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**EFFECTS OF DIFFERENT TYPES OF EXPLOSIVE  
EFFORTS IN CARDIOVASCULAR AND  
HORMONAL PARAMETERS OF SUBJECTS WITH  
DIFFERENT LEVELS OF PHYSICAL  
PERFORMANCE**

**Doctoral Thesis**

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## List of Publications

This doctoral thesis is a compendium of the following publications:

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**Study III.** Castro-Sepulveda M, Ramirez-Campillo R, Abad-Colil F, Monje C, Peñailillo L, Cancino J and Zbinden-Foncea H (2018). Basal mild dehydration increase salivary cortisol after a friendly match in young elite soccer players. *Front. Physiol.* 9:1347. doi: 10.3389/fphys.2018.01347

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## Resumen

La presente tesis doctoral es un compendio de tres estudios diferentes que subrayan el propósito general de examinar los efectos de diferentes tipos de esfuerzos explosivos en los parámetros cardiovasculares y hormonales de sujetos con diferentes niveles de rendimiento físico. El objetivo del estudio 1 fue comparar los efectos agudos del entrenamiento pliométrico de intensidad baja, moderada, alta y combinada sobre la frecuencia cardíaca (FC), la presión arterial sistólica (PAS), la presión arterial diastólica (PAD) y respuestas cardiovasculares del producto de presión-presión en sujetos normotensos masculinos y femeninos. Quince (8 mujeres) sujetos normotensos físicamente activos participaron en este estudio (edad  $23.5 \pm 2.6$  años, índice de masa corporal  $23.8 \pm 2.3$  kg.m<sup>-2</sup>). Utilizando un diseño cruzado aleatorio, los ensayos se realizaron con intervalos de descanso de al menos 48 horas. Cada prueba comprendió 120 saltos, usando cajas de 20, 30 y 40 cm para baja, moderada y alta intensidad, respectivamente. Para la intensidad combinada, se combinaron las 3 alturas. Las mediciones se tomaron antes y después (es decir, cada 10 minutos durante un período de 90 minutos) de cada prueba. Cuando se combinaron las respuestas de hombres y mujeres, se observó una reducción media en PAS, PAD y producto de presión-presión después de todas las intensidades pliométricas. No se observaron diferencias significativas antes o después del ejercicio (en cualquier momento) para FC, PAS, PAD o producto de presión-presión cuando se compararon los ensayos de intensidad baja, moderada, alta o combinada. No se observaron diferencias significativas entre los hombres y las mujeres, excepto por una mayor reducción de la PAS en las mujeres (212%) en comparación con los hombres (27%) después del ensayo de alta intensidad. Aunque hubo diferencias menores entre los puntos de tiempo posteriores al ejercicio, colectivamente, los datos demostraron que todas las intensidades de entrenamiento pliométrico pueden inducir un efecto hipotensor agudo después del ejercicio en sujetos jóvenes normotensos masculinos y femeninos.

El objetivo del estudio 2 fue comparar los efectos de un programa de entrenamiento de salto, con o sin carga de mano tipo haltera, en el rendimiento del ejercicio de máxima intensidad. Los jugadores de fútbol juveniles ( $12.1 \pm 2.2$  años) fueron asignados a un grupo de entrenamiento de salto (JG, n = 21), un grupo de entrenamiento de salto más carga manual de tipo haltera (LJG, n = 21), o un grupo de control que solo siguió entrenamiento de fútbol (CG, n = 21). Los atletas fueron evaluados para medidas de rendimiento de máxima intensidad antes y después de 6 semanas de entrenamiento, durante un período de entrenamiento en temporada. El CG logró un cambio significativo en la velocidad máxima de patada solamente (ES = 0.11–0.20). Ambos grupos de entrenamiento de salto mejoraron en la pierna derecha (ES = 0.28–0.45) e izquierda para salto de contramovimiento horizontal con los brazos (ES = 0.32–0.47), el salto de contramovimiento horizontal con ambas piernas y con los brazos (ES = 0.28–0.37), el salto de contramovimiento vertical con los brazos (ES = 0.26), índice de fuerza reactiva de salto de caída de 20 cm (ES = 0.20–0.37) y velocidad máxima de patada (ES = 0.27–0.34). Sin embargo, en comparación con el CG, solo el LJG exhibió mayores mejoras en todas las pruebas de rendimiento. Por lo tanto, la carga manual de tipo haltera mejora aún más las adaptaciones de rendimiento durante el entrenamiento de salto en jugadores de fútbol juveniles.

Respecto del estudio 3, un partido de fútbol induce cambios en biomarcadores de estrés fisiológico como la testosterona (T), cortisol (C) y relación testosterona:cortisol (T: C). El estado de hidratación también puede modular estas hormonas y, por lo tanto, pueden alterar el equilibrio anabólico / catabólico en respuesta al partido de fútbol. El papel del estado de hidratación antes del

partido en estos biomarcadores aún no se ha informado. Por tanto, el objetivo del estudio 3 fue comparar las respuestas salivales de la relación T, C y T:C después de dos partidos amistosos en jóvenes jugadores de fútbol masculino de élite bien hidratados y moderadamente deshidratados (MD). Diecisiete jugadores (edad,  $16.8 \pm 0.4$  años;  $VO_{2max}$   $57.2 \pm 3.6$  ml. kg.min) se dividieron en dos equipos. Antes de los partidos, se evaluó el nivel de hidratación de los atletas mediante el método de gravedad específica de la orina y se dividió para el análisis en grupos bien hidratado (WH; n = 9; USG <1.010 g.mL) y moderadamente deshidratado (MD; n = 8; USG 1.010 a 1.020 g.mL). Se recogieron hormonas antes y después de cada partido mediante muestras de saliva. La frecuencia cardíaca media (FC media) y máxima (FCmax) se midieron a lo largo de los partidos. Se usó un ANOVA de dos vías para comparar T, C y T: C entre y dentro de los grupos. Similares valores de FC media (WH,  $83.1 \pm 4.7\%$ ; MD,  $87.0 \pm 4.1$ ;  $p = 0.12$ ) y FCmax (WH,  $93.2 \pm 4.4\%$ ; MD,  $94.7 \pm 3.7\%$ ;  $p = 0.52$ ) fueron observados para ambos grupos durante los partidos. No se encontraron diferencias entre los grupos para T ( $p = 0.38$ ), C ( $p = 0.66$ ), ni T: C ( $p = 0.38$ ). No se encontraron cambios en ninguno de los grupos para T (WH,  $p = 0.20$ ; MD,  $p = 0.36$ ) y T: C (WH,  $p = 0.94$ ; MD,  $p = 0.63$ ). Con respecto a la C, solo el grupo MD mostró aumentos (28%) después de los partidos (MD,  $p = 0.03$ ; WH,  $p = 0.13$ ). En conclusión, el grupo MD exacerbó la respuesta del C a los partidos amistosos en jugadores de fútbol masculinos jóvenes de élite, lo que sugiere que la deshidratación antes del partido puede ser un estrés adicional a considerar.

## Summary

The present doctoral dissertation is a compendium of three different studies underling the general purpose of examine the effects of different types of explosive efforts in cardiovascular and hormonal parameters of subjects with different levels of physical performance.

The aim of the study 1 was to compare the acute effects of low-, moderate-, high-, and combined-intensity plyometric training on heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP), and rate-pressure product (RPP) cardiovascular responses in male and female normotensive subjects. Fifteen (8 women) physically active normotensive subjects participated in this study (age  $23.5 \pm 2.6$  years, body mass index  $23.8 \pm 2.3$  kg.m<sup>-2</sup>). Using a randomized crossover design, trials were conducted with rest intervals of at least 48 hours. Each trial comprised 120 jumps, using boxes of 20, 30, and 40 cm for low, moderate, and high intensity, respectively. For combined intensity, the 3 height boxes were combined. Measurements were taken before and after (i.e., every 10 minutes for a period of 90 minutes) each trial. When data responses of men and women were combined, a mean reduction in SBP, DBP, and RPP was observed after all plyometric intensities. No significant differences were observed pre- or postexercise (at any time point) for HR, SBP, DBP, or RPP when low-, moderate-, high-, or combined-intensity trials were compared. No significant differences were observed between male and female subjects, except for a higher SBP reduction in women (212%) compared with men (27%) after high-intensity trial. Although there were minor differences across postexercise time points, collectively, the data demonstrated that all plyometric training intensities can induce an acute postexercise hypotensive effect in young normotensive male and female subjects.

The aim of the study 2 was to compare the effects of a jump training program, with or without haltere type handheld loading, on maximal intensity exercise performance. Youth soccer players ( $12.1 \pm 2.2$  y) were assigned to either a jump training group (JG, n = 21), a jump training group plus haltere type handheld loading (LJG, n = 21), or a control group following only soccer training (CG, n = 21). Athletes were evaluated for maximal intensity performance measures before and after 6 weeks of training, during an in-season training period. The CG achieved a significant change in maximal kicking velocity only (ES = 0.11–0.20). Both jump training groups improved in right leg (ES = 0.28–0.45) and left leg horizontal countermovement jump with arms (ES = 0.32–0.47), horizontal countermovement jump with arms (ES = 0.28–0.37), vertical countermovement jump with arms (ES = 0.26), 20-cm drop jump reactive strength index (ES = 0.20–0.37), and maximal kicking velocity (ES = 0.27–0.34). Nevertheless, compared to the CG, only the LJG exhibited greater improvements in all performance tests. Therefore, haltere type handheld loading further enhances performance adaptations during jump training in youth soccer players.

A soccer match induce changes in physiological stress biomarkers as testosterone (T), cortisol (C), and testosterone:cortisol (T:C) ratio. Hydration state may also modulate these hormones, and therefore may alter the anabolic/catabolic balance in response to soccer match. The role of hydration status before the match in these biomarkers has not yet been reported. The aim of the study 3 was to compare the salivary T, C, and the T:C ratio responses after two friendly matches in well-hydrated and mild-dehydrated (MD) elite young male soccer player. Seventeen players (age,  $16.8 \pm 0.4$  years; VO<sub>2</sub>max  $57.2 \pm 3.6$  ml.kg<sup>-1</sup>.min<sup>-1</sup>) were divided into two teams. Before the matches the athletes were assessed for hydration level by the urine specific gravity method and divided for the analysis into well-hydrated (WH; n = 9; USG < 1.010 g/mL<sup>-1</sup>) and milddehydrated

(MD; n = 8; USG 1.010 to 1.020 g/mL<sup>-1</sup>) groups. Hormones were collected before and after each match by saliva samples. The mean (HR<sub>mean</sub>) and maximal (HR<sub>max</sub>) heart rate were measured throughout the matches. A two-way ANOVA was used to compare T, C, and T:C between and within groups. Similar HR<sub>mean</sub> (WH, 83.1 ± 4.7%; MD, 87.0 ± 4.1; p = 0.12) and HR<sub>max</sub> (WH, 93.2 ± 4.4%; MD, 94.7 ± 3.7%; p = 0.52) were found for both groups during the matches. No differences were found before the matches in the T (p = 0.38), C (p = 0.66), nor T:C (p = 0.38) between groups. No changes within groups were found after matches in neither group for T (WH, p = 0.20; MD, p = 0.36), and T:C (WH, p = 0.94; MD, p = 0.63). Regarding the C, only the MD group showed increases (28%) after the matches (MD, p = 0.03; WH, p = 0.13). In conclusion, MD exacerbate the C response to friendly matches in elite young male soccer players, suggesting that dehydration before match may be an added stress to be considered.

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## **LIST OF ABBREVIATIONS**

A: acyclic.

ANOVA: analysis of variance.

AU: arbitrary unit.

BDJ: bounce drop jump.

BMI: body mass index.

C: cortisol.

CG: control group.

COM: combined-intensity (i.e. low, moderate and high).

DBP: diastolic blood pressure.

DJ: drop jump.

ES: effect sizes.

HCMJA: horizontal countermovement jump with arms.

HI: high-intensity.

HR: heart rate.

HRmax: maximal heart rate.

HRmean: mean heart rate.

HTHL: hand-held loaded jump training.

ICC: intra-class correlation coefficient.

JG: jump training group.

LHCMJA: left leg horizontal countermovement jump with arms.

LI: low-intensity.

LJG: loaded jump-training group.

MD: mild dehydration.

MI: moderate-intensity.

MKV: maximal kicking velocity.

PEH: post-exercise hypotension.

PHV: peak height velocity.

RHCMJA: right leg horizontal countermovement jump with arms.

RPP: rate-pressure product.

RSI: reactive strength index.

SBP: systolic blood pressure.

SD: standard deviation.

SSC: stretch-shortening cycle.

T: testosterone.

T:C: testosterone-cortisol ratio.

U17: under-17 years old.

USG: urine specific gravity.

VCMJA: vertical countermovement jump with arms.

VDJ20: vertical twenty centimeter drop jump reactive strength index.

VO<sub>2</sub>max: maximal oxygen consumption

WH: well-hydration.

## 1. INTRODUCTION

Plyometric training is a strength training method commonly used to increase explosive performance, with the advantage of requiring reduced physical space, time and equipment to complete the training sessions, hence integrating easily during regular training. High intensity drop jumps (DJ) are the most common exercises used during plyometric training, including bounce drop jumps (BDJ). A BDJ involves a sudden eccentric muscle action, which activates a reflex contraction and higher muscle activity, as well as involves a rapid coupling between an eccentric and concentric muscle action (i.e. stretch-shortening cycle - SSC) (9). Training exercises based on a SSC are established techniques for enhancing athletic performance. This type of training may lead preferentially to adaptations such as increased rate of activation of motor units (34, 35), with little or null impact on muscle hypertrophy, which may be important in sports where endurance and/or muscle power relative to body weight is relevant to performance. However, although commonly accepted as an effective training method, previous studies have not established its effects on cardiovascular responses.

### 1.1. Plyometric training: its effect on cardiovascular responses

Plyometric training is an explosive-type strength training method based on the stretch-shortening cycle [SSC] muscle performance. Plyometric training may induce long-term improvements in sprint (88), strength (87) and several sport-related explosive performance measures (21, 53). In addition to these chronic effects, plyometric training may also induce acute effects on physical performance (1, 13, 93), muscle activation (91), metabolic (13, 94) and hormonal (13) variables, effects that may have potential implications to establish optimum plyometric training designs. However, the acute effects of plyometric training on cardiovascular variables are poorly known.

*Post-exercise hypotension* (PEH) (43) is an acute cardiovascular phenomenon commonly observed after endurance (14, 71) and traditional low-speed strength exercises (10, 14, 71), which may have the potential to positively affect athlete's maximal oxygen consumption ( $VO_2max$ ), ventilatory threshold and hearth rate reserve (57). Regarding plyometric training, only two studies have examined this phenomenon, one showing PEH (5) but not the other (3). Aside from the conflict results on PEH, little is known regarding the effects of plyometric training intensity on cardiovascular responses. Although training intensity during endurance and low-speed strength training have shown to affect cardiovascular responses (8, 31), only one study have compared the acute effects of different intensities of plyometric training on cardiovascular variables in male subjects, reporting no significant differences between low, moderate or high intensity (5). However, the acute effects of plyometric training intensity on cardiovascular responses of female's subjects are unknown.

Although males and females may not exhibit a different acute PEH effect after endurance (16) or low-speed resistance training (74), the possibility exists for an interaction between gender and acute cardiovascular responses to plyometric training (11), considering that the mechanisms may be gender specific (74, 89), probably related with their different gender-hormone environments (20), as estrogen levels may modulate vascular reactivity (64).

## 1.2. Plyometric training: its effect after hand-held loading

Aside from the lack of information regarding the acute effects of plyometric training on cardiovascular responses, the optimal application of plyometric training is yet to be determined. In this sense, new and improved methods of plyometric training are needed, particularly for athletes in need for explosive power, such as soccer players.

Maximal intensity short-duration actions are important determinants of successful performance in both adult (84) and youth soccer (17). Although these actions only represent a small fraction (~10%) (28) of activity during an entire youth soccer game (17), most crucial moments, such as winning ball possession, scoring, or conceding goals depend on them (28, 84). It has been proposed that the high degree of plasticity in neuromuscular development at younger ages combined with timely implementation and progression of adequate neuromuscular training (e.g., supplemental training combining general and specific strength-power exercises, such as jump training exercises) may allow for optimized physical development that contributes favorably to athleticism into adulthood (67), and reduce the risk of injury (85). Therefore, the selection of relevant training methods capable of contributing to the proper development of maximal intensity short-duration soccer activities since the younger categories (60), must be on the forefront of the practitioner's strategies to increase the chance of the player's success (45).

Unloaded (body weight) jumping may be a relevant training stimuli in youth soccer players (25, 55, 61, 62), affecting maximal sport-specific muscle power (60). However, only a few studies offer optimal jump training design in relation to exercise selection for youth soccer players (24, 77, 79). Recently, an acute kinematic and kinetic augmentation of jump performance was observed using haltere type handheld loading (23). The augmentation might be explained by an improvement in the height/distance of the center of mass at takeoff, and its respective velocity (58), an increased jump duration, peak vertical ground reaction force, and vertical and horizontal impulse (23), an improvement in jumping technique (58), or a combination of factors. These findings suggest further longitudinal research is required to determine possible chronic adaptations that may result from repeated exposure to this type of training.

## 1.3. Physiological stressor in soccer

Although plyometric training may induce favourable effects on youth soccer players, may also induce an important stress. If basal (and soccer-related) stress of youth soccer players is not considered, the application of plyometric training (or any other additional or complementary training method) in youth soccer players may impose an unnecessary risk. Indeed, determination of potential stress markers is an important research area in youth soccer that need to be developed.

Hormonal response to sport competition is a controversial matter in sports sciences. The hormones of greatest interest are testosterone (T), which is an anabolic hormone, and cortisol (C), which is a catabolic hormone (90). At the same time, their ratio (T:C) is considered a physiological stress indicator associated to overtraining (38).

Specifically in soccer, the responses of T and C after a match are controversial. Some studies such as Peñailillo et al. (2015) showed a decrease on T, without changes on C (70), whereas others such as Thorpe et al. (2012) describe increases on both hormones (92). The differences between studies

can be explained due to differences in match intensity, biological, psychological, social factors and/or due to the degree or years of training, the latter being highly dependent to the age of the athletes (18). In addition, it has been shown that the hormonal response during exercise might depend on hydration state (86). For instance, it has been observed greater C concentration on hypohydrated subjects before and after running at 70% of maximal oxygen consumption ( $VO_{2max}$ ) in comparison to euhydrated runners (51). However, the relationship of hydration state before a soccer match and its effect on hormonal response after the match in young male soccer players is unknown. Therefore, the aim of this investigation was to assess the effects of hydration level before a soccer match on the T, C, and T:C response after the match in young elite soccer players.

Given the relevance of cardiovascular and explosive performance to the competitive capacity of athletes, particularly youth soccer athletes, together to the few data regarding the acute plyometric training effects on cardiovascular reactivity, acute plyometric training variables manipulation (i.e. hand-held loading), and the pre-training (and competition) physiological stress in young soccer players, the present doctoral thesis is composed of three separate studies (I-III). Therefore, the objectives and hypotheses are as follows:

## **2. OBJECTIVES OF THE THESIS**

Objective I. To compare the acute effects of low intensity (20-cm drop jumps), moderate intensity (30-cm drop jumps), high intensity (40-cm drop jumps) and combined intensity (combination of 20, 30, and 40-cm drop jumps) plyometric training on heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP) and rate-pressure product (RPP) in physically active normotensive male and female subjects.

Objective II. To investigate the effects of a jump training program, with or without haltere type handheld loading, on youth soccer player's performance.

Objective III. To assess the effects of hydration level before a soccer match on the T, C, and T:C response after the match in young elite soccer players.



### **3. HYPOTHESES OF THE THESIS**

Hypothesis I. Plyometric training would induce acute post-exercise hypotension in both groups.

Hypothesis II. Handheld loading will induce greater adaptations to plyometric training.

Hypothesis III. Pre-training hydration level should be considered a complementary stress marker.

## 4. METHODS

### 4.1. Study I

#### 4.1.1. Experimental Approach to the Problem

This study was designed to compare the acute effects of four different plyometric training intensities (i.e., LI, MI, HI and COM) on HR, SBP, DBP and RPP in physically active normotensive (i.e., SBP <120 mmHg; DBP <80 mmHg) male and female subjects. Using a randomized crossover design, the four plyometric intensity trials were conducted with 48-72 h of rest between them. Cardiovascular measures were performed before and also every 10 minutes for 90 minutes after each plyometric trial. Sample size was computed according to the changes observed in SBP ( $d = 10$  mmHg;  $SD = 8$ ) in a group of normotensive male subjects participating in very similar plyometric training intensities as the ones performed in this study (5). A total of 6 participants per group would yield a power of 80% and  $\alpha = 0.05$ .

#### 4.1.2. Subjects

Initially, 22 healthy young (19.6 to 28.4 years of age) male and female (i.e., female were tested during the luteal phase of their menstrual cycle) subjects volunteered for this study. Subjects were recruited from a physical education university course, free of cardiovascular problems confirmed by physician. Subjects were familiar with plyometric exercises as they participate in recreationally sports. Exclusion criteria included participants with (a) potential medical and cardiovascular problems (i.e., including hypertension) or a history of ankle, knee, or back pathology that compromised their participation or performance in the study, (b) any lower extremity reconstructive surgery in the past two years or unresolved musculoskeletal disorders, (c) use of drugs (i.e., including hypertensive drugs) that could influence cardiovascular responses at rest, during or after exercise. To be included in the final analyses, subjects were required to complete all of the familiarization sessions, control and plyometric trial sessions and test measurements, which resulted in 15 subjects (eight females) being included for the final analyses. Descriptive characteristics of participants are provided in Table 1.

Table 1. Descriptive characteristics of participants

	Females (n = 8)	Males (n = 7)	Total (n = 15)
<b>Age (y)</b>	23.4 ± 3.1	23.7 ± 2.1	23.5 ± 2.6
<b>Body mass (kg)</b>	58.6 ± 8.7 <sup>b</sup>	71.4 ± 7.0	64.6 ± 10.4
<b>Height (m)</b>	1.58 ± 0.04 <sup>b</sup>	1.71 ± 0.06	1.64 ± 0.08
<b>Body mass index (kg/m<sup>2</sup>)</b>	23.5 ± 2.9	24.3 ± 1.5	23.8 ± 2.3
<b>Heart rate (beats/min)</b>	76.1 ± 6.7 <sup>b</sup>	63.9 ± 10.2	70.4 ± 10.4
<b>Systolic blood pressure (mmHg)</b>	106.6 ± 5.9 <sup>b</sup>	119.1 ± 6.4	112.5 ± 8.8
<b>Diastolic blood pressure (mmHg)</b>	66.0 ± 4.2	62.9 ± 5.8	64.5 ± 5.1
<b>Rate pressure product<sup>a</sup> (AU)</b>	8124 ± 931	7575 ± 1151	7868 ± 1040

a: the product of heart rate × systolic blood pressure; b: denotes significant difference compared to males ( $p < 0.05$ ); AU: arbitrary unit.

Subjects were informed about the experimental procedures and about possible risks and benefits associated with participation in the study and signed an informed consent before any of the tests

were performed. The study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board for use of human subjects of the University.

#### 4.1.3. Testing Procedures

Participants were familiarized with the cardiovascular test procedures two weeks prior to the initial assessment to reduce a *white-coat* effect. Also, during familiarization sessions standing height (m) and body mass (kg) were assessed according to international standards for anthropometric assessment (52). To evaluate height and body mass, a stadiometer/mechanical scale (SECA, model 220, Germany) with precisions of 0.1 cm and 0.1 kg, respectively, was used. Subjects were tested while wearing light clothing (shoes were removed). The body mass index (BMI) was calculated ( $\text{kg}/\text{m}^2$ ). In addition, during these sessions subjects were instructed in the proper form and technique of the plyometric exercises to be used.

Cardiovascular measurements were always administered in the same order, at the same time of the day (i.e., between 13:00 and 15:00 h) and by the same experimented investigator, who was blinded to the trial group of the participants. Participants were instructed to maintain their sleeping, eating and drinking habits during the study process. Also, participants were instructed to take a light meal (i.e., <500 kcal) two hours before experimental trials, to avoid physical exercise and alcohol for at least 48 h, and to avoid smoking, caffeine, or other substances (e.g., energetic or caffeinated drinks, Guarana or ginseng related supplements) that could influence cardiovascular responses at rest, during or after exercise for  $\geq 12$  h before trials. Tests were conducted indoors, in a calm environment without noise, with a room temperature and humidity of approximately 25°C and 50%, respectively. Throughout testing, an investigator subject ratio of 1:1 was maintained. During measurements, subjects maintained a sitting position and used a spontaneous breathing pattern. For resting blood pressure, two measurements were taken after ten min of seated rest in five visits to the laboratory. A mean value of the ten measurements was calculated (74).

*Systolic and diastolic blood pressure.* For SBP (mmHg) and DBP (mmHg) measurements, a digital blood pressure monitor was used (Omron®, HEM-742, Japan), following a previously described protocol (72).

*Heart rate and rate pressure product.* For HR (beats/min) measurements, a cardiac monitor (Polar®, RS200, Finland) was used, following previously established criteria (11). The RPP was calculated as  $\text{HR (bpm)} \times \text{SBP (mmHg)}$ , which is considered a reliable predictor of myocardial oxygen demand (3, 4). RPP is represented as a single number of magnitude in arbitrary units.

#### 4.1.4. Trials

The plyometric trials (i.e., LI, MI, HI, COM) were based on previous recommendations (3, 5), where intensity has been defined as the amount of landing force after a drop jump (5, 50), thus, with a higher drop jump height, the intensity would be increased (41, 50).

A 10 min standard warm-up (i.e., sub maximal running with change of direction in a 40-m indoor track, 20 vertical and 10 horizontal sub-maximal jumps) was executed before each trial. During each plyometric trial, six sets of 20 valid drop jump repetitions were completed. A 20-cm, 30-cm, and 40-cm height box was used during LI, MI and HI plyometric trails, respectively. For the COM

trial, the three height boxes were randomly used, and two sets of 20 repetitions were completed from each box to equal volume between trials. Participants were instructed to use the same athletic shoes and clothes during all trials. Plyometric exercises were completed on a wooden floor. Rest intervals of 15 s (83) and 120 s (5) between repetitions and sets were used during plyometric trials, respectively. Each plyometric trial lasted approximately 50 min.

Aside from the drop height, the same technique was used for all plyometric exercises. Basically, subjects began by standing on a box with their arms akimbo and then step off the box with the supporting leg straight to avoid any initial upward propulsion or sinking, ensuring a drop height of 20, 30, or 40 cm (i.e., depending on the trial protocol). Upon landing with two feet on the ground, participants were instructed to maximize jump height and minimize ground contact time (i.e., bounce drop jump). Take-off and landing were standardized to full knee and ankle extension on the same spot. To assure that subjects performed exercises with maximal volitional effort, jump height and contact time during the plyometric exercises repetitions were measured with an electronic contact mat system (Ergojump, Globus®, Codogne, Italy) and the reactive strength index (RSI) was calculated (27). The reliability of this measure has been described elsewhere (27, 54). Valid repetitions were considered if participants achieved  $\geq 80\%$  of their maximal RSI. All exercises were supervised to assure proper technique with an investigator to participant ratio of 1:1, and particular attention was paid to demonstration and execution, giving maximal motivation to participants during each jump. Four basic techniques were stressed during jumps i) correct posture (i.e., spine erect and shoulders back) and body alignment (e.g., chest over knees), ii) jumping straight up with no excessive side-to side or forward-backward movement, iii) instant recoil for the concentric part of the jump. Phrases such as “on your toes”, “straight as a stick”, “light as a feather” and “recoil like a spring” were used as verbal and visualization cues during jumps.

#### 4.1.5. Statistical Analyses

All values are reported as mean  $\pm$  standard deviation (SD). Normality and homoscedasticity assumptions were checked with Shapiro-Wilk and Levene tests, respectively. For variables not normally distributed, logarithmization procedures were performed. To determine the acute effect of trials on dependent variables, a two-way variance analysis with repeated measurements (four trials  $\times$  two times) was performed. When a significant F value was achieved across time or between trials, Bonferroni post hoc procedures were performed to locate the pairwise differences between the means. The  $\alpha$  level was set at  $p < 0.05$  for statistical significance. All statistical calculations were performed using STATISTICA statistical package (Version 8.0; StatSoft, Inc, Tulsa). Data on cardiovascular measurements obtained during a control trial was used to determine reliability through the intraclass correlation coefficient (ICC). A ICC below 0.40 was considered poor, 0.40 to 0.59 fair, 0.60 to 0.74 good, and 0.75 to 1.00 excellent (48). We obtained excellent ICC for the different cardiovascular measurements.

## 4.2. Study II

### 4.2.1. Participants

Sixty-three youth male soccer players participated in this study, randomly (i.e., computer-based) assigned to a jump training group (JG, n = 21), a HTHL jump training group (LJG, n = 21) or a control group following only soccer training (CG, n = 21). Exclusion criteria included participants with (a) potential medical problems or a history of ankle, knee, or back pathology that compromised their participation or performance in the study, and (b) any lower extremity reconstructive surgery in the past two years or unresolved musculoskeletal disorders. Although participants were between ten and sixteen years of age, no significant differences were noted between group characteristics at baseline (Table 2) and developmental stages (i.e., maturity status), which were determined using predicted years from age of peak height velocity (i.e., PHV offset) (65), were equally distributed among groups.

Table 2. Descriptive data of the control group (CG; n = 21), jump training group (JG; n = 21), and hand-held loaded jump training group (LJG; n = 21).

	CG	JG	LJG
Age (y)	12.0 ± 2.2	12.3 ± 2.3	12.1 ± 2.1
Height (cm)	151 ± 15.1	150 ± 14.9	150 ± 13.6
Sit height (cm)	80.7 ± 9.0	78.5 ± 7.6	79.1 ± 7.4
Body mass (kg)	47.8 ± 13.0	47.3 ± 11.9	45.0 ± 12.4
Body muscle (%)	50.1 ± 6.9	50.4 ± 6.7	49.6 ± 7.7
Bone mass (kg)	9.8 ± 1.9	9.6 ± 1.7	9.9 ± 1.8
Session rating of perceived exertion	411 ± 176	335 ± 148	376 ± 175
Soccer experience (y)	3.8 ± 1.8	3.9 ± 2.5	3.9 ± 2.1
Weekly non-soccer sport practice (h)	1.7 ± 1.7	1.8 ± 1.9	1.5 ± 2.0
Weekly physical education-related activity (min/week <sup>-1</sup> )	106 ± 35.6	109 ± 29.8	108 ± 31.1

Participants (and their respective parents or guardians) were informed about the experimental procedures, possible risks, and benefits associated with participation in the study. They then signed informed assent and consent forms, respectively, before performing any of the tests and training sessions. The present study is in accordance with the ethical standards of the International Journal of Sports Medicine (37) and was approved by the ethical review board for use of human participants from the responsible institutional department.

### 4.2.2. Experimental design

Participants were familiarized with the test procedures two weeks prior to the initial assessment. Measurements were undertaken one week before and after the intervention. Athletes underwent seventy two hours of rest between the last training session and measurement session. Also, no competitive games were performed the week preceding performance measurements. Tests were completed under highly standardized conditions. Throughout testing, an investigator participant ratio of 1:1 was maintained. Ten minutes of standard warm-up was executed before testing.

The standing height, sitting height, body mass, body composition, right leg horizontal countermovement jump with arms (RHCMJA), left leg horizontal countermovement jump with arms (LHCMJA), horizontal countermovement jump with arms (HCMJA), vertical countermovement jump with arms (VCMJA), vertical twenty centimeter drop jump reactive strength index (VDJ20), and the maximal kicking velocity (MKV) test were completed. Anthropometric measurements were obtained using a stadiometer (Bodometer 206; SECA ®, Hamburg, Germany) and an electrical scale (BF100\_Body Complete ®; Beurer, Ulm, Germany).

The horizontal jump tests were completed with arm swings and measured to the nearest centimeter using a tape measure secured to the floor. The vertical jump tests were performed as previously described (77), on an electronic contact mat system (Ergojump; Globus ®, Codogne, Italy). Athletes were instructed to maximize jump height and to use their arms during jumps; except for the VDJ20, where participants kept their arms akimbo and were instructed to maximize jump height and minimize ground contact time after dropping down from a twenty centimeter drop box.

For the MKV test a previously described protocol was used (79). Participants performed a maximal instep kick with their dominant leg after a run up of two strides, using a size five soccer ball (Nike Seitiro ®, FIFA certified). Maximal velocity was measured using a radar gun (Speed Gun SR3600; Sports Radar ®, Homosassa, FL). Participants were given two practice and three valid maximal trials for all jump and kicking tests, with one minute of rest between trials.

### 4.2.3. Training protocol

The current experiment was completed during the in-season. Athletes in the JG and LJG performed jump drills as a substitute for some soccer drills, twice per week for six weeks. The CG maintained its typical soccer training schedule. Before (and during) intervention, participants were instructed regarding correct jump exercises technique. All training sessions were supervised using a trainer to player ratio of 1:4. The jump drills were performed immediately after the warm-up and separated with a minimum of forty-eight hours (including games).

The JG and the LJG performed the same unipedal and bipedal horizontal and vertical exercises using arm swing during jumps, and combining cyclic and acyclic jumps. Participants were asked to achieve maximal vertical height and horizontal distance for acyclic jumps, and with minimal ground contact time for cyclic jumps. Maximal intensity during training was verified in a randomly assigned subsample of participants (two from each jump group;  $n = 4$ ) during two randomly assigned training sessions by measuring contact times, height, and distance of jumps drills using the same procedures as those used in testing. A detailed description of the training program is depicted in Table 3.

Table 3. Six week jump training program.

Exercises	Set × Repetitions (mode of execution)		
	Week 1-2	Week 3-4	Week 5-6
Horizontal left leg	2 × 4 (C)	2 × 6 (C)	2 × 8 (C)
	2 × 4 (A)	2 × 6 (A)	2 × 8 (A)
Horizontal right leg	2 × 4 (C)	2 × 6 (C)	2 × 8 (C)
	2 × 4 (A)	2 × 6 (A)	2 × 8 (A)
Vertical left leg	2 × 4 (C)	2 × 6 (C)	2 × 8 (C)
	2 × 4 (A)	2 × 6 (A)	2 × 8 (A)
Vertical right leg	2 × 4 (C)	2 × 6 (C)	2 × 8 (C)
	2 × 4 (A)	2 × 6 (A)	2 × 8 (A)
Bipedal vertical	2 × 4 (C)	2 × 6 (C)	2 × 8 (C)
	2 × 4 (A)	2 × 6 (A)	2 × 8 (A)
Bipedal horizontal	2 × 4 (C)	2 × 6 (C)	2 × 8 (C)
	2 × 4 (A)	2 × 6 (A)	2 × 8 (A)

C: cyclic; A: acyclic.

The order of exercises was randomized in each training session. The JG and the LJG completed the same amount of total jumps, with the same rest intervals between jumps (i.e., fifteen seconds for acyclic jumps) and series (i.e., sixty seconds), used the same surface for training, and trained at the same time of the day. The only difference between jump training groups was the HTHL used by the LJG during acyclic jumps.

The technique for the acyclic HTHL jumps used in this study has been previously described in detail (23), involving maximum effort jumps with the respective optimal haltere loads (i.e., 0 to ~15% of participants body mass - total/cumulative loads for both hands) (23). For the horizontal jumps, the participants were instructed to swing the loads up to “head-height” at the termination of the propulsive phase and to jump “as far as possible.” For the vertical jumps the participants were instructed to swing the loads up to “over head-height” at the termination of the propulsive phase and to jump “as high as possible”. For bipedal and unipedal jumps, participants landed on both feet and one foot, respectively.

The session rating of perceived exertion was determined during intervention, as previously described (40), and is reported in Table 2. Total training load was calculated as rating of perceived exertion × training session duration (i.e., minutes). Although we did not control for the potential

influence of age on session rating of perceived exertion (33), as the age (Table 2) and PHV offset (65) were equally distributed among groups, this potential confounding variable was minimized.

#### 4.2.4. Statistical analysis

Statistical analyses employed the STATISTICA statistical package (Version 8.0; StatSoft, Inc., Tulsa). All values are reported as the means  $\pm$  standard deviations. Relative changes (%) in performance and Cohen's d effect sizes (ES) are expressed with 90% confidence limits. Normality assumptions made for all data before and after intervention were assessed using the Kolmogorov-Smirnov test. Groups were compared using two-way ANOVA with repeated measures, with Tukey post hoc analysis. In addition, a between-groups one-way ANOVA compared performance changes (i.e., the difference between scores before and after the intervention). The  $\alpha$  level was set at  $p < 0.05$  for statistical significance. Threshold values for ES were 0.20, 0.60, 1.2, and 2.0 for small, moderate, large, and very large, respectively (39). For the magnitudes of differences in training effects between groups, if the confidence interval overlapped thresholds for substantial positive and negative values, the effect was deemed unclear (i.e., trivial). The effect was otherwise clear and reported as the magnitude of the observed value with a qualitative probability as noted above (i.e., small, moderate, large, and very large) (39).

### 4.3. Study III

#### 4.3.1. Participants

Seventeen male soccer players (age:  $16.8 \pm 0.4$  years; body mass:  $67.5 \pm 7.5$  kg; height:  $173 \pm 6.8$  cm;  $VO_2\text{max}$ :  $57.2 \pm 3.6$  ml/kg<sup>-1</sup>/min<sup>-1</sup>), from a South American under-17 (U17) soccer national team, participated in this study. According to the hydration level assessed by the urine specific gravity (USG) before the matches, the subjects were divided for the analysis into two groups: well-hydration (WH) (USG  $< 1.010$  g/mL<sup>-1</sup>, n=9) and mild dehydration (MD) (USG from 1.010 to 1.020 g/mL<sup>-1</sup>, n=8) (15). Injured players and goalkeepers were excluded. The legal guardians of the players signed an informed consent, while the players give their verbal assent, after the potential benefits, and risks were explained to them. The study was developed in accordance to the latest version of the Helsinki Declaration, and was approved by the Ethics Committee of the responsible Institution.

#### 4.3.2. Measurements

The evaluated soccer team was divided into two teams (A and B). The first match (team A) was played at 11:00 am, and the second match (team B) at 11:30 pm (the same day), in preparation for the FIFA U17 World Championship 2015, carried out in Chile. Climatic conditions were similar between matches. The both friendly matches were played against a professional soccer team of the Chilean professional league. The assessed teams won both matches (first match, 2-0; second match, 3-1). During the two matches, the players were asked to play as it was an official match. The matches follow international rules (FIFA). The USG was assessed 30 minutes before the match with a portable Refractometer (Robinair, model SPX, USA) in triplicate according to previous suggestions (19). Nutritional recommendations were not made prior to matches and during the friendly matches players consumed water ad libitum. According to Peñailillo et al. (2015) (70), for the assessment of the T and C, saliva was collected 30 minutes before each match (Pre-), and 5-10



minutes after each match (Post-). Briefly, the players were sat, with their eyes open, their head slightly tilted forward and making minimal orofacial movement. All saliva ( $\pm 3$  ml) was collected for about two minutes. The saliva samples were centrifuged at 1,500 g for 15 minutes and stored at  $-20^{\circ}$  C until analysis. The T and C were determined by enzyme immunoassay using a commercial kit (Salimetrics, PA, USA). The optical density was determined with a microplate reader (Multiskan, Thermo®) at 450 nm. All analyses were performed in duplicate according to the manufacturer's procedures. The intra-assay coefficient of variation was 2.5% and 2.8% for the T and C, respectively. Only players that played  $>80$  minutes were considered. The mean (HR<sub>mean</sub>) and maximal (HR<sub>max</sub>) heart rate was measured throughout the match using the Polar Team® system. The heart rate values were reported as relative values, according to age-expected maximum values (220-age).

#### **4.3.3. Statistical analysis**

Data is shown as mean  $\pm$  standard deviation (SD). The normality of the data was analyzed by the Shapiro-Wilk test, showing that data was normally distributed. An unpaired T-test was used to compare hydration level and HR during matches between groups. A two-way ANOVA was used for the comparison of the T, C and T:C between WH and MD groups, with a Tukey post-hoc test when significant main effect was found. The alpha value was set at  $p < 0.05$ . Statistical analyses were performed in GraphPad Prism® 6.0 (Graphpad Software, San Diego, CA, USA).

## 5. RESULTS

### 5.1. Study I

A significant ( $P < 0.05$ ) mean reduction of HR was observed (Table 4) after COM trial in male, in addition to a significant ( $P < 0.05$ ) mean reduction of SBP and DBP after all plyometric intensities (except for SBP and DBP after MI and LI, respectively) (Table 4). Also, male subjects showed a mean reduction of RPP after all plyometric intensities, although achieve significance ( $P < 0.05$ ) only after LI and COM trials (Table 4).

Table 4. Males ( $n = 7$ ) - acute cardiovascular responses before and after different intensities of plyometric training.

	Before	After			Mean
		10 min	60 min	90 min	
<b>HR (beats/min)</b>					
Low intensity	66.3 ± 11.3	70.1 ± 12.1	66.7 ± 7.0	63.0 ± 6.7	65.5 ± 7.1
Moderate intensity	74 ± 14.3	83.4 ± 14.6 <sup>a</sup>	72.2 ± 12.4 <sup>b, c</sup>	70.9 ± 11.9 <sup>b</sup>	68.3 ± 9.1
High intensity	73.5 ± 10.4	83.7 ± 11.1 <sup>a</sup>	71.3 ± 8.4 <sup>b</sup>	70.6 ± 9.2 <sup>b</sup>	69.4 ± 4.7
Combined	78.9 ± 10.6	82.7 ± 13.0	71.3 ± 8.1 <sup>b</sup>	69.9 ± 11.1 <sup>a, b</sup>	67.3 ± 2.8 <sup>a</sup>
<b>SBP (mmHg)</b>					
Low intensity	118.3 ± 10.3	113.3 ± 10.4	107.5 ± 8.4 <sup>a</sup>	110.9 ± 9.7	113.9 ± 8.8 <sup>a</sup>
Moderate intensity	116.3 ± 12.1	114.2 ± 10.3	107.3 ± 9.5	109.3 ± 8.2	109.2 ± 7.9
High intensity	119.5 ± 8.9	113.3 ± 11.1	106.6 ± 11.1 <sup>a</sup>	107.9 ± 9.2 <sup>a</sup>	116.5 ± 7.0 <sup>a</sup>
Combined	118.9 ± 10.8	113.6 ± 11.0	107.7 ± 10.2 <sup>a</sup>	109.3 ± 9.0 <sup>a</sup>	116.1 ± 9.7 <sup>a</sup>
<b>DBP (mmHg)</b>					
Low intensity	66.2 ± 6.2	66.9 ± 6.0	65.7 ± 7.4	68.5 ± 6.4	64.5 ± 4.7
Moderate intensity	70.3 ± 5.1	69.0 ± 5.6	64.5 ± 5.2	67.3 ± 4.7	65.3 ± 4.5 <sup>a</sup>
High intensity	70.4 ± 6.1	68.1 ± 4.7	65.9 ± 6.9	66.9 ± 6.1	65.4 ± 4.8 <sup>a</sup>
Combined	70.2 ± 7.0	68.9 ± 4.4	65.5 ± 4.0	66.6 ± 5.9	66.1 ± 4.9 <sup>a</sup>
<b>Rate pressure product (AU)</b>					
Low intensity	8619 ± 1418	8965 ± 1874	7582 ± 1221	7615 ± 1228	7487 ± 1212 <sup>a</sup>
Moderate intensity	8607 ± 1923	9470 ± 1561	7716 ± 1314 <sup>b</sup>	7719 ± 1239 <sup>b</sup>	7494 ± 1824
High intensity	8785 ± 1408	9441 ± 1188	7569 ± 1007 <sup>b</sup>	7607 ± 1091 <sup>b</sup>	8099 ± 773
Combined	9406 ± 1313	9317 ± 1321	7557 ± 824 <sup>a, b</sup>	7479 ± 1068 <sup>a, b</sup>	7739 ± 785 <sup>a</sup>

<sup>a, b, c</sup>; denotes significant ( $p < 0.05$ ) difference compared with Before, 10 min and 20 min, respectively; HR: heart rate; SBP: systolic blood pressure; DBP: diastolic blood pressure; AU: arbitrary unit.

Similarly, in female subjects a significant ( $P<0.05$ ) mean reduction of HR was observed (Table 5) after COM trial, in addition to a significant ( $P<0.05$ ) mean reduction of SBP and DBP after all plyometric intensities (except for DBP after LI and COM) (Table 5). A significant ( $P<0.05$ ) mean reduction of RPP was observed after all plyometric intensities (except after LI) (Table 5).

Table 5. Females (n = 8) acute cardiovascular responses before and after different intensities of plyometric training.

	Before	After				Mean
		10 min	60 min	90 min		
<b>HR (beats/min)</b>						
Low intensity	79.0 ± 8.8	87.5 ± 16.6	74.0 ± 12.9 <sup>b, c, d</sup>	74.3 ± 13.3 <sup>b, c, d</sup>	78.5 ± 13.6	
Moderate intensity	81.5 ± 11.5	90.4 ± 13.8	78.8 ± 11.6 <sup>b</sup>	75.6 ± 12.3 <sup>b</sup>	80.2 ± 10.7	
High intensity	76.8 ± 5.9	86.6 ± 5.9 <sup>a</sup>	74.1 ± 5.1 <sup>b</sup>	73.3 ± 7.7 <sup>b</sup>	77.2 ± 11.1	
Combined	83.5 ± 10.4	89.0 ± 13.7	76.3 ± 8.0 <sup>b</sup>	76.1 ± 11.2 <sup>b</sup>	79.2 ± 10.2 <sup>a</sup>	
<b>SBP (mmHg)</b>						
Low intensity	113.8 ± 9.3	107.9 ± 3.1	101.8 ± 4.6 <sup>a</sup>	105.9 ± 7.4	104.4 ± 4.3 <sup>a</sup>	
Moderate intensity	110.0 ± 6.8	109.1 ± 7.9	102.0 ± 5.7	104.1 ± 5.2	103.5 ± 4.2 <sup>a</sup>	
High intensity	115.1 ± 7.6	105.4 ± 6.8 <sup>a</sup>	99.6 ± 10.0 <sup>a</sup>	101.8 ± 5.9 <sup>a</sup>	101.3 ± 5.2 <sup>a</sup>	
Combined	113.3 ± 8.2	107.1 ± 6.7	102.0 ± 8.3 <sup>a</sup>	104.9 ± 6.9 <sup>a</sup>	102.8 ± 6.7 <sup>a</sup>	
<b>DBP (mmHg)</b>						
Low intensity	68.3 ± 6.1	67.6 ± 6.7	65.9 ± 7.4	69.8 ± 7.5	67.2 ± 5.9	
Moderate intensity	69.6 ± 4.9	70.6 ± 6.5	65.1 ± 3.7	68.6 ± 4.5	67.2 ± 3.4 <sup>a</sup>	
High intensity	69.8 ± 6.7	67.5 ± 5.3	65.4 ± 7.7	65.5 ± 5.1	65.4 ± 4.8 <sup>a</sup>	
Combined	69.1 ± 7.9	68.3 ± 5.3	64.4 ± 3.8	67.6 ± 5.3	65.9 ± 3.9	
<b>Rate pressure product (AU)</b>						
Low intensity	8982 ± 1225	9440 ± 1828	7528 ± 1358 <sup>a, b, c, d</sup>	7832 ± 1343 <sup>b</sup>	8190 ± 1398	
Moderate intensity	8982 ± 1523	9825 ± 1457	8047 ± 1352 <sup>b</sup>	7862 ± 1250 <sup>b</sup>	8309 ± 1124 <sup>a</sup>	
High intensity	8884 ± 1895	9095 ± 1307	7376 ± 1259 <sup>a, b</sup>	7444 ± 1043 <sup>a, b</sup>	7800 ± 1013 <sup>a</sup>	
Combined	9463 ± 1446	9525 ± 159	7759 ± 914 <sup>a, b</sup>	7932 ± 912 <sup>a, b</sup>	8129 ± 1029 <sup>a</sup>	

<sup>a, b, c, d, e</sup>: denotes significant ( $p<0.05$ ) difference compared with Before, 10 min, 20 min, 30 min and 40 min values, respectively; HR: heart rate; SBP: systolic blood pressure; DBP: diastolic blood pressure; AU: arbitrary unit.

When data of male and female subjects was combined, a similar pattern of results was observed, with a significant ( $P<0.05$ ) mean reduction of HR after COM trial (Table 6), in addition to a significant ( $P<0.05$ ) mean reduction of SBP and DBP after all plyometric intensities (except for DBP after LI) (Table 6). Also, a significant ( $P<0.05$ ) mean reduction of RPP was observed after all plyometric intensities (except after MI) (Table 6).

Table 6. Participants ( $n = 15$ ) acute cardiovascular responses before and after different intensities of plyometric training.

	Before	After			Mean
		10 min	60 min	90 min	
<b>HR (beats/min)</b>					
Low intensity	73.1 ± 11.7	79.4 ± 16.8 <sup>a</sup>	70.6 ± 10.9 <sup>b</sup>	69.0 ± 15.0 <sup>b, c, d</sup>	72.4 ± 12.6
Moderate intensity	74.0 ± 14.3	83.3 ± 14.6 <sup>a</sup>	72.2 ± 12.4 <sup>b, c</sup>	70.9 ± 11.9 <sup>b, c</sup>	74.6 ± 11.4
High intensity	73.5 ± 10.4	83.7 ± 11.1 <sup>a</sup>	71.3 ± 8.4 <sup>b, c</sup>	70.6 ± 9.2 <sup>b, c</sup>	73.6 ± 9.3
Combined	78.9 ± 10.6	82.7 ± 13.0	71.3 ± 8.1 <sup>a, b</sup>	69.9 ± 11.1 <sup>a, b, c</sup>	73.7 ± 9.6 <sup>a</sup>
<b>SBP (mmHg)</b>					
Low intensity	118.3 ± 10.3	113.3 ± 10.4	106.7 ± 6.1 <sup>a</sup>	110.9 ± 9.7 <sup>a</sup>	108.9 ± 8.2 <sup>a</sup>
Moderate intensity	116.3 ± 12.1	114.2 ± 10.3	107.3 ± 9.5 <sup>a, b</sup>	109.3 ± 8.2 <sup>a</sup>	106.2 ± 13.9 <sup>a</sup>
High intensity	119.5 ± 8.9	113.3 ± 11.1 <sup>a</sup>	106.6 ± 11.1 <sup>a, b</sup>	107.9 ± 9.2 <sup>a</sup>	108.4 ± 9.8 <sup>a</sup>
Combined	118.9 ± 10.8	113.6 ± 11.0	107.7 ± 10.2 <sup>a</sup>	109.3 ± 9.0 <sup>a</sup>	102.8 ± 6.7 <sup>a</sup>
<b>DBP (mmHg)</b>					
Low intensity	66.2 ± 6.2	66.9 ± 6.0	65.7 ± 7.4	68.5 ± 6.4	66.0 ± 5.4
Moderate intensity	70.3 ± 5.1	69.0 ± 5.6	64.5 ± 5.2 <sup>a</sup>	67.3 ± 4.7	66.3 ± 3.9 <sup>a</sup>
High intensity	70.4 ± 6.1	68.1 ± 4.7	66.9 ± 6.9	66.9 ± 6.1	66.1 ± 4.8 <sup>a</sup>
Combined	70.2 ± 7.0	68.9 ± 4.4	65.5 ± 4.0	66.6 ± 5.9	65.9 ± 3.9 <sup>a</sup>
<b>Rate pressure product (AU)</b>					
Low intensity	8619 ± 1448	8304 ± 1643	7582 ± 1221 <sup>a, b</sup>	7615 ± 1228 <sup>a, b</sup>	7862 ± 1318 <sup>a</sup>
Moderate intensity	8607 ± 1923	9470 ± 1561 <sup>a</sup>	7716 ± 1314 <sup>b, c</sup>	7719 ± 1239 <sup>a, b, c</sup>	7929 ± 1495
High intensity	8758 ± 1408	9441 ± 1188	7569 ± 1007 <sup>a, b, c</sup>	7607 ± 1091 <sup>a, b, c</sup>	7940 ± 890 <sup>a</sup>
Combined	9406 ± 1313	9317 ± 1321	7557 ± 824 <sup>a, b</sup>	7479 ± 1068 <sup>a, b, c</sup>	7947 ± 913 <sup>a</sup>

<sup>a, b, c, d, e</sup>: denotes significant ( $p<0.05$ ) difference compared with Before, 10 min, 20 min, 30 min and 40 min values, respectively; HR: heart rate; SBP: systolic blood pressure; DBP: diastolic blood pressure; AU: arbitrary unit.

The intensity × group effect analyses indicate no significant differences at pre or post-exercise (at any time point) for HR, SBP, DBP or RPP when LI, MI, HI or COM trials were compared in males (Table 4), females (Table 5) or males-females combined (Table 6).

No significant differences were observed for mean relative (%) change between male and female subjects (Table 7), except for a significantly higher SBP reduction in females compared to males after HI plyometric training (-12% and -7%, respectively;  $P < 0.05$ ).

Table 7. Mean<sup>a</sup> acute effects (with 90% confidence limits) for cardiovascular variables in males (n = 7), females (n = 8) and all (n = 15) subjects after low (LI), moderate (MI), high (HI) or combined (COM) intensity plyometric training

	LI	MI	HI	COM
<b>Heart rate</b>				
All	-0.4 (-5.3, 4.3)	2.0 (-2.2, 6.1)	0.7 (-3.6, 5.1)	-6.3 (-9.6, -3.1)
Males	-0.1 (-6.4, 6.2)	5.6 (-1.3, 12.5) <sup>c</sup>	0.1 (-7.9, 8.1)	-7.8 (-13.9, -1.8)
Females	-0.8 (-9.2, 7.6)	-1.3 (-6.7, 4.2)	1.3 (-4.7, 7.2)	-5.0 (-9.3, -0.8)
<b>Systolic blood pressure</b>				
All	-7.8 (-9.7, -5.9)	-4.8 (-8.3, -1.3)	-9.3 (-11.7, -6.9)	-8.2 (-10.5, -6.0)
Males	-7.7 (-9.8, -5.5)	-3.7 (-11.9, 4.5)	-6.5 (-8.4, -4.6)	-7.3 (-11.7, -2.8)
Females	-7.9 (-11.4, -4.3)	-5.8 (-8.1, -3.4)	-11.8 (-15.7, -7.9) <sup>d</sup>	-9.1 (-11.9, -6.3)
<b>Diastolic blood pressure</b>				
All	0.1 (-3.8, 4.0)	-5.4 (-8.3, -2.4)	-5.8 (-8.5, -3.0)	-5.5 (-8.7, -2.4)
Males	1.6 (-6.1, 9.3)	-7.7 (-13.2, -2.2)	-5.7 (-8.5, -3.0)	-7.2 (-12.3, -2.1)
Females	-1.3 (-5.9, 3.4)	-3.3 (-6.7, 0.1)	-5.8 (-11.1, -0.6)	-4.1 (-8.7, 0.5)
<b>Rate pressure product</b>				
All	-8.0 (-13.6, -2.4)	-2.5 (-9.3, 4.4) <sup>c</sup>	-8.4 (-13.8, -2.9)	-15.0 (-18.1, -11.9)
Males	-7.7 (-14.5, -0.9)	2.6 (-12.2, 17.4) <sup>c</sup>	-6.2 (-14.7, 2.4)	-16.7 (-21.4, -12.0)
Females	-8.3 (-18.3, 1.8)	-6.9 (-11.8, -1.9)	-10.3 (-18.8, -1.8)	-13.6 (-18.4, -8.8)

<sup>a</sup>: mean relative (%) change across 90 min post-exercise; <sup>c</sup>: denotes significant difference compared with COM ( $p < 0.05$ ); <sup>d</sup>: denotes significant difference compared with males.

## 5.2. Study II

The intraclass correlation coefficient and coefficient of variation for the RHCMJA (0.94 and 4.1%, respectively), LHCMJA (0.94 and 4.5%, respectively), HCMJA (0.95 and 3.8%, respectively), VCMJA (0.98 and 3.0%, respectively), VDJ20 (0.91 and 5.9%, respectively), and MKV test (0.93 and 4.0%, respectively) suggest high measurement reliability for all dependent variables.

No significant differences were noted between groups' descriptive data (Table 2). Additionally, before training, no significant differences were observed between groups in RHCMJA, LHCMJA, HCMJA, VCMJA, VDJ20, and MKV (Table 8).

Table 8. Training effects for the performance variables for the control group (CG; n = 21), jump training group (JG; n = 21), and hand-held loaded jump training group (LJG; n = 21).

	Baseline Mean ± SD	Performance change (%)	Magnitude of training effect
<b>Right leg horizontal countermovement jump with arms (cm)</b>			
CG	135 ± 22.7	2.6 (-2.1, 7.6)	0.16 (-0.13, 0.45)
JG	137 ± 30.7	6.3 (4.0, 8.8) <sup>a</sup>	0.28 (0.18, 0.39) <sup>*</sup>
LJG	129 ± 26.0	10.1 (7.6, 12.6) <sup>c,d</sup>	0.45 (0.34, 0.56) <sup>*</sup>
<b>Left leg horizontal countermovement jump with arms (cm)</b>			
CG	134 ± 27.7	3.8 (-0.5, 8.3)	0.18 (-0.03, 0.39)
JG	137 ± 33.5	7.7 (5.8, 9.7) <sup>b</sup>	0.32 (0.24, 0.39) <sup>*</sup>
LJG	130 ± 32.4	12.1 (8.2, 16.2) <sup>c,d</sup>	0.47 (0.32, 0.62) <sup>*</sup>
<b>Horizontal countermovement jump with arms (cm)</b>			
CG	156 ± 24.8	2.7 (-0.9, 6.4)	0.18 (-0.06, 0.42)
JG	159 ± 35.7	6.1 (4.1, 8.2) <sup>b</sup>	0.28 (0.19, 0.37) <sup>*</sup>
LJG	155 ± 30.9	7.7 (5.6, 9.9) <sup>c,d</sup>	0.37 (0.27, 0.47) <sup>*</sup>
<b>Countermovement jump with arms (cm)</b>			
CG	31.4 ± 6.3	1.5 (-0.2, 3.1)	0.08 (-0.01, 0.16)
JG	31.7 ± 9.0	4.3 (3.0, 5.6) <sup>c</sup>	0.26 (0.21, 0.30) <sup>*</sup>
LJG	29.8 ± 7.3	7.2 (5.6, 8.8) <sup>c,d</sup>	0.26 (0.21, 0.32) <sup>*</sup>
<b>20 cm drop jump reactive strength index (mm/ms<sup>-1</sup>)</b>			
CG	1.19 ± 0.4	2.6 (-1.0, 6.3)	0.06 (-0.03, 0.15)
JG	1.05 ± 0.4	8.8 (6.9, 10.7) <sup>c,d</sup>	0.20 (0.16, 0.24) <sup>*</sup>
LJG	0.95 ± 0.5	19.0 (13.3, 25.0) <sup>c,d,e</sup>	0.37 (0.27, 0.48) <sup>*</sup>
<b>Maximal kicking velocity (km/h<sup>-1</sup>)</b>			
CG	61.9 ± 14.7	4.0 (2.9, 5.1) <sup>c</sup>	0.15 (0.11, 0.20)
JG	64.7 ± 15.9	6.8 (5.6, 8.0) <sup>c,d</sup>	0.27 (0.22, 0.32) <sup>*</sup>
LJG	60.8 ± 13.3	8.3 (7.4, 9.2) <sup>c,d</sup>	0.34 (0.31, 0.38) <sup>*</sup>

\* small standardized effect; <sup>a, b, c</sup>: denote significant difference pre to post training (p<0.05, p<0.01 and p<0.001, respectively); <sup>d</sup>: denote significant difference with the CG post training (p<0.05); <sup>e</sup>: denote significant difference with the JG post training (p<0.05). Values in brackets represent 90% confidence limits.

After intervention, the CG exclusively achieved a performance improvement in MKV (Table 8). On the other hand, the JG and the LJG exhibited performance improvements in all dependent variables (Table 8). Nevertheless, compared with the CG, only the LJG exhibited greater performance improvements in all dependent variables. Moreover, in the VDJ20, the LJG exhibited greater performance improvements compared with the JG (Table 8).

### 5.3. Study III

In A team were found 5 players in WH condition and 4 players in MD condition and in B team were found 4 players in WH condition and 4 players in MD condition. As expected, the USG was lower ( $p < 0.0001$ ) in the WH group ( $1.006 \pm 0.002 \text{ g/mL}^{-1}$ ) compared to the MD group ( $1.014 \pm 0.002 \text{ g/mL}^{-1}$ ). No differences were found between groups in basal characteristics (age: WH  $16.3 \pm 0.7$  and MD  $16.9 \pm 0.5$  years,  $p = 0.61$ ; body mass: WH  $68.5 \pm 8.6$  and MD  $67.3 \pm 8.4$  kg,  $p = 0.68$ ; height: WH  $171 \pm 9.1$  and MD  $175 \pm 8.0$  cm,  $p = 0.21$ ;  $\text{VO}_2\text{max}$ : WH  $56.3 \pm 3.8$  and MD  $57.8 \pm 7.2 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ,  $p = 0.74$ ). Neither were found differences between groups in HRmean (WH  $83.1 \pm 4.7\%$  and MD  $87.0 \pm 4.1\%$ ;  $p = 0.12$ ) or HRmax (WH  $93.2 \pm 4.4\%$  and MD  $94.7 \pm 3.7\%$ ;  $p = 0.52$ ) during the matches. After the matches no differences between groups were found in body mass loss (WH  $1.6 \pm 0.3$  kg and MD,  $1.4 \pm 0.7$  kg;  $p = 0.33$ ).

Before the match no differences were found between groups in salivary T (WH  $33.41 \pm 22.56 \text{ pg/mL}^{-1}$  and MD  $43.70 \pm 18.52 \text{ pg/mL}^{-1}$ ;  $p = 0.38$ ), C (WH  $3.17 \pm 0.19 \text{ ng/mL}^{-1}$  and MD  $3.22 \pm 0.18 \text{ ng/mL}^{-1}$ ;  $p = 0.66$ ), nor T:C (WH  $10.41 \pm 6.96$  and MD  $13.52 \pm 5.65$ ;  $p = 0.38$ ). The within-group analysis show that salivary T did not change after matches in WH ( $49.4 \pm 18.6 \text{ pg/mL}^{-1}$ ,  $p = 0.20$ ) nor MD ( $57.1 \pm 22.5 \text{ pg/mL}^{-1}$ ,  $p = 0.36$ ) (Figure 1A).

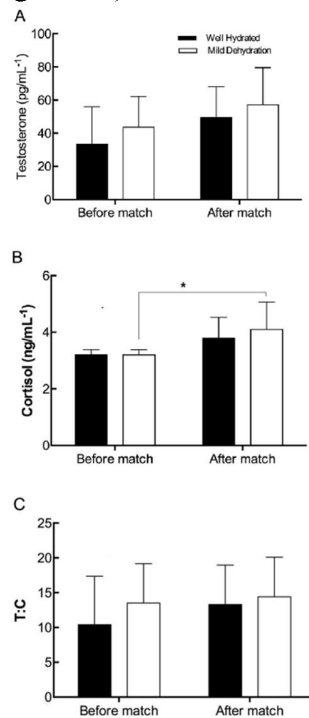


Figure 1. Comparison within well and mild dehydration groups in testosterone (A), cortisol (B), and their quotient (C) before and after a friendly match in elite young soccer players. \*:  $p < 0.05$ .

With respect to the C level after the match (MD  $4.1 \pm 0.9 \text{ pg/mL}^{-1}$ ; WH  $3.8 \pm 0.7 \text{ pg/mL}^{-1}$ ), the MD group exhibit an increase (28%;  $p = 0.03$ ), while no significant change was observed in the WH group ( $p = 0.13$ ) (Figure 1B). Regarding the T:C ratio, no changes were found within group in neither group after matches (WH  $13.3 \pm 5.7$ ,  $p = 0.94$ ; MD  $14.4 \pm 5.7$ ,  $p = 0.63$ ) (Figure 1C). No relationship was found between HR during matches and changes in C (HRmean vs changes in C,  $r = 0.13$ ,  $p = 0.37$ ; HRmax vs changes in C,  $r = 0.25$ ,  $p = 0.17$ )

## 6. DISCUSSION

### 6.1. Study I

For study I, the objective was to compare the acute effects of LI, MI, HI and COM intensity of plyometric training on HR, SBP, DBP and RPP in healthy males and females, hypothesizing that plyometric training trials would induce an acute PEH, without significant effects between intensities. According to our hypothesis, we observed a significant acute PEH after all plyometric trials, with no significant differences when LI, MI, HI or COM trials were compared. Therefore, although plyometric training is usually conducted with the aim of achieving neuromuscular adaptations, its potential cardiovascular effects should not be overlooked.

Previously, an increased HR have been observed after plyometric training, although at 90 min post exercise HR was significantly reduced compared to pre-exercise values (5). Our results (female and male subjects combined) showed a very similar pattern, with a significant increase at 10 min post exercise (except after COM), and then a significant (i.e., COM) or a tendency to significant (i.e., LI, MI, HI) reduction towards the end of the post-exercise measurement period. Regarding SBP, DBP and RPP, a mean reduction was observed after all plyometric intensities (except for DBP and RPP after LI and MI, respectively). These results are similar to those reported after traditional strength training (2) and also after plyometric training (5). However, others have reported no significant changes in blood pressure after high (11) or moderate (4) intensity plyometric training, although in these studies only a 10-min and a 3-min post-exercise measurement period was used, respectively, which probably reduced the chances to observe PEH (12, 69), as blood pressure values tend to decrease with the advance of the post-exercise recovery period (3, 29).

Regarding the effect of plyometric intensity, our results showed no significant effect for HR, SBP, DBP or RPP in all subjects, female subjects or male subjects. These results are similar to those reported previously in male subjects participating in plyometric training with different intensities (5). Although with others training strategies - as traditional low-speed resistance training (26) or aerobic training (8, 31) - the acute post-exercise cardiovascular effects may be affected by intensity, with physiological mechanisms relatively well known [(i.e., centrally mediated decreases in sympathetic nerve activity; reduced signal transduction from sympathetic nerve activation into vasoconstriction; local vasodilatation - (36)], if these mechanisms operate after plyometric training remains to be elucidated. Interestingly, in a previous study a significant acute increase in RPP have been observed after plyometric training in male athletes (3). The difference with our results can be related with the training status of subjects (i.e., athletes compared to non-athletes) (22), as chronic training may induce hemodynamic adjustments (66) that may potentially interfere with the PEH after strength training (32). Because RPP is a reliable predictor of myocardial oxygen demand (3, 4), our results are novel, indicating that low, moderate, high and combined plyometric training intensity can be used to induce a reduction in myocardial stress, potentially helping to positively affect athlete's maximal oxygen consumption ( $VO_{2max}$ ), ventilatory threshold and hearth rate reserve (57).

Regarding the effect of gender, at basal condition, women exhibit a significantly higher heart rate and lower systolic blood pressure, differences probably related to reduce muscle sympathetic nerve activity or an increased baroreflex sensitivity influenced by estrogen (6). After plyometric training,



both genders showed similar acute cardiovascular responses with all intensities, except for a higher SBP reduction in females after HI. These results are similar to those obtained after endurance (16) or low-speed resistance training (74). Therefore, because male and female subjects do not exhibit a different acute PEH effect, the possibility exists for no interaction between gender and acute cardiovascular responses to plyometric training, although the mechanisms behind the PEH may be gender specific (74, 89), probably related with their different gender-hormone environments (20), as estrogen levels may modulate vascular reactivity (64). However, because no physiological measurements were performed in this study, this may limit final conclusions. Future studies must look at the acute physiological mechanism behind male and female PEH effect after different intensities of plyometric training to better understand its underlying mechanism.

As a practical application, external validity is an important aspect of any research, especially if its applicability to the clinical field is considered. Hypertensive individuals should follow training programs that elicit lower increases in blood pressure (32) and strength training sets with a reduced number of repetitions of high intensity should be preferred to longer sets with lower intensity (44). Considering that compared to other strength methods plyometric training induces a relatively small increase in blood pressure (4, 11), and because its adaptive effects have been observed in hypertensive population with as low as three repetitions per set (78), therefore the external validity of our experimental approach can be regarded as high in this context. Thus, the results of this study may help to better define the optimal intensity during plyometric training regarding its potentially blood pressure lowering effects in male and female subjects.

## 6.2. Study II

The results of this study show that both jump training interventions can improve jump and kicking performance in youth soccer players. Nevertheless, HTHL further enhances performance adaptations during jump training.

The JG and the LJG showed an increase in horizontal, vertical, unipedal, and bipedal jumps ( $ES = 0.26$  to  $0.47$ ). Among youth soccer players, it seems relatively common to observe improvements in countermovement jump after short-term jump training (25, 61, 95), which might be related to several neuromuscular adaptations (53). However, a novel finding of this study is that only the LJG achieved greater horizontal, vertical, unipedal, and bipedal jump improvement compared with the CG. Despite the existence of HTHL jumping dates back nearly three thousand years (i.e., eighteenth ancient Olympiad) (63), its performance-augmentation effects during both vertical and horizontal jumping with arm swing have only been investigated in recent years (58). These effects may suggest several theoretical foundations (i.e., pull theory, joint torque augmentation theory, hold back theory, and takeoff angle theory), underlying mechanisms that affect the mechanics of the body during a given jump, thus increasing the height/distance of the centre of mass at takeoff, and its respective velocity (58). In fact, HTHL appears to elicit an increase in jump distance accompanied by an increase in jump duration, peak vertical ground reaction force, and vertical and horizontal impulse (23). More so, handheld loaded jumps with arm swing might have helped to reinforce jumping technique (58). Actually, the technique-reinforcement component of HTHL jump drills potentially increases the technical ability to apply force in the direction of intended movement; and this component may be of greater importance than the magnitude of the applied force applied (59). The kinematic and kinetic augmentation effects induced by HTHL could have a number of interesting long-term performance-enhancement training implications that for the first

time have been demonstrated in this study. Further longitudinal investigation should establish what physiological and biomechanical adaptations may induce these functional adaptations after long-term HTHL training.

Both jump training groups improved the VDJ20 (ES = 0.20–0.37). Several neuromuscular adaptations (53) might underlie the commonly observed improvements in this functional index among youth soccer players after short-term jump training (75, 77, 79-82). Considering the necessity to produce a high rate of force development in explosive actions among youth soccer players and its connection with the reactive strength index (60), the improvement in VDJ20 potentially enhanced physical parameters of sport-specific performance. In fact, reactive strength index may predict the change of direction ability (97), which can be considered as an important aspect of a successful performance among adult (84) and youth soccer players (17). Moreover, an increased neuromuscular ability (e.g., VDJ20) may be transferred to an improved running economy (56), which may affect the endurance capacity independently of the maximal oxygen uptake, lactate threshold, and other “aerobic” indicators (68, 96). It should be noted that, although both jump training groups improved VDJ20 performance, the LJG achieved a greater increase compared with the JG. It is quite likely that jumps performed with HTHL have provided a substantial horizontal and vertical neuromuscular training stimulus during both the vertical concentric phase (23) and, specifically, the eccentric landing phase of the jumps (58); thus stimulating an increase in lower limb muscle strength (76). Considering the relationship between leg strength and drop jump performance (7), athletes from the LJG might have increased lower limb muscle strength which may have contributed to a greater improvement in VDJ20 performance after HTHL jump training.

Both the JG and the LJG improved MKV performance after six weeks of jump training (ES = 0.27–0.34). Although the CG also improved, the jump training groups experienced a greater performance improvement compared with the CG. As jump training may induce motor coordination adaptations related to the specificity of movements used during training (25, 49), the unipedal nature of the instep kick may help to explain the jump training effects observed after the athletes trained with unipedal jump drills. Improvements in MKV performance might be attributed to neuromuscular adaptations such as increased strength and explosiveness of the leg extensor muscles (62), which can affect the instep kick in soccer (46, 47). It is possible that these neuromuscular and strength-explosive adaptations had an effect on the biomechanical factors related to kicking performance, such as maximum linear velocity of the toe, ankle, knee, and hip at ball contact (46, 47), which potentially contribute to produce a higher MKV, cumulatively or individually. Improvements in kicking performance after jump training have been previously observed in youth soccer players (62, 79, 80). However, to the best of the authors’ knowledge, this is the first study that compared the effects of jump training versus jump training plus HTHL on MKV in youth soccer players. Although HTHL exercises might be postulated as a potentiation training strategy to increase neural drive and power output for another activity (e.g., ball kicking) (58), current results suggest that similar ball kicking velocity performance enhancement can be achieved after jump training programs with or without added handheld loading. Further studies should analyze the effects of jump training with HTHL, where jump drills may be alternated in the same set with soccer-specific ball kicking drills.

Of practical significance, the findings from study II presented herein indicate that HTHL can induce positive functional adaptations for both fast stretch-shortening and slow stretch-shortening muscle actions. Such performance adaptations may be particularly important during the in-season

soccer period, where the maintenance/development of lower limb muscle power might require special attention due to the increased level of fatigue caused by the accumulation of matches and specific training sessions (which possibly compromise the functionality of the stretch-shortening cycle) (73). Further studies should be conducted to test the effects of combining HTHL with other types of traditional strength-power training models (i.e., traditional weightlifting or training at the optimum power zone) on the functional performance of top-level soccer players.

### 6.3. Study III

The aim of this study was to assess the effects of hydration level before a soccer match on the T, C, and T:C after two friendly matches in young elite soccer players. The main results indicate that MD group showed an exacerbated increase in cortisol response after the matches. These results suggest that C response to soccer match is sensitive to hydration state.

With respect to T, different to our results (no changes after match) Peñailillo et al. (2015) showed a decrease in T after a friendly match in elite soccer players (70). It is possible that the different results with the former study, is the difference in years of training experience of athletes. In Peñailillo et al. (2015) the players had an average of 26 years of age, while in our study, subjects averaged 17 years of age. Therefore, the greater chronological age, hence greater training experience of soccer players in the study of Peñailillo et al. (2015), could have induced a reduced hormone response during a competitive match compared to the less experienced U17 soccer players recruited in our study. In addition, several other factors have been identified to play a role in the hormone response to a soccer match (10).

Similar to Peñailillo et al. (2015) our results showed no changes in C after match in WH group it is possible that this match was not a sufficient physiological stressor for these professional elite footballers. Thus, the absence of cortisol response could possibly be due to their high fitness level. With respect to T:C did not change at the end of the match, this may be due to the fact that T:C only decreases during periods of high intensity training, but remains stable during periods of competition (30).

Our study is the first to assess the effects of mild dehydration on the response of T, C and T:C in young elite male soccer players. Our results showed that MD group increase the C response after two friendly matches without alters in effort intensity during matches evaluated by heart rate. Sensibility of C to hydration state was observed previously in seven adults with different levels of hydration completed a strength test. They showed that the most dehydrated subjects (-5% of body weight) showed greater increases in C after exercise, with no changes in T (42). Moreover, another study showed that C increased in hypohydrated ( $USG = 1,034 \pm 0.001 \text{ g/mL}^{-1}$ ) young cross-country athletes (51). Therefore, our results confirm previous findings, expanding the knowledge for young elite male soccer players, regarding the effects of dehydration on stress response (i.e., cortisol increase) during a soccer match. The C concentrations is a well-recognized physiological stress marker. This steroid hormone plays an important role in response to stress because of the activation of the hypothalamic-pituitary-adrenocortical axis.

## 7. CONCLUSIONS

1. Different intensities of plyometric training may induce an acute post-exercise hypotensive effect in young normotensive male and female subjects (Study I).
2. The combination of soccer drills and a short-term jump training program improves jump and kicking velocity performance in youth soccer players during the in-season. However, only the use of HTHL during some jump drills adds further performance adaptations over those induced by soccer training only, and proves to be a safe (and fun) training approach (Study II).
3. Mild dehydration before soccer match increase cortisol response after match. These results suggest that C response to soccer match is sensitive to hydration state which suggests that dehydration may be an added stress to be considered (Study III).

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## 9. APPENDIX (published studies)

### 9.1 Study I

# MEN AND WOMEN EXHIBIT SIMILAR ACUTE HYPOTENSIVE RESPONSES AFTER LOW, MODERATE, OR HIGH-INTENSITY PLYOMETRIC TRAINING

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#### ABSTRACT

Ramírez-Campillo, R, Abad-Colil, F, Vera, M, Andrade, DC, Caniuqueo, A, Martínez-Salazar, C, Nakamura, FY, Arazi, H, Cerda-Kohler, H, Izquierdo, M, and Alonso-Martínez, AM. Men and women exhibit similar acute hypotensive responses after low, moderate, or high-intensity plyometric training. *J Strength Cond Res* 30(1): 93–101, 2016—The aim of this study was to compare the acute effects of low-, moderate-, high-, and combined-intensity plyometric training on heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP), and rate-pressure product (RPP) cardiovascular responses in male and female normotensive subjects. Fifteen (8 women) physically active normotensive subjects participated in this study (age  $23.5 \pm 2.6$  years, body mass index  $23.8 \pm 2.3$  kg·m<sup>-2</sup>). Using a randomized crossover design, trials were conducted with rest intervals of at least 48 hours. Each trial comprised 120 jumps, using boxes of 20, 30, and 40 cm for low, moderate, and high intensity, respectively. For combined intensity, the 3 height boxes were combined. Measurements were taken before and after (i.e., every 10 minutes for a period of 90 minutes) each trial. When data responses of men and women were combined, a mean reduction in SBP, DBP, and RPP was observed after all plyometric intensities. No significant differences were observed pre- or postexercise (at any time point) for HR, SBP, DBP, or RPP when low-, moderate-,

high-, or combined-intensity trials were compared. No significant differences were observed between male and female subjects, except for a higher SBP reduction in women (–12%) compared with men (–7%) after high-intensity trial. Although there were minor differences across postexercise time points, collectively, the data demonstrated that all plyometric training intensities can induce an acute postexercise hypotensive effect in young normotensive male and female subjects.

**KEY WORDS** stretch-shortening cycle, postexercise hypotension, systolic blood pressure, diastolic blood pressure, heart rate

#### INTRODUCTION

Plyometric training is an explosive-type strength training method based on stretch-shortening cycle muscle performance. Plyometric training may induce long-term improvements in sprint (50), strength (49), and several sport-related explosive performance measures (18,37). In addition to these chronic effects, plyometric training may also induce acute effects on physical performance (1,12,53), muscle activation (52), and metabolic (12,54) and hormonal (12) variables, effects that could have potential implications on establishing optimum plyometric training designs. However, the acute effects of plyometric training on cardiovascular variables are poorly known.

*Postexercise hypotension* (31) is an acute cardiovascular phenomenon commonly observed after endurance (13,43) and traditional low-speed strength exercises (8,13,43), which may have the potential to positively affect athlete's maximal oxygen consumption, ventilatory threshold, and heart rate (HR) reserve (39). Although nonconsensual, postexercise hypotension might be explained by an increased parasympathetic tone

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TABLE 1. Descriptive characteristics of participants.\*

	Women (n = 8)	Men (n = 7)	Total (n = 15)
Age (y)	23.4 ± 3.1	23.7 ± 2.1	23.5 ± 2.6
Body mass (kg)	58.6 ± 8.7†	71.4 ± 7.0	64.6 ± 10.4
Height (m)	1.58 ± 0.04†	1.71 ± 0.06	1.64 ± 0.08
Body mass index (kg·m <sup>-2</sup> )	23.5 ± 2.9	24.3 ± 1.5	23.8 ± 2.3
Heart rate (b·min <sup>-1</sup> )	76.1 ± 6.7†	63.9 ± 10.2	70.4 ± 10.4
Systolic blood pressure (mm Hg)	106.6 ± 5.9†	119.1 ± 6.4	112.5 ± 8.8
Diastolic blood pressure (mm Hg)	66.0 ± 4.2	62.9 ± 5.8	64.5 ± 5.1
Rate-pressure product‡ (AU)	8,124 ± 931	7,575 ± 1,151	7,868 ± 1,040

\*AU = arbitrary unit.

†Significant difference compared with men ( $p \leq 0.05$ ).‡The product of heart rate  $\times$  systolic blood pressure.

(14) in vascular regulation (28), reduced systemic vascular resistance (24), decreased left ventricular end-diastolic volume, stroke volume, and cardiac output (9). Despite endurance (13,43) and low-speed strength training (8,13,43) having been shown to affect cardiovascular responses, different types of exercises may have different acute effects on postexercise hypotension (13,34). Regarding plyometric training, only 2 studies have examined its effects on cardiovascular responses, of which one presented postexercise hypotension (5), but not the other (3). Aside from the conflicting results on postexercise hypotension, little is known regarding the effects of plyometric training intensity on cardiovascular responses. Although training intensity during endurance and low-speed strength training has been shown to affect cardiovascular responses (7,23,24), only one study has compared the acute effects of different intensities of plyometric training on cardiovascular variables in male subjects, reporting no significant differences between low, moderate, and high intensity (5). Because a lower predominance of the sympathetic nervous system may occur after not-to muscle failure strength training (23), plyometric training might be especially suited to induce postexercise hypotension, because of its explosive not-to muscle failure nature, as previously suggested (32). However, the acute effects of plyometric training intensity on cardiovascular responses of female subjects have never been investigated.

Although men and women may not exhibit a different, acute postexercise hypotension effect after endurance (15) or low-speed resistance training (45), the possibility exists for an interaction between gender and acute cardiovascular responses to plyometric training (10), considering that the mechanisms may be gender specific (45,51), probably related to the different gender-hormone environments (16), as estrogen levels may modulate vascular reactivity (40). However, similar sprint (effect size = 0.36–0.37) (50) and endurance (~4%) (46) responses have been observed in men and women after plyometric training exercises, independent of

differences in physical performance (46), hormonal markers (26), or basal cardiovascular activity (e.g., HR, systolic blood pressure [SBP]) (6).

Given the previously cited conflicting results and limitations, our aim was to compare the acute effects of low-intensity (20-cm drop jumps), moderate-intensity (30-cm drop jumps), high-intensity (40-cm drop jumps), and combined-intensity (combination of 20, 30, and 40-cm drop jumps) plyometric training on HR, SBP, diastolic blood pressure (DBP), and rate-pressure product (RPP) in physically active normotensive male and female subjects, hypothesizing that plyometric training would induce acute postexercise hypotension in both groups.

## METHODS

### Experimental Approach to the Problem

This study was designed to compare the acute effects of low, moderate, high, and combined plyometric training intensities on HR, SBP, DBP, and RPP in physically active normotensive (i.e., SBP < 120 mm Hg; DBP < 80 mm Hg) male and female subjects. Using a randomized crossover design, 4 plyometric intensity trials were conducted with 48–72 hours of rest between them. Cardiovascular measures were taken before and every 10 minutes for 90 minutes after each plyometric trial. Sample size was computed according to the changes observed in SBP ( $d = 10$  mm Hg,  $SD = 8$ ) in a group of normotensive male subjects participating in very similar plyometric training intensities to those performed in this study (5). A total of 6 participants per group would yield a power of 80% and  $\alpha = 0.05$ .

### Subjects

Initially, 22 healthy young (19.6–28.4 years old) male and female (i.e., women were tested during the luteal phase of their menstrual cycle) subjects volunteered for this study. Subjects were recruited from a physical education university course and were confirmed to be free of cardiovascular problems by a physician. Subjects were familiar with plyometric exercises as they participated in recreational sports. Exclusion criteria included participants with (a) potential medical and cardiovascular problems (i.e., including hypertension) or a history of ankle, knee, or back pathology that compromised their participation or performance in the study, (b) any lower extremity reconstructive surgery in the previous 2 years or unresolved musculoskeletal disorders, and (c) use of drugs (i.e., including hypertensive drugs) that could

**TABLE 2.** Men ( $n = 7$ )—acute cardiovascular responses before and after different intensities of plyometric training.\*

	Before	After				Mean
		10 min	60 min	90 min		
<b>HR (<math>b \cdot \text{min}^{-1}</math>)</b>						
Low intensity	66.3 ± 11.3	70.1 ± 12.1	66.7 ± 7.0	63.0 ± 6.7	65.5 ± 7.1	
Moderate intensity	74 ± 14.3	83.4 ± 14.6 <sup>a</sup>	72.2 ± 12.4 <sup>b,c</sup>	70.9 ± 11.9 <sup>b</sup>	68.3 ± 9.1	
High intensity	73.5 ± 10.4	83.7 ± 11.1 <sup>a</sup>	71.3 ± 8.4 <sup>b</sup>	70.6 ± 9.2 <sup>b</sup>	69.4 ± 4.7	
Combined	78.9 ± 10.6	82.7 ± 13.0	71.3 ± 8.1 <sup>b</sup>	69.9 ± 11.1 <sup>a,b</sup>	67.3 ± 2.8 <sup>a</sup>	
<b>SBP (mm Hg)</b>						
Low intensity	118.3 ± 10.3	113.3 ± 10.4	107.5 ± 8.4 <sup>a</sup>	110.9 ± 9.7	113.9 ± 8.8 <sup>a</sup>	
Moderate intensity	116.3 ± 12.1	114.2 ± 10.3	107.3 ± 9.5	109.3 ± 8.2	109.2 ± 7.9	
High intensity	119.5 ± 8.9	113.3 ± 11.1	106.6 ± 11.1 <sup>a</sup>	107.9 ± 9.2 <sup>a</sup>	116.5 ± 7.0 <sup>a</sup>	
Combined	118.9 ± 10.8	113.6 ± 11.0	107.7 ± 10.2 <sup>a</sup>	109.3 ± 9.0 <sup>a</sup>	116.1 ± 9.7 <sup>a</sup>	
<b>DBP (mm Hg)</b>						
Low intensity	66.2 ± 6.2	66.9 ± 6.0	65.7 ± 7.4	68.5 ± 6.4	64.5 ± 4.7	
Moderate intensity	70.3 ± 5.1	69.0 ± 5.6	64.5 ± 5.2	67.3 ± 4.7	65.3 ± 4.5 <sup>a</sup>	
High intensity	70.4 ± 6.1	68.1 ± 4.7	65.9 ± 6.9	66.9 ± 6.1	65.4 ± 4.8 <sup>a</sup>	
Combined	70.2 ± 7.0	68.9 ± 4.4	65.5 ± 4.0	66.6 ± 5.9	66.1 ± 4.9 <sup>a</sup>	
<b>Rate-pressure product (AU)</b>						
Low intensity	8,619 ± 1,418	8,965 ± 1,874	7,582 ± 1,221	7,615 ± 1,228	7,487 ± 1,212 <sup>a</sup>	
Moderate intensity	8,607 ± 1,923	9,470 ± 1,561	7,716 ± 1,314 <sup>b</sup>	7,719 ± 1,239 <sup>b</sup>	7,494 ± 1,824	
High intensity	8,785 ± 1,408	9,441 ± 1,188	7,569 ± 1,007 <sup>b</sup>	7,607 ± 1,091 <sup>b</sup>	8,099 ± 773	
Combined	9,406 ± 1,313	9,317 ± 1,321	7,557 ± 824 <sup>a,b</sup>	7,479 ± 1,068 <sup>a,b</sup>	7,739 ± 785 <sup>a</sup>	

\*HR = heart rate; SBP = systolic blood pressure; DBP = diastolic blood pressure; AU = arbitrary unit. Superscript letters <sup>a, b, c</sup> denote significant ( $p \leq 0.05$ ) difference compared with before, 10-, and 20-minute values, respectively.

influence cardiovascular responses at rest, during, or after exercise. To be included in the final analyses, subjects were required to complete all familiarization sessions, control and plyometric trial sessions, and test measurements. Six subjects were excluded because of failure to complete all plyometric trial sessions and 1 subject was excluded because of hypertension, which resulted in 15 subjects (8 women) being included in the final analyses. Descriptive characteristics of the participants are provided in Table 1. Subjects were informed about the experimental procedures and about possible risks and benefits associated with participation in the study and signed an informed consent before any of the tests were performed. The study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board of the University for use of human subjects.

#### Testing Procedures

Participants were familiarized with the cardiovascular test procedures 2 weeks before the initial assessment to reduce a *white coat* effect. During the familiarization sessions, standing height (meters) and body mass (kilograms) were assessed according to the international standards for anthropometric assessment (36). To evaluate height and body mass, a stadiometer/mechanical scale (model 220; SECA, Hamburg, Germany) with precisions of 0.1 cm and 0.1 kg, respectively, was used. Subjects were tested while wearing light clothing

(shoes were removed). The body mass index was calculated (kilograms per square meter). In addition, during these sessions, the subjects were instructed on the proper form and technique of the plyometric exercises to be used.

Cardiovascular measurements were always administered in the same order, at the same time of the day (i.e., between 1300 and 1500 hours), and by the same experienced investigator, who was blinded to the trial group of the participants. Participants were instructed to maintain their sleeping, eating, and drinking habits during the study process. In addition, participants were instructed to eat a light meal (i.e., <500 kcal) 2 hours before the experimental trials, to avoid physical exercise and alcohol for at least 48 hours, and to avoid smoking, caffeine, or consumption of other substances (e.g., energetic or caffeinated drinks, Guarana or ginseng-related supplements) that could influence cardiovascular responses at rest, during, or after plyometric exercise trials, for  $\geq 12$  hours before the trials. Tests were conducted indoors in a calm environment without noise, with a room temperature and humidity of approximately 25°C and 50%, respectively. Throughout testing, an investigator to subject ratio of 1:1 was maintained. During measurements, subjects maintained a sitting position, breathing spontaneously. For resting blood pressure, 2 measurements were taken after 10 minutes of seated rest on 5 visits to the laboratory. A mean value of the 10 measurements was calculated (45).

**TABLE 3.** Women ( $n = 8$ )—acute cardiovascular responses before and after different intensities of plyometric training.\*

	Before	After			Mean
		10 min	60 min	90 min	
<b>HR (<math>b \cdot \text{min}^{-1}</math>)</b>					
Low intensity	79.0 $\pm$ 8.8	87.5 $\pm$ 16.6	74.0 $\pm$ 12.9 <sup>b,c,d</sup>	74.3 $\pm$ 13.3 <sup>b,c,d</sup>	78.5 $\pm$ 13.6
Moderate intensity	81.5 $\pm$ 11.5	90.4 $\pm$ 13.8	78.8 $\pm$ 11.6 <sup>b</sup>	75.6 $\pm$ 12.3 <sup>b</sup>	80.2 $\pm$ 10.7
High intensity	76.8 $\pm$ 5.9	86.6 $\pm$ 5.9 <sup>a</sup>	74.1 $\pm$ 5.1 <sup>b</sup>	73.3 $\pm$ 7.7 <sup>b</sup>	77.2 $\pm$ 11.1
Combined	83.5 $\pm$ 10.4	89.0 $\pm$ 13.7	76.3 $\pm$ 8.0 <sup>b</sup>	76.1 $\pm$ 11.2 <sup>b</sup>	79.2 $\pm$ 10.2 <sup>a</sup>
<b>SBP (mm Hg)</b>					
Low intensity	113.8 $\pm$ 9.3	107.9 $\pm$ 3.1	101.8 $\pm$ 4.6 <sup>a</sup>	105.9 $\pm$ 7.4	104.4 $\pm$ 4.3 <sup>a</sup>
Moderate intensity	110.0 $\pm$ 6.8	109.1 $\pm$ 7.9	102.0 $\pm$ 5.7	104.1 $\pm$ 5.2	103.5 $\pm$ 4.2 <sup>a</sup>
High intensity	115.1 $\pm$ 7.6	105.4 $\pm$ 6.8 <sup>a</sup>	99.6 $\pm$ 10.0 <sup>a</sup>	101.8 $\pm$ 5.9 <sup>a</sup>	101.3 $\pm$ 5.2 <sup>a</sup>
Combined	113.3 $\pm$ 8.2	107.1 $\pm$ 6.7	102.0 $\pm$ 8.3 <sup>a</sup>	104.9 $\pm$ 6.9 <sup>a</sup>	102.8 $\pm$ 6.7 <sup>a</sup>
<b>DBP (mm Hg)</b>					
Low intensity	68.3 $\pm$ 6.1	67.6 $\pm$ 6.7	65.9 $\pm$ 7.4	69.8 $\pm$ 7.5	67.2 $\pm$ 5.9
Moderate intensity	69.6 $\pm$ 4.9	70.6 $\pm$ 6.5	65.1 $\pm$ 3.7	68.6 $\pm$ 4.5	67.2 $\pm$ 3.4 <sup>a</sup>
High intensity	69.8 $\pm$ 6.7	67.5 $\pm$ 5.3	65.4 $\pm$ 7.7	65.5 $\pm$ 5.1	65.4 $\pm$ 4.8 <sup>a</sup>
Combined	69.1 $\pm$ 7.9	68.3 $\pm$ 5.3	64.4 $\pm$ 3.8	67.6 $\pm$ 5.3	65.9 $\pm$ 3.9
<b>Rate-pressure product (AU)</b>					
Low intensity	8,982 $\pm$ 1,225	9,440 $\pm$ 1,828	7,528 $\pm$ 1,358 <sup>a,b,c,d</sup>	7,832 $\pm$ 1,343 <sup>b</sup>	8,190 $\pm$ 1,398
Moderate intensity	8,982 $\pm$ 1,523	9,825 $\pm$ 1,457	8,047 $\pm$ 1,352 <sup>b</sup>	7,862 $\pm$ 1,250 <sup>b</sup>	8,309 $\pm$ 1,124 <sup>a</sup>
High intensity	8,884 $\pm$ 1,895	9,095 $\pm$ 1,307	7,376 $\pm$ 1,259 <sup>a,b</sup>	7,444 $\pm$ 1,043 <sup>a,b</sup>	7,800 $\pm$ 1,013 <sup>a</sup>
Combined	9,463 $\pm$ 1,446	9,525 $\pm$ 159	7,759 $\pm$ 914 <sup>a,b</sup>	7,932 $\pm$ 912 <sup>a,b</sup>	8,129 $\pm$ 1,029 <sup>a</sup>

\*HR = heart rate; SBP = systolic blood pressure; DBP = diastolic blood pressure; AU = arbitrary unit. Superscript letters <sup>a, b, c, d</sup> denote significant ( $p \leq 0.05$ ) difference compared with before, 10-, 20-, 30-, and 40-minute values, respectively.

**Systolic and Diastolic Blood Pressure.** For SBP (mm Hg) and DBP (mm Hg) measurements, a digital blood pressure monitor was used (HEM-742; Omron, Kyoto, Japan), after a previously described protocol (44).

**Heart Rate and Rate-Pressure Product.** For HR ( $b \cdot \text{min}^{-1}$ ) measurements, a cardiac monitor (RS200; Polar, Kempele, Finland) was used, after previously established criteria (10). The RPP was calculated as HR ( $b \cdot \text{min}^{-1}$ )  $\times$  SBP (mm Hg), which is considered a reliable predictor of myocardial oxygen demand (3,4). The RPP is represented as a single number in arbitrary units.

Data on cardiovascular measurements obtained during a control trial were used to determine reliability through the intraclass correlation coefficient. An intraclass correlation coefficient below 0.40 was considered poor, 0.40–0.59 fair, 0.60–0.74 good, and 0.75–1.00 excellent (33). An excellent intraclass correlation coefficient was observed for SBP, DBP, HR, and RPP measurements, ranging between 0.96 and 0.99.

#### Trials

The jump heights (20, 30, and 40 cm) used during the plyometric trials (i.e., low-, moderate-, high-, and combined-intensity trials) were based on previous recommendations (3,5), where intensity was defined as the amount of landing

force after a drop jump (5,35); thus, with a higher drop jump height, the intensity would be increased (30,35). Although we did not directly measure plyometric intensity, higher forces (29,30) and lower muscle activation (21) were expected with higher drop jump heights, therefore, higher plyometric intensity (i.e., muscle stress).

A 10-minute standard warm-up (i.e., submaximal running with change of direction on a 40-m indoor track, 20 vertical and 10 horizontal submaximal jumps) was executed before each trial. During each plyometric trial, 6 sets of 20 valid drop jump repetitions were completed. Height boxes of 20, 30, and 40 cm were used during low-, moderate-, and high-intensity plyometric trials, respectively. For the combined-intensity trial, the 3 height boxes were used randomly and 2 sets of 20 repetitions were completed for each box to equal volume between trials. Participants were instructed to use the same athletic shoes and clothes during all trials. Plyometric exercises were completed on a wooden floor. Rest intervals of 15 (48) and 120 seconds (5) between repetitions and sets were used during plyometric trials, respectively. Each plyometric trial lasted approximately 50 minutes.

Aside from the drop height, the same technique was used for all plyometric exercises. Basically, subjects began by standing on a box with their arms akimbo and then stepped off the box with the supporting leg straight to avoid any

**TABLE 4.** Participants ( $n = 15$ )—acute cardiovascular responses before and after different intensities of plyometric training.\*

	Before	After				Mean
		10 min	60 min	90 min		
<b>HR (<math>b \cdot \text{min}^{-1}</math>)</b>						
Low intensity	73.1 ± 11.7	79.4 ± 16.8 <sup>a</sup>	70.6 ± 10.9 <sup>b</sup>	69.0 ± 15.0 <sup>b,c,d</sup>	72.4 ± 12.6	
Moderate intensity	74.0 ± 14.3	83.3 ± 14.6 <sup>a</sup>	72.2 ± 12.4 <sup>b,c</sup>	70.9 ± 11.9 <sup>b,c</sup>	74.6 ± 11.4	
High intensity	73.5 ± 10.4	83.7 ± 11.1 <sup>a</sup>	71.3 ± 8.4 <sup>b,c</sup>	70.6 ± 9.2 <sup>b,c</sup>	73.6 ± 9.3	
Combined	78.9 ± 10.6	82.7 ± 13.0	71.3 ± 8.1 <sup>ab</sup>	69.9 ± 11.1 <sup>ab,c</sup>	73.7 ± 9.6 <sup>a</sup>	
<b>SBP (mm Hg)</b>						
Low intensity	118.3 ± 10.3	113.3 ± 10.4	106.7 ± 6.1 <sup>a</sup>	110.9 ± 9.7 <sup>a</sup>	108.9 ± 8.2 <sup>a</sup>	
Moderate intensity	116.3 ± 12.1	114.2 ± 10.3	107.3 ± 9.5 <sup>ab</sup>	109.3 ± 8.2 <sup>a</sup>	106.2 ± 13.9 <sup>a</sup>	
High intensity	119.5 ± 8.9	113.3 ± 11.1 <sup>a</sup>	106.6 ± 11.1 <sup>ab</sup>	107.9 ± 9.2 <sup>a</sup>	108.4 ± 9.8 <sup>a</sup>	
Combined	118.9 ± 10.8	113.6 ± 11.0	107.7 ± 10.2 <sup>a</sup>	109.3 ± 9.0 <sup>a</sup>	102.8 ± 6.7 <sup>a</sup>	
<b>DBP (mm Hg)</b>						
Low intensity	66.2 ± 6.2	66.9 ± 6.0	65.7 ± 7.4	68.5 ± 6.4	66.0 ± 5.4	
Moderate intensity	70.3 ± 5.1	69.0 ± 5.6	64.5 ± 5.2 <sup>a</sup>	67.3 ± 4.7	66.3 ± 3.9 <sup>a</sup>	
High intensity	70.4 ± 6.1	68.1 ± 4.7	66.9 ± 6.9	66.9 ± 6.1	66.1 ± 4.8 <sup>a</sup>	
Combined	70.2 ± 7.0	68.9 ± 4.4	65.5 ± 4.0	66.6 ± 5.9	65.9 ± 3.9 <sup>a</sup>	
<b>Rate-pressure product (AU)</b>						
Low intensity	8,619 ± 1,448	8,304 ± 1,643	7,582 ± 1,221 <sup>ab</sup>	7,615 ± 1,228 <sup>ab</sup>	7,862 ± 1,318 <sup>a</sup>	
Moderate intensity	8,607 ± 1,923	9,470 ± 1,561 <sup>a</sup>	7,716 ± 1,314 <sup>b,c</sup>	7,719 ± 1,239 <sup>ab,c</sup>	7,929 ± 1,495	
High intensity	8,758 ± 1,408	9,441 ± 1,188	7,569 ± 1,007 <sup>ab,c</sup>	7,607 ± 1,091 <sup>ab,c</sup>	7,940 ± 890 <sup>a</sup>	
Combined	9,406 ± 1,313	9,317 ± 1,321	7,557 ± 824 <sup>ab</sup>	7,479 ± 1,068 <sup>ab,c</sup>	7,947 ± 913 <sup>a</sup>	

\*HR = heart rate; SBP = systolic blood pressure; DBP = diastolic blood pressure; AU = arbitrary unit. Superscript letters <sup>a, b, c, d, e</sup> denote significant ( $p \leq 0.05$ ) difference compared with before, 10-, 20-, 30-, and 40-minute values, respectively.

initial upward propulsion or sinking, ensuring a drop height of 20, 30, or 40 cm (i.e., depending on the trial protocol). On landing with 2 feet on the ground, participants were instructed to maximize jump height and minimize ground contact time (i.e., bounce drop jump). Takeoff and landing were standardized to full knee and ankle extension on the same spot. To assure that the subjects performed the exercises with maximal volitional effort, jump height and contact time during the plyometric exercise repetitions were measured with an electronic contact mat system (Ergo-jump; Globus, Codogne, Italy), and the reactive strength index was calculated (20). The reliability of this measure has been described elsewhere (20,38). Valid repetitions were considered if participants achieved  $\geq 80\%$  of their maximal reactive strength index. To assure proper technique, all exercises were supervised with an investigator to participant ratio of 1:1. Technique was visually assessed by the same highly experienced investigator, and particular attention was paid to demonstration and execution, giving maximal motivation to participants during each jump. Four basic techniques were stressed during jumps (a) correct posture (i.e., spine erect and shoulders back) and body alignment (e.g., chest over knees), (b) jumping straight up with no excessive side-to-side or forward-backward movement, and (c) instant recoil for the concentric part of the jump.

Phrases such as “on your toes,” “straight as a stick,” “light as a feather,” and “recoil like a spring” were used as verbal and visualization cues during jumps. Between jumps, subjects were also instructed to concentrate on maximal performance and to focus on breathing deeply.

#### Statistical Analyses

All values are reported as mean  $\pm$  SD. Normality and homoscedasticity assumptions were checked with the Shapiro-Wilk and Levene's tests, respectively. For variables not normally distributed, logarithmization procedures were performed. To determine the acute effect of trials on dependent variables, a 2-way variance analysis with repeated measurements (4 trials  $\times$  2 times) was performed. When a significant  $F$  value was achieved across time or between trials, Bonferroni post hoc procedures were performed to locate the pairwise differences between the means. The  $\alpha$  level was set at  $p \leq 0.05$  for statistical significance. All statistical calculations were performed using STATISTICA statistical package (Version 8.0; StatSoft, Inc., Tulsa, OK, USA).

#### RESULTS

A significant ( $p \leq 0.05$ ) mean reduction in HR was observed (Table 2) after the combined-intensity trial in men, in addition to a significant ( $p \leq 0.05$ ) mean reduction

**TABLE 5.** Mean\* acute effects (with 90% confidence limits) for cardiovascular variables in men ( $n = 7$ ), women ( $n = 8$ ), and all ( $n = 15$ ) subjects after low- (LI), moderate- (MI), high- (HI), or combined- (COM) intensity plyometric training.

	LI	MI	HI	COM
<b>Heart rate</b>				
All	-0.4 (-5.3 to 4.3)	2.0 (-2.2 to 6.1)	0.7 (-3.6 to 5.1)	-6.3 (-9.6 to -3.1)
Men	-0.1 (-6.4 to 6.2)	5.6 (-1.3 to 12.5)†	0.1 (-7.9 to 8.1)	-7.8 (-13.9 to -1.8)
Women	-0.8 (-9.2 to 7.6)	-1.3 (-6.7 to 4.2)	1.3 (-4.7 to 7.2)	-5.0 (-9.3 to -0.8)
<b>Systolic blood pressure</b>				
All	-7.8 (-9.7 to -5.9)	-4.8 (-8.3 to -1.3)	-9.3 (-11.7 to -6.9)	-8.2 (-10.5 to -6.0)
Men	-7.7 (-9.8 to -5.5)	-3.7 (-11.9 to 4.5)	-6.5 (-8.4 to -4.6)	-7.3 (-11.7 to -2.8)
Women	-7.9 (-11.4 to -4.3)	-5.8 (-8.1 to -3.4)	-11.8 (-15.7 to -7.9)‡	-9.1 (-11.9 to -6.3)
<b>Diastolic blood pressure</b>				
All	0.1 (-3.8 to 4.0)	-5.4 (-8.3 to -2.4)	-5.8 (-8.5 to -3.0)	-5.5 (-8.7 to -2.4)
Men	1.6 (-6.1 to 9.3)	-7.7 (-13.2 to -2.2)	-5.7 (-8.5 to -3.0)	-7.2 (-12.3 to -2.1)
Women	-1.3 (-5.9 to 3.4)	-3.3 (-6.7 to 0.1)	-5.8 (-11.1 to -0.6)	-4.1 (-8.7 to 0.5)
<b>Rate-pressure product</b>				
All	-8.0 (-13.6 to -2.4)	-2.5 (-9.3 to 4.4)†	-8.4 (-13.8 to -2.9)	-15.0 (-18.1 to -11.9)
Men	-7.7 (-14.5 to -0.9)	2.6 (-12.2 to 17.4)†	-6.2 (-14.7 to 2.4)	-16.7 (-21.4 to -12.0)
Women	-8.3 (-18.3 to 1.8)	-6.9 (-11.8 to -1.9)	-10.3 (-18.8 to -1.8)	-13.6 (-18.4 to -8.8)

\*Mean relative (percent) change across 90 minutes after exercise.

†Significant difference compared with COM ( $p \leq 0.05$ ).

‡Significant difference compared with men.

in SBP and DBP after all plyometric intensities (except for SBP and DBP after moderate and low intensities, respectively) (Table 2). Also, male subjects presented a mean reduction in RPP after all plyometric intensities, although this was significant ( $p \leq 0.05$ ) only after low- and combined-intensity trials (Table 2).

Similarly, in female subjects, a significant ( $p \leq 0.05$ ) mean reduction in HR was observed (Table 3) after the combined-intensity trial, in addition to a significant ( $p \leq 0.05$ ) mean reduction in SBP and DBP after all plyometric intensities (except for DBP after low and combined intensities) (Table 3). A significant ( $p \leq 0.05$ ) mean reduction in RPP was observed after all plyometric intensities (except after low intensity) (Table 3).

When data of male and female subjects were combined, a similar pattern of results was observed, with a significant ( $p \leq 0.05$ ) mean reduction in HR after the combined-intensity trial (Table 4), in addition to a significant ( $p \leq 0.05$ ) mean reduction in SBP and DBP after all plyometric intensities (except for DBP after low intensity) (Table 4). Also, a significant ( $p \leq 0.05$ ) mean reduction in RPP was observed after all plyometric intensities (except after moderate intensity) (Table 4).

The intensity  $\times$  group effect analyses indicated no significant differences before or after exercise (at any time point)

for HR, SBP, DBP, or RPP when low-, moderate-, high-, or combined-intensity trials were compared in men (Table 2), women (Table 3), or men and women combined (Table 4).

No significant differences were observed for mean relative (percent) change between male and female subjects (Table 5), except for a significantly higher SBP reduction in women compared with men after high-intensity plyometric training (-12 and -7%, respectively;  $p \leq 0.05$ ).

## DISCUSSION

Our research objective was to compare the acute effects of low-, moderate-, high-, and combined-intensity plyometric training on HR, SBP, DBP, and RPP in healthy men and women, hypothesizing that plyometric training trials would induce acute postexercise hypotension, without significant effects between intensities. Confirming our hypothesis, we observed a significant acute postexercise hypotension after all plyometric trials, with no significant differences when low-, moderate-, high-, or combined-intensity trials were compared. Therefore, although plyometric training is usually conducted with the aim of achieving neuromuscular adaptations, its potential cardiovascular effects should not be overlooked.

An increased HR has been observed previously after plyometric training, although at 90 minutes postexercise HR



was significantly reduced compared with pre-exercise values (5). Our results (female and male subjects combined, Table 4) demonstrated a very similar pattern, with a significant increase at 10 minutes after exercise (except after combined intensity) and then a significant (i.e., after combined intensity) or a tendency to significant (i.e., after low, moderate, and high intensities) reduction toward the end of the post-exercise measurement period (Table 4). Regarding SBP, DBP, and RPP, a mean reduction was observed (Table 4) after all plyometric intensities (except for DBP and RPP after low and moderate intensities, respectively). These results are similar to those reported after traditional strength training (2) and also after plyometric training (5). However, others have reported no significant changes in blood pressure after high- (10) or moderate- (4) intensity plyometric training, although in these studies only 10- and 3-minute postexercise measurement periods were used, respectively. Therefore, as our results demonstrated (Tables 2, 3, and 4), for significant acute reduction in cardiovascular variables to occur after a plyometric training session (3), as with other types of exercises (11,22,42), more than 10 minutes of recovery is usually required.

Regarding the effect of plyometric intensity, our results presented no significant effect for HR, SBP, DBP, or RPP in all subjects (Table 4), female subjects (Table 3), or male subjects (Table 2). Therefore, the acute cardiovascular responses were similar after low, moderate, high, or combined plyometric training intensities. To our knowledge, this is the first study to analyze the effects of combined plyometric training intensity on acute cardiovascular responses in men and women. Our results are comparable with those reported previously in male subjects participating in plyometric training with different intensities (5). Although with other training strategies, such as traditional low-speed resistance training (19) or aerobic training (7,24), the acute post-exercise cardiovascular effects may be affected by intensity, with relatively well-known physiological mechanisms (i.e., centrally mediated decreases in sympathetic nerve activity, reduced signal transduction from sympathetic nerve activation into vasoconstriction, local vasodilatation; 27); whether these mechanisms operate after plyometric training remains to be elucidated. Interestingly, in a previous study, a significant acute increase in RPP was observed after plyometric training in male athletes (3). The difference in our results could be related to the training status of the subjects (i.e., athletes compared with nonathletes) (17), as chronic training may induce hemodynamic adjustments (41) that potentially interfere with the postexercise hypotension after strength training (25). Because RPP is a reliable predictor of myocardial oxygen demand (3,4), our results are novel, indicating that low, moderate, high, and combined plyometric training intensities can be used to induce a reduction in myocardial stress.

To the best of our knowledge, this is the first study to compare acute cardiovascular responses in men and women

after an acute session of low-, moderate-, high-, or combined-intensity plyometric training. Although at baseline women exhibited a significantly higher HR and lower SBP (Table 1), the differences were probably related to reduced muscle sympathetic nerve activity or increased baroreflex sensitivity influenced by estrogen (6). After plyometric training, both genders showed similar acute cardiovascular responses at all intensities (Table 5), except for a higher SBP reduction in women after high intensity. These results are similar to those obtained after endurance (15) or low-speed resistance training (45). Therefore, because male and female subjects do not exhibit different acute postexercise hypotension, the possibility exists for no interaction between gender and acute cardiovascular responses to plyometric training, although the mechanisms behind postexercise hypotension may be gender specific (45,51), probably related to their different gender-hormone environments (16), as estrogen levels may modulate vascular reactivity (40). However, as no physiological measurements were performed in this study, the final conclusions might be limited. Future studies should look at the acute physiological mechanism behind the male and female postexercise hypotension effect after different intensities of plyometric training to better understand its underlying mechanism.

In conclusion, different intensities of plyometric training may induce an acute postexercise hypotensive effect in young normotensive male and female subjects.

#### PRACTICAL APPLICATIONS

External validity is an important aspect of any research, especially if its applicability in the clinical field is considered. Hypertensive individuals should follow training programs that elicit lower increases in blood pressure (25), and strength training sets with a reduced number of high-intensity repetitions should be preferred to longer sets with lower intensity (32). Considering that compared with other strength methods plyometric training induces a relatively small increase in blood pressure (4,10) and because its adaptive effects have been observed in a hypertensive population with as low as 3 repetitions per set (47), the external validity of our experimental approach can be regarded as high in this context. In addition, as in the general population, hypertension is one of the most prevalent cardiovascular disorders in athletes and, even in the absence of structural or functional heart damage, may negatively affect maximal oxygen consumption, ventilatory threshold, and HR reserve (39). Thus, the results of this study may help to better define the optimal intensity of plyometric training, regarding its potential to lower blood pressure in male and female subjects.

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## 9.2. Study II.

Training & Testing

# Jump Training in Youth Soccer Players: Effects of Haltere Type Handheld Loading

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Key words: muscle strength, muscle contraction, sports, football

**Abstract**  
The aim of this study was to compare the effects of a jump training program, with or without haltere type handheld loading on maximal force, maximal kicking velocity only (ES=0.11–0.20). Both jump training groups improved in right leg (ES=0.28–0.45) and left leg horizontal counter-movement jump with arms (ES=0.32–0.47), horizontal counter-movement jump with arms

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## 9.3. Study III



# Basal Mild Dehydration Increase Salivary Cortisol After a Friendly Match in Young Elite Soccer Players

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A soccer match induce changes in physiological stress biomarkers as testosterone (T), cortisol (C), and testosterone:cortisol (T:C) ration. Hydration state may also modulate these hormones, and therefore may alter the anabolic/catabolic balance in response to soccer match. The role of hydration status before the match in this biomarkers has not yet been reported. The aim of this study was to compare the salivary T, C, and the T:C ratio responses after two friendly matches in well-hydrated and mild-dehydrated (MD) elite young male soccer player. Seventeen players (age,  $16.8 \pm 0.4$  years;  $VO_2\max$   $57.2 \pm 3.6$  ml/kg<sup>-1</sup>/min<sup>-1</sup>) were divided into two teams. Before the matches the athletes were assessed for hydration level by the urine specific gravity method and divided for the analysis into well-hydrated (WH;  $n = 9$ ; USG  $< 1.010$  g/mL<sup>-1</sup>) and mild-dehydrated (MD;  $n = 8$ ; USG  $1.010$  to  $1.020$  g/mL<sup>-1</sup>) groups. Hormones were collected before and after each match by saliva samples. The mean (HRmean) and maximal (HRmax) heart rate were measured throughout the matches. A two-way ANOVA was used to compare T, C, and T:C between and within groups. Similar HRmean (WH,  $83.1 \pm 4.7\%$ ; MD,  $87.0 \pm 4.1$ ;  $p = 0.12$ ) and HRmax (WH,  $93.2 \pm 4.4\%$ ; MD,  $94.7 \pm 3.7\%$ ;  $p = 0.52$ ) were found for both groups during the matches. No differences were found before the matches in the T ( $p = 0.38$ ), C ( $p = 0.66$ ), nor T:C ( $p = 0.38$ ) between groups. No changes within groups were found after matches in neither group for T (WH,  $p = 0.20$ ; MD,  $p = 0.36$ ), and T:C (WH,  $p = 0.94$ ; MD,  $p = 0.63$ ). Regarding the C, only the MD group showed increases (28%) after the matches (MD,  $p = 0.03$ ; WH,  $p = 0.13$ ). In conclusion MD group exacerbate the C response to friendly matches in elite young male soccer players, suggesting that dehydration before match may be an added stress to be considered.

**Keywords:** hydration, hormone, endocrine, saliva, catabolic, football, recovery, sport health

## INTRODUCTION

Hormonal response to a soccer match is a hot topic, with anabolic (i.e., testosterone [T]) and catabolic (i.e., cortisol [C]) hormones potentially influencing the performance and health status of the athlete (Slimani et al., 2017). At the same time, their ratio testosterone:cortisol (T:C) is considered a physiological stress indicator associated to overtraining (Hayes et al., 2015).

Some studies such as Penailillo et al. (2015) showed a decrease on T, without changes on C, whereas others such as Thorpe and Sunderland (2012) describe increases on both hormones. The differences between studies can be explained due to differences in match intensity, biological, psychological, social factors and/or due to the degree or years of training, the latter being highly dependent to the age of the athletes (Casto and Edwards, 2016). In addition, it has been shown that the hormonal response during exercise might depend on hydration state (Roy et al., 2001). For instance, it has been observed greater C concentration on hypohydrated subjects before and after running at 70% of maximal oxygen consumption ( $VO_2\text{max}$ ) in comparison to euhydrated runners (Maresh et al., 2006). Although in male soccer players, salivary cortisol, T, and T:C have been assessed as physiological stress biomarkers after the match, the role of hydration status before the match in this biomarkers has not yet been reported. This information could increase our understanding of the physical stress induced by a football match, which could improve the preparation and strategy to protect and/or enhance elite performance in subsequent matches. Therefore, the aim of this investigation was to assess the effects of hydration level before a soccer match on the T, C, and T:C response after the match in young elite soccer players.

## MATERIALS AND METHODS

### Subjects

Seventeen male soccer players (age:  $16.8 \pm 0.4$  years; body mass:  $67.5 \pm 7.5$  kg; height:  $173 \pm 6.8$  cm;  $VO_2\text{max}$ :  $57.2 \pm 3.6$  ml/kg<sup>-1</sup>/min<sup>-1</sup>), from a South American under-17 (U17) soccer national team, participated in this study. According to the hydration level assessed by the urine specific gravity (USG) before the matches, the subjects were divided for the analysis into two groups: well-hydration (WH) (USG < 1.010 g/mL<sup>-1</sup>,  $n = 9$ ) and mild dehydration (MD) (USG from 1.010 to 1.020 g/mL<sup>-1</sup>,  $n = 8$ ) (Casa et al., 2000). Injured players and goalkeepers were excluded. The legal guardians of the players signed an informed consent, while the players give their verbal assent, after the potential benefits, and risks were explained to them. The study was approved by the ethics committee of the Universidad Finis Terrae and conformed to the principles outlined in the Declaration of Helsinki.

### Study Design

One week before the matches  $VO_2\text{max}$  was assessed to all participants with an incremental test. In the matches day the evaluated soccer team was divided into two teams (A and B). The first match (team A) was played at 11:00 am, and the second match (team B) at 11:30 pm (the same day), in preparation for the FIFA U17 World Championship 2015, carried out in Chile. Climatic conditions were similar between matches. The both friendly matches were played against a professional soccer team of the Chilean professional league. The assessed teams won both matches (first match, 2-0; second match, 3-1).

During the two matches, the players were asked to play as it was an official match. The matches follow international rules (FIFA). The USG was assessed 30 min before the match with a portable Refractometer (Robinair, model SPX, United States) in triplicate according to previous suggestions (Castro-Sepulveda et al., 2015). Nutritional recommendations were not made prior to matches and during the friendly matches players consumed water *ad libitum*. According to Penailillo et al. (2015), for the assessment of the T and C, saliva was collected 30 min before each match (Pre-), and 5–10 min after each match (Post-). Briefly, the players were sat, with their eyes open, their head slightly tilted forward and making minimal orofacial movement. All saliva ( $\pm 3$  ml) was collected for about 2 min. The saliva samples were centrifuged at 1,500 g for 15 min and stored at  $-20^\circ\text{C}$  until analysis. The T and C were determined by enzyme immunoassay using a commercial kit (Salimetrics, State College, PA, United States). The optical density was determined with a microplate reader (Multiskan, Thermo®) at 450 nm. All analyses were performed in duplicate according to the manufacturer's procedures. The intra-assay coefficient of variation was 2.5 and 2.8% for the T and C, respectively. Only players that played >80 min were considered. The mean (HRmean) and maximal (HRmax) heart rate was measured throughout the match using the Polar Team system (MARCA, PAIS). The heart rate values were reported as relative values, according to age-expected maximum values (220-age).

### Maximal Oxygen Uptake

The  $VO_2\text{max}$  was determined by a breath-by-breath pulmonary gas exchange system (Ergocard, Medisoft, Belgium) during an incremental treadmill test. The starting speed was 3 km<sup>-1</sup>/h<sup>-1</sup>, with speed increments of 1 km<sup>-1</sup>/h<sup>-1</sup> every 60 s. Prior to the tests, the gas analyser was calibrated using gases of known concentrations ( $VO_2 = 16.0\%$  and  $VCO_2 = 4.0\%$ ), and the airflow was calibrated using a 3-liter syringe (Hans Rudolph, Kansas, MO, United States).

### Body Mass Loss

Body mass loss (kg) was calculated by measuring body mass before and after matches (body mass after match – body mass before match) using the same scale (SECA model M20812, Germany), with a precision of 0.1 kg.

### Statistical Analysis

Data is shown as mean  $\pm$  standard deviation (SD). The normality of the data was analyzed by the Shapiro–Wilk test, showing that data was normally distributed. An unpaired *t*-test was used to compare hydration level, basal characteristics, body mass loss, and HR during matches between groups. A two-way ANOVA was used for the comparison of the T, C, and T:C between and within of WH and MD groups, with a Tukey *post hoc* test when significant main effect was found. The alpha value was set at  $p < 0.05$ . Statistical analyses were performed in GraphPad Prism® 6.0 (GraphPad Software, San Diego, CA, United States).

## RESULTS

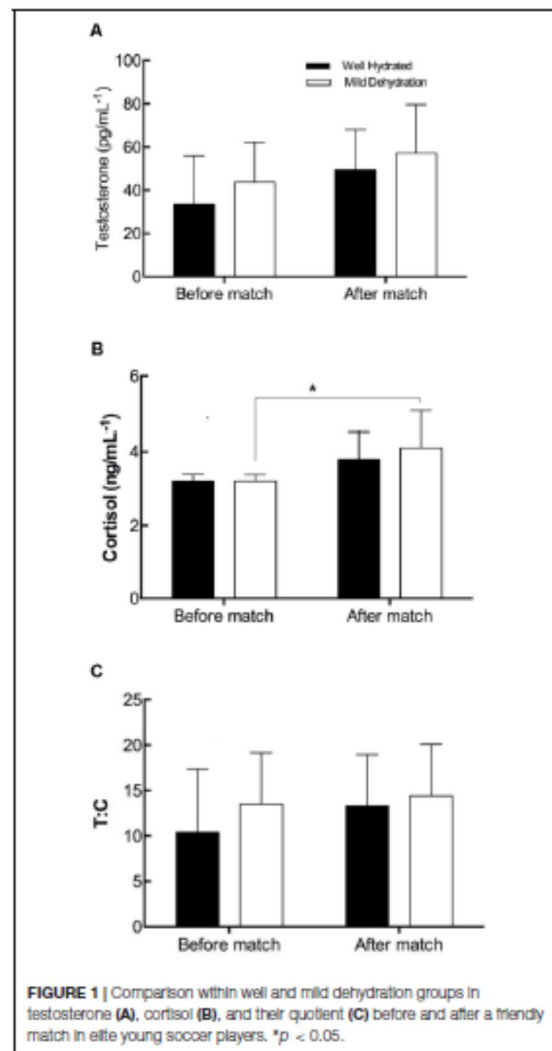
In A team were found five players in WH condition and four players in MD condition and in B team were found four players in WH condition and four players in MD condition. As expected, the USG was lower ( $p < 0.0001$ ) in the WH group ( $1.006 \pm 0.002 \text{ g/mL}^{-1}$ ) compared to the MD group ( $1.014 \pm 0.002 \text{ g/mL}^{-1}$ ). No differences were found between groups in basal characteristics (age: WH  $16.3 \pm 0.7$  and MD  $16.9 \pm 0.5$  years,  $p = 0.61$ ; body mass: WH  $68.5 \pm 8.6$  and MD  $67.3 \pm 8.4$  kg,  $p = 0.68$ ; height: WH  $171 \pm 9.1$  and MD  $175 \pm 8.0$  cm,  $p = 0.21$ ;  $\text{VO}_2\text{max}$ : WH  $56.3 \pm 3.8$  and MD  $57.8 \pm 7.2 \text{ ml/kg}^{-1}/\text{min}^{-1}$ ,  $p = 0.74$ ). Neither were found differences between groups in HRmean (WH  $83.1 \pm 4.7\%$  and MD  $87.0 \pm 4.1\%$ ;  $p = 0.12$ ) or HRmax (WH  $93.2 \pm 4.4\%$  and MD  $94.7 \pm 3.7\%$ ;  $p = 0.52$ ) during the matches. After the matches no differences between groups were found in body mass loss (WH  $1.6 \pm 0.3$  kg and MD,  $1.4 \pm 0.7$  kg;  $p = 0.33$ ).

Before the match no differences were found between groups in salivary T (WH  $33.41 \pm 22.56 \text{ pg/mL}^{-1}$  and MD  $43.70 \pm 18.52 \text{ pg/mL}^{-1}$ ;  $p = 0.38$ ), C (WH  $3.17 \pm 0.19 \text{ pg/mL}^{-1}$  and MD  $3.22 \pm 0.18 \text{ pg/mL}^{-1}$ ;  $p = 0.66$ ), nor T:C (WH  $10.41 \pm 6.96$  and MD  $13.52 \pm 5.65$ ;  $p = 0.38$ ). The within-group analysis show that salivary T did not change after matches in WH ( $49.4 \pm 18.6 \text{ pg/mL}^{-1}$ ,  $p = 0.20$ ) nor MD ( $57.1 \pm 22.5 \text{ pg/mL}^{-1}$ ,  $p = 0.36$ ) (Figure 1A). With respect to the C level after the match (MD  $4.1 \pm 0.9 \text{ pg/mL}^{-1}$ ; WH  $3.8 \pm 0.7 \text{ pg/mL}^{-1}$ ), the MD group exhibit an increase (28%;  $p = 0.03$ ), while no significant change was observed in the WH group ( $p = 0.13$ ) (Figure 1B). Regarding the T:C ratio, no changes were found within group in neither group after matches (WH  $13.3 \pm 5.7$ ,  $p = 0.94$ ; MD  $14.4 \pm 5.7$ ,  $p = 0.63$ ) (Figure 1C). No relationship was found between HR during matches and changes in C (HRmean vs. changes in C,  $r = 0.13$ ,  $p = 0.37$ ; HRmax vs. changes in C,  $r = 0.25$ ,  $p = 0.17$ ).

## DISCUSSION

The aim of this study was to assess the effects of hydration level before a soccer match on the T, C, and T:C after two friendly matches in young elite soccer players. The main results indicate that MD group showed an exacerbated increase in C response after the matches. These results suggest that C response to soccer match is sensitive to hydration state.

With respect to T, different to our results (no changes after match) Penailillo et al. (2015) showed a decrease in T after a friendly match in elite soccer players. It is possible that the different results with the former study, is the difference in years of training experience of athletes. Penailillo et al. (2015) the players had an average of 26 years of age, while in our study, subjects averaged 17 years of age. Therefore, the greater chronological age, hence greater training experience of soccer players in the study of Penailillo et al. (2015), could have induced a reduced hormone response during a competitive match compared to the less experienced U17 soccer players recruited in our study. In addition, several other factors have



**FIGURE 1 |** Comparison within well and mild dehydration groups in testosterone (A), cortisol (B), and their quotient (C) before and after a friendly match in elite young soccer players. \* $p < 0.05$ .

been identified to play a role in the hormone response to a soccer match (Kobayashi and Miyazaki, 2015; Slimani et al., 2017).

Similar to Penailillo et al. (2015) our results showed no changes in C after match in WH group. However, our results indicate that the MD group showed a significant increase after the match. Considering that no relationship was found between HR during matches and changes in C ( $r = 0.13$ – $0.25$ ,  $p = 0.17$ – $0.37$ ), and that  $\text{VO}_2\text{max}$  (i.e., fitness level) was similar in the WH and the MD groups ( $56.3$  and  $57.8 \text{ ml/kg}^{-1}/\text{min}^{-1}$ ,  $p = 0.74$ ), it is unlikely that potential differences in physiological stress during match or differences in fitness level explain the increase in C observed in the MD group and the lack of increase in the WH group. Therefore, the hydration level probably played a key

significant role. Regarding the T:C, this marker did not change at the end of the match. This may be due to the fact that T:C only decreases during periods of high intensity training, but remains stable during periods of competition (Filaire et al., 2001). In this sense, the role of the hydration level probably played a minor role on the T:C ratio as compared to its role on C levels after the match.

Our study is the first to assess the effects of MD before the match on the response of T, C, and T:C in young elite male soccer players. Our results showed that MD group increase the C response after two friendly matches without alters in effort intensity during matches evaluated by heart rate. Sensibility of C to hydration state was observed previously in seven adults with different levels of hydration completed a strength test. They showed that the most dehydrated subjects ( $-5\%$  of body weight) showed greater increases in C after exercise, with no changes in T (Judelson et al., 2008). Moreover, another study showed that C increased in hypohydrated ( $USG = 1,034 \pm 0.001 \text{ g/mL}^{-1}$ ) young cross-country athletes (Maresh et al., 2006). Finally, a recent study reported that body weight loss during the match (dehydration) was associated with the increases in C levels in male professional tennis players (López-Samanes et al., 2018). Therefore, our results, in a collective sport, confirm previous findings, expanding the knowledge for young elite male soccer players, regarding the effects of dehydration on stress response (i.e., C increase) during a soccer match.

The C concentrations is a well-recognized physiological stress marker. This steroid hormone plays an important role in response to stress and skeletal muscle recovery after exercise because of the activation of the hypothalamic-pituitary-adrenocortical axis. This finding may be of utmost importance for coaches and medical staff of football teams to consider, since MD before training or competition is very common in football soccer players (Castro-Sepulveda et al., 2015). A previous study in Cushing's syndrome shows a relationship between

HR and C (Chandran et al., 2013). Our results do not show this relationship, this inconsistency could be explained by the different mechanisms that modify the HR in the Cushing's syndrome and during the exercise.

One of the limitations of this study is not having evaluated other variables that influence C levels and responses as (1) sleep quality before the matches (Bassett et al., 2015) and (2) natural daily response of C (Pritchard et al., 2017). Another potential limitation is that players were free to hydrate during the match. This, although ethically sound, may have altered the after-match hydration level. However, after the match no differences between groups were found in body mass loss (WH,  $1.6 \pm 0.3$ ; MD,  $1.4 \pm 0.7$ ;  $p = 0.33$ ).

## CONCLUSION

In conclusion, MD before soccer match increase C response after match. These results show that C response to soccer match is sensitive to hydration state which suggests that dehydration before match may be an added stress to be considered.

## AUTHOR CONTRIBUTIONS

MC-S and HZ-F designed the study. MC-S, RR-C, and HZ-F collected and analyzed the data. MC-S, RR-C, FA-C, CM, LP, JC, and HZ-F interpreted the data and prepared the manuscript. All authors approved the final version of the paper.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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