

# Physiological and performance effects of glycerol hyperhydration and rehydration

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*Studies have shown that beverages containing glycerol can enhance and maintain hydration status and may improve endurance exercise performance by attenuating adverse physiological changes associated with dehydration. Improvements to performance include increased endurance time to exhaustion by up to 24%, or a 5% increase in power or work. However, some studies have found no performance benefits during either prolonged exercise or specific skill and agility tests. In studies that have shown benefits, the improvements have been associated with thermoregulatory and cardiovascular changes. These include increased plasma volume and sweat rates, as well as reduced core temperature and ratings of perceived exertion. In a very small number of subjects, glycerol consumption has been associated with side-effects including nausea, gastrointestinal discomfort, dizziness, and headaches. In summary, while glycerol and fluid ingestion results in hyperhydration, the documented benefits to exercise performance remain inconsistent.*

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## INTRODUCTION

Glycerol is the structural 3-carbon frame of triacylglycerides and it plays an important role in fat metabolism.<sup>1–3</sup> It is also an osmotically active solute that is rapidly absorbed after ingestion and evenly dispersed throughout the body's water compartments (except the cerebrospinal fluid and aqueous humor).<sup>2,4,5</sup> This increases the osmolality within these compartments, enhancing the retention of fluid with which glycerol is ingested. The result is an expansion of these compartments with a concomitant reduction in urine volume, causing a transient state of hyperhydration.<sup>6–22</sup> During exercise, this extra water is available to attenuate fluid lost in sweat and breathing, so that total body water is maintained for longer. In a separate article, guidelines are provided for glycerol use in pre-exercise hyperhydration and rehydration during and after exercise; these are summarized in Table 1.<sup>23</sup>

The importance of adequate hydration for the endurance athlete is indicated by the extensive physiological changes that accompany dehydration, including the following: 1) decreased cardiac output due to a reduction in stroke volume<sup>24–30</sup>; 2) decreased systemic and cutaneous vascular conductance<sup>28,29,31</sup> further decreasing cardiovascular function<sup>24,29</sup>; and 3) reduction in heat dissipation due to decreasing cutaneous<sup>24,28,29,32</sup> and muscle blood flow<sup>24,28,29</sup> and sweat rates,<sup>27,31–35</sup> which results in increasing body core temperature.<sup>24,26,27,29,33–36</sup> Dehydration associated with a reduction in body weight (BW) amounting to as little as 1–2%, may increase the body's core temperature by 0.2–0.3°C<sup>24,32,37–39</sup> and significantly decrease stroke volume or cardiac output.<sup>24,38</sup> A loss of approximately 2% BW has also proved sufficient to reduce endurance performance in both mild<sup>40,41</sup> and warm/hot conditions.<sup>37,42–45</sup> When considering that sweat rates for athletes exercising in hot/humid environments can be as

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**Table 1 Guidelines for glycerol use in pre-exercise hyperhydration and rehydration during and after exercise.**

Purpose of glycerol use	Guideline
Pre-exercise hyperhydration	<ul style="list-style-type: none"> <li>• Only undertake a pre-exercise glycerol hyperhydration protocol if the exercise is likely to induce a reduction in BW of &gt;2%.</li> <li>• Consume a glycerol dose of 1.2 g/kg BW with a volume of fluid equal to 26 mL/kg BW. If this volume is too high for an individual, then consider personalizing the protocol by consuming a smaller volume of fluid and glycerol closer to the commencement of activity.</li> <li>• Consume the glycerol solution over a period of 60 min.</li> <li>• Use the normal choice of beverage. Carbohydrate-electrolyte beverages with a relatively high sodium content might provide an additional advantage; however, this requires further investigation.</li> <li>• Commence exercise approximately 30 min after the total hyperhydration fluid volume has been consumed.</li> <li>• Consider the increased metabolic cost associated with undertaking weight-bearing exercise with elevated BW.</li> </ul>
Glycerol rehydration during exercise	<ul style="list-style-type: none"> <li>• If pre-exercise hyperhydration has taken place, then consume 0.125 g/kg BW of glycerol in a volume equal to 5 mL/kg BW when exercise is of sufficient duration to dehydrate by &gt;2% BW.</li> <li>• Drinking fluid at a rate greater than that required to replace sweat loss (leading to a net weight gain during exercise) should be avoided. Therefore, if an athlete is hyperhydrated before exercise lasting 75 min or less, very little fluid would be needed during exercise under most conditions, and the consumption of glycerol with any fluid is not recommended.</li> <li>• If no pre-exercise hyperhydration has taken place, then a larger dose of glycerol with fluid during exercise is warranted. Therefore, we recommend athletes consume 0.4 g/kg BW glycerol with fluid during each of the first 4 h of exercise. After 4 h individuals should consume fluid alone where necessary. Again, fluid replacement should be based on sweat rate, and drinking to a level that would promote weight gain should be avoided.</li> </ul>
Glycerol rehydration post-exercise	<ul style="list-style-type: none"> <li>• Consume 1.5 L of fluid for each 1 kg of weight loss.</li> <li>• Add 1.0 g/kg BW of glycerol to each 1.5 L of fluid consumed when a subsequent bout of exercise will be undertaken within a few hours (this will provide similar glycerol doses with fluid volumes as those used in hyperhydration).</li> <li>• If there is a long duration between successive exercise bouts, then rehydrate with water and meals and follow the pre-exercise hyperhydration recommendations before the next exercise session.</li> </ul>
General recommendations	<ul style="list-style-type: none"> <li>• Try using glycerol in training before attempting to use it during competition.</li> <li>• If side effects are encountered, discontinue use or try to personalize the above recommendations by consuming a small dose of glycerol.</li> </ul>

high as 3.0 L/h,<sup>46</sup> fluid losses of 2% BW or greater can be accrued rapidly.

Considerable dehydration may also contribute to exertion-induced heatstroke, which has the potential to be fatal.<sup>47–49</sup> Therefore, maintaining the hydration status of an athlete is vital. Pre-exercise hyperhydration and maintenance of fluid balance during exercise may provide a mechanism by which the detrimental physiological processes associated with dehydration may be reduced, delayed, or prevented.<sup>50</sup> Glycerol, being a substance that increases fluid retention and prolongs the state of hyperhydration or euhydration,<sup>23</sup> could have significant beneficial effects on exercise performance and provide some protection against heat-related illness. This review is focused on the effects of pre-exercise glycerol hyperhydration on physiological variables and endurance performance. The use of glycerol during exercise and in rehydration is also discussed.

## ORIGINAL INVESTIGATIONS

For this mainly narrative review, the Medline database was searched for relevant publications up to October 31, 2008, using PubMed. The key search terms used were glycerol, exercise, hyperhydration, rehydration, and performance. The search identified 21 original investigations that were analyzed for this review. Tables were created to detail the fluid regimens and protocols of the studies (Table 2) as well as their results (Table 3).<sup>6–8,10,13,14,16–18,20–22,51–59</sup>

The glycerol doses used were similar among the studies (1.0–1.2 g/kg BW during pre-exercise hyperhydration, 0.7–0.94 g/kg BW during exercise, and 1.0 g/kg BW during rehydration); however, the total volumes of fluid differed considerably (1440–2500 mL during pre-exercise hyperhydration, 647–1000 mL during exercise, and 2103–2487 mL during rehydration). There was also considerable variation in the environmental conditions

Table 2 Fluid regimens and protocols of exercise performance tests following glycerol ingestion in hyperhydration, during exercise, or in rehydration.

Reference	Treatment	Glycerol dose (g/kg BW)	Fluid volume (mL/kg BW)	Total fluid volume (mL)	Hydration regimen	Exercise environment	Exercise protocol	Exercise duration (min)	Subject training status
Pre-exercise glycerol hyperhydration Lyons et al. (1990) <sup>6</sup>	- GLYC - Placebo 1 (large fluid) - Placebo 2 (small fluid)	1.0 in 3.3 mL/kg OJ in 1 h + 0.1 in 0.1 mL/kg OJ at 2, 3, and 4 h	Initial bolus 21.4 in 1 h, total of 28.4 after 4 h	1498 in 1 h* 1988 after 4 h*	Initial glycerol bolus at time 0; then fluid consumed over 60 min. Exercise began 90 min after final pre-exercise fluid intake. Additional glycerol and fluid given at the 2, 3, and 4 h marks after time = 0.	42°C, 25% RH, air velocity 100 m/min	Treadmill walking at 60% VO <sub>2</sub> max, with 5 min rest every 30 min (lab-based)	90	Not specified <sup>d</sup>
Meyer et al. (1995) <sup>51</sup>	- H <sub>2</sub> O - 5% CHO-electrolyte drink - 4% CHO-electrolyte drink with 1% GLYC	Unknown -estimated at 0.125 g/h over 60 h	26 <sup>f</sup>	1742 <sup>f</sup>	Initial glycerol solution consumed over 90 min; then additional glycerol solution and fluid consumption variable over 60 h; exercise variable over 60 h.	35–45°C, 20% RH	Treadmill walking – 3 × 40 min protocols at 4.8 km/h daily (carrying army pack weighing ~ 16.5 kg) (lab-based)	120 (daily)	Healthy males (not specified further)
Monther et al. (1996) <sup>13</sup> ; Series I	- GLYC - H <sub>2</sub> O	1.2	26	1749	Initial glycerol solution consumed over 30 min; then additional glycerol dose at 60 min mark; total fluid ingestion over 120 min; exercise began 60 min after final fluid intake.	24°C, 26% RH, air velocity 2.03 m/sec, altitude = 1600 m	Cycle to exhaustion at 61% Wmax (lab-based)	77–93	Cycled minimum 75 miles/week
Monther et al. (1996) <sup>13</sup> ; Series II	- GLYC - H <sub>2</sub> O	1.2	26 + 3 every 20 min during exercise	1749 + 605.6 mL per h during exercise	Same pre-exercise hyperhydration regime as for Series I. Additional fluid consumed during exercise.	24°C, 26% RH, air velocity 2.03 m/sec, altitude = 1600 m	Cycle to exhaustion at 61% Wmax (lab-based)	99–123	Cycled minimum 75 miles/week
Latzka et al. (1997) <sup>52</sup>	- Euhydration - H <sub>2</sub> O - H <sub>2</sub> O + rehydration - GLYC - GLYC + rehydration	1.2 g/kg LBM over 30 min	29.1 mL/kg LBM	1862 + 2190–2360 during exercise in euhydration and rehydration trials	Initial glycerol bolus at time 0; then fluid consumed over 30 min; pre-exercise fluid intake. Additional fluid consumed during exercise for euhydration and both rehydration trials.	35°C, 45% RH, air velocity = 1 m/sec	Treadmill walking at 1.56–1.65 m/s at 4–9% grade (= 45% VO <sub>2</sub> max) (lab-based)	120	Endurance-trained, heat-acc
Latzka et al. (1998) <sup>53</sup>	- Euhydration - H <sub>2</sub> O - GLYC	1.2 g/kg LBM over 30 min	29.1 mL/kg LBM	1862	Initial glycerol bolus at time 0; then fluid consumed over 30 min; exercise began 30 min after final fluid intake.	35°C, 45% RH, air velocity = 1 m/sec	Treadmill walking to exhaustion at 1.56–1.65 m/s at 4–9% grade wearing chemical protective clothing (= 55% VO <sub>2</sub> max) (lab-based)	29–34	Endurance-trained, heat-acc
Hitchins et al. (1999) <sup>7</sup>	- GLYC with CHO - Placebo (CHO)	1.0	22	1628	Glycerol solution consumed over 30 min; exercise began 120 min after final fluid intake.	32°C, 58% RH, air velocity 5.5 m/sec	Cycle – 30 min fixed power output + 30 min self-paced variable power output (lab-based)	60	Well-trained cyclists, non-acc
Monther et al. (1999) <sup>14</sup>	- GLYC pre-exercise + 5% glucose during exercise - GLYC pre-exercise + 0.5% GLYC, 5% glucose during exercise - GLYC pre-exercise + 1.5% GLYC, 5% glucose during exercise - Control (H <sub>2</sub> O pre-exercise + 5% glucose during exercise)	1.0 initial + 0.2 at 60 min	26 pre-exercise + 5 every 20 min during exercise	1768 + 1700 during exercise	Initial glycerol solution consumed over 30 min, then additional glycerol dose at 60 min mark. Total fluid ingestion over 120 min from the start of the glycerol solution intake; exercise began immediately after final pre-exercise fluid intake. Additional fluid consumed during exercise.	24°C, 26% RH, altitude = 1600 m	Cycle at 44% VO <sub>2</sub> max for 110 min (lab-based)	110	Not specified
Anderson et al. (2001) <sup>8</sup>	- GLYC - H <sub>2</sub> O	1.0	20 + CHO-electrolyte drink during exercise	1440 + 1764 during exercise	Glycerol solution consumed over 15 min; exercise began 120 min after final pre-exercise fluid intake. Additional fluid consumed during exercise.	35°C, 30% RH, wind speed not specified	Cycle at 98% LT for 90 min, followed by max effort for 15 min (lab-based)	105	Endurance-trained

Coutts et al. (2002) <sup>16</sup>	- GLYC - Placebo	1.2	25	1955	Glycerol solution consumed over 60 min; exercise began 70 min after final fluid intake.	Hot = 30.5°C, 46% RH Warm = 25.4°C, 52% RH Testing conducted in field	ODT (1.5 km swim, 40 km cycle, 10 km run) (field-based)	139–142	Well-trained, heat-acc triathletes
Maqal et al. (2003) <sup>17</sup>	Three phases 1) hyperhydration with/without GLYC 2) dehydration 3) rehydration with/without GLYC	1) 1.0 2) - 3) 0.5	1) 22 2) 10 3) 11	1) 1703 2) 774 3) 851	Glycerol solution consumed over 15 min; then fluid consumed over the next 135 min. Exercise began immediately after final fluid intake. Additional fluid consumed during exercise. During rehydration, glycerol solution consumed over 15 min with total fluid consumption over 90 min.	31°C, 75% RH; testing conducted in field, (court temp 33°C)	Specific skill and agility tests followed by 75-min tennis match (field-based)	120	Advanced-level tennis players
Marino et al. (2003) <sup>18</sup>	- GLYC - Placebo	1.2	21	1655	Glycerol solution consumed over 150 min; exercise began immediately after final pre-exercise fluid consumption. Additional fluid consumed during exercise.	34.5°C, 63.4% RH, air velocity 3 m/sec	Cycle – 60 min with aim to complete greatest distance possible. 1 min sprints at 10, 20, 30, 40, 50, 60 min marks (lab-based)	60	Moderately to well-trained
Wingo et al. (2004) <sup>19</sup>	- H <sub>2</sub> O pre- but not during exercise - H <sub>2</sub> O pre- + during exercise - H <sub>2</sub> O + GLYC pre- + H <sub>2</sub> O during exercise	1.0	2.8% BW	2153 (+up to 1200 mL per 10 mile loop (3600 mL total ad libitum)) <sup>9</sup>	Glycerol solution consumed over 120 min; exercise began after final pre-exercise fluid intake. Additional fluid consumed ad libitum during exercise.	30°C, testing conducted in field	3 × 10 mile loop mountain bike race (8 min break between loops) (field-based)	~155–160	Well-trained, heat-acc
Goulet et al. (2006) <sup>20</sup>	- GLYC - H <sub>2</sub> O	1.2	26 mL/kg BW before exercise + 500 mL/h during exercise	1781 + 830 during exercise	Glycerol and fluid intake over 110 min (glycerol solution given at 0, 40, and 80 min marks, H <sub>2</sub> O given at 20 and 60 min marks); exercise began 10 min after final pre-exercise fluid consumption. Additional fluid consumed during exercise.	25°C, 38–42% RH, low air velocity	Cycle at 65% VO <sub>2</sub> max for 120 min, followed by 5 min break then an incremental cycle to exhaustion (lab-based)	120 + ~12	Endurance-trained
Easton et al. (2007) <sup>21</sup>	- Placebo/ placebo - Placebo/GLYC - Creatine/ placebo - Creatine/GLYC	1.0	~ 28.6 / day*	2000 mL <sup>1</sup>	On day of trial, glycerol solution consumed over 60 min; then additional fluid over the next 180 min; further 60 min until exercise began. Additional fluid given during exercise	30°C, 70% RH, air velocity ~1.8 m/sec	Cycle at 63% WR <sub>max</sub> for 40 min, followed by 16.1 km (10 mile) time trial (lab-based)	40 + ~23	Endurance-trained, non-acc
Nishijima et al. (2007) <sup>24</sup> Experiment 2	- Placebo - Glycerol	1.2	25	1651	Placebo solution consumed over 90 min. Glycerol was 1.0 g/kg BW glycerol in 8 mL/kg BW fluid bolus within 30 min; then additional fluid over the next 60 min (with additional glycerol 0.2 g/kg BW ingested with fluid 60 min after starting hydration).	30°C, 51% RH	Cycle for 30 min fixed power output, + 30 min self-paced variable power output (lab-based)	60	Middle-distance runners
Dini et al. (2007) <sup>55S</sup>	- H <sub>2</sub> O pre- + H <sub>2</sub> O during exercise - GLYC pre- + H <sub>2</sub> O during exercise - GLYC pre- + GLYC during exercise	1.0 pre- + 1.0 during exercise	28.5 pre- + 4.5 during exercise	2500 pre- + 400 during exercise	Pre-exercise solution consumed over 90 min; then 180 min until exercise began. Additional 2 × 200 mL solutions consumed at ~30 and 60 min marks during exercise.	36°C, 30% RH	Rowing protocol: 6 × 3 min blocks, with 1 min rest between each, starting at 250 W and ↑ to 400 W in 30 W increments; repeated 3 times with 15 min break between each (lab-based)	89 (including 30 min rest)	National-level oarsman

Table 2 Continued

Reference	Treatment	Glycerol dose (g/kg BW)	Fluid volume (mL/kg BW)	Total fluid volume (mL)	Hydration regimen	Exercise environment	Exercise protocol	Exercise duration (min)	Subject training status
Goulet et al. (2008) <sup>36</sup>	- Glycerol - Control (euhydration)	1.2	26 pre- + 12.5 during exercise	1776 pre- + 853 during exercise	At 0 and 20 min, subjects drank 9 and 6 mL/kg BW fluid, each with 0.6 g/kg BW glycerol; then drank 6 mL/kg BW H <sub>2</sub> O at 40 and 60 min marks; further 30 min until exercise.	26–27°C, 55% RH, air velocity low	Cycle for 120 min at 65% VO <sub>2</sub> max with 5 × 2 min intervals at 80% VO <sub>2</sub> max performed at 12, 32, 52, 72, and 92 min marks); then incremental test to exhaustion (lab-based)	120 + –13	Endurance-trained
Glycerol consumption during exercise** Murray et al. (1991) <sup>57</sup>	- H <sub>2</sub> O - 10% GLYC - 6% CHO - 6% CHO + 4% GLYC	1.2 g/kg LBM (= 0.94)	3/kg LBM	647	Glycerol solution consumed over initial 60 min of exercise.	30°C, 45% RH	Cycle at 51.8% VO <sub>2</sub> peak for 90 min (lab-based)	90	Non-athletes
Siegler et al. (2008) <sup>58††</sup>	- Glycerol (5.2% solution) - Placebo	-0.7	13.4	1000	500 mL of glycerol or placebo solution consumed within 30 min before training; then a further 500 mL consumed after 30 min of training.		Variable-intensity training drills followed by intermittent endurance test (field-based)	60 + –20	Experienced soccer players
Glycerol use in rehydration†† Scheett et al. (2001) <sup>32</sup>	- Glycerol - GLYC - Placebo	1.0	3% BW (100% fluid lost during dehydration)	2487	Glycerol solution (30% of fluid) consumed in 1 <sup>st</sup> 30 min; then remaining 70% of fluid in 5 volumes (each 14% of total) consumed over the next 150 min; exercise began immediately after final fluid consumption.	40°C, 33% RH	Cycle to exhaustion at 50% VO <sub>2</sub> peak, cadence 60 rpm (lab-based)	38–42.8	Non-acc
Kavouras et al. (2005) <sup>59</sup>	- GLYC - H <sub>2</sub> O - No fluid	1.0	3% BW (75% of fluid lost during dehydration)	2103	Glycerol solution (1/3 of fluid) consumed over initial 15 min; then remaining 2/3 of fluid consumed in equal volumes from min 20 to 80; exercise began 30 min after final fluid consumption.	36.8°C, 48.1% RH, air velocity 2.54 m/sec	Cycle to exhaustion at 74% VO <sub>2</sub> peak, cadence 80–100 rpm (lab-based)	18.9–32.5	Endurance-trained, heat-acc cyclists

**Abbreviations:** BW, body weight; CHO, carbohydrate; GLYC, glycerol; H<sub>2</sub>O, water; heat-acc, heat acclimated; LBM, lean body mass; LT, lactate threshold; non-acc, non-heat acclimated; ODT, Olympic distance triathlon (1.5 km swim, 40 km bicycle, 10 km run); OJ, orange juice; RH, relative humidity; VO<sub>2</sub>max, maximum oxygen consumption; Wmax, maximal workload; WR<sub>max</sub>, maximal work rate.

\* Assume average subject weight of 70 kg.

† Mean VO<sub>2</sub>max of 42.1 mL/kg/min indicates not highly trained.

‡ Subjects consumed fluid ad libitum during 60 h of simulated desert conditions.

§ The full 1200 mL of fluid was consumed at the end of the loop on only three occasions out of the 72 trials. Average fluid consumed ranged from 693 mL to 842 mL across the trials. Average total fluid consumed was 4458 mL for water trial, 4643 mL for glycerol trial, and 2240 mL for no fluid during exercise trial.

¶ Easton et al.<sup>21</sup> gave 2 × 500 mL treatment solution daily for 6 days pre-trial + 500 mL of treatment solution with 2 × 500 mL H<sub>2</sub>O on day of trial.

\*\* Magal et al.<sup>17</sup> also gave glycerol during rehydration following hyperhydration and dehydration in their three-part study.

†† Lyons et al.,<sup>6</sup> Montner et al.,<sup>14</sup> and Dini et al.<sup>55</sup> also gave glycerol during exercise following glycerol-induced hyperhydration.

‡‡ Sieglar et al.<sup>58</sup> also gave 500 mL of glycerol solution before exercise.

**Table 3 Cardiovascular and thermoregulatory effects during performance tests following glycerol ingestion in hyperhydration, during exercise or in rehydration.**

Reference	Plasma volume	Heart rate	Sweat rate	Rectal temp	Skin temp	O <sub>2</sub> consumption	[Lactate]	FBF	Subjective parameter	Performance
Pre-exercise glycerol hyperhydration Lyons et al. (1990) <sup>61</sup>	→ (but not measured until 20–30 min post-exercise)	→ (↓ to 17 bpm – not significant)	↑ (~33%)	↓ (–0.9°C)	NM	NM	NM	NM	NM	NM
Meyer et al. (1995) <sup>51</sup>	NM (but Hb and Hct not different)	↑ (11–18 bpm) in exercise session 1, day 1	↑ (by up to 44% in exercise sessions 2 and 3 on day 2 and sessions 3 on day 3)	→	→	→ (RER ↓ with glycerol during all 3 exercise sessions on day 1)	NM	NM	RPE →	NM
Montner et al. (1996) <sup>13</sup> , Series I	NM (but Hb and Hct not different)	↓ ~4 bpm ¼ of the way through performance (tended to stay ↓ throughout)	→	→	NM	NM (RER →)	→	NM	RPE → Thirst →	Endurance time significantly ↑ to 21% with GLYC.
Montner et al. (1996) <sup>13</sup> , Series II	NM	↓ 4.4 bpm (mean throughout)	NM	→	NM	NM	NM	NM	RPE → Thirst →	Endurance time significantly ↑ to 24% with GLYC.
Latzka et al. (1997) <sup>52</sup>	→	→	→	→	→	→	→	NM	NM	NM
Latzka et al. (1998) <sup>53</sup>	NM	→	→	→	→	→	NM	NM	NM	Endurance time to exhaustion with GLYC was significant ↑ (13%) over control trial, and non-significant ↑ (~8%) over water hyperhydration.
Hitchins et al. (1999) <sup>7</sup>	→	↑ (4–5 bpm) during first 10 min of variable power phase with GLYC	→	→	→	→	→	NM	RPE → Thirst → Thermal →	Total work during variable power phase significantly ↑ by 5% with GLYC.
Montner et al. (1999) <sup>64</sup>	NM	↓ ~6 bpm (and SV ↑ 7%) with continued GLYC ingestion during exercise	NM	NM	NM	NM	NM	NM	NM	NM
Anderson et al. (2001) <sup>8</sup>	Not presented due to analytical problems	Significant ↓ (~7 bpm) with GLYC at 90 min mark. Tended to be ↓ throughout	NM	Significant ↓ (–0.4°C) with GLYC at 90 min mark. Tended to be ↓ throughout.	→ (tended to ↓ –0.4°C with GLYC throughout (muscle temp →))	→	→	Significant ↑ (~40%) with GLYC at 78 min mark	RPE →	Significant ↑ (5%) in work performed with GLYC.
Coutts et al. (2002) <sup>16</sup>	↑ 5% more during hyperhydration in GLYC group on hot day than placebo	NM	→	NM	NM	NM	NM	NM	NM	GLYC significantly ↓ (by ~10 min) the ↑ in completion time between hot and warm conditions for Olympic distance triathlon (mostly through 11% ↓ in run time with GLYC). Overall triathlon time significantly ↓ (7:49 min) with GLYC on hot day.

Table 3 Continued

Reference	Plasma volume	Heart rate	Sweat rate	Rectal temp	Skin temp	O <sub>2</sub> consumption	[Lactate]	FBF	Subjective parameter	Performance
Magal et al. (2003) <sup>17*</sup>	↑ (~7% following hyperhydration, dehydration and rehydration)	NM	NM	NM	NM	NM	NM	NM	NM	→
Marino et al. (2003) <sup>18</sup>	NM (ABV% ↑ with GLYC at 60 min exercise)	↑ (~10–20 bpm) with GLYC during 1 min sprints at 10, 20, 30, 40, and 60 min	↑ (~50%) in GLYC trial	→	→	NM	→	NM	RPE →	→ (with GLYC: power output non-significantly ↑ (~30–40 W) during 1-min sprints at mins 20, 30, and 50; however, total distance cycled non-significantly ↓ ~2.7%). → (time to complete final loop ↓ 5 min (~9%) with GLYC, but this was not significant).
Wingo et al. (2004) <sup>19</sup>	NM	→	→	→	NM	NM	→	NM	RPE → Thermal ↓ Thirst ↓ ESQ ↓	→
Goulet et al. (2006) <sup>20</sup>	NM	→	→	→	NM	→	NM	NM	RPE → Thirst →	→
Easton et al. (2007) <sup>21</sup>	→	↓	↑ (~14%)	↓ with creatine and creatine + GLYC	→	→	→	NM	RPE → Perceived dyspnea ↓ with GLYC in creatine trial	→
Nishijima et al. (2007) <sup>25</sup> Experiment 2	NM	→	NM	NM	NM	NM	→	NM	→	→ Average power ↑ 9% in the variable power phase in the glycerol condition, but this was not significant.
Dini et al. (2007) <sup>27†</sup>	NM	←	NM	NM	NM	NM	→	NM	NM	→ During the 2 <sup>nd</sup> and 3 <sup>rd</sup> performance blocks, work at the anaerobic threshold was maintained in the pre-exercise hyperhydration with additional glycerol during exercise condition, but ↓ in the control and pre-exercise hyperhydration without additional glycerol during exercise conditions. Glycerol ↑ endurance time (~12.5%) and peak power output (~5%).
Goulet et al. (2008) <sup>26</sup>	NM	↓	→	→	NM	NM	NM	NM	Thirst ↓	→



Glycerol consumption during exercise* Murray et al. (1991) <sup>27</sup> ↑ (10% GLYC >4% GLYC >CHO >water) <sup>c</sup>	↑	→ (non-significant ↑ ~20% in CHO trial over GLYC trials)	NM (esophageal temp →)	NM	NM (RER ↑ in CHO trial)	↑	NM	RPE → Thirst ↓	NM
Siegler et al. (2008) <sup>58</sup> ↑	↑	NM	→	NM	NM	↑	NM	NM	Time to exhaustion non-significantly ↓ (5.6%) with GLYC (19 vs 20 min).
Glycerol use in rehydration <sup>†</sup> Scheett et al. (2001) <sup>22</sup> – Greater ↑ (3–6%) during rehydration – Greater ↓ (4.3%) following exercise	↑	→	→ (non-significant ↓ (-0.4°C) during rehydration) → during exercise	NM	NM	NM	NM	NM	Time to exhaustion ↑ (12.6%) with GLYC.
Kavouras et al. (2005) <sup>59</sup> – Greater ↑ (3% vs H <sub>2</sub> O, 8% vs no fluid) during rehydration – ↑ maintenance of PV during exercise	→	→ (between GLYC and H <sub>2</sub> O; both ↓ vs no fluid)	→ (between GLYC and H <sub>2</sub> O; both ↓ vs no fluid)	→	→	↓ vs. no fluid (non-significant ↓ vs. H <sub>2</sub> O)	↑ (vs. both H <sub>2</sub> O and no fluid)	NM	Time to exhaustion ↑ (19%) with GLYC compared to H <sub>2</sub> O and ↑ (72%) compared to no fluid.

**Abbreviations:** ↑, increased in glycerol trials; →, not significantly different between treatments; ↓, decreased in glycerol trials; ΔBV%, percent change in blood volume; bpm, beats per minute; CHO, carbohydrate; ESQ, environmental systems questionnaire; FBF, forearm blood flow; GLYC, glycerol; Hb, hemoglobin; Hct, hematocrit; HR, heart rate; [Lactate], lactate concentration; NM, not measured; O<sub>2</sub> consumption, oxygen consumption; RER, respiratory exchange ratio; RPE, rating of perceived exertion; SV, stroke volume; temp, temperature.  
\* Magal et al.<sup>17</sup> also gave glycerol during rehydration following hyperhydration and dehydration in their three-part study.  
† Lyons et al.,<sup>6</sup> Montner et al.,<sup>14</sup> and Dini et al.<sup>55</sup> also gave glycerol during exercise following glycerol-induced hyperhydration.

and the exercise protocols used in the studies. While most of the studies included subjects who were well-trained, the heat acclimation of subjects varied across trials (Table 2). In a separate article on guidelines for glycerol use, we evaluated the quality of these studies and this was accounted for in the following discussions.<sup>23</sup> Seven of the studies documented in the other article were not included in the current review for the following reasons: 1) they did not include exercise protocols<sup>9,11,12,15</sup>; 2) they studied glycerol hyperhydration in cold rather than warm/hot environments<sup>19,60</sup>; or 3) they provided insufficient fluid to be regarded as a hyperhydration study.<sup>61</sup>

## PHYSIOLOGICAL EFFECTS OF PRE-EXERCISE GLYCEROL HYPERHYDRATION

The increased total body water with pre-exercise glycerol hyperhydration has resulted in physiological effects attributable to thermoregulatory, cardiovascular, and/or central nervous system mechanisms.

### Cardiovascular benefits

Cardiovascular effects have been studied as a mechanism for enhanced endurance performance following pre-exercise glycerol hyperhydration in 17 studies (Table 3). Eight of these showed benefits, including reductions in exercising heart rate,<sup>8,13,14,21,56</sup> increased cardiac filling and stroke volume, and better maintenance of plasma volume.<sup>16–18</sup> Combined, these effects indicate that glycerol hyperhydration attenuates exercise-induced cardiovascular strain.<sup>8,16,18</sup> Theoretically, the reduced cardiovascular strain could allow increased oxygen and nutrient delivery to working muscles, coupled with enhanced removal of waste products.<sup>62</sup> In contrast, however, it should be noted that three studies showed higher heart rates following glycerol ingestion.<sup>7,18,51</sup> In two of these, the higher heart rates coincided with power outputs that were,<sup>7</sup> or tended to be,<sup>18</sup> higher in the glycerol trials.

The most likely explanation for these benefits is that the extra retained fluid maintains mean arterial pressure and prevents the large decreases in plasma and stroke volumes that occur with prolonged exercise.<sup>8</sup> In this way, cardiovascular efficiency may be improved during endurance performance following pre-exercise glycerol hyperhydration. Others have argued that benefits resulting directly from a small increase in blood volume are unlikely because blood volume is the smallest of the body water compartments,<sup>14</sup> representing only 12% of total body water.<sup>11</sup> If an average of 500 mL of additional fluid is retained during hyperhydration, and distributed evenly throughout the total body water, blood volume could be expected to increase by 60 mL. This constitutes a



variation of only approximately 1–2% given that the average female or male adult has 4000 or 5000 mL of blood, respectively.<sup>11</sup> However, it could be expected that the additional fluid retained in other compartments would be used to support plasma volume once it starts to decrease during exercise.

### Thermoregulatory benefits

Thirteen of the 21 studies included in this review investigated aspects of thermoregulation following glycerol-induced hyperhydration; six of these showed benefits including increased sweat rates,<sup>6,18,21,51</sup> reductions in rectal temperature,<sup>6,8,21</sup> and increased forearm blood flow<sup>8</sup> (Table 3).

Increases in sweat rates of 14–50% were seen in 4 of the 12 studies that measured sweat rate following pre-exercise glycerol hyperhydration.<sup>6,18,21,51</sup> Such increases should promote evaporative cooling, allowing an avenue to reduce core temperature. Indeed, two of the four studies showed a concomitant reduction in core temperature and an increased sweat rate.<sup>6,21</sup> The largest reduction in core temperature (~0.9°C) was seen with a large increase in sweat rate (~33%) in dry heat (42°C, 25% relative humidity [RH]).<sup>6</sup> In contrast, another study showing large increases in sweat rates (~50%) following glycerol consumption did not show reductions in rectal temperature or mean skin temperature.<sup>18</sup> The failure of this enhanced potential for evaporative heat loss to influence core temperature may be related to the higher humidity (63%) in that study.<sup>18</sup> Eight other studies reported no difference in sweat rate between glycerol and non-glycerol conditions (Table 3).<sup>7,10,13,16,20,52,53,56</sup> Of these, three studied exercise in relatively thermoneutral environments,<sup>13,20,56</sup> one studied exercise of a short duration,<sup>53</sup> one studied exercise at a relatively low intensity,<sup>52</sup> and two allowed rest periods during the performance tests (Tables 2 and 3).<sup>10,20</sup> These factors may have contributed to the observed lack of effect of glycerol on sweat rate in these studies.

Another of the observed thermoregulatory benefits is increased forearm blood flow, indicating increased distribution of circulating blood volume to the periphery. This would enhance heat dissipation via radiation, convection, and conduction. Only one study was identified that measured this parameter during exercise following glycerol hyperhydration.<sup>8</sup> Forearm blood flow was nearly 40% higher towards the end of the exercise period in the glycerol trials. However, the authors noted that the difference between trials was due to a decrease in the control trials rather than an augmented increase with glycerol. During exercise, the body attempts to maintain mean arterial pressure as a priority over skin blood flow.<sup>8</sup> Dehydration results in a greater relative

proportion of the total blood volume being required centrally to maintain mean arterial pressure. The result is a relative decrease in the amount of blood shunted to the periphery.<sup>8</sup> With pre-exercise hyperhydration, extra water within the body would be available to maintain blood volume for longer, and thus delay the point at which peripheral blood flow is compromised in favor of central blood volume. Anderson et al.<sup>8</sup> showed that maintenance of forearm blood flow in the glycerol trials occurred while both rectal temperature and skin temperature tended to be lower, suggesting the possibility that glycerol increased evaporative cooling via this mechanism.<sup>8</sup>

### Perceived exertion, thirst sensation, and thermal sensation

Undertaking long-duration, high-intensity exercise in the heat induces both severe physiological stress on the body and severe psychological stress. Higher ratings of perceived exertion (RPE) have been seen at the end of 60 min of exercise in the heat (32°C, 60% RH) in individuals dehydrated by 1.8% BW.<sup>42</sup> Because glycerol does not cross the blood-brain barrier, it is unlikely to have a direct central nervous system effect on subjective measures. However, by reducing the severity of dehydration, in conjunction with improving cardiovascular and thermoregulatory functions, glycerol hyperhydration may reduce the perceived effort of exercise. Eight studies have measured RPE during performance following pre-exercise glycerol ingestion (Table 3).<sup>7,8,10,13,18,20,21,51</sup> Three of these showed significant performance improvements<sup>7,8,13</sup> and two showed non-significant performance benefits (>5%).<sup>10,18</sup> In all of these studies, RPE was similar between conditions even though total work,<sup>7</sup> power output,<sup>8,18</sup> time to exhaustion,<sup>13</sup> or race time<sup>10</sup> was improved in the glycerol trials. Together, these findings indicate that subjects were able to maintain a higher work rate without feeling like they were working any harder.<sup>7,10</sup>

Other perceptual markers, including thirst sensation and environmental symptoms questionnaire scores, have also been diminished following glycerol hyperhydration, suggesting decreased perception of thermal strain.<sup>10,56</sup> Importantly, however, it can also be argued that reduced thirst may be a negative consequence for individuals exercising in the heat, as reduced inclination to consume fluids may exacerbate ensuing dehydration.<sup>57</sup> Athletes, therefore, need to be aware of their expected sweat rate during an exercise bout. As body water losses of up to 2% BW are generally well tolerated, the goal of fluid replacement during exercise should be to replace sufficient fluid so that BW is maintained above this level.

## PERFORMANCE EFFECTS OF GLYCEROL HYPERHYDRATION

Thirteen of the studies discussed in the previous section also investigated the effects of pre-exercise glycerol hyperhydration on aspects of endurance exercise performance (Tables 2 and 3). Six of the 13 (including one with two series of experiments) showed significant improvements in endurance performance parameters,<sup>7,8,13,16,53,56</sup> while seven did not.<sup>10,17,18,20,21,54,55</sup> However, one of those seven did show a potentially practical, albeit non-significant, performance improvement of 9%.<sup>10</sup> One further study showed improved power at the anaerobic threshold with pre-exercise glycerol hyperhydration and additional glycerol during exercise, as discussed in the next section.<sup>55</sup> Importantly, none of the studies showed a decrement in endurance performance following glycerol hyperhydration. All of the studies are discussed in more detail below, and the differences in study designs are used to highlight factors leading to implications for the use of glycerol to improve performance.

### Timing of exercise performance

A hyperhydration protocol should be most beneficial when exercise coincides with maximal fluid retention. However, establishing a definitive timeframe for peak hyperhydration is difficult because a number of complex physiological mechanisms affect fluid uptake and body water. With dehydration and rehydration, many markers of hydration status undergo rapid changes. Unfortunately, it is common to have relatively long periods of time between successive analyses of these markers in hydration research. It is consequently possible to miss transient changes, adding to the difficulty of determining when maximal hyperhydration occurred.

Peak fluid retention<sup>6,7,9,11,19</sup> and changes in plasma volume<sup>7,11,19,52</sup> have been seen 30–120 min following fluid ingestion. Tables 2 and 3 demonstrate that performance improvements have been seen with exercise protocols starting within this period. Exercise time to exhaustion was increased when the performance test began 30,<sup>53</sup> 60,<sup>13</sup> or 120 min<sup>56</sup> after hyperhydration ended. Similarly, performance under racing conditions improved when starting 35 min<sup>10</sup> or 120 min<sup>16</sup> post-hyperhydration. Furthermore, power output was increased in three studies when performance started 120 min after hyperhydration.<sup>7,8,56</sup> Of the five studies that showed thermoregulatory benefits with glycerol ingestion, as discussed in the previous section, four initiated the exercise protocol at least 60 min after the hyperhydration period ended.<sup>6,8,21,51</sup>

Starting exercise too soon will not allow enough time for fluid absorption. Three studies began performance tests within 10 min of the hyperhydration period.<sup>17,18,20</sup> All

three showed no difference in performance between conditions, although one did show thermoregulatory benefits.<sup>18</sup> However, waiting too long before starting exercise will also reduce the effectiveness of this technique. Free water clearance and urine flow are seen to peak 30–120 min following hyperhydration.<sup>7,9,11,19</sup> Therefore, long delays between hyperhydration and performance will result in greater excretion of the ingested fluid and a lower relative state of hyperhydration when exercise begins. Several studies have mapped fluid retention for periods of up to 3 h post ingestion, without the confounding of an exercise protocol beginning within this period. After 2 and 3 h, respectively, 60–80% and 45–60% of the ingested fluid was still retained in the glycerol trials.<sup>6,9,11</sup>

### Exercise mode, intensity, and performance task

Exercise trials that induce a 2% BW loss and impose a large strain on the thermoregulatory system allow a greater opportunity for the effects of glycerol hyperhydration to be witnessed. In four of the six exercise studies demonstrating enhanced performance,<sup>8,13,16,56</sup> and the single study showing non-significant improvements,<sup>10</sup> a long-duration (>75 min), high-intensity exercise trial was used (Table 2).

In the studies that did not show significant performance improvements, four allowed rest periods during the exercise protocols,<sup>6,10,20,55</sup> one studied exercise at a relatively low intensity,<sup>52</sup> two studied exercise for relatively short durations,<sup>18,21</sup> one investigated specific skill and agility tasks,<sup>17</sup> one used exercise to exhaustion (a protocol that has been associated with poor reliability and a coefficient of variation in individuals of up to 27%<sup>63–65</sup>),<sup>20</sup> and one used a protocol that has failed to show benefits with other supplementation strategies (Table 2).<sup>18</sup> However, two of the studies that showed benefits also utilized exercise protocols of relatively short duration ( $\leq 60$  min)<sup>7,53</sup> and three studied exercise to exhaustion<sup>13,53,56</sup> (one at a relatively low intensity<sup>53</sup>).

Allowing rest periods during the exercise protocols seems to consistently limit the potential to see performance benefits since the thermal load on the body is reduced through enhanced heat dissipation. As a result, the thermoregulatory and cardiovascular systems become less stressed. Only one of the studies with rest periods (for urine collection during initial steady-state exercise) showed significant performance improvements during subsequent incremental exercise to exhaustion.<sup>56</sup> One other showed a non-significant 5 min (~9%) improvement under race conditions.<sup>10</sup> It could be speculated that this difference may have become significant if rest periods were not allowed. Four studies that incorporated rest periods into the performance task also measured cardiovascular and thermoregulatory mechanisms and two

showed no benefits.<sup>10,20</sup> However, subjects in the study of Wingo et al.<sup>10</sup> did have a lower perceived thermal sensation, thirst sensation, and environmental symptoms questionnaire score. In contrast, Lyons et al.<sup>6</sup> allowed a 5-min rest period every 30 min and subjects still showed significant increases in sweat rate with concomitant reductions in core temperature following glycerol ingestion. These differences can likely be explained by the fact that the environmental conditions used by Wingo et al.<sup>10</sup> (30°C) and Goulet et al.<sup>20,56</sup> (25–27°C, 38–55% RH) would allow heat dissipation during resting conditions, while the 42°C, 25% RH used by Lyons et al.<sup>6</sup> was not conducive to enhanced heat dissipation, even when resting.

### Environmental conditions

The detrimental effects of dehydration on physiological processes are exaggerated in thermally stressful environments. Gonzalez-Alonso et al.<sup>66,67</sup> report that when presenting separately, dehydration (–4% BW) and hyperthermia each reduce stroke volume by 7–8% and increase systemic vascular resistance by approximately 2%. However, when presenting together, dehydration (–4% BW) and hyperthermia elicit a synergistic effect and reduce stroke volume by more than 20%<sup>66,67</sup> while increasing systemic vascular resistance by more than 10%.<sup>66</sup> Thus, this level of dehydration in a thermally stressful environment seriously compromises the thermoregulatory and cardiovascular systems.

Four of the six studies showing significant performance improvements following glycerol hyperhydration conducted exercise in thermally stressful conditions of 30–35°C and 30–60% RH.<sup>7,8,16,53</sup> The specific influence of environmental conditions on performance was displayed by Coutts et al.,<sup>16</sup> who had subjects complete a triathlon following glycerol ingestion or a placebo in both warm (25°C, 52% RH) and hot (30.5°C, 46% RH) conditions. Performance was 1:47 min slower on the hot day compared to the warm day after glycerol consumption but 11:40 min slower on the hot day compared to the warm day in the placebo condition. The overall time was also significantly faster (5.7%; 7:49 min) with glycerol compared to placebo on the hot day.<sup>16</sup>

The significant variation in environmental conditions found in the exercise protocols may also contribute to the inconsistent results witnessed for the previously discussed thermoregulatory parameters. High ambient temperatures may enhance the benefits in hyperhydrated subjects by exacerbating dehydration in control subjects. Imposing a greater thermal load on the body would potentially increase the benefits associated with having extra water available to maintain plasma volume and assist in heat dissipation. Two of the four studies showing significant improvements in sweat rate were conducted in

very hot, dry conditions (42°C, 25% RH<sup>6</sup> and 35–45°C, 20% RH<sup>51</sup>) while the other two were in warm/hot, humid conditions (34.5°C, 64% RH<sup>18</sup> and 30°C, 70% RH<sup>21</sup>). Similarly, the most significant reductions in rectal temperature occurred with exercise conducted in dry heat (42°C, 25% RH<sup>6</sup> and 35°C, 30% RH<sup>8</sup>) or warm/hot, humid conditions (30°C, 70% RH<sup>21</sup>). These high ambient air temperatures would limit heat dissipation to evaporation, while exercise in moderate conditions, as used in a number of other studies (24°C, 26% RH,<sup>13,14</sup> 25°C, 38–42% RH<sup>20</sup> and 26–27°C, 55% RH<sup>56</sup>), would allow heat loss via all avenues (evaporation, convection, conduction, and radiation).<sup>13</sup> Given these extra avenues for heat dissipation in moderate temperatures, the lack of thermoregulatory benefits in these studies is not surprising.

### GLYCEROL CONSUMPTION WITH FLUID DURING EXERCISE

Hyperhydration increases body water to delay the progression of dehydration during exercise. However, most athletes also consume additional fluid while they are competing. The following sections discuss the effect of glycerol ingestion with fluid during exercise and the effect of consuming fluid without additional glycerol during exercise.

#### Glycerol ingestion with fluid during exercise

It is reasonable to expect that the osmotic properties of glycerol may also enhance the retention of fluid ingested after exercise has begun. To date, only five studies have employed protocols that include glycerol in beverages consumed during exercise. One of these showed a benefit to anaerobic performance<sup>55</sup> while each of the other four showed one or more significant benefits to thermoregulatory or cardiovascular variables.<sup>6,14,57,58</sup>

Only two of the studies reviewed provided glycerol with fluid during exercise, without pre-exercise glycerol hyperhydration (Tables 2 and 3).<sup>57,58</sup> To our knowledge, Murray et al.<sup>57</sup> are the only researchers who have studied the specific effects of glycerol on hydration during exercise without any pre-exercise glycerol and fluid ingestion, since Siegler et al.<sup>58</sup> gave 500 mL at 30 min pre-exercise and a further 500 mL during exercise. These studies are not hyperhydration studies because only a small total volume of fluid was consumed (647<sup>57</sup>–1000 mL<sup>58</sup>). The two studies provided glycerol solutions with concentrations ranging from 4 to 10% (4% glycerol with 6% CHO and 10% glycerol in one<sup>57</sup> and 5.2% glycerol with 4% CHO in the other<sup>58</sup>). The resulting glycerol doses were 0.38 and 0.94 g/kg BW for the 4% and 10% solutions of Murray et al.<sup>57</sup> and 0.7 g/kg BW for the 5.2% beverage of Siegler et al.<sup>58</sup>

During 90 min of cycling at 50%  $\text{VO}_2$  peak (in 30°C, 45% RH environment) both the 4% and 10% glycerol solutions reduced thirst sensation and attenuated the decrease in plasma volume seen with the water placebo and sports drink solutions.<sup>57</sup> From 60 to 80 min during exercise, the 10% glycerol solution provided better maintenance of plasma volume than did the 4% solution. In fact, the 10% glycerol solution caused plasma volume to increase above resting euhydrated levels at 70 and 80 min of exercise. However, the results were similar at all other time points and there were no differences between treatments for heart rate, sweat rate, and esophageal temperature. It is possible that the total volume of fluid consumed (647 mL) may have been too small to translate into thermoregulatory benefits. Overall, mean ratings of perceived thirst were also lower for the glycerol treatments; however, the authors note this may be a negative consequence for individuals exercising in the heat.<sup>57</sup> The protocol of Siegler et al.<sup>58</sup> resulted in a 40% reduction in BW loss and a 55% reduction in the change in plasma volume over 60 min of exercise, compared to the sports drink. Thus, the addition of glycerol to beverages consumed during exercise has the potential to maintain fluid balance to a greater extent than sports drinks or water alone.

The three other studies investigating glycerol consumption during exercise did so after pre-exercise glycerol hyperhydration.<sup>6,14,55</sup> Subjects in the study of Dini et al.<sup>55</sup> maintained work rate at the anaerobic threshold when additional glycerol was consumed with fluid during exercise, while power output dropped at the anaerobic threshold in the trials without. Lyons et al.<sup>6</sup> showed increased sweat rates and reduced core temperature in the glycerol trials. However, they had no pre-exercise hyperhydration trial without additional glycerol during exercise. It is, thus, impossible to differentiate the effects of glycerol before exercise alone, as compared to before and during exercise. Montner et al.<sup>14</sup> showed that continued glycerol and fluid intake during exercise increased stroke volume and reduced exercising heart rate, compared to the intake of fluid alone, even though exercise was performed at a low intensity in mild environmental conditions. Together, these studies indicate that ingestion of glycerol along with fluid during exercise may maintain hydration; however, the practical benefits resulting from this enhanced rehydration are inconclusive and require further investigation.

### **Pre-exercise glycerol hyperhydration with additional fluid during exercise**

Most other hyperhydration studies that gave glycerol before exercise also provided fluid without additional glycerol during the performance tests.<sup>8,10,13,14,17,18,20,21,51,52,55</sup>

This more realistically reflects the behavior of athletes. The provision of additional fluid may further explain the lack of thermoregulatory or cardiovascular benefits seen in some studies discussed in the previous section. Hyperhydration delays the development of body water deficits when sweat losses are not replaced.<sup>52</sup> However, if sufficient fluid is available during exercise to maintain hydration, the physiological stress associated with dehydration will be limited and pre-exercise hyperhydration would ostensibly have a negligible effect.<sup>10,52</sup>

Vast differences in the designs of the 11 studies examining this effect make it difficult to determine the extent to which potential benefits associated with hyperhydration are negated by ingesting extra fluid during exercise. In five studies, subjects ingested large volumes of fluid during exercise (i.e., >1200 mL).<sup>8,10,13,14,52</sup> In three of these studies, subjects showed cardiovascular benefits,<sup>8,13,14</sup> but thermoregulatory benefits were only seen in one.<sup>8</sup> Five studies reported the temperature of the ingested fluid; four provided beverages cooler than body temperature (4°C<sup>13,14</sup>–25°C<sup>10,20</sup>) while one gave beverages at body temperature.<sup>52</sup> It can be surmised that providing cooler fluid may allow an extra avenue for heat dissipation, thereby reducing the influence of hyperhydration on thermoregulation. However, several studies have observed significant physiologic and thermoregulatory benefits with glycerol hyperhydration even though fluid was available during exercise (Tables 2 and 3).<sup>6,8,13,14,17,21,51</sup> Benefits seen include increased sweat rates,<sup>6,18,21,51</sup> increased plasma volume<sup>17,18</sup> and forearm blood flow,<sup>8</sup> and reduced rectal temperature<sup>6,8,21</sup> and heart rate.<sup>8,13,14,21</sup> Two studies also showed significantly enhanced endurance performance.<sup>8,13</sup>

### **GLYCEROL USE IN REHYDRATION AFTER EXERCISE**

Many athletes, in both individual and team sports, complete multiple, intense training sessions on a daily basis. Many of these athletes also compete at events where they are expected to repeat maximal performance in competitions over successive days. Whether in training or in competition, it is imperative that these athletes maximize their recovery between sessions. Rehydration is a vital component of recovery and may be enhanced by the inclusion of glycerol in rehydration beverages.<sup>17,22,59</sup> To date, only three studies have explored the potential benefit of incorporating glycerol in beverages ingested while rehydrating from a state of exercise-induced dehydration.<sup>17,22,59</sup> Scheett et al.<sup>22</sup> and Kavouras et al.<sup>59</sup> dehydrated subjects via exercise in the heat to –3% and –4% BW, respectively. They then rehydrated the subjects (3% BW each) with or without glycerol (1 g/kg BW) before evaluating the effectiveness of the rehydration protocols via a performance test. Magal et al.<sup>17</sup> initially hyperhydrated subjects with or



without glycerol and then dehydrated them via exercise before rehydrating them with or without glycerol. The effect of glycerol was, thus, observed during both hyperhydration and rehydration.

Both studies investigating glycerol use exclusively in rehydration found significant performance improvements in the glycerol trials (Table 2).<sup>22,59</sup> Cycling time to exhaustion with glycerol increased by 12.6%<sup>22</sup>–19%<sup>59</sup> when compared to rehydrating with water alone. Additionally, all studies using glycerol in rehydration showed that plasma volume was more rapidly and completely restored when rehydrating with glycerol solutions compared to water. Glycerol rehydration was also associated with higher forearm blood flow during the performance test in one study,<sup>59</sup> and a non-significant decrease in rectal temperature ( $\sim 0.4^{\circ}\text{C}$ ) during the rehydration period in another.<sup>22</sup> However, no other thermoregulatory benefits were observed (Table 3).

## ADDITIONAL CONSIDERATIONS

### Metabolic benefits

In addition to providing thermoregulatory and/or cardiovascular benefits, glycerol may potentially enhance endurance performance by maintaining blood glucose via liver gluconeogenesis,<sup>1,68–72</sup> thereby decreasing the utilization of muscle and liver glycogen during exercise. While sound in theory, ingestion of glycerol in a small volume of fluid (400–500 mL) has proved ineffective for improving endurance performance.<sup>68–71,73</sup> It has been shown that the human liver, while possessing high levels of glycerol kinase,<sup>1,68,72,74,75</sup> does not have the gluconeogenic capacity to rapidly convert glycerol to glucose for metabolism during exercise.<sup>8,70,71,73</sup> There is some evidence in humans that glycerol may contribute a small amount to total  $\text{CO}_2$  production and increase blood glucose,<sup>1,68,69,73</sup> and, in rats, glycerol feeding significantly prolonged exercise time to exhaustion by 32%.<sup>72</sup> However, when looking at all studies on this topic, at best, glycerol may provide a minor energy substrate during exercise.

### Potential negative effects of glycerol hyperhydration

The BW gain associated with glycerol hyperhydration may have a detrimental effect on performance during weight-bearing activity of a relatively short duration or in non-stressful environmental conditions. It has been shown that the primary factor determining the metabolic cost of walking or running is the force required to support BW.<sup>76–79</sup> Supporting BW and accelerating the center of gravity account for 28% and 45% of the metabolic cost of walking, respectively.<sup>77</sup> The energy required

to support BW is even higher when running, increasing to 74% of the net metabolic cost.<sup>76</sup> Additionally, increasing BW has a slightly greater than proportional effect on increasing metabolic rate.<sup>76</sup> Adding 1 kg to a 68.85 kg individual is enough to increase metabolic rate by 1.18 W when walking.<sup>77</sup> Thus, the increased energy associated with carrying the additional fluid retained with pre-exercise hyperhydration may be more substantial than any potential benefits arising from reduced thermal strain. A further confounding factor is that an increased metabolic rate will act to elevate core temperature, because 80% of metabolic energy is converted to heat.<sup>80</sup> One study showed that 10-m sprint times tended to be slightly higher immediately following glycerol hyperhydration when subjects were approximately 1 kg heavier.<sup>17</sup> Another showed a non-significant (5%) reduction in exercise time during a short incremental field test when athletes were approximately 0.6 kg heavier.<sup>58</sup> However, in a situation of long-duration, high-intensity exercise in a thermally stressful environment, where the dehydration is likely to be well in excess of 2% BW, then the benefit of additional fluid may be more important.

### Side effects associated with glycerol consumption

Glycerol consumption is very well tolerated. In hydration research, glycerol administration has been investigated with fluid before, during, or after exercise in 28 studies. Three studies reported side-effects after rapidly administering the glycerol as a concentrated bolus followed by fluid ingestion.<sup>8,52,53</sup> In two of these studies, a total of four subjects were nauseous after glycerol ingestion, resulting in cancellation of the trial on that day<sup>52,53</sup>; in the other study, two subjects developed diarrhea in the 24-h period after the trial.<sup>8</sup> A small number of subjects in four other studies reported side-effects that did not affect participation; these included gastrointestinal distress (bloating/nausea)<sup>16,21,57</sup> or lightheadedness.<sup>57</sup>

In subjects reporting side-effects, glycerol was given both as a concentrated bolus administered rapidly followed by fluid ingestion<sup>8,52,53</sup> and in a larger volume of a less concentrated solution over a longer duration.<sup>16,21</sup> Other investigators have replicated these methods of administration without incident.<sup>6,9,11,13,15,17–20,22,59</sup> Lower total concentrations of glycerol, and its administration in divided doses, may have accounted for the lack of side-effects reported in some studies.<sup>7,10,13,14,18,20</sup> Glycerol ingestion over longer periods of up to 49 h has also been well tolerated.<sup>12</sup>

Of the two studies using glycerol during exercise without pre-exercise hyperhydration, side-effects were seen in one<sup>57</sup> both not the other.<sup>58</sup> Murray et al.<sup>57</sup> gave a higher dose with less fluid, which, when combined with a reduction in gastrointestinal blood flow and delayed

gastric emptying with exercise, may account for the symptoms reported. Three other studies hyperhydrated with glycerol pre-exercise and then provided additional glycerol with fluid while exercising and reported no side-effects.<sup>6,13,55</sup>

While glycerol has been used clinically to reduce cerebral edema, intracranial hypertension, and intraocular pressure by drawing water out of the cerebrospinal fluid and brain,<sup>3,74,81</sup> there is very little chance of these events occurring when glycerol is consumed with large volumes of fluid. However, there are certain populations for which glycerol ingestion is not recommended due to actions on liver gluconeogenesis, kidney filtration, cardiovascular homeostasis, and hydration homeostasis. These include individuals with diabetes, renal disease, migraine and headache disorders, cardiovascular disease, liver disorders, and those who are pregnant.<sup>9</sup>

### CONCLUSION

In a separate article, we conclude that glycerol ingestion promotes fluid retention and delays dehydration.<sup>23</sup> In this review, we discuss how the translation of these effects into performance benefits has not been consistently demonstrated. The effects of glycerol hyperhydration on thermoregulatory and cardiovascular physiology include higher sweat rates, reduced heart rates and core temperatures, increased forearm blood flow, and favorable subjective responses including reduced thermal sensation, thirst sensation, environmental symptoms questionnaire scores, and RPE. These effects have been associated with performance improvements including increased endurance time to exhaustion (up to 24%), increased work and power (up to 5%), and improved Olympic distance triathlon performance (approximately 10 min). However, others have found no benefits affecting endurance and/or specific skill and agility tasks. Presently, no studies have shown a detrimental effect of pre-exercise glycerol hyperhydration on endurance performance. Only two studies have provided glycerol with fluid during exercise, without pre-exercise hyperhydration. Each provided only a small volume of fluid with the glycerol, which may have reduced its effectiveness. Plasma volume was better maintained in the glycerol trials; however, no thermoregulatory or cardiovascular benefits were seen. When used during rehydration, glycerol has also been shown to enhance the restoration of plasma volume, reduce core temperature during rehydration, increase forearm blood flow, and improve subsequent exercise time to exhaustion by 12.6–19%.

Glycerol hyperhydration is most likely to have a beneficial effect when used as a preparatory aid for subsequent continuous exercise of a long duration and high intensity in thermally stressful conditions. Under these

conditions, the thermoregulatory and cardiovascular systems will be highly stressed and extra fluid will be most useful.

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### REFERENCES

1. Lin EC. Glycerol utilization and its regulation in mammals. *Annu Rev Biochem.* 1977;46:765–795.
2. Robergs RA, Griffin SE. Glycerol. *Biochemistry, pharmacokinetics and clinical and practical applications.* *Sports Med.* 1998;26:145–167.
3. Frank MS, Nahata MC, Hilty MD. Glycerol: a review of its pharmacology, pharmacokinetics, adverse reactions, and clinical use. *Pharmacotherapy.* 1981;1:147–160.
4. Nelson JL, Robergs RA. Exploring the potential ergogenic effects of glycerol hyperhydration. *Sports Med.* 2007;37:981–1000.
5. Lyons TP, Riedesel ML. Glycerol-induced hyperhydration: its effects on fluid compartments in the rat. *Life Sci.* 1993;53:1779–1787.
6. Lyons TP, Riedesel ML, Meuli LE, Chick TW. Effects of glycerol-induced hyperhydration prior to exercise in the heat on sweating and core temperature. *Med Sci Sports Exerc.* 1990;22:477–483.
7. Hitchins S, Martin DT, Burke L, et al. Glycerol hyperhydration improves cycle time trial performance in hot humid conditions. *Eur J Appl Physiol Occup Physiol.* 1999;80:494–501.
8. Anderson MJ, Cotter JD, Garnham AP, Casley DJ, Febbraio MA. Effect of glycerol-induced hyperhydration on thermoregulation and metabolism during exercise in heat. *Int J Sport Nutr Exerc Metab.* 2001;11:315–333.
9. Riedesel ML, Allen DY, Peake GT, Al-Qattan K. Hyperhydration with glycerol solutions. *J Appl Physiol.* 1987;63:2262–2268.
10. Wingo JE, Casa DJ, Berger EM, Dellis WO, Knight JC, McClung JM. Influence of a pre-exercise glycerol hydration beverage on performance and physiologic function during mountain-bike races in the heat. *J Athl Train.* 2004;39:169–175.
11. Freund BJ, Montain SJ, Young AJ, et al. Glycerol hyperhydration: hormonal, renal, and vascular fluid responses. *J Appl Physiol.* 1995;79:2069–2077.
12. Koenigsberg PS, Martin KK, Hlava HR, Riedesel ML. Sustained hyperhydration with glycerol ingestion. *Life Sci.* 1995;57:645–653.
13. Montner P, Stark DM, Riedesel ML, et al. Pre-exercise glycerol hydration improves cycling endurance time. *Int J Sports Med.* 1996;17:27–33.
14. Montner P, Zou Y, Robergs R, et al. Glycerol hyperhydration alters cardiovascular and renal function. *J Exerc Physiol (Online).* 1999;2:1–10.
15. Melin B, Jimenez C, Koulmann N, Allevard AM, Gharib C. Hyperhydration induced by glycerol ingestion: hormonal and renal responses. *Can J Physiol Pharmacol.* 2002;80:526–532.



16. Coutts A, Reaburn P, Mummery K, Holmes M. The effect of glycerol hyperhydration on olympic distance triathlon performance in high ambient temperatures. *Int J Sport Nutr Exerc Metab.* 2002;12:105–119.
17. Magal M, Webster MJ, Sistrunk LE, Whitehead MT, Evans RK, Boyd JC. Comparison of glycerol and water hydration regimens on tennis-related performance. *Med Sci Sports Exerc.* 2003;35:150–156.
18. Marino FE, Kay D, Cannon J. Glycerol hyperhydration fails to improve endurance performance and thermoregulation in humans in a warm humid environment. *Pflugers Arch.* 2003;446:455–462.
19. O'Brien C, Freund BJ, Young AJ, Sawka MN. Glycerol hyperhydration: physiological responses during cold-air exposure. *J Appl Physiol.* 2005;99:515–521.
20. Goulet ED, Robergs RA, Labrecque S, Royer D, Dionne JJ. Effect of glycerol-induced hyperhydration on thermoregulatory and cardiovascular functions and endurance performance during prolonged cycling in a 25 degrees C environment. *Appl Physiol Nutr Metab.* 2006;31:101–109.
21. Easton C, Turner S, Pitsiladis YP. Creatine and glycerol hyperhydration in trained subjects before exercise in the heat. *Int J Sport Nutr Exerc Metab.* 2007;17:70–91.
22. Scheett TP, Webster MJ, Wagoner KD. Effectiveness of glycerol as a rehydrating agent. *Int J Sport Nutr Exerc Metab.* 2001;11:63–71.
23. van Rosendal SP, Osborne MA, Coombes JS. Guidelines for glycerol use in hyperhydration and rehydration associated with exercise. *Sports Medicine.* In press.
24. Nadel ER, Fortney SM, Wenger CB. Effect of hydration state of circulatory and thermal regulations. *J Appl Physiol.* 1980;49:715–721.
25. Fortney SM, Vroman NB, Beckett WS, Permutt S, LaFrance ND. Effect of exercise hemoconcentration and hyperosmolality on exercise responses. *J Appl Physiol.* 1988;65:519–524.
26. Hamilton MT, Gonzalez-Alonso J, Montain SJ, Coyle EF. Fluid replacement and glucose infusion during exercise prevent cardiovascular drift. *J Appl Physiol.* 1991;71:871–877.
27. Sawka MN, Montain SJ, Latzka WA. Hydration effects on thermoregulation and performance in the heat. *Comp Biochem Physiol A Mol Integr Physiol.* 2001;128:679–690.
28. Gonzalez-Alonso J, Calbet JA, Nielsen B. Muscle blood flow is reduced with dehydration during prolonged exercise in humans. *J Physiol.* 1998;513:895–905.
29. Gonzalez-Alonso J, Mora-Rodriguez R, Below PR, Coyle EF. Dehydration reduces cardiac output and increases systemic and cutaneous vascular resistance during exercise. *J Appl Physiol.* 1995;79:1487–1496.
30. Fortney SM, Wenger CB, Bove JR, Nadel ER. Effect of blood volume on forearm venous and cardiac stroke volume during exercise. *J Appl Physiol.* 1983;55:884–890.
31. Kamijo Y, Okumoto T, Takeno Y, et al. Transient cutaneous vasodilatation and hypotension after drinking in dehydrated and exercising men. *J Physiol.* 2005;568:689–698.
32. Buono MJ, Wall AJ. Effect of hypohydration on core temperature during exercise in temperate and hot environments. *Pflugers Arch.* 2000;440:476–480.
33. Sawka MN, Young AJ, Francesconi RP, Muza SR, Pandolf KB. Thermoregulatory and blood responses during exercise at graded hypohydration levels. *J Appl Physiol.* 1985;59:1394–1401.
34. Fortney SM, Nadel ER, Wenger CB, Bove JR. Effect of blood volume on sweating rate and body fluids in exercising humans. *J Appl Physiol.* 1981;51:1594–1600.
35. Armstrong LE, Maresh CM, Gabaree CV, et al. Thermal and circulatory responses during exercise: effects of hypohydration, dehydration, and water intake. *J Appl Physiol.* 1997;82:2028–2035.
36. Greenleaf JE, Castle BL. Exercise temperature regulation in man during hypohydration and hyperhydration. *J Appl Physiol.* 1971;30:847–853.
37. Below PR, Mora-Rodriguez R, Gonzalez-Alonso J, Coyle EF. Fluid and carbohydrate ingestion independently improve performance during 1 h of intense exercise. *Med Sci Sports Exerc.* 1995;27:200–210.
38. Gonzalez-Alonso J, Mora-Rodriguez R, Coyle EF. Stroke volume during exercise: interaction of environment and hydration. *American J Physiology.* 2000;278:H321–H330.
39. Chevront SN, Haymes EM. Thermoregulation and marathon running: biological and environmental influences. *Sports Med.* 2001;31:743–762.
40. Armstrong LE, Costill DL, Fink WJ. Influence of diuretic-induced dehydration on competitive running performance. *Med Sci Sports Exerc.* 1985;17:456–461.
41. Chevront SN, Carter R 3rd, Castellani JW, Sawka MN. Hypohydration impairs endurance exercise performance in temperate but not cold air. *J Appl Physiol.* 2005;99:1972–1976.
42. Walsh RM, Noakes TD, Hawley JA, Dennis SC. Impaired high-intensity cycling performance time at low levels of dehydration. *Int J Sports Med.* 1994;15:392–398.
43. Craig EN, Cummings EG. Dehydration and muscular work. *J Appl Physiol.* 1966;21:670–674.
44. Pichan G, Gauttam RK, Tomar OS, Bajaj AC. Effect of primary hypohydration on physical work capacity. *Int J Biometeorology.* 1988;32:176–180.
45. Nybo L, Jensen T, Nielsen B, Gonzalez-Alonso J. Effects of marked hyperthermia with and without dehydration on VO(2) kinetics during intense exercise. *J Appl Physiol.* 2001;90:1057–1064.
46. Rehrer NJ. Fluid and electrolyte balance in ultra-endurance sport. *Sports Med.* 2001;31:701–715.
47. Hubbard RW. The role of exercise in the etiology of exertional heatstroke. *Med Sci Sports Exerc.* 1990;22:2–5.
48. Coris EE, Ramirez AM, Van Durme DJ. Heat illness in athletes: the dangerous combination of heat, humidity and exercise. *Sports Med.* 2004;34:9–16.
49. Armstrong LE, Casa DJ, Millard-Stafford M, Moran DS, Pyne SW, Roberts WO. American College of Sports Medicine position stand. Exertional heat illness during training and competition. *Med Sci Sports Exerc.* 2007;39:556–572.
50. Wagner DR. Hyperhydrating with glycerol: implications for athletic performance. *J Am Diet Assoc.* 1999;99:207–212.
51. Meyer LG, Horrigan DJ, Jr, Lotz WG. Effects of three hydration beverages on exercise performance during 60 hours of heat exposure. *Aviat Space Environ Med.* 1995;66:1052–1057.
52. Latzka WA, Sawka MN, Montain SJ, et al. Hyperhydration: thermoregulatory effects during compensable exercise-heat stress. *J Appl Physiol.* 1997;83:860–866.
53. Latzka WA, Sawka MN, Montain SJ, et al. Hyperhydration: tolerance and cardiovascular effects during uncompensable exercise-heat stress. *J Appl Physiol.* 1998;84:1858–1864.
54. Nishijima T, Tashiro H, Kato M, et al. Alleviation of exercise-induced dehydration under hot conditions by glycerol hyperhydration. *Int J Sport Health Sci.* 2007;5:32–41.
55. Dini M, Corbianco S, Rossi B, Lucacchini A. Hyperhydrating with glycerol: effects on thermoregulation, hydration and athletic performance during specific exergonic exercise in a warm-humid environment. *Sport Sci Health.* 2007;2:1–7.

56. Goulet ED, Rousseau SF, Lamboley CR, Plante GE, Dionne IJ. Pre-exercise hyperhydration delays dehydration and improves endurance capacity during 2 h of cycling in a temperate climate. *J Physiol Anthropol.* 2008;27:263–271.
57. Murray R, Eddy DE, Paul GL, Seifert JG, Halaby GA. Physiological responses to glycerol ingestion during exercise. *J Appl Physiol.* 1991;71:144–149.
58. Siegler JC, Mermier CM, Amorim FT, Lovell RJ, McNaughton LR, Robergs RA. Hydration, thermoregulation, and performance effects of two sport drinks during soccer training sessions. *J Strength Cond Res.* 2008;22:1394–1401.
59. Kavouras SA, Armstrong LE, Maresh CM, et al. Rehydration with glycerol: endocrine, cardiovascular and thermoregulatory responses during exercise in the heat. *J Appl Physiol.* 2006;100:442–450.
60. Arnall DA, Goforth HW Jr. Failure to reduce body water loss in cold-water immersion by glycerol ingestion. *Undersea Hyperb Med.* 1993;20:309–320.
61. Inder WJ, Swanney MP, Donald RA, Prickett TC, Hellems J. The effect of glycerol and desmopressin on exercise performance and hydration in triathletes. *Med Sci Sports Exerc.* 1998;30:1263–1269.
62. Green HJ, Jones LL, Painter DC. Effects of short-term training on cardiac function during prolonged exercise. *Med Sci Sports Exerc.* 1990;22:488–493.
63. Jeukendrup A, Saris WH, Brouns F, Kester AD. A new validated endurance performance test. *Med Sci Sports Exerc.* 1996;28:266–270.
64. Palmer GS, Dennis SC, Noakes TD, Hawley JA. Assessment of the reproducibility of performance testing on an air-braked cycle ergometer. *Int J Sports Med.* 1996;17:293–298.
65. McLellan TM, Cheung SS, Jacobs I. Variability of time to exhaustion during submaximal exercise. *Can J Appl Physiol.* 1995;20:39–51.
66. Gonzalez-Alonso J. Separate and combined influences of dehydration and hyperthermia on cardiovascular responses to exercise. *Int J Sports Med.* 1998;19(Suppl 2):S111–S114.
67. Gonzalez-Alonso J, Mora-Rodriguez R, Below PR, Coyle EF. Dehydration markedly impairs cardiovascular function in hyperthermic endurance athletes during exercise. *J Appl Physiol.* 1997;82:1229–1236.
68. Bortz WM, Paul P, Haff AC, Holmes WL. Glycerol turnover and oxidation in man. *J Clin Invest.* 1972;51:1537–1546.
69. Burelle Y, Massicotte D, Lussier M, Lavoie C, Hillaire-Marcel C, Peronnet F. Oxidation of [(13)C]glycerol ingested along with glucose during prolonged exercise. *J Appl Physiol.* 2001;90:1685–1690.
70. Miller JM, Coyle EF, Sherman WM, et al. Effect of glycerol feeding on endurance and metabolism during prolonged exercise in man. *Med Sci Sports Exerc.* 1983;15:237–242.
71. Maughan RJ, Gleeson M. Influence of a 36 h fast followed by refeeding with glucose, glycerol or placebo on metabolism and performance during prolonged exercise in man. *Eur J Appl Physiol Occup Physiol.* 1988;57:570–576.
72. Terblanche SE, Fell RD, Juhlin-Dannfelt AC, Craig BW, Holloszy JO. Effects of glycerol feeding before and after exhausting exercise in rats. *J Appl Physiol.* 1981;50:94–101.
73. Gleeson M, Maughan RJ, Greenhaff PL. Comparison of the effects of pre-exercise feeding of glucose, glycerol and placebo on endurance and fuel homeostasis in man. *Eur J Appl Physiol Occup Physiol.* 1986;55:645–653.
74. Tourtellotte WW, Reinglass JL, Newkirk TA. Cerebral dehydration action of glycerol. I. Historical aspects with emphasis on the toxicity and intravenous administration. *Clin Pharmacol Ther.* 1972;13:159–171.
75. Landau BR, Wahren J, Previs SF, Ekberg K, Chandramouli V, Brunengraber H. Glycerol production and utilization in humans: sites and quantitation. *Am J Physiol.* 1996;271(6 Pt 1):E1110–E1117.
76. Teunissen LP, Grabowski A, Kram R. Effects of independently altering body weight and body mass on the metabolic cost of running. *J Exp Biol.* 2007;210:4418–4427.
77. Grabowski A, Farley CT, Kram R. Independent metabolic costs of supporting body weight and accelerating body mass during walking. *J Appl Physiol.* 2005;98:579–583.
78. Chang YH, Huang HW, Hamerski CM, Kram R. The independent effects of gravity and inertia on running mechanics. *J Exp Biol.* 2000;203:229–238.
79. Chang YH, Kram R. Metabolic cost of generating horizontal forces during human running. *J Appl Physiol.* 1999;86:1657–1662.
80. Sawka MN, Burke LM, Eichner ER, Maughan RJ, Montain SJ, Stachenfeld NS. American College of Sports Medicine position stand. Exercise and fluid replacement. *Med Sci Sports Exerc.* 2007;39:377–390.
81. McCurdy DK, Schneider B, Scheie HG. Oral glycerol: the mechanism of intraocular hypotension. *Am J Ophthalmol.* 1966;61:1244–1249.