ORIGINAL ARTICLE

D-Leucine protects oocytes from chronic psychological stress in mice

Ai Tsuji [iD] | Yuka Ikeda | Mutsumi Murakami | Yasuko Kitagishi | Satoru Matsuda

Department of Food Science and Nutrition, Faculty of Human Life and Environment, Nara Woman's University, Nara, Japan

Correspondence
Ai Tsuji, Department of Food Science and Nutrition, Faculty of Human Life and Environment, Nara Woman's University, Nara 630-8506, Japan.
Email: atsuji@cc.nara-wu.ac.jp

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Abstract

Purpose: Psychological stress could negatively influence female reproductive ability. D-Leucine (D-Leu) is a D-type amino acid found in foods and mammalian tissues. We have examined the protective effects of D-Leu on oocyte abnormality induced by psychological stress.

Methods: Female mice (6-week-old) were divided into three groups: control, restraint stress (RS), and RS/D-Leu. The RS and RS/D-Leu mice were holed for 3 hours daily during 14 days. RS/D-Leu mice were fed 0.3% D-Leu diet. The oocyte maturation failure was analyzed by shapes of spindles and chromosomes. In addition, levels of heme-oxygenase-1 (HO-1) and superoxide dismutase (SOD) expression in the ovaries were also examined. Whether D-Leu reduces the generation of reactive oxygen species (ROS) in cultured cells, K562 cells were treated with D-Leu, and then ROS in K562 were analyzed.

Results: Oocyte maturation failure was increased in RS mice. D-Leu reduced abnormal oocytes to control level. The expression levels of HO-1 and SOD2 increased in RS/D-Leu mice compared to those of RS mice. ROS levels were decreased in K562 cells with D-Leu in a dose-dependent manner.

Conclusions: We concluded that D-Leu protects oocytes from psychological stress through the induction of HO-1 and SOD2 expression then by reducing oxidative stress.

KEYWORDS
heme-oxygenase-1, leucine, oocyte, psychological stress, superoxide dismutase

1 | INTRODUCTION

Excess stress might induce infertility and abortion. It has been reported that Japanese women have experienced psychological stress than men.[1,2] Some studies have suggested that psychological stress could have harmful effects on the reproduction system.[3,4] Psychological stress increases oocyte aneuploidy and impairs oocyte meiosis, which has been shown to disrupt the development of fertilized eggs in animals,[5,6] and reduces successful fertilization in vitro.[7] Accordingly, protection from psychological stress may be important to keep the normal reproductive function. Psychological stress induces the production of reactive oxygen species (ROS; eg, $O_2 \bullet -$, H_2O_2, $OH\bullet$, $^1O_2$) in oocytes.[5] Vigorous production of ROS can damage mitochondria, DNA, and

protein. The increase in ROS levels induces oocyte maturation failure by apoptosis.[8] Heme-oxygenase-1 (HO-1) and superoxide dismutase (SOD) protect cells from ROS. Nuclear factor E2-related factor 2 (Nrf2) is a transcription factor that regulates the expression of HO-1 and several SODs. HO-1 degrades heme and generates the antioxidant molecules biliverdin and carbon monoxide (CO). These products contribute to the suppression of inflammation, apoptosis, and oxidative damage to DNA and other cellular molecules, including membrane lipids and proteins.[9,10] The SODs (SOD1, SOD2, and SOD3) are antioxidant enzymes that play a role in the cellular defense system against ROS. SOD1, SOD2, and SOD3 localize to intercellular cytoplasmic compartments, the mitochondria of aerobic cells, and extracellular elements, respectively.

D-amino acids are stereoisomers of widely observed L-amino acids, which are contained in certain types of food, beverages, and intestinal bacteria [11,12] and the mammalian brain.[13,14] Recently, much attention has focused on the physiological functions of D-amino acids. For example, D-aspartic acid stimulates testosterone synthesis in rat Leydig cells.[15] D-aspartic acid also plays a role in luteinizing hormone and estrogen secretion in rat and frog brains.[16,17] D-serine binds to a glycine-binding site in the N-methyl-D-aspartate receptor and may be useful as a therapeutic agent for schizophrenia.[18,19]

D-Leucine (D-Leu) has been found in the pituitary gland and the pineal gland [20] and is present in foods such as sour milk, Emmentaler cheese, and carrot juice.[21] Maesawa et al reported that D-Leu is able to induce immature ovaries in asexual worms at picomolar levels.[22] D-Lue has also been reported to suppress seizures in mice.[23] We think, however, the biological functions of D-Leu remain unknown. The mechanism of oocyte meiosis failure induced by a high-fat diet is similar to that induced by psychological stress. A high-fat diet also increases oocyte ROS levels, and oocyte meiosis failure.[24,25] We tested the effects of D-Leu on oocyte meiosis failure induced by a high-fat diet. The percentage of morphologically abnormal oocytes was reduced in mice fed a D-Leu-supplemented high-fat diet. In this report, we suggest a function of D-Leu in oocyte protection from psychological stress.

2 | MATERIALS AND METHODS

2.1 | Mice

Female Crl:ICR mice (6-week-old) were obtained from Japan SLC, Inc (Shizuoka, Japan). The mice were housed in an environmentally controlled room, at approximately 20°C and 60% humidity with a 12-h light/dark cycle (lights on at 07:00 and off at 19:00). The mice in the control and stress groups were fed a commercial powder diet [MF diet, Oriental Yeast Co., Ltd]. The care and treatment of the experimental animals conformed to the guidelines for the ethical treatment of laboratory animals established by Nara Women's University (Nara, Japan) (Approval No. 19-02).

2.2 | Food and restraint stress

Mice were randomly divided into three groups; control (n = 13), restraint stress (RS; n = 10), and RS/D-Leu (n = 10). Mice were acclimated to the environment for 1 week before use. The mice in the RS/D-Leu group were fed an MF diet containing 0.3% D-Leu. D-Leu was purchased from Tokyo Chemical Industry Co., LTD. The body weight and food intake of mice were measured daily. Animals were allowed free access to food and water. To induce restraint stress, the mice in the RS and RS/D-Leu groups were placed into 50-mL plastic tubes for 3 hours (9:00-12:00) each day for 14 consecutive days.

2.3 | Estrus cycle

The estrus cycle was evaluated by performing daily vaginal smears. The vaginal smears were collected at 8:30 before the restraint stress treatment. The smears were stained using 3.2% Giemsa stain solution (Fujifilm Wako Pure Chemicals Co.). The method for determining the estrus cycle was described in a previous report.[26] Consecutive 4- or 5-day cycles were considered to represent a regular estrus cycle, whereas ≥6-days cycles were considered to represent an irregular estrus cycle.

2.4 | Oocyte and ovary collection

The method used for oocyte collection was described in a previous report.[26] To collect oocytes, the mice were intraperitoneally injected with 5 IU pregnant mare serum gonadotropin (PMSG; product No. L816A; ASUKA Pharmaceutical Co., Ltd.) on Day 12. Then, the mice were intraperitoneally injected with 5 IU human chorionic gonadotrophin (hCG; product No. L239A; ASUKA Pharmaceutical Co., Ltd.), 48 h after PMSG priming. Oocyte-cumulus cell complexes and ovaries were collected from mice at 8:30 on Day 15. The cumulus cells were removed by hyaluronidase treatment (Sigma-Aldrich Inc). Ovulating oocytes were counted and classified as normal or abnormal, according to morphology. The ovaries were weighed and stored at −80°C until analysis.

2.5 | Serum collection and measurement of AST and ALT

Immediately after the stress treatment for 3 hours on Day 14, whole blood samples were collected into 1.5-mL tubes. The samples were centrifuged at 3000 g for 15 min at 4°C to obtain serum. The serum samples were stored at −80°C until analysis. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels in the serum were measured using a Wako GOT/GPT CII-test, according to the manufacturer's instructions (FUJIFILM Wako Pure Chemical Corporation).

2.6 | Immunofluorescence

The method used to perform immunofluorescence analysis was described in a previous report.[23] Spindles in oocytes were stained with a 1:4000 dilution of anti-tubulin antibody (Cell Signaling Technology, Inc), followed by 1:500 dilution of goat anti-mouse IgG with Alexa Fluor-499, after fixation with 4% paraformaldehyde. Chromosomes in the oocytes were stained with propidium iodide. The spindles and chromosomes were observed by confocal fluorescence microscopy (C2; Nikon Instech Co., LTD.). The method used to classify oocytes as normal or abnormal was described in a previous report.[27]

2.7 | Expression of genes in the ovary

Total RNA was isolated from ovary samples using RNAiso Plus (Takara Bio Inc) and transcribed into cDNA using ReverTra Ace® qPCR RT Master Mix (TOYOBO Co. LTD.). The analysis was performed by real-time reverse-transcriptase-polymerase chain reaction (RT-PCR) on a Light cycler® Nano (Roche diagnostics KK) using Thunderbird SYBR qPCR Mix (TOYOBO Co. LTD.). The expression levels of each target mRNA were calculated and normalized against the expression level of β-actin using the $2^{-\Delta\Delta CT}$ method. Primer sequences were as follows: heme-oxygenase-1 (*Ho-1*), sense 5′-CAGAG CCGTC TCGAG CATAG-3′, antisense 5′-CAAAT CCTGG GGCAT GCTGT-3′; Superoxide dismutase 1 (*Sod1*), sense 5′-AAGAG AGGCA TGTTG GAGAC C-3′, antisense 5-CGGCC AATGA TGGAA TGCTC-3′; *Sod2*, sense 5′-TGGAG AACCC AAAGG AGAGT TG-3′, antisense 5′-CAGGC AGCAA TCTGT AAGCG-3′; Sod3, sense 5′-CTGACAGGTGCAGAGAACCTC-3′, antisense 5′-GCGTGTCGCCTATCTTCTCA-3′; β-actin, sense 5′-TTCTA CAATG AGCTG CGTGT G-3′, antisense 5′-CTTTT CACGG TTGGC CTTAG-3′. Each primer was designed using nucleotide BLAST in NCBI.

2.8 | Cell culture and western blotting

Human K562 leukemia cells were cultured in RPMI-1640 medium (Fujifilm Wako Pure Chemical Co.) supplemented with 3% fetal bovine serum, penicillin, and streptomycin for 48 h, followed by incubation with various concentration of D-Leu (0-10 μmol/L) for 24 h at 37°C in 5% CO_2. The cells were collected analyzed by western blotting. Membranes were immunostained with primary antibodies against Nrt2 (1:2000 dilution, Cusabio Technology LLC.), HO-1 (1:8000 dilution, Gene Tex Inc), SOD2 (1:4000 dilution, Cusabio LLC.), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH, 1:8000 dilution, Fujifilm Wako Pure Chemical Co.), followed by incubation with anti-rabbit IgG horseradish peroxidase (HRP)-conjugated secondary antibody (Cell Signaling Technology Inc) for 1 hour at room temperature. Immunoreactive bands were visualized using a Luminescence Reagent Set (Wako Pure Chemical Industries, Ltd) and detected with Image Quant LAS500 (GE Healthcare Japan Com., Tokyo, Japan). The intensities of the detected bands were calculated using ImageJ software.

2.9 | Detection of intercellular ROS levels

L-Leu has been reported to increase Nrf2 and SOD2 mRNA expression in fish and piglets.[27,28] D-Leu is converted into L-Leu via DAO and BCAT. K562 cells are a human leukemia-derived cell line. DAO and BCAT expression levels are very low in K562 cells. Therefore, we used K562 cells for evaluating the effects of D-Leu alone on intracellular ROS levels, as well as HO-1 and SOD2 expression. To detect intercellular ROS, K562 cells treated with D-Leu for 24 h were incubated with 5-(6)-chloromethyl-2′,7′-dichlorodihydrofluoresce in diacetate acetyl ester (CM-H2DCFDA, Setareh Biotech LLC) for 20 min at 37°C in 5% CO_2. The media were removed, and the cells were washed with phosphate-buffered saline (PBS). The fluorescence of CM-H2DCFDA was detected using a Floid™ cell imaging station.

2.10 | Statistical analyses

Animal data are expressed as the mean ± standard error (SE). Cell data are expressed as the mean ± standard deviation (SD). Differences in body weight gain among groups were analyzed by two-way analysis of variance (ANOVA). The frequency of abnormal oocytes was analyzed using the Chi-square test. Other data were analyzed by one-way ANOVA, followed by Tukey's multiple comparison tests. The level of significance was set at $P < .05$. All statistical analyses were performed using GraphPad Prism version 5.0 (GraphPad Software, Inc).

3 | RESULTS

3.1 | Effects of chronic physiological stress on body weight gain and estrus cycle

Body weight gain is described in Figure 1. Body weight gain was significantly suppressed in the RS and RS/D-Leu group compared with the control group from Day 5 to Day 14. On the other hand, there was no difference in body weight gain between RS mice and RS/D-Leu.

The estrus cycle in mice averages 4-5 days. The estrus cycle was delayed in RS mice, albeit not significantly ($P = .0971$, control vs RS) (Table 1). In RS/D-Leu mice, also, the estrus cycle also tended to be delay compared with Control group ($P = .102$, control vs RS/D-Leu). There was no difference in the estrus cycle days between RS mice and RS/D-Leu mice. Thus, D-Leu did not affect body weight gain and estrus cycles.

3.2 | AST and ALT levels in the serum

The AST and ALT levels in serum are shown in Figure 2. It has been reported that AST and ALT are increased in RS mice by oxidative stress.[29] The AST levels were approximately 1.5 times higher in the RS group than that in the control group. The increase in AST levels were suppressed in RS/D-Leu mice. On the other hand, ALT levels tended to be higher in the RS and RS/D-Leu groups than in the control group (control vs RS groups; $P = .0814$, control vs RS/D-Leu groups; $P = .0651$). There was no difference in the ALT levels between RS mice and RS/D-Leu mice.

3.3 | Ovulation and oocyte meiosis

The numbers of ovulated oocytes and the percentages of dead oocytes did not differ among the three groups (Table 1). Abnormal oocytes (spindle defects and chromosomally misaligned oocytes) were frequently observed in the RS group (Figure 3A). The percentage of abnormal oocytes was significantly higher in the RS group than that in control groups, as determined by Chi-square test (Figure 3B, $P = .0474$). The percentage of abnormal oocytes was deceased to the level observed in the control group in the RS/D-Leu group.

3.4 | Expression of antioxidation genes ovaries

Elevated ROS levels induce oocyte maturation failure.[30] HO-1, SOD1, and SOD2 are associated with intercellular and extracellular ROS removal. The expression levels of these genes in ovaries are
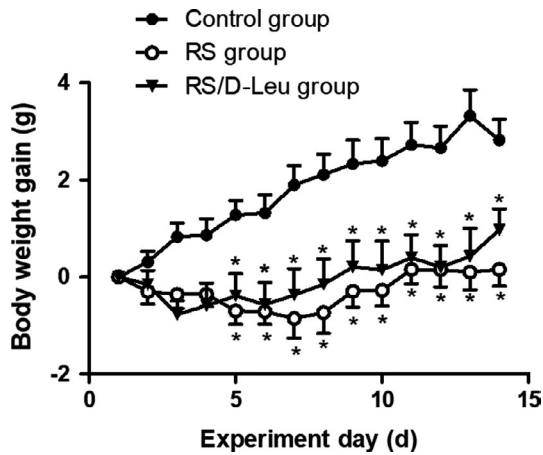

FIGURE 1 Effects of RS and D-Leu intake on body weight gain in mice. Body weights were measured before exposure to restraint stress every day during the experiment. Control group (closed circle), RS group (open circle), and RS/D-Leu group (closed triangle). Values are expressed as the mean ± SE, n = 10/group. The data were tested by two-way ANOVA. *$P < .05$ vs Control group

TABLE 1 Body and tissue weights, and estrus cycle

	Control group	RS group	RS/D-Leu group
Final boy weight (g)	29.9 ± 0.5	28.0 ± 0.6	27.9 ± 0.7
Food intake (g/d)	4.34 ± 0.23	3.98 ± 0.13	4.13 ± 0.14
Uterus weight (g)	0.148 ± 0.011	0.117 ± 0.013	0.143 ± 0.012
Estrus cycle (day)	4.5 ± 0.2	7.4 ± 1.3	7.1 ± 1.2
Ovaries weight (g)	0.019 ± 0.003	0.020 ± 0.003	0.021 ± 0.002
Oocytes			
Ovulated oocytes (number/mouse)	34 ± 5	39 ± 8	44 ± 8
Dead oocyte (%)	12.0 ± 3.7	5.7 ± 2.0	9.4 ± 3.0

Note: Values are mean ± SE; control, n = 13; RS group, n = 10; RS/D-Leu group, n = 10 (Estrus cycle; n = 5/group). The data were analyzed by one-way ANOVA, followed by Tukey's multiple comparison test. No significant differences were observed among the three groups.
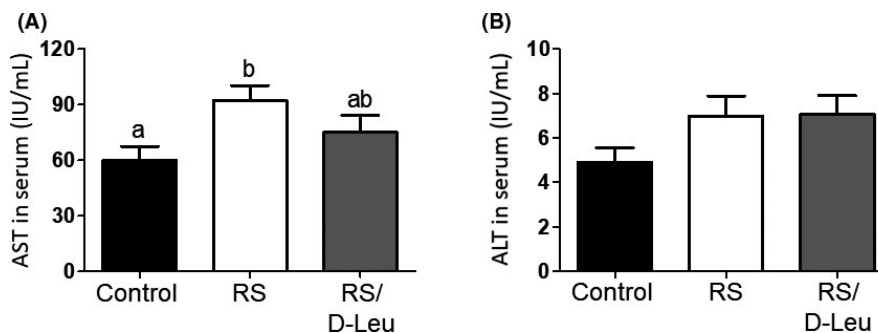

FIGURE 2 Effects of RS and D-Leu intake on AST and ALT levels in the serum. Serum was collected just after the termination of restriction stress on Day 14. Serum AST (A) and ALT (B) levels were analyzed. Black, white, and gray bars indicate data for the control, RS, and RS/D-Leu groups, respectively. Values are expressed as the mean ± SE, n = 5 or 8/group. The data were tested by one-way ANOVA. A different letter indicates a significant difference at $P < .05$
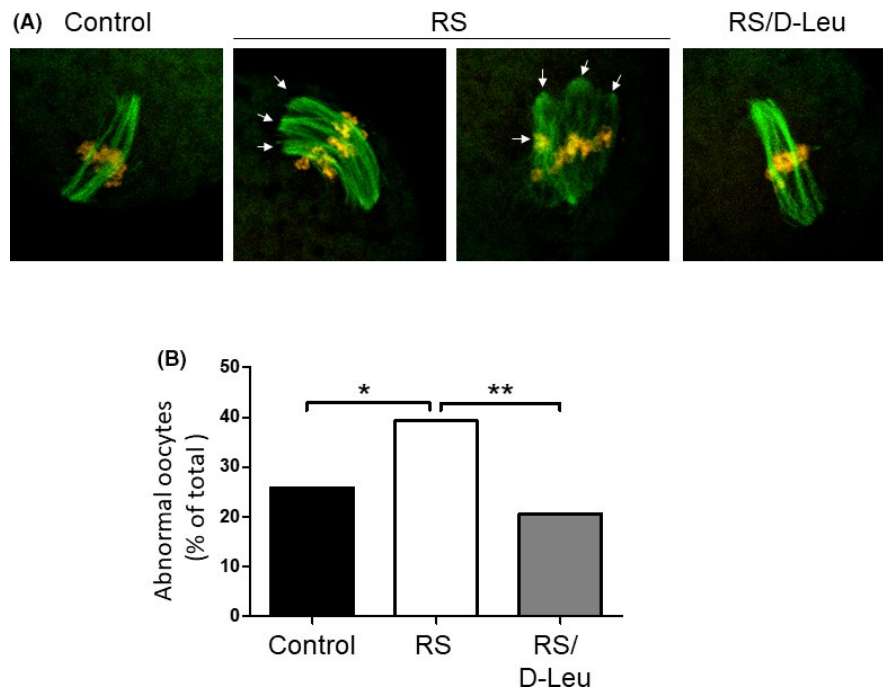
FIGURE 3 Effects of RS and D-Leu intake on oocyte meiosis. Tubulin and chromosomes were stained using an immunofluorescence stain and propidium iodide, respectively. Oocytes were classified as normal or abnormal oocytes (the latter included those oocytes with spindle defects, chromosomal misalignments, or that were immature) (A). The percentage of abnormal oocytes was calculated (number of abnormal oocytes/number of total oocytes × 100) (B). Black, white, and gray bars indicate the data for the control, RS, and RS/D-Leu groups, respectively. Values are expressed as the mean ± SE, Control group, n = 86; RS group, n = 107; RS/D-Leu group, n = 117. The oocytes were collected from 5 mice in each group. Data were analyzed using the Chi-square test (*P < .05, **P < .01) [Colour figure can be viewed at wileyonlinelibrary.com]

FIGURE 4 Ovarian mRNA expression of *Ho-1*, *Sod1*, and *Sod2*. The ovaries from mice in each group were collected, and mRNA was isolated. The mRNA levels of *Ho-1* (A), *Sod1*, and *Sod2* (B) were measured. The gene expression levels were normalized against that for *β-actin*. Black, white, and gray bars indicate the data for the control, RS, and RS/D-Leu groups, respectively. Values are expressed as the mean ± SE, n = 3/group. The data were tested by one-way ANOVA. A different letter indicates a significant difference at P < .05
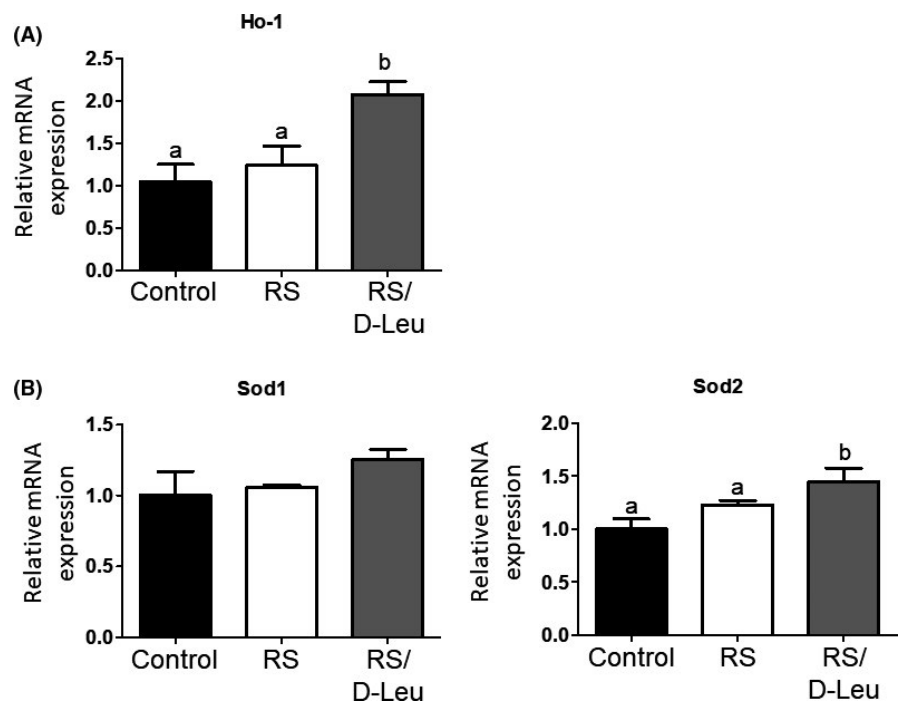
shown in Figure 4. The expression levels of these genes in the ovaries did not change in the RS group compared with the control groups. On the other hand, *Ho-1* and *Sod2* expression levels in the RS/D-Leu group were markedly elevated, by 2-fold and 1.4-fold, respectively, compared with those in the control and RS groups. *Sod1* expression level tended to be higher in the RS/D-Leu group than that in the RS group (P = .0577). Sod3 expression levels in ovary did not differ among the group (data not shown).
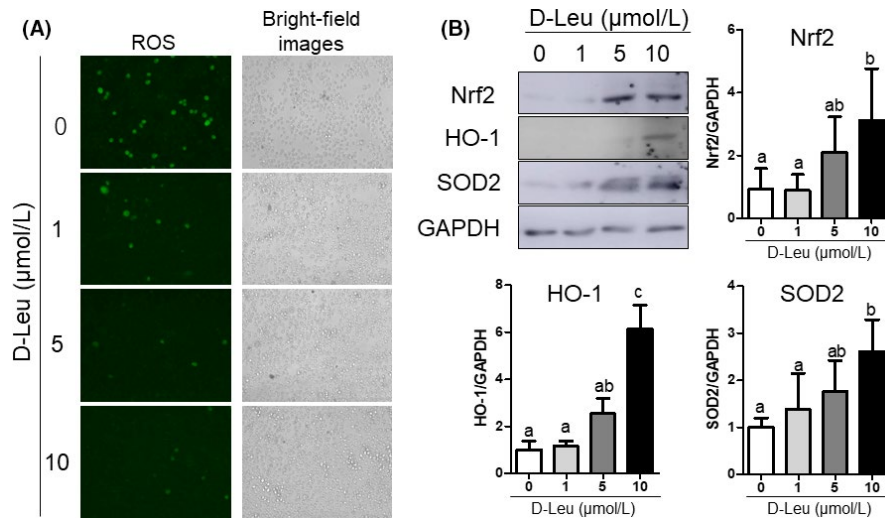
FIGURE 5 Intercellular ROS, Nrf2, HO-1, and SOD2 levels in K562 cells stimulated with D-Leu. The K562 cells were incubated with various concentrations of D-Leu for 24 h, and the levels of ROS, HO-1, and SOD2 were detected in the treated cells. The ROS levels were determined by fluorescence staining with CM-H2DCFDA (A). The green dots in the left panels indicate the ROS in K562 cells and the right panel comprises the bright-field images when ROS was detected. Western blot analyses of Nrt2, HO-1, and SOD2 protein in K562 cells (B). Values are expressed as the mean ± SD, n = 3-5. The data were tested by one-way ANOVA. A different letter indicates a significant difference at $P < .05$ [Colour figure can be viewed at wileyonlinelibrary.com]

3.5 | Intracellular ROS levels and the expression of proteins associated with antioxidation

To evaluate the ROS elimination efficacy of D-Leu, we incubated K562 cells with a medium containing D-Leu for 24 hours. The intercellular ROS in K562 was detected using CM-H2DCFDA (Figure 5A). D-Leu treatment dose-dependently reduced ROS levels in K562 cells. Amazingly, Nrt2, HO-1, and SOD2 protein expression levels were also increased dose-dependently with D-Leu treatment (Figure 5B). These results suggest that D-Leu upregulates the expression of HO-1 and SOD2 via Nrf2.

4 | DISCUSSION

Psychological stress can exert detrimental effects on oocytes in women. Anxiety- and depression-like behaviors are observed in RS animals.[31] Therefore, RS has been used to inflict psychological stress in experimental animals.[32,33] Several stress hormones (cortisol, adrenaline, and noradrenaline) are released from the adrenal glands via the activation of corticotrophin-releasing hormone and adrenocorticotropic hormone in hypothalamus and pituitary gland. Stress hormones could elevate ROS levels and activate apoptosis system in oocytes and mural granulosa cells.[5,34,35] Furthermore, it has been shown that ROS production downregulates the expression of spindle assembly checkpoint proteins.[3,5] As a result, the number of oocytes with aneuploidy and spindle defects increases, and then embryo development becomes impaired in stressed animals.[3,36] In these studies, animals are typically exposed to strong and acute psychological stress for relatively short times (24-48 hours). In our study, we chronically stressed the mice to mimic daily stress and to

verify the effects of D-Leu on abnormal oocyte meiosis caused by chronic psychological stress. Suppression of body weight gain, an increase in AST level were observed in RS mice. It has been reported that a suppression of body weight gain and an increase in AST level are observed in psychological stress model animals.[29,37] Therefore, we consider that our experiment mice were placed under psychological stress by RS treatment.

In our study, D-Leu improved the oocyte maturation failure and increase in AST levels induced by RS. Additionally, we showed that D-Leu upregulated the *Ho-1* and *Sod2* expression levels in the ovaries. These effects of D-Leu were not observed in mice fed 0.3% diet without RS treatment (Figure S1 and Table S1). Oxidative stress can be caused by the generation of excess ROS and nitrogen species. HO-1 and the SODs are key proteins that serve as the antioxidant defense system in cells. Under oxidative stress conditions, HO-1 expression is promoted by Nrf2 signaling pathway to protect cells from oxidative stress. Perkins et al reported increased levels of SOD1 and SOD2 suppress the meiotic segregation errors in Drosophila aging oocytes.[38] To the best of our knowledge, there are no studies about the association between oocyte meiosis failure and HO-1. However, HO-1 has been reported to may improve chronic kidney disease, and allergic disease.[39,40]

We showed increased Nrf2, HO-1, and SOD2 protein expression levels, and decreased intercellular ROS levels were observed in D-Leu-stimulated K562 cells. D-amino acid is metabolized to L-amino acid by DAO and BCAT. These enzymes are hardly expressed in K562 cells. Therefore, almost all D-Leu are not metabolized to L-Lue in K562 cells. This result suggests that induction of HO-1 and SOD2, and extinction of ROS is caused by D-Leu but not L-Leu. The gene expression of *Ho-1*, *Sod1*, and *Sod2* are regulated by the Keap1/Nrf2 pathway.[9] D-Leu may protect oocytes from oxidative

stress through the activation of HO-1 and SOD2 via the Keap1/Nrf2 pathway. Interestingly, the Ho-1 and Sod2 mRNA expression levels did not change in ovaries from mice fed a 0.3% D-Leu diet without RS treatment (Figure S1). It has been reported that SOD1 and SOD2 expression levels are increased in ovaries from psychologically stressed mice.[41] In our study, the Sod2 mRNA expression levels in the RS group tended to increase compared to the control group ($P = .0940$). RS stimulates ovarian ROS levels. The Keap1/Nrf2 pathway is activated by an increase in ROS levels. Subsequently, the transcription of SOD2 and HO-1 is upregulated in order to protect ovary cells from oxidative stress. We hypothesized that D-Leu may have a potentiating effect on the Keap1/Nrf2 pathway. However, the potentiating effect of D-Leu may not occur when the Keap1/Nrf2 pathway is not activated. Additional studies remain necessary to determine the molecular mechanisms through which D-Leu activates the Keap1/Nrf2 pathway.

RS treatment for 6 hours induced severe oxidative stress and hepatic injury, as evidenced by the marked elevation of serum AST and ALT levels in mice.[29] AST is contained in myocardium, liver, kidney, and erythrocyte. The AST levels in the RS/D-Leu group tended to be lower than those in the RS group, which indicated that the anti-oxidative and anti-inflammatory effects of D-Leu not only affected oocytes but also affected the other tissues.

Delayed estrus cycles were unaffected by D-Leu. Estrus is regulated by multiple sex hormones, such as gonadotropin-releasing hormone (GnRH), follicle-stimulation hormone (FSH), and luteinizing hormone (LH). The suppression of FSH and LH release from the pituitary gland has been reported in psychological stress model animals.[42,43] D-Leu has been reported to be contained in the pituitary gland [20]; however, we considered that D-Leu had no or weak effects on the release of sex hormones.

In summary, the present studies suggested that D-Leu protected oocytes from chronic physiological stress. Additionally, we demonstrated the possibility that D-Leu may have anti-oxidative and anti-inflammatory effects through the expression of HO-1 and SOD2 in ovaries. Few studies have examined D-Leu in terms of intestinal absorption, tissue metabolism, or other functions. D-amino acid oxidase produces H_2O_2.[44] Excess intake of D-amino acids could promote DNA damage and oxidative stress via the production of ROS in intracellular.[17,45] Therefore, additional studies are necessary to determine the safety and dose-dependent effects of D-Leu from a multilateral perspective.

CONFLICT OF INTEREST

Ai Tsuji, Yuka Ikeda, Mutsumi Murakami, Yasuko Kitagishi, Satoru Matsuda have no conflicts of interest.

HUMAN AND ANIMAL RIGHTS

This article does not describe any experiments involving human participants. All of the institutional and national guidelines for the care and use of laboratory animals were followed.

ORCID

Ai Tsuji https://orcid.org/0000-0003-1619-7592

REFERENCES

1. Comprehensive Survey of Living Conditions 2019. Tokyo: Ministry of Health, Labour and Welfare; 2020.
2. Special Survey on Industrial Safety and Health 2018. Tokyo: Ministry of Health, Labour and Welfare; 2019.
3. Zhou P, Lian HY, Cui W, et al. Maternal-restraint stress increases oocyte aneuploidy by impairing metaphase I spindle assembly and reducing spindle assembly checkpoint proteins in mice. *Biol Reprod*. 2012;86:1-14.
4. Liu YX, Cheng YN, Miao YL, et al. Psychological stress on female mice diminishes the developmental potential of oocytes: a study using the predatory stress model. *PLoS One*. 2012;7:e48083.
5. Li C-Y, Li Z-B, Kong Q-Q, et al. Restraint-induced corticotrophin-releasing hormone elevation triggers apoptosis of ovarian cells and impairs oocyte competence via activation of the Fas/FasL system. *Biol Reprod*. 2018;99 828-837.
6. Liang B, Wei DL, Cheng YN, et al. Restraint stress impairs oocyte developmental potential in mice: role of CRH-induced apoptosis of ovarian cells. *Biol Reprod*. 2013;89:64.
7. Klonoff-Cohen H, Chu E, Natarajan L, Sieber W. A prospective study of stress among women undergoing in vitro fertilization or gamete intrafallopian transfer. *Fertil Steril*. 2001;76:675-687.
8. Chaube SK, Prasad PV, Thakur SC, Shrivastav TG. Hydrogen peroxide modulates meiotic cell cycle and induces morphological features characteristic of apoptosis in rat oocytes cultured in vitro. *Apoptosis*. 2005;10:863-874.
9. Loboda A, Damulewicz M, Pyza E, Jozkowicz A, Dulak J. Role of Nrf2/HO-1 system in development, oxidative stress response and diseases: an evolutionarily conserved mechanism. *Cell Mol Life Sci*. 2016;73:3221-3247.
10. Abed DA, Goldstein M, Albanyan H, Jin H, Hu L. Discovery of direct inhibitors of Keap1-Nrf2 protein-protein interaction as potential therapeutic and preventive agents. *Acta Pharm Sin B*. 2015;5:285-299.
11. Friedman M. Chemistry, nutrition, and microbiology of D-amino acids. *J Agric Food Chem*. 1999;47:3457-3479.
12. Brückner H, Jaek P, Langer M, Godel H. Liquid chromatographic determination of D-amino acids in cheese and cow milk. Implication of starter cultures, amino acid racemases, and rumen microorganisms on formation, and nutritional considerations. *Amino Acids*. 1992;2:271-284.
13. Hamase K, Morikawa A, Zaitsu K. D-Amino acids in mammals and their diagnostic value. *J Chromatogr B*. 2002;781:73-91.
14. Morikawa A, Hamase K, Inoue T, Konno R, Zaitsu K. Alterations in D-amino acid levels in the brains of mice and rats after the administration of D-amino acids. *Amino Acids*. 2007;32:13-20.
15. Nagata Y, Homma H, Lee JA, Imai K. D-Aspartate stimulation of testosterone synthesis in rat Leydig cells. *FEBS Lett*. 1999;444:160-164.
16. Burrone L, Santillo A, Pinelli C, Baccari GC, Di Fiore MM. Induced synthesis of P450 aromatase and 17β-estradiol by D-aspartate in frog brain. *J Exp Biol*. 2012;215(Pt 20):3559-3565.
17. D·Aniello A, Di Fiore MM, Fisher GH, et al. Occurrence of D-aspartic acid and N-methyl-D-aspartic acid in rat neuroendocrine tissues and their role in the modulation of luteinizing hormone and growth hormone release. *FASEB J*. 2000;14:699-714.

18. Kleckner NW, Dingledine R. Requirement for glycine in activation of NMDA-receptors expressed in Xenopus oocytes. *Science.* 1988;241:835-837.

19. Tsai GE, Yang P, Chang YC, Chong MY. D-alanine added to antipsychotics for the treatment of schizophrenia. *Biol Psychiatry.* 2006;59:230-234.

20. Hamase K, Inoue T, Morikawa A, Konno R, Zaitsu K. Determination of free D-proline and D-leucine in the brains of mutant mice lacking D-amino acid oxidase activity. *Anal Biochem.* 2001;298:253-258.

21. Brückner H, Mausch M. Gas chromatographic detection of D-amino acids as common constituents of fermented foods. *Chromatographia.* 1989;28:487-492.

22. Maezawa T, Tanaka H, Nakagawa H, et al. Planarian D-amino acid oxidase is involved in ovarian development during sexual induction. *Mech Dev.* 2014;132:69-78.

23. Hartman AL, Santos P, O'Riordan KJ, Stafstrom CE, Marie HJ. Potent anti-seizure effects of D-leucine. *Neurobiol Dis.* 2015;82:46-53.

24. Luzzo KM, Wang Q, Purcell SH, et al. High fat diet induced developmental defects in the mouse: oocyte meiotic aneuploidy and fetal growth retardation/brain defects. *PLoS One.* 2012;7:e49217.

25. Hou YJ, Zhu CC, Duan X, Liu HL, Wang Q, Sun SC. Both diet and gene mutation induced obesity affect oocyte quality in mice. *Sci Rep.* 2016;6:18858.

26. Tsuji A, Nakamura T, Shibata K. Biotin-deficient diet induces chromosome misalignment and spindle defects in mouse oocytes. *Biosci Biotechnol Biochem.* 2015;79:292-299.

27. Deng Y-P, Jiang W-D, Liu Y, et al. Differential growth performance, intestinal antioxidant status and relative expression of Nrf2 and its target genes in young grass carp (*Ctenopharyngodon idella*) fed with graded levels of leucine. *Aquaculture.* 2014;434:66-73.

28. Chen X, Xiang L, Jia G, Liu G, Zhao H, Huang Z. Effects of dietary leucine on antioxidant activity and expression of antioxidant and mitochondrial-related genes in longissimus dorsi muscle and liver of piglets. *Anim Sci J.* 2019;90:990-998.

29. Kim HG, Lee JS, Lee JS, Han JM, Son CG. Hepatoprotective and antioxidant effects of Myelophil on restraint stress-induced liver injury in BALB/c mice. *J Ethnopharmacol.* 2012;142:113-120.

30. Zhang X, Wu XQ, Lu S, Guo YL, Ma X. Deficit of mitochondria-derived ATP during oxidative stress impairs mouse MII oocyte spindles. *Cell Res.* 2006;16:841-850.

31. Chiba S, Numakawa T, Ninomiya M, Richards MC, Wakabayashi C, Kunugi H. Chronic restraint stress causes anxiety- and depression-like behaviors, downregulates glucocorticoid receptor expression, and attenuates glutamate release induced by brain-derived neurotrophic factor in the prefrontal cortex. *Prog Neuropsychopharmacol Biol Psychiatry.* 2012;39:112-119.

32. Govindaraj S, Shanmuganathan A, Rajan R. Maternal psychological stress-induced developmental disability, neonatal mortality and stillbirth in the offspring of Wistar albino rats. *PLoS One.* 2017;12:e0171089.

33. Matsuhisa F, Kitamura N, Satoh E. Effects of acute and chronic psychological stress on platelet aggregation in mice. *Stress.* 2014;17:186-192.

34. Flaherty RL, Owen M, Fagan-Murphy A, et al. Glucocorticoids induce production of reactive oxygen species/reactive nitrogen species and DNA damage through an iNOS mediated pathway in breast cancer. *Breast Cancer Res.* 2017;19:35.

35. Prasad S, Tiwari M, Pandey AN, Shrivastav TG, Chaube SK. Impact of stress on oocyte quality and reproductive outcome. *J Biomed Sci.* 2016;23:36.

36. Yuan H-J, Han X, He N, et al. Glucocorticoids impair oocyte developmental potential by triggering apoptosis of ovarian cells via activating the Fas system. *Sci Rep.* 2016;6:24036.

37. Cooper B, Fuchs E, Flügge G. Expression of the axonal membrane glycoprotein M6a is regulated by chronic stress. *PLoS One.* 2009;4:e3659.

38. Perkins AT, Greig MM, Sontakke AA, Peloquin AS, McPeek MA, Bickel SE. Increased levels of superoxide dismutase suppress meiotic segregation errors in aging oocytes. *Chromosoma.* 2019;128:215-222.

39. Lever JM, Boddu R, George JF, Agarwal A. Heme oxygenase-1 in kidney health and disease. *Antioxid Redox Signal.* 2016;25:165-183.

40. Chen L, Zhong JL. MicroRNA and heme oxygenase-1 in allergic disease. *Int Immunopharmacol.* 2020;80:106132.

41. Kala M, Nivsarkar M. Role of cortisol and superoxide dismutase in psychological stress induced anovulation. *Gen Comp Endocrinol.* 2016;225:117-124.

42. Wagenmaker ER, Moenter SM. Exposure to acute psychosocial stress disrupts the luteinizing hormone surge independent of estrous cycle alterations in female mice. *Endocrinology.* 2017;158:2593-2602.

43. Wang S-F, Chen X-H, He B, et al. Acute restraint stress triggers progesterone withdrawal and endometrial breakdown and shedding through corticosterone stimulation in mouse menstrual-like model. *Reproduction.* 2019;157:149-161.

44. Pollegioni L, Diederichs K, Molla G, et al. Yeast D-amino acid oxidase: structural basis of its catalytic properties. *J Mol Biol.* 2002;324:535-546.

45. Matlashov ME, Belousov VV, Enikolopov G. How much H_2O_2 is produced by recombinant D-amino acid oxidase in mammalian cells? *Antioxid Redox Signal.* 2014;20:1039-1044.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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