CHAPTER 4

RESULTS

Characteristics of subjects

From the characteristics of all sedentary men, no difference existed in BMI and CBC screening tests in either the control or NAC supplement group (Table 1). Characteristics of all 30 sedentary men were healthy; and the body mass index (BMI) in the control group (n=13) and NAC supplement group (n=16) was 21.14 ± 2.73 and 21.34 ± 3.14 kg/m^2, respectively. Basic results such as WBC, RBC, Hb, Hct or Plt in the complete blood count (CBC) showed healthy status in both groups.

Oxidative Stress

At baseline at day 0, there was no difference between the NAC supplement group and control group; TAC (2.27 ± 0.11 mmol Trolox/L) (Figure 4), GSH (57.18 ± 2.55 μmol/g Hb) (Figure 5), Nitrite (78.3 ± 15.3 nmol/L) (Figure 6), and MDA (0.63 ± 0.08 μmol/L) (Figure 7). Then, after short heavy exercise, all parameters changed significantly; TAC (1.96 ± 0.09 mmol Trolox/L), GSH (52.92 ± 2.15 μmol/g Hb), Nitrite (210.0 ± 48.4 nmol/L) and MDA (0.91 ± 0.07 μmol/L). On the other hand, all resting parameters changed significantly after NAC supplementation for 7 days at day 8; TAC (2.45 ± 0.11 mmol Trolox/L), GSH (59.58 ± 1.81 μmol/g Hb) and MDA (0.43 ± 0.05 μmol/L). The nitrite level was higher (98.68 ± 16.9 nmol/L) than

that at day 0 (78.3 ± 15.3 nmol/L), but not statistically different. Before NAC supplementation at day 0, and after short heavy exercise, results showed a significant change in detail; TAC (2.27 ± 0.11 to 1.96 ± 0.09 mmol Trolox/L), GSH (57.18 ± 2.55 to 52.92 ± 2.15 µmol/g Hb), Nitrite (78.3 ± 15.3 to 210.1 ± 48.4 nmol/L) and MDA (0.63 ± 0.08 to 0.91 ± 0.07 µmol/L). Interestingly, results at day 8 after supplementation and short heavy exercise showed that all parameters did not differ significantly from those at pre-exercise; TAC (2.47 ± 0.04 mmol Trolox/L), GSH (55.10 ± 1.85 µmol/g Hb), Nitrite (192.9 ± 46.9 nmol/L) and MDA (0.44 ± 0.07 µmol/L). However, these parameters differed significantly in the control group from those in the resting data of pre-exercise periods at day 0 and day 8.

Table 1. Characteristics and Completed blood count test before study in both the control and NAC supplement group.

	Control (n=13)	**NAC (n=16)**	**Reference value**
Aged, yr	19.49 ± 3.58 (18 - 23)	20.01 ± 0.79 (18.33 - 30.47)	-
BMI, kg/m^2	21.14 ± 2.73 (17.5 - 28.4)	21.34 ± 3.14 (18.3 - 30.5)	-
WBC, 10^3/µL	6.83 ± 1.19 (4.2 - 9.0)	6.04 ± 1.15 (4.8 - 7.9)	5.0 - 10.0
RBC, 10^6/µL	5.68 ± 0.60 (4.9 - 6.6)	5.49 ± 0.39 (4.97 - 6.19)	3.80 - 5.30
Hb, gm/dL	14.65 ± 1.37 (11.7 - 16.8)	15.45 ± 0.7 (14.1 - 16.4)	10 - 16
Hct, %	45.31 ± 3.5 (37.1 - 50.9)	47.48 ± 2.13 (43.7 - 50.9)	40 - 50
Plt, 10^3/µL	318.84 ± 64.7 (185 - 428)	246.5 ± 49.68 (155 - 354)	140 - 440

Table 2. All oxidative stress parameters; total antioxidant capacity (TAC) (mmol Trolox/L), glutathioine (GSH) (µmol/g Hb), nitrite (nmol/L), and malondialdehyde (MDA) (µmol/L) in the control and NAC supplement group

	Control (n =13)				**NAC (n = 16)**			
	day 0		**day 8**		**day 0**		**day 8**	
	Pre-exs	**Post-exs**	**Pre-exs**	**Post-exs**	**Pre-exs**	**Post-exs**	**Pre-exs**	**Post-exs**
TAC	2.09 ± 0.12 (1.70 – 2.89)	1.88 ± 0.11 # (1.58 - 2.03)	2.09 ± 0.12 (1.08 - 2.82)	1.98 ± 0.04 # (2.17 - 2.54)	2.27 ± 0.11 (1.05 - 2.70)	1.96 ± 0.09 # (0.98 - 2.60)	2.45 ± 0.11 ** (2.31 - 2.67)	2.47 ± 0.04 † (2.20 - 2.79)
GSH	54.92 ± 2.80 (32.71 - 69.26)	51.50 ± 2.38 # (31.59 - 70.51)	53.59 ± 2.01 (36.74 - 66.83)	50.69 ± 2.05 # (34.63 - 65.88)	57.18 ± 2.55 (39.98 – 80.06)	52.92 ± 2.15 # (37.86 - 71.33)	59.58 ± 1.81 ** (40.51 - 94.00)	55.10 ± 1.85 † (39.11 - 63.57)
Nitrite	78.8 ± 16.9 (30 - 206)	213.9 ± 53.7 # (69 - 807)	64.4 ± 18.7 (29 - 167)	239.9 ± 52.1 # (25 - 690)	78.3 ± 15.3 (26 - 315)	210.1 ± 48.4 # (50 - 680)	98.68 ± 16.9 (40 - 345)	192.9 ± 46.9 (30 - 542)
MDA	0.58 ± 0.09 (0.01 – 1.33)	0.90 ± 0.08 # (0.44 - 1.46)	0.61 ± 0.05 (0.17 - 1.64)	0.93 ± 0.08 # (0.12 - 1.80)	0.63 ± 0.08 (0.09 - 1.04)	0.91 ± 0.07 # (0.49 - 1.35)	0.43 ± 0.05 ** (0.14 - 0.68)	0.44 ± 0.07 † (0.14 - 0.98)

Values are mean (SE.) and (Min-Max), ** $p < 0.05$ compared to pre-exercise period at day 0 in the NAC supplement group, # $p < 0.05$ compared to pre-exercise period on the same day, and † $p < 0.05$ compared to post-exercise period at day 0.

From plasma TAC results (Figure 4) at day 0, there was no difference between the NAC supplement group and control group; TAC (2.27 ± 0.11 mmol Trolox/L). Then, after short heavy exercise, the TAC changed significantly; TAC (1.96 ± 0.09 mmol Trolox/L). On the other hand, plasma TAC resting parameters changed significantly after NAC supplementation for 7 days at day 8; TAC (2.45 ± 0.11 mmol Trolox/L). Before NAC supplementation at day 0, and after short heavy exercise, results showed a significant change in TAC (2.27 ± 0.11 to 1.96 ± 0.09 mmol Trolox/L). Interestingly, results at day 8 after supplementation and short heavy exercise showed that TAC did not significantly differ from that at pre-exercise: TAC (2.47 ± 0.04 mmol Trolox/L). However, the plasma TAC differed significantly in the control group from that in the resting data of pre-exercise periods at day 0 and day 8.

From erythrocyte GSH results (Figure 5) at day 0, the NAC supplement group showed no difference from the control group; Erythrocyte GSH (57.18 ± 2.55 μmol/g Hb). Then, after short heavy exercise, erythrocyte GSH changed significantly; GSH (52.92 ± 2.15 μmol/g Hb). On the other hand, Erythrocyte GSH changed significantly after NAC supplementation for 7 days at day 8; GSH (59.58 ± 1.81 μmol/g Hb). Before NAC supplementation at day 0, and after short heavy exercise, results showed a significant change in GSH (57.18 ± 2.55 to 52.92 ± 2.15 μmol/g Hb). Interestingly, results after supplementation at day 8 and short heavy exercise showed that Erythrocyte GSH did not significantly differ from that at pre-exercise: GSH (55.10 ± 1.85 μmol/g Hb). However, Erythrocyte GSH differed significantly in the control group from that in the resting data of pre-exercise periods at day 0 and day 8.

From plasma nitrite results (Figure 6) at day 0, there was no difference between the NAC supplement group and control group; nitrite (78.3 ± 15.3 nmol/L). Then, after short heavy exercise, Plasma nitrite changed significantly; nitrite (210.0 ± 48.4 nmol/L). On the other hand, the nitrite level was higher (98.68 ± 16.9 nmol/L) after NAC supplementation for 7 days at day 8 than that at day 0 (78.3 ± 15.3 nmol/L), but this was not statistically different. Before NAC supplementation at day 0, and after short heavy exercise, results showed a significant change in nitrite (78.3 ± 15.3 to 210.1 ± 48.4 nmol/L). Interestingly, results at day 8 after supplementation and short heavy exercise showed no significant difference in plasma nitrite from that at pre-exercise: nitrite (192.9 ± 46.9 nmol/L). However, plasma nitrite differed significantly in the control group from that in the resting data of pre-exercise periods at day 0 and day 8.

From plasma MDA results (Figure 7) at day 0, there was no difference between the NAC supplement group and control group; MDA (0.63 ± 0.08 μmol/L). Then, after short heavy exercise, plasma MDA changed significantly; MDA (0.91 ± 0.07 μmol/L). On the other hand, plasma MDA resting parameters changed significantly after NAC supplementation for 7 days at day 8; MDA (0.43 ± 0.05 μmol/L). Before NAC supplementation at day 0, and after short heavy exercise, results showed a significant change in MDA (0.63 ± 0.08 to 0.91 ± 0.07 μmol/L). Interestingly, results after supplementation at day 8 and short heavy exercise showed no significant difference in plasma MDA from that at pre-exercise: MDA (0.44 ± 0.07 μmol/L). However, plasma MDA differed significantly in the control group from that in the resting data of pre-exercise periods at day 0 and day 8.

From the IL-2 results (Figure 8), short heavy exercise depleted the IL-2 release in the control group at both day 0 (3.2 ± 0.65 to 2.36 ± 0.37 pg/ml) and day 8 (3.11 ± 0.38 to 2.31 ± 0.52 pg/ml). However, the IL-2 level increased after short heavy exercise at day 8 (7.19 ± 0.59 from 3.01± 0.44 pg/ml) in the NAC supplement group, when compared to day 0 (3.09 ± 0.71 and 2.25 ± 0.36 pg/ml), due to the supplementation. Moreover, the time taken on the treadmill, until the heart rate reached 85% of MHR, did not change significantly in the control group (14.13 ± 0.46 and 13.94 ± 0.56 min), whereas, the time slightly increased (15.45 ± 0.41 to 15.95 ± 0.39 min) in the NAC supplement group, but with no difference in statistical analysis. This study found no adverse effects from NAC supplementation at 1,200 mg per day.

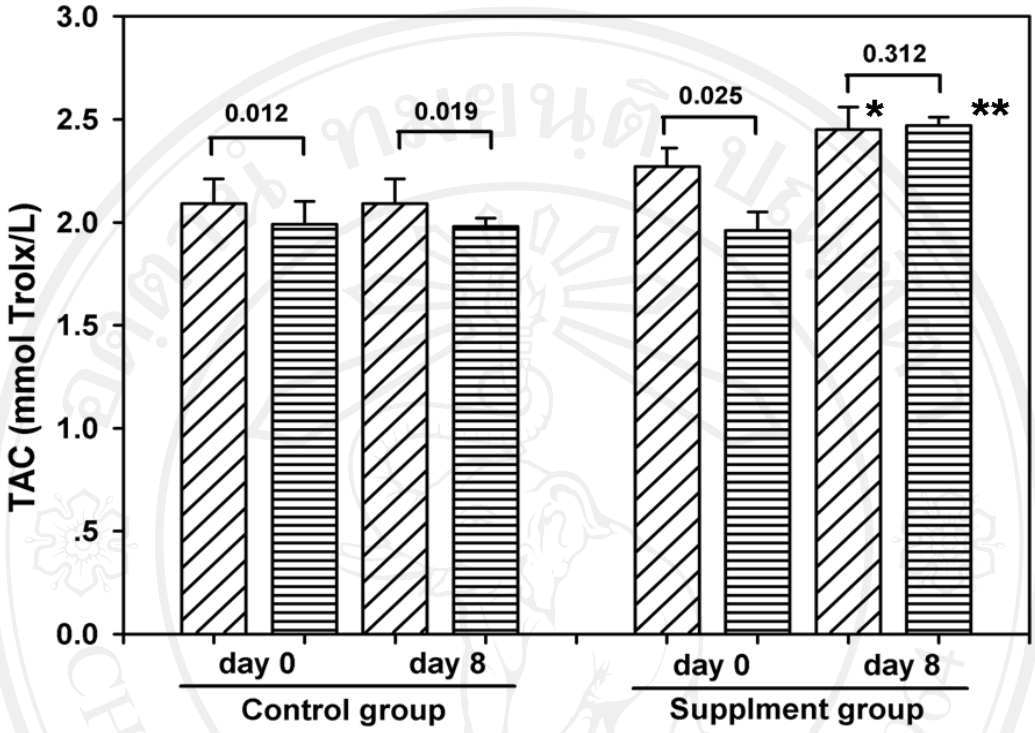

Figure 4. Plasma TAC (mmol Trolox/L) at the pre exercise (oblique line bar) and post-exercise test (horizontal line bar) between the control group (n = 13) and supplement group taking NAC at 1,200 mg daily (n = 16) before day 0 and after 7 days (day 8). TAC was assayed by the ABTs decolorization method. Each box plot represents the mean and standard error of mean (SE). Repeated measurement with two factors and LSD was tested with a General linear model at the $p<0.05$ level. (*, compared to before-exercise at day 0 and day 8 in both groups, and **, compared to post-exercise at day 0 and day 8 in both groups)

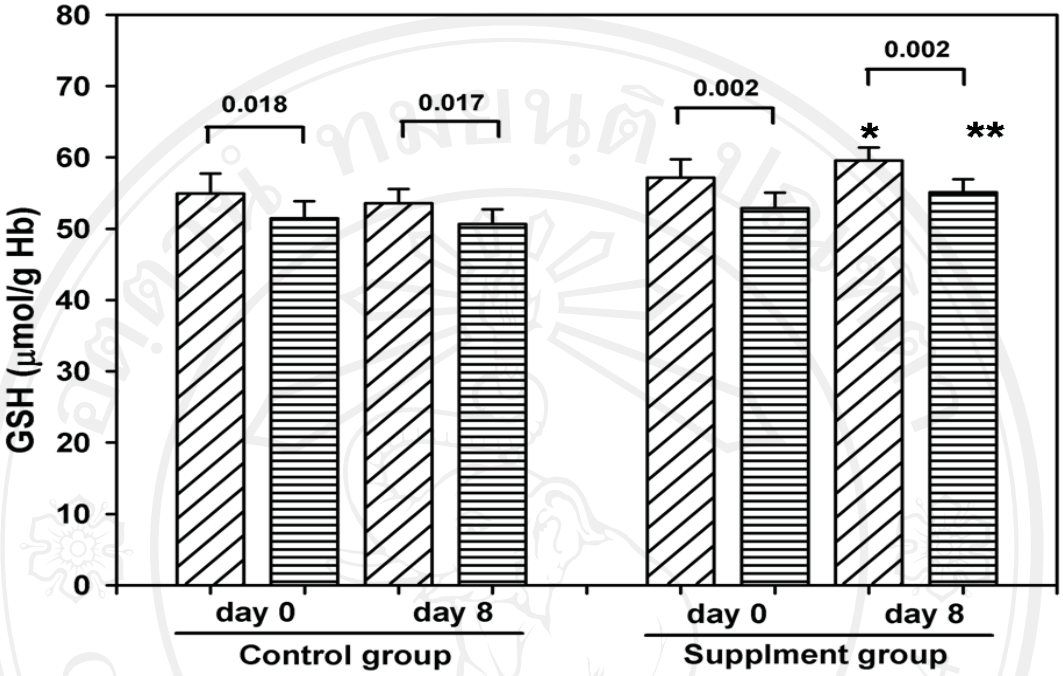

Figure 5. Erythrocyte GSH (µmol/g Hb) at pre exercise (oblique line bar) and post-exercise test (horizontal line bar) between the control group (n = 13) and supplement group taking NAC at 1,200 mg daily (n = 16) before day 0 and after 7 days (day 8). GSH was assayed with a DTNB reagent. Each box plot represents the mean and standard error of mean (SE). Repeated measurement with two factors and LSD was tested with a General linear model at the $p<0.05$ level. (*, compared to before-exercise at day 0 and day 8 in both groups, and **, compared to post-exercise at day 0 and day 8 in both groups)

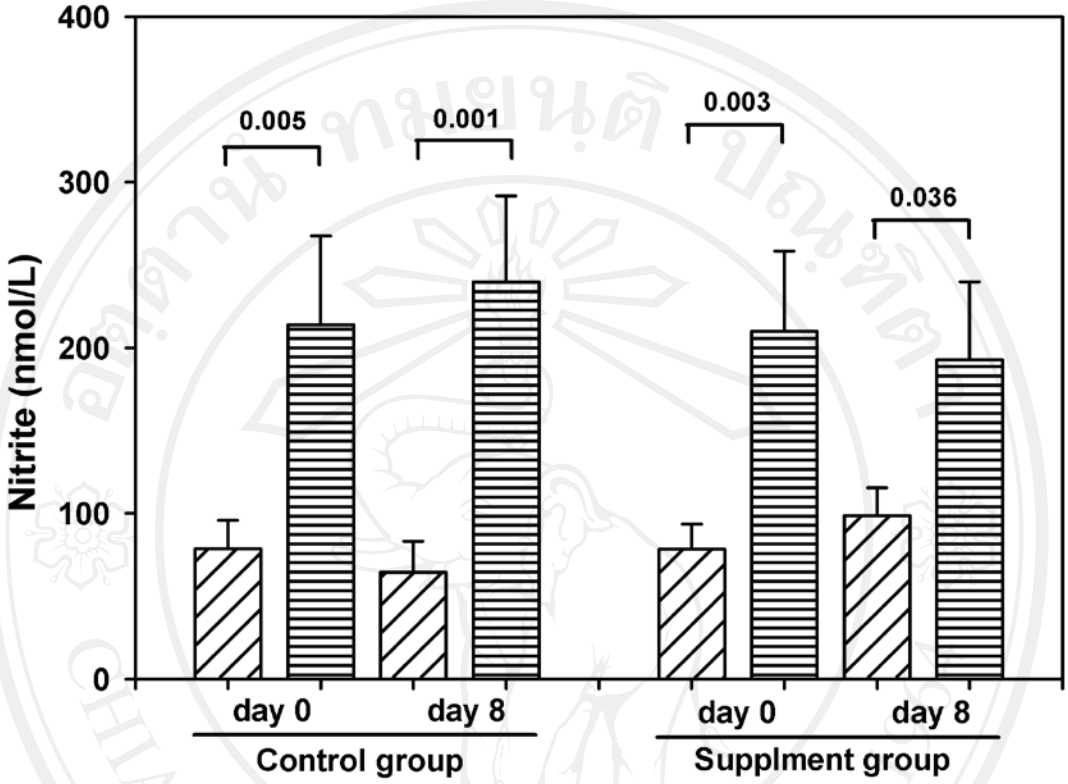

Figure 6. Plasma nitrite (nmol/L) at pre exercise (oblique line bar) and post-exercise test (horizontal line bar) between the control group (n = 13) and supplement group taking NAC at 1,200 mg daily (n = 16) before day 0 and after 7 days (day 8). Nitrite was assayed with a Griess reagent. Each box plot represents the mean and standard error of mean (SE). Repeated measurement with two factors and LSD was tested with a General linear model at the $p<0.05$ level. (*, compared to before-exercise at day 0 and day 8 in both groups, and **, compared to post-exercise at day 0 and day 8 in both groups)

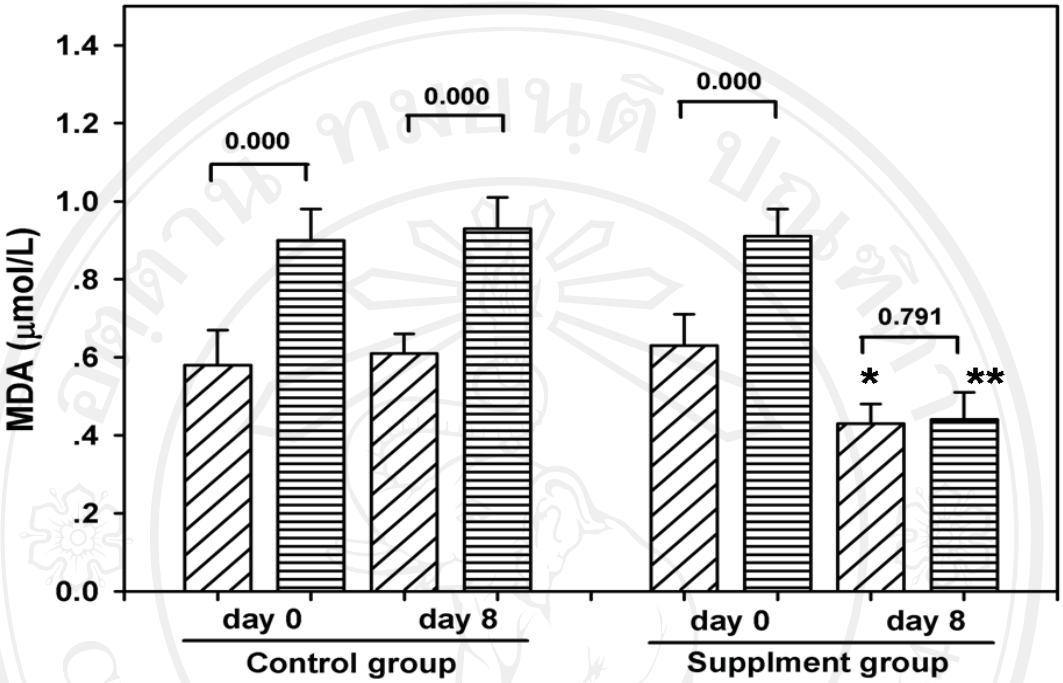

Figure 7. Plasma MDA (µmol/L) at pre exercise (oblique line bar) and post-exercise test (horizontal line bar) between the control group (n = 13) and supplement group taking NAC at 1,200 mg daily (n = 16) before day 0 and after 7 days (day 8). MDA was assayed with a TBA reagent. Each box plot represents the mean and standard error of mean (SE). Repeated measurement with two factors and LSD was tested with a General linear model at the $p<0.05$ level. (*, compared to before-exercise at day 0 and day 8 in both groups, and **, compared to post-exercise at day 0 and day 8 in both groups)

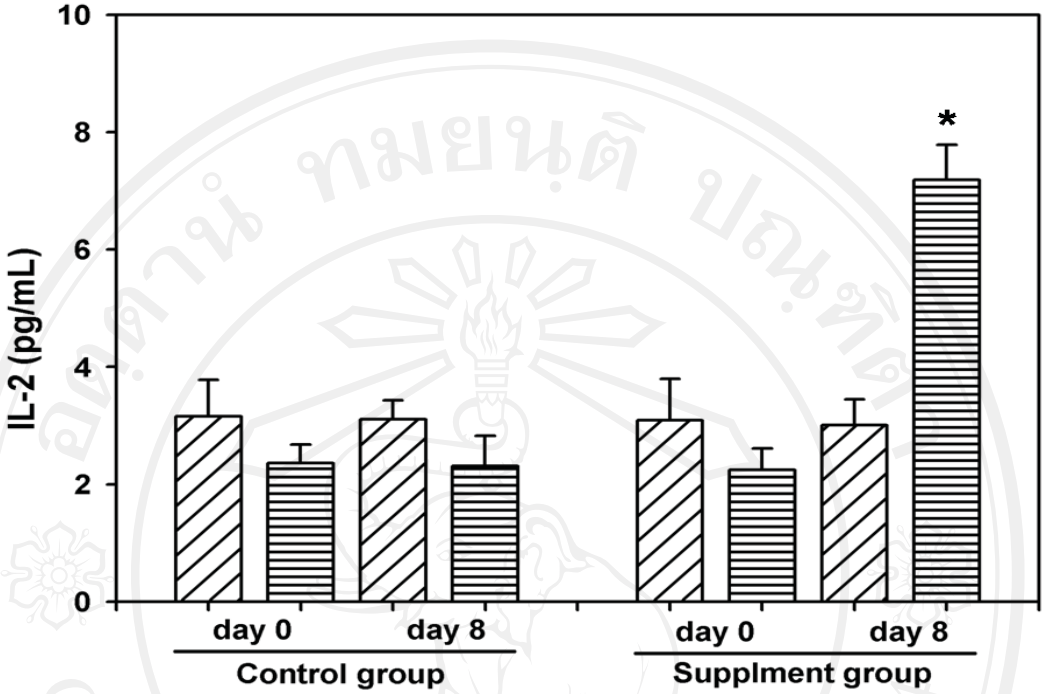

Figure 8. Plasma interleukin-2 (pg/mL) at pre exercise (oblique line bar) and post-exercise test (horizontal line bar) between the control group (n = 13) and supplement group taking NAC at 1,200 mg daily (n = 16) before day 0 and after 7 days (day 8). IL-2 was assayed using an ELISA-kit. Each box plot represents the mean and standard error of mean (SE). Repeated measurement with two factors and LSD was tested with a General linear model at the $p<0.05$ level. (*, compared to before-exercise and post-exercise at day 0 in control group, and before supplementation in supplement group at day 0 and before-exercise at day 8)

Maximal running time

From this study, we evaluated the maximal running time of all subjects at before and after 7 days in both groups. The target of both group is set up at 85% of MHR, and RPE was not more than 15. Although, the mean age in both group was not significant difference ($p > 0.05$), but when calculated the target heart rate between was significant difference. The result of this study found the statistical difference in maximal running time until heart rate reaching to 85% of MHR at before 7 days.

Table 3. Target heart rate, Rate of perceived exertion (RPE), and Maximal running times between control and supplement groups.

	Control group (n = 13)		**Supplement group (n = 16)**	
	Day 0	**day 8**	**day 0**	**day 8**
Target HR (bpm)	193.88 ± 1.21 (198-202)	164.79 ± 9.48 (168.3-171.7)	199.31 ± 0.79 (197-200)	199.31 ± 0.79 (197-200)
RPE	14.26 ± 0.79 (12-15)	14.67 ± 0.86 (13-15)	13.62 ± 0.80 * (12-15)	13.34 ± 0.92 (12-15)
Maximal running Time (sec)	14.13 ± 1.65 (11.67-18.08)	13.94 ± 2.02 (10.86-17.95	15.45 ± 1.65 * (12.66-19.12)	15.94 ± 1.55 # (13.18-18.7)

*, $p < 0.05$ compared at day 0 in control group, #, $p < 0.05$ compared to at day 8 in control group, Two-ways ANOVA was used to statistical analysis and LSD was compared between groups.