RESEARCH ARTICLE

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Dietary D-Lactate Intake Facilitates Inflammatory Resolution by Modulating M1 Macrophage Polarization

Yongheng Yan, Xiu Li, Qin Yang, Hao Zhang, Kasper Hettinga, Haitao Li, and Wei Chen**

Scope: Given the D-lactate dehydrogenase (D-LDH) deficiency, L- but not D-lactate is assumed to be the physiological isomer in mammals. Paradoxically, many fermented foods (e.g., yogurt, sauerkraut, cheeses) often contain substantial amounts of D-lactate. In the present study, dietary D-lactate may be a previously unrecognized nutrient aiding in inflammatory resolution is hypothesized.

Methods and results: The anti-inflammatory properties of D-lactate are evaluated in experimental colitis and endotoxemia. Oral administration of D-lactate favorably affects acute inflammation in two different mouse models. Analysis of lactate—the lactate receptor (the hydroxycarboxylic acid receptor 1 HCA1, formerly GPR81) signal axis in inflammation is performed in primary peritoneal macrophages and wild-type (WT) or GPR81 knockout (KO) mice. GPR81 KO mice are susceptible to endotoxic shock than WT mice, while D-lactate exerts its anti-inflammatory activities in a GPR81-dependent manner. Mechanistically, the activation of lactate-GPR81 axis may suppress LPS-TLR4 signaling to modulate M1 macrophage polarization. Although D-LDH deficiency in mammals impairs D-lactate clearance, it might prolong its plasma terminal half-life, and thus provide a pharmacokinetic advantage of D-lactate over L-lactate.

Conclusion: This study highlights housekeeping function of the lactate-GPR81 axis in inflammation control, and suggests that dietary intake of D-lactate may underlie Metchnikoff's probiotic yogurt theory of life prolongation.

1. Introduction

Yogurt, the most popular fermented dairy product worldwide, has been consumed since ancient times.[1] The Nobel laureate Elie Metchnikoff firstly put forward the theory that yogurt might be responsible for the longevity of the people in Eastern Europe in 1907. Being debated and kept dormant for nearly a century, but his hypothesis is appreciated by modern biomedical science now. Furthermore, accumulating epidemiological studies suggest an inverse relationship between yogurt intake and the risk of obesity, diabetes, metabolic syndrome, allergy, and colorectal cancer.[1-3] Nevertheless, mechanism by which yogurt confers those health benefit remains largely unclear.

Lactate, a naturally occurring carboxylic acid with a pK_a of 3.86, is a major ingredient of yogurt. In addition to its pivotal role in anaerobic metabolism, lactate might serve as an energy source or signaling molecule under diverse physiological or pathophysiological conditions.[4–7] In this case, lactate is a ligand for the hydroxycarboxylic acid receptor 1 (HCA, formerly GPR81). In view of its clinical potential in detection of organ injury and acute inflammation, lactate is receiving extensive attention from both food and pharmaceutical industries.

Despite its simple chemical structure, lactate has a chiral center.[8] Lactate exists as two optical iomers: L-lactate and D-lactate. Given the deficiency of D-lactate dehydrogenase (D-LDH) in mammals, D-lactate might be not metabolized as readily as L-lactate and thus might increase the risk of hyperlactacidemia. To this end, L- but not D-lactate is generally regarded as a physiological isomer, and dietary intake of D-lactate has even been strictly limited by FAO/WHO in the past.[9,10] However, D-lactate is widely present in nature, especially in fermented foods (e.g., yogurt, sauerkraut, cheeses). For example, *Lactobacillus bulgaricus* could convert 90% of the pyruvate into D-lactate in yogurt production.[11] Hence, therein lays a paradox for nutritionists that yogurt and other fermented foods contain substantial amounts of D-lactate.

Acute inflammatory response in nature serves as a protective mechanism in host defence. Either rapid induction or timely resilience of an acute inflammatory response is essential for

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host homeostasis.[12] Given the fact that blood lactate is clinically linked with the severity of critical illness, lactate supplementation has long been a controversial issue for those patients. However, such concern has been challenged by recent studies in which lactate therapy undeniably improved the outcome of systemic inflammation in acute pancreatitis.[13,14] In addition, studies so far were focused on L-lactate, while the importance of D-lactate has not been ascertained yet. In the present study, we questioned whether dietary D-lactate may be a previously unrecognized nutrient protecting against inflammation.

2. Experimental Section

2.1. Chemicals and Reagents

Primary antibodies against cyclooxygenase-2 (COX-2, #12282), i-NOS (#13120), and ikBα (#4814) were purchased from Cell Signaling Technology (Beverly, MA, USA). Primary antibody against glutamine synthetase (ab64613) was obtained from Abcam (Cambridge, UK). Enzyme immunoassay kits for plasma tumor necrosis factor-a (TNF-a), interleukin-6 (IL-6), malondialdehyde (MDA), myeloperoxidase (MPO), and reduced Glutathione (GSH) were purchased by R&D systems. Lipopolysaccharide (LPS), L- and D-lactate, GPR81 agonist 3-chloro-5-hydroxy benzoic acid, and anti-GPR81-S296 antibody were from Sigma-Aldrich unless indicated otherwise.

2.2. Mice

C57BL/6 GPR81 heterozygous mice were from Beijing Vitalstar Biotechnology Co., Ltd. GPR81 knockout (KO) and the wild-type (WT) control mice were bred and maintained in colony cages with a light/dark cycle (12 h/12 h) and controlled temperature (22 ± 1 °C). For genotyping, duplex PCR were performed on tail biopsies. Littermate mice used for experiments were between 6 and 8 weeks of age unless otherwise specified. All animal care and experimental studies were approved by the Institutional Animal Ethics Committee of Jiangnan University (protocol number JN. Noc0360605, JN. Noc0701202, JN.No0400502, JN. NoS0281102, JN.Noc0400918).

2.3. Pharmacokinetic Properties of D-Lactate

For pharmacokinetic analysis, 6-week-old male C57BL/6 mice (n = 6 per group) were given a single oral dose of sodium L-lactate and D-lactate (2000 mg kg^{-1}), as described earlier.[15] Blood samples were obtained at 0, 5, 10, 15, 30, 60, 120, 240, 360 min post dose. And plasma samples were analyzed by HPLC. Pharmacokinetic parameters were determined using the WinNonlin Professional software 2.0.

2.4. Primary Peritoneal Macrophages

Murine primary peritoneal macrophages were isolated as described.[16] Primary peritoneal macrophages were adjusted to 2 × 10^6 cells mL^{-1} in the tissue culture medium. After incubation for 2 h, the culture medium was changed to discard nonadherent cells. Isolated primary peritoneal macrophages were used for subsequent macrophage polarization by LPS (200 ng mL^{-1}) or IL-4 (20 ng mL^{-1}) stimulation for 8 h. Total RNA was extracted with TRIzol reagent. The primer sequences for real-time PCR were summarized in Table S1, Supporting Information. After treatment, cells were lysed in RIPA buffer, and obtained protein samples (20 μg) were analyzed by Western blotting.

Table 1. Score of disease activity index (DAI).

Score	Weight loss	Stool consistency	Rectal bleeding
0	None	Normal	Normal
1	1–5%		
2	5–10%	Loose stools	Slight bleeding
3	10–20%		
4	>20%	Diarrhea	Gross bleeding

2.5. DSS-Induced Colitis Model

For acute colitis induction, 6-week-old male C57BL/6 mice (n = 10 per group) were subjected to DSS administration through drinking water (3%) for total 7 days. Sodium D-lactate (1000 mg kg^{-1}) was administered orally once daily throughout the experiment, starting 7 days before colitis induction. The body weight, the stool consistency, and gross bleeding were monitored through the whole study. The disease activity index (DAI) was calculated as described criteria (**Table 1**).[17,18]

2.6. LPS-Induced Systemic Inflammation Model

For endotoxicity studies, 6-week-old male C57BL/6 mice wild-type (WT) or GPR81 KO mice were challenged intraperitoneally (i.p.) with lipopolysaccharides (LPS, *Escherichia coli* 0127:B8, 10 μg) as described previously.[19] Two hours later, half mice were euthanized and blood samples were collected for pro-inflammatory cytokine determination, the other half mice were sacrificed after 24 h of treatment. In endotoxic shock, mice were injected with 200 μg LPS and 8 mg D-galactosamine per mice and were monitored for survival for the ensuing 24 h (n = 10). D-lactate (1000, 2000 mg kg^{-1}) was administered orally once daily throughout the experiment, starting 7 days before the LPS challenge. Sandwich ELISAs were used to measure natural and recombinant mouse Interleukin 6 (IL-6) and TNF-α (R&D Systems, #DY406-05 and #DY410-05).

2.7. Dosage Information

In vitro, the choice of dose and the route of lactate treatments were made in agreement with the literature.[20] Lactate could be generated by the intestinal microbiota, the concentration that was typically achieved in the colon from around 10 to 30 mM.[21] Lactate concentration could also be possible rose under certain

pathophysiologic situations, such as intestinal ischemia or sepsis which indicating that the concentration was physiologically reasonable.[22,23]

In vivo, the administration time and the doses of sodium lactate were optimized on the basis of previous studies.[8,24] Commercial yogurt products typically contain 0.7% D-lactate and 1% L-lactate (Figure S1, Supporting Information). Therefore, mice were given oral L- or D-lactate doses (1000 mg kg^{-1}), equivalent to a 65 kg human consuming about 770 g of yogurt daily which according to Dietary Guidelines means this dose was considered to be acceptable and achievable through regular diet.[13] Detailed dosages were described in individual experiments.

2.8. GC/MS and HPLC Analysis

Six kinds of SCFAs of acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, and isovaleric acid were analyzed by gas chromatography–mass spectrometry (GC/MS). One-step derivatization was carried out using 100 µL of propyl chloroformate (PCF) in a reaction system of water, isopropanol, and pyridine (V/V/V = 8:3:2) by two-step extraction with hexane and GC/MS analysis. This method can simultaneously measure six kinds of SCFAs of acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, and isovaleric acid.

Yogurt were centrifuged for 20 min at 8000 r min^{-1} and the supernatant fractions were filtered through a 0.45 µm membrane. The samples were then analyzed by HPLC on chiral columns with a PDA detector at 254 nm. High separation efficiency was achieved by using a Chirex 3126 (D)-penicillami column (4.6 mm ID × 250 mm L, 5 µm) and a mobile phase consisting of CuSO4 (2 mmol L^{-1}) and isopropyl alcohol-water (5:95).

2.9. Western Blot Analysis

Cells or colonic tissues were lysed in RIPA buffer, and protein concentration of cell lysates was determined by Bradford assay. Protein samples (20 µg) were analyzed by Western blot. And final protein bands were visualized using enhanced chemiluminescence reagent.

2.10. Real-Time qPCR

Total RNA were extracted from cells or tissues using Trizol. Sample was then reverse-transcribed with a high-capacity cDNA reverse transcription Kit (Shenggong, Shanghai, China). The mRNA levels were expressed as relative values compared with β-actin mRNA levels. The primer sequences for Real-time PCR were listed in Table S1, Supporting Information.

2.11. Statistical Analysis

Statistical analysis was performed using GraphPad Prism 6.0 Software. Multiple group comparisons were done by a one-way analysis of variance (ANOVA), followed by the Bonferroni procedure for comparison of means. Student's *t*-test was used to compare two independent groups. Data are the means ± S.E.M. Differences were regarded as statistically significant if $p \leq 0.05$ versus Control or WT.

3. Results

3.1. Efficacy of D-Lactate in Inflammation Control

Acute inflammation is often triggered by tissue injury or microbial infection.[14] We accordingly evaluated the anti-inflammatory activity of D-lactate in two different inflammation models. By using a well-established model of intestinal injury (**Figure 1**A), we observed that the mice body weight dramatically dropped from the fifth day of dextran sodium sulfate (DSS) administration (Figure 1B). D-lactate protected mice from experimental colonic colitis, as evidenced by the multiple preclinical scores such as body weight loss, an average daily disease activity index (DAI) and colon length (Figure 1C,D). Accordingly, the response of oxidative stress including MDA, LPO, and GSH in colon tissues was significantly changed in DSS-treated mice compared with the control mice. Compared with the DSS group, D-lactate administration significantly suppressed colonic MDA and LPO production and increased the activities of colonic GSH (Figure 1E). In addition, D-lactate protected mice from the infiltration of inflammatory cells in colon (Figure 1F).

We observed a similar trend in an endotoxin-induced acute inflammation model (**Figure 2**A). Upon lipopolysaccharide challenge (LPS, 20 mg kg^{-1}, ip), mice developed the systemic inflammation phenotype, showing splenomegaly, neutrophil accumulation as well as a pro-inflammatory cytokine storm (Figure 2B–D). All of those inflammatory signs could be significantly improved by D-lactate. Acute inflammation is normally self-limited and thus could be divided into initiation and resolution.[12,25–27] Sub-lethal LPS challenge evoked a self-limited host response in which the peripheral neutrophil count reached its maximum at 12 h, followed by decline in resolution (Figure 2E). In this connection, we observed that D-lactate reduced maximal neutrophil count and shortened the resolution interval. Taken together, D-lactate favorably inhibited acute inflammation.

3.2. Critical Roles of GPR81 in M1 Macrophage Polarization

The kinetic participation of macrophage plays a critical role in inflammatory response.[16,28] Analysis of the expression pattern of lactate biosynthesis, transport, and sensor unexpectedly revealed that the pro-inflammatory M1 macrophage polarization was accompanied by a pronounced elevation of the L-lactate dehydrogenase (LDHA), the monocarboxylate transporter 1 (MCT1) as well as lactate accumulation. In contrast, the lactate receptor GPR81 was markedly down-regulated during M1 macrophage polarization (**Figure 3**A). To clarify the role of GPR81 in macrophage polarization, GPR81 KO mice were generated. We observed that the deficiency of GPR81 in macrophage boosted LPS-triggered induction of M1 marker expression (TNFα, IL6, and NOS_2) but downregulated M2 marker expression (YM1) in response to LPS and IL-4 stimulation (Figure 3B). Importantly, the GPR81 selective agonist (3-chloro-5hydroxybenzoic acid) suppressed pro-inflammatory M1 macrophage polarization (Figure 3B). However, LPS-induced lactate production in macrophages was not affected by GPR81 knockout (Figure 3C). Consistent with their mRNA dynamics, genetic deletion of GPR81 reduced both inducible nitric oxide synthase (iNOS) and Arg-1 protein levels

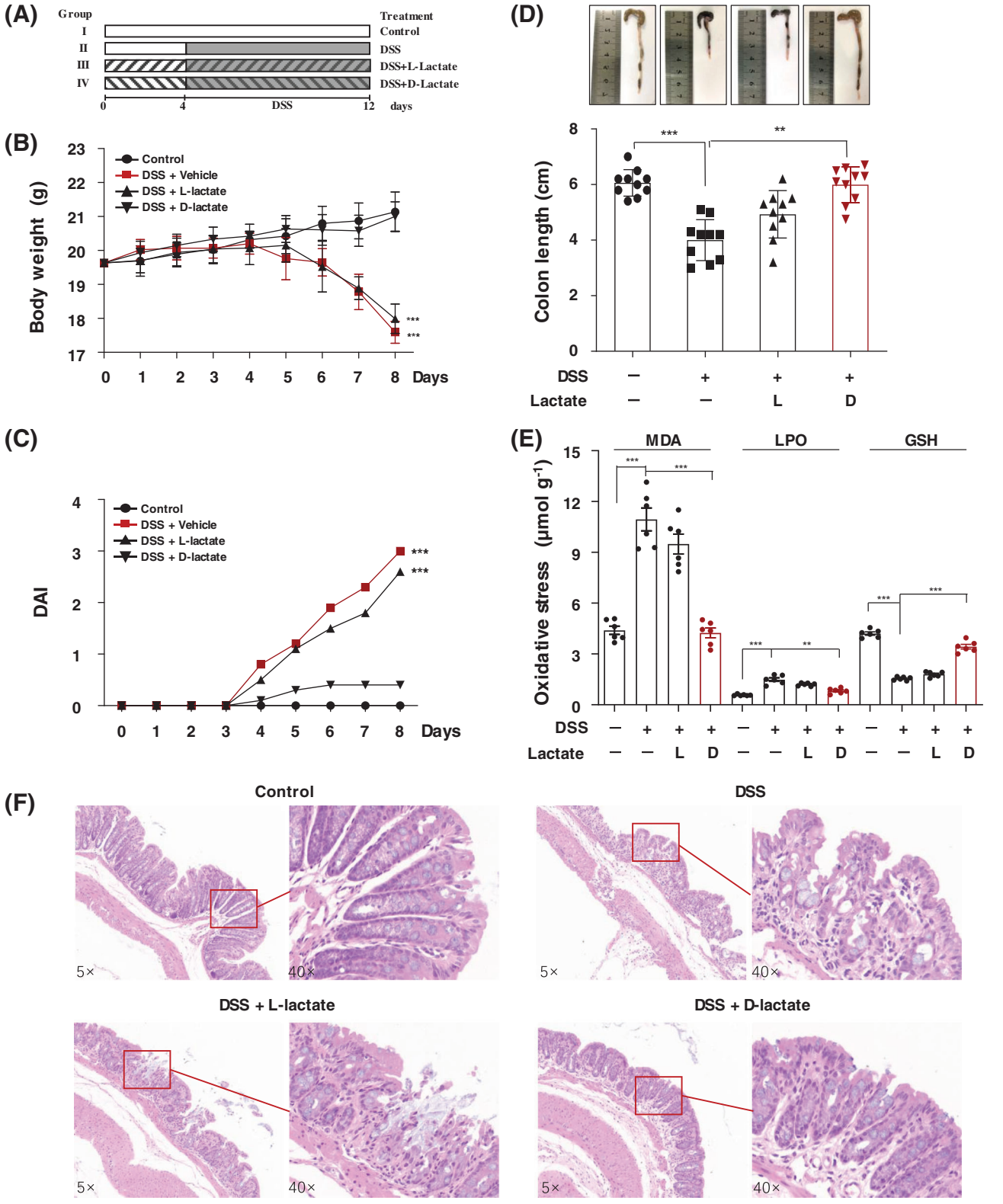


Figure 1. D-lactate protected mice from experimental colitis. A) Experimental design. C57/BL6 mice were treated with sodium L-lactate or sodium D-lactate (1000 mg kg^{-1}, $N = 10$) and with 3% DSS in drinking water daily starting on day 1 and continued through day 8 when they were sacrificed. B) Body weight and C) Disease activity index (DAI) was assessed daily. D) Colon lengths were measured on day 8. E) Colon tissues from mice were stained with hematoxylin and eosin (H&E). F) Protein levels of inducible nitric oxide synthase (iNOS) and phos-p38 were analyzed by Western blotting from colon tissues. Data are the means ± S.E.M. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to control or DSS vehicle.

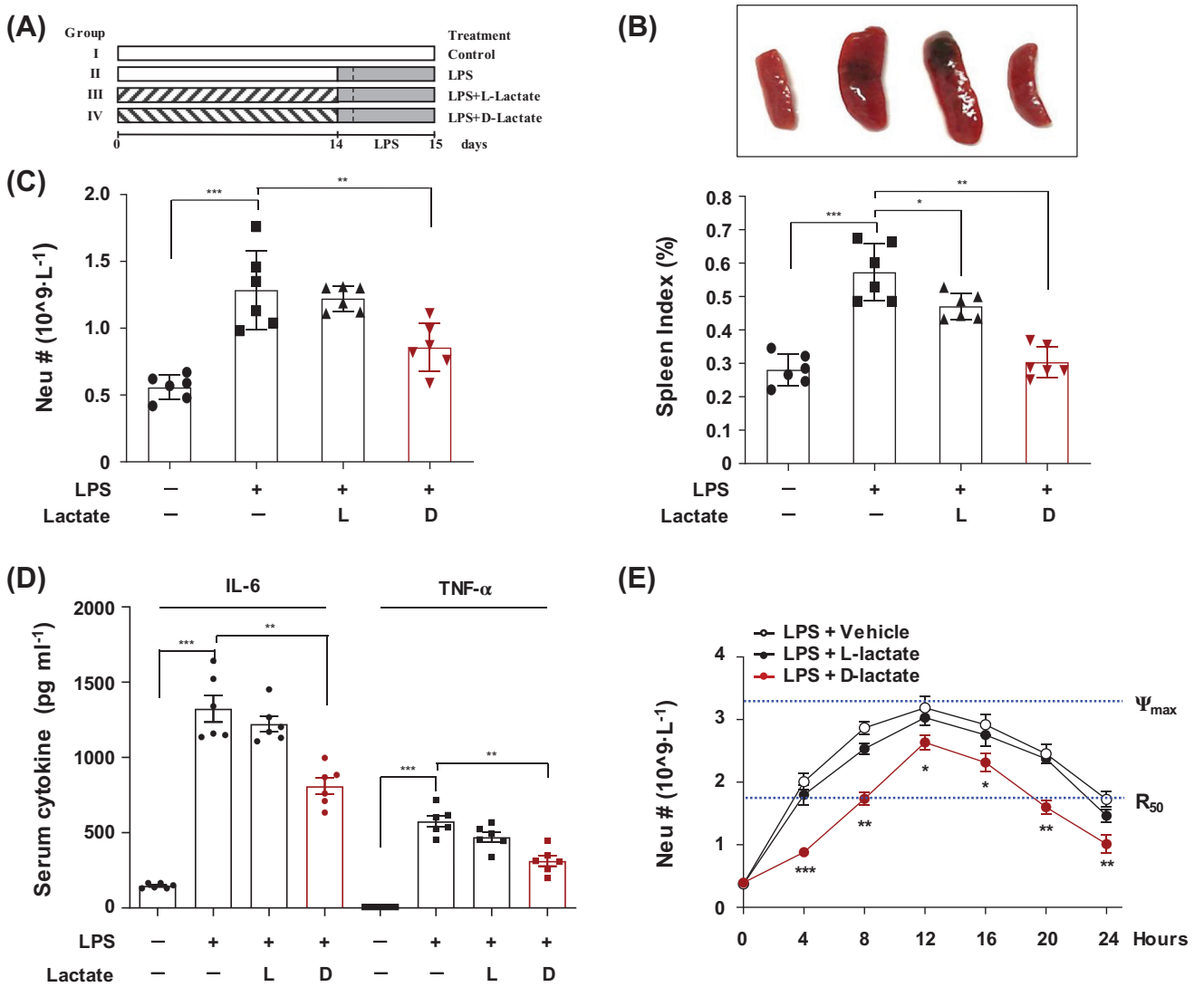


Figure 2. D-lactate protected against LPS-induced acute inflammation. A) Experimental design. C57BL/6 mice were given sodium lactate (1000 mg kg^{-1}) or vehicle control by oral gavage once daily for 14 days, followed by LPS challenge injection (10 μg per mouse) for another 2 h or 24 h. B) Spleen size and weight. C) Neutrophil counts in blood. D) Serum TNF-α and IL-6 levels. E) Profile of neutrophil accumulation in LPS-induced inflammation. Data are the means ± S.E.M. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to control.

(Figure 3D). What's more, same as the lower GPR81 transcriptional regulation, the protein stability of GPR81 that was also greatly decreased by LPS stimulation (Figure 3E,F). Collectively, GPR81 negatively modulated M1 macrophage polarization.

3.3. Anti-Inflammatory Effects of D-Lactate is Mediated Partly by GPR81

We next questioned whether GPR81 mediated the anti-inflammatory effect of D-lactate. To examine such possibility, we firstly checked the influence of D-lactate on macrophage polarization. Data clearly showed that D-lactate suppressed pro-inflammatory M1 macrophage polarization, but promoted anti-inflammatory M2 macrophage polarization (**Figure 4**A). Consistently, D-lactate lowered LPS-induced activation of NF-κB and MAPK as well as the following pro-inflammatory cytokine production in GPR81 WT macrophages (Figure 4B,C). Notably, these protective effects of D-lactate were completely absent in GPR81-deficiency macrophages .We eventually assessed the anti-inflammatory effect of D-lactate by using a more rigorous endotoxin shock model. Although GPR81 KO mice are of normal size, fertile, and appear healthy, they are much more susceptible to endotoxic shock than WT mice (**Figure 5**A). At 10 h after LPS challenge, seven of the 10 WT mice whereas none of the GPR81 KO mice had survived. Consistent with that, in another relatively mild stimulation GPR81KO mice produced significantly higher pro-inflammatory cytokines than WT mice (Figure 5B). Once again, we observed that D-lactate treatment might provide a protective effect only in WT but not in GPR81KO mice. Taken together, D-lactate exerted its anti-inflammatory effects dependently of GPR81.

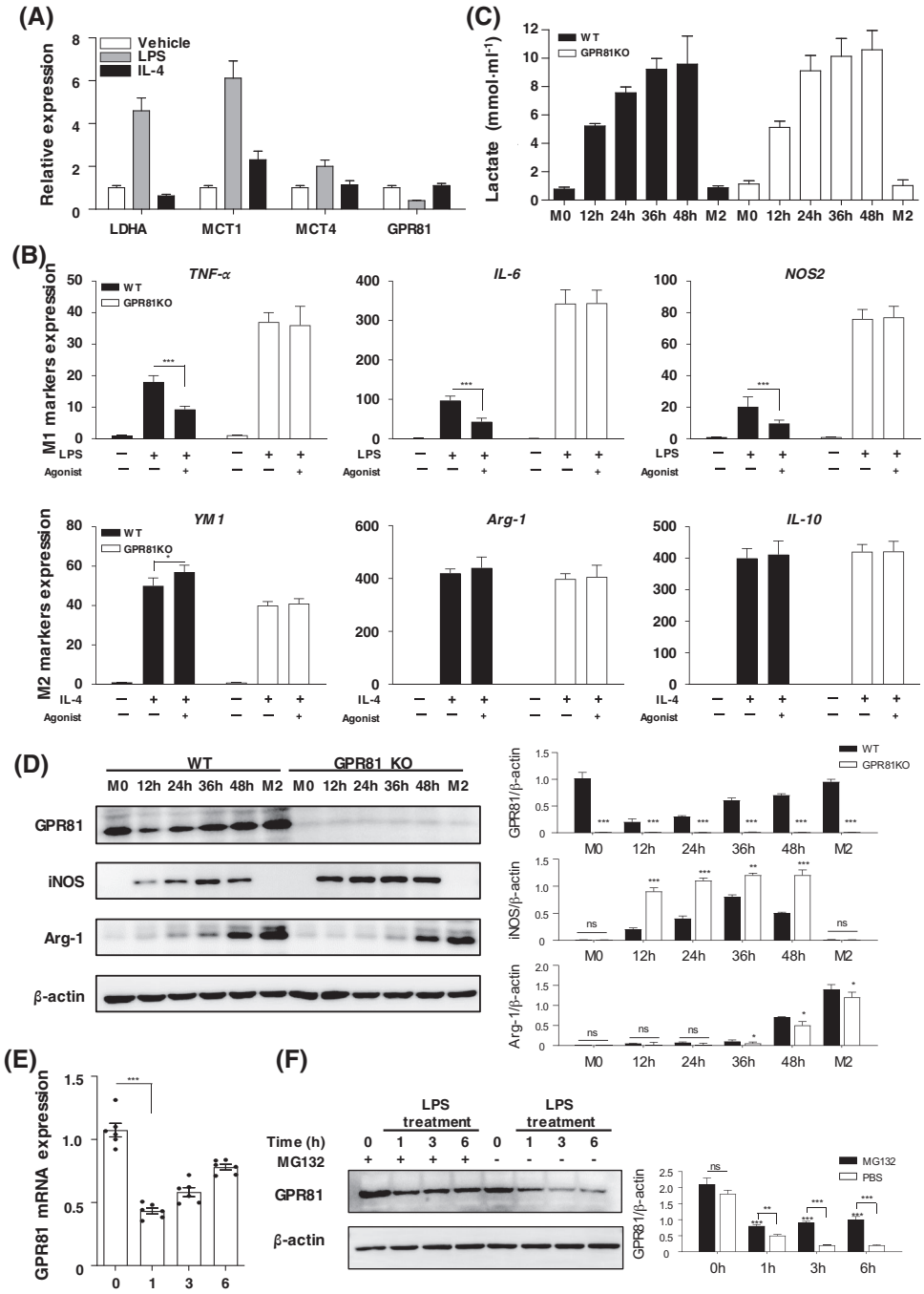


Figure 3. Role of GPR81 in macrophage polarization. A) Expression of LDHa, MCT1, MCT4, and GPR81 receptors in macrophages in response to LPS or IL-4 treatment. B) Effect of GPR81 agonist on quantitative RT-PCR mRNA levels of M1 marker and M2 marker expression on macrophage in response to LPS and IL-4 treatment. C) GPR81 deficiency did not affect lactate production of macrophage. D) Protein levels of GPR81, iNOS, and Arg-1 were analyzed by Western blotting from macrophage activated stimuli. LPS induced lactate accumulation in primary peritoneal macrophages. E) GPR81 mRNA expression in WT macrophages stimulated with LPS for 6 h. F) Effects of LPS stimulation on GPR81 stability. Macrophages were pretreated with MG132 (10 μM, 4 h) followed by LPS treatment for 6 h. GPR81 was verified using Western blotting. These data sets are representative of three independent experiments. Data are the means ± S.E.M. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to WT group.

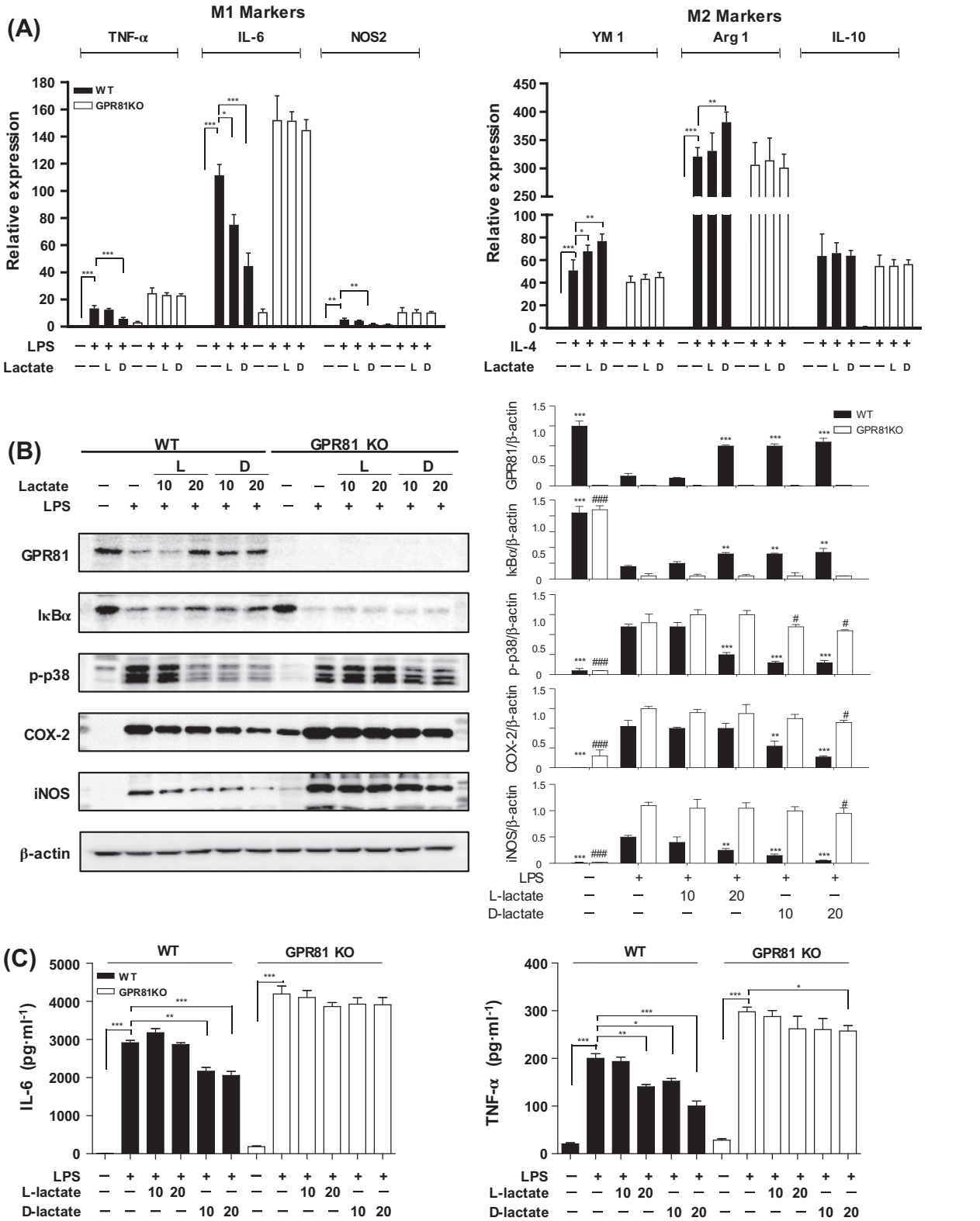


Figure 4. D-lactate reduced M1 macrophage polarization dependently of GPR81. A) Quantitative RT-PCR mRNA levels of M1 marker and M2 marker expression on macrophage after L-or D-lactate pretreatment (20 mM, 2 h). B) Effect of D-lactate on LPS-Toll-like receptor 4 (TLR4) signaling transduction in macrophage. C) Pro-inflammatory cytokine productions in macrophages. Cytokines in cell culture supernatant above were measured by ELISA. These data sets are representative of three independent experiments. Data are the means ± S.E.M. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ compared to WT+LPS group. $\#p < 0.05$, $\#\#p < 0.01$, $\#\#\#p < 0.001$ compared to KO+LPS group.

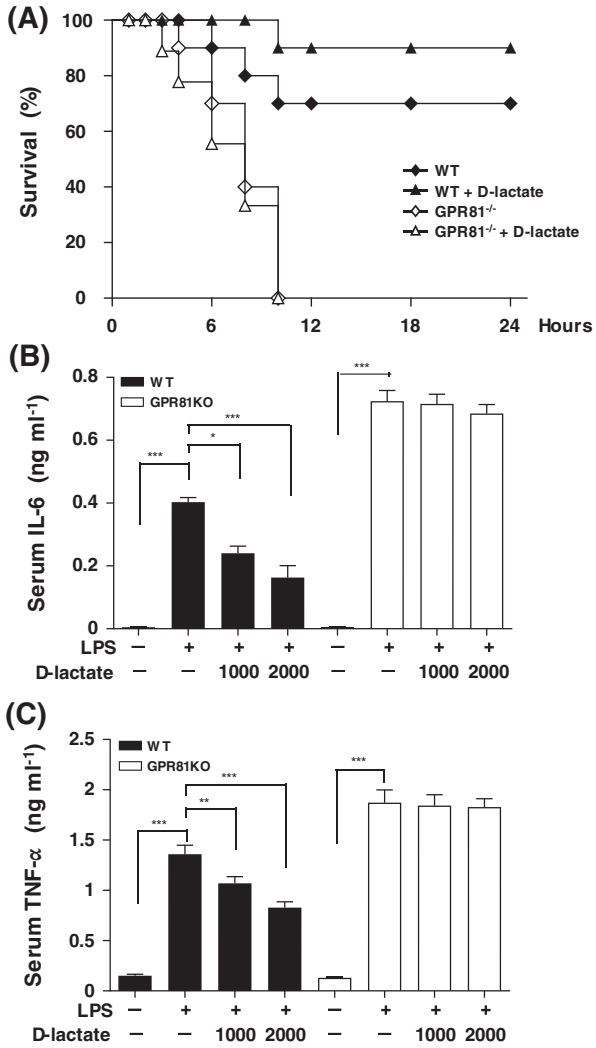


Figure 5. D-lactate exerted its anti-inflammatory effects dependently of GPR81. A) D-lactate protected from endotoxin shock in WT mice. Compared with WT mice, GPR81-deficient mice were more susceptible to endotoxic shock. Wild-type (WT) or GPR81 KO mice (n = 10/group) were given sodium D-lactate (1000 mg kg^{-1}) or vehicle control for 7 days, challenged intraperitonealy with 200 μg of LPS and 8 mg D-gallactosmine and then monitored for up to 24 h. B) Serum TNF-a and C) IL-6 levels in mice. Mice were fed with D-lactate (1000, 2000 mg kg^{-1} bw) or vehicle control for 7 days, followed by a non-lethal LPS challenge injection (10 μg) for another 2 h. Data are the means ± S.E.M. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to control.

3.4. Pharmacokinetic Proprieties of D-Lactate in Mice

Pharmacokinetic evaluation of D-lactate in mice [orally (p.o.) 1000 mg kg^{-1}] yielded a plasma $t_{1/2}$ of 5.5 h, oral time of maximum plasma concentration (T_{max}) of 1.0 h, and oral maximum concentration (C_{max}) in the plasma [$C_{max(plasma)}$] of 2.1 mM (**Figure 6**). The plasma terminal half-life ($T_{1/2}$) of D-lactate was 9-fold longer than that of L-lactate. Our data indicated that D-lactate was not metabolized as readily as L-lactate. As such, these findings not only ruled out the safety concerns about D-lactate, but also indicated that it might be a novel immune modulator.

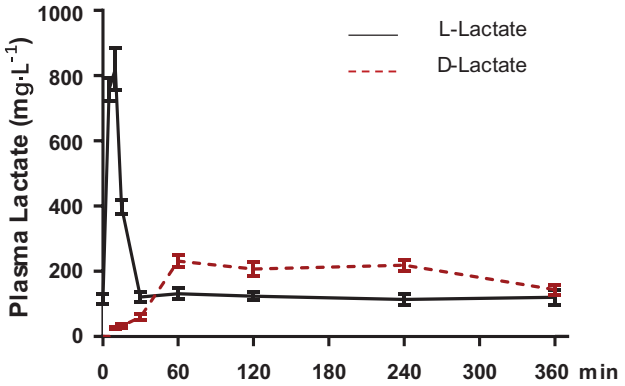
4. Discussion

In the present study, we provided evidence to support that dietary D-lactate may be a previously unrecognized nutrient aiding in resolution of acute inflammation. Findings in this study might provide an insight into how fermented foods profoundly influence the host's health and thereby give an interpretation of Metchnikoff's probiotic theory of life prolongation. If this is the case, the formation of D-lactate might be an important goal of lactic acid fermentations in the future.

The concept that L-lactate is the only lactate enantiomer possessing physiological action has been challenged. In addition to being an energy source, lactate might serve as a signaling molecule. Since either L- or D-lactate is natural ligand for GPR81, it is not surprising to find that D-lactate might possess immunemodulatory and/or anti-inflammatory activities. Unexpectedly, data in this study strongly suggested that the anti-inflammatory properties of D-lactate might be potent than of L-lactate. In theory, the efficacy of a drug is not only up to its binding affinity to a defined target but also the related binding kinetics profile.[29] In this regards, we noticed that the plasma terminal half-life ($T_{1/2}$) of D-lactate was 9-fold longer than that of L-lactate. Bearing those in mind, we proposed that D-LDH deficiency in mammals might coincidentally provide a pharmacokinetic advantage of D-lactate over L-lactate, and thus improve its efficacy in vivo. In other words, D-lactate might be superior to L-lactate, when serving as signalling molecular.

Under inflammatory conditions, macrophage infiltration and activation leads to increased secretion of proinflammatory cytokines.[30] Classically activated M1 macrophages secrete proinflammatory cytokines, such as TNF-α and IL-6. Alternatively activated M2 macrophages are thought to provide anti-inflammatory response in response to IL-4 signaling.[31,32] Similar to the previous study on Nature, we found that Arg-1, a representative indicator of M2 polarization, could also be activated by LPS.[33] Following LPS stimulation in vitro, macrophages are polarized to an M1 phenotype with high expression of p38 and NF-κb. Phosphorylation of ERK and p38 can induce iNOS and COX-2 expressions.[34] Consistent with previous studies above, in the present study, we identified a physiological role of the lactate-GPR81 axis in LPS-induced macrophage polarization. Our data further suggest that GPR81 deficiency might sensitize mice to experimental endotoxic shock. Thus, GPR81 definitely provide survival advantage in acute inflammation. What's more, the anti-inflammatory property of lactate may extend beyond the macrophage and acute inflammation. If lactate is serving as a signaling molecule, such a lactate-GPR81 axis should be conserved. Indeed, lactate had been reported to affect bone marrow neutrophil mobilization, tumor-associated macrophage polarization as well as Kupffer cells.[6,7,35,36] Most likely, inflammation leads to a profound metabolic alteration triggering lactatemia, whereas the lactate-GPR81 axis might serve as an ancient mechanism to aid in inflammatory resolution.

Although findings in this study seem promising, there are several issues raised. For example, the first issue is the



Parameter	Lactate enantiomers	
	L-lactate (2000mg kg^{-1})	D-lactate (2000mg kg^{-1})
C_{max} (mg L^{-1})	820± 92.9	231.55± 86.4
T_{max} (min)	10.02± 2.5	60± 9.1
$T_{1/2}$ (min)	36.9± 6.2	327± 21.2
CL/F (L h^{-1})	141.65± 25.4	478.2± 67.4
Vd /F (L kg^{-1})	7.55± 1.9	4.03± 1.2
MRT (min)	147± 22.7	182.36± 29.03
AUC_{0-t} (hr μg ml^{-1})	880.5± 89.4	1063.48± 121.6

Figure 6. Pharmacokinetic analysis of D-lactate. Mice ($n = 8$) were given a single oral dose of L- or D-lactate at 2000 mg kg^{-1}, and then blood lactate concentrations were analyzed by HPLC.

possibility of bench-to-bedside translation. To this end, the most important question is how much and how often fermented foods should a person to take to reach the doses reported in this study. Our data clearly showed that commercial yogurt products normally contain 10‰ L-lactate and 7‰ D-lactate (Figure S1, Supporting Information). As such, mice were given an oral dose of D-lactate (1000 mg kg^{-1}), which is equivalent to about consume 770 g yogurt daily for a 65 kg human. Secondly, although our findings together with previous studies by others indicated the effectiveness of lactate against acute inflammation, it should be applied judiciously for critically ill hospitalized patients as they might be at risk of fatal lactic acidosis.[1,24,37] Given the critical role of lactate in immunosuppression in tumors, D-Lactate or D-lactate based probiotic intervention might not be suited for cancer patients, too. Perhaps its most intriguing target disease state should be the chronic low-grade inflammation which has been implicated in obesity, metabolic syndrome as well as diabetes.[27,38-40] And our previous study did support the protective effect of lactate against high-fat-diet-induced obesity, dyslipidemia as well as hyperglycemia in mice.[41] Thirdly, the mechanism underlying D-lactate modulating inflammatory response remains unclear. For example, given that blood lactate levels are normally delicately controlled at rather narrow range, the turnover flux of lactate may be underestimated. We could not exclude the role of the L-lactate endogenous released in response to LPS treatment. To further clarify the anti-inflammatory action of D-lactate in vivo, the isotopic tracer studies definitely provide direct evidence and thus should be performed in future. In addition to immune cells, GPR81 is highly expressed in adipose cells. To dissect the molecular nature of how GPR81 modulates inflammatory response, more rigorous studies should be performed in conditional knockout mice. And ablation of GPR81 in bone marrow (BM)-derived cells seems be an essential step forward. Short chain fatty acids (SCFAs) have been implicated in host health, but our data showed that D-lactate treatment did not affect fecal SCFA concentration in two different inflammation mouse models (Figure S2, Supporting Information). During the course of this study, we also noticed that GPR81 was markedly down-regulated during M1 macrophage polarization. Interestingly, a similar inhibitory effect of LPS on GPR81 has been also reported in endothelial cells.[42,43] Nevertheless, the interpretation of such phenomenon was still missing.

In summary, this study highlights the importance of dietary D-lactate intake in promoting and maintaining human health, and offers reliable evidence for its efficacy in controlling acute inflammation. We also identified a physiological role of the lactate-GPR81 axis in macrophage polarization. Maintaining or augmenting macrophage GPR81 activation might represent a promising strategy in the management of inflammatory diseases. All in all, these insights may explain the frequently reported benefits of fermented food in chronic inflammatory diseases as reported in epidemiological studies.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

H.L., H.Z., and W.C. designed research; Y.Y., X.L., and Q.Y. performed research; Y.Y., X.L., Q.Y., and H.L. analyzed data; Y.Y, X.L. B.J., H.Z., H.L., K.H., and W.C. interpreted data; and Y.Y., H.L., and K.H. wrote the paper.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request. Some data may not be made available because of privacy or ethical restrictions.

Keywords

D-Lactate, GPR81, inflammation, macrophage polarization, yogurt

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