

Effects of Isokinetic versus Isotonic Training and its Cessation on Total Leukocytes and Lymphocytes Count in Adolescent State-level Weightlifters

Abstract

Background: The study investigated the effects of isokinetic versus isotonic training among adolescent state-level weightlifters in terms of total leukocytes, total lymphocytes, and its subsets following 24 sessions of training program and a month following training program cessation. **Methods:** Nineteen adolescent state-level weightlifters were assigned into isokinetic or isotonic groups. All participants were recruited from a pool of weightlifters with standardized training program provided by their coach. Series of immunological tests were carried out before the commencement, immediately upon the completion, and a month after the cessation of the additional training program to evaluate total leukocytes and lymphocytes count. **Results:** The results revealed a significant time and group interaction and main effects of time on mean total leukocytes ($P < 0.05$). Mean total leukocytes count at posttest decreased in both groups. In isotonic group, it was further decreased following 1 month of training cessation ($P < 0.05$) but not in the isokinetic group. However, the decrement was not high and the values were in the normal range. No significant time and group interaction was observed in total lymphocytes and its subsets count. **Conclusions:** Eight weeks of isokinetic and isotonic additional training with emphasis on shoulder joint only affect mean total leukocytes count in state-level adolescent weightlifters.

Keywords: Adolescent, cell count, resistance training, rotator cuff, weight lifting

Introduction

Exercise is a form of a physical activity, and it induces a certain level of stress upon the immune system. The stress will cause the immune system to respond in a certain sequence of mechanism collectively called immune response.^[1] The immune responses are largely dependent on the type, intensity and duration of exercise, participant's fitness level, environmental factors, and duration of measurement.^[2]

Isotonic and isokinetic are types of muscle contraction. Typical resistance exercise training involves isotonic muscle contraction by lifting a fixed weight such as biceps and triceps curl with dumbbell. In isotonic muscle contraction, the applied resistance remains constant throughout the range of motion of involved joint with varied joint angular velocity.^[3,4] Hence, loading occurs at the weakest point in the system, while the rest of the system is functioning at less than its capacity.^[4] On the other hand, isokinetic training mode provides

proportional resistance following the amount of force that being exerted, which allows constant angular velocity of the involved joint across its range of motion.^[5] Hence, a maximal effort can be achieved during isokinetic muscle contraction due to the application of maximal load throughout the range of motion. However, isokinetic muscle contraction can only be achieved by using a dynamometer.

Immune cells, especially macrophages (i.e., mature form of monocytes) have an important role in muscles' repair following muscle damage induced by resistance training.^[6] Muscle repair is initiated by inflammatory responses mediated by systemic and local cytokines produced by macrophages. These pro-inflammatory cytokines act to remove cellular debris from damaged myofibers.^[7] Following that, macrophages involve in downregulation of inflammation by secreting anti-inflammatory cytokines to prevent secondary damage and further permit the growth of muscle tissues.^[8] At later stage, cell growth was

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promoted by macrophages arginine metabolism and its byproducts.^[9] This macrophage stimulation is not necessarily occurring due to muscle injury. In the absence of muscle damage following adaptation to training, macrophages predominate to produce growth factors that are needed for hypertrophy.

Resistance training may cause muscle damage and alter immune responses.^[10] Furthermore, it could impose a significant change on immune system despite its shorter duration of effort than endurance training.^[11] Previous studies showed that isotonic resistance training induced changes of leukocytes concentration immediately after exercise and during recovery period.^[12-18] Leukocytes mediate the rejuvenation of muscle tissue after resistance training.^[8,19] It was also reported that the amount of lymphocytes, natural killer cell, and monocytes increased following resistance training.^[14]

There were several studies that highlighted the usage of isokinetic exercise to investigate the immune responses such as inflammation by measuring the level of cytokines.^[20-22] However, study with regard to total leukocytes and lymphocytes count is scarce. Furthermore, majority of previous studies on the effects of resistance training on immune functions were designed to evaluate the immune parameters only after a short-term training period or following a single bout of resistance exercise.^[16,18,20,23] The participants of these previous research were recreationally active males,^[23] male adults with an average of 9 years of weight training experience,^[18] and healthy but nonstrength-trained women.^[16,20]

To date, no studies have been conducted to evaluate the effects of isotonic versus isokinetic training and its cessation on immune responses among advanced (i.e., state level) adolescent weightlifters. Therefore, the purpose of the present study is to examine the effects of isokinetic and isotonic training and its cessation on total leukocytes and lymphocytes count of state-level adolescent weightlifters. We hypothesized that both types of training may induce stress on immune system; hence, total leukocytes and lymphocytes count may increase following training but ceased after its attenuation. We aim to fill the gap in literature regarding different modes of muscle contraction training for advanced level of weightlifters and its cessation effects on immune responses.

Methods

Study participants

A priori sample size calculation indicated that eight participants in each group were adequate to yield 0.8 power of the study with large effect size (0.6) according to Cohen.^[24] In this study, 23 participants with minimum 2 years of experience in competitive weightlifting and without history of shoulder injury were included. They were adolescent athletes with age between 13 and 17 years

old. Participants were omitted from the study if they had any musculoskeletal injury within the past 2 years or unable to adhere to at least 85% of the training program.

Participants were provided with detailed explanation regarding the methodology of the study. Upon agreement, their written consent form was collected. For participants under 18 years old, assent was obtained from their guardians. The participation in the present study was voluntary. Participants were randomly assigned into either isokinetic or isotonic training groups with their weight matched. Both groups went through 24 sessions of training program three times per week for 8 weeks. Ethical approval was acquired from Human Ethical Committee of Universiti Sains Malaysia (USM/JEPeM/14110457) with protocols in compliance to Declaration of Helsinki 1975.

Study protocol

The entire experiment was conducted during preparatory phase of the weightlifters' training cycle. All participants were recruited from the same pool of weightlifters with standardized training program provided by their coach. Their program included five strength training sessions and one technical session per week. The duration of their standardized training session was 2 h/session.

Meanwhile, the training program prescribed in the current study (i.e., isokinetic and isotonic rotator cuff resistance training) was an additional training for the athletes. The athletes adhere to their mandatory training program while attending to the prescribed training program of the current study. Series of tests to evaluate the immunological responses were conducted (1) before the commencement, (2) immediately after the completion, and (3) 1 month following the cessation of the additional training program.

Participants arrived at the laboratory at 8.30 am after an overnight fasting. For each participant, 3 ml of blood were withdrawn and collected into an EDTA tube. The EDTA tube containing the whole blood was brought to the immunology laboratory and processed on the same day of collection. Blood samples in the EDTA vacutainer were used for hematological analysis including total leukocytes and total lymphocytes count using an automated hematology analyzer (Sysmex XS-800i, Illinois, USA). Lymphocyte subsets of serum T lymphocytes (CD3+), B lymphocytes (CD19+), and NK cells (CD16⁺ CD56⁺) absolute counts were determined by using the flow cytometer (BD FACS Cantor II, Becton Dickinson, USA) with a four-color direct immunofluorescence reagent kit (BD Multitest™ IMK, Becton Dickinson, USA).

First, 20 μ L of BD Multitest 6-color TBNK reagent (i.e., fluorochrome) was pipetted into the bottom of the tube followed by 50 μ L of well-mixed, anticoagulated whole blood. The tube was capped and gently vortexed to mix. It was then incubated in the dark at room

temperature (20°C–25°C) for 15 min. After the incubation period, 450 µL of 1X BD FACS lysing solution was added to the tube. Again, the tube was mixed and incubated for 15 min in the dark at room temperature (20°C–25°C). After that, the sample was analyzed on the flow cytometer.

The fluorochrome-labeled antibodies in the reagent bind precisely to leukocyte surface antigens following addition of whole blood to the reagent. During acquisition, the cells move past the laser beam and scatter the laser light while the stained cells fluoresce. These scatter and fluorescence signals were identified by the instrument thus provided information about the cell’s size, its relative fluorescence intensity, and internal complexity.

Percentage of differences of measured variables for each test was determined using the formula as follows:

$$\text{Percentage of difference}_1 = \frac{\text{posttest value} - \text{pretest value}}{\text{pretest value}} \times 100$$

$$\text{Percentage of difference}_2 = \frac{\text{one month after - test value} - \text{posttest value}}{\text{posttest value}} \times 100$$

Training program

The duration of the training program was 8 weeks. The isokinetic training group had their training sessions three times per week at sports science laboratory of a local university. The isokinetic training was conducted using a dynamometer (Multi-joint System Biodex Pro, Shirley, NY, USA). For familiarization, all participants completed one set of 12 reciprocal internal and external shoulder rotations in concentric mode. The training program was initiated following a minimum 3 days of recovery.

For every training sessions, participants performed 10 min of warmup and shoulder joint stretching followed by a minute of active rest. The dynamometer was calibrated with known weight before each training sessions. A correction for the lever arm system and mass of the limb was made for all torque curves. The preparation of participant on the dynamometer followed the manufacturer’s guide closely to ensure safety of the participants.

Training was progressive in terms of the number of sets, angular velocities applied, and changes of body position to resemble upper limb’s movement during weightlifting. For

the first until the eighth sessions, participants were trained with 45° of shoulder abduction in seated position, with 12–15 repetitions for two sets at 120°.s⁻¹ angular velocity. For the ninth to the 16th sessions, participants were trained with 90° of shoulder abduction in seated position, with 10–12 repetitions for three sets at 240°.s⁻¹ angular velocity. Finally, for the 17th to 24th sessions, training was conducted in standing position while diagonally lifting the bar. The angular velocity was set at 360°.s⁻¹ with 8–10 repetitions for four sets. The training program has been published elsewhere.^[10,25]

A minute of rest interval between sets was provided for all training positions. Training program was conducted for both sides of upper limb. For cool down, the participants stretched their shoulder and applied ice pack on the shoulder for 10 min to ease muscle soreness. Training was progressive in terms of the number of sets, angular velocity applied, and changes of body position to resemble upper limb’s movement during weightlifting. Training sessions including warming up and stretching were completed in 1 h.

The isotonic group had similar lifting positions, number of sets, duration of rest interval between sets, and repetitions with isokinetic group. However, instead of using dynamometer, their training was conducted using a constant weight which is about half of their upper limb’s weight. The participants’ upper limb weight was measured using isokinetic device during familiarization phase. In isotonic group, the angular velocity was not fixed. The training program is summarized in Table 1.

Statistical analysis

The count of total leukocytes, total lymphocytes, and its subsets were compared between isokinetic and isotonic training groups at three time points (e.g., before training commencement, after 24 sessions of training, and 1 month following training cessation) using general linear model-mixed ANOVA with repeated measure to determine the interaction between groups and intervention phase. Main time effects and interaction effects between group and time were of interest. *Post hoc* analyses with Bonferroni correction were conducted when there were significant effects of between and within groups. The accepted level of significance was set at *P* < 0.05. All data were expressed as mean ± standard deviation. Data analyses were conducted using SPSS statistical software (version 22.0, SPSS Inc., Chicago, USA).

Table 1: Characteristics of prescribed isokinetic and isotonic training

Sessions	Body position	Repetitions	Sets	Rest (min)	Velocity (°/s)
1-8	Seated with 45° of shoulder abduction	12-15	2	1	120
9-16	Seated with 90° of shoulder abduction	10-12	3	1	240
17-24	Standing while lifting the bar diagonally overhead	8-10	4	1	360

Velocity was applied for isokinetic training only

Results

In this study, a total of 23 participants (19 males, 4 females) with age range between 13 and 17 years old were recruited from a state-level weightlifting team. However, only 19 participants (16 males, 3 females) successfully completed all training sessions and participated in the pre-, post-, and 1 month after training cessation tests. Four participants (3 males, 1 female) dropped out due to personal problems and injury. All the remaining participants' data were included in the subsequent analyses.

The participants had a mean age of 14.74 ± 1.37 years, mean height of $158 \text{ cm} \pm 9.61 \text{ cm}$ (range, 142 cm–174 cm), and mean weight of $62.82 \pm 16.24 \text{ kg}$ (range, 45–98 kg). Table 2

Table 2: Physical characteristics of participants (mean±standard deviation)

	Isokinetic group (n=8)	Isotonic group (n=11)
Age (years)	14.36±1.06	14.82±1.60
Height (m)		
Pretest	1.59±0.07	1.58±0.11
Posttest	1.60±0.07	1.59±0.11
1-month posttest	1.61±0.07	1.59±0.11
Body weight (kg)		
Pretest	70.82±19.63	57.00±10.79
Posttest	73.24±19.40*	58.25±10.88*
1-month posttest	74.49±19.97 [#]	65.00±25.28 [#]
Body fat percentage (%)		
Pretest	33.87±11.30	24.93±5.86
Posttest	36.31±10.57	27.96±6.14
1-month posttest	36.31±10.58	26.74±7.18
Fat-free mass (kg)		
Pretest	45.44±8.64	46.63±15.47
Posttest	45.54±9.47	45.46±13.59
1-month posttest	46.27±9.44	46.67±14.01

*Significantly different from its respective pretest value ($P < 0.05$),

[#]Significantly different from its respective posttest value ($P < 0.05$)

summarizes the descriptive data of physical characteristics of participants. The general linear model-mixed ANOVA with repeated measure design revealed significant time x group interaction ($df = 2$, $F = 7.137$, $P < 0.05$) and significant main time effect ($df = 2$, $F = 57.19$, $P < 0.05$) on mean body weight. An increment of body weight was observed in both groups at posttest compared to the pretest, and at 1 month after training cessation-test compared to the pretest.

Table 3 summarizes the results of mean total leukocytes, total lymphocytes, and lymphocytes subsets (CD3+, CD19+, CD16+ CD56+) after 24 sessions of either isokinetic or isotonic training. The general linear model-mixed ANOVA with repeated measure design revealed significant time x group interaction ($df = 2$, $F = 4.742$, $P = 0.016$) and significant main time effects ($df = 2$, $F = 2.485$, $P = 0.039$) on mean total leukocytes. There were significant differences in mean total leukocytes at 1 month after training cessation-test compared to posttest in isokinetic ($P = 0.033$) and isotonic groups ($P = 0.004$). Similarly, significant differences in mean total leukocytes were observed in isotonic group ($P = 0.027$) but not in isokinetic group ($P = 0.755$) at 1 month after training cessation-test compared to pretest.

There was no significant time x group interaction and main time effects on mean total lymphocytes, mean T lymphocytes (CD3+), and NK cells (CD16+ CD56+). However, B lymphocytes (CD19+) showed significant main time effects ($df = 2$, $F = 3.693$, $P = 0.038$).

Discussion

We found that leukocytes count decrease significantly from pretest following 8 weeks of both isotonic and isokinetic training in advanced level of adolescent weightlifters. In isotonic group, it was further decreased following 1 month of training cessation but not in the isokinetic group. The decreased of total leukocytes count is due to a

Table 3: Total leukocytes, total lymphocytes, and lymphocytes subsets count across isokinetic and isotonic groups (mean±standard deviation)

Groups	Pretest ($10^3/\mu\text{L}$)	Posttest ($10^3/\mu\text{L}$)	1-month posttest ($10^3/\mu\text{L}$)	Percentage of difference ₁	Percentage of difference ₂
Isokinetic (n=8)					
Leukocytes	8.35±2.12	7.17±1.87	8.16±2.95 [#]	-14.13	13.81
Lymphocytes	3.27±0.79	2.88±0.68	3.06±0.76	-11.93	6.25
CD3+	2.09±0.49	1.88±0.48	1.95±0.51	-10.05	3.72
CD19+	0.41±0.14	0.36±0.05	0.35±0.06	-12.19	-2.78
CD16+CD56+	0.61±0.27	0.52±0.22	0.58±0.26	-14.75	11.54
Isotonic (n=11)					
Leukocytes	7.93±2.04	7.86±1.34*	6.59±1.16 [#]	-0.88	-16.16
Lymphocytes	3.04±0.95	2.99±0.62	2.68±0.67	-1.64	-10.37
CD3+	1.85±0.55	1.88±0.48	1.68±0.46	1.62	-10.64
CD19+	0.34±0.09	0.37±0.11	0.30±0.09	8.82	-18.92
CD16+CD56+	0.72±0.37	0.63±0.29	0.56±0.27	-12.5	-11.11

*Significantly different from its respective pretest value ($P < 0.05$), [#]Significantly different from its respective posttest value ($P < 0.05$)

delayed leukocytosis by the release of newly synthesized neutrophils from the bone marrow.^[26,27] On the other hand, the increased total leukocytes count is caused by the release of leukocytes from the marginal pools due to the increased of blood flow.^[28,29] It should be noted that despite decrement and increment of the total leukocytes count in the present study, all values were within the normal range of leukocytes count. On the other hand, no significant changes were observed in total lymphocytes and its subsets count in both training groups.

Even though the present study found that the total leukocytes count diminished following concentric isotonic training, it was reported in previous studies that the count increased after performing an eccentric isotonic exercise.^[30,31] This is because eccentric muscle contraction induces more damage and inflammation in human muscle tissues compared to concentric muscle contraction.^[32] These minor injuries may cause stronger inflammatory response and lead to the activation of immune system.^[19] Therefore, different types of muscle contraction (i.e., concentric versus eccentric), training intensity, and participants' level of expertise could be the reason of contradictory findings of the present study from previous studies.

In the present study, it was shown that there were no significant differences of total lymphocytes and its subsets (T-lymphocyte, B-lymphocyte, NK cell) count following 24 sessions of additional training program. Previously, it was reported that total leukocytes, lymphocytes and its subsets, eosinophils, neutrophils, and monocytes increased following six sets of six repetitions eccentric isokinetic elbow flexion at velocity of $30^{\circ}.s^{-1}$ in recreationally active adult male participants.^[31] Contrary to the previous study, the number of sets, repetitions, and angular velocity of the current study protocol may not be sufficient to elicit a significant response of the immune system other than leukocytes count. In addition, the mode of muscle contraction applied in the study^[31] was eccentric compared to the concentric mode of the present study. Furthermore, our participants were advance (i.e., state level) adolescent weightlifters compared to their recreationally active male adult participants. In elite athletes, particularly those participating in aerobic endurance sports, white blood cell counts can be lower than other population.^[33]

A significant interaction of time and intervention was observed in body weight. The data showed that body weight increases in both groups at post- and 1 month after training cessation tests compared to their respective baseline values. On the other hand, there were no interaction between time (e.g., pre-, post-, 1 month after training cessation-test) and intervention (e.g., isokinetic and isotonic training) for body fat percentage and fat-free mass. Our findings suggest that both interventions did not cause significant changes of body fat percentage and fat-free mass. Therefore, we suggest that the cause of increased in body weight was due to the

normal growth and development among the adolescent athletes.

Studies regarding the effects of isokinetic and isotonic resistance training and its cessation on immune responses were scarce. Furthermore, less studies were involved with advanced level of weightlifters who may have different physiological adaptations toward resistance training compared to novice and recreationally active population.^[34] Our findings showed that except for a small decrement, 24 sessions of isokinetic and isotonic training among adolescent state-level weightlifters did not extensively suppressed immune function in terms of total leukocytes and lymphocytes count. Further studies are recommended with different intensities, angular velocities, duration of training program, levels of expertise, and additional assessment of cytokines concentration to discover possible beneficial effects of isotonic and isokinetic resistance training on immune responses.

The findings of the present study are limited to advance level of adolescent weightlifters. Moreover, we did not include anti-inflammatory cytokines such as interleukin (IL)-6, IL-10, C-reactive protein, and tumor necrosis factor, which also mediate leukocytes function following exercise.^[35] For example, it was reported that a significant increase of IL-6 concentration was observed in power athletes compared to endurance athletes following concentric isokinetic knee extension at $180^{\circ}.s^{-1}$.^[23] Hence, the evaluation of cytokines concentration in future studies is recommended to understand the immune response following isokinetic and isotonic training.

Conclusions

Eight weeks of isokinetic and isotonic additional training with emphasis on shoulder joint only affect mean total leukocytes count in state-level adolescent weightlifters.

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Conflicts of interest

There are no conflicts of interest.

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