Effect of the Long Term 'Training and Competitive' Cycle on Urinary Protein and Creatinine in Elite Male Triathletes in Malaysia – A Pilot Study

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Abstract— **Background: Strenuous exercise may cause DNA, skeletal muscle as well as renal damage. Triathletes experience strenuous muscular activity both during competition and training. Studies have shown changes in renal function after competition which returns to normal after few days. However, these finding have only focused on a single event. There is no literature studying the cumulative effect of "training and competition" cycles over a season of triathlon competitions. The objective of this study was to evaluate the cumulative effect of "training and competition" on renal function in elite male triathletes using urine protein and creatinine.**

Methods: Seven male elite triathletes were recruited for the study. They were on a standardized training regimen and competed in at least one endurance event every month for the past 3-4 years. They were followed up for nine months. Urine samples were collected at the beginning (Phase 1) and at the end of the triathlon season (Phase 2). Urine protein and creatinine levels were estimated using spectrophotometric methods and compared with a Student's t test ($p<0.05$ was considered significant).

Results: The urine protein was 5.26 ± 3.99 mg/dl and 11.48 ± 6.87 mg/dl and urine creatinine was 11.67 ± 5.16 mmol/L and 19.09 ± 7.15 mmol/L for phase 1 and phase 2 respectively. There was a statistically significant difference in urine protein and creatinine ($p<0.05$) between phase 1 and phase 2.

Discussion: Urine protein and creatinine are considered markers of renal function. Our results show that at the end of the "training and competition" cycle of an elite triathlete, there is two-fold increase in urinary protein and creatinine. This finding provides evidence to the cumulative effect of training and competition over a period of 9 months.

Keywords— **DNA damage, Triathlon, Urinary protein, Urinary creatinine.**

I. Introduction

Triathlon is an endurance sport which involves three different disciplines (swimming, running and cycling) which are continuous and sequential. Triathlon races vary in distance and a typical standard or intermediate race involves 1.5 kilometers (0.93 mi) swimming, 40 kilometers (25 mi) biking and 10 kilometers (6.2 mi) running to the finish line. There are events which have longer race distances called the ultra-distance, most commonly called the ironman triathlons [1]. The race typically starts in the morning (7.00 am) and ends around midnight lasting approximately 17 hours. Triathlon training also involves strenuous exercise and it requires the athlete to divide training time between the three disciplines. Training usually involves reaching similar endurance levels as a race but of lesser duration, however more importantly on day to day basis. Hence, for a competitive elite triathlete it would mean periods of "high endurance training" and "high endurance competitions" throughout one's career. Triathletes are also busy the whole year around with a constant "competition and training" cycles every 2 months.

The race on its own is a strenuous exercise [2] and it is well documented in literature that at the end of a triathlon race, there is evidence of muscle damage [3]. A strong body evidence exists which shows marked DNA damage [4] due to increased oxidative stress [5]. High intensity training which every triathlete undergoes training too contributes to the effects of strenuous exercise on the body. This also may lead to excessive urinary excretion of protein and this may vary from 18 to 100% [6]. Proteinuria after exercise is usually depended on the type and intensity of the exercise rather than the duration of the exercise and is caused due to changes in the glomerular permeability and filtration ratio. [7,8] However proteinuria after exercise is usually transient and it returns to physiological levels following few hours of resting [9]. Another marker of renal function and hemodynamics is urinary creatinine. Creatinine is an enzymatic breakdown product of creatine mainly from the skeletal muscle following exercise induced skeletal muscle degradation. Creatinine is excreted through urine and its increased excretion indicates compromised renal function [10].

Though many studies have established the effects of participation in a triathlon event on the renal function and hemodynamics, the contribution of training as a factor has not been investigated. Thus the aim of this study was to investigate the cumulative effect of "training and competition" cycle on the renal function using urinary protein and creatinine measures.

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II. METHODS

A. Subjects

Seven male triathletes with an average age of 17.71 ± 3.59 years volunteered to take part in this study. They are members of a professional triathlon team and undergo a standardised structured and rigorous training program. Table 1 provides the anthropometric characteristics of the subjects. The subjects were informed of the potential benefits and risks involved in this study and informed consent was obtained. The study was conducted in accordance with the Declaration of Helsinki and the guidelines of Resolution on 198/96 of the National Health Council, and was approved by the Institutional Ethics Committee of the National Defence University of Malaysia.

B. Training schedule

The triathletes have on average consistently trained for 4.43 ± 1.27 years. They trained regularly between 9-15 hours of training per week. For 9 months this included 3-5 swim sessions, 2-3 bike sessions and 3-4 running sessions. The training sessions were periodised into 4 x 4 training blocks, with the 4^{th} week being a lower volume 'recovery' week. The average distance completed by the triathlete was 144.8 km/week (including swimming, biking and running). Apart from this, strength and conditioning sessions were also carried out.

C. Experimental Design

The subjects were recruited at the beginning of the triathlon season (February). They were asked to report to the lab for the baseline anthropometric measurement and urine sample collection (Phase 1). The subjects were advised not to take part in vigorous physical activity for at least 24 hours prior to the day of the sample collection. They were also advised to avoid caffeinated and alcoholic drinks 48 hours prior to the sample collection. Body composition measurements were carried out using a N2O segmental body composition analyser (U. Healthcare System, Singapore). Urine samples were collected in sterile containers and stored at -80°C until analysis. After the baseline collection, the athletes were asked to begin their training. The training was monitored under the supervision of a professional coach and the athletes were encouraged to take part in at least 6 triathlon events during this period. At the end of the triathlon season, the triathletes were once again asked to report to the lab (Phase 2). All samples were collected 14 days after the subject had participated in the last triathlon. Anthropometry and urine collections were repeated at this phase of the study.

D. Urinary Protein and Creatinine Assay

Urinary protein was estimated using the protein dye-binding spectrophotometric method. In this method, an improved pyrogallol red-molybdate protein dye-binding was used (BioAssay Systems, Hayward, CA 94545, USA). In this single step method, the color developed at the end of the assay was measured at 600nm and using a calibration curve, urine protein was estimated and expressed as mg/dl. Urinary creatinine was measured using a spectrophotometric assay. In this assay, creatinine is converted to creatine by creatininase, creatine is converted to sarcosine, which is specifically oxidized to produce a product which reacts with a probe to generate red color (Biovision, Milpitas, CA 95035 USA). The intensity of the red color which was measured at 570nm was proportional to the creatinine concentration. Using a calibration curve, the concentration of creatinine in urine was estimated and was expressed as nmol/L. In order to eliminate the effect of hydration on these assays, urine specific gravity was measured. The specific gravity of all the urine samples were within normal limits.

E. Statistical Analysis

All data were expressed as means ± standard deviation. To study the effect of 'training and competition' cycle on renal function, student's t-test was used to determine the difference between baseline (Phase 1) and end of triathlon season (Phase 2) for both urine protein and creatinine measurements. Statistical significance was accepted at $p < 0.05$.

III. RESULTS

Figure 1 and 2 shows the change in urine protein and urine creatinine between phase 1 and phase 2. We found a statistically significant increase in urinary protein. There was almost a 2-fold increase in urinary protein between phase 1 and phase 2. Urinary protein is an indicator of changes in glomerular permeability and filtration. This is usually a transient process seen in athletes after strenuous exercise; however in our study we find urinary protein showing a cumulative increase. Similarly, urinary creatinine also showed a significant increase between phase 1 and phase 2. Urinary creatinine is measure of skeletal muscle breakdown. Like urinary protein, increase in urinary creatinine after strenuous exercise is a transient phenomenon which should return to baseline in few days. However similar to urinary protein, we find a cumulative increase in urinary creatinine in our triathletes.

Table 1: Anthropometric characteristics of triathletes
All data are presented in mean ± SD

Item	Triathletes
Height(cm)	166.09±10.83
Weight (Kg)	55.13±10.65
BMI (Kg/m^2)	19.80±2.21
Fat free Mass(Kg)	45.96±9.23
Percent Body Fat (%)	16.71±3.88
Skeletal Muscle Mass(Kg)	24.77±5.47

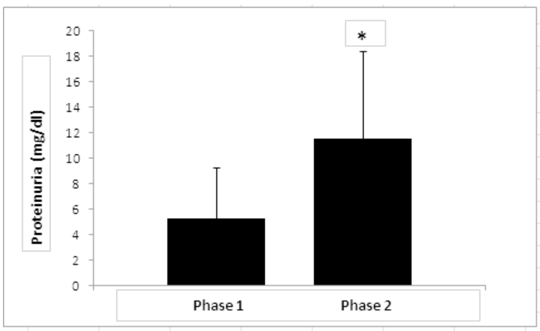

Fig. 1: Urinary protein (mg/dl) in Triathletes at phase 1 and Phase 2. *= p<0.05

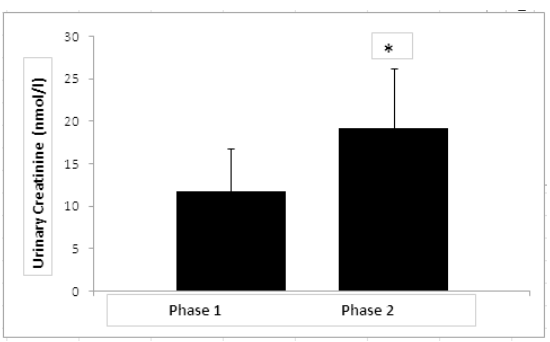

Fig. 2: Urinary creatinine (nmol/l) in Triathletes at phase 1 and Phase 2. *= p<0.05

IV. Discussion

The main aim of this study was to establish whether a 'training and competitive' cycle for one complete triathlon season (9 months) has a cumulative effect on renal function. This was achieved by measuring renal function markers such as urinary protein and creatinine in triathletes before and after a triathlon season. During this season, the triathletes underwent a structured systematic training session as well as took part in at least 6 triathlon events at regular intervals. In this study we used proteinuria as a measure of renal function. Strenuous physical activity causes increased excretion of protein in the urine and this has been termed sport proteinuria. This is due to the changes in the glomerular membrane permeability. In normal conditions, glomerular membrane acts as filter and allows selective permeability of molecules of different sizes [7]. This prevents filtration of plasma proteins. However, strenuous physical activity causes changes in the glomerular membrane permeability as well as decrease in protein re-absorption in proximal tubules. This causes increase in protein excretion leading to proteinuria. One of the important determining factors for proteinuria is the intensity of exercise rather than the duration. Presence of proteinuria immediately after a triathlon race has been very well documented. In a study on triathletes before and after a half-ironman triathlon, Puggina et al showed an 8 fold increase in urinary protein levels [8]. In a study on Japanese triathletes, a significant increase in urinary protein was noted [9]. However, most of these studies were conducted to show the effect of a single race or event on the urinary protein excretion. The long term effect of training and competition was not accessed. In one study, effect of 12 week of training on urinary protein in triathletes showed no significant increase in urinary protein [8]. This study looked at only one training and competition cycle. We have not come across any literature to show the effect of one complete season of triathlon on urinary protein excretion. In our study we find a significant increase in protein excretion (Fig.1). We feel there is a cumulative effect of long term 'training and competition' cycle on the glomerular permeability which causes increased proteinuria. This could be an indication of the strenuous nature of the training and competition as well as insufficient recovery period for the triathletes.

Urinary creatinine excretion is a good measure of glomerular filtration ratio since creatinine is excreted at a constant rate in a day. Strenuous physical activity causes increase in blood flow to the kidney, increasing the glomerular filtration ratio and thereby resulting in increased excretion of creatinine. Studies have shown this phenomenon in athletes from different sports [11,12,13]. Increased urinary creatinine excretion in triathletes after a triathlon race/event has also been very well documented [8,14] . Similar to proteinuria, few studies have looked into the effect of training on urinary creatinine excretion. In the same study, Puggina et al did not find a increase in creatinine excretion after 12 weeks of training, but found a significant increase after the triathlon race [8]. Again this study was limited to only one 'training and competition cycle'. In our study we found a statistically significant increase in urinary creatinine excretion (Fig.2) similar to urinary protein, showing a cumulative effect of multiple training and competition cycles.

The present results confirm that multiple ‘training and competition’ cycles have a cumulative effect on the urinary protein and creatinine excretion, thus providing evidence for changes in renal hemodynamics following long term strenuous physical activity. Our study did have certain limitations. The sample size of this study was low mainly due to the following factors: Our study required for the athlete to be followed up for an entire season (9 months) and also for the athletes to take part in triathlon events at a regular intervals, which caused a drop out of at-least 50% of our subjects. Secondly, since we needed all the athletes to follow a similar training regimen, we had to limit ourselves to one coach and his team. All these factors lead to the low sample size. The other main drawback of this study was non-inclusion of control subjects. Since in this study we only wanted to see the pre and post effect of training and competition of triathletes on renal function, we used the “one group pre-test and post test” experimental design. Since our measurement parameters were urine protein and creatinine, we presumed that there we be will no change in these parameters in non- triathlete subjects and hence did not include control subjects. We were also not able to collect 24 hour urine protein excretion mainly due to logistical reasons. However studies have shown that spot urine protein measurements and comparable to 24 urine protein measurement and can be used to assess renal marker for glomerular permeability.

V. Conclusion

In conclusion, it appears that there is a cumulative effect on continuous training and competition on the renal function as assessed by urinary protein and creatinine excretions. Thus for coaches, the findings from our study could provide good justification for proper recovery periods for the triathletes, in order to avoid a permanent renal damage.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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