Characterizing the Lipid-Lowering Effects and Antioxidant Mechanisms of Tomato Paste

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Lycopene is known to decrease cardiovascular risks. The objective of this study was to investigate the molecular mechanisms of tomato paste containing approximately 0.1% lycopene in regulating lipid metabolism and oxidation. Hamsters fed 3% or 9% tomato paste containing 0.2% cholesterol were subjected to total cholesterol (TC), low density lipoprotein (LDL), high density lipoprotein (HDL), and triglyceride (TG) measurements. Our results showed reduced rates of serum TC and LDL levels due to 9% tomato paste were 14.3% and 11.3% respectively. Concentrations of 3% and 9% of tomato paste after 8 weeks of feeding significantly increased serum HDL levels, by 19.4% and 28.8% respectively. After ingestion of tomato paste for 8 weeks, the plasma malondialdehyde (MDA) levels significantly decreased, by 80.18% and 89.33% respectively, as compared to the cholesterol group. MDA and diene conjugation assays indicated the potent antioxidant activity of the tomato paste. The increased activities of superoxide dismutases (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px), further supported the antioxidant effects of the tomato paste. Two dimension-gel electrophoresis (2-DE) analysis revealed that carbonic anhydrase III (CAIII) and adenylate kinase 2 (AK2) may be two important regulators involved in the anti-lipid and antioxidant effects of tomato paste, opening new insight into the nutritional value of tomato in public health promotion.

Key words: tomato paste; atherogenesis; cholesterol; oxidation

Hypercholesterolemia is the major risk factor for atherosclerosis.1–4 It increases the levels of ROS and thus creates an oxidative environment in the blood.5–7 Based on epidemiological and experimental data, increasing levels of low density lipoproteins of cholesterol (LDL-C) and total cholesterol are closely related to atherogenesis. Moreover, many studies have suggested that oxidized form of LDL-C (ox-LDL-C) is an atherogenic agent.8,9 The increased levels of LDL-C were prone to be oxidized by reactive oxygen species (ROS).10 The ox-LDL-C scavenged by macrophages can result in foam cell formation and the accumulation of numerous adhesion factors, neutrophils, and monocytes on the vascular lumen, which in turn contributes to the development of a bulk of atherosclerotic plaques and cell death.10–12

Under conditions of a high-calorie, high-lipid diet and lack of exercise, some normal physiological conditions can malfunction, resulting in obesity, hypertension, hyperlipidemia, hyperglycemia, and hyperuricemia. Among these, hyperlipidemia, resulting in cardiovascular disease, has been rated one of the leading causes of death worldwide. Some antioxidants, such as probucol and lovastatin, have been indicated as hypocholesterolemic agents with established inhibitory effects on LDL-C oxidation.13 A natural antioxidant lycopene, which is present in great amounts in tomato (Lycopersicum esculentum), has been shown to have a similar effect in preventing atherosclerosis.14–17 It has been reported that a supplement of tomato juice boosted the plasma lycopene level in renal transplant recipients,17

Abbreviations: AK2, adenylate kinase 2; CAT, catalase; CAIII, carbonic anhydrase III; GSH-Px, glutathione peroxidase; HDL, high density lipoprotein; HPLC, high performance liquid chromatography; ICAT, isotope-coded affinity tags; IEF, isoelectric focusing; LDL, low density lipoprotein; LDL-C, low density lipoproteins of cholesterol; MALDI, matrix-assisted laser desorption ionization; MDA, malondialdehyde; MS, mass spectrometry; ox-LDL-C, oxidized form of LDL-C; ROS, reactive oxygen species; SOD, superoxide dismutases; TBA, thiobarbituric acid; TC, total cholesterol; TCA, trichloroacetic acid; TG, triglyceride; TOF, time of flight; TP, tomato paste; 2-DE, two dimension-gel electrophoresis; U, units

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but the levels of LDL-C oxidation did not show significantly differences as between supplementation with tomato juice and orange drink. Increasing plasma lycopene levels might not reduce the susceptibility of LDL to oxidation. In a recent healthy-human study, subjects were asked to follow a controlled carotenoid-poor diet for a week and then to take a standardized three-week menu supplemented with tomato products. Consumption of tomato products for 3 weeks led to increased plasma levels of carotenoids and lycopene and prevented lipid peroxidation. This observation is in agreement with an earlier multi-center case-control study whose subjects enrolled from 10 European countries, showing a strong preventive effect of lycopene on coronary heart disease.

Other than lycopene, tomato contains a significant amount of other nutrients, such as folate, vitamin A, vitamin C, vitamin E, and other carotenoids, as well as phytochemicals such as polyphenols. These components of tomatoes have all been suggested to be beneficial for cardiovascular function. Although the antioxidant properties of tomato have been extensively studied for the prevention of cardiovascular disease, the lipid lowering effect, another protective factor in coronary heart disease, has been studied to a lesser extent. In the present study, we aimed to whether fresh ripe tomato paste can regulate serum lipid levels, as well as oxidation status in hypercholesterolemic animals. The molecular mechanism of tomato paste on anti-lipid oxidation was also elucidated. These findings may provide insight into the preventive effects of tomato in hyperlipidemic impact on vascular function.

Materials and Methods

Preparation of tomato paste. The preparation of tomato paste proceeds according to a standard operation protocol kindly provided by AGV (Chiayi, Taiwan), and is briefly described as follows. Fresh ripe tomato is sheared and homogenized under 150 kg/cm² pressure, and then passed through a 20-mesh filter. The filtrate is then supplemented with sodium citrate, alanine, and sucrose, followed by autoclaving. The tomato juice is then subjected to dry freezing to granulize it. The condensation rate of tomato extract used in this study was 9.92–10.4%. According to high performance liquid chromatography (HPLC) analysis, the lycopene content in the tomato paste was approximately 0.11%.

Animal models. Thirty-two Golden Syrian hamsters with an average body weight of 0.1 kg were housed in a laboratory animal care facility under an environmentally controlled atmosphere (25°C) and a 12/12 h light/dark cycle. Food and water were provided ad libitum. All procedures were performed according to the guidelines for the Care and Use of Laboratory Animals of the Chinese Society for Laboratory Animal Science (Taiwan). After a 2-week adaptation period, hamsters were randomly assigned to four experimental groups, including normal control, 0.2% cholesterol, and tomato paste (3% or 9%). The normal control group was fed a regular chow diet (10 g/d). The cholesterol group received a high-fat atherogenic diet consisting of chow enriched with 10% corn oil and 0.2% cholesterol. The experimental groups were fed the high fat atherogenic diet supplemented with 3% or 9% tomato paste. After 8 weeks of feeding, the hamsters were sacrificed by overdoses of pentobarbital for blood withdraw from cardiac punctation for biochemical studies.

Copper-induced oxidation of LDL. LDL was then isolated by single-step nonequilibrium density gradient ultracentrifugation (Beckman TL-100, Beckman, Palo Alto, CA) using a TLV-100 vertical rotor at 100,000 rpm for 30 min at 10°C. The isolated LDL fraction was desalted by passing it through a gel filtration column (Econo-Pac 10 DG, Bio Rad Laboratories, Hercules, CA). LDL was collected in 0.7 ml of PBS. The protein concentration of the eluate was measured using bovine...
serum albumin as standard. For the oxidation experiment, the LDL preparations were diluted with PBS to contain 0.05 g/l protein (about 0.1 μmol/l LDL). Oxidation was started by adding 10 μl of freshly prepared 0.167 mmol/l CuSO$_4$ to 1.0 ml of LDL solution in a 1-cm quartz cuvette. Oxidation was determined as the production of hydroperoxides with conjugated double bonds (conjugated dienes) by continuously (at 1 min intervals for 8 h) monitoring the change in absorbance at 234 nm at 37 °C. Several indices were obtained from the absorbance versus time curves. The initial absorbance of the sample was recorded to assess the baseline level of conjugated dienes (BDC, μmol/l) formed in the circulation prior to the isolation of LDL. The lag time (min) was determined from intercept of lines drawn through the linear portions of the lag phase and the propagation phase.

**TBARS level.** Malondialdehyde (MDA) in plasma was determined by a modification of the method of Ohkawa et al.\(^ {20} \) The assay mixture consisted of 25 μl of the plasma, 2.5 μl of 60 mM CuSO$_4$, and 22.5 μl of H$_2$O, and was incubated for 4 h at 37°C. The mixture was then mixed with 0.35 ml of 20% TCA and 0.35 ml of 0.67% TBA, and was heated for 30 min at 70 °C.\(^ {22} \) After centrifugation at 10,000 rpm for 2.5 min, the supernatant was assayed spectrophotometrically at 540 nm. Since other aldehydic compounds in the sample can be measured, the plasma MDA content was expressed as TBA reactive substance (TBARS, MDA equivalent) in nmol.

**Measurement of enzymatic activities of superoxide dismutases (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) in the liver.** Liver proteins for enzymatic activity assay were extracted from tissue treated with or without tomato paste, according to previously published protocols.\(^ {23} \) SOD activity was assayed with a commercial kit Ransel (Randox, San Diego, CA). The enzyme activity of SOD was defined as 1 U inhibiting the reducing rate of 2-(iodophenyl)-3-(4-nitrophenoxy)-5-phenyltetrazolium chloride by 50%. SOD activity was denoted as U/mg protein. GSH-Px activity was assayed with a commercial kit Ransod (Randox, San Diego, CA). The enzyme activity of GSH-Px was defined as 1 U oxidizing 1 μmol NADPH in 1 min. GSH-Px activity was denoted as μU/mg protein. The assay of CAT was based on the protocol published by Aebi.\(^ {24} \) CAT activity was denoted as U/mg protein.

**Two dimension-gel electrophoresis (2-DE).** Liver protein was extracted from tissue treated with or without tomato paste according to the previously published protocols.\(^ {23} \) The protein sample was loaded onto Readystrip IPG strips (Bio Rad Laboratories) in a pH range of 3-10NL (nonlinear), and layered with 0.8 ml of cover oil to prevent the gel from drying and urea crystalization. The gel was run on the PROTEAN IEF cell (Bio Rad Laboratories) at 30 V to rehydrate the gel strip for 12 h, followed by the running programs: 30 V for 12 h with 360 Vh, 500 V for 1 h with 500 Vh, 1,000 V for 1 h with 1,000 Vh, and 8,000 V for 2 h with 1,600 Vh. The voltage ramped automatically based on the increasing resistance from the strip as excess ions moved out of the strip. After first-dimension IEF, the strip was washed to remove the cover oil, and then equilibrated in 5 ml of equilibration buffer containing 50 mM Tris–HCl, pH 8.8, 6 M urea, 2% SDS, 30% glycerol, and 1% DTT for 12–15 min. The strip was then subjected to a second equilibration with 5 ml of equilibration buffer with 1.5% iodoacetamide substituted for the DTT for an additional 12–15 min, followed by SDS electrophoresis at 200 V for 4 h. At the end of electrophoresis, the gel was stained with 0.25% (w/v) coomassie brilliant blue (Amersham Biosciences, Buckinghamshire, UK), 50% (v/v) methanol, and 10% (v/v) acetic acid for 30 min, followed by destaining with 10% (v/v) methanol, 0.07% (v/v) acetic acid to remove excessive stains. The spots with differential expression level between control and experimental groups were picked out manually and digested by trypsin with the subsequent MALDI-TOF-TOF analysis.

**Matrix-assisted laser desorption ionization (MALDI)-time of flight (TOF)-TOF analysis.** MALDI-TOF-TOF allowed high-throughput mass spectrometry (MS) analysis. The isotope-coded affinity tags (ICAT)\(^ {25} \) labeling reagent for MS analysis was composed of three functional elements: a reactive group that binds to Cys residues of proteins, an biotin-containing affinity tag that binds irreversibly to avidin, and a linker region that contains eight stable isotopes (8 hydrogen (H\(^ \text{1} \)) atoms vs. 8 deuterium (D\(^ \text{2} \)) atoms in the light version and 8 deuterium (D\(^ \text{2} \)) atoms vs. 8 deuterium (D\(^ \text{2} \)) atoms in the heavy version).\(^ {25,26} \) Based on our ICAT-labeling strategy, identical peptides derived from each of the paired samples were modified with either the light version or the heavy version of ICAT. Hence, they differed in mass by 8 Da, and appeared as doublets in the MS spectra. By measuring the ratios of the peak intensities, we were able to quantify relative protein expression levels due to tomato treatment. To define the identity of the peptide of interest, we subjected it to a second MS analysis (MS/MS, peptide MS fingerprinting) to get sequence information on the peptide. Then the database (MASCOT)\(^ {27} \) was searched to identify the most probable protein, consistently with the given peptide sequence.

**Western blot analysis.** Liver protein was extracted in the way as described in 2-DE section. Total protein was resolved in SDS-polyacrylamide gel electrophoresis, followed by blotting a PVDF (polyvinylidene fluoride) membrane. Ponceau S was used to identify the successful transfer of proteins to the membrane. The membrane was then incubated in PBS buffer containing 0.2% Tween-20 and 5% non-fat milk for 1 h. Primary antibodies against CAIII and AK2 reconstituted in PBS containing 0.1% Tween-20 were added and the membrane was incubated overnight at 4 °C. After washing
Values were presented as mean associated with the followed by Tukey’s Student Rank test. Differences evaluated by one-way analysis of variance (ANOVA) computer program. Differences in mean values were analyzed by the SPSS (ver. 10) (SPSS, Chicago, Il).

Results

Body weight

No differences in weight gain at the end of the experimental period was found among the experimental groups. For the 8-week period of feeding, all animals gained about 30% of original body weight (Table 1).

Effects of tomato paste on plasma level of TC, TG, LDL, and HDL in the hamster model

The hyperlipidemic hamster model was successfully established since the plasma levels of TC, TG, and LDL increased significantly on 8-week feeding of the 0.2% of cholesterol diet (Table 2). In the present study, we found that TC was significantly reduced by, 9% not 3% of the tomato paste diets after 8 weeks of feeding, whereas TG was not affected by either concentration of tomato paste (Table 2). Similarly to the results of TC, LDL also decreased by 9% not 3% of tomato paste diets after 8 weeks of feeding. The reducing rates of serum TC and LDL by feeding 9% of tomato paste diet were 14.3% and 11.3% respectively (Table 2). Both concentrations, of 3% and 9%, of tomato paste after 8 weeks of feeding significantly increased serum HDL levels, by 19.4% and 28.8% respectively (Table 2).

Table 1. Effects of Tomato Paste on the Body Weight after 8 Weeks of High-Cholesterol Feeding

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Cholesterol</th>
<th>3% TP</th>
<th>9% TP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body wt (g)</td>
<td>88.75 ± 2.57</td>
<td>84.58 ± 3.57</td>
<td>90 ± 6.89</td>
<td>83.33 ± 1.33</td>
</tr>
<tr>
<td>Final body wt (g)</td>
<td>128.25 ± 5.69*</td>
<td>128.00 ± 1.81*</td>
<td>128.75 ± 1.86*</td>
<td>127.63 ± 1.79*</td>
</tr>
</tbody>
</table>

Values were mean ± SD (n = 8).
Statistical difference was determined by Student’s t test.
*Significantly different as compared with the initial body weight for each group, P < 0.05.

Table 2. Effects of Tomato Paste on Serum Levels of TG, TC, LDL, and HDL after 8 Weeks of High-Cholesterol Feeding

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Cholesterol</th>
<th>3% TP</th>
<th>9% TP</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG (mg/dl)</td>
<td>76.17 ± 7.50***</td>
<td>110.33 ± 8.62</td>
<td>102.67 ± 4.35</td>
<td>103.50 ± 4.56</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>124.83 ± 7.42***</td>
<td>248.17 ± 6.05</td>
<td>240.17 ± 7.16</td>
<td>212.67 ± 6.76***</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>75.10 ± 4.23</td>
<td>74.88 ± 2.67</td>
<td>89.43 ± 2.74***</td>
<td>96.47 ± 3.24***</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>12.33 ± 1.99***</td>
<td>82.50 ± 2.29</td>
<td>82.00 ± 3.17</td>
<td>73.17 ± 2.56***</td>
</tr>
</tbody>
</table>

Values were mean ± SD (n = 8).
Statistical difference was determined by Student’s t test.
**Significantly different as compared with the cholesterol group, P < 0.001.

with PBS containing 0.1% Tween-20, the protein expressing level was illuminated on X-ray film by the ECL reaction (Amersham, Arlington Heights, IL).

Statistical analysis. Statistical evaluations of the data were analyzed by the SPSS (ver. 10) (SPSS, Chicago, Il) computer program. Differences in mean values were evaluated by one-way analysis of variance (ANOVA) the followed by Tukey’s Student Rank test. Differences associated with P < 0.05 were considered significant. Values were presented as mean ± SE.

Table 3. Lag Time of Serum LDL Oxidation Affected by Tomato Paste

<table>
<thead>
<tr>
<th></th>
<th>Cholesterol</th>
<th>3% TP</th>
<th>9% TP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lag time (min)</td>
<td>208.33 ± 22.05</td>
<td>321.67 ± 43.43</td>
<td>398.38 ± 9.28***</td>
</tr>
</tbody>
</table>

Values were mean ± SD (n = 8).
Statistical difference was determined by Student’s t test.
**Significantly different as compared with the cholesterol group, P < 0.001.

Antioxidization effects of tomato paste on lag time of serum LDL oxidation and MDA formation

Polyunsaturated fatty acid in serum LDL can be modified by reactive oxygen species to form conjugated dienes. The lag time indicates the delay of conjugated diene formation the reflecting antioxidant activity.28) As Table 3 shows, the lag time for conjugated diene formation was significantly increased by 9% but not by 3% of tomato paste after 8 weeks of feeding as compared to the cholesterol group. The plasma MDA level was significantly increased by 8 weeks of cholesterol feeding, by 15.59-fold as compared to the normal controls (Fig. 1). After ingestion of 3% or 9% of tomato paste for 8 weeks, plasma MDA levels were significantly decreased, 80.18% and 89.33%, respectively, as compared to the cholesterol group.

Effects of tomato paste on SOD, CAT, and GSH-Px activities

The values for SOD, CAT and GSH-Px measurements for the different groups are shown in Fig. 2. CAT and GSH-Px activities were significantly increased in the 9% tomato paste group in comparison with the cholesterol group (P < 0.01), but no significant alteration in SOD activity was detected in the 9% tomato paste group as compared to the cholesterol group (P > 0.05).
Liver protein profiles analysis by 2-DE-MALDI-TOF/TOF peptide fingerprinting

Liver proteins extracted from the control, cholesterol, and 9% tomato groups were separated by 2-DE and visualized by coomassie brilliant blue staining. Two protein spots were identified by PDQuest 2-DE analysis software, as their expression levels were significantly increased in the 9% tomato paste group as compared to the cholesterol group (Fig. 3A). As shown in Fig. 3B, the shaded area in the MALDI-TOF/TOF MS spectra indicates the noise signals with a probability score of under 36. Both the highest scores, of 94 and 176, in two individual tests by database searching were identified as adenylate kinase isozyme 2 (AK2) and carbonic anhydrase III (CA III) with molecular weights of 25-KDa.

Western blot assay to confirm the stimulatory effects of tomato paste on protein expression levels

Based on 2-DE and MALDI TOF/TOF MS results, CAIII and AK2 were found to be activated by tomato paste. To further confirm the protein expression levels of CAIII and AK2 affected by, 9% of tomato paste, western blot analysis was performed to delineate the relationship between the tomato paste and these proteins. With β-
actin normalization, our results indicates that CAIII and AK2 protein expression levels were increased by 9% of tomato paste by 4 and 3.5 folds respectively (Fig. 4).

Discussion

Lycopene, an important component of tomato paste is known to be beneficial in preventing atherosclerosis.14–16) In a large-scale investigation comprising 1,028 mid-age males performed in Finland, it was found that low concentrations of plasma lycopene conferred vascular atherosclerosis.12) This was also evident in a report showing that cultured macrophages containing 10μM lycopene significantly inhibited cellular cholesterol synthesis from [3H]-acetate, by 73%.29) In the same study applied to human subjects, continued intake of 60 mg/d of lycopene significantly reduced plasma LDL-C levels, by 14%.29) That study also reported that HMG-CoA reductase, a key enzyme in the biosynthesis of cholesterol, was inhibited by lycopene. These reports are in agreement with previous studies indicating that circulating lycopene might play a pivotal role in blocking the early development of atherosclerosis.15,16)

Following to those reports of the effects of lycopene on lipid metabolism, we attempted to determine whether tomato paste containing lycopene is beneficial in a hyperlipidemic hamster model. The tomato paste contained approximately 0.11% of lycopene. Other than lycopene, several other constituents of tomato paste are listed in Table 4. Two different concentrations of tomato paste (3% and 9%) were evaluated as to their lipid-lowering effects at 8 weeks of high-cholesterol diet feeding. Our results indicate that the hypolipidemic effects of tomato paste were different at various concentrations. In hamsters, the serum levels of TC and LDL-C were significantly reduced by 9% but not by 3% of tomato paste at 8 weeks of feeding, but TG levels were not significantly affected by either concentration of tomato paste. These results indicate that tomato paste is effective in reducing total cholesterol levels only at
higher concentrations. The serum HDL-C levels were increased by 3% of tomato paste (19.4%) and 9% of tomato paste (28.8%). These results are consistent with previous studies, in which the serum LDL-cholesterol and LDL-cholesterol/HDL-cholesterol ratio decreased at 6 weeks in rabbits fed 1% proanthocyanidin,30) and plasma HDL-C concentrations in high-cholesterol rats fed polyphenolic natural products, such as quercetin, morin, and tannic acid, were increased at 4 weeks.31)

It is commonly known that high plasma lipid levels are an important risk factor for atherogenesis. There is a close relationship between atherosclerosis and lipid oxidization.10) Among various lipoproteins, LDL-C is much more prone to be oxidized by reactive oxygen species (ROS), resulting in foam-cell formation when macrophages scavenge oxidized LDL-C. In addition, oxidized LDL-C is also toxic to the vascular endothelium, and is a potent promoter of smooth muscle cell proliferation and inflammation.6,7,16) If oxidation of lipoproteins or fatty acids persists, a toxic aldehyde (MDA) may be formed, resulting in malfunction of normal cells.

In the present study, lipid peroxidation was evaluated as MDA production (TBARS). As seen in Fig. 1, increases in plasma MDA was evident at 8 weeks of the high-fat atherogenic diet. Significant reductions in the MDA level were observed at 8 weeks of 3% and 9% of tomato paste feeding in hamsters. This observation is consistent with our finding that serum lycopene concentrations were significantly increased after 3% and 9% of tomato paste feeding in hamsters (0.28 mol/l for 3%, 0.41 mol/l for 9%, and 0.07 mol/l for cholesterol control; data not shown). The contribution of lycopene in tomato paste in reducing lipid peroxidation is implied in this regard. The lag time for LDL-C oxidation in the 9% tomato paste group was significantly increased as compared with the cholesterol group. These data suggest that high concentrations of tomato paste can provide protective effects on oxidative stress. Our results are in agreement with a recent report indicating that regular

<table>
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<tr>
<th>Constituents of Tomato Paste</th>
<th>Amount per 8 g of tomato paste</th>
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<tbody>
<tr>
<td>Lycopene</td>
<td>8.7 mg</td>
</tr>
<tr>
<td>Resistant maltodextrin</td>
<td>3 g</td>
</tr>
<tr>
<td>Total carbohydrate</td>
<td>4 g</td>
</tr>
<tr>
<td>Dietary fiber</td>
<td>420 mg</td>
</tr>
<tr>
<td>Sodium</td>
<td>28.5 mg</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>15 mg</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>30 mg</td>
</tr>
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</table>

Table 4. Constituents of Tomato Paste

Fig. 4. Western Blot Assay to Confirm the Stimulatory Effects of Tomato Paste on Protein Expression Levels.

A. Protein expression levels of CA III and AK2 affected by normal diet (N), cholesterol diet (C), and 9% of tomato paste. B. Fold induction of CA III and AK2 by 9% TP with β-actin normalization. Results are expressed as the means ± SD (n = 3). **P < 0.001 as compared with the cholesterol group by Student’s t test.
intake of tomato products over an extended period of time can prevent lipid peroxidation.\(^{18}\)

In terms of its lipid-lowering effect as well as its antioxidant effects, tomato paste is expected to cope with atherosclerosis. This was evident in increased protein levels of antioxidant enzymes in the liver, including SOD, CAT, and GSH-Px. As a food supplement, tomato paste can exert an antiatherogenic effect, and hence these findings should draw attention to the clinical benefits of tomato paste in cardiovascular protection.

CA III and AK2 were increased by 9% tomato paste as shown by 2-DE and MS analysis. CA is a zinc metalloenzyme that catalyzes the hydration of carbon dioxide to bicarbonate, and CA III is the major soluble protein in rat liver and muscle. Its function is probably to protect the proteins of these tissues from oxidation catalyzed by iron-containing degradation products of hemoglobin and myoglobin.\(^{32,33}\) AK catalyzes the reversible transfer of the \(\gamma\)-phosphate group from ATP to AMP, releasing two molecules of ADP. Three AK isoforms have been identified in mammals. They are involved in energy metabolism and nucleic acid synthesis. AK2 is the protein present in the mitochondrial intermembrane space.\(^{34}\) It has also been found that AK2 specifically binds to insulin in mouse liver cells.\(^{35}\) 2-DE analysis has revealed that CA III and AK2 are two important regulators involved in the anti-lipid and antioxidant effects of tomato paste, giving a new insight into the nutritional value of tomato in public health promotion.

In conclusion, our data suggest that tomato paste is effective in lowering the serum levels of TC and LDL-C in cholesterol-fed hamsters. An appropriate concentration is required to reach the optimal effects of tomato paste on lipid metabolism. A further study can be designed on human subjects to evaluate the health-promoting effects of tomato paste on hyperlipidemia and atherosclerosis.

**Acknowledgments**

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