Aim: Improving cholesterol efflux capacity (CEC) of high-density lipoprotein (HDL) has been regarded as a novel target for preventing cardiovascular disease. HDL reportedly has antioxidant properties which may contribute to its functions. We investigated changes in CEC with intake of the Japan Diet (JD) recommended by the Japan Atherosclerosis Society and the relationship of these changes to serum antioxidant concentrations.

Methods: A randomized parallel controlled clinical trial on JD intake was performed in Japanese patients with dyslipidemia. Ninety-eight participants were randomly divided into the JD (n=49) or the partial JD (PJD) (n=49) group. Nutrition education, based on each diet at baseline and at 3 months, was provided and the participants were followed up for 6 months.

Results: Mean CEC was 1.05 in total and correlated positively with HDL-cholesterol (p<0.001) at baseline. CEC did not change while oxygen radical absorbance capacity (ORAC) was decreased in both groups (p<0.001). Although serum total carotenoid increased in both groups, serum α-tocopherol decreased in the JD group as compared to the PJD group (p<0.05). CEC correlated positively with HDL ORAC at baseline (p=0.021) and with serum total carotenoid at 3 and 6 months (p=0.005, 0.035). Changes in CEC correlated positively with changes in HDL ORAC at 3 months and serum total tocopherol at 3 and 6 months (p<0.001).

Conclusion: CEC was not changed by JD education in Japanese patients with dyslipidemia who already had normal CEC at baseline. CEC was suggested to be positively associated with serum α- and γ-tocopherol and HDL ORAC.

Clinical trial registration number: UMIN000022955

Key words: Cholesterol efflux capacity, Diet, Oxygen radical absorbance capacity, Tocopherol, Carotenoid

Introduction

A low level of plasma high-density lipoprotein cholesterol (HDL-C) has been regarded as a strong and independent negative risk factor for cardiovascular disease (CVD). However, the classic hypothesis that high HDL-C concentrations lead to CVD risk reduction has been challenged by intervention studies. Cholesterol efflux capacity (CEC) is one of the major functions of HDL, which contributes to the first step of the reverse cholesterol transport pathway by promoting efflux of excess cellular cholesterol.
CEC has been demonstrated to be an important factor preventing CVD\(^5\), \(^6\). Therefore, improving CEC of HDL particles, rather than increasing HDL-C levels, is considered to be a novel target for the prevention of CVD. Although HDL has been shown to have antioxidant properties\(^7\), \(^8\) and certain antioxidant foods reportedly increase CEC\(^9\), \(^10\), there have been few studies focusing on the relationships of CEC with oxygen radical absorbance capacity (ORAC) and lipophilic dietary antioxidant levels in serum\(^11\), \(^12\).

Healthy dietary patterns are considered to be beneficial for the prevention of cardiovascular events. The Mediterranean diet recommends olive oil, nuts and vegetables, and emphasizes consuming large amounts of foods containing antioxidants such as \(\alpha\)-tocopherol, polyphenols and carotenoids to protect against oxidation, and this diet has been reported to be beneficial for both preventing CVD\(^13\) and increasing CEC\(^14\).

The Japan Diet (JD) recommended by the Japan Atherosclerosis Society (JAS) for the prevention of atherosclerotic cardiovascular diseases\(^15\), \(^16\) features consuming more fish, soy and soy-products, vegetables, seaweed, mushrooms, konjak, and unpolished cereals instead of refined cereals, while concurrently consuming less animal fat and fatty meat and poultry, sweets and alcoholic drinks. We previously reported that a nutritional education program focusing on JD intake improved metabolic parameters in patients with dyslipidemia\(^17\). However, there have been no reports on the effects of the JD on CEC.

**Aim**

We investigated changes in CEC and the relationships of CEC with ORAC, and serum concentrations of tocopherols and carotenoids as lipid-soluble antioxidants, during a JD education program in patients with hyperlipidemia.

**Materials and Methods**

The study design, methods, and subjects were described in detail in our previous report\(^17\). Briefly, the recruited subjects were Japanese patients with dyslipidemia, between 30 and 65 years of age, with a body mass index (BMI) over 18.5 kg/m\(^2\), non-smokers, receiving consistent drug regimens and permitted to participate in the program by doctors certified by the JAS.

This study was conducted at Japan Women’s University according to the guidelines of the Declaration of Helsinki, and all procedures were approved by The Ethics Committee for Experimental Research involving Human Subjects of Japan Women’s University (No.246). Written informed consent was obtained from all subjects prior to participation. The clinical trial registration number is UMIN000022955.

This was a 6-month randomized parallel-group clinical trial. Participants were allocated to either the JD group or the Partial JD (PJD) group. Face-to-face nutritional education for each diet, at baseline and at 3 months, was provided by three registered dietitians from Japan Women’s University who had been specially trained for this study and we followed up the participants at 3 and 6 months.

For both diet groups, reductions in the intakes of animal fat, meat and poultry with fat, sweets, desserts and snacks, and alcoholic drinks were recommended. In addition, consuming more fish (especially fatty fish), soybeans and soy products (especially natto, a fermented soy product), vegetables including green and yellow vegetables, seaweed, mushrooms, konjak and unrefined cereals including barley were recommended for the JD group.

Three-day (two weekdays and 1 weekend day) weighted dietary records were kept at baseline, and at the ends of the 3- and 6-month follow-up periods. Nutrient intakes were calculated employing Excel-Eiyokun Ver.8.0, (Kenpaku-sha Co., Ltd., Tokyo, Japan) software.

At baseline, 3 months and 6 months, height, weight and blood pressure were measured and BMI was calculated as weight(kg)/height(m\(^2\)). Fasting blood collections were conducted in the morning following a 12-hour fast and the samples were centrifuged to obtain serum or plasma at each facility. Then, the serum or plasma samples were stored at \(-80^\circ\)C until analyses. The lipid parameters measured were total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), HDL-C, triglyceride (TG) and malondialdehyde-modified low-density lipoprotein (MDA-LDL).

**Analysis**

**Preparation of Apolipoprotein B (ApoB)-Depleted Plasma/Serum**

Plasma/serum samples were incubated with 20% polyethylene glycol solution in 0.2 mol/L glycine buffer (pH 7.4) for 20 minutes and these samples were centrifuged at 10,000 rpm and 4°C for 10 minutes. The supernatants were apoB-depleted plasma/serum.

**Cholesterol Efflux Capacity**

The protocol for CEC was previously described\(^5\), \(^18\). J774.1 cells (RIKEN, Saitama, Japan) cultured in Roswell Park Memorial Institute media containing
10% fetal bovine serum were kept under constant conditions of 5% carbon dioxide and 37°C. After the J774.1 cells had been seeded into 24-well plates and grown to 80% confluence, they were radiolabeled with 2 µCi/mL 3H-cholesterol in the presence of acyl-CoA: cholesterol acyltransferase (ACAT) inhibitor (2 µg/mL Sandoz 58-035, Sigma-Aldrich Corp., St. Louis, Missouri, USA) and 0.3 mM 8-Br-cAMP (Sigma-Aldrich) for up-regulating ATP-binding cassette transporter (ABCA1). Subsequently, an efflux medium containing 2.8% apoB-depleted plasma was added, followed by incubation for 4 hours. A liquid scintillation counter was used to quantify the efflux of radioactive cholesterol from the cells. The quantity of radioactive cholesterol incorporated into the cells was counted twice, through hexane: isopropanol (v:v, 1:1) extraction in control wells not exposed to the serum. Relative efflux was calculated using the following formula: (cpm of 3H-cholesterol in media containing 2.8% apoB-depleted serum - cpm of 3H-cholesterol in serum-free media)/(cpm of 3H-cholesterol before the efflux step). All assays were performed in duplicate. The CEC of patient plasma samples were expressed as the relative values to those of the pooled serum from six healthy volunteers.

**Oxygen Radical Absorbance Capacity**

The ORAC assay estimates the ability of serum to resist oxidative damage, reflecting the combined effects of all antioxidants in the serum, rather than any individual antioxidant. We measured serum ORAC and HDL ORAC using apoB-depleted serum.

Serum or apoB-depleted serum samples were diluted with phosphate buffer. Fluorescein (3’, 6’-dihydroxy-spiro-3-one; 0.15 µmol/L, Sigma-Aldrich Corp., St. Louis, Missouri, USA) was added to the serum or apoB-depleted sera as a target of free radical attack with the water soluble peroxy radical generator 2, 2’-azobis dihydrochloride (AAPH; 60 µmol/L, Sigma-Aldrich). Starting immediately after the addition of AAPH, fluorescence was measured every 2 minutes using the fluorescence spectrophotometer (TriStar LB942 Multimode Reader, Berthold Technologies GmbH & Co., KG, Bad Wildbad, Germany) for 90 minutes. The excitation wavelength was 485 nm, the emission wavelength 535 nm and the experimental temperature 37°C. A water-soluble Vitamin E analog, Trolox (Sigma-Aldrich), was used to establish a standard curve and the data were expressed as Trolox equivalents per volume. All assays were performed six times each.

**Tocopherols and Carotenoids**

Serum tocopherols and carotenoids were extracted according to the method reported by Johnson et al., and by Khachik et al. Serum was treated with ethanol to precipitate the proteins. Echinonone and tocol (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) dissolved in ethanol were added as internal standards for the carotenoid and tocopherol analyses, respectively. The mixture was extracted twice with hexane containing 20 mg/L butylated hydroxytoluene. The serum extracts were redissolved in 200 µL of ethanol, vortexed and passed through a 0.45 µm membrane filter. A 20 µL aliquot was used for HPLC analysis.

The HPLC system consisted of a Chromaster 5110 Pump (Hitachi Co., Tokyo Japan), Chromaster Oven (Hitachi), Chromaster 5440 FL Detector (Hitachi) (α-tocopherol and γ-tocopherol were monitored at Ex 297 and Em 327 nm, respectively), Chromaster 5430 Diode Array Detector (Hitachi) (lutein, zeaxanthin, β-cryptoxanthin, α-carotene, and β-carotene were monitored at UV 450 nm and lycopene was monitored at UV 480 nm), a C30 carotenoid column (3 µm 150×4.6 mm, YMC), and a guard column (YMC-PARTS XPGCH-W1). The HPLC mobile phase and gradient procedure according to Yeum et al. was methanol: methyl-tert-butyl ether: water (83:15:2, by vol, with 1.5% ammonium acetate in the water; solvent A) and methanol: methyl-butyl-ether: water (8:90:2, by vol, with 1% ammonium acetate in the water; solvent B). The gradient procedure, at a flow rate of 1 mL/min as follows: 1) 90% solvent A and 10% solvent B for 5 min, 2) a 12-min linear gradient to 5% solvent A, 3) a 12-min linear gradient to 95% solvent B, 4) a 5-min hold at 95% solvent B, and 5) a 1-min gradient back to 90% solvent A and 10% solvent B. Total tocopherol and carotenoid concentrations were calculated as the sum of α-tocopherol and γ-tocopherol and the sum of lutein, zeaxanthin, β-cryptoxanthin, α-carotene, and β-carotene and lycopene, respectively.

**Statistical Analysis**

Statistical analyses were carried out using SPSS for Windows (version 26; IBM Japan, Inc.). Normality was assessed by applying the Shapiro-Wilk test. All values are presented as means with standard deviation for the normal distribution and as medians (25th percentile, 75th percentile) for non-normal distributions. The t test or the Mann-Whitney U test was used to compare mean differences between the PJD and JD groups. The paired t test or Wilcoxon’s signed-rank test was used to analyze differences from the baseline concentrations. The statistical significances of correlations were assessed using the Pearson or non-parametric Spearman’s ranked test. P
change in response to the intervention, though HDL ORAC and serum ORAC were decreased in both groups \( (p<0.001\) and there was no difference between the two groups (Table 1).

Serum total tocopherol, especially \( \alpha \)-tocopherol, was decreased at 6 months in the JD group as compared to the PJD group \( (p<0.05\). Serum total carotenoid increased in both groups after the dietary interventions. Changes in serum \( \beta \)-carotene, which accounted for more than 40% of all carotenoids, tended to be larger in the JD group than in the PJD group at 3 months \( (p<0.05)\) (Table 2).

Food and nutrient intakes during the intervention were described in detail in our previous report. Briefly, intakes of energy, protein and fat were decreased at 6 months in both groups. There were no changes in either group’s tocopherol intake. Though \( \alpha \) and \( \beta \)-carotene intake was lower at baseline in the JD group than in the PJD group, changes in \( \beta \)-carotene \( (p<0.001)\), \( \alpha \)-carotene and \( \beta \)-cryptoxanthin \( (p<0.05)\) intakes in the JD group ware larger than those in the PJD group at 3 months \( (p=0.055)\) (Table 2).

Food and nutrient intakes during the intervention were described in detail in our previous report. Briefly, intakes of energy, protein and fat were decreased at 6 months in both groups. There were no changes in either group’s tocopherol intake. Though \( \alpha \) and \( \beta \)-carotene intake was lower at baseline in the JD group than in the PJD group, changes in \( \beta \)-carotene \( (p<0.001)\), \( \alpha \)-carotene and \( \beta \)-cryptoxanthin \( (p<0.05)\) intakes in the JD group ware larger than those in the PJD group at 3 months (Supplemental Table 2).

In total, CEC showed a positive correlation with HDL-C at baseline, 3 months, and 6 months \( (r=0.72, 0.75 and 0.63, \text{ respectively})\). Furthermore, CEC correlated negatively with BMI and TG at all cross-sectional points and MDA-LDL at 3 and 6 months. CEC correlated positively with serum \( \beta \)-cryptoxanthin, \( \alpha \)-carotene, and \( \beta \)-carotene at baseline \( (p<0.05)\), and the correlations with serum lutein+zeaxanthin and total carotenoid became significant at 3 and 6 months.

<0.05 was considered to indicate a statistically significant difference.

## Results

The characteristics and clinical backgrounds of the 98 participants \( (\text{PJD: } n=49, \text{JD: } n=49)\), imputations were used to replace missing data, employing the last observation carried forward method) with dyslipidemia at baseline and the changes in clinical parameters after the dietary interventions were detailed in our previous report\(^ {17} \). At the 6-month follow-up, 43 participants \( (87.8\%)\) in each of the diet groups had completed the study. Of these 43 participants, 79% answered that they had adhered to the recommended diet. Briefly, mean HDL-C at baseline was 62 mg/dL \( (\text{range: } 24 \text{ to } 114 \text{ mg/dL})\) and 7% of participants had hypo-alpha lipoproteinemia defined as HDL-C levels less than 40 mg/dL, representing a minority with abnormal HDL metabolism based on quantity. After the intervention, BMI decreased in both groups \( (p<0.001)\). TC, LDL-C and TG concentrations were decreased at 6 months in the JD group as compared to the PJD group \( (p<0.01)\). HDL-C did not change in either group (Supplemental Table 1).

Mean CEC was 1.05 \( (\text{min } 0.76, \text{ max } 1.62)\) and the number of participants whose CEC was equal to or greater than 1.0 was 58 \( (60\%)\) in total at baseline. CEC at baseline was independent of the presence/absence of lipid lowering therapy. CEC did not

### Table 1. Changes in cholesterol efflux capacity and oxygen radical absorbance capacity

|                      | PJD group \( (n=49) \) | JD group \( (n=49) \) | Between-group comparisons at 3 and 6 months \( (\text{JD - PJD}) \) |
|----------------------|------------------------|------------------------|---------------------------------------------------------------|
|                      | Mean \( (\text{SD}) \) | Mean \( (\text{SD}) \) | Difference \( (95\% \text{ CI}) \) |
| CEC\(^g\)            |                        |                        | \( p^f \)                                                     |
| Baseline             | 1.05 \( (0.14) \)      | 1.04 \( (0.16) \)      | -0.01 \( (-0.05, 0.03)\) \( p=0.58 \)                        |
| 3-month              | 1.06 \( (0.17) \)      | 1.04 \( (0.16) \)      | 0.00 \( (-0.05, 0.05)\) \( p=0.91 \)                        |
| 6-month              | 1.03 \( (0.18) \)      | 1.02 \( (0.15) \)      | -0.01 \( (-0.05, 0.03)\) \( p=0.58 \)                        |
| HDL ORAC (TE \( \mu \text{mol/L} \)) |                        |                        | \( p^f \)                                                     |
| Baseline             | 176 \( (26) \)         | 176 \( (24) \)         | 1 \( (-6, 8)\) \( p=0.73 \)                                  |
| 3-month              | 173 \( (30) \)         | 174 \( (28) \)         | 1 \( (-6, 8)\) \( p=0.73 \)                                  |
| 6-month              | 161 \( (33) \)         | 160 \( (29) \)         | 1 \( (-10, 8)\) \( p=0.84 \)                                 |
| Serum ORAC (TE \( \mu \text{mol/L} \)) |                        |                        | \( p^f \)                                                     |
| Baseline             | 276 \( (30) \)         | 276 \( (35) \)         | -6 \( (-13, 2)\) \( p=0.15 \)                               |
| 3-month              | 271 \( (29) \)         | 265 \( (36) \)         | -6 \( (-13, 2)\) \( p=0.15 \)                               |
| 6-month              | 257 \( (39) \)         | 253 \( (40) \)         | -3 \( (-14, 8)\) \( p=0.54 \)                               |

CEC, Cholesterol efflux capacity; ORAC, oxygen radical absorbance capacity; TE, trolox equivalent.
\(^p\) values within-group comparisons from baseline are analyzed by the paired \( t \) test.
\(^f\) values between-group comparisons are analyzed by the unpaired \( t \) test.
\(^g\) CEC is expressed as the ratio of efflux in the sample, normalized to a reference sample.
\(^f\) Values are expressed as means (95\% CI).
Table 2. Changes in serum tocopherol and carotenoid concentrations

|                       | PJD group (n = 49) | JD group (n = 49) | Between-group comparisons at 3 and 6 months (JD - PJD) |
|-----------------------|--------------------|------------------|------------------------------------------------------|
|                       | Median (25th percentile, 75th percentile) | Median (25th percentile, 75th percentile) | Difference (95% CI) | p²        |
| **Total tocopherol** |                    |                  |                                                      |           |
| Baseline              | 32.3 (25.7, 37.0)  | 30.2 (28.1, 34.4) | -1.3 (-3.0, 0.5) § | 0.055     |
| 3-month               | 32.6 (26.6, 36.9)  | 29.8 (26.8, 32.2) | 0.008                                               |           |
| 6-month               | 30.6 (26.7, 36.3)  | 28.4 (26.4, 30.9) | 0.000                                               |           |
| **α-tocopherol**      |                    |                  |                                                      |           |
| Baseline              | 28.5 (23.4, 33.0)  | 27.3 (24.8, 30.4) | -1.1 (-2.7, 0.6) § | 0.091     |
| 3-month               | 28.4 (24.0, 33.0)  | 26.6 (23.5, 29.1) | 0.008                                               |           |
| 6-month               | 28.1 (24.4, 32.2)  | 25.6 (23.5, 27.1) | 0.000                                               |           |
| **γ-tocopherol**      |                    |                  |                                                      |           |
| Baseline              | 3.1 (2.6, 4.2)     | 3.3 (2.5, 4.0)    | -0.2 (-0.6, 0.2) § | 0.22      |
| 3-month               | 3.3 (2.4, 4.1)     | 3.2 (2.6, 3.9)    | 0.31                                                |           |
| 6-month               | 3.0 (2.4, 4.3)     | 3.3 (2.6, 4.0)    | 0.16                                                |           |
| **Total carotenoid**  |                    |                  |                                                      |           |
| Baseline              | 2.97 (2.29, 4.64)  | 2.73 (2.06, 4.26) | 0.27 (-0.18, 0.72) § | 0.14      |
| 3-month               | 3.44 (2.29, 4.89)  | 3.25 (2.21, 4.63) | 0.063                                               |           |
| 6-month               | 3.97 (2.41, 5.57)  | 3.04 (2.04, 4.74) | 0.061                                               |           |
| **Lutein + zeaxanthin** |                |                  |                                                      |           |
| Baseline              | 0.59 (0.53, 0.77)  | 0.55 (0.48, 0.64) | 0.00 (-0.07, 0.06) § | 0.81      |
| 3-month               | 0.62 (0.48, 0.89)  | 0.58 (0.47, 0.74) | 0.063                                               |           |
| 6-month               | 0.70 (0.56, 0.90)  | 0.62 (0.49, 0.72) | 0.061                                               |           |
| **β-cryptoxanthin**   |                    |                  |                                                      |           |
| Baseline              | 0.23 (0.13, 0.36)  | 0.20 (0.13, 0.34) | -0.05 (-0.12, 0.01) § | 0.13      |
| 3-month               | 0.24 (0.15, 0.37)  | 0.21 (0.15, 0.46) | 0.069                                               |           |
| 6-month               | 0.29 (0.18, 0.45)  | 0.20 (0.16, 0.35) | 0.16                                                |           |
| **α-carotene**        |                    |                  |                                                      |           |
| Baseline              | 0.30 (0.20, 0.45)  | 0.30 (0.19, 0.43) | -0.01 (-0.09, 0.07) § | 0.11      |
| 3-month               | 0.32 (0.21, 0.63)  | 0.31 (0.20, 0.51) | 0.63                                                |           |
| 6-month               | 0.38 (0.23, 0.54)  | 0.26 (0.18, 0.45) | 0.44                                                |           |
| **β-carotene**        |                    |                  |                                                      |           |
| Baseline              | 1.28 (0.61, 2.00)  | 0.98 (0.68, 1.94) | 0.15 (-0.07, 0.38) § | 0.055     |
| 3-month               | 1.32 (0.64, 2.42)  | 1.25 (0.72, 2.27) | 0.000                                               |           |
| 6-month               | 1.59 (0.67, 2.47)  | 1.27 (0.62, 2.51) | 0.000                                               |           |
| **Lycopene (trans+cis)** |                |                  |                                                      |           |
| Baseline              | 0.58 (0.36, 0.97)  | 0.55 (0.30, 0.84) | 0.01 (-0.15, 0.16) § | 0.88      |
| 3-month               | 0.63 (0.35, 0.94)  | 0.51 (0.33, 0.79) | 0.36                                                |           |
| 6-month               | 0.68 (0.41, 0.90)  | 0.51 (0.32, 0.81) | -0.08 (-0.25, 0.08) § | 0.11      |

1p values within-group comparisons from baseline are analyzed by the Wilcoxon signed-rank test.

2p values between-group comparisons are analyzed by the Mann–Whitney U test.

3Values are expressed as means (95% CI).

4Total tocopherols are the sum of α-tocopherol and γ-tocopherol.

5Total carotenoids are the sum of lutein, zeaxanthin, β-cryptoxanthin, α-carotene, β-carotene and lycopene.

The CEC change correlated positively with those in HDL-C, TG, both serum α- and γ-tocopherol, and HDL ORAC (Table 3, Fig.1). The HDL ORAC correlated positively with HDL-C at baseline and at 3 months. Change in HDL ORAC correlated positively with changes in serum total tocopherol at 3 and 6 months and with α-tocopherol at 3 months (Table 4). Serum α-tocopherol showed positive correlations with TC, LDL-C, TG, and MDA-LDL at all cross-sectional points (p < 0.01) and α-tocopherol change correlated positively with all lipid parameters including HDL-C (p < 0.01).
Fig. 1. Correlation between changes in CEC and serum α and γ-tocopherol concentrations at 3 and 6 months

\( n = 98 \) (PJD group, \( n = 49 \); JD group, \( n = 49 \))

**Table 3.** Correlation with CEC at baseline, 3 and 6 months and their changes

|                           | baseline | 3-month | 6-month | Δ3-0 month | Δ6-0 month |
|---------------------------|----------|---------|---------|------------|------------|
| Body Mass Index           | -0.319** | -0.359** | -0.291** | 0.117      | 0.023      |
| LDL-cholesterol           | 0.113    | 0.058   | -0.031  | -0.045     | 0.042      |
| HDL-cholesterol           | 0.720**  | 0.749** | 0.627** | 0.418**    | 0.463**    |
| Triglyceride              | -0.281** | -0.286** | -0.265** | 0.217*     | 0.191      |
| MDA-LDL                  | -0.036   | -0.214* | -0.337** | -0.051     | 0.051      |
| Total tocopherol\(^1\)    | 0.184    | 0.123   | 0.173   | 0.428**    | 0.346**    |
| α-tocopherol              | 0.168    | 0.131   | 0.162   | 0.361**    | 0.334**    |
| γ-tocopherol              | 0.105    | 0.051   | 0.122   | 0.446**    | 0.231*     |
| Total carotenoids\(^2\)   | 0.185    | 0.281** | 0.215*  | 0.134      | 0.026      |
| Lutein + zeaxanthin       | 0.196    | 0.309** | 0.250*  | 0.138      | 0.047      |
| β-cryptoxanthin           | 0.241*   | 0.201*  | 0.146   | -0.153     | 0.029      |
| α-carotene                | 0.265**  | 0.304** | 0.221*  | 0.159      | 0.123      |
| β-carotene                | 0.219*   | 0.313** | 0.212*  | 0.094      | 0.035      |
| Lycopene (trans+cis)      | -0.080   | -0.017  | 0.065   | 0.103      | -0.027     |
| HDL ORAC                  | 0.232*   | 0.186   | 0.040   | 0.289**    | 0.188      |
| serum ORAC                | -0.022   | -0.010  | -0.079  | -0.004     | 0.109      |

Values are expressed in correlation coefficients; \( r \) or \( r_s \) (\( p \) values).

Values are analyzed using Pearson’s or Spearman’s rank correlation. \(* p < 0.05; ** p < 0.01\)

\(^1\)Total tocopherols are the sum of α-tocopherol and γ-tocopherol.

\(^2\)Total carotenoids are the sum of lutein, zeaxanthin, β-cryptoxanthin, α-carotene, β-carotene and lycopene.
whether or not a PPAR-α agonist was being taken, and none of the subjects had been prescribed a PPAR-β/δ agonist at baseline. Though no difference in mean CEC was observed, MDA-LDL and TG were shown to correlate negatively with CEC at cross-sectional points in this study. Oxidized LDL measured by the ELISA method using an oxidized LDL antibody was revealed to have a negative association with CEC in obese Austrian adults\(^23\), an observation consistent with the MDA-LDL results in this study. Reported correlations between CEC and circulating TG are not consistent. TG was reported to be positively associated with CEC in subjects with HDL-C ≤ 40 mg/dL but there was no association in subjects with HDL-C ≥ 40 mg/dL in the PHINOX study\(^24\). On the other hand, TG did not differ between groups of Japanese coronary artery disease patients with impaired versus enhanced CEC\(^25\) and were not related in control participants in the EPIC-Norfolk study\(^26\). ABCA1-CEC, which is calculated as ABCA1 up-regulated CEC minus non up-regulated CEC, was higher in patients with TG ≥ 300 mg/dL than in healthy controls\(^27\). A positive association between TG and ABCA1-CEC was also reported in participants including those with coronary heart disease\(^28\). Although reasons for the mixed results due to lipid levels are unclear, different CEC methodologies, few subjects with TG ≥ 300 mg/dL in

### Discussion

This is the first study to examine the effects of the JD on CEC in Japanese patients with dyslipidemia. We found that JD did not change CEC despite significant reductions in serum lipids in response to the JD\(^17\).

CEC has been measured by several methods, which differ in the cells used, the transporters up-regulated such as ABCA1, ATP binding cassette subfamily G member 1 (ABCG1) and scavenger receptor class B member 1 (SRB1), and the types of reagents used for labeling. In this study, J774 cells were radiolabeled with \(^3\)H-cholesterol in the presence of an ACAT inhibitor and 8-Br-cAMP to up-regulate ABCA1. Mean CEC at baseline was greater than 1.0, and CEC showed a high positive correlation with HDL-C, indicating CEC to not be impaired or to even be better than in healthy controls. We found that the JD did not change CEC despite serum lipids being significantly reduced by the JD\(^17\), because the lack of change in CEC in response to the diet was partly due to the relatively good CEC at baseline. In previous reports employing the same method as this study, a PPAR-α agonist\(^22\) and a PPAR-γ agonist increased CEC, while an HMG-CoA reductase inhibitor had no effect on CEC\(^9\). In this study, CEC did not differ among the participants regardless of whether or not a PPAR-α agonist was being taken, and none of the subjects had been prescribed a PPAR-γ agonist at baseline.

Though no difference in mean CEC was observed, MDA-LDL and TG were shown to correlate negatively with CEC at cross-sectional points in this study. Oxidized LDL measured by the ELISA method using an oxidized LDL antibody was revealed to have a negative association with CEC in obese Austrian adults\(^23\), an observation consistent with the MDA-LDL results in this study. Reported correlations between CEC and circulating TG are not consistent. TG was reported to be positively associated with CEC in subjects with HDL-C ≤ 40 mg/dL but there was no association in subjects with HDL-C ≥ 40 mg/dL in the PHINOX study\(^24\). On the other hand, TG did not differ between groups of Japanese coronary artery disease patients with impaired versus enhanced CEC\(^25\) and were not related in control participants in the EPIC-Norfolk study\(^26\). ABCA1-CEC, which is calculated as ABCA1 up-regulated CEC minus non up-regulated CEC, was higher in patients with TG ≥ 300 mg/dL than in healthy controls\(^27\). A positive association between TG and ABCA1-CEC was also reported in participants including those with coronary heart disease\(^28\). Although reasons for the mixed results due to lipid levels are unclear, different CEC methodologies, few subjects with TG ≥ 300 mg/dL in

### Table 4. Correlation with HDL ORAC at baseline, 3 and 6 months and their changes

|                        | baseline | 3-month | 6-month | Δ3-0 month | Δ6-0 month |
|------------------------|----------|---------|---------|------------|------------|
| Body Mass Index        | 0.018    | 0.007   | 0.007   | -0.046     | 0.134      |
| LDL-cholesterol        | 0.182    | -0.039  | -0.033  | 0.079      | 0.064      |
| HDL-cholesterol        | 0.217*   | 0.207*  | 0.150   | 0.222*     | 0.118      |
| Triglyceride           | 0.088    | 0.058   | 0.058   | 0.051      | 0.069      |
| MDA-LDL                | 0.082    | 0.065   | 0.030   | 0.088      | 0.071      |
| Total tocopherol       | 0.087    | 0.100   | 0.035   | 0.256*     | 0.202*     |
| α-tocopherol           | 0.083    | 0.106   | 0.003   | 0.255*     | 0.196      |
| γ-tocopherol           | 0.093    | -0.030  | 0.098   | 0.119      | 0.185      |
| Total carotenoid\(^†\) | -0.038   | 0.023   | -0.116  | 0.216*     | -0.056     |
| Lutein + zeaxanthin    | 0.023    | 0.139   | -0.032  | 0.161      | -0.034     |
| β-cryptoxanthin        | -0.019   | 0.157   | -0.040  | 0.022      | 0.020      |
| α-carotene             | -0.043   | -0.128  | -0.179  | 0.025      | 0.092      |
| β-carotene             | -0.047   | -0.019  | -0.113  | 0.200*     | -0.074     |
| Lycopene (trans+cis)   | 0.040    | -0.052  | -0.160  | -0.021     | -0.060     |
| serum ORAC             | 0.380**  | 0.473** | 0.382** | 0.175      | 0.202*     |

\(n=98\) (PJG group, \(n=49\); JD group, \(n=49\))

CEC, Cholesterol efflux capacity; ORAC, oxygen radical absorbance capacity; MDA-LDL, malondialdehyde modified-low density lipoprotein.

Values are expressed correlation coefficients; \(r\) or \(r_s\) (\(p\) values).

Values are analyzed using Pearson's or Spearman's rank correlation. \(*p<0.05\); \(**p<0.01\)

\(^†\)Total tocopherols are the sum of α-tocopherol and γ-tocopherol.

\(^\dagger\)Total carotenoids are the sum of lutein, zeaxanthin, β-cryptoxanthin, α-carotene, β-carotene and lycopene.
the present study, and higher CEC values at baseline may account for the differences.

Regarding nutrients, serum α-tocopherol concentrations decreased in the JD group without changes in tocopherol intake. Around 60–90% of serum α-tocopherol is distributed to very low-density lipoprotein (VLDL) and LDL and reportedly to be involved in the secretions of α-tocopherol in states of low LDL without α-tocopherol supplementation. Therefore, serum tocopherol concentration might be due to the decreases in LDL-C and TG in response to JD intake. α-tocopherol is consumed as an antioxidant for prevention of lipid peroxidation. It has also been reported that the higher the degree of unsaturation, the greater the need for α-tocopherol. The JD group participants were recommended to increase intake of fish, which contains polyunsaturated fatty acids such as eicosapentaenoic acid and docosahexaenoic acid, possibly resulting in the need for more α-tocopherol for protection from oxidation than in the PJD group. Furthermore, the serum α-tocopherol concentration might have been decreased in the JD group.

As previously reported, supplementation of α-tocopherol increased CEC in patients with end-stage renal disease, a finding consistent with that in patients with type 1 diabetes. CEC changes correlated positively with serum α-tocopherol and γ-tocopherol in this study. In cell and animal experiments, ABCA1 and SR-B1 were reported to be involved in the secretions of α-tocopherol and γ-tocopherol from tissues and in their intestinal absorption. In addition, α-tocopherol has been shown to activate the ABCA1 transporter in apoE-/- mice, which may affect the capacity for cholesterol efflux via ABCA1. We consider our results to be supported by these previous studies.

It is noteworthy that, in this study, not only serum α-tocopherol but also γ-tocopherol appeared to have a positive correlation with CEC. The serum γ-tocopherol concentration did not change whereas α-tocopherol decreased with serum lipid reduction. The usefulness of γ-tocopherol has not been emphasized in terms of its physiological activity or lipid peroxidation reactivity. However, the γ-tocopherol level has been reported to be inversely correlated with coronary heart disease and supplementation of γ-tocopherol alone or with α-tocopherol reduced biomarkers of oxidative stress in patients with metabolic syndrome. Intake of γ-tocopherol, which is contained in vegetable oils, nuts and seeds, soybeans, and so on, was the highest among tocopherol analogues such as α, β, γ and δ. Therefore, serum γ-tocopherol might serve a compensatory function in response to decreased α-tocopherol in states of low LDL without α-tocopherol supplementation.

Higher circulating ORAC has been reported in subjects on the Mediterranean and Dietary Approaches to Stop Hypertension (DASH) diets, and ORAC increases with phytochemical supplementation from oat porridge, white mushrooms and coffee. We predicted that JD intake would improve circulating oxidative stress in patients with dyslipidemia. Although a weak but positive correlation between CEC and HDL ORAC was present at baseline, total and HDL ORAC decreased in both of our study groups after the interventions. Decreases in ABCA1-CEC and HDL ORAC have been reported after weight loss in overweight and obese women, findings consistent with those of our present study. Nutrients such as tocopherol, vitamin C and flavonoids are well known to have radical scavenging capacity, as reflected by ORAC. The oxidized state of HDL has been suggested to be related to CEC, but only serum tocopherol concentrations were measured in this study. We found HDL ORAC to not be related to the serum tocopherol concentration at cross sectional points but we did detect relationships between changes in HDL ORAC which showed moderately positive correlations with tocopherol and β-carotene. Changes in serum α-tocopherol and ORAC were reported to be positively related in the DASH-sodium trial, while the diet high in fruits and vegetables trial showed no relationship between serum α-tocopherol and ORAC.

Multiple antioxidant systems might work synergistically to enhance total antioxidant capacity. Further study is needed to elucidate the relationship between antioxidant capacity and CEC.

Carotenoids were measured as parameters of vegetable intake, and it was confirmed that green-yellow vegetables and serum carotenoids increased due to the JD intervention. Serum carotenoid levels correlated positively with CEC at cross-sectional measurements. In a previous study on the relationship between CEC and carotenoids, using RAW264.7 cells, β-carotene was shown to be associated with increased CEC, and 9-cis β-carotene in particular increased the RNA expression levels of ABCA1 and ABCG1. It has been suggested that CEC might be increased by retinoic acid converted from β-carotene, which reportedly improves CEC. On the other hand, in an in vivo trial, β-cryptoxanthin or lycopene, which are non-provitamin A carotenoids, increased ABCA1. Although it can be said that carotenoids are related to CEC, the carotenoid concentration in our study was lower than those in the aforementioned in vivo studies and the correlation with CEC was weak.
This study has major limitations. First, our CEC measuring method employed a variety of cells, up-regulated transporters and reagent types used for labeling. Second, CEC values in our participants were normal at baseline. Third, since we did not measure antioxidants closely related to HDL such as PON1 or the antioxidants reflected in ORAC such as vitamin C and flavonoids, the relationship between CEC and antioxidant capacity could not be fully evaluated. Finally, our study's sample size was not sufficient for multiple regression analysis. However, we did obtain results suggesting that dietary components such as tocopherol and carotenoids affect CEC in patients with dyslipidemia.

Conclusion

HDL function, i.e., CEC, was not changed by the JD in Japanese patients with dyslipidemia who already had normal CEC at baseline. CEC has been suggested to be positively associated with serum α and γ-tocopherol concentrations and HDL ORAC. Further studies are needed on the JD education program to improve CEC in subjects with severe dyslipidemia.

Acknowledgements

The authors thank all patients who participated in the present study, and all staff members who supported the survey. The authors also appreciate all nutrition study team members of the laboratory of Nutrition Education and Clinical Nutrition of Japan Women's University, including Ms. Kanako Kamoshita, Ms. Seina Komine, Ms. Sayaka Hasegawa, Ms. Rina Ichiki, Ms. Kanako Chibai, Ms. Chieko Fukuda, Ms. Miyu Oshika, Ms. Sia SuHuai, Ms. Moe Matsumoto, Ms. Saaya Yamada, Ms. Mariko Nakazawa, Ms. Yui Nishikata, Ms. Sayuri Igawa, Ms. Hazuki Kitayama, Ms. Mayuka Kodama, Ms. Kirika Fujitani, Ms. Aoi Tokunaga, Ms. Akari Yasuda, Ms. Hinako Omata, Ms. Saori Toyota, Ms. Kana Kinugawa, Ms. Mai Murano, Ms. Chisato Ogawa, and Ms. Nana Mihara, all of whom made major contributions to calculating the nutrient intakes in this study.

Financial Support

This study was supported by a research grant from the SKYLARK Food Science Institute and Rice Stable Supply Support Organization. This study was also supported by gifts of canned mackerel from Maruha Nichiro corporation, Tokyo, retort-packed rice with barley, Mochimugi Gohan from Hakubaku Co., Ltd. Tokyo and Omugi Gohan from Otsuka Pharmaceutical Co., Ltd. The SKYLARK Food Science Institute, Rice Stable Supply Support Organization, Maruha Nichiro corporation and Hakubaku Co., Ltd. had no role in the design, analysis or writing of this article.

Conflict of Interest

The authors report the following disclosures: Masako Waki has received clinical research funding from AstraZeneca KK and Eli Lilly Japan KK. Tamio Teramoto has received clinical research funding from Dai-ichi Sankyo KK. Chizuko Maruyama has received scholarship grant from Rice Stable Supply Support Organization. Ariko Umezawa, Yasuhiro Endo, Yumiko Suenaga, Yuri Shijo, Noriko Kameyama, Aisa Sato, Ai Nishitani, Makoto Ayaori, Katsunori Ikewaki have no conflicts of interest.

References

1) Wilson PW, Abbott RD, and Castelli WP: High density lipoprotein cholesterol and mortality. The Framingham Heart Study. Arteriosclerosis, 1988; 8: 737-741
2) Talbot CPJ, Plat J, Ritsch A, and Mensink RP: Determinants of cholesterol efflux capacity in humans. Prog Lipid Res, 2018; 69: 21-32
3) van Capelleveen JC, Brewer HB, Kastelein JJ, and Hovingh GK: Novel therapies focused on the high-density lipoprotein particle. Circ Res, 2014; 114: 193-204
4) de Goma EM, de Goma RL, and Rader DJ: Beyond high-density lipoprotein cholesterol levels evaluating high-density lipoprotein function as influenced by novel therapeutic approaches. J Am Coll Cardiol, 2008; 51: 2199-2211
5) Khera AV, Cuchel M, de la Llera-Moya M, Rodrigues A, Burke MF, Jafri K, French BC, Phillips JA, Mucksavage ML, Wilensky RL, Mohler ER, Rader DJ: Cholesterol efflux capacity, high-density lipoprotein function, and atherosclerosis. N Engl J Med, 2011; 364: 127-135
6) Rohatgi A, Khera A, Berry JD, Givens EG, Ayers CR, Wedin KE, Neeland IJ, Yuhanna IS, Rader DR, de Lemos JA, and Shaul PW: HDL cholesterol efflux capacity and incident cardiovascular events. N Engl J Med, 2014; 371: 2383-2393
7) Eren E, Yilmaz N, and Aydin O: High density lipoprotein and its dysfunction. Open Biochem J, 2012; 6: 78-93
8) Barter PJ, Nicholls S, Rye KA, Anantharamaiah GM, Navab M, and Fogelman AM: Antiinflammatory properties of HDL. Circ Res, 2004; 95: 764-772
9) Zhu Y, Huang X, Zhang Y, Wang Y, Liu Y, Sun R, and Xia M: Anthocyanin supplementation improves HDL-associated paraoxonase 1 activity and enhances cholesterol efflux capacity in subjects with hypercholesterolemia. J Clin Endocrinol Metab, 2014; 99: 561-569
10) Hernández A, Fernández-Castillejo S, Farràs M, Catalán Ú, Subirana I, Montes R, Solà R, Muñoz-Aguayo D, 889
Gelabert-Gorgues A, Díaz-Gil Ó, Nyysönen K, Zunft HJ, de la Torre R, Martín-Peláez S, Pedret A, Remaley AT, Covas MI, and Fitó M: Olive oil polyphenols enhance high-density lipoprotein function in humans: a randomized controlled trial. Arterioscler Thromb Vasc Biol, 2014; 34: 2115-2119

11) Mune M, Uto-Kondo H, Iteya I, Fujii Y, Ikeda S, and Ikewaki K: Vitamin E supplementation improves high-density lipoprotein and endothelial functions in end-stage kidney disease patients undergoing hemodialysis. Clin Nephrol, 2018; 90: 212-221

12) Costacou T, Levy AP, Miller RG, Snell-Bergeon J, Asleh R, Farbstein D, Fickley CE, Pambianco G, de la Vega R, Evans RW, and Orchard TJ: Effect of vitamin E supplementation on HDL function by haptoglobin genotype in type 1 diabetes: results from the HapE randomized crossover pilot trial. Acta Diabetol, 2016; 53: 243-250

13) Ros E, Martínez-González MA, Estruch R, Salas-Salvadó J, Fitó M, Martinez JA, and Corella D: Mediterranean diet and cardiovascular health: Teachings of the PREDIMED study. Adv Nutr, 2014; 5: 3305-336S

14) Hernández A, Castañer O, Elosua R, Pintó X, Estruch R, Salas-Salvadó J, Corella D, Arós F, Serra-Majem L, Fiol M, Ortega-Calvo M, and Ros E, Martínez-González MA, de la Torre R, López-Sabater MC, Fitó M: Mediterranean diet Improves high-density lipoprotein function in high-cardiovascular-risk individuals: A randomized controlled trial. Circulation, 2017; 135: 633-643

15) Teramoto T, Sasaki J, Ishibashi S, Birou S, Daida H, Ohishi S, Egusa G, Hiro T, Hirobe K, Iida M, Kihara S, Kinoshita M, Maruyama C, Ohta T, Okamura T, Yamashita S, Yokode M, and Yokote K: Treatment A) lifestyle modification: executive summary of the Japan Atherosclerosis Society (JAS) guidelines for the diagnosis and prevention of atherosclerotic cardiovascular diseases in Japan--2012 version. J Atheroscler Thromb, 2013; 20: 835-849

16) Kinoshita M, Yokote K, Arai H, Iida M, Ishigaki Y, Ishibashi S, Umemoto S, Egusa G, Ohmura H, Okamura T, Kihara S, Koba S, Saito I, Shoji T, Daida H, Tsukamoto K, Deguchi J, Dohi S, Dobashi K, Hamaguchi H, Hara M, Hiro T, Biro S, Fujioka Y, Maruyama C, Miyamoto Y, Murakami Y, Yokode M, Yoshida H, Rakugi H, Watai T, and Yamashita S: Committee for Epidemiology and Clinical Management of Atherosclerosis: Japan Atherosclerosis Society (JAS) guidelines for prevention of atherosclerotic cardiovascular diseases 2017. J Atheroscler Thromb, 2018; 25: 846-984

17) Maruyama C, Shijo Y, Kameyama N, Umezawa A, Sato A, Nishitani A, Ayaoi M, Ikewaki K, Waki M, and Teramoto T: Effects of dietary education program for the Japan Diet on metabolic and chronic inflammation parameters: a randomized controlled trial. J Atheroscler Thromb, 2021; 28: 1035-1051

18) Ishikawa T, Ayaoi M, Uto-Kondo H, Nakajima T, Mutoh M, and Ikewaki K: High-density lipoprotein cholesterol efflux capacity as a relevant predictor of atherosclerotic coronary disease. Atherosclerosis, 2015; 242: 318-322

19) Johnson EJ, Qin J, Krinsky NI, and Russell RM: Beta-carotene isomers in human serum, breast milk and buccal mucosa cells after continuous oral doses of all-trans and 9-cis beta-carotene. J Nutr, 1997; 127: 1993-1999

20) Khachik F, Spangler CJ, Smith JC Jr, Canfield LM, Steck A, and Pfander H: Identification, quantification, and relative concentrations of carotenoids and their metabolites in human milk and serum. Anal Chem, 1997; 69: 1873-1881

21) Yeum KJ, Booth SL, Sadowski JA, Liu C, Tang G, Krinsky NI, and Russell RM: Human plasma carotenoid response to the ingestion of controlled diets high in fruits and vegetables. Am J Clin Nutr, 1996; 64: 594-602

22) Khra AV, Millar JS, Ruotolo G, Wang MD, and Rader DJ: Potent peroxisome proliferator-activated receptor-α agonist treatment increases cholesterol efflux capacity in humans with the metabolic syndrome. Eur Heart J, 2015; 36: 3020-3022

23) Marsche G, Zelzer S, Meinitzer A, Kern S, Meissl S, Pregartner G, Weghuber D, Almer G, and Manzge H: Adiponectin predicts high-density lipoprotein cholesterol efflux capacity in adults Irrespective of body mass index and fat distribution. J Clin Endocrinol Metab, 2017; 102: 4117-4123

24) Weibel GL, Drazul-Schrader D, Shivers DK, Wade AN, Rothblat GH, Reilly MP, and de la Llera-Moya M: Importance of evaluating cell cholesterol influx with efflux in determining the impact of human serum on cholesterol metabolism and atherosclerosis. Arterioscler Thromb Vasc Biol, 2014; 34: 17-25

25) Hisauchi I, Ishikawa T, Ayaoi M, Uto-Kondo H, Koshikawa Y, Ukaji T, Nakamura H, Mizutani Y, Taguchi I, Nakajima T, Mutoh M, and Ikewaki K: High-density lipoprotein cholesterol efflux capacity as a novel prognostic surrogate for coronary artery disease. J Atheroscler Thromb, 2021; 28: 696-702

26) Saleheen D, Scott R, Javad S, Zhao W, Rodrigues A, Picataggio A, Lukmanova D, Muckavage ML, Luben R, Billheimer J, Kastelein JJ, Boekholdt SM, Khaw KT, Wareham N, and Rader DJ: Association of HDL cholesterol efflux capacity with incident coronary heart disease events: a prospective case-control study. Lancet Diabetes Endocrinol, 2015; 3: 507-513

27) Asztalos BF, Horvath KV, Mehan M, Yokota Y, and Schaefer EJ: Influence of HDL particles on cell-cholesterol efflux under various pathological conditions. J Lipid Res, 2017; 58: 1238-1246

28) Asztalos BF, Horvath KV, and Schaefer EJ: High-density lipoprotein particles, cell-cholesterol efflux, and coronary heart disease risk. Arterioscler Thromb Vasc Biol, 2018; 38: 2007-2015

29) Bjorronson LD, Kayden HJ, Miller E, and Mosshell AN: The transport of alpha-tocopherol and beta-carotene in human blood. J Lipid Res, 1976; 17: 343-352

30) Lewis JS, Pian AK, Baer MT, Acosta PB, and Emerson GA: Effect of long-term ingestion of polyunsaturated fat, age, plasma cholesterol, diabetes mellitus, and supplemental tocopherol upon plasma tocopherol. Am J Clin Nutr, 1973; 26: 136-143

31) Raederstorff D, Wyss A, Calder PC, Weber P, and Eggersdorfer M: Vitamin E function and requirements in stage kidney disease patients undergoing hemodialysis. Br J Nutr, 2015; 114: 1113-1122

32) Jessup W, Rankin SM, De Whalley CV, Hoult JR, Scott J, and Leake DS: Alpha-tocopherol consumption during relation to PUFA. Br J Nutr, 2015; 114: 1113-1122
low-density-lipoprotein oxidation. Biochem J, 1990; 265: 399-405

33) Oram JE, Vaughan AM, and Stocker R: ATP-binding cassette transporter A1 mediates cellular secretion of alpha-tocopherol. J Biol Chem, 2001; 276: 39898-39902

34) Reboul E, Trompier D, Moussa M, Klein A, Landrier JF, Chimini G, and Borel P: ATP-binding cassette transporter A1 is significantly involved in the intestinal absorption of alpha- and gamma-tocopherol but not in that of retinyl palmitate in mice. Am J Clin Nutr, 2009; 89: 177-184

35) Reboul E, Klein A, Bietrix F, Gleize B, Malezet-Desmoulins C, Schneider M, Margotat A, Lagrost L, Collet X, and Borel P: Scavenger receptor class B type I (SR-BI) is involved in vitamin E transport across the enterocyte. J Biol Chem, 2006; 281: 4739-4745

36) Mardones P, Strobel P, Miranda S, Leighton F, Quiñones V, Amigo L, Rozowski J, Krieger M, and Rigotti A: Alpha-tocopherol metabolism is abnormal in scavenger receptor class B type I (SR-BI)-deficient mice. J Nutr, 2002; 132: 443-449

37) Tang F, Lu M, Zhang S, Mei M, Wang T, Liu P, and Wang H: Vitamin E conditionally inhibits atherosclerosis in ApoE knockout mice by anti-oxidation and regulation of vasculature gene expressions. Lipids, 2014; 49: 1215-1223

38) Brigelius-Flohé R, and Traber MG: Vitamin E: function and metabolism. FASEB J, 1999; 13: 1145-1155

39) Kamal-Eldin A, and Appelqvist LA: The chemistry and antioxidant properties of tocopherols and tocotrienols. Lipids, 1996; 31: 671-701

40) Ohvall M, Sundlöf G, and Vessby B: Gamma, but not alpha, tocopherol levels in serum are reduced in coronary heart disease patients. J Intern Med, 1996; 239: 111-117

41) Devaraj S, Leonard S, Traber MG, and Jialal I: Gamma-tocopherol supplementation alone and in combination with alpha-tocopherol alters biomarkers of oxidative stress and inflammation in subjects with metabolic syndrome. Free Radic Biol Med, 2008; 44: 1203-1208

42) Kolomvotsou AI, Rallidis LS, Mountzouris KC, Lekakis J, Koutelidakis A, Efstathiou S, Nana-Anastasiou M, and Zampelas A: Adherence to Mediterranean diet and close dietetic supervision increase total dietary antioxidant intake and plasma antioxidant capacity in subjects with abdominal obesity. Eur J Nutr, 2013; 52: 37-48

43) Miller ER 3rd, Erlinger TP, Sacks FM, Svetkey LP, Charleston J, Lin PH, and Appel LJ: A dietary pattern that lowers oxidative stress increases antibodies to oxidized LDL: results from a randomized controlled feeding study. Atherosclerosis, 2005; 183: 175-182

44) Pavadhgul P, Bhumrungpert A, Harjani Y, and Kurilich A: Oat porridge consumption alleviates markers of inflammation and oxidative stress in hypercholesterolemic adults. Asia Pac J Clin Nutr, 2019; 28: 260-265

45) Calvo MS, Mehrrota A, Beelman RB, Nadkarni G, Wang L, Cai W, Goh BC, Kalaras MD, and Uribarri J: A retrospective study in adults with metabolic syndrome: diabetic risk factor response to daily consumption of agaricus bisporus (White Button Mushrooms). Plant Foods Hum Nutr, 2016; 71: 245-251

46) Martínez-López S, Sarriá B, Mateos R, and Bravo-Clemente L: Moderate consumption of a soluble green/roasted coffee rich in caffeoylquinic acids reduces cardiovascular risk markers: results from a randomized, cross-over, controlled trial in healthy and hypercholesterolemic subjects. Eur J Nutr, 2019; 58: 865-878

47) Aicher BO, Haser EK, Freeman LA, Carneie AV, Stonik JA, Wang X, Remaley AT, Kato GJ, and Cannon RO 3rd: Diet-induced weight loss in overweight or obese women and changes in high-density lipoprotein levels and function. Obesity (Silver Spring), 2012; 20: 2057-2062

48) Corral-Aguayo RD, Yahia EM, Carrillo-Lopez A, and González-Aguilar G: Correlation between some nutritional components and the total antioxidant capacity measured with six different assays in eight horticultural crops. J Agric Food Chem, 2008; 56: 10498-10504

49) Niki E: Role of vitamin E as a lipid-soluble peroxyl radical scavenger: in vitro and in vivo evidence. Free Radic Biol Med, 2016; 94: 3-12

50) Cao G, Sofic E, and Prior RL: Antioxidant and prooxidant behavior of flavonoids: structure-activity relationships. Free Radic Biol Med, 1997; 22: 749-760

51) Shao B: Site-specific oxidation of apolipoprotein A-I impairs cholesterol export by ABCA1, a key cardioprotective function of HDL. Biochim Biophys Acta, 2012; 182: 490-501

52) Cao G, Booth SL, Sadowski JA, and Prior RL: Increases in human plasma antioxidant capacity after consumption of controlled diets high in fruit and vegetables. Am J Clin Nutr, 1998; 68: 1081-1087

53) Cooney RV, Custer LJ, Okinaka L, and Franke AA: Effects of dietary sesame seeds on plasma tocopherol levels. Nutr Cancer, 2001; 39: 66-71

54) Bechor S, Zolberg Relevy N, Harari A, Almog T, Kamari Y, Ben-Amotz A, Harats D, and Shaish A: 9-cis β-carotene increased cholesterol efflux to HDL in macrophages. Nutrients, 2016; 8: 435

55) Argmann CA, Sawyez CG, McNeil CJ, Hegele RA, and Huff MW: Activation of peroxisome proliferator-activated receptor gamma and retinoid X receptor results in net depletion of cellular cholesterol esters in macrophages exposed to oxidized lipoproteins. Arterioscler Thromb Vasc Biol, 2003; 23: 475-482

56) Zhou W, Lin J, Chen H, Wang J, Liu Y, and Xia M: Retinoic acid induces macrophage cholesterol efflux and inhibits atherosclerotic plaque formation in apoE-deficient mice. Br J Nutr, 2015; 114: 509-518

57) Matsumoto A, Mizukami H, Mizuno S, Umegaki K, Nishikawa J, Shudo K, Kagechika H, and Inoue M: beta-Cryptoxanthin, a novel natural RAR ligand, induces ATP-binding cassette transporters in macrophages. Biochem Pharmacol, 2007; 74: 256-264

58) Palozza P, Simone R, Catalano A, Parrone N, Monego G, and Ranelletti FO: Lycopene regulation of cholesterol synthesis and efflux in human macrophages. J Nutr Biochem, 2011; 22: 971-978
**Supplemental Table 1.** Changes in anthropometric variables and lipid parameters of the patients in the Partial Japan Diet and Japan Diet groups

|                              | PJD group (n = 49) | JD group (n = 49) | Between-group comparisons at 3 and 6 months (JD - PJD) |
|------------------------------|--------------------|-------------------|-------------------------------------------------------|
|                              | Mean (SD)          | Mean (SD)         | Difference (95% CI)                                   | p         |
| **Body Mass Index (kg/m²)**  |                    |                   |                                                       |           |
| Baseline                     | 23.9 (3.3)         | 24.4 (3.7)        |                                                       |           |
| 3-month                       | 23.5 (3.2)         | 24.0 (3.8)        | 0.1 (-0.2, 0.4)                                      | 0.50      |
| 6-month                       | 23.3 (3.3)         | 24.0 (3.7)        | 0.1 (-0.2, 0.5)                                      | 0.43      |
| **Total cholesterol (mg/dL)**|                    |                   |                                                       |           |
| Baseline                     | 224 (38)           | 213 (29)          |                                                       |           |
| 3-month                       | 219 (38)           | 204 (25)          | -4 (-14, 5)                                           | 0.38      |
| 6-month                       | 223 (36)           | 202 (27)          | -10 (-19, -1)                                         | 0.033     |
| **LDL-cholesterol (mg/dL)**  |                    |                   |                                                       |           |
| Baseline                     | 128 (33)           | 123 (24)          |                                                       |           |
| 3-month                       | 124 (30)           | 116 (20)          | -3 (-11, 5)                                           | 0.45      |
| 6-month                       | 129 (32)           | 115 (22)          | -9 (-17, 0)                                           | 0.043     |
| **MDA-LDL (U/L)**            |                    |                   |                                                       |           |
| Baseline                     | 94 (71, 126)       | 88 (74, 107)      |                                                       |           |
| 3-month                       | 86 (71, 107)       | 86 (69, 105)      | 4 (-7, 16)                                            | 0.76      |
| 6-month                       | 91 (67, 122)       | 79 (69, 98)       | -4 (-16, 8)                                           | 0.29      |
| **HDL-cholesterol (mg/dL)**  |                    |                   |                                                       |           |
| Baseline                     | 63 (18)            | 61 (17)           |                                                       |           |
| 3-month                       | 63 (21)            | 60 (15)           | -2 (-4, 1)                                            | 0.26      |
| 6-month                       | 64 (20)            | 60 (16)           | -2 (-4, 1)                                            | 0.25      |
| **Triglyceride (mg/dL)**     |                    |                   |                                                       |           |
| Baseline                     | 90 (63, 173)       | 103 (77, 155)     |                                                       |           |
| 3-month                       | 91 (61, 152)       | 92 (68, 127)      | -9 (-30, 13)                                          | 0.21      |
| 6-month                       | 87 (64, 153)       | 86 (60, 131)      | -10 (-33, 13)                                         | 0.023     |

*P* values within-group comparisons from baseline are analyzed by the paired *t* test or Wilcoxon signed-rank test.

*P* values between-group comparisons are analyzed by the unpaired *t* test or Mann–Whitney U test.

MDA-LDL, malondialdehyde modified-low density lipoprotein.

*Values are expressed as means (95% CI).

*Values are expressed as medians (25th percentile, 75th percentile).
**Supplemental Table 2.** Changes in energy and nutrients in the Partial Japan Diet and Japan Diet groups

|                           | PJD group (n = 49) | JD group (n = 49) | Between-group comparisons at 3 and 6 months (JD - PJD) |
|---------------------------|--------------------|-------------------|------------------------------------------------------|
|                           | Median (25th percentile, 75th percentile) | Median (25th percentile, 75th percentile) | Value (95% CI) |
| **Energy (kcal)**         | p                 | p                 | p                       | p                 |
| Baseline                  | 1873 (493)        | 1919 (440)        | 0.63                    | -83 (-220, 54)    | 0.23                |
| 3-month                   | 1786 (446)        | 1749 (303)        | 0.089                   | 1749 (303)        | 0.001               |
| 6-month                   | 1726 (503)        | 1650 (415)        | 0.001                   | 1650 (415)        | 0.000               |
| **Protein (g)**           | p                 | p                 | p                       | p                 |
| Baseline                  | 70.2 (62, 84)     | 70.2 (60, 84)     | 0.85                    | 70.2 (62, 84)     | 0.85                |
| 3-month                   | 72.8 (59, 84)     | 72.7 (62, 81)     | 0.48                    | 72.7 (62, 81)     | 0.48                |
| 6-month                   | 64.7 (56, 81)     | 66.6 (60, 82)     | 0.56                    | 66.6 (60, 82)     | 0.56                |
| **Fat (g)**               | p                 | p                 | p                       | p                 |
| Baseline                  | 63 (20)           | 65 (20)           | 0.45                    | 65 (20)           | 0.45                |
| 3-month                   | 60 (19)           | 58 (18)           | 0.94                    | 58 (18)           | 0.94                |
| 6-month                   | 55 (19)           | 55 (19)           | 0.000                   | 55 (19)           | 0.000               |
| **Carbohydrate (g)**     | p                 | p                 | p                       | p                 |
| Baseline                  | 237 (73)          | 237 (59)          | 0.99                    | 237 (73)          | 0.99                |
| 3-month                   | 225 (65)          | 219 (46)          | 0.004                   | 219 (46)          | 0.004               |
| 6-month                   | 225 (72)          | 204 (56)          | 0.000                   | 204 (56)          | 0.000               |
| **α-tocopherol (mg)**     | p                 | p                 | p                       | p                 |
| Baseline                  | 8.2 (6.3, 9.2)    | 7.3 (5.4, 9.7)    | 0.44                    | 7.3 (5.4, 9.7)    | 0.44                |
| 3-month                   | 7.5 (6.1, 9.3)    | 8.0 (6.4, 9.7)    | 0.33                    | 8.0 (6.4, 9.7)    | 0.33                |
| 6-month                   | 7.4 (6.3, 9.3)    | 7.6 (5.5, 9.6)    | 0.59                    | 7.6 (5.5, 9.6)    | 0.59                |
| **β-tocopherol (mg)**     | p                 | p                 | p                       | p                 |
| Baseline                  | 0.4 (0.3, 0.5)    | 0.4 (0.3, 0.5)    | 0.11                    | 0.4 (0.3, 0.5)    | 0.11                |
| 3-month                   | 0.4 (0.3, 0.5)    | 0.4 (0.3, 0.5)    | 0.81                    | 0.4 (0.3, 0.5)    | 0.81                |
| 6-month                   | 0.4 (0.3, 0.5)    | 0.4 (0.3, 0.5)    | 0.60                    | 0.4 (0.3, 0.5)    | 0.60                |
| **γ-tocopherol (mg)**     | p                 | p                 | p                       | p                 |
| Baseline                  | 12.0 (9.5, 15.5)  | 12.0 (9.3, 14.9)  | 0.92                    | 12.0 (9.3, 14.9)  | 0.92                |
| 3-month                   | 11.7 (8.6, 15.2)  | 11.9 (8.7, 15.0)  | 0.83                    | 11.9 (8.7, 15.0)  | 0.83                |
| 6-month                   | 11.2 (7.9, 14.7)  | 10.8 (8.8, 15.7)  | 0.78                    | 10.8 (8.8, 15.7)  | 0.78                |
| **δ-tocopherol (mg)**     | p                 | p                 | p                       | p                 |
| Baseline                  | 2.8 (2.1, 3.6)    | 2.7 (1.7, 3.4)    | 0.60                    | 2.7 (1.7, 3.4)    | 0.60                |
| 3-month                   | 2.5 (1.8, 3.4)    | 2.7 (1.9, 4.0)    | 0.11                    | 2.7 (1.9, 4.0)    | 0.11                |
| 6-month                   | 2.5 (2.1, 3.6)    | 3.0 (1.9, 3.7)    | 0.19                    | 3.0 (1.9, 3.7)    | 0.19                |
| **β-carotene (µg)**       | p                 | p                 | p                       | p                 |
| Baseline                  | 3434 (2064, 4541) | 2293 (1458, 3118) | 0.006                   | 2293 (1458, 3118) | 0.006               |
| 3-month                   | 2830 (1915, 4508) | 4090 (2076, 5711) | 0.000                   | 4090 (2076, 5711) | 0.000               |
| 6-month                   | 3002 (2116, 5143) | 4073 (2426, 6276) | 0.000                   | 4073 (2426, 6276) | 0.000               |
| **α-carotene (µg)**       | p                 | p                 | p                       | p                 |
| Baseline                  | 570 (357, 1036)   | 426 (139, 795)    | 0.043                   | 426 (139, 795)    | 0.043               |
| 3-month                   | 480 (199, 971)    | 712 (303, 1306)   | 0.012                   | 712 (303, 1306)   | 0.012               |
| 6-month                   | 640 (360, 1086)   | 682 (271, 1384)   | 0.032                   | 682 (271, 1384)   | 0.032               |
| **β-cryptoxanthin (µg)**  | p                 | p                 | p                       | p                 |
| Baseline                  | 72 (32, 129)      | 44 (27, 88)       | 0.25                    | 44 (27, 88)       | 0.25                |
| 3-month                   | 47 (27, 77)       | 54 (34, 146)      | 0.10                    | 54 (34, 146)      | 0.10                |
| 6-month                   | 47 (34, 105)      | 45 (31, 80)       | 0.34                    | 45 (31, 80)       | 0.34                |

1. *P* values within-group comparisons from baseline are analyzed by the paired t test or Wilcoxon signed-rank test.

2. *P* values between-group comparisons are analyzed by the unpaired t test or Mann–Whitney U test.

3. Values are expressed as means (SD).

4. Values are expressed as means (95% CI).