Prognostic consideration of postoperative skeletal muscle proteolysis in patients underwent cardiovascular surgery

（心臓血管外科術後における筋タンパク分解と機能的予後に関する研究）

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リハビリテーション療 法 専 攻

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Abstract

[Background]

Systemic muscle weakness is common in patients undergone cardiac surgery, and is associated with decreased 6-mininute walking distance (6MWD) after surgery. Elevation of inflammatory cytokine production after surgery enhances systemic catabolic states, and leads to muscle proteolysis. These observations suggest that muscle weakness after surgery may be affected by muscle proteolysis *via* postoperative hyper-catabolism, but the interrelationships of these factors remain unclear. We postulated that postoperative muscle weakness may be associated with urinary 3-methylhistidine (3-MH) excretion, which is well known as a marker of skeletal muscle proteolysis, and elevation of inflammatory cytokine production.

[Objectives]

The aim of this study was to clarify postoperative skeletal muscle proteolysis by measuring 3-MH, and to explore the factors underlying increased muscle proteolysis by measuring the urinary 3-methylhistidine/creatinine ratio (3-MH/Cr) and the relationship between 3-MH/Cr and 6MWD in patients who had recently undergone cardiac surgery.

[Methods]

Sixty-nine patients undergone elective cardiac surgery participated in this study. A 24 hour urinary 3-MH/Cr was measured for the first five days post-surgery. Grip strength (GS), maximum inspiratory pressure (MIP), maximum expiratory pressure (MEP), and isometric knee-extensor strength (IKES) were measured before surgery and on postoperative days (POD) 7 and 14, and 6MWD was tested on POD 14. The serum level of interleukin (IL)-6, cortisol, insulin-like growth factor (IGF)-1 / growth hormones (GH), and branched chain amino acids (BCAA) / aromatic amino acids (AAA) were also measured to confirm a hyper-catabolism immediately after the operation. We examined the relationships among the mean change in muscle strength, IL-6 and 3-MH/Cr, and the relationship between 3-MH/Cr and 6MWD in patients who had recently undergone cardiac surgery.

[Results]

We observed a marked increase in 3-MH/Cr from POD 3 to 5, and its peak occurred on POD 4. Cumulative 3-MH/Cr (cum3-MH/Cr) during 5 days after surgery significantly correlated with IL-6 and the changes in skeletal muscle strengths. In multivariate analysis, factors associated with an increased cum3-MH/Cr for three days after surgery were: grip strength ($\beta = -0.31$), BMI ($\beta = -0.30$), immediate postoperative IL-6 ($\beta = 0.29$), haemoglobin ($\beta = -0.24$) and cardiopulmonary bypass time ($\beta = 0.18$). In stepwise logistic regression analysis, preoperative grip strength (OR = 0.88, 95%CI = 0.80 – 0.96), cum3-MH/Cr (OR = 1.09, 95%CI = 1.03 – 1.14) were extracted as independent predictors of a postoperative 6MWD < 300 m.

[Conclusions]

Our findings suggest that postoperative muscle proteolysis is facilitated by preoperative catabolic accelerators, and is related to a postoperative 6MWD decline in patients undergoing cardiac surgery.

Muscle proteolysis immediately after surgery may be a novel therapeutic target in rehabilitation intervention.

要旨

【背景】

心臓外科(心外)術後早期は骨格筋の筋力低下が発生し、術後回復や予後を不良にすることは広く知られている。術後筋力低下の要因として術後の骨格筋減少も一因として考えられるが、筋タンパク分解の程度や術後筋力変化ならびに異化作用との関連については明らかでない。一方、心外術後は 6 分間歩行距離(6MWD)が減少するとされ、300m 未満では予後不良とされている。6MWD の関連因子のひとつに筋力低下が挙げられるが、心外術後の筋タンパク分解が 6MWD に関連するかは不明である。これらの関連が明らかになれば、心外術後の予後改善につながる介入策構築の一助になると考えられる。

【目的】

心外術患者において、①筋タンパク分解と術後筋力変化ならびに異化指標の関連について明らかにすること、②筋タンパク分解増大の予測因子、ならびに筋タンパク分解と 6MWD の関連について明らかにすること。

【方法】

厚生連海南病院で待機的に心外術を受けた連続症例のうち、術前に研究への参加同意が得られた者で、中枢神経疾患、運動器疾患、呼吸器疾患、肝機能障害、腎機能障害、術後 24 時間以上の人工呼吸器装着例、再開胸術施行例、術後プログラム逸脱例を除外した 69 例を対象とした。指標は動脈血採取により、interleukin-6(IL-6)、cortisol、成長ホルモン(GH)、インスリン様成長因子 1(IGF-1)、分岐鎖アミノ酸(BCAA)、芳香族アミノ酸(AAA)を測定した(術前、術直後、術後 1

日目）。また筋タンパク分解の指標として 24 時間蓄尿による尿中 3-methylhistidine と creatinine の比 3-MH/Cr を測定した(術後 5 日間 41 例、術後 3 日間 28 例)。筋力は、呼吸筋力、握力、等尺性膝伸展筋力(術前、術後 7、14 日目)、さらに 6MWD(術後 14 日目)をそれぞれ測定した。

【結果】

3-MH/Cr を術後 5 日目まで測定した 41 例の検討では、3-MH/Cr は術後 3 日目より有意に上昇し 4 日目でピークとなった。術後累積 3-MH/Cr 値は術直後 IL-6 ならびに術後筋力低下量と有意な正の相関を認めた。術後は、IL-6 とコルチゾールの上昇ならびに BCAA/AAA と IGF-1/GH の低下を認め、異化亢進状態であることが示された。重回帰分析の結果、術後の累積 3-MH/Cr 増大の予測因子として、握力($\beta=-0.31$)、BMI ($\beta=-0.30$)、術直後 IL-6 ($\beta=0.29$)、ヘモグロビン($\beta=-0.24$)、体外循環時間 ($\beta=0.18$) が抽出された。また、術後 6MWD300m 未満の予測因子について、ロジスティック回帰分析を行なった結果、術前握力(OR = 0.88, 95%CI = 0.80–0.96)、累積 3MH/Cr (OR = 1.09, 95%CI = 1.03–1.14) が抽出された。

【結語】

心外術後の予後指標となる6MWDには術侵襲による筋タンパク分解が関連する。心外術患者において、筋タンパク分解増大の予測因子が存在する例では、3-MH/Crが極値となる術後4日以前、すなわち術後3日間に異化作用を抑制する方策を講じる必要のあることが示唆された。

Ⅰ. INTRODUCTION

The deterioration of skeletal muscles due to surgery is a morbidity with serious complications, which occurs during the postoperative period [1,2]. Persistent muscle weakness is also a major cause of general fatigue after surgery [3], which causes functional limitations in daily living after discharge. Prophylaxis of muscle deterioration, therefore, is an important clinical issue in rehabilitation intervention for surgical patients. However, systemic muscle weakness is still common in patients undergone cardiac surgery, even in patients with early mobilisation.

Skeletal muscle atrophy is widely recognised as a complication of the acute phase after cardiac surgery [4], and is thought to be dependent on skeletal muscle catabolism induced by the surgery [5]. We previously reported that muscle weakness on postoperative day (POD) 7 or 14 was associated with interleukin (IL)-6 productions immediately after cardiac surgery [6]. This finding suggests that muscle proteolysis is enhanced via postoperative hyper-catabolism, which is induced by postoperative elevation of inflammatory cytokine production [7-9]. To clarify this enhancement, the urinary excretion of 3-methylhistidine (3-MH), an established marker of skeletal muscle proteolysis [10,11], should be examined to establish its relationship with IL-6 or muscle weakness.

On the other hand, preoperative loss of body tissue, physical inactivity, altered metabolism and aging are the factors linked to loss of skeletal muscle mass and have been reported to be associated with increased risk of postoperative complications and length of stay in patients undergone cardiac surgery [12-14]. These observations suggest that preoperative factors linked to skeletal muscle loss may facilitate muscle proteolysis immediately after surgery.

Six-minute walking distance (6MWD) is widely used to assess the functional status of patients with cardiopulmonary disease, including postsurgical cardiac patients. Several studies have indicated that 6MWD is a reliable measure of increased mortality among cardiac patients, with a distance < 300 m being a strong indicator of a poor prognosis [15-17]. Reduced walking speed is usually associated with systemic muscle weakness after cardiac surgery [18,19], suggesting that reduced postoperative 6MWD may be partially caused by surgery-induced muscle proteolysis. Thus, taking our previous findings into consideration, we hypothesised that muscle proteolysis induced by cardiac surgery may play a key role in the reduction of postsurgical 6MWD.

In this study, we aimed (1) to examine the relationship among urinary 3-MH concentration, inflammatory cytokine production, and skeletal muscle weakness in patients undergone cardiac surgery (Study 1) (Fig 1), and (2) to explore the factors that contribute to facilitated muscle catabolism indicated by 3-MH, and to examine the relationship between 3-MH and 6MWD in patients undergone cardiac surgery (Study 2) (Fig 2).

Ⅱ. STUDY 1

Methods

Subjects

Forty-one consecutive patients who undergone elective cardiac surgery between April 2011 and March 2012, involving median sternotomy and cardiopulmonary bypass (CPB), were enrolled into this study. Patients requiring mechanical ventilation beyond 24 h post-surgery, reoperation, or with comorbidities of respiratory failure, chronic kidney disease (estimated glomerular filtration rate < 30 mL/min/1.73 m^2), and central nervous system dysfunction, were excluded. After cardiac surgery, all patients remained intubation and continued artificial ventilation until the patient recovered sufficient spontaneous respiration in the intensive care unit. During this time, patients had infused with fentanyl at 0.01 – 0.02 μg/kg/h for sedation.

After receiving approval from the Nagoya University Ethics Committee (approval number: 1086), written informed consent was obtained from each patient who participated in this study.

Data collections

Study protocol are indicated in Fig 3.

Blood samples

Blood samples were taken at three intervals for each patient, as follows: sample 1, the day before surgery; sample 2, 4 h after surgery in the ICU; and sample 3, POD 1. The samples were immediately centrifuged at 4°C and stored at -80°C until assayed. The serum level of IL-6 was measured using an automated chemiluminescent enzyme immunoassay (CLEIA) system (Lumipulse; Fujirebio Inc., Tokyo, Japan). To confirm the catabolic/anabolic balance, plasma levels of branched-chain amino acids (BCAA) and aromatic amino acids (AAA) were measured by use of the liquid chromatography/mass spectrometry (LC/MS) (LCMS2020; Shimadzu, Kyoto, Japan). Plasma levels of cortisol, growth hormone (GH), and insulin-like growth factor 1 (IGF-1) were also determined using a commercially available direct competitive radioimmunoassay (RIA) (automatic γ-counter; Wallac, Turku, Finland).

Urine samples

24-hour urine was collected from pre-operation and sampled every morning for 5 days after surgery. The enzyme method was performed to measure the level of urinary creatinine (Cr), and urinary 3-MH was measured by high performance liquid chromatography using an automated amino acid analyser (L-8500; Hitachi, Tokyo, Japan). The amount of muscle proteolysis was calculated as the urinary 3-MH to Cr ratio (3-MH/Cr). The urinary excretion of 3-MH is proportional to muscle mass as is urinary Cr excretion. Consequently, the 3-MH/Cr has a smaller inter-individual variation than does 3MH itself.

Muscle strength measurements

Muscle strength measurements of grip strength (GS), isometric knee extensor strength (IKES), maximum inspiratory pressure (MIP), and maximum expiratory pressure (MEP) were performed within 3 days prior to the operation and then again on POD 7 and 14. The magnitude of the change (Δ) in muscle strength was determined by subtracting the postoperative values from the preoperative values.

GS was measured using a JAMAR hand-held dynamometer (Biometrics Ltd., Ladysmith, USA). The patients were seated with the elbow flexed at 90° and the forearm in neutral position, and were asked to squeeze the handle as much as possible. Each hand was measured three times and the highest value was recorded. IKES was measured with the patient seated at the edge of the bed with the hip and knees flexed at 90º. The hand-held dynamometer (μ Tas F-1; Anima Corporation; Tokyo, Japan) was placed on distally anterior of the tibia to against the isometric knee extension contractions. KEIS were expressed in kg and as a percentage of body weight. The highest value from two measurements was recorded. MIP and MEP were measured using a respiratory dynamometer (Vitalopower KH-101; Chest, Tokyo, Japan) as an index of respiratory muscle strength. The patients seated position and sustained maximum effort against an interrupted airway for each test. The best of three consecutive attempts was recorded for MIP and MEP.

Statistical methods

The relationships among IL-6 production immediately after surgery, cumulative 3-MH/Cr during 5 days after surgery and Δ muscle strength on POD 7 and POD 14 were examined by Pearson's correlation coefficient. Changes in IL-6, BCAA, AAA, GH, IGF-1, 3-MH/Cr, and muscle strength were examined by repeated-measures analysis of variance (ANOVA). When the ANOVA showed significant differences, a post hoc analysis was performed using Tukey's test. $P < 0.05$ was considered statistically significant. Data for IL-6, IGF-1, GH, and 3-MH/Cr were logarithmically transformed in all analyses because of the skewed distribution in this study. Data are expressed as the mean ± SD. Statistical evaluation of the data was completed with SPSS 19.0J (SPSS Japan Inc., Tokyo, Japan).

Results

Patient characteristics are listed in Table 1.

Change in catabolic and anabolic indicators

Interleukin-6 and cortisol, the catabolic factors, were significantly increased at 4 hours post-surgery and returned to baseline on POD 1 (Fig. 4 A, B). In contrast, anabolic indicators of the BCAA-to-AAA ratio (BCAA/AAA) and the IGF-1-to-GH ratio (IGF-1/GH) were significantly decreased on POD 1 (Fig. 4 C, D).

Urinary 3-MH/Cr during 5days after surgery

A marked increase in urinary 3-MH/Cr was shown from POD 3 to 5 with its peak on POD 4 (Fig. 5).

The log cumulative 3-MH/Cr was positively associated with the log IL-6 production (Fig. 6).

Relationship between the change in muscle strength and 3-MH/Cr after surgery

The time-course of the mean percentage changes in each muscle strength exhibited similar trends. The rate of decrease in GS, MIP, MEP and KEIS were 28.2, 41.6, 40.4, and 51.7% on POD 7, and 13.5, 17.3, 22.0, 39.6% on POD 14, respectively (Fig. 7). The decrease in GS, MIP, and MEP on POD 7 (Fig. 8A), as well as in GS, MIP, KEIS on POD 14, were positively associated with log cumulative 3-MH/Cr (Fig. 8B).

Discussion

We found that a marked increase in urinary 3-MH/Cr, an established marker of muscle proteolysis, occurred until POD 5 and was associated with postoperative inflammation and systemic skeletal muscle weakness. This finding suggests that muscle proteolysis via postoperative hyper-catabolism may be responsible for the functional decline that is observed in patients undergone cardiovascular surgery.

A few studies have conducted to focus on the mechanism of skeletal muscle wasting during postoperative phase, but not using distinctive biochemistry [5]. In the present study, we determined that urinary 3-MH/Cr remarkably increased on POD 3 and reached a peak on POD 4. Because of 24-hour urine collection and the fact that 3-MH cannot be reutilised for protein synthesis [10,11], the urinary 3-MH/Cr in this study can be considered to reflect muscle proteolysis that occurred on the previous day. Based on this understanding, the finding of urinary 3-MH/Cr indicates that muscle proteolysis accelerated 24 hours post-operation (POD 2). This suggests that 48 hours following the operation will become a target period for interventions to preserve skeletal muscle mass.

The findings for the postoperative inflammatory reaction in this study are consistent with previous studies that demonstrated an immediate increase in pro-inflammatory cytokine such as tumour necrosis factor (TNF)-α and IL-6 after surgery [6-8]. The systemic inflammatory reaction may have occurred in response to surgical trauma, extracorporeal circulation, and perioperative hypothermia [21]. TNF-α has been regarded as an important proinflammatory factor to activate the synthesis of cytokines, including IL-6 [22] and to elicit direct muscle protein breakdown through multiple proteolytic mechanisms, such as the nuclear factor-kappa B signalling pathway [23,24]. On the other hand, IL-6 is responsible for the coordination of the acute phase response that consists of fever, leukocytosis, altered vascular permeability, and increased production of acute phase proteins [22]. Therefore, in this study, IL-6 may be considered as an indicator that reflects a catabolic phase, instead of as a factor that directly regulates muscle proteolysis during the postoperative acute phase [23-25].

The catabolic state was also evident in our results from hormonal changes and abnormalities in amino acid metabolism. In our study, we observed an increased serum cortisol level, which promotes proteolysis and lipolysis to produce gluconeogenic precursors [26,27] and the resistance of IGF-1 response to GH secretion. These metabolic effects of the hormonal changes indicate increased catabolism, which mobilises substrates to provide energy sources [28-30]. Finally, a reduced BCAA to AAA ratio also indicated the catabolic state in the early postoperative period [31] because BCAA are the greatest producers of energy in conditions under severe stress. These findings suggested that protein breakdown in the muscle was, in part, due to the metabolic response to surgical stress [32]. Furthermore, from the observation of a moderate correlation among immediate postoperative IL-6 production, urinary 3-MH/Cr and skeletal muscle weakness, we speculated that the postoperative skeletal muscle weakness was most likely the result of catabolism-induced muscle proteolysis due to cardiac surgery, not mere skeletal muscle deconditioning.

Our study has several limitations. Firstly, our study is single-centre, relatively small and observational in nature, making it prone to bias. Secondly, our study may have been subjected to a selection bias; for example, factors enhanced the catabolic response, such as chronic kidney disease, and prolonged mechanical ventilation was eliminated. Furthermore, inactivity or immobilization immediately after surgery may have individually differed and may have been confounded in muscle catabolism. Finally, we did not measure other catabolic factors, such as TNF-α, because of the rapid and slight change during operation. Further investigations are warranted to clarify the precise mechanism of postoperative muscle proteolysis.

Summary of study 1

The results of the present study suggested that postoperative anabolic/catabolic imbalance may be a key consideration for postoperative muscle weakness after surgery. A clinical approach to counteract this possible mechanism will be necessary to prevent muscle weakness after cardiac surgery.

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Ⅲ. STUDY 2

Methods

Subjects

In study 1, we demonstrated, by analysing 24-h urine samples for 5 days, that the peak of increased urinary 3-MH/Cr after cardiac surgery occurred on POD4. We also found a strong correlation (r = 0.873, $P < 0.0001$) between cumulative 3-MH/Cr (cum3-MH/Cr) excretion over 3 days and that over 5 days after cardiac surgery. Based on these findings, we measured the 3-MH/Cr up to POD3 to provide an index of total postoperative muscle proteolysis following cardiac surgery. Consequently, we analysed sixty-nine patients who measured 3-MH/Cr during 3 days after surgery between 2011 and March 2013.

Data collections

6MWD were measured on POD14 in addition to the data in study 1 (Fig 3). 6-min walking test (6MWT) was performed according to the ATS guidelines [20] in a 30m long passageway situated in our centre. Participants were encouraged to walk as much distance as they could, with supervision by a physiotherapist during testing. Patients were permitted to stop walking when they developed either dyspnoea or fatigue. This test was performed two times on POD 14 and the better of the two tests was adopted.

Statistics

Bivariate analyses between the clinical variables and level of the cum3-MH/Cr during the three days after surgery were tested by Pearson's correlation. Multivariate linear regression analysis was performed to identify predictive factors associated with the cum3-MH/Cr. When comparing means and frequencies, the unpaired *t* test and chi-square test were used. Stepwise logistic regression analysis was applied to determine the significant factors for 6MWD < 300 m on POD 14. Potential factors of interest with bivariate $P < 0.2$ were entered into multivariable models; age and gender were entered into these models as exceptions, since muscle mass is known to be affected by both aging and gender. Statistical evaluation of the data was performed with SPSS 19.0J (SPSS Japan Inc., Tokyo, Japan). Data are expressed as the means ± standard deviation or absolute numbers and percentages. A two-tailed *P*-value less than 0.05 was considered to indicate statistical significance.

Results

Patient characteristics

Patient characteristics are listed in Table 2. Preoperative average muscle strength in GS, IKES, MIP and MEP were 32.8 ± 11.1 kgf, 0.38 ± 0.19 kgf/kg, 58.6 ± 18.1 cmH_2O and 62.2 ± 21.3 cmH_2O, respectively. The rates of decrease in GS, IKES, MIP and MEP were 29.7%, 18.2%, 23.0% and 19.8% on POD 14, respectively.

Factors linked to increased cum3-MH/Cr during 3 days after surgery

We found correlations between cum3-MH/Cr and preoperative GS ($r = –0.528$, $P < 0.0001$) and immediate postoperative IL-6 ($r = 0.505$, $P < 0.0001$) (Table 3). Using multivariate analysis, BMI, CPB time, level of haemoglobin, preoperative GS and immediate postoperative IL-6 excretion were independently associated with an increase in 3-MH/Cr (adjusted $R^2 = 0.53$, $P < 0.0001$) (Table 4).

Relationship between cum3-MH/Cr and 6MWD < 300 m

The 3-MH/Cr gradually and significantly increased from 148.2 ± 44.2 on POD1 to 208.9 ± 68.2 on POD2 and 319.5 ± 93.8 on POD 3, and the mean value of the cum3-MH/Cr over the 3-day period was 676.7 ± 169.0. The 6MWD test on POD 14 was well-tolerated in all patients with a mean walking distance of 328.0 ± 78.8 m. The absolute value of distance walked was inversely related to the cum3-MH/Cr after surgery ($r = –0.696$, $P < 0.0001$). A comparison of the clinical variables of patients with postoperative 6MWD ≥ 300 m and < 300m is shown in Table 5. Significant differences were observed for gender, BMI, preoperative GS, immediate postoperative IL-6 levels and the cum3-MH/Cr between the groups. Stepwise logistic regression analysis showed two significant predictors for 6MWD < 300 m: preoperative grip strength (OR = 0.88, 95%CI = 0.80 – 0.96, $P = 0.007$), and the cum3-MH/Cr (OR = 1.09, 95%CI = 1.03 – 1.14, $P = 0.002$) (Table 6).

Discussion

This study has demonstrated that reduced 6MWD after cardiac surgery was affected not only by preoperative muscle weakness, but also by muscle proteolysis induced by surgery. To our knowledge, this is the first study to indicate that muscle proteolysis following surgery can be one of the determinants of 6MWD, and in turn worse prognoses [33], especially in patients with a low tolerance for exercise. These findings also suggest that, in patients with lower 6MWD, muscle proteolysis immediately after cardiac surgery is a novel therapeutic target in rehabilitation intervention.

The results of multiple regression analysis selected both operative factors (longer CPB time and immediate postoperative IL-6 elevation) and prognostic factors (lower GS, BMI and Hb) as independent predictors of the cum3-MH/Cr. The operative factors selected here are known to be considerable accelerating factors of the catabolic state [8], suggesting that these factors also up-regulated muscle proteolysis after surgery. The immediate postoperative catabolic state in this study was evident from significantly enhanced IL-6 production and suppressed anabolic state as indicated by reduction in IGF-1/GH and BCAA/AAA [29-31]. Our previous study indicated that lower GS and BMI was associated elevated IL-6 levels immediately after surgery [6]. CPB has also been reported as a factor facilitating the immune response during cardiac surgery. Hb levels are also known to be greatly reduced by hepcidin synthesis through activation of inflammatory cytokines [34]. These reports suggested that postoperative 3-MH excretion may be determined by surgery-induced catabolism and preoperative factors such as enhanced inflammation. Thus, patients with predictive parameters for increased postoperative muscle proteolysis, may be candidates for preventive interventions in the perioperative phase.

In the present study, the average 6MWD was equivalent to previous studies [33,35], whilst the present study confirmed the independent relationship of preoperative GS and the cum3-MH/Cr to postoperative 6MWD < 300 m. Notably, reduced preoperative GS has been reported to be associated with functional decline, disability, mortality and increased postoperative complications in surgical patients [36], and is regarded as a preoperative catabolic indicator. In addition, our previous study indicated that surgery-induced muscle proteolysis was related to decreases in GS, IKES and respiratory muscle strength after cardiac surgery [37]. These data suggest that elevation of muscle proteolysis induced by surgical stress may intervene between preoperative catabolic factors, such as low GS, and postoperative muscle mass, which are linked to reduced walking speed after surgery. Furthermore, our preoperative data showing decreased IGF-1/GH and BCAA/AAA in patients with postoperative 6MWD < 300 m indicated that these patients were already in a hyper-catabolic state before surgery, which may contribute towards accelerated postoperative catabolism. This suggests that patients in the preoperative catabolic state should be screened and provided preoperative intervention for postoperative weakness.

This study had several limitations. First, this was a single-center study with a relatively small cohort. Second, our study may have been subject to selection bias. These results may not be generalizable to high-risk populations. Finally, we did not measure preoperative 6MWD or walking speed in the participants of this study, because of the mobility limitation induced by cardiac symptom before surgery. Further studies are warranted to clarify the precise mechanism of postoperative muscle proteolysis and the relationship between muscle proteolysis and low 6MWD after cardiac surgery. Nevertheless, our findings provide a fundamental source for underlying mechanisms of postoperative functional decline in patients undergone cardiac surgery.

Summary of study 2

In this study 2, reduced postoperative 6MWD may be caused by a poor preoperative functional status and postoperative muscle proteolysis. It may therefore be necessary to develop clinical approaches to counteract the postoperative triggers of muscle proteolysis in these patients.

Ⅳ. CONCLUSIONS

The findings in this study suggested that immediate postoperative muscle catabolism may affect muscle weakness and low 6MWD after cardiac surgery.

To date, postoperative muscle weakness has been generally regarded as muscle deconditioning after cardiac surgery and it was impossible to improve the immediate postoperative functional weakness by conventional rehabilitation intervention. In this study, we indicated that operative invasion-induced muscle proteolysis may, at least in part, cause muscle weakness and postoperative low 6MWD related to worsen prognosis. In addition, the augmentation of postoperative muscle proteolysis was prolonged at least during 5 days after surgery. These facts suggest that, firstly, the patients in the preoperative catabolic state should be screened and provided preoperative and/or immediate postoperative intervention for muscle catabolism. As the perioperative management designed to correct the catabolic/anabolic imbalance, we propose that improvements in surgical techniques, anaesthesia, perioperative nutrition therapy, early mobilization, and muscle contraction such as a neuromuscular electrical stimulation therapy.

In conclusions, the findings of the present study suggest that postoperative anabolic/catabolic imbalance may be a key consideration for postoperative functional status and provide a new framework of preventive approach against perioperative muscle metabolism, particularly in patients with poor prognosis.

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Table 1: Baseline characteristics and procedural details in 41 patients

Male, n (%)	31 (75.6)
Age, years	68.1 ± 9.7
Body mass index, kg/m^2	22.6 ± 3.2
Brain natriuretic peptide, pg/ml	197.5 ± 174.6
Hemoglobin, g/dl	12.7 ± 1.51
Hemoglobin A1c, %	6.11 ± 0.85
Surgery, n (%)	
CABG	20 (48.8)
VR	17 (41.4)
CABG+VR	4 (9.8)
Operation time, min	216.6 ± 82.7
CPB duration, min	118.6 ± 67.4
Aortic cross-clamp duration, min	86.7 ± 41.4
Preoperative comorbidities, n	
Hypertension;	22
Dyslipidemia;	13
Chronic heart failure;	12
Diabetes Mellitus;	11
Chronic kidney disease;	1
Left ventricle ejection fraction, %	60.97 ± 14.0
Preoperative medication, n (%)	
Statin	32 (78.0)
βblockers	30 (73.2)
ACE inhibitors or ARBs	18 (43.9)
Calcium channel blockers	12 (29.3)
Diuretics	11 (26.8)

CABG=coronary artery bypass grafting. VR=valve replacement. CPB=cardiopulmonary bypass. ACE=angiotensin-converting enzyme. ARBs=angiotensin-Ⅱ-receptor blockers. Continuous data are shown as mean ± standard deviation.

Table 2: Patient characteristics and procedural details in 69 patients

Male, n (%)	53 (76.8)
Age, years	69.1 ± 8.8
Body mass index, kg/m^2	23.1 ± 4.1
Brain natriuretic peptide, pg/ml	197.5 ± 174.6
Hemoglobin, g/dl	12.7 ± 1.51
Hemoglobin A1c, %	6.11 ± 0.85
Surgery, n (%)	
CABG	34 (49.3)
VR	26 (37.7)
CABG+VR	9 (13.0)
Operation time, min	216.6 ± 82.7
CPB duration, min	118.6 ± 67.4
Aortic cross-clamp duration, min	86.7 ± 41.4
Preoperative comorbidities, n (%)	
Hypertension;	37 (53.6)
Dyslipidemia;	22 (31.9)
Chronic heart failure;	20 (29.0)
Diabetes Mellitus;	18 (26.1)
Left ventricle ejection fraction, %	63.75 ± 13.3
Preoperative medication, n (%)	
Statin	54 (78.3)
β-blockers	50 (72.5)
ACE inhibitors or ARBs	30 (43.5)
Calcium channel blockers	20 (29.0)
Diuretics	18 (26.1)

CABG, coronary artery bypass grafting; VR, valve replacement; CPB, cardiopulmonary bypass; ACE, angiotensin-converting enzyme; ARBs, angiotensin-Ⅱ-receptor blockers. Continuous data are shown as mean ± standard deviation.

Table 3. Correlation of clinical variable with cum3-MH/Cr during 3 days after surgery

Variable	*r* (95%CI)	*P*-value
Preoperative		
Age	0.07 (-0.19, 0.302)	0.565
Female	0.067 (-0.173, 0.98)	0.589
BMI	-0.432 (-0.60, -0.218)	0.0002
LVEF	0.145 (-0.095, 0.369)	0.233
HbA1c	0.066 (-0.174, 0.298)	0.591
CPB time, min	0.16 (0.080, 0.382)	0.189
CRP, mg/dl	0.239 (0.002, 0.45)	0.048
Hemoglobin	-0.255 (0.020, 0.464)	0.034
Grip strength, kg	-0.528 (-0.68, -0.333)	<0.0001
IKES, kgf/kg	0.056 (-0.183, 0.289)	0.648
MIP, mmHg	-0.036 (-0.27, 0.202)	0.769
MEP, mmHg	-0.078 (-0.309, 0.162)	0.525
IL-6, pg/ml	0.158 (-0.08, 0.38)	0.195
Cortisol, μg/ml	-0.079 (-0.31, 0.161)	0.520
IGF-1 / GH	-0.039 (-0.273, 0.199)	0.748
BCAA / AAA	-0.194 (-0.412, 0.04)	0.109
Immediate postoperative		
IL-6, pg/ml	0.505 (0.305, 0.663)	<0.0001
Cortisol, μg/ml	0.103 (-0.307, 0.331)	0.401
IGF-1 / GH	0.121 (-0.119, 0.348)	0.320
BCAA / AAA	-0.03 (-0.269, 0.204)	0.780

Cum3-MH/Cr, cumulative 3-methylhistidine; BMI, body mass index; Hb, hemoglobin; LVEF, left ventricular ejection fraction; CPB, cardiopulmonary bypass; IL-6, interleukin-6; IGF, insulin-like growth factor; GH, growth hormone; BCAA, branched chain amino acid; AAA, aromatic amino acid

Table 4. Multiple regression models for independent predictors of cum3-MH/Cr

Variable	*β* (SE)	95%CI	*P* value	Adjusted R^2
Preoperative				0.531
BMI	-14.57 (4.25)	-23.2, -6.07	0.001	
CPB time, min	-0.511 (0.25)	-1.01, -0.002	0.049	
CRP, mg/dl	27.14 (14.78)	-2.41, 56.7	0.071	
Hemoglobin	25.66 (9.14)	7.37, 43.9	0.007	
IL-6, pg/ml	0.094 (1.24)	-2.38, 2.57	0.940	
BCAA / AAA	-21.89 (26.23)	-74.3, 30.6	0.407	
Grip strength, kg	-4.66 (1.51)	-7.67, -1.64	0.003	
Immediate postoperative				
IL-6, pg/ml	0.410 (0.13)	0.15, 0.66	0.002	

cum3-MH/Cr, cumulative 3-methylhistidine; BMI, body mass index; LVEF, left ventricular ejection fraction; CPB, cardiopulmonary bypass; IL-6, interleukin-6; IGF, insulin-like growth factor; GH, growth hormone; BCAA, branched-chain amino acid; AAA, aromatic amino acid

Table 5. Comparison of the laboratory data and functional status based on postoperative 6MWD

	6MWD <300m (n=27)	6MWD ≧300m (n=42)	P value
Preoperative			
Age, year	70.5±7.9	68.7±9.3	0.307
Female ,n (%)	12 (44.4)	4 (9.5)	0.002
BMI, kg/m²	21.4±2.2	24.1±3.7	0.002
LVEF, %	64.7±10.0	63.1±10.3	0.636
Hemoglobin, g/dl	13.1±1.7	12.8±1.6	0.449
Hemoglobin A1c ,%	6.05±0.70	5.94±0.77	0.538
IL-6, pg/ml	7.71±17.9	3.85±5.14	0.283
IGF-1 / GH	284.5±256.8	895.9±1114.8	0.001
BCAA / AAA	3.09±0.48	3.61±0.61	0.003
Chronic heart failure, n (%)	11 (40.7)	9 (21.4)	0.146
Diabetes mellitus, n (%)	9 (33.3)	9 (21.4)	0.413
Grip strength, kg	24.9±8.3	37.8±9.7	0.0001
IKES, kgf/kg	0.377±0.22	0.384±0.16	0.891
MIP, mmHg	57.1±18.4	59.5±18.1	0.582
MEP, mmHg	59.4±20.0	64.0±22.1	0.382
Immediate postoperative			
IL-6, pg/ml	256.1±138.2	180.0±100.4	0.015
IGF-1 / GH	312.2±347.7	335.0±368.4	0.798
BCAA / AAA	3.10±0.47	3.41±0.75	0.035
Postoperative day 1			
IL-6, pg/ml	75.4±45.0	62.2±49.2	0.332
IGF-1 / GH	81.7±158.2	105.2±167.6	0.564
BCAA / AAA	2.00±0.28	2.29±0.41	0.008
During 3days after surgery			
Cum3-MH/Cr	781.8±150.0	609.1±145.5	0.0003
Postoperative day 14			
Grip strength, kg	21.8±8.1	33.1±13.9	0.0006
IKES, kgf/kg	0.274±0.15	0.336±0.17	0.128
MIP, mmHg	41.8±18.7	55.1±18.8	0.009
MEP, mmHg	40.4±18.6	48.2±18.8	0.104

6MWD, 6 minutes walking distance; BMI, body mass index; LVEF, left ventricular ejection fraction; IL-6, interleukin-6; IGF-1, insulin-like growth factor-1; GH, growth hormone; BCAA, branched-chain amino acid; AAA, aromatic amino acid; IKES, isometric knee extensor strength; MIP, maximum inspiratory pressure; MEP, maximum expiratory pressure; cum3-MH/Cr, cumulative 3-methylhistidin/creatinine.

Table 6. Results of stepwise logistic regression analysis for predicting of 6MWD <300m

Variable	OR	95%CI	*P*
Preoperative grip strength, kg	0.88	0.80, 0.96	0.007
Cumulative 3-methylhistidine/creatinine	1.09	1.03, 1.14	0.002

Variables included in regression model: age; gender; body mass index; preoperative insulin-like growth factor -1 / growth hormone; preoperative and postoperative branched-chain amino acid / aromatic amino acid; preoperative grip strength; immediate postoperative interleukin-6; and cumulative 3-methylhistidine/creatinine. Abbreviation: 95% CI, 95% confidence interval; OR, odds ratio.

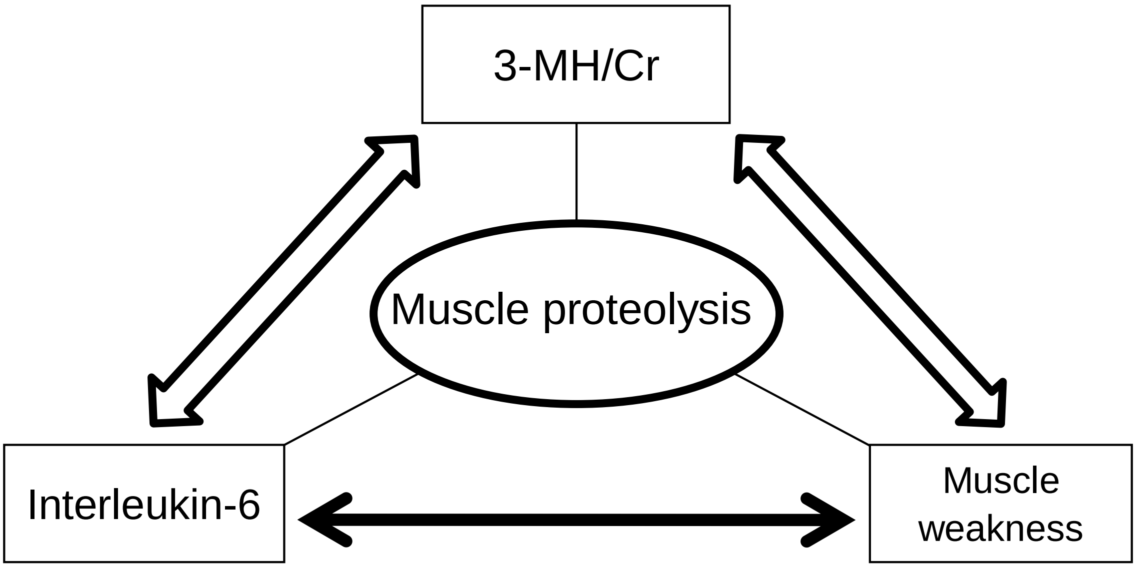


Figure 1. Study 1 was aimed to examine the relationship among urinary 3-methylhistidine/creatinine (3-MH) concentration, interleukin-6 as an inflammatory cytokine, and skeletal muscle weakness in patients undergone cardiac surgery.

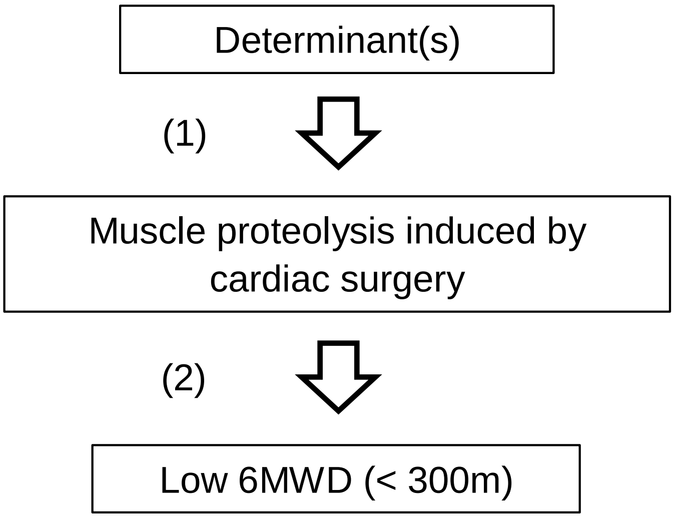


Figure 2. Study 2 was performed to explore (1) the factors underlying increased muscle proteolysis, and (2) the relationship between muscle proteolysis and 6-min walking distance (6MWD) in patients undergone cardiac surgery.

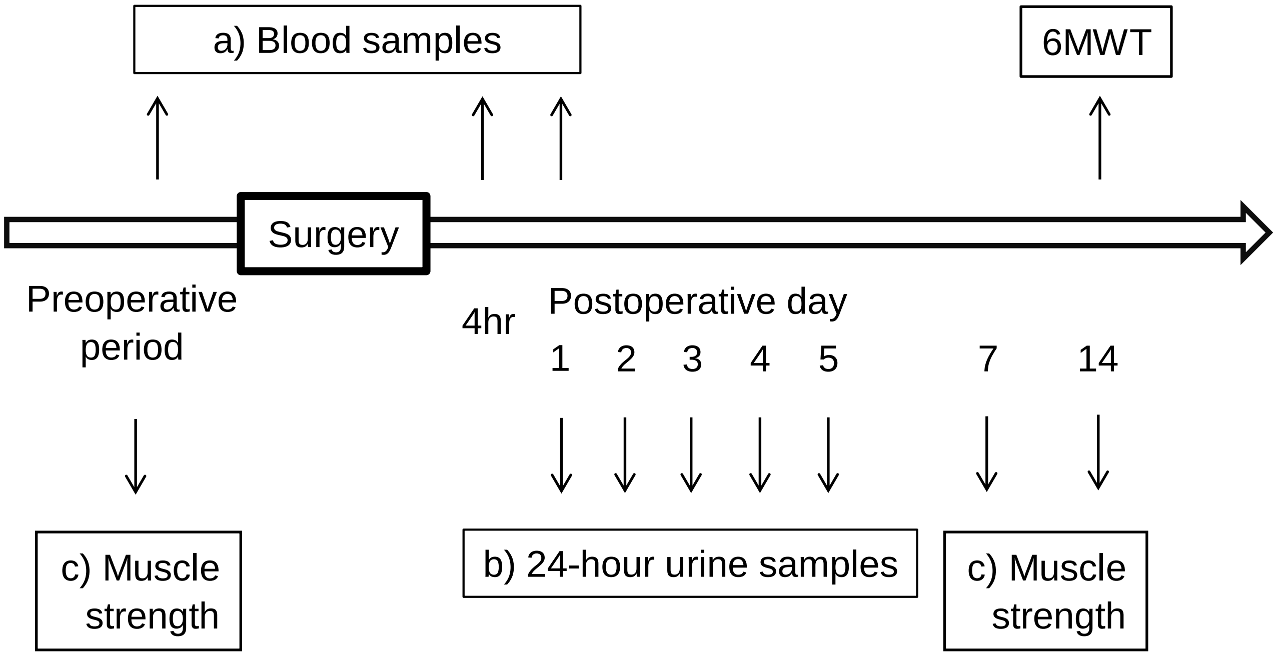


Figure 3. Study protocol and data collection.

The data in this study were collected from, (a) blood samples; IL-6, cortisol, IGF-1, GH, BCAA, and AAA, (b) 24-hour urine samples; 3-methylhistidine and creatinine, and (c) muscle strength; grip strength, isometric knee extensor strength, maximum inspiratory pressure, and maximum expiratory pressure. IL-6, interleukin-6; IGF-1, insulin-like growth factor-1; GH, growth hormone; BCAA, branched-chain amino acid; AAA, aromatic amino acid.

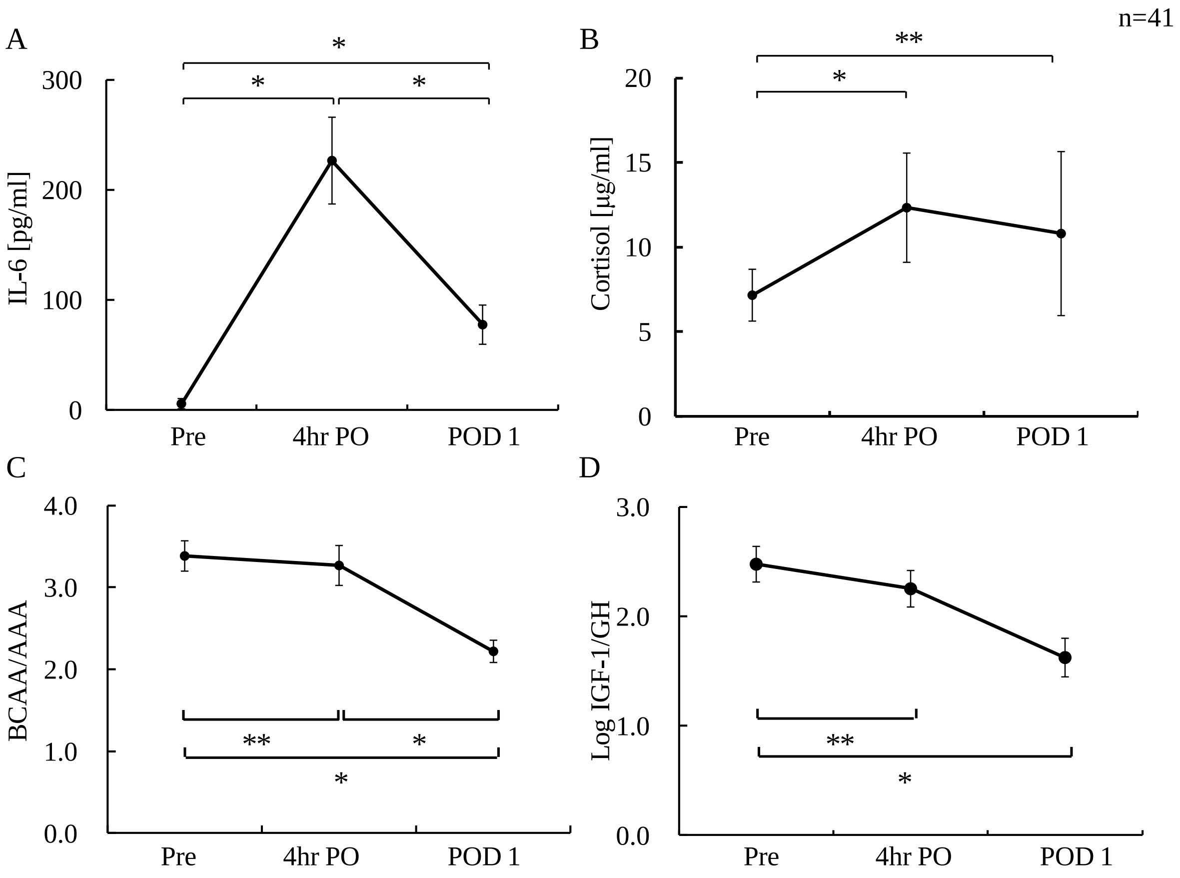


Figure 4. Immediate postoperative time courses of IL-6, BCAA/AAA, Cortisol, and Log IGF-1/GH

Interleukin-6 (A) and cortisol (B) were significantly increased at 4 hours post-surgery. The BCAA to AAA ratio (BCAA/AAA) (C) and IGF-1 to growth hormone ratio (IGF-1/GH) (D) were significantly decreased at post-operative day 1. *p<0·01. **p<0·05.

IL-6, Interleukin-6; BCAA, branched chain amino acids; AAA, aromatic amino acids; IGF-1, insulin-like growth factor-1; Pre, preoperative; 4 hr PO,4 hour postoperative; POD1, postoperative day 1.

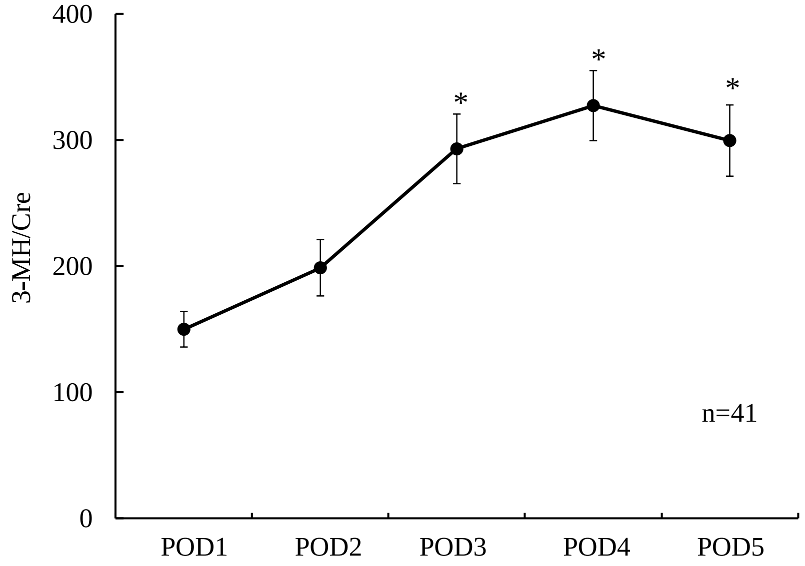


Figure 5. Postoperative time course of 3-MH/Cre during the 5 days after surgery

*p < 0·01 vs POD1. 3-MH, 3-methylhistidine; Cre, creatinine; POD, postoperative day.

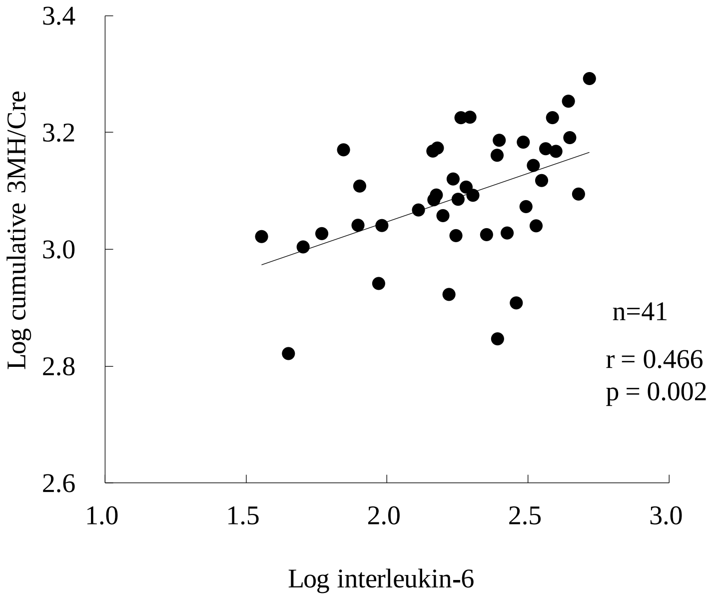


Figure 6. Relationship between immediate postoperative interleukin-6 production and cumulative 3-MH/Cre during the 5 days after surgery

Log 3-MH/Cre, logarithmic 3-methylhistidine/creatinine; Log interleukin-6, logarithmic interleukin-6.

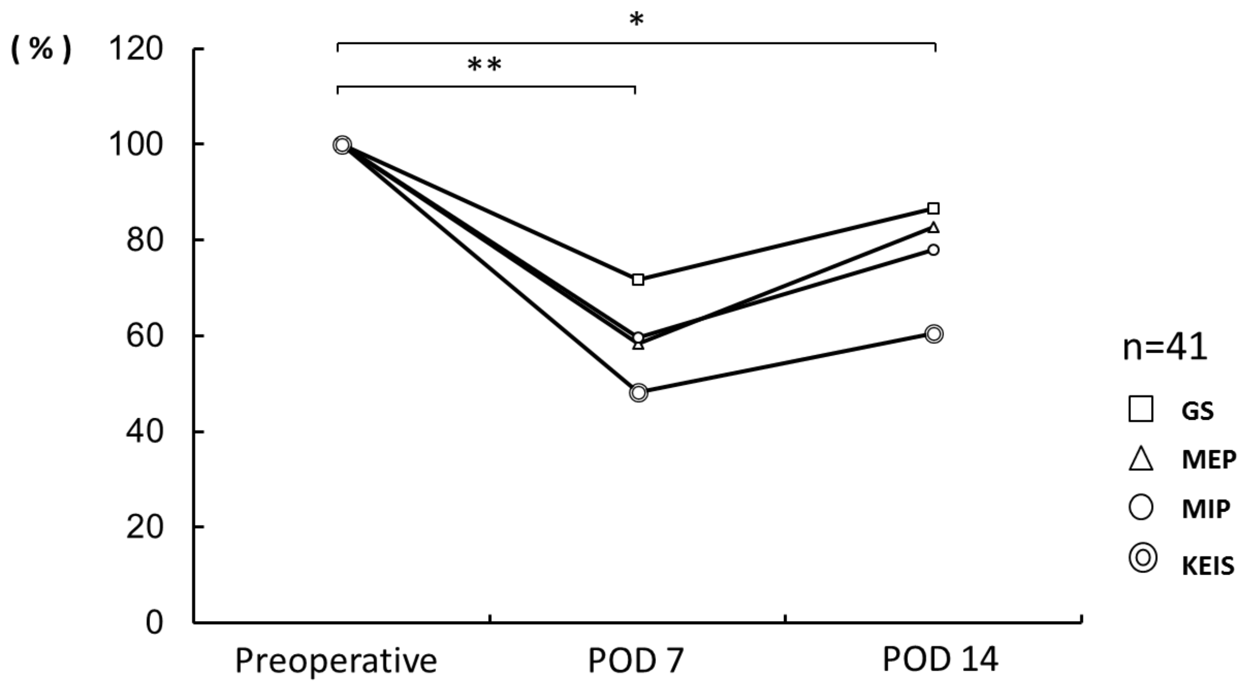
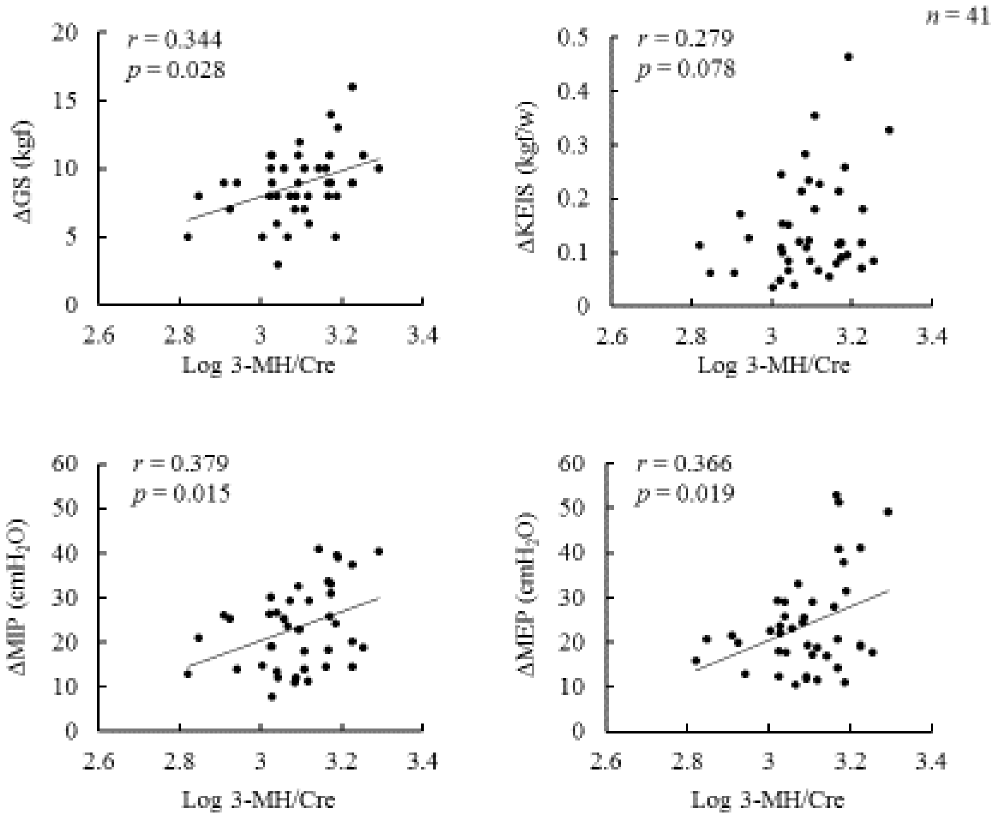


Figure 7. Mean percent change in muscle strengths after surgery (POD 7 and 14). Absolute peak grip strength measured in kilogram, maximum inspiratory and expiratory pressure measured in centimeter H_20, and knee extensor isometric strength measured in kgf/kg were used in the calculation of percent changes. *$P < 0.01$. **$P < 0.05$. POD, post-operative day; GS, grip strength; MIP, maximum inspiratory pressure; MEP, maximum expiratory pressure; IKES, knee extensor isometric strength.

A


n = 41
r = 0.344
p = 0.028
ΔGS (kgf)
Log 3-MH/Cre
r = 0.279
p = 0.078
ΔKEIS (kgf/w)
Log 3-MH/Cre
r = 0.379
p = 0.015
ΔMIP (cmH2O)
Log 3-MH/Cre
r = 0.366
p = 0.019
ΔMEP (cmH2O)
Log 3-MH/Cre

B

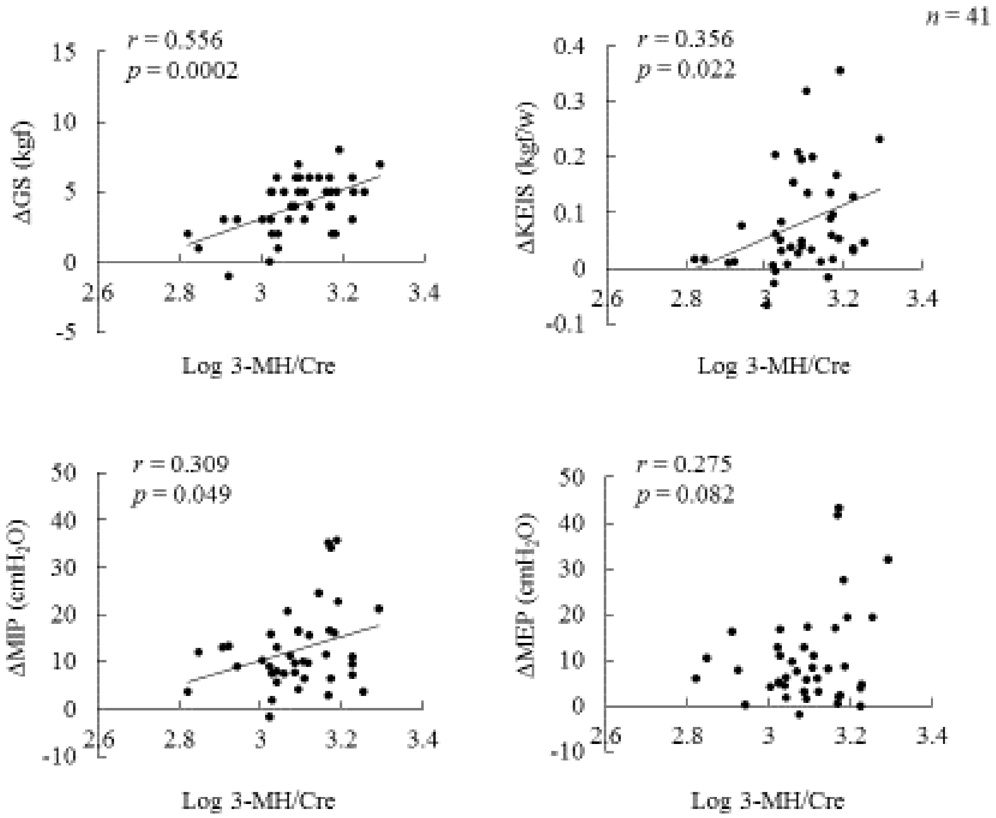

n = 41
r = 0.556
p = 0.0002
ΔGS (kgf)
Log 3-MH/Cre
r = 0.356
p = 0.022
ΔKEIS (kgf/w)
Log 3-MH/Cre
r = 0.309
p = 0.049
ΔMIP (cmH2O)
Log 3-MH/Cre
r = 0.275
p = 0.082
ΔMEP (cmH2O)
Log 3-MH/Cre

Figure 8. Relationship between cumulative 3-MH/Cre during the 5 days after surgery and decrease in skeletal muscle strength on POD 7 (A), and on POD 14 (B). Log cumulative 3-MH/Cre, logarithmic cumulative 3-methylhistidine/creatinine; GS, grip strength; IKES, isometric knee extensor strength; MIP, maximum inspiratory pressure; MEP, maximum expiratory pressure.

References

1. Convertino VA, Bloomfield SA, Greenleaf JE. An overview of the issues: physiological effects of bed rest and restricted physical activity. Med Sci Sports Exerc 1997; 29: 187–90.
2. Convertino VA. Cardiovascular consequences of bed rest: effect on maximal oxygen uptake. Med Sci Sports Exerc 1997; 29: 191–6.
3. Christensen T, Kehlet H. Postoperative fatigue. World J Surg 1993; 17: 220–5.
4. van Venrooij LM, Verberne HJ, de Vos R, Borgmeijer-Hoelen MM, van Leeuwen PA, de Mol BA. Postoperative loss of skeletal muscle mass, complications and quality of life in patients undergoing cardiac surgery. *Nutrition* 2012;28:40–5.
5. Bloch SA, Lee JY, Wort SJ, Polkey MI, Kemp PR, Griffiths MJ. Sustained elevation of circulating growth and differentiation factor-15 and a dynamic imbalance in mediators of muscle homeostasis are associated with the development of acute muscle wasting following cardiac surgery. *Crit Care Med* 2013;41:982–9.
6. Iida Y, Yamada S, Nishida O, Nakamura T. Body mass index is negatively correlated with respiratory muscle weakness and interleukin-6production after coronary artery bypass grafting. J Crit Care 2010; 25: 172.e1–8.
7. Sander M, von Heymann C, von Dossow V, Spaethe C, Konertz WF, Jain U, et al. Increased interleukin-6 after cardiac surgery predicts infection. Anesth Analg 2006; 102: 1623–9.
8. Diegeler A, Doll N, Rauch T, Haberer D, Walther T, Falk V, et al. Humoral immune response

during coronary artery bypass grafting: A comparison of limited approach, “off-pump” technique, and conventional cardiopulmonary bypass. Circulation 2000; 102(19 Suppl 3): S95–100.

9. Tsujinaka T, Ebisui C, Fujita J, Kishibuchi M, Yano M, Monden M. Muscle Wasting and IL-6. Basic Appl Myol 1998; 8: 361–70.

10. Sugawara T, Ito Y, Nishizawa N, Suzuki H, Kobayashi H, Nagasawa T. Measurement of the rate of myofibrillar protein degradation using the arteriovenous difference in plasma 3-methylhistidine concentration of rats. J Nutr Sci Vitaminol 2009; 55: 381–4.

11. Vesali RF, Cibicek N, Jakobsson T, Klaude M, Wernerman J, Rooyackers O. Protein metabolism in leg muscle following an endotoxin injection in healthy volunteers. Clin Sci 2010; 118: 421–7.

12. van Venrooij LM, de Vos R, Borgmeijer-Hoelen MM, Haaring C, de Mol BA. Preoperative unintended weight loss and low body mass index in relation to complications and length of stay after cardiac surgery. *Am J Clin Nutr* 2008;87:1656–61.

13. van Venrooij LM, de Vos R, Zijlstra E, Borgmeijer-Hoelen MM, van Leeuwen PA, de Mol BA. The impact of low preoperative fat-free body mass on infections and length of stay after cardiac surgery: a prospective cohort study. *J Thorac Cardiovasc Surg* 2011;142:1263–9.

14. Makary MA, Segev DL, Pronovost PJ, Syin D, Bandeen-Roche K, Patel P et al. Frailty as a predictor of surgical outcomes in older patients. *J Am Coll Surg* 2010;210:901–8.

15. Roul G, Germain P, Bareiss P. Does the 6-minute walk test predict the prognosis in patients with NYHA class II or III chronic heart failure? *Am Heart J* 1998;136:449–57.
16. Rostagno C, Olivo G, Comeglio M, Boddi V, Banchelli M, Galanti G et al. Prognostic value of 6-minute walk corridor test in patients with mild to moderate heart failure: comparison with other methods of functional evaluation. *Eur J Heart Fail* 2003;5:247–52.
17. Cacciatore F, Abete P, Mazzella F, Furgi G, Nicolino A, Longobardi G et al. Six-minute walking test but not ejection fraction predicts mortality in elderly patients undergoing cardiac rehabilitation following coronary artery bypass grafting. *Eur J Prev Cardiol* 2012;19:1401–9.
18. Afilalo J, Eisenberg MJ, Morin JF, Bergman H, Monette J, Noiseux N et al. Gait speed as an incremental predictor of mortality and major morbidity in elderly patients undergoing cardiac surgery. *J Am Coll Card* 2010;56:1668–76.
19. De Feo S, Tramarin R, Faggiano P, Ambrosetti M, Riccio C, Diaco T et al. The inability to perform a 6 minute walking test after cardio-thoracic surgery is a marker of clinical severity and poor outcome. Data from the ISYDE 2008 Italian survey. *Int J Cardiol* 2011;151:115–6.
20. ATS committee on proficiency standards for clinical pulmonary function laboratories. ATS statement: guidelines for the six-minute walk test. *Am J Respir Crit Care Med* 2002;166:111–7.
21. Ascione R, Lloyd CT, Underwood MJ, Lotto AA, Pitsis AA, Angelini GD. Inflammatory response after coronary revascularization with or without cardiopulmonary bypass. Ann Thorac Surg 2000; 69: 1198–204.

22. Rangel-Frausto MS, Pittet D, Costigan M, Hwang T, Davis CS, Wenzel RP. The natural history of the systemic inflammatory response syndrome (SIRS). JAMA 1995; 273:117–23.

23. Chai J, Wu Y, Sheng ZZ. Role of ubiquitin-proteasome pathway in skeletal muscle wasting in rats with endotoxemia. Crit Care Med 2003; 31: 1802–7.

24. Li H, Malhorta S, Kumar A. Nuclear factor-kappa B signalling in skeletal muscle atrophy. J Mol Med 2008; 86: 1113–26.

25. Williams A, Wang JJ, Wang L, Sun X, Fischer JE, Hasselgren PO. Sepsis in mice stimulates muscle proteolysis in the absence of IL-6. Am J Physiol 1998; 275: 1983–91.

26. Desborough JP. The stress response to trauma and surgery. Br J Anaesth 2000; 85: 109–17.

27. Peeters GMEE, van Schoor NM, van Rossumt EFC, Visser M, Lips P. The relationship between cortisol, muscle mass and muscle strength in older persons and the role of genetic variations in the glucocorticoid receptor. Clin Endcrinol 2008; 69: 673–82.

28. Petersson B, Wernerman J, Waller SO, von der Decken A, Vinnars E. Elective abdominal surgery depresses muscle protein synthesis and increases subjective fatigue: effects lasting more than 30 days. Br J Surg 1990; 77: 796–800.

29. Lang CH, Frost RA. Role of growth hormone, insulin-like growth factor-I, and insulin-like growth factor binding proteins in the catabolic response to injury and infection. Curr Opin Clin Nutr Metab Care 2002; 5:271–9.

30. Freund H, Yoshimura N, Lunetta L, Fischer JE. The role of the branched-chain amino acids in

decreasing muscle catabolism in vivo. Surgery. 1978; 83: 611–8.

31. Vente JP, von Meyenfeldt MF, van Eijk HM, van Berlo CL, Gouma DJ, van der Linden CJ, et al. Plasma-amino acid profiles in sepsis and stress. Ann Surg 1989; 209: 57–62.

32. Uehara M, Plank LD, Hill GL. Components of energy expenditure in patients with severe sepsis and major trauma: a basis for clinical care. Crit Care Med 1999; 27: 1295–302.

33. Opasich C, De Feo S, Pinna GD, Furgi G, Pedretti R, Scrutinio D et al. Distance walked in the 6-minute test soon after cardiac surgery: toward an efficient use in the individual patients. *Chest* 2004;126:1796–801.

34. Theurl I, Mattle V, Seifert M, Mariani M, Marth C, Weiss G et al. Dysregulated monocyte iron homeostasis and erythropoietin formation in patients with anemia of chronic disease. *Blood* 2006;107:4142–8.

35. Fiorina C, Vizzardi E, Lorusso R, Maggio M, De Cicco G, Nodari S et al. The 6-min walking test early after cardiac surgery. Reference values and the effects of rehabilitation programme. *Eur J Cardiothorac Surg* 2007;32:724–9.

36. Lee DH, Buth KJ, Martin BJ, Yip AM, Hirsch GM. Frail patients are at increased risk for mortality and prolonged institutional care after cardiac surgery. *Circulation* 2010;121:973–8.

37. Iida Y, Yamazaki T, Kawabe T, Usui A, Yamada S. Postoperative muscle proteolysis affects systemic muscle weakness in patients undergoing cardiac surgery. *Int J Cardiol* 2014;172:595–7.