Technical Note

Lymphocyte Apoptosis in Smokers and Non-Smokers Following Different Intensity of Exercises and Relation with Lactate

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ABSTRACT

Int J Exerc Sci 4(3) : 204-216, 2011. Purposes of this study were 1) to examine the exercise intensity where lymphocyte apoptosis index (AI) is significantly increased in smokers and non-smokers, 2) to find out whether AI is associated with level of lactate (L). Fourteen healthy untrained smokers (≥ 1 pack year, n=7) and non-smokers (n=7) aged 18 to 26 were recruited. Each subject conducted three treadmill runs at different intensities randomly. Running distance for all three runs was equivalent to 30 minute run at 70% VO_{2max}. AI and L were analyzed at rest (Pre), immediately after (Post), and 1 h following (1 h post) each run. Data was analyzed using two way repeated measures ANOVA. Smokers showed higher AI than non-smokers at Post in 60% (12.5±0.62% vs. 9.97±0.51, p<.008) and 70% VO_{2max} running trials (17.53±0.57% vs. 15.6±0.41, p<.018). All L values at post showed significantly higher than Pre and 1 h post, but there was no significant difference between smokers and non-smokers. The strong positive relationship between AI and L was detected (r=.739, smokers vs. r=.793, non-smokers). Smokers tend to have higher AI than non-smokers following runs at 60% and 70% $VO_{2max,}$ but not following a run at 80% VO_{2max}. An increase in AI following a run at 60% VO_{2max} indicates that lymphocyte apoptosis can be increased following moderate intensity exercise. Since L and AI at post were increased in dose-dependent manner to exercise intensity, it is suggested that an increase in lactate production during exercise might contribute to the increase in lymphocyte apoptosis.

KEY WORDS: Lymphocyte apoptosis, lactate, exercise intensity, smoker

INTRODUCTION

Cigarettes contain more than 6,000 components, and many factors can induce DNA damage and oxidative stress in a diversity of cell types (6, 17, 18), which refers to a disturbance in the oxidant and antioxidant balance resulting in potential cell damage (2, 22, 29). In response to oxidative stress by smoking, pro-inflammatory mediators and cytokines such as TNF-α, IL-1, and IL-8 can activate the transcription factors such as activator protein-1 and NF-κB, and finally can induce apoptosis (2, 8). Exercise, which is a well-known stress factor, can induce lymphocyte apoptosis (15, 20). Lymphocytopenia is a condition where the level of lymphocytes in the blood is abnormally low, and this can be caused by intense physical exercise (31).

Generally, the number of lymphocytes increases during exercise and falls below resting levels rapidly depending on the intensity, duration and type of exercise (23, 31). Recent studies suggested that the mechanisms responsible for exercise-induced lymphocytopenia may be at least partly due to lymphocyte apoptosis, and this reduces immunity (14, 20, 25). However, the exercise-induced lymphocyte apoptosis in smokers who are under chronic oxidative stress has not been elucidated.

During exercise, significantly higher percentages of lymphocyte apoptosis have been reproduced in humans, in most cases, following an exhaustive exercise trial (20, 25, 26, 38). However, most studies were designed to compare lymphocyte apoptosis between moderate and high intensity exercise instead of finding the specific intensity at which lymphocyte apoptosis is significantly increased. Only one study increased exercise intensity stepwise and found that a significant increase in the level of apoptosis occurred at 60% of VO_{2max} or greater (27). However, it is possible that level of lymphocyte apoptosis might be affected by exercise duration instead of exercise intensity which denotes that lymphocyte apoptosis occurred earlier than that precise point. In addition, no studies have been conducted to assess if differences exist between smokers and non-smokers regarding the level of lymphocyte apoptosis following identical exercise.

L-lactate is the final product of anaerobic glucose metabolism in muscle tissue (32). During rest, L-lactate is consistently produced from pyruvate via the enzyme lactate degydrogenase (LDH) (30). The level of blood lactate is normally 1-2 mmol/L at rest (32). However, the concentration of lactate can be increased to over 13 mmol/L after intense exercise (34). Hyperlactatemia, not regarded as a serious condition, decreases the blood pH range to 7.35-7.45 concomitant with an increased level of lactate to the range of 2-5 mmol/L. In contrast, the level of lactate rising above 5 mmol/L and blood pH values falling below 7.25, a condition called lactic acidosis, is considered a serious condition indicative of tissue hypoxia, hypoperfusion, and possible damage (19, 32).

An increasing number of studies have investigated the relationship between the level of lactate and apoptosis (13, 24, 37). Cause and effect are still equivocal as well as its mechanisms. However, lactate production is associated with the loss of mitochondrial membrane potential suggesting that the elevated level of lactate might be an early indicator of apoptosis (37). Another research study showed that over-expression of the LDH-A gene and increased LDH-A gene activity produced more lactate which altered the lactate production to glucose consumption molar ratio, and this increased apoptosis (13). Recent reports demonstrated that an increase in LDH-A expression or LDH-A activity sensitizes cells to pro-apoptotic stimuli (13, 35). Also, acidification can initiate a previously latent set of enzymes with pH optima below 7.0, such as the acid endonuclease (DNases II) which stimulates DNA fragmentation (24). However, previous research studies do not exist focusing on direct relationships between the levels of lactate and apoptosis during exercise in human subjects.

Exercise can affect immunity both positively and negatively depending on

frequency, intensity and duration of exercise (11). Exercise-induced apoptosis is believed to be a normal regulatory process that serves to remove certain damaged cells without a pronounced inflammatory response, thus ensuring optimal body function. In contrast, following intense exercise, immunity is suppressed and susceptible to infection (5, 7). Intense exercise is known to disturb immune-homeostasis and induce immune cell apoptosis (20, 25). It is also known that moderate intensity exercise enhances immune function and antioxidant defense systems in non-smokers (21). However, we do not know whether moderate intensity exercise enhances immunity in chronic cigarette smokers. Since chronic smokers have antioxidant imbalance and immune suppression (2, 22, 29) an additional stress (exercise) may aggravate their condition instead of inducing potentially beneficial adaptation. In addition, the exercise intensity for untrained healthy people to avoid from immune suppression is equivocal. Therefore, this study was designed 1) to investigate the exercise intensity where lymphocyte apoptosis is significantly increased in smokers and non-smokers, 2) to find out whether lymphocyte apoptosis is associated with level of lactate during exercise.

METHODS

Participants

After approval from the Texas A&M International University Institutional Review Board (#2009-07-01), all subjects were informed of the purpose of the study. Healthy, untrained smokers and non-smokers aged 18 to 26 (20.67±.96 yr smokers vs. 20.17 ±.26 yr non-smokers) were recruited from the University student population. Participants were untrained, not taking any medication which could influence metabolic, cardiovascular function, or immune function and had no musculoskeletal limitations. Subjects' physical characteristics are shown in Table 1. Smokers showed higher % body fat than non-smokers (18.2 ±1.11% smokers vs. 13.0±1.14 non-smokers, $p<.007$). VO_{2max} was measured at least one week before other tests. Active smokers were selected who had a history of smoking in the previous 1 year and smoked at least 1 pack year (16). The first seven smokers and seven non-smokers VO_{2max} ranged between average and well above average (42.5-48.2 $ml \cdot kg^{-1} \cdot min^{-1}$) which indicated that they were untrained were selected for this study (10).

Table 1. Subjects' Physical Characteristics

Variables	Smokers	Non smokers
Age (yrs)	20.7±0.97	20.2±0.26
Height (m)	1.69±0.13	1.71±0.22
Weight (kg)	78.3±2.39	74.1±2.95
% Body fat	18.2±1.11*	13.0±1.14
VO_{2max} ($ml \cdot kg^{-1} \cdot min^{-1}$)	45.9±0.68	47.8±0.27
Pack year	2.72 ± 0.68*	

Protocol

Each participant had an orientation meeting with the principal investigator prior to the VO_{2max} test. During this meeting, participants were informed of the purpose and procedures of the study. All subjects signed informed consent forms before participation. Participants were asked to refrain from any strenuous exercise for two days prior to the exercise tests.

VO_{2max} test: VO_{2max} was measured with continuous, progressive, treadmill (Noramco NF4600C, Willis, TX, USA)

running protocol. It was initiated with a speed of 4.5 mph (7.2 kmh) at 0% grade. Speed and grade was increased by 0.5 mph (0.8 kmh) every two minutes up to 7.5 mph (12 kmh). This speed was maintained until exhaustion. Further increases in exercise intensity were achieved by changing the grade by 2% increments every 2 minutes. VO_{2max} was assumed if subjects achieved at least two of the criteria: volitional fatigue, increased workload with no increase in VO_2, RER greater than 1.15, heart rate near the estimated maximum based on age (220-age). VO_2 was measured continuously, heart rate was obtained at the end of every minute, and rating of perceived exertion (RPE) was recorded every 2 minutes. VO_{2max} was analyzed by an automated gas analysis system (MAX-2 metabolic cart; Naperville, IL, USA). Heart rate was monitored by telemetrically (Polar; Oy, Finland).

Exercise Tests

After conducting VO_{2max} testing, subjects randomly performed three running trials at different intensities: 60%, 70%, and 80% VO_{2max}. The test day and time were identical for each subject and had one week interval among tests. The running distance for three running trials was identical to the running distance for 30 minutes at 70% VO_{2max}. Once subjects entered the laboratory, each subject was fitted with a telemetric heart rate monitor and rested 20 minutes in a chair. Heart rate was measured (Polar; Oy, Finland), and RPE were recorded. Blood samples were collected from antecubital veins before (Pre), immediately after (Post), and an hour after exercise (1 h post) with 21 gauge blood collection needles (Greiner Bio-One, Kremsmünster, Austria) and used to measure apoptotic index and level of lactate.

Level of lactate

An amperometic method using an enzymatic reaction was used to measure level of lactate with lactate test meter and test strip (Arkary inc, Kyoto, Japan). A test strip contains 1.92 units lactate oxidase and 0.096 mg of potassium ferricyanide. Five µl of blood was drawn into the test strip. When the blood sample reaches to the reaction layer, lactate in the sample reacts with lactate oxidase in the reaction layer, and potassium ferricyanide is reduced into potassium ferrocyanide. Cumulated potassium ferrocyanide is oxidized into potassium ferricyanide and induces electrical currents which can be converted to the lactate concentration (mmol/L).

Lymphocyte apoptotic index

The morphological method which allows fast fixation of cells was used to measure lymphocyte apoptosis (28). Five µl of whole blood was pipetted onto microscope slides (VWR scientific, West Chester, PA, USA). After the slide samples dried in air, blood was stained with May-Grünwald Gimesa (Sigma-Aldrich Inc., St. Louis, MO, USA). The May-Grünwald Gimesa staining was carried out on the same day when blood was collected. Slides were immersed in May-Grünwald stain for 4 min, subsequently placed in phosphate buffer saline (Sigma-Aldrich Inc., St. Louis, MO, USA) for 3 min. Slides were submerged in Gimsa stain for 2 min, and rinsed with deionized water briefly. Slides were allowed to air dry before evaluation. Blood films were evaluated under a light microscope using the oil objective lens (Vista vision, VWR Scientific, West Chester, PA, USA). Lymphocytes were identified

and classified as either normal or apoptotic. If the lymphocyte cell displayed an approximately circular shape with a smooth cell membrane, the lymphocyte cell was considered normal. In contrast, a lymphocyte was determined apoptotic, if the lymphocyte displayed a decreased in cell volume (cell condensed) with hyperchromatic staining, exhibited membrane blebbing, or contained the formation of apoptotic bodies (12). A minimum of 100 lymphocytes per slide was counted. Apoptosis was recorded as the apoptotic index (AI), defined as the number of apoptotic lymphocytes divided by the total number of lymphocytes counted and expressed as a percentage. Slides were counted in duplicate and averaged to give the AI. If duplicate counts were discrepant more than 5%, the third slide was evaluated.

Statistical Analysis

Data was presented as means ± *SE*. The statistical package for social sciences (SPSS) 13 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The characteristics of smokers and non-smokers were compared using *t*-test with *p* value of <.05. Changes over three different running trials (60, 70, and 80% VO_{2max}), times (Pre, Post, and 1 h post) and between variables (smokers and non-smokers) were analyzed using *split-plot* design (two way repeated measures) ANOVA. *P*<.05 was inferred to be statistically significant. If significance was indicated, significant difference location was determined, *post hoc* test using Bonferroni's correction was performed. The Pearson's *r* was calculated to find out the relationship between level of lactate and lymphocyte apoptosis, *p*<.05.

RESULTS

Exercise Intensity and Level of Lactate

Level of lactate was measured by a calorimetric method with an enzymatic reaction. Results are displayed in Figure 1 and 2. In both smokers and non-smokers, level of lactate was significant increased immediately after (Post) at 60% VO_{2max} (3.11±0.28mmol/L smokers vs. 2.93±0.38mmol/L non-smokers, p<.05) as compared to Pre and 1 h post following exercise (Pre: 1.66±0.28mmol/L smokers vs. 1.33±0.16mmol/L non-smokers, 1 h post: 1.21±0.09mmol/L smokers vs. 1.41±1.35mmol/L non-smokers). There was no significant difference found between Pre and 1 h post. In 70% VO_{2max} running trial, smokers and non-smokers showed significantly higher level at Post (4.76±0.35mmol/L smokers vs. 5.26±0.74mmol/L non-smokers, p<.001) than Pre (1.43±0.10mmol/L smokers vs. 2.03±0.33mmol/L non-smokers) and 1 h post (1.60±0.21mmol/L smokers vs. 1.50±0.22mmol/L non-smokers). During 80% VO_{2max} running trial, level of lactate was significantly increased at Post in smokers and non-smokers (7.21±0.63mmol/L smokers vs. 7.70±0.81mmol/L non-smokers, p<.0001) compared to Pre (1.26±0.13mmol/L smokers vs. 1.46±0.21mmol/L non-smokers) and 1 h post (1.99±0.32mmol/L vs. 1.50±0.14mmol/L). There were no significant differences between smokers and non-smokers in level of lactate at each trial and time. Among Post measurements in smokers and non-smokers, level of lactate was significantly higher at Post following 80% VO_{2max} run as compared to Post following 60% VO_{2max} and 70% VO_{2max} runs (p<.05). However, post following 70% of VO_{2max} run did not show any significant difference as compared to Post following

60% of VO_{2max}. There were no significant differences in 1 h post values following 60%, 70%, and 80% VO_{2max} runs in smokers. In smokers, significantly higher level of lactate was found at 1 h post following 80% VO_{2max} (1.99±0.32mmol/L, p<.031) than 1 h post at 60% of VO_{2max} (1.21±0.86mmol/L) which did not show up in non-smokers. There were no significant differences among Pre values in three different trials in smokers and non-smokers.

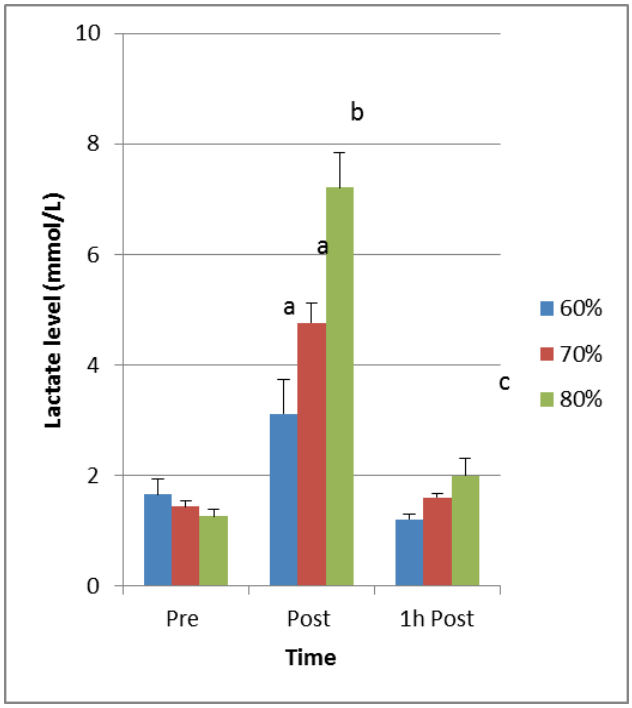

Figure 1. Level of lactate during 60%, 70% and 80% of VO_{2max} running trials at Pre, Post, and 1 h post in smokers. a, Significantly higher than Pre and 1 h post (p<.05). b, Significantly higher than Post 60% and 70% of VO_{2max} runs (p<.014). c. significantly higher than Post 60% of VO_{2max} (p<.031) Values are *mean ± SE.*

Exercise Intensity and Lymphocyte Apoptotic Index

The percentage of lymphocyte apoptosis at Pre, Post, and 1 h post during three running trials at different exercise intensities was measured by morphological identification with May-Grünwald and giemsa stain. Lymphocyte apoptosis showed *mean ± SE* % (Fig. 3-5). Between smokers and non-smokers, significant differences in lymphocyte apoptotic index were found at Post following 60% (12.5±0.62% smokers vs. 9.97±0.51 non-smokers, p<.008) and 70% runs (17.53±0.57% smokers vs. 15.6±0.41% non-smokers p<.018) (Fig. 3). There was no difference was found at Post following 80% VO_{2max} run between smokers and non-smokers. During the 60, 70, and 80% of VO_{2max} running trials, a significant rise in the lymphocyte apoptotic index was noted at Post compared to Pre and 1 h post in both Smokers and non-smokers (Fig. 4,5). Significant differences were also found at Post among three trials at different intensities. Data showed that the higher exercise intensity is, the greater lymphocyte apoptotic index is in both smokers and non-smokers (p<.0001).

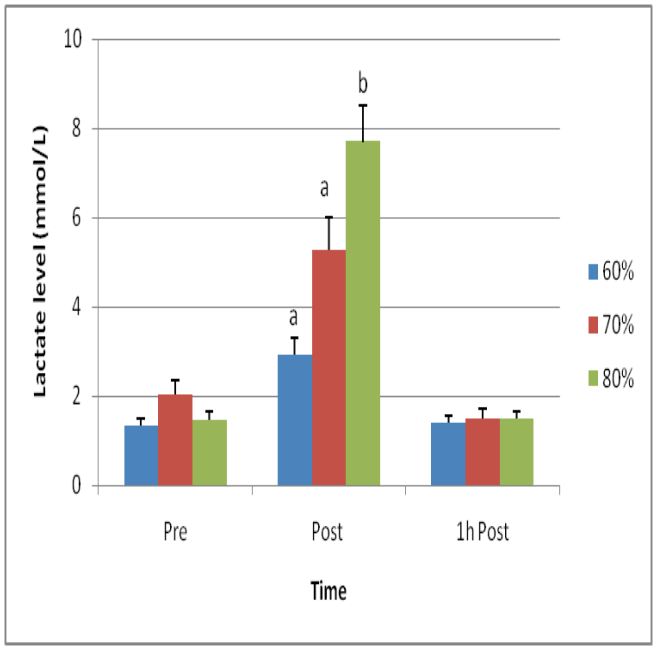

Figure 2. Level of lactate during 60%, 70% and 80% of VO_{2max} running trials at Pre, Post, and 1 h post in non-smokers. a, Significantly higher than Pre and 1 h post (p<.001). b, Significantly higher than Post 60% and 70% of VO_{2max} runs (p<.05). Values are *mean ± SE.*

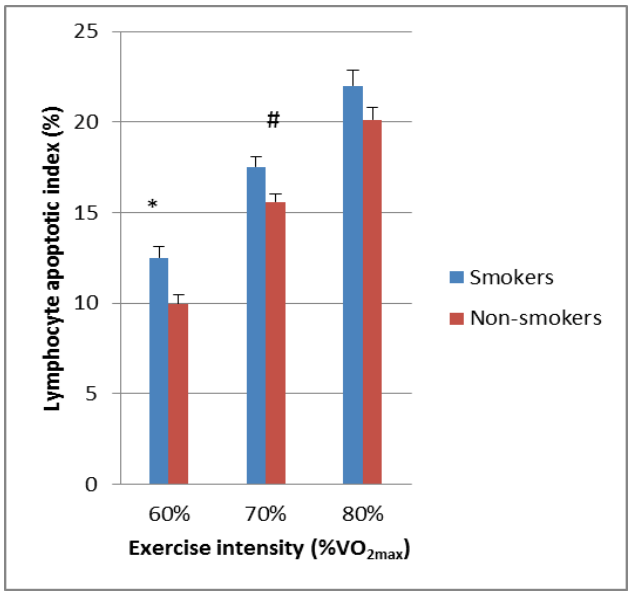

Figure 3. Lymphocyte apoptotic index (%) at Post during 60, 70 and 80% VO_{2max} running trials in smokers and non-smokers. *, Significantly higher level in smokers than non-smokers (p<.008). #, Significantly higher level in smokers than non-smokers (p<.018). Values are mean ± *SE*.

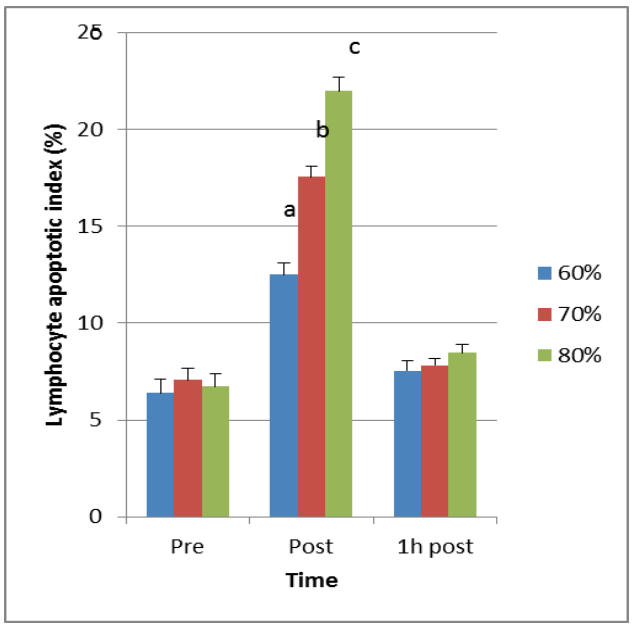

Figure 4. Lymphocyte apoptotic index (%) during 60%, 70%, and 80% VO_{2max} running trials in smokers. a, Significantly higher than Pre and 1 h post (p<.001). b, Significantly higher than Post 60% VO_{2max} runs (p<.0001). c, Significantly higher than Post 70% VO_{2max} (p<.0001). Values are *mean* ± *SE*.

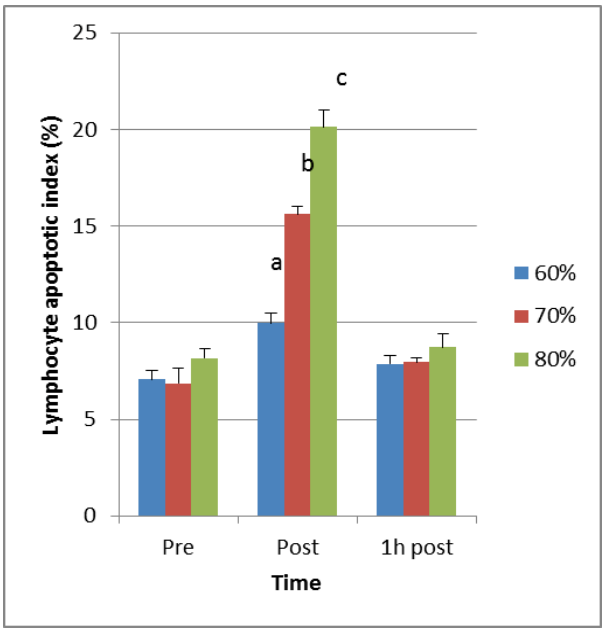

Figure 5. Lymphocyte apoptotic index (%) during 60%, 70%, and 80% VO_{2max} running trials in non-smokers. a, Significantly higher than Pre and 1 h post (p<.001). b, Significantly higher than Post 60% VO_{2max} runs (p<.0001). c, Significantly higher than Post 70% VO_{2max} runs (p<.0001). Values are *mean* ± *SE*.

Correlation between Level of Lactate and Lymphocyte Apoptotic Index

Pearson correlation r was used to indicate the relationship between level of lactate and lymphocyte apoptotic index at post following three running trials at different intensities in smokers and non-smokers. Level of lactate and lymphocyte apoptotic index (%) showed strong positive relation in smokers (r=.739 and p<.0001, Fig. 6) and non-smokers (r=.793 and p<.0001, Fig. 7).

DISCUSSION

Lymphocyte apoptosis during exercise in smokers and non-smokers

It was reported that lymphocyte apoptosis was significantly elevated at a high

intensity of exercise, which is above 80% VO_{2max} (20, 25, 38) or 75% VO_{2max} (36) indicating that moderate exercise did not induce lymphocyte apoptosis. To date, one study suggested that lymphocyte apoptosis can be significantly increased between 40% and 60 % VO_{2max} when exercise test was performed gradually (27). This result corresponded with present study data in that lymphocyte apoptosis was significantly increased at 60% VO_{2max} in both smokers and non-smokers (p<.01). Also, results indicated that exercise at higher intensities induced greater lymphocyte apoptosis.

Previous studies assessed lymphocyte apoptosis after 1 h (25, 26, 27), and 24 h (20, 38) following exercise. Mars et al. (20) reported that 86% of cells still showed lymphocyte apoptotic pattern of DNA distribution at 24 h after exercise. However, Wang & Huang (38) showed that the lymphocyte apoptotic pattern of DNA fragmentation returned to basal levels within 24 h. Other studies showed that lymphocyte apoptosis had returned to baseline levels in 1 h (25, 26, 27). The present study also found that elevated lymphocyte apoptosis decreased to resting level at 1 h after following all three different intensity running trials in both smokers and non-smokers (p<.012). This discrepancy between two results can probably be attributed to the differences in number of subjects, exercise mode and blood treatment method. In the study by Mars et al. (20), only three subjects were recruited with a high variation with the pretest values of apoptotic cells and exercise restriction before and after exercise testing was not controlled.

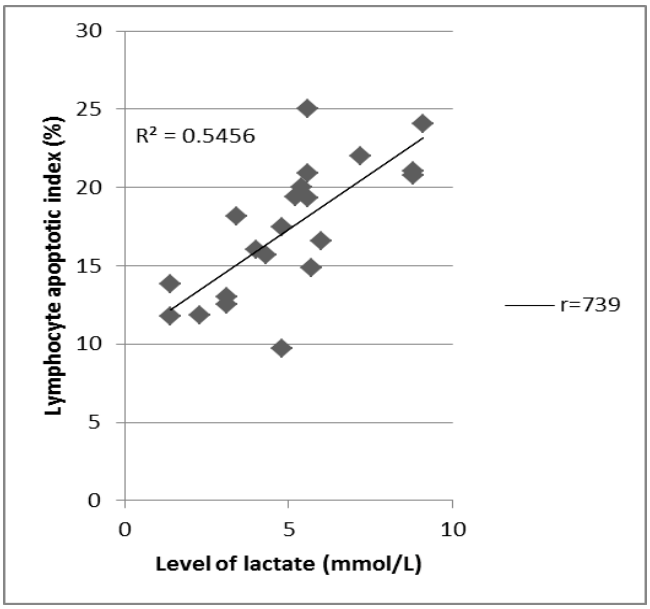

Figure 6. The correlation between level of lactate and lymphocyte apoptotic index in smokers measured at Post following three running trials at 60%, 70%, and 80% of VO_{2max} (p<.0001).

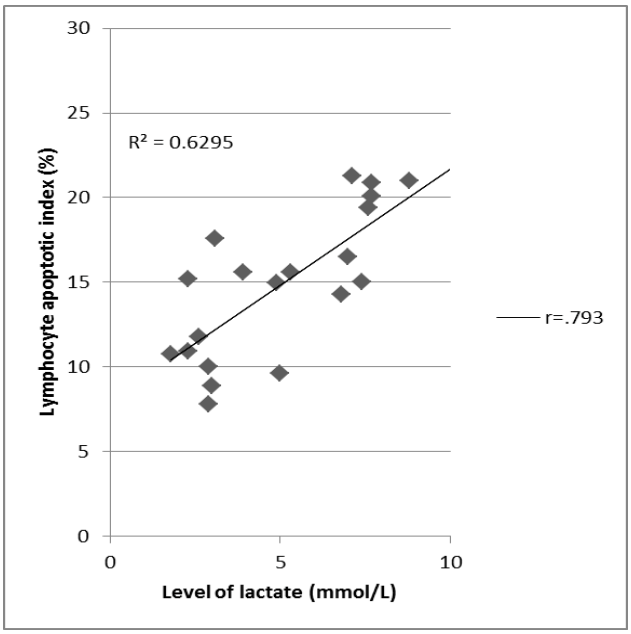

Figure 7. The correlation between level of lactate and lymphocyte apoptotic index in non-smokers measured at Post following three running trials at 60%, 70%, and 80% of VO_{2max} (p<.0001).

To our knowledge there are no studies which directly examine human lymphocyte apoptosis in smokers who are under chronic oxidative stress by cigarette

smoking. However, previous research studies have suggested that cigarette smoke can induce apoptosis in various tissues in human and animal lung (1, 2, 3). Cumulating studies reported oxidative stress and inflammatory factors in smokers in cell culture (8, 22). Reddy Thavanati et al. (29) found that lymphocyte DNA damage in smokers was twofold higher than that in non-smokers and this result was associated with a reduced antioxidant capacity in smokers. In the present study, smokers and non-smokers did not show any significant difference in lymphocyte apoptosis at rest, however, smokers showed higher lymphocyte apoptosis immediately after the run at 60% VO_{2max} (p<.008) and the run at 70% VO_{2max} (p<.018). This difference was not found following the run at 80% VO_{2max}. Thus, at exercise intensity under 80% VO_{2max}, smokers are more prone to have significantly higher lymphocyte apoptotic index than non-smokers. This might indicate that smokers tend to show immune suppression resulting from disturbed oxidative homeostasis due to cigarette smoking. In addition, a significant increase in lymphocyte apoptosis at 60% VO_{2max} indicates minimal exercise intensities where lymphocyte apoptosis significantly increased might be lower than 60% VO_{2max} in smokers. Moreover, a significantly higher level of lymphocyte apoptosis in smokers showed that exercise-induced lymphocyte apoptosis in smokers might occur earlier than non-smokers.

Although oxidant and antioxidant capacity were not measured in this study, the response of higher lymphocyte apoptosis in smokers following runs at 60% and 70% VO_{2max} may be attributed to oxidative stress in smokers. Smokers have been known to be exposed to large quantities of reactive oxygen species from cigarette smoking which agitate the antioxidant defense system, and these can lead to oxidative stress indicating increased lipid peroxidation and disturbance of oxidants and antioxidants (4, 9). In a cell culture study with human fetal lung fibroblasts, the exposure to cigarette smoke extract caused a dose-dependent increase in cellular oxidative stress and reduction of intracellular glutathione and these were correspondent with increased apoptosis and DNA damage (2). A study with human blood lymphocytes reported that even low levels of cigarette smoke activated NF-□B through an increase in oxidative stress (8), which decreased anti-oxidant capacity caused lymphocyte apoptosis (38). In the present study there was no significant difference in lymphocyte apoptosis at rest between smokers and non-smokers which might indicate that lymphocyte apoptosis is well regulated in smokers and non-smokers during rest even though smokers might have a higher oxidant and lower antioxidant status. In addition, oxidative stress with 80% VO_{2max} run might surpass the oxidative stress that was accumulated by cigarette smoke which induced greater lymphocyte apoptosis in smokers at 60% and 70% VO_{2max}. However, this study did not uncover the mechanism. Thus, further investigation is needed to examine the level of oxidative stress in smokers and non-smokers to observe the effects of cigarette smoking on lymphocyte apoptosis at rest and following exercise.

Level of lactate during exercise in smokers and non-smokers

It has been shown that level of lactate in muscle and blood can be increased during the intense exercise (30, 33, 34). The present study measured the level of lactate from

blood to provide evidence if there was difference in lactate concentration in smokers and non-smokers at rest and following running trials at different intensities. Results showed that level of lactate is not varied between smokers and non-smokers at rest, following exercise, and after 1 h recovery. The present study demonstrated that the level of lactate increased depending on exercise intensity, which corresponds with previous studies that reported an increase in lactate following high intensity exercise and an accompanying return to rest value within 1 h (33, 34). The present study showed significant higher level of lactate at 1 h post following the run at 80% VO_{2max} in smokers than 1 h post 60% VO_{2max} (p<.031). Since there is no significant difference in level of lactate between 1 h post and Pre in 80% VO_{2max} trial in smokers, this phenomena could not be considered a noteworthy finding caused by a type-2 error.

Lymphocyte apoptosis and level of lactate during exercise in smokers and non-smokers

It has been reported that an increase in level of lactate can induce apoptosis (13, 37). Klearchos et al. (13) found that increased lactate production by increasing LDH-A activity induced apoptosis indicated by measuring PARP cleavage. The possible mechanism which links the level of lactate and apoptosis is acidification resulting from accumulation of the lactate product. Tiefenthaler et al. (37) showed that increased lactic acid was related with loss of mitochondrial membrane potential which was associated with apoptosis. Disturbance of mitochondrial membrane potential resulted from an increase in lactic acid associated with acidification. (37). Another mechanism is that acidification can activate acid endonucleases which have optimal pH below 7.0 (24). It has been well reported that increased level of lactate is associated with decreased pH along with the mechanism of lactic acidosis (30, 33, 34). After termination of exhaustive exercise, the pH level fell to 6.60 in muscle samples whereas level of lactate in muscle and blood were increased 113 mmol/kg and 13 mmol/L, respectively (34). Also, exhaustive exercise is reported to decrease pH level to 6.96 in blood with an increase in level of lactate to 18 mmol/L (33).

A significant correlation (p<.0001) between increased lactate and lymphocyte apoptosis might suggest that level of lactate can be an indicator of lymphocyte apoptosis. However, there were no significant differences in level of lactate between smokers and nonsmokers in all three running trials and time while lymphocyte apoptotic index was significantly higher in smokers at Post following 60% and 70% VO_{2max} runs. There were no varied level of lactate between smokers and non-smokers even though the higher lymphocyte apoptosis level in smokers during 60% and 70% VO_{2max} might be associated with total work load. The total work load performed by non-smokers was higher than smokers even though the present study tried to assign an identical metabolic intensity of exercise. This unexpected result may be due to the higher running speed at similar %VO_{2max} in non-smokers. Therefore, the difference in lymphocyte apoptosis at Post between smokers and non-smokers could be explained by other mechanisms untested in this study. A well designed study controlling total work load and exercise intensity is needed to find out the relationship between lymphocyte apoptosis and level of lactate.

The present study has shown that lymphocyte apoptosis was significantly increased following exercise at 60% VO_{2max} in both smokers and non-smokers. Results of the present study did not exhibit a minimal exercise intensity where lymphocyte apoptosis appears to increase (apoptotic threshold). However, it is implied that the lymphocyte apoptotic threshold might occur under 60% VO_{2max}, and smokers may have a lower threshold at which lymphocyte apoptosis increases significantly. It is suggested that smokers tend to have higher lymphocyte apoptosis following exercise at 60% and 70% VO_{2max} at least partly due to increased oxidative stress caused by cigarette smoking. However, the mechanism for this difference between smokers and non-smokers was not investigated. Further study with assessing oxidant and antioxidant status is needed to elucidate the potential mechanisms.

The present study posited that lymphocyte apoptosis might be associated with increased levels of lactate during exercise. A strong relation between lymphocyte apoptosis and level of lactate suggests level of lactate might be one of the factors associated with exercise-induced lymphocyte apoptosis. However, level of lactate from this study was unvaried between smokers and non-smokers at any time points. Therefore, difference in lymphocyte apoptosis at Post between smokers and non-smokers should be explained by other mechanisms than were measured in this study.

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