Myristic Acid Stimulates Endothelial Nitric-oxide Synthase in a CD36- and an AMP Kinase-dependent Manner*

Weifei Zhu and Eric J. Smart†

From the Department of Pediatrics, University of Kentucky, Lexington, Kentucky 40536

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Dietary free fatty acids have been reported to have various effects on the endothelium including the generation of nitric oxide. The goal of the current study was to determine the mechanism whereby free fatty acid causes an increase in nitric oxide synthesis. The specific hypothesis tested was that free fatty acid association with CD36, a class B scavenger receptor, induces the activation of endothelial nitric-oxide synthase (eNOS). A human microvascular endothelial cell line and a transfected Chinese hamster ovary cell system were used to determine which free fatty acids stimulate eNOS. Surprisingly, only myristic acid, and to a lesser extent palmitic acid, stimulated eNOS. The stimulation of eNOS was dose- and time-dependent. Competition experiments with other free fatty acids and with a CD36-blocking antibody demonstrated that the effects of myristic acid on eNOS required association with CD36. Further mechanistic studies demonstrated that the effects of myristic acid on eNOS function were not PI 3-kinase, Akt kinase, or calcium. Studies and dominant negative constructs were used to demonstrate that myristic acid stimulation of eNOS activity was dependent on AMP kinase. These data demonstrate an unexpected link among myristic acid, CD36, and AMP kinase activity.

The specific effects of free fatty acids on eNOS activity have been controversial. The controversy is in part because dietary fatty acids affect multiple systems (e.g. lipid metabolism, angiogenesis (20), a process known to involve nitric oxide generation (21)). Interestingly, CD36 and eNOS are both localized to caveolae in endothelial cells, which may provide a localized and specific signaling environment for fatty acids. Endothelial nitric-oxide synthase has a surprisingly large number of regulatory mechanisms for controlling the synthesis of nitric oxide. An increase in intracellular calcium followed by calcium-calmodulin binding to eNOS is a major mechanism for stimulating nitric oxide generation. Ceramide (24), subcellular localization (5), acylation (7, 8), chaperone proteins (25), cofactor availability (26), and cholesterol (27) have all been shown to modulate eNOS activity. Numerous studies have also demonstrated that eNOS can be activated by direct phosphorylation at serine 1179 (28). Activation of the PI 3-kinase/Akt kinase pathway will induce eNOS phosphorylation and activation (29). In addition, activation of AMP kinase can result in eNOS phosphorylation and activation (29, 30).

AMP kinase is a major regulator of cellular energy metabolism. AMP kinase is activated by phosphorylation when the ATP levels in the cell decrease and the AMP levels rise. Upon phosphorylation and activation, AMP kinase phosphorylates numerous downstream targets, many of which are involved in restoring ATP levels, such as acetyl-CoA carboxylase (ACC), hydroxymethylglutaryl-CoA reductase, glycogen synthase, and eNOS (31). The exact mechanisms responsible for AMP kinase

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† To whom correspondence should be addressed: Dept. of Pediatrics, University of Kentucky, 423 Sanders-Brown, 800 Limestone St., Lexington, KY 40536-0230. Tel.: 859-323-6412; Fax: 859-257-2120; E-mail: ejsmart@uky.edu.
‡ The abbreviations used are: eNOS, endothelial nitric-oxide synthase; ACC, acetyl-CoA carboxylase; BAPTA-AM, 1,2-bis(aminophenoxy)ethane-N,N,N',N'-tetraacetic acid-acetoxymethyl; CHO, Chinese hamster ovary; HME, human microvascular endothelial; t-NNA, N-nitro-l-arginine; PI 3-kinase, phosphatidylinositol 3-kinase; PVDF, polyvinylidene difluoride; TBS, Tris-buffered saline; cAMPS, adenosine 3',5'-cyclic phosphorothioate.

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phosphorylation are not clear. A recent study by Thors et al. (32) demonstrated that thrombin and histamine stimulation of AMP kinase and subsequent activation of eNOS was independent of the PI 3-kinase/Akt kinase pathway. In contrast, Ouchi et al. (33) demonstrated that adiponectin-induced eNOS activation required cross-talk between AMP kinase and the PI 3-kinase/Akt kinase pathway. Interestingly, Zou et al. (34) have shown that peroxynitrite can stimulate AMP kinase and eNOS phosphorylation via a Src kinase-mediated mechanism.

In this study, we investigated the effect of free fatty acids on eNOS activity. In addition, we defined a novel signaling pathway involving free fatty acid association with CD36 which resulted in the activation of AMP kinase and the subsequent activation of eNOS.

**EXPERIMENTAL PROCEDURES**

**Materials—**M199 medium, glutamine, trypsin-EDTA, BME vitamin mix, and penicillin/streptomycin were from Invitrogen. The Bradford assay kit was from Bio-Rad. The free fatty acids, wortmannin, PVDF membrane, ethanol, and actin IgG were purchased from Sigma. The anti-CD36 IgM was from Biodesign International (Kennebunk, ME). The non specific mouse IgM (MOPC-104E) was from Sigma. The phosho-Akt kinase, phosho-eNOS (serine 1179) AMP kinase, and phosho-AMP kinase (threonine 172) IgGs were from Cell Signaling Technologies (Beverly, MA). The ACC IgG was from Upstate Biotechnology, Inc. Ionomycin, BAPTA-AM, H89, R, cAMPS, acetylcholine, bradykinin, 4-amino-5-(4-chloroanilino)-7-(6-(2-buty1)pyrazolo[3,4-d]pyrimidine, herbinicin A, and genistein were from Calbiochem. Super Signal chemiluminescent substrate was purchased from Pierce. [3H]Arginine was from DuPont (Wilmington, Del). AMP kinase adenoviral constructs were a gift from K. Walsh (Boston University, MA).

**Buffers—**Sample buffer (5×) consisted of 0.31 M sucrose (w/v), 50 mM Tris, 150 mM NaCl, 2 mM L-glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, and 0.125% (v/v) buffered saline (TBS) consisted of 20 mM Tris, pH 7.6, and 137 mM NaCl.

**Cell Culture—**Human microvascular endothelial cells were cultured in M199 medium supplemented with 10% fetal calf serum. On day 0, 5,000 cells were placed into 12-well plates and used on day 3 at ~60% confluence. The human eNOS cDNA was stably transfected into CHO cells, and cell lines stably expressing eNOS were obtained by G418 selection. The CHO cell lines stably expressing eNOS, CD36 and eNOS were established in Ham’s F-12 medium containing 10% fetal bovine serum, 2 mM l-glutamine, 100 units/ml penicillin, 50 μg/ml streptomycin, 0.3 mg/ml G418.

**Preparation of Fatty Acid-Albumin Complex—**Fatty acids were delivered to endothelial cells in a complex with albumin using sheep serum albumin. To generate the fatty acid-albumin complex, fatty acids were added slowly to Kreb-Ringer buffer containing 5% sheep serum albumin as described previously (35). Fatty acids were added from concentrated stock (200 mM in ethanol). Previous studies have demonstrated that the ratio of 0.5 fatty acids to albumin is sufficient to deliver free fatty acids to cells without causing nonspecific perturbation of the plasma membrane.

**Activation of NOS in Intact Cells—**NOS activation was determined in intact cells as described previously (36). Briefly, the cells were plated into 12-well plates at 5,000 cell/well and grown to 60% confluence. The medium was replaced with serum-free medium for 16 h and then placed for 2 h in phosphate-buffered saline, at 37 °C. After the preincubation period, the phosphate-buffered saline was removed from the wells and replaced with 400 μl of phosphate-buffered saline containing 0.75 μCi/ml of [3H]Arginine and the indicated treatments. The cells were incubated at 37 °C for 30 min. The NOS reaction was terminated by adding 500 μl of ice-cold 1 N trichloroacetic acid to each well. The cells were freeze-fractionated twice in liquid nitrogen for 2 min with thawing at 37 °C for 5 min and scraped with a rubber spatula. The contents of each well were transferred to ice-cold glass test tubes. Ethanol extraction was performed three times with water-saturated ether to remove the trichloroacetic acid. The samples were neutralized with 1.5 ml of 25 mM HEPES, pH 8, applied to Dowex AG 50WX-8 (Tris form) columns, and eluted with 1 ml of 40 mM HEPES buffer, pH 5.5, containing 2 mM EDTA and 2 mM EGTA. l-[3H]Citrulline was collected in scintillation vials and quantified by liquid scintillation counting. In individual experiments performed in 12-well plates, 3 wells were used for each treatment group. Findings were confirmed in at least six independent experiments. NOS activation in the intact cells was inhibited by 1 mM N-nitro-l-arginine (l-NNA). To ensure that the treatments did not affect the loading of the cells with l-[3H]Arginine, the amount of l-[3H]Arginine associated with the cells was determined.

**Electrophoresis and Immunoblots—**Samples were concentrated by trichloroacetic acid precipitation and washed in acetone. Pellets were suspended in sample buffer that contained 1.2% (v/v) β-mercaptoethanol and heated at 95 °C for 3 min before being loaded onto gels. Proteins were separated in a 12.5% SDS-polyacrylamide gel using the method of Laemmli (37). The separated proteins were then transferred to PVDF membrane. The membrane was blocked in TBS that contained 5% dry milk for 1 h at room temperature. Primary antibodies were diluted in TBS that contained 1% dry milk and incubated with the membrane for 1 h at room temperature. The membrane was washed four times, 10 min each in TBS + 1% dry milk. The secondary antibodies (all conjugated to horseradish peroxidase) were diluted 1/20,000 in TBS + 1% dry milk and incubated with the membrane for 1 h at room temperature. The membrane was then washed and the bands visualized by chemiluminescence.

**Statistical Analysis—**One-way analysis of variance was used to evaluate the data with regard to treatment, time, and their interaction using the analysis of variance procedure of Statistica. When appropriate, treatment means within a given time were compared using the Tukey’s honestly significant difference test. Means were considered significant at p < 0.05.
myristic acid affected the uptake of \[^{3}H\]arginine. HME cells were treated with 0.75 Ci of \[^{3}H\]arginine and 10 \(\mu M\) myristic acid or buffer for 2 min or 20 min in the presence of 1 mM L-NNA. The cells were washed, and the amount of \[^{3}H\]arginine associated with the cells was determined. Fig. 2 demonstrates that the amount of \[^{3}H\]arginine associated with the cells was maximal by 2 min and was not affected by the presence of myristic acid.

We next determined whether the large increase in eNOS activity, compared with the ionomycin control, was unique to myristic acid or whether other eNOS agonists also induced the same magnitude of activation. HME cells were incubated with \[^{3}H\]arginine and myristic acid as described above. Additional sets of cells were treated with 1 \(\mu M\) acetylcholine, 1 \(\mu M\) bradykinin, or 2 \(\mu g/ml\) ionomycin. Fig. 3 demonstrates that acetylcholine, bradykinin, and ionomycin all stimulated eNOS activity to the same extent. The addition of myristic acid to acetylcholine, bradykinin, and ionomycin did not enhance the stimulation caused by myristic acid alone. These data suggest that myristic acid maximally stimulates eNOS.

**Association with CD36 Is Required for Myristic Acid to Increase eNOS Activity**—The CHO cell lines used in Fig. 1 strongly suggest that CD36 is necessary for myristic acid to stimulate eNOS activity. To determine whether the effects of myristic acid were dependent upon CD36, HME cells were pretreated for 30 min at 37 °C with 20 \(\mu g/ml\) of an established CD36-blocking antibody or 20 \(\mu g/ml\) of a nonrelevant, isotype-matched antibody (38, 39). The cells were then incubated with 10 \(\mu M\) myristic acid and 0.75 Ci/ml \[^{3}H\]arginine for 30 min at room temperature. Control cells that were incubated with myristic acid but did not receive any antibodies were stimulated 8–10-fold over cells not receiving myristic acid (Fig. 4A). The addition of a nonrelevant antibody had no effect on the ability of myristic acid to stimulate eNOS (Fig. 4A). In contrast, preincubation of cells with a CD36-blocking antibody inhibited myristic acid-induced eNOS activity (Fig. 4A).
induced nitric oxide generation. The cells were incubated with 0.75

Each experiment included controls using 1 mM L-NNA to demonstrate
demonstrate that the generated citrulline was the result of eNOS ac-
activity. The data are from four independent experiments, with triplicate

demonstrates that increasing concentrations of stearic acid
core the ability of myristic acid to stimulate eNOS.

To confirm further that the effect of myristic acid on eNOS
time-dependent manner. Myristic acid stimulated eNOS

demonstrates that the positive control, platelet-derived
activity, and therefore, ionomycin (Fig. 5A). To confirm

Fig. 2. Myristic acid stimulates eNOS in a concentration-and
time-dependent manner. The cells were incubated with 0.75

Myristic acid maximally stimulates eNOS. The cells were

Myristic acid stimulates eNOS in a concentration- and
time-dependent manner. First, HME cells were pretreated

mechanisms. The ability of myristic acid to stimulate eNOS

Myristic acid does not stimulate eNOS activity. The cells were

Fig. 3. Myristic acid maximally stimulates eNOS. The cells were incubated with 0.75 μCi/ml [3H]arginine, buffer, 10 μM myristic acid, 1 μM acetylcholine, 1 μM bradykinin, 2 μg/ml ionomycin, 10 μM myristic acid + 1 μM acetylcholine, 10 μM myristic acid + 1 μM bradykinin, and 10 μM myristic acid + 2 μg/ml ionomycin. The cells were incubated for 30 min at room temperature with the above reagents and then pro-
cessing to quantify the amount of [3H]citrulline generated. The data are from three independent experiments with triplicate measurements, mean ± S.E. A, Ach, acetylcholine; BK, bradykinin; open bars, the indicated treatment + L-NNA. C, effect of myristic acid on [3H]arginine uptake. The cells were incubated with 0.75 μCi/ml [3H]arginine and 10 μM myristic acid for 2 or 20 min in the presence of 1 mM L-NNA. The amount of [3H]arginine associated with the cells was quantified. The data are from three independent experiments with triplicate measurements in each experi-

Because myristic acid induced the phosphorylation of eNOS

Before quantifying the production of [3H]citrulline. Fig. 4B
demonstrates that wortmannin did not inhibit the stimulatory ef-
faction data we examined the phosphorylation

cycles 1179 in eNOS induced responses (28). Two methods were used to
to further the possible involvement of Akt kinase, we used commercially available antibodies to de-

Fig. 4B shows that the positive control, platelet-derived
growth factor, stimulated Akt kinase phosphorylation and that

We next determined whether myristic acid stimulates AMP

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Myristic acid stimulates eNOS via a calcium-dependent mechanism we first loaded the

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Fatty Acids and Nitric Oxide

myristic acid to the cells. Fig. 5A shows that the positive control, platelet-derived

Fatty Acids and Nitric Oxide
eNOS by direct phosphorylation of the enzyme. To test the potential involvement of AMP kinase in myristic acid-induced eNOS activity, A, the indicated cells were treated with 20 μg/ml CD36-blocking antibody or 20 μg/ml irrelevant antibody for 30 min at 25 °C. Without removing the antibodies, the cells were then incubated with 0.75 μCi/ml [3H]arginine, only, 10 μM myristic acid, or 2 μg/ml BAPTA-AM. Cells were then processed to quantify the amount of [3H]citrulline generated. Each experiment included controls using 1 μM L-NNA to demonstrate that the generated citrulline was the result of eNOS activity. The data are from five independent experiments, with triplicate measurements in each experiment, mean ± S.E., *p < 0.01. Black bars, the indicated treatment; open bars, the indicated treatment.

Fig. 4. Association with CD36 is required for myristic acid stimulation of eNOS activity. A, the indicated treatment; open bars, the indicated treatment.

Because CD36 has been shown to stimulate Src kinases (19) and because AMP kinase can be activated by Src kinases (42), we determined whether myristic acid stimulates AMP kinase via a Src kinase. HME cells were pretreated for 15 min at 37 °C with different Src kinase inhibitors: 10 μM genistein, 0.1 μg/ml herbimycin A, or 10 μM 4-amino-5-(4-chlorophenyl)-7-(4-butylnitripyrazolo[3,4-d]pyrimidine. The cells were then incubated with 10 μM myristic acid and 0.75 μCi of [3H]arginine for 30 min at room temperature. Additional sets of cells were treated with buffer only or with 2 μg/ml ionomycin. Each experiment also included duplicate samples containing 1 mM L-NNA. Fig. 9 demonstrates that the three different Src kinase inhibitors did not prevent myristic acid-induced stimulation of eNOS. Higher concentrations of the Src kinase inhibitors and longer or shorter pretreatment times did not affect myristic acid-induced stimulation of eNOS (data not shown). Finally, the addition of myristic acid did not alter the AMP:ATP ratio in the cells,
FIG. 6. Myristic acid stimulation of eNOS is independent of Akt kinase. A, the indicated cells were pretreated with 100 nM wortmannin for 30 min at 25 °C. The cells were then incubated with 0.75 μCi/mI [1H]arginine, buffer only, or the indicated concentrations of myristic acid for 30 min at 25 °C. The cells were then processed to quantify the amount of citrulline generated. Each experiment included controls using 1 mM t-NAME to demonstrate that the generated citrulline was the result of eNOS activity (data not shown). The data are from three independent experiments, with triplicate measurements in each experiment, mean ± S.E. B, the cells were incubated with 67 ng/ml platelet-derived growth factor (PDGF), 100 nM wortmannin (pretreated 30 min), or 10 μM myristic acid for 30 min at 25 °C. The cells were washed, lysates generated, and cellular proteins resolved by SDS-PAGE (10 μg). The separated proteins were transferred to PVDF and Western blotted with actin and phospho-Akt antibodies. The Western blots were developed by the method of chemiluminescence (1–2-min exposures). The data are representative of three independent experiments.

suggesting that myristic acid did not stimulate AMP kinase through alterations in energy metabolism.

We have defined a novel signaling pathway that couples exogenous free fatty acids to eNOS activation of AMP kinase. One of the current study is the remarkable specificity with which free fatty acids stimulate eNOS activity, examined, myristic acid, was the most effective at stimulating eNOS, whereas palmitic acid did not cause a significant increase in eNOS activity (data not shown). The lack of activity of stimulating eNOS. Experiments with blocking antibodies, CD36-minus cell lines, and competition studies clearly demonstrated that the effect of myristic acid depended on the presence of CD36.

CD36 is a fatty acid-binding protein and is involved in the uptake of long chain fatty acids, although long chain fatty acid uptake is not absolutely dependent on the presence of CD36 (15–17). In addition, CD36 can interact with and facilitate the uptake of short chain fatty acids; however, short chain fatty acids cross the plasma membrane at a sufficiently high rate that CD36 does not appear to contribute significantly to this process (43). The molecular mechanisms and regulation involved in CD36-mediated fatty acid uptake are not completely understood. Recently, Bonen et al. (44) demonstrated that in myocytes, contraction and insulin caused an intracellular pool of CD36 to translocate to the cell surface, which caused an increase in fatty acid uptake without synthesis of new CD36. In addition, Pohl et al. (17) have shown that CD36-dependent uptake of long chain fatty acids in 3T3-L1 adipocytes requires the presence of intact lipid rafts. In endothelial cells, caveolae (a type of lipid raft) contain CD36 and eNOS and may form a functional signaling complex that couples exogenous fatty acids to eNOS activity. Our current data do not distinguish conclusively between a direct binding to CD36 or after translocation to the cell surface. The effects of myristic acid depend on the presence of CD36 and not CD36-mediated processes based on the following. First, myristic acid require CD36 for maximal uptake into the cell; however, the presence or absence of CD36 does not significantly alter the amount of myristic acid associated with the cells. Therefore, the effects of myristic acid are absolutely dependent on the presence of CD36. Second, CD36-blocking antibodies and competition studies with stearic acid prevented the stimulatory effects of myristic acid. These reagents will not affect the amount of myristic acid taken up by the cell, again because myristic acid moves across the membrane independently of CD36 (43). Third, activation of eNOS is specific for myristic acid, which argues against membrane perturbations and detergent-like effects of excessive fatty acid accumulation. However, it is feasible that CD36 mediates the uptake and delivery of a small amount of myristic acid to caveolae where it may possibly have direct effects on AMP kinase and/or eNOS.

We think it is more likely that myristic acid association with CD36 activates a signaling cascade that results in AMP kinase activation and subsequently eNOS activation. Recently, Medeiros et al. (18) have shown that in mouse macrophages apolipoprotein C-II can activate the Src-like kinase, Lyn, in a CD36-dependent manner. In addition, the activation of Lyn resulted in p44/p42 mitogen-activated protein kinase activation and ultimately an inhibition in the expression of tumor necrosis factor-α (19). Macrophages isolated from CD36 null mice did not respond to apolipoprotein C-II treatment. In a different study, Lee et al. (45) demonstrated that caveolin-1 and c-Src are functionally coupled in caveolae. The available data sug-
and AMP kinase and because AMP kinase has been shown to regulate eNOS function directly, independent of CD36 and eNOS, and may not always disrupt caveolae functions (46). Furthermore, the use of small interfering RNA to eliminate caveolin-1 is problematic because caveolin-1 is known to regulate eNOS function directly, independent of CD36 and myristic acid. Elucidating the possible role of caveolae in this process is important and will require numerous approaches to generate a reliable conclusion.

Studies using the calcium chelator BAPTA-AM demonstrated that myristic acid did not stimulate eNOS by causing an increase in intracellular calcium. In addition, we used the PI 3-kinase inhibitor, wortmannin, and phospho-Akt antibodies to confirm these findings further we used a dominant negative AMP kinase or empty control adenovirus at a multiplicity of infection of 50 for 24 h as described previously (41). The cells were then incubated with 0.75 μCi/ml [3H]arginine, buffer only, 10 μM myristic acid, or 2 μg/ml ionomycin for 30 min at 25 °C. The cells were processed to quantify the amount of citrulline generated. Each experiment included controls using 1 mM L-NNA to demonstrate that the generated citrulline was the result of eNOS activity. The data are from three independent experiments, with triplicate measurements in each experiment, mean ± S.E., p < 0.01. The adenoviral infections did not significantly increase cell death (data not shown).

Histone inhibitors do not prevent myristic acid stimulation of eNOS. HME cells were pretreated for 15 min with different Src kinase inhibitors: 10 μM genistein, 0.1 μg/ml herbimycin A, or 10 μM 4-amino-5-(4-chlorophenyl)-7-(t-butyl)pyrazolo[3,4-d]pyrimide. Cells were then incubated with 10 μM myristic acid and 0.75 μCi of [3H]arginine for 30 min at room temperature. Additional sets of cells were treated with 2 μg/ml ionomycin or buffer. Duplicate samples also contain L-NNA. The data are from three independent experiments with triplicate measurements in each experiment, mean ± S.E. Black bars, the indicated treatment; open bars, indicated treatment + L-NNA.

FIG. 7. Myristic acid stimulation of eNOS requires AMP kinase. A, HME cells were treated with buffer only, 20 μM Rp-cAMPS for 15 min. Myristic acid (10 μM) and Rp-cAMPS were then added for 30 min at 25 °C. To confirm these findings further we used a dominant negative AMP kinase or empty control adenovirus at a multiplicity of infection of 50 for 24 h as described previously (41). The cells were then incubated with 0.75 μCi/ml [3H]arginine, buffer only, 10 μM myristic acid, or 2 μg/ml ionomycin for 30 min at 25 °C. The cells were processed to quantify the amount of citrulline generated. Each experiment included controls using 1 mM L-NNA to demonstrate that the generated citrulline was the result of eNOS activity. The data are from three independent experiments, with triplicate measurements in each experiment, mean ± S.E., p < 0.01. The adenoviral infections did not significantly increase cell death (data not shown).

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FIG. 8. A dominant negative AMP kinase prevents myristic acid stimulation of eNOS. HME cells were infected with an adenovirus construct containing dominant negative AMP kinase or empty control adenovirus at a multiplicity of infection of 50 for 24 h as described previously (41). The cells were then incubated with 0.75 μCi/ml [3H]arginine, buffer only, 10 μM myristic acid, or 2 μg/ml ionomycin for 30 min at 25 °C. The cells were processed to quantify the amount of citrulline generated. Each experiment included controls using 1 mM L-NNA to demonstrate that the generated citrulline was the result of eNOS activity. The data are from three independent experiments, with triplicate measurements in each experiment, mean ± S.E., p < 0.01. The adenoviral infections did not significantly increase cell death (data not shown).
transfect cells and examine their response to myristic acid. The cells transfect with a control adenovirus, and uninfected cells, both displayed a stimulatory response to myristic acid and ionomycin. However, the cells transfect with the dominant negative AMP kinase construct did not respond to myristic acid, further demonstrating that AMP kinase is in the signaling pathway leading to eNOS activation in these cells. It is important to emphasis that agonist-induced stimulation of AMP kinase and eNOS may vary between different types of cells. For instance, Fleming et al. (47) demonstrated that insulin stimulates eNOS via AMP kinase in platelets but not in endothelial cells. The mechanistic explanation for this difference is not known.

Myristic acid is the third most common saturated fat in the diet with ~8 g consumed per day in the United States (48). Saturated fatty acid increases plasma high and low density lipoprotein levels in humans (1). Of the dietary fatty acids, myristic acid causes the largest increase in plasma low density lipoprotein levels. Although an increase in high density lipoprotein levels is considered cardioprotective, an increase in low density lipoprotein levels is considered proatherogenic (1). Saturated fatty acid increases plasma high and low density lipoprotein levels is considered proatherogenic (1). Myristic acid is the third most common saturated fat in the diet with only a small increase in risk for coronary heart disease (47). However, analysis of data from the Nurses’ Health Study indicated the conclusion that saturated fats were highly pro-disease.

Our current data demonstrate that a specific fatty acid, myristic acid, may offset the myristic acid-induced increase in plasma cholesterol levels. Both platelet and uninfected cells, transfected with a control adenovirus, and uninfected transfect cells and examine their response to myristic acid. The signaling pathway leading to eNOS activation in these cells. It is important to emphasis that agonist-induced stimulation of AMP kinase and eNOS may vary between different types of cells. For instance, Fleming et al. (47) demonstrated that insulin stimulates eNOS via AMP kinase in platelets but not in endothelial cells. The mechanistic explanation for this difference is not known.

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