

Glucose Infusion Suppresses Surgery-induced Muscle Protein Breakdown by Inhibiting Ubiquitin-proteasome Pathway in Rats

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Background: **It appears to have been well established that after surgery, protein catabolism is accelerated and glucose infusion suppresses the catabolic reactions. However, in the early postoperative period, the effects of surgical stress and glucose infusion on muscle protein catabolism and the related mechanisms remain unclear.**

Methods: **Rats undergoing laparotomy were infused with acetated Ringer's solution (10 ml · kg^{-1} · h^{-1}) without glucose (control) or containing 1% or 5% glucose. The infusion was continued for a further 4 h after the surgical treatment. The catabolic index, excretion of urinary nitrogen and 3-methylhistidine, and release of tyrosine and 3-methylhistidine from isolated muscle were determined. Furthermore, muscular mRNA expression of proteolytic-related genes (atrogin-1/MAFbx, muscle ring finger-1, μ- and m-calpain, and cathepsin L and H) and phosphorylation of components of insulin signaling (forkhead box O3 and protein kinase B) were evaluated.**

Results: **Surgery increased the catabolic index, and this increase was suppressed by glucose infusion (both 1% and 5%). In the control group, mRNA expression of atrogin-1/MAFbx and muscle ring finger-1 was increased, and they were suppressed in the two glucose groups. Furthermore, insulin signaling (phosphorylation of protein kinase B and forkhead box O3) in muscles was stimulated by glucose infusion.**

Conclusion: **The present study indicates that glucose infusion, even at a relatively low rate, suppresses muscle protein breakdown in the early postoperative period. The mechanism of this effect is related to the suppression of the ubiquitin-proteasome pathway, accompanied by activation of insulin signaling.**

SURGICAL stress causes protein catabolism,[1] which is one of the most important factors affecting postoperative convalescence.[2] It has been demonstrated that skeletal muscle is the main source of degraded protein after surgery,[3] and it appears to have been well established that glucose infusion after surgery suppresses protein catabolism.[4–6] A few studies have investigated the effect of glucose infusion on protein catabolism during surgery. Glucose infusion during abdominal surgery in humans decreased oxidation of leucine, as assessed by a stable isotope tracer technique using L-[1-13C] leucine,[7] and decreased plasma levels of branched-chain amino acids[8]; both of these findings indicate a protein-sparing effect. However, in the early postoperative period, little is known about the effect of surgical stress and/or glucose infusion on muscle protein breakdown.

Urinary excretion of 3-methylhistidine (3-MeHis) has been previously used as an index of muscle protein breakdown,[9,10] since this amino acid is localized in the main myofibrillar protein and undergoes quantitative urinary excretion.[11] The release of tyrosine and 3-MeHis from incubated muscle indicates degradation of total protein and myofibrillar protein,[12] respectively. Increased protein breakdown occurs in response to different catabolic conditions, such as burn injury,[13] starvation,[14,15] and sepsis,[12] and it is more pronounced in the myofibrillar protein pool than in the total protein pool. Whether surgical stress and/or glucose infusion affect total and/or myofibrillar protein breakdown in skeletal muscle has not been clarified.

In skeletal muscle, intracellular degradation of protein in some catabolic conditions is regulated by three proteolytic pathways: lysosomal-dependent, calcium-dependent, and ubiquitin-proteasome pathways.[16–18] The ubiquitin-proteasome pathway in particular contributes to muscle protein breakdown.[19] Two ubiquitin ligases, atrogin-1/MAFbx (atrogin-1) and muscle ring finger-1 (MuRF1), play a key role in the ubiquitin-proteasome pathway.[20,21] Therefore, increases in atrogin-1 and MuRF1 expression are known to be related to skeletal muscle protein degradation.[22] It has been shown that activation of insulin signaling in skeletal muscle, particularly phosphorylation of protein kinase B (Akt) and forkhead box O3 (Foxo3), suppresses the expression of atrogin-1 and MuRF1, which is accompanied by suppression of the catabolic reaction.[23–26] Thus, increased mRNA expression of atrogin-1 and MuRF1 suggests that the ubiquitin-proteasome pathway may be activated in skeletal muscle. Glucose infusion may suppress mRNA expression by activating insulin signaling.

The present study was designed to examine whether surgical stress causes muscle protein breakdown, and

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whether glucose infusion is effective in suppressing this protein breakdown in rats subjected to laparotomy. To clarify which proteolytic pathways are involved, the mRNA expression of proteolytic pathway-related genes in skeletal muscle were examined. In addition, we examined the effect of glucose infusion on phosphorylation of Akt and Foxo3 in skeletal muscle.

Materials and Methods

Animals

The following surgical and experimental procedures were approved by the Committee on the Care and Use of Laboratory Animals of Otsuka Pharmaceutical Factory (Tokushima, Japan). Male Sprague-Dawley rats (n = 37) from Charles River Japan (Yokohama, Japan), weighing 260 to 300 g, were maintained under conditions of constant humidity and temperature (22 ± 2°C) on a 12:12-h light-dark cycle. The rats had free access to a standard Charles River Formula-1 diet (Oriental Yeast Co., Tokyo, Japan) and water.

Experiment Design

A total of 37 rats were stratified by body weight, and were then randomized into three treatment groups (n = 10/group) and one nonsurgical group (n = 7). The three treatment groups were subjected to laparotomy for 4 h, and were infused with test solution for 8 h (during and after laparotomy), as described below. One group of animals (n = 7) was not subjected to the surgical treatment, anesthesia, or infusion (nonsurgical treatment group).

Laparotomy and Infusion

The rats were fasted for 16 h and then anesthetized by pentobarbital sodium (IV 45 mg/kg, after which a silicon catheter for continuous infusion of pentobarbital sodium (15 $mg \cdot kg^{-1} \cdot h^{-1}$) was inserted into the left jugular vein. The rats were then subjected to laparotomy; the intestine was gently rubbed for 3 min every hour after opening the abdomen, and after 4 h, the abdomen was closed and the pentobarbital infusion was stopped. It took about 30 or 45 min for the rats to recover from anesthesia. During and after laparotomy, the rats were continuously infused with acetated Ringer's solution (140 mM Na^+, 4 mM K^+, 115 mM Cl^-, 2 mM Mg^+, 1.5 mM Ca^{2+} and 25 mM $acetate^-$) without glucose (control), or containing 1% or 5% glucose *via* the tail vein. As the infusion flow rate was 10 $ml \cdot kg^{-1} \cdot h^{-1}$, infusion of 1% and 5% glucose solutions corresponded to a glucose administration of 0.1 and 0.5 $g \cdot kg^{-1} \cdot h^{-1}$, respectively. Body temperature was maintained at 36 to 37°C using a temperature controller (NS-TC10; Neuroscience Inc.; Tokyo, Japan) during laparotomy. Rats in the nonsurgical treatment group were fasted for 16 h, and then individually set in a cage for 8 h while fasting.

Sample Collection

Blood was collected from the right jugular vein before surgery (5 min after the induction of anesthesia) and 8 h after the induction of anesthesia. Plasma was obtained by centrifugation at 1,800 × *g* for 20 min at 4°C, and was maintained at −80°C until analysis. Urine was collected during the infusion of test solution. During anesthesia, we obtained urine from the penis with a microtube. After recovery from anesthesia, we placed a plastic cup under the cage. The gastrocnemius and extensor digitorum longus (EDL) muscles were isolated 8 h after the induction of anesthesia. Urine and muscles from the nonsurgical treatment group were also obtained.

Assessment of Total and Myofibrillar Protein Breakdown

The EDL muscles were immediately preincubated for 30 min in oxygenated (O_2/CO_2 = 95:5) Krebs-Henseleit bicarbonate buffer (pH 7.4) with 10 mM glucose. After preincubation, the muscles were transferred to fresh medium containing cycloheximide (0.5 mM) and incubated for 2 h. Protein breakdown rates were determined by net release of tyrosine and 3-MeHis into the incubation medium, as described previously.[12] Tyrosine levels were measured using a high-performance liquid chromatography system (2690 Alliance separation module; Waters, Milford, MA). Twenty microliters of medium was separated isocratically on a Cadenza CD-C18 column (2.0 × 150 mm, 3 μm; Imtakt Co.; Kyoto, Japan) using a mobile phase of 50 mM SDS/acetonitrile/phosphoric acid at 610: 390: 3 at a flow rate of 0.2 ml/min. An ultraviolet detector (2487, Waters) was used for detection at 213 nm. Concentrations of 3-MeHis were measured using high-performance liquid chromatography, similarly as for tyrosine, after the medium was concentrated fivefold. Concentrations of tyrosine and 3-MeHis were determined by comparing peak height of samples with those of external standards. The linear ranges of the tyrosine and 3-MeHis assays are 1 to 50 nmol/ml and 0.125 to 2 nmol/ml, respectively. The precision and accuracy of the assay is about 1.3 to 4.5% and 2.8 to 6%, respectively.

Western Blot Analyses

The gastrocnemius muscles were homogenized on ice in 0.5 ml of lysis buffer (Pierce Biotechnology, Rockford, IL) containing the proteasome inhibitor epoxomicin (Peptide Institute Inc., Osaka, Japan), phosphatase inhibitor cocktail, and protease inhibitor cocktail (Pierce Biotechnology). Skeletal muscle extracts (40 μg protein/lane) were subjected to SDS-8%-polyacrylamide gel electrophoresis and were transferred to polyvinylidene fluoride membranes. Primary antibodies were as follows: anti-Akt (BD PharMingen, San Jose, CA), antiphospho-Akt (Cell Signaling Technology Inc., Danvers, MA), and anti-Foxo3 or antiphospho-Foxo3 (Upstate Biotechnology, Lake Placid, NY). Bound antibodies were detected

Table 1. Primers for Polymerase Chain Reaction

Target Gene	Sequence	Length (bp)
Atrogin-1		
Forward	5'-AGATCCGCAAGCGATTGATC-3'	84
Reverse	5'-TTGGGTAACATCGCACAAGC-3'	
Probe	5'-TCTGACAAAGGGCAGCTGGATTGGA-3'	
MuRF1		
Forward	5'-TGAGCCACAAGTTTGACGC-3'	102
Reverse	5'-CGATGAAGTCCAGCTTCTCCT-3'	
Probe	5'-CTCTACGCCATCCTGGACGAGAAGAAGAGTG-3'	
μ-calpain		
Forward	5'-CAAAGACCTGCGCACTAACG-3'	77
Reverse	5'-CATTACCGTCTCGATCCATGAG-3'	
Probe	5'-CAGCCTGGAGTCGTGCCGCA-3'	
m-calpain		
Forward	5'-CAACTTTGTGCGGTGTTTGGT-3'	103
Reverse	5'-CCACGAGATAAGGTCGAGCTGTA-3'	
Probe	5'-TCAAGCAGCTGGACCCTGAGAACACTG-3'	
Cathepsin H		
Forward	5'-CAATCATGGCTGCCAAGGA-3'	76
Reverse	5'-CTCTCCCATGATGCCCTTGT-3'	
Probe	5'-CCAGCCAGGCCTTCGAGTACATCCT-3'	
Cathepsin L		
Forward	5'-GCTGAGTATGCTGTGGCTAACG-3'	121
Reverse	5'-ACGGATGGCTTGCATCCAT-3'	
Probe	5'-CCCTCATGAAGCCTGTAGCGACGGT-3'	

Atrogin-1 = atrogin-1/MAFbx; bp = base pair; MuRF1 = muscle ring finger-1.

using a secondary antirabbit immunoglobulin G antibody (Amersham Biosciences, Piscataway, NJ) and the enhanced chemiluminescence system (Amersham Biosciences). Signals were quantified by densitometric analysis. Protein concentrations were determined by detergent-compatible protein assay based on Lowry's method (Bio-Rad Laboratories Inc., Tokyo, Japan) with bovine serum albumin as a standard.

Quantitative Real-time Reverse Transcription-polymerase Chain Reaction

Total RNAs were extracted from gastrocnemius muscles using the RNeasy Mini Kit (Qiagen Co., Hilden, Germany) according to the manufacturer's instructions. Quantitative real-time reverse transcription-polymerase chain reaction with TaqMan probes was performed using an ABI PRISM 7700 (Applied Biosystems, Foster City, CA), as described previously.[27] The primers for specific genes are shown in table 1. For each sample, glyceraldehyde-3-phosphate dehydrogenase was used as an internal control after amplification using glyceraldehyde-3-phosphate dehydrogenase-specific primers (Applied Biosystems).

Measurement of Other Biochemical Markers

Urinary nitrogen levels were measured with a total nitrogen analyzer (TN-100; Dia Instruments Co., Kanagawa, Japan). Concentrations of 3-MeHis in urine were measured using high-performance liquid chromatography, as described above, after the urine was hydrolyzed with 6 N hydrochloric acid at 110°C for 2 h. The linear range of the 3-MeHis assay is 10 to 1000 nmol/ml. Plasma glucose concentrations were measured using the glucose oxidase method[28] with a DRI-CHEM 5500 analyzer (Fujifilm Co., Tokyo, Japan). Plasma insulin concentrations were measured using an enzyme-linked immunosorbent assay kit (Shibayagi Co., Gumma, Japan), with rat recombinant insulin as a standard. Plasma amino acid levels were measured using an automatic amino acid analyzer (L-8800A; Hitachi High-Technologies Co., Tokyo, Japan), as described previously.[27]

Statistical Analysis

Sample size (n = 8–10) determination was based on detecting the differences in urinary 3-MeHis between the control and glucose infusion groups ($P = 0.05$, power = 0.8) in a preliminary study. Values for each group are presented as means ± SD. Data were analyzed using EXSAS 7.5.2.2 software (SAS Institute Japan Inc., Tokyo, Japan). Plasma levels of glucose, insulin, and amino acid were compared using a two-way ANOVA, followed by a Tukey-Kramer test or Steel-Dwass test (leucine and isoleucine) among the groups. One-way ANOVA or a Kruskal-Wallis test was used to determine whether other parameters differed among the groups, and was followed by a Tukey-Kramer test or Steel-Dwass test (urinary nitrogen, tyrosine release, MuRF1, cathepsin L, and phosphorylation of Foxo3), as appropriate. The Steel-Dwass test was used as a nonparametric version of the Tukey-Kramer test. Statistical significance was defined as $P < 0.05$.

Results

Effects of Surgical Stress and/or Glucose Infusion on Excretion of Urinary Nitrogen and 3-MeHis

Excretion of urinary nitrogen and 3-MeHis in the control group increased to 170% and 150%, respectively, as compared with the nonsurgical treatment group (fig. 1, A and B). Intravenous infusion of 1% or 5% glucose significantly suppressed the increases in excretion of urinary nitrogen and 3-MeHis. The inhibitory effects did not differ between the two glucose groups.

Effects of Surgical Stress and/or Glucose Infusion on Muscle Protein Breakdown

To examine, in detail, the effect of glucose infusion and/or surgical stress on muscle protein breakdown, the release of tyrosine and 3-MeHis from incubated EDL muscles isolated 8 h after the induction of anesthesia was examined. The release of tyrosine did not change significantly among the groups (fig. 2A). In contrast,

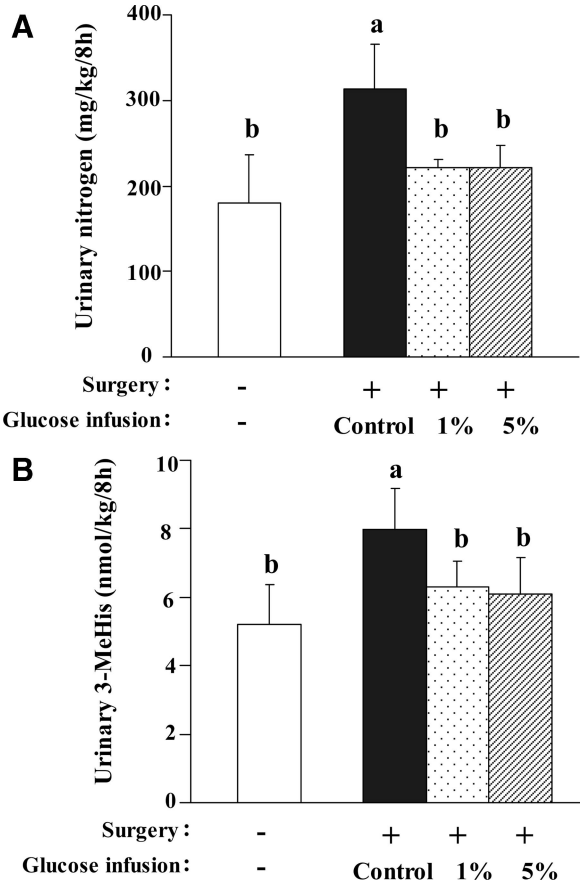

Fig. 1. Glucose infusion effectively inhibited excretion of urinary nitrogen and 3-methylhistidine (3-MeHis) caused by surgery. *A* and *B*: Fasted rats were subjected to laparotomy for 4 h under anesthesia. During and for 4 h after laparotomy, the rats were continuously infused with acetated Ringer's solution without glucose (control), or containing 1% or 5% glucose. Urine was collected during infusion. (*A*) Urinary nitrogen and (*B*) 3-MeHis levels were measured. A group of rats that did not undergo surgery or infusion was also prepared. Values are means ± SD, n = 10 or 7 (nonsurgical treatment group). Means with different superscripts differ significantly ($P < 0.05$).

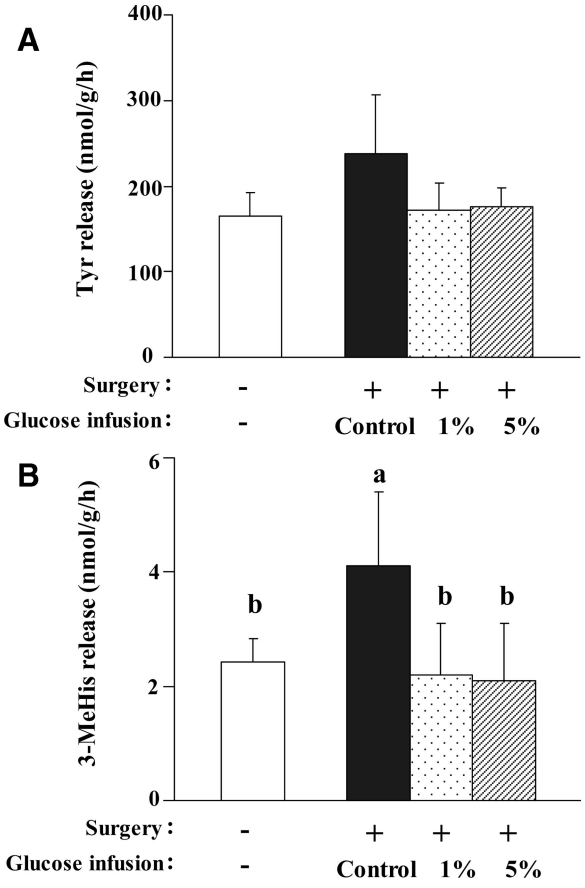

Fig. 2. Glucose infusion suppressed the release of protein breakdown markers from isolated rat skeletal muscles. *A* and *B*: Fasted rats were subjected to laparotomy for 4 h under anesthesia. During and for 4 h after laparotomy, the rats were continuously infused with acetated Ringer's solution without glucose (control), or containing 1% or 5% glucose. Extensor digitorum longus (EDL) muscles were isolated 8 h after the induction of anesthesia and were incubated with Krebs-Ringer's solution. (*A*) The amounts of free tyrosine (tyr) and (*B*) 3-methylhistidine (3-MeHis) released from the EDL muscles were measured. A group of rats that did not undergo surgery or infusion was also prepared. Values are means ± SD, n = 10 or 7 (nonsurgical treatment group). Means with different superscripts differ significantly ($P < 0.05$).

consistent with the excretion of urinary nitrogen and 3-MeHis, the release of 3-MeHis was higher in the control group than in the nonsurgical treatment group (fig. 2B).

Effects of Glucose Infusion on Plasma Amino Acids Levels

Plasma-branched chain amino acid levels, particularly leucine levels, and total essential amino acid levels reflect the rate of whole body protein breakdown.[29] In the present study, the levels of plasma-branched chain amino acid and total essential amino acids were significantly decreased in each glucose group, as compared with the control group, in a dose-dependent manner (table 2).

Effects of Glucose Infusion on Plasma Glucose and Insulin Levels

Plasma glucose levels were increased in a dose-dependent manner (table 3): the 1% glucose infusion increased plasma glucose level to a moderate extent, whereas the 5% glucose infusion increased plasma glucose levels to 160% *versus* the control group. The 5% glucose infusion significantly increased the plasma insulin levels, as compared with the control group (table 3).

Stimulation of Muscle Insulin Signaling by Glucose Infusion

The effects of glucose infusion on insulin signaling (phosphorylation of Akt and Foxo3) were examined in the gastrocnemius muscles, as stimulation of insulin signaling down-regulates mRNA expression of atrogin-1 and MuRF1,[23–26] along with a suppression of protein breakdown. The phosphorylation of Akt and Foxo3 in the muscle did not change significantly in the control group *versus* the nonsurgical treatment group. The ratio of

Table 2. Plasma-branched Chain Amino Acid and Total Amino Acids Levels

	Before Surgery	8 h
Ile (μM)		
Control	100 ± 20	117 ± 11
1% glucose	95 ± 15	80 ± 12*
5% glucose	100 ± 12	44 ± 2*†
Leu (μM)		
Control	152 ± 31	204 ± 20
1% glucose	144 ± 21	141 ± 16*
5% glucose	156 ± 23	84 ± 5*†
Val (μM)		
Control	194 ± 35	239 ± 27
1% glucose	185 ± 23	165 ± 16*
5% glucose	197 ± 23	101 ± 9*†
Total essential amino acids (μM)		
Control	1,476 ± 179	1,287 ± 76
1% glucose	1,363 ± 151	1,075 ± 58*
5% glucose	1,427 ± 137	890 ± 56*†
Total amino acids (μM)		
Control	3,779 ± 406	3,025 ± 189
1% glucose	3,684 ± 241	2,757 ± 83*
5% glucose	3,737 ± 288	2,488 ± 196*†

Blood was collected before surgery and at 8 h after the induction of anesthesia. Values are means ± SD, n = 10.

* $P < 0.05$ *versus* control. † $P < 0.05$ *versus* 1% glucose.

Ile = isoleucine; Leu = leucine; Val = valine.

phosphorylated Akt to the respective total protein in the muscles was increased in each glucose group, as compared with the control and nonsurgical treatment groups. The ratio of phosphorylated Foxo3 to the respective total protein in the muscles was increased in the 1% glucose group, as compared with the control and nonsurgical treatment groups (fig. 3A and B).

Expression of Proteolytic Pathway-related Genes in Glucose-infused Rats

The mRNA expression of proteolytic pathway-related genes was examined in the gastrocnemius muscles. Although the mRNA expression of μ-calpain, m-calpain, and cathepsin H did not change among the groups, the mRNA expression of atrogin-1, MuRF1, and cathepsin L was significantly increased in the control group when compared with the nonsurgical treatment group (fig. 4).

Table 3. Plasma Glucose and Insulin Levels

	Before Surgery	8 h
Glucose (mg/dl)		
Control	115 ± 9	110 ± 13
1% glucose	121 ± 12	140 ± 10*
5% glucose	125 ± 13	174 ± 12*†
Insulin (ng/ml)		
Control	0.4 ± 0.2	1.7 ± 0.7
1% glucose	0.5 ± 0.4	2.4 ± 1.0†
5% glucose	0.3 ± 0.2	3.1 ± 1.5*

Blood was collected before surgery and at 8 h after the induction of anesthesia. Values are means ± SD, n = 10.

* $P < 0.05$ *versus* control. † $P < 0.05$ *versus* 1% glucose.

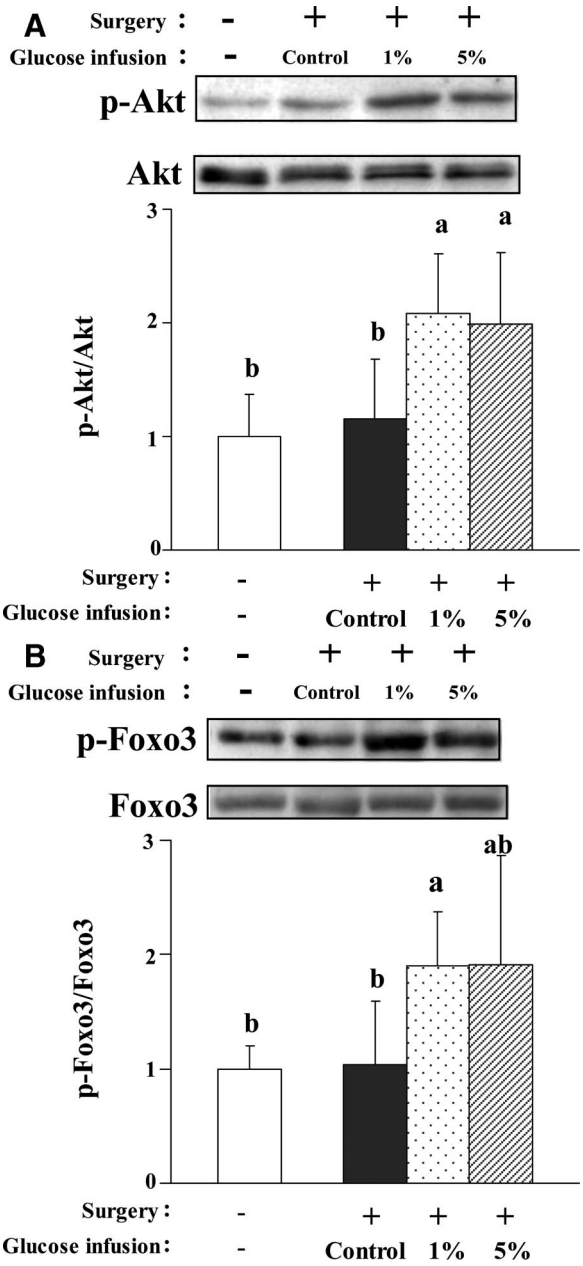

Fig. 3. Glucose infusion up-regulated phosphorylation of Akt and Foxo3 in rat gastrocnemius muscles. *A* and *B*: Fasted rats were subjected to laparotomy for 4 h under anesthesia. During and for 4 h after laparotomy, the rats were continuously infused with acetated Ringer's solution without glucose (control), or containing 1% or 5% glucose. The gastrocnemius muscles were isolated 8 h after the induction of anesthesia. Homogenates (40 μg protein/lane) from the gastrocnemius muscle were subjected to SDS-8%- polyacrylamide gel electrophoresis. (*A*) Immunoblottings for protein kinase B (Akt) and phosphorylated Akt (p-Akt) and forkhead box O3 (Foxo3) and (*B*) phosphorylated Foxo3 (p-Foxo3) were performed. The intensity ratios of phosphorylated Akt and Foxo3 against the respective total proteins were calculated and compared with the values of the control group. Values are means ± SD, n = 10 or 7 (nonsurgical treatment group). Means with different superscripts differ significantly ($P < 0.05$).

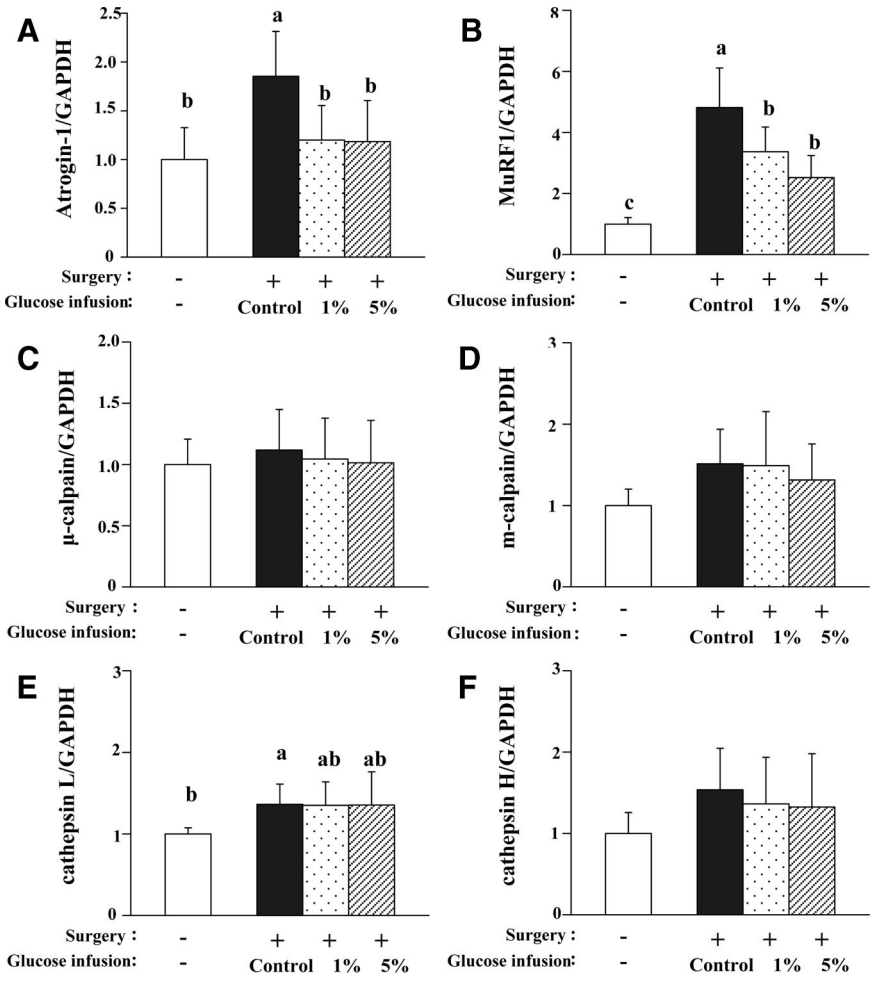

Fig. 4. Glucose infusion suppressed the mRNA expression of atrogin-1/MAFbx (atrogin-1) and muscle ring finger-1 (MuRF1) in gastrocnemius muscles. *A-F* Fasted rats were subjected to laparotomy for 4 h under anesthesia. During and for 4 h after laparotomy, the rats were continuously infused with acetated Ringer's solution without glucose (control), or containing 1% or 5% glucose. Total RNA was extracted from gastrocnemius muscles isolated 8 h after the induction of anesthesia. Total RNA (1 μg) was subjected to real-time reverse transcription-polymerase chain reaction analyses for muscle proteases and related genes. The fluorescence ratio of the cDNA of the target genes to that of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was calculated. Values are means ± SD, n = 10 or 7 (nonsurgical treatment group). Means with different superscripts differ significantly ($P < 0.05$).

The mRNA expression of atrogin-1 and MuRF-1 was lower in each glucose group than in the control group, but there were no significant differences between the two glucose groups.

Discussion

The present study demonstrates that surgical stress causes muscle protein breakdown and is suppressed by glucose infusion in rats. Glucose infusion down-regulates mRNA expression of atrogin-1 and MuRF1 (ubiquitin-proteasome pathway-related genes), and is accompanied by stimulating insulin signaling.

It has been shown that, after surgery, protein catabolism increases and is accompanied by muscle protein breakdown, while it has been well established that glucose infusion has a protein-sparing effect.[4–6] However, in the early postoperative period, it is not fully understood whether surgical stress causes protein breakdown or whether glucose infusion suppresses the catabolic reaction, and, if so, how these effects are mediated. When in the surgical procedure of the present study the intestine was gently rubbed for 3 min, a similar degree of stress as gastrectomy was induced.[30] In this study protocol, the plasma levels of corticosterone were increased, as compared with before surgery (data not shown). To assess the effect of surgical stress on protein breakdown, we also used a nonsurgical treatment group. To align the sampling times, rats in the nonsurgical treatment group were fasted similarly as other groups. In the preliminary study, some catabolic indices did not differ between fasting rats and rats fasting for a further 8 h (data not shown).

Excretion of urinary nitrogen and 3-MeHis was significantly increased in the control group *versus* the nonsurgical treatment group. These results demonstrate that surgical stress caused loss of whole body protein accompanied by muscle protein breakdown in the early postoperative period. To elucidate the effects of surgical stress on muscle protein breakdown, release of tyrosine and 3-MeHis from isolated EDL muscles, which indicate the breakdown of total muscle protein and myofibrillar protein, respectively,[12] was examined. In various catabolic states, myofibrillar protein breakdown contributes to muscle protein breakdown.[12–15] In the present study, release of 3-MeHis from isolated EDL muscles in the control group increased significantly to 170%, and release of tyrosine in the control group did not signifi-

cantly increase, as compared with the nonsurgical treatment group. Thus, surgical stress induced myofibrillar protein breakdown, rather than total protein breakdown in skeletal muscle. However, the increase in urinary nitrogen and 3-MeHis excretion was suppressed by 1% or 5% glucose infusion. Glucose infusion was also found to suppress myofibrillar protein breakdown in skeletal muscle. Interestingly, these catabolic indices did not decrease in the glucose infusion groups when compared with the nonsurgical treatment group, which was not given any extra energy. The efficacies of 1% ($0.1\ g \cdot kg^{-1} \cdot h^{-1}$) and 5% ($0.5\ g \cdot kg^{-1} \cdot h^{-1}$) glucose infusion on these catabolic indices were comparable. These results suggest that glucose infusion suppressed increases in protein breakdown caused by surgery. Although the prolonged effects after surgery are not clear, a low rate of glucose infusion is sufficient for suppression of protein breakdown in the early postoperative period.

It has been shown that plasma-branched chain amino acid levels, particularly leucine levels, and the total essential amino acid level reflect the rate of whole body protein breakdown.[29] In the present study, these plasma amino acid levels were significantly decreased by glucose infusion *versus* the control group. Unlike other catabolic indices, the effect of glucose infusion on theses plasma amino acid levels was dose-dependent. Insulin stimulates the transmembrane transport of some amino acids.[31] Thus, the decrease in these plasma amino acid levels may reflect a decrease in protein breakdown and an increase in the incorporation of these amino acids.

Another aim of the present study was to elucidate the mechanisms of the anticatabolic effects of glucose infusion on muscle protein breakdown in the early postoperative period. Surgical stress induced significant mRNA expression of atrogin-1 and MuRF1 (ubiquitin-proteasome pathway-related genes). These results suggest that at least the ubiquitin-proteasome pathway contributes to the catabolic reaction. Several proteolytic pathways, particularly the ubiquitin-proteasome proteolytic pathway, contribute to muscle protein breakdown in various catabolic states.[19] Recent investigations have demonstrated that atrogin-1 and MuRF1 are rate-limiting enzymes for skeletal muscle protein breakdown.[20–22] Therefore, it is important to inhibit the surgery-induced mRNA expressions of atrogin-1 and MuRF1. Interestingly, glucose infusion was found to effectively inhibit this mRNA expression. These results suggest that the ubiquitin-proteasome pathway is activated in the early postoperative period, and that this activation is inhibited by glucose infusion. In contrast, mRNA expression of cathepsin L (lysosomal-dependent proteolytic pathway) was also increased in the control group, as compared with the nonsurgical treatment group. This suggests the possibility that the lysosomal proteolytic pathway is also activated by surgical stress. However, the proteolytic pathway was not related to the anticatabolic effects of glucose, as mRNA expression did not change with glucose infusion.

More recent investigations have demonstrated that activation of insulin signaling in skeletal muscle, particularly phosphorylation of Akt and Foxo3, suppresses the expression of atrogin-1 and MuRF1, along with suppressing protein breakdown.[23–26] Thus, activation of insulin signaling is very important for the suppression of protein breakdown, although surgery occasionally induces insulin resistance, or so-called surgical diabetes.[32–35] Therefore, the present study focused on insulin signaling in skeletal muscle to elucidate the anticatabolic effects of glucose infusion. Interestingly, phosphorylation of Akt and Foxo3 in skeletal muscle did not decrease in the control group as compared with the nonsurgical treatment group, although the mRNA expression of atrogin-1 and MuRF1 increased in the control group. It has been shown that activation of nuclear factor κB also induces muscle protein breakdown through induction of several proteolytic genes.[36] Thus, the induction of atrogin-1 and MuRF1 mRNA expressions in the present study may be regulated by mechanisms other than insulin signaling, such as nuclear factor κB. In contrast, phosphorylation of Akt and Foxo3 increased in the glucose infusion groups as compared with the control group, which is consistent with the suppression of atrogin-1 and MuRF1 expression by glucose infusion. Thus, the inhibitory effect of glucose infusion on protein breakdown in skeletal muscle is related to activation of insulin signaling.

It has been reported that glucose infusion caused severe hyperglycemia during surgery as a result of insulin resistance.[33] Hyperglycemia is also a risk factor for complications after surgery.[32] In the present study, the 2 glucose infusions did not induce severe hyperglycemia over 200 mg/dl, including during the intraoperative period (data not shown), but plasma glucose levels were dose-dependently increased. Although glucose infusion requires careful attention during surgery, the present results indicate that glucose infusion suppresses protein catabolism. However, the efficacy of glucose infusion is not dose-dependent in the present study. In contrast, the protein sparing effect of glucose is dose-dependent during fasting.[37] Although further study is needed, we consider that this discrepancy may reflect decreased glucose tolerance during the early postoperative period. On the other hand, it has been reported that preoperative glucose infusion or perioperative glucose and insulin infusion reduced postoperative insulin resistance,[38,39] thus insulin resistance may have been greater in the control group than in the glucose infusion group in the present study. To assess the effects of glucose infusion on insulin resistance after surgery, further study is needed. During surgery, it has been reported that infusion of 0.1 g glucose/kg BW/h has positive effects, including preventing glycogen depletion[30] and preventing lipolysis and ketosis,[40] and these effects are not dose-dependent. Con-

sidering all of these findings, a low-rate glucose infusion of 0.1 $g \cdot kg^{-1} \cdot h^{-1}$ appears to have clinical potential to suppress muscle protein breakdown. However, the protocol of the present study was not designed to examine the effects of glucose infusion on postoperative outcome, which is an issue that requires further study.

In conclusion, our findings suggest that muscle protein breakdown is induced in the early postoperative period. Glucose infusion suppresses protein catabolism accompanied by activation of insulin signaling and inhibition of the ubiquitin-proteasome pathway.

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