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Abstract

Objective: To determine the effect size of wearing sports garments treated with microscopic titanium particles (AQUA TITAN) during recovery from strenuous running on restoration of running economy during subsequent exercise. Design: A double-blind crossover was used to determine the effect of AQUA TITAN on running metabolic cost in 10 healthy men. Participants performed 40 min of treadmill running comprising 2 x (10 min at 5% and 10 min at -10% grade), followed by random allocation to skin-tight nylon polyurethane AQUA TITAN treated or non-treated placebo garments covering the torso, limbs, and feet. Garments were worn continuously throughout the next 48-h, during which time participants rested (day 2) then completed a graded treadmill run to determine metabolic outcome (day 3). Method: Body-weight normalised running metabolic cost was evaluated by indirect calorimetry and the effect size referenced against the smallest meaningful change in economy (0.9%) for improvement in distance running performance. Results: The fatigue effect while wearing control garments on metabolic cost at 48-h was small (2.2% 95%CL ±1.2%). In contrast, AQUA TITAN garments most certainly reduced running metabolic cost (-3.1% ±0.9%) vs control. Additionally, AQUA TITAN increased the respiratory exchange ratio (0.011 ±0.005) and lowered minute ventilation at intensities below the ventilator threshold (-4.0% ±0.9%). Conclusions: AQUA TITAN garments worn during recovery from strenuous exercise improved subsequent running economy to a magnitude likely to restore endurance performance. Future research should verify the magnitude of garment effects on performance outcomes, and on identifying the acute or passive neural, musculotendinous or metabolic mechanisms responsible.

Key words. recovery, tendon stiffness, short latency reflex, running economy, sports garments, performance

Introduction

Sports garments are potential mediums for the application of compounds that may modulate the physiological response to exercise for the purpose of aiding performance and enhancing recovery. One such compound is AQUA TITAN, which results from dispersion of microtitanium particles in water[1]. AQUA TITAN is subsequently utilised as a dye in the manufacturing process where microtitanium particles are bonded into the fabric becoming an integral component of the garment. There is evidence to suggest that when applied close to cells or tissues, AQUA TITAN imparts physiological effects on the nervous system[2,3] and on muscle and tendon[4-6]. In humans, nylon-polyurethane AQUA TITAN-treated garments worn for 4 days following a bout of intermittent high-intensity running increased joint range of motion (ROM) and possibly lowered the metabolic cost of running, relative to non-treated placebo[6]. These observations are of interest because running economy is one of the strongest predictors of distance running performance[7].

A number of physiological factors determine running economy, including muscle fibre characteristics, body mass, muscle-tendon elasticity and neuromuscular efficiency[8]. Of these factors, muscle contractility (secondary to fiber type) and tendon stiffness are thought to be important determinants of musculotendinous contributions, with the optimal tendon stiffness a component of successful specific adaptation to loading (trained optima)[9]. Because the rate of force development is an important determinant of tendon-muscle performance in running and jumping tasks[10], altered neuromuscular firing rates could also affect contractile performance via improved neuromuscular coordination[11]. Therefore, improved neuromuscular coordination, attenuated reduction in tendon stiffness[12], and reduced economy[13] after loading stress has the potential to improve skeletal muscle contractile function following strenuous exercise common to athletic training.

Building evidence suggests AQUA TITAN garments may improve musculotendinous function following strenuous running, and that this may improve muscle contractile performance and running economy. Indeed, AQUA TITAN tape applied to the triceps surae during recovery from a bout of strenuous treadmill running increased stretch-reflex response time and attenuated the fatigue-induced decline in tendon stiffness[4]. Therefore, we hypothesized that strenuous running would increase the metabolic cost of subsequent running (lower economy), but this effect would be attenuated with AQUA TITAN garments.

Method

Ten healthy active men participated with mean (SD) age 29.2 y (7.1), weight 78.0 kg (1.5), stature of 181.3 m (9.3), and maximum oxygen uptake (VO_{2max}) of 65.1 $mL\cdot kg^{-1}\cdot min^{-1}$ (7.9). Inclusion criteria were aged 18 to 45 y, regular participation in a sport involving running, and VO_{2max} >50 $mL\cdot kg^{-1}\cdot min^{-1}$. Exclusion criteria were history or complaints of tendinopathy or lower limb trauma, illness, or current use of analgesic medication. All participants were informed of potential risks and gave informed written consent according to the protocol approved by Massey University Research Ethics Committee.

The study design is illustrated in Figure 1. On the first visit to the laboratory, participants completed a VO_{2max} test on a calibrated motorised treadmill according to Wadsworth et al.[6], with the exception of external breath-by-breath respiratory gas collection (Sensormedics Vmax, San Diago, CA). Ventilatory threshold (VT) was determined using the ventilatory equivalence method[14], where VT was the treadmill running speed corresponding to the first sustained rise in the ventilatory equivalent of O_2 (VE/VO_2) without a concurrent rise in the ventilatory equivalent of CO_2 (VE/VCO_2).

The second visit 2-3 days later comprised familiarisation of the testing procedures and baseline measurement of running economy that comprised a continuous protocol of 4 min of walking at 4 $km\cdot h^{-1}$, 6 min running at 9 $km\cdot h^{-1}$, and 5 x 6-min running stages beginning -1.5 $km\cdot h^{-1}$ of the VT speed and increasing 0.5 $km\cdot h^{-1}$ for each progressive stage. The protocol determined the fourth stage occurred at VT and the final stage at 0.5 $km\cdot h^{-1}$ above VT. This protocol provided 3 estimates below the VT where steady-state VO_2 is likely, and 2 at or above VT where a higher percentage of type-II muscle fibres are likely to be recruited associated lower relative oxygen economy[25]. The same protocol was used for the recovery running economy measurement. Body weight was measured following toileting prior to running under standardised clothing and shoe conditions. Expired gas was collected using the metabolic cart. Running economy was the metabolic cost of exercise expressed as the energy equivalent for oxygen per min adjusted for on-the-day body weight, which was determined using the final 2 min data from each stage. Metabolic power ($J\cdot kg^{-1}\cdot min^{-1}$) was calculated using the non-protein respiratory quotient[15].

Subsequent trials involved two experimental blocks in a crossover with 7-day washout: a fatigue inducing run, rest day, and then recovery measure of running economy 48 h later

(Figure 1). Participants completed one experimental block wearing AQUA TITAN-treated garments and the other wearing identical placebo garments allocated by double-blind randomization. Garments were worn after the loading run, continuously during recovery, including sleeping and all exercise tests. The AQUA TITAN garments were made by Phiten Co. Ltd. (Kyoto, Japan) as described previously[6]. Phiten Co. Ltd had no other involvement in, nor right to approve or disapprove the current publication. The clothing was tight fitting, 81% Nylon:19% polyurethane, black, and covered the entire torso to the neck and the limbs; feet and ankles were covered with tennis length socks. To control for a potential confounding influence of different fuel substrate availability and training pre-load, participants standardised both training and diet by recording habit from 48-h prior to beginning the first block; this regimen was repeated for the second block.

On day one of the experimental block (Figure 1B) lower limb fatigue leading to reduced subsequent running economy[16] was induced by a 40-min treadmill run at a speed eliciting 70% VO_{2max} during flat running. The run comprised 2 x (10 min at 5% and 10 min at -10%) modified by adding 10 min from the protocol of Braun and Dutto16113 . Participants reported near maximal effort on the uphill sections. Laboratory environment was 20°C, 44-50% humidity. Participants were fan cooled during running.

Sample size (n=10) was generated on the basis of sufficient power to declare a likely substantial change in running economy[17] drawn from the mean effect of AQUA TITAN garments on metabolic power (2.2%)[6] and typical error of 2.5% for metabolic power determined in our laboratory. All outcome variables were log-transformed before modelling to reduce nonuniformity of error and to express outcomes as percentages[17]. Outcomes were estimated with mixed linear modelling (Proc Mixed, SAS Version 9.1; SAS Institute, Cary, NC) of the breath-by-breath data comprising the interaction of trial order, treatment, and stage (fixed effects); the random effect was subject identity.

Population values of statistics were estimated by magnitude-based inference[17]. For reference to the primary outcome the smallest important change in metabolic cost with the likelihood of improving endurance running performance was 0.9%. This value was derived from a linear model of the physiological factors determining endurance-running performance[18], where a 5% improvement in running economy corresponded to a 3.8% improvement in performance, and where a 0.7% improvement in performance was considered worthwhile[6, 19]. Consequently, the denominator for calculations of effect size on metabolic cost was the threshold value divided by 0.3 corresponding to the qualifiers: trivial=0.0–0.3, small=0.3–0.9, moderate=0.9–1.6, large=1.6–2.5, very large=2.5–4.0, and extremely large >4.0[17]. For the other variables, the standardised difference was used (trivial=0.0–0.2, small=0.2–0.6, moderate=0.6–1.2, large=1.2–2.0, very large=2.0–4.0, and extremely large >4.0)[17]. The thresholds for assigning qualitative terms to probability of a substantial effect were: <0.5%, most certainly not; <5%, very unlikely; <25%, unlikely; <75%, possible; >75%, likely; >95%, very likely; >99.5%, most certain. An effect was *unclear* if the uncertainty included both substantial increases and decreases (i.e. >5%)[17].

Results

To provide a point of reference to the loading run effect size on the subsequent metabolic cost, we determined running economy in the placebo garments at 48-h relative to that with normal untreated running clothing worn during the baseline run. Here, bodyweight normalized overall mean metabolic power very likely increased (2.2% 95%CL ±0.8%, p=5.00E-9); in contrast, the mean AQUA TITAN minus baseline reduction was likely trivial (-0.9% ±0.7%, p=0.02). Therefore, the primary outcome at 48-h was a reduction of metabolic cost with AQUA TITAN garments, relative to control (Figure 2A-B, Table 1). AQUA TITAN garments lowered minute ventilation when the running speed was below the VT (stages 1-3) by 4.0% (±0.7%, p<1.00E-18, standardized difference -0.17 ±0.03); when above the VT (stage 5), the effect was trivial (0.7% ±1.2%, p=0.34, 0.03 ±0.04) (Figure 2D). The increase in respiratory exchange ratio with AQUA TITAN was trivial (Table 1), but minute ventilation per unit O_2 consumption adapted to the lower oxygen cost of exercise with AQUA TITAN (Figure 2C) with a trivial decrease (-0.3 ±0.5%, p=0.36, -0.01 ±0.03).

Discussion

The purpose of the study was to determine the effect AQUA TITAN treated garments worn during the 48-h recovery period of recovery from a bout of strenuous running on subsequent running economy. AQUA TITAN garments lowered the metabolic cost of subsequent running by 3.1% ±6%, relative to non-treated garments. This running economy finding is consistent with a previous report[6]. Braun and Dutto[16] previously reported the mean fatigue effect of a hilly run on 48-h metabolic cost during subsequent running was 3.2%. Coupled with our estimate of the non-treated fatigue effect, these data suggest the AQUA TITAN garments enabled a restoration of running economy near to the pre-loaded condition, with the garments assisting in maintenance of initial muscle contractile efficiency inferred by 166 relative restoration of the metabolic cost of subsequent exercise.

With the physiological assumption of sample equivalence between studies based on conservation of bioenergetics and VO_{2max}, Di Prampero et al.'s[18] linear model for bioenergetic predictors of running performance[17,19] provided for a prediction of the magnitude of change in distance running performance attributable to the AQUA TITAN garments. The model suggests the mean improvement in economy corresponded to a moderate 2.4% improvement in performance. Therefore, AQUA TITAN garments during recovery may be a useful intervention for athletes competing in multiple-day events where rapid recovery of muscle performance is an important component of outcome. Our study design, however, did not allow determination of whether treatment effect was derived from physiological responses that occurred while wearing AQUA TITAN during the 48-h recovery period or from acute effects during subsequent exercise, or if the leggings, socks or upper garments were responsible.

Changes to the mechanical properties of the musculotendon unit may explain the improvement in metabolic economy. Energy storage and return is thought to be optimal when the muscle contracts isometrically while the tendon lengthens[20]. This process works best with a compliant tendon, but if the tendon is too compliant it will impair force transmission to the bone[21]. For any movement, therefore, there is an optimal tendon stiffness based on the demands of the task. If we assume that running patterns have developed to produce maximal performance from the normal rested (i.e. pre-trial) tendon stiffness, any change in tendon stiffness would decrease running economy. AQUA TITAN tape[4] and garments[6] have been shown to produce small increases in joint range of motion (ROM) which could improve musculotendinous efficiency through altered gait mechanics (although we lack the data to confirm this is the case). In our companion study[4], achilles tendon stiffness did not decrease following fatiguing exercise when AQUA TITAN tape was applied to the tendon. A similar effect may explain the present results.

A second possible mechanism to explain the AQUA TITAN effect is improved repair of damaged muscle tissue. It is well known that muscle damage follows strenuous eccentric exercise[16,22]; such tissue insult could impair stride efficiency by reducing stride length[16], reduce muscle and joint ROM[23] and decrease knee extensor torque[24] thereby producing impaired contractile function and reduced running economy. Braun and Dutto[16] reported that reduced stride length is inversely correlated to running energy cost, suggesting damage to the preferred type-I muscle fibre pool increases relative recruitment of type-II fibres. Type-II specific myosin ATPase isoforms require 1.6- to 2.1-fold more ATP per unit force production than type-I[25] and therefore require a proportionately higher oxidative phosphorylation. We observed 4% lower minute ventilation during sub-VT exercise with AQUA TITAN. Minute ventilation is coupled to muscle contraction by locomotor muscle afferents[26]. Lower minute ventilation with AQUA TITAN, although possibly trivial in effect size, offers some physiological support for relatively higher relative type-I fibre recruitment profile[27]. Histologically, damaged muscle undergoes regeneration and repair mediated by inflammatory process associated wound healing responses followed by enhanced myogenesis[28]. A recent cell culture experiment suggests a possible damage repair mechanism. Ishizaki et al.[5] reported that AQUA TITAN coated rubber upregulated expression of myofibril components (vinculin, type I and III collagen) and accelerated myocyte and osteoblast adhesion and growth, thereby demonstrating a potential mechanism for the effect we observed.

A final perspective suggests a neural mechanism may be involved. Disrupted reflex activity of the lower limb is known to impair running gait[29]. Cronin et al.[29] reported that impaired triceps surae short latency response results in less efficient transfer of force during running. This is a significant mechanistic consideration with respect to the current study because downhill running reduces the short latency reflex response of the triceps surae[30]. Indeed, AQUA TITAN tape applied to the triceps surae for 48-h following high-intensity running decreased the short latency response and increased achilles tendon stiffness and ROM[4]; the subsequent reduced yield and stiffer tendon[29] could perhaps have been sufficient to improve joint efficiency during the gait cycle and therefore improve running economy. Further investigation is required to determine if improved neuromuscular coordination and recovery of tendon stiffness are the primary causal mechanisms for restoration of running economy with AQUA TITAN garments following strenuous exercise.

Conclusion

AQUA TITAN garments worn during recovery from strenuous exercise restored subsequent running economy. Current evidence for a mechanism supports improved musculotendinous contractile function via faster short latency response regulating or restoring tendon stiffness towards the pre-loaded adapted optima for running[4]. Further research is warranted to investigate the potential modifications of cellular repair mechanisms relating to muscle and connective tissue integrity and function during recovery, determine dose response, and examine whether effects are mediated by physiological changes during the passive recovery period or during exercise. Regardless of mechanism, the magnitude and certainty of the running economy outcome implies that AQUA TITAN could have a meaningful impact on muscle contractile function during periods of intense training and in multiday competitive events.

Practical implications

- Strenuous running exercise induces fatigue and impairs running economy
- Sports garments treated with uniquely-processed microtitanium particles restored running economy, which could also contribute towards restoration of running performance
- Wearing AQUA TITAN treated sports apparel may benefit 247 recovery in tournament or repeated heavy training sessions

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References

1. Hirata Y, Ueda Y, Takase H et al., inventors. High functional water containing titanium and method and apparatus for producing the same. New Zealand patent NZ 522431. 2004.
2. Korte M. Influence of Aquatitan Tape on nerve cells of the central nervous system. *J Clin Biochem Nutr*. 2008;43:1-4.
3. Aoi W, Takanami Y, Kawai Y et al. Relaxant effect of microtitan via regulation of autonomic nerve activity in mice. *Life Sci*. 2009;85:408-411.
4. Hughes JD, Graham DF, Fink PW et al. Microtitanium impregnated adhesive tape applied to the triceps surae during recovery from strenuous running shortens subsequent short latency response and maintains Achilles tendon stiffness *J Electromyo and Kinesiol*. 2012;in review: In review.
5. Ishizaki K, Sugita Y, Iwasa F et al. Nanometer-thin TiO_2 enhances skeletal muscle

cell phenotype and behavior. *Int J Nanomedicine*. 2011;6:2191-2203. Epub 2011/11/25.

6. Wadsworth DP, Walmsley A, Rowlands DS. Aquatitan garments extend joint range of motion without effect on run performance. *Med Sci Sports Exerc*. 2010;42(12): 2273-2281.
7. Pollock ML. Submaximal and maximal working capacity of elite 274 distance runners. Part I: Cardiorespiratory aspects. *Ann N Y Acad Sci*. 1977;301:310-322.
8. Saunders PU, Pyne DB, Telford RD et al. Factors affecting running economy in trained distance runners. *Sports Med*. 2004;34(7):465-485.
9. Lichtwark GA, Wilson AM. Optimal muscle fascicle length and tendon stiffness for maximising gastrocnemius efficiency during human walking and running. *J Theor Biol*. 2008;252(4):662-673.
10. Fukashiro S, Komi PV, Järvinen M et al. In vivo achilles tendon loading during jumping in humans. *Eur J Appl Physiol*. 1995;71(5):453-458.
11. Ishikawa M, Komi PV. The role of the stretch reflex in the gastrocnemius muscle during human locomotion at various speeds. *J Appl Physiol*. 2007;103(3):1030-1036.
12. Kay AD, Blazevich AJ. Isometric contractions reduce plantar flexor moment, Achilles tendon stiffness, and neuromuscular activity but remove the subsequent effects of stretch. *J Appl Physiol*. 2009;107(4):1181-1189.
13. Dutto DJ, Braun WA. DOMS-associated changes in ankle and knee joint dynamics during running. *Med Sci Sports Exerc*. 2004;36(4):560-566.
14. Gaskill SE, Ruby BC, Walker AJ et al. Validity and reliability of combining three methods to determine ventilatory threshold. *Med Sci Sports Exerc*. 2001;33(11):1841-1848. Epub 2001/11/02.
15. Jeukendrup A, Wallis G. Measurement of substrate oxidation during exercise by means of gas exchange measurements. *Int J Sports Med*. 2005;26:S1-S10.
16. Braun WA, Dutto DJ. The effects of a single bout of downhill running and ensuing delayed onset of muscle soreness on running economy performed 48 h later. *Eur J Appl Physiol*. 2003;90(1-2):29-34. Epub 2003/06/05.
17. Hopkins WG, Marshall SW, Batterham AM et al. Progressive statistics for studies in sport medicine and exercise science. *Med Sci Sports Exerc*. 2009;41:3-13.
18. Di Prampero PE, Capelli C, Pagliaro P et al. Energetics of best performances in middle-distance running. *J Appl Physiol*. 1993;74(5):2318-2324.
19. Hopkins WG, Hewson DJ. Variability of competitive performance 302 of distance

runners. *Med Sci Sports Exerc*. 2001;33:1588-1592.

20. Lichtwark GA, Wilson AM. Is Achilles tendon compliance optimised for maximum muscle efficiency during locomotion? *J Biomech*. 2007;40(8):1768-1775.
21. Alexander RM. Tendon elasticity and muscle function. *Comp Biochem Physiol: A Physiol*. 2002;133:1001-1011.
22. Eston RG, Mickleborough J, Baltzopoulos V. Eccentric activation and muscle damage: biomechanical and physiological considerations during downhill running. Br *J Sports Med*. 1995;29(2):89-94. Epub 1995/06/01.
23. Hamill J, Freedson P, Clarkson P et al. Muscle soreness during running biomechanical and physiological considerations. *Int J Sport Biomech*. 1991;7(2):125-137.
24. Eston RG, Finney S, Baker S et al. Muscle tenderness and peak torque changes after downhill running following a prior bout of isokinetic eccentric exercise. *J Sports Sci*. 1996;14(4):291-299.
25. Reggiani C, Bottinelli R, Stienen GJM. Sarcomeric myosin isoforms: fine tuning of a molecular motor. *Physiology*. 2000;15(1):26-33.
26. Dempsey JA. Bayliss-Starling Memorial Lecture - 2012 "New perspectives concerning feedback influences on cardio-respiratory control during rhythmic exercise and on exercise performance". *J Physiol*. 2012.
27. Franch J, Madsen K, Djurhuus MS et al. Improved running economy following intensified training correlates with reduced ventilatory demands. *Med Sci Sports Exerc*. 1998;30(8):1250-1256.
28. Tidball JG, Villalta SA. Regulatory interactions between muscle and the immune system during muscle regeneration. *Am J Physiol Reg Int Comp Physiol*. 2010;298(5):R1173-R1187.
29. Cronin NJ, Carty CP, Barrett RS. Triceps surae short latency stretch reflexes contribute to ankle stiffness regulation during human running. *PLoS ONE*. 2011;6(8):e23917.
30. Avela J, Komi PV. Reduced stretch reflex sensitivity and muscle 329 stiffness after long lasting stretch-shortening cycle exercise in humans. *Eur J Appl Physiol Occ Physiol*. 1998;78(5):403-410.

Tables

Table 1. Statistical summary of the effect of AQUA TITAN garments worn for 48-h during recovery from strenuous hill running on the metabolic cost of movement during subsequent running.

Outcome[1]	AQUA TITAN minus Control Effect (%) ±95%CL[2]	Effect Size[4] ±95%CL[2]	P-value	Effect Magnitude[4]	Inference[4]
	Normalised oxygen consumption[3]				
Stages 1-3	-3.9 ±0.9	-0.9 ±0.2	1.0E-17	Moderate	most certain
Stage 4	-1.9 ±1.4	-0.4 ±0.3	0.01	Small	likely
Stage 5	-2.8 ±1.5	-0.7 ±0.4	3.6E-04	Small	very likely
All Stages	-3.3 ±0.6	-0.8 ±0.2	6.0E-21	Small	most certain
	Normalized metabolic cost[3]				
Stages 1-3	-3.7 ±0.9	-0.9 ±0.2	1.0E-17	Moderate	most certain
Stage 4	-2.1 ±1.4	-0.5 ±0.3	0.003	Small	likely
Stage 5	-2.5 ±1.5	-0.6 ±0.3	8.8E-05	Small	likely
All Stages	-3.1 ±0.6	-0.7 ±0.2	3.0E-21	Small	most certain
	Respiratory exchange ratio[3]				
Stages 1-3	0.014 ±0.004	0.12 ±0.04	2.5E-06	Trivial	most certain
Stage 4	-0.007 ±0.007	-0.07 ±0.06	0.122	Trivial	most certain
Stage 5	0.020 ±0.08	0.18 ±0.07	8.1E-05	Trivial	possible
All Stages	0.011 ±0.003	0.10 ±0.03	1.5E-06	Trivial	most certain

[1]Stages 1-3 < ventilatory threshold (VT), stage 4 approximately VT, stage 5 > VT.

[2]Add or subtract this value as a factor of the mean to obtain the upper and lower confidence limits.

[3]Oxygen ($VO_2 \cdot kg^{-1}$) and metabolic cost ($kJ \cdot kg^{-1} \cdot km^{-1}$) data are percentage estimates, while the respiratory exchange ratio are raw unit estimates.

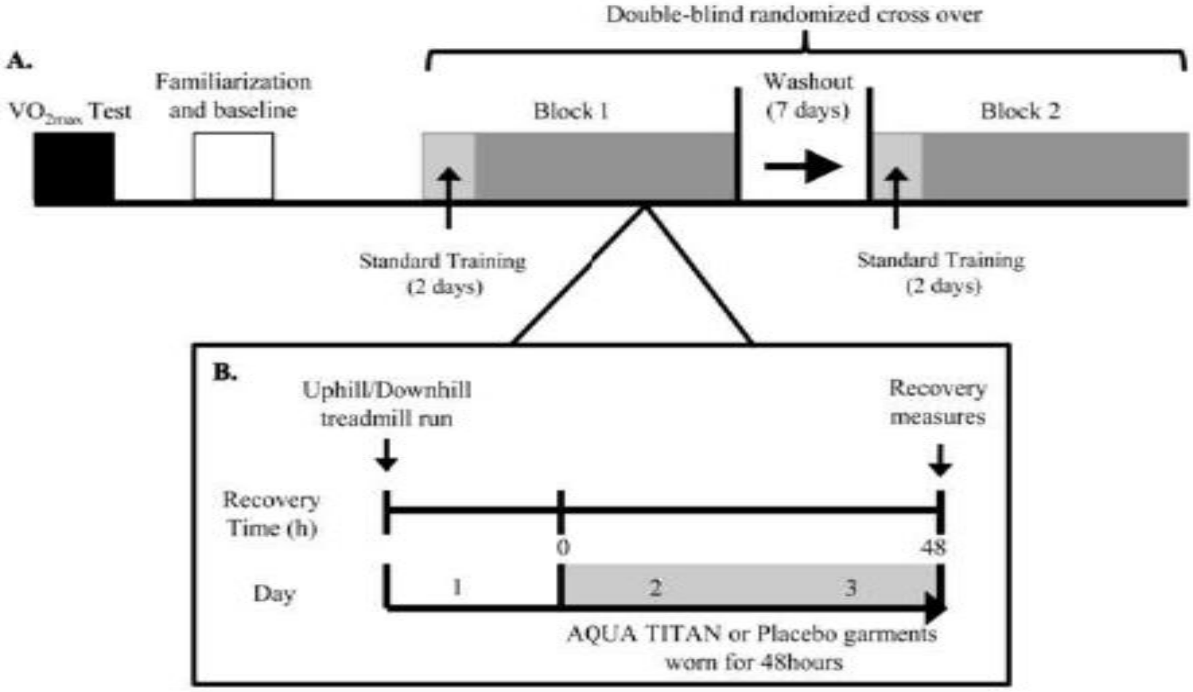
[4]Magnitude-based inferences about the true value for outcomes were qualified using the within-subject effect size for measures of running metabolic cost, where 1.3% was the threshold for smallest worthwhile change in economy with reference to endurance running performance[18]. See Methods for further detail.

Figure captions

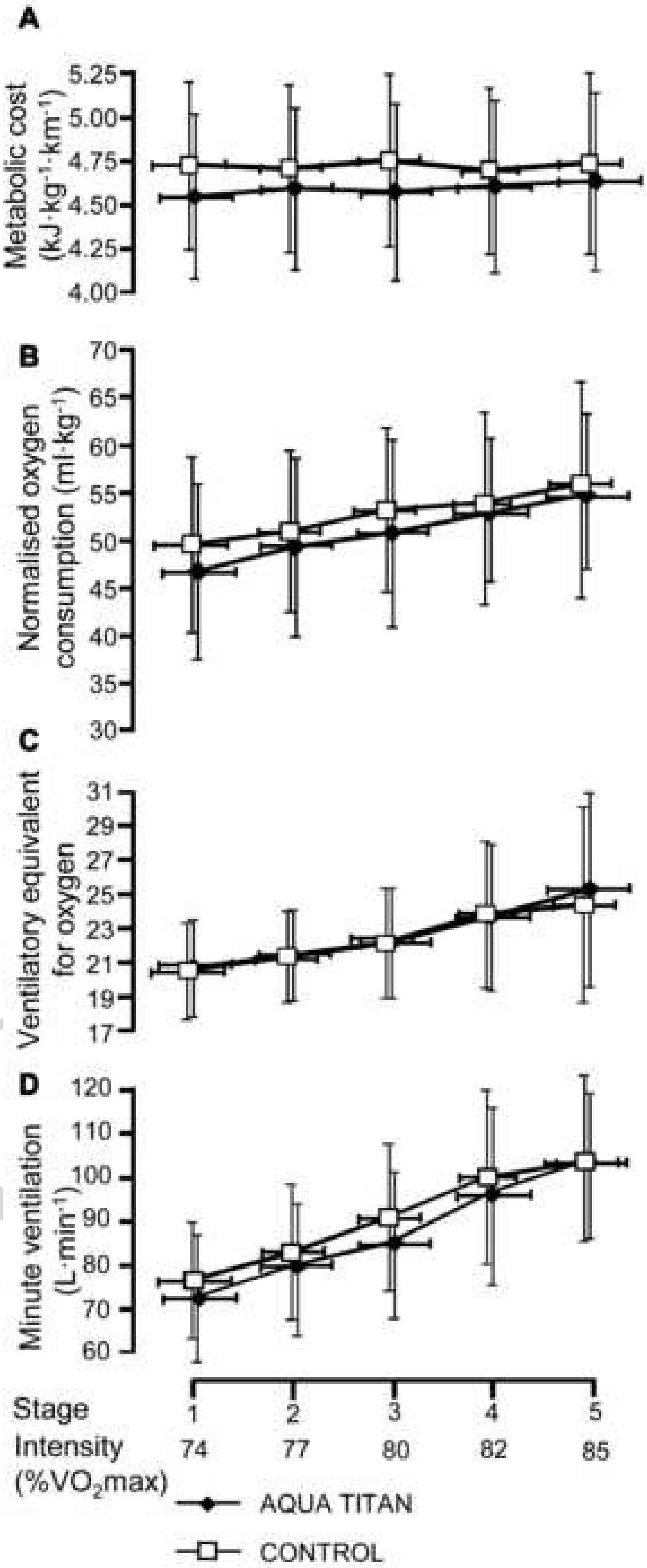
Figure 1. A. experimental crossover design following baseline and familiarisation measures. B. detail of one of the two experimental blocks.

Figure 2. Effect of AQUA TITAN treated garments on (A) normalised metabolic cost, (B) running oxygen consumption, (C) ventilatory equivalent for oxygen, and (D) ventilatory minute volume 48-h following a bout of strenuous hill running. Data are raw means and bars standard deviations; bars in the x-axis represent the SD for exercise intensity (%VO_{2max}).

Figure 1


Double-blind randomized cross over
A.
VO_{2max} Test
Familiarization
and baseline
Block 1
Washout
(7 days)
Block 2
Standard Training
(2 days)
Standard Training
(2 days)
B.
Uphill/Downhill
treadmill run
Recovery
measures
Recovery
Time (h)
0
48
Day
1
2
3
AQUA TITAN or Placebo garments
worn for 48hours

419 Figure 2



420