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1 **Abstract**

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

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

25
26

27 **Introduction**

28 Sports garments are potential mediums for the application of compounds that may modulate
29 the physiological response to exercise for the purpose of aiding performance and enhancing
30 recovery. One such compound is AQUA TITAN, which results from dispersion of
31 microtitanium particles in water¹. AQUA TITAN is subsequently utilised as a dye in the
32 manufacturing process where microtitanium particles are bonded into the fabric becoming an
33 integral component of the garment. There is evidence to suggest that when applied close to
34 cells or tissues, AQUA TITAN imparts physiological effects on the nervous system^{2,3} and on
35 muscle and tendon⁴⁻⁶. In humans, nylon-polyurethane AQUA TITAN-treated garments worn
36 for 4 days following a bout of intermittent high-intensity running increased joint range of
37 motion (ROM) and possibly lowered the metabolic cost of running, relative to non-treated
38 placebo⁶. These observations are of interest because running economy is one of the strongest
39 predictors of distance running performance⁷.

40

41 A number of physiological factors determine running economy, including muscle fibre
42 characteristics, body mass, muscle-tendon elasticity and neuromuscular efficiency⁸. Of these
43 factors, muscle contractility (secondary to fiber type) and tendon stiffness are thought to be
44 important determinants of musculotendinous contributions, with the optimal tendon stiffness
45 a component of successful specific adaptation to loading (trained optima)⁹. Because the rate of
46 force development is an important determinant of tendon-muscle performance in running and
47 jumping tasks¹⁰, altered neuromuscular firing rates could also affect contractile performance
48 via improved neuromuscular coordination¹¹. Therefore, improved neuromuscular coordination,
49 attenuated reduction in tendon stiffness¹², and reduced economy¹³ after loading stress has the
50 potential to improve skeletal muscle contractile function following strenuous exercise common
51 to athletic training.

52

53 Building evidence suggests AQUA TITAN garments may improve musculotendinous function
54 following strenuous running, and that this may improve muscle contractile performance and
55 running economy. Indeed, AQUA TITAN tape applied to the triceps surae during recovery
56 from a bout of strenuous treadmill running increased stretch-reflex response time and
57 attenuated the fatigue-induced decline in tendon stiffness⁴. Therefore, we hypothesized that
58 strenuous running would increase the metabolic cost of subsequent running (lower economy),
59 but this effect would be attenuated with AQUA TITAN garments.

60

61 **Method**

62 Ten healthy active men participated with mean (SD) age 29.2 y (7.1), weight 78.0 kg (1.5),
63 stature of 181.3 m (9.3), and maximum oxygen uptake ($\text{VO}_{2\text{max}}$) of $65.1 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ (7.9).
64 Inclusion criteria were aged 18 to 45 y, regular participation in a sport involving running, and
65 $\text{VO}_{2\text{max}} > 50 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$. Exclusion criteria were history or complaints of tendinopathy or
66 lower limb trauma, illness, or current use of analgesic medication. All participants were
67 informed of potential risks and gave informed written consent according to the protocol
68 approved by Massey University Research Ethics Committee.

69
70 The study design is illustrated in Figure 1. On the first visit to the laboratory, participants
71 completed a $\text{VO}_{2\text{max}}$ test on a calibrated motorised treadmill according to Wadsworth et al.⁶,
72 with the exception of external breath-by-breath respiratory gas collection (Sensormedics
73 Vmax, San Diego, CA). Ventilatory threshold (VT) was determined using the ventilatory
74 equivalence method¹⁴, where VT was the treadmill running speed corresponding to the first
75 sustained rise in the ventilatory equivalent of O_2 (VE/VO_2) without a concurrent rise in the
76 ventilatory equivalent of CO_2 (VE/VCO_2).

77
78 The second visit 2-3 days later comprised familiarisation of the testing procedures and baseline
79 measurement of running economy that comprised a continuous protocol of 4 min of walking
80 at $4 \text{ km}\cdot\text{h}^{-1}$, 6 min running at $9 \text{ km}\cdot\text{h}^{-1}$, and 5 x 6-min running stages beginning $1.5 \text{ km}\cdot\text{h}^{-1}$ of
81 the VT speed and increasing $0.5 \text{ km}\cdot\text{h}^{-1}$ for each progressive stage. The protocol determined
82 the fourth stage occurred at VT and the final stage at $0.5 \text{ km}\cdot\text{h}^{-1}$ above VT. This protocol
83 provided 3 estimates below the VT where steady-state VO_2 is likely, and 2 at or above VT
84 where a higher percentage of type-II muscle fibres are likely to be recruited associated lower
85 relative oxygen economy²⁵. The same protocol was used for the recovery running economy
86 measurement. Body weight was measured following toileting prior to running under
87 standardised clothing and shoe conditions. Expired gas was collected using the metabolic cart.
88 Running economy was the metabolic cost of exercise expressed as the energy equivalent for
89 oxygen per min adjusted for on-the-day body weight, which was determined using the final 2
90 min data from each stage. Metabolic power ($\text{J}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) was calculated using the non-protein
91 respiratory quotient¹⁵.

92
93 Subsequent trials involved two experimental blocks in a crossover with 7-day washout: a
94 fatigue inducing run, rest day, and then recovery measure of running economy 48 h later

95 (Figure 1). Participants completed one experimental block wearing AQUA TITAN-treated
96 garments and the other wearing identical placebo garments allocated by double-blind
97 randomization. Garments were worn after the loading run, continuously during recovery,
98 including sleeping and all exercise tests. The AQUA TITAN garments were made by Phiten
99 Co. Ltd. (Kyoto, Japan) as described previously⁶. Phiten Co. Ltd had no other involvement in,
100 nor right to approve or disapprove the current publication. The clothing was tight fitting, 81%
101 Nylon:19% polyurethane, black, and covered the entire torso to the neck and the limbs; feet
102 and ankles were covered with tennis length socks. To control for a potential confounding
103 influence of different fuel substrate availability and training pre-load, participants standardised
104 both training and diet by recording habit from 48-h prior to beginning the first block; this
105 regimen was repeated for the second block.

106

107 On day one of the experimental block (Figure 1B) lower limb fatigue leading to reduced
108 subsequent running economy¹⁶ was induced by a 40-min treadmill run at a speed eliciting 70%
109 VO_{2max} during flat running. The run comprised 2 x (10 min at 5% and 10 min at -10%) modified
110 by adding 10 min from the protocol of Braun and Dutton¹⁶. Participants reported near
111 maximal effort on the uphill sections. Laboratory environment was 20°C, 44-50% humidity.
112 Participants were fan cooled during running.

113

114 Sample size (n=10) was generated on the basis of sufficient power to declare a likely substantial
115 change in running economy¹⁷ drawn from the mean effect of AQUA TITAN garments on
116 metabolic power (2.2%)⁶ and typical error of 2.5% for metabolic power determined in our
117 laboratory. All outcome variables were log-transformed before modelling to reduce
118 nonuniformity of error and to express outcomes as percentages¹⁷. Outcomes were estimated
119 with mixed linear modelling (Proc Mixed, SAS Version 9.1; SAS Institute, Cary, NC) of the
120 breath-by-breath data comprising the interaction of trial order, treatment, and stage (fixed
121 effects); the random effect was subject identity.

122

123 Population values of statistics were estimated by magnitude-based inference¹⁷. For reference
124 to the primary outcome the smallest important change in metabolic cost with the likelihood of
125 improving endurance running performance was 0.9%. This value was derived from a linear
126 model of the physiological factors determining endurance-running performance¹⁸, where a 5%
127 improvement in running economy corresponded to a 3.8% improvement in performance, and
128 where a 0.7% improvement in performance was considered worthwhile^{6, 19}. Consequently, the

129 denominator for calculations of effect size on metabolic cost was the threshold value divided
130 by 0.3 corresponding to the qualifiers: trivial=0.0–0.3, small=0.3–0.9, moderate=0.9–1.6,
131 large=1.6–2.5, very large=2.5–4.0, and extremely large >4.0¹⁷. For the other variables, the
132 standardised difference was used (trivial=0.0–0.2, small=0.2–0.6, moderate=0.6–1.2,
133 large=1.2–2.0, very large=2.0–4.0, and extremely large >4.0)¹⁷. The thresholds for assigning
134 qualitative terms to probability of a substantial effect were: <0.5%, most certainly not; <5%,
135 very unlikely; <25%, unlikely; <75%, possible; >75%, likely; >95%, very likely; >99.5%, most
136 certain. An effect was *unclear* if the uncertainty included both substantial increases and
137 decreases (i.e. >5%)¹⁷.

138

139 **Results**

140 To provide a point of reference to the loading run effect size on the subsequent metabolic cost,
141 we determined running economy in the placebo garments at 48-h relative to that with normal
142 untreated running clothing worn during the baseline run. Here, bodyweight normalized overall
143 mean metabolic power very likely increased (2.2% 95%CL \pm 0.8%, $p=5.00E-9$); in contrast,
144 the mean AQUA TITAN minus baseline reduction was likely trivial (-0.9% \pm 0.7%, $p=0.02$).
145 Therefore, the primary outcome at 48-h was a reduction of metabolic cost with AQUA TITAN
146 garments, relative to control (Figure 2A-B, Table 1). AQUA TITAN garments lowered minute
147 ventilation when the running speed was below the VT (stages 1-3) by 4.0% (\pm 0.7%, $p<1.00E-$
148 18, standardized difference -0.17 ± 0.03); when above the VT (stage 5), the effect was trivial
149 (0.7% \pm 1.2%, $p=0.34$, 0.03 ± 0.04) (Figure 2D). The increase in respiratory exchange ratio with
150 AQUA TITAN was trivial (Table 1), but minute ventilation per unit O₂ consumption adapted
151 to the lower oxygen cost of exercise with AQUA TITAN (Figure 2C) with a trivial decrease (-
152 0.3 \pm 0.5%, $p=0.36$, -0.01 ± 0.03).

153

154 **Discussion**

155 The purpose of the study was to determine the effect AQUA TITAN treated garments worn
156 during the 48-h recovery period of recovery from a bout of strenuous running on subsequent
157 running economy. AQUA TITAN garments lowered the metabolic cost of subsequent running
158 by 3.1% \pm 6%, relative to non-treated garments. This running economy finding is consistent
159 with a previous report⁶. Braun and Dutto¹⁶ previously reported the mean fatigue effect of a
160 hilly run on 48-h metabolic cost during subsequent running was 3.2%. Coupled with our
161 estimate of the non-treated fatigue effect, these data suggest the AQUA TITAN garments
162 enabled a restoration of running economy near to the pre-loaded condition, with the garments

163 assisting in maintenance of initial muscle contractile efficiency inferred by 166 relative
164 restoration of the metabolic cost of subsequent exercise.

165

166 With the physiological assumption of sample equivalence between studies based on
167 conservation of bioenergetics and VO_{2max} , Di Prampero et al.'s¹⁸ linear model for bioenergetic
168 predictors of running performance^{17,19} provided for a prediction of the magnitude of change in
169 distance running performance attributable to the AQUA TITAN garments. The model suggests
170 the mean improvement in economy corresponded to a moderate 2.4% improvement in
171 performance. Therefore, AQUA TITAN garments during recovery may be a useful
172 intervention for athletes competing in multiple-day events where rapid recovery of muscle
173 performance is an important component of outcome. Our study design, however, did not allow
174 determination of whether treatment effect was derived from physiological responses that
175 occurred while wearing AQUA TITAN during the 48-h recovery period or from acute effects
176 during subsequent exercise, or if the leggings, socks or upper garments were responsible.

177

178 Changes to the mechanical properties of the musculotendon unit may explain the improvement
179 in metabolic economy. Energy storage and return is thought to be optimal when the muscle
180 contracts isometrically while the tendon lengthens²⁰. This process works best with a compliant
181 tendon, but if the tendon is too compliant it will impair force transmission to the bone²¹. For
182 any movement, therefore, there is an optimal tendon stiffness based on the demands of the task.
183 If we assume that running patterns have developed to produce maximal performance from the
184 normal rested (i.e. pre-trial) tendon stiffness, any change in tendon stiffness would decrease
185 running economy. AQUA TITAN tape⁴ and garments⁶ have been shown to produce small
186 increases in joint range of motion (ROM) which could improve musculotendinous efficiency
187 through altered gait mechanics (although we lack the data to confirm this is the case). In our
188 companion study⁴, achilles tendon stiffness did not decrease following fatiguing exercise when
189 AQUA TITAN tape was applied to the tendon. A similar effect may explain the present results.

190

191 A second possible mechanism to explain the AQUA TITAN effect is improved repair of
192 damaged muscle tissue. It is well known that muscle damage follows strenuous eccentric
193 exercise^{16,22}; such tissue insult could impair stride efficiency by reducing stride length¹⁶, reduce
194 muscle and joint ROM²³ and decrease knee extensor torque²⁴ thereby producing impaired
195 contractile function and reduced running economy. Braun and Dutto¹⁶ reported that reduced
196 stride length is inversely correlated to running energy cost, suggesting damage to the preferred

197 type-I muscle fibre pool increases relative recruitment of type-II fibres. Type-II specific
198 myosin ATPase isoforms require 1.6- to 2.1-fold more ATP per unit force production than
199 type-I²⁵ and therefore require a proportionately higher oxidative phosphorylation. We observed
200 4% lower minute ventilation during sub-VT exercise with AQUA TITAN. Minute ventilation
201 is coupled to muscle contraction by locomotor muscle afferents²⁶. Lower minute ventilation
202 with AQUA TITAN, although possibly trivial in effect size, offers some physiological support
203 for relatively higher relative type-I fibre recruitment profile²⁷. Histologically, damaged muscle
204 undergoes regeneration and repair mediated by inflammatory process associated wound
205 healing responses followed by enhanced myogenesis²⁸. A recent cell culture experiment
206 suggests a possible damage repair mechanism. Ishizaki et al.⁵ reported that AQUA TITAN
207 coated rubber upregulated expression of myofibril components (vinculin, type I and III
208 collagen) and accelerated myocyte and osteoblast adhesion and growth, thereby demonstrating
209 a potential mechanism for the effect we observed.

210

211 A final perspective suggests a neural mechanism may be involved. Disrupted reflex activity of
212 the lower limb is known to impair running gait²⁹. Cronin et al.²⁹ reported that impaired triceps
213 surae short latency response results in less efficient transfer of force during running. This is a
214 significant mechanistic consideration with respect to the current study because downhill
215 running reduces the short latency reflex response of the triceps surae³⁰. Indeed, AQUA TITAN
216 tape applied to the triceps surae for 48-h following high-intensity running decreased the short
217 latency response and increased achilles tendon stiffness and ROM⁴; the subsequent reduced
218 yield and stiffer tendon²⁹ could perhaps have been sufficient to improve joint efficiency during
219 the gait cycle and therefore improve running economy. Further investigation is required to
220 determine if improved neuromuscular coordination and recovery of tendon stiffness are the
221 primary causal mechanisms for restoration of running economy with AQUA TITAN garments
222 following strenuous exercise.

223

224 **Conclusion**

225 AQUA TITAN garments worn during recovery from strenuous exercise restored subsequent
226 running economy. Current evidence for a mechanism supports improved musculotendinous
227 contractile function via faster short latency response regulating or restoring tendon stiffness
228 towards the pre-loaded adapted optima for running⁴. Further research is warranted to
229 investigate the potential modifications of cellular repair mechanisms relating to muscle and
230 connective tissue integrity and function during recovery, determine dose response, and

231 examine whether effects are mediated by physiological changes during the passive recovery
232 period or during exercise. Regardless of mechanism, the magnitude and certainty of the running
233 economy outcome implies that AQUA TITAN could have a meaningful impact on muscle
234 contractile function during periods of intense training and in multiday competitive events.

235

236 **Practical implications**

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

243

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249

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332 **Tables**

333

334 Table 1. Statistical summary of the effect of AQUA TITAN garments worn for 48-h during
 335 recovery from strenuous hill running on the metabolic cost of movement during subsequent
 336 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

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

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

337

³Oxygen ($\text{VO}_2 \cdot \text{kg}^{-1}$) and metabolic cost ($\text{kJ} \cdot \text{kg}^{-1} \cdot \text{km}^{-1}$) data are percentage estimates, while the respiratory exchange ratio are raw unit estimates.

⁴Magnitude-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 performance¹⁸. See Methods for further detail.

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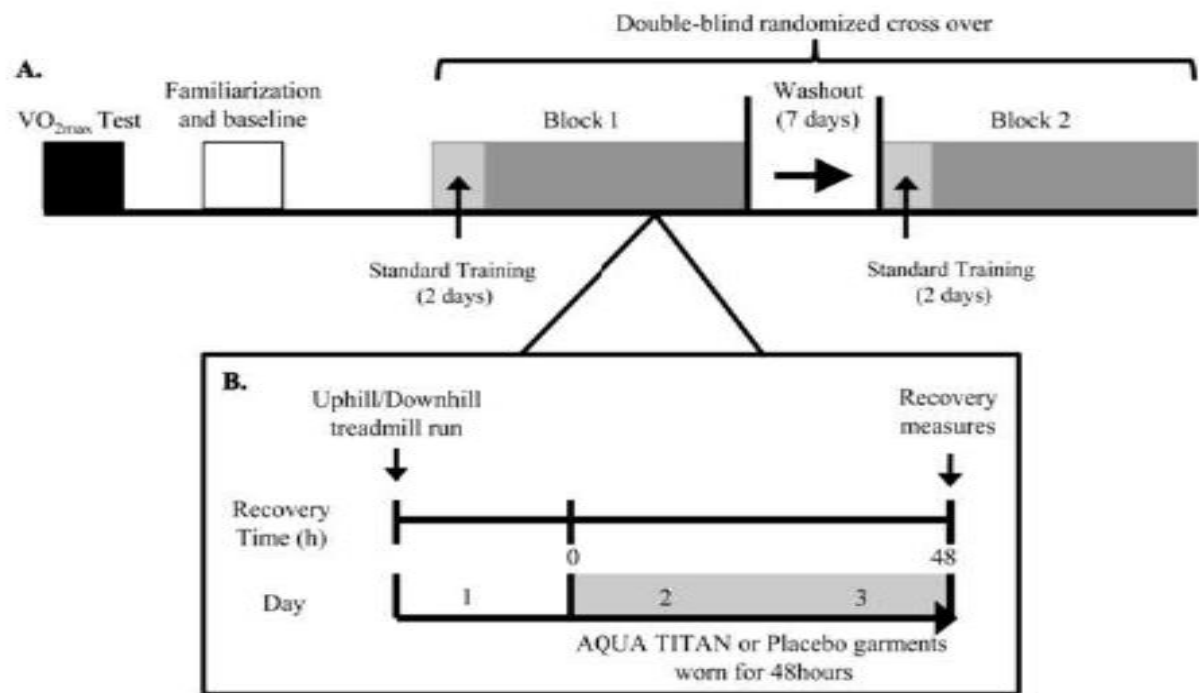
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Figure captions

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 (% $\text{VO}_{2\text{max}}$).

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