Lactate Promotes Survival of Peripheral Blood Mononuclear Cells by Decreasing Apoptotic Events

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INTRODUCTION

Moderate exercise can increase the efficiency of the immune system and reduce the risk of infection.[1] In contrast, strenuous exercise, such as intense and prolonged training, can inhibit immune function and increase the risk for upper respiratory tract infections.[2] Strenuous exercise can cause lymphopenia, a reduction in the proportions of white blood cells, depending on the type and intensity of exercise.[3] Lymphopenia could be due to decreased flow of lymph into blood, or by immune cell death by necrosis or apoptosis.[4] The biochemical mechanism of action by which exercise modifies the immune system is not completely understood. During anaerobic exercise, working muscles utilize glycogen, leading to the formation of lactate, which can spill over into the blood. Traditionally, lactate has been viewed as a by-product of exercise that can lead to fatigue. In 2007, however, lactate was found to actually provide protective effects to rat skeletal muscles, suggesting that it can enhance cell survival.[5]

Cells typically die by two ways: necrosis or apoptosis. Necrosis is characterized as a passive cell death following environmental perturbations, whereas apoptosis is a regulated process of 'programmed' cell death. Lymphocyte necrosis and apoptosis were higher immediately following a 24-hour period after a high intensity treadmill exercise test, as they were compared to the values obtained after an exercise of moderate intensity.[6,7]

We reasoned that lactate could be one of the factors acting as a bridge between exercise and the immune system. Lactic acid produced during exercise is converted in the form of its conjugated base lactate. Lactate accumulates in muscles where it is mostly consumed, and some enters the circulation. In an oxygen-poor environment, such as in muscle cells during exercise, lactate can be produced from pyruvate catalysed by the enzyme lactate dehydrogenase (LDH) where pyruvate + NADH + H^+ ➔ lactate + NAD^+. If lactate builds up in muscles, it will stimulate pain sensation and promote damage to the tissue, and it will also cause lactate levels to increase in the blood. The clearance of blood lactate can be improved by endurance training.[8]

Lactate is metabolized by the heart, neurons in the brain, and hepatic cells, which can convert lactate into glucose via the Cori cycle.[9] However, what is the effect of blood lactate on the immune system? In the current study, lactate was explored as a possible chemical factor linking together effects of exercise and immune responses. Peripheral blood mononuclear cells (PBMCs) were used for this study as a source of human immune cells. This blood fraction, containing a mix of lymphocytes and monocytes that belong to the white blood cell family, is commonly used for in vitro research on the immune system.

MATERIALS AND METHODS

PBMC ISOLATION AND CELL CULTURE

The study was conducted using human PBMCs. Whole human blood was obtained by venipuncture (approved by the Concordia University Human Research Ethics Committee). The Blood samples were diluted in a solution of 1X phosphate buffered saline (PBS) and a ratio of 1:2 Ficoll to separate blood into different layers. After 30 minutes of centrifugation at 1800 rpm (700 X g) on the Thermo Scientific CL10 series with a 0-G26/1 swing-out rotor, four layers were observed: PBS with plasma as the top layer, followed by a PBMCs layer, then Ficoll, and finally erythrocytes as bottom layer. The PBMCs layer was carefully removed with a transfer pipette, placed into fresh conical tube, diluted with PBS, and centrifuged for 15 minutes at 1500 rpm (480 X g) with the same centrifuge. PBMCs formed a pellet, and can be used after washing twice with PBS at 1200 rpm (310 X g).[10]

Six different media were prepared from a stock of Roswell Park Memorial Institute media (RPMI; Wisent Bioproducts) with L-glutamine, penicillin-streptomycin and 2mg/mL glucose. The first medium was a lactate-free medium. The other five media contained lactate at 0.5mM, 1.0mM, 3.5mM, 7mM, and 14mM, respectively. Lactate was purchased from SIGMA Aldrich. The pH values measured for the media containing lactate at 0mM, 0.5mM, 1.0mM, 3.5mM, 7.0mM, and 14mM were, respectively, 7.56, 7.51, 7.49, 7.42, 7.35, and 7.08. The PBMCs (at $4.0x10^6$ cells/mL) were added to each medium in a 96 well round-bottom sterile plate. Two duplicates were made to account for within-test variance.

FLOW CYTOMETRY AND APOPTOSIS MEASUREMENTS

The culture plate containing PBMCs was incubated at 37°C and cell counting was performed by flow cytometry using fluorescent dyes, including a propidium iodide analogue (7AAD) and annexin V. Percentages for live, necrotic, and apoptotic cells were obtained using the Acurri BD C6 Plus software by placing a quadrant that falls between the populations of cells. Samples without any dye added were used as a negative control to set the quadrants correctly.

STATISTICS

The data was processed for removal of within subject variations, according to Loftus et al.[11] ANOVA analysis was done on each data set from day 1, day 2, and day 3, and a post-hoc test (Tukey's multiple comparison) test was performed.

RESULTS

Samples of PBMCs were exposed in vitro to a range of lactate concentrations intended to simulate biologically relevant blood lactate concentrations. The samples were analysed each day for a total of three days to determine the percentages for live, necrotic, and apoptotic cells using flow cytometry (Figure 1). 7AAD is a measure that indicates necrosis and late apoptosis, and annexin V is a measure of early apoptosis. If a cell has holes in its membrane, then it will be positive for 7AAD and positive for annexin V. Thus, necrosis is 7AAD-positive and annexin V positive, and apoptosis is 7AAD-negative annexin V positive, as shown in the figure 1 quadrant analysis.

On day 1 of the analysis, which corresponded to 24 hours after starting the incubation, there was no significant difference in the percentage of live cells in the cultures (Figure 2A). On day 2, the survival was still the same for all lactate concentrations, although the overall amount of live cells appeared to be lower than on day 1, suggesting that the cells were slowly dying in culture (Figure 2B). By day 3, there were significantly higher survival rates in the 3.5mM and 7.0mM lactate concentrations as compared to the culture without lactate (Figure 2C). Necrosis was not significantly different between the lactate concentrations at any of the times, although there was an overall trend of increasing necrosis from day 1 to day 2, and from day 2 to day 3 (Figure 2D-F). Interestingly on day 2, the percentage of apoptosis was lower for the 3.5mM lactate as compared to no lactate, and on day 3, apoptosis was lower for the 3.5mM and 7.0mM lactate compared to the control group without lactate (Figure 2G-I).

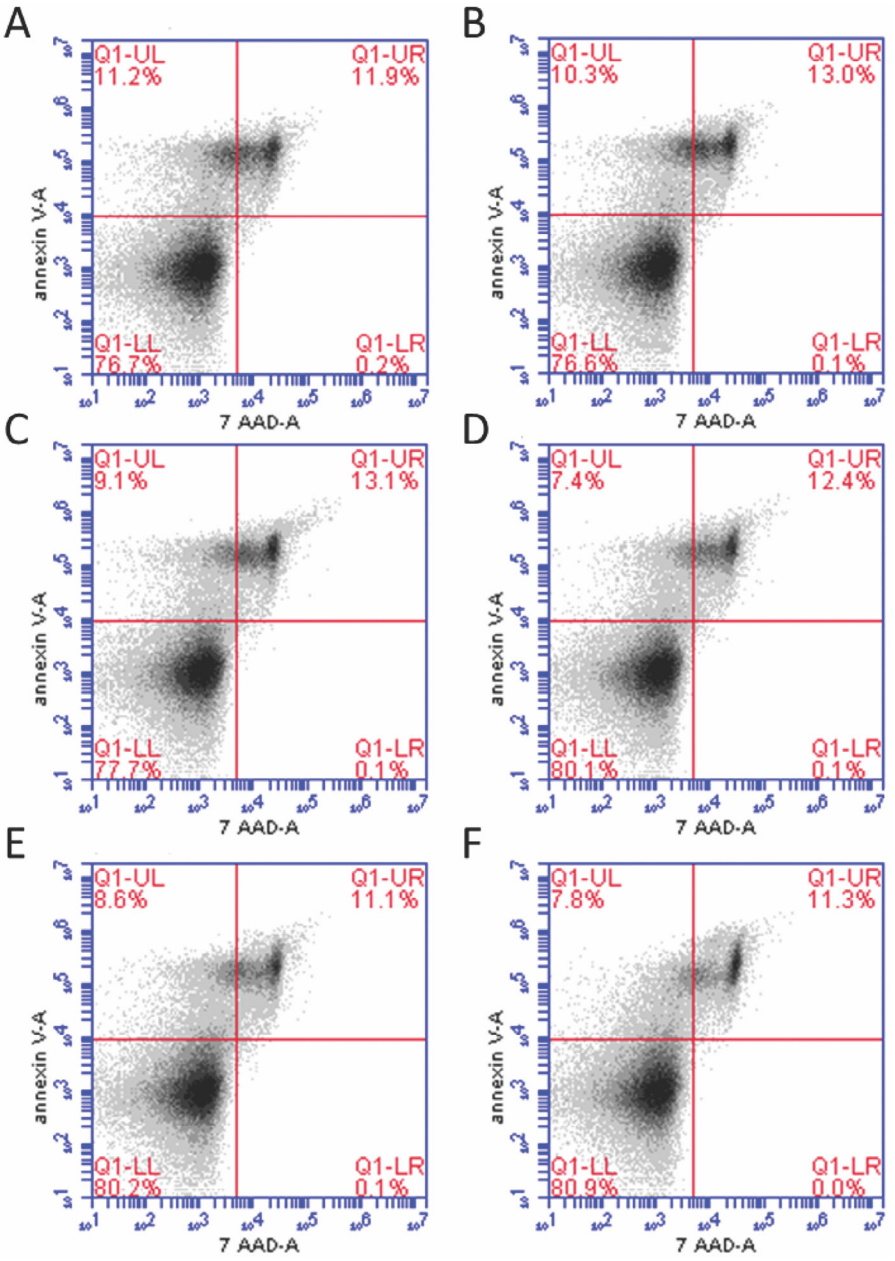
Looking at trends for peak values on each day, the highest percentage of live cells was present in the 7mM lactate medium for day 1, the 3.5mM lactate medium for day 2, and the 7mM lactate medium for day 3 (Figure 2A-C). The highest percentage of necrotic cells was present for the 1mM lactate treatment for day 1, the 1mM lactate for day 2, and the 14mM lactate for day 3 (Figure 2D-F). The highest percentage of apoptotic cells was present in the 0.5mM lactate medium for day 1 and the 0mM for day 2 and day 3 (Figure 2G-I). This analysis is consistent with the notion that cells are surviving better at 3.5mM and 7mM lactate compared to the lower values of lactate or the higher value of 14mM. The enhanced survival is due to less apoptosis occurring when PBMCs have 3.5mM or 7.0mM of lactate. With 14mM of lactate, the PBMCs had a non-significant trend towards surviving more and having less apoptosis than the 0mM condition.

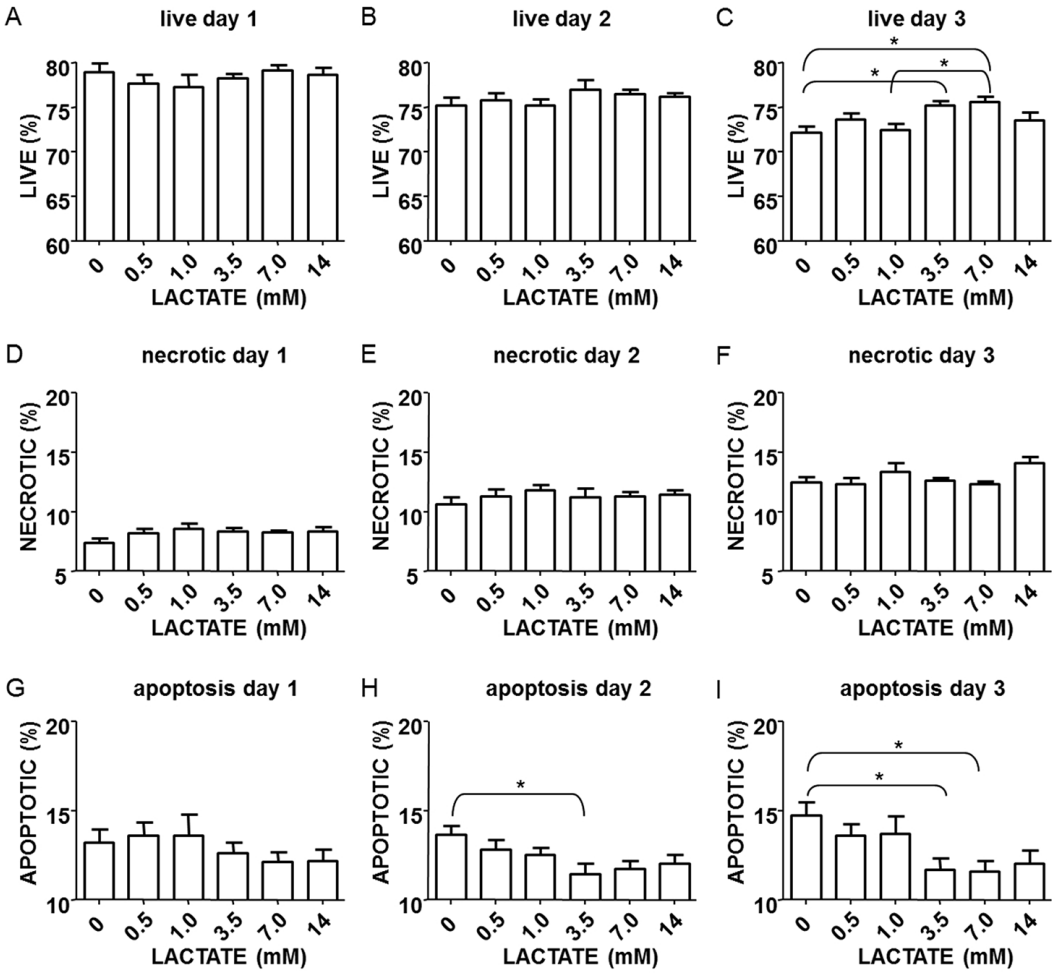
Figure 1: Example flow cytometry data and quadrant analysis

PBMCs were untreated (A), or treated with lactate at 0.5mM (B), 1.0 mM (C), 3.5 mM (D), 7.0 mM (E), and 14 mM (F). Shown is example data of a day 3 analysis. Cells were stained with annexin V and 7AAD, then analysed by flow cytometry. Each plot is divided into four quadrants by the cross, and the percentage of cells that is in each quadrant is shown. The lower left (LL) quadrants are live cells, the upper left (UL) are apoptotic cells, and the upper right (UR) are necrotic cells. Data is representative of 5 experiments.

FIGURE 2: Summary of results from PBMCs treated with various concentrations of lactate

The percentage of cells is shown for live, necrotic, and apoptotic cells, as determined based on the quadrant analysis criteria in Figure 1. The data is pooled from 5 healthy people's PBMC samples. The samples had been treated in vitro with lactate for 1 day, 2 days, or 3 days. *p<0.05





DISCUSSION

The underlying mechanism of action by which exercise affects the immune system remains unclear. In this study, blood lactate was explored as a factor that may affect lymphocyte survival using an in vitro modelling approach. The amount of lactate we chose to add to the PBMCs was based on biologically relevant values of lactate concentration in the blood that has been recorded during various levels and modes of exercise. The values represent increasing intensities of exercise. The 0.5mM and 1.0mM values correspond to plasma lactate concentrations when a person is at rest.[12] The small amount of blood lactate at rest is produced by red blood cells from anaerobic respiration.[13] The 3.5mM lactate value reflects lactate concentrations measured at a work rate corresponding to low-intensity exercise.[14] The 7.0mM lactate value corresponds to the amount of lactate present in the blood 5 minutes after exercising at 60% VO_2 max, equivalent to a medium intensity.[15] The 14.0mM lactate corresponds to the amount present in the blood after exercising at 90% VO_2 max, equivalent to a high-intensity exercise involving arm cranking or knee extension in healthy men.[14,16] The 14.0mM concentration also represents a high value recorded after maximum-intensity treadmill running by trained men subjects aged 22 to 29 years.[17] By recreating these values of lactate, we modelled in vitro what effect they have on lymphocytes. This reductionist approach is advantageous in that we can control the amount of lactate precisely and minimize other confounding variables that exist when a person is exercising.

Our results demonstrate that immune cells survive better in vitro at the higher lactate concentrations that represent low to medium intensity exercise (3.5mM, 7.0mM), and high intensity exercise to an extent (14.0mM). The lower lactate concentrations that represent resting levels of lactate had little effect on PBMC survival. The enhanced survival with the higher lactate concentrations was due to decreased apoptosis, rather than necrosis. It was surprising that PBMCs tolerated the 14mM lactate condition well. Although there was a trend of more necrosis on day 3 with the 14mM lactate, the live cells were similar to the control with no lactate.

Being an acid in fluid means that lactate will lower the pH of the growth media. Despite the high concentration (14mM) of lactate added to the media, we measured a neutral pH (7.0), as compared to the control media, which was measured at a pH of 7.5. The minimal difference in pH in the various lactate medias we prepared is due to the strong bicarbonate buffer that is present in the media purchased from the manufacturer. Blood also contains a bicarbonate buffer, which can counteract acids, so the media we used closely matches that of blood.

The fact that lactate promoted cell survival suggests that it may provide a source of carbohydrate which the cells can use for energy. In theory, immune cells would require a lactate transporter in their membranes and lactate dehydrogenase to convert the lactate back into glucose. Moncarboxylate transporters are found in human lymphocytes[18]. More research is required to determine the extent to which white blood cells might use lactate for energy.

There were some limitations to this study. The PBMCs used were a mixture of cell types, so it is not possible to say definitively that the cell measures are coming from a particular type of mononuclear cell or all mononuclear cell types, including T cell, B cell, monocytes, NK cells, and other rare subsets. Cell sorting would be one way to address this issue in future experiments. Another limitation that is inherent to reductionist experiments is that it does not necessarily translate into an actual exercise paradigm. While these experiments were done in vitro, they do shed light on the in vivo interaction between the immune system and exercise. We exposed PBMCs to lactate and examined the effects over a period of three days. We chose this time frame because apoptosis typically occurs over the course of approximately three days, and we wanted to ensure we captured any effect on cell survival.

In conclusion, lactate at levels reflecting low to moderate or strenuous exercise promoted survival of PBMCs. Considering the evidence that strenuous exercise induces apoptosis, it seems unlikely that lactate is the reason for apoptosis in the context of exercise. On the contrary, immune cells appeared to benefit from lactate. In fact, it could be that lactate production is one of the reasons that moderate exercise enhances the immune system.

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