Hydration and urinary pseudoephedrine levels after a simulated team game

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ABSTRACT

This study investigated the influence of dehydration on urinary levels of pseudoephedrine (PSE) after prolonged repeated effort activity. Fourteen trained male athletes performed a simulated team game circuit (STGC) outdoors over 120 min under three different hydration protocols: hydrated (HYD), dehydrated (DHY) and dehydrated + post-exercise fluid bolus (BOL). In all conditions, a 60 mg dose of PSE was administered 30 min before each trial and at half time of the STGC. Urinary PSE levels were measured before drug administration and at 90 min post-exercise. Additionally, body mass (BM) changes and urinary specific gravity (USG), osmolality (OSM), creatinine (Cr) and pH values were recorded. No differences in PSE levels were found 90 min post-exercise between conditions (HYD: $208.5 \pm 116.5 \mu\text{g}\cdot\text{ml}^{-1}$; DHY: $238.9 \pm 93.5 \mu\text{g}\cdot\text{ml}^{-1}$; BOL: $195.6 \pm 107.3 \mu\text{g}\cdot\text{ml}^{-1}$), although large variations were seen within and between participants across conditions (range: 33 to $475 \mu\text{g}\cdot\text{ml}^{-1}$; ICC $r = 0.03-0.16$, $p > 0.05$). There were no differences between conditions in USG, OSM, pH or PSE/Cr ratio. The %BM lost due to exercise had no association with post-recovery PSE concentrations ($p > 0.05$; $r = 0.156-0.396$) in any condition. In conclusion, hydration status did not influence urinary PSE levels after prolonged repeated effort activity, with ~70% of samples greater than the WADA limit ($>150 \mu\text{g}\cdot\text{ml}^{-1}$) and ~30% under. Due to the unpredictability of urinary PSE values, athletes should avoid taking any medications containing PSE during competition.

Key Words: dehydration; fluid-bolus; specific gravity; creatinine

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LIST OF ABBREVIATIONS

ADH – anti-diuretic hormone	ml – millilitres
ASADA – Australian Sports Anti-Doping Agency	mmol - millimoles
BLa – blood lactate	mOsm - milliosmoles
BM – body mass	MS – mass spectrometry
BOL – bolus protocol	N - Newtons
bpm – beats per minute	NE – norepinephrine
CNS – central nervous system	OSM - osmolality
Cr – creatinine	PLA – placebo
°C – degrees Celsius	P _{osm} – plasma osmolality
DHY – dehydrated protocol	PSE – pseudoephedrine
g - grams	RPE – rating of perceived exertion
GC – gas chromatography	s - seconds
h – hours	SBP – systolic blood pressure
HPLC – high performance liquid chromatography	SNS – sympathetic nervous system
HR – heart rate	STGC – simulated team game circuit
HYD – hydrated protocol	S _{osm} – serum osmolality
kJ - kilojoules	TBW – total body water
km – kilometre	U _{osm} – urine osmolality
L - litres	USG – urine specific gravity
µg – micrograms	W - Watts
m – metres	WADA – World Anti-Doping Agency
mg – milligrams	WAFL – West Australian Football League
min - minutes	1RM – one repetition maximum

Chapter 1

Introduction

1.1 Introduction

In 2004, the World Anti-Doping Agency (WADA) removed a prohibition on the use of the stimulant pseudoephedrine (PSE) during athletic competition, due both to a lack of conclusive evidence that PSE is performance enhancing and its widespread use as a nasal and sinus decongestant. However, in 2010 PSE was reintroduced onto the WADA list of prohibited and restricted substances, at a urinary concentration of above 150 micrograms per millilitre ($\mu\text{g}\cdot\text{ml}^{-1}$).

On September 19th, 2010 the Western Australian Football League (WAFL) grand final was played. After the game, ASADA officials obtained a urine sample from one of the players, who acknowledged that he had taken a therapeutic dose of Sudafed (an over the counter medication containing PSE). In this instance, the tested player had taken one Sudafed tablet before the game, and a second tablet during the half time break (each tablet containing 60 mg of PSE). When required to produce a urine sample after the game, the player had extreme difficulty, most likely due to the warm, sunny conditions on the day and inadequate hydration during the match. As a result, during the time period between match completion and finally producing a urine sample (~90 min later), the player consumed a considerable amount of fluid (mostly water) in order to facilitate the test sample collection.

The results of the urine analysis recorded higher than permitted levels of PSE, and the player duly received a two year ban from competition. However, the method of analysis for PSE concentration in urine does not include any criterion measure of hydration status, other than the simple assessment of urine specific gravity (USG); a measure which is easily influenced by recent fluid intake. As a result, it is possible that the PSE

concentration in this player's urine was increased (excessively) as a consequence of dehydration, in spite of the low dose ingested.

Therefore, the aim of this study was to examine the influence of differing levels of hydration status on the post-exercise concentrations of urinary PSE. Here, 14 trained male team sport athletes were recruited to perform a simulated team game circuit on three separate occasions. Each player was given one Sudafed tablet (60 mg PSE) 30 min before the commencement of the circuit, and one Sudafed tablet during the half time break. In each trial, participants were randomly assigned to one of three hydration conditions: a well hydrated condition, where players consumed fluid at a rate and volume considered desirable for Australian footballers during and after competition; a dehydrated condition, where fluids were heavily restricted; a bolus condition, where fluid ingested was the same as for the dehydrated condition during the circuit, but the participants then consumed a large fluid bolus 60 min after the conclusion of exercise, as was done by the aforementioned player during the grand final. In all conditions body mass, USG, creatinine, and PSE levels were assessed at regular intervals.

The significance of this investigation is that, if it can be determined dehydration has a significant effect on urinary PSE concentrations, there may be potentially important implications for the testing of this substance in sporting competitions in the future.

1.1 Statement of Problem (Aim of Study)

The aim of this study was to determine whether hydration status (and a fluid bolus) influences the urinary levels of PSE obtained after prolonged simulated team game activity in warm conditions.

1.3 Hypothesis

It was hypothesized that after consuming a medicinal dose of 120 mg of PSE and performing prolonged, exhaustive exercise, participants would record significantly higher levels of urinary PSE when in a dehydrated state than when adequately hydrated. Furthermore, it was also hypothesized that when a fluid bolus was provided to a dehydrated athlete in the post-exercise period, USG would decrease with a corresponding reduction in urinary PSE concentration.

1.4 Limitations

- All players participated on a voluntary basis; therefore, motivation levels may have affected exercise performance during testing procedures, possibly influencing results.
- Conclusions that may arise from results of the study may be valid only with respect to trained team game players, and not to other populations.

1.5 Delimitations

- Participants were restricted to well-trained team sport players.
- A total dose of only 120 mg of PSE was used in the study.
- No (potential) performance enhancement of PSE was assessed in this study, only the influence of hydration status was examined.

Chapter 2

Literature Review

2.1 Overview

This literature review is divided into six sections. The first briefly reports on general information about the drug pseudoephedrine (PSE); in particular, its common medical uses and side effects. The second section summarizes the research examining the potential ergogenic effects of PSE. This is not designed to be an exhaustive examination of this topic, but rather provide context for its use by athletes. The third section examines research on the urinary excretion of PSE and common factors which may influence this. The fourth section describes the different methods of measuring hydration status, with particular focus on urinary measures such as urine specific gravity (USG), which is routinely used in doping control. The fifth section examines the possible impact of changes in hydration on the urinary measurement of PSE. The final section briefly discusses the current Australian Sports Anti-Doping Agency (ASADA) testing protocols and the further potential for erroneous doping results.

It is important to note that the purpose of this review is not to evaluate whether or not PSE is performance enhancing. Rather, the section on the potential ergogenic effects of PSE provides the sporting context in which PSE may be used and the likely changing nature of an athlete's hydration status when a post-competition urine sample is required for doping control. Similarly, this review does not draw any conclusions about the validity of urine tests for PSE measurement post-competition, but rather examines the potential for error not addressed by the current testing protocols.

2.2 Introduction

The use of PSE as a nasal decongestant is widespread, due to it causing vasoconstriction in the respiratory tract. This decongestant effect is evident in relatively small doses (~30-60 mg), although it has been used by athletes in larger doses for possible ergogenic benefits, especially when not listed by the World Anti-Doping Agency (WADA) on the list of prohibited substances. Research to date suggests that these ergogenic effects are small and only evident at doses of 180 mg or greater.

In doping tests urinary PSE levels are commonly measured, with the hydration status of the athlete at the time of testing also assessed by measuring USG. Although generally reliable, USG has been shown to lag behind the actual hydration status of the athlete, especially when dehydrated. The excretion of PSE normally peaks around 2-4 h after ingestion (Delbeke & Debackere, 1991; Gillies et al., 1996), although if urinary pH is reduced, this time is reduced, and vice versa (Delbeke & Debackere, 1991; Strano-Rossi et al., 2009). Therefore, it is possible that post-exercise urinary PSE measurements do not always accurately reflect the dose of PSE consumed, especially after prolonged exercise in warm-hot and humid conditions where large amounts of sweating (fluid loss) occur.

2.3 Pseudoephedrine

Pseudoephedrine is one of a class of drugs known as sympathomimetic amines. These drugs mimic the actions of adrenaline and noradrenaline in the sympathetic nervous system. Most commonly, PSE has a therapeutic use as a decongestant, causing vasoconstriction in the respiratory tract, in particular resulting in a reduction of nasal congestion and secretions (Wellington & Jarvis, 2001). Empey et al. (1980) reported

that the nasal decongestant qualities of the drug were effective at a dose of 60 mg, without the presence of any associated side effects. Some possible side effects of PSE ingestion include dry mouth, insomnia and headache, although research has not found the incidence of these side effects to be consistent or widespread (Wellington & Jarvis, 2001).

Other effects of PSE ingestion are commonly reported in the circulatory system. A meta-analysis (24 studies, 1285 participants) by Salerno, Jackson & Berbano (2005) reported that doses ranging from 15 mg to 240 mg of PSE lead to small, but significant increases in systolic blood pressure (SBP) (~1 mmHg) and heart rate (HR) (~3 bpm), with these changes amplifying as the administered dose increased. They also found that the magnitude of side effects varied depending on the type of medication taken, in addition to the size of the dose. Additionally, immediate release formulations had a greater effect on SBP and HR than sustained release, and shorter duration studies (range 2 h to 7 days) also led to greater side effects than longer duration studies (greater than 7 days). However, these authors made no comment on possible reasons for this, despite there being a likely physiological adaptation to PSE, with prolonged use of the drug leading to a reduction in the magnitude and frequency of side effects.

Other reported side effects are suggested to stem from central nervous system (CNS) stimulation, including increased nervousness, higher rates of sweating and respiration, and increased endocrine gland secretion (Martin et al., 1971; Wellington & Jarvis, 2001; Hodges et al., 2006). It has been hypothesised that due to some of these side effects, PSE is potentially performance enhancing (Bouchard, Weber & Geiger, 2002), and consequently there has been much research into its possible ergogenic effects. Largely because of its CNS stimulatory effects, PSE is currently on the WADA banned-substance list, although its status has changed over the years. Since 2010 PSE has been

banned above a urinary cut-off level of $150 \mu\text{g}\cdot\text{ml}^{-1}$, although between 2004 and 2010 it was not sanctioned at all. Prior to 2004, it was on the banned list, but at a urinary level of $>25 \mu\text{g}\cdot\text{ml}^{-1}$.

2.4 Pseudoephedrine & Exercise Performance

It has been theorised that due to its stimulation of the sympathetic nervous system (SNS), PSE can reduce fatigue and increase blood flow to active muscles (Gill et al., 2000), which could improve exercise performance. Research has examined this hypothesis using a variety of dosing regimens and modes of exercise, but to date, evidence for an ergogenic effect of PSE is equivocal.

2.4.1 Endurance Performance

Several studies have investigated the effects of PSE on endurance (time-trial) performance (Gillies et al., 1996; Hodges et al., 2006; Pritchard-Peschek et al., 2010; Berry & Wagner, 2012) but with conflicting findings. Gillies et al. (1996) had trained male cyclists complete 40 km time trials (on different days) 2 h after ingesting 120 mg of PSE or placebo. No differences were found in time-trial performance between conditions (PSE: 58.7 min; placebo: 58.1 min), nor in post-exercise blood lactate (BLa) accumulation.

In contrast, Pritchard-Peschek et al. (2010) used a 180 mg dose of PSE (or placebo) with trained male cyclists performing time trials of approximately 30 min duration (time to complete a fixed amount of work), commencing 60 min after ingestion. As shown in Figure 1, a significant improvement in time trial performance (mean of 5.1%) was

observed after PSE supplementation (29.0 ± 4.4 min) compared to placebo (30.5 ± 4.6 min). There was no effect of PSE supplementation on BLa accumulation, but norepinephrine (NE) levels were significantly increased post-exercise in the PSE condition, supporting an effect of PSE on the SNS. The higher dose of PSE used by Pritchard-Peschek et al. (2010) in contrast to Gillies et al. (1996), may be important in explaining these divergent results on cycling time trial performance.

Figure 1 - Effect of pseudoephedrine (PSE) & placebo (PLA) on individual time trial performance (Pritchard-Peschek et al., 2010).

Evidence is also mixed when examining the impact of PSE on shorter exercise durations. Hodges et al. (2006) administered either PSE in a dose of 2.5 mg.kg^{-1} of body mass (BM) (approx. 180 mg), or a placebo to male runners 90 min before a 1500 m time trial. Completion time with PSE (273.9 ± 4.4 s) was significantly faster than with placebo (279.7 ± 4.4 s), with no difference in BLa or heart rate (HR) observed between trials. This is one of the few studies to observe a performance enhancing effect of PSE, possibly because, similar to Pritchard-Peschek et al. (2010), participants were

administered a large (~3 times the normal therapeutic) dose. Hodges et al. (2006) suggested that a possible mechanism for the noted performance enhancement might be an increase in CNS stimulation, and therefore a reduction in perceived effort.

Recently, Berry & Wagner (2012) sought to confirm the results of Hodges et al. (2006) and Pritchard-Peschek et al. (2010) by assessing the impact of ingesting 2.5 mg.kg⁻¹ BM of PSE administered 90 min prior to an 800 m time trial by female college athletes. Here, no differences in run time (PSE: 159.5 ± 9.6 s; placebo: 159.4 ± 9.6 s) or in post-exercise HR (PSE: 177 ± 19 bpm; placebo: 177 ± 15 bpm) were found. Although this contradicts the findings of Hodges et al. (2006) and Pritchard-Peschek et al. (2010), who both showed a positive impact of PSE on endurance performance, it is possible the duration of exercise in this study (~ 2.7 min) is below that at which an ergogenic effect may be likely. Additionally, the use of female athletes may have contributed to this different finding, as potential gender differences in the effect of PSE on performance have not been studied to date.

2.4.2 Aerobic Power

Swain et al. (1997) measured VO_{2max} and time to exhaustion in male cyclists during a graded cycling test 60 min after the ingestion of PSE (either 1 mg.kg⁻¹ or 2 mg.kg⁻¹ BM doses) or a placebo. No significant differences in either VO_{2max} (placebo: 57.7 ± 9.6 ml.kg⁻¹.min⁻¹; 1 mg.kg⁻¹ of PSE: 60.0 ± 13.4 ml.kg⁻¹.min⁻¹; 2 mg.kg⁻¹ of PSE: 59.5 ± 13.5 ml.kg⁻¹.min⁻¹) or time to exhaustion (placebo: 832.7 ± 128.5 s; 1 mg.kg⁻¹ of PSE: 854.1 ± 157.8 s; 2 mg.kg⁻¹ of PSE: 822.3 ± 138.7 s) were found. The authors suggested that a larger dose may be needed to demonstrate an effect, even though the 2 mg.kg⁻¹ dose (~150 mg) is similar to that used in the studies by Hodges et al. (2006) and Pritchard-Peschek et al. (2010). Swain et al. (1997) also postulated that the 60 min delay

between PSE administration and exercise used here may have been insufficient time for the PSE to be fully absorbed. Immediate post-exercise urinary PSE was $67.3 \pm 36.2 \mu\text{g.ml}^{-1}$ after 1 mg.kg^{-1} of PSE, and $106.4 \pm 54.4 \mu\text{g.ml}^{-1}$ after 2 mg.kg^{-1} . Comparatively, PSE concentrations immediately post-exercise reported by Gillies et al. (1996) averaged $45 \mu\text{g.ml}^{-1}$, and after 1 h, $114 \mu\text{g.ml}^{-1}$, suggesting that the immediate post-exercise urinary PSE levels measured by Swain et al. (1997) were not peak values.

Chester, Reilly, & Mottram (2003) examined the effects of smaller (60 mg) but multiple ($n=6$) doses of PSE administered over a 36 h period on male runners performing a 5000 m time trial and 20 min of steady state running at 70% of VO_2max . Neither the time trial performance, nor the steady state VO_2 , HR or BLa showed any difference between the PSE or placebo conditions. Although the final dose of PSE was taken here 4 h prior to exercise, the total amount taken over the 36 h period (360 mg) suggests that sufficient levels of PSE existed in the system to produce an ergogenic effect. However, the authors did note that they failed to achieve statistical power in the study due to the unexpected withdrawal of participants.

Unfortunately, urinary PSE was not measured in this study, but in a companion paper, Chester, Mottram, Reilly & Powell (2003) used the same PSE administration protocol, and found that urinary PSE peaked during the dosing regimen at $149 \pm 72 \mu\text{g.ml}^{-1}$. Once supplementation was completed, PSE excretion peaked again (at $70 \pm 28 \mu\text{g.ml}^{-1}$) after 4 h. Importantly, these two studies replicate the therapeutic use of PSE, when taken for its nasal decongestant properties. In contrast, studies that have reported improved endurance performance have used a much larger dose before exercise (Gill et al., 2000; Hodges et al., 2006; Pritchard-Peschek et al., 2010).

In conclusion, it is evident that further research is required to determine whether or not PSE is performance enhancing for endurance exercise and aerobic power. Research to

date suggests that large pre-exercise doses (~180 mg) are required to gain an ergogenic effect for aerobic exercise (Hodges et al., 2006; Pritchard-Peschek et al., 2010), rather than smaller doses (Gillies et al., 1996), or multiple smaller doses over time (Chester, Reilly, & Mottram, 2003). Further, it appears that there is no performance enhancing effect for shorter (<3 min) bouts of exercise (Berry & Wagner, 2012).

2.4.3 Strength & Power Performance

There is a smaller body of research examining the effect of PSE on strength and power performance. As part of their cycling time trial study, Gillies et al. (1996) also performed maximum voluntary contraction (MVC) tests both before and after the cycling time trials (the first MVC was 90 min after PSE ingestion). Their results found no change in isometric knee extension torque in either test after consuming 120 mg of PSE (before; PSE: $247 \pm 18 \text{ Nm}^{-1}$; placebo: $251 \pm 21 \text{ Nm}^{-1}$ and after; PSE: $238 \pm 21 \text{ Nm}^{-1}$; placebo: $232 \pm 16 \text{ Nm}^{-1}$). Gill et al. (2000) proposed that this lack of effect was due to the delay between PSE administration and the torque measures (the second trial was approximately 3 h after PSE administration). Therefore, they examined the effect of a larger (180 mg) dose of PSE on isometric knee extension, one repetition maximum (1RM) bench press, 70% 1RM bench press (performed to failure), and a 30 s cycling sprint test in male team sport athletes. In contrast to Gillies et al. (1996), testing commenced 45 min after the drug or placebo administration. Here, no effect was found in 1RM bench press (PSE: 88.9 kg; placebo: 88.0 kg) or 70% 1RM bench press repetitions (PSE: 10.9; placebo: 11.1). However, isometric leg extension (PSE: 321.1 Nm^{-1} ; placebo: 295.7 Nm^{-1}) and cycling peak power (PSE: 1262.5 W; placebo: 1228.4 W) were significantly improved after PSE ingestion.

Gill et al. (2000) proposed that the shorter delay between PSE administration and exercise (45 min) could have contributed to the ergogenic effects noted. However, peak plasma PSE concentration occurs approximately 2-3 h after ingestion (Gillies et al., 1996), with urinary excretion peaking 2-4 h after ingestion (Chester, Mottram, Reilly, & Powell, 2003). Therefore, it is unlikely that peak PSE concentrations were present during their exercise tasks. Consistent with the effect seen with endurance exercise performance, it may be that a larger dose (~180 mg), as was used by Gill et al. (2000), is required to obtain a strength or power benefit. In contrast, Chu et al. (2002), found no effect on performance when replicating the 30 s maximal cycling test used by Gill et al. (2000), when performed 2 h after ingesting 120 mg of PSE. Chu et al. (2002) also tested grip strength and ankle dorsiflexion, and again found no difference between PSE and placebo conditions. However, the strength test selection of tasks such as grip strength and ankle dorsiflexion limits the application of these results to an actual sporting environment.

In summary, to date only Gill et al. (2000) have shown PSE to have an effect on strength or power, using a large dose (180 mg), and with a short delay before exercise (45 min). These findings have not yet been replicated, and more research needs to be conducted to determine if PSE ingestion can improve strength or power.

2.5 Urinary Excretion of Pseudoephedrine

Up to 96% of PSE is excreted through the urine unchanged, while most of the rest is converted into cathine (norpseudoephedrine), an inactive metabolite also excreted in urine (Wellington & Jarvis, 2001). Cathine is also prohibited by WADA (at levels above 5 $\mu\text{g}\cdot\text{ml}^{-1}$), as it is an indicator of PSE use, although the correlation between the

levels of these two substances in urine samples is weak (Deventer et al., 2009). Gas chromatography (GC) and/or mass spectrometry (MS) is generally used to determine the amount of PSE present in a urine sample. More recently, high performance liquid chromatography (HPLC) has also been shown to be an accurate measurement method (Gmeiner et al. 2002).

Delbeke & Debackere (1991) performed preliminary research on the excretion of PSE using GC. Participants were each given a 60 mg PSE dose, before multiple urine samples were collected over a 72 h period. Peak urinary PSE concentration ($103 \pm 27 \mu\text{g}\cdot\text{ml}^{-1}$) was observed 3-4 h after ingestion, with some PSE still detected 72 h after administration. This data suggests that a 60 mg dose of PSE would be unlikely to result in a positive doping test; however it is difficult to draw firm conclusions from this study due to the small sample size ($n=4$). Additionally, this finding is not relevant outside of a laboratory setting, since PSE will usually be consumed in higher doses when used for exercise performance enhancing reasons, or when repeated therapeutic doses are used. Further, exercise metabolism and/or dehydration may alter the uptake and excretion of PSE (Gillies et al., 1996); however, the data of Delbeke and Debackere (1991) only relates to resting conditions.

More recent research has demonstrated that urinary PSE levels are highly variable in different individuals, even with the same dose and timing of ingestion. Gillies et al. (1996) showed that although plasma PSE levels tended to peak approximately 2 h after supplementation, there were large individual differences in the rate of urinary excretion of PSE. In samples taken 1 h after the completion of a cycling time trial (4 h after a single dose of 120 mg) urinary PSE concentration was $114 \pm 27 \mu\text{g}\cdot\text{ml}^{-1}$; however, the individual results ranged from 7 to $261 \mu\text{g}\cdot\text{ml}^{-1}$. This data suggests that accurately

determining (or estimating) the amount of PSE consumed by an athlete from a single urine sample may be very difficult.

Chester, Mottram, Reilly & Powell (2003) used a total of 6 PSE (therapeutic) doses of 60 mg, consumed over 36 h. Here, the mean maximum urinary concentration of PSE recorded was $149 \pm 72 \mu\text{g}\cdot\text{ml}^{-1}$, approximately 4 h after the final dose and being nearly 6 times greater than the (then) allowed limit of $25 \mu\text{g}\cdot\text{ml}^{-1}$. Similar to Gillies et al. (1996), there was also a large range of individual values reported ($62.8\text{-}294.4 \mu\text{g}\cdot\text{ml}^{-1}$), making it difficult to predict at what dose an athlete would exceed the allowed limit of urinary PSE (both then and now). However, participants in this study were also not exercising, so extrapolating these results to athletes and competition performances is again problematic.

Strano-Rossi et al. (2009) examined the rate of PSE excretion and its metabolite, cathine, using a variety of dosing schedules (from 60 mg to 360 mg over a 12-24 h period). In support of the results of Gillies et al. (1996), large inter-individual differences in urinary PSE concentrations were observed with all doses. For example, after a 120 mg dose peak urinary PSE was $160 \pm 69.4 \mu\text{g}\cdot\text{ml}^{-1}$, with a range of $34\text{-}291 \mu\text{g}\cdot\text{ml}^{-1}$. A similar marked variability was also observed with cathine concentrations. In contrast to previous research showing peak PSE excretion after 2-4 h (Delbecke & Debackere, 1991; Gillies et al., 1996; Chester, Mottram, Reilly & Powell, 2003), peak excretion here occurred at 10.9 ± 2.9 h, although the varying administration schedules used between participants could also have contributed to this difference.

The large inter-individual differences seen in urinary PSE excretion within all the aforementioned studies suggest that it is very difficult to determine (or estimate) the dose of PSE that an athlete may have consumed based on a urine sample. This broad individual variability has been observed when using both GCMS (Strano-Rossi et al.,

2009) and HPLC (Gillies et al., 1996; Chester, Mottram, Reilly, & Powell, 2003) methodologies. Currently, there are no reports suggesting that PSE is metabolised differently between individuals, as most of the ingested drug is excreted in urine unchanged (Delbecke & Debackere, 1991; Wellington & Jarvis, 2001; Chester, Mottram, Reilly, & Powell, 2003). However, it appears that individual pharmacokinetics (in regard to the timing of peak uptake and excretion of PSE) are broad and varied, and will affect PSE excretion in the urine far more than the size of the administered dose. As yet, there is no proposed mechanism for this effect within different individuals, but exercise metabolism and/or dehydration may be influencing factors.

2.6 Measurement of Hydration Status

Under WADA protocols, when an athlete provides a urine sample for doping analysis it is tested for USG. This measure of the concentration of solutes in the urine gives an indication of the hydration status of the athlete at the time of the test. Although USG is used in drug testing procedures, in the field, athletes commonly use urine colour or BM change (Δ BM) as an indicator of hydration, and in a laboratory setting, measures of plasma and/or urinary osmolality can be used. Of these variables, urine colour is least preferred, because it is less accurate (Armstrong, 2005), being easily influenced by illness, vitamin supplementation and medication. For ease and convenience of measurement, urinary measures also tend to be more favoured than plasma variables. Armstrong et al. (1994) also proposed that plasma variables were not sensitive to early changes in hydration (BM change of <3%), although this has not been demonstrated in more recent research (Popowski et al., 2001; Cheuvront et al., 2010).

Despite their convenience, urinary measures of hydration also have some weaknesses. Both USG and urine osmolality (U_{osm}) have been shown to lag behind the actual hydration state of the body (Popowski et al., 2001; Armstrong, 2007), primarily for two reasons. Firstly, the contents of the bladder at any one time represent an average of the urine production since the previous voiding. Fluid consumption before, during and/or after an event can therefore influence the urine composition within the bladder. Secondly, plasma osmolality (P_{osm}) is a stimulus for the release of antidiuretic hormone (ADH), such that during periods of dehydration an increase in P_{osm} will increase ADH secretion, thereby causing the kidneys to conserve extra-cellular fluid, resulting in the production of more concentrated urine. Previously, Popowski et al. (2001) observed only moderate correlations between P_{osm} and USG ($r = 0.46$) and U_{osm} ($r = 0.43$), when assessed at multiple stages (-1%, -3%, and -5% ΔBM) of dehydration.

Despite this, urinary measures of hydration correlate well with each other. Popowski et al. (2001) reported that USG and U_{osm} correlated strongly ($r = 0.82$) during both progressive dehydration and post-exercise rehydration. Armstrong et al. (1994) found an even stronger relationship ($r = 0.97$) during a range of studies when participants were at rest, exercising in a laboratory and playing competitive outdoor tennis, concluding that USG and U_{osm} could be used interchangeably to indicate and measure dehydration levels. This was subsequently confirmed by Armstrong et al. (1998), who found a nearly perfect correlation ($r = 0.99$) between these two variables during exercise, dehydration, and subsequent rehydration.

Larger variance between USG and U_{osm} has been shown during rehydration after high intensity exercise in hot conditions (Armstrong et al., 1998). This variation may be due to differences in the volume of fluid consumed during recovery, and the fluid choices of individual athletes. Therefore, it is possible that an athlete rehydrating with a large bolus

of plain water to facilitate urine production will present somewhat misleading USG and U_{osm} results in subsequent urine samples. Quickly consuming a large bolus of water is common practice when asked to provide a urine sample for doping control purposes after exercise. Therefore, it is possible that a USG measure could then indicate an adequate level of hydration (due to the kidney's rapid filtration of the ingested fluid) when in fact the total body water content may still be in considerable deficit (Opplinger et al., 2005; Popowski et al., 2001).

Kovacs, Senden, & Brouns (1999) reported similar inaccuracies during rehydration following cycling in a climate-controlled chamber (28°C and 50-60% relative humidity). After 3% BM loss had been achieved, participants were permitted to drink *ad libitum* (mineral water, caffeinated soft drink, or a sports drink) during the first two hours (only) of a 6 h recovery period. Only weak correlations were found between net fluid consumption (expressed as a percentage of BM lost during exercise) and urine colour ($r = -0.18$) and U_{osm} ($r = -0.12$) across all conditions, with the authors concluding that much of the fluid consumed was not absorbed by the body, but was quickly passed out as urine during recovery. However, following the initial 2 h of (*ad libitum*) recovery no further fluid intake was allowed; alternatively, had participants been permitted to continue drinking, better rehydration could have been achieved, and a stronger correlation with U_{osm} may have resulted.

A common method of assessing fluid loss in sporting environments is ΔBM (Sawka et al., 2007), and this variable remains the most accurate way of assessing short term changes to hydration status, other than laboratory tests (e.g., deuterium oxide dilution) of total body water (ΔTBW). Baker, Lang & Kenny (2009) assessed a variety of common hydration measures against ΔBM during a 2 h running trial using a variety of hydration strategies (maintenance of BM, -2% ΔBM , -4% ΔBM , and +2% ΔBM). Here,

there was no significant difference between ΔTBW and ΔBM ($p = 0.29$; $\text{ICC} = 0.76$) in any of the hydration strategies used. Further, ΔTBW also correlated significantly with P_{osm} ($r = 0.68$), U_{osm} ($r = 0.61$), and USG ($r = 0.63$). This appears to be the only study comparing these variables to a laboratory-based gold standard measure.

Cheuvront, Elu, Kenefick & Sawka (2010) concluded that P_{osm} is the only practical measurement of hydration that can accurately assess hydration status against a euhydrated standard, without a series of repeated measures (e.g. USG) over time. This is due to the wide range of values that euhydrated individuals can present for USG , U_{osm} , urine colour, and saliva osmolality (S_{osm}). However, these authors did conclude that these measures were appropriate for the assessment of dynamic hydration measured at multiple time points (e.g. across exercise and post-exercise rehydration), suggesting using two or more methods is preferable for diagnostic confidence. Regardless, this is currently not required by WADA testing protocols, where only a single USG measure is taken.

Of most relevance in the context of doping control are urinary measures of hydration that can be performed in the field. Plasma measures of hydration are less practical, and do not provide instant results, while ΔBM can be affected by factors (that may not significantly affect dehydration) such as food intake and defecation. While USG is not the most accurate measure for hydration at any single time point, it remains practical and convenient, and is current WADA practice. Furthermore, USG correlates well with other measures of hydration (such as U_{osm}), although it can lag behind the actual hydration status (TBW) of an athlete.

2.7 Influence of Hydration on Pseudoephedrine Levels

The potential influence of hydration in the measurement of urinary PSE levels is not well understood. Delbeke and Debackere (1991) reported that consuming a 1.5 L fluid (water) bolus in the 15–20 min immediately after taking 60 mg of PSE (at rest) decreased urinary PSE concentration 4-fold, from $67.3 \pm 25.3 \mu\text{g}\cdot\text{ml}^{-1}$ to $11.0 \pm 1.4 \mu\text{g}\cdot\text{ml}^{-1}$, without any change in the rate of excretion. Additionally, in the control condition an inverse relationship between urinary pH and urinary PSE levels was reported, such that as one increased, the other decreased. This relationship between urinary pH and PSE excretion has been replicated in other research (Wang, Feng, Wang & Zhu, 2008; Strano-Rossi et al., 2009). To this end, it should be considered that since a lower urinary pH often accompanies dehydration (Ylitalo, 1991) it is possible that quickly ingesting a large bolus of water (or an alkaline solution) could potentially lower urinary PSE concentrations in doping tests when athletes are dehydrated. (Delbeke & Debackere, 1991).

It has been previously hypothesised that prolonged exercise and/or accompanying dehydration may influence PSE measurement post-exercise. Gillies et al. (1996) found that after a cycling time-trial, participants recorded significantly higher urine PSE concentrations than when compared to a non-exercising control condition, and significantly lower urine volumes during both the exercise and recovery periods (suggesting that dehydration was present). However, the rate of PSE excretion was unchanged between conditions. Additionally, a negative relationship was found between average urine pH and average PSE concentration with exercise ($r = -0.76$, $p < 0.05$), but this could not be replicated at rest (see Figure 2).

Therefore, it appears plausible that urinary PSE concentration could be influenced by hydration status, possibly via two potential mechanisms. Firstly, since urine production

is decreased during and after exercise, especially when dehydrated (Armstrong et al., 1998), PSE levels may be relatively more concentrated. Secondly, a lowered urinary pH (which is usual after exercise and when dehydrated), has been demonstrated to increase the rate of renal clearance of PSE (Strano-Rossi et al., 2009). This relationship between urinary pH and PSE levels has been observed in studies examining the effect of PSE on exercise performance (Gillies et al., 1996) and the clearance of PSE at rest (Chester, Mottram, Reilly & Powell, 2003), although more research is required to confirm and extend these findings.

Figure 2 - Urine volume, pH and pseudoephedrine (PSE) content and concentration post-exercise versus control (rest) (Gillies et al., 1996). Drug was ingested at time 0, exercise began at 120 min for ~ 1 h, * Exercise vs. control, $p < 0.05$. ** Exercise vs. control, $p < 0.001$.

2.8 WADA Testing Procedures

Several variables could influence the collection (and result) of a doping test under current WADA procedures. One such variable is the time between the completion of competition and collection of the urine sample. Currently, the athlete subjected to doping control is entitled to perform a cool down, fulfil media commitments, receive medical treatment, and participate in a victory celebration, amongst other potential delays, before a sample is provided (ASADA Athlete Testing Guide, 2012). If the athlete in question is unable to provide a sample immediately when requested, it could mean a delay of 1-2 h between the end of competition and collection of the sample. Given that peak urinary PSE concentration occurs approximately 2-4 h after consumption (Delbecke & Debackere, 1991; Gillies et al, 1996; Chester, Mottram, Reilly & Powell, 2003), this potentially could significantly affect a test result.

Additionally, during the delay between the end of an event and provision of a urine sample, athletes may use varied recovery and rehydration strategies. Athletes may choose to rehydrate with a variety of fluids, for example, plain water or electrolyte based solutions, and will likely consume these at different rates. This introduces further variation to the hydration status of the athlete, and potentially any subsequent USG measurements.

Although a reasonably accurate and convenient field measure, USG has been found to lag behind the gold standard laboratory measures of hydration (Baker, Lang & Kenney, 2009). Further, field measures of hydration tend to be less accurate during rehydration following exercise (Kovacs, Senden, & Brouns, 1999), in spite of (often) strong agreement between measures. This suggests that when a bolus of fluid is consumed quickly following an event (for example, in order to facilitate production of a urine

sample), some of this fluid may pass rapidly through the body, producing a dilute urine sample that does not truly reflect the actual hydration status (total body water content) of the athlete.

In the context of doping control, the findings of Cheuvront et al. (2010) are important. Although supporting previous research showing agreement between the urinary measures of hydration, broad individual differences in readings were seen at a given level of hydration (ranging from euhydration to 7% of BM loss). Cheuvront et al. (2010) concluded that these field measures of hydration can be useful when taken across multiple time points, but their value is much reduced when only a single reading is taken. In doping control a single USG measurement is used for convenience, but this may not adequately reflect the overall body hydration status.

The variables discussed above (potential delay in providing a urine sample, athletes' differing rehydration strategies, and the inherent weaknesses of USG as a measure of hydration) increase the potential inaccuracies in doping control. When combined with the unpredictable nature of PSE excretion, and its widely available status, some potential for inadvertent doping violation is present.

Additionally, other research has shown that high doses of PSE (>180 mg) are required to elicit an ergogenic effect (Gill et al., 2000; Hodges et al., 2006; Pritchard-Peschek et al., 2010), yet the current cut-off limit of $>150 \mu\text{g}\cdot\text{ml}^{-1}$ in urine does not adequately reflect this, since this urinary concentration may be achieved with much smaller doses in many individuals. This introduces the potential for more positive tests, and a greater negative impact (damage to reputation, loss of athletes' income and competitive opportunities, etc.) than the benefit of controlling PSE at the current level (Kayser, Mauron & Miah, 2007), particularly in light of the weaknesses in the testing procedures discussed above.

2.9 Conclusions

Although far from unanimous, some research shows that taking PSE may lead to modest performance benefits in endurance events when taken in large doses (>180 mg). The normal therapeutic dose of PSE (60 mg) is well below that which seems to be required to obtain a performance benefit. However, the potential for positive doping tests remains strong, because of the great individual variation in PSE levels after the same dose. The therapeutic use of the drug by athletes is made more difficult by these wide variations, although the firm guideline of not using PSE on competition days should override these concerns.

Chu et al. (2002) suggest that if a threshold dose response can be found, a more accurate (and potentially higher) urinary excretion limit can be tied to this, allowing more precise differentiation of therapeutic doses from performance enhancing doses, thereby reducing the number of inadvertent positive tests. Nevertheless, it is strongly recommended that athletes avoid taking medications containing PSE on competition days, given the unpredictable nature of the metabolism and excretion of the drug.

Another factor contributing to this unpredictability is the influence of hydration. It has been established that urinary PSE concentration is affected by urinary pH. During and after exercise, urine volume and pH tends to decrease, while PSE excretion rates may remain high, potentially increasing urinary PSE concentrations. However, more research needs to be conducted on the effect of exercise and changes to hydration status on urinary PSE concentrations. That said, common measures of hydration used in WADA sanctioned drug tests to monitor hydration status can be misleading. For example, there is a wide variation in USG scores that a euhydrated athlete can provide, and thus adjustments for dehydration could be made when inappropriate. Additionally, when an athlete is required to produce a urine sample for doping officials, the rapid consumption

of a fluid bolus is common. However, this could affect the accuracy of USG measurement to the potential detriment of the athlete. Therefore, a range of factors need to be considered in future research when investigating the use of PSE in sport, and the potential monitoring strategies employed by the anti-doping authorities.

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Chapter 3

Hydration and urinary pseudoephedrine levels after a simulated team game

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Title

Hydration and urinary pseudoephedrine levels after a simulated team game

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3.1 Abstract

This study investigated the influence of dehydration on urinary levels of pseudoephedrine (PSE) after prolonged repeated effort activity. Fourteen athletes performed a simulated team game circuit (STGC) outdoors over 120 min under three different hydration protocols: hydrated (HYD), dehydrated (DHY) and dehydrated + post-exercise fluid bolus (BOL). In all trials, a 60 mg dose of PSE was administered 30 min before each trial and at half time of the STGC. Urinary PSE levels were measured before drug administration and at 90 min post-exercise. Additionally, body mass (BM) changes and urinary specific gravity (USG), osmolality (OSM), creatinine (Cr) and pH values were recorded. No differences in PSE levels were found 90 min post-exercise between conditions (HYD: 208.5 ± 116.5 ; DHY: 238.9 ± 93.5 ; BOL: 195.6 ± 107.3 $\mu\text{g}\cdot\text{ml}^{-1}$), although large variations were seen within and between participants across conditions (range: 33 to 475 $\mu\text{g}\cdot\text{ml}^{-1}$: ICC $r = 0.03-0.16$, $p > 0.05$). There were no differences between conditions in USG, OSM, pH or PSE/Cr ratio. In conclusion, hydration status did not influence urinary PSE levels after prolonged repeated effort activity, with ~70% of samples greater than the WADA limit (>150 $\mu\text{g}\cdot\text{ml}^{-1}$), and ~30% under. Due to the unpredictability of urinary PSE values, athletes should avoid taking any medications containing PSE during competition.

Key Words: dehydration, bolus, specific gravity, creatinine

3.2 Introduction

Pseudoephedrine (PSE) is a sympathomimetic amine known for its stimulant properties, mimicking the actions of noradrenaline and adrenaline released from the sympathetic nervous system (Bouchard, Weber & Geiger, 2002; Hodges, Hancock, Currell, Hamilton & Jeukendrup, 2006). Commonly, PSE is prescribed for relief of nasal and sinus congestion, as provided in over-the-counter medications (Empey, Young, Letley, Smith, McDonnell, Bagg, & Hughes, 1980). Additionally, PSE also stimulates the central nervous system (CNS), promoting feelings of alertness, reduced fatigue and euphoria (Martin, Sloan, Sapira & Jasinski, 1971), factors which may assist athletic performance. Consequently, PSE currently appears on the WADA list of prohibited and restricted substances, at a urinary concentration of $>150 \mu\text{g}\cdot\text{ml}^{-1}$.

Research investigating the influence of PSE on exercise performance suggests that any ergogenic effect does not manifest when taken in medicinal doses ($\leq 120 \text{ mg}$) during sub-maximal and maximal running or during maximal strength activities (Chester, Reilly & Mottram, 2003; Chu, Doherty, Parise, Milheiro & Tarnopolsky, 2002). In these studies, PSE doses of 60-120 mg resulted in urine concentrations of 30-200 $\mu\text{g}\cdot\text{ml}^{-1}$ (Chester et al., 2003; Gillies et al., 1996), underscoring a wide individual variation in its metabolism and excretion. Alternatively, with doses of $\geq 180 \text{ mg}$, exercise performance may be enhanced during 30 s maximal cycling (Gill, Shield, Blazeovich, Zhou & Weatherby, 2000), 1500 m track running (Hodges et al., 2006), and ~30 min cycling time-trials (Pritchard-Peschek, Jenkins, Osbourne & Slater, 2010). However, no blood or urine samples were obtained during these investigations to quantify circulating concentrations of PSE.

Highly variable individual responses to the metabolism and excretion of PSE at rest (Delbeke & Debackere, 1991; Strano-Rossi, Leone, Torre & Botre, 2009) and during

exercise (Chester et al., 2003; Gillies et al., 1996) have been commonly reported. This inherent variability does not seem depend on body mass, gender or the dose administered (Strano-Rossi et al., 2009). However, Gillies et al., (1996) suggesting that urinary PSE excretion may be influenced by urine acidity, such that a lower pH is associated with a greater urinary PSE output. Consequently, another factor which may potentially influence PSE metabolism and excretion, especially during long duration exercise and in warm, hot environments is hydration status, since dehydration is associated with low urinary pH levels (Eggleton, 1947). Currently, WADA doping control methods use urine specific gravity (USG) measures as the standard for athlete hydration assessment, with some correction of urinary concentrations possible for endogenous (but not exogenous) substances (WADA Technical Document TD2004EAAS). However, USG measures are easily influenced by the recent ingestion of plain water, and when measured soon after the consumption of hypotonic fluid, USG levels can misleadingly indicate an optimal hydration state due to the kidney's rapid filtration of the ingested fluid (Opplinger, Magnes, Popowski & Gisolfi, 2005; Popowski et al., 2001).

Regardless, the influence of post-exercise hydration status on urinary PSE levels is not well understood. Previously, Delbecke and Debackere (1991) reported that ingesting 1.5 L of mineral water at rest over 15-20 min resulted in a four-fold decrease in urinary PSE concentration (from $67 \mu\text{g}\cdot\text{ml}^{-1}$ to $11 \mu\text{g}\cdot\text{ml}^{-1}$) for up to 4 h after consumption. By extension, exercise induced dehydration could possibly increase post-exercise urinary PSE concentrations (above those likely when euhydrated), relative to the PSE dose administered.

Our interest in this possibility was sparked by the case of a state-level Australian footballer who tested positive for excessive levels of PSE in his system after the 2010

Western Australian Football League (WAFL) Grand Final. In a statutory declaration, he stated that 2 x 60 mg “Sudafed” capsules (containing PSE) were consumed (for symptoms of nasal congestion); one taken ~ 30 min pre-game and another ~ 90 min later at half-time. Match conditions were sunny and warm (dry bulb temperature 24-26 °C). The athlete played the majority of the game, but reported drinking “very little” during the match, potentially incurring a large fluid deficit. Once notified about the doping test (~30 min post-game), he was allowed to continue with post-match celebrations, consuming both food and a mix of water, carbonated drinks and electrolyte fluids. Subsequently, he struggled to produce a urine sample, ingesting a large bolus of fluid before a sample was eventually produced ~90 min post-game. After analysis, he tested positive to excessive PSE levels (~230 µg·ml⁻¹) and subsequently served-out a 2 year suspension. In his hearing it was argued that exercise induced dehydration and post-exercise fluid consumption could influence urinary PSE levels in doping tests. However, there is insufficient evidence in the literature to support such a claim.

Therefore, this study aimed to replicate the specific circumstances of this case (as much as possible) to determine whether hydration status influences post-exercise urinary PSE levels. It was hypothesized that after consuming 2 x 60 mg PSE tablets and performing prolonged, exhaustive exercise in warm conditions, significantly higher urinary PSE levels would be recorded when in a dehydrated state than when adequately hydrated. Additionally, it was hypothesized that consuming a large fluid bolus post-exercise would quickly decrease USG, with a corresponding reduction in urinary PSE levels. Any potential ergogenic effect of PSE on exercise performance was not investigated in this study.

3.3 Materials & Methods

Fourteen trained male team sport athletes were recruited for this study. Participants had recently completed a competitive season, primarily in the WAFL. None were taking any medication containing PSE. All provided written informed consent prior to commencement. Ethical approval was granted by the Human Research Ethics Committee of the University of Western Australia.

Each participant performed three (outdoor) experimental trials, completing a 120 min (4 quarters) simulated team game circuit (STGC) designed to mimic the physical demands of Australian football. Participants were instructed to eat and drink in the 24 h period before each trial as if they were preparing for a competitive game, and to replicate this pattern of eating and drinking for each subsequent trial. Each trial was followed by a 90 min post-exercise recovery period, to replicate the delay experienced by the player in producing a urine sample for the doping assessment. These trials involved three different hydration interventions, with the order randomized between participants. On each testing day, multiple hydration conditions (across participants) were represented. To best match the game environmental conditions, testing was postponed when conditions were unfavourable due to cool ($< 20^{\circ}\text{C}$), hot ($> 35^{\circ}\text{C}$), windy ($> 3 \text{ m}\cdot\text{s}^{-1}$) or wet weather. The anticipated dry bulb temperatures for the testing days were $\sim 25^{\circ}\text{C}$. Environmental temperatures were monitored by a weather station (model QuestTemp - 32; Quest Technologies, Oconomowoc, WI) and wind speed was measured using a digital anemometer (model AM-4203HA; Lutron, Taipei, Taiwan). Measurements were taken at the beginning of the STGC, at half time, and immediately upon finishing.

3.3.1 Simulated Team Game Circuit (STGC)

Prior to commencing the STGC, players performed a 10 min standardized game-day warm-up. The STGC comprised 80 (4 sets x 20) repetitions (starting every minute) of a pre-determined exercise circuit, as established by Singh, Guelfi, Landers, Dawson & Bishop (2010). Each repetition took approximately 40 s (allowing ~20 s recovery between repetitions), and involved periods of walking, jogging, striding, sprinting, agility and jumping. Additionally, during every fifth repetition, participants took a padded tackling bag to ground with maximal force and received three contacts to each side of the body from bump pads. After the first and third sets of 20 circuits were completed, a 10 min (quarter-time) break was permitted, with a 20 min (half-time) break allowed after 40 repetitions.

3.3.2 Hydration Protocols

Before and throughout the STGC and post-exercise recovery period, fluid intake was manipulated using practically relevant fluid volumes and choices representative of those used during the aforementioned situation. The aim was to assess the influence of three typical post-exercise hydration states on urinary PSE concentrations, and included:

1. Hydrated (HYD): Participants consumed 250 ml of water and 250 ml of Powerade (Powerade Isotonic, Coca-Cola Amatil, Sydney, Australia: 133 kJ per 100 ml, 7.6 g of carbohydrate, 28 mg of sodium) during the 60 min prior to the STGC commencement. Water was provided during each quarter (200 ml) and at halftime (150 ml). Powerade was provided at the end of the first and third quarter (100 ml), and at halftime (150 ml). On conclusion of the STGC, 50 ml of water and 50 ml of Powerade was consumed

every 10 min for 90 min. Total fluid ingestion was 2650 ml; designed to provide a practically relevant 'optimal fluid replacement' schedule.

2. Dehydrated (DHY): Participants consumed 125 ml of water and 125 ml of Powerade during the 60 min before STGC commencement. They received no fluid during each quarter of the STGC, and 50 ml of Powerade after the first and third quarters. At halftime they consumed 50 ml water and 50 ml of Powerade. After completing the STGC, they received 25 ml of Powerade every 20 min until the conclusion of the recovery period. Total fluid ingestion in DHY was 550 ml; designed to provide a representation of an exercise-induced dehydrated athlete.

3. Bolus (BOL): This condition followed the same hydration schedule as for DHY until the final 30 min of the 90 min post-exercise recovery period. Here, participants received a bolus of 1475 ml of water, and a 375 ml can of Coca Cola[®] (Coca-Cola Amatil, Sydney, NSW. 180 kJ per 100 ml, 10.6 g carbohydrate, 10 mg sodium), and consumed this over the next 20 min. Total fluid ingestion in BOL was 2375 ml; designed to replicate the actual practices of the aforementioned WAFL player during and after the game.

On testing days, participants arrived at 1300 h, where an initial urine sample was collected. At 1330 h, they ingested one 60 mg PSE tablet (Sudafed[®], Johnson & Johnson, USA). At 1350 h, a second urine sample was collected and body mass measured. At 1400 h the warm-up and STGC was commenced. During half time, participants provided a third urine sample and consumed another 60 mg PSE tablet (to match the PSE consumption practices of the player, as stated in his statutory declaration). After completing the STGC, body mass was again measured, and a fourth urine sample collected. Participants then remained in the laboratory for a further 90 min. After 30 min they were fed with a standardized meal (2 x Be Natural Snacks muesli

bars, Charmhaven, NSW: 500 kJ per bar, 2.4 g protein, 2.7 g fat, 19.3 g carbohydrate, 51 mg sodium) to replicate the specific post-game food consumption as reported by the player. After 90 min of recovery, a final urine sample was provided and body mass re-measured. No further assessments were made beyond 90 min post-exercise, since this time point matched the duration required for the player to produce a urine sample for doping assessment.

3.3.3 Heart Rate & Rating of Perceived Exertion

Heart Rate (HR) was continuously monitored during the STGC (Polar RS200, Finland). Ratings of perceived exertion (RPE) (6-20 scale; Borg, 1982) were recorded immediately after finishing each half of the STGC.

3.3.4 Body Mass

Body mass (BM) was used as an indicator of hydration status and sweat loss. Participants were weighed nude (± 10 g; D-7470, August Sauter GmbH, Germany), with excess post-exercise sweat towelled off the skin. Changes in BM (accounting for urine volumes excreted during and after the STGC and fluid volumes ingested) were used for sweat loss estimation at selected time points.

3.3.5 Urine Specific Gravity (USG)

Urine samples (mid-stream) were collected into sterile 1 L containers at pre-ingestion, pre-exercise, half time, immediately post-exercise and 90 min post-exercise and tested immediately for USG using a hand-held refractometer (Atago, Japan). After USG was

measured at pre-ingestion and 90 min post-recovery, these samples were placed into two sterilised sample jars, then frozen, prior to being transported for analysis of PSE, creatinine, pH and osmolality at a commercial laboratory (ChemCentre, WA).

3.3.6 Osmolality (OSM)

Urine samples were batch tested for OSM using a freezing point depression osmometer (Fiske 110, John Morris Scientific, Australia). Repeatability of these measures was assessed using a NaCl standard at ± 2 mOsm $\cdot$ kg H₂O⁻¹ (1 SD) between 0 and 400 mOsm $\cdot$ kg H₂O⁻¹, $\pm 0.5\%$ (1 SD) between 400 and 1500 mOsm $\cdot$ kg H₂O⁻¹ and $\pm 1\%$ (1 SD) between 1500 and 2000 mOsm $\cdot$ kg H₂O⁻¹.

3.3.7 Creatinine (Cr), pH, & Pseudoephedrine (PSE)

Urine samples were batch analysed in the laboratory after thawing. Urinary pH was measured on a Hanna instruments pH meter calibrated in the range of pH 4 – 10 using commercial calibration solutions. Creatinine was measured on a Beckman Coulter UniCel[®] Dx_C 600 using the Beckman Coulter SYNCHRON[®] Systems CR-S creatinine reagent kit, which employs a modified rate Jaffé reaction, as per the manufacturer's instructions. Precision specification for this measurement is ± 2.0 mg $\cdot$ dl⁻¹ or $\pm 2\%$. Pseudoephedrine was quantitatively analysed by gas chromatography-mass spectrometry using an Agilent 5975 gas chromatography-mass spectrometry system following liquid-liquid extraction and derivatisation. The pseudoephedrine response was linear across the range 0 - 300 μ g $\cdot$ ml⁻¹ ($R^2 = 0.999$) with the intra-and inter-day precision (%RSD) $\sim 12\%$ across the range. Samples exceeding this range were diluted with Milli Q water. Values were expressed in μ g $\cdot$ ml⁻¹, and indexed against Cr to account

for hydration status. For this comparison, both PSE and Cr were converted to mmol l^{-1} , and the index expressed as a ratio. Our rationale for measuring creatinine here was to allow the calculation of the Cr/PSE ratio, because its excretion is usually consistent under a range of different conditions (Guyton & Hall, 2000).

3.3.8 Statistical Analysis

All results are expressed as mean $\pm$ SD. A repeated measures ANOVA calculated time, trial and interaction effects between the HYD, DHY and BOL protocols. Pairwise comparisons were made post-hoc where applicable. Intra-class correlation coefficients were calculated to assess the variation in PSE levels between conditions. Statistical significance was accepted at $p \leq 0.05$. All statistical tests were conducted using the software package SPSS for Windows Version 18.

3.4 Results

Mean dry bulb temperature for the three experimental trials was $24.3 \pm 3.8^{\circ}\text{C}$, relative humidity $41.2 \pm 16.2\%$ and wind speed $1.45 \pm 0.73 \text{ m s}^{-1}$. No significant differences in ambient weather conditions were recorded between the hydration conditions (HYD vs. DHY: $p=0.86$; HYD vs. BOL: $p=0.70$; DHY vs. BOL: $p=0.53$).

3.4.1 Exercise Responses

There were no significant differences between trials in HR ($p=0.97$) or RPE ($p=0.77$) during the STGC (Table 1). However, both variables significantly increased over time in DHY (HR: $p=0.01$; RPE: $p=0.02$), and significant increases in RPE were reported in

BOL ($p=0.01$). In contrast, no changes were evident over time during HYD (HR: $p=0.84$, RPE: $p=0.17$).

Table 1. Mean ($\pm$ SD) heart rate and rating of perceived exertion values across time and hydration conditions.

Condition	Measure	Half time	Post-recovery
Hydrated	Heart Rate (bpm)	175	175
		(9)	(9)
	RPE	15	15
		(1.4)	(1.3)
Dehydrated	Heart Rate (bpm)	171	179 ^b
		(12)	(11)
	RPE	15	16 ^a
		(1.5)	(0.9)
Bolus	Heart Rate (bpm)	173	176
		(10)	(8)
	RPE	14	15 ^b
		(1.6)	(1.6)

^a Significantly different from half time ($p<0.05$).

^b Significantly different from half time ($p<0.01$).

3.4.2 Body Mass

Significant BM losses were recorded between pre- and post-exercise in all hydration conditions (HYD: $p=0.01$; DHY: $p=0.01$; BOL: $p=0.01$; Table 2). There was also a significant BM increase in all conditions from post-exercise to 90 min post-recovery (HYD: $p=0.01$; DHY: $p=0.01$; BOL: $p=0.01$). Specifically, the %BM lost between pre- and post-exercise was significantly lower in HYD compared to both DHY and BOL ($p=0.01$). Additionally, the %BM regained between post-exercise and 90 min post-

recovery was significantly lower in DHY compared to HYD and BOL ($p=0.01$). There was no difference in the %BM regained between HYD and BOL ($p=0.12$). Urine volumes from the start of exercise to 90 min post-recovery BM and USG measurements were not different ($p=0.08$) between conditions, being 0.25 ± 0.12 L in HYD, 0.18 ± 0.13 L in DHY and 0.17 ± 0.11 L in BOL.

Table 2. Mean ($\pm$ SD) body mass (BM) changes across time and hydration conditions.

Condition	Pre-exercise (kg)	Post-exercise (kg)	%BM Lost	Post-recovery (kg)	%BM Regained
Hydrated	85.34	83.64 ^{a c}	1.97 ^c	84.40 ^b	0.91 ^c
	(8.48)	(8.17)	(0.58)	(8.18)	(0.13)
Dehydrated	84.89	82.35 ^a	2.99	82.49 ^{b c}	0.17 ^c
	(8.93)	(8.72)	(0.53)	(8.71)	(0.06)
Bolus	84.95	82.37 ^a	3.05	84.23 ^b	2.28 ^c
	(8.76)	(8.64)	(0.52)	(8.64)	(0.25)

^a Significantly different from Pre-exercise ($p<0.05$).

^b Significantly different from both Pre-exercise and Post-exercise ($p<0.05$).

^c Significantly different to other conditions ($p<0.001$).

3.4.3 Urinary Measures

No PSE was found in the pre-ingestion urine samples of any participant. No significant differences in post-recovery PSE values were found between conditions ($p=0.45$) or with test order ($p=0.51$). In all conditions, mean PSE values were above the WADA legal limit of $150 \mu\text{g}\cdot\text{ml}^{-1}$ (Table 3). Individually, across all conditions, 29/42 samples ($\sim 70\%$) were above the WADA limit. Six (of 14) participants recorded values $>150 \mu\text{g}\cdot\text{ml}^{-1}$ in all three conditions, while eight tested below this level in one or more

conditions. Only one participant recorded PSE levels below the WADA limit in all three conditions. Individual PSE responses are shown in Figure 1, displaying an overall range of 33 to 475 $\mu\text{g}\cdot\text{ml}^{-1}$. Intra-class correlation coefficients between PSE values in all conditions ranged between $r=0.03-0.16$ ($p>0.05$).

Figure 3. Pseudoephedrine (PSE) concentration in individual participants within and between conditions (HYD = hydrated; DHY = dehydrated; BOL = bolus).

Table 3. Mean ($\pm$ SD) urine specific gravity (USG), osmolality (OSM), pH, creatinine (Cr), and pseudoephedrine (PSE) values across time and hydration conditions.

Measure	Time	Hydrated	Dehydrated	Bolus
USG	Pre-ingestion	1.011 (0.008)	1.012 (0.008)	1.012 (0.008)
	Pre-exercise	1.008 (0.008)	1.009 (0.008)	1.007 (0.007)
	Half time	1.007 (0.006)	1.010 (0.006)	1.010 (0.006)
	Post-exercise	1.017 (0.005)	1.019 (0.005)	1.019 (0.004)
	Post-recovery	1.019 (0.007)	1.023 (0.005)	1.024 (0.006)
OSM	Pre-ingestion	415 (296)	451 (299)	422 (328)
(m _{osm} kg ⁻¹)	Post-recovery	610 (232)	751 (161)	731 (198)
pH	Pre-ingestion	8.04 (0.69)	7.79 (1.01)	7.63 (0.77)
	Post-recovery	5.88 (0.53)	5.89 (0.46)	5.93 (0.63)
Cr	Pre-ingestion	83.5 (76.2)	86 (75.1)	92.2 (85.9)
	Post-recovery	216.6 (106.3)	294.7 (93.2)	288.8 (118.7)
PSE	Pre-ingestion	0	0	0
	Post-recovery	208.5 (116.5)	238.9 (92.5)	195.6 (107.3)
PSE/Cr	Post-recovery	0.075 (0.042)	0.056 (0.017)	0.060 (0.049)

^a Significantly different from pre-ingestion (p<0.05).

^b Significantly different from prior measurement (p<0.05)

No significant differences in USG were found between conditions ($p=0.14$), although there were significant increases observed over time ($p=0.01$) (across both exercise and recovery periods) in all three conditions (Table 3). In HYD only, USG significantly decreased from pre-ingestion to pre-exercise ($p=0.04$). All conditions showed a significant increase in USG from half-time to post-exercise (HYD: $p=0.01$; DHY: $p=0.01$; BOL: $p=0.01$), while only DHY and BOL showed an increase from post-exercise to 90 min post-recovery (DHY: $p=0.01$; BOL: $p=0.01$). Urinary OSM increased significantly between pre-ingestion and 90 min post-recovery measurements across all trials (HYD: $p=0.03$; DHY: $p=0.01$; BOL: $p=0.01$), with no differences observed between conditions ($p=0.18$).

Urinary pH was not different between conditions pre-ingestion (range: 7.63-8.04; HYD vs. DHY: $p=0.42$; HYD vs. BOL: $p=0.19$; DHY vs. BOL: $p=0.63$). Significant decreases over time were then evident in all conditions ($p=0.01$), but no differences between conditions were present at 90 min post-recovery (5.88-5.93; HYD vs. DHY: $p=0.96$; HYD vs. BOL: $p=0.78$; DHY vs. BOL: $p=0.74$). Similarly, urinary Cr increased significantly in all conditions between pre-ingestion and 90 min post-recovery ($p=0.01$), but no significant differences existed between conditions at either time point. There was no difference in the PSE/Cr ratio between conditions ($p=0.35$).

3.5 Discussion

The purpose of this study was to investigate the influence of hydration status on urinary PSE levels following prolonged, repeated sprint activity. Here, no differences in PSE values were found between the three hydration conditions; however, there were large inter- and intra-individual responses to the 2 x 60 mg PSE dose administered. To our

knowledge, this is the first study to measure urinary PSE levels after prolonged, exhaustive exercise with manipulated hydration levels. Previously (after ingesting 120 mg of PSE 3 h before exercise), PSE levels were shown to be higher 1 h after endurance exercise as compared to rest (Gillies et al., 1996), demonstrating that the urinary excretion of PSE is altered by exercise. These authors concluded that urinary drug concentrations were increased with exercise, although total excretion (i.e. urine content) was not affected. The rationale for this change was largely attributed to a reduction in urine volume and an increase in urine pH as a result of the exercise stimulus. Furthermore, Delbecke & Debackere (1991) showed that a fluid bolus of 1.5 L consumed at rest over a 15 to 20 min period significantly lowered urinary PSE concentration over the next 4 h. Although a similarly sized fluid bolus was consumed over a similar time-frame in the current investigation, our bolus was delivered post-exercise to dehydrated athletes, and measures were only taken for an additional 30 min after ingesting the fluid. As a result, it may be that a fluid bolus consumed at rest when euhydrated may quickly lower urinary PSE levels, but if taken post-exercise when dehydrated, a much smaller impact is seen, at least in the initial 30 min after consuming the fluid.

Prior research also suggests that post-exercise urinary PSE values are commonly higher than if recorded during exercise. Gillies et al. (1996) reported slightly higher urinary PSE levels (approximately $120 \mu\text{g}\cdot\text{ml}^{-1}$) measured 60 min after a 40 km cycling time trial as compared to those recorded at the immediate completion of exercise (approximately $90 \mu\text{g}\cdot\text{ml}^{-1}$). Based on these findings, our 90 min post-exercise urinary PSE values are likely to have been higher than any value measured during or upon finishing exercise, and the longer time frame increased the opportunity for any influence of the fluid manipulations to materialise. However, the results here showed no differences in urinary PSE levels between the three hydration conditions, showing that

the combination of exercise and fluid restriction did not significantly impact on urinary PSE excretion.

The wide individual variability in PSE levels found here is also consistent with previous research involving both resting (Chester, Mottram, Reilly & Powell, 2003; Strano-Rossi et al., 2009) and exercise (Gillies et al., 1996; Swain, Harsha, Baenziger & Saywell, 1997) trials. This variability does not seem to be related to the degree of dilution (Strano-Rossi et al., 2009) or concentration (Gillies et al., 1996) of the urine samples, which is supported by our results when a change in body mass of ~3% is evident. However, the effects of larger changes in body mass (reflecting greater levels of dehydration), on urinary PSE excretion remain to be determined. Regardless, the factors that underscore this individual variability in the pharmacokinetics of PSE are currently unknown. Strano-Rossi et al., (2009) suggested that body mass, gender and the PSE dose administered appear unrelated to its metabolism and excretion. However, when investigating the urinary excretion of other banned substances (i.e. Nadrolone metabolites), Watson, Judkins, Houghton, Russell & Maughan, (2009) explained that the large variations seen in peak urinary levels and the % recovery of the initial dose consumed were due to differences in cumulative urine volume, the rate of urine production, and the degree of lean tissue the individual possessed (with the potential for an increased space for distribution in leaner athletes). As such, some of these mechanisms may also be implicated in the large degree of variability in PSE excretion rates seen here and in other investigations; but such possibilities remain to be fully elucidated. Regardless, the widespread inter- and intra-individual variability across both individuals and trials, makes any prediction of likely urinary PSE concentration based on an administered dose virtually impractical, either at rest, or from an athletic (and doping) perspective, during and after exercise.

The variability in PSE concentrations found here is also not explained by the usual tolerance applied to this measurement in doping cases. Although the stated detection limit for a doping violation is $150 \mu\text{g}\cdot\text{ml}^{-1}$, it is stated that $170 \mu\text{g}\cdot\text{ml}^{-1}$ represents the WADA decision limit (WADA Technical Document TD2013DL). This tolerance approximates the 12% error associated with the analysis method used here. For endogenous substances, WADA allows for a correction based on USG values (WADA Technical Document TD2004EAAS), but this does not apply to exogenous substances such as PSE. In examining the variability of urinary PSE values, Strano-Rossi et al. (2009) previously applied these corrections to urinary PSE concentrations based on the USG values obtained, finding the corrections caused increases in PSE concentration in some cases (low USG) and decreases in others (higher USG). They found the same trend when corrections were made for creatinine concentrations. Based on these results (and given that PSE is an exogenous substance), we have not made similar corrections here, to allow an interpretation of the raw results.

As mentioned above, in anti-doping testing, the USG of the sample is measured and recorded for possible use in correcting the concentration of illegal substances. Our results show no reduction in USG values from post-exercise to 90 min post-recovery in either the HYD or BOL conditions, suggesting that this measure, while simple and inexpensive for field use (Armstrong, 2007; Baker, Lang & Kenney, 2009), may not be sensitive to rapid rehydration strategies after exercise. However, it is possible that with a longer (than 90 min) post-exercise recovery period, and with multiple urine samples collected, the present study may have produced greater differences in USG, OSM, and pH between conditions, which may then have implication to its use for correcting urinary PSE levels during anti-doping assessment.

A possible limitation of this study was the relatively short time period between post-exercise and post-recovery urine measurements (i.e. 90 min). Peak urinary PSE concentration has been shown to occur between 4 and 10 h after administration (Chester et al., 2003; Delbeke & Debackere, 1991; Strano-Rossi et al., 2009). It was measured here 4.5 h after the ingestion of the initial 60 mg dose, and 2.5 h after the second 60 mg dose, so it is possible that urinary PSE concentration was still increasing at this point. Further changes in urinary hydration indicators (USG, OSM, and creatinine) also may have occurred between the various conditions if monitored over a longer post-exercise recovery period. Any effect of this may have been most magnified in the BOL condition, since the final post-recovery urine sample was taken only 30 min after the consumption of the bolus, so any potential changes in USG, OSM, or PSE concentration after this point were not observed. Our rationale for taking a final sample only 30 min after consumption was to match the likely time frame which would occur with a dehydrated athlete who may ingest a large fluid bolus quickly in the post-exercise period when having difficulty in producing a urine specimen for drug testing purposes.

In conclusion, this study found no effect of hydration status on urinary PSE concentration. Although the hydration protocols used elicited marked dehydration, urinary hydration measures seemed to lag behind, and showed no significant differences between the HYD, DHY and BOL conditions during the post-exercise recovery period. Due to the large inter- and intra- variation in individual PSE concentrations found here, it appears impossible to accurately predict the resultant urinary PSE levels after ingesting a medicinal (120 mg) dose of the drug. Even though such doses are below those commonly producing a performance enhancement, this large degree of variability in urinary PSE measures presents a strong possibility of a positive test result. Even at the relatively low doses used here, 13 out of 14 participants would have returned a positive PSE test in at least one trial, and overall, 29 out of 42 trials (~70 %) resulted in

a PSE level above 150 $\mu\text{g}\cdot\text{ml}^{-1}$. For this reason, it is prudent for athletes to avoid taking PSE (at any dose) during competition.

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Appendix A

Participant Information Sheet

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Effect of Hydration Status on Urinary Pseudoephedrine Concentration Following Simulated Team Game Activity

— Participant Information Sheet —

Purpose

This study will aim to investigate the effect of varying hydration states on the post-exercise urinary concentrations of pseudoephedrine ("Sudafed": an "over the counter", non-prescription medication commonly used to alleviate nasal and sinus congestion).

Procedures

Your participation in this investigation will require you to undertake three experimental trials, each inclusive of a 120 min simulated team game circuit (STGC), which will be designed to mimic the physical demands you commonly encounter during a game of Australian rules football.

On the day of each testing session, you will be required to attend the School of Sport Science, Exercise and Health, located within the University of Western Australia at 1300 h. Initially, you will be asked to provide a baseline urine sample. Over the next 50 min, you will be required to consume 1 L of fluid (500 ml water + 500 ml Powerade)

spread evenly over this period of time. Additionally, 30 min after your arrival, you will be required to ingest one Sudafed tablet, which contains 60 mg of pseudoephedrine (PSE). Twenty minutes after the ingestion of this Sudafed tablet, you will be asked to provide a second urine sample, and your body mass will then be measured. Subsequent to the attainment of these measures, you will then commence a 10 min warm-up that will be standardized and specific to the ensuing activity. After this warm-up period, the 120 min STGC will commence.

The STGC will comprise four 20 min quarters, separated by two 10 min quarter time breaks, and one 20 min half time break. During the half time rest period, you will be asked to provide a third urine sample, and also to consume a second Sudafed tablet. At the conclusion of the STGC, your body mass will again be measured, and you will be asked to provide a fourth urine sample. Subsequently, you will be required to remain at the laboratory for a further 90 min. At 30 min after the completion of the STGC, you will be fed with a standardized meal. After 90 min of elapsed recovery time, you will be asked to provide a final urine sample, and your body mass will be collected one final time.

Throughout the STGC and during the 90 min recovery period that follows, the fluid intake that you will be allowed will be manipulated in order to assess the influence of hydration status on your urinary concentrations of PSE. To achieve this, the three experimental conditions that you will partake in will include:

1. A Well Hydrated Trial (HYD)

In the hydrated trial, you will consume 500 ml of water and 500 ml of an electrolyte drink during the 60min prior to the STGC commencement. The electrolyte drink will also be provided at the end of the first and third quarter (100 ml), at halftime (150 ml), and then in 50 ml aliquots every 10 min after the conclusion of the STGC. Water will be

provided to you during each quarter (200 ml), at halftime (150 ml), and then in 50 ml aliquots every 10 min after the conclusion of the STGC.

2. A Dehydrated Trial (DYH)

In the dehydrated trial, you will consume 500 ml of water and 500 ml of an electrolyte drink during the 60 min prior to the STGC commencement. Subsequently, you will receive no fluid during each quarter of the STGC, and only 50 ml of an electrolyte drink will be given to you at the end of the first and third quarters. At halftime you will receive 50 ml of water and 50 ml of an electrolyte drink. At the conclusion of the STGC, you will receive only 25 ml of electrolyte drink every 20 min until the conclusion of the trial.

3. A Bolus Trial (BOL)

The bolus condition will follow the same hydration schedule as the dehydrated trial until the final 30 min of the 90 min post-exercise rest period. At this point you will receive a bolus of fluid (1.47 L of water, and 1 x 375 ml can of Coca Cola) to be consumed rapidly during this 30 min period.

Please Note: In the **24 h** prior to testing, it is important that you refrain from any form of structured exercise training or heavy manual labour.

Urine Collection

The urine samples required from you during this investigation will be collected mid-stream into a small sterile container. You will be provided with the sterile collection cup, and directed to a rest room facility where you can produce the sample in complete privacy.

Risks

This study requires maximal effort testing and therefore risks are involved. These include, but are not limited to, injuries to muscles and tendons of the body. Every effort will be made to minimise these risks by ensuring you perform a thorough warm up and cool down during each testing session. However, the requirements will not exceed those experienced during normal training and competition.

Benefits

Individual: You will receive a full assessment of your STGC performance during the investigation. You may find such results interesting and useful to your training program.

Community: The results of this study may help to critically analyse the current testing procedures of the World Anti-Doping Authority (WADA), thereby providing information as to the efficacy of their current testing protocols.

Confidentiality of Data

Personal details and test results from this testing will be treated confidentially at all times. Individual data will not be identifiable, but collective results may be published. No data will be stored on public computers within the department. All data will be stored on the chief investigator's computer only, in a secure location.

Subject Rights

Participation in this research is voluntary and you are free to withdraw from the study at any time and for any reason, without prejudice in any way. If you withdraw from the study and you are an employee or student at the University of Western Australia (UWA) this will not prejudice your status and rights as an employee or student of UWA. Your participation in this study does not prejudice

any right to compensation that you may have under the statute of common law. Further information regarding this study may be obtained from Professor Brian Dawson on telephone number 6488 2276.

The committee for Human Rights at the University of Western Australia requires that all participants are informed that, if they have any complaint regarding the manner, in which a research project is conducted, it may be given to the researcher or, alternatively to the Secretary, Human Research Ethics Committee Registrar's Office, University of Western Australia, Crawley, WA 6009 (telephone number 6488 3703). All study participants will be provided with a copy of the information sheet and consent form for their personal records.

Appendix B

Participant Consent Form

THE UNIVERSITY OF
WESTERN AUSTRALIA
Achieving International Excellence

Professor Brian Dawson
School of Sport Science, Exercise and Health
The University of Western Australia
35 Stirling Highway
Crawley WA, 6009
Phone +61 8 6488 2276

Effect of Hydration Status on Urinary Pseudoephedrine Concentration Following Simulated Team Game Activity

— Participant Consent Form —

As a subject, you are free to withdraw your consent to participate at any time without prejudice. The researchers will answer any questions you may have in regard to the study at any time.

I _____ (participants name) acknowledge that I have read the above statement and information sheet, which explains the nature, purpose and risks of the investigation and that any questions I have asked have been answered to my satisfaction. I agree to participate in this study realising that I may withdraw at any time without prejudice.

I understand that all information provided is treated as strictly confidential and will not be released by the investigator unless required to do so by law.

I agree that research data gathered for the study may be published provided my name or other identifying information is not used.

Participant

Date

The committee for Human Rights at the University of Western Australia requires that all participants are informed that, if they have any complaint regarding the manner, in which a research project is conducted, it may be given to the researcher or, alternatively to the Secretary, Human Research Ethics Committee Registrar's Office, University of Western Australia, Crawley, WA 6009 (telephone number 6488 3703). All study participants will be provided with a copy of the information sheet and consent form for their personal records.

Appendix C

Ethics Approval

THE UNIVERSITY OF
WESTERN AUSTRALIA
Achieving International Excellence

**Research Ethics and Biosafety Office
Research Services**

Phone: +61 8 6488 3703
Fax: +61 8 6488 8775
email: hreo-research@uwa.edu.au
MBDP: M459

Our Ref: RA/4/1/5036

10 October 2011

Winthrop Professor Brian Dawson
Sport Science, Exercise & Health (School of)
MBDP: M408

Dear Professor Dawson

HUMAN RESEARCH ETHICS APPROVAL - THE UNIVERSITY OF WESTERN AUSTRALIA

Effect of Hydration States on Urinary Pseudoephedrine Concentration Following Simulated Team Game Activity

Student(s): Daniel Jolley - MSc - 19710283

Ethics approval for the above project has been granted from 10 October 2011 to 01 November 2012 in accordance with the requirements of the *National Statement on Ethical Conduct in Human Research* (National Statement) and the policies and procedures of The University of Western Australia.

You are reminded of the following requirements:

1. The application and all supporting documentation form the basis of the ethics approval and you must not depart from the research protocol that has been approved.
2. The Human Research Ethics Office must be approached for approval in advance for any requested amendments to the approved research protocol.
3. The Chief Investigator is required to report immediately to the Human Research Ethics Office any adverse or unexpected event or any other event that may impact on the ethics approval for the project.
4. The Chief Investigator must inform the Human Research Ethics Office as soon as practicable if a research project is discontinued before the expected date of completion, providing reasons.

Any conditions of ethics approval that have been imposed are listed below:

Special Conditions

None specified

The University of Western Australia is bound by the National Statement to monitor the progress of all approved projects until completion to ensure continued compliance with ethical standards and requirements.

Please note that the maximum period of ethics approval for this project is five (5) years from the date of this notification. However, ethics approval is conditional upon satisfactory progress reports being received by the designated renewal date for continuation of ethics approval.

The Human Research Ethics Office will forward a request for a Progress Report approximately 60 days before the due date. A further reminder will be forwarded approximately 30 days before the due date.

If your progress report is not received by the due date for renewal of ethics approval, **your ethics approval will expire**, requiring that all research activities involving human participants cease immediately.

If you have any queries please do not hesitate to contact the Human Research Ethics Office (HREO) at hreo-research@uwa.edu.au or on (08) 6488 3703.

Please ensure that you quote the file reference – RA/4/1/5036 – and the associated project title in all future correspondence.

Yours sincerely

A handwritten signature in black ink, appearing to read 'Peter Johnstone', written in a cursive style.

Peter Johnstone
Manager
Human Research Ethics Committee

Appendix D

Raw Data

HYDRATED CONDITION																									
Participant	Trial	USG1	Osm1 (m _{osm} ·kg ⁻¹)	PSE1 (μg·ml ⁻¹)	pH1	Cr1 (μg·ml ⁻¹)	USG2	BM1 (kg)	HR1 (bpm)	RPE1	USG3	HR2 (bpm)	RPE2	USG4	BM2 (kg)	BM Lost (%)	Tot Urine Vol (L)	BM3 (kg)	USG5	Osm5 (m _{osm} ·kg ⁻¹)	PSE5 (μg·ml ⁻¹)	pH5	Cr5 (μg·ml ⁻¹)	Sweat Loss (kg)	BM Regain (%)
1	2	1.022	775	<LOR	7.49	184.4	1.013	88.75	163	15	1.007	174	16	1.022	86.85	2.14	0.18	87.60	1.019	647	164.5	5.71	214.0	3.02	0.86
2	2	1.007	210	<LOR	7.30	38.5	1.004	83.00	178	18	1.011	179	18	1.016	80.85	2.59	0.34	81.55	1.018	467	109.4	6.51	142.9	3.12	0.87
3	2	1.009	330	<LOR	8.26	61.0	1.006	85.60	175	16	1.007	180	17	1.020	84.30	1.52	0.17	85.05	1.017	539	247.8	6.08	187.9	2.43	0.89
4	1	1.027	1021	<LOR	8.82	270.2	1.033	89.80	183	-	1.028	172	-	1.021	88.20	1.78	0.11	89.10	1.029	984	470.4	5.41	416.5	2.79	1.02
5	1	1.019	744	<LOR	8.81	125.7	1.011	99.55	168	16	1.008	169	16	1.014	97.90	1.66	0.23	98.60	1.016	558	134.6	6.18	169.1	2.72	0.72
6	3	1.003	142	<LOR	6.62	21.1	1.003	100.70	169	13	1.005	169	13	1.013	97.70	2.98	0.58	98.55	1.020	677	208.5	5.23	179.9	3.73	0.87
7	2	1.008	326	<LOR	8.20	45.5	1.008	86.10	173	15	1.009	182	14	1.014	84.95	1.34	0.15	85.75	1.015	556	161.1	5.29	170.7	2.30	0.94
8	2	1.002	90	<LOR	8.35	13.9	1.003	69.70	159	15	1.003	155	15	1.013	68.30	2.01	0.34	69.00	1.008	258	93.6	6.49	83.9	2.36	1.02
9	3	1.018	731	<LOR	8.94	148.0	1.002	78.45	186	15	1.003	188	15	-	76.45	2.55	0.33	77.30	1.022	710	338.9	5.56	322.3	2.98	1.11
10	2	1.008	274	<LOR	8.58	58.0	1.003	82.50	188	16	1.003	188	16	1.017	80.90	1.94	0.25	81.60	1.023	745	284.1	5.29	310.7	2.65	0.87
11	1	1.014	548	<LOR	7.25	114.1	1.011	84.40	186	-	1.005	176	-	-	82.60	2.13	0.16	83.50	1.026	838	239.6	6.15	356.3	2.94	1.09
12	1	1.004	177	<LOR	8.26	18.9	1.004	87.05	171	15	1.005	166	16	1.020	84.90	2.47	0.25	85.55	1.017	509	106.1	5.76	157.4	3.20	0.77
13	3	1.009	348	<LOR	7.55	59.3	1.002	72.90	173	13	1.003	176	15	1.005	72.40	0.69	0.29	73.10	1.005	144	42.9	6.95	42.0	1.51	0.97
14	2	1.002	101	<LOR	8.06	10.0	1.003	86.30	180	16	1.007	183	16	1.023	84.70	1.85	0.21	85.30	1.026	910	317.7	5.74	278.9	2.69	0.71

DEHYDRATED CONDITION																									
Participant	Trial	USG1	Osm1 (m _{osm} ·kg ⁻¹)	PSE1 (μg·ml ⁻¹)	pH1	Cr1 (μg·ml ⁻¹)	USG2	BM1 (kg)	HR1 (bpm)	RPE1	USG3	HR2 (bpm)	RPE2	USG4	BM2 (kg)	BM Lost (%)	Tot Urine Vol (L)	BM3 (kg)	USG5	Osm5 (m _{osm} ·kg ⁻¹)	PSE5 (μg·ml ⁻¹)	pH5	Cr5 (μg·ml ⁻¹)	Sweat Loss (kg)	BM Regain (%)
1	3	1.020	740	<LOR	5.70	130.3	1.007	88.20	166	13	1.013	169	15	1.025	86.35	2.10	0.13	86.50	1.021	739	87.0	6.44	165.9	1.92	0.17
2	3	1.007	258	<LOR	7.86	42.7	1.004	82.75	174	16	1.005	183	16	-	80.10	3.20	0.25	80.20	1.016	521	201.8	6.44	198.7	2.60	0.12
3	1	1.012	425	<LOR	7.38	67.8	1.012	85.35	163	-	1.007	187	-	1.014	82.50	3.34	0.51	82.60	1.026	814	260.0	6.23	409.1	2.54	0.12
4	3	1.019	728	<LOR	7.75	120.3	1.020	90.20	156	13	1.023	180	15	1.026	88.55	1.83	0.08	88.70	1.027	933	298.5	5.53	278.4	1.77	0.17
5	2	1.008	318	<LOR	8.60	52.4	1.004	98.95	163	15	1.007	165	15	1.014	95.50	3.49	0.29	95.60	1.024	720	242.9	5.66	320.8	3.36	0.10
6	1	1.002	92	<LOR	7.91	14.1	1.004	101.20	174	15	1.011	169	15	1.018	98.10	3.06	0.19	98.20	1.027	878	166.4	5.19	246.4	3.12	0.10
7	1	1.027	972	<LOR	5.49	282.8	1.024	85.05	186	12	1.019	191	17	1.017	82.25	3.29	0.06	82.50	1.020	633	297.5	5.20	290.1	2.94	0.30
8	3	1.007	256	<LOR	8.56	31.1	1.003	68.70	164	17	1.004	166	17	1.024	66.10	3.76	0.22	66.20	1.030	905	386.7	6.10	292.2	2.59	0.15
9	2	1.021	828	<LOR	8.57	172.0	-	76.90	186	16	-	195	16	-	74.70	2.86	0.00	74.90	1.014	467	125.8	6.46	243.2	2.40	0.27
10	1	1.006	227	<LOR	8.48	39.4	1.003	81.75	188	16	1.005	192	17	1.018	79.50	2.75	0.18	79.60	1.025	805	331.5	5.34	317.6	2.28	0.13
11	3	1.013	495	<LOR	7.93	114.9	-	84.10	165	15	1.009	173	16	-	81.40	3.21	0.15	81.60	1.027	876	375.2	5.67	536.5	2.75	0.25
12	2	1.004	117	<LOR	7.89	17.1	1.003	87.20	150	15	1.004	174	16	1.007	84.20	3.44	0.28	84.30	1.017	522	134.8	6.10	206.4	2.93	0.12
13	2	1.003	109	<LOR	8.26	17.9	1.003	71.30	184	16	1.006	182	16	1.016	69.35	2.73	0.07	69.50	1.021	751	201.5	6.22	329.9	2.08	0.22
14	1	1.017	748	<LOR	8.73	101.2	1.019	86.75	180	16	1.019	192	18	1.025	84.30	2.82	0.08	84.45	1.027	951	235.0	5.86	291.1	2.58	0.18

Participant	Trial	BOLUS CONDITION																							
		USG1	Osm1 (m _{osm} ·kg ⁻¹)	PSE1 (μg·ml ⁻¹)	pH1	Cr1 (μg·ml ⁻¹)	USG2	BM1 (kg)	HR1 (bpm)	RPE1	USG3	HR2 (bpm)	RPE2	USG4	BM2 (kg)	BM Lost (%)	Tot Urine Vol (L)	BM3 (kg)	USG5	Osm5 (m _{osm} ·kg ⁻¹)	PSE5 (μg·ml ⁻¹)	pH5	Cr5 (μg·ml ⁻¹)	Sweat Loss (kg)	BM Regain (%)
1	1	1.015	587	<LOR	8.47	74.2	1.004	88.20	174	16	1.005	180	17	1.020	85.75	2.78	0.22	87.65	1.018	320	32.6	7.10	61.5	2.43	2.22
2	1	1.009	339	<LOR	8.23	61.4	1.005	82.60	176	16	1.005	176	17	1.014	79.85	3.33	0.24	81.65	1.014	452	84.2	7.31	156.0	2.72	2.25
3	3	1.018	705	<LOR	6.69	138.8	1.006	85.50	178	16	1.008	171	16	1.025	83.10	2.81	0.11	84.90	1.028	863	195.6	5.64	422.2	2.49	2.17
4	2	1.017	422	<LOR	6.21	183.1	-	90.05	169	14	1.020	169	15	-	87.65	2.67	0.07	89.55	1.029	902	196.3	5.41	524.0	2.53	2.17
5	3	1.005	194	<LOR	7.91	31.4	1.006	99.45	163	13	1.010	169	13	1.016	96.45	3.02	0.15	98.30	1.021	659	154.6	5.53	245.6	3.05	1.92
6	2	1.007	208	<LOR	8.30	29.1	1.006	100.90	154	13	1.009	170	13	1.022	97.90	2.97	0.31	99.75	1.022	795	155.9	5.75	182.9	2.90	1.89
7	3	1.026	1037	<LOR	7.28	215.1	1.027	84.75	176	15	1.023	176	15	1.015	82.40	2.77	0.06	84.30	1.017	585	102.5	5.35	219.0	2.49	2.31
8	1	1.003	96	<LOR	8.38	15.6	1.003	68.75	176	18	1.005	175	18	1.025	65.95	4.07	0.24	67.80	1.031	844	475.4	6.69	330.4	2.77	2.81
9	1	1.009	375	<LOR	8.04	67.0	-	77.05	198	13	-	198	15	-	74.30	3.57	0.00	76.20	1.023	779	172.1	5.97	303.6	2.95	2.56
10	3	1.007	271	<LOR	8.25	55.2	1.003	82.80	178	15	1.009	183	16	1.020	80.95	2.23	0.20	82.85	1.024	778	249.5	5.61	263.8	1.85	2.35
11	2	1.028	1061	<LOR	6.88	299.6	-	84.10	171	16	-	171	16	-	82.10	2.38	0.00	83.85	1.031	1024	247.2	5.80	418.3	2.20	2.13
12	3	1.004	168	<LOR	7.89	22.7	1.004	85.80	173	13	1.005	169	16	1.014	82.90	3.38	0.20	84.80	1.021	593	141.9	5.57	264.3	2.90	2.29
13	1	1.011	320	<LOR	6.51	79.7	1.011	72.45	180	-	1.013	180	-	1.016	70.30	2.97	0.20	72.15	1.023	669	235.7	5.54	327.9	2.15	2.63
14	3	1.004	126	<LOR	7.78	17.8	1.003	86.85	166	16	1.004	184	18	1.026	83.60	3.74	0.37	85.50	1.031	974	294.7	5.80	323.4	3.09	2.27

Weather															
Condition	Pre STGC					Half Time					Post STGC				
	Dry Bulb (°C)	Wet Bulb (°C)	Globe (°C)	Relative Humidity (%)	Wind Speed (m.s ⁻¹)	Dry Bulb (°C)	Wet Bulb (°C)	Globe (°C)	Relative Humidity (%)	Wind Speed m.s ⁻¹	Dry Bulb (°C)	Wet Bulb (°C)	Globe (°C)	Relative Humidity (%)	Wind Speed (m.s ⁻¹)
Hydrated	24.3	18.6	36.3	37.2	1.28	23.5	18.6	33.1	41.4	1.55	23.0	17.9	30.5	45.7	1.53
Dehydrated	23.9	18.8	35.1	39.6	1.24	23.1	18.8	31.6	44.7	1.35	23.0	18.4	31.0	47.1	1.38
Bolus	24.7	19.1	36.8	38.6	1.18	24.2	19.2	34.9	39.4	1.61	23.7	18.4	32.4	42.8	1.42