidine	1.8±0.4[a]	2.8±0.4[a]	2.6±0.6[a]	19.8±1.5[a]
water+alanine	1.6±0.6[a]	2.7±0.7[a]	2.4±0.5[a]	21.3±0.9[a]
water+carnosine	1.7±0.4[a]	2.6±0.5[a]	2.5±0.8[a]	20.5±1.3[a]
high fat diet				
water alone	3.2±0.8[b]	5.8±0.7[c]	5.3±1.3[c]	35.8±2.2[c]
water+histidine	2.9±1.0[b]	4.3±0.5[b]	4.1±0.6[b]	27.4±1.6[b]
water+alanine	3.3±0.9[b]	5.5±1.2[c]	5.6±1.0[c]	33.9±1.5[c]
water+carnosine	2.6±0.6[b]	4.0±0.8[b]	3.7±0.7[b]	25.3±1.7[b]

[a-c]Means in a column without a common letter differ, $P<0.05$.

Fig. 1. mRNA expression of malic enzyme, fatty acid synthase (FAS) and 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase in liver and epididymal white adipose tissue from mice treated with control diet (Con) or high fat diet (HF) with water, histidine, alanine or carnosine for 8 weeks. Data are mean ± S.D. (n=10). *P<0.05 vs. control diet with water group. $^{\#}P$<0.05 vs. high fat diet with water group.


liver
relative mRNA expression (%)
350
300
250
200
150
100
50
0
malic enzyme
FAS
HMG-CoA
Con+water
Con+histidine
Con+alanine
Con+carnosine
HF+water
HF+histidine
HF+alanine
HF+carnosine

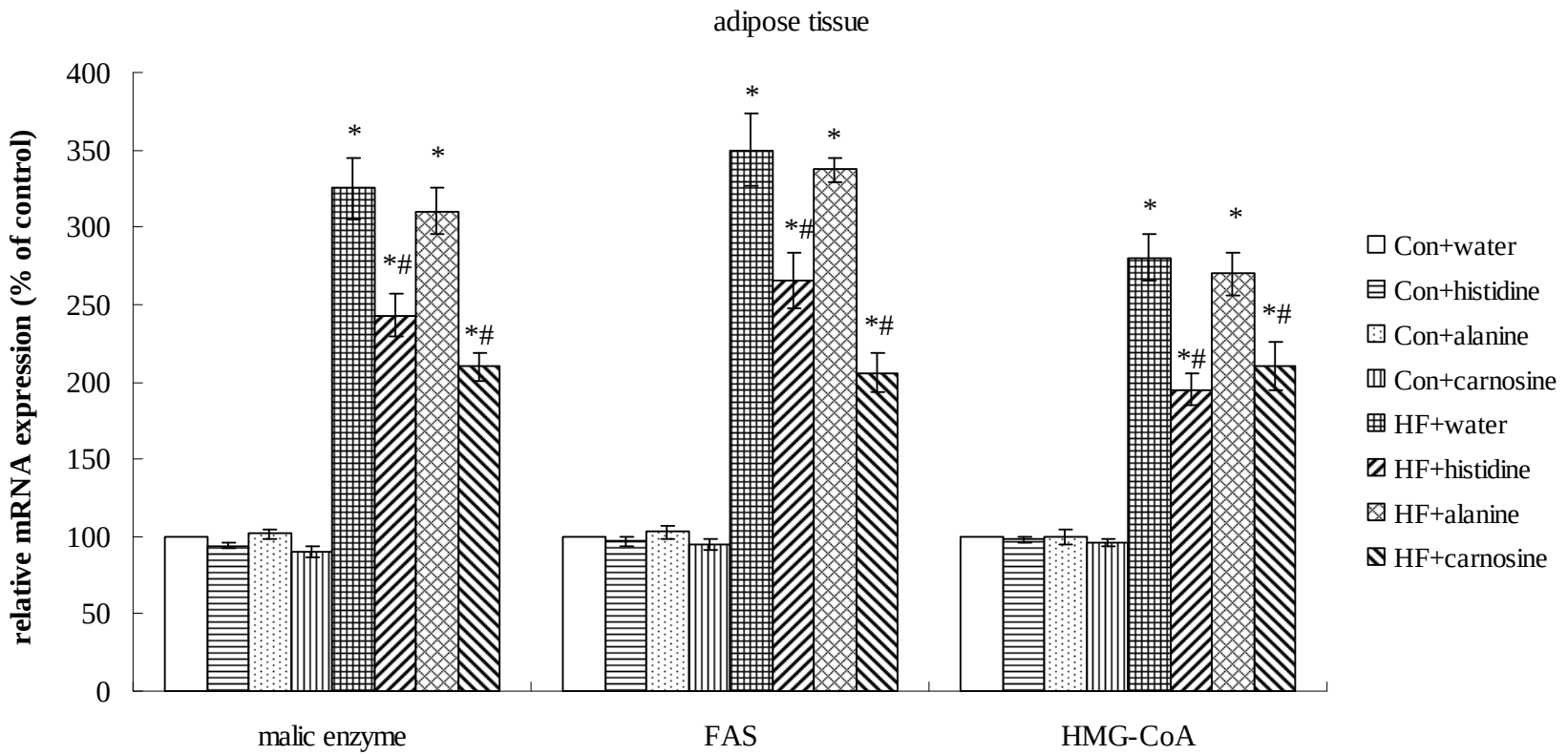
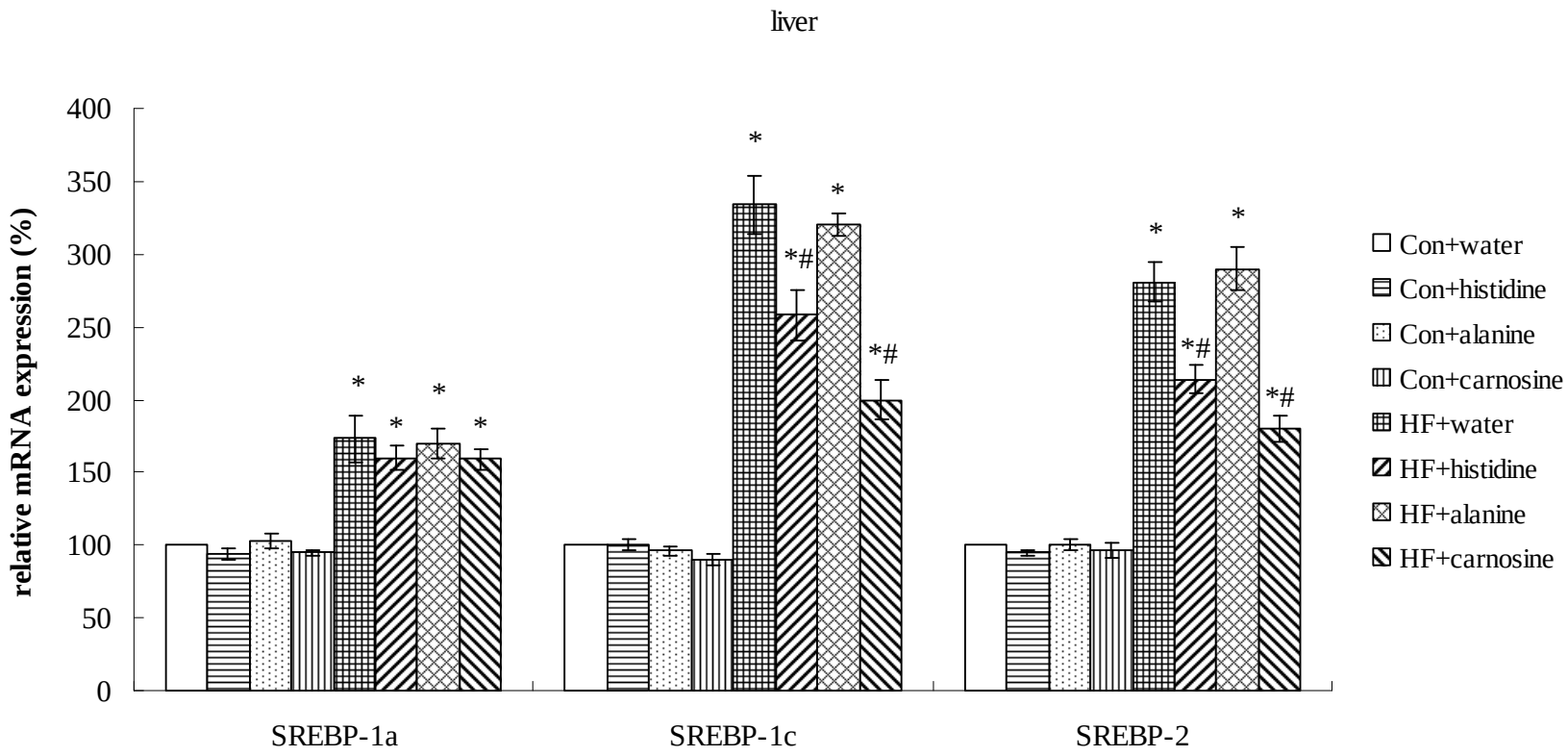

adipose tissue
relative mRNA expression (% of control)
400
350
300
250
200
150
100
50
0
malic enzyme
FAS
HMG-CoA
Con+water
Con+histidine
Con+alanine
Con+carnosine
HF+water
HF+histidine
HF+alanine
HF+carnosine

Fig. 2. mRNA expression of sterol regulatory element-binding proteins (SREBPs), SREBP-1a, SREBP-1c and SREBP-2, in liver and epididymal white adipose tissue from mice treated with control diet (Con) or high fat diet (HF) with water, histidine, alanine or carnosine for 8 weeks. Data are mean ± S.D. (n=10). **P*<0.05 vs. control diet with water group. $^{\#}$*P*<0.05 vs. high fat diet with water group.


liver
relative mRNA expression (%)
400
350
300
250
200
150
100
50
0
SREBP-1a
SREBP-1c
SREBP-2
Con+water
Con+histidine
Con+alanine
Con+carnosine
HF+water
HF+histidine
HF+alanine
HF+carnosine

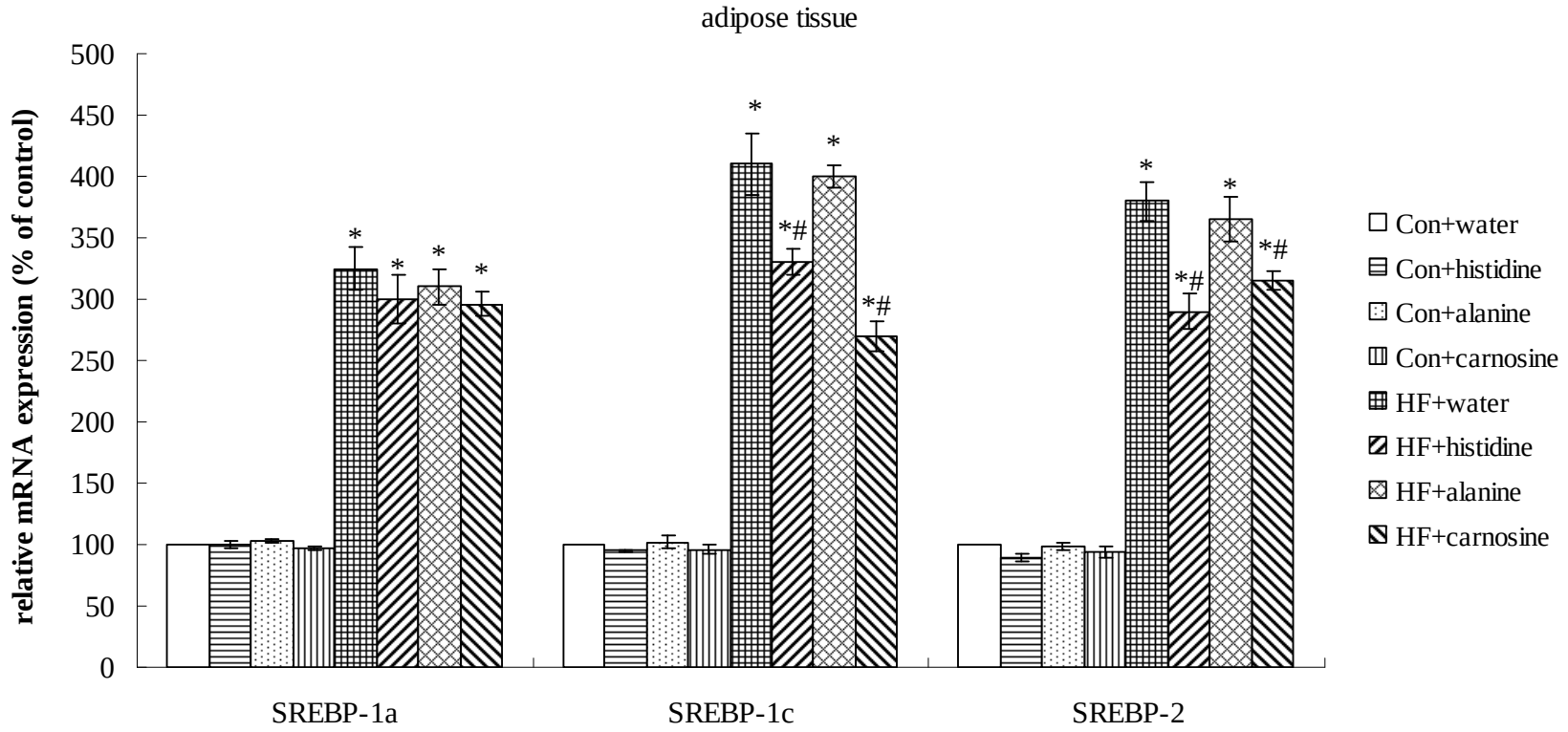

adipose tissue
relative mRNA expression (% of control)
500
450
400
350
300
250
200
150
100
50
0
SREBP-1a
SREBP-1c
SREBP-2
Con+water
Con+histidine
Con+alanine
Con+carnosine
HF+water
HF+histidine
HF+alanine
HF+carnosine

Table 6

Level of insulin, insulin resistance (determined as HOMA-IR), leptin, adiponectin and ghrelin in plasma from mice treated with control diet or high fat diet with water, histidine, alanine or carnosine for 8 weeks. Values are mean ± S.D., n=10.

	Insulin nmol/l	HOMA-IR	Leptin ng/ml	Adiponectin μg/ml	Ghrelin fmol/ml
control diet					
water alone	16.1±1.9[a]	4.4±1.0[a]	3.1±0.6[a]	7.4±1.1[b]	146±17[b]
water+histidine	15.7±2.1[a]	5.0±0.8[a]	2.3±0.3[a]	8.5±1.5[b]	138±24[b]
water+alanine	16.4±1.4[a]	3.9±0.9[a]	2.7±0.7[a]	7.0±1.3[b]	150±19[b]
water+carnosine	15.8±1.5[a]	4.1±1.1[a]	2.2±0.4[a]	8.1±1.0[b]	133±20[b]
high fat diet					
water alone	28.8±3.7[c]	37.8±4.5[c]	5.8±1.0[b]	4.7±0.8[a]	93±8[a]
water+histidine	21.6±2.9[b]	19.6±3.6[b]	5.2±0.8[b]	5.1±1.4[a]	100±13[a]
water+alanine	27.7±4.1[c]	34.9±5.1[c]	6.0±1.2[b]	4.5±1.0[a]	89±10[a]
water+carnosine	20.8±2.5[b]	18.3±4.0[b]	5.5±0.9[b]	5.2±1.2[a]	95±12[a]

[a-c]Means in a column without a common letter differ, $P<0.05$.

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