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ORIGINAL ARTICLE
Heart Failure

Oral Administration of *Euglena Gracilis Z* Alleviates Constipation and Cardiac Dysfunction in a Mouse Model of Isoproterenol-Induced Heart Failure

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Background: Patients with heart failure (HF) often experience gastrointestinal problems such as constipation, diarrhea, and disturbances to drug absorption. In HF, hypoperfusion and congestion cause structural and functional changes in the gut, which, in turn, lead to impaired cardiac function. *Euglena gracilis* Z (hereafter "Euglena"), called *Midorimushi* in Japanese, is a microalga that is used as a food or nutritional supplement. It is unclear whether Euglena is beneficial for bowel habitus and cardiac function in subjects with HF.

Methods and Results: We injected C57BL/6 male mice subcutaneously with isoproterenol (ISO) (20 mg/kg/day) for 7 days to examine bowel movement in HF. Euglena was orally administered to mice on an *ad libitum*-feeding to a normal chow containing 2% dietary mixture. ISO induced a decrease in bowel movement and an increase in fecal retention in the cecum, as well as a decrease in left ventricular (LV) contraction. Euglena accelerated intestinal transit, relieved fecal retention, and prevented the alterations in gut pathology in ISO-treated mice. Euglena also suppressed ISO-induced decreases in LV contraction, although it had no significant effect on LV hypertrophy.

Conclusions: The results suggested that oral administration of Euglena alleviated constipation and cardiac dysfunction in a mouse model of ISO-induced HF, and highlight the potential clinical benefit of Euglena in patients with HF in preventing constipation and contractile deterioration.

Key Words: Cardiac dysfunction; Constipation; Euglena; Isoproterenol; Mouse model

Heart failure (HF) is a clinical syndrome of a serious and progressive condition in which the heart is unable to pump sufficient amounts of blood and oxygen to meet the demands of the body.[1,2] HF is mostly caused by a wide spectrum of underlying diseases, such as hypertension, ischemic heart disease, cardiomyopathies, valvular heart diseases, and arrhythmias. Hemodynamic decompensation is associated with tissue ischemia due to hypoperfusion and tissue edema due to congestion, and thereby causes symptoms such as dyspnea, malaise, swelling, and/or decreased exercise capacity. Gastrointestinal function is also altered in HF patients.[3–5] Microcirculatory disturbances impair gut epithelial function, which may not only hamper the absorption of nutrients and orally administered drugs, but also induce bacterial translocation and possibly aggravate HF by activating inflammatory responses.[3–5] In addition, emerging evidence indicates that the composition of the gut microbiota is altered (dysbiosis) in HF patients.[6–8] In particular, HF-associated gut dysbiosis is associated with an imbalance in gut microbe-derived metabolites such as short-chain fatty acids (SCFAs),[6] and the aberrant production of microbe-derived metabolites may contribute to the pathogenesis of HF.[9–11]

Meanwhile, it has been reported that 25–30% of HF patients experience constipation.[12] A restriction of water intake, the use of diuretics, sedentary behavior due to decreased exercise capacity, adverse drug effects, and other factors contribute, in combination, to the high prevalence of constipation among HF patients. The symptom burden of constipation on quality of life (QOL) is considerably high in HF patients. Furthermore, strain at stool may trigger blood pressure elevation and increase the risk of

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Table. Nutritional Composition of the Control and Euglena Diets

	Control diet	Euglena diet
Casein (%)	20.0	20.0
L-Cystine (%)	0.3	0.3
Corn starch (%)	39.7	39.7
α-Corn starch (%)	13.2	13.2
Sucrose (%)	10.0	10.0
Soybean oil (%)	7.0	7.0
AIN93 mineral mixture (%)	3.5	3.5
AIN93 vitamin mixture (%)	1.0	1.0
Choline bitartrate (%)	0.3	0.3
Cellulose (%)	5.0	3.0
Euglena (%)	0.0	2.0

cardiovascular events.[13] A large population-based cohort study revealed that constipation was associated with an increased risk of HF.[14] Therefore, it is important to manage and prevent constipation in HF patients.

Euglena gracilis Z (hereafter "Euglena"), called *Midorimushi* in Japanese, is a microalga, and has features of both animals and plants. Euglena has recently been used as a food or nutritional supplement; it is rich in nutrients such as vitamins, minerals, amino acids, and unsaturated fatty acids.[15] Euglena also produces and accumulates paramylon, a β-1,3-glucan, within its cells.[15] Experiments in rodents revealed that the oral administration of Euglena or paramylon was beneficial in a variety of disease models such as hepatic injury,[16] atopic dermatitis,[17] rheumatoid arthritis,[18] type 2 diabetes,[19] influenza virus infection,[20] and chronic kidney disease.[21] It is also possible that oral administration of Euglena may maintain healthy gut microbiota and ameliorate intestinal function, because some components of Euglena stimulate the growth of butyrate-producing bacteria in the gut.[22]

In this study we examined the effects of Euglena on constipation and cardiac dysfunction in a mouse model of isoproterenol (ISO)-induced HF.

Methods

Mice, Transthoracic Echocardiography, and Blood Pressure Measurements

All animal experiments were approved by the Ethics Committee for Animal Experiments of the University of Tokyo, and adhered strictly to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (https://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf [accessed April 2017]).

Male C57BL/6J mice (7 weeks old) were purchased from CLEA Japan (Tokyo, Japan). Mice were housed 3–4 per cage in rooms maintained at a mean (±SD) temperature of 24±1°C and 55–60% humidity under a 12-h light-dark cycle and specific pathogen-free conditions. Mice were allowed free access to water and chow. To induce HF, the non-selective β-adrenergic receptor agonist ISO was dissolved in normal saline and 8-week-old mice were injected subcutaneously with 20 mg/kg/day ISO once a day for 7 days.[23]

To evaluate cardiac dimensions and contractility, transthoracic echocardiography using a Vevo 2100 System (FUJIFILM VisualSonics, Toronto, Canada) was performed in mice that had been anesthetized with 2% isoflurane. Blood pressure and pulse rates were measured in conscious mice by the tail-cuff method (MK-2000ST; Muromachi Kikai, Tokyo, Japan). Thoracic echocardiography and measurement of blood pressure and pulse rates were performed on Day 8 (the next day after the final ISO injection). Mice were killed by intraperitoneal injection of sodium pentobarbital (50 mg/kg) and tissue samples were collected.

Preparation of Euglena

Euglena is a powdered product provided by Euglena Co. Ltd. (Tokyo, Japan). The culture liquid of *Euglena gracilis* Z was concentrated using ultrafiltration and centrifugation, and the concentrated suspension was dried using a spray dryer. According to a nutiritional analysis performed by Euglena Co. Ltd., Euglena comprises 51.0% carbohydrates, 32.5% protein, 8.8% fat, 4.0% ash, and 3.7% water. To create the Euglena-supplemented diet used in this study, Euglena (2%) was mixed into the AIN93G diet (Oriental Yeast, Tokyo, Japan), according to previous studies using rodent disease models.[18–20] The composition of the AIG93G (control) and Euglena-supplemented diets is listed in the **Table**.

Mice were randomly assigned to 4 groups: control/saline (control diet, saline treatment), Euglena/saline (Euglena diet, saline treatment), control/ISO (control diet, ISO treatment), Euglena/ISO (Euglena diet, ISO treatment). Mice were fed the Euglena or control diet for 8 weeks, and then injected subcutaneously with ISO or saline for 7 days.

Intestinal Transit Analysis

Gastrointestinal transit was measured as described previously.[24] After fasting for 2 h with free access to water, mice were given an oral gavage of 150 μL of 0.5% Trypan blue solution. Mice were killed 30 min after the administration of the solution, and the small intestine was dissected out. For each mouse, intestinal transit was calculated as the percentage of the distance traveled by the Trypan blue relative to the total length of the small intestine.

Measurement of Fecal Water Content

Fecal water content was determined in fresh feces discharged from each mouse on the day of blood pressure measurement. After weighing the collected feces, the samples were dried at 90°C for >24 h and weighed again. The fecal water content was calculated as the change in weight from before to after drying.

Real-Time Quantitative Reverse Transcription-Polymerase Chain Reaction (PCR)

Total RNA was extracted from the left ventricle (LV) of the heart using TRIzol reagent (Thermo Fisher Scientific, Waltham, MA, USA), and single-stranded cDNA was reverse transcribed for subsequent real-time PCR using qPCR RT Master Mix (TOYOBO, Osaka, Japan) according to the manufacturer's instructions. Gene expression was assessed using KOD SYBR qPCR Mix (TOYOBO). PCRs were performed using a LightCycler 480 system (Roche Diagnostics K.K., Tokyo, Japan) with initial denaturation for 10 min at 95°C followed by 40 cycles of denaturation at 95°C for 10 s, annealing at 60°C for 30 s, and extension at 72°C for 60 s. Gene expression levels were standardized to

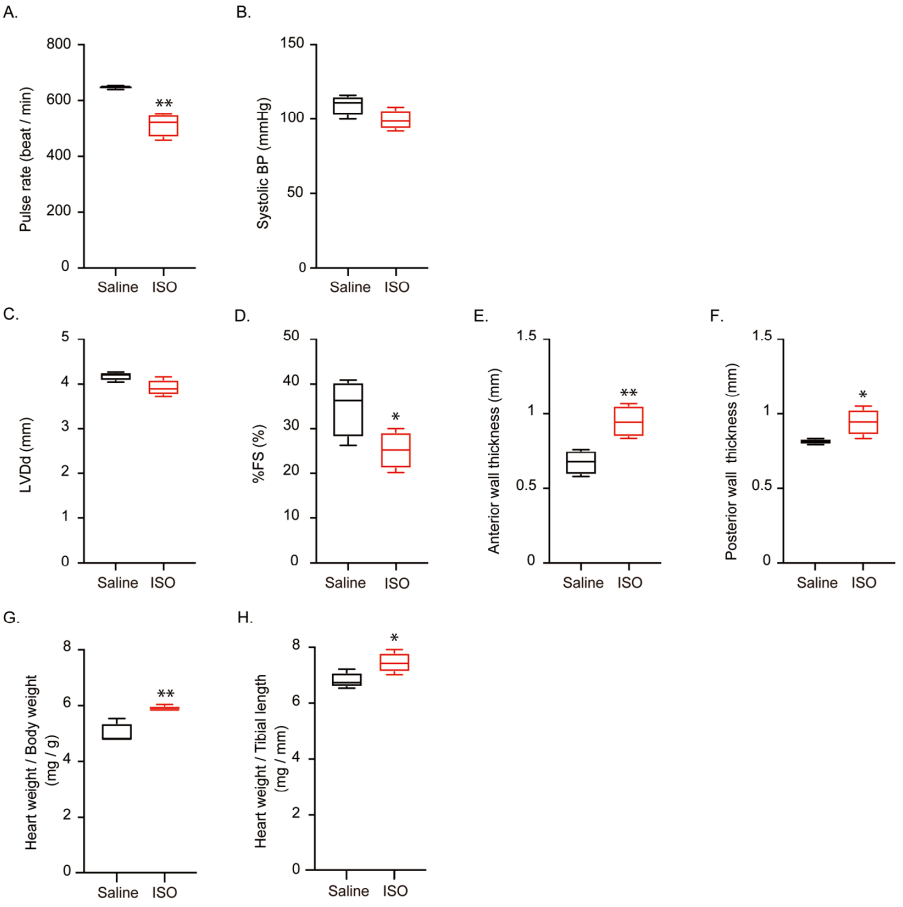

Figure 1. Cardiac structure and function in a mouse model of isoproterenol (ISO)-induced heart failure (HF). (**A**) Pulse rate and (**B**) systolic blood pressure (BP) in ISO- and saline-treated mice. (**C**–**F**) Echocardiographic measurements of left ventricular end-diastolic dimension (LVDd; **C**), fractional shortening (%FS; **D**), anterior wall thickness (**E**), and posterior wall thickness (**F**) in ISO- and saline-treated mice. (**G**,**H**) Heart weight to body weight (**G**) and heart weight to tibial length (**H**) ratios in ISO- and saline-treated mice. Data are presented as box-and-whisker plots, with upper and lower whiskers representing maximum and minimum values, respectively, the boxes representing the upper and lower quartiles, and horizontal lines within boxes indicating the median (n=4 in each group). *P<0.05, **P<0.01 compared with saline-treated mice.

that of glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*) using the comparative ΔC_t method. The following PCR primers were used: natriuretic peptide A (*Nppa*), 5'-CACAGATCTGATGGATTTCAAGA-3' (forward) and 5'-CCTCATCTTCTACCGGCATC-3' (reverse); myosin heavy chain 7 (*Myh7*), 5'-CGCATCAAGGAGCTCACC-3' (forward) and 5'-CTGCAGCCGCAGTAGGTT-3' (reverse); ATPase sarcoplasmic/endoplasmic reticulum Ca^{2+} transporting 1 (*Atp2a1*), 5'-TGGAACAACCCGGTAAAGAGT-3' (forward) and 5'-CACCAGGGGCATAATGAGCAG-3' (reverse); collagen type I α 1 chain (*Col1a1*), 5'-AGACATGTTCAGCTTTGTGGAC-3' (forward) and 5'-GCAGCTGACTTCAGGGATG-3' (reverse); *Gapdh*, 5'-GGCAAGTTCAATGGCACAGT-3' (forward) and 5'-TGGTGAAGACGCCAGTAGACTC-3' (reverse).

Histological Analysis

For histological analyses, the heart and gut were excised from all mice, fixed immediately in 10% neutralized formalin (Sakura Finetek Japan, Tokyo, Japan), and embedded in paraffin. Serial sections (5 μm) were stained with hematoxylin-eosin (HE) for morphological analysis and Masson's trichrome for the detection of collagen fibers. To assess cardiomyocyte cross-sectional area, heart sections were stained with Invitrogen wheat germ agglutinin (WGA), Alexa Fluor 594 conjugate (1:100 dilution in phosphate-buffered saline [PBS]; Thermo Fisher Scientific) for 60 min at room temperature, washed 3 times with PBS for 5 min each time, and then mounted with Invitrogen Fluoromount-G Mounting Media, with 4',6'-diamidino-2-phenylindole (Thermo Fisher Scientific). Images were acquired with an all-in-one fluorescence microscope (BZ-9000; Keyence, Osaka, Japan). The cross-sectional areas of cardiomyocytes, as well as the length of villi and the thickness of the tunica muscularis in the gut, were measured using National Institutes of Health ImageJ (http://imagej.nih.gov/ij/).

Blood Analysis

Serum total cholesterol, blood urea nitrogen, creatinine, aspartate transaminase (AST), and alanine transaminase (ALT) concentrations were measured in the laboratory of SRL, Inc. (Tokyo, Japan).

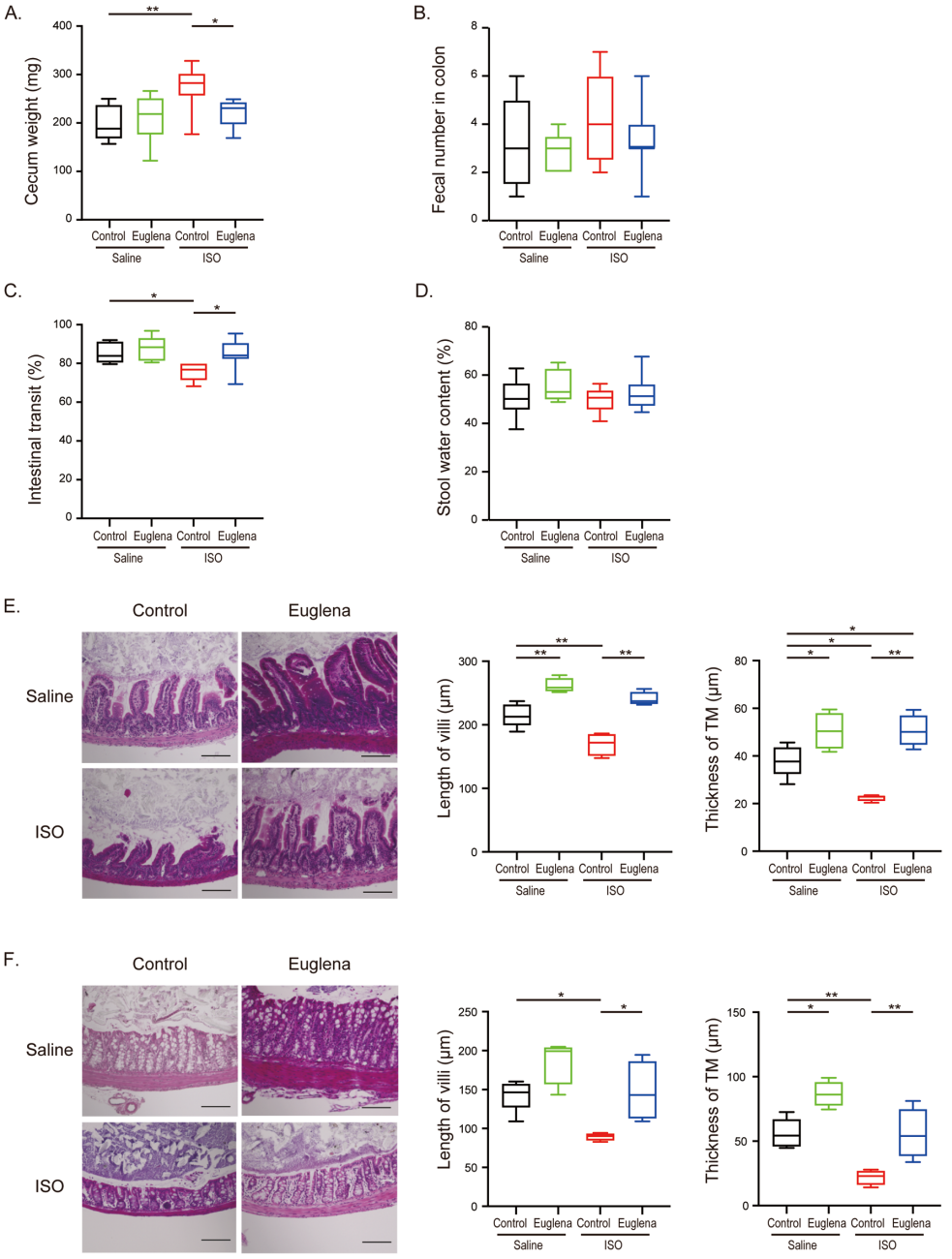

Figure 2. Effects of Euglena on constipation and gut pathology in a mouse model of isoproterenol (ISO)-induced heart failure (HF). The amount of residual feces in the cecum (**A**) and the number of residual faces in the colon (**B**) of ISO- and saline-treated mice fed either a control or Euglena diet (n=8–9 in each group). (**C**) Intestinal transit (n=6–7 in each group) and (**D**) fecal water content (n=8–9 in each group) in ISO- and saline-treated mice fed either a control or Euglena diet. (**E**,**F**) Hematoxylin-eosin staining (**Left panels**) with quantification of villi length and thickness of the muscularis layer (**Right panels**) in the ileum (**E**) and colon (**F**). Scale bars, 100 μm. Data are presented as box-and-whisker plots, with the upper and lower whiskers representing maximum and minimum values, respectively, the boxes representing the upper and lower quartiles, and horizontal lines within boxes indicating the median. *P<0.05, **P<0.01.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism version 8.3.1 (GraphPad Software, San Diego, CA, USA). All data are presented as box-and-whisker plots. Comparisons between 2 groups were made using unpaired Student's t-tests. Multiple-group comparisons were made by 1-way analysis of variance (ANOVA) with Bonferroni's post hoc test for parametric analyses and by the Kruskal-Wallis test with Dunn's multiple comparisons test for non-parametric analyses to identify differences between specific groups. Two-sided P<0.05 was considered statistically significant.

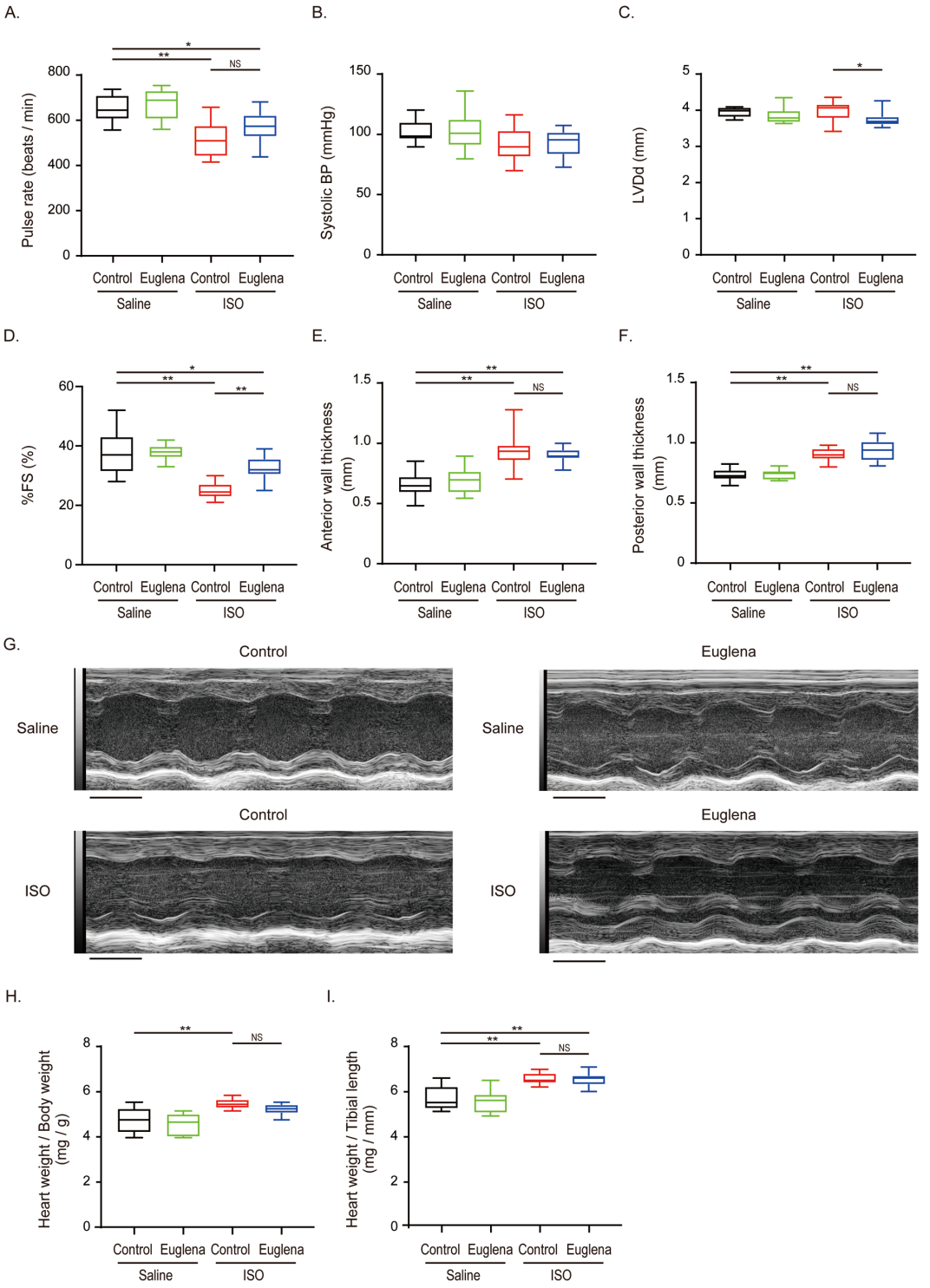

Figure 3. Effects of the Euglena diet on cardiac dysfunction in a mouse model of isoproterenol (ISO)-induced heart failure (HF). (**A**) Pulse rate and (**B**) systolic blood pressure (BP) in ISO- and saline-treated mice fed either a control or Euglena diet (n=12–13 in each group). (**C**–**F**) Echocardiographic measurements of left ventricular end-diastolic dimension (LVDd; **C**), fractional shortening (%FS; **D**), anterior wall thickness (**E**), and posterior wall thickness (**F**) in ISO- and saline-treated mice fed either a control or Euglena diet (n=12–13 in each group). (**G**) Representative 2-dimensional M-mode echocardiograms in the parasternal short-axis view at the papillary muscle level in ISO- and saline-treated mice fed either a control or Euglena diet. Scale bars, 100 ms. (**H**,**I**) Heart weight to body weight (**H**) and heart weight to tibial length (**I**) ratios in ISO- and saline-treated mice fed either a control or Euglena diet (n=8–9 in each group). Data are presented as box-and-whisker plots, with the upper and lower whiskers representing maximum and minimum values, respectively, the boxes representing the upper and lower quartiles, and horizontal lines within boxes indicating the median. *P<0.05, **P<0.01.

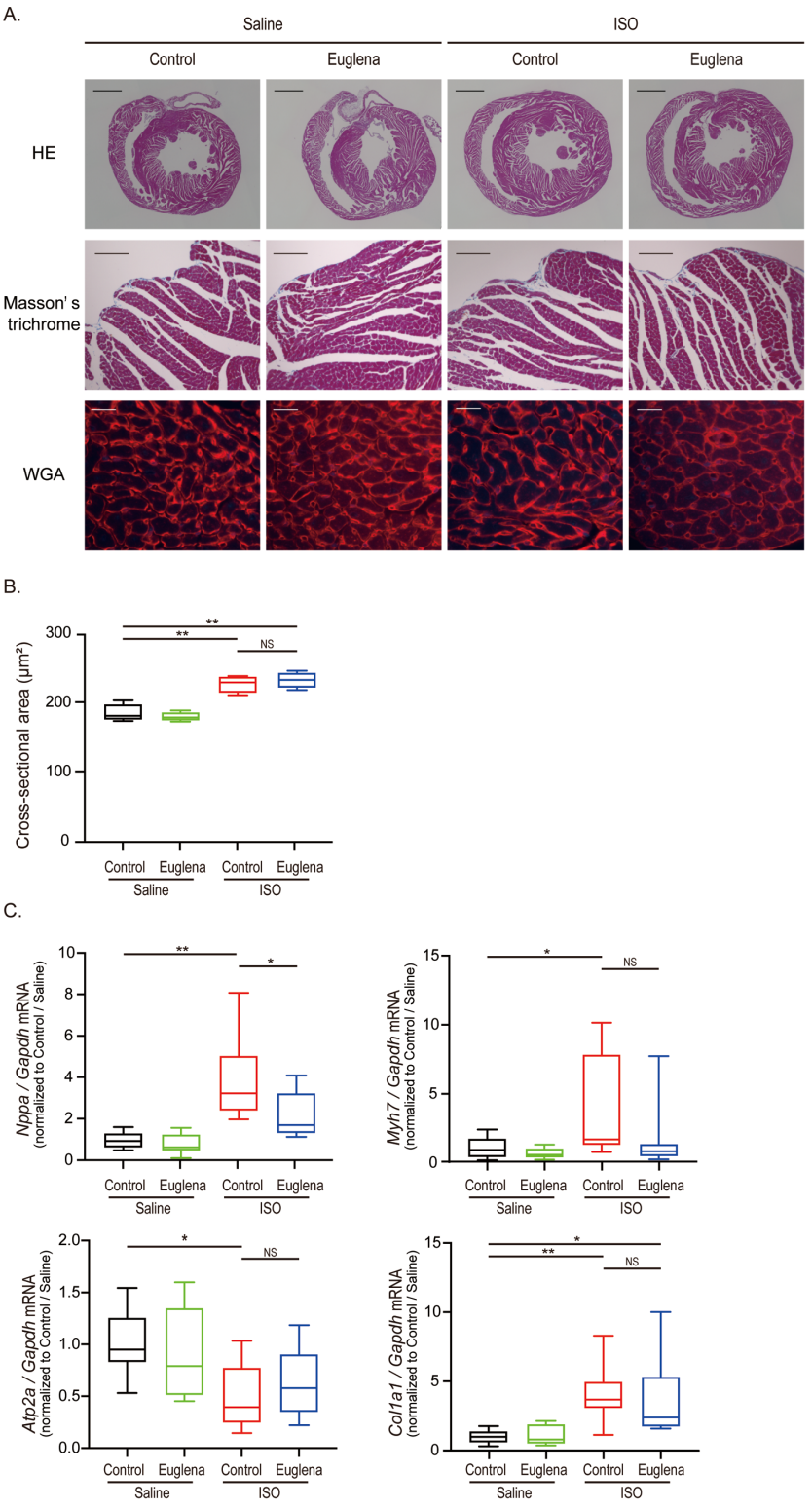

Figure 4. Histological analysis and quantitative reverse transcription-polymerase chain reaction analysis of the heart in isoproterenol (ISO)- or saline-treated mice after feeding a Euglena or control diet. (**A**) Representative images following hematoxylin-eosin (HE) staining of the heart (scale bars=100 μm), Masson's trichrome staining of the heart (scale bars=100 μm), or wheat germ agglutinin (WGA) staining of the heart (scale bars=20 μm). (**B**) Quantification of cardiomyocyte cross-sectional area (n=456–520 cardiomyocytes from 4 mice in each group). (**C**) Expression of natriuretic peptide A (*Nppa*), myosin heavy chain 7 (*Myh7*), ATPase sarcoplasmic/endoplasmic reticulum Ca^{2+} transporting 1 (*Atp2a1*), and collagen type I alpha 1 chain (*Col1a*) mRNA (n=9 in each group). Data are presented as box-and-whisker plots, with the upper and lower whiskers representing maximum and minimum values, respectively, the boxes representing the upper and lower quartiles, and horizontal lines within boxes indicating the median. *P<0.05, **P<0.01.

Results

Cardiac Structure and Function in a Mouse Model of ISO-Induced HF

To induce HF, mice were injected with ISO (20 mg/kg/day, s.c.) for 7 days. With this treatment, conscious mice had a significantly lower pulse rate, as reported previously,[25] but there was no significant change in systolic blood pressure (SBP; **Figure 1A,B**). Transthoracic echocardiography showed that fractional shortening was significantly decreased in

ISO-treated mice, but there was no change in LV end-diastolic dimension (LVDd), indicating that ISO impaired LV systolic function in mice (**Figure 1C**,**D**). In addition, the significant increase in the thickness of the anterior and posterior walls in ISO-treated mice (**Figure 1E**,**F**) indicated that ISO induced LV hypertrophy. Consistent with the echocardiographic measurement of LV dimension and wall thickness, the heart weight to body weight and heart weight to tibial length ratios were significantly increased after ISO treatment (**Figure 1G**,**H**).

Effects of Oral Administration of Euglena on Bowel Movement in a Mouse Model of ISO-Induced HF

Little is known about the changes in bowel movement in mouse models of HF. In the present study, ISO-treated mice had a higher amount of residual feces in the cecum than saline-treated mice (**Figure 2A**). ISO-treated mice also tended to have more stool pellets in the colon than mice in the control group, although the difference was not statistically significant (**Figure 2B**). In addition, intestinal transit was significantly slower in ISO-treated mice (**Figure 2C**). The accumulation of hard feces also contributes to constipation, but the stool water content in ISO-treated mice did not differ significantly from that in the saline-treated mice (**Figure 2D**). These results suggest that the constipation in ISO-treated mice may be caused primarily by a decrease in bowel movement, like in patients with HF. We found that the Euglena diet accelerated intestinal transit and decreased fecal retention in the cecum and colon (**Figure 2A**–**C**), suggesting that Euglena alleviates constipation in a mouse model of ISO-induced HF.

We next examined changes in gut pathology in ISO-treated mice. Notably, the villi were shorter and the muscularis layer was thinner in both the ileum and colon of ISO-treated compared with saline-treated mice (**Figure 2E**,**F**). The Euglena diet not only increased the length of the villi and the thickness of muscularis layer in saline-treated mice, but also significantly prevented shortening of the villi and thinning of the muscularis layer in ISO-treated mice (**Figure 2E**,**F**), indicating beneficial effects of Euglena on HF-associated gut pathology.

Effects of Oral Administration of Euglena on Cardiac Structure and Function in a Mouse Model of ISO-Induced HF

We further examined the effects of the Euglena diet on cardiac structure and function. Although there was no significant change in pulse rate, SBP, and LV wall thickness, the Euglena diet significantly attenuated the decrease in fractional shortening after ISO treatment compared with the control diet (**Figure 3A**–**G**). The Euglena diet also decreased LVDd in ISO-treated mice, but not in saline-treated mice (**Figure 3C**,**G**). There was no significant difference in the heart weight to body weight and heart weight to tibial length ratios between mice fed the Euglena diet and those fed the control diet (**Figure 3H**,**I**).

Consistent with these findings, HE staining of the histological sections of hearts revealed an ISO-induced increase in LV wall thickness, which was not attenuated by the Euglena diet (**Figure 4A**). Masson's trichrome staining showed no significant change in interstitial fibrosis in hearts from ISO-treated mice (**Figure 4A**). WGA staining revealed pronounced cardiomyocyte hypertrophy in ISO-treated mice, with a 1.2-fold increase in the cross-sectional area of cardiomyocytes compared with saline-treated mice; the Euglena diet did not attenuate ISO-induced cardiomyocyte hypertrophy (**Figure 4A**,**B**).

We next examined the mRNA expression of several molecular markers related to cardiac hypertrophy and HF. ISO treatment significantly increased the expression of the *Nppa*, *Myh7*, and *Col1a1* genes, and decreased expression of *Atp2a* (**Figure 4C**); these changes are indicative of a myocardial response to hypertrophic stimuli and intracellular Ca^{2+} dysregulation.[26] The Euglena diet significantly attenuated ISO-induced changes in *Nppa* expression, but not in the expression of *Myh7*, *Atp2a*, and *Col1a1*, although there was a tendency for the Euglena diet to suppress ISO-induced changes in the mRNA expression of these genes (**Figure 4C**).

Discussion

In this study, oral administration of Euglena increased intestinal transit and decreased fecal retention in a mouse model of ISO-induced HF. Notably, the Euglena diet not only improved laxation, but also attenuated ISO-induced cardiac dysfunction. These results may support the concept of the heart-gut axis, proposing a role for the gut in the pathogenesis of HF,[5,27] and highlight the potential use of Euglena to improve QOL and prognosis in HF patients.

Euglena is a promising candidate as a biomass source of food and other products (fiber, feed, fertilizer, and fuel), and Euglena-containing food products are available.[28] Euglena is rich in the β-glucan paramylon, as well as nutrients such as vitamins, minerals, amino acids, and unsaturated fatty acids.[15] Beta-glucans are insoluble dietary fibers that have pleiotropic biological effects, such as scavenging reactive oxygen species, preventing the absorption of cholesterol, and promoting egestion.[29]

The mechanisms underlying the beneficial effects of Euglena on comorbid constipation and HF remain to be determined. We previously analyzed the composition of gut microbiota in HF patients using *16S* ribosomal RNA gene sequencing and demonstrated that gut dysbiosis in HF was characterized by a significant reduction in the number of bacteria producing SCFAs such as acetate and butyrate.[6] SCFAs regulate bowel movement by G-protein-coupled receptor 43 (GPR43)-mediated release of 5-hydroxytryptamine from enteroendocrine cells and mucosal mast cells,[30,31] and by modulating neuronal excitability in enteric neurons through the inhibition of histone deacetylase activity.[32] It has also been reported that SCFA levels in stools are decreased in patients with mixed refractory constipation.[33] In an in vitro model culture system simulating the microbiota of the human colon, the addition of Euglena enhanced butyrate production by stimulating the growth of *Faecalibacterium*.[22] In mice, SCFA levels in the feces and serum exhibited a nominal increase after feeding of the Euglena diet, although the effect did not reach statistical significance.[34] Euglena may alleviate constipation in ISO-treated mice in part by restoring the production of SCFAs in the gut. SCFAs are a major nutrient source for colonic epithelial cells, and play an essential role in maintaining intestinal barrier function.[35,36] In the present study, we confirmed that the Euglena diet had beneficial effects on HF-associated gut pathology (**Figure 2E**,**F**). Among the SCFAs, butyrate also enhances the differentiation of colonic regulatory T cells through the inhibition of histone deacetylases, and thereby inhibits inflammation.[35–37] We speculate that an increase in SCFA production by feeding

of the Euglena diet may prevent aggravation of cardiac dysfunction by maintaining gut epithelial function and inhibiting bacterial translocation, which causes inflammatory responses.

Recently, it was reported that Euglena and the β-glucan paramylon induced Ca^{2+} signaling in intestinal epithelial, immune, and neural cells.[38] Because stimulated intestinal epithelial cells produce cytokines and peptide hormones such as ghrelin, serotonin, cholecystokinin, peptide tyrosine tyrosine, glucagon-like peptide-1, and glucose-dependent insulinotropic peptide,[39] Euglena may modulate cardiovascular homeostasis by the gut-immune-brain axis via secretion of various cytokines and hormones. It is also possible that fatty acids, vitamins, or other substances contained in Euglena may contribute to the improvement in cardiac function through actions on multiple organs and lipid metabolism. However, we did not observe significant differences in liver and epididymal fat weights (**Supplementary Figure 1**) or serum total cholesterol, blood urea nitrogen, creatinine, AST, and ALT concentrations (**Supplementary Figure 2**).

The main limitation of the present study is that we are not able to determine the mechanism by which Euglena protects against constipation and HF. Although some specific ingredients of Euglena may be responsible, it is more likely that multiple ingredients in a complex combination may contribute to the beneficial effects of Euglena on the heart-gut axis. Future studies, including in-depth multi-omics profiling of the microbiota and metabolites in the feces and serum, as well as gene regulatory networks in the heart, will be required to elucidate the precise mechanisms underlying the protective effects of Euglena on the gut and heart.

This study demonstrated that oral administration of Euglena alleviated constipation and cardiac dysfunction in a mouse model of ISO-induced HF. The effectiveness of Euglena on constipation and cardiac dysfunction will be translated into practice in a clinical setting. Daily ingestion of Euglena increases bowel movement frequency and stool volume in healthy human subjects.[22] However, the causes of constipation in HF patients are multifactorial, with contributing factors including immobility due to hospitalization and bed rest, intestinal dehydration due to the excessive use of diuretics and restricted ion of water intake, and adverse effects of several medications, such as calcium channel blockers and anti-arrhythmics, which cause relaxation of the intestinal smooth muscle.[40] Future studies using different models of HF, such as pressure overload, volume overload, and myocardial infarction, will strengthen the rationale for the potential use of Euglena to relieve symptoms associated with constipation and cardiac dysfunction in HF patients.

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Disclosures

A.N., K.Y., K.S. are salaried employees of Euglena Co. Ltd, which produced some of the *Euglena* used in this study. There are no patents, products in development, or marketed products to declare. This does not alter the authors' adherence to all the *Circulation Reports* policies on sharing data and materials. H.A. has received research funding from euglena Co. Ltd and scholarship funding from Takeda Pharmaceutical, Daiichi Sankyo, Shionogi & Co. Ltd, Nippon Boehringer Ingelheim, Bristol-Myers Squibb K.K., MSD K.K., Sanofi K.K., and Sumitomo Dainippon Pharma. H.A. , I.K. are members of *Circulation Reports*' Editorial Team. The remaining authors have no conflicts of interest to declare.

IRB Information

All animal experiments in this study were approved by the Ethics Committee for Animal Experiments of the University of Tokyo.

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Supplementary Files

Please find supplementary file(s);
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