Suppressive Effect of Globin Digest on Postprandial Hyperlipidemia in Male Volunteers\(^1,2\)

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ABSTRACT We have reported previously that various edible protein digests inhibit dietary hyperlipidemia in mice, rats, pigs and dogs. Of the various digests tested, globin digest had the most potent inhibitory activity, and a tetrapeptide extracted from globin digest, Val-Val-Tyr-Pro, had activity 7000-fold greater than that of the parent digest. In this clinical study, we investigated the influence of globin digest on serum chylomicron triglyceride concentrations as an indicator of the effect of globin digest on fat absorption and catabolism in humans. Parallel and crossover trials were conducted in which men consumed a control high fat diet (25 g fat, 7.6 g carbohydrate, 1.9 g protein and 0.7 g sodium chloride) or the same diet supplemented with globin digest. The supplemented dosages were 1 and 4 g globin digest. In the parallel trial, 22 men were divided into three groups: control, globin digest 1 g and globin digest 4 g. The increases in chylomicron triglyceride concentrations at 1 h after ingestion of 1 or 4 g globin digest were significantly lower (\(P < 0.05\)) compared with the control group. The crossover trial involved six subjects who consumed the control high fat diet and the same diet supplemented with 4 g globin digest. Serum chylomicron triglyceride levels increased in both groups at 1 and 2 h after ingestion, but when subjects consumed 4 g globin digest the increases were suppressed to 75 (\(P < 0.05\)) and 42% (\(P < 0.05\)) of the increases in controls at the corresponding times, respectively. The areas under the curves of chylomicron and serum total triglyceride concentrations during the 4 h after ingestion of 4 g globin digest were 46 (\(P < 0.05\)) and 34% (\(P < 0.05\)) lower, respectively, than when the men consumed the high fat control diet. In these trials, globin digest reduced the increase in serum chylomicron triglyceride concentrations as a result of the ingestion of a high fat diet. This hypotriglyceridemic effect of globin digest may be valuable for preventing obesity and in lowering the incidence of cardiovascular diseases. J. Nutr. 128: 56–60, 1998.

KEY WORDS: • humans • hypotriglyceridemic effect • chylomicron • oligopeptide • globin digest

Serum cholesterol concentration is one of the risk factors for developing cardiovascular disease. In many epidemiologic studies (Austin 1989, Carlson et al. 1979, Carlson and Böttlinger 1985; Castelli 1986), however, serum triglyceride level is regarded as an independent risk factor for coronary heart disease. Furthermore, a high postprandial triglyceride level may also be a risk factor for atherosclerotic disease (Groot et al. 1991, Patsch et al. 1992, Simons et al. 1987). These findings suggest that lowering the serum triglyceride level may be more important than lowering cholesterol concentration in the prevention of cardiovascular disease and obesity.

In our studies of lipid absorption, we have been trying to identify effective hypotriglyceridemic products. Recently, some oligopeptides having 3–8 amino acid residues were shown to be hypotriglyceridemic (Kagawa 1990). Oligopeptides were prepared by suitable protease digestion of various edible proteins such as globin, casein or soybean (EU patent no. WO 89/06970). Globin digest (GD)\(^4\) demonstrated hypotriglyceridemic function superior to that of the other protein digests after fat ingestion in mice, rats and dogs in minute doses compared with the usual protein intake. A peptide, Val-Val-Tyr-Pro, which is present in GD, had 7000-fold greater hypotriglyceridemic activity than GD (Kagawa et al. 1996). Globin digest and Val-Val-Tyr-Pro inhibited fat absorption from the digestive tract and enhanced the activity of hepatic triglyceride lipase (EC 3.1.1.3) in mice. Oral administration of GD and olive oil enhanced the hepatic free fatty acid (FFA) concentrations compared with that found in mice administered olive oil (Kagawa et al. 1996). However, neither repression of intestinal peristaltic movement nor the delaying of...


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\(^4\) Abbreviations used: AUC, area under the curve; C, control high fat diet; FFA, free fatty acid; GD, globin digest; GD-1, the control diet supplemented with 1 g globin digest; GD-4, the control diet supplemented with 4 g globin digest; HTGL, hepatic triglyceride lipase.
gastric emptying was caused by GD intake (Kagawa 1990, Kagawa et al. 1996).

A single oral administration of GD (±10 g/kg body weight) showed no toxic effects in male or female mice. A lethal dose of GD in mice was >10 g/kg body weight (Kagawa, K & Hasegawa, S., unpublished results). When globin digest [4 g/kg body wt d] was administered orally to male rats for 3 mo, no toxic reactions were observed during or after administration (Kagawa, K & Hasegawa, S., unpublished results).

In this study, the suppressive effect of GD on postprandial hyperlipidemia was examined in male human volunteers.

SUBJECTS AND MATERIALS

Volunteers and diet. This study was conducted in compliance with the Declaration of Helsinki. All clinical investigations were conducted in the Second Department of Medical Biochemistry, School of Medicine, Ehime University. Healthy male volunteers aged 20–24 y signed an informed consent form. After overnight fasting (~12 h), the subjects were given 100 mL of thick cream soup to which was added 24 g butter (control diet) or the same meal supplemented with GD. The control diet N-hydroxyethylaminoanilidine as a substrate. concentrations were determined by enzymatic methods using commercial kits (Triglyceride E-test, NEFA C-test, Cholesterol E-test, Wako Pure Chemicals, Osaka, Japan).

Serum triglyceride concentrations of 22 subjects were measured on the day before the parallel trial. Participants were divided into three groups: control (C), GD-1 and GD-4 on the basis of the triglyceride concentrations. On the morning of the clinical trial, blood was collected again for a base-line analysis from the participants who had fasted for 12 h. Groups did not differ significantly (P > 0.05).

Abbreviations used: FFA, free fatty acids; GOT, glutamate oxaloacetate transaminase; GPT, glutamate pyruvate transaminase; γ-GTP, γ-glutamyl-transpeptidase; GD-1, 1 g globin digest; GD-4, 4 g globin digest.

Results and Discussion

Characteristics of the subjects involved in the parallel study are summarized in Table 1. Serum lipid composition did not

<table>
<thead>
<tr>
<th>TABLE 1</th>
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<tr>
<td><strong>Base-line analysis of serum lipids and hepatic function of study groups before the parallel trial</strong></td>
</tr>
<tr>
<td><strong>Serum lipids</strong></td>
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<tr>
<td><strong>Triglyceride</strong></td>
</tr>
<tr>
<td><strong>Diet</strong></td>
</tr>
<tr>
<td><strong>GD-1</strong></td>
</tr>
<tr>
<td><strong>GD-4</strong></td>
</tr>
<tr>
<td><strong>C</strong></td>
</tr>
</tbody>
</table>

1 Values are means ± SEM.

2 Control diet (C) consisted of 25 g fat, 1.9 g protein, 7.6 g carbohydrate and 0.7 g sodium chloride (total energy 1.15 MJ). The two experimental diets were the same diet supplemented with 1 g (GD-1) and 4 g (GD-4) globin digest, respectively.

Pharmacodynamic analysis of elimination velocity of chylomicron triglyceride. Absorption and elimination rates of chylomicron triglyceride in the crossover were calculated with the use of equations of the one-compartment model (Benet and Sheiner 1985).

Statistical analysis. Data were expressed as means ± SEM. Changes in triglyceride concentration from initial levels in both parallel and crossover trials were analyzed by repeated measures ANOVA. One-way (diet) ANOVA was used in the parallel design trial; if the F test was significant, Fisher’s protected least significant difference test (Steel and Torrie 1980) was used to detect significantly different means. These analyses were performed by the statistics programs of Yanai and Nagata (1994) using macro commands of Lotus 1-2-3 (Lotus Development, Cambridge, MA). The analysis of the crossover design trial was accomplished by two-way (diet and time) ANOVA (Wagner 1975). Differences in the elimination and absorption rate constants were analyzed by the parallel line assay method (Finney 1964). Differences were considered significant at P < 0.05.
Serum total triglyceride concentrations at 1 and 2 h after ingestion of the control diet were significantly lower than the value for the control group, $P < 0.05$; significantly greater than baseline (time 0 h), $P < 0.05$.

differ among the three groups. The activities of serum glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, and $\gamma$-glutamyltranspeptidase, indicators of hepatic function, were within normal ranges. There were four subjects with hyperlipidemia. Two individuals had hypercholesterolemia (range, 5.70–6.44 mmol/L) and another two had hypertriglyceridemia (range, 1.47–1.72 mmol/L). These two subjects were separated into the control and GD-4 groups.

Serum total and chylomicron triglyceride concentrations at 1–3 h after ingestion of the control diet were significantly increased relative to baseline values ($P < 0.05$) (Fig. 1). Triglyceride concentrations peaked 2–3 h after ingestion. When subjects consumed the GD-1 or GD-4 diet, the serum total and chylomicron triglyceride concentrations at 2 and 3 h, respectively, were significantly increased relative to baseline values ($P < 0.05$). The increases in chylomicron triglyceride concentrations at 1 h after ingestion of the GD-1 or GD-4 diet were significantly lower ($P < 0.05$) than the increase in subjects who consumed the control diet. No significant differences in the magnitude of changes in serum total triglyceride concentrations were observed among the three groups.

Serum lipids and hepatic enzyme activities of participants in the crossover trial were within normal ranges (Table 2). Serum total triglyceride concentrations at 2 and 3 h and chylomicron triglyceride concentrations at 1–3 h after ingestion of the control diet were significantly increased relative to baseline values ($P < 0.05$) (Fig. 2). When subjects consumed the GD-4 diet, serum total and chylomicron triglyceride concentrations at 2 and 3 h, respectively, were significantly increased relative to baseline values ($P < 0.05$). The increases in chylomicron triglyceride concentration were suppressed to 75 ($P < 0.05$) and 42% ($P < 0.05$) of the increases that occurred when they consumed the control high fat diet at 1 and 2 h postingestion, respectively. The increase in serum total triglyceride concentrations at 1 h after ingestion of the GD-4 diet were significantly lower ($P < 0.05$) than the increase in subjects who consumed the control diet.

Areas under the triglyceride concentration curves for 4 h (AUC0–4 h) were calculated for subjects in both the parallel and crossover trials (Table 3). In the parallel trial, no significant differences among groups were observed in the AUC of serum total or chylomicron triglycerides because of large variation among the participants. However, the AUC of serum total and chylomicron triglyceride concentrations when subjects consumed the GD-4 diet in the crossover trial were significantly lower, by 34 ($P < 0.05$) and 46% ($P < 0.05$), respectively, than when they consumed the control diet.

To determine the influence of GD on chylomicron metabolism, absorption and elimination rate, constants were calculated.
lateral from the chylomicron triglyceride concentrations in subjects in the crossover trial by using the one-compartment model (Table 4). No significant differences in magnitude of changes for the absorption and elimination rate constants of chylomicron triglycerides were observed between subjects that consumed the control and GD-4 diets.

Postprandial serum levels of remnant lipoproteins are regarded as an indicator of hepatic uptake of chylomicron remnants. High chylomicron remnant level after fat ingestion is a risk factor for cardiovascular disease (Campos et al. 1992, Groot et al. 1991, Patsch et al. 1991, Simons et al. 1987). Chylomicron remnant concentrations in the most subjects were too low to quantify. However, changes in chylomicron remnant concentrations were detected in two subjects (Fig. 3). At 1 and 2 h after ingestion of the GD-4 diet, chylomicron remnant concentrations in these two men appeared to be lower than when they consumed the high fat control diet. Oral administration of GD in mice did not affect lipoprotein lipase activity (Fukuhama et al. 1991). We have also reported that GD induced hepatic triglyceride lipase (HTGL) activity in mice (Kagawa et al. 1996). Chylomicron remnant was digested by HTGL and resulted in the formation of FFA, which were observed in vitro and in vivo in human studies (Murase and Itakura 1981, Nicoll and Lewis 1980). Ingestion of a high fat diet with GD may increase hepatic FFA concentrations. Oral administration of GD with olive oil to mice enhanced hepatic FFA concentration in the early phase (Kagawa et al. 1996).

### Table 3

**Areas under the curves of serum total and chylomicron triglycerides in men consuming high fat control and/or globin digest–supplemented diet in parallel and crossover trials**

<table>
<thead>
<tr>
<th>Trial</th>
<th>Diet</th>
<th>n</th>
<th>Serum total triglycerides</th>
<th>Chylomicron triglycerides</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>µmol/(L·4 h)</td>
<td>% of Control</td>
</tr>
<tr>
<td>Parallel3</td>
<td>C</td>
<td>8</td>
<td>1196 ± 396</td>
<td>100 ± 33</td>
</tr>
<tr>
<td></td>
<td>GD-1</td>
<td>6</td>
<td>944 ± 296</td>
<td>79 ± 25</td>
</tr>
<tr>
<td></td>
<td>GD-4</td>
<td>6</td>
<td>785 ± 286</td>
<td>66 ± 24</td>
</tr>
<tr>
<td>Crossover4</td>
<td>C</td>
<td>6</td>
<td>1250 ± 508</td>
<td>100 ± 41</td>
</tr>
<tr>
<td></td>
<td>GD-4</td>
<td>6</td>
<td>828 ± 363</td>
<td>66 ± 29</td>
</tr>
</tbody>
</table>

1. Values are means ± SEM. Areas under the curves of Figure 1 (parallel study) and Figure 2 (crossover study) were calculated from 0 to 4 h by geometrical method according to Wolever et al. (1991).
2. Control diet (C) consisted of 25 g fat, 1.9 g protein, 7.6 g carbohydrate and 0.7 g sodium chloride (total energy 1.15 MJ). The experimental diets, GD-1 and GD-4, were the same diet supplemented with 1 (GD-1) or 4 g globin digest (GD-4).
3. Differences between control and GD-1 and GD-4 diets in the parallel study were analyzed by one-way (diet) ANOVA.
4. Differences between control and GD-4 diets in the crossover study were analyzed by two-way (time and diet) ANOVA. P values refer to percentage of control values of areas under the curves.

### Table 4

**Absorption and elimination rate constants calculated by using the one-compartment model from chylomicron triglyceride concentrations in men who consumed a high fat control and a globin digest supplemented diet (GD-4) in the crossover trial**

<table>
<thead>
<tr>
<th></th>
<th>Absorption rate2 (0–2 h)</th>
<th>Elimination rate2 (2–4 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>GD-4</td>
</tr>
<tr>
<td>K3</td>
<td>−0.710</td>
<td>−0.669</td>
</tr>
<tr>
<td>C0 (µmol/L)</td>
<td>1518</td>
<td>1288</td>
</tr>
<tr>
<td>r2</td>
<td>0.967</td>
<td>0.957</td>
</tr>
</tbody>
</table>

1. Each parameter was calculated from the equation of the one-compartment model (Wagner 1975) as follows: Concentration of chylomicron triglyceride ([C] = C0abs·e−kabs·t + Ce−kEL·t) Symbols C0, kabs, kEL and r represent the initial concentration, coefficient of absorption, coefficient of elimination and coefficient of regression curve, respectively. The crossover trial was taken between a control diet (Control) and the same diet supplemented with 4 g globin digest (GD-4). The control diet consisted of 25 g fat, 1.9 g protein, 7.6 g carbohydrate and 0.7 g sodium chloride (total energy 1.15 MJ).
2. Absorption and elimination rates were calculated from 0–2 h and 2–4 h of chylomicron triglyceride concentrations (means of 6 subjects showed in Figure 2), respectively.
3. Differences in elimination and absorption rate constants were analyzed by the parallel line assay method (Finney 1964). No differences were significant.
4. Coefficient of regression curve.
We have also observed that the repeated administrations of GD accelerated oxidation of hepatic FFA to carbon dioxide in mice (Kagawa, K., Matsutaka, H., Fukuhara, C. & Fujino, H., unpublished data). In these clinical studies, FFA concentrations in serum were measured not only at 0 h but also 1–4 h postingestion in the parallel and crossover trials. However, no significant differences in the magnitude of changes in serum FFA concentrations were observed between the control and GD-supplemented group (data not shown).

In this study, we examined the influence of GD on absorption and catabolism of dietary triglyceride. GD may reduce diet-induced hypertriglyceridemia through the suppression of lipid absorption. However, the elimination rate constant of chylomicron triglycerides was not affected by GD ingestion. The mechanism of action of GD remains to be determined.

LITERATURE CITED


