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Review article

Generation of short chain aldehydes and glyceraldehyde 3-phosphate dehydrogenase (GAPDH)

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Abstract

Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is an enzyme involved in the glycolytic pathway. From the substrates of GAPDH, toxic aldehydes, such as glyceraldehyde and methylglyoxal, can be generated. Inhibition of GAPDH in HCT116 cells by koningin acid increased glyceraldehyde. Animal experiments demonstrated that high fat diet-feeding significantly decreased mice liver GAPDH in protein level with increase of lipid peroxidation, liver damage, and shortage of liver cysteine. On the other hand, some food compounds, a water extract of chlorella and its constituent (phenethylamine), ameliorated high fat diet-induced decrease of GAPDH and following pathological events. Treatment with these compounds significantly decreased liver methylglyoxal. Cysteine can react with short chain glyceraldehydes including methylglyoxal and convert them to nontoxic metabolites. These facts indicate that decrease of GAPDH can increase toxic aldehydes in liver and induce liver damage and some food compounds can ameliorate liver damage via recovering GAPDH.

***KEY WORDS*:** glyceraldehyde 3-phosphate dehydrogenase (GAPDH), glyceraldehyde, methylglyoxal, short-chain aldehydes, high fat diet

1. Background.

Most organisms have a glycolytic system that metabolizes glucose to pyruvate. The glycolytic system can produce ATP without the need for oxygen. In glycolysis, glyceraldehyde 3-phosphate, an intermediate product, is oxidized by the coenzyme, nicotinamide adenine dinucleotide (NAD^+), and concurrently bound with a new phosphate, resulting in 1,3-diphosphoglycerate, from which the phosphate group at position 1 is then released to yield 3-phosphoglyceric acid. Conjugated to this reaction, two molecules of ATP are synthesized per molecule of glucose. The enzyme catalyzing the formation of 1,3-diphosphoglycerate is glyceraldehyde 3-phosphate dehydrogenase (GAPDH). GAPDH has traditionally been considered a housekeeping gene product and used as an internal standard in Western blot and qPCR analyses. However, GAPDH has been found to have moon-light functions other than being a glycolytic enzyme, such as inducing apoptosis [1)]. It has also been reported that the expression of GAPDH in the liver fluctuates with a high fat diet (HFD) [2), 3)]. Therefore, the use of GAPDH as an internal standard has been questioned.

If GAPDH as a glycolytic enzyme decreases, glyceraldehyde 3-phosphate and its isomer dihydroxyacetone phosphate, which are substrates of GAPDH, would accumulate without being metabolized. It is known that short-chain aldehydes (SCAs), *i.e.*, highly reactive glyceraldehyde (GA) and methylglyoxal (MGO), are formed from glyceraldehyde 3-phosphate and dihydroxyacetone phosphate [4)]. These SCAs are thought to react with amino and thiol groups of proteins, thereby reducing protein function [5)]. The reactants (advanced glycation endproducts: AGEs) bind to receptors (RAGE) in vascular endothelial cells and immune-response cells, and induce inflammatory responses, so-called glycative stress [6)]. Therefore, a decrease in GAPDH in the glycolytic system or a decrease in its activity may cause glycative stress. However, the relationship between

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GAPDH and SCAs has not received much attention, as only an inverse correlation between GAPDH activity and MGO production has been reported in erythrocytes[7]. This report presents recent *in vitro* and *in vivo* findings on the relation between GAPDH and the formation of SCAs.

2. Glyceraldehyde and methylglyoxal content in vivo

MGO, a dicarbonyl compound, has been determined with high sensitivity by derivatization with 2,3-diaminonaphthalene (DAN)[8]. MGO has been shown to be present in the liver in amounts of several tens of μmol/kg, but in plasma it is present at the detection limit (low μmol/L). It has also been shown that plasma MGO is increased in diabetic patients[4,9]. In contrast, highly sensitive determination of GA, a monocarbonyl compound, has been difficult. Recently, a stable and sensitive determination of GA in biological sample became possible by optimizing the conditions for 1-phenyl-3-methyl-5-pyrazolone (PMP) derivatization[10]. Using this technique, GA in plasma of healthy subjects and type 2 diabetics has been reported. The content varies 2 - 20 μM and a strong correlation with blood glucose has been reported in type 2 diabetes patients[10]. Compared to blood glucose (about 100 mg/dL), the plasma GA is naturally much lower. However, most glucose has a hemiacetal structure, and the linear aldehyde form in solution (0.001% - 0.003% of total glucose)[11] is considerably less than GA[10]. Therefore, glycative stress on blood vessels is higher for GA, one of SCAs, than for blood glucose. Previously, immunostaining has shown that GA-derived AGEs are increased in animal models and patients with diabetes. Based on these findings, it has also been proposed that this AGE derived from GA is called toxic AGE (TAGE)[12,13]. The aforementioned result that plasma GA is higher than aldehyde-type glucose in the diabetic patients strongly supports the above hypothesis that SCAs play an important role in glycative stress.

3. In vitro GAPDH activity and short-chain aldehyde formation

As mentioned above, an inverse correlation has been shown between GAPDH activity and the generation of MGO derived from GAPDH substrates in erythrocytes[7]. *In vitro* experiments using HCT116 cells derived from human colon adenocarcinoma cells also investigated the relation between GAPDH activity and formation of GA. When koningin acid (KA), an inhibitor of GAPDH, was added to the medium of HCT116 cells up to 100 μM the intracellular concentration of GA increased up to 20 μM KA as shown in ***Fig. 1-a, b***. GA release to the extracellular space was also observed with the addition of KA. Intracellular GA was rather reduced at KA concentrations above 20 μM, while, as shown in ***Fig. 1-d, e***, HCT116 cells floated up at these concentrations, suggesting that cell death reduced GA production. Therefore, *in vitro* experiments have shown that inhibition of GAPDH produces MGO or GA.

4. Formation of short-chain glyceraldehyde by diet.

Since it is now possible to measure GA content in body, the formation of GA and other SCAs by feeding has been investigated. In healthy subjects, no significant changes in plasma GA were observed after consumption of 100 g of rice[10]. In mice and rats, GA in liver (about 80 - 90 μmol/kg) has been shown to be higher than in plasma (about 8 - 9 μM). Overnight fasting significantly decreased GA in the liver, while no significant difference in plasma was found[10]. This result is consistent with the aforementioned human study in which plasma GA did not change significantly after meal ingestion. When mice (CB57/b6) were fed a long-term (8 weeks) HFD (60% on a caloric basis), GA in the liver was not significantly different from that in the normal diet group, however unexpectedly, plasma GA was significantly increased (***Fig. 2-a, b***). This rearing condition induced liver damage and caused a significant increase in plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (***Fig. 2-c, d***)[10]. These results indicate that although a considerable amount of GA is produced in hepatocytes through metabolism of diet-derived components, its release into the blood is suppressed under physiological conditions. However, it can be assumed that GA is leaked from hepatocytes in the event of hepatic dysfunction[10]. This hypothesis is appealing because it could explain the phenomenon of hepatic dysfunction being associated with the progression of diabetes mellitus. Later, it will be necessary to prove this hypothesis using stable isotope tracers.

As mentioned above, sustained HFD feeding to CB57/b6 mice results in liver dysfunction. During this process, antioxidant enzymes in the liver, superoxide dismutase (SOD) and glutathione peroxidase (GPX) activity were significantly decreased (***Fig. 3-a, b***)[14]. To examine the expression of both enzymes at the protein level, Western blot analysis was used. The analysis, which initially used GAPDH as an internal standard, showed no significant differences in the protein content of SOD and GPX, however GAPDH in the mouse liver was significantly reduced by HFD (***Fig. 3-c, d, e***). Therefore, we examined the GA and NGO contents in the liver and found that long-term HFD did not significantly alter GA (***Fig. 2-a***) or MGO in the liver (***Fig. 4-a***). This may be partly due to the higher proportion of carbohydrates in the normal diet compared to HFD. As shown in the next section, a functional food and its ingredient restores the GAPDH and decreases MGO in the liver of mice fed a high fat diet[14]. These facts indicate that, decrease of GAPDH in liver under same carbohydrate loading condition can increase of MGO.

MGO is also known to produce oxidative stress[15,16]. When malondialdehyde, an oxidized product of linoleic acid in the liver, was quantified as 2-thiobarbituric acid reactive substances (TBARS), TBARS was significantly increased by HFD (***Fig. 4-b***)[14]. Therefore, it is possible that MGO may further promote the formation of other SCAs as GAPDH is reduced by HFD. The reason for the increased formation of MGO over GA in the liver due to decreased GAPDH is unknown. Next, whether MGO is produced via GA needs to be examined.

Short-chain aldehydes (SCAs), *i.e.*, MGO, are enzymatically metabolized and detoxified, and are also known to react with cysteine to form stable thiazoline

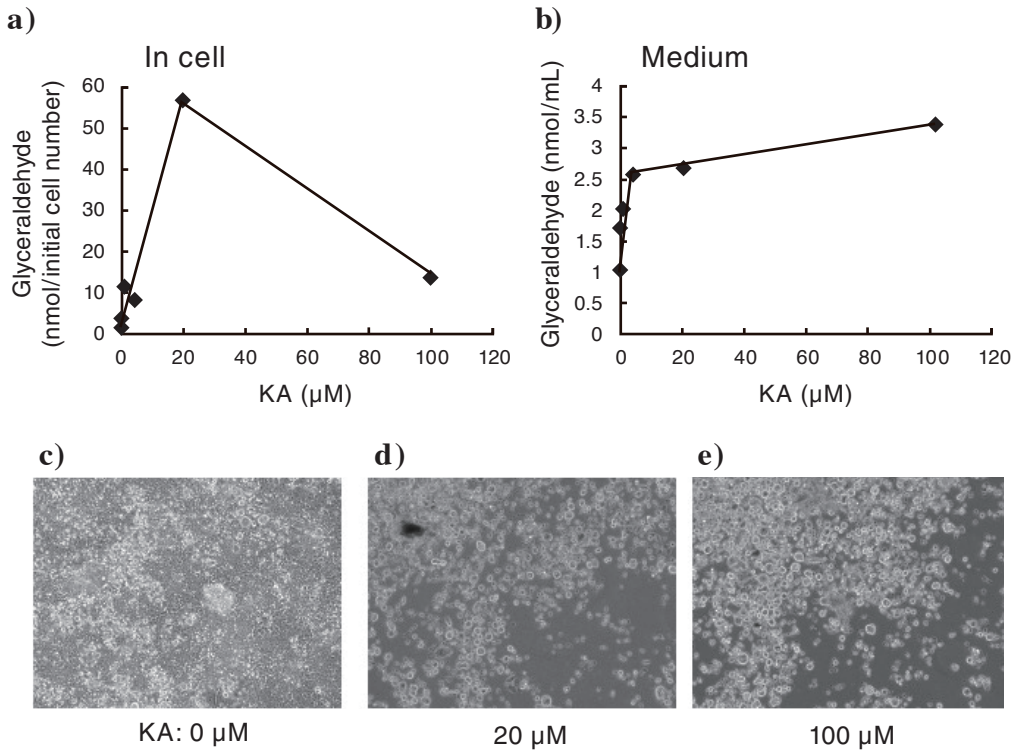

Fig. 1. **Effect of KA on HCT116 cell intracellular and medium GA.**

HCT116 cells (1×10^5) were inoculated on 24 well plate and incubated in a high glucose DMEM medium containing 10 % fetal bovine serum. KA was added to the medium containing one day after inoculation. Cells were harvested 7 days after inoculation and washed with the medium. Intracellular GA was extracted with 75% ethanol. The intracellular (**a**) and medium (**b**) GA was quantified by the method of Martin-Morales *et al*. (Reference 10). Cells after 6-day incubation with KA (**c**: 0 μM, **d**: 20 μM, and **e**: 100 μM) were photographed (lower). Non-published data. KA, koningin acid; GA, glyceraldehyde.

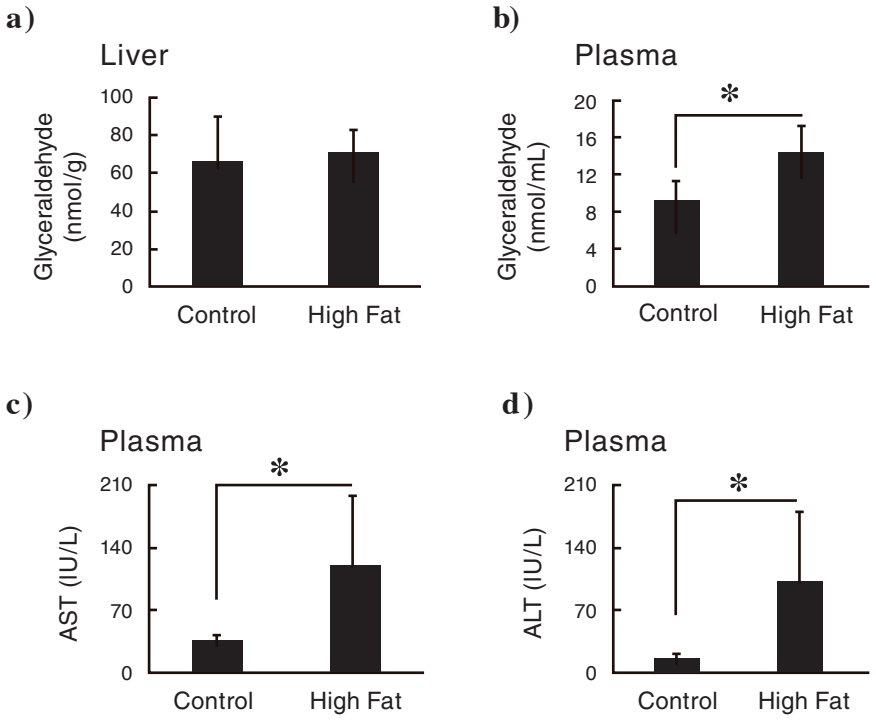

Fig. 2. **Effects of long-term HFD on CB57/b6 mice.**

Liver (**a**) and blood plasma (**b**) GA contents in mice fed control diet and HFD (60 % in calorie) for 8 weeks with plasma AST (**c**) and ALT (**d**). The figure was adapted with permission from Martin-Morales *et al*. (Reference 10). HFD, high fat diet; GA, glyceraldehyde; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

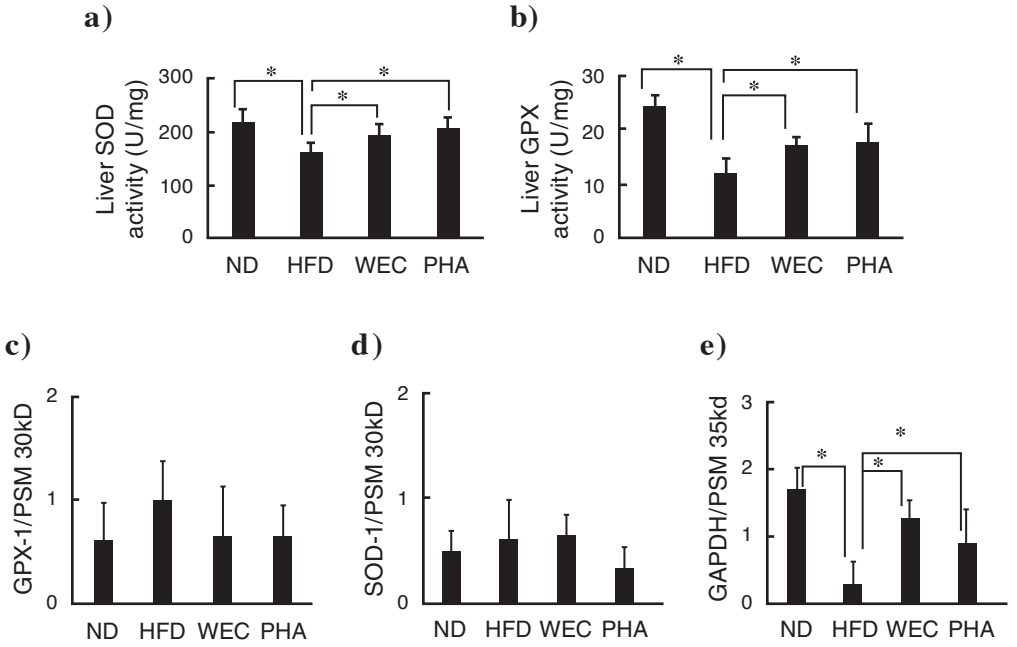

Fig. 3. **Effects of WEC and PHA on liver dysfunction in HFD feeding mice: Changes of SOD, GPX and GAPDH.**

Liver SOD (**a**) and GPX (**b**) activities and their protein levels (**c**, **d**) with protein levels of GAPDH (**e**) in mice fed ND, HFD, HFD with 100 mg/kg body weight of WEC, and HFD with 10 μg/kg body weight of PHA for 12 weeks. The figure was adapted with permission from Zheng *et al*. (Reference 14). SOD, superoxide dismutase; GPX, glutathione peroxidase; HFD, high fat diet; ND, normal diet; WEC, hot water extract of chlorella; PHA, phenethylamine; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

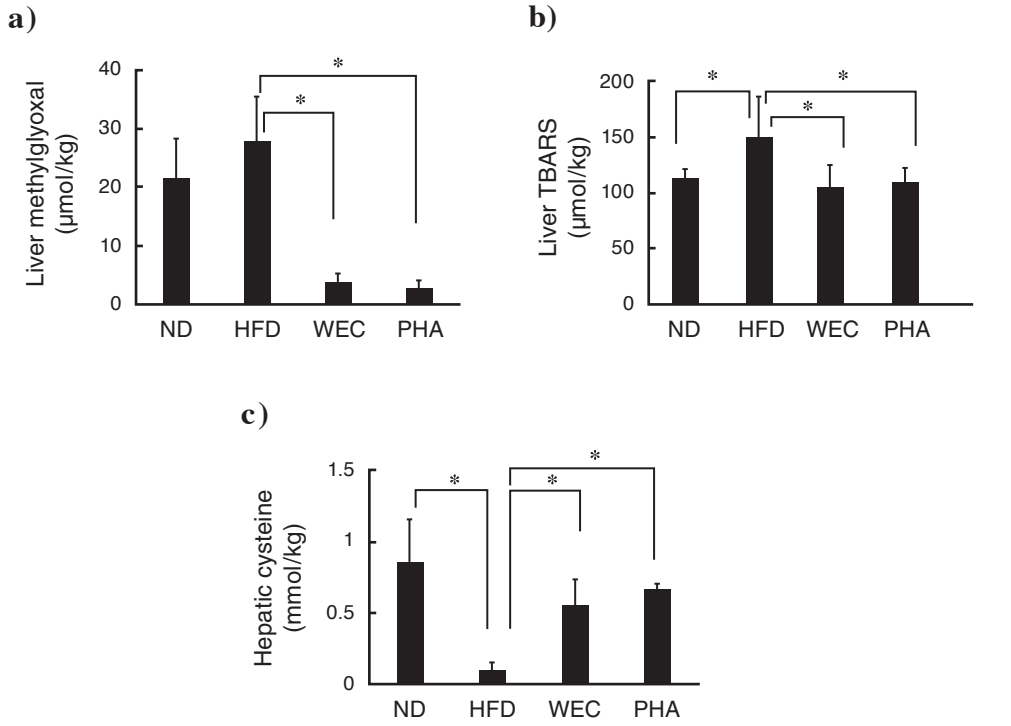

Fig. 4. **Effects of WEC and PHA on liver dysfunction in HFD feeding mice: Changes of MGO, TBARS and cysteine.**

Liver MGO (**a**), TBARS (**b**), and cysteine (**c**) levels in mice fed ND, HFD, HFD with 100 mg/kg body weight of WEC, and HFD with 10 μg/kg body weight of PHA for 12 weeks. The figure was adapted with permission from Zheng *et al*. (Reference 14). MGO, methylglyoxal; TBARS, malondialdehyde; HFD, high fat diet; ND, normal diet; WEC, hot water extract of chlorella; PHA, phenethylamine.

compounds[17]. This reaction is considered to be one of the detoxification actions of SCAs. Therefore, the cysteine content in liver was determined by labeling the thiol and amino groups with 4-vinylpyridine and 6-amino quinolyl-*N*-hydroxysuccinimidyl carbamate (AccQ), respectively. The results showed that cysteine in the liver was drastically reduced to about 1/10 in HFD (***Fig. 4-c***)[14]. These findings suggest that MGO and malondialdehyde produced by decreased GAPDH deplete the cysteine necessary for detoxification, resulting in the inability to detoxify SCAs, which in turn causes decreased liver function.

5. Inhibition of short-chain aldehyde formation by food ingredients

Hot water extract of chlorella is known to improve lipid metabolism[18]. Hot water extract of chlorella has also been found to prolong the survival period of short-lived *Drosophila* flies in which the SOD gene, *Sod*, has been knocked down[19].

Phenethylamine, a decarboxylated form of phenylalanine, has recently been identified as a substance that prolongs survival in *Sod*-knockdown *Drosophila*[19]. When hot water extract of chlorella (WEC) and phenethylamine (PHA) were administered orally at 100 mg/kg and 10 μg/kg, respectively, to CB57/b6 mice fed HFD, both doses resulted in a decrease in plasma LDL-C and TC (***Fig. 5-a, b***)[14].

Furthermore, administration of PHA significantly reduced the markers of liver damage (AST, ALT) (***Fig. 5-c, d***), and TBARS levels in the liver (***Fig. 4-b***), indicating that oxidative stress in the liver was improved and liver function was preserved. Interestingly, administration of WEC and PHA restored GAPDH, which had been depleted by HFD (***Fig. 3-e***), resulting in a decrease in MGO in the liver (***Fig. 4-a***). Furthermore, the depletion of cysteine, which reacts with SCAs, was significantly improved (***Fig. 4-c***)[14]. These results support the hypothesis, discussed in the previous section, that a decrease in GAPDH leads to the SCA formation, resulting in liver dysfunction. They also indicate that food components can protect liver function by suppressing the GAPDH decrease.

6. Future issues

In this report, animal experiments using mice have shown that a decrease in GAPDH, which is highly abundant in cells, causes an increase of MGO, which is derived from dihydroxyacetone phosphate, an isomer of substrate of GAPDH, resulting in increased oxidative stress and impaired liver function. The limiting enzyme in the glycolytic system is kinases, *i.e.*, phosphofructokinase. Nevertheless, the reason for the presence of large amounts of GAPDH in cells has not been fully understood. The facts presented in this paper suggest that excess GAPDH is present to prevent the formation of toxic SCAs from GAPDH substrates. Whether this phenomenon occurs in other organs or in other animal models, especially in the human liver fed HFD, needs to be investigated in the future. If HFD in humans also causes a decrease in GAPDH and an increase in the production of SCAs in the liver, direct and indirect "hits" by SCAs may

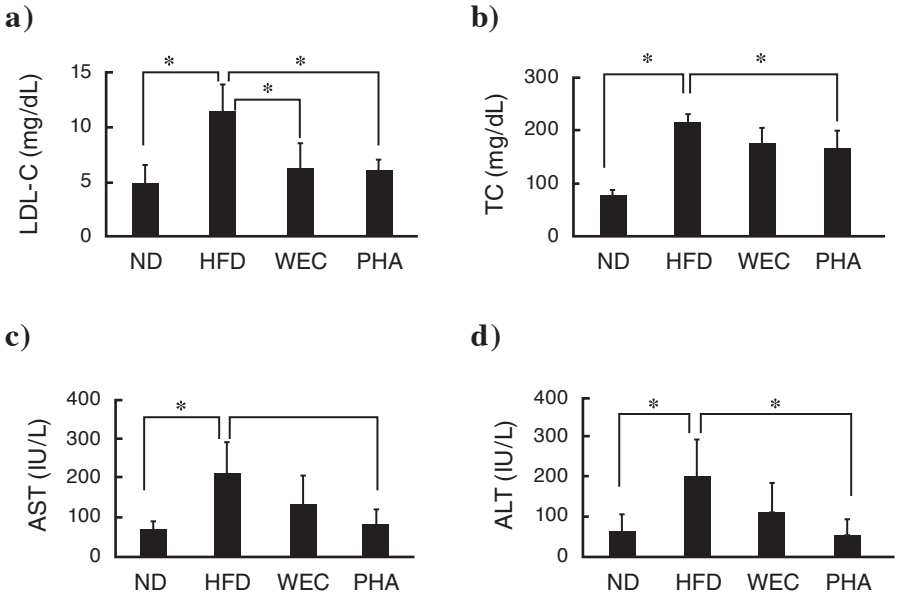

Fig. 5. **Effects of WEC and PHA on liver dysfunction in HFD feeding mice: Changes of blood plasma LDL-C, TC, AST, and ALT.**

Blood plasma LDL-C (**a**) and TC (**b**), AST (**c**), and ALT (**d**) levels in mice fed ND, HFD, HFD with 100 mg/kg body weight of WEC, and HFD with 10 μg/kg body weight of PHA for 12 weeks. The figure was adapted with permission from Zheng *et al*. (Reference 14). LDL-C, low-density lipoprotein-cholesterol; TC, total cholesterol; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HFD, high fat diet; ND, normal diet; WEC, hot water extract of chlorella; PHA, phenethylamine.

occur resulting in development of nonalcoholic hepatitis and other age-related diseases. The maintenance of cysteine in cells may also be an indicator of the "hits."

The reason why GAPDH is decreased in the liver of HFD feeding mice is unknown. The cysteine residue in GAPDH is known to undergo modifications such as nitrosylation by nitric oxide (NO)[20] and succinylation by fumaric acid[21]. It is not known whether such modifications are caused by HFD, but such modifications may be involved in the reduction of GAPDH. Also, as mentioned above, SCAs react with the thiol group of cysteine. The resulting SCAs may have modified GAPDH. These modifications of the thiol group of GAPDH may be the direct cause of the decrease in GAPDH, while it is also possible that the decrease in GAPDH due to other factors increased the SCAs, thus causing further GAPDH decrease. Alternatively, a very low dose (10 μg/kg body weight) of phenethylamine maintains GAPDH in the liver of mice fed HFD. The reason for this is also unknown. Elucidation of the mechanisms of these changes will provide important insights into glucose metabolism and homeostasis of liver function.

Conflict of interest declaration

The authors have no conflicts of interest to declare.

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