

Citrulline increases cholesterol efflux from macrophages *in vitro* and *ex vivo* via ATP-binding cassette transporters

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Reverse cholesterol transport (RCT) is a mechanism critical to the anti-atherogenic property of HDL. Although citrulline contributes to the amelioration of atherosclerosis via endothelial nitric oxide production, it remains unclear whether it affects RCT. This study was undertaken to clarify the effects of citrulline on expressions of specific transporters such as ATP binding cassette transporters (ABC)A1 and ABCG1, and the cholesterol efflux from macrophages to apolipoprotein (apo) A-I or HDL *in vitro* and *ex vivo*. Citrulline increased ABCA1 and ABCG1 mRNA and protein levels in THP-1 macrophages, translating into enhanced apoA-I- and HDL-mediated cholesterol efflux. In the human crossover study, 8 healthy male volunteers (age 30–49 years) consumed either 3.2 g/day citrulline or placebo for 1 week. Citrulline consumption brought about significant increases in plasma levels of citrulline and arginine. Supporting the *in vitro* data, monocyte-derived macrophages (MDM) differentiated under autologous post-citrulline sera demonstrated enhancement of both apoA-I- and HDL-mediated cholesterol efflux through increased ABCA1 and ABCG1 expressions, compared to MDM differentiated under pre-citrulline sera. However, the placebo did not modulate these parameters. Therefore, in addition to improving endothelium function, citrulline might have an anti-atherogenic property by increasing RCT of HDL.

Key Words: citrulline, cholesterol, macrophage, HDL, reverse cholesterol transport

HDL has been shown to be inversely associated with the risk of atherosclerotic cardiovascular disease (CVD) and is thus considered to be an anti-atherogenic lipoprotein.^(1,2) However, failure in recent clinical trials with HDL-C raising agents such as nicotinic acid and the cholesteryl ester transfer protein inhibitor torcetrapib to provide cardiovascular benefits despite raising HDL-C levels, indicate that the focus should be on quality (functionality), and not necessarily quantity (HDL-C levels) regarding therapeutic targets.⁽³⁾ Anti-atherogenic functions of HDL include anti-inflammatory,⁽⁴⁾ anti-oxidative,⁽⁵⁾ anti-coagulative,⁽⁶⁾ and endothelial-improving functions,⁽⁷⁾ as well as enhancement of macrophage reverse cholesterol transport (RCT), a process by which cholesterol is transported from macrophages to the liver for ultimate fecal excretion. Although these functions may comprehensively be involved in the prevention of atherosclerosis progression,^(8,9) RCT itself has been postulated to play a major role in HDL-mediated atheroprotection.⁽¹⁰⁾ Indeed, quantitative measures of macrophage RCT have been shown to be more strongly associated with atherosclerosis than plasma HDL-C concentrations in mice and humans.^(11,12) The first critical step of

macrophage-derived foam cell RCT involves efflux of cellular cholesterol to circulating HDL particles.

Research in recent years has documented a specific role for the macrophage transporters ATP-binding cassette subfamilies (ABC) A1 and ABCG1 in cholesterol efflux. ABCA1 has been shown to play an important role in apolipoprotein (apo) A-I-mediated cholesterol efflux from peripheral cells and macrophages while ABCG1 promotes cholesterol efflux from macrophages to HDL particles.^(13,14) These findings have stimulated efforts to target the macrophage at the cellular level as a means of enhancing overall RCT.

Endothelial dysfunction is a key feature of early atherosclerotic lesions in both humans and animal models and elevated plasma concentrations of risk factors for CVD.^(15–18) Citrulline is unusually abundant in watermelon (*Citrullus vulgaris*) and arginine is synthesized from it in endothelial cells.⁽¹⁹⁾ The conversion of citrulline to nitric oxide (NO) via arginine forms part of a recycling pathway.^(20–22) NO is synthesized by NO synthase (NOS), which produces citrulline, a precursor of arginine. Chronic consumption of citrulline-rich food has been known to be effective in increasing plasma concentrations of arginine in healthy humans,⁽²³⁾ and Hayashi *et al.*⁽²⁴⁾ reported that citrulline decreased the progression of atherosclerosis in rabbits fed a high cholesterol diet, via endothelial NOS (eNOS). Although citrulline contributes to anti-atherosclerosis via endothelial nitric oxide, it remains unclear whether it affects the anti-atherogenic properties of HDL-mediated RCT functions, or how it might do this. Therefore, the present study was undertaken to investigate the effects of citrulline on ABCA1 and ABCG1 expressions and HDL-mediated cholesterol efflux from macrophages, both *in vitro* and *ex vivo* situations.

Materials and Methods

Materials. Actinomycin D (ActD) and human apoA-I were purchased from Sigma (St. Louis, MO). *N*^G-nitro-L-arginine methyl ester (L-NAME) and phorbol 12-myristate 13-acetate (PMA) were purchased from Wako Pure Chemical (Tokyo, Japan). HDL was isolated by sequential ultracentrifugation and acetylated LDL (acLDL) was prepared according to the previously reported methods.^(25,26) L-Citrulline for the *in vitro* and *in vivo* study was purchased from Kanto Chemical CO., INC. (Tokyo, Japan). Placebo and citrulline for the *ex vivo* study were kindly

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donated by Kyowa Hakko Bio Co., Ltd. (Tokyo, Japan).

Cell culture. THP-1 cells (Riken Cell Bank, Tsukuba, Japan) were maintained in RPMI 1640 (Sigma) containing 10% fetal bovine serum (FBS). The differentiation of THP-1 monocytes into macrophages was induced in the presence of 320 nM of PMA for 72 h. RAW264.7 cells (Riken Cell Bank, Tsukuba, Japan) were maintained in Dulbecco's modified Eagle's medium (DMEM) (Sigma) containing 10% FBS.

Quantitative real-time PCR. At the indicated hours after treatment with the compounds, total RNA was extracted from the cells, and first-strand cDNA was synthesized from the total RNA (250 ng) by placing in a Reverse Transcription Reagent (Applied Biosystems, Foster City, CA). Quantitative real-time PCR was performed using an ABI 7900 PCR machine, TaqMan PCR master mix and FAM-labeled TaqMan probes (Assays-on-Demand, Applied Biosystems) for human ABCA1, ABCG1, scavenger receptor class B type I (SR-BI) and 18S ribosomal RNA. The expression data were normalized for 18S levels.

Western blot analyses. The cells were harvested and protein extracts prepared as previously described.^(25–27) They were then subjected to Western blot analyses (10% SDS-PAGE; 30 µg protein per lane) using rabbit anti-ABCG1 (Novus Biologicals, Littleton, CO) and anti-β-actin (Santa Cruz Biotechnology, Santa Cruz, CA) antibodies. Rat anti-ABCA1 was kindly donated by Dr. S. Yokoyama. The proteins were visualized and quantified using a chemiluminescence method (ECL Plus Western Blotting Detection System; GE Healthcare UK Ltd., Buckinghamshire, UK) and the NIH image analysis software program.

Determination of cholesterol efflux. Cholesterol efflux experiments were performed as previously described.^(25–27) The cells were labeled with [³H] cholesterol (1.0 µCi/ml) in media containing 0.1% bovine serum albumin (BSA) for 24 h. The cells were washed with phosphate buffered saline (PBS) and incubated in RPMI 1640 containing 0.1% BSA in the presence and absence of apoA-I (10 µg/ml) or HDL (50 µg/ml) for 24 h. The percentage cholesterol efflux was calculated by dividing the media-derived radioactivity by the sum of the radioactivity in the media and the cells.

Human study. Eight healthy male volunteers (age 30–49 years) were recruited for a crossover study. After an overnight (12 h) fast, the study subjects consumed placebo or 1.6 g citrulline twice daily for 1 week.⁽²⁸⁾ Blood samples were taken at 0 and 1 week after consumption of placebo or citrulline. All subjects provided written informed consent. The study was approved by the Ethical Committee of the National Defense Medical College.

Human monocyte isolation and macrophage differentiation. Human monocytes were isolated as previously described.^(25–27,29) The remaining blood was drawn into heparinized blood collection tubes. Human peripheral blood monocytes were isolated using the method of Fogelman *et al.*⁽³⁰⁾ with Ficoll/Hypaque gradient centrifugation. The mononuclear cells were re-suspended in RPMI 1640 (Sigma) supplemented with 20% autologous serum, plated on to serum-treated 10-cm dishes and incubated for 2 h. Non-adherent cells were removed by washing three times with PBS, and adherent cells were then detached by incubation in PBS containing 10% autologous serum and 0.02% EDTA at 4°C for 30 min. The adherent cells were then washed extensively and re-suspended in RPMI 1640 supplemented with 10% autologous serum. They were then plated on 12-well plates and incubated for 10 days so that they would differentiate into monocyte derived macrophages (MDM). The culture media was changed every 3–4 days during this 10-days period.

Analysis of citrulline and arginine in plasma. Blood samples were rapidly centrifuged and deproteinized with a 5% (w/v) sulfosalicylic acid solution. The supernatant fractions were stored at –80°C for analysis of amino acids. Amino acids were separated and quantified by ion exchange chromatography using an amino acid autoanalyser (Amino Tac, JLC-300; Jeol Ltd, Tokyo,

Japan) and the detection sensitivity was 125–500 nM. Amino acid analysis was performed using an LC-R-6 column (6.0 × 90 mm I.D., Mitsubishi Chemical Co., Tokyo, Japan). The mobile phase was performed using lithium citrate buffer solution.⁽²⁸⁾ The auto-analyzer system consisted of the reaction coil (0.25 × 10 m I.D.) and a 570 and 440 nm at 132°C.

Serum TC, TG, LDL-C, HDL-C, glucose and NO₃. Serum total cholesterol (TC), triglycerides (TG), LDL-cholesterol (LDL-C) and HDL-C levels were determined by standard enzymatic methods (Measure L, Kyowa Mediceed, Tokyo, Japan). The serum concentration of glucose was determined using an Olympus AU 600 analyser. Blood nitrate (NO₃) was quantified by the HPLC-UV method.^(31,32)

Statistical analysis. Statistical analyses were performed using the Stat View ver. 5.0 software package (SAS Institute Inc., NC). For the cell culture study, two-way factorial ANOVA (Fig. 1A and B) or one-way ANOVA, followed by post-hoc analysis using Bonferroni/Dunn test, with a value of $p < 0.05$ considered to be significant. All results were expressed as the mean ± SEM.

Results

Upregulation of ABCA1 and ABCG1 expressions by citrulline. The effect of citrulline on ABCA1 and ABCG1 mRNA levels in THP-1 macrophages was first investigated. As shown in Fig. 1A and B, 1 mM of citrulline increased ABCA1 and ABCG1 mRNA levels by $74.5 \pm 21.5\%$ ($p < 0.05$) and $58.3 \pm 22.0\%$ ($p < 0.05$), respectively. In contrast, citrulline did not affect SR-BI expression in THP-1 macrophages (data not shown). To further investigate an association between ABCA1 and ABCG1 expressions and NOS, we performed experiments using the NOS inhibitor L-NAME. To our surprise, citrulline-induced ABCA1 and ABCG1 mRNA expression with or without 5 mM of L-NAME in THP-1 macrophages (Fig. 1C and D), indicating that citrulline-induced ABCA1 and ABCG1 expressions were NO-independent. Mirroring the increased mRNA levels, citrulline dose-dependently increased the protein levels of ABCA1 and ABCG1 (Fig. 1E).

Effect of ABCA1 and ABCG1 mRNA stability by citrulline. To further explore the mechanisms by which the citrulline increased ABCA1 and ABCG1 mRNA levels in the macrophages, we investigated whether they transcriptionally induced these genes using Act D. As shown in Fig. 2A and B, treatment with 10 µg/ml of ActD did not abolish the inducible effects of citrulline on ABCA1 and ABCG1 expression in THP-1 macrophages. Further, ABCA1 and ABCG1 mRNA decay induced by ActD were inhibited in the presence of the citrulline (Fig. 2C and D), thus indicating they enhanced mRNA stability of ABCA1 by $44.5 \pm 7.0\%$ ($p < 0.05$) and ABCG1 by $39.1 \pm 5.5\%$ ($p < 0.05$). These data indicated that citrulline enhanced mRNA stability of ABCA1 and ABCG1.

Citrulline enhanced ApoA-I and HDL-mediated cholesterol efflux in THP-1 macrophages. Next, we investigated the effect of citrulline on cholesterol efflux from THP-1 macrophages mediated by apoA-I or HDL. Citrulline enhanced both apoA-I- and HDL-mediated cholesterol efflux from THP-1 macrophages by $96.1 \pm 26.1\%$ ($p < 0.05$) and $14.4 \pm 2.5\%$ ($p < 0.05$), respectively, compared to control (Fig. 3A and B).

Citrulline increases serum citrulline and arginine, but has no effects on serum TC, TG, LDL-C, HDL-C, glucose or NO₃.

Our next objective was to clarify the matter of whether the above *in vitro* observations translate into human physiology. As shown in Fig. 4, 1 week of citrulline consumption markedly increased its concentration, by 595% ($p < 0.05$). Further, arginine and ornithine, which are metabolically linked with citrulline through the Krebs-Henseleit cycle,⁽³³⁾ were also increased, by 44.7% ($p < 0.05$) and 12.4%, respectively. As expected, placebo

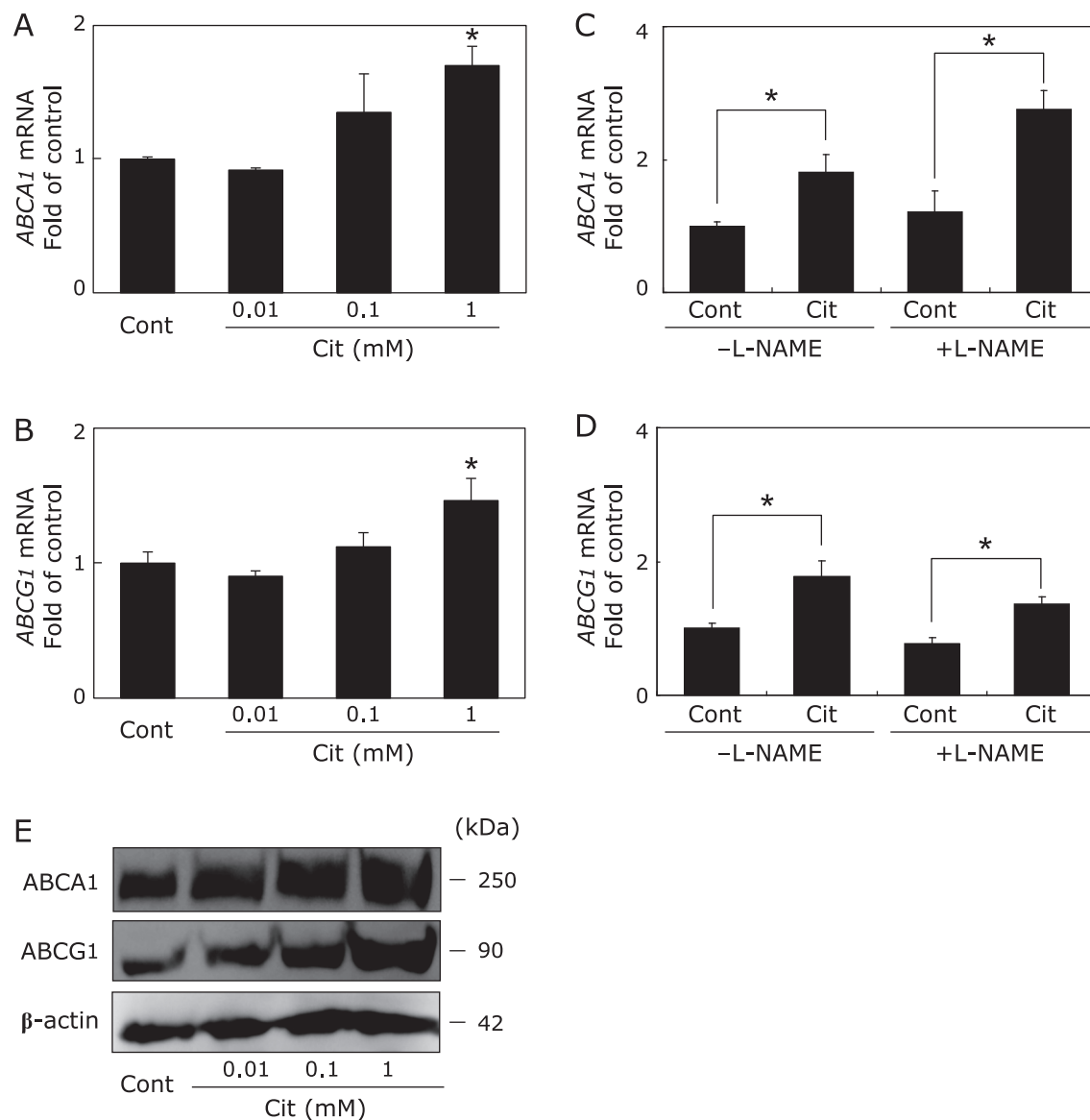


Fig. 1. Citrulline increases expression of ABCA1 and ABCG1 in macrophages. A, B, THP-1 macrophages were treated with control (Cont) or 0.01, 0.1, 1 mM of citrulline (Cit) for 6 h. RNA extraction and quantitative real-time PCR were performed as described in Materials and Methods. The results from 3 separately performed experiments are expressed relative to the vehicles and presented as the mean \pm SEM. Within each treatment group, means without a common letter, $p < 0.05$. C, D, THP-1 macrophages were treated with Cont or 1 mM of Cit with or without 5 mM of L-NAME for 6 h. The results from 3 separately performed experiments are expressed relative to Cont and presented as the mean \pm SEM. *Different from Cont, $p < 0.05$. RNA extraction and quantitative real-time PCR were performed as described in Materials and Methods. E, THP-1 macrophages were lysed and subjected to Western blot analysis 24 h after treatment with Cont or the indicated doses of Cit. The results are from 3 separately performed experiments that yielded similar results.

consumption did not produce any changes. We also found that 1 week of citrulline consumption did not change other parameters, including plasma lipids, glucose, and NO_3^- (a metabolite of NO) (data not shown).

Increased ABCA1 and ABCG1 expression in MDM in presence of post-citrulline sera. Individual changes in ABCA1 and ABCG1 mRNA levels in the MDM differentiated using autologous sera obtained before and after citrulline or placebo consumption are shown in Fig. 5. Although there was some individual variation, ABCA1 and ABCG1 mRNA levels were increased in the MDM treated with post-citrulline sera as compared to pre-citrulline cells and sera combinations by 95.9% ($p < 0.05$) and 162% ($p < 0.05$), respectively, in most study subjects. As expected, placebo did not affect the expression of the MDM

genes. As shown in Fig. 6, parallel increases in ABCA1 and ABCG1 protein levels were observed. ABCA1 and ABCG1 protein levels in MDM cultured under post-citrulline sera were significantly increased, by $85.7 \pm 24.1\%$ ($p < 0.05$) and $34.7 \pm 10.5\%$ ($p < 0.05$), respectively (Fig. 6C and D). Again, there was no difference between pre- and post-placebo cells and sera (Fig. 6A and B).

Enhanced ApoA-I and HDL-mediated cholesterol efflux in MDM treated with post-citrulline sera. Finally, we performed an HDL-mediated cholesterol efflux assay using human MDM cultured in media containing sera obtained before and after citrulline consumption. Consistent with the increased ABCA1/G1 expressions observed above, ApoA-I and HDL-mediated cholesterol effluxes were also significantly increased, by 78.0%

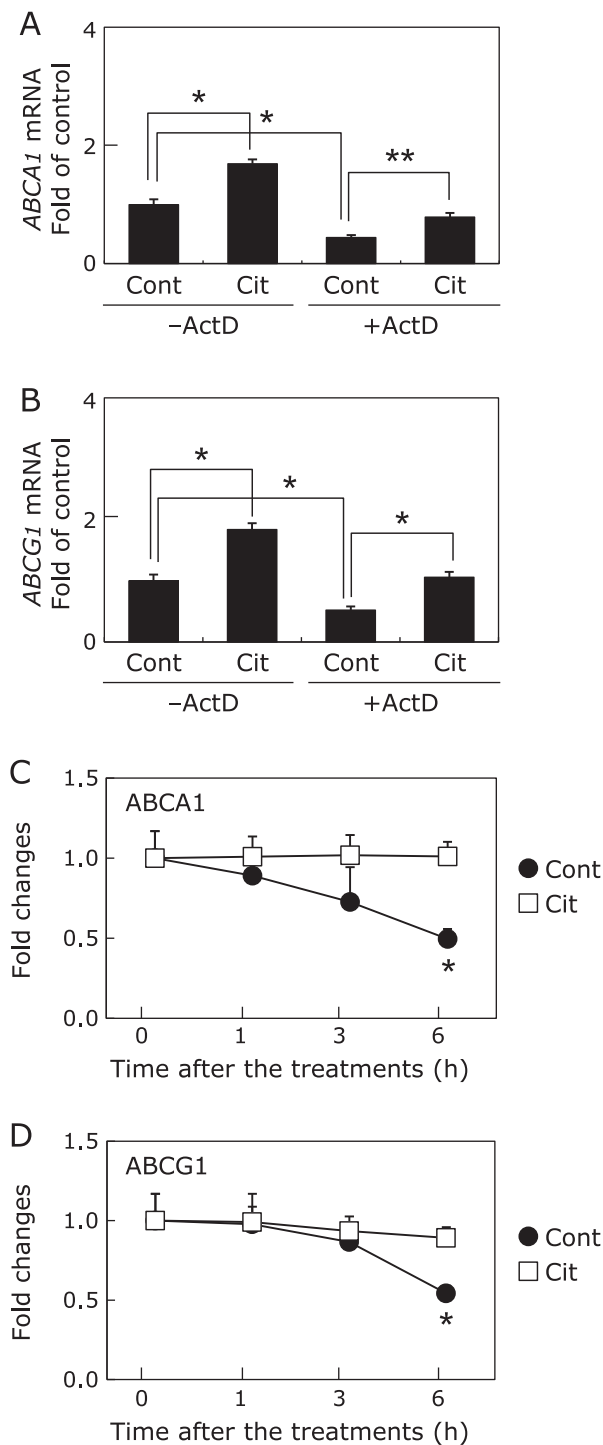


Fig. 2. ABCA1 and ABCG1 mRNA stability enhanced by citrulline in macrophages. A, B, THP-1 macrophages were pretreated with 10 $\mu\text{g/ml}$ of ActD 0.5 h before the treatment with control (Cont) or 1 mM of citrulline (Cit) with or without 10 $\mu\text{g/ml}$ of ActD for 6 h. The results from 3 separately performed experiments are expressed relative to the vehicles and presented as the mean \pm SEM. *Different from Cont or ActD, $p < 0.05$, $p < 0.05$. RNA extraction and quantitative real-time PCR were performed as described in Materials and Methods. C, D, THP-1 macrophages were pretreated with 10 $\mu\text{g/ml}$ of ActD 0.5 h before the treatment with Cont or 1 mM of Cit with 10 $\mu\text{g/ml}$ of ActD for 1, 3 and 6 h. The results from 3 separately performed experiments are expressed relative to the vehicles and presented as the mean \pm SEM. *Different from Cont, $p < 0.05$. RNA extraction and quantitative real-time PCR were performed as described in Materials and Methods.

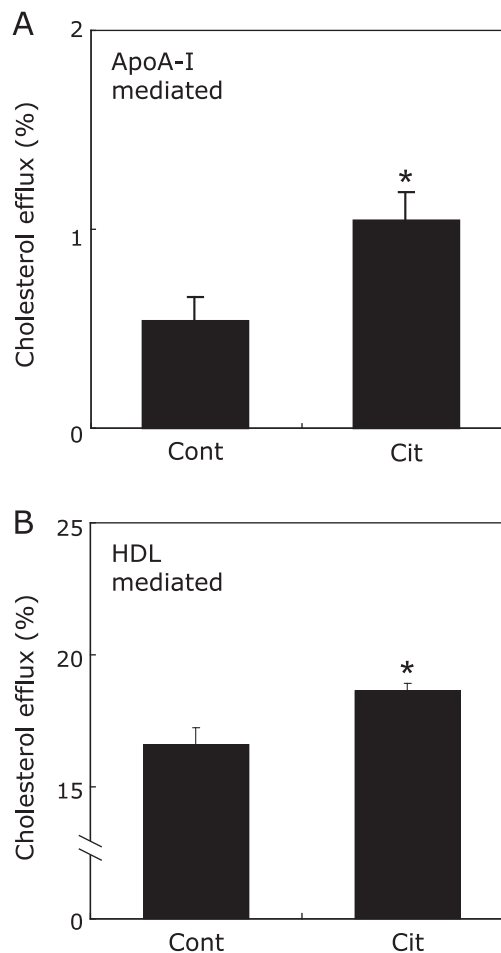


Fig. 3. Citrulline enhances ApoAI- and HDL-mediated cholesterol efflux in macrophages. The indicated doses of citrulline (Cit) or control (Cont) were added to the cultures after [^3H] cholesterol labeling, which were then incubated in the presence of 10 $\mu\text{g/ml}$ of human apoA-I (A) or 50 $\mu\text{g/ml}$ of HDL (B) for 24 h. Determination of cholesterol efflux from the cells was performed as described in Materials and Methods. The results for 6 samples are presented as the mean \pm SEM. * $p < 0.05$ vs Cont.

($p < 0.05$) and 11.0% ($p < 0.05$), respectively, in MDM cultured under post-citrulline sera, as compared to pre-citrulline cells and sera (Fig. 7A and B). Again, no changes were observed for placebo.

Discussion

There is a strong inverse correlation between plasma concentrations of HDL-C and risk of atherosclerotic cardiovascular disease.⁽³⁴⁾ Although the mechanism by which HDL may exert a direct protective effect against the development of atherosclerosis is not yet well understood, it has been postulated that HDL facilitates the efflux of cholesterol from peripheral tissues and transports it back to the liver in a process called RCT.⁽³⁵⁾ Here we showed, for the first time, that citrulline consumption lead to increased ApoA-I- and HDL-mediated cholesterol efflux from macrophages by inducing ABCA1 and ABCG1 expressions, both in an *in vitro* and *ex vivo* study.

With regard to mechanisms by which citrulline-mediated induction of ABCA1 and ABCG1 gene expression is transcriptionally regulated, preliminary results showed that citrulline did not increase these promoter activities of well-defined human ABCA1

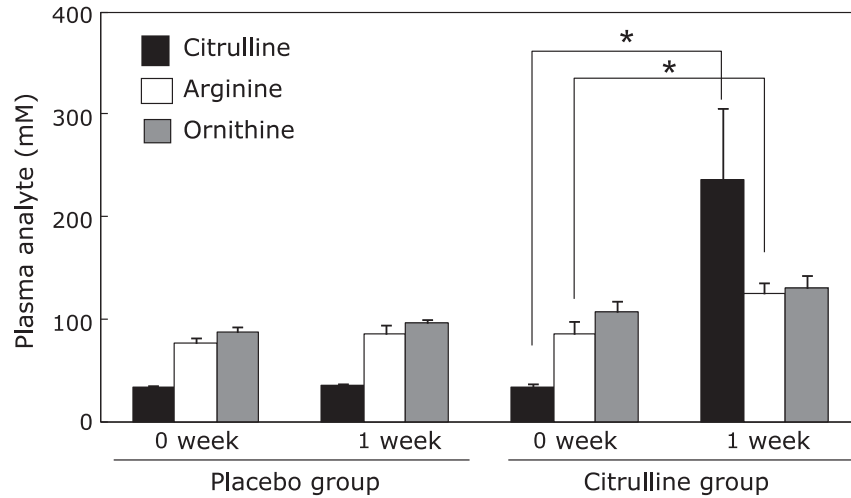


Fig. 4. Citrulline consumption results in increased levels of citrulline/arginine in human plasma. A, plasma was isolated from blood obtained from 8 subjects before and 1 week after consumption of placebo or citrulline. The plasma concentrations of citrulline, arginine and ornithine were determined by amino acid analyzer as described in Materials and Methods. The results are presented as the mean \pm SEM. *Different from 0 week, $p < 0.05$.

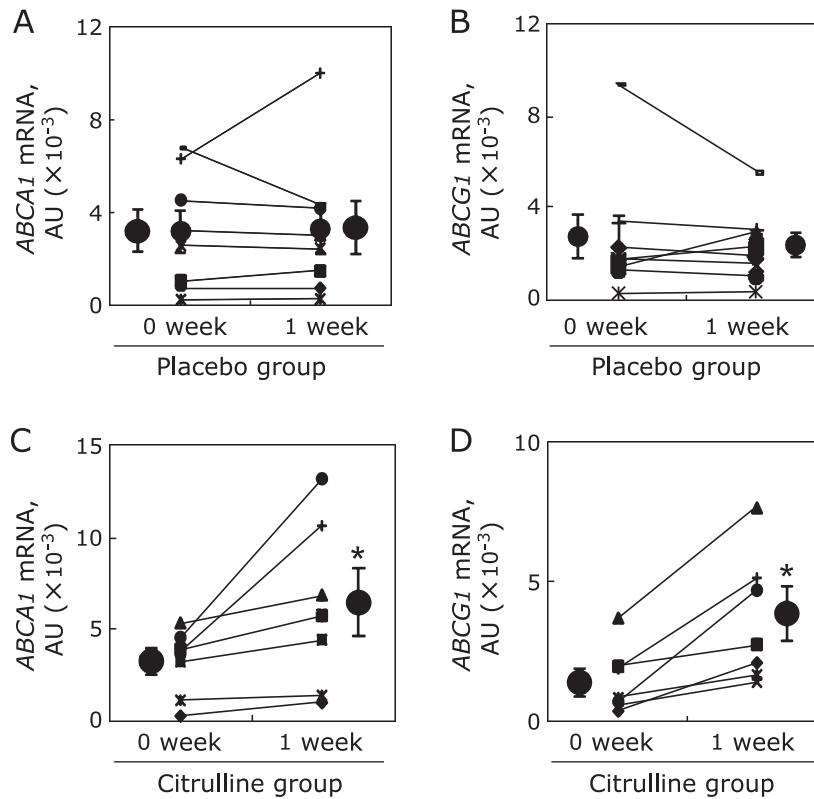


Fig. 5. Citrulline consumption results in increased mRNA levels of ABCA1 and ABCG1 in human monocyte derived macrophages (MDM). The monocytes were cultured and differentiated into macrophages in the presence of autologous sera obtained from 8 subjects before and 1 week after placebo (A, B) or citrulline (C, D) consumption. RNA extraction and quantitative real-time PCR were performed as described in Materials and Methods. The results are expressed as the mean \pm SEM. *Different from 0 week-citrulline, $p < 0.05$.

(-940 to +110 bp relative to transcription start site) and ABCG1 (-1104 to +38 bp relative to transcription start site in exon 1) or (-1180 to +144 bp in exon 5) promoter-luciferase constructs (data not shown).^(27,36,37) We then investigated effects of citrulline on mRNA stability of ABCA1 and ABCG1 by using ActD to find that citrulline enhanced ABCA1 and ABCG1 mRNA stability. Indeed, we showed that citrulline enhanced ABCA1 and ABCG1

mRNA stability (Fig. 2C and D), thus resulting in increased steady-state expression of these genes in the macrophage.

Consistent with the *in vitro* findings, the *ex vivo* human study showed that MDM differentiated under autologous post-citrulline sera enhanced apoA-I- and HDL-mediated cholesterol efflux by increasing ABCA1 and ABCG1 expression without changing plasma HDL-C levels. In theory, since *ex vivo* experiments can

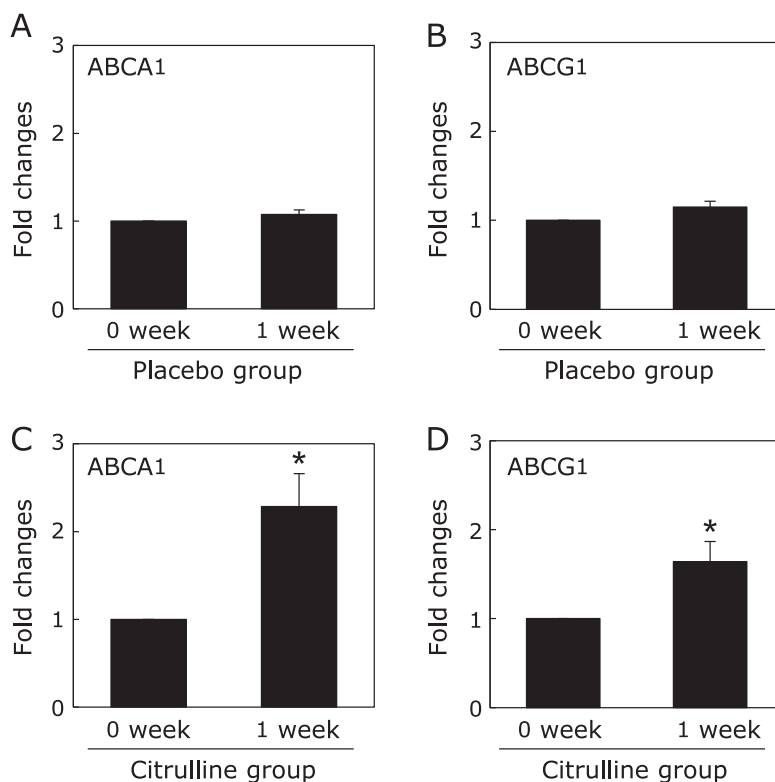


Fig. 6. Citrulline results in increased protein levels of ABCA1 and ABCG1 in human monocyte derived macrophages (MDM). The monocytes were cultured and differentiated into macrophages in the presence of autologous sera obtained from 8 subjects before and 1 week after placebo or citrulline consumption as describe in Materials and Methods. MDM were lysed and subjected to a Western blot analysis. Protein levels were quantified as described in Materials and Methods. Independent experiments from 8 different subjects are expressed as the mean \pm SEM. *Different from 0 week-citrulline, $p < 0.05$.

provide a situation that is closer to the *in vivo* situation in humans than the *in vitro* setting, comparable results from *in vitro* and *in vivo* studies would provide a reasonable basis for hypothesizing that citrulline promotes RCT by enhancing cholesterol efflux via ABCA1/G1 pathways in humans *in vivo*. This has been highlighted as an important concept in the recent study by Rader *et al.*⁽¹¹⁾ which found that quantitative measures of macrophage RCT are more strongly associated with atherosclerotic disease morbidity than plasma HDL-C concentrations. Enthusiasm concerning cholesterol efflux and its modulation began about 10 years ago when ABCA1 was discovered as the mutation causing human HDL deficiency in Tangier disease. However, although research efforts have been intensely focused on enhancing ABCA1 function, drugs that achieve this are still not available. In the meantime, natural products that enhance cholesterol efflux might be realistic alternatives.

Several studies have reported an association between citrulline and anti-atherosclerosis. The activation of adhesion molecules elicited by oxidized LDL and the uptake of oxidized LDL by macrophages are key steps in the progression of atherothrombotic cardiovascular diseases.⁽³⁸⁾ L-citrulline itself is an efficient radical scavenger and is a strong antioxidant.⁽³⁹⁾ In addition, NO has potent antioxidant properties that exert anti-inflammatory effects and can inhibit LDL oxidation.⁽⁴⁰⁾ Recently, Morita *et al.*⁽⁴¹⁾ reported that 800 mg/day of L-citrulline intake for 8 weeks reduced serum oxidized LDL and lectin-like oxidized LDL receptor 1 (LOX-1) ligand containing ApoB, an indicator of the biological activity of oxidized lipoprotein binding to LOX-1 in humans with vasospastic angina. NO is a widespread signaling molecule in the cardiovascular system, which functions in

multiple ways to protect against the initiation and progression of atherosclerosis.⁽⁴²⁻⁴⁴⁾ Interestingly, in our study, citrulline selectively induced ABCA1 and ABCG1 independently of L-NAME. Also, citrulline consumption did not induce any changes in serum NO₃ levels (data not shown), indicating that citrulline increases HDL-mediated cholesterol efflux from macrophages via an NOS-independent pathway. Thus, overall, our results do not completely exclude the possibility that enhanced cholesterol efflux due to citrulline is NO dependent. We will therefore need to perform an *in vivo* RCT assay using NOS knockout mice as a further study.

There are several limitations in the present study. First, as mentioned above, the subjects in the human study were healthy volunteers. Therefore, it is difficult to generalize the findings to dyslipidemic patients who are at increased risk for atherosclerosis. Second, 1 week of oral citrulline represents an acute effect, which may differ from the effect of taking it habitually. Third, the present study did not test for the presence of other factors potentially affecting cholesterol efflux nor factors along the pathway by which citrulline consumption could affect NO. Likewise, arginine might increase cholesterol efflux by citrulline administration because citrulline administration increased serum arginine concentration, although arginine concentration is very lower than citrullin concentration. Finally, we did not directly examine whether citrulline actually removed oxidized cholesterol, oxidized fatty acids or oxidized phospholipids from peripheral tissues. Such interesting a posteriori considerations deserve future studies.

In conclusion, the comparable results from the *in vitro* and *ex vivo* studies indicate that potential anti-atherogenic properties of citrulline could be explained, at least in part, as being due to up-regulated cholesterol efflux from macrophages mediated through

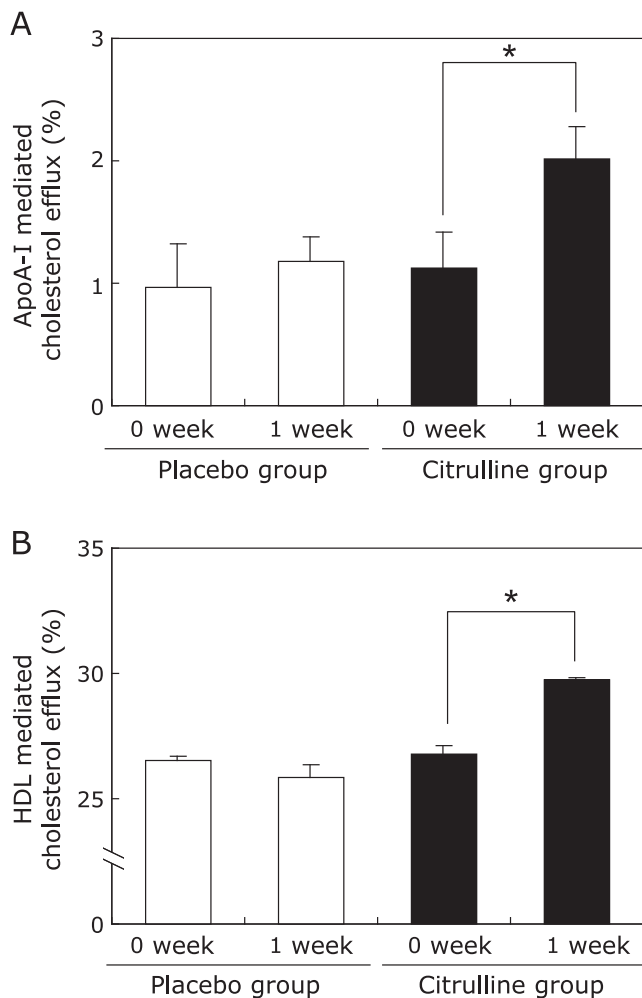


Fig. 7. Citrulline consumption enhances ApoAI- and HDL-mediated cholesterol efflux in human monocyte derived macrophages (MDM). The monocytes were cultured and differentiated into macrophages under pooled sera obtained before and 1 week after placebo or citrulline consumption as described in Materials and Methods. After [3 H] cholesterol labeling, MDM were incubated in the presence of 10 μ g/ml of ApoA-I (A) and 50 μ g/ml of HDL (B) for 24 h. Determination of cholesterol efflux from the cells was performed as described in Materials and Methods. The results for HDL-mediated efflux in quadruplicate and ApoA-I-mediated efflux in sextuplicate are presented as the mean \pm SEM. *Different from 0 week-citrulline, $p < 0.05$.

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ABCA1 and ABCG1 pathways. Combined with its previously-proven endothelial NO-related properties, this favorable effect of citrulline on HDL suggests great synergistic potential with regard to cardioprotective properties.

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Abbreviations

ABCA1	ATP-binding cassette subfamily A member
ABCG1	ATP-binding cassette subfamily G member 1
acLDL	acetylated LDL
ActD	actinomycin D
Apo	apolipoprotein
BSA	bovine serum albumin
cAMP	cyclic AMP
CVD	cardiovascular disease
eNOS	endothelial nitric oxide synthase
L-NAME	N^G -nitro-L-arginine methyl ester
LOX-1	lectin-like oxidized LDL receptor 1
LXR	liver X receptor
MDM	monocyte derived macrophages
NO	nitric oxide
NO ₃	nitrate
NOS	nitric oxide synthase
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PMA	phorbol 12-myristate 13-acetate
PPAR	peroxisome proliferator-activated receptor
RCT	reverse cholesterol transport
SR-BI	scavenger receptor class B type I
TC	total cholesterol
TG	triglycerides

Conflict of Interest

No potential conflicts of interest were disclosed.

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