Original Article

Differential effects of saturated and unsaturated fatty acids on vascular reactivity in isolated mesenteric and femoral arteries of rats

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ABSTRACT Free fatty acid (FFA) intake regulates blood pressure and vascular reactivity but its direct effect on contractility of systemic arteries is not well understood. We investigated the effects of saturated fatty acid (SFA, palmitic acid), polyunsaturated fatty acid (PUFA, linoleic acid), and monounsaturated fatty acid (MUFA, oleic acid) on the contractility of isolated mesenteric (MA) and deep femoral arteries (DFA) of Sprague–Dawley rats. Isolated MA and DFA were mounted on a dual wire myograph and phenylephrine (PhE, 1–10 μ M) concentration-dependent contraction was obtained with or without FFAs. Incubation with 100 μ M of palmitic acid significantly increased PhE-induced contraction in both arteries. In MA, treatment with 100 μ M of linoleic acid decreased 1 μ M PhE-induced contraction while increasing the response to higher PhE concentrations. In DFA, linoleic acid slightly decreased PhE-induced contraction while 200 μ M oleic acid significantly decreased it. In MA, oleic acid reduced contraction at low PhE concentration (1 and 2 μ M) while increasing it at 10 μ M PhE. Perplexingly, depolarization by 40 mM KCl-induced contraction of MA was commonly enhanced by the three fatty acids. The 40 mM KCl-contraction of DFA was also augmented by linoleic and oleic acids while not affected by palmitic acid. SFA persistently increased alpha-adrenergic contraction of systemic arteries whereas PUFA and MUFA attenuated PhE-induced contraction of skeletal arteries. PUFA and MUFA concentration-dependent dual effects on MA suggest differential mechanisms depending on the types of arteries. Further studies are needed to elucidate underlying mechanisms of the various effects of FFA on systemic arteries.

INTRODUCTION

Cardiovascular disease (CVD) remains the leading cause of death worldwide [1]. Each year 17.3 million deaths are caused by CVD, which is expected to increase to more than 23.6 million by 2030 [2]. CVD is caused by non-modifiable risk factors such as aging, sex, and family history; however, modifiable factors have a greater impact on CVD development, including hypertension, tobacco use, physical inactivity, diabetes, unhealthy diet, overweight, and obesity [3].

An unhealthy diet, including excessive intake of salt, fats, or alcohol, and lack of fruit and vegetable consumption, is associated with increased risk of developing chronic disease. An unhealthy diet has been associated with coronary heart disease [4], type 2 diabetes [5], and metabolic syndrome [6]. These risk factors have been associated with endothelial dysfunction through various complex mechanisms [7]. Impairment of endothelial function is crucial in increasing vascular tone [8]. Vascular endothelium plays a significant role in regulating vascular function via production of vasoactive factors [9].

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According to the World Health Organization, salt intake lower than 5 g per day is recommended for preventing CVD [10]. In 2006, the American Heart Association also encouraged regulating fat intake to prevent CVD, recommending limiting the intake of saturated fat to 7% of total energy, trans fat to 1%, and cholesterol to 300 mg per day by choosing lean meats and vegetable alternatives, and fat-free (skim) or low-fat (1% fat) dairy products [11]. The most recent American Heart Association guidelines recommend a dietary pattern that achieves 5% to 6% of calories from saturated fat [12].

The risk of CVD is increased by saturated fatty acid (SFA) intake [13], and CVD risk reduction is observed with decreased SFA intake [14]. However, monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) may have a positive effect on the cardiovascular system. Most recent intervention studies suggest that replacing SFAs with PUFAs is beneficial in reducing the risk of CVD [15–17]. However, the effects of replacing SFAs with MUFAs are still unclear because of the studies' small sample size [14,18].

Elevated levels of fatty acids may cause vascular dysfunction by reducing nitric oxide (NO) or enhancing the oxidative stress in endothelial cells [19,20]. Animal and human studies suggest that free fatty acids (FFAs) in both high and physiological concentrations cause vascular dysfunction through impaired endothelium-dependent relaxation. Effects of FFAs have been reported in various vascular beds such as mesenteric arteries [21], femoral arteries [22], aortic arteries [23], and from human leg or forearm [24–27]. FFAs may act as either proinflammatory or anti-inflammatory agents depending on their chemical structure [28]. The effect of physiological plasma concentrations of FFAs on systemic arteries, especially the smooth muscle contractility, has not been fully understood. Among all body organs, the digestive system and skeletal muscles occupy the largest volume. Therefore, the tone and reactivity of mesenteric and skeletal arteries may represent the overall response of systemic circulation to FFAs. Therefore, the purpose of this study was to investigate the effects of representative SFA (palmitic acid), PUFA (linoleic acid), and MUFA (oleic acid) on the contractility of isolated mesenteric (MA) and deep femoral arteries (DFA) of rats.

METHODS

Animals and arterial ring preparation

This animal study was approved by the Institutional Animal Care and Use Committee (IACUC) in Chung-Ang University (IACUC approval No. 2015-00012). Adult male Sprague–Dawley rats (7–8 weeks old) were used in this experiment. Pentobarbital sodium (60–100 mg/kg) was injected intraperitoneally to anesthetize the rats before the operation. Full anesthesia was confirmed by limb withdrawal after toe pinching. The intestine and

proximal hind legs were quickly removed and placed in a normal tyrode (NT) solution at 4°C. The second and third branches of the mesenteric arteries (MAs) and deep femoral arteries (DFAs) were isolated rapidly and cut into 2.5–3 mm long segments (inner diameter: 200–300 μ m).

Isometric tension protocol

The arterial rings were mounted on two 25 μ m tungsten wires of a multiwire myograph system (620 M; DMT, Aarhus, Denmark). Each myograph chamber was filled with 5 ml of physiological salt solution (PSS). A segment of the arterial ring was stabilized in PSS with continuous gas bubbling (21% O₂, 5% CO₂, and N₂ balanced at 37°C) to maintain pH at 7.4. The isometric tension was recorded by a data acquisition system (Lab Chart Pro version 8.0; ADInstruments, Sydney, Australia). The arterial rings were tested for the validity of segment preparation by determining their response to a high concentration of potassium chloride (80 mM KCl-PSS). A high concentration of 80 mM KCl was prepared by replacing NaCl with equimolar KCl. The rings were washed with the PSS solution, and we waited until their resting tone returned. Endothelium-dependent relaxation was observed by full relaxation to 10 μ M of acetylcholine (ACh) in the presence of pre-constriction to 10 μ M of α -adrenergic agonist (phenylephrine, PhE). The dose-response curve of PhE was obtained after 30 min of incubation in PSS with or without palmitic acid (100 μ M), linoleic acid (100 μ M), and oleic acid (200 μ M) with different concentration increments at 5-min intervals (0.05 μ M, 0.1 μ M, 1 μ M, 2 μ M, 5 μ M, and 10 μ M).

Nonreceptor-mediated contraction to high concentration of potassium chloride (40 mM KCl) was investigated after the arterial segments were incubated in PSS with or without palmitic acid (100 μ M), linoleic acid (100 μ M), and oleic acid (200 μ M) for 30 min. Arterial segments were exposed to 40 mM KCl for 2 min and then washed out with PSS.

Solutions and chemicals

The composition of the NT solution was as follows (in mmol/L): NaCl 140, KCl 5.4, NaH₂PO₄ 0.33, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid 10, glucose 10, MgCl₂ 1, and CaCl₂ 1.8 (adjusted with NaOH to pH 7.4). The composition of the PSS solution was as follows (in mmol/L): NaCl 118, KCl 4, MgSO₄ 1, NaH₂PO₄ 0.44, NaHCO₃ 24, glucose 5.6, and CaCl₂ 1.8. The 40 mM KCl-PSS contained the following (in mmol/L): NaCl 82, KCl 40, MgSO₄ 1, NaH₂PO₄ 0.44, NaHCO₃ 24, glucose 5.6, and CaCl₂ 1.8. A high concentration of 40 mM KCl was prepared by replacing NaCl with equimolar KCl. All the solutions and chemicals were purchased from Sigma (St. Louis, MO, USA).

Statistical analysis

Statistical analysis was conducted using OriginPro version 8.0 for Windows (Origin Lab, Northampton, MA, USA). The response to PhE and 40 mM KCl is expressed as percent of the 80 mM KCl-PSS induced maximum constriction. Data are presented as mean $\pm$ standard error of the mean. Statistical comparisons were performed using unpaired Student's *t*-test, and *p* < 0.05 was considered statistically significant.

RESULTS

In each vessel, standard contraction by 80 mM KCl-induced depolarization was initially confirmed. Data were excluded if the contraction to 80 mM KCl-induced maximum constriction was less than 1 g. Then, 10 μ M PhE-induced maximum contraction was also confirmed, followed by endothelium-dependent relaxation by applying 10 μ M ACh. We used the endothelium-intact arterial rings, i.e. > 60% relaxation by 10 μ M ACh. After confirming the stable basal tone by washout of PhE and ACh, the concentration-dependent PhE-induced contractions of MA and DFA were obtained by cumulative increase of PhE from 0.05 to 10

μ M (Fig. 1A, D).

Pretreatment with 100 μ M palmitic acid alone did not produce any effect on the basal tone of MA and DFA but significantly augmented PhE-induced contractions in both arteries (Fig. 1B, C, E, F). In DFA, it was notable that palmitic acid incubation revealed the PhE-contraction even at 0.1 μ M (Fig. 1E, F).

Pretreatment with 100 μ M linoleic acid alone did not change the basal tone of MA and DFA (Fig. 2). However, it blunted the 1 μ M PhE-induced contraction of MA while increasing it at 2 μ M PhE and above (Fig. 2A–C). In contrast, pretreatment of DFA with linoleic acid tended to decrease PhE-induced contractions. However, this effect was not statistically significant (Fig. 2D–F).

Pretreatment with 200 μ M oleic acid alone did not change the basal tone of MA and DFA (Fig. 3), but reduced PhE-induced contraction of MA at 1 and 2 μ M PhE while augmenting it at 10 μ M (Fig. 3A–C). In contrast, pretreatment of DFA with oleic acid significantly suppressed PhE-induced contractions (1 μ M to 10 μ M) (Fig. 3D–F).

To get a mechanistic clue for the changes of vascular contractions by FFAs, we examined the effects of FFAs on membrane depolarization-induced contractions of MA and DFA. Increasing the extracellular K⁺ concentration from 4 to 40 mM would induce moderate depolarization to around –15 mV, which activates L-

Fig. 1. Effect of saturated fatty acid on phenylephrine (PhE)-induced contractile response in mesenteric arteries (MAs) and deep femoral arteries (DFAs) of rat. Original trace of the dose-dependent response curve of PhE in control isolated MAs and DFAs. Representative trace of PhE-induced contraction in MAs (A, B) and DFAs (D, E) with and without palmitic acid. After 30 min of exposure to physiological salt solution with or without palmitic acid, dose-response curves of different PhE concentrations (0.05 μ M, 0.1 μ M, 1 μ M, 2 μ M, 5 μ M, and 10 μ M) are shown. Summaries of PhE dose-response curve in MAs (C) and DFAs (F) are presented as means $\pm$ standard error of the mean. Endo, endothelial cells; ACh, acetylcholine. **p* < 0.05, ***p* < 0.01, ****p* < 0.001.

Fig. 2. Effect of polyunsaturated fatty acid on phenylephrine (PhE)-induced contractile response in mesenteric arteries (MAs) and deep femoral arteries (DFAs) of rat. Original trace of the dose-dependent response curve of PhE in control isolated MAs and DFAs. Representative trace of PhE-induced contraction in MAs (A, B) and DFAs (D, E) with and without linoleic acid. After 30 min of exposure to physiological salt solution with or without palmitic acid, dose-response curves of different PhE concentrations (0.05 μ M, 0.1 μ M, 1 μ M, 2 μ M, 5 μ M, and 10 μ M) are shown. Summaries of PhE dose-response curve in MAs (C) and DFA (F) are presented as mean $\pm$ standard error of the mean. Endo, endothelial cells; ACh, acetylcholine. *** p < 0.001.

type voltage dependent Ca^{2+} channels (VDCC) in smooth muscle. The 40 mM KCl-induced contraction (40K-contraction) was $55.06 \pm 2.53\%$ and $76.68 \pm 5.03\%$ of 80 mM KCl-induced contraction in MA and DFA, respectively (Fig. 4). In MA, the 40K-contraction was increased by pretreatment with palmitic acid ($67.16 \pm 2.17\%$), linoleic acid ($71.31 \pm 2.38\%$), and oleic acid ($87.76 \pm 1.94\%$) (Fig. 4A). In DFA, the 40K-contraction was increased by pretreatment with oleic acid ($95.03 \pm 3.48\%$) and linoleic acid ($101.68 \pm 4.20\%$). However, palmitic acid pretreatment did not affect DFA 40K-contraction (Fig. 4B).

DISCUSSION

The present study showed that the dose-dependent PhE-induced contraction was enhanced in the presence of palmitic acid with a physiological concentration in MAs and DFAs. Furthermore, the application of oleic acid attenuated PhE-induced contraction in both types of arteries. Additionally, incubation with linoleic acid in the artery induced different responses to PhE-induced contraction in MAs and DFAs. The FFAs used in our study are the primary fatty acids circulating in blood plasma concentration [21,29]. The FFAs may have different effects de-

pending on the vessel types perfusing different organs and tissues [19,21,30]. The present study aimed to extend our previous findings investigating the effects of actual nutrient rich (termed nutrition full, NF) solution in vessels [31]. These results may elucidate the specific fatty acids responsible for the previous inconsistent results observed using NF solution in mesenteric and femoral arteries [31].

SFAs, such as palmitic acid, are positively associated with an increased risk of CVD [13,32]. However, the underlying mechanism of how SFAs can increase vascular tone and CVD is unknown [33]. *In vitro*, palmitic acid exposure attenuated the response to endothelium-dependent relaxation in both physiological and high concentrations [21,22,34], which suggested that FFAs may impair the production of endothelium-derived NO. Elevated FFAs induced NO production in endothelial cells from rat aorta [35], and it is well known that NO released from the endothelium has a significant function in regulating vascular tone as a vasodilator [9,36]. NO and superoxide (O_2^-) play a crucial role in the production of peroxynitrite (ONOO^-) [37].

The incubation of linoleic acid, one of PUFA, showed different responses between MAs and DFAs in the present study. A previous study showed that the application of linoleic acid induces uncoupled endothelial NO synthase (eNOS) leading to increased O_2^-

Fig. 4. Effect of fatty acids on the contractile response induced by high K⁺ in mesenteric arteries (MAs) and deep femoral arteries (DFAs) of rat. The contractile response to 40 mM of KCl in the presence or absence of palmitic acid (PA), linoleic acid (LA), oleic acid (OA) in MAs (A) and DFAs (B). *p < 0.05, **p < 0.01, ***p < 0.001.

production, which may react with NO to form the highly reactive intermediate ONOO⁻ responsible for vascular dysfunction [19]. On the other hand, other studies demonstrated that linoleic acid inhibits PhE-induced contraction in rat aorta [38] and induces vascular relaxation and hyperpolarization via activation of the Na⁺/K⁺-ATPase pump in the coronary artery [39]. These various effects of linoleic acid on vascular contractility may explain the different arterial responses observed in the present study.

Fig. 3. Effect of monounsaturated fatty acid on phenylephrine (PhE)-induced contractile response in mesenteric arteries (MAs) and deep femoral arteries (DFAs) of rat. Original trace of the dose-dependent response curve of PhE in control isolated MAs and DFAs. Representative trace of PhE-induced contraction in MAs (A, B) and DFAs (D, E) with and without oleic acid. After 30 min of exposure to physiological salt solution with or without palmitic acid, dose-response curves of different PhE concentrations (0.05 μM, 0.1 μM, 1 μM, 2 μM, 5 μM, and 10 μM) are shown. Summaries of PhE dose-response curve in MAs (C) and DFA (F) of PhE are presented as mean ± standard error of the mean. Endo, endothelial cells; ACh, acetylcholine. *p < 0.05, **p < 0.01, ***p < 0.001.

MUFAs, such as oleic acid in physiological concentration decreased PhE-induced contraction in the present study. Therefore, the application of oleic acid may prevent or improve vascular function by maintaining or increasing NO production by the endothelium [40]. A previous study demonstrated that a high-fat diet rich in unsaturated fatty acids does not decrease PhE sensitivity of the thoracic aorta in rats [41]. Additionally, oleic acid does not induce insulin resistance in cultured cardiovascular cells and it even protects against insulin resistance caused by palmitic acid or inflammatory factors, such as tumor necrosis factor α [42,43]. However, there is evidence that oleic acid attenuates endothelium-dependent relaxation in MAs [21]. Further, the direct exposure to oleic acid enhances vascular smooth muscle cell proliferation and migration from the rat aorta [30,44]. These conflicting results might be due to differences in experimental conditions, animal strains, and vessels.

The present study also showed effects of FFAs on high potassium induced contraction. High potassium levels induce membrane depolarization, which activates VDCC [45]. The increase in intracellular calcium levels might be responsible for the increase of high K⁺-induced contraction in the presence of palmitic acid, linoleic acid, and oleic acid in our study. A previous study found that linoleic acid increases the activation of intracellular calcium

levels in endothelial cells from the pulmonary artery [19]. Even though the incubation with oleic acid decreased sensitivity of alpha adrenergic constriction, high K^+ -induced contraction was increased in the presence of oleic acid. The increase in high K^+ -induced contraction with unsaturated fatty acids may be due to VDCC, which induces the contraction by regulating intracellular calcium release [19]. However, the vascular constriction in response to alpha adrenergic agonists is regulated by a more complex mechanism including receptor operated calcium influx via Rho-kinase or protein kinase C (PKC) activation [46]. Alpha adrenergic agonists such as PhE can activate phospholipase C which leads to the formation of inositol triphosphate and diacylglycerol [47], which induces phosphorylation of PKC and intracellular calcium release [46]. A previous study showed that the infusion of FFAs increases PKC activity in rat aortic endothelium [35]. Thus, increased PKC activity might contribute to the enhancement of PhE-induced contraction in the present study. Further investigation is required to clarify the obtained results.

In the present study, we found that SFAs in physiological concentration augments sensitivity to PhE-induced contraction in rat MAs and DFAs, whereas MUFAs improve vascular relaxation. On the basis of our finding, we suggest that the consumption of foods containing SFAs may increase the risk of CVD. Furthermore, a diet that replaces saturated fat with unsaturated fat within normal range may prevent the increase of vascular tension.

In summary, we conclude that both saturated and unsaturated fatty acids have diverse effects on vascular function depending on vascular beds. Our findings may serve as basic evidence for exploring the underlying mechanism of the effect of FFAs on vascular contractility.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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