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Extra virgin olive oil increases uncoupling protein 1 content in brown adipose tissue and enhances noradrenaline and adrenaline secretions in rats☆

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Abstract

The effects of extra virgin olive oil (EV-olive oil) on triglyceride metabolism were investigated by measuring the degree of thermogenesis in interscapular brown adipose tissue (IBAT) and the rates of noradrenaline and adrenaline secretions in rats, both in vivo and in situ. In Experiment 1 (in vivo), rats were given an isoenergetic high-fat diet (30% fat diet) containing corn oil, refined olive oil, or EV-olive oil. After 28 days of feeding, the final body weight, weight gain, energy efficiency, perirenal adipose tissue and epididymal fat pad and plasma triglyceride concentrations were the lowest in the rats fed the EV-olive oil diet. The content of uncoupling protein 1 (UCP1) in IBAT and the rates of urinary noradrenaline and adrenaline excretions were the highest in the rats fed the EV-olive oil diet. In Experiment 2 (in situ), the effects of the extract of the phenolic fraction from EV-olive oil and a compound having excellent characteristics as components of EV-olive oil, hydroxytyrosol, on noradrenaline and adrenaline secretions were evaluated. The intravenous administration of the extract of the phenolic fraction from EV-olive oil significantly increased plasma noradrenaline and adrenaline concentrations, whereas that of hydroxytyrosol had no effect. These results suggest that phenols except hydroxytyrosol in EV-olive oil enhance thermogenesis by increasing the UCP1 content in IBAT and enhancing noradrenaline and adrenaline secretions in rats.

Keywords: Extra virgin olive oil; Phenols; Uncoupling protein 1; Noradrenaline; Adrenaline

1. Introduction

Olive oil is an integral ingredient of the Mediterranean diet. Accumulating evidence suggests that it may have health benefits that include the reduction in the risk of coronary heart disease, the prevention of several varieties of cancers and the modification of immune and inflammatory responses [1–7]. Olive oil appears to be a functional food with various components such as monounsaturated fatty acids that may have health benefits and is also a good source of phytochemicals, including polyphenolic compounds [1,3–10]. In particular, olive oil is a source of at least 30 phenolic compounds [7–9], which are strong antioxidants and radical scavengers [6–12]. Recent findings show that olive oil phenols are powerful antioxidants, both in vitro and in vivo, and possess other potent biological activities that could partially account for the observed health effects of the Mediterranean diet [1,7,9–12]. However, there have been few reports on the nutritional effects of olive oil on triglyceride catabolism. Furthermore, the constituents of olive oil that are effective in enhancing triglyceride catabolism have not been clarified yet. Triglyceride metabolism is stimulated by catecholamines (noradrenaline and

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Table 1
Fatty acid compositions of corn oil, olive oil and EV-olive oil used in Experiment 1

	Corn oil[a]	r-Olive oil[b]	EV-Olive oil[c]
Fatty acid (%)			
16:0	11.4	10.5	10.7
16:1	0.2	1.1	0.6
18:0	2.2	3.9	2.9
18:1	32.0	78.6	76.0
18:2	51.9	4.3	8.0
18:3 (n-3)	1.6	0.9	0.7
20:0	0	0	0.5
20:1	0	0	0.3
20:2	0	0	0.2

[a] Oriental Yeast, Tokyo, Japan.
[b] Refined olive oil; Wako Pure Chemical Ind., Tokyo, Japan.
[c] Filippo Berio EV-olive oil.

adrenaline) released through the stimulation of the activities of the sympathetic nervous system and the subsequent thermogenesis [13–16]. Noradrenaline secretion, in response to sympathetic nervous system stimulation, likely plays a major role in regulating thermogenesis in brown adipose tissue (BAT) [17–19]. Sympathetic nervous stimulation has been reported to regulate thermogenesis by increasing uncoupling protein (UCP) content, particularly UCP of subtype 1 (UCP1) but not UCP2 or UCP3, in BAT [17,19–21]. In a recent study, the influences of four dietary lipid sources (ie, olive oil, sunflower oil, palm oil and beef tallow) on UCP1, UCP2 and UCP3 contents and the mRNA expression of three proteins in several tissues of rats have been compared [22]. Olive oil feeding induces the highest levels of UCP1, UCP2 and UCP3 mRNA expressions in interscapular BAT (IBAT). Also, olive oil may induce the up-regulation of UCP mRNA, which is probably not mediated by systemic metabolic changes but is rather related to local effects on IBAT and skeletal muscle. However, there is as yet no information available on the effects of the components of olive oil on UCP content and mRNA expression level. In particular, extra virgin olive oil (EV-olive oil) contains various minor components (phenolic compounds) that produce a particular aroma and taste. Hydroxytyrosol [2-(3,4-dihydroxyphenyl)ethanol], the major phenolic compound in EV-olive oil, may contribute to the antioxidative activities and other beneficial effects of EV-olive oil [23,24]. Therefore, this study was carried out to identify the constituents of EV-olive oil that are effective in enhancing triglyceride catabolism. Two experiments were performed, in vivo (Experiment 1) and in situ (Experiment 2), in rats to determine whether EV-olive oil stimulates triglyceride catabolism through a comparison of corn oil and refined olive oil (r-olive oil), particularly to determine the effects of the phenol contents in r-olive oil (0 mg/kg) and EV-olive oil (141 mg/kg) on the expression of UCP1 in IBAT, and to determine whether the extract of the phenolic fraction of EV-olive oil and the excellent characteristics of the components of EV-olive oil, i.e., hydroxytyrosol, stimulate triglyceride catabolism.

2. Methods

2.1. Animal care

Male Sprague–Dawley rats (Japan SLC, Shizuoka, Japan) were housed individually in stainless steel wire-bottom cages in a room maintained at 22°C–24°C and about 50% relative humidity. The room was lit from 07:00 h to 19:00 h. Tap water was freely available. Four-week-old rats for Experiment 1 and 7-week-old rats for Experiment 2 were purchased and given a commercial diet (CE-2, Japan Clea, Tokyo, Japan) for 3 days before starting the experiments. This study was approved by the Institutional Animal Care and Use Committee of Kobe Women's University, Faculty of Home Economics.

2.2. Materials

The fatty acid compositions of corn oil, refined olive oil (r-olive oil) and EV-olive oil used in the experimental diets in Experiment 1 are shown in Table 1. Corn oil was purchased from Oriental Yeast, Tokyo, Japan. r-Olive oil was purchased from Wako Pure Chem. Ind., Osaka, Japan. EV-olive oil (Fillippo Berio EV-olive oil, Carbonail, Italy) was obtained from J-Oil Mils, Tokyo, Japan. The phenolic contents in r-olive oil and EV-olive oil were 0 and 141 mg/kg, respectively, as measured by the method of Favati et al. [25]. The phenolic fraction in EV-olive oil was extracted by the method of Favati et al. The extracted phenolic fraction was used as the extract of the phenolic fraction from EV-olive oil in Experiment 2.

Table 2
Compositions of experimental diets (Experiment 1)

(g/kg)	Corn oil diet	r-Olive oil diet	EV-Olive oil diet
Casein[a]	250	250	250
Corn oil[a]	300	0	0
r-Olive oil[b]	0	300	0
EV-olive oil[c]	0	0	300
Vitamins[d]	17	17	17
Minerals[e]	50	50	50
Cellulose[a]	40	40	40
Sucrose[a]	300	300	300
α-Cornstarch[a]	43	43	43
Energy density (MJ/kg)[f]	21.21	21.21	21.21

[a] Oriental Yeast Co.
[b] Refined olive oil; Wako Chemical Ind., Tokyo, Japan.
[c] Filippo Berio EV-olive oil from Carbonail, Italy.
[d] Purchased from Oriental Yeast Co. The vitamin mixture contained (in mg/kg diet) retinyl acetate 17, cholecalciferol 0.0425, all-rac-α-tocopherol acetate 85, menandione 88.4, thiamin-HCl 20.4, riboflavin 68, pyridoxine-HCl 13.6, vitamin B_{12} 0.0085, vitamin C 510, D-biotin 0.34, folic acid 3.4, Ca pantothenate 85, *P*-aminobenzoic acid 85, nicotinic acid 102, inositol 102, choline chloride 3,400 and cellulose powder 12,419.809.
[e] Purchased from Oriental Yeast Co. The mineral mixture contained (in mg/kg diet) $CaHPO_4 \cdot H_2O$ 7,280, KH_2PO_4 12,860, $NaH_2PO_4 \cdot H_2O$ 4,675, NaCl 2,330, Ca lactate 17,545, Fe citrate 1,590, $MgSO_4$ 3,585, $ZnCO_3$ 55, $MnSO_4 \cdot 4{\sim}5H_2O$ 60, CuSO4 · 5H2O 15 and KI 5.
[f] The energies for the respective components are as follows: starch, soluble carbohydrates and protein (16.70 MJ/kg); fat (37.70 MJ/kg).

Table 3
Effects of different phenol contents in corn oil, olive oil and EV-olive oil on body weight and weights of perirenal adipose tissue and epididymal fat pad, liver weight, urinary creatinine excretion and plasma concentrations of triglyceride, free fatty acids and total cholesterol in rats for 28 days (Experiment 1)[1]

	Corn oil diet	r-Olive oil diet	EV-Olive oil diet
Initial body weight (g)	108.9±5.66	108.5±4.2	108.4±3.7
Final body weight (g/28 days)	280.4±2.5[a]	270.3±3.2[ab]	269.3±1.9[b]
Weight gain (g/28 days)	171.5±2.4[a]	161.9±3.1[ab]	160.8±2.3[b]
Energy intake (MJ/28 days)	8.357	8.357	8.357
Energy efficiency (g gain/MJ consumed)	20.5±0.3[a]	19.4±0.4[ab]	19.2±0.3[b]
Weight of perirenal adipose tissue (g)	2.98±0.22[a]	1.74±0.12[b]	1.64±0.10[b]
Weight of epididymal fat pad (g)	7.34±0.20[a]	5.42±0.37[b]	4.91±0.23[b]
Liver weight (g)	10.1±0.3[a]	11.7±0.3[a]	11.0±0.3[a]
Urinary creatinine excretion (μmol/day)	44.2±4.9[a]	44.8±4.8[a]	45.3±5.0[a]
Triglyceride concentration (mmol/L plasma)	10.72±0.79[a]	7.43±1.89[a,b]	7.01±0.54[b]
Free fatty acids concentration (μmol/L plasma)	22.0±2.0[a]	29.0±3.0[a]	27.0±2.2[a]
Total cholesterol concentration (mmol/L plasma)	0.51±0.09[a]	0.69±0.06[a]	0.52±0.02[a]

[1] Values are expressed as mean±S.E.M. ($n=6$ or 7). Within a row, values with different superscripts are significantly different ($P<.05$).

2.3. Chemicals

The rats were anesthetized using α-chloralose and urethane [26], which were purchased from Wako Pure Chem. Ind. (Osaka, Japan) and Tokyo Chemical (Tokyo, Japan), respectively. Hydroxytyrosol [2,(3,4-dihydroxyphenyl)ethanol] was synthesized by the method of Bai et al. [27] and purified to approximately 96%.

2.4. Experiment 1 (in vivo)

The experimental diets used in Experiment 1 were three high-fat diets (30% fat diet) containing corn oil (corn oil diet), r-olive oil (r-olive oil diet) or EV-olive oil (EV-olive oil diet), as shown in Table 2. Rats weighing 80–90 g were separated into three groups of 6–7 rats and each given a shortening diet, a lard diet, or an EV-olive oil diet for 28 days. Each group of rats was given the respective diet in amounts such that the three groups consumed equal metabolizable energies during the experimental period, and the food consumption in all the three diet groups was approximately equivalent to the maximal amounts of the experimental diets that the rats can consume under these conditions. At the end of the experimental period, the rats were transferred to individual metabolic cages, where urine and feces from the rats after feeding were separately collected for 1 day. Previously, we confirmed that the daily urinary excretions of noradrenaline and adrenaline and feed intake are not affected by stress caused by placing rats in a metabolic cage. Each urinary sample was collected in a bottle containing 1 ml of 6 mol/L HCl and stored at −40°C until analysis. Urinary total noradrenaline and adrenaline excretions were determined by the method of Davidson and Fitzpatrick [28]. Urinary creatinine excretion was measured by the method of Clark and Thompson [29]. After the collection, the rats were anesthetized by intraperitoneal injections of α-chloralose and urethane (75 and 750 mg/kg body weight, respectively). Blood samples were collected from the abdominal aorta, and plasma was separated by centrifugation (3,000×g for 15 min). After collecting blood samples, the liver, kidney, perirenal adipose tissue, epididymal fat pad and IBAT were immediately excised and weighed. All samples were stored at −40°C until analysis. Plasma triglyceride and free fatty acid concentrations were determined enzymatically using commercial kits [triglycerides, Triglyceride G-test (Wako Chemical); free fatty acids, NEFA C-Test (Wako Chemical)]. Plasma total cholesterol concentration was measured according to the method of Pearson et al. [30].

The method of IBAT UCP1 content analysis by Western blotting was performed as reported previously [31]. IBAT was removed from the rats, immediately frozen in liquid nitrogen and stored at −40°C until analysis. From our previous study [31], we confirmed that the analysis of UCP1 content is not affected by the freezing of IBAT tissue. IBAT mitochondria were isolated as reported previously [32], and the total protein content in IBAT was measured with a DC protein assay kit (Bio-Rad). The mitochondrial fraction (5 μg) isolated from the IBAT of each rat was subjected to reducing sodium dodecyl sulfate–polyacrylamide gel electrophoresis, transferred onto a nylon membrane and reacted with anti-rat UCP1 serum [31]. Its specificity has been reported elsewhere [33]. UCP1 content was determined by Western blotting, as previously described [34]. These membranes were then incubated with a pig anti-rabbit IgG antibody conjugated with horseradish peroxidase (Dako Japan, Kyoto, Japan). UCP1 was thus quantified by densitometric and image analyses and expressed as a relative value for each group of rats.

2.5. Experiment 2 (in situ)

Rats weighing about 250 g were anesthetized as described in Experiment 1, and their rectal temperature was maintained between 36.5°C and 37.5°C using a direct-current heating pad. Six to seven rats were used in the evaluation of the extract of the phenolic fraction from EV-olive oil and hydroxytyrosol and for comparison with rats that received vehicle injection alone (9 g/L NaCl solution containing 2% ethanol and 0.5% Tween 80). Each rat was administered 1 ml of the vehicle containing 0 (vehicle alone), 1.41, 2.82, or

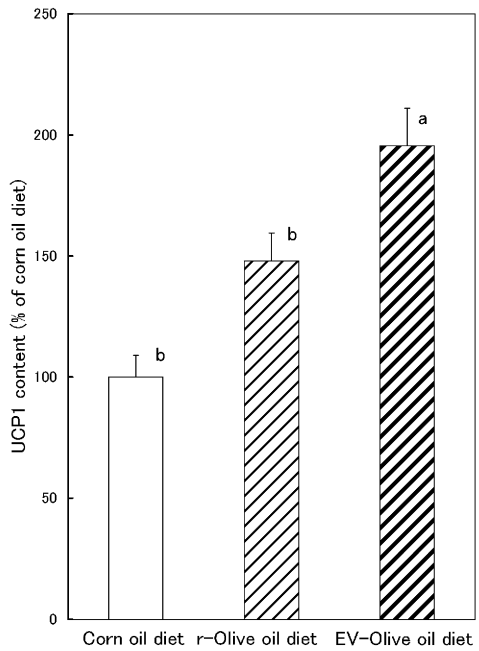

Fig. 1. Effects of different phenol concentrations in corn oil, r-olive oil and EV-olive oil on uncoupling protein 1 (UCP1) content in IBAT in rats for 28 days (Experiment 1). The UCP1 content in IBAT was calculated as counts per tissue from Western blotting data. Values are expressed relative to that of the control rats fed the corn oil diet and are means±S.E.M. ($n=6$ or 7). Means without a common superscript are significantly different ($P<.05$).

4.23 mg as phenol content (corresponding to 0, 10, 20, or 30 g of EV-olive oil, respectively) of the extract of the phenolic fraction from EV-olive oil into the right femoral vein over a period of 1 min. Blood samples were collected from the abdominal aorta after 10 min.

To investigate the effects of hydroxytyrosol, the rats were individually administered 1 ml of the vehicle containing 0 (0 mg, vehicle alone), 10 (1.54 mg), or 30 mmol/L (4.63 mg) hydroxytyrosol into the right femoral vein over a period of 1 min.

Blood samples from each rat were collected into heparinized tubes, and plasma was obtained by centrifugation. Plasma noradrenaline and adrenaline were purified with aluminum oxide and assayed by high-performance liquid chromatography (HPLC) with electrochemical detection, as described previously [35].

2.6. Statistical analysis

All data are presented as mean±S.E.M. Statistical analyses were carried out with Statistical Package for Social Sciences (SPSS13.0 for Windows, SPSS, Chicago, IL, USA). Data were analyzed by one-way analysis of variance, and significant differences between means were evaluated by the Bonferroni post hoc test. Differences were considered significant at $P<.05$.

3. Results

3.1. Experiment 1

After 28 days of dietary treatment, the final body weight, weight gain and energy efficiency in the rats fed the EV-olive oil diet were significantly lower than those in the rats fed the corn oil diet. The weights of perirenal adipose tissue and epididymal fat pad in the rats fed the r-olive oil and EV-olive oil diets were significantly lower than those in the rats fed the corn oil diet. There were no significant

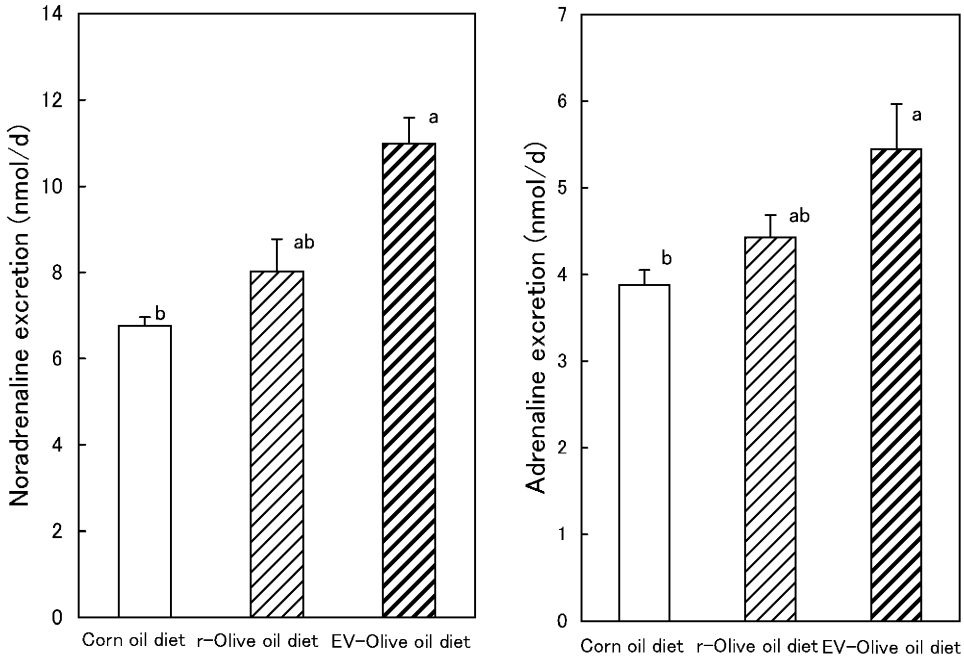

Fig. 2. Effects of different phenol concentrations in corn oil, r-olive oil or EV-olive oil on urinary noradrenaline and adrenaline excretions in rats for 28 days (Experiment 1). Values are means±S.E.M. ($n=6$ or 7). Means without a common superscript are significantly different ($P<.05$).

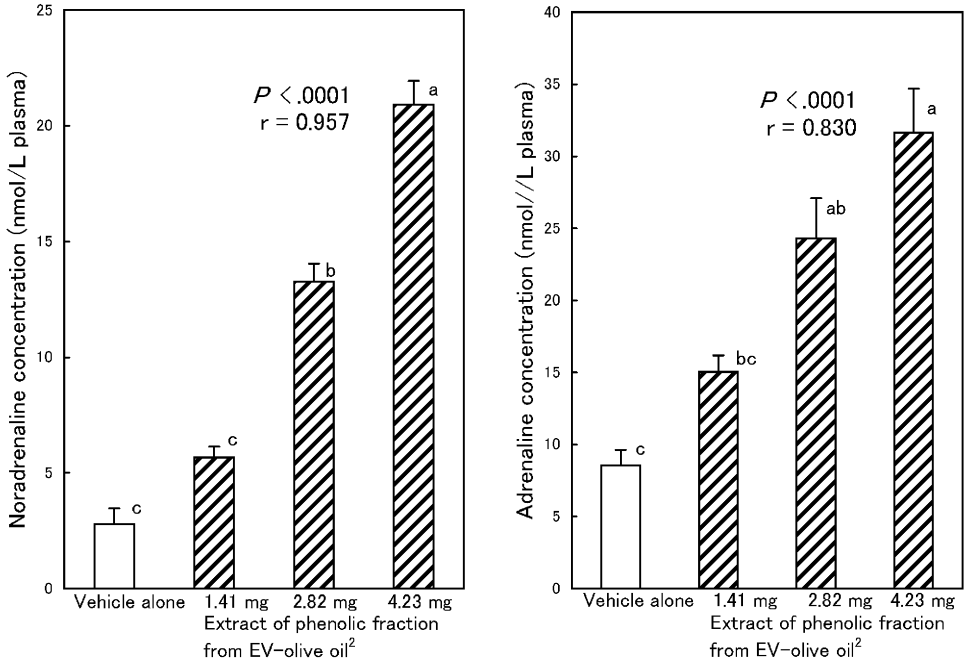

Fig. 3. Dose response with respect to plasma noradrenaline and adrenaline concentrations in rats following administration of extract of phenolic fraction from EV-olive oil (Experiment 2[1]). Values are mean±S.E.M. (n=6 or 7). Within a column, values with different superscripts are significantly different (P<.05). [2]Each rat was administered 1 ml of the vehicle containing 0 (vehicle alone), 1.41, 2.82 or 4.23 mg of phenol (corresponding to 0, 10, 20 or 30 g of EV-olive oil, respectively) of the extract of the phenolic fraction from EV-olive oil into the right femoral vein over a period of 1 min.

differences in liver weight or urinary creatinine level between the rats fed the corn oil and the EV-olive oil diets (Table 3). The plasma triglyceride concentrations were significantly lower in the rats fed the EV-olive oil diet than in those fed the corn oil diet. There were no significant differences in plasma free fatty acid or total cholesterol concentration between the rats fed the corn oil and EV-olive oil diets (Table 3). The IBAT UCP1 content in the rats fed the EV-olive oil diet was significantly higher than that in the rats fed the corn oil and r-olive oil diets (Fig. 1). The urinary noradrenaline and adrenaline excretions were significantly higher in the rats fed the EV-olive oil diet than in those fed the corn oil diet (Fig. 2).

3.2. Experiment 2

The dose responses of plasma noradrenaline and adrenaline concentrations in the rats following the administration of the extract of the phenolic fraction from EV-olive oil are shown in Fig. 3. The plasma concentrations of both noradrenaline and adrenaline significantly increased in the rats that received 2.82 or 4.23 mg (corresponding to 20 or 30 g of EV-olive oil, respectively) of the extract of the phenolic fraction from EV-olive oil, as compared with those in the rats that received the vehicle alone. The increases were dose-dependent, and there was a significant positive correlation between noradrenaline and adrenaline concentrations and the dose of the extract of the phenolic fraction from EV-olive oil [noradrenaline, P<.0001 (r=.957), adrenaline P<.0001 (r=.830)].

The dose-response relationships with respect to plasma noradrenaline and adrenaline concentrations in the rats following the hydroxytyrosol administration are shown in Table 4. Hydroxytyrosol did not significantly affect the plasma concentration of noradrenaline or adrenaline in the rats.

4. Discussion

EV-olive oil contains a considerable amount of phenolic compounds, e.g., hydroxytyrosol and oleuropein, which are responsible for its peculiar taste and high stability [1]. There

Table 4
Dose responses of plasma noradrenaline and adrenaline concentrations in rats following hydroxytyrosol administration (Experiment 2)[1]

	Plasma concentration (nmol/L)	
	Noradrenaline	Adrenaline
Vehicle alone[2]	2.958±0.623[a]	8.547±1.070[a]
Hydroxytyrosol[2]		
10 mmol/L (1.54 mg)	3.126±0.839[a]	10.012±1.268[a]
20 mmol/L (3.08 mg)	3.417±0.785[a]	11.209±0.626[a]
30 mmol/L (4.63 mg)	3.379±1.037[a]	10.955±1.857[a]

[1] Values are means±S.E.M. (n=6 or 7). Within a column, values with different superscripts are significantly different (P<.05).

[2] Each rat was administered an infusion of 1 ml of the vehicle (9 g/L NaCl solution containing 2% ethanol and 0.5% Tween 80) or 1 ml of the vehicle containing 0 (vehicle alone), 10 (1.54 mg), 20 (3.08 mg) or 30 (4.63 mg) hydroxytyrosol into the right femoral vein over a period of 1 min. An abdominal aortic blood sample was collected 10 min after the infusion.

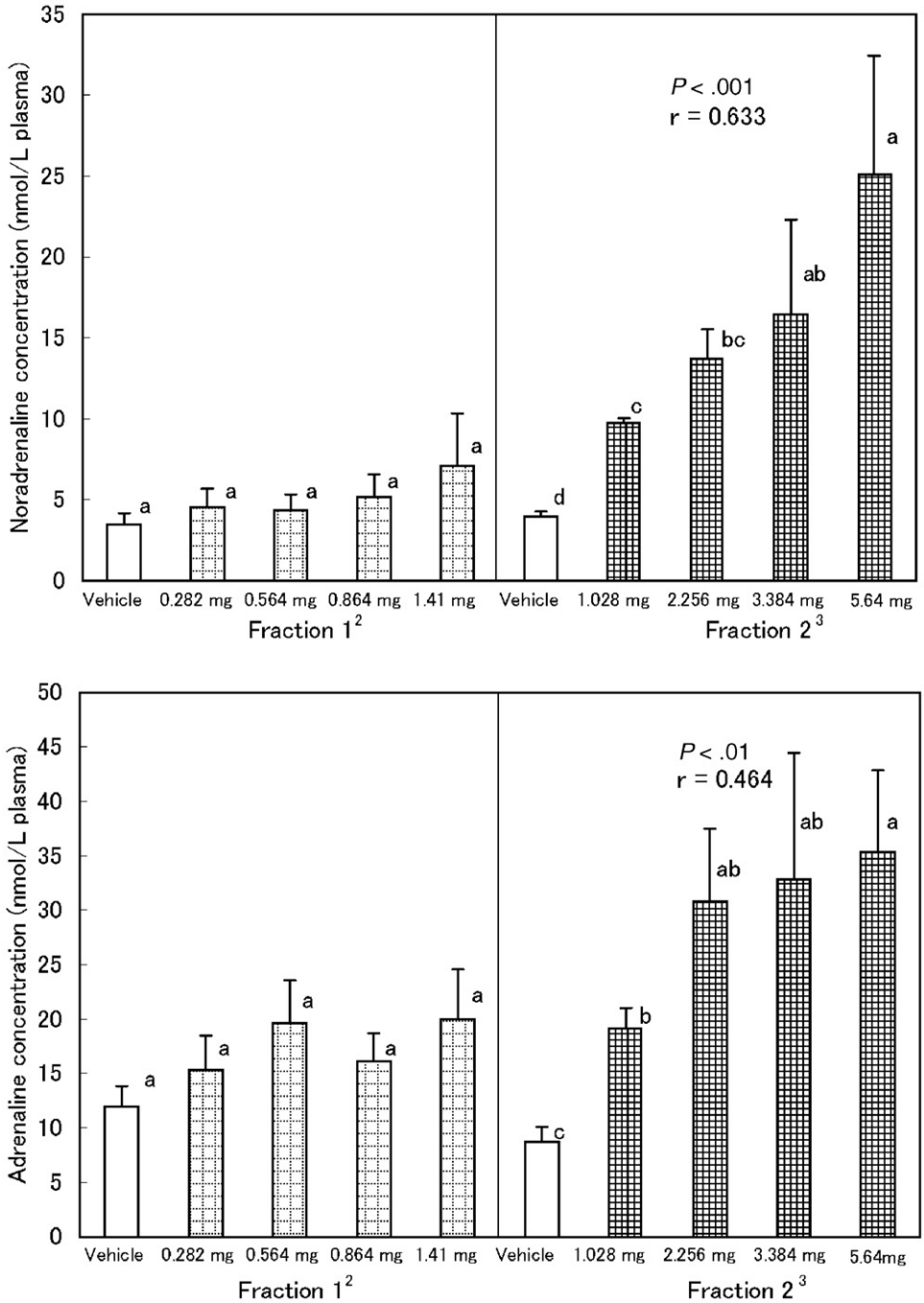

Fig. 4. Dose response with respect to plasma noradrenaline and adrenaline concentrations in rats following administration of Fraction 1 or Fraction 2[1]. Values are mean±S.E.M. ($n=6$ or 7). Within a column, values with different superscripts are significantly different ($P<.05$). [2]Each rat was administered 1 ml of the vehicle containing 0 (vehicle alone), 0.282, 0.564, 0.864, or 1.41 mg of phenol content (corresponding to 0, 10, 20, 30, or 50 g of EV-olive oil, respectively) of Fraction 1 into the right femoral vein over a period of 1 min. [3]Each rat was administered 1 ml of the vehicle containing 1.028, 2.256, 3.384, or 5.641mg of phenol content (corresponding to 0, 10, 20, 30, or 50 g of EV-olive oil, respectively) of Fraction 2 into the right femoral vein over a period of 1 min.

is accumulating evidence that olive oil phenolics are powerful antioxidants, both in vitro and in vivo, and that they exert other potent biological activities that could partially account for the observed healthful effects of the Mediterranean diet [1]. In this study, we examined the effects of EV-olive oil on triglyceride metabolism by comparing r-olive oil and corn oil, with the aim of identifying the effective constituents of EV-olive oil.

In Experiment 1, it was suggested that a higher degree of thermogenesis occurs in the rats fed the EV-olive oil diet than in those fed the other diets. The long-term overeating of a highly palatable cafeteria diet, which is generally high in

fat content, was reported to induce BAT hypertrophy [18], and the three diets (i.e., corn oil diet, r-olive oil diet and EV-olive oil diet) used in this study are possible models of highly palatable greasy diets with a sweet taste. There is considerable interest in determining what dietary macronutrients, with particular emphasis on dietary lipids of various degrees of saturation, affect thermogenesis and whole-body energy flux [36–38]. With respect to the influence of the type of dietary fat on thermogenesis, our findings of different responses in rats fed three high-fat diets are consistent with the findings of other researchers [36–39]. The phenol concentration in EV-olive oil varies from 50–800 mg/kg, with a mean of approximately 180 mg/kg for commercial olive oil [5]. In this study concerning the phenol concentrations in r-olive oil and EV-olive oil, although the fatty acid compositions of EV-olive oil and r-olive oil were almost the same, the total phenol concentration in EV-olive oil was 141 mg/kg, whereas that in r-olive oil was 0 mg/kg. The results indicate that phenols in EV-olive oil enhance urinary noradrenaline and adrenaline excretions and suppress body fat accumulation, as well as decrease the weights of perirenal adipose tissue and epididymal fat pads, by increasing triglyceride catabolism through the enhancement of thermogenesis in IBAT via an increase in UCP1 content. In this study, the average total amount of phenols consumed by the EV-olive oil diet (containing 30% EV-olive oil)-fed rats (in the case of diet intake of 17 g per day) was about 20 mg per rat during the 28-day experimental period. Therefore, it appears that thermogenesis is facilitated by phenols in EV-olive oil and that the intake of about 20 mg of phenols in EV-olive oil for 28 days induced accelerated thermogenesis in the rats fed the EV-olive oil diet.

In Experiment 2, to identify the constituents of EV-olive oil that enhance triglyceride catabolism and thermogenesis, the effects of EV-olive oil on plasma noradrenaline and adrenaline concentrations were investigated in anesthetized rats in situ. We confirmed whether an extract of the phenolic fraction from EV-olive oil and hydroxytyrosol act as the major signal for the enhancement of hormonal secretion (the stimulation of noradrenaline and adrenaline secretions). Results indicate that an extract of the phenolic fraction from EV-olive oil (total phenols in EV-olive oil) enhances noradrenaline and adrenaline secretions. In addition, hydroxytyrosol administrations did not affect noradrenaline or adrenaline secretion, suggesting that hydroxytyrosol does not affect thermogenesis. From these results, it was suggested that hydroxytyrosol did not act as the major signal for the enhancement of hormonal secretion. The hydroxytyrosol concentration in EV-olive oil has been reported to be 14.42 ± 3.01 mg/kg [8]. On the basis of this data, the hydroxytyrosol doses in this study could therefore be considered as equivalent to the physiological levels consumed during the normal dietary intake of EV-olive oil. Visioli et al. [40] demonstrated that, after olive oil ingestion, 4-hydroxyphenylethanol and hydroxytyrosol are dose-dependently absorbed in humans and excreted in the urine, mostly as glucuronide conjugates. Vissers et al. [41] suggested that humans absorb a large part of ingested olive oil phenols, mainly in the small intestine, and the absorbed phenols are extensively modified in the body. Therefore, in this study, it could be considered that phenols in EV-olive oil were absorbed in the rats and enhanced thermogenesis as a result of the increased UCP1 content in IBAT via the stimulation of noradrenaline and adrenaline secretions. Previously, we reported that, in rats, the pungent principles of allyl-containing sulfides in garlic enhance thermogenesis by increasing the UCP1 content in IBAT and by enhancing noradrenaline and adrenaline secretions [42]. In this study, we suggest that phenols in EV-olive oil enhance triglyceride catabolism and thermogenesis by a similar mechanism, whereas hydroxytyrosol in phenols do not affect thermogenesis, i.e., phenols except hydroxytyrosol in EV-olive oil are responsible for the enhancement of noradrenaline and adrenaline secretions as well as for enhanced thermogenesis, as indicated by the increased UCP1 content in IBAT.

In a preliminary experiment, to identify effective compounds in the phenolic fractions, the extract of the phenolic fraction from EV-olive oil was divided into two fractions, Fraction 1 (mainly containing hydroxytyrosol, tyrosol, vanillic acid and ferulic acid) and Fraction 2 (the oleuropein fraction, mainly containing oleuropein glycoside and oleuropein aglycone), from the retention time based on a chromatogram obtained by HPLC. We then performed the same in situ experiment with the administration of Fraction 1 or 2 (Fig. 4). The plasma concentrations of both noradrenaline and adrenaline significantly increased in the rats that received Fraction 2 (Fig. 4). The increases were dose-dependent, and there were significant positive correlations between noradrenaline ($r = .633$, $P < .001$) and adrenaline ($r = .464$, $P < .01$) concentrations and the dose of Fraction 2 (Fig. 4). In contrast, the administration of Fraction 1 did not affect plasma noradrenaline or adrenaline concentration (Fig. 4). Therefore, it was suggested that Fraction 2, the oleuropein fraction, enhances noradrenaline and adrenaline secretions.

Further investigation is necessary to determine the effects of phenols, particularly, oleuropein (both oleuropein glycoside and oleuropein aglycone), in EV-olive oil on thermogenesis. These studies are now in progress.

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