In the present study, we investigated the beneficial and risky effects of exercise intended to prevent or treat lifestyle-related diseases on insulin sensitivity, lactic acid utilization, lipid metabolism, hepatic and renal oxidative stress, hepatic selenoprotein P and renal function in obese and glucose-intolerant rats with renal failure. We fed normal rats a 20% casein diet while the glucose-intolerant, obese rats received a high-fructose diet, and after then rats received single injection of vancomycin at a dose of 400 mg/kg for constructing the duplicative state of renal failure and diabetes mellitus. They were forced to run for 1 h/day, 6 days/week, for 10 weeks. Exercise reduced visceral fat and ameliorated insulin sensitivity in the high-fructose group, improved lactic acid usage efficiency, however, increased hepatic oxidative stress and complicated renal dysfunction in the normal and high-fructose fed groups with renal failure. Additionally, exercise upregulated hepatic selenoprotein P expression and enhanced renal antioxidative system in both groups. It is concluded that strictly controlled exercise conditions must be adapted to patient health states especially in view of kidney protection, and supplemental therapy is also recommended in parallel with exercise, using nutrients and vitamins for kidney protection.

Key Words: exercise effect, energy metabolism, renal failure, glucose intolerance, selenoprotein P

Type 2 diabetes is controlled and treated by the three main therapeutic strategies such as diet, exercise, and pharmacotherapy. Among them, regular exercise has been reported to substantially improve the development of diabetes, hypertension, and dyslipidemia. Additionally, it would maintain overall health condition, prevent lifestyle-related diseases, and prolong healthy life expectancy, which includes the increments of muscle mass and insulin sensitivity and improvements for obesity and cardiopulmonary function.

However, certain patients are known to possess low exercise tolerance and capacity mainly because of mutations in their energy production pathways. At most, 20% of all patients with type 2 diabetes present with inconsequent decreases in blood glucose level even after regular exercise. With regard to this report, it was recently reported that selenoprotein P, one of the selenium (Se)-containing proteins, is associated with exercise performance and diabetic status, where blood selenoprotein P concentrations were elevated in diabetic patients. Selenoprotein P eliminates reactive oxygen species (ROS) generated during exercise and may reversely induce type 2 diabetes through reductive stress, suggesting its possible contribution to exercise intolerance by alleviating the health-promoting effects of exercise.

Additionally, exercise is closely associated with biological trace metals such as iron, manganese, copper, zinc, and selenium which are cofactors in either antioxidative enzymes or glucose-metabolizing enzymes. Both the deficiency and excess of these metal cations may cause functional impairment. Determination of the exercise-induced fluctuations in biological trace metals may answer to evaluate exercise performance and diagnose pathological conditions. However, few studies have investigated the relationship between in vivo trace elements and exercise.

In our previous paper, we have indicated that the salutary and beneficial effects of exercise were substantially more pronounced in healthy condition than in glucose intolerance and obesity condition induced by high-fructose diet. Exercise for health maintenance was found to affect much differently on either healthy or diseased rats. Therefore, exercise conditions and regimens are strongly suggested to be adapted to health states of individual, which includes nutritional status, in advance before starting the systematic exercise training intended to prevent or treat lifestyle-related diseases.

From other studies, exercise has been reported to increase both the insulin sensitivity by enhancing insulin-independent glucose uptake and type I skeletal muscle fiber density rich in GLUT4 and mitochondrial mass, and exercise-mediated skeletal muscle transformation would also enhance insulin sensitivity. This mechanism is mediated by muscle contraction-induced activation of AMP-activated protein kinase (AMPK) and glucose transporter 4 (GLUT4) which govern transmembrane glucose transport.

There are many papers in which even diabetic patients with renal failure were improved at a certain level by exercise, while there are other opinions that the exercise strength should be more systematically customized and optimized for each patient.

Therefore, in this study, we tried to examine the merits and demerits of exercise effect under the different physiological and pathophysiological condition for the purpose to spread a wide
target. To this end, we used rats with the duplicative state of renal failure and diabetes mellitus (DM) and compared several parameters with healthy animals, where renal failure model was induced by single injection of vancomycin and, obesity and glucose intolerance were induced by a high-fructose diet (HF).

Here, we studied both the beneficial and risky effects of exercise on biological responses such as the in vivo energy metabolism, oxidative stress in organs, selenoprotein P expression, in vivo biological trace metals, and renal function in normal and diseased experimental animals.

Materials and Methods

Animal care. Male Wistar rats aged 4 weeks were purchased from CLEA Japan (Tokyo, Japan). All animals were housed in a temperature-controlled (22 ± 2°C) environment under a 12-h light/dark cycle and had ad libitum food and water access. The rats were fed a standard MF diet (Oriental Yeast, Tokyo, Japan) for the first week followed by either a 20% (w/w) casein diet (CA) or a high-fructose diet [HF; 58% (w/w) fructose] from ages 5–15 weeks.

The rats were divided into the following four groups at 5 weeks as follows; (1) 20% casein diet + no exercise [CA (–) without renal failure; normal group]; (2) 20% casein diet + no exercise [CA (–) with renal failure]; (3) 20% casein diet + exercise [CA (ex) with renal failure]; (4) high-fructose diet + no exercise [HF (–) with renal failure]; and (5) high-fructose diet + exercise [HF (ex) with renal failure]. Each group was fed the corresponding diet for 10 weeks.

After then, according to the experimental protocol (Fig. 1), vancomycin (VCM; vancomycin hydrochloride for intravenous infusion, Shionogi & Co., Ltd., Osaka) was once injected into jugular vein of rats for making renal failure model in rats at 11-week old. VCM dissolved in saline was injected at a dose of 400mg/kg body weight, which was known as the renal failure dose. The water supply was removed for 24 h before and after the VCM administration.

Body weight and food intake were measured weekly. At 15 weeks, the rats were euthanized by isoflurane after 16 h fasting. Blood was drawn from the abdominal aorta and transferred to heparin-coated vials. Plasma was prepared (1,000 × g, 10 min) and stored at −30°C until further analysis. Liver, kidney, pancreas, and adipocytes were collected and then stored at −30°C for measuring those weights. A part of liver tissue samples was frozen in liquid nitrogen and stored at −80°C prior to PCR and western blotting analyses.

All animal experiments were conducted in accordance with the Guidelines for Animal Experimentation of Kobe Women’s University, Kobe, Japan. The research protocol was approved by the Animal Experiment Committee of Kobe Women’s University, Kobe, Japan (Approval No. A208).

Exercise training. Rats aged 5 weeks in the exercise training groups were subjected to 10 weeks (6 days/week) of exercise. They ran for 1 h/day on a treadmill (MK-680; Muromachi Kikai, Tokyo, Japan) inclined at 0° grade and moving at 30 m/min.

Blood biochemical analyses. The plasma samples were measured by an automatic dry-chemistry analyzer (Fuji Dri-Chem 3500V; Fujifilm Medical, Tokyo, Japan). The measurement items were as follows: plasma high-density lipoprotein cholesterol (HDL-cho), total cholesterol (T-cho), triglyceride (TG), blood urea nitrogen (BUN), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein (TP), creatine phosphokinase (CPK), creatinine (CRE), and glucose (GLU). Moreover, plasma insulin levels were determined with a Morniga Ultrasensitive Rat Insulin ELISA Kit (Morniga Institute of Biological Science, Kanagawa, Japan) and lactic acid levels were determined with Lactate Pro2 (Arkray, Kyoto, Japan).

Trace element analysis. Fifty μl of plasma and 30 mg each of the liver, kidney, and pancreas samples were placed in 50 ml tall beakers and heated on hotplates to 150°C. Then 2 ml of 60% (v/v) nitric acid (HNO₃; Kanto Chemical, Tokyo, Japan) was added. Next time, 2 ml of 60% (v/v) perchloric acid (HClO₄; Kishida Chemical, Osaka, Japan) was added. Then, 2 ml of 30% (v/v) hydrogen peroxide (H₂O₂; Kishida Chemical) was added. This process was repeated thrice. Then another 2 ml of 30% (v/v) H₂O₂ was added and the samples were heated until digestion was complete. The liquid was evaporated, and the sample residues were cooled. Then 9 ml of 5% (v/v) HNO₃ was added and the residues were dissolved for 3 h. The solutions were then transferred to sample cups. All beakers and sample cups used in this experiment were washed with 1% (v/v) HNO₃ to avoid metal contamination. The trace elements [calcium (Ca), magnesium (Mg), copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), chromium (Cr), and Se] in the solutions were identified and quantitated by inductively coupled plasma-mass spectrometry (ICP-MS; Agilent 7700/Agilent Technologies, Santa Clara, CA). Standard curves were plotted by preparing 1,000 μg/ml (ppm) standard solutions of Ca, Mg, Cu, Fe, Mn, Zn, Cr, and Se (FujiFilm Wako Pure Chemical Industries Ltd., Osaka, Japan) and diluting them in 5% (v/v) HNO₃ to final metal concentrations of 0, 1, 5, 10, 50, 100, and 500 ng/ml (ppb). For quality control, 1 ng/ml (ppb) of a reference internal standard, indium (In), was measured along with the samples.

Measurement of artificial superoxide anion production. Superoxide anion (O₂⁻^•) was generated from the reaction between hypoxanthine and xanthine oxidase. For the hepatic and renal superoxide anion measurements, 2-methyl-6-p-methoxyphenylethylnilimidazo[pyrazinone (MPEC) was used to induce oxidation by superoxide anion as a substrate. Xanthine oxidase and hypoxanthine were prepared in phosphate buffer (0.1 M KH₂PO₄ buffer, pH 7.5). The reaction solution for the superoxide anion scavenging activity test consisted of 60 μl crude hepatic or renal

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**diagram**

![Fig. 1. Experimental protocol.](image-url)
enzyme solution, 10 μl of 300 μM MPEC, 170 μl of 0.1 M
KH₂PO₄ buffer, 60 μl xanthine oxidase solution (0.1 U/ml) and
50 μl of 3.6 mM hypoxanthine/KH₂PO₄. Crude hepatic or renal
gene solutions were obtained from sample homogenates pre-
pared in phosphate-buffered saline (PBS) as follows: 0.1 g liver or
kidney isolates were homogenized in 500 μl of 0.1 M PBS
(pH 7.3) and the homogenized samples were centrifuged at
1,800 g and 4°C for 15 min. The supernatants were collected
and used as crude hepatic or renal gene solutions. The reaction
was initiated by adding hypoxanthine. Fifty μl of the reaction
solution was placed in each Röhren tube (5 ml; 75 mm × 12 mm;
Sarstedt, Nümbrecht, Germany) and MPEC light emission
was measured with a luminometer (Lumat3 LB9508; Berthold
Technologies, Bad Wildbad, Germany).

RNA isolation, cDNA synthesis, and real-time PCR for
hepatic selenoprotein P. Frozen liver tissue stored at −80°C
was soaked in RNA later-ICE frozen tissue transition solution
(Thermo Fisher Scientific, Waltham, MA) at −20°C for ≥16 h.
Total RNA was isolated with a high pure RNA tissue kit (Roche
Diagnostics, Basel, Switzerland). The cDNA synthesis and PCR
were performed with a One Step SYBR PrimeScript Plus RT-PCR
Kit (Takara Bio Inc., Shiga, Japan) according to the manufacturer’s
instructions. The mRNA expression of selenoprotein P was
analyzed in a LightCycler Nano (Roche Diagnostics). The PCR
amplicons were quantified with LightCycler software (Roche
Diagnostics). Data were internally normalized to the β-actin
housekeeping gene whose primer sequences were previously
described. Target primers were designed with the Universal Probe
Library Assay Design Center (Roche Diagnostics). Their specifici-
ties were confirmed by melting curve analysis. Following are the
target primer sequences: rat β-actin: R: GCCGCACTGTCGCACT
CTC, L: GGGAAATCGTGCGTGCACTT; rat selenoprotein P: R:
TGTCACGACCAAATGTGGAG, L: GGAGAAAAGAGA

Results

General remarks and behavior. In renal failure groups, the
body weights were smaller compared with normal group. Addition-
ally, the exercise further made the body weights decrease
(Table 1). Compared to the normal group, in the CA (−) with
the renal failure group, the liver weights were reduced, and this
reduction was recovered in the CA (ex) with the renal failure
group. In both HF groups with the renal failure, the liver weights
tended to increase (Table 1). In both the CA and HF groups with
the renal failure, the kidney weights increased compared to the
normal group. Especially, in the HF (ex) group, the kidney weight
showed the significant increment, suggesting that the duplicative
state of renal failure and DM are overload to the kidneys (Table1).
Moreover, even in both the CA and the HF groups with the renal
failure, the adipose tissue weights were significantly reduced by
exercise (Table 1).

Determination of blood parameters. Blood insulin levels
were lower in the exercised than the non-exercised rats in both
diagnostic diet groups. Especially, the insulin levels were
highest in the HF (−) group with renal failure and then those
levels significantly decreased by exercise in HF (ex) group (Table 2).
Lactic acid levels significantly decreased in the exercised than
the non-exercised rats in both experimental diet groups. Additionally,
the levels in both exercise groups were significantly lower than
the normal group (Table 2). In the HF groups with renal failure, the T-cho level increased in
the HF (−) group compared to the normal group and this increment
was reduced by the exercise in the HF (ex) group (Table 2).
In both the CA and HF groups with renal failure, the TG levels were
lower than the normal group, and the exercise more reduced TG in

Table 1. General characteristics of animals

| Renal function | Groups                  |
|----------------|-------------------------|
| Experimental diet | Normal       | Renal failure |
|                | CA (−)     | CA (ex)     | HF (−)     | HF (ex)    |
| Body weight (g) | 385 ± 17  | 363 ± 26    | 357 ± 20    | 352 ± 17   | 341 ± 14*  |
| Liver (g/100 g body weight) | 3.0 ± 0.1 | 2.8 ± 0.1   | 2.9 ± 0.1   | 3.2 ± 0.2** | 3.1 ± 0.2†* |
| Kidney (g/100 g body weight) | 0.32 ± 0.01 | 0.40 ± 0.04 | 0.40 ± 0.04 | 0.44 ± 0.04* | 0.52 ± 0.06**††§,# |
| Genital adipose tissue (g/100 g body weight) | 1.9 ± 0.3 | 1.7 ± 0.2   | 1.2 ± 0.1**±,†,# | 1.7 ± 0.2   | 1.3 ± 0.1**±,†,# |

Data are means ± SD. *p<0.05 and **p<0.01 vs CA (−) in normal group. †p<0.05 and ‡p<0.01 vs CA (−) with renal failure group. §p<0.01 vs CA (ex) with renal failure group. †p<0.05 vs HF (−) with renal failure group. CA (−) in normal group, CA (−), CA (ex) and HF (−) with renal failure, n = 4; HF (ex) with renal failure, n = 5.
with renal failure significantly decreased compared to the normal group (Table 2). In the renal failure groups, hepatic CRE levels showed the significant decrease in the CA (–) groups compared to CA and HF groups with renal failure, renal Ca levels showed the increment and the exercise tended to increase the GPx1 level, however, in the HF diet with renal failure, the exercise tended to reversely decrease the GPx1 level (Fig. 4). These results suggested that the exercise in the duplicative state of renal failure and DM would provide the oxidative damage to the liver.

### Discussion

We investigated both the beneficial and risky effects of exercise intended to prevent and ameliorate disease on the in vivo energy metabolism, oxidative stress in organs, selenoprotein P expression, in vivo biological trace metals, and renal function in glucose intolerance condition and renal failure. In this study, rats were exercised 6 days/week for 10 weeks (Fig. 1). Since glucose intolerance and obesity are characteristic symptoms of type 2 diabetes, rats were fed 58% (w/w) high-fructose diets to induce the diabetic model. Fructose is rapidly metabolized in the liver, and does not induce the insulin secretion but causes insulin resistance and lipid metabolism disorders.\(^{22}\) Additionally, rats were received intravenous single injection of VCM to induce the renal failure model.\(^{21}\) It was recently reported that VCM-induced nephrotoxicity and renal dysfunction are based on free radical injury and that VCM-induced apoptosis occurs in renal tubular cells through the production of mitochondrial ROS and peroxidation of mitochondrial membrane phospholipid.\(^{24,25}\) Exercise is also known to activate enzymatic reactions in the mitochondria and generate superoxide anion.

Endurance exercise at both the 50–70% of the maximum oxygen uptake for at least 3–5 days/week.\(^{27}\) It corresponded to treadmill running at 30 m/min and 0° inclination for 1 h/day and 6 days/week for 10 weeks.

For both the CA and HF diet groups with renal failure, rat body weights and food intake decreased in response to exercise. These parameters markedly decreased for the exercised group with HF diet. Here, rats in the exercised groups were assumed to lose body weights because of the exercise, digestive hormones, and stress response reducing food intake.\(^{28,29}\) Additionally, continuous exercise significantly decreased epididymal adipose mass in both

| Table 2. Blood biochemical analyses |
|------------------------------------|
| **Renal function**                 |
| **Exercise diet**                  |
| **Groups**                         |
| **Normal**                         |
| **Renal failure**                  |
| **Exercise training**              |
| **(-)**                            |
| **(-)**                            |
| **(ex)**                           |
| **(-)**                            |
| **(ex)**                           |
| **GLU (mg/dl)**                    |
| 172 ± 26                           |
| 144 ± 19                           |
| 206 ± 92                           |
| 201 ± 33                           |
| 167 ± 34                           |
| **Insulin (ng/ml)**                |
| 0.47 ± 0.13                        |
| 0.40 ± 0.12                        |
| 0.34 ± 0.04*                       |
| 0.86 ± 0.34*                       |
| 0.24 ± 0.04*                       |
| **Lactic acid (mmol/L)**           |
| 2.0 ± 0.5                          |
| 2.2 ± 0.4                          |
| 1.1 ± 0.2*                         |
| 2.2 ± 0.4                          |
| 1.1 ± 0.1*                         |
| **HDL-cho (mg/dl)**                |
| 58 ± 2                             |
| 60 ± 5                             |
| 55 ± 9*                            |
| 71 ± 4                             |
| 60 ± 8                             |
| **T-cho (mg/dl)**                  |
| 86 ± 5                             |
| 86 ± 8                             |
| 80 ± 13*                           |
| 101 ± 6                            |
| 85 ± 11                            |
| **TG (mg/dl)**                     |
| 163 ± 60                           |
| 75 ± 13*                           |
| 70 ± 9*                            |
| 103 ± 47                           |
| 64 ± 22**                          |
| **BUN (mg/dl)**                    |
| 17 ± 3                             |
| 26 ± 4                             |
| 24 ± 8                             |
| 26 ± 5                             |
| 31 ± 8*                            |
| **AST (U/L)**                      |
| 57 ± 9                             |
| 56 ± 4                             |
| 63 ± 3                             |
| 55 ± 0                             |
| 66 ± 7                             |
| **ALT (U/L)**                      |
| 26 ± 3                             |
| 24 ± 2                             |
| 25 ± 4                             |
| 23 ± 0                             |
| 24 ± 2                             |
| **TP (g/dl)**                      |
| 6.8 ± 0.2                          |
| 6.5 ± 0.2                          |
| 6.2 ± 0.2                          |
| 6.3 ± 0.5                          |
| 6.0 ± 0.2**                        |
| **CPK (U/L)**                      |
| 89 ± 5                             |
| 89 ± 17                            |
| 106 ± 6*                           |
| 79 ± 10                            |
| 92 ± 11                            |
| **CRE (mg/dl)**                    |
| 0.23 ± 0.05                        |
| 0.38 ± 0.05                        |
| 0.43 ± 0.13                        |
| 0.23 ± 0.05                        |
| 0.32 ± 0.13                        |

Data are means ± SD. *p<0.05 and **p<0.01 vs CA (-) in normal group. *p<0.01 vs CA (-) with renal failure group. *p<0.05 and **p<0.01 vs HF (-) with renal failure group. CA (-) in normal group, CA (-), CA (ex) and HF (-) with renal failure, n = 4; HF (ex) with renal failure, n = 5.

The BUN levels increased in the renal failure groups, and further the BUN level in HF group was significantly increased by exercise compared to the normal group, suggesting that the exercise may make the renal function worse in the duplicative state of renal failure and DM (Table 2). Similarly, the CRE levels in all groups with renal failure, except for the HF (–) group, showed the increment and the exercise would make the CRE levels more increase (Table 2). Additionally, the TP levels were lower in the renal failure groups than normal group and the exercise significantly reduced the TP level in HF group compared to normal group (Table 2).

Both the AST and CPK values were increased by the exercise (Table 2), according with the well-known previous reports.

### Measurement of trace elements in plasma and organs.

There were no significant differences in plasma and pancreas data among groups (Table 3). In the renal failure groups, hepatic Cr and Mn levels were changed slightly (Table 3). In both the CA and HF groups with renal failure, renal Ca levels showed the clear increment but renal Se levels tended to decrease and then showed the significant decrease in the CA (–) groups compared to the normal group. Additionally, renal Cu levels in the HF groups with renal failure significantly decreased compared to the normal group (Table 3).

### Superoxide anion production measurement.

Artificial superoxide anion production in hepatic solution tended to increase in renal failure groups compared to the normal group. In the HF (ex) group with renal failure, the increment of artificial superoxide anion production was significant (Fig. 2A). On the other hand, artificial superoxide anion production in renal solution significantly increased only in the HF (–) group compared to the normal group, and this increment was significantly improved by exercise (Fig. 2B).

### RNA isolation, cDNA synthesis, and PCR analysis of selenoprotein P.

Hepatic selenoprotein P mRNA increased in the exercised groups. Additionally, it was upregulated in the exercised groups compared to the nonexercised groups, particularly for rats on the CA diet (Fig. 3).

### GPx1 protein expression in the liver.

The expression levels of GPx1 in the liver were determined by western blotting. Hepatic GPx1 showed the lower expression in the renal failure groups than the normal group (Fig. 4). Especially, in the CA (–) and HF (ex) groups with renal failure, the expression levels further decreased compared to the normal group. In the CA diet with renal failure, the expression tended to increase the GPx1 level, however, in the HF diet with renal failure, the expression tended to reversely decrease the GPx1 level (Fig. 4). These results suggested that the exercise in the duplicative state of renal failure and DM would provide the oxidative damage to the liver.
Table 3. Biological trace metal concentrations in plasma (A), liver (B), kidney (C), and pancreas (D)

| A          | Normal (µg/ml) | Renal failure (µg/ml) | HF (µg/ml) |
|------------|----------------|-----------------------|------------|
| Plasma     |                | (-)                   | (ex)       | (-)        |
| Mg         | 22.0 ± 3.7     | 23.8 ± 3.4            | 25.2 ± 3.5 | 21.5 ± 3.0 |
| Ca         | 109.4 ± 10.8   | 112.1 ± 17.2          | 109.4 ± 11.6 | 105.4 ± 8.8 |
| Cr         | 0.06 ± 0.01    | 0.06 ± 0.01           | 0.07 ± 0.02 | 0.05 ± 0.01 |
| Mn         | 0.04 ± 0.01    | 0.03 ± 0.01           | 0.03 ± 0.00 | 0.03 ± 0.00 |
| Fe         | 2.2 ± 0.2      | 3.0 ± 0.5             | 2.8 ± 0.7  | 2.3 ± 0.2  |
| Cu         | 1.6 ± 0.3      | 1.6 ± 0.1             | 1.7 ± 0.3  | 1.4 ± 0.1  |
| Zn         | 4.4 ± 2.3      | 1.9 ± 0.3             | 2.2 ± 0.7  | 1.8 ± 0.5  |
| Se         | 0.15 ± 0.09    | 0.18 ± 0.16           | 0.31 ± 0.10 | 0.31 ± 0.08 |
| B          |                | (-)                   | (ex)       | (-)        |
| Liver      | CA (µg/g wet weight) | (-) | (ex) | (-)       |
| Mg         | 243.0 ± 4.0    | 237.7 ± 7.0           | 234.8 ± 19.4 | 250.2 ± 8.2 |
| Ca         | 53.4 ± 2.5     | 77.4 ± 14.2           | 55.5 ± 1.2  | 67.8 ± 11.0 |
| Cr         | 0.13 ± 0.01    | 0.12 ± 0.03           | 0.17 ± 0.01* | 0.10 ± 0.02 |
| Mn         | 2.13 ± 0.09    | 2.05 ± 0.10           | 1.90 ± 0.29* | 2.50 ± 0.16 |
| Fe         | 200.0 ± 20.2   | 249.8 ± 22.7          | 236.7 ± 6.2 | 229.1 ± 30.3 |
| Cu         | 5.2 ± 0.2      | 5.9 ± 0.9             | 5.8 ± 0.2  | 5.4 ± 0.1  |
| Zn         | 39.3 ± 4.0     | 46.4 ± 6.9            | 39.9 ± 7.5 | 39.3 ± 2.7 |
| Se         | 0.30 ± 0.19    | 0.35 ± 0.32           | 0.34 ± 0.27 | 0.41 ± 0.24 |
| C          |                | (-)                   | (ex)       | (-)        |
| Kidney     | CA (µg/g wet weight) | (-) | (ex) | (-)       |
| Mg         | 190.9 ± 5.4    | 222.8 ± 36.5          | 216.6 ± 37.6 | 190.1 ± 11.6 |
| Ca         | 132.6 ± 43.2   | 896.1 ± 599.0*        | 374.2 ± 222.7 | 410.2 ± 188.6 |
| Cr         | 0.12 ± 0.02    | 0.16 ± 0.04           | 0.18 ± 0.05 | 0.13 ± 0.01 |
| Mn         | 0.79 ± 0.07    | 0.74 ± 0.17           | 0.67 ± 0.01 | 0.68 ± 0.07 |
| Fe         | 80.2 ± 5.3     | 90.4 ± 16.1           | 82.5 ± 9.9  | 63.7 ± 14.7 |
| Cu         | 11.0 ± 2.3     | 8.0 ± 2.2             | 8.1 ± 2.5  | 5.7 ± 1.5* |
| Zn         | 41.4 ± 6.7     | 40.4 ± 5.5            | 42.7 ± 1.8  | 39.9 ± 8.3 |
| Se         | 1.04 ± 0.01    | 0.25 ± 0.17**         | 0.60 ± 0.15 | 0.63 ± 0.27 |
| D          |                | (-)                   | (ex)       | (-)        |
| Pancreas   | CA (µg/g wet weight) | (-) | (ex) | (-)       |
| Mg         | 289.2 ± 26.8   | 335.7 ± 36.6          | 294.9 ± 28.4 | 303.5 ± 17.9 |
| Ca         | 118.6 ± 30.0   | 152.5 ± 27.6          | 134.5 ± 25.3 | 132.0 ± 9.6 |
| Cr         | 0.14 ± 0.01    | 0.20 ± 0.01           | 0.17 ± 0.03 | 0.15 ± 0.02 |
| Mn         | 1.37 ± 0.17    | 1.43 ± 0.18           | 1.31 ± 0.07 | 1.52 ± 0.09 |
| Fe         | 17.3 ± 2.6     | 21.4 ± 2.5            | 17.4 ± 0.8  | 19.2 ± 2.8  |
| Cu         | 1.4 ± 0.1      | 1.6 ± 0.2             | 1.4 ± 0.1  | 1.2 ± 0.1  |
| Zn         | 40.4 ± 12.0    | 47.0 ± 8.8            | 45.3 ± 8.5  | 36.3 ± 5.7  |
| Se         | 0.15 ± 0.11    | ud                     | 0.07 ± 0.07 | ud         |

Data are means ± SD. *p<0.05 and **p<0.01 vs CA (-) in normal group. †p<0.05 vs CA (-) with renal failure group. ‡p<0.01 vs CA (ex) with renal failure group. §p<0.01 vs HF (-) with renal failure group. ud, under determine. CA (-) in normal group, CA (-), CA (ex) and HF (-) with renal failure, n = 4; HF (ex) with renal failure, n = 5.

diet groups with renal failure (Table 1).

However, simultaneously, the exercise significantly increased the kidney weight in the HF (ex) group with renal failure (Table 1), suggesting that the strenuous exercise regimen may cause more damage to kidney by oxidative stress in the duplicative state of renal failure and DM. In a similar way, BUN and CRE levels were higher in the renal failure groups (Table 2) which reflect kidney function. Moreover, the BUN level in HF group and CRE level in both diet groups were increased by continuous exercise compared to the normal group (Table 2), suggesting that the strenuous exercise may exacerbate the renal dysfunction in the duplicative state of renal failure and DM.
On the other hand, blood insulin was highest in the HF (–) group with renal failure among all groups and then exercise significantly reduced those levels lower than normal, indicating that exercise improves insulin resistance even in the glucose intolerance with renal failure (Table 2). Lactic acid is a glycogen catabolite formed by anaerobic respiration in the muscle after hard exercise. In the present study, the significant decrement in plasma lactic acid was observed in both exercised groups, indicating that exercise increases the lactic acid utilization ratio even in the case of renal failure (Table 2). Additionally, exercise decreased T-cho and TG which participate in lipid metabolism, however, the reductions of these lipid metabolism parameters in the plasma levels were relatively small (Table 2), suggesting that enhancement of glucose and lipid metabolism based on AMPK activation is reduced in response to continuous exercise in the case of DM with renal failure.

Earlier studies on obese rats reported that exercise increased blood and pancreatic zinc levels and altered hepatic element distribution. We determined biological trace metals in the plasma, liver, kidney, and pancreas by ICP-MS because several trace metals are associated with exercise and diabetes. However, we observed no significant differences in plasm and organ metal levels between the exercised- and non-exercised groups (Table 3). Instead, Ca was ascending but both Cu and Se were descending in the kidney of rats in response to the renal failure (Table 3D).

Exercise was reported to increase organ oxidative stress which, in turn, would lead to upregulate the antioxidant enzymes such as superoxide dismutase (SOD) that protects the cells, tissues, and organs against superoxide anion. In the present study, the measured hepatic and renal artificial O₂⁻ products disclosed that exercise increases hepatic oxidative stress but reversely decreases renal oxidative stress (Fig. 2). Acute exercise increases ROS such as O₂⁻ and generally may enhance oxidative damage and inflammation. Therefore, relative to non-exercised rats, exercised rats showed increments in hepatic oxidative stress levels (Fig. 2A). On the other hand, exercise produced improvement in terms of renal oxidative stress even in such a situation as glucose intolerance and obesity with renal failure (Fig. 2B). These results suggested contiguous exercise may act on upregulating the antioxidant enzymes such as SOD and GPx through the golden mean production of ROS.

Hepatic selenoprotein P as a hepatokine is secreted to blood circulation from the liver, transports its abundant Se to all peripheral tissues, and protects against oxidative stress by upregulating glutathione peroxidase (GPX) activity. However, when its antioxidant activity is excessive through the overexpression and secretion, it may cause diabetes or exercise intolerance. Although it is unclear whether exercise affects hepatic selenoprotein P expression, the increase of selenoprotein P may be caused by a response to exercise-induced oxidative stress. The present study suggested that renal failure and further exercise upregulated hepatic selenoprotein P in both dietary groups and much remarkably in the CA diet-fed rats (Fig. 3). In the first place, renal failure greatly upregulated hepatic selenoprotein P which would transport Se to kidney, and this may be caused from the reason why kidney requires antioxidant co-factors such as Se in order to induce an antioxidant system. In fact, renal Se levels were found to greatly decrease by renal damage (Table 3D).

We also measured hepatic GPx1 expression (Fig. 4) and found lower expression levels in the state of renal failure. Previous studies reported that exercise upregulated hepatic GPx. In the present study, the exercise upregulated hepatic GPx1 especially in the CA diet group, however, it downregulated hepatic GPx1.
reversely in the HF diet group, being consistent with the results of hepatic oxidative stress levels (Fig. 2A).

In conclusion, in the duplicative state of renal failure and DM, the beneficial effects of exercise were shown in improvements of insulin sensitivity, lactic acid utilization, adipose mass reduction (obesity improvement), and lipid metabolism, however, the risky effects of exercise were revealed that the renal function caught the more damage as the compensation. In other words, the present results are like the relations of trade-off. Additionally, in the state with renal failure, exercise further induced hepatic selenoprotein P which transports Se and turns out to enhance the antioxidative system in the kidney. Therefore, the strong exercise treatment should be avoided, and strictly controlled exercise conditions must be adapted to patient health states especially in view of protection of kidney and its function. Moreover, supplemental therapy is considered to need in parallel with exercise, using nutrients and vitamins which are recommended for the kidney protection, for example, vitamin D.(36)

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Conflict of Interest

KC is an employee of CycloChem Bio Co. Ltd., KT is representative director of CycloChem Bio Co. Ltd.

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