博 士 論 文

IL-1 supports strenuous masseter muscle activity via induction of IL-6

（**IL-1** は **IL-6** の誘導を介して激しい咬筋活動を支える）

千 葉　航

平成二十六年度提出

東 北 大 学

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ABSTRACT

Objective: Exercise has been suggested, but not confirmed, to stimulate IL-1 production within skeletal muscles, while muscle-derived IL-6 has been suggested to support exercise by regulating glucose homeostasis. Tsuchiya previously found in mice that strenuous activity in masseter muscles (MMs) stimulates IL-6 production by the MMs themselves. Here, I examined the relationship between IL-1 and IL-6 in MM activity.

Design: When restrained within a narrow cylinder blocked at the front end with a thin plastic strip (a condition termed "R+"), a mouse gnaws away the strip to escape (termed "G+"). The absolute weight of plastic gnawed away serves as an index of MM activity. I analyzed MM activity in this R+G+ model using mice lacking both IL-1α and IL-1β (IL-1-KO mice).

Results: R+G+ rapidly fatigued IL-1-KO-MMs. The blood glucose level was not causally related to this fatigue. Glycogen stores in IL-1-KO-MMs were strongly depleted by R+G+. During R+G+, glucose uptake by IL-1-KO-MMs was less than by the MMs of wild-type mice (WT). In WT-MMs, R+G+ increased IL-1β mRNA (but not IL-1α mRNA) and tended to increase IL-1β protein. Interestingly, IL-1α was constitutively present in the perimysium of WT-MMs. After R+G+, the IL-6 protein level was lower in IL-1-KO-MMs than in WT-MMs. Intravenous injection of IL-1β into WT mice increased IL-6 in MMs.

Conclusions: The IL-1β produced in MMs by strenuous activity, although small in amount, helps to support that activity via the production of IL-6. We discuss the roles of IL-1α in MMs and of IL-1β in temporomandibular disorders.

Keywords: masseter muscle, IL-1α, IL-1β, IL-6, exercise, temporomandibular diseases

1. Introduction

Strenuous, unaccustomed, or prolonged physical exercise results in fatigue, stiffness, and pain in the working skeletal muscles, and the dysfunction of masticatory muscles seen in

temporomandibular disorders (TMD) is believed to result from strenuous, improper, or abnormal occlusion, including bruxism and/or prolonged clenching.[1] It is supposed that anti-fatigue mechanisms may operate in opposition to such muscle fatigue, serving to prevent exhaustion and/or to protect the working muscles against their own destruction. However, the molecular mechanisms behind such fatigue, as well as behind anti-fatigue, in skeletal muscles remain to be clarified.

Prolonged exercise must be metabolically supported by an adequate supply of energy or glucose to the working muscles. IL-6 (recognized as a typical “myokine”) has been suggested to play an important role as an “energy sensor” in exercise-related metabolic changes.[2-4] Tsuchiya reported that in mice, strenuous activity of masseter muscles (MMs) led to the production and release of IL-6, and he suggested that the MM-derived IL-6 helped to support the functional activity of the MMs by maintaining glucose homeostasis.[5]

Prolonged or strenuous exercise induces damage or inflammation in skeletal muscles,[6] and exercise has been shown to increase the plasma levels of various proinflammatory cytokines.[2-4] IL-1 is a representative proinflammatory cytokine, and it exhibits a variety of metabolic effects, including effects on glucose metabolism,[7] lipid metabolism,[8] and muscle protein breakdown.[9] It has also been shown that synovial fluid sampled from the temporomandibular joints of TMD patients contains raised levels of various proinflammatory cytokines, including IL-1β, and these have been suggested to be involved in the pathology of TMD.[10,11] However, the plasma level of IL-1 has been shown to be increased very little, if at all, by exercise.[2,4] Thus, it is not yet clear (a) whether exercise does indeed stimulate skeletal muscle fibers, or some related cell-types, to produce IL-1, and thus (b) whether IL-1 really is involved in muscle activity, damage (or inflammation), and/or fatigue. Here, to address these questions, I carried out experiments involving MM activity in mice deficient in both IL-1α and IL-1β (IL-1-KO mice), and compared these mice with their control wild-type (WT) mice.

2. Materials and methods

2.1. Animals and materials

WT control BALB/c mice were obtained from CLEA-Japan (Tokyo, Japan). Homozygous BALB/c IL-1-KO mice (deficient in both IL-1α and IL-1β) were established from original IL-1α-KO and IL-1β-KO mice by back-crossing to BALB/c mice,[12] and the required IL-1-KO mice were raised in my laboratory. No physical or health abnormalities were apparent in any of these mice.[13] The mice used for the present experiments were males 7 to 8 weeks old. All mice were allowed standard food pellets (LabMR Stock; Nihon Nosan Inc, Yokohama, Japan) and tap water *ad libitum* in an air-conditioned room at 23 ± 1°C and 55 ± 5% relative humidity with a standard cycle of 12 h light and 12 h dark (lights on at 07:00 a.m.). Recombinant mouse IL-1β was purchased from BioLegend (San Diego, CA, USA).

2.2. Evaluation of MM activity

When a mouse is restrained (R+) within a cylinder, the mouse gnaws (G+) away the plastic strip blocking one end in an attempt to escape (a condition called "R+G+") (Fig. 1A).[14,15] Hence, the strip's weight-reduction can be used as an index of MM activity. In the present study, the strip's weight-reduction in the hour it was in place is shown as the MM activity for that period, with a decline in that parameter during prolonged R+G+ providing an index of fatigue.[15] The following two groups were used as controls: "R+G–" (the tail was fastened by tape to the cylinder at such a position that the mouse was unable to reach the strip) and "R–G–" (the mice were kept in their home cages without food or water for the duration of the experimental period). The R+G+ experiments were performed in the evening, up to midnight.

2.3. Blood glucose

After the tail vein had been pierced, the blood extruded (about 5 μl) was directly applied to a

glucometer (Accu-Chek Advantage, Roche Diagnostics K.K., Tokyo, Japan).

2.4. Glycogen in MMs

Following the method of van Handel,[16] glycogen was quantified as described previously.[5] Briefly, glycogen was separated from MMs and hydrolyzed to glucose (using H_2SO_4 at 100 °C for 2 h). After neutralization of the reaction mixture with NaOH, glucose was measured using a glucose-determining kit (Glucose-CII-test-Wako; Wako Pure Chemical Industries, Osaka, Japan).

2.5. Glucose uptake by MMs

Since 2-deoxy-D-glucose (2DG) is taken up via glucose transporters, but not metabolized by the glycolytic pathway, 2DG has been used as a marker for evaluating the amount of glucose uptake. Here, ^{14}C-2DG (5 μCi/ml) was intraperitoneally injected (0.1 ml/10 g body weight), and 30 min later MMs were removed and subjected to analysis of their content of ^{14}C-2DG using the method reported by Ferré *et al.*[17]

2.6. Quantitative polymerase chain reaction analysis

Total RNA was extracted from masseter muscle tissue using Trizol reagent (Invitrogen, CA, USA). cDNA was reverse transcribed using a Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics, Minneapolis, MN, USA) with oligo-dT primers. The cDNA was subjected to qPCR amplification in a BioRad CFX96 qPCR system (Bio-Rad, Hercules, CA, USA). The relative expression of target genes was determined by the $2^{-\Delta\Delta CT}$ method.[18] Primers and their sequences were as follows. IL-1α: F5'-GAC CGA CCT TTT CTT CTG-3' and R5'-AGG TGC ACC CGA CTT TGT TCT T-3'; IL-1β: F5'-GCA CCT TCT TTT CCT TCA TCT TTG-3' and R5'-GTT GTT CAT CTC GGA GCC TGT-3'; IL-6: F5'-AAC CAC

GGC CTT CCC TAC TT-3' and R5'-CCA TTG CAC AAC TCT TTT CTC ATT-3'; EF1α1 (internal reference control): F5'-ATT CCG GCA AGT CCA CCA CAA-3' and R5'-CAT CTC AGC AGC CTC CTT CTC AAA C -3'.

2.7. Measurement of IL-1 and IL-6 proteins

Tissue extracts, prepared as described previously,[19] were subjected to measurement of cytokines. ELISA kits were used for measuring IL-1α (R&D Systems, Inc, Minneapolis, MN, USA), and IL-1β and IL-6 (Thermo Fisher Scientific Inc, Rockford, IL, USA).

2.8. Immunostaining for IL-1α

Masseter muscles were fixed in 4% paraformaldehyde overnight at 4°C. Samples were then dehydrated and embedded in paraffin. Serial sections were used for immunostaining as described previously.[20] After deparaffinization, antigen retrieval was performed using proteinase K solution for 5 minutes at 37℃, followed by incubation with the primary antibody for IL-1α (1: 40 dilution; Abcam, Cambridge, MA, USA, #ab7632) at 4℃ overnight. Next, the sections were incubated with biotinylated goat anti-rabbit antibody (1: 1,000 dilution; Vector Laboratories, Tokyo, Japan), and then visualized using the Vectastain ABC kit (Vector Laboratories).

2.9. Data analysis

Experimental values are each given as the mean ± standard error (S.E.). The statistical significance of differences was assessed either by a Student's unpaired *t*-test (for comparing two means) or by a Bonferroni multiple-comparison test after two-way ANOVA (for comparing three or more means). For this, SPSS v.14 software (Chicago, IL, USA) was used. *P* values less than 0.05 were considered to indicate significance.

3. Results

3.1. IL-1-KO-MMs are easily fatigued by R+G+ activity

First, I compared MM activity in the R+G+ condition between IL-1-KO and WT mice. The MM activity of WT mice declined steadily with time (Fig. 1B). In contrast, the MM activity of IL-1-KO mice fell rapidly between 1 and 2 h after the start of R+G+, and then remained at a very low level. These results suggest that IL-1-KO-MMs are easily fatigued by R+G+ activity. It should be noted, however, that there was no significant difference in MM activity between WT and IL-1-KO mice during the initial 1 h of R+G+.

3.2. Blood glucose level is mot causally involved in the fatigability of IL-1-KO-MMs

To explore the mechanism behind the fatigability of IL-1-KO-MMs, I first compared the blood glucose level between WT and IL-1-KO mice during R+G+. Despite the marked difference in MM activity (Fig. 1B), there was no significant difference in blood glucose levels between the two groups of mice during 3 h of R+G+ (Fig. 2A, lower panel). It should also be noted that the changes with time in the blood glucose level in these mice were similar between R+G– (Fig. 2A, upper panel) and R+G+. These results suggest that (i) the blood glucose level may not be causally involved in the fatigability of IL-1-KO-MMs, and (ii) the blood glucose change seen during R+G+ is due to R+ (i.e., to restraint itself).

3.3. Glycogen stores in IL-1-KO-MMs are strongly depleted by R+G+

As described above, MM activity during the initial 1 h of R+G+ was similar between WT and IL-1-KO mice. So, in the following experiments, in which I compared fatigue-related factors between these mice, 1 h of R+G+ was imposed. I first compared glycogen content in MMs between WT and IL-1-KO mice. As shown in Fig. 2B, 1 h R+G+ tended to reduce glycogen in WT-MMs, although not significantly. However, a much greater reduction was evident in

IL-1-KO-MMs, suggesting that glycogen stores in IL-1-KO-MMs are easily and strongly depleted by R+G+, and/or that glycogen consumption during R+G+ is greater in IL-1-KO-MMs.

3.4. Glucose uptake during 1 h R+G+ is less in IL-1-KO-MMs than in WT-MMs

Next, the uptake of glucose by MMs during 1 h R+G+ was compared between WT and IL-1-KO mice. As shown in Fig. 2C, the uptake of 2DG during 1 h R+G+ was less in IL-1-KO-MMs than in WT-MMs, although there was no such difference during R-G- or R+G-.

3.5. R+G+ stimulates the production of IL-1β (but not of IL-1α), and IL-1α is constitutively present in MMs

As described above, IL-1 deficiency increases the fatigability of MMs. So, I examined whether R+G+ stimulates IL-1 production in MMs. As shown in Fig. 3A-left, 0.5 h R+G+ did not stimulate the production of IL-1α mRNA in WT-MMs. Moreover, no significant increase in IL-1α protein was detected after 1.5 h R+G+ in either WT- or IL-1-KO-MMs (Fig. 3B-upper). In contrast, IL-1β mRNA increased markedly in WT-MMs in response to 0.5 h R+G+ (Fig. 3A-right). However, no significant increase in IL-1β protein could be detected in WT-MMs after 1.5 h R+G+ (Fig. 3B-lower), although it tended to be increased. In a repeat of that experiment (except that this time, 2 h R+G+ was imposed), similar results were obtained (data not shown). In Fig. 3B, it should be noted that (i) IL-1β protein levels were similar between WT-MMs and IL-1-KO-MMs, and (ii) the ordinate values for IL-1β are almost 20-times greater than those for IL-1α. These results indicate that the MMs of both WT and IL-KO mice contain a large amount of unknown protein(s) that react with the anti-IL-1β

antibody supplied in the present assay kit. Thus, an increase in IL-1β protein, if quite small, might be masked by such a non-specific reaction. Collectively, the results described above suggest that R+G+ stimulates the production of IL-1β (but not of IL-1α) in WT-MMs, even though an increase in its protein level could not be detected.

Interestingly, the levels of IL-1α protein, even under the R-G- and R+G- conditions, were much higher in WT-MMs than in IL-1-KO-MMs (Fig. 3B-upper), indicating that IL-1α protein is constitutively present in WT-MMs. I found strong IL-1α-staining in the perimysium of WT-MM (Fig. 3C-b), but not in that of IL-1-KO-MM (Fig. 3C-c), and there was no clear staining among muscle fibers in either WT- or IL-1-KO-MMs, indicating that IL-1α is present in the perimysium of WT-MMs.

3.6. IL-1 is involved in R+G+-induced IL-6 production in MMs

Tsuchiya previously reported that 0.5 h R+G+ stimulated the production of IL-6 in MMs, leading to a slight but significant elevation of the serum level of IL-6, and he presented evidence that such IL-6 could support strenuous MM activity by maintaining glucose homeostasis.[5] Since IL-1 reportedly stimulates the production of IL-6,[21,22] I examined whether IL-6 is produced in WT- and/or IL-1-KO-MMs in response to R+G+. As shown in Figs. 4A and 4B, in WT-MMs, 1 h R+G+ increased both IL-6 mRNA and IL-6 protein. However, in IL-1-KO-MMs, their increases in the R+G+ group were small and were not significantly different from those in the R+G- group. It should be noted that I detected large amounts of IL-6 protein even in the R-G- condition, although at present I do not know whether this is truly due to IL-6 protein itself. To my knowledge, no one has ever determined the IL-6 protein level in any skeletal muscle. Collectively, although they are not conclusive the above results suggest that IL-1 may be causally involved in the R+G+-induced IL-6 production in WT-MMs.

3.7. Intravenous IL-1β injection stimulates IL-6 production in MMs

To explore the possible involvement of IL-1 in R+G+-induced IL-6 production in MMs, I examined whether injection of IL-1β might induce IL-6 production in MMs. As shown in Fig. 5A, an intravenous injection of recombinant mouse IL-1β markedly increased IL-6 protein in WT-MMs. This was evident even at 1 h after the injection, and the level was much greater at the 2-h time-point. It should be noted that for IL-6, the unit is "μg" in Fig. 5A, but "ng" in Fig. 4B. These results indicate that IL-1β is a very powerful inducer of IL-6 in MMs. As shown in Fig. 5B, intravenous IL-1β increased IL-6 in quadriceps femoris muscles, too, indicating that the response is essentially the same between the masseter and quadriceps femoris muscles.

4. Discussion

As described in Introduction, the exercise-induced increase in the plasma level of IL-1α and/or IL-1β is very small, if it exists at all. Here, I found that R+G+ stimulates IL-1β production within WT-MMs. In the present and previous[5] studies, I demonstrated that R+G+ stimulates IL-6 production in MMs. In the present study, I found that after 1 h R+G+, the IL-6 protein level was lower in IL-1-KO-MMs than in WT-MMs. IL-1 reportedly stimulates IL-6 production in cultured skeletal muscle cells.[23] Here, I found that intravenously injected IL-1β markedly increased IL-6 in MMs, and in quadriceps femoris muscles. These findings suggest that the R+G+-induced IL-6 production seen here in MMs may be mediated by R+G+-induced IL-1β.

Recent studies have shown that IL-1α is constitutively expressed in some cell types and is released upon cell damage, and that it then stimulates other cell types (in a paracrine manner) to produce IL-1β.[24,25] Interestingly, I detected a significant amount of IL-1α in WT-MMs under both R-G- and R+G- conditions, as well as under the R+G+ condition (Fig. 3B-upper),

and I observed IL-1α immunostaining in the perimysium, but not among muscle fibers (Fig. 3C). The perimysium is rich in blood vessels, neurons, and mast cells.[26] Thus, it would be of interest to examine in future studies whether R+G+ (or exercise) can induce a release of IL-1α from the perimysium of MMs. A translocation of IL-1α between compartments within MM might exert important effects on the skeletal muscle fibers, but such a translocation would not be detectable as an increased *content* of this protein in the whole MM. My findings -- viz. IL-1α in the perimysium in the R-G- condition (Fig. 3C) and an absence of a significant increase in its MM content in the R+G+ condition (Fig. 3B) -- are consistent with this idea.

IL-1-KO-MMs were easily, and rapidly fatigued by R+G+ activity, and my results indicate that the level of blood glucose by itself was not causally involved in this fatigability. Exogenously injected IL-1 (either IL-1α or IL-1β) induces transient hypoglycemia.[27-29] Thus, if IL-1 were to be released into the blood stream from the working skeletal muscles during exercise, the blood glucose might be lowered, as has been observed in mice injected with lipopolysaccharide.[7] However, I found that R+G+ did not reduce blood glucose (Fig. 2A). This suggests that R+G+-induced IL-1 production is local, or possibly limited to MMs alone, and in either case might play a local role(s) within the MMs themselves, perhaps supporting their functional activity. IL-6 enhances (a) lipolysis in adipose tissues, (b) glucose output from the liver, and (c) the utilization of fatty acids and glucose by skeletal muscles.[30] Thus, my findings support the view that an exercise-induced local release of IL-6 from skeletal muscles may occur, and may support the muscle's endurance against exercise-induced fatigue or exhaustion by helping to maintain their glucose homeostasis.

The etiology of TMD is believed to be multi-factorial, and stiffness and pain are the main symptoms of TMD.[31-33] Such symptoms are believed to result, at least in part, from strenuous, improper, or abnormal occlusion, including bruxism and/or prolonged clenching. Interestingly,

IL-1β and IL-6 are the most prevalent cytokines detected in synovial fluid sampled from the temporomandibular joints of TMD patients.[34,35] However, it is unclear how they are produced. Although further studies are required, the present findings suggest that (i) the raised levels of IL-1β and IL-6 reported in the synovial fluid of TMD joints might be a reflection of fatigue of MMs, and (ii) chronically increased IL-1β and IL-6 might become causal to the production of chronic inflammation in TMD.

In conclusion, the present findings, together with previous findings, suggest that IL-1β produced in MMs by their strenuous activity (possibly via release of IL-1α from MMs) may help to support continuing MM activity via the production of IL-6. The findings may also provide an insight into the importance of the elevations of IL-1β and IL-6 observed in the synovial fluid of the temporomandibular joints in TMD patients.

Funding

This study was supported by the following grants from the Japan Society for the Promotion of Science: 23390439 (to M. Watanabe), and 24390429 and 25670813 (both to M. Tsuchiya).

Competing interest

The author declares that there are no conflicts of interest.

Ethical approval

The Ethical Review Board of Tohoku University Graduate School of Dentistry approved the experimental procedures (2011DNA-36).

Acknowledgements

The author is grateful to Dr. Robert Timms (Birmingham, UK) for English language editing.

謝　辞

稿を終えるにあたり，終始御懇篤な御指導と御校閲を賜りました本学口腔機能形態学講座 加齢歯科学分野 服部佳功教授に深甚なる謝意を表します．また，本論文の御校閲を賜りました口腔分子制御学分野 菅原俊二教授に感謝の意を表します．本研究を遂行するにあたり終始御教示，御指導を賜りました東北福祉大学の渡邉誠教授，土谷昌広先生，ならびに遠藤康男先生，黒石智誠先生，田中志典先生をはじめとする口腔分子制御学分野の教室員の皆様に深く感謝致します．

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Figure legends

Fig. 1 – (A) The R+G+ model used in the present study for evaluating MM activity. A mouse is restrained (R+) within a cylinder (internal diameter 2.5 cm), the front end of which is blocked with a thin plastic strip (width 1.5 cm, thickness 1.0 mm, length 10 cm). A mouse that can reach the strip gnaws it away (G+) to escape. It is possible to make a given mouse either able to or unable to gnaw the strip by taping its tail to the cylinder at such a position that the mouse can or cannot reach the strip. We termed the former condition R+G+ and the latter R+G–, while R–G– means that mice are put in a home cage without restraint. The reduction in the weight of such a strip reflects the MM activity for the 1-h period the strip is in place, and the decline with time that occurs in that parameter gives a fatigue index for the MM. **(B) IL-1KO-MMs fatigue easily in the R+G+ condition.** WT and IL-1-KO mice (each 12 mice) were subjected to R+G+, and MM activity was measured as described in Methods. Experimental values are given as the mean ± S.E. $*P < 0.05$ *vs.* WT at same time-points.

Fig. 2 – Effects of R+G+ on blood glucose, glycogen stores in MMs, and uptake of glucose by MMs. (A) There was no difference in blood glucose levels between WT and IL-1-KO mice during R+G+. WT and IL-1-KO mice (n = 4 or 5) were subjected to R+G+ (lower panel) or R+G– (upper panel), and blood was taken from the tail vein for measuring blood glucose, as described in Methods. Experimental values are given as the mean ± standard deviation. **(B) Glycogen stores in IL-1-KO-MMs were strongly depleted by R+G+.** WT and IL-1-KO mice (n = 12-16) were subjected to 1 h R+G+ or R+G–, and the glycogen in their MMs was measured. MMs from R–G– (i.e., resting, unrestrained) mice were also analyzed as a control. **(C) Glucose uptake by IL-1-KO-MMs was less than by WT-MMs during 1 h R+G+.** WT and IL-1-KO mice (n = 10-12) were intraperitoneally injected with ^{14}C-2DG, and 30 min later MMs were removed. Experimental values in B and C are given as the mean ± S.E. **P* < 0.05, ***P* < 0.01.

Fig. 3 – IL-1 levels and production of IL-1 by MMs in response to R+G+. (A) R+G+ increased the mRNA level of IL-1β (but not that of IL-1α) in WT-MMs. WT mice were subjected to 0.5 h R+G– or R+G+, and IL-1α and IL-1β mRNAs in MMs were analyzed (n = 6-8). **(B) IL-1α is constitutively present in WT-MMs, and R+G+ tended to increase the level of IL-1β protein (but not of IL-1α protein) in WT-MMs.** WT and IL-1-KO mice were subjected to 1.5 h R+G– or R+G+, and the IL-1α and IL-1β proteins in their MMs were analyzed. (Please note: measurements were made in IL-1-KO-MMs to evaluate proteins that react non-specifically with the antibodies against IL-1α and IL-1β provided in the assay kits for IL-1α and IL-1β.) In these experiments, MMs from resting, unrestrained mice (R–G–) were also analyzed, as a control. Experimental values in A and B are given as the mean ± S.E. (n = 10-15). **P* < 0.05, ****P* < 0.001. **(C) IL-1α is present in the perimysium of WT-MMs.**

Control (R–G–) WT-MMs and IL-1-KO-MMs were subjected to hematoxylin and eosin (HE) staining as well as immunostaining for IL-1α.

Fig. 4 – R+G+ stimulates IL-6 production in WT-MMs, but not in IL-1-KO-MMs. WT and IL-1-KO mice were subjected to 1 h R+G+ or R+G–, and then the IL-6 mRNA (A) and IL-6 protein (B) in their MMs were measured. MMs from R–G– (i.e., resting, unrestrained) mice were also analyzed. Experimental values in A and B are given as the mean ± S.E. (n = 6 for A and n = 4-8 for B). **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

Fig. 5 – IL-1β injection induces IL-6 production in WT-MMs. Recombinant mouse IL-1β was intravenously injected into WT mice at a dose of 50 μg/kg. Then, 1 or 2 h later the mice were killed by decapitation, and MMs were subjected to measurement of IL-6 protein. In this experiment, quadriceps femoris muscles were also analyzed, confirming similar effects of IL-1β on skeletal muscles other than MMs. Experimental values are given as the mean ± S.E. (n = 5-6). **P* < 0.05, ***P* < 0.01.

Fig. 1

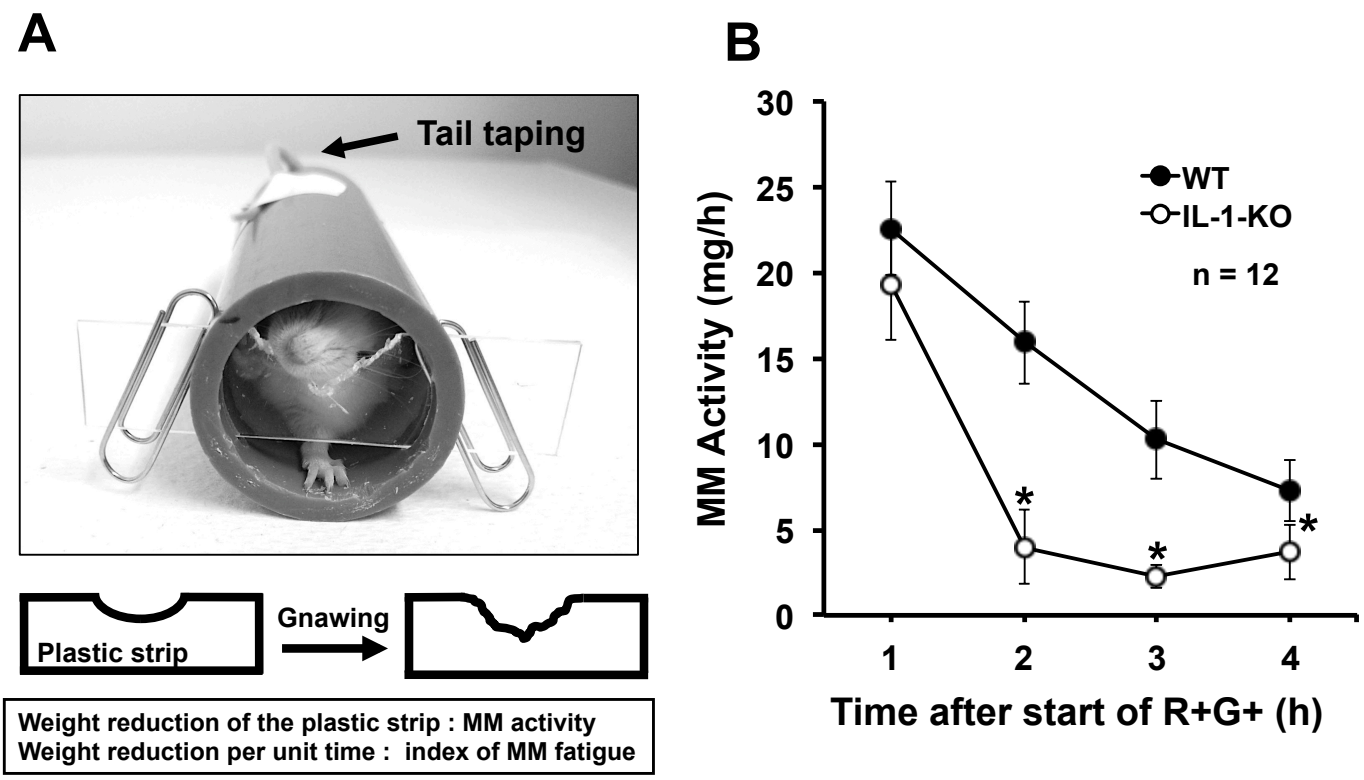

A
Tail taping
Plastic strip
Gnawing
Weight reduction of the plastic strip : MM activity
Weight reduction per unit time : index of MM fatigue
B
MM Activity (mg/h)
30
25
20
15
10
5
0
WT
IL-1-KO
n = 12
*
*
*
1
2
3
4
Time after start of R+G+ (h)

Fig. 2

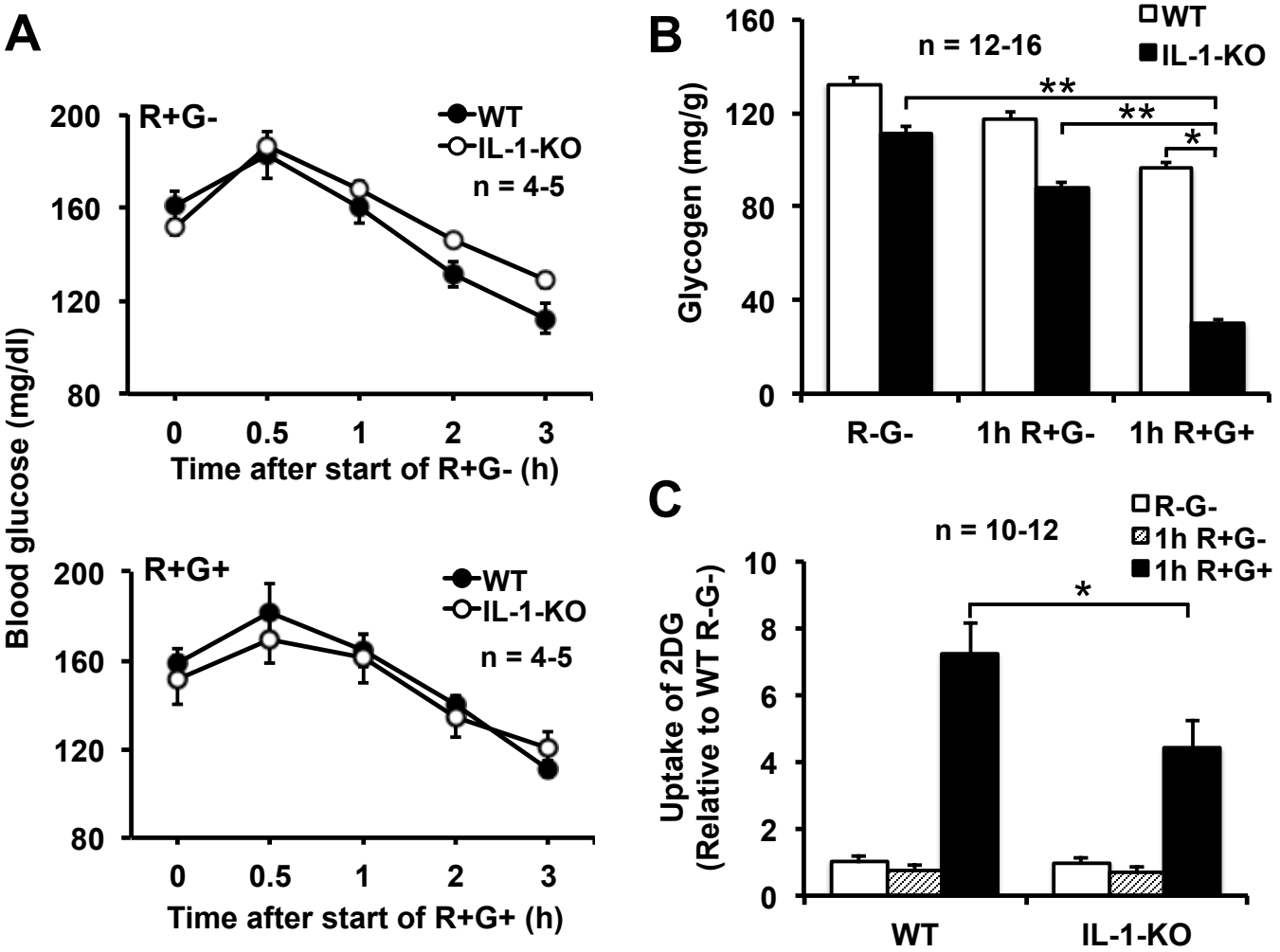

A
R+G-
WT
IL-1-KO
n = 4-5
Blood glucose (mg/dl)
200
160
120
80
0
0.5
1
2
3
Time after start of R+G- (h)
R+G+
WT
IL-1-KO
n = 4-5
Time after start of R+G+ (h)
B
n = 12-16
WT
IL-1-KO
Glycogen (mg/g)
160
120
80
40
0
**
**
*
R-G-
1h R+G-
1h R+G+
C
n = 10-12
R-G-
1h R+G-
1h R+G+
Uptake of 2DG
(Relative to WT R-G-)
10
8
6
4
2
0
*
WT
IL-1-KO

Fig. 3

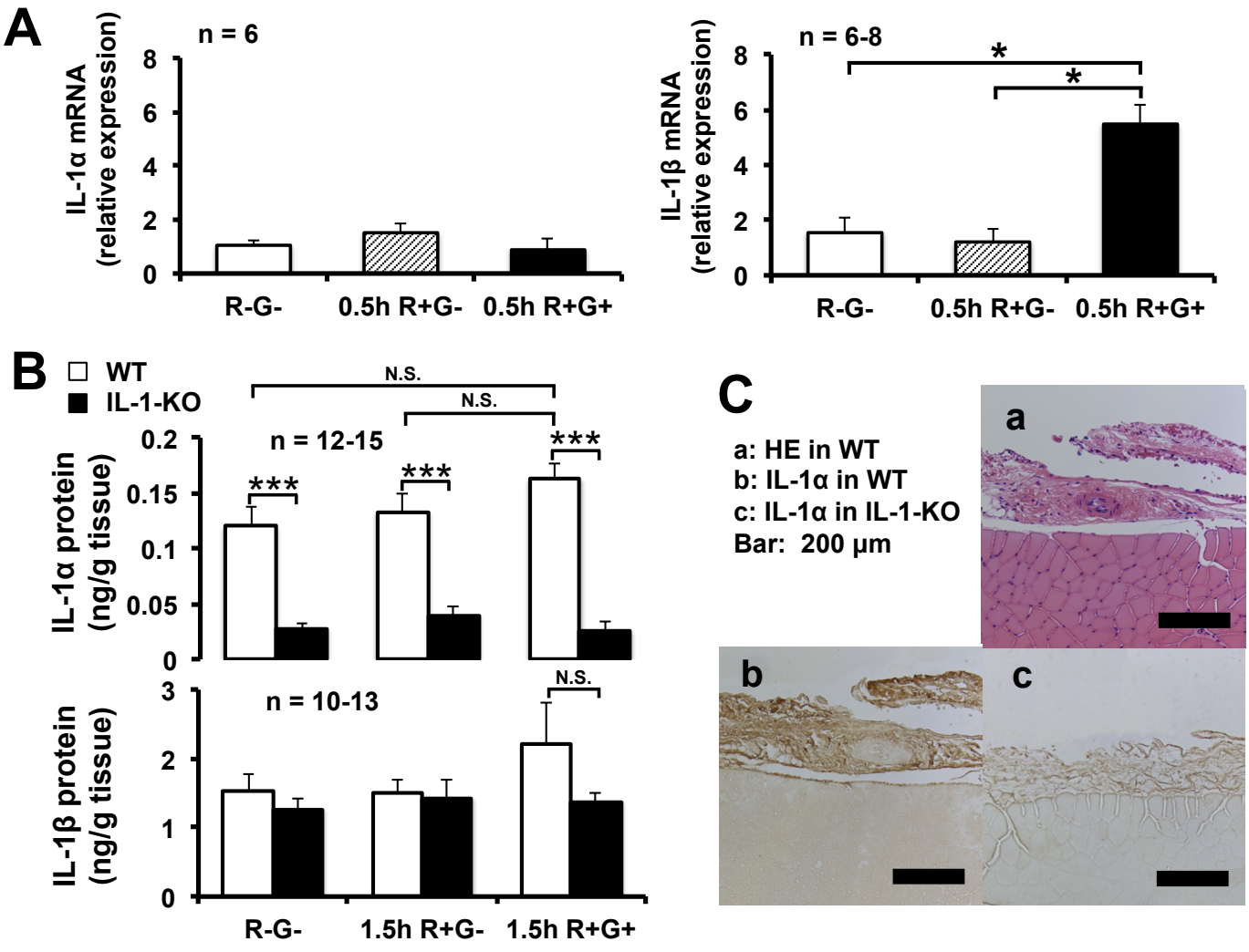

A
n = 6
IL-1α mRNA (relative expression)
8
6
4
2
0
R-G-
0.5h R+G-
0.5h R+G+
n = 6-8
IL-1β mRNA (relative expression)
*
*
B
WT
IL-1-KO
N.S.
N.S.
n = 12-15
***
***
***
IL-1α protein (ng/g tissue)
0.2
0.15
0.1
0.05
0
n = 10-13
N.S.
IL-1β protein (ng/g tissue)
3
2
1
0
R-G-
1.5h R+G-
1.5h R+G+
C
a: HE in WT
b: IL-1α in WT
c: IL-1α in IL-1-KO
Bar: 200 μm
a
b
c

Fig. 4

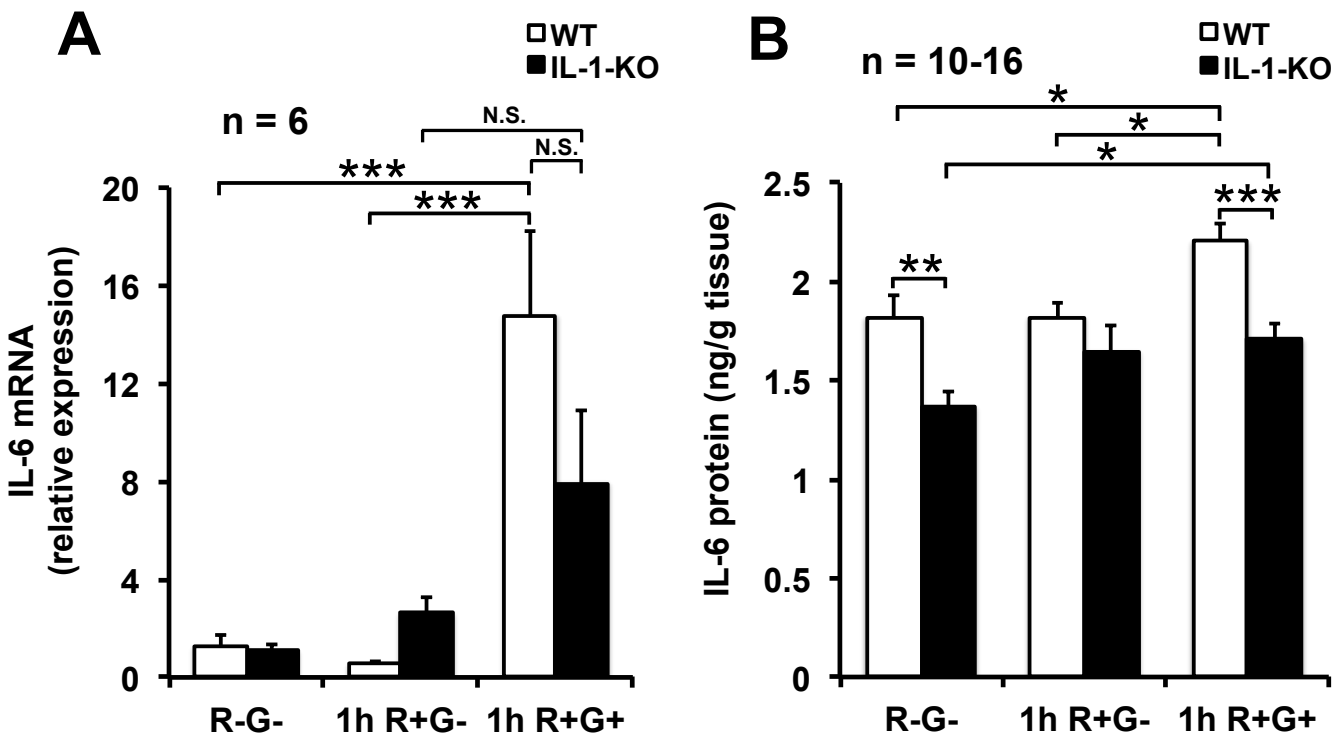
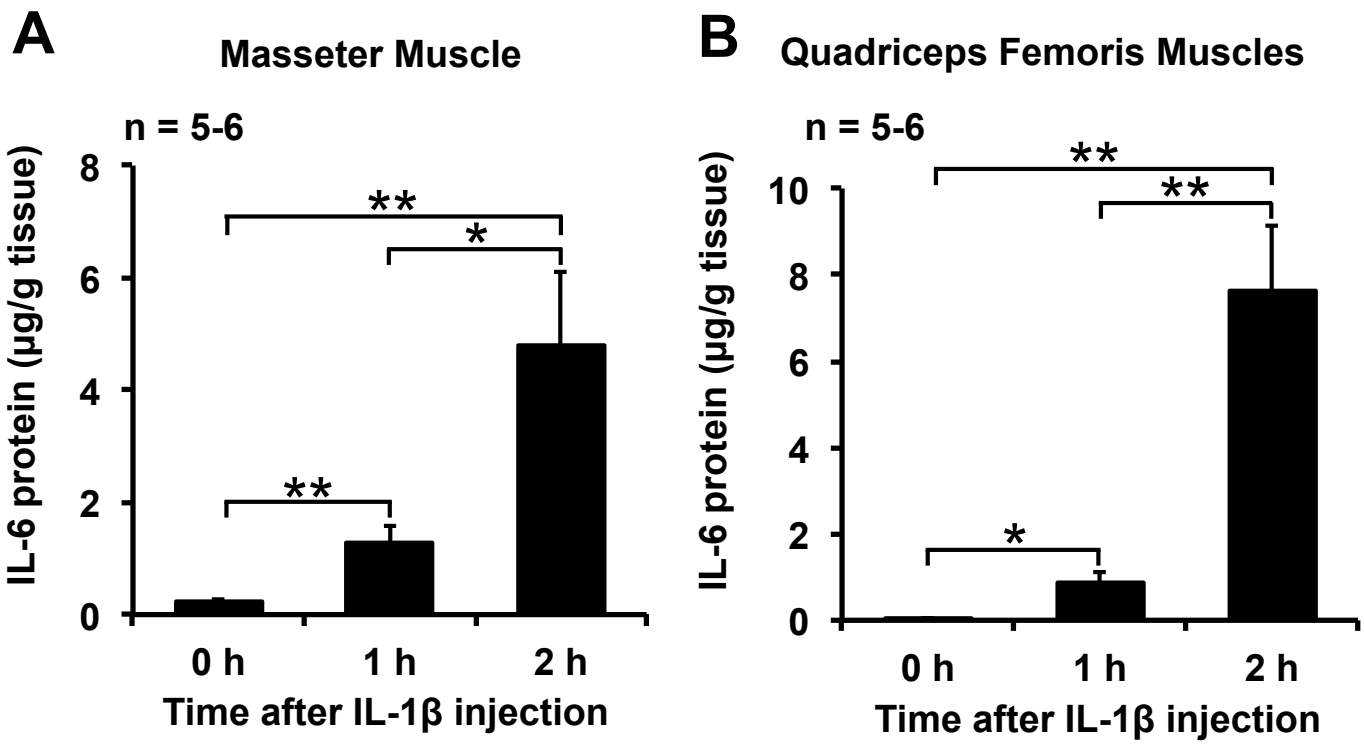

A
WT
IL-1-KO
n = 6
N.S.
N.S.
***
***
IL-6 mRNA
(relative expression)
20
16
12
8
4
0
R-G-
1h R+G-
1h R+G+
B
WT
IL-1-KO
n = 10-16
*
*
*
**
***
IL-6 protein (ng/g tissue)
2.5
2
1.5
1
0.5
0
R-G-
1h R+G-
1h R+G+

Fig. 5


A
Masseter Muscle
n = 5-6
IL-6 protein (μg/g tissue)
8
6
4
2
0
**
*
**
0 h
1 h
2 h
Time after IL-1β injection
B
Quadriceps Femoris Muscles
n = 5-6
IL-6 protein (μg/g tissue)
10
8
6
4
2
0
**
**
*
0 h
1 h
2 h
Time after IL-1β injection