Differences of Matrix Metalloproteinase 2 Expression between Left and Right Ventricles in Response to Nandrolone Decanoate and/or Swimming Training in Mice

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Abstract

Background: Matrix metalloproteinase (MMP)-2 plays an important role in the remodeling of left ventricles (LVs) and right ventricles (RVs). We investigated the differences of MMP-2 expression between LV and RV in response to nandrolone decanoate (ND), swimming training (ST), and combined ND and ST (NS) in mice, based on their structural, functional, and biochemical characteristics.

Methods: Totally 28 male C57B1 mice (6 weeks old; 20–23 g) were divided into four groups, including the control (n = 7), ND (n = 6), ST (n = 8), and NS (n = 7) groups. After respective treatments for 8 weeks, echocardiographic examination was used to assess the cardiac structure and function. Van Gieson stain was used to examine the fibrosis of LV and RV in response to different treatments, and Western blotting analysis was performed to explore different MMP-2 expressions between LV and RV in response to ND and/or ST. Analysis of variance was used for comparing the four groups.

Results: At 8 weeks, right ventricular dimension/body weight in the ND group was larger than the other three groups (F = 7.12, P < 0.05) according to the echocardiographic examination. Fibrosis induced by ND administration was increased more in RV (2.59%) than that in LV (2.21%). MMP-2 expression of the ND group in RV was significantly greater than the control and NS groups in RV and the corresponding ND group in LV.

Conclusion: The experimental data support the hypothesis that ND administration induces greater MMP-2 expression increase in RV compared to LV, leading to consequent RV dilation.

Key words: Fibrosis; Matrix Metalloproteinase 2; Nandrolone Decanoate; Swimming Training

Introduction

Nandrolone decanoate (ND) is a synthetic derivative of testosterone related to heart function impairment, when used in high dose among young athletes.[¹] When combined with exercise, ND predisposes to pathophysiological cardiac hypertrophy,[²,³] myocardial injury, and more severely, complications such as ventricular fibrillation, cardiac dysfunction, or sudden cardiac death.[⁴,⁵] Long-term use of testosterone was shown to affect RV hypertrophic response to load stress by altering myocyte size and increasing fibrosis in mice.[⁸]

The matrix metalloproteinases (MMPs) play an important role in the process of cardiac remodeling by regulating
structural integrity of the extracellular matrix.\textsuperscript{[9]} MMP-2 is one of the predominant MMPs expressed in the cardiac ventricles. The altered expression of MMP-2 is shown to contribute to structural remodeling in pathological heart tissue.\textsuperscript{[10,11]} The purpose of the present study was to investigate the differences of MMP-2 expression between LVs and RVs in response to ND, swimming training (ST), and combined ND and ST (NS) in mice, based on their structural, functional, and biochemical characteristics.

**Methods**

**Animal model and experimental protocol**

We raised 6-week-old male C57B1 mice with initial body weight of 20–23 g in a room maintaining temperature of 22 ± 1°C and humidity of 55–65%. Free water and chow were served and free movements were allowed in their cages. The experimental procedures were approved by the Institutional Animal Care and Use Committee of Capital Medical University (No. SYXK [Jing] 2016-0027).\textsuperscript{[12]}

The mice (n = 28) were randomly divided into four groups according to different treatment methods, which were the control (n = 7), ND (n = 6), ST (n = 8), and NS (n = 7) groups. The control group received intramuscular arachidis oil, twice per week (i.e., Monday and Thursday) for 8 weeks; the ND group received ND (Jiangxi Luoshi Biotechnology Development Co., Ltd., China), which was administrated intramuscularly at a dose of 10 mg/kg, twice per week (i.e., Monday and Thursday) for 8 weeks with arachidis oil as vehicle; the ST group received exercise administration of Capital Medical University (No. SYXK [Jing] 2016-0027).\textsuperscript{[12]}

The physical training was executed five times a week for 8 weeks, in a swimming system (water temperature at 32°C) for 60 min, with a gradual increase of work load (tail weight-percentage body weight [BW]) until it reached 5% of BW. This low-intensity, long-training protocol was considered effective for promoting cardiovascular adaptations and increasing muscle oxidative capacity; the NS group received both ND administration and ST illustrated as above.

**Echocardiographic examination**

Vivid 7 (GE Co., Inc., Schenectady, New York, USA) was used to perform B-mode, M-mode, and Doppler transthoracic echocardiographic investigations on the mice. Offline software (GE Co., Inc., Schenectady, New York, USA) was used to measure all digitally stored images. We performed all the echocardiographic studies at baseline and 8 weeks later as previously proposed.\textsuperscript{[14-16]} Interventricular septum thickness, LV end-diastolic dimension, LV end-systolic dimension (LVDs), and LV ejection fraction were measured from the M-mode of left parasternal short-axis standard views at the level of papillary muscle. Right ventricular end-diastolic dimension (RVD), E-velocity, E/A ratio, and E deceleration time (E-DT) were obtained from the M-mode of parasternal long- and short-axis standard views. All dimensions used for analysis were indexed to BW.

**Determination of heart weight and left and right ventricular weights**

The whole heart and separately dissected LV and RV were weighed immediately after being quickly removed. Then, the ratios of these weights to the corresponding BWs (heart weight [HW]/BW, LV weight [LVW]/BW, and right ventricular weight [RVW]/BW) were calculated for analysis. After that, they were frozen in liquid nitrogen at −80°C or fixed in 10% formalin to prepare for histological studies.

Reagent Kit (Beijing Yili Chemical Co., Ltd., China) was used for Van Gieson stain.\textsuperscript{[17,18]} To determine the degree of collagen fiber accumulation, we randomly selected twenty fields in three individual sections and calculated the ratios of the areas of Van Gieson-stained interstitial fibrosis to the total ventricular area using software Image-Pro Plus 6.0 (Media Cybernetics, Maryland, USA).\textsuperscript{[17,19]}

**Statistical analysis**

The number of mice in each group was indicated with n values. Continuous values were expressed as mean ± standard error (SE). Analysis of variance with LSD method was used to compare the four groups. A value of P < 0.05 was considered as statistical significance. All analyses were performed using SPSS 21.0 (SPSS Inc., Chicago, IL, USA).

**Results**

**Structural examination in vivo**

The BW at baseline was similar among all groups [Table 1]. At the end of 8 weeks, BW of the ND group had a larger increase than the other three groups [Table 2]. As for HW, no significant difference was seen among the four groups. When normalized by BW, HW/BW of the NS group was significantly greater than the control and ND groups, but similar to the ST group.

After being adjusted by BW, the ST group showed significantly larger LVDs/BW compared to the control group [Table 3 and Figure 1]. The ND group had larger RVD/BW than the other three groups (F = 7.12, P < 0.05). RVD/BW in the NS group was larger than the control group, but similar to the ST group. E-DT of the NS group was greater than the other three groups (F = 15.31, P < 0.05).

**Fibrosis changes after different treatments**

Administration of ND to the mice increased fibrosis formation both in LV and RV, but with greater effects on
Table 1: Comparison of Baseline and 8-week body weight within and between groups

| Group    | Number | Baseline  | 8 weeks | Difference between baseline and 8 weeks | t   | P     |
|----------|--------|-----------|---------|----------------------------------------|-----|-------|
| Control  | 7      | 21.86 ± 0.34 | 29.38 ± 0.81 | 7.53 ± 1.14 | 6.61 | 0.001 |
| ND       | 6      | 21.67 ± 0.33 | 31.72 ± 0.76 | 10.05 ± 0.71 | 14.10 | <0.001 |
| ST       | 8      | 21.63 ± 0.26 | 29.24 ± 0.71 | 7.62 ± 0.69 | 11.00 | <0.001 |
| NS       | 7      | 21.71 ± 0.42 | 27.82 ± 0.51 | 6.11 ± 0.62 | 9.92 | <0.001 |

No significant difference between each other at baseline; *P<0.05 from the control, ST and NS groups at 8 weeks. ND: Nandrolone decanoate; ST: Swimming training; NS: Combined nandrolone decanoate and swimming training.

Table 2: Cardiac parameters of four different treatment groups at 8 weeks

| Groups   | BW (g)      | HW (g)    | LVW (g)  | RVW (g)  | HW/BW (mg/g)  | LVW/BW (mg/g) | RVW/BW (mg/g) |
|----------|-------------|-----------|----------|----------|---------------|---------------|---------------|
| Control  | 29.386 ± 0.813 | 0.174 ± 0.009 | 0.102 ± 0.007 | 0.023 ± 0.004 | 0.595 ± 0.027 | 0.351 ± 0.019 | 0.080 ± 0.012 |
| ND       | 31.716 ± 0.756 | 0.188 ± 0.006 | 0.097 ± 0.002 | 0.026 ± 0.004 | 0.594 ± 0.022 | 0.315 ± 0.001 | 0.084 ± 0.010 |
| ST       | 29.244 ± 0.710 | 0.194 ± 0.008 | 0.103 ± 0.007 | 0.028 ± 0.001 | 0.668 ± 0.035 | 0.358 ± 0.030 | 0.096 ± 0.001 |
| NS       | 27.824 ± 0.508 | 0.198 ± 0.008 | 0.106 ± 0.007 | 0.027 ± 0.003 | 0.710 ± 0.024 | 0.379 ± 0.014 | 0.095 ± 0.008 |
| F        | 4.73        | 1.59      | 0.28     | 0.39     | 3.83          | 1.45          | 0.74          |
| P        | 0.010       | 0.219     | 0.841    | 0.762    | 0.203         | 0.278         | 0.547         |

*P<0.05 from the control, ST, and NS groups; †P<0.05 from NS group. ND: Nandrolone decanoate; ST: Swimming training; NS: Combined nandrolone decanoate and swimming training; BW: Body weight; HW: Heart weight; LVW: Left ventricular weight; RVW: Right ventricular weight.

Table 3: Echocardiographic parameters in four different treatment groups at 8 weeks

| Groups   | LV dimension and function | RV dimension and function | E/A ratio |
|----------|---------------------------|---------------------------|-----------|
|          | LVDd/BW (cm/g) | LVDs/BW (cm/g) | E/A ratio | RVD/BW (cm/g) | E-velocity§ | E-DT (s) | E/A ratio |
| Control  | 1.140 ± 0.040 | 0.630 ± 0.060 | 2.250 ± 0.036 | 0.530 ± 0.058 | 30.000 ± 0.580 | 0.130 ± 0.006 | 2.000 ± 0.039 |
| ND       | 1.380 ± 0.070 | 0.880 ± 0.080 | 2.390 ± 0.052 | 0.620 ± 0.014† | 55.000 ± 15.000* | 0.160 ± 0.010 | 4.500 ± 2.500 |
| ST       | 1.390 ± 0.140 | 1.020 ± 0.140* | 2.490 ± 0.391 | 0.570 ± 0.017 | 33.000 ± 3.330 | 0.140 ± 0.010 | 1.560 ± 0.560 |
| NS       | 1.250 ± 0.050 | 0.830 ± 0.060 | 1.790 ± 0.1550 | 0.580 ± 0.005* | 59.250 ± 3.590* | 0.280 ± 0.025§ | 3.720 ± 0.610 |
| F        | 0.18        | 2.22      | 1.43     | 7.12     | 8.30          | 15.31         | 2.23          |
| P        | 0.359       | 0.139     | 0.283    | 0.005    | 0.008         | 0.001         | 0.163         |

*P<0.05 compared with control group; †P<0.05 compared with the other three groups; §ND (n = 2), and ST (n = 3) for calculation of E-velocity. ND: Nandrolone decanoate; ST: Swimming training; NS: Combined nandrolone decanoate and swimming training; LV: Left ventricle; RV: Right ventricle; BW: Body weight; LVDd: Left ventricular end-diastolic dimension; LVDs: Left ventricular end-systolic dimension; RVD: Right ventricular end-diastolic dimension; E-DT: E deceleration time.

Figure 1: Diagrams of mouse echocardiography at the papillary level of short-axis view in four different treatment groups at 8 weeks. RVD in the ND group was larger compared to the other three groups (arrows). (a) Control group; (b) ND group; (c) ST group; (d) NS group. ND: Nandrolone decanoate; ST: Swimming training; NS: Combined nandrolone decanoate and swimming training; RV: Right ventricle; RV: Right ventricle end-diastolic dimension.

Figure 2: Matrix metalloproteinase-2 expression in the left and right ventricles in response to different treatments. MMP-2 expression in LVs and RVs at 8 weeks was shown in Figure 4. In RV, the ND group had significantly greater MMP-2 expression than the control and NS groups, but it appeared similar when the ST and NS groups were compared to the control group. MMP-2 expression in RV was greater.

RV (LV vs. RV: 2.21% increase vs. 2.59% increase). In contrast, the ST group had no significantly different fibrosis accumulation between LV and RV, so did the NS group. In addition, the ND, ST, and NS groups showed increased fibrosis formation in both LV and RV compared to the control group. Fibrosis changes caused by different treatments were shown in Figures 2 and 3.
**Figure 3:** Relative levels of fibrosis in left and right ventricles in four different treatment groups at 8 weeks. *P < 0.05 compared to the ND group of the left ventricle; †P < 0.05 compared to the corresponding control group of the left ventricle; ‡P < 0.05 compared to the corresponding control group of the right ventricle. ND: Nandrolone decanoate; ST: Swimming training; NS: Combined nandrolone decanoate and swimming training; LV: Left ventricle; RV: Right ventricle.

than LV in the control, ND, and ST groups, while the difference between the two ventricles in the NS group was not significant.

**DISCUSSION**

In this study, RV dilation was induced by high-dose administration of ND, ST, and NS after 8-week treatment, with the strongest effects caused by ND and the weakest effects caused by NS. Increase of MMP-2 expression estimated by Western blotting was consistent with the RV dilation changes examined *in vivo* in the four treatment groups.
To minimize age-related influence on the structural, functional, and biochemical changes of the ventricles, we used the mice of the same week age among the four groups.[21] BW in the ND group increased more significantly than the other three groups. However, BW in the NS group was lower compared to the control group at 8 weeks. No significant difference was seen in the LVW among the four groups, regardless of whether they were adjusted by BW or not. This greater BW gain induced by ND administration was similar to some studies,[22,23] but opposite to other studies showing lower BW gain in the ND group compared with the control group or non-ND group.[24,25] A possible explanation was that 8-week administration of ND and/or ST was not long enough to produce LV remodeling, as suggested by previous research.[26]

It was well accepted that steroid played an important role in cardiac pathology, especially when combined with exercises,[7,25] It was argued that their combination could produce pathological cardiac hypertrophy, myocardial infarction, and even cardiac failure. However, there were some debates on their related effects. For example, some studies showed that exercise would prevent the adverse effects caused by nandrolone,[22,27] which were consistent with this study.

In this study, ND induced larger RV dilation than NS according to RVD/BW based on echocardiographic examination. MMP-2 was one of the predominant MMPs shown to play a key role in cardiac ventricle remodeling and was considered to be an important fibrotic marker.[9,10] The level of MMP-2 expression (confirmed by Western blotting) was upregulated by ND, showing consistent results with RV dilation after 8 weeks of high-dose ND. Therefore, we made an assumption that MMP-2 expression was stimulated by ND. It was assumed that this stimulation led to a destructive effect on the RV and the consequent dilation in the present study. Though ND was also supposed to regulate LV remodeling by stimulating MMPs expression, the effect seemed weaker than that in RV after 8-week administration. The novel finding of the present study is that 8-week administration of ND induced different levels of MMP-2 expression between LV and RV. Nonetheless, the report was controversial to one previous study reporting an inhibition effect of ND on MMP-2 expression in the LV of rats after 7-week administration.[28]

Finally, although ND was shown to have a stimulative effect on MMP-2 expression in RV, the underlying mechanism was not revealed by the present study. In general, this was only a descriptive and superficial study, though MMP-2 expression was examined. Further studies are needed to make a deeper molecular mechanical exploration on the relationship between ND administration and RV remodeling caused by MMP-2 expression regulation.

In summary, the experimental data support the hypothesis that MMP-2 expression in RV increases more significantly than LV induced by high-dose ND administration, leading to consequent RV dilation. Further studies are needed to explore the time course of right ventricular failure and the changes of MMP-2 expression. This will help understand the consequences of high-dose ND administration in the RV with regard to structural and functional alteration, and thus promote the development of new therapeutic approaches.

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Conflicts of interest
There are no conflicts of interest.

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