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Tesis Doctoral Internacional

Effects of moderate alcohol consumption in response to a high-intensity interval training in healthy adults. The BEER-HIIT study.

Efecto del consumo moderado de alcohol en respuesta a un programa de entrenamiento de alta intensidad en adultos sanos. Estudio BEER-HIIT.

PROGRAMA DE DOCTORADO EN PSICOLOGÍA

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FACULTAD DE PSICOLOGÍA

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UNIVERSIDAD DE GRANADA

Cristina Molina Hidalgo

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“Estoy floreciendo fuerte, tan fuerte, que ya no necesitaré primaveras”

-Paloma de la Paz-

A quienes me dan la oportunidad de crecer, de equivocarme y avanzar en este camino. En especial a vosotros, mis pilares y ejemplo de vida, mamá y hermano.

“Nada ha cambiado, yo he cambiado, todo ha camnbiado”

-Marcel Proust-

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RESEARCH PROJECTS AND FUNDING

The present project was partially supported by the Centro de Información Cerveza y Salud (CICS, Madrid, Spain) by an unrestricted grant (29 870 €).

ABBREVIATIONS

ANCOVA: analysis of covariance.

ANOVA: analysis of variance.

BC: body composition.

BMI: body mass index.

CI: confidence interval.

ERP: event-related brain potentials.

HIIT: High-intensity interval training.

fMRI: functional magnetic resonance imaging.

LM: lean mass.

LMI: lean mass index.

MRI: magnetic resonance imaging.

RT: reaction time.

VO_{2max}: maximum oxygen consumption or maximal aerobic capacity.

ABSTRACT

Physical activity is an integral part of a healthy lifestyle and physical exercise is an excellent tool to preserve health, enhance well-being, and improve physical and mental performance. For that, the most effective physical exercises are those leading to improvements in physical fitness and, consequently, are part of a structured training program. High intensity interval training (**HIIT**) is a novel form of training particularly effective and efficient since it allows achieving results with less time investment. HIIT leads to improvements in body composition, reducing body fat levels, increasing fat free mass, and bone mineral content. HIIT also elicits improvements in aerobic capacity, resistance, and muscle strength. Indeed, physical activity enhances cognitive function including better processing speed, attention, and memory, also it shows positive associations with self-perceptions, self-esteem, and mental well-being in young adults. Recent studies have reported the positive effects of HIIT on cognitive function, however contradictory results have been found related to performance on the executive function tasks. Although gender differences are obvious in physical and metabolic mechanisms concerning exercise, this issue has not generally received much attention, and data derived from men should not be directly applicable to women.

Although the multiple and negative effects of high intakes of alcohol have been widely studied and demonstrated, the effect of moderate alcohol doses after exercise is not clear and it is currently under debate. Some studies indicate that post-exercise alcohol intake may have detrimental effect on muscle recovery. Others investigations have suggested that beer consumption could be associated with increased waist circumference. Further, some research has showed that alcohol containing beverages may decrease performance, specifically affecting reaction time, fine motor control, levels of arousal, and judgement. However, we can find contradictory and inconclusive results.

The main aims of this International Doctoral Thesis are to evaluate the combined effects of a highly demanding training program and moderate alcohol/beer consumption on physical performance (i.e. body composition and cardiorespiratory fitness and muscular strength (**Section 1**), on cognitive performance (**Section 2**), and on psychosocial parameters and mood state (**Section 3**).

The present results show that 10 weeks of HIIT did not influence body weight, but significantly decreased fat mass and fat mass percentage while increasing lean mass. These positive effects were not affected by the concomitant regular intake of beer, or its alcohol equivalent, in moderate amounts (**Study 1**). Additionally, our 10-week HIIT program improved cardiorespiratory fitness and hand grip strength, while no influence determined by the concurrent daily intake of beer or ethanol in moderate amount was observed (**Study 2**). Although improvements were found in reaction time (**Study 3**) and cognitive function (**Study 4**) we cannot conclude that those benefits were elicited by our HIIT intervention. Interestingly, moderate alcohol consumption did not negatively influence those beneficial adaptations on cognition. Finally, a 10-week HIIT program improved overall psychosocial parameters and mood adaptations in healthy young adults (**Study 5**). Also, those benefits were not affected by moderate alcohol consumption.

Collectively, the results from the present International doctoral Thesis enhance our understanding of the effects of a highly demanding training program on several physical variables, such as body composition and physical fitness, and psychosocial parameters. However, inconclusive results were found regarding to the effects of a high intensity training on cognitive performance in healthy young adults. Moreover, it provides novel information regarding to the role of moderate beer or ethanol consumption while training in healthy young adults.

RESUMEN

La actividad física es parte integral de un estilo de vida saludable y el ejercicio físico es una excelente herramienta para preservar la salud, mejorar el bienestar y aumentar el rendimiento físico y mental. En este sentido, los ejercicios físicos más efectivos son aquellos que conducen a mejoras en la condición física y, en consecuencia, forman parte de un programa de entrenamiento estructurado. El entrenamiento interválico de alta intensidad (HIIT) es una novedosa forma de entrenamiento particularmente eficaz y eficiente ya que permite lograr resultados con una menor inversión de tiempo. El HIIT determina mejoras en la composición corporal, reduciendo los niveles de grasa corporal, aumentando la masa magra, así como el contenido mineral óseo. El HIIT también provoca mejoras en la capacidad aeróbica, la resistencia y la fuerza muscular. Por otra parte, la actividad física mejora la función cognitiva, lo que incluye una mejor velocidad de procesamiento, atención y memoria. En adultos jóvenes, también se ha demostrado la existencia de una relación positiva entre el nivel de actividad física y la autopercepción, la autoestima y el bienestar mental. Estudios recientes también han puesto de manifiesto los efectos positivos del HIIT sobre la función cognitiva, sin embargo, los resultados contradictorios en lo relacionado con el desempeño en las tareas de la función ejecutiva. Aunque las diferencias de género son obvias en los mecanismos físicos y metabólicos relacionados con el ejercicio, este tema generalmente no ha recibido adecuada atención y los datos derivados de hombres no deberían ser directamente aplicables a mujeres.

Aunque los múltiples y negativos efectos de la ingesta elevada de alcohol se han estudiado y demostrado ampliamente, el efecto de dosis moderadas de alcohol, en particular después de realizar ejercicio, no está claro y es objeto de debate. Algunos estudios indican que la ingesta de alcohol después del ejercicio puede tener un efecto perjudicial sobre la recuperación muscular. Otras investigaciones han sugerido que el consumo de cerveza podría estar asociado con una mayor acumulación de grasa abdominal. Por otro lado, algunas investigaciones han demostrado que las bebidas que contienen alcohol pueden disminuir el rendimiento, afectando específicamente el tiempo de reacción, el control motor fino, los niveles de excitación y el juicio. Sin embargo, los resultados que muestra la literatura científica son contradictorios y, por tanto, no concluyentes.

Los principales objetivos de la presente Tesis Doctoral Internacional son evaluar los efectos combinados de un programa de entrenamiento muy exigente y un consumo moderado de alcohol / cerveza sobre el rendimiento físico (es decir, la composición corporal y la aptitud cardiorrespiratoria y la fuerza muscular (**Sección 1**), sobre el rendimiento cognitivo (**Sección 2**). y sobre parámetros psicosociales y el estado de ánimo (**Sección 3**).

Nuestros resultados muestran que 10 semanas de HIIT no influyeron en el peso corporal, pero disminuyen significativamente la masa grasa y el porcentaje de masa grasa al tiempo que aumentan la masa magra. Estos efectos positivos no se vieron afectados por la ingesta habitual y concomitante de cerveza, o su equivalente en alcohol, siempre en cantidades moderadas (**Estudio 1**). Además,

nuestro programa HIIT de 10 semanas mejoró la capacidad cardiorrespiratoria y la fuerza de agarre manual, no observándose ningún efecto asociado a la ingesta diaria simultánea de cerveza o etanol en cantidad moderada (**Estudio 2**). Por otro lado, aunque se encontraron mejoras en el tiempo de reacción (**Estudio 3**) y la función cognitiva (**Estudio 4**), no podemos concluir que esos beneficios fueran consecuencia de la intervención con HIIT. Curiosamente, el consumo moderado de alcohol tampoco influyó negativamente en esas adaptaciones beneficiosas sobre la cognición. Finalmente, un programa HIIT de 10 semanas mejoró los parámetros psicosociales generales y las adaptaciones del estado de ánimo en adultos jóvenes sanos (**Estudio 5**). Además, esos beneficios no se vieron afectados por el consumo moderado de alcohol.

GENERAL INTRODUCTION

Chapter 1

Physical exercise, lifestyle, health, and fitness

Ejercicio físico, estilo de vida, salud y condición física

GENERAL INTRODUCTION

Physical activity is an integral part of a healthy lifestyle and physical exercise is an excellent tool to preserve health, enhance well-being, and improve performance. For that, the most effective physical exercise is the one leading to improvements in physical fitness and, consequently, is part of a structured training program [1]. High-intensity interval training (**HIIT**) is a novel form of training particularly effective and efficient since it allows achieving results with less time investment [2,3]. Training in general and HIIT in particular, represent a significant overload or stress for the body, an overload that is repetitive and sustained over time. For exercise, this overload is mainly of a physical nature, but, in the case of HIIT, it is also a mental stress given the psychological pressure while having to perform multiple highly demanding tasks in short periods of time. Adequate overload is a requisite to achieve improvements. Without stress, or with very little stress, there will be no adaptation or improvement [4]. If the stress is excessive, it ends on negative consequences in terms of health, well-being, and/or performance.

To achieve improvements, in addition to stress, adequate recovery is mandatory. Recovery requires sufficient rest, adequate nutrition, and the absence of toxic elements that might hinder it. Stress, here exercise, determines not only energy consumption but also a wear and tear of functional structures. Rest and proper nutrition allow the recovery of energy reserves but also, and more important, the clearance, regeneration, and repair of damaged or worn structures, and even its reinforcement or protection against future stress. With that, the integrity and functionality of the involved structures not only returns to the baseline situation but they are brought to a better, more prepared, or resistant state. This phenomenon is known as supercompensation where the body is in a better position to face new stresses, both at a functional and structural level. This occurs for physical and psychological stress, and, therefore, has positive consequences both at a physical and psychological level. This is the physiological basis of training and its multiple and positive effects in terms of health, well-being, and performance [4,5].

Beer intake accompanying meals (for example, in the context of the Mediterranean diet) or after exercise, is common practice for many physically active individuals and it is also frequently consumed after practicing many sports. Due to its alcohol content, beer consumption during the recovery phase, or on a regular basis with meals, can be questioned in physically active people. In addition to water and alcohol, other beer components, such as polyphenols, lupulins, dextrin, vitamins, or minerals can also influence the recovery process, either physically or mentally. Therefore, it is important to clarify the existing controversy regarding the consumption of beer while practicing exercise or, even more, while following a highly demanding training program [6–10].

Understanding important concepts…

Physical activity, physical exercise, physical fitness, and training

It is necessary to clarify and differentiate several concepts that, although inter-related and mutually influential, are clearly different. Thus, the terms physical activity, physical exercise, physical fitness, and training are often used interchangeably and even confusingly; however, even though they are closely related terms, they should not be used synonymously. It is also necessary to differentiate these concepts of an active lifestyle as opposed to a sedentary lifestyle.

Physical activity refers to any body movement produced by skeletal muscle that is associated with energy consumption. Physical activity can be the movements while performing the tasks of daily life (daily life physical activity), while working (labor physical activity), or that carried out during leisure time (leisure time physical activity). The latter is the most beneficial since is voluntary and performed as an alternative to other types of activities frequently sedentary.

Sedentary activities are those performed while sitting, reclining, or standing, without moving or moving little. Being sedentary is not simply the opposite of being physically active since a person can be physically active, if practicing exercise (i.e., one hour a day), and also be sedentary, or leading a sedentary lifestyle, if expending most of the time in a sitting position, for instance working, reading, driving, or using screens. A sedentary lifestyle is, in itself, a risk factor for various diseases, such as obesity, cardio-metabolic diseases, or even degenerative processes associated with aging.

Physical exercise is defined as a physical activity that is planned, structured, systematic, and intentionality. That intentionality can simply be the reward determined by its practice or more specific, such as maintaining health, improving physical fitness or performance, socializing… If physical exercise adheres to rules or norms, we speak of sport.

Physical fitness is the capacity to perform physical activity and/or physical exercise at its highly attainable level. For this purpose, it integrates most of the body structures and functions involved in body movement, such as the locomotor, cardiorespiratory, hematocirculatory, endocrine-metabolic, and psycho-neurological systems. We can speak of physical fitness in relation to (sports) performance or physical fitness in relation to health. In this last case, the objective is not performance but simply being better or more capable, which obviously entails, or can lead to improving, or at least preserving, physical and mental performance.

Training is any program of physical exercise systematized and individualized which is carried out in order to improve general performance, in a specific sporting activity or simply to improve physical fitness, in all or some of its different components (i.e., aerobic capacity, strength, endurance, agility, coordination, body composition - **BC**). There are an enormous number and forms of training. It is beyond the scope of this thesis to analyze the different forms of training. We will just focus in one specific modality of them, which is highly oriented to obtain results with less dedication of time, which in today's world and for many people has a value in itself. It is the **HIIT**.

Exercise for fitness, health, and well-being

Physical activity and diet are fundamental factors of the lifestyle and, when adequate, are excellent tools to maintain and improve health, increase the level of well-being, enhance performance, and even mitigate the inevitable consequences of aging. Moreover, maintaining and, if possible, improving physical fitness are of paramount importance as determinants of both physical and mental well-being and performance. When done properly, the benefits of exercise always occur, regardless of age, health, and fitness. The available scientific evidence has shown this unequivocally [1,11–13]. The practice of exercise has beneficial effects on most, if not all, organic functions, helping to maintain that functionality and even improve it. In a specific and direct way, physical exercise maintains and improves musculoskeletal, osteoarticular, cardio-circulatory, respiratory, endocrine-metabolic, immunological, and psycho-neurological functions [14]. It has also been shown that physically active individuals live longer and better (with a higher quality of life) than less active or sedentary [1,12,15]. Despite the undoubted benefit of staying active and exercising, a significant number of individuals, both young and old, lead lives that can be considered as sedentary nowadays.

Health-related physical

Physical fitness integrates various components among which we can highlight the cardiorespiratory fitness or aerobic capacity, muscular strength, psychokinetic characteristics including coordination, gait, static, and dynamic balance, flexibility, posture, or perception-reaction time. Finally, as established by the World Health Organization, BC is another component of physical fitness [16].

Physical fitness integrates the different physical qualities that a person requires for daily life, work activities, and exercise [17]. Its assessment integrates all the functions and structures implicated in the adequate performance of physical activity or exercise, representing an integrated measurement of the capacity the person to carry them out. With age, there is a progressive decline of that capacity, which occurs at a rate of 10% per decade [17]. Physical fitness is a powerful health marker from childhood and adolescence until the end of life [1,18]. It is important, therefore, to control the level of physical fitness throughout life in order to determine its decline, propose strategies to maintain, or improve it and adapt the physical demands to the real possibilities of each individual, for instance establishing individualized training programs, one of which is undoubtedly HIIT.

Body composition as a health indicator

BC, apart from its aesthetic component, is an indicator of health and physical fitness. BC assessment is important to design any exercise program, as it serves as a guide for mapping out an individualized training strategy based on the need to lose fat, build muscle, or both. BC can be calculated or estimated in different ways, among which are: anthropometry (with measurement of the thickness of skin folds at certain points on the body, body perimeters, weight, height, body mass index...), analysis of electrical bio-impedance, or whole-body dual energy x-ray absorptiometry (DEXA), among others. This low energy X-ray technique is capable of evaluating the individual's lean body mass, fat mass, bone mineral content and density. This method is one of the most accurate and reliable, being considered a “gold standard” in the study of BC [25].

Lifestyle and aging cause changes in BC, such as an increase in fat mass, a decrease in muscle mass, and a reduction in bone mass [25]. This can lead to the development of various diseases, such as obesity, sarcopenia, and osteoporosis, a triad that, for example, gives rise to the osteosarcopenic obesity syndrome [26]. One of the most common health problems in people with osteosarcopenic obesity is the high risk of falls and fractures, which has a significant impact on social, economic and social costs, and represent an important cause of morbidity and mortality in the elderly [27].

Aerobic capacity as a health parameter

The most important component of physical fitness is aerobic capacity and the physiological variable that best defines it is the maximum oxygen consumption (**VO_{2max}**), which can be measured directly or indirectly (as heart rate), performing tests of maximum or sub-maximum effort [1].

Solid scientific evidence confirms that VO_{2max} is a powerful and independent predictor of risk of death from all causes. The existence of an inverse relationship between aerobic capacity and cardiovascular and cancer mortality has been repeatedly demonstrated, regardless of age, alcoholic habit, diabetes mellitus, or even tobacco consumption; it also has been found that VO_{2max} is an important determinant of insulin sensitivity, and low aerobic capacity is associated with the presence of metabolic syndrome. Likewise, a good aerobic capacity is associated with a decrease in neuronal loss that occurs with age and protects against cognitive dysfunction in aging [1,19].

Muscular strength as a health parameter

Another component of physical fitness is muscular strength, which can be measured using various techniques, among which manual dynamometry stands out for its ease and reliability. Muscle strength has been independently related to health status becoming another powerful predictor of mortality and life expectancy [1,20]. The decrease in muscle mass, muscle strength, and function of the musculoskeletal system is a functional disability (sarcopenia), which is directly related to a high risk of morbidity and mortality [21]. Interestingly, muscle strength, such as handgrip strength, has been found to be a predictor of bone mass in some specific population [22]. The ability to perform activities of daily life, such as walking speed or balance, is strongly associated not only to maximal muscle strength but also with explosive strength production of the leg extensors [17]. Furthermore, increased maximal and explosive muscle strength has indirect effects on improved postural control and, thereafter, balance due to possible changes in explosive strength characteristics [23,24].

Understanding the relationship between…

Physical activity and cognition

Physical activity has been associated with the reduction of a number of physical and mental disorders across the adult lifespan [12,28]. In addition, a growing body of literature has linked physical activity with improvements in brain function and cognition [18]. This favorably influence could be based on the fundamental neurobiological principle that cellular and molecular events in the brain are amenable to modification by environmental enrichment [29]. Neurobiological effects of this environmental enrichment have been found in rodents which are translated to enhanced learning and memory, indicating that physically active behaviours influence cognitive function and the supporting brain structures [30,31]. Those positive effects have lead questions about its potential for positive effects in humans. Although some studies have found promising results; several reviews and meta-analyses have concluded that the effect of physical activity on cognitive and brain health remains unclear because of the inconsistencies in the measurements of physical activity or cognition and the quality of the study designs [32].

The scientific investigation of this relationship between physical activity and cognition in human began in the 1930s. The early findings indicated that physically active older adults had faster psychomotor speed on reaction time (RT) tasks, relative to sedentary adults. Interestingly, no such relationship was observed in comparable groups of younger adults [33–36]. Hence, clearly more research is needed to determine the effects in these an other age-groups and to examine the magnitude of the benefit in psychomotor speed and RT. Recently the exercise–cognition relation in older adults has been strengthened by a small but growing number of randomized intervention studies that examined whether fitness training has a positive effect on different aspects of perception and cognition [18]. The central question is whether individuals who participate in an aerobic training regime show larger gains in cognition than wait-list control subjects or control subjects who participate in non-aerobic regimes, such as toning and stretching. Previous studies have reported that aerobic exercise training may potentially provide substantial benefits on cognitive function in children and older adults [13,18,37]. Actually, a few meta-analyses have determined a positive relationship between physical activity training and improvements on cognition in normal and older patient adults, suggesting that physical activity can have positive effect on a wide range of cognitive functions [38,39]. Furthermore, it has been suggested that the greatest effects are observed for higher cognitive functions, such as executive function, with fewer effects on lower level functions [40]. Executive functions refers to domains of cognitive function that involve executive control, including control planning, scheduling, working memory, interference control, and task coordination [41]; also, they are considered critical for performance in novel situations or when an individual is required to inhibit a previously learned response [42].

Regarding to children and young adults (aged 4-18 yr) a beneficial relation between physical activity and cognitive performance has been observed [43]. In this way, it seems that early interventions might be important for the improvement and/or maintenance of cognitive health and function throughout the adult lifespan, since stronger relationship between physical activity and cognitive performance has been found in the younger children (ranges of 4-7 and 11-13 yr) [43]. As regard the effects of physical activity on cognition and brain outcomes in young a middle-adults (18-59 yr), there is a dearth of high-quality data available in this population. Nonetheless, several reports have investigated physical activity on cognition across the adult life span, but the majority of the studies included older population (>60 yr), individuals with clinical disorders, or they just focused on acute physical activity effects [29]. One obvious reason for this absence of literature is that cognitive health peaks during young adulthood, which suggests that there is no space for exercise-related improvement to cognitive function during this period of the lifespan [18].

Physical fitness and cognition

Multiple studies have demonstrated that engaging in regular exercise reduce the risk of many chronic diseases [12]. In fact, physical parameters such as body composition (i.e., BMI) have demonstrated a negative relation to academic achievement, whereas physical fitness scores (i.e., cardiovascular endurance) have been related to achievement in mathematics and reading in school-aged [18,44]. It has been reported that cognitive function in the elderly is improved even when moderate intensity exercise training is performed for 6 months or longer [45]. Similar improvements has been found in visual-spatial memory and short-term memory after 6-12 weeks of exercise intervention in young adults [46,47]. It was also suggested that aerobic training improves performance on tasks which demand greater executive control processes, a phenomenon known as the "selective improvement" hypothesis [48,49]. The selective nature of the improvements produced by aerobic exercise, which affect only executive control processes supported by frontal and prefrontal regions of the brain, might explain the ambiguity of previous studies relating aerobic fitness with improved neurocognitive function. These improvements seem to require only small increases in aerobic fitness [48]. The positive effects of fitness on perceptual, cognitive, and motor processes may reflect a predisposition of the exercisers toward fast and accurate responding rather than a benefit of aerobic fitness achieved through exercise. An interesting question for future research concerns the manner in which these performance changes are supported by changes in patterns of brain activation, as inferred from positron emission tomography, functional magnetic resonance imaging (**fMRI**), and optical imaging. Clearly, changes in cognitive performance must be mediated by changes in neural activation. However, it is unclear precisely what role cardiovascular fitness might play in instantiating these changes [49]. Longitudinal assessments of cardiovascular changes and neurocognitive functioning would allow one to test the role that dedifferentiation plays in normal aging more directly. Such assessments would enable researchers to determine whether improvements in cognitive function that result from enhanced cardiovascular fitness would lead older adults to become more dissimilar from younger adults in their patterns of brain activation (i.e., increased dedifferentiation) [49].

Despite these impressive results, an important unanswered question concerns why some studies find improvements in cognitive performance with enhanced aerobic fitness while other studies have failed to observe such a relationship [50]. Definitely, some of the ambiguity in the results obtained in the human studies could be the result of methodological factors, such as (i) widely varying age groups, (ii) different nature, intensity, and length of the aerobic fitness manipulations, (iii) the type of fitness measures employed, (iv) the general health and fitness level of the participants at the beginning of the study, (v) subjects' gender, (vi) the tasks used to measure aspects of cognition, and (vii) the nature of the control groups. However, studies of the influence of fitness training on cognition have differed not only with regard to these methodological concerns, but also with regard to the theoretical framework in which they have been conducted [49]. Differences in theoretical framework to select cognitive tests, in turn, influence the tasks selected to index fitness effects [49].

Socio-emotional mechanisms of exercise

These terms refer to mental states and higher-order behaviors that may be influenced by exercise and thus contribute to some of its salutary effects on brain and cognition. Mood, in particular less depressive symptomology, is one likely psychosocial mechanism of exercise [51]. In population samples of healthy individuals, higher levels of objectively measured physical activity are associated with lower levels of depressive symptoms and better cognitive function in children and adolescents (ages 5–17 yr) [52,53], as well as in older adults [54]. Emerging literature also suggests that physical activity and fitness may have a positive effect on mental health outcomes for youth (i.e., depression and anxiety) [55,56]. Most of the causal evidence for mood as a mechanism of exercise comes from studies of patients diagnosed with a mental disorder (i.e., major depression). Recent meta-analyses of exercise interventions have concluded that exercise is an effective additive treatment (compared with treatment as usual) to reduce symptoms of depression in adolescents, young adults, and older adults with depressive or psychiatric disorders [57,58]. Moreover, exercise concurrently improves the cognitive symptoms typical in these disorders, supporting that exercise-induced changes in mood may at least partially underlie similar improvements in cognitive function in patient populations, which may occur through molecular and structural brain changes [57]. The mechanisms linking exercise to cognition and depressive symptoms may therefore be bidirectional. Furthermore, mood is linked to cognitive performance and physical activity. While several studies have considered mood along with other behavioral or socioemotional factors in statistical models assessing mechanisms of physical activity, virtually none have considered the unique or independent contribution of mood to the physical activity-cognition relationship [51]. These results of the studies of physical activity and mood reviewed indicate that we do not yet understand whether, to what extent, and how changes in mood mediate the effects of physical activity on cognition, which suggests that there are likely many other possible behavioral mechanisms, such as self-efficacy, motivation, fatigue, or pain, that should be investigated in future work.

However, despite there is strong evidence indicating that HIIT can elicit a positive effect on depression [59] and emotional well-being [60] in a range of adult population groups (i.e., older adults, cancer patients, and cancer survivors), little is known regarding the effect of HIIT on cognitive and mental health outcomes in young and middle-aged populations. Therefore, additional studies including both psychological and neurocognitive functioning as outcome variables are needed to enhance our understanding of this level of analysis.

HIIT AS AN EFFICIENT TRAINING

HIIT is a modern form of training more similar to what has been the physical activity traditionally carried out by humans (or their closest neighbors on the phylogenetic scale), where the physical activity carried out was aimed at individual and collective survival, as well as to the preservation of the species. In fact, in natural adult life, such as the one that our ancestors have led until relatively few years ago, the determining factors for the practice of physical activity with a certain level of intensity have been searching for food or shelter, defending against attacks or attacking, facing adverse environmental circumstances, seeking reward, following instincts, and reproduction. In this regard, HIIT is a type of activity very similar to what we were supposed to do naturally and to meet those needs. This activity is characterized by bouts of high or very high intensity, performed at "near maximal" or "submaximal" intensity (≥80% maximal heart rate), short duration, and varied in nature. These activities allowed small intervals of rest or post-effort recovery and were presented in accumulated sessions lasting less than one hour, with the necessary breaks between sessions given their intensity. HIIT can serve as an effective alternative to traditional resistance training, causing similar, or even higher, physiological adaptations in both healthy or unhealthy individuals [61]. This fact is important from the point of view of public health and could be considered when promoting training as a strategy for improving functional capacity and performance, and even for promoting health or with anti-aging purpose, especially in sedentary individuals who do not perform other exercises and lack of time [62]. Some operational advantages that HIIT offers including: (i) substantial physiological stimulus possible in a short time period; (ii) the potential to impact multiple components of fitness simultaneously; (iii) is typically performed using a single exercise mode; and (iv) an ability to target upper- and lower-body function [63].

The most relevant acute responses or effects that occur during and after HIIT sessions are: marked increases in heart rate, increases in plasma lactate concentration, release of catecholamines, growth hormone, cortisol, and various metabolic changes including decrease in blood glucose, and therefore insulin, as well as greater lipid mobilization. As a consequence of the muscle contraction, there is a decrease in muscle phosphagens (ATP, PCr), glycogen, and, finally, a variable parasympathetic reactivation after exertion [64]. All this underlines the important level of overload to which the person is exposed, although it must always be adapted to the individual characteristics and possibilities. Indeed, HIIT induces numerous physiological adaptations including improved aerobic and anaerobic capacity [61,63]. It also improves glycemic control and insulin sensitivity, endothelial function and some components of blood pressure and tissue perfusion, as well as an increase in fatty acid oxidative muscle capacity, resting glycogen storage and gene transcription. These effects explain why HIIT could be applied in the treatment, control, and prevention of certain cardio-metabolic pathologies, such as heart failure [65], hypertension [66], coronary diseases [67], type II diabetes [68], obesity [3], and metabolic syndrome [69]. Another possible application would be in pathologies with impaired lung function.

HIIT performed several months elicits improvements in VO_{2max} in normal weight and overweight/obese population, and the conventional wisdom is that intervals of 3–5 minutes are especially effective in evoking increases in exercise capacity [61,70,71]. Also, it has been found these improvements in VO_{2max} are greater for less fit adults, demonstrating that aerobic training has an adaptive effect that favours the less fit [71]. Further to this, HIIT has a clear beneficial effect on the aerobic fitness of healthy young to middle-aged adults when compared with no exercise, which could be moderate by initial fitness as the training benefits individuals with lower initial fitness [70,71]. It is well accepted that the increases in VO_{2max} with training are due to increases in cardiac output and peripheral oxygen extraction. HIIT is able to increase VO_{2max} rapidly via increasing mitochondrial density, resulting in the generation of more ATP for working muscles, thereby producing greater force generation for a longer duration [61]. Although, the potential for HIIT to increase muscle mass remains largely unknown, emerging evidence suggests that HIIT increases explosive muscular power, assessed via leg extension [72] and standing broad jump [73]. These findings reaffirm the potential for HIIT as a training strategy capable of improving cardiorespiratory and muscular fitness simultaneously. In addition to that, and as can be easily understood, HIIT leads to improvements in body composition (reducing body fat levels even in the absence of weight loss), anthropometric measures resistance, and strength [14,61]. Possible mechanisms underlying HIIT-induced fat loss include generation of catecholamines that increased fat oxidation and fat release from visceral fat stores, decreased post-exercise appetite and increased excess post-exercise oxygen consumption resulting in an elevated fat loss state [61]. One intriguing finding is the absence of weight loss despite observed decrease in body fat, this is likely to be a consequence of gain in muscle mass. In summary, HIIT is a highly valuable and effective training method, that can be used not only as a means to improve fitness and performance, but also to prevent and treat diseases related to a sedentary lifestyle, and prevent or delay the consequences of aging [1,15,74,75]. The challenge therefore for future research is to identify the optimal length and work-to-rest ratio of HIIT that would provide maximum health benefit.

Regarding to the effects of HIIT on cognitive function, recent studies have found positive effects of HIIT on information processing speed, which is a lower level of cognitive process [76]. However, contradictory results have been found related to performance on the executive function tasks [76]. Some authors have found acute positive effect of HIIT on inhibitory control, which was sustained for 30 min following training [77]. The authors argued that alteration in brain lactate metabolism, due to higher blood lactate during the post-HIIT period, may be associated with prolonged improvements, which might also be associated with larger elevation in catecholamines and brain-derived neurotrophic factor following HIIT [77]. Specifically, HIIT exhibited larger and longer increases in blood lactate, which coincided with prolonged improvement in inhibitory control [78]. Indeed, improvements in RT have been observed following HIIT in a sample of young adults, suggesting that this type of exercise enhanced response speed associated with inhibitory control [78]. Additionally, a selective effect during task conditions requiring greater amounts of inhibitory control was reported [78]. Likewise, improvements in anxiety and depression have been seen following HIIT and these results seem to be consistent across a range of ages and across healthy populations [14]. Finally, HIIT may be a viable strategy for obtaining positive psychological responses, revealing that HIIT sessions could be perceived more enjoyable than moderate-to-vigorous intensity continuous training [79]. However, it is generally agreed that high intensity exercises above the ventilatory threshold could evoke displeasure feelings towards exercise, which has been translated in a negative relationship between exercise intensity and affect [79]. The inconsistent findings of exercise enjoyment responses between studies could largely be attributed to differences in the chosen exercise protocols (i.e., work-to-rest ratio, exercise intensity and the total exercise duration) and traits of the participants (i.e., health and fitness conditions) [79]. Cardiovascular fitness has been proposed as a potential mediating factor in the enhancement of cognitive performance [13,48,49]. However, the studies investigating the effects of acute exercise on cognitive processing have been divided as to whether physical activity facilitates cognitive function. Hence, the question regarding the effect of HIIT on cognition is a matter of interest.

Gender differences…

Although gender differences are obvious in physical and metabolic performance regarding exercise, this issue has not been generally considered in the literature during the past decade [80]. However, authors have concluded that data derived from male subjects should not be directly applicable to women.[81] Apparently, there is a gender difference in protein use which is related to reduced glycogen and/or enhanced fat use in women, perhaps as a result of differing hormonal responses [81]. In addition, gender differences related to metabolic substrates use and metabolism have led to discussion regarding macro- and micronutrient recommendations [80]. Further, important physiological and functional differences have been noted between the male and female responses to dynamic exercise. In addition sex differences have been reported for most of the major determinants of exercise capacity [82]. Women typically have smaller lung volumes and maximal expiratory flow rates even when corrected for height relative to men. Furthermore, differences in resting and exercising ventilation across the menstrual cycle, although the functional significance remains unclear [82].

On the other hand, previous studies have revealed the importance of sex as a moderator of the association between exercise and cognition [83]. Specifically, greater cognitive performance of executive functions have been found in women than in men [83]. The possible sex difference in the cognitive enhancing effects of different exercise interventions may be related to sex steroid hormones, which could play a role in neuroplasticity and preservation of cognitive function [83]. Regarding gender differences with respect to the association between exercise regulations and behavior, it has been found that introjected regulation may be more positively associated with exercise among females than males [84]. Indeed, it has been suggested that more active males might respond more negatively to social pressures to exercise related to their skills in external regulation [84].

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Chapter 2

Recovery process, physical exercise, and beer/alcohol consumption

Proceso de recuperación, ejercicio físico y consumo de cerveza/alcohol

Recovering process, physical exercise, and beer/alcohol consumption

Exercise / stress, rest, and recovery

Intense physical exercise represents an overload or stress for the body. This determines an important energy consumption derived from stored energy substrates among which glycogen, can be a limiting factor [1]. Energy depletion determines a decrease in functional capacity, only recovered with rest and adequate supply of nutrients that replenish the energy stores. On the other hand, high intensity exercise induces a wear and tear of functional structures involved in the neuro-muscular activity [2]. These wear and tear processes greatly contribute to the decrease in functional capacity while exercising. The worn and damaged functional structures must be adequately replaced during the post-stress recovery process [3]. This recovery must be adequate and require sufficient rest and appropriate nutrition. Four important processes occur during the recovery process [4]. First, the removal of damaged structures; second, the replacement of damaged structures; third, the repair of those structures that can be repaired; and fourth, the protection or hypertrophy of these structures so that they can cope with a new overload in better conditions [5]. This process is known as adaptation or supercompensation [6], and constitutes the theoretical basis of the training process. From a functional point of view, it implies not only the recovery of the work capacity but also the adaptation to a better response. To achieve this supercompensation state, it is necessary to replace the water and glycogen losses produced during the exercise session [7]. In addition, it is necessary to eliminate the waste products for which the liver and kidney play a fundamental role and require the absence of hepatotoxic or nephrotoxic factors and an adequate hydration that facilitates elimination through urine or even bile [8]. During this process, it is also necessary to normalize the neuro-vegetative functions, both at the peripheral and central nervous system, the normalization of bioelectric processes, and the restoration of the capacity for accumulation and use of energy and neurotransmitters, particularly those corresponding to the structures more demanded during the exercise sessions [9].

Quality of rest and sleep

Sleep is an active state of unconsciousness produced by the body when the brain is in a relative state of rest [10]. It is a biological need that allows restoring the physical, mental and psychological functions essential to start a new day. It allows the body to repair and replace the cellular components necessary for biological functions that deteriorate throughout the day. Various functions such as muscle repair, tissue growth, protein synthesis [11], and the release of hormones and growth factors take place mainly during sleep [12]. Rest and sleep are essential for health and basic for a good quality of life.

Scarce, non-restorative sleep affects humans at a physical level (presentation or worsening of diseases such as diabetes, obesity and cardiovascular diseases), at a cognitive level (memory, learning and concentration alteration), at an emotional level (mood alteration, depression and irritability) and also behavioral (fatigue, tiredness). Various studies suggest that insufficient or non-restorative sleep affects mental and physical health, deteriorating the quality of life [13], and negatively influences the adaptive response to any training program, especially if it is highly demanding as is the case with HIIT.

Nutrition and hydration during the recovery process

The traditional Mediterranean diet has been the basis of diet for millennia in many countries of the Mediterranean basin. However, the traditional Mediterranean diet is changing due to globalization and westernization. Although it is presented and understood as a dietary pattern, it really encompasses much more than diet, it is a way of life including multiple sociological elements and a healthy lifestyle [14].

The dietary pattern of the Mediterranean is based on foods of vegetable origin, whose main pillars are use of olive oil as the main cooking fat and dressing; abundant greens, vegetables and fruits, mainly seasonal; cereals (bread, pasta, rice...) as a the main source of carbohydrates; legumes and tubers as ingredients in traditional dishes; dairy products ingested in moderate quantities, traditionally in the form of yogurt, cheese and other fermented products; extensive and varied use of spices, aromatic herbs, garlic and onion to introduce a variety of smells and flavors to dishes and consequently reduce the addition of salt; olives, nuts and seeds; little amounts of foods of animal origin notand used as the main ingredient of the main meal, but rather as a source of flavor, prioritizing the consumption of fish and eggs instead of meat products; in general, the size of the portions being moderate [14]. A characteristic component is the moderate consumption of wine and other fermented beverages, such as beer, mainly accompanying the main meals of the day. To be considered healthy, the consumption of these drinks, as in all of the above items, the ingested amounts must be moderate, taking as a general reference: one drink a day for women and two drinks a day for men. A specific characteristic that incorporates the Mediterranean diet is socialization, providing a cultural and social value to the process of eating, being generally considered as a relaxed activity. The consumption of moderate amounts of wine or beer may help in this regard [14,15].

The great variety of health benefits of the Mediterranean diet are popularly known, especially against chronic diseases [14,16–18]. The Mediterranean diet pattern has been shown to have a positive effect on the prevention and management of cardiovascular diseases, hypertension, type II diabetes, cognitive decline, dementia, and cancer; as well as reducing the risk of fractures due to falls and an increase longevity [1,19]. However, there are no data in the scientific literature that analyze the effects of the Mediterranean diet pattern on recovery after exercise or in the supercompensation effect of training [20]. In this sense, the habitual intake of beverages with alcoholic content, even in moderate amounts, can have a negative influence by affecting the rehydration process given the diuretic and anti-dipsogenic effect of alcohol among other deleterious effects [21–24]. Water loss due to exercise-induced perspiration can alter the water and electrolyte balance, hinder thermoregulation, and even lead to dehydration [25]. Consequently, it represents a health risk or, at least, cause a decrease in performance. The rehydration phenomenon is regulated by the sensation of thirst, this being a phenomenon with great variations among individuals and circumstances [26]. In today humans in the current living conditions of occidental countries, the intake of fluids, and therefore water, is not only determined by the sensation of thirst, other factors are possibly more determinants than thirst itself.Among them are: (i) the reward or pleasure determined by the intake of certain beverages, (ii) the ready availability of water and other beverages, (iii) the regular intake of food, generally accompanied by water or other drinks, (iv) several social factors, including the personal time schedule, which frequently leads to drink even in anticipation of feeling thirsty. In any case, it is essential to assure adequate rehydration particularly after carrying out physical activity in order to preserve health and guarantee well-being. pleasant [27].

Beer/alcohol and physical exercise

Currently, beer is the second or third most consumed beverage in the world, depending on the area, and the first with alcoholic content [15,28,29]. It is ingested when thirsty, accompanying food, or even having a beer after finishing exercise is a common practice for many physically active people and is also characteristic of many sports [14]. In particular, its consumption is common after certain recreational sports or team activities as a social aspect of sporting events, for example to facilitate team bonding and as part of post-match celebration and relaxation [30]. Beer contains 90-94% water; therefore, it has the ability to rehydrate, but also beer contains around 4-8% alcohol [31,32]. This can have negative effects on the rehydration process and in the post-effort recovery process [33], as discussed below. It also provides a series of nutrients that can influence the tolerance to exertion, the recovery process, by influencing rest and sleep, and even specifically by preventing the adequate clearing, repairing, and protection of damaged functional structures [15]. This can have consequences both at physical and mental level. Among the substances likely to be of influence, in addition to water and alcohol, are polyphenols, antioxidants, vitamins, potassium, and other minerals, as well as other beer components derived from the three main ingredients with which beer is made: water, malted barley, and hops, which is a cannabis plant [15]. It also contains carbon dioxide derived from natural fermentation. This gas may cause stomach bloating, and influence fluid intake. In any case, the most controversial and dangerous component of beer is alcohol, which represents between 4% and 8% of the total volume, depending on the type of beer.

Alcohol has a thirst-quenching effect and an inhibitory effect on antidiuretic hormone. This determines a diuretic effect leading to a negative water balance. In addition, alcohol can have an effect on the central nervous system [34,35]. Studies of the effects of alcohol on cardiovascular, respiratory, and muscular function have provided conflicting results, suggesting that ingestion of small amounts of alcohol has been reported not to significantly alter the cardiorespiratory and metabolic responses to submaximal exercise [35]. In general, it has been concluded that the acute ingestion of alcohol has no beneficial effects on aspects of muscle function and performance tasks because it is not used as a source of energy by muscles [36,37]. Therefore, exercise does not increase alcohol metabolism, as generally believed by lay people [37]. Overall alcohol is thought to impair muscular work capacity and to result in a decrease in overall performance levels, to impair temperature regulation during exercise, and to increase the onset of fatigue during high-intensity exercise [37]. It is known to inhibit glutamatergic activation, which is excitatory, and also behaves as a GABAergic agonist, and as such determining a relaxing or depressant effect [33]. This, together with the effect of lupulins (derived from hops), can influence the wear and tear and recovery process by reducing stress, improving the response to overload, and facilitating rest [35]. Alcohol can also negatively influence the process of reconstruction, repair, and protection of structures damaged as a result of exercise. Consequently, beer intake is controversial in particular when linked to the practice of physical activity, either recreational or associated to work [21,24,25,38]. Indeed, since alcohol is an energy-dense nutrient, frequent episodes of heavy alcohol intake, which is commonly called binge drinking, are generally accompanied by weight gain [39–41]. However, weekend binge drinkers tend to maintain their food consumption, since alcohol does not seem to regulate total energy intake in the short term [33].

Various investigations have focused on the role of beverages and their effect on hydration and dehydration during physical exercise [7,21,34,35,42–44]. Professional athletes do not usually consume alcohol, but the situation is the opposite in amateur athletes for whom beer is the preferred drink [29]. There are some suggested mechanisms by which alcohol might affect sports performance (see Figure 1). Alcohol inhibits the production of antidiuretic hormone (ADH), potentially leading to increased diuresis, and subsequent water and electrolyte imbalance [45,46]. Additionally, energy metabolism, psychomotor skills, and performance can be impaired by alcohol in a dose dependent manner [35,37,47,48]. Furthermore, glycogen restoration is crucial for sports performance, but it is suggested that alcohol may interfere with activation and inhibition of glycogenolysis and gluconeogenesis, respectively [42]. Similarly, it is believed that alcohol can affect muscle recovery processes by means of various mechanisms, including the immune system [21,22,49,50]. To what extent these mechanisms affect the recovery processes in amateur athletes deserve to be studied.

Besides the deleterious effects of excessive consumption of alcohol on overall health [15], alcohol use has a complex association with physical and mental health [51]. While some studies have suggested that low alcohol consumption could even have protective effects on cognitive function decline [51,52], other researchers have found negative associations, even at moderate levels [53]. Earlier studies on different types of alcohol containing beverages on abdominal obesity have also shown conflicting results [54]. The mechanism for the association between alcohol intake and abdominal fat deposition is not completely understood, but effects of alcohol on the endocrine system may be important. Previous hypotheses have shown that regular alcohol intake stimulates the hypothalamic-pituitary-adrenalin axis, also it seems that alcohol directly stimulate the release of glucocorticoids from the adrenals and an increased secretion of cortisol has been associated with an altered fat distribution with a pronounced intra-abdominal fat deposition [40].

The consumption of beer with food and after exercising is a common practice in physically active individuals and in amateur athletes, particularly when the level of perspiration is important [35,42,55]. Generally, the ergogenic benefits of alcohol intake immediately before and during exercise are psychologically driven. Alcohol has been used to decrease sensitivity to pain, improve confidence, and to remove other psychological barriers to performance [35]. It may also be used to stimulate the cardiovascular system or to lessen the tremor and stress-induced emotional arousal in fine motor control sports [35]. Alcohol may also positively modulate the ratings of perceived exertion. A schematic view of the effect of alcohol on physical performance through physiological and psychological factors is presented in Figure 1. The available evidence showed a detrimental effect of small to moderate amounts of alcohol on reaction time, hand–eye coordination, accuracy, balance, and complex skilled tasks, with no evidence cited to support the purported beneficial effects of reduced tremor [35]. However, it is not only negative side effects that are observed with alcohol consumption. In moderate dosage, alcohol may have some advantages through psychobiological mechanisms [37]. It has, however, been proposed that the ingestion of small amounts of alcohol may result in a greater feeling of self-confidence in athletes [37]. The discrepancies in the literature can be explained on several grounds: (i) differences in the experimental design, (ii) the type of exercise performed, (iii) the absolute and relative intensity of exercise used, as well as its duration, (iv) the nature of performance measured, (v) the training level (for both exercise and alcohol consumption!) and (vi) methodological issues [37].

To the best of our knowledge, there are no scientific studies that have studied the effect that the moderate but systematic intake of beer, either with or without alcohol, may have on the adaptive response to an intensive training program that involves significant physical and mental overload. Given that the consumption of beer is common practice for many people exposed to significant physical efforts or training programs, it is worth to investigate its effects.

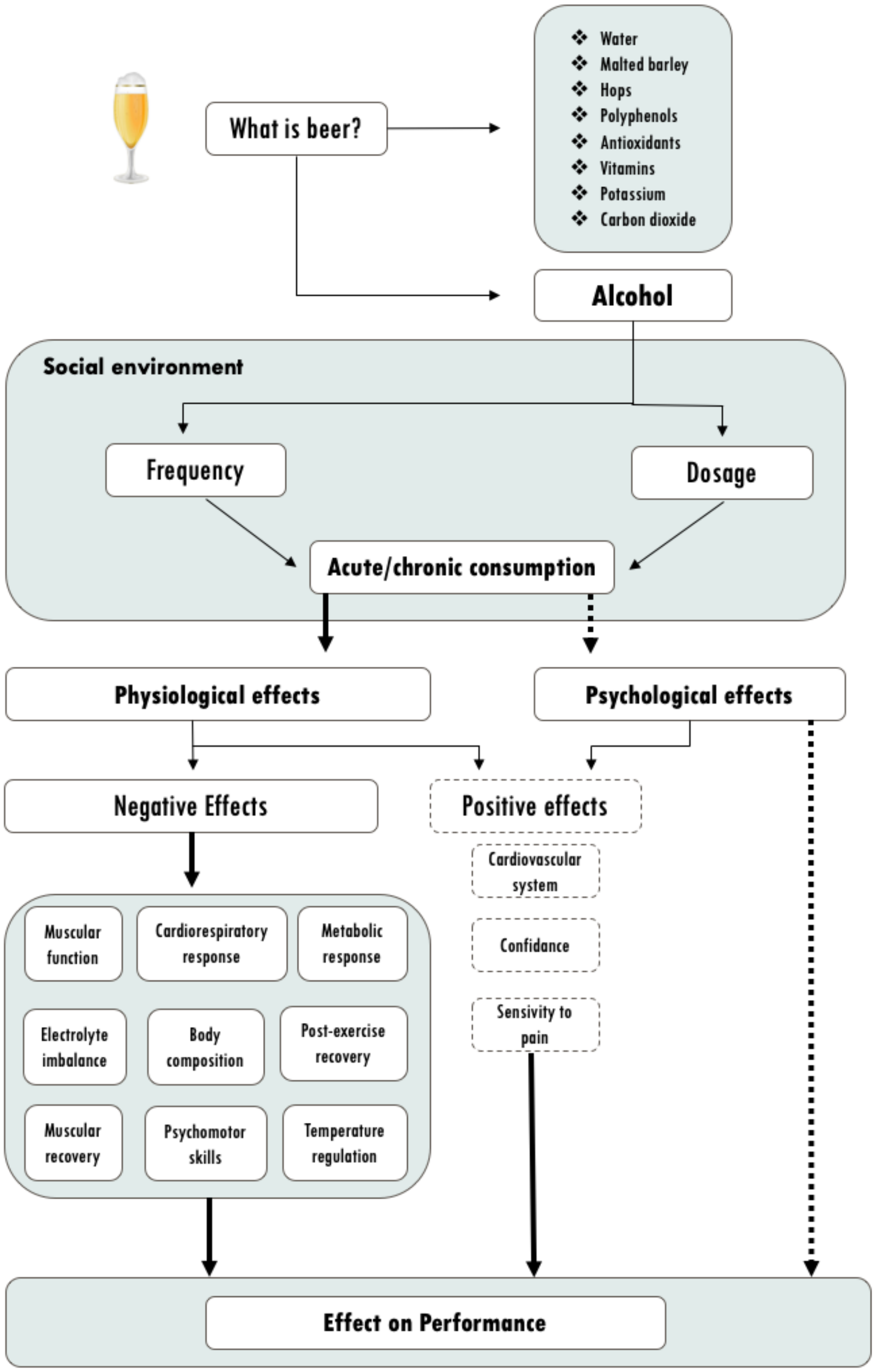

Figure 1. Schematic view of the numerous physiological and psychological effects induced by alcohol consumption and their potential impacts on physical performance. Solid lines, solid evidence; discontinuous lines, probable influence.

Gender differences…

Gender differences in alcohol effects and consumption must be considered [56]. Some studies have found that women tend to drink less, whereas men often drink higher quantities and prefer spirits [57]. Researches on biological gender differences have shown that alcohol has differing effects on the female and the male body. It has been observed that whereas blood alcohol is similar between genders when it was given intravenously, higher levels are found in women when taken orally [60]. These gender differences could potentially affect physiology and performance. For instance, by affecting upper body muscle mass and strength, endurance capacity in isometric and dynamic exercise at relatively low intensities, resting metabolic rate, and heart rate measured during different exercise modalities [58]. Due to the greater average content of lipids and the smaller average content of water in women's bodies, the same amount of alcohol per body weight, consumed in the same length of time, leads to higher blood alcohol levels for women than for men [56]. Indeed, ethanol metabolism has been suggested as another biological reason for women's greater vulnerability to the effects of ethanol due to the fact that women have lower total gastric alcohol dehydrogenase isozymes in the stomach. This leads to a significantly lesser activity for the Class III enzymes, which play an important role in the metabolism of primary alcohols [56].

On the other hand, some differences between alcohol consumption and central obesity (BMI and waist circumference) have been found among women only with high alcohol intake, whereas in men the association was more linear [59]. However, other studies have found a positive association between alcohol consumption and abdominal fat in men only, who reported higher spirits consumption than women [40]. In this regard, moderate alcohol use is being associated with better cardiovascular prognosis in hypertensive patients, consumption levels of <30 g/d, that is 2.5 drinks/d for men and <15 g/d, that is one drink/d for women. [31]. Different beverages may also differently affect men and women [57]. The strong difference in the effects of different types of alcoholic beverages seems to suggest that ingredients other than ethanol contribute to a beneficial effect of fermented beverages on dementia, such as flavonoids acting as antioxidants or vasoactive polyphenols, not typically contained in spirits. Most alcohol-related cancers (85-90%), in fact, are due to heavy drinking (>2 drinks per day in men, >1in women), even moderate drinking is associated to about 50-60,000 cancer cases and 30-40,000 deaths per year worldwide [15].

As previously presented, there are clearly gender-related differences in consumption patterns, but age, socio-economic background, and geographical location also influence drinking habits. Indeed, the rate at which ethanol is cleared by the liver varies widely between individuals, and the response of the individual will depend on the amount of ethanol consumed in relation to the habitual intake. Therefore, gender must be considered while studying the role played by beer intake on the response to a highly demanding training program.

SUMMARIZING…

The present study has been designed to shed light on the existing controversy about the moderate beer and alcohol consumption in relation to exercise or, more particularly, while training, in healthy individuals [21,24,38,61]. The primary aim of the study is to evaluate the effects of a HIIT program on physical performance (such as cardiorespiratory fitness and body composition), as well as, mental health (psychological and psychosocial aspects), and cognitive function; and subsequently, to assess whether those effects are influenced by the regular consumption of beer, or its equivalent alcohol content, in moderate amounts. One of the novelties of this study lies in the evaluation of psychological parameters such as motivation, mood state, self-perception, quality of life, or positive-negative emotional perception, while training in conjunction with moderate and regular alcohol consumption, reflecting real-life conditions.

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AIMS

The overall aims of this International Doctoral Thesis were:

SECTION 1: Combined effects of a highly demanding traning program and moderate alcohol/beer consumption on physical performance

- **General objective 1:** To evaluate the effects of a HIIT program on physical performance (such as cardiorespiratory fitness, body composition, and cardiovascular adaptations), and to assess whether those effects are influenced by the regular consumption of beer, or its equivalent alcohol content, in moderate amounts, in young healthy adults.
    - **Specific objective 1.1:** To study the effects of a HIIT program on body composition and to asses the potential influence on those effects of regular, but moderate, beer/alcohol consumption **(Study 1).**
    - **Specific objective 1.2:** To study the effects of a HIIT program on cardiorespiratory fitness and muscular strength, and to asses the potential influence on those effects of regular, but moderate, beer/alcohol consumption **(Study 2).**

SECTION 2: Combined effects of a highly demanding traning program and moderate alcohol/beer consumption on cognitive performance

- **General objective 2:** To evaluate the effects of a HIIT program on conginitve function (such as psychomotor fucntion, memory, attention, verbal fluency, and working memory), and to assess whether those effects are influenced by the regular consumption of beer, or its equivalent alcohol content, in moderate amounts, in young healthy adults.
    - **Specific objective 2.1:** To study the effects of a HIIT program on psychomotor performance and to asses the potential influence on those effects of regular, but moderate, beer/alcohol consumption ***(Study 3).***
    - **Specific objective 2.2:** To study the effects of a HIIT program on verbal memory, attention, verbal fluency, and working memory, and to asses the potential influence on those effects of regular, but moderate, beer/alcohol consumption ***(Study 4).***

SECTION 3: Combined effects of a highly demanding traning program and moderate alcohol/beer consumption on psychosocial variables and mood state

- **General objective 3:** To evaluate the changes on psychosocial variables (such as depression, satisfaction with life, subjective happiness, positive and negative expentacies, and perceived stress) and mood state (positive and negative emotions, emotional intelligence, and positive and negative affect), while performing a HIIT program, and to assess whether those effects are influenced by the regular consumption of beer, or its equivalent alcohol content, in moderate amounts, in young healthy adults ***(Study 5).***

MATERIAL AND METHODS

All the content regarding the material and methods of the present International Doctoral Thesis is in course of being published in a methodological article on the BEER-HIIT study [1], in which the author of the present Doctoral Thesis appears as the first co-author.

DESIGN

The present doctoral thesis was conducted under the framework of the BEER-HIIT study, an exercise-based registered controlled trial (ClinicalTrials.gov ID: NCT03660579). The study protocols, experimental design, and informed consent procedure were approved by the Ethics Committee on Human Research at the University of Granada (321/CEIH/1617). Prior to any data collection, all participants provided written informed consent after having received and understood the details of the study assessments and intervention. Following baseline testing, participants were allocated into two different groups, based on individual preferences: (1) a Non-Training group or (2) a training group. High intensity interval training (HIIT) Training consisted in two HIIT sessions per week, during 10 consecutive weeks (see below). Those going for training then choose whether they preferred to be included in a group who would ingest from Monday to Friday, during those 10 weeks, an ethanol containing beverage (5.4% alcohol content) or an alcohol-free beverage. Men ingested 330 ml of that beverage with lunch and 330 ml with dinner. Women ingested 330 ml with dinner. All of the baseline and post-intervention measurements were performed in the same setting [Sport and Health University Research Institute (iMUDS) at the University of Granada]. The study followed the last revised Ethical Principles for Medical Research Involving Human Subjects comprised in the Declaration of Helsinki [2] and was conducted from February to May 2018 in Granada, Spain. The BEER-HIIT study had highly standardized protocols for selection criteria, recruitment, exercise training program, outcomes measurements, and data collection.

Participants and eligibility

The participants are healthy Caucasian adults, aged between 18-40 years old, living in Granada (Spain). The inclusion and exclusion criteria are listed in Table 1. All participants had a health history and a medical examination done prior to the intervention program to minimize risks by ruling out contraindications to the testing and training protocols. If any participant suffered any injury or medical problem, a medical evaluation was performed and, if necessary, excluded from the study.

Table 1. Eligibility criteria

Inclusion criteria	Exclusion criteria
- Age: 18-40 yr	- History of cardiovascular disease or diabetes
- Body Mass Index: 18.5-30 kg/m2	- Personal or family history of alcohol related problems
- Not having a family history of alcholism	- Pregnancy or planning to get pregnant during the study period
- Not engaged in a structured training program or weight-loss program (last 5 months)	- Beta blockers or benzodiazepines use
- Stable weight over the last 5 months (body weight changes < 3kg)	- Taking any medication of thyroid
- Normal electrocardiogram	- Other significant conditions that are life-threatening or that can interfere with or be aggravated by exercise
- The participants must be capable and willing to provide consent, understand the exclusion criteria, and accept the randomized group assignment	- Unwillingness to either complete the study requirements or to be assigned to the training groups

BMI: Body mass index.

Prior to the enrollment, all potential individuals completed a health history revision and were medically examined to identify any pathological condition and current medication that could affect the ability to complete the required assessment protocols and intervention exercise programs. If any medical problems appeared during the intervention, participants were referred for medical evaluation and, if necessary, dropped from the study. Prior to the evaluations the participants were asked: to avoid moderate exercise (24 h), and strenuous exercise (48 h); to avoid the consumption of alcohol, drug or medication during the previous 48 h; to avoid the consumption of stimulants such as coffee or caffeine containing beverages the previous 12 h; to avoid the consumption of food 2 h before the evaluation and to have a standardized breakfast the evaluation day. Additionally, all the participants were asked to report their usual frequency of alcohol intake in seven possible response categories using the Beverage Intake Questionnaire (BEVQ) [3], before and after the 10-week intervention. This questionnaire was developed to estimate mean daily intake of water, sugar-sweetened beverages, and alcohol beverages. To score the BEVQ, frequency (“How often”) is converted to the unit of times per day, and then multiplied by the amount consumed (“How much each time”) to provide average daily and weekly intake beverage consumption. Total alcohol consumption was quantified through the sum of beverage categories containing alcohol (i.e. beer, wine, spirits, and cocktails). In addition, the physical activity levels were registered before and after the intervention program by self-report.

Recruitment

The recruitment of participants was performed using different strategies including the use of social networks, local media, word of mouth, and posters at different points of Granada. Furthermore, information meetings were organized at the Sport and Health University Research Institute (iMUDS) of Granada. People interested contacted the research staff through phone and/or e-mail to provide general information about the study. They visited the research center to receive a thorough explanation about the study aims, inclusion and exclusion criteria, assessments to be performed, study requirements of the participants, and types and characteristics of the intervention program. They were clearly informed about the risks of alcohol intake, the problems associated to excessive consumption, and the need of commitment for maintaining a healthy and stable lifestyle during the duration of the study. After clarification by the research staff of any participant's questions or doubts, the potentially interested participants volunteering to participate and meeting the inclusion criteria were invited to a second orientation session. In this session, the participants received detailed written information about the study methodology, and were asked to sign the informed consent. Finally, the participants were cited and informed of all necessary preconditions for their first assessment day.

Figure 2 shows the participants flow diagram from the recruitment to the randomization stages of this study.

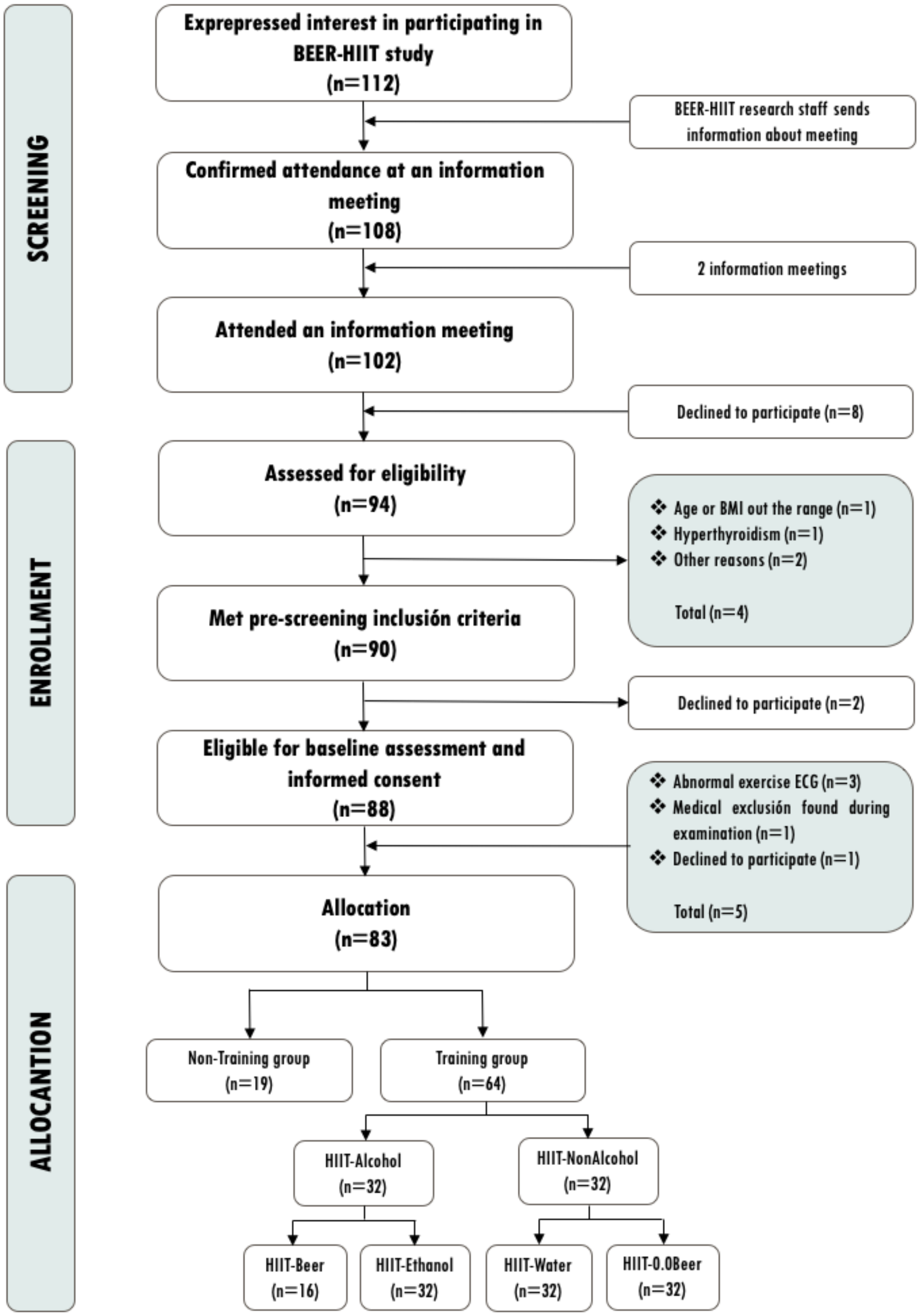

Figure 2. Flow diagram of the BEER-HIIT study participants. BMI: Body mass index; ECG: Electrocardiogram; HIIT: High intensity interval training.

Allocation and randomization

Once included in the study, the participants received an internal number to be de-identified. After completing the baseline measurements, the eligible participants choose whether they prefer to be included in a Training (T) or Non-Training group. Training consisted in two HIIT sessions per week, during 10 consecutive weeks (see below). Those going for training then choose whether they prefer to be included in a group who ingest from Monday to Friday, during those 10 weeks, an ethanol containing beverage (5.4% alcohol content) or an alcohol-free beverage. Men ingested 330 ml of that beverage with lunch and 330 ml with dinner. Women ingested 330 ml with dinner. Training (T) participants choosing ethanol were randomly allocated either to beer (HIIT-Beer) or to sparkling water with added vodka ethanol (HIIT-Ethanol). Those choosing non-alcohol were randomly allocated either to alcohol-free beer (HIIT-0.0 Beer) or to sparkling water (HIIT-Water). Each group is composed of 8 men and 8 women. This type of non-random (based on individual preference) and random allocation of the participants was done following ethical considerations and advice made by the ethical committee (321-CEIH-2017), since drinking alcohol or participating in a highly demanding training program should be a personal choice. Due to the nature of interventions, the research staff conducting exercise training sessions was not blinded. Each participant was specifically informed of their assigned group. In this sense, to ensure masking of the assessment staff, participants were frequently reminded to omit their assigned group and not talk about their interventions during follow-up measurements session

Sample size

The determination of the sample size and power of the study were based on the data of a pilot study [4]. We have considered cardiorespiratory fitness (which was considered the main outcome in the design of the study) levels differences between pre- and post-treatment in order to assess the sample size requirements for the three-way ANOVA [5]. We expected to detect an effect change of 2 ml/kg/min in cardiorespiratory fitness considering a type I error of 0.05 with a statistical power of 0.85 if we considered a minimum of 13 participants per group. Assuming a maximum loss at follow-up of 20%, we have decided to recruit 16 participants (≈50% women) for each study group. A total of 80 participants (≈50% women) were enrolled in the BEER-HIIT study. Based on previous RCT and on our own experience [6–8], we estimated that the sample size was realistic and accessible.

Participant retention and adherence

The participants were allowed to withdraw at any time throughout the intervention study; however, in order to reduce drop-out cases and to maintain adherence to the training program, every effort was made to provide a positive training environment: (i) all sessions were accompanied by music that participants could choose, and were held on an airy, well equipped, and well-lighted gym; (ii) qualified and certificated trainers were carefully supervising every session; (iii) the training sessions were conducted in small groups (≤8 persons) to ensure that participants were performing the exercises correctly at an adequate intensity; (iv) different training schedules were offered in all groups to fit each participant's needs; (v) if attendance to sessions fell below target (i.e., 90%), the exercise trainer worked with the participant to identify barriers and increase adherence; (vi) the intervention program was carried out from February to May to avoid vacations periods that might interfere with participant's availability; (vii) we used phone calls to inquire for any adverse events if a participant missed a session. These and other strategies such as positive reinforcement, regular follow-up, and support of the participants were used by training specialists and the other study staff to enhance study adherence.

High-intensity interval training - HIIT Program

HIIT refers to repeated bouts of short-to-moderate duration of vigorous and intense exercise, interspersed with passive or active recovery periods [9]. There are many HIIT protocols, and the specific physiological adaptations induced by this training modality are related to exercise stimulus (i.e., intensity, duration, or number of intervals performed among others), as well as the duration and activity patterns during recovery [6]. Similar to other studies, our project will add a small intervention effect for psychological well-being in the intervention HIIT protocol: each session will be designed to meet participants' psychological needs such as choice of music, provision of challenging and progressive workouts, working in groups, and support to professional trainers [10].

Intensity

The HIIT intensity was based on scientific evidence [9,11,12]. The participants performed different sessions of HIIT with short rest intervals, with an intensity >8 RPE [13], which has a positive linear relationship with a HR and VO_{2max} (maximal oxygen uptake) [14,15].

Traditionally, HIIT has been recommended 3 times/week [16,17]. However, considering the fitness level of the participants (untrained individuals) and based in our previous experience [6,8,18], we have decided to reduce the training frequency (twice per week) to ensure a correct training recovery after the HIIT session [16].

Type of exercise

The participants performed eight self-loading exercises in circuit form (i.e. frontal plank, high knees up, TRX horizontal row, battle rope, squat, dead lift, push up, and burpees) with a passive rest between exercises, and twice per set with an active rest (an intensity of 6 RPE, which corresponded with 60% VO2max [15,19], following the periodization described in Table 2.

Training session

The training sessions were performed in the late afternoon or early evening from Monday to Friday allowing at least two-day rest between consecutive sessions. The participants started with a dynamic standardized warm-up, including several muscle activations exercises (i.e. child's pose breathing, pelvis bridge, pelvic anteversion-retroversion, upper back rotation, front and side plank, toe walks, high knee walks, walking and side lunges, monster and sumo walk and anti-rotational stability press). In addition, the training sessions ended with a cooling-down protocol (active global stretching) including 7 anterior or posterior chain exercises (i.e. pigeon pose, lying twist, figure four stretch, lunging hip flexor stretch, biceps stretch and trapezius neck stretch).

Training periodization

The training periodization of the program is shown in Table 2. It was divided into two different phases, starting with a familiarization phase to learn the main movement patterns as previously suggested [6,8,20].

(i) **Familiarization phase**: The intensity selected for the first two weeks was 80% of VO_2max, completing 6-7 sets of 4 minutes (2 minutes work/2 minutes rest) with a maximal duration of 14 minutes/session in session type A. In session type B, the participants completed 2 sets (8-10.5 min) of 16 exercises (15-20 seconds work/ 15-20 seconds rest) with an active rest of 5 min at 60% VO_2max between each set, and a maximal duration of 21 min/session. In the third and fourth week both intensity and volume were increased. Thus, the intensity selected was 90% of VO_2max, completing 8-9 sets of 4 minutes (2 minutes work/ 2 minutes rets) with a maximal duration of 18 minutes/session in session type A. The session type B maintained the same volume of work and rest in the previous two weeks.

(ii) **Phase I**: The intensity selected was >95% VO_2max, completing 8-10 sets of 4 minutes (2 minutes work/2 minutes rest) with a maximal duration of 20 minutes/session in session type A. In session type B, the intensity was 120% VO_2max, completing 2 sets (8-16 min) of 16 exercises (15-30 seconds work/ 15-30 seconds rest) with an active rest of 5 min at 60% VO_2max between each set, and a maximal duration of 32 min/session.

(iii) **Phase II**: In this phase, the intensity was the same compared to phase I in both types of sessions (>95% VO_2max, and 120% VO_2max respectively). However, training volume was higher than phase I but not exceeding 65 minutes/week. In session type A, the participants completed 6-8 sets of 5 minutes (3 minutes work/2 minutes rest) with a maximal duration of 24 minutes/session. In session type B, the participants completed 3 sets (8-13.5 min) of 16 exercises (15-30 seconds work/ 15-30 seconds rest) with an active rest of 5 min at 60% VO_2max between each set, and a maximal duration of 40.5 min/session.

Table 2. HIIT training program periodization for 10 weeks

			FAMILIARIZATION PHASE			
Week	**1**		**2**		**3**	
Session (Type)	**1**	**2**	**3**	**4**	**5**	**6**
Exercises	8x2 =16 (W-B)	8x2 =16 (W-B)	8x2 =16 (W-B)	8x2 =16 (W-B)	8x2 =16 (W-B)	8x2 =16 (W-
Volume	16 min	16 min	21 min	21 min	16 min	16 min
Intensity	8 RPE	8 RPE	8 RPE	8 RPE	9 RPE	9 RPE
Sets	2	2	2	2	2	2
Set duration	8 min	8 min	10.5 min	10.5 min	8 min	8 min
Work exercise	15 sec	15 sec	16 sec	16 sec	15 sec	15 sec
Rest exercise	15 sec	15 sec	16 sec	16 sec	15 sec	15 sec
Rest between sets	5 min (6 RPE)	5 min (6 RPE)	5 min (6 RPE)	5 min (6 RPE)	5 min (6 RPE)	5 min (6 RPE
			PHASE I			
Week	**5**		**6**		**7**	
Session (Type)	9	10	11	12	13	14
Exercises	8x2 =16 (W-B)	8x2 =16 (W-B)	8x2 =16 (W-B)	8x2 =16 (W-B)	8x2 =16 (W-B)	8x2 =16 (W-
Volume	16 min	16 min	21 min	21 min	27 min	27 min
Intensity	10 RPE	10 RPE	10 RPE	10 RPE	10 RPE	10 RPE
Sets	2	2	2	2	2	2
Set duration	8 min	8 min	10.5 min	10.5 min	13.5 min	13.5 min
Work exercise	15 sec	15 sec	16 sec	16 sec	25 sec	25 sec
Rest exercise	15 sec	15 sec	16 sec	16 sec	25 sec	25 sec
Rest between sets	5 min (6 RPE)	5 min (6 RPE)	5 min (6 RPE)	5 min (6 RPE)	5 min (6 RPE)	5 min (6 RPE

		PHASE II	
Week	**9**		
Session (Type)	8x2 =16 (W-B)	8x2 =16 (W-B)	8x2 =16 (W-B)
Exercises	24 min	24 min	31.5 min
Volume	10 RPE	10 RPE	10 RPE
Intensity	3	3	3
Sets	8 min	8 min	10.5 min
Set duration	15 sec	15 sec	16 sec
Work exercise	15 sec	15 sec	16 sec
Rest exercise	24 min	24 min	31.5 min
Rest between sets	5 min (6 RPE = 60% VO_{2max})	5 min (6 RPE = 60% VO_{2max})	5 min (6 RPE = 60% VO_{2max})

Abbreviations: HIIT; High Intensity Interval Training: W-B: Weight-bearing exercises; VO_{2max}: Maximal oxygen uptake; Min: Minutes;

Beverage Intake Protocol

The beverages ingested daily from Monday to Friday. The volumes of fluid ingested were the same in all groups (660 ml for men and 330 ml for women), men ingested 330 ml with lunch and 330 ml with dinner, women ingested 330 ml with dinner: (i) T-Beer group ingested regular Lager Beer (5.4% alcohol-Alhambra Especial, Granada, Spain); (ii) T-0.0Beer group ingested alcohol-free beer (0.0% alcohol-Cruzcampo®, Sevilla, Spain); (iii) T-Water group ingested sparkling water (Eliqua 2®, Font Salem, Spain); (iv) T-Ethanol group ingested sparkling water with exactly the same amount of distilled alcohol added. The distilled alcoholic beverage used in our study was branded vodka because of the purity of its composition (37.5% ethanol and 62.5% water). Table 3 shows the characteristics of the drinks. We are based on scientific evidence to select the amount of alcohol ingested by the participants, which define a moderate amount as two or three drinks/day or 24-36 grams of ethanol/day for men and one to two drinks/day or 12-24 grams of ethanol/day for women [21,22]. The only alcohol consumption allowed from Monday to Friday was that supplied by the investigators. Specifically, the beverages were coded and provided by a staff member of our research laboratory on the beginning of each week. The investigators who did the evaluations were not aware of the group assignment of the participant. During the week-end, those participants included in alcohol groups were requested to drink no more than a moderate amount of alcohol during the Saturday and Sunday (i.e., 660 ml/day for men and 330 ml/day for women). Those included in non-alcohol groups were requested to refrain from alcohol also during the weekend. Additionally, the alcohol intakes were registered before and after the intervention.

Table 3. Nutritional characteristics of drinks

Characteristics	0.0% beer	Alcohol beer	Sparkling water	Vodka
Energy (Kj/Kcal)	84.0 / 16.0	166.0 / 49.0	0.0/0.0	508 / 121
Fat (g)	0.0 / 0.0	0.0 / 0.0	0.0	0.0
Carbohydrates (g)	4.7 / 1.8	4.2 / 0.3	0.0	0.0
Protein (g)	0.0	0.3	0.0	0.0
Salt (g)	<0.01	0.00	0.00	0.00
Sodium (mg/L)	0.0	0.0	5.1	0.0

*Values expressed per 100 mL.

Non-Training group

We provided general advices to the Non-Training group about the importance for health and well-being of having an active lifestyle and we explained the current physical activity recommendations from the World Health Organization (Samuelson, 2004). For these concerns, informative meeting presided by graduates in Sport Sciences and Human Nutrition and Dietetics were organized. The participants included in this group were encouraged to keep an active lifestyle and, if desired, participate in sport activities; but they were strictly requested not to engage in any kind of structured training program during the period of the study.

Assessment period

The primary and secondary outcomes of our study include physical fitness components, body composition and anthropometric measurements, psychomotor and cognitive variables, health-related quality of life, and psychological profile. Also, sociodemographic information was collected, such as dietary pattern, exercise habits, educational level, and occupational activity.

The measurements were organized on one day, following the following sequence: 1. Medical examination (anamnesis and physical exam including blood pressure measurement). 2. Anthropometric assessment (weight, height, waist, hip and neck circumferences). 3. Verbal memory assessment. 4. Body composition assessment by full body Dual emission X-ray absorptiometry. 5. Vertical jump assessment. 7. Psychomotor function assessment. 6. Handgrip strength assessment. 7. Working memory assessment. 8. Cardiorespiratory fitness assessment. 9. Attention assessment. 10. Verbal Fluency assessment. In addition, at different time frames, a battery test of questionnaires was completed at home by each participant (Table 4).

Table 4. Time frames of questionnaire battery

	TIME FRAMES		
QUESTIONNAIRES	**Baseline**	**Every 2 weeks**	**After 10-week**
Quality of Life and Psychosocial			
SWLS	X		X
SHS	X		X
LOT-R	X		X
PSS	X	X	X
BDI-II	X		X
Mood State			
POMS	X		X
TMMS-24	X	X	X
PANAS	X	X	X
EVEA	X	X	X
Exercise habits			
PREDIMED	X		X
BEVQ	X		X
BREQ-2	X		X
GOES	X		X
Quality of sleep			
Pittsburgh Sleep Quality Index	X		X

Abbreviations: SWLS, Satisfaction with Life; SHS, Scale of Subjective Happiness; LOT-R, Life Orientation Test Questionnaire; PSS, Perceived Stress Scale; BDI-II, Beck Depression Inventory; POMS, Profile of Mood States Questionnaire; TMMS-24, Trait Meta-Mood Scale-24; PANAS, The Positive and Negative Affect Schedule; EVEA, Scale of Assessment of Mood; PREDIMED, Questionnaire on Adherence to the

Mediterranean Diet; BEVQ, Beverage Intake Questionnaire; BREQ-2, Scale of Regulation of the Behaviour in Physical Exercise; GOES, Scale of Measurement of the Orientations of Goals in the Exercise.

OUTCOME MEASURES

Physical fitness assessment (PFa)

Anthropometric and body composition assessment (BCa)

The body weight, height, hip circumference, and waist circumference were determined following the recommended standardization procedures from the International Society for the Advancement of Kinanthropometry (ISAK) [32,33]. The fat mass, fat free mass, lean body mass, visceral adipose tissue, and bone mineral density were evaluated by conducting a full-body Dual-energy X-ray absorptiometry scan (Discovery Wi, Hologic, Inc., Bedford, MA, USA). The measurement was done according to the instructions provided by the manufacturer.

Cardiorespiratory fitness

Cardiorespiratory fitness was measured through a maximum treadmill test (H/P/Cosmos Pulsar treadmill, H/P/ Cosmos Sport & Medical GMBH, Germany) applying the modified Balke protocol [23], which has been widely used and validated [6,8,24]. We measured O_2 uptake and CO_2 production with a breath by breath gas analyzer (CPX Ultima CardioO_2, Medical Graphics Corp, St Paul, USA) calibrated with known gas mixtures and environmental air immediately before the test. During each trial, the participants were strongly encouraged to invest maximum effort. The criteria for achieving VO_{2max} were a respiratory exchange ratio ≥1.1, a plateau in VO_2 /change of <100 ml/min in the last three consecutive 10s stage), a heart rate within 10 beats/min of the aged predicted maximal heart rate (168-0.7*age) [25,26], and if these criteria were not met, the peak oxygen uptake value during the exercise test was considered [27]. The exercise electrocardiogram was continuously monitored during the test. We also controlled the blood pressure before the test, one minute after the beginning of the test, and every five minutes during the treadmill test. The participants were previously instructed and asked about the following pre-conditions: (i) to refrain from stimulant substances at least 24 hours before the test, (ii) to fast for 3 hours and (iii) to avoid any physical activity of moderate and/or vigorous intensity for 24/48 hours before the test, respectively.

Muscular strength

We measured muscular strength by the handgrip strength. The handgrip strength was measured using a digital dynamometer (TKK 5101 Grip-D; Takey, Tokyo, Japon). This measurement has been suggested as a valid test to predict muscular strength and endurance with a simpler equipment and minimizing effort from subjects, also it has been associated with whole body and upper body strength [28]. The participants maintained the standard bipedal position during the entire test with the arm in complete extension without touching any part of the body with the dynamometer except the hand being measured. Each subject performed (alternately with both hands) the test twice allowing a 1-minute rest period between measures. The grip span was determined following the procedures described previously by Ruiz et al. [29].

Muscular power

The evaluation of the power of the extensor muscles of the lower limbs was done through the "Ergo Jump Bosco System®" (Globus, Treviso, Italy). This device has a digital timer (± 0.001 s) connected by a cable to a resistive (or capacitive) platform. The timer is triggered by the feet of the subject at the moment of release from the platform and stopped at the moment of touch down. Thus, the flight time of the subject during the jump is recorded [30].

The test consists on a series of maximum vertical jumps strictly standardized (see below). This test offers a simple and intuitive method for evaluating the anaerobic (mechanical) power output from the leg extensor muscles [30]. The participants received feedback from the researcher to indicate the position of the legs, arms and trunk during the test. To familiarize the subjects with the test, the participants performed some practice trials before the assessment [31].

Four types of jumps were performed in the following order. 1. Squat Jump (SJ), the participant performs the vertical jump from a starting position of 90° knees angle without allowing any counter movement; the hands being held on the hips during the jump, thus avoiding any arm swing. The SJ evaluates the explosive power of the lower limbs. 2. Counter movement Jump (CMJ), the participant performs this jump standing with straight legs and with the hands always on the hips during the jump to avoid any effect of arm-swing. The participant performs a jump beginning with a counter movement down to a knee angle of 90°. The CMJ evaluates the explosive power with the reuse of elastic energy. 3. Abalakov-Jump (ABKJ), this jump is similar to the CMJ, but the participant can coordinate the movement of the arms to achieve higher height in the vertical jump. The ABKJ evaluates the explosive power of the lower limbs, the elastic component and the intramuscular coordination capacity. 4. Drop-Jump (DJ), the participant performs this test jumping from a standardized height. The participant stands on the box, drop down off the box onto mat, bending the knees on landing (until 90°), then immediately going into a maximal vertical jump. The participant jumps vertically as high as possible, and back on the mat with both feet landing at the same time. The DJ jump evaluates the explosive power of the lower limbs with use of the myotatic reflex.

The results from the above tests allow the calculation of relevant muscle-strength related index, such as: the elasticity index (elastic energy=({CMJ-SJ}/CMJ) x100; the upper limbs coordination index ((ABK-CMJ)/ABK) x100; and the percentage of fast-twitch fibers [30].

Cognitive performance (COGa)

Psychomotor function assessment

The analysis of the psychomotor function was measured with the Vienna Test System (Schuhfried GmbH Mödling, Austria). We selected the determination test Turkish form, S16 (DT). The DT is used to measure reactive stress tolerance, attention and reaction speed in situations requiring continuous, swift and varying responses to rapidly changing visual and acoustic stimuli [34–36]. The test requires the respondents to use their cognitive skills to distinguish different colours and sounds, to memorize the relevant characteristics of stimulus configurations, response buttons and assignment rules, and to select the relevant responses according to the assignment rules laid down in the instructions and/or learned in the course of the test. The difficulty of DT lies in the production of continuous, sustained rapid and varied reactions to rapidly changing stimuli [34,36].

Verbal memory

We use the Spanish-Complutense Verbal Learning Test (TAVEC) to evaluate the episodic verbal memory, as well as its codification processes, data storage and retrieval. The reliability, validity and psychometric properties of this test were established in a previous study [37]. The test comprises three lists. The first (list A) contains 16 items from four different categories (fruit, spices, items of clothing and tools) presented five times. After each presentation, subjects were assessed according to the number of words remembered correctly. We measured the number of words after the first trial, after the fifth trial, the total number of words in the five trials (learning rate), the number of intrusions (words recited by the subject but that do not feature in list A), the number of perseverations (repetition of words, both correct ones and intrusions) and the use during recall of semantic strategies/clusters (grouping words according to categories) and/or series-based strategies/clusters (grouping words by the order in which they are presented). The second list (list B) comprises 16 different items to those in list A, which are also taken from different categories; its aim is to cause interference after the fifth attempt to learn list A. After administration of list B, subjects were assessed on their short-term free recall of list A. Following a 16-min rest period, during which other tests were administered for the purpose of distraction, subjects' long-term free recall was assessed (list A). Finally, a third list comprising 44 words (including the 16 from list A) was presented in order to measure subjects' recognition.

Attention

We use the standard version of D2 Test to evaluate the attention capacity. This version is a one-page paper-and-pencil cancellation test, consisting of 14 rows (trials), each with 47 interspersed “p” and “d” characters [38]. The characters have one to four dashes that are configured individually or in pairs above and/or below each letter. The target symbol is a “d” with two dashes (hence “d2”), regardless of whether the dashes appear both above the “d”, both below the “d”, or one above and one below the “d”. Thus, a “p” with one or two dashes and a “d” with more or less than two dashes are distracters. The participant's task is to cancel out as many target symbols as possible, moving from left to right, with a time limit of 16 s/trial. No pauses were allowed between trials.

Verbal fluency

The verbal fluency test is a short test of verbal functioning. It typically consists of two tasks: category fluency and letter fluency (sometimes called phonemic fluency) [39]. In the standard versions of the tasks, participants have 1 minute to produce as many unique words as possible within a semantic category (category fluency) or starting with a given letter (letter fluency). The participant's score in each task is the number of unique correct words. For this study, we use phonemic fluency: /f/, /a/, /s/, /m/, /r/, /p/ and the category verbal fluency test: /animals/ and /fruits/.

The phonemic part is applied in the following order in the evaluation of pre-values: /p/, /r/, /m/, /s/, /a/, /f/, and in opposite order in the evaluation of post-values. In addition, the presentation of two categories is different, /animals/ and /fruits/ in pre-values evaluation, and vice versa in post-values evaluation.

Working memory

We use the Letter-Number Sequencing Test to evaluate the spatial span forward. The test is administered according to the rules in the WAIS-IV manual [40]. The examiner reads aloud a number and letter sequence and require from the participant to recall the numbers in ascending order and letters in alphabetical order. Span scores range from 0 to 7 and total scores, from 0 to 21.

Mental health and mood state (MHa)

A set of questionnaires were used to assess mental health and psychosocial parameters. In order to control the evolution of the variables measured during the intervention process, the time frames are: at baseline and after 10-weeks intervention (pre- and post-intervention) and every two weeks (see Table 4).

Quality of life and Psychosocial measurements

Satisfaction with life (SWLS), is a 5-item self-report instrument for the measurement of global life satisfaction as a cognitive-judgmental process [41]; Subjective Happiness Scale (SHS), is a 4-item self-report measure in which a single composite score for global subjective happiness is computed by averaging responses to the four items [42]; Life Orientation Test Questionnaire (LOT-R), is an 8-item self-report instrument (plus four filler items) assessing generalized expectancies for positive versus negative outcomes [43]; Perceived Stress Scale (PSS), is a 14-item self-report instrument for measurement of the degree to which situations in one's life are appraised as stressful [44]; and Beck Depression Inventory (BDI-II), is a 21-item self-report test used to assess mainly cognitive symptoms of depression such as mood, sense of failure, lack of social withdrawal, and indecisiveness [45,46].

Mood state

Profile of Mood States Questionnaire (POMS), is a 65-item self-report instrument used to measure six general affect areas: tension-anxiety, depression-dejection, anger-hostility, vigor-activity, fatigue-inertia, and confusion-bewilderment [47–49]; Trait Meta-Mood Scale-24 (TMMS-24), is 24-item test which measures three emotional intelligence components: attention, clarity, and repair [50]; The Positive and Negative Affect Schedule (PANAS) Questionnaire, is a 20-item self-report measure of positive and negative affect [51]; and Scale of Assessment of Mood (EVEA), is a 16-item self-report instrument designed for measuring transitory moods [52,53].

METHODOLOGICAL OVERVIEW OF THE STUDIES INCLUDED

The present International Doctoral Thesis is composed of a total of five studies. They are classified in three different sections: Section 1 focuses on the effects of a 10-week HIIT intervention program and moderate alcohol consumption on physical fitness and body composition, (**Studies 1 and 2**); Section 2 focuses on the effect of a 10-week HIIT intervention program and moderate alcohol consumption on psychomotor speed and cognitive function (**Studies 3 and 4**); and Section 3 focuses on the effects of a 10-week HIIT intervention program and moderate alcohol consumption on psychosocial variables and mood state adaptations (**Study 5**). All studies contain data from the participants enrolled in the BEER-HIIT study. Table 8 shows an overview. Figure 3 shows an overview of the design, participants, and outcomes included in each study contained in the present International Doctoral Thesis.

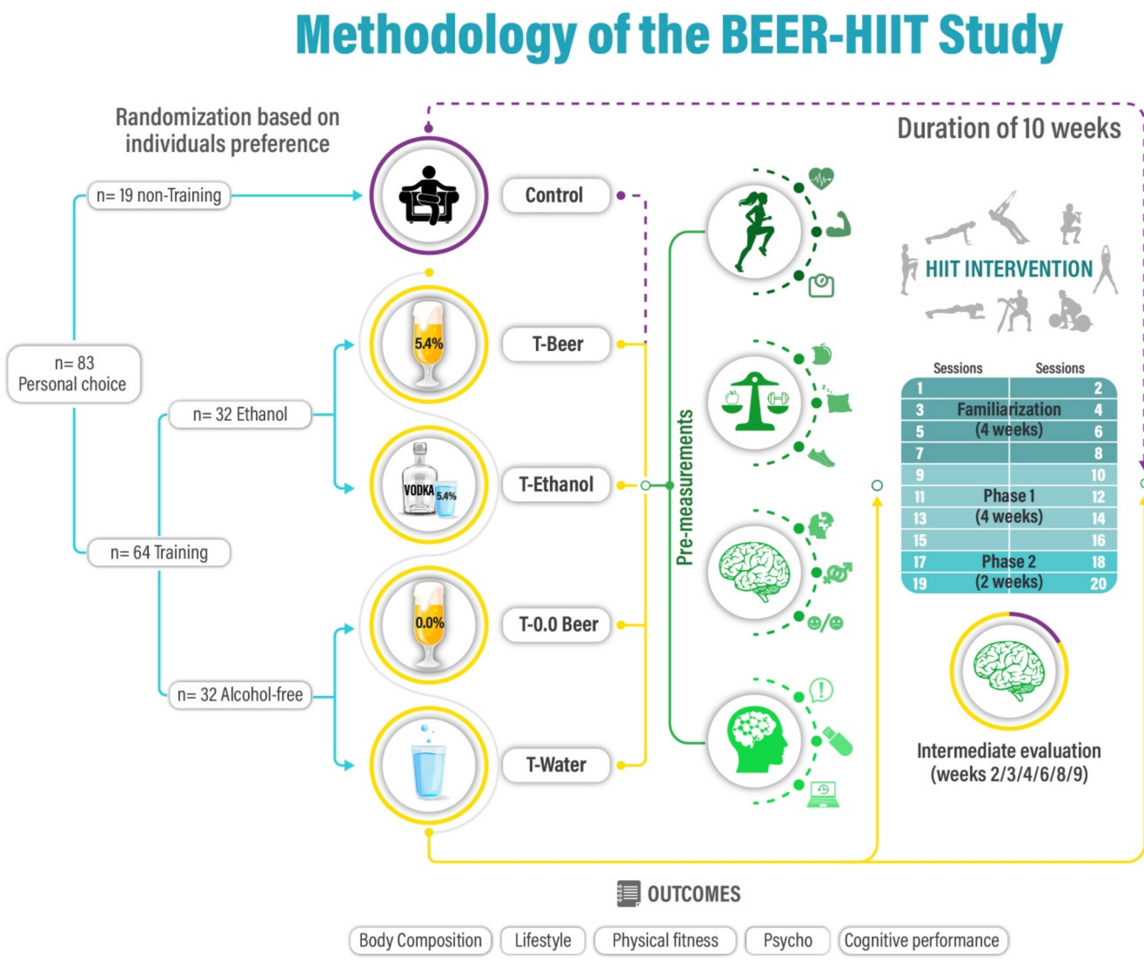

Figure 3. Graphical description of the methodology of the BEER-HIIT study.

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RESULTS AND DISCUSSION

SECTION 1

Combine effects of a highly demanding traning program and moderate alcohol/beer consumption on physical performance

Chapter 3

Beer or ethanol effects on the body composition response to high-intensity interval training. The BEER-HIIT study (Study 2)

Abstract

Background: High-intensity interval training (HIIT) is promoted as a time-efficient strategy to improve body composition but concomitant beer intake, which is common among physically active individuals, may interfere with these effects. The primary aim of this study is to determine the effects of a 10-weeks (2 days/week) HIIT program on anthropometric and body composition measurements, and to assess whether those effects are influenced by the moderate consumption of beer (at least 5 days/week), or its alcohol equivalent.

Methods: Young (24±6 years old) healthy adults (n=72, 35 females) volunteered for a non-training group or for HIIT training. Those going for training choose whether they preferred to receive alcohol or not. Those choosing alcohol were randomly allocated for receiving beer (5.4%) or the equivalent amount of alcohol (vodka) in sparkling water. Those choosing no-alcohol were randomly allocated for receiving alcohol-free beer (0.0%) or sparkling water. From Monday through Friday, men ingested 330 ml of the beverage with lunch and 330 ml with dinner; women ingested 330 ml with dinner. Before and after the intervention, anthropometry and body composition, through dual-emission X-ray absorptiometry, were measured.

Results: No changes in body weight, waist circumference, waist/hip ratio, visceral adipose tissue or bone mineral density occurred in any of the groups. By contrast, in all the training groups, significant decreases in fat mass together with increases in lean mass (all $P<0.05$) occurred. These positive effects were not influenced by the regular intake of beer or alcohol.

Conclusions: In conclusion, in young healthy adults, moderate beer intake does not blunt the positive effect of 10-week HIIT on body composition.

Background

Physical exercise is an integral component of a healthy life-style, with strong evidence supporting the notion that it can help to lose weight and improve body composition [1]. The international physical activity recommendations provided by the World Health Organization (WHO) include 150 minutes of aerobic exercise at moderate intensity or 75 minutes of aerobic exercise at vigorous intensity per week [2]. In addition, strength and power exercises are recommended in order to increase muscle mass or to prevent age associated sarcopenia [3]. However, it has been shown that most adults failed to meet the recommendations of physical activity [1], noting the lack of time as the main barrier to follow an exercise training program [4]. Recently, high-intensity interval training (HIIT), which consists in alternate short bursts of high-intensity exercise and recovery periods, has emerged as an alternative to the traditional exercise recommendations because of its time efficiency [5]. In this sense, HIIT has demonstrated to be effective in the improvement of body composition, by reducing fat mass [5,6] and increasing muscle mass [7], in normal-weight and also in overweight-obese individuals [8]. Actually, there is robust and growing evidence showing that HIIT may elicit greater benefits that moderate-intensity continuous training across a range of health markers [5]. Among them, HIIT has demonstrated to be effective in reducing total, abdominal, and visceral fat mass in both males and females [8]. Similarly, different HIIT protocols have resulted in modest increases in total body and trunk lean mass [9–11].

Beer is a widely consumed beverage in western countries and the most consumed alcoholic beverage in the world [12]. It is consumed by many healthy adults to quench thirst in preference to other beverages particularly after a hard day work, as part of social relationship, or after practicing exercise [13,14]. This is particularly the case in a recreational context, where having a beer after a match is considered part of the social aspect of many sport activities [15–17]. It has been reported that beer is the most popular alcoholic drink among athletes and sport administrators, with over 90% naming it as their preferred alcoholic beverage [16]. The intake of beer in the exercise recovery phase has been questioned due to its alcohol content [13,18]. Similarly, there is controversy regarding the influence of beer consumption on fat distribution and body composition [19–24]. Some authors have reported that moderate beer consumption is not associated with changes in body weight or body composition parameters [20,22–24], while others have reported that alcohol consumption is associated with increased adiposity in adults [19,21]. Moreover, alcohol intake can have influence not only in fat mass (FM) but also in lean mass (LM) [25]. It has been reported that alcohol can suppress the anabolic response to physical exercise through the reduction of muscle protein synthesis [25].

To the best of our knowledge, there are no previous studies that investigated the influence of a moderate beer, or alcohol, consumption in the body composition response to a highly demanding physical training precisely designed to induce changes in body composition, in conditions similar to those occurring in real life situations. Therefore, the present study aimed (i) to evaluate the effects of a HIIT program on body composition parameters in healthy adults, and (ii) to analyze whether those effects are influenced by frequent but moderate beer consumption or its alcohol equivalent. We hypothesize that HIIT may improve body composition, but these effects may be blunted by the concomitant intake of beer even in moderate amounts, being these deleterious effects attributed to its alcohol content.

Materials and Methods

Study Design and Participants

A total of 72 healthy adults (35 females), aged between 18-40, years were enrolled in this registered controlled trial (ClinicalTrials.gov ID: NCT03660579). The study was in accordance with the latest revision of the Helsinki Declaration, and was approved by the Ethics Committee on Human Research at the University of Granada (CEI-Granada) [321-CEIH-2017]. The participants were recruited from the province of Granada (Spain) using social networks, local media, and posters. Interested individuals were screened via telephone, and/or e-mail. Several appointments were made in order to provide detailed explanation about the study objectives, design, inclusion criteria, assessments to be undertaken, exercise program intervention, and types of beverages to be ingested. Volunteers were included based on the following inclusion criteria: (i) body mass index (BMI) from 18.5 to 30 kg/m^2, (ii) nor engaged in a training program, (iii) having a stable body weight during the last 5 months, (iv) being free of disease, pregnant or lactating women; (iv) not taking any medication for chronic diseases, and (v) no pain, recent lesions or other problems preventing strenuous physical activity. Prior to the study all participants completed a health exam and gave their written informed consent. All the baseline and follow-up tests were performed at the same setting [Instituto Mixto Universitario Deporte y Salud (IMUDS) at the University of Granada].

As shown in Fig. 1, 94 individuals attended an information meeting and were assessed for eligibility. A total of 83 individuals met the inclusion criteria, and, after completing the baseline measurements, they chose whether they preferred to be included in a training (T) or a non-training (N-T) group. Training consists in two HIIT sessions per week, during 10 consecutive weeks (see below). Those going for training then chose whether they preferred to be included in a group ingesting from Monday to Friday an ethanol containing beverage (5.4% alcohol content) or an alcohol-free beverage. Men ingested 330 ml of that beverage with lunch and 330 ml with dinner. Women ingested 330 ml with dinner. Participants choosing ethanol were randomly allocated either to beer (T-Beer) or to sparkling water with added vodka ethanol (T-Ethanol). The distilled alcoholic beverage used for our study was vodka because of the purity of its composition (37.5% ethanol and 62.5% water). Those choosing non-alcohol were randomly allocated either to alcohol-free beer (T-0.0Beer) or to sparkling water (T-Water). Each group was composed of 8 men and 8 women. This type of non-random (based on individual preference) and random allocation of the participants was conducted following ethical considerations and advice made by the ethical committee [321-CEIH-2017]. The assessment staffs were blinded to the participants' randomization assignment.

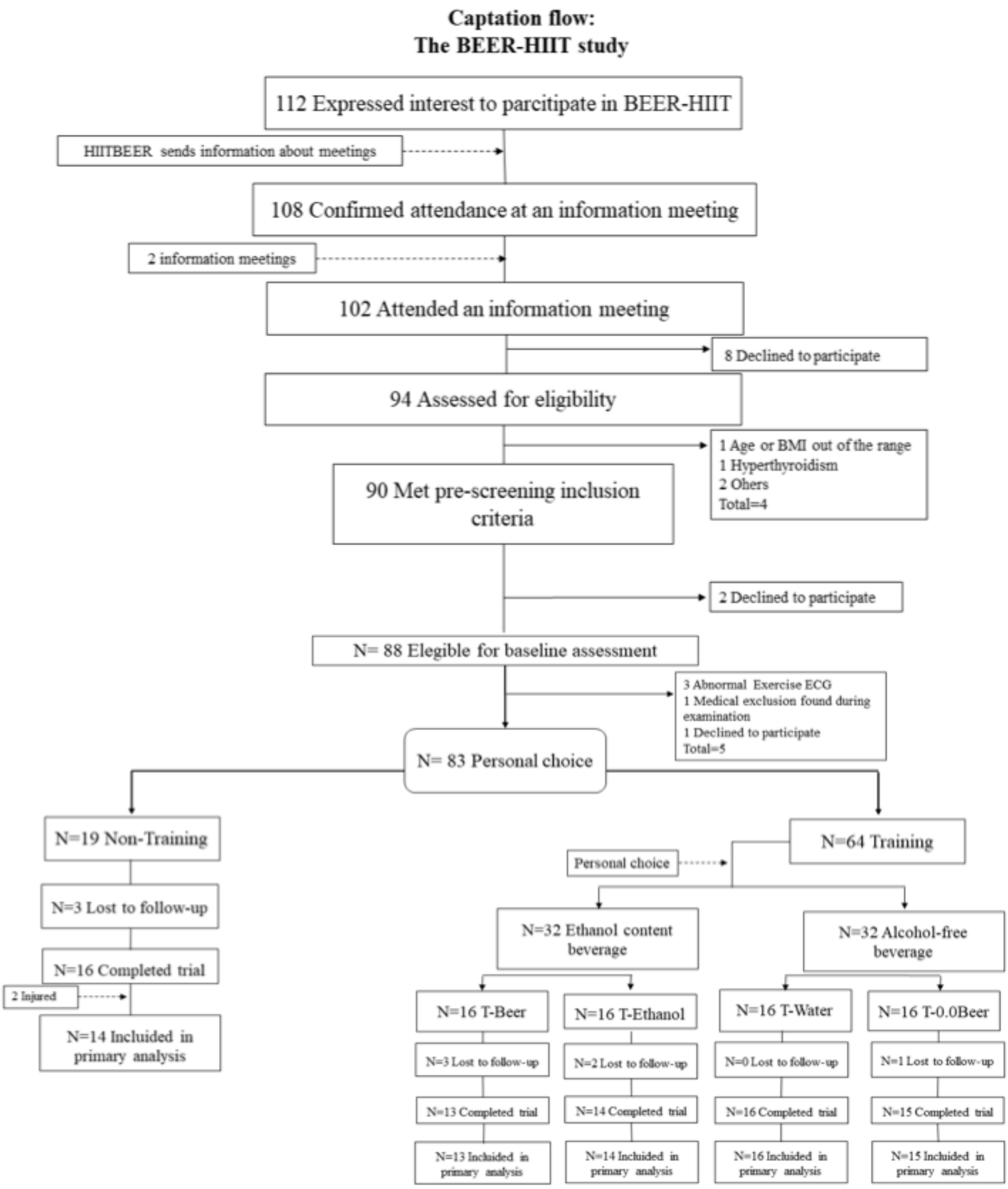

Figure 1. Flow-chart diagram. Abbreviations: BMI, body mass index; ECG, electrocardiogram; N-T, non-training group; T-Beer, group that performed HIIT and consumed alcohol beer; T-0.0Beer, group that performed HIIT and consumed non-alcoholic beer; T-Water, group that performed HIIT and consumed sparkling water; T-Ethanol, group that performed HIIT and consumed sparkling water with alcohol added.

Beverage intake and physical activity levels were registered before and after the intervention program by the Beverage Intake Questionnaire (BEVQ [26]) and self-reported, respectively. In the background questionnaire, participants were asked to report their usual frequency of alcohol intake in seven possible response categories: The lack of adherence to the training protocol (<20% no-shows for training sessions) and/or not complaining with the stipulated beverage intake were excluded from the study.

Training protocol

A 10-week registered controlled trial was conducted. The training sessions took place 2 days/week in the late afternoon or early evening from Monday to Friday, leaving a rest period of, at least, 48 hours between training sessions. The training volume was 40-65 min/week following the methodology described by previous studies [27–29]. It was divided into two different phases, starting with a familiarization phase to learn the main movement patterns as previous studies have suggested [30–32].

The HIIT intensity was programmed considering the updated scientific evidence [28,33,34]. In all cases, the intensity was >8 Rating of Perceived Exertion {0-10 RPE scale} [35], which has a positive linear relationship with heart rate (HR) and VO_{2max} [36,37]. The training sessions started with a dynamic standardized warm-up, including several muscle activation exercises (i.e. child's pose breathing; pelvis bridge; cat camel; upper back rotation; front and side planks; arms Ts; arms Ys; toe walks; high knee walks; walking lunges; side lunges; monster walk; sumo walk and anti-rotational stability press). The participants performed eight weight-bearing exercises in circuit form twice per set (i.e. frontal plank, high knees up, horizontal row, battle rope, squat, dead lift, push up, and burpees). There was a passive rest between exercise, and an active rest between sets (an intensity of 6 RPE, which corresponds with 60% VO_{2max} [36,38]), following the periodization described in previous studies [30]. The training sessions ended with a cooling-down protocol (active global stretching) including anterior and posterior chain exercises (i.e. pigeon pose; lying twist; figure four stretch; lunging hip flexor stretch; biceps stretch, and trapezius neck stretch).

Beverage intake protocol

The beverages were ingested daily from Monday to Friday. The volumes of fluid ingested were the same in all groups (660 ml for men and 330 ml for women), men ingested 330 ml with lunch and 330 ml with dinner, women ingested 330 ml with dinner: (i) T-Beer group ingested regular Lager Beer (5.4% alcohol-Alhambra Especial, Granada, Spain); (ii) T-0.0Beer group ingested alcohol-free beer (0.0% alcohol-Cruzcampo®, Sevilla, Spain); (iii) T-Water group ingested sparkling water (Eliqua 2®, Font Salem, Spain); (iv) T-Ethanol group ingested sparkling water with exactly the same amount of distilled alcohol added. The distilled alcoholic beverage used in our study was branded vodka because of the purity of its composition (37.5% ethanol and 62.5% water). We based on scientific evidence to select the amount of alcohol ingested by the participants, which define a moderate amount as two or three drinks/day or 24-36 grams of ethanol/day for men and one to two drinks/day or 12-24 grams of ethanol/day for women [23]. The beverages were coded and provided by a staff member of our research laboratory on the beginning of each week. The investigators who did the evaluations were not aware of the group assignment of the participant. For Saturday and Sunday, the participants of the alcohol groups were strictly instructed that if they drank alcohol during the weekend to do it in moderate amounts. Those included in non-alcohol groups were requested to refrain from alcohol also during the weekend.

Anthropometric parameters and body composition assessment

Anthropometric parameters and body composition assessment were conducted before and after the 10-week intervention program. Body weight (BW) and height were measured without shoes and with light clothing, using a pre-validated scale and stadiometer (model 799, Electronic Column Scale, Hamburg, Germany), and the body mass index was calculated (weight/ height^2) [39].

Waist circumference (WC) and hip circumference (HC) were measured in triplicate with a non-elastic tape (Seca 200, MWS Ltd, Scalesmart) to the nearest 0.1 cm and the mean of the 3 measurements was used in the analyses. WC was measured at the mid-point between the bottom of the rib cage and the iliac crest at the end of a normal expiration [40]. HC was measured around the widest portion of the buttocks [40]. Waist-hip ratio (WHiR) was calculated by dividing waist by hip values.

FM, visceral adipose tissue (VAT), LM, and bone mineral density (BMD) were measured by a dual-energy X-ray absorptiometry (DXA) scanner (Discovery Wi, Hologic, Inc., Bedford, MA, USA). The whole-body scan was used to obtain all body composition parameters. We conducted the quality controls, the positioning of the participants, and the analyses of the results following the manufacturer's recommendations. An automatic delineation of the anatomic regions was performed by the software APEX 4.0.2. FM was also expressed as percentage of body weight.

Statistical analysis

Sample size calculations were based on a minimum predicted 15% change in BMI, FM and LM between the intervention groups and the control group, with an expected standard deviation of 15%. A sample size of 13 participants was predicted to provide a statistical power of 85% considering a type I error of 0.05 [41], based on a pilot study. However, we recruited a minimum of 16 participants per group (a total of 80) to accommodate for a maximum loss of 20% at follow-up.

Data were checked for normality with use of Shapiro-Wilk test and visual inspection of Q-Q plots. We conducted repeated-measures analysis of variance in order to study changes in BW, BMI, WC, HC, WHiR, FM, FM percentage, VAT, LM, and BMD across time, between groups, and the interaction (time*group). An analysis of covariance (ANCOVA) was conducted to determine the effect of the groups (fixed factor) on anthropometric and body composition outcomes, i.e. post-FM minus pre-FM (dependent variable), adjusting for the baseline values (model 1). We conducted the same analysis for changes in WC, FM percentage, VAT, LM, and BMD. Bonferroni post hoc test with adjustment for multiple comparisons were also performed. The level of statistical significance was defined at $P<0.05$. Additional models were conducted controlling for baseline values and sex (model 2), and for baseline values and age (model 3) (see Supplementary material, Table S1). The statistical analyses were conducted in Statistical Package for Social Science (SPSS, V. 25.0, IBM SPSS Statistics, IBM

Corporation), and the graphical plots were conducted in GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA).

Results

Participants flow-chart is presented in Figure 1. During the study period a total of 11 participants (6 men and 5 women) dropped out due to insufficient attendance to the training sessions or the difficulty to fulfil the protocol requirements. The baseline characteristics of the study participants can be observed in Table 1. There were no differences among groups at baseline. The reported intakes of alcohol in the different groups were also similar (P=0.144; See Table 1). The distribution in the number of men and women was nearly equal in each group.

Table 1 shows BW, BMI, WC, HC and WHiR before and after the intervention study. Repeated measures ANOVA revealed no effect of training neither of type of beverage in BW (P=0.849), BMI (P=0.842), and HC (P=0.900), while a significant effect of training and of the beverage type was observed in WHiR and HC (P=0.029, and P=0.003, respectively).

Body composition variables (FM, FM percentage, VAT, LM and BMD) before and after the intervention study are shown in Table 1. Repeated measures ANOVA revealed a significant effect of training and of the beverage type in FM, FM percentage, and LM (P=0.048, P=0.003, and P<0.001, respectively), while no effect of the training neither of type of beverage was observed in VAT and BMD (P=0.342, and P=0.645, respectively).

Figure 2 shows changes in FM, WC, and LM after the intervention study among the five groups. ANCOVA revealed no significant differences among groups in FM (P=0.156; Figure 1A), whereas significant differences were noted in WC and LM (P=0.007, and P≤0.001, respectively; Figure 1B and 1C). The results remained after controlling by sex and age in FM (all P>0.05; see Supplementary Material, Table S1), also in WC and LM (all P<0.05; see Supplementary Material, Table S1). A significantly higher LM post-intervention was noted in all training groups compared with the control group (HIIT-AB, P≤0.001; HIIT-NAB, P=0.005; HIIT-SW, P≤0.001; and HIIT-ASW, P≤0.001).

Figure 2 also shows changes in FM percentage, VAT, and BMD after the intervention study among the five groups. ANCOVA also revealed significant differences between groups in FM percentage (P=0.009; Figure 2D), whereas no significant differences were noted neither in VAT nor in BMD (P=0.627, and P=0.474, respectively; Figure 1E and 1F). The results remained after controlling by sex in FM percentage (all P≤0.013; see Supplementary Material, Table S1), also in VAT and BMD (all P>0.05; see Supplementary Material, Table S1). A significantly lower FM percentage post-intervention was noted in HIIT-AB, HIIT-SW and HIIT-ASW compared with the control group (all P<0.05; Figure 3D).

RESULTS AND DISCUSSION

	Non-Training (n=14)				T-Beer (n=13)				T-0.0Beer (n=15)				T-Water (n=16)			
Age (years)	20.1 (2.4)				24.5 (5.6)				24.5 (5.6)				24.6 (6.6)			
Sex (%)																
Men	7 (50.0)				6 (46.2)				8 (53.3)				9 (56.3)			
Women	7 (50.0)				7 (53.8)				7 (46.7)				7 (43.8)			
Beverage intake questionnaire (BVQ)																
Total Alcohol Intake (cc/week)	957.3 (732.1)				566.2 (770.9)				1500.0 (1255.9)				927.5 (1041.1)			
Test of change over time																
	PRE	POST	%	P	PRE	POST	%	P	PRE	POST	%	P	PRE	POST	%	P
Anthropometric variables																
Body mass	64.7 (10.8)	65.0 (11.5)	9.4	0.294	70.1 (15.6)	70.9 (15.8)	1.2	0.050	71.5 (16.9)	71.9 (15.8)	0.6	0.302	69.6 (10.1)	70.5 (10.8)	1.3	0.087
BMI (kg/m^2)	22.1 (2.0)	22.2 (2.1)	0.5	0.335	24.0 (4.3)	24.3 (4.3)	1.2	0.048	25.0 (3.7)	25.1 (3.8)	0.6	0.279	24.9 (3.4)	25.2 (3.5)	1.2	0.088
Wc	74.1 (0.1)	75.6 (6.0)	2.0	0.143	80.1 (11.9)	80.5 (11.9)	0.5	0.668	85.8 (11.5)	80.5 (9.9)	-6.2	0.014	82.2 (10.1)	81.8 (10.0)	-0.5	0.627
Hc	95.4 (5.3)	95.6 (6.0)	0.2	0.846	97.0 (9.8)	98.1 (9.5)	1.1	0.338	98.8 (10.1)	100.2 (7.4)	1.4	0.444	99.4 (6.9)	100.2 (6.1)	1.4	0.373
WHiR	0.7 (0.1)	0.8 (0.0)	2.1	0.189	0.8 (0.1)	0.7 (0.1)	-0.7	0.529	0.9 (0.2)	0.8 (0.1)	-8.6	0.063	0.8 (0.1)	0.7 (0.1)	-1.2	0.306
Body composition variables																
FM (kg)	17.0 (5.9)	17.3 (5.7)	1.9	0.327	20.0 (7.4)	18.7 (7.0)	-6.8	0.005	22.4 (5.7)	20.9 (5.9)	-6.2	0.004	22.1 (7.0)	20.8 (6.7)	-6.2	0.009
FM (%)	26.93 (8.9)	27.42 (8.7)	1.8	0.378	29.0 (7.6)	26.4 (7.2)	-8.8	<0.001	32.2 (5.4)	29.8 (5.6)	-7.3	0.001	32.0 (7.7)	29.8 (6.9)	-7.7	<0.00

VAT (g)	212.3 (69.6)	213.5 (91.3)	0.5	0.908	282.5 (161.2)	254.5 (162.8)	- 9.9	0.094	320.1 (154.4)	280.8 (174.3)	- 12.3	0.015	321.4 (170.5)	283.2 (169.6)	- 11.9	0.004
LM (kg)	44.3 (10.1)	44.1 (10.6)	- 0.4	0.740	46.2 (10.7)	48.6 (10.4)	5.1	**<0.001**	45.4 (11.9)	47.4 (12.3)	4.5	**0.001**	43.8 (7.7)	46.2 (7.7)	5.5	**<0.00**
BMD (g/cm²)	1.1 (0.1)	1.2 (0.1)	- 0.7	0.920	1.1 (0.1)	1.2 (0.1)	0.8	0.091	1.1 (0.1)	1.0 (0.1)	0.5	0.271	1.1 (0.1)	1.2 (0.1)	0.8	0.107

Values of main variables before and after the 10-week intervention program for the control and intervention groups. Change score
p-values are for tests of an overall change over time among all subjects, and for tests of a treatment effect between different inte
indicate significance differences (P < 0.05). Only participants with complete intervention program were included in the compl
centimetre intake at week; BMI, body mass index; WHiR, waist-hip ratio; FM, fat mass (kg); FM %, fat mass percentage; VAT, v
bone mineral density BMI, body mass index; T-Beer, group that performed HIIT and consumed alcohol beer; T-0.0Beer, group that
beer; T-Water, group that performed HIIT and consumed sparkling water; T-Ethanol, group that performed HIIT and consumed spar

RESULTS AND DISCUSSION

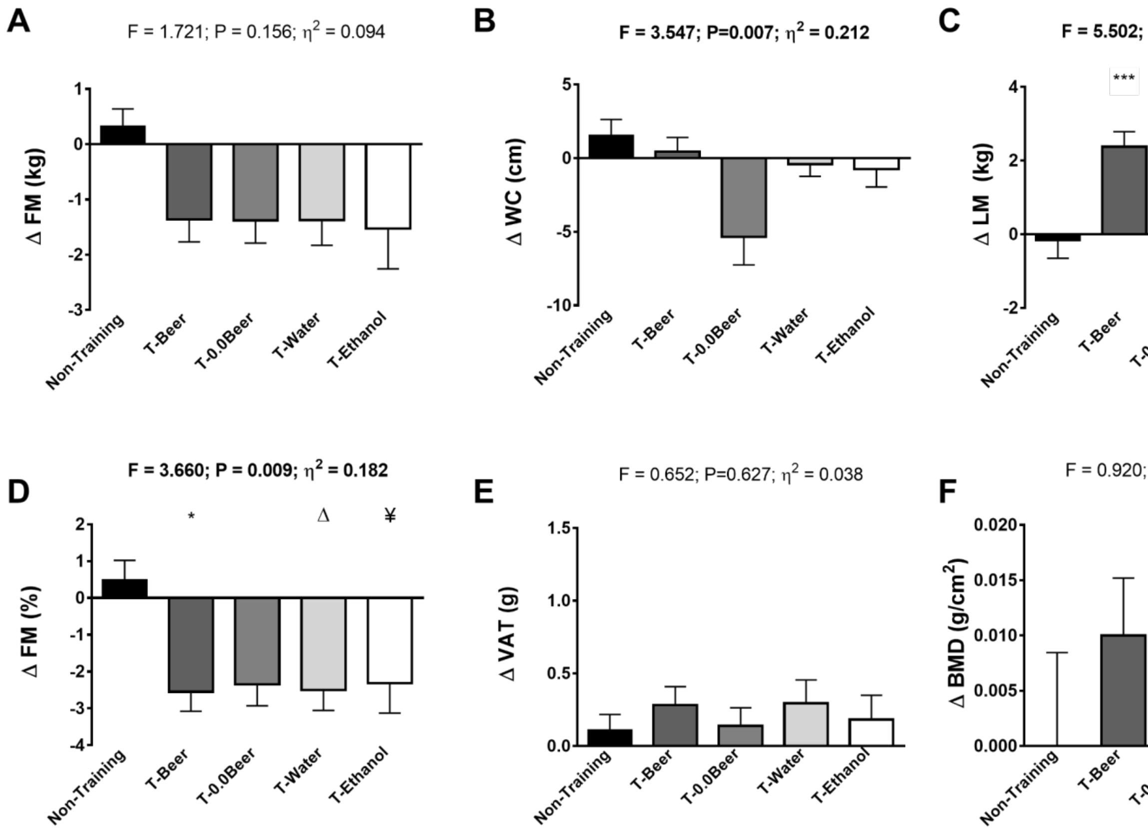

Figure 2. Changes in fat mass (kg) (A), waist circumference (B), lean mass (C), fat mass (%) (D), visceral adipose tissue (E), and bo study among the five groups. Significant differences between groups applying an analysis of covariance adjusting by baseline valu are indicated as: Non-training group vs T-Beer (* P<0.05), (*** P≤0.001); Non-training group vs. T-0.0Beer (εεε P<0.01); Non-tr P≤0.001); and Non-training group vs T-Ethanol (¥ P<0,05), (¥¥¥ P≤0.001). Data are shown as means ± standard error of the m waist circumference; LM, lean mass; FM %, fat mass percentage; VAT, visceral adipose tissue; BMD, bone mineral density; N- performed HIIT and ingested alcohol beer; T-0.0Beer, group that performed HIIT and ingested non-alcoholic beer; T-Water, group water; T-Ethanol, group that performed HIIT and ingested sparkling wa

Discussion

The primary findings of our study are that 10-weeks of HIIT did not have influence on body weight, but this type of training significantly decreased FM and FM percentage and increased LM in healthy adults. These positive effects were not affected by the concomitant regular intake of beer, or its alcohol equivalent, in moderate amounts. Neither HIIT nor beer or alcohol intake influenced adipose tissue distribution or BMD. The lack of effect on BW or BMI was the result of the simultaneous decrease in FM and increase in LM.

The role of HIIT program on body composition parameters have been analyzed previously in several studies [42]. Some recent meta-analysis have reported that different HIIT protocols did no modify BMI and WC in normal-weight individuals [6,43], which concurred with the findings of this study. In addition, current systematic reviews have concluded that a HIIT program appears to be effective on FM reduction after a ~10-weeks training program [5,6]. These results are quite similar to those obtained by Maillard et al. [8] in a meta-analysis that studied the efficacy of HIIT in reducing total, abdominal and visceral fat mass, concluding that HIIT reduces FM (-6%) in sedentary individuals with a BMI ranged from 18.5 to 35 kg/m^2. In addition, the meta-analysis of Verheggen et al. [42] confirmed that diet or training alone can significantly alter VAT, although exercise training tends to show a larger decrease compared to caloric restriction (-6.1%). Our results agree in this line, since HIIT showed a significant decrease of FM and FM percentage (all $P<0.05$) in T-0.0Beer and T-Water groups, also a significant decrease of VAT (all $P<0.05$); whereas no significant changed were found on BMI or WHiR (all $P>0.05$) after the HIIT intervention in any group (see Table1).

Moreover, it has been previously reported that HIIT is a time efficient and effective method to stimulate muscle size adaptations in individuals with a BMI between 25-45 kg/m^2 after 3 sessions/week (14% increase in muscle cross-sectional area) during 3-weeks intervention [6], and in non-obese young adult women after 3 sessions/week (5-6% increase in LM) during 12-weeks HIIT intervention [43]. These results agree with our findings, since a significant increase of LM (+5%) were obtained in T-0.0Beer and T-Water groups (see Table 1). Our results are in accord with a previous study that compared 12-weeks of a moderate intensity continuous exercise program vs. 12-weeks of a HIIT exercise program in untrained men, that found no significant changes in BMD in both cases [44]. In addition, our findings did not concur with two previous studies that showed that 7 and 12 weeks of a HIIT exercise program improved BMD in untrained subjects and in young females, respectively [6,45]. However, exercise programs that combine high impact activity with resistance training are the most effective in augmenting BMD [46,47]. The low number of cases in our groups may be the cause of not finding a statistically significant effect.

The effects of alcohol consumption on body composition have been debatable. The relationship between alcohol consumption and central adiposity has not been clearly or consistently reported in the literature with some studies reporting a positive association between alcohol consumption and waist circumference or WHiR [21,48,49], while others have reported an inverse relationship [24,50]. A previous study observed that alcohol ingestion was not associated with BMI, WHiR and WC in adults aged between 35 and 64 years in a daily low-to-moderate alcohol consumption [21]. Actually, a cross-sectional study found a inversely association between drinking frequency and BMI, where the lowest odds of being obese was observed among the most frequent drinkers [24]. Our results agree with previous findings, since none group showed changes in BMI, WHiR and WC, independently of the beverage ingested during the intervention. Some studies have reported that alcohol consumption is associated with an increase in VAT [21,48,49], and could stimulate lipogenesis and inhibit lipolysis on healthy adults [21]. Notwithstanding, the effect of alcohol on fat metabolism remains obscure. Moreover, alcohol consumption has been associated with lower insulin levels and increased insulin sensitivity [51]. These results have been confirmed by Hong et al. [52], who found that alcohol consumption increased insulin sensitivity without affecting body fat levels in mice. Our results agree with those obtained by Kim et al [49], who found that the participants decreased in subcutaneous adipose tissue in spite of their alcohol intake. Further, our results in FM concur with those obtained by Brandhagen et al. [19], since all participants including alcohol consumption groups, decreased in FM % (see Figure 2A and 2D). In addition, T-Beer and T-Ethanol groups showed decrease in VAT without statistical differences after the 10-weeks intervention (-9.9% and -10.2% respectively; see Table 1).

Moreover, it has been reported that alcohol consumption, particularly beer consumption, is directly associated with body fat, especially abdominal fat [53]. In addition, it is believed that beer consumption is associated with increased waist circumference or WHiR, particularly in men, a phenomenon popularly referred to as 'beer belly' [54]. This belief might be supported by cross-sectional research, reporting abdominal obesity as being associated with beer consumption [55]. However, some prospective studies have shown inconsistent results, such as the study of Schütze et al. [54]. These authors have reported only limited evidence for a site-specific effect of beer drinking on waist circumference [54]. Our findings do not agree, since all interventions groups, T-Beer and T-0.0Beer groups included, showed no negative changes in VAT, WC or WHiR. Furthermore, T-0.0Beer group showed a significant decrease in VAT and WC, and a clear trend decrease in WHiR, while T-Beer group did not show impairment in either of variables.

On the other hand, it seems clear that alcohol consumption could reduce muscle protein synthesis, supressing the anabolic response in skeletal muscle [25]. In this line, Coulson et al. [48] found that those groups consuming three or more alcoholic drinks on usual drinking days had lower lean mass than non-drinkers, and this association was not attenuated by adjustment for physical activity levels. Thus, it could be expected that the positive effects of an exercise program on LM could be attenuated by the alcohol consumption. However, a moderate and habitual alcohol consumption during an exercise training intervention has been not previously studied. In that sense, our results do not agree with these previous findings, since T-Beer and T-Ethanol groups increased their LM (+5%; see Table1), improving similarly in all interventions groups independently of the type of beverage intake. Therefore, the moderate consumption of alcohol did not seem to influence the anabolic response of a HIIT program of 10-weeks. Our results agree with the data obtained by Viena et al. [56] in a recent meta-analysis, who concluded that HIIT is a useful tool to reduce FM and FM percentage. In our study, all intervention groups showed a reduction in FM and FM percentage after a 10-weeks HIIT program, and these positive effects were not influenced by the concomitant regular intake of beer, or its alcohol equivalent, in moderate amounts. Apart from that, while the evidence regarding the impact of alcohol use on BMD is inconclusive, longitudinal studies have shown that exercise with high-impact load may provide an effective osteogenic stimulus [44]. However, Nybo et al. [44] did not find a significant increase in BMD after 12-weeks intervention, suggesting that a training program with a high intensity did not provide the adequate stimulus for enhancing bone mass. Our results agree with that, since no changes were found in BMD neither in T-Beer and T-Ethanol groups nor T-0.0Beer and T-Water groups. The lack of changes in BMD in any of groups could be due to the short duration of the intervention program, since longer programs are needed to induce improvements in BMD [57,58].

The present study has some limitations. Firstly, the sample size was relatively small to study the influence of different alcohol beverages in moderate amounts during an exercise training intervention on body composition considering both sexes separately, although no interaction effects were observed. Considering that we compared a total of four different types of beverages ingested, our study could be underpowered to note statistical differences in specific body composition-related parameters between them. Therefore, further studies are needed to clarify the long-term effects (>10 weeks) of HIIT and other training modalities with the ingestion of a moderate dose of alcohol on body composition parameters. Secondly, we just control the levels of physical activity and beverage intake before and after the intervention program, not during the study period. In addition, physical activity of the non-training group was not monitored with trial-axial accelerometer for movement registration. Despite we did not collect dietary data during the intervention, we assessed the adherence to the Mediterranean Diet by PREDIMED questionnaire before the intervention, finding that our sample had not adherence to Mediterranean diet. Therefore, our results could be extrapolated to other dietary patterns. Further studies are needed to clarify what is the role of the dietary pattern on body composition parameters during an exercise program intervention combined with moderate alcohol consumption. Finally, participants were not purely randomized, they were asked to choose their preferences about being included in a training or in a non-training group, or in an alcohol or an alcohol-free group, basically due to ethical considerations, but also reflecting the common reality of the daily life.

Conclusion

In conclusion, our results show that, in healthy adults, a 10-weeks HIIT program improves body composition by decreasing FM and increasing LM, and these positive effects are not influenced by the concomitant intake of beer, or its alcohol equivalent, in moderate amounts. In addition, the intake of beer, or its alcohol equivalent, while exercising does not affect body fat distribution.

Supplementary Materials

Author Contributions: Conceptualization, Manuel J. Castillo; Formal analysis, Cristina Molina-Hidalgo and Lucas Jurado-Fasoli; Methodology, Alejandro De-la-O and Lucas Jurado-Fasoli; Writing – original draft, Cristina Molina-Hidalgo and Lucas Jurado-Fasoli; Writing – review & editing, Lucas Jurado-Fasoli, Francisco J. Amaro-Gahete, Alejandro De-la-O and Manuel J. Castillo.

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Table S1. Changes in body composition outcomes adjusted by baseline values (Model 1) adjusted by baseline values and sex (Model 2), by baseline values and age (Model 3).

		Analysis of covariance P value		
		F	P	η^2
Fat mass (kg)	Model 1	1.721	0.156	0.094
	Model 2	1.698	0.161	0.095
	Model 3	1.943	0.114	0.107
Waist Circumference	Model 1	3.053	0.023	0.156
	Model 2	2.774	0.034	0.146
	Model 3	3.064	0.022	0.159
Lean mass (kg)	Model 1	6.683	≤0.001	0.288
	Model 2	6.604	≤0.001	0.289
	Model 3	6.283	≤0.001	0.279
Fat mass (%)	Model 1	3.660	0.009	0.182
	Model 2	3.459	0.013	0.175
	Model 3	3.697	0.009	0.185
Visceral adipose tissue (g)	Model 1	0.652	0.627	0.038
	Model 2	0.503	0.733	0.030
	Model 3	0.688	0.603	0.041
Bone mineral density (g/cm^2)	Model 1	0.572	0.684	0.034
	Model 2	0.563	0.690	0.033
	Model 3	0.634	0.640	0.038

Chapter 4

Influence of daily beer or ethanol consumption on physical fitness in response to a high-intensity interval training program. The BEER-HIIT study (Study 1)

Abstract

Background: High-intensity interval training (HIIT) is an effective approach to improve physical fitness, but consuming beer, which is a regular practice in many physically active individuals, may interfere with these effects. The purposes of this study were to investigate the effects of a 10-week (2 days/week) HIIT program on cardiorespiratory fitness, muscle strength and power parameters, and also to assess the possible influence on them of a moderate consumption of beer (at least from Monday to Friday) or its alcohol equivalent.

Methods: Young (24 ± 6 years old) healthy adults (n = 73, 35 females) were allocated to five groups. Four groups participated in the HIIT intervention program while the fifth group was a control Non-Training group (n = 15). Participants in the training groups chose whether they preferred receiving alcohol or alcohol-free beverages. Those choosing alcohol were randomized to either beer or ethanol intake: (i) T-Beer group (alcohol beer, 5.4%; n = 13) or (ii) T-Ethanol (sparkling water with vodka, 5.4%; n = 14). Those choosing alcohol-free intake were randomized to (iii) T-Water group (sparkling water, 0.0%; n = 16), or (iv) T-0.0Beer group (alcohol-free beer, 0.0%; n = 15). Men ingested 330 ml of the beverage at lunch and 330 ml at dinner; women ingested 330 ml at dinner. Before and after the intervention, maximal oxygen uptake in absolute and relative terms (VO2max.), maximal heart rate, total test duration, hand grip strength and four types of vertical jumps were measured.

Results: HIIT induced significant improvements in absolute and relative values of VO2max, and total test duration (all $p < 0.05$) in all the training groups; also, clinical improvements were found in hand grip strength. These positive effects were not influenced by the regular intake of beer or alcohol. No changes in the vertical jumps occurred in any of the groups.

Conclusions: A moderate beer or alcohol intake does not mitigate the positive effect of a 10-week HIIT on physical fitness in young healthy adults.

Background

Physical fitness is the ability to do a physical activity and/or physical exercise using most of the body structures [1,2]. Physical fitness integrates several components [1], such as cardiorespiratory fitness and muscular strength, which are widely recognized as powerful markers of sport performance, health-related outcomes and relevant determinants of current and future health status as well as important predictors of all-cause mortality [1,3,4]. Emerging evidence suggests that high-intensity interval training (HIIT), which involves repeated bouts (< 45 secs) of intense exercise (85-95% maximal heart rate) interspersed with periods of rest or lower-intensity active recovery, is an effective strategy to get important improvements in cardiorespiratory fitness [5–9] and muscular strength [5–10]. Furthermore, HIIT overcomes one of the most common personal barriers to physical exercise training as it is the lack of time, since HIIT is a less time-consuming training methodology which allows to maximize the potential benefits induced by exercise [11,12].

After training, optimal resting and adequate nutrition are crucial to allow not only the recovery of energy reserves, but also the removal, regeneration, repair and protection of damaged or worn structures [13,14]. This brings the functional structures to a state of adaptation with supercompensation, such as an increased skeletal muscle oxidative capacity, increased resting glycogen content, a reduced rate of glycogen utilization and lactate production, or increased capacity for whole-body and skeletal muscle lipid oxidation, among others. These adaptations improve exercise performance, promote health as well as well-being [15–18] and represent the physiological basis of the multiple and positive benefits of moderate-to-vigorous exercise training [7].

One common post-exercise nutritional practice is the consumption of beer, which is one of the most consumed beverages in the world [19,20] and is frequently consumed by adults after exercise in Western countries [21]. Actually, consumption of beer and, more rarely, other alcohol-containing beverages is often encouraged as a social aspect of sport activities, particularly in the case of recreational contexts, or to facilitate team bonding as part of a post-match celebration and relaxation [22,23]. Beer content is mainly water (90%) but also contains a certain amount of alcohol (range approximately from 3.5 to 7%) [24]. There is a concern about the effects that beer can have on hydration, recovery, and exercise performance due to the effects of alcohol [25]. These negative effects can be associated with a reduced production of testosterone and its subsequent effects on protein synthesis and muscular adaptation/regeneration, and with an increase in the diuretic response of the body due to the inhibitory effect of ethanol on vasopressin release [26–28]. Even though the damages associated with a high intake of alcohol have been widely studied and are beyond doubt [28], the effects of moderate doses remain under debate [24,29]. Some authors conclude that alcohol consumed after an exercise session could interfere with some aspects of the performance, such as strength recovery [28]. However, a low dose of alcohol seems not to affect strength recovery [30], neither power recovery in men [31].

The differences in exercise modalities, alcohol doses, types of beverages and participant characteristics, including their habitual alcohol intake, may explain the contradictory and inconclusive results found among studies [28]. However, there are no studies that investigate the influence of a moderate consumption of beer or alcohol on the physical fitness response to a highly demanding training program in conditions reflecting the real-life situation of healthy adults. Thus, the purpose of this study was to evaluate the effects of a HIIT program on physical fitness parameters in healthy young adults, and to analyze the possible influence of daily but moderate beer consumption or its alcohol equivalent on it. The primary hypothesis was that HIIT would improve cardiorespiratory fitness, muscular strength and power outcomes, but the intake of beer, even in moderate amounts, may mitigate these positive effects due to its alcohol content.

Methods

Study Design and Participants

Following recruitment via social networks, local media, and posters, a total of 83 healthy adults (35 females) from Granada (Spain) were assessed for eligibility. This study is a registered controlled trial (ClinicalTrials.gov ID: NCT03660579) and follows the latest revision of the Helsinki Declaration. The Ethics Committee on Human Research of the University of Granada (321/CEIH/2017) approved the study design, the study protocols, and the informed consent procedure. Prior to the enrolment, all potential individuals completed a medical examination to identify any pathological condition, provided a written informed consent, and were fully informed about the study objectives, design, inclusion criteria, assessments to be undertaken, exercise program intervention, and types of beverages to be ingested. The inclusion criteria were as follows: (i) having a body mass index (BMI) from 18.5 to 30 kg/m^2, (ii) not being engaged in a concurrent or previous structured training program or a weight-loss program, (iii) having a stable body mass during the last 5 months (body mass changes < 3 kg), (iv) being free of disease, (v) not being pregnant or lactating; (vi) not taking any medication for chronic diseases, and (vii) not suffering pain, recent injuries, or other problems preventing strenuous physical activity. All participants were informed about the physical activity recommendations of the World Health Organization during the intervention program, as well as the benefits of practicing physical activity [32], and they were instructed to maintain their usual physical activity levels and not to engage in other additional structured exercise outside of the intervention program.

As shown in Figure 1, 94 individuals attended an information meeting and were assessed for eligibility. A total of 83 individuals met the inclusion criteria and, after the baseline measurements, the participants were allocated to a training (T) or a Non-Training group based on personal preferences. Those allocated to the T group then chose whether they preferred to be included in a group ingesting an ethanol-containing beverage (5.4% alcohol content) or an alcohol-free beverage from Monday to Friday. Participants choosing ethanol were randomly allocated to either a group consuming beer (T-Beer) or to a group consuming sparkling water with added vodka ethanol (T-Ethanol). Those choosing non-alcoholic beverages were randomly allocated to either an alcohol-free beer group (T-0.0Beer) or to a sparkling-water group (T-Water). Each group was composed of 8 men and 8 women. This type of non-random (based on individual preference) and random allocation of the participants was conducted following ethical considerations and advice suggested by the ethical committee (321-CEIH-2017), since drinking alcohol, or participating in a highly demanding training program should be a personal choice. The assessment staff was blinded to the participants' randomization assignment.

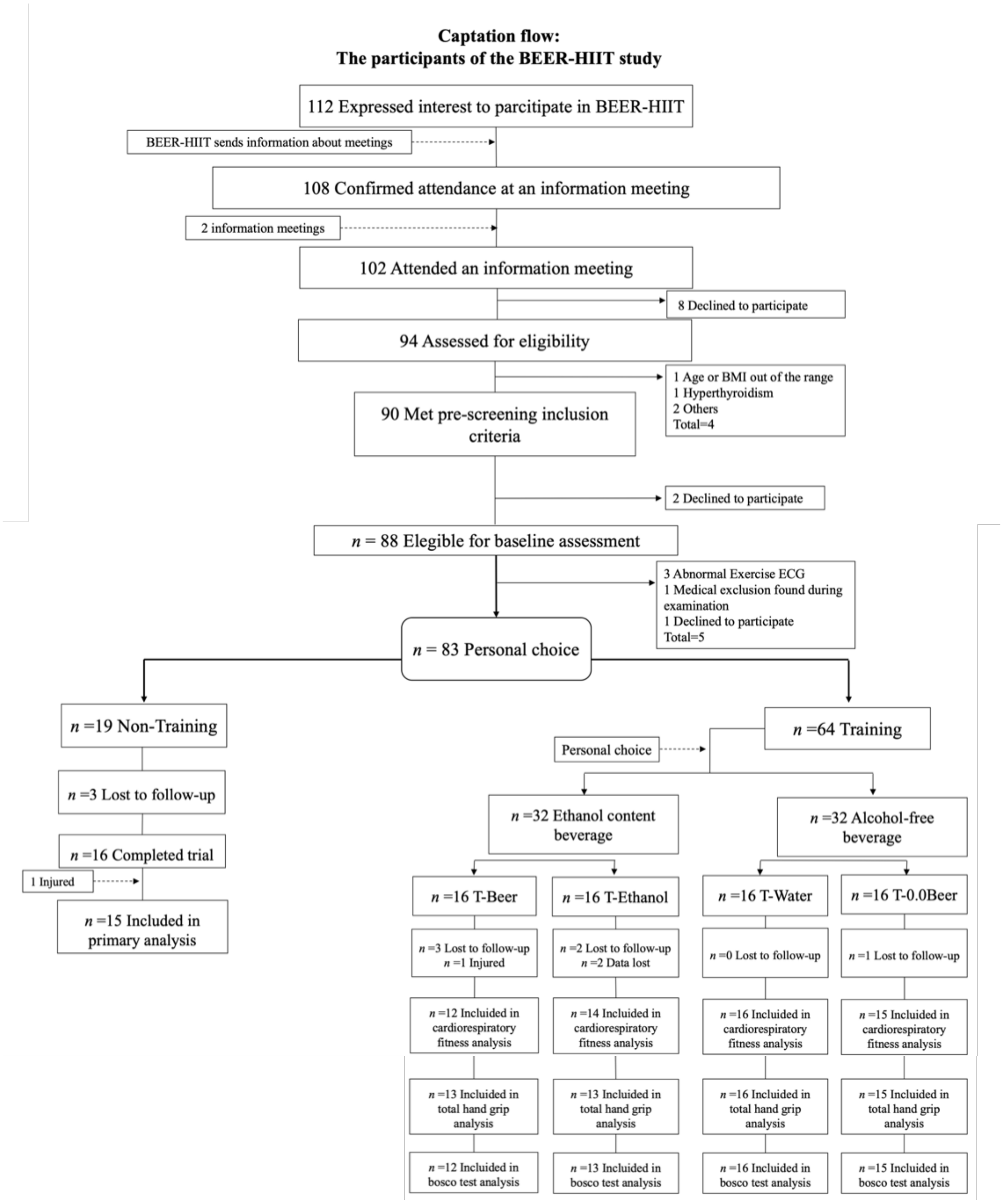

Figure 1. Flow-chart diagram.

The participants were asked to report their usual frequency of alcohol intake in seven possible response categories using the Beverage Intake Questionnaire (BEVQ) before and after the intervention. This questionnaire was developed to estimate mean daily intake of water, sugar-sweetened beverages, and alcohol beverages. To score the BEVQ, frequency ("How often") is converted to the unit of times per day, and then multiplied by the amount consumed ("How much each time") to provide average daily and weekly intake beverage consumption. Total alcohol consumption was quantified through the sum of beverage categories containing alcohol (i.e. beer, wine, spirits, and cocktails). In addition, the physical activity levels were registered before and after the intervention program by self-report. A total of 10 participants dropped out between the allocation and the follow-up due to (i) lack of adherence to the training program (n=5), (ii) physical reasons (n=3), or (iii) other reasons (n=2).

Training Protocol

A detailed description of each exercise of the training program can be found elsewhere and the proposed exercises are based on our previous experience [33,34]. Attending at least 80% of sessions was required to be included in the final analysis. All training sessions were supervised by qualified and certified personal trainers and performed in groups of ~8 participants. An intensity gradual progression was also scheduled in order to ensure a good adherence of the intervention group.

The HIIT intervention groups completed a total of 2 sessions per week in the late afternoon or early evening from Monday to Friday for 10 weeks with at least 48 h of recovery between each session. A gradual progression was proposed to avoid injuries and drop outs. Training sessions started with a volume of 40 min per week and an intensity of 8-9 RPE following previous studies [33,35,36]. Subsequent increments of both volume and intensity were established in Phase I (50 min per week and 10 RPE) and in Phase II (65 min per week and 10 RPE).

The HIIT intervention was divided into two different phases, starting with a familiarization phase to learn the main movement patterns. In all cases, the intensity of the training was > 8 Rating of Perceived Exertion (0–10 RPE scale) [37], which has a positive linear relationship with heart rate and VO_2max (maximal oxygen uptake) [38,39]. There was a passive rest between exercises and an active rest between sets (an intensity of 6 RPE, which corresponds with 60% VO_2max [38,40]), following the periodization described in previous studies [41]. Eight self-loading exercises were performed in a circuit form twice per set (i.e., frontal plank, high knees up, TRX horizontal row, squat, deadlift, side plank, push up, and burpees) with a passive rest between exercises and an active rest between sets (6 RPE intensity).

A dynamic standardized warm up and an active global-stretching cooling-down protocols were completed at the beginning and at the end of each training session, respectively, in all intervention groups. An extra effort was made to elicit better adherence to the training protocol. For instance, the sessions were rescheduled when a participant was unable to attend due to work, personal issues, or illness. The participants were constantly encouraged throughout each training session and were instructed to reach the specific target intensity.

Beverage Intake Protocol

The volumes of ingested fluid were 660 ml for men and 330 ml for women from Monday to Friday during the 10-week intervention. Men ingested 330 ml at lunch and 330 ml at dinner, and women ingested 330 ml at dinner: (i) the T-Beer group ingested regular Lager Beer (5.4% alcohol-Alhambra Especial®, Granada, Spain); (ii) the T-0.0Beer group ingested alcohol-free beer (0.0% alcohol-Cruzcampo®, Sevilla, Spain); (iii) the T-Water group ingested sparkling water (Eliqua 2®, Font Salem, Spain); (iv) the T-Ethanol group ingested sparkling water with exactly the equivalent amount of distilled alcohol added. The distilled alcoholic beverage used in our study was branded vodka because of the purity of its composition (37.5% ethanol and 62.5% water). The amount of alcohol selected to ingest was based on scientific evidence, which defines a moderate amount as two or three drinks/day or 24–36 g of ethanol/day for men and one to two drinks/day or 12–24 g of ethanol/day for women [28,36]. The only alcohol consumption allowed from Monday to Friday was that provided by the investigators. During the weekend, those participants included in alcohol groups were requested to drink no more than a moderate amount of alcohol (i.e., 660 ml/day for men and 330 ml/day for women). All the beverages were coded and provided by a staff member of our research laboratory at the beginning of each week to maintain blinded the group assignment of the participants to the investigators who did the evaluations. Additionally, the alcohol intakes were registered before and after the intervention.

Physical Fitness Assessment

Cardiorespiratory fitness

A maximum treadmill (H/P/Cosmos Pulsar treadmill, H/P/ Cosmos Sport & Medical GMBH, Germany) exercise test applying a previously validated protocol [33,35,42] (i.e., the modified Balke protocol [43]) was used to determine VO_2max. We conducted a warm up (walking at 3.5 km/h for 1 min and at 4 km/h for 2 min) followed by an incremental protocol which maintained a speed of 5.3 km/h at 0% grade for 1 min. Participants walked on a treadmill at a constant speed (5.3 km/h) with increases of 1% every minute until volitional exhaustion. We measured O_2 uptake and CO_2 production with an indirect calorimeter using an oronasal mask (model 7400, Hans Rudolph Inc., Kansas City, MO, United States) equipped with a prevent TM metabolic flow sensor (Medgraphics Corp., MN, United States). We calibrated the gas analyzer using two standard gas concentrations and we performed a flow calibration with a 3-L calibration syringe before the test every day. The Breeze Suite software (version 8.1.0.54 SP7, MGC Diagnostic®) was used to assess averages for oxygen uptake (VO_2) and respiratory exchange ratio (the ratio between carbon dioxide and oxygen percentage) every minute. In addition, heart rate was continuously measured throughout the exercise test (Polar RS800, Kempele, Finland), and ratings of perceived exertion (RPE) were assessed at each stage and at exhaustion (during the last 15 s) using the 6-20 Borg scale [44]. A familiarization process with the RPE scale was conducted before the exercise test. During each trial, the participants were strongly encouraged to make their utmost effort. The criteria for achieving VO_2max were: (i) a respiratory exchange ratio ≥ 1.1, a plateau in VO_2 /change of < 100 ml/min in the last three consecutive 10 s stage), (ii) a heart rate within 10 beats/min of the aged predicted maximal heart rate (168-0.7*age) [45,46], and (iii) if these criteria were not met, the peak oxygen uptake value during the exercise test was considered [46].

The participants were asked to refrain from stimulant substances and from ingesting food 24 h and 2 h before the exercise test, respectively, and not to perform any physical activity of moderate (previous 24 h) and/or vigorous intensity (previous 48 h).

Muscular strength

Hand grip strength (kg) was assessed with a digital hand dynamometer (T.K.K. 5401 Grip-D; Takey, Tokyo, Japan). This measurement has been suggested as a valid test to predict muscular strength and endurance with a simpler equipment and minimizing efforts from subjects. Also, it has been associated with whole body and upper body strength [47], although greater benefits for strength improvements would have been obtained by using a battery of strength tests such as those used in the weight-bearing exercises practiced during the training sessions. Participants maintained a standard bipedal position during the entire test with the arm in complete extension preventing the dynamometer from coming in contact with any part of the body except the hand being measured. Two attempts were made for each hand, with a 1-min rest between each trial. We instructed the participants to continuously squeeze for 2–3 s and they were asked to exert their maximal force in every attempt. Following previous studies, we fixed the grip spam of the dynamometer at 5.5 cm for men and a validated equation was used for women [48]. We considered total hand grip strength as the sum of the best attempt for both the left and right hand.

Muscular power

The anaerobic power of lower limbs was evaluated through the "Ergo Jump Bosco System®" (Globus, Treviso, Italy). The participants performed two trials for each jumping exercise and the best score was recorded [49]. Four types of jumps were performed in the following order: squat jump, SJ; countermovement jump without arm-swing, CMJ; with arm-swing, Abalakov jump, ABKJ; and drop jump, DJ. The participants received specific feedback from the investigators indicating leg, arm and trunk position during the test. The participants performed some practice trials before the assessment [50]. The results from the above-mentioned tests allowed the calculation of relevant muscle-strength-related indexes: elasticity index (elastic energy=({CMJ-SJ}/CMJ)x100), upper limbs coordination index ((ABKJ-CMJ)/ABKJ)x100), and percentage of fast-twitch fibers [51].

Statistical Analysis

Sample size calculations were based on a minimum predicted change of 15% in VO_2max, hand grip strength and SJ jump (with a 15% of estimated standard deviation) between the intervention groups and the control Non-Training group. Considering the results of a pilot study, 13 participants per group were necessary to provide a statistical power of 85% (type I error = 0.05) [52]. However, we recruited a minimum of 16 participants per group (a total of 80) to accept a maximum loss of 20% at the follow up [53].

Data were checked for normality using a Shapiro–Wilk test and a visual inspection of Q-Q plots. We conducted repeated measure analyses of variance to study changes in cardiorespiratory fitness and muscular strength parameters (i.e. VO_2max. in absolute and relative terms, maximal heart rate, total test duration, total hand grip, SJ, CMJ, ABKJ and DJ) across time, between groups, and their interaction (time × group). We applied the Student's t-test to paired values to determine intragroup differences in cardiorespiratory fitness and muscular strength parameters before and after the 10-week intervention.

An analysis of covariance (ANCOVA) was conducted to determine the effect of the groups (fixed factor) on cardiorespiratory fitness and muscular strength outcomes, i.e., post-VO_2max. minus pre-VO_2max. (dependent variable), adjusting for the baseline values (model 1). We conducted the same analysis for changes in VO_2max. in relative terms, maximal heart rate, total test duration, total hand grip, SJ, CMJ, ABKJ and DJ. Bonferroni post hoc tests with adjustment for multiple comparisons. Additional models were conducted controlling for baseline values and sex (model 2) and for baseline values and age (model 3) (see Additional file 3).

The level of statistical significance was defined at $p < 0.05$. The statistical analyses were conducted in Statistical Package for Social Science (SPSS, V. 25.0, IBM SPSS Statistics, IBM Corporation), and the graphical plots were conducted in GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA).

Results

A total of 73 participants (35 women) were included in the analyses after a loss to follow up of 12% (see Figure 1).

BMI, body mass index; ECG, electrocardiogram; T-Beer, the group that performed HIIT and consumed alcohol beer; T-0.0Beer, the group that performed HIIT and consumed non-alcoholic beer; T-Water, the group that performed HIIT and consumed sparkling water; T-Ethanol, the group that performed HIIT and consumed sparkling water with alcohol added.

The characteristics of the study population by sex are shown in Table 1. No differences were observed in the baseline values, neither in the training background existed between groups. The reported intakes of alcohol in the different groups were also similar (p = 0.144; See Table 1). The distribution of the number of men and women was nearly equal in each group.

Table 1. Descriptive parameters before the intervention program.

	Non-Training (n=15)		T-Beer (n=13)		T-0.0Beer (n=15)		
	Men (n=8)	Women (n=7)	Men (n=6)	Women (n=7)	Men (n=8)	Women (n=7)	Men (n=9)
Age (years)	19.9 ± 2.3	20.1 ± 2.9	25.2 ± 5.5	23.9 ± 8.1	25.4 ± 8.1	23.4 ± 4.0	26.9 ± 7
Body Mass (kg)	72.0 ± 9.8	57.3 ± 4.5	80.3 ± 16.3	61.3 ± 8.5	82.3 ± 15.3	59.2 ± 7.4	73.6 ± 6
Body Mass Index (kg/m^2)	22.7 ± 2.2	21.4 ± 1.2	26.4 ± 4.4	22.0 ± 3.2	26.8 ± 3.4	22.9 ± 2.9	25.6 ± 2
Weekly Alcohol Intake (liters)	0.9 ± 0.9	1.1 ± 0.6	0.2 ± 0.5	0.9 ± 0.9	1.0 ± 0.9	2.0 ± 1.4	1.0 ± 1.
Cardiorespiratory fitness	Men (n=7)	Women (n=7)	Men (n=6)	Women (n=6)	Men (n=8)	Women (n=7)	Men (n=9)
VO_2 max. (ml/min)	3091 ± 515	2144 ± 297	3230 ± 419	2232 ± 382	3154 ± 587	2219 ± 317	2699 ± 2
VO_2 max. (ml/kg/min)	44 ± 10	37 ± 6	41 ± 8	35 ± 4	38 ± 5	37 ± 4	37 ± 6
Maximal heart rate (b/min)	188 ± 13	190 ± 14	188 ± 18	188 ± 10	185 ± 7	189 ± 12	177 ± 1
Total test duration (s)	1207 ± 321	1041 ± 175	1231 ± 295	1035 ± 108	1074 ± 136	1025 ± 150	1086 ± 2
Muscular Strength	Men (n=8)	Women (n=7)	Men (n=6)	Women (n=7)	Men (n=8)	Women (n=7)	Men (n=9)
Total hand grip (kg)	73.5 ± 12.5	59.9 ± 6.1	82.2 ± 13.7	57.6 ± 6.4	78.2 ± 19.0	50.5 ± 6.3	75.8 ± 2
Muscular Power	Men (n=7)	Women (n=7)	Men (n=6)	Women (n=6)	Men (n=8)	Women (n=7)	Men (n=9)
Squat Jump (cm)	29.8 ± 6.3	21.4 ± 5.3	29.5 ± 3.5	20.9 ± 6.9	26.9 ± 10.5	19.6 ± 2.8	26.7 ± 7
Counter-movement Jump (cm)	32.0 ± 8.1	21.8 ± 4.4	31.1 ± 1.9	22.1 ± 6.2	24.3 ± 4.4	18.5 ± 1.4	28.4 ± 8
Abalakov Jump (cm)	35.4 ± 7.6	24.2 ± 5.2	36.9 ± 4.3	24.1 ± 6.1	31.6 ± 10.1	22.4 ± 4.2	34.1 ± 9
Drop Jump (cm)	36.3 ± 8.1	23.0 ± 5.1	35.2 ± 3.1	25.4 ± 5.8	28.7 ± 4.8	21.1 ± 2.7	33.4 ± 9

Data are shown as mean ± standard deviation. T-Beer, the group that performed HIIT and consumed alcohol beer; T-0.0Beer, th non-alcoholic beer; T-Water, the group that performed HIIT and consumed sparkling water; T-Ethanol, the group that performe alcohol added.

RESULTS AND DISCUSSION

Figure 2 shows cardiorespiratory fitness-related variables before and after the intervention study. A significant time x group interaction was found in VO_2max. in absolute and relative values ($p = 0.004$ and $p = 0.002$, respectively), whereas no significant time x group interaction was found in maximal heart rate and total test duration (all $p > 0.05$). VO_2max. in absolute and relative terms increased in all the intervention groups with no influence of the beverage intake (2730 ± 658 vs. 3192 ± 784 ml/min for T-Beer, 2718 ± 670 vs. 3134 ± 757 ml/min for T-0.0Beer, 2466 ± 351 vs. 2855 ± 563 ml/min for T-Water, 2554 ± 593 vs. 2994 ± 709 ml/min for T-Ethanol in absolute VO_2max.; all $p < 0.001$, Figure 2A; and 38 ± 7 vs. 44 ± 7 ml/kg/min for T-Beer, 38 ± 4 vs. 43 ± 5 ml/kg/min for T-0.0Beer, 36 ± 5 vs. 41 ± 7 ml/kg/min for T-Water, and 37 ± 4 vs. 43 ± 4 ml/kg/min for T-Ethanol in relative VO_2max.; all $p < 0.001$, Figure 2B). Similarly, the total test duration significantly increased in all the intervention groups without influence of the beverage intake (1125 ± 229 vs. 1269 ± 261 seconds for T-Beer, 1051 ± 140 vs. 1189 ± 155 seconds for T-0.0Beer, and 1067 ± 192 vs. 1165 ± 211 seconds for T-Ethanol; all $p < 0.05$, Figure 2D). No statistical differences were noted in the Non-Training group in any case (all $p > 0.05$).

Figure 3 shows muscular strength and power-related variables before and after the intervention study. A significance time x group interaction was found in CMJ ($p = 0.029$, Figure 3), whereas no significance was observed in total hand grip strength, SJ, ABKJ and DJ (all $p > 0.05$, Figure 3). Total hand grip performance only increased significantly in the T-0.0Beer group (65.3 ± 20.1 vs. 68.9 ± 19.3 kg; $p = 0.0041$, Figure 3A), whereas no statistically significant improvements were found in the rest of the intervention groups. SJ, CMJ and DJ performance increased in the T-0.0Beer group (21.9 ± 4.7 vs. 24.2 ± 4.4 cm for SJ, 21.6 ± 4.4. vs. 24.0 ± 4.8 cm for CMJ, and 25.2 ± 5.5 vs. 28.3 ± 4.3 cm for DJ; all $p < 0.05$, Figure 3B, 3C and 3D) as well as in the T-Water group (24.6 ± 8.5 vs. 26.6 ± 8.3 cm for SJ, and 28.8 ± 9.8 vs. 32.0 ± 9.3 cm for DJ; all $p < 0.05$, Figure 3B and 3E). No statistical differences were noted in the Non-Training group for SJ, ABKJ and DJ (all $p > 0.05$), whereas a significant decrease was observed in CMJ (26.9 ± 8.2 vs. 25.3 ± 7.9 cm; $p = 0.022$, Figure 3B).

Figure 4 shows changes in the cardiorespiratory fitness-related variables after the intervention study between the five groups (total, men, and women). The T-Beer, the T-0.0Beer, the T-Water, and the T-Ethanol intervention groups similarly increased VO_2max. in absolute and relative terms compared with the Non-Training group (all $p < 0.05$), with no significant differences between them (all $p > 0.05$). The results persisted in the T-Beer and the T-0.0Beer women groups after analyzing data divided by sex (see Additional file 1B and 1D). All intervention groups showed similar increases in the total test duration compared with the Non-Training group, with statistical differences between the T-Beer women group vs. the Non-Training women group (see Additional file 1H). The results persisted when the analyses were additionally adjusted for sex and age (see Additional file 3).

Figure 5 shows changes in muscular strength and power-related variables after the intervention study among the five groups. All the intervention groups similarly improved total hand grip, SJ, CMJ, ABKJ and DJ performance compared with the Non-Training group, with no statistical differences between them (all $p > 0.05$). The results persisted in all cases when sex and age were included as a covariate (see Additional file 3).

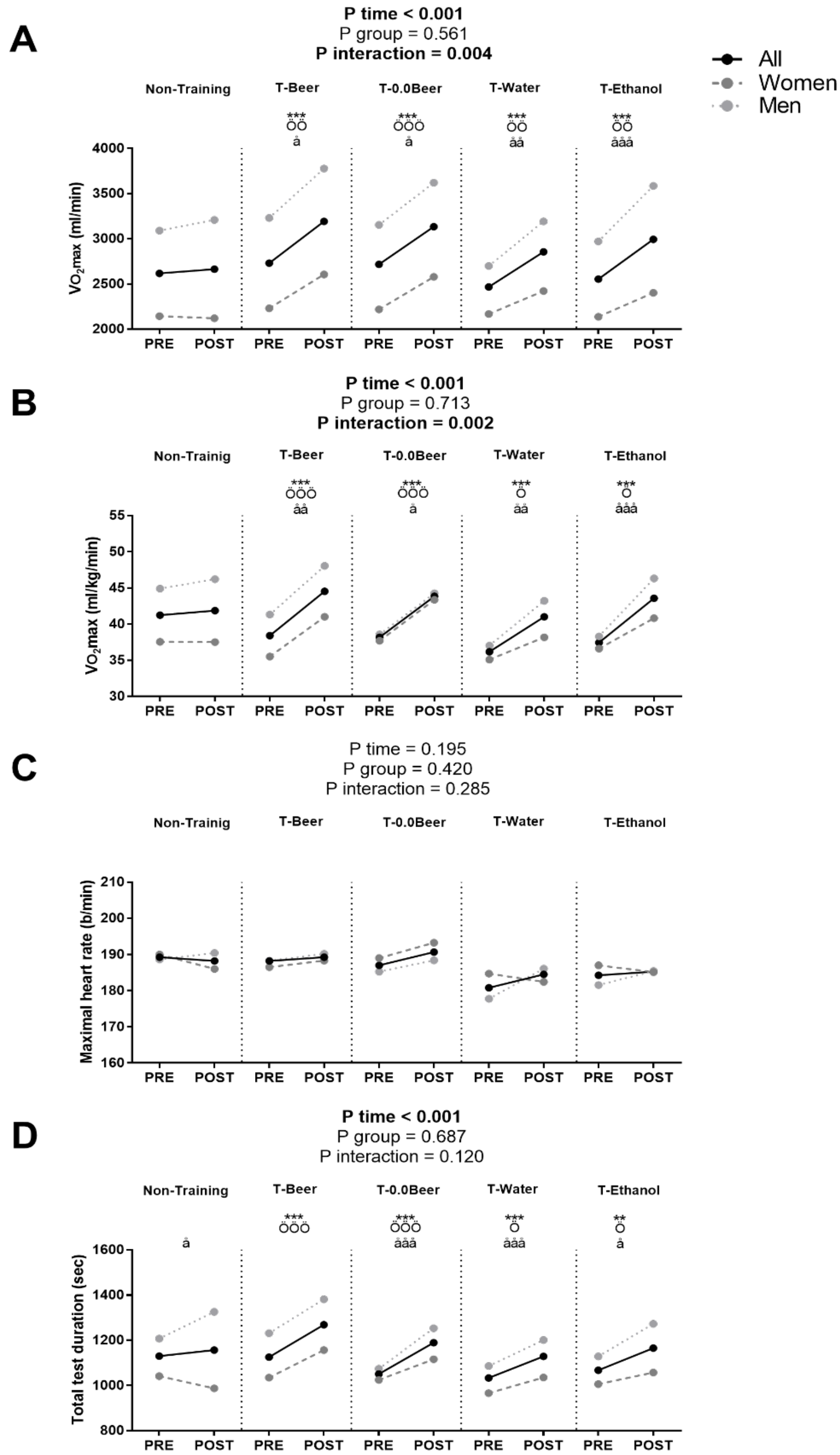

Figure 2. Changes in maximum oxygen uptake (VO2max.) in absolute (A) and relative terms (B), maximal heart rate (C), and total test duration (D) before and after the intervention study. P-value [time, group, and interaction (time x group)] of repeated measure analysis of variance. ** $p < 0.01$, *** $p < 0.001$ for total sample, ö $p < 0.05$, öö $p < 0.01$, ööö, $p < 0.001$ for women, å $p < 0.05$, åå $p < 0.01$, ååå $p < 0.001$ for men, obtained by Student's paired t-test. Data are shown as means. Abbreviations: T-Beer, group that performed HIIT and consumed alcohol beer; T-0.0Beer, the group that performed HIIT and consumed non-alcoholic beer; T-Water, the group that performed HIIT and consumed sparkling water; T-Ethanol, the group that performed HIIT and consumed sparkling water with added alcohol.

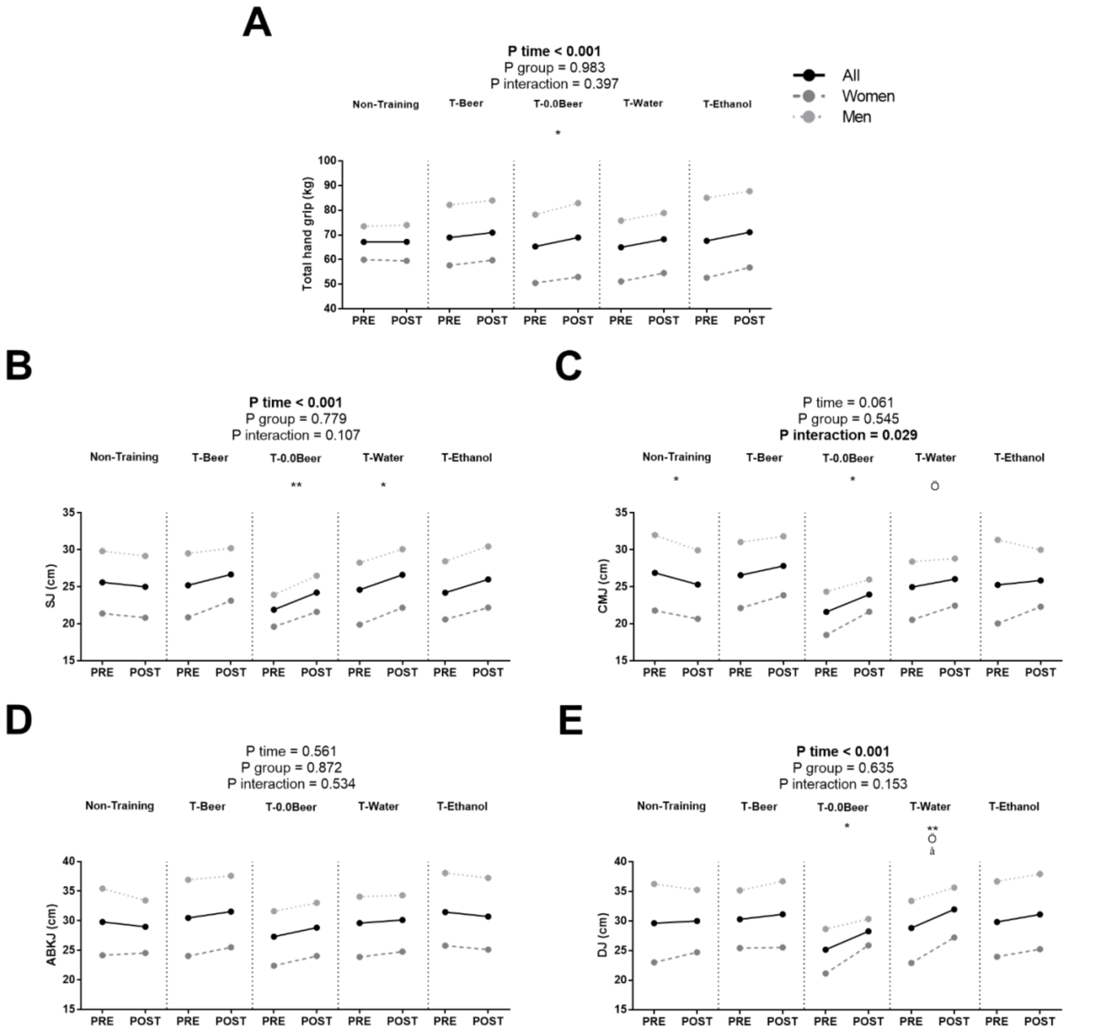

Figure 3. Changes in total hand grip (A), squat jump (B), counter-movement jump (C), Abalakov jump (D), and drop jump (E) before and after the intervention study. P-value [time, group, and interaction (time x group)] of repeated measure analysis of variance. * $p < 0.05$; ** $p < 0.01$ for total sample, ö $p < 0.05$, for women, å $p < 0.05$ for men, obtained by Student's paired t-test. Data are shown as means. Abbreviations: SJ, squat jump; CMJ, counter-movement jump; ABKJ, Abalakov jump; DJ, drop jump; T-Beer, the group that performed HIIT and consumed alcohol beer; T-0.0Beer, the group that performed HIIT and consumed non-alcoholic beer; T-Water, the group that performed HIIT and consumed sparkling water; T-Ethanol, the group that performed HIIT and consumed sparkling water with alcohol added.

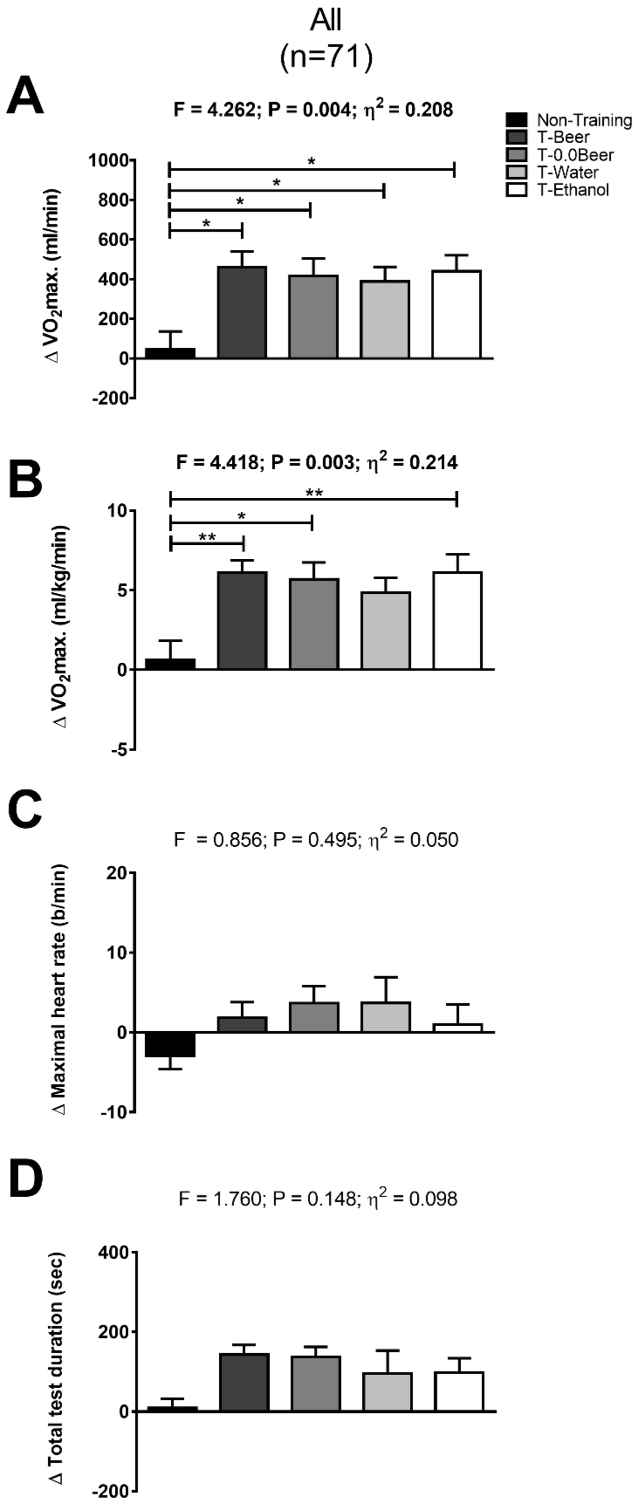

Figure 4. Changes in maximum oxygen uptake (VO2max.) in absolute (A) and relative terms (B), maximal heart rate (C), and total test duration (D), after the intervention study between the five groups. Significant differences between groups applying an analysis of covariance adjusting for baseline values with post hoc Bonferroni-corrected t-test are indicated as: * $p < 0.05$ ** $p < 0.01$, *** $p < 0.001$. Data are shown as means ± standard error of the mean. Abbreviations: η2, partial eta squared; T-Beer, the group that performed HIIT and consumed alcohol beer; T-0.0Beer, the group that performed HIIT and consumed non-alcoholic beer; T-Water, the group that performed HIIT and consumed sparkling water; T-Ethanol, the group that performed HIIT and consumed sparkling water with alcohol added.

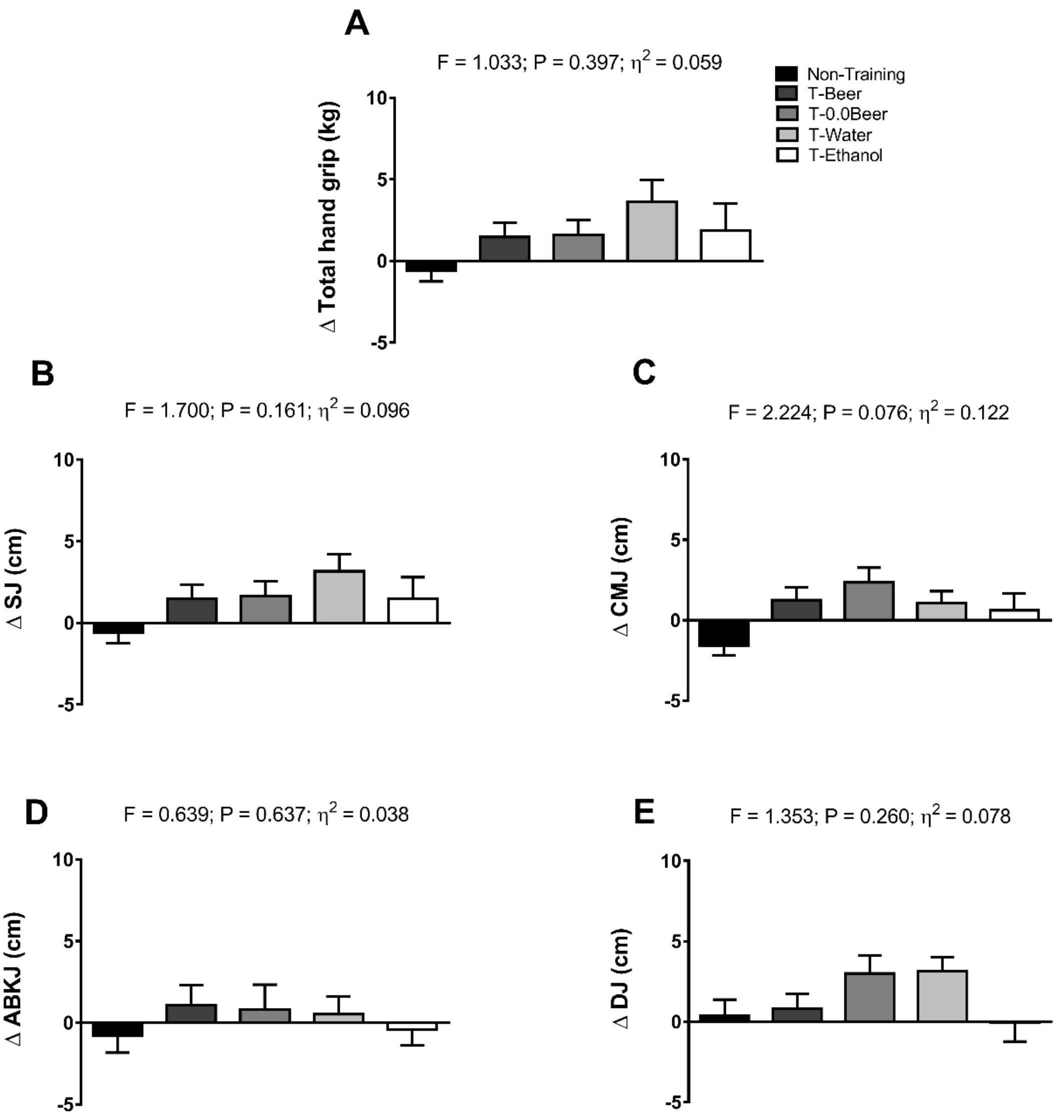

Figure 5. Changes in total hand grip (A), squat jump (B), counter-movement jump (C), Abalakov jump (D), and drop jump (E), after the intervention study between the five groups. Data are shown as means ± standard error of the mean. Abbreviations: $\eta 2$, partial eta squared; SJ, squat jump; CMJ, counter-movement jump; ABKJ, Abalakov jump; DJ, drop jump; T-Beer, the group that performed HIIT and consumed alcohol beer; T-0.0Beer, the group that performed HIIT and consumed non-alcoholic beer; T-Water, the group that performed HIIT and consumed sparkling water; T-Ethanol, the group that performed HIIT and consumed sparkling water with alcohol added.

Discussion

This study shows that a 10-week structured and highly demanding exercise intervention improves cardiorespiratory fitness and hand grip strength in healthy adults. This was not influenced by the concurrent daily intake of beer or ethanol in moderate amounts. No significant improvements were found in muscular power variables. Therefore, moderate and daily beer consumption accompanying meals, like in the present study, appears not to be an issue of concern affecting physical fitness parameters such as cardiorespiratory fitness and muscular strength after a HIIT program in healthy young adults.

Numerous studies have reported a robust evidence that HIIT induces improvements in cardiorespiratory fitness [5–8]. In a recent meta-analysis, Weston et al. [7] concluded that a low-volume HIIT produces moderate improvements in the VO_2max. of active non-athletic and sedentary subjects. These findings concur with those obtained by Milanović et al. [8], who reported in their systematic review and meta-analysis that HIIT elicits improvements in the VO_2max. of healthy, young to middle-aged adults, showing greater gains following HIIT than with continuous endurance training. In line with this, we showed that the T-0.0Beer and the T-Water groups increased their VO_2max. in absolute and relative terms after a 10-week HIIT intervention program. Thus, our results are of great value for public health due to the strong and positive association between better VO_2max. and reduced morbidity and mortality risk [1,3], which improves health, well-being and exercise performance [15–18].

Although most research has focused on the relationship between cardiorespiratory fitness and health outcomes, some studies have recently concentrated on muscular fitness. Recent researches have shown that high levels of muscular fitness are associated with a decreased cardiovascular risk and it has also been favorably correlated with improved bone health, enhanced self-esteem, and decreased adiposity [3]. A recent study found that a 12-week HIIT intervention, using the body weight as load, improved muscle strength in young women [54]. These results concur with those obtained by Amaro-Gahete et al., who found that a 12-week body-weight HIIT intervention improved extension and flexion peak torque and hand grip strength in middle-aged adults [55]. The present findings suggest that a 10-week HIIT intervention improves muscular strength (~ 5%, in the T-0.0Beer and the T-Water groups), measured by the hand grip test. On the other hand, Weston et al. [7] suggested that a low-volume HIIT has an unclear effect on muscular power that could be, at most, either a moderately beneficial or a mildly harmful effect. Our results showed no statistically significant increases from 5% to 12% in the three jump types of the Bosco test, showing lighter improvements in ABKJ (1.8% for the T-0.0Beer group, and 5.6% for the T-Water group) after the 10-week of HIIT intervention.

The majority of literature has been unable to establish a significant cause-effect relationship of alcohol with aerobic or anaerobic performance. While some studies have not found significant consequences of alcohol for submaximal endurance performance [56,57], other research have suggested that alcohol is detrimental to endurance performance [19,58]. In line with this, our results showed that a daily but moderate consumption of beer, or the equivalent ethanol amount, did not have deleterious effects on cardiorespiratory fitness-related variables, since the T-Beer and the T-Ethanol groups showed similar improvements in VO_2max. in absolute (17% for the T-Beer and 17.7% for the T-Ethanol) and in relative terms (16.3% and 16.8%, respectively) in relation to the free-alcohol intervention groups. Actually, it is under debate whether the possible beneficial effects of fermented beverages depend on the alcoholic or non-alcoholic components. A narrative review concluded that the protective effects resulting from the moderate consumption of wine or beer are due to both their alcohol and polyphenol components [59]. Some researchers have investigated the impact of acute alcohol consumption on skeletal muscle recovery and its effects on protein synthesis [28]. Poulsen et al. [60] suggested that muscular strength was not affected by moderate alcohol intoxication in healthy subjects. Our results agree with these findings since no detrimental effects were found in the T-Beer and T-Ethanol groups; contrarily to expected, these alcohol-consuming groups showed increases of ~ 3.5 kg in hand grip strength, but without statistically significant differences. On the other hand, it has been reported that large amounts of alcohol consumed immediately after prolonged exercise were associated with impairments of carbohydrates and lipid metabolism, which suggests an indirect deleterious effect of alcohol on muscle glycogen synthesis [22]. However, some authors found that low doses of alcohol ingested after strenuous damaging exercise had no impact on the exercise-induced muscle-damage losses in muscular performance [61], also that alcohol could have little or no effect on the resynthesis of muscle glycogen after exercise in male athletes [28]. Further, our previous results showed that a daily consumption of moderate amounts of beer or alcohol did not impair the gain of lean mass after a 10-week HIIT intervention [62]. Interestingly, the T-Beer, the T-0.0Beer and the T-Water women groups showed improvements with no statistical significance in SJ, Abalakov, and DJ, while the T-Ethanol group showed a decrease in all of them, although with no statistical significance. Similar impairments were found in CMJ for the T-Ethanol men group. Contrary to our results, Levitt et al. [63] found that consuming alcohol after strenuous-eccentric resistance exercise did not affect performance recovery for young recreationally resistance-trained women. Further, these authors [31] found that consuming a moderately high dose of alcohol following strenuous resistance exercise did not affect recovery of vertical jump height in resistance-trained men. In line with our findings, Barnes et al. [30] found that consuming a moderately high dose of alcohol (1 g ethanol kg^{-1} body mass) after eccentric exercise affected negatively the strength recovery in healthy men. The results of the present study are inconclusive since no significant improvements in muscle power have been found in the other intervention groups. Further studies are therefore required to examine the effectiveness of HIIT self-load program on muscular power and the possible concomitant effect of alcohol intake. In addition, future studies are needed to clarify if alcohol consumption affects differently women and men.

Although our study has demonstrated that a 10-week HIIT intervention can elicit improvements in muscular strength as well as cardiorespiratory fitness and that these improvements are not mitigated by the intake of beer or ethanol, it presents some limitations. Firstly, the sample size was relatively small to study the influence of different alcohol beverages in moderate amounts during an exercise-training intervention on physical-fitness variables. Considering that we compared a total of four different types of beverages ingested, future studies may replicate this study in larger clinical trials. Secondly, the participants included in our study are young, moderately active, healthy adults. Further research is required to clarify the effects on other populations such as older adults, sedentary, overweight or sport/athlete people; also, future studies are needed to clarify the effects in different atmospherics' conditions (i.e., lower or higher temperature). Thirdly, although physical activity levels were registered before and after the intervention program, they were subjectively assessed / self-reported. Finally, participants were not purely randomized, basically due to ethical considerations, and, given the organoleptic characteristics of the different types of beverages, a real double-blind design, placebo controlled for alcohol, was not possible.

Conclusions

This study shows that a 10-week structured and highly demanding exercise intervention improves cardiorespiratory fitness and hand grip strength in healthy adults. This was not influenced by the concurrent intake of beer in moderate amounts. Although we did not find deleterious effects of post-exercise alcohol consumption neither on cardiorespiratory fitness nor on muscular strength, the use of alcohol after strenuous exercise should be managed carefully. Therefore, more information is required if recommendations on appropriate alcohol use during the post-event period are to be made.

Declarations

Ethical approval and consent to participate: The Ethics Committee on Human Research of the University of Granada (321/CEIH/2017) approved the study design, the study protocols, and the informed consent procedure.

Consent for publication: Not applicable.

Availability of data and materials: All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Competing Interests: The authors have no conflicts of interest that are directly relevant to the content of this article. Manuel J. Castillo is a former member of the Scientific Advisory Board of the CICS. The funders had no role in the design of the study, in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

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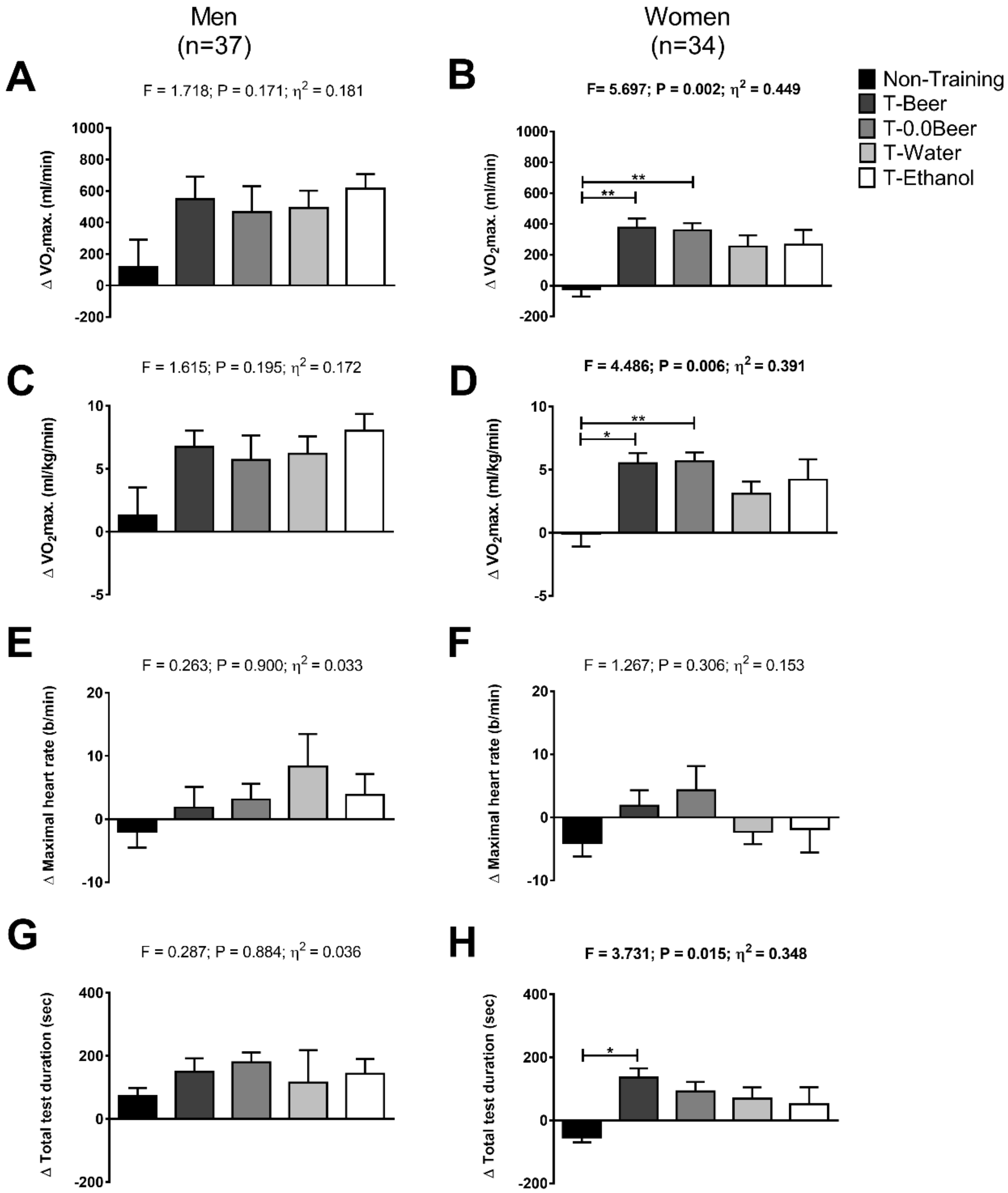
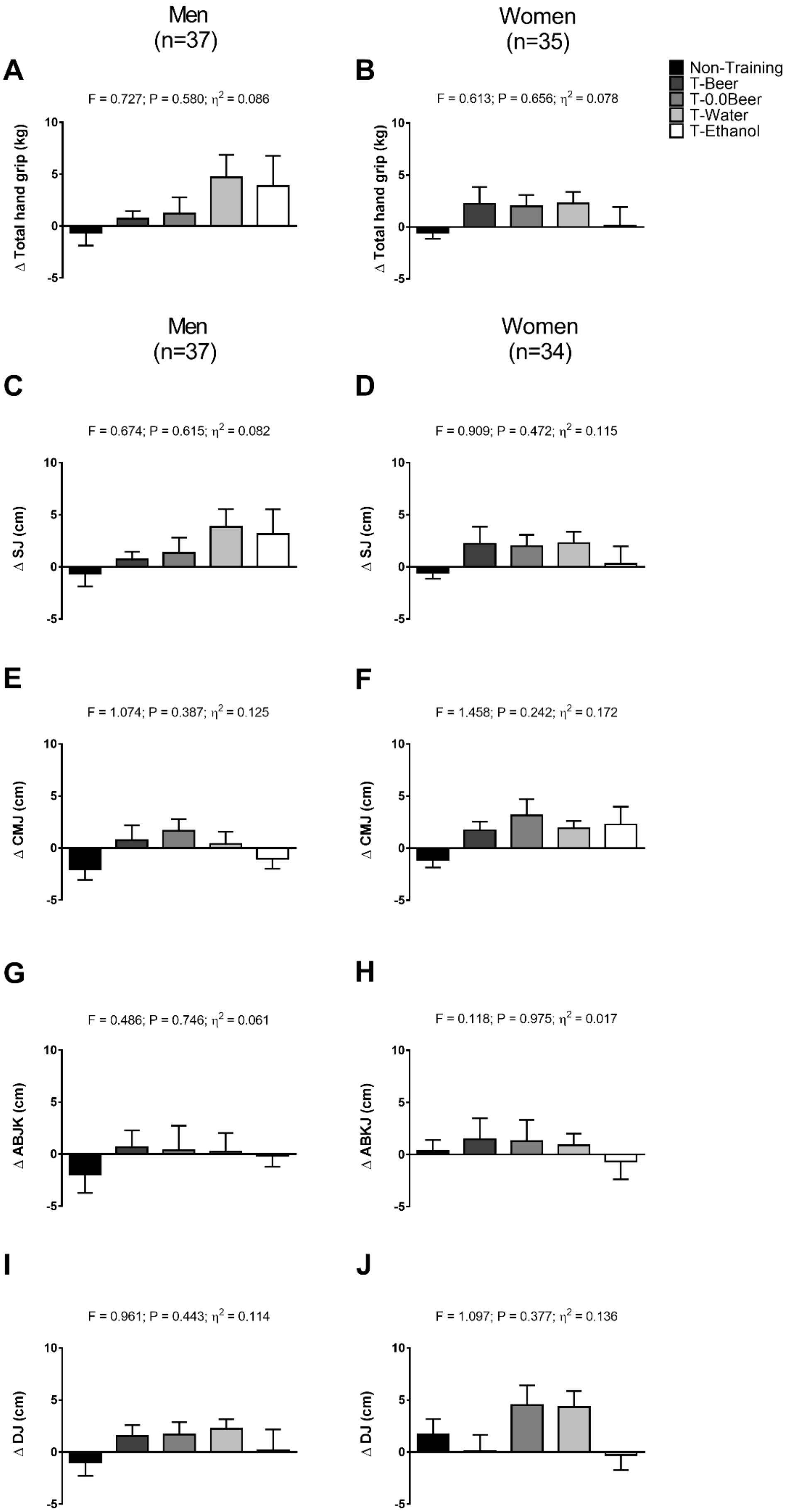


Additional File 1. Changes in maximum oxygen uptake (VO2max.) in absolute (A for men and B for women) and relative terms (C for men and D for women), maximal heart rate (E for men and F for women), and total test duration (G for men and H for women), after the intervention study between the five groups. Significant differences between groups applying an analysis of covariance adjusting for baseline values with post hoc Bonferroni-corrected t-test are indicated as: * $p < 0.05$ ** $p < 0.01$. Data are shown as means ± standard error of the mean. Abbreviations: η2, partial eta squared; T-Beer, the group that performed HIIT and consumed alcohol beer; T-0.0Beer, the group that performed HIIT and consumed non-alcoholic beer; T-Water, the group that performed HIIT and consumed sparkling water; T-Ethanol, the group that performed HIIT and consumed sparkling water with alcohol added.



Men
(n=37)
Women
(n=35)
Non-Training
T-Beer
T-0.0Beer
T-Water
T-Ethanol
A
F = 0.727; P = 0.580; η2 = 0.086
Δ Total hand grip (kg)
B
F = 0.613; P = 0.656; η2 = 0.078
Δ Total hand grip (kg)
Men
(n=37)
Women
(n=34)
C
F = 0.674; P = 0.615; η2 = 0.082
Δ SJ (cm)
D
F = 0.909; P = 0.472; η2 = 0.115
Δ SJ (cm)
E
F = 1.074; P = 0.387; η2 = 0.125
Δ CMJ (cm)
F
F = 1.458; P = 0.242; η2 = 0.172
Δ CMJ (cm)
G
F = 0.486; P = 0.746; η2 = 0.061
Δ ABJK (cm)
H
F = 0.118; P = 0.975; η2 = 0.017
Δ ABKJ (cm)
I
F = 0.961; P = 0.443; η2 = 0.114
Δ DJ (cm)
J
F = 1.097; P = 0.377; η2 = 0.136
Δ DJ (cm)

Additional File 2. Changes in total hand grip (A for men and B for women), squat jump (C for men and D for women), counter-movement jump (E for men and F for women), Abalakov jump (G for men and H for women), and drop jump (I for men and J for women), after the intervention study between the five groups. Data are shown as means ± standard error of the mean. Abbreviations: $\eta 2$, partial eta squared; SJ, squat jump; CMJ, counter-movement jump; ABKJ, Abalakov jump; DJ, drop jump; T-Beer, the group that performed HIIT and consumed alcohol beer; T-0.0Beer, the group that performed HIIT and consumed non-alcoholic beer; T-Water, the group that performed HIIT and consumed sparkling water; T-Ethanol, the group that performed HIIT and consumed sparkling water with alcohol added.

Additional file 3. Changes in physical fitness outcomes adjusted by baseline values (Model 1) adjusted by baseline values and sex (Model 2), by baseline values and age (Model 3).

		Analysis of covariance P value		
		F	P	η^2
VO_2max. (ml/min)	Model 1	4.262	0.004	0.208
	Model 2	4.671	0.002	0.226
	Model 3	5.391	0.001	0.252
VO_2max. (ml/kg/min)	Model 1	4.418	0.003	0.214
	Model 2	4.381	0.003	0.215
	Model 3	5.511	0.001	0.256
Maximal heart rate (b/min)	Model 1	0.856	0.495	0.050
	Model 2	0.853	0.497	0.051
	Model 3	1.090	0.369	0.064
Total test duration (sec)	Model 1	1.760	0.148	0.098
	Model 2	1.803	0.139	0.101
	Model 3	1.800	0.140	0.101
Total hand grip (kg)	Model 1	1.033	0.397	0.059
	Model 2	1.176	0.330	0.067
	Model 3	0.913	0.463	0.053
Squat jump (cm)	Model 1	1.700	0.161	0.096
	Model 2	1.540	0.201	0.089
	Model 3	1.582	0.190	0.091
Counter-movement jump (cm)	Model 1	2.224	0.076	0.122
	Model 2	2.257	0.073	0.125
	Model 3	4.793	0.002	0.223

Additional file 1 continued

Abalakov jump (cm)	Model 1	0.639	0.637	0.038
	Model 2	0.541	0.706	0.033
	Model 3	0.726	0.577	0.044
Drop jump (cm)	Model 1	1.353	0.260	0.078
	Model 2	1.161	0.336	0.069
	Model 3	1.338	0.266	0.078

SECTION 2

Combine effects of highly demanding training program and moderate alcohol/beer consumption on cognitive performance

Chapter 5

Effect of moderate alcohol intake during a high-intensity interval training intervention on choice reaction time task in healthy adults. The BEER-HIIT study (Study 3)

Abstract

Purpose. The aim of this investigation is threefold: (i) to determine the effects of a high intensity interval training (HIIT) program on reaction time (RT) performance in healthy young adults, (ii) to assess whether those effects are influenced by moderate alcohol consumption, and (iii) to examine the practice effects over several test administrations on RT performance.

Methods. We conducted a 10-week HIIT program and compared four type of beverages with/without alcohol content (consumed at least 5 days/week). A total of 74 healthy adults (18-40 years; 49% females) were randomly assigned to either a control Non-Training group or a HIIT program group (2 days/week, >8 RPE (Rating of Perceived Exertion)). Using a block randomization, participants in the HIIT program group were further allocated to a HIIT-Alcohol group (alcohol beer or sparkling water with vodka added, 5.4%) or a HIIT-NonAlcohol group (sparkling water or non-alcohol beer, 0.0%). Outcomes included changes from baseline to week 10 in Median RT and On time reactions in three different interval presentation and Total RT.

Results. Participants in the intervention groups improved speed and accuracy RT in Attempt 1 and 2 (all $p \leq 0.001$), independently of sex and alcohol consumption, with no statistical differences between groups in any attempt (all $p > 0.050$). A practice effect was detected in the successive tests ($F_{3,178} = 178.4$, $\eta^2 = 0.715$, $p \leq 0.00$). We also found that practice effects were higher at baseline in all groups (all $p \leq 0.001$).

Conclusions. These findings suggest a protector effect of HIIT on speed and accuracy RT in healthy young adults, independently of alcohol consumption. Furthermore, our results indicate that retesting may produce an improvement in RT task execution.

Introduction

Reaction time (RT) is a traditionally widely used measurement of cognitive functioning.[1,2] It has also been considered as a putative element of executive functions (EFs),[3] including processing or perceptual speed, motor and sensory components, and inhibitory control.[4,5] EFs are essential skills for mental and physical health, as well as for cognitive, social, and psychological development since they control impulsive or premature responses.[2] Answer accuracy and speed play a key function in RT performance tests,[6] both having an important role in the possibility of speed-accuracy trade-offs, which refers to achieving increases in speed at cost of decreases in accuracy, an vice-versa.[7] While RT reflects the time spent to respond to an item, independently from its correctness, accuracy indicates the number of correctly completed items within time span. It has been shown that practicing not only may improve performance but also reduce RTs.[4] This is the so-called retest effects, practice effects, or testing effects, which is explained by the automatization of psychomotor skills or the habituation process.[4] Accordingly to this issue, the influence of retest effects decreases when the participant is retested multiple times, being expected to be greater in the first repetitions of the test.[4,8] Similarly, a reduction of construct-irrelevant factors, such as anxiety and rule comprehension, has been found to take plateau from the first to second test.[8] Interestingly, in longitudinal studies retested (pre- and post-measurements) is a criterion task to evaluate the intervention effectiveness.[4] In these cases, retest effects should be taken into account because those effects might contaminate the measurement of cognitive abilities.[4]

There is converging evidence that regular physical exercise across the lifespan has beneficial effects on cognition enhancing neuroplasticity and preventing cognitive age-related decline.[9–11] Although little is known about the effects of exercise training on RT, it has been previously shown that regular physical exercise is effective at improving cognitive functions.[9,12,13] Furthermore, previous studies suggest that a high physical fitness level might be positively involved in the relationship between exercise and EFs in children and older adults.[11,14,15] However, adhering to an exercise training program may be challenging owing to the lack of time in developed societies. Along these lines, high-intensity interval training (HIIT) is a time-efficient exercise training modality that has emerged as an effective tool not only for improving maximum physical fitness[16] or cardiometabolic health,[17–19] but also for enhancing information processing speed measured by RT tasks in older adults.[20] Yet, the effects of this exercise training modality on EFs in young and middle-aged adults remains still uncertain.

Remarkably, there is a strong link between physically active people and alcohol consumption,[21–23] which is often observed in a social and relaxed context. In fact, beer — one of the most consumed beverage in the world [24] — is usually consumed for thirst quenching, accompanying meals (in a Mediterranean diet) or after exercise.[25,26] However, there is limited scientific knowledge regarding the effect of moderate alcohol consumption on motor control and performance of skilled tasks.[22] It has been suggested that the behavioural effects of alcohol consumption on the central nervous system may be dependent of the pattern of intake and the doses ingested.[27] Although no cognitive impairments have been shown in response to an acute low dose of alcohol intake,[27,28] a significant decline of cognitive function has been observed after the ingestion of high alcohol doses.[7] However, data are less clear regarding the effect of moderate alcohol consumption on cognitive function (i.e., RTs) while engaged in an exercise training intervention.

The purpose of this investigation is threefold. First, to determine the effects of a HIIT program on RT performance in healthy young adults. Second, to assess whether those effects are influenced by moderate alcohol consumption. Finally, to examine the practice effects over several test administrations and throughout three intervals of the stimulus presentation on RT performance in young healthy adults. We hypothesised that (i) HIIT would improve RT performance, (ii) improvements in RT performance would be dependent of alcohol consumption, and (ii) a reduction in RTs and improvements in accuracy would be found as a consequence of retesting, being those improvements more noticed in the shortest interval stimulus presentation (834 ms, RTI2).

Material and Methods

Subjects

A total of 74 healthy Caucasian volunteers (36 women) aged 18-40 years were recruited and enrolled in the current study. The study, with BEER-HIIT acronym, is a registered controlled trial (ClinicalTrials.gov ID: NCT03660579). It was conducted in accordance with the last revised guidelines of the Declaration of Helsinki and approved by the Ethics Committee on Human Research of the University of Granada [321/CEI/2017]. Prior to their enrolment, all participants completed a health exam and gave their oral and written informed consent. Similarly, they accepted to maintain their dietary and lifestyle habits, to be abstained from consuming alcoholic beverages unrelated to those provided by the research staff, and not to exceed the corresponding amounts to a moderate consumption. The inclusion criteria were: (i) to have a body mass index (BMI) from 18.5 to 30 kg/m^2, (ii) not being engaged in an structured training program or a weight-loss program, (iii) having a stable body weight during the last 5 months (body weight changes < 3 kg), (iv) being free of disease, pregnant, or lactating women; (iv) not taking any medication for chronic diseases, and (v) not suffering pain, recent lesions, or other problems preventing strenuous physical activity.

All participants were informed about the physical activity recommendations from the World Health Organization during the intervention program, as well as the benefits of practicing physical activity. They were also instructed to maintain their usual physical activity levels and not to engage in other additional structured exercise outside of the intervention program. Furthermore, they were asked to report, before and after the 10-week intervention program, their usual frequency of alcohol intake through seven possible response categories using the Beverage Intake Questionnaire (BEVQ [29]). Similarly, dietary habits were assessed by the PREDIMED questionnaire.[30]

Incompliance with these conditions, lack of adherence to the protocol or the training program (> 20% no-shows for training sessions), or the detection of health problems during the intervention were exclusion criteria.

Design

This study was a registered controlled trial primarily designed to assess the effects of a 10-week HIIT intervention program, as compared to a control/Non-Training group, on cognitive performance (i.e. EFs). Intervention effects on primary outcomes depending on moderate intake of beer and its alcohol equivalent content, as compared with non-alcohol beer and sparkling water consumption, were also assessed. In addition, we measured the effect of test-retest at the baseline and after the 10-week intervention program.

After completing baseline measurements, selected participants were assigned to a Non-Training group or a HIIT intervention program group based on individual preferences. Training sessions consisted in two high intensity and demanding training sessions per week, during 10 consecutive weeks. Participants included in the training groups were asked to choose about their drinking preference for alcoholic or non-alcoholic beverages due to ethical considerations suggested by the ethical committee (321-CEIH-2017). Those participants willing to consume alcohol at baseline were randomly assigned to the training group with beer intake (5.4% alcohol) or to the sparkling water group with exactly the same amount (5.4% alcohol) of distilled alcohol added. Participants not willing to consume alcohol were randomly assigned to a training group with alcohol-free beer (0.0% alcohol) or a training group with sparkling water. We used a computer random number generator with a block size of eight with a one-to-one ratio. This blocked randomisation method is considered methodologically valid and ethically acceptable, as it confers adequate balance (on the stratified factors) between groups.[31] This type of non-random (based on individual preference) and random allocation of the participants was conducted following ethical considerations and advices provided by the ethical committee of the University of Granada [321-CEIH-2017], since drinking alcohol or participating in a highly demanding training program should be a personal choice. The study personnel responsible for the data collection was blinded to participants' allocation assignment.

Baseline and follow-up assessments were performed at the same setting [Sport and Health University Research Institute (iMUDS) at the University of Granada].

Methodology

Training Protocol. The HIIT intervention program consisted on 2 sessions/week performed in the late afternoon or early evening from Monday to Friday during 10 consecutive weeks with at least 48 h of recovery between each session. The training volume was 40-65 min/week following similar procedures than those described by previous studies,[32–34] and the training periodization was divided into two different phases starting with a familiarization phase.[35–37] Training intensity was fixed at > 8 Rating of Perceived Exertion (0–10 RPE scale)[38] which has a positive linear relationship with heart rate and VO_2max.[39,40] There was a passive rest between exercises and an active rest between sets (an intensity of 6 RPE).[34,39,41]

Participants performed a total of eight body-weight exercises in a circuit form (i.e., frontal plank, high knees up, horizontal row, squat, dead lift, side plank, push up, and burpees).[41] A detailed description of each exercise of the training program can be found elsewhere.[41] All sessions started with a dynamic standardized warm-up, including several muscle activations exercises, and ended with an active cooling-down protocol.[41] An extra effort was made to promote maximal attendance. Sessions were rescheduled when a participant was unable to attend due to work, personal issues, or illness. Participants were constantly motivated throughout each training session and were instructed to reach the specific target intensity.

Beverage Intake Protocol. The volume of fluid ingested were the same in all groups (i.e. 660 ml for men and 330 ml for women). The beverages were coded; thus, the research staff was unaware of contents until analyses completion. All beverages were given to participants at the beginning of each week. The amount of alcohol selected to ingest by participants was based on previous evidence.[42,43] The beverage intake was programmed from Monday to Friday; men consumed the beverages twice per day (330 ml with lunch time and 330 ml with dinner), and women ingested 330 ml with dinner: (i) HIIT-Alcohol group ingested regular Lager Beer (5.4% alcohol-Alhambra Especial®, Granada, Spain) or sparkling water with exactly the same amount of distilled alcohol added, and (ii) HIIT Non-Alcohol group ingested alcohol-free beer (0.0% alcohol-Cruzcampo®, Sevilla, Spain) or sparkling water (Eliqua 2®, Font Salem, Spain). The only alcohol consumption allowed from Monday to Friday was that provided by the investigators. During the weekend, those participants included in the alcohol group were requested to drink the same amount of alcohol than those ingested during the weekdays.

Choice reaction time. The RT outcomes were evaluated by the Vienna Test System (Schuhfried GmbH, Mödling, Austria). We selected the determination test (DT) Turkish form, S16. The DT is used to measure reactive stress tolerance, attention, and its associated response speed in several situations requiring continuous, swift, and varying responses to rapidly changing visual and acoustic stimuli.[44–46] The test requires the respondents to use their cognitive skills: (i) to distinguish different colours and sounds, (ii) to memorize the relevant characteristics of stimulus configurations, response buttons, and assignment rules, and (iii) to select the relevant responses according to the assignment rules laid down in the instructions or learned in the course of the test.

Prior to the test, all participants received the corresponding comprehensive instructions. Consecutively, a familiarization and instruction phase were performed in a task of 20 stimuli with 3000 milliseconds (ms) of duration, and three subtests in reaction modes with fixed presentation time per stimulus. Each stimulus was presented for three fixed periods of time: (i) 1078 ms in Interval 1 (RTI1), (ii) 834 ms in RTI2, and (iii) 948 ms in RTI3. A total of 120 stimulus were presented for each condition. Moreover, the system generated the total mode reaction during the three intervals to a total of 360 stimuli (Total RT). There were five visual stimuli coloured white, yellow, red, green and blue, which appeared in an upper and a lower row. Manual response (circles buttons) was required to react to visual circle stimuli, also manual response (rectangular buttons) was required to two different acoustic stimuli, finally, pedal response was required to react to visual rectangular stimuli (see Figure 1).

The primary outcome of our study is RT to multiple stimuli. The variables studied were: (i) Median of the time from the appearance of a stimulus to the pressing of a button (Median RT, measured in seconds) and (ii) On Time reactions if it was correct and occurred within the stimulus presentation time. On time reactions were expressed as ponderations.

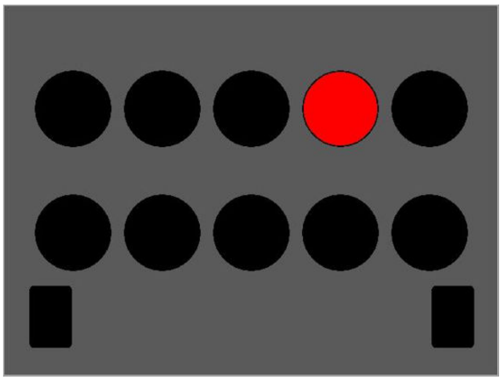


Figure 1. Picture of the test equipment used for the Vienna determination test.
The participants had to respond manually to coloured circles that appeared on the screen by pressing the coloured buttons at the upper centre of the response board. The participants had to respond to two different acoustic tones by pressing the black or grey bars at the centre of the board and to two grey rectangles that appeared at the lower left or right side of the screen by pressing the foot pedals with the left or right foot, respectively.

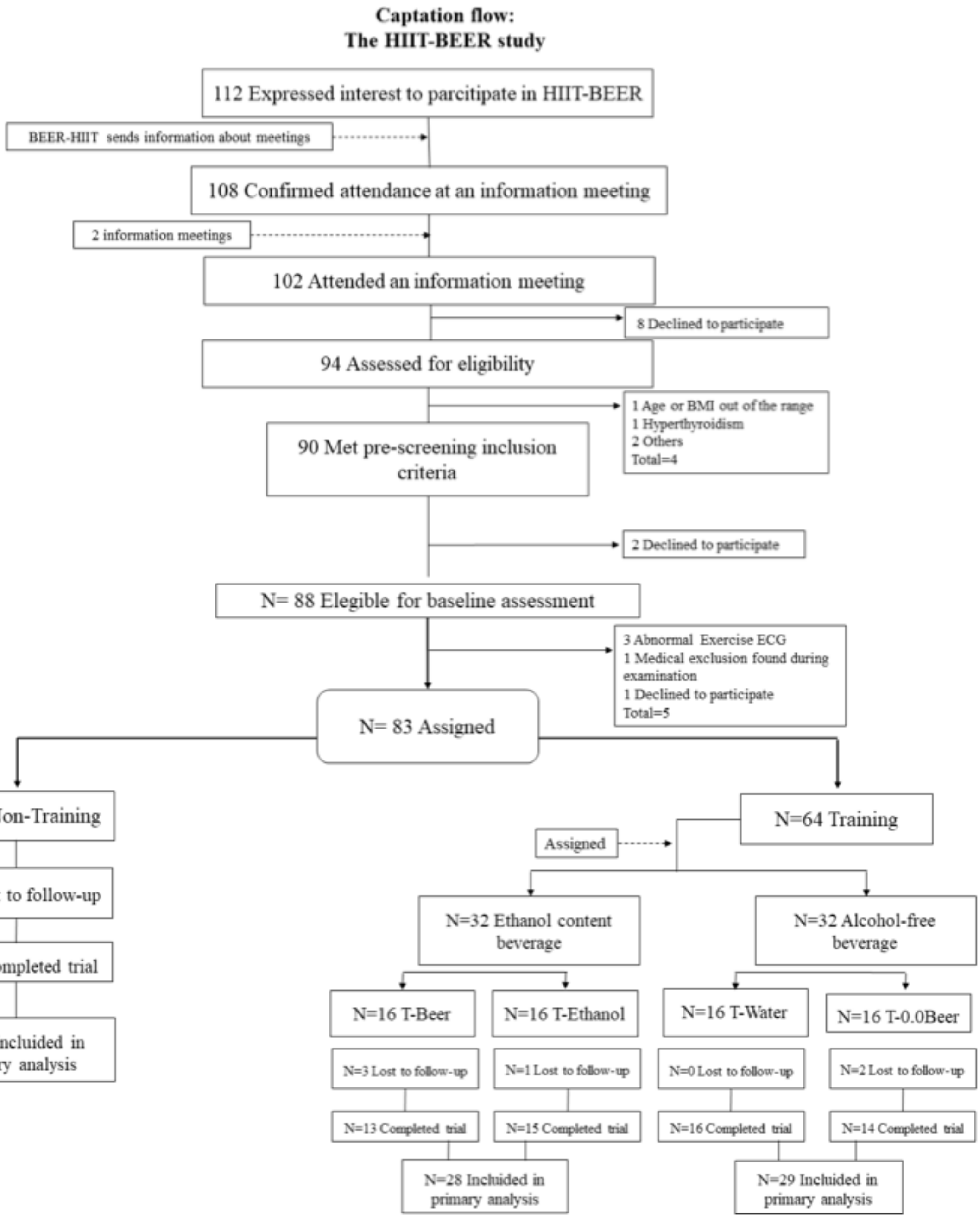


Figure 2. Flow-chart diagram. Abbreviations: BMI, body mass index; ECG, electrocardiogram; HIIT-Alcohol, group that performed HIIT and ingested alcohol beer; HIIT-NonAlcohol, group that performed HIIT and ingested non-alcoholic beer.

Statistical analysis

Sample size calculations were made based on the data of a pilot choice RT task sample.[47] We considered RTs differences between the baseline and after intervention to assess the sample size requirements for the three-way ANOVA.[48] As a result, we expected to detect an effect size of 0.25 in cognitive function (i.e., RT) considering a type I error of 0.05 with a statistical power of 0.85 including a minimum of 13 participants per group. Assuming a maximum loss at follow-up of 20%, we decided to recruit a total of 80 participants (16 participants per group; ~50% women). As no significant interaction was obtained by sex, we fitted all models including men and women together.

Descriptive parameters are reported as mean and standard deviation, except for longitudinal changes which are expressed as means and standard errors; 95% confidence intervals (CIs) being estimated for practice effect calculations. Normality was tested using Shapiro-Wilk tests, visual check of histograms, and Q-Q plots. Differences in baseline characteristics between groups were evaluated through analyses of variance (ANOVA). Practice effect was analysed using repeated-measures analysis of variance to determine changes in Median RT and On time reaction across attempts (Attempts 1-2 Baseline and Attempts 1-2 After 10-week), between groups, and the interaction term (attempt*group). Changes in Median RT and On time reactions were analysed using a mixed model design [Time (2: Baseline, After 10-week) as within-subjects' factors, and groups (Non-Training, HIIT-Alcohol, and HIIT-NonAlcohol) as between-subject factor, and the interaction term (time*group)]. Also, a repeated-measures analysis of variance was performed to evaluate differences in dependent variables before and after the intervention programme within attempts. We examined the effect of group (fixed factor) on all variable changes using an analysis of covariance (ANCOVA), i.e. post-Median RT seconds minus pre-Median RT seconds (dependent variable), adjusting for baseline values. The same analyses were conducted for changes in On time reactions. Additional models were conducted controlling for baseline values and sex (model 2), and for baseline values and age (model 3) (see Supplementary material, Table S1). We performed Bonferroni post hoc tests with adjustment for multiple comparisons to determine differences between all intake modality groups. The level of statistical significance was defined at $P < .05$.

All analyses were performed using the Statistical Package for Social Sciences (SPSS, v. 25.0, IBM SPSS Statistics, IBM Corporation). The graphical presentations were prepared using GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA).

Results

A total of 74 participants (36 women) were included in the main analyses after a loss to follow up of 13% (see Fig. 2). Baseline characteristics of the study participants are shown in Table 1. No differences in alcohol intake were observed between groups at baseline (p=0.144; See Table 1

Table 1. Baseline characteristics of participants

	Non-Training (n=17)		HIIT-Alcohol (n=28)		HIIT-NonAlcohol (n=29)	
Age (years)	20.1 (2.5)		25.3 (6.0)		24.5 (6.4)	
Sex (%)						
Men	10 (58.8)		13 (46.4)		15 (51.7)	
Women	7 (41.2)		15 (53.6)		14 (48.3)	
Total Alcohol Intake	1.0 (40.7)		0.7 (0.8)		1.2 (1.1)	
Educational level (%)						
Primary Education	--		3.7%		--	
Secondary Education	47.1%		40.7%		22.5%	
Vocational Education and Training	23.5%		18.5%		22.5%	
University Degree or Certificate of Higher Education	29.5%		37.3%		54.8%	
Reaction time						
Total	Attempt 1	Attempt 2	Attempt 1	Attempt 2	Attempt 1	Attempt 2
Median RT (sec)	0.671±0.05	0.614±0.05	0.662±0.07	0.612±0.06	0.687±0.04	0.621±0.03
On Time reactions	0.928±0.05	0.971±0.02	0.930±0.07	0.970±0.03	0.927±0.04	0.973±0.02
RTI1 (1078 ms)						
Median RT (sec)	0.673±0.06	0.616±0.05	0.669±0.07	0.610±0.07	0.694±0.05	0.623±0.03
On Time reactions	0.983±0.01	0.991±0.01	0.984±0.01	0.990±0.01	0.983±0.01	0.996±0.01
RTI2 (834 ms)						
Median RT (sec)	0.607±0.06	0.602±0.05	0.663±0.09	0.610±0.06	0.686±0.05	0.613±0.04
On Time reactions	0.853±0.11	0.951±0.04	0.844±0.16	0.939±0.06	0.836±0.10	0.947±0.04
RTI3 (948 ms)						
Median RT (sec)	0.667±0.05	0.622±0.05	0.656±0.06	0.618±0.06	0.681±0.05	0.629±0.04
On Time reactions	0.941±0.05	0.971±0.02	0.954±0.05	0.980±0.02	0.949±0.04	0.973±0.02

Values of main variables before the 10-week HIIT intervention program for the control and intervention groups. Data are expressed as mean ± standard deviation. Only participants with complete intervention program were included in the complete analysis. **Abbreviations:** RT, reaction time; Sec, seconds; Ms, milliseconds; RTI1, reaction time interval 1; RTI2, reaction time interval 2; RTI3, reaction time interval 3; HIIT-Alcohol, group that performed HIIT and consumed an alcohol beverage (5.4% ethanol); HIIT-NonAlcohol, group that performed HIIT and consumed a free alcohol beverage.

Intervention effects of a HIIT program on RT performance

Fig. 3A shows Median RT and On time reaction values in Attempt 1 (left) and Attempt 2 (right) before and after 10 weeks. Median RT decreased in both attempts after 10 weeks in the Non-Training, HIIT-Alcohol, and HIIT-NonAlcohol groups (all $p \leq 0.001$, see Fig. 3A), whereas a significant increment in On time reactions in both Attempt 1 and 2 was noted in Non-Training, HIIT-Alcohol and HIIT-NonAlcohol groups (all $p<0.050$; see Fig. 3B). Furthermore, no differences between groups were observed in Median RT or On time reactions (all $p>0.050$; see Figure 3C, D). The results persisted when the analyses were adjusted for sex and age (see Table S1).

Median RT and On time reactions values obtained in Interval 1 (RTI1), Interval 2 (RTI2), and Interval 3 (RTI3) before and after the intervention are included in the Supplementary Material (see Fig. S1 and Fig. S2).

Changes in Median RT and On time reactions obtained in the three different intervals are showed in Fig. 4. No statistical differences between groups were found in the changes of Median RT neither in Attempt 1 nor in Attempt 2 (all $p>0.050$; see Figure 4A). Similar results were observed in On time reactions in both attempts (all $p>0.050$; see Figure 4B). When comparing intragroup changes in response speed (Median RT) and accuracy (On time reactions), we found that all groups showed greater increments in Median RT and On time reactions in Attempt 1 than in Attempt 2 (improvements calculated from after 10-week Attempt 1 minus baseline values for Attempt 1, and from after 10-week Attempt 2 minus baseline values for Attempt 2; all $p \leq 0.001$; see Figure 4C, D). The results persisted when the analyses were adjusted for sex and age (see Table S1).

RESULTS AND DISCUSSION

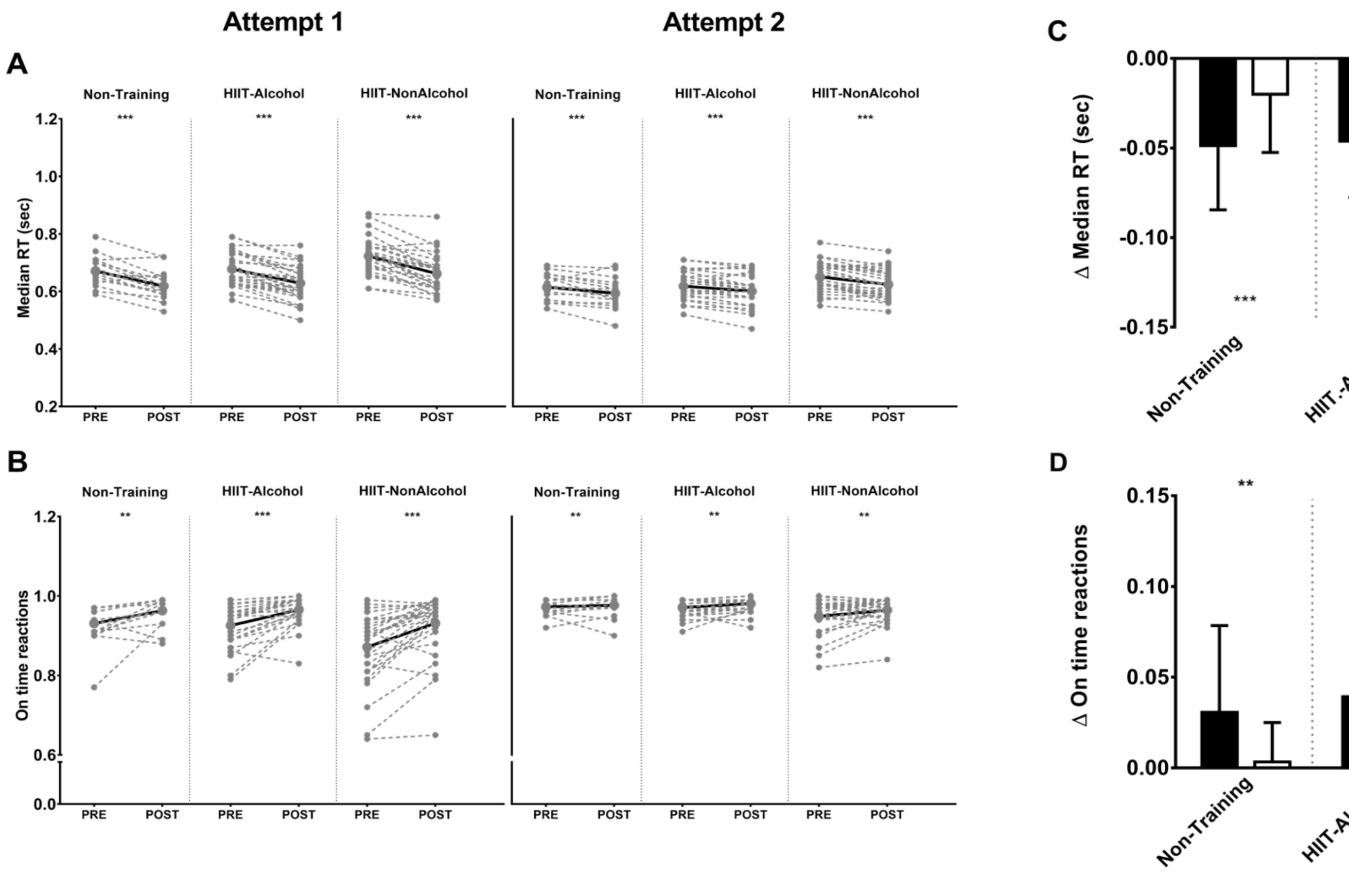


Figure 3. Median reaction time (seconds; A) and On time reactions (B) values in Total RT, before and after the intervention stud Changes in Median RT (seconds) (C), and On time reactions (D) Total RT values, after the intervention study among the five g applying of Mixed-effect model test are indicated as: ** P ≤ .01; *** P ≤ .001. Data are shown as means ± standard deviation. Alcohol, group that performed HIIT and ingested alcohol beer; HIIT-NonAlcohol, group that performed H

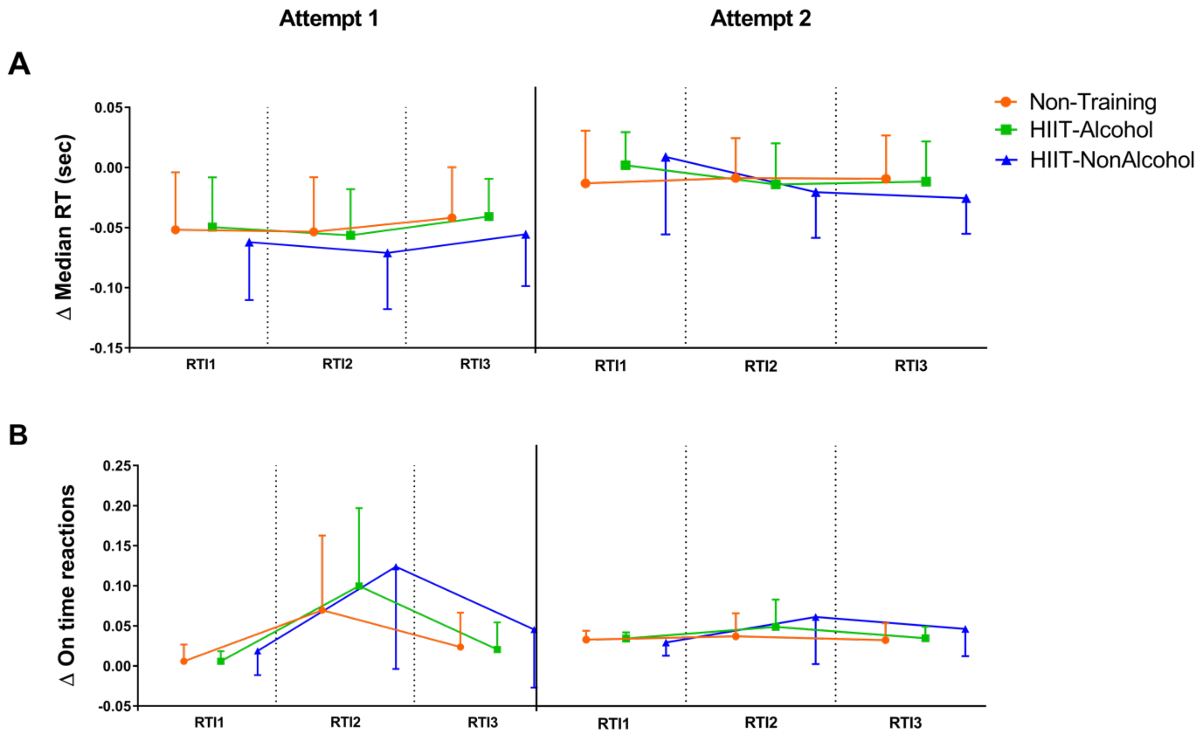


Figure 4. Changes in Median reaction time (seconds) (A), and On time reactions (B) in the Interval values, after the intervention study among the five groups comparing Attempt 1 (left) vs. Attempt 2 (right). Significant differences within groups applying of Mixed-effect model test are indicated as: * P ≤ .05; ** P ≤ .01; *** P ≤ .001. Data are shown as means ± standard deviation. Abbreviations: RTI1, reaction time interval 1 (1078 Msec); RTI2, reaction time interval 2 (834 Msec); RTI3, reaction time interval 3 (948 Msec); T-Beer, group that performed HIIT and ingested alcohol beer; T-0.0Beer, group that performed HIIT and ingested non-alcoholic beer.

Effects of retesting or practice effects

With regard to the practice effect, Median RT was shorter in the successive tests ($F_{3,178}$=178.4, η^2=0.715, p≤0.001). We found statistically significant differences among the 4 attempts (Attempt 1 baseline, Attempt 2 baseline, Attempt 3 after 10-week, and Attempt 4 after 10-week; all p≤0.001; see Fig. 5A), except between Attempt 2 Baseline vs. Attempt 1 After 10-week (p>0.05, see Fig. 5B). Similar improvements were found in On time reactions ($F_{2,148}$=60.6, η^2=0.460, p≤0.001). We also observed statistically significant differences among the 4 attempts in On time reactions (all p≤0.001; see Fig. 5B).

The practice effect in Median RT and On time reactions observed in Interval 1 (RTI1), Interval 2 (RTI2), and Interval 3 (RTI3) are included in Supplementary Material (see Fig. S3 and Fig. S4).

The practice effect in Median RT and On time reactions at the Baseline and After 10-week values in the 3 different intervals stimulus presentation (RTI1, RTI2, and RTI3) are showed in Fig. 6A, B (left for Baseline changes and right for After 10-week changes). No differences between groups were observed in Median RT (all p>0.05; see Fig. 6A) neither at the Baseline nor After 10-week changes. However, differences in On time reactions were found between Non-Training and HIIT-Alcohol groups (p=0.030, 95%CI: 0.001-0.033) and between HIIT-Alcohol and HIIT-NonAlcohol (p=0.018, 95%CI: 0.002-0.033) in the longest interval stimulus presentation (RTI1) in the Baseline changes Differences in On time reactions were also observed between Non-Training and HIIT-NonAlcohol groups (p=0.047, 95%CI: -0.053-0.001) in the Interval 3 (RTI3) in After 10-week changes.

Further, intragroup changes in the practice effect Baseline vs. After 10-week in Total RT are showed in Fig. 6C, D. Larger changes in Median RT Baseline than After 10-week were found in all groups (all p≤0.001; see Fig. 6C). Similar results were found in On time reactions, showing bigger changes in Baseline than After 10-week in all groups (all p≤0.01; see Fig. 6D). Differences in On time reactions were also found between Non-Training group and HIIT-NonAlcohol (p=0.044, 95%CI: 0.001-0.070) and between HIIT-Alcohol and HIIT-NonAlcohol (p=0.034, 95%CI: 0.002-0.062) in Baseline changes.

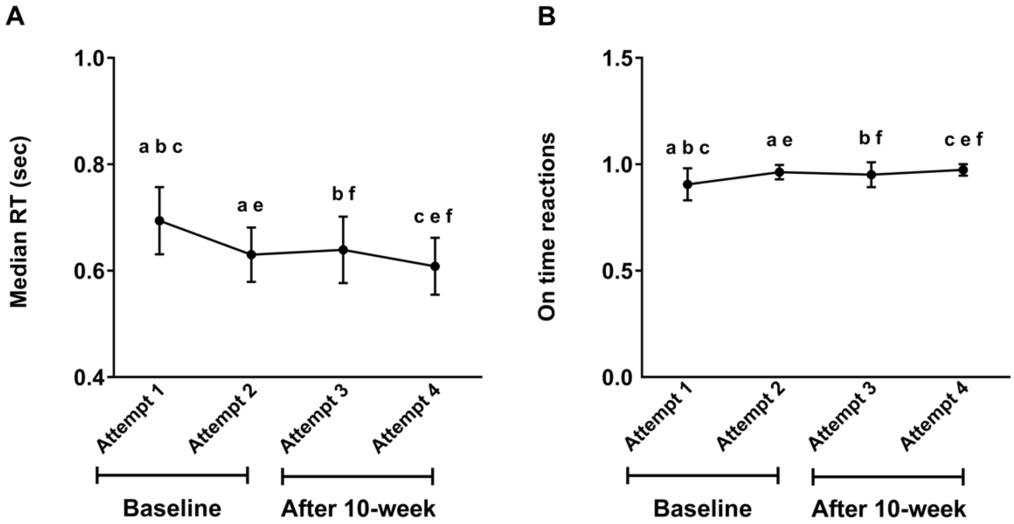


Figure 5. Changes in Median reaction time (seconds) (A) and On time reactions (B) Total RT values, tested at Attempt 1 Baseline, Attempt 2 Baseline, Attempt 1 After 10-week, and Attempt 2 After 10-week. Significant differences between trials applying of Mixed-effect model test are indicated as: Attempt 1 vs. Attempt 2 at baseline (a $P < .05$), Attempt 1 at baseline vs. Attempt 1 at post-intervention (b $P < .05$), Attempt 1 at baseline vs. Attempt 2 at post-intervention (c $P < .05$); Attempt 2 at baseline vs. Attempt 1 at post-intervention (d $P < .05$); Attempt 2 at baseline vs. Attempt 2 at post-intervention (e $P < .05$); Attempt 1 at post-intervention vs. Attempt 2 at post-intervention (f $P < .05$). **Abbreviations:** RT, reaction time total.

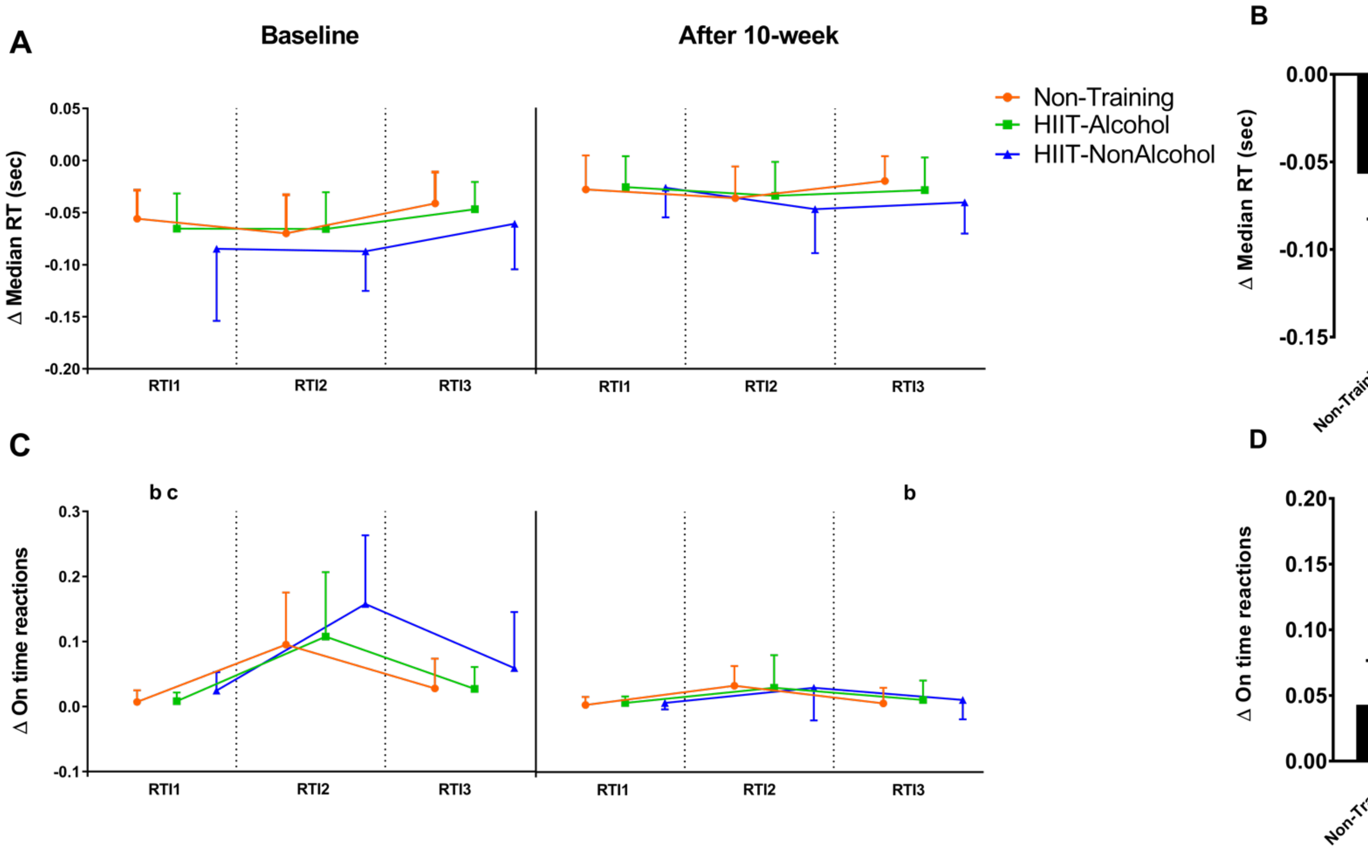


Figure 6. Changes among attempts (re-test effect) in Median reaction time (seconds) (A), and On time reactions (C) Interval values, week (Trial 4 minus Trial 3) intervention study between the three groups. Significant differences between groups applying an a values, with post hoc Bonferroni-corrected test, are indicated as: Non-Training group vs. HIIT-NonAlcohol group (b P < .05); HIIT-Alc .05). Changes in Median reaction time (seconds) (B), and On time reactions (D) Total RT values, Baseline vs. After 10-week the Significant differences within groups applying of Mixed-effect model test are indicated as: * P < .05; ** P ≤ .01; *** P ≤ .0 applying an analysis of covariance adjusting by baseline values, with post hoc Bonferroni-corrected test, are indicated as: Non-Trai .05); HIIT-Alcohol group vs. HIIT-NonAlcohol group (c P < .05). Data are shown as means ± standard deviation. Abbreviations: RTI1 reaction time interval 2 (834 Msec); RTI3, reaction time interval 3 (948 Msec); HIIT-Alcohol, group that performed HIIT and ingeste performed HIIT and ingested

Discussion

The present study provides evidence on the chronic effect of a HIIT program on RT, and the potential concomitant effect of a moderate alcohol consumption. Furthermore, this study examines the test-retest effect in a choice RT task. Our primary hypothesis was that a highly demanding training program may improve the RT performance, although these positive effects could be blunted by habitual alcohol intake. Interestingly, this hypothesis was not fully supported by the present results since all intervention groups decreased RT and increased accuracy, independently of the beverage intake. Our secondary hypothesis was that retesting will produce an improvement in RT task performance, which was supported by our findings since we found improvements in RT and accuracy over the several test administrations.

Longitudinal effects of a HIIT program on RT performance

Previous studies have found enhances in processing speed after exercise training. [17,49] Moreover, exercise-induced facilitation on inhibitory control following HIIT[43] and aerobic exercise at moderate intensity has also been previously found.[50,51] However, our findings indicated a decrease of RT in all groups after 10-week, suggesting enhancements in response speed associated with inhibitory control. Along these lines, these improvements seem to be not attributed to the HIIT intervention since the Non-Training group presented similar enhancements as well. Such inconsistent results could be explained by the specific characteristics of our sample: (i) intervention studies in young and middle-aged adults are rare and have reported rather contradictory results; (ii) our Non-Training group included active and young adults. Thus, we might speculate that there is less capacity for improvement in younger and high-functioning adults ("ceiling effect") than in other populations, such as children or older people. Similarly, being active in the youth could be enough to generate positive effects on cognitive functioning at this stage, although this activity would not be framed into a training program. Indeed, a certain learning effect cannot be totally discarded in the psychomotor test used, [52] as we presented below. Therefore, future research is needed to identify the specific role of physical exercise on cognition in young and middle adults. Similarly, it is important to highlight that the studies previously cited including young and middle adults as participants used training interventions relatively short (i.e., <12 weeks) than those studies conducted in both children and older adults.[13,19,20,53,54] Thus, it is plausible that longer and more intense trainings would result in similar improvements than those obtained in younger and older adults.

Prolonged periods of abusing alcohol are undoubtedly harmful to the brain impairing inhibitory control or divided attention among other cognitive functions. [43,55,56] Furthermore, alcohol in acute doses causes general and progressive impairment, disorganisation, retardation, and depression of central nervous system functioning.[27,57] In this regard, previous studies observed that high-alcohol doses have negative effects on RT.[56,58] However, Poli et al. (2013) and Gaetano et al. (2016) described that moderate beer consumption in healthy individuals had not harmful consequences for major chronic conditions, even providing some benefits for cardiovascular disease.[24,43] Therefore, these cognitive impairments seem to be dependent of the alcohol dose ingested.[27] Our results concurred with these findings since we did not find significant impairments on RT in any intervention group, including alcohol consumers that performed a HIIT program who also improved the speed and accuracy of RT. Our findings support the notion that physical exercise training at high intensity not only exerts a positive effect on executive functions such as RT,[18,59] but also may attenuate the potential impairments of a moderate dose of alcohol consumption during this type of intervention.

Furthermore, a significant effect of stimulus presentation times was observed, all groups showing a greater improvement in RT speed and accuracy when working under pressure (i.e. Interval 2, 834 ms). These results suggest that effortful or attentionally demanding tasks may be more sensitive to undergo the beneficial effects of exercise compared to other tasks that require minimal effort.[50] In summary, the direction of the trends found in the present study suggest that task characteristics including task difficulty and the amount of stress within a task, should be manipulated in future studies to deeply understand this point.[60]

Effects of retesting or practice effects

Retesting processing speed has been shown to improve RT over several studies with different test-retest intervals and research settings.[4] There is also scientific evidence on the declining of the automatization with the length of the test-retest interval. However, RT reductions are less pronounced in more complex tasks, this being more evidenced in healthy samples.[8] Our results concurred with the above-mentioned rationale since RT decreased with the number of test administrations in our sample of healthy young adults. Indeed, accordingly to previous findings the length of the test-retest interval has a significant influence,[8] since a loss of the practice effect after a long temporal difference (i.e. Attempt 2 baseline vs. Attempt 1 after 10-week) was detected. The significant linear effect of presentation interval showed that subjects react faster when the pressure becomes higher (i.e. Interval 2, 834 ms). These results agreed with those obtained by Nederhof et al. [60], who found that well-training and young adults react faster under pressure. Indeed, no significant between-group changes were found on RT when examining the practice effect. However, a significant difference was observed between the HIIT-NonAlcohol and the Non-Training groups on the accuracy respond when examining the practice effect after the intervention. These results suggest that 10-week of HIIT could have facilitated the learning process due to retesting in the HIIT-NonAlcohol group. However, HIIT-Alcohol group showed similar practice effects to the Non-Training group which could bring to light a possible attenuation of the exercise training because of the alcohol consumption.

Moreover, we found that the practice effect was greater at baseline (Attempt 1 vs. Attempt 2) than after 10-week (Attempt 1 vs. Attempt 2) assessments, suggesting a loss of retest effects over the several test administrations, which could be interpreted as a "ceiling or plateau effect". In summary, our findings support the "power law of practice" which assumes that retest effects decrease with the number of test administrations.[4,61] These findings are in accordance with those found in a previous meta-analysis conducted by Scharfen et al.,[8] who reported that a growth of test scores reaching a plateau after a few tests. Therefore, future research is required to determine the number of administrations to reach a plateau and the length that this practice effect is retained.

Limitations

The present study had a number of limitations: (i) the control group was not purely sedentary, they were asked to report the physical exercise they performed, but it was not controlled by any objective test (i.e. accelerometer system), (ii) our study only included healthy adults, and hence we cannot extend the results to older individuals or patients, (iii) participants were not purely randomized since they chose whether they preferred to be included in an alcohol or in an alcohol-free group; consequently our results cannot be extrapolated to the abstemious population. Further studies are needed to clarify the long-term effects (>10 weeks of HIIT interventions on RT outcomes). Subsequently, future studies are required to determine the effects of the same training intervention in other samples to identify effective public health strategies that promote a healthy lifestyle and to deeply understand the concomitant effect of fermented beverages in moderate amounts co-ingested with meals.

Conclusions

The present study suggests that exercise training can have a protector effect on cognitive function. However, we cannot conclude that a HIIT intervention program elicits cognitive improvements in healthy young adults. In addition, it shows that a habitual but moderate alcohol intake does not have negative effects on the improvements of a training program. Moreover, this study indicates that retesting produced an improvement in RT task execution (speed and accuracy) showing a loss of this practice effect when the temporal difference was greater. Additionally, we found that subjects have faster responses when the pressure becomes higher.

Due to the fact that limited studies have evaluated the impact of engaging in HIIT on cognition in young adults, our study provides some interesting answers to the current paradigm. This research is closely related to the society at large since the consumption of alcohol beverages as part of the social interaction is a commonly practice for physically active people.

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Authors' contributions

CMH participated in the design of the study, contributed to data collection, data reduction/analysis, and interpretation of results, and contributed to the manuscript writing – original draft; FAG participated in the design of the study, contributed to the interpretation of results and participated in the manuscript writing – original draft; ACB participated in the manuscript writing – review and editing; MC participated in the design of the study and participated in the manuscript writing – review and editing; AC participated in to the manuscript writing – review and editing.

Competing interest

The authors declare that they have no competing interests.

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Chapter 6

Interaction of high intensity interval training and daily alcohol intake: effects on cognitive function in young healthy adults. The BEER-HIIT study (Study 4)

Abstract

Purpose. The main purpose of this investigation was to determine the effects of a High Intensity Interval Training (HIIT) intervention parallel to a moderate alcohol consumption on cognitive performance in healthy young adults.

Methods. We conducted a 10-week HIIT program taking four type of beverages with/without alcohol content. A total of 75 healthy adults (18-40 years; 46% females) were randomly assigned to either a control Non-Training group or a HIIT program group (2 days/week). Using block randomization, participants in the HIIT group were further allocated to a HIIT-Alcohol group (alcohol beer or sparkling water with vodka added, 5.4%) or a HIIT-NonAlcohol group (sparkling water or non-alcohol beer, 0.0%). The control group were asked to maintain an active lifestyle, but did not complete any regular training. A comprehensive battery of cognitive tests was used to evaluate memory, working memory, processing speed, inhibitory control, and verbal fluency. Changes from baseline to week 10 were included in the main analyses.

Results. All groups improved learning process, memory, processing speed, inhibitory control, and verbal fluency (all $p \leq 0.001$), independently of sex and alcohol consumption, with no statistical differences between groups (all $p > 0.050$). Further, positive associations were found between changes in maximal oxygen uptake and changes in processing speed, inhibitory control, and verbal fluency (all $p < 0.050$).

Conclusions. Although the improvements found in cognition cannot be attributed to the HIIT intervention, no significant impairments in cognitive functions were noted due to alcohol intake in moderate amounts.

Introduction

Exercise training is currently considered a powerful tool to improve cognitive function in both children and older adults.[1–3] In fact, it has been reported that cognitive function in the elderly is improved when a moderate intensity exercise intervention is performed for 6 months or longer.[4] Similarly, it has been found notorious enhancements of visual-spatial and short-term memory after 6 to 12 weeks of programmed exercise in young adults.[5,6] Importantly, a recent study has suggested that repeated high-intensity interval exercise (i.e., high-intensity interval training [HIIT]) could provide additional physiological and psychological adaptations in higher-order cognitive functions and flexibility cognitive performance compared with lower intensity exercise in older adults.[7] Similar improvements in cognitive flexibility have been found after 7 week of an interval training intervention in young active individuals.[8] Complementary research indicates that an acute bout of interval exercise training can also positively impact on cognitive function in healthy middle-aged individuals.[9] Nevertheless, the effect of exercise training on cognition in young and middle adults has received considerably less attention and there is still controversy regarding the optimal exercise training intensity to improve cognitive performance at this stage.[10] One obvious reason for this absence of scientific literature is that cognitive health peaks during young adulthood and therefore, there seems to be no space for exercise-related improvement on cognitive function during this period of the lifespan.[2]

Lifestyle habits, such as alcohol consumption, have been considered to play an important role in the development of dementia and others neurodegenerative diseases.[11] Alcohol intake, specially beer, is a common practice for many physically active people.[12,13] Besides the deleterious effects of excessive consumption of alcohol on general health,[11] uncertain results have been reported regarding the effects of low to moderate alcohol consumption on cognitive health.[14] While Mehlig et al. suggest that low to moderate alcohol consumption increase the risk of suffering cognitive impairments and dementia[15], others studies have found no or minimal effects associated with alcohol consumption,[16,17]. In addition, it has been also reported significant improvements on cognitive function associated with low to moderate alcohol intake.[18,19]. Due to the high heterogeneity of the above-mentioned results, investigating whether a moderate alcohol consumption could influence the potential positive effects of exercise training on cognitive function is of scientific and clinical interest. To the best of our knowledge, there is no study exploring the combination of a HIIT intervention and moderate alcohol consumption in healthy individuals, which is widely common in a social context for active people.

The primary purpose of the present study was to test the hypothesis that a highly demanding training intervention would improve cognitive function (i.e., memory, working memory, processing speed, inhibitory control, and verbal fluency) in healthy young adults. The secondary purpose of the study was to assess whether those potential positive effects could be influenced by moderate alcohol consumption. Finally, the third purpose of our study was to test whether changes on physical fitness (as measured through maximal oxygen uptake (VO_{2max}), handgrip strength, and body composition) after 10-week HIIT intervention would be associated with changes on cognitive function.

Methods

Trial Design

The BEER-HIIT study is a registered controlled trial (ClinicalTrials.gov ID: NCT03660579) with a follow-up of 10 weeks. A detailed study protocol description has been published elsewhere .[20] The procedures were designed accordingly with last revised Declaration of Helsinki and approved by the Ethics Committee on Human Research of the University of Granada (321/CEIH/2017).

Participants

Potential participants of the BEER-HIIT project were healthy young adults who lived in the province of Granada, Spain. The study was announced via social networks, local media, and posters. Prior to the enrolment, all individuals completed a medical examination, provided a written informed consent, and were fully informed about the study objectives, design, inclusion criteria, assessments to be undertaken, exercise program intervention, and types of beverages to be ingested. Subjects who met the inclusion criteria (i.e. (i) having a body mass index (BMI) from 18.5 to 30 kg/m^2, (ii) not being engaged in a previous structured training program or a weight-loss program (last 5 months), (iii) having a stable body weight during the last 5 months (body weight changes < 3 kg), (iv) being free of disease, (v) not being pregnant or lactating, (vi) not taking any medication for chronic diseases, and (vii) not suffering pain, recent injuries, or other problems preventing strenuous physical activity) were invited to an information meeting in which the research staff gave specific information about healthy dietary patters and the physical activity recommendations provided by the World Health Organization.[21]

Randomisation and follow-up

After the baseline measurements, a total of 83 individuals were allocated to a training (i.e., HIIT) or a Non-Training group based on personal preferences. Participants in the Non-Training group were instructed to maintain their usual physical activity levels and not to be engaged in a structured exercise program. Those participants included into the training group were subsequently allocated in an ethanol-containing beverage (i.e. 5.4% alcohol) group or an alcohol-free beverage group. The participants willing to consume ethanol were randomly allocated to either a group consuming alcohol beer (HIIT-Beer) or to a group consuming sparkling water with added vodka ethanol (HIIT-Ethanol). The participants choosing non-alcoholic beverages were randomly allocated to either an alcohol-free beer group (HIIT-0.0Beer) or to a sparkling-water group (HIIT-Water). This type of non-random (i.e., based on individual preference) and random allocation of the participants was conducted following ethical considerations and advices provided by the ethical committee (321-CEIH-2017), since drinking alcohol or participating in a highly demanding training program should be a personal choice.

Intervention

The HIIT intervention consisted on 2 sessions/week performed from Monday to Friday during 10 consecutive weeks with at least 48 h of recovery between sessions. The training intervention was divided into two different phases, starting with a familiarization phase to learn the main movement patterns aiming to avoid injuries or potential drop outs. The volume and intensity of the sessions in the familiarization phase was fixed at 40 min/week and 8-9 Rating of Perceived Exertion (0-10 RPE), respectively.[22,23] Subsequent increments of both volume and intensity were established in Phase I (50 min/week and 10 RPE) and in Phase II (65 min/week and 10 RPE). Eight self-loading exercises were performed in a circuit form twice per set (i.e., frontal plank, high knees up, TRX horizontal row, squat, deadlift, side plank, push up, and burpees) with a passive rest between exercises and an active rest between sets (i.e., 6 RPE intensity, which corresponds with 60% VO_2max).[23,24] A dynamic standardized warm up and an active global-stretching cooling-down protocols were completed at the beginning and at the end of each training session, respectively. A detailed description of each exercise of the training program can be found elsewhere.[20]

During the intervention, the alcohol consumption allowed were 330 ml of the respective beverage at lunch and 330 ml at dinner for men, and 330 ml at dinner for women, from Monday to Friday: (i) HIIT-Alcohol group ingested randomly assigned alcohol beer (5.4% alcohol-Alhambra Especial®, Granada, Spain) or sparkling water with exactly the equivalent amount of distilled alcohol added (Vodka, 37.5% ethanol and 62.5% water), and (ii) HIIT-NonAlcohol group randomly assigned ingested alcohol-free beer (0.0% alcohol-Cruzcampo®, Sevilla, Spain) or sparkling water (Eliqua 2®, Font Salem, Spain). The amount of alcohol selected to ingest was based on scientific evidence (i.e. 2-3 drinks/day or 24–36 g of ethanol/day for men and 1-2 drinks/day or 12–24 g of ethanol/day for women).[25,26] During weekends, participants were requested to respect the beverage intake condition (i.e., moderate alcohol consumption and non-alcohol consumption). All beverages were coded and provided by a blinded staff member of our research laboratory at the beginning of each week. Additionally, the alcohol intakes were registered to control its consumption before and during the intervention.

Cognitive function

Cognitive variables were taken at baseline and after 10-week of the supervised HIIT program. We used the Spanish-Complutense Verbal Learning Test (TAVEC) to evaluate the episodic verbal memory, as well as its codification processes, data storage, and retrieval.[27] The main outcomes of this test were: (i) Learning process (defined as the sum of correctly recalled words across all five presentations), (ii) Short-term memory (free recall of list A after an interference list previously presented), (iii) Delay memory (free recall of list A after 20-min of rest period), and (iv) Recognition (defined as total correct score (tc) and discriminability index calculated as the difference between correctly detected old words minus false alarms to new words (id). Working memory was assessed using the Letter-Number sequencing test according to the instructions of the WAIS-IV manual,[28] where the span score ranges from 0-7 and total scores from 0-21. We measured processing speed and inhibitory control using the one-page paper-and-pencil cancellation test version of D2 test.[29] In addition, we used a standard verbal fluency test to measure the phonetic, semantic, and total verbal fluency.[30]

Age, sex, and occupational activity were also registered by a self-report demographic questionnaire before the intervention.

Statistical analyses

Considering the results of a pilot study, the sample size calculations revealed that 13 participants per group were needed to detect an effect size of 0.25 in TAVEC immediate memory scores with an α error of 0.05, and a power of 0.85.[31] However, we recruited a minimum of 16 participants per group (a total of 80) considering a maximum loss of 20% at the follow up.[32]

Standard statistical methods were used for the calculation of means and standard deviations. Normal Gaussian distribution of the data was verified by the Shapiro–Wilk test, visual check of histograms, Q-Q, and box plots. Homoscedasticity was verified by the modified Levene Test. The compound symmetry, or sphericity, was checked by the Mauchly test. When the assumption of sphericity was not met, the significance of F-ratios was adjusted according to the Greenhouse–Geisser procedure when the epsilon correction factor was < 0.75, or according to the Huyn–Feld procedure when the epsilon correction factor was > 0.75.

Composite scores for memory (immediate, short-term and delayed recall, and recognition scores; α = .900), working memory (direct and processing scores; α = .922), processing speed (total productivity, correct work, and concentration index scores; α = .986), inhibitory control (total effectiveness score), and verbal fluency (total score) were calculated by averaging z scores for their individual components. A recategorization of the occupational activity measure was performed into three levels showing a relatively high internal consistency (α = .705). We combined the responses of computed and classified occupational activities and categorized them as level 1 (unemployment, homemaker, and student), level 2 (primary sector services, retail or catering services, and administrative clerk), and level 3 (support and scientific/intellectual technicians and professionals, and business administration and management).

Age, sex, and occupational activity were used as potential confounders in the analyses. Given that we did not observe a sex interaction, we conducted the analysis including men and women together. Similarly, since no beverage interaction was observed between groups in any outcome, we conducted the analysis including both alcohol beer and sparkling water with ethanol HIIT groups in the same group (i.e. HIIT-Alcohol group) and both 0.0% alcohol beer and sparkling water HIIT groups in the same group (i.e. HIIT-NonAlcohol group).

A repeated-measured analysis of variance (2 x 2 ANOVA; time x training group; and time x beverage) was conducted to test differences between groups. Post-hoc Bonferroni corrections were conducted for multiple comparisons. The significance level was set at $p < 0.05$ for all analyses. Sensitivity analyses were performed to examine whether the lowest baseline levels on cognitive functions were the reason to be more benefited from the exercise intervention. We subsequently compared averages performance on the three composite scores for memory, working memory, and processing speed, and for the z-scores of verbal fluency and inhibitory control taken before and after the intervention (baseline, after 10-week) x 4 (quartile: low, middle-low, middle-high, high).

Finally, simple and multiple linear regression analyses were performed to examine the association between changes in physical fitness (i.e. maximum oxygen uptake, hand grip strength, and body composition, such as BMI, FMI, and LMI) with changes in cognitive outcomes (i.e. memory, working memory, processing speed, inhibitory control, and verbal fluency) after the intervention. In addition, multiple linear regression analyses models were built to investigate the above-mentioned associations after controlling for potential confounders (i.e., age, sex, and occupational activity). Potential confounders were selected based on statistical procedures (i.e., hierarchical regressions) and theoretical bases.

All analyses were conducted using the Statistical Package for Social Sciences (SPSS, v. 25.0, IBM SPSS Statistics, IBM Corporation). Graphical presentations were prepared using GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA).

Results

A total of 74 participants (34 women) were included in the final analyses after a loss to follow up of 10% (see Fig. 1). The characteristics of the participants are presented in Table 1. There were no significant differences between groups at the baseline values (all $p > 0.05$), excepting for age and occupational activity ($p = 0.006$ and $p = 0.005$, respectively; see Table 1).

Processing Speed

Table 1. Baseline characteristics of participants.

	Non-Training (n = 17)			HIIT-Alcohol (n = 27)		
	Mean ± SD					
Sex (men% / women%)	64.7%	/	35.3%	48.1%	/	51.8%
Age	20.2	±	1.3	24.2	±	1.5
Alcohol Ingested (ml/week)	1144.1	±	831.9	696.7	±	857.4
Body mass index (kg/m²)						
Educational level (%)						
Primary Education	--			3.7%		
Secondary Education	47.1%			40.7%		
Vocational Education and Training	23.5%			18.5%		
University Degree or Certificate of Higher Education	29.5%			37.3%		
Occupational activity (%)						
Level 1	100%†			55.6%		
Level 2	--			18.5%		
Level 3	--			25.9%		
Learning Process						
Trial 1	8.8	±	2.3	8.3	±	2.0
Trial 2	11.2	±	2.7	12.2	±	2.1
Trial 3	12.9	±	2.3	13.1	±	2.2
Trial 4	14.4	±	1.3	13.4	±	2.1
Trail 5	14.5	±	1.3	13.8	±	1.7
Memory						
Immediate memory	61.8	±	7.4	60.9	±	7.7.
Short-term memory	13.6	±	1.2	13.9	±	1.8
Delay memory	13.9	±	1.4	14.2	±	1.8
Recognition (tc)	15.3	±	0.9	15.6	±	0.6
Recognition (id)	97.7	±	2.7	98.2	±	1.9
Working Memory						
Direct Score	13.0	±	1.6	11.8	±	2.1
Processing Score	5.8	±	0.8	5.6	±	0.9

RESULTS AND DISCUSSION

Total productivity	516.6	±	88.0	514.5	±	76.5	5
Correct work	208.7	±	43.4	203.9	±	39.0	2
Concentration Index	208.4	±	43.5	203.4	±	39.4	2
Inhibitory Control							
Total Effectiveness	502.9	±	86.7	498.3	±	77.5	4
Verbal Fluency							
Phonologic	86.1	±	23.2	88.4	±	20.5	7
Semantic	40.5	±	8.0	40.1	±	6.5	3
Total Score	126.6	±	28.0	128.4	±	23.8	1

Data expressed as mean ± standard deviation. Differences in baseline characteristics between the different groups were c
(ANOVA). † Occupational activity: 53% unemployment, 47% student. *Boldface values indicate significance differences (P <
group that performed high-intensity interval training and consumed alcoholic beverage; HIIT-NonAlcohol, group that perfo
and consumed non-alcoholic beverage; Level 1, unemployment, homemaker, student; Level 2, agriculture, livestock and
service, administrative clerk; Level 3, support technicians and professionals, scientific and intellectual technicians and profes
management.

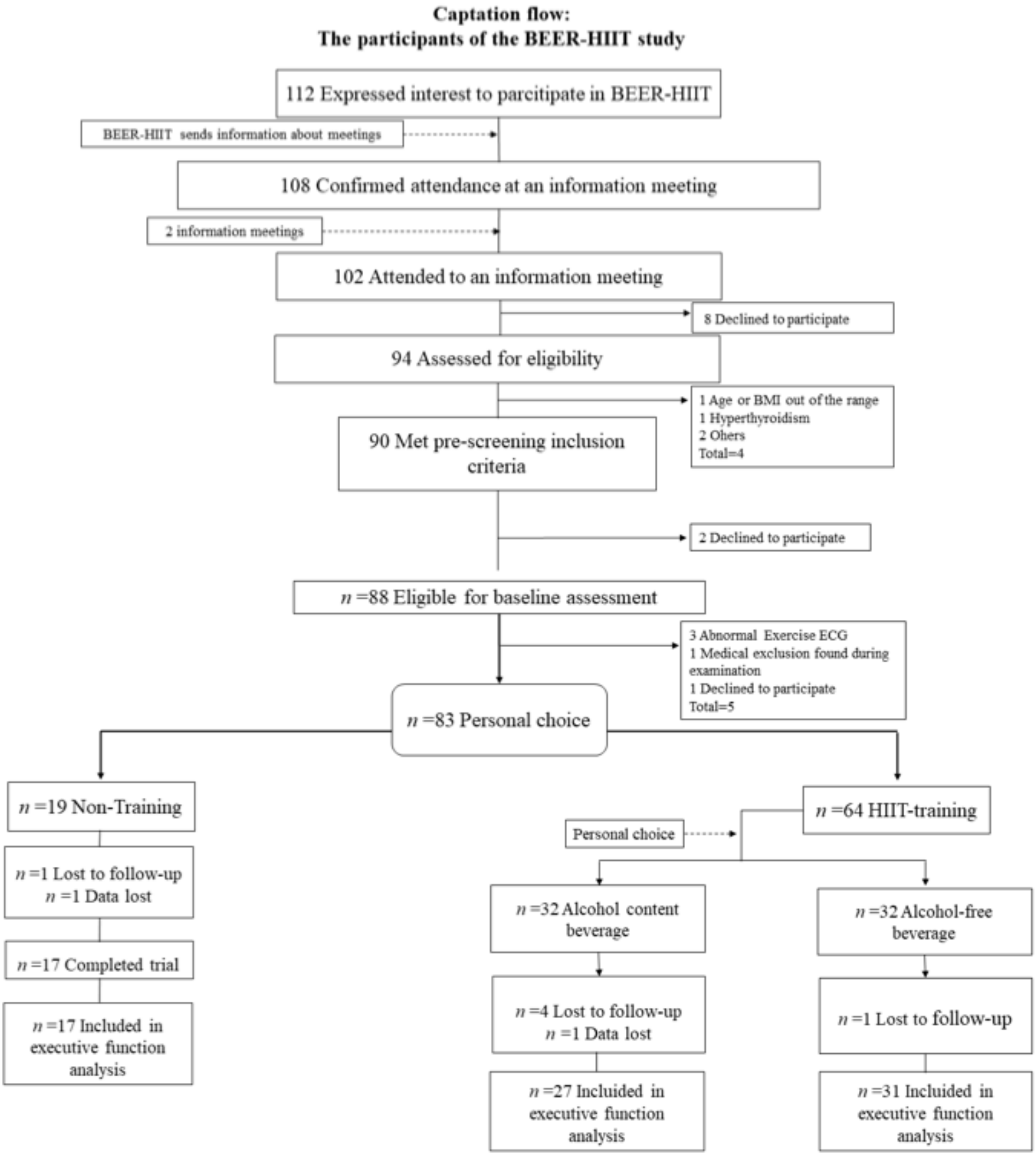

Figure 1. Flow of participants in the BEER-HIIT study.
Abbreviations: HIIT, high-intensity interval training; BMI, body mass index; ECG, electrocardiogram.

Intervention effects of a HIIT program on cognitive function

Fig. 2 shows changes in learning process (number of the recalled words of first and firth trials; to see the number of the recalled words across all five trials; see Fig. S1) tested at baseline and post-intervention. A significant increment was observed in the number of recalled words across trials at baseline and post-intervention measures independently of the intervention group (all $p \leq 0.001$; see Fig. 2). Significant differences in the learning process were also noted between baseline and post-intervention measures in Non-Training group, HIIT-Alcohol group, and HIIT-NonAlcohol group (all $p \leq 0.001$; see Fig. 2). Further, statistically significant differences between trial 1 and trial 2 (all $p < 0.05$; see Fig. S1) in Non-Training group, HIIT-Alcohol group, and HIIT-NonAlcohol group at baseline and post-intervention measures.

Table 2 presents changes in the raw scores and z-transformed cognitive outcomes before and after the intervention. The z scores are interpreted as the change from baseline in standard deviations. The Non-Training, HIIT-Alcohol, and HIIT-NonAlcohol groups showed significant improvements in memory (i.e. immediate, short-term, and delayed recall scores) and recognition (all $p \leq 0.001$; see Table 2) after the intervention. Similarly, all groups showed significant enhancements in processing speed, inhibitory control, and verbal fluency (all $p \leq 0.01$; see Table 2). There were no intragroup differences in the memory, working memory, and processing speed composite z scores in any of the groups (all $p > 0.05$; see Table 2), neither in the inhibitory control and verbal fluency z scores (all $p > 0.05$; see Table 2).

The analysis of covariance of raw scores and z-transformed cognitive outcomes, adjusting for baseline values (Model 0), is shown in Table 2. No significant between-group differences were observed in any cognitive function variables (all $p > 0.05$). These results persisted after controlling by potential confounders (i.e., age, sex, and occupational activity; see Table S2).

Repeated-measures ANOVA with the second factor between subjects showed a significant time x quartile interaction for memory ($F_{(2, 72)} = 23.74$, $p < .001$, $\eta^2 = .40$) and for working memory ($F_{(2, 72)} = 10.53$, $p < .001$, $\eta^2 = .23$). No significant interaction was found in any cognitive variable when we investigate the time x group x quartile, although we found a lightly tendency for memory ($p = .056$). No interaction effects of the level at baseline were found for processing speed, verbal fluency, or inhibitory control.

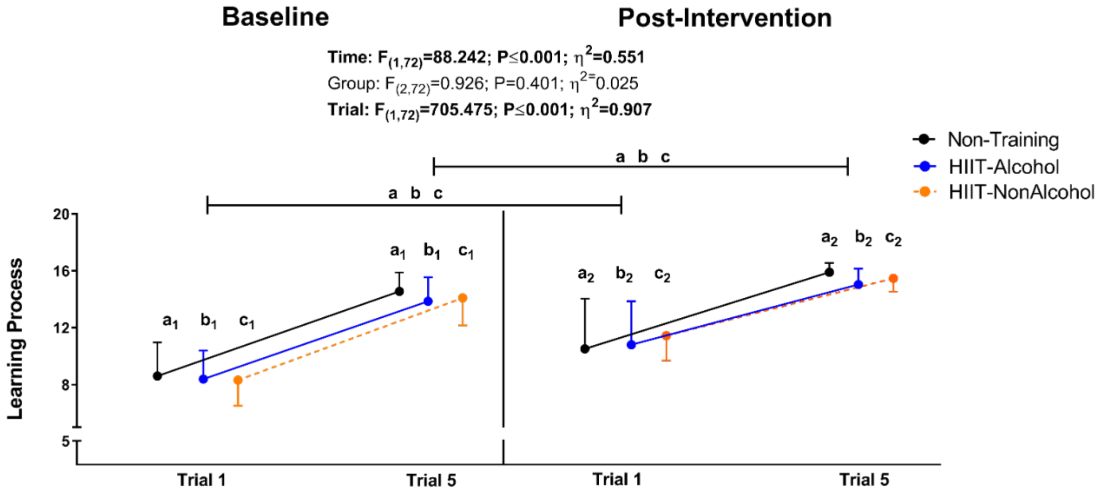

Figure 2. Changes in learning process (Trial 1 and 5) measured by Spanish-Complutense Verbal Learning Test (TAVEC), tested at Baseline and Post-Intervention study. F, p, and η of repeated-measures analysis of variance (ANOVA) for time, group, and trial. Intragroup changes obtained by repeated-measures ANOVA (group x trial x time, adjusting by trial), with a post hoc Bonferroni-corrected test, are indicated as: Trial 1 vs. Trial 5 (a_1 $p < 0.05$) for Non-Training group; Trial 1 vs. Trial 5 (b_1 $p < 0.05$) for HIIT-Alcohol group; Trial 1 vs. Trial 5 (c_1 $p < 0.05$) for HIIT-NonAlcohol group. Intragroup trials' changes before and post-intervention obtained by repeated-measures ANOVA (group x trial x time, adjusting by time), with a post hoc Bonferroni-corrected test, are indicated as: a $p < 0.05$ for Non-Training group, b $p < 0.05$ for HIIT-Alcohol group, and c $p < 0.05$ for HIIT-NonAlcohol group. Raw data are presented as mean and standard deviation. Abbreviations: HIIT-Alcohol, group that performed high-intensity interval training and consumed alcoholic beverage; HIIT-NonAlcohol, group that performed high-intensity interval training and consumed non-alcoholic beverage.

RESULTS AND DISCUSSION

Table 2. Changes before and after the intervention study and changes over time in Cognitive Scores

	Non-Training			HIIT-Alcohol			HIIT-NonA	
	Baseline	Post-Intervention		Baseline	Post-Intervention		Baseline	Post-In
	Mean ± SD							
Memory			P value			P value		
Immediate Memory	61.82 ± 7.38	70.06 ± 5.85	≤ 0.001	60.93 ± 7.68	70.15 ± 5.56	≤ 0.001	59.55 ± 9.26	67.94
Short-Term Memory	13.59 ± 1.23	15.29 ± 0.85	≤ 0.001	13.85 ± 1.81	14.89 ± 1.58	0.004	12.87 ± 2.47	14.77
Delay Memory	13.88 ± 1.41	15.47 ± 0.62	≤ 0.001	14.15 ± 1.83	14.93 ± 1.62	0.0014	13.29 ± 2.18	15.00
Recognition (tc)	15.29 ± 0.92	16.00 ± 0.00	0.005	15.56 ± 0.64	15.89 ± 0.32	0.105	14.97 ± 1.38	15.68
Recognition (id)	97.73 ± 2.67	99.60 ± 0.89	0.014	98.23 ± 1.93	98.82 ± 1.93	0.321	96.92 ± 4.09	99.12
Composite score	0.082 ± 0.204	0.277 ± 0.174	0.315	0.195 ± 0.162	-0.002 ± 0.162	0.201	-0.219 ± 0.151	-0.125
Working Memory								
Direct Score	13.00 ± 1.62	13.06 ± 3.47	0.928	11.78 ± 2.08	11.96 ± 3.11	0.722	11.74 ± 2.49	11.77
Processing Score	5.82 ± 0.81	5.88 ± 1.27	0.845	5.63 ± 0.88	5.41 ± 1.12	0.354	5.52 ± 0.89	5.32 ±
Composite score	0.349 ± 0.820	0.307 ± 1.020	0.852	-0.042 ± 0.186	-0.050 ± 0.183	0.966	-0.117 ± 0.173	-0.113
Processing Speed								
Total productivity	516.59 ± 87.93	576.59 ± 72.84	≤ 0.001	514.52 ± 76.49	566.07 ± 67.83	≤ 0.001	510.87 ± 67.316	571.8
Correct work	208.71 ± 43.44	244.82 ± 39.72	≤ 0.001	203.85 ± 39.04	235.15 ± 36.43	≤ 0.001	200.68 ± 36.54	237.2
Concentration Index	208.41 ± 43.46	242.94 ± 41.62	≤ 0.001	203.44 ± 39.37	234.85 ± 36.50	≤ 0.001	200.00 ± 36.58	236.9
Composite score	0.102 ± 0.245	0.136 ± 0.240	0.783	0.007 ± 0.194	-0.075 ± 0.180	0.399	-0.067 ± 0.181	-0.007
Inhibitory Control								
Total Effectiveness	502.82 ± 86.61	565.18 ± 74.533	≤ 0.001	498.26 ± 77.45	552.22 ± 66.00	≤ 0.001	492.61 ± 68.01	554.8
Verbal Fluency								
Phonologic	86.12 ± 23.15	95.71 ± 24.51	0.003	88.37 ± 20.55	96.89 ± 16.70	≤ 0.001	77.10 ± 14.93	84.42
Semantic	40.47 ± 8.02	39.88 ± 10.78	0.705	40.11 ± 6.47	42.37 ± 6.03	0.070	37.48 ± 8.55	37.48
Total Verbal Fluency	126.59 ± 27.97	135.59 ± 33.55	0.016	128.41 ± 23.76	139.26 ± 21.09	≤ 0.001	114.58 ± 19.25	121.9

P value of intragroup changes before and post-intervention obtained by repeated-measures ANOVA.
F, p value, and η^2 of analysis of covariance adjusting by baseline values.
*Boldface values indicate significance differences ($P < 0.05$). Abbreviations: HIIT-Alcohol, group that performed high-intensity interval training and consumed
performed high-intensity interval training and consumed non-alcoholic beverage; tc, total number of correct responses in the recognition test; id, index of discrimi

Relationships of changes in physical fitness and body composition with cha cognitive function after the intervention

A positive relationship was found between changes over the 10-week HIIT intervention in p speed (composite score) and changes in VO_2max (ml/kg/min) (β = 0.273, R^2 = 0.0 0.020; see Fig. 3A), which persisted after adjusting for sex, age, and occupational activity 0.012; see Fig. 3A). Changes in inhibitory control were positively related to changes in (ml/kg/min) (β = 0.249, R^2 = 0.049, p = 0.034; see Fig. 3B), which remained after adjusting age, and occupational activity (all $p \leq 0.040$; see Fig. 3B). In addition, a positive associa observed between changes in verbal fluency and changes in VO_2max (ml/kg/min) (β = 0.3 0.115, p = 0.002; see Fig. 3C), which persisted after controlling for sex, age, and occu activity (all $p \leq 0.004$; see Fig. 3C). Finally, changes in verbal fluency were positively r changes in VO_2max (ml/min) (β = 0.313, R^2 = 0.085, p = 0.007; see Table S2), which after adjusting for sex, age, and occupational activity (all $p \leq 0.013$; see Table S2).

A significant negative association was detected between changes in working memory (c score) and changes in body mass index (BMI) (β = -0.282, R^2 = 0.066, p = 0.016; see which persisted after adjusting for sex, age, and occupational activity (all $p \leq 0.019$; see Fig

No significant association was seen between changes in cognitive outcomes and changes in h strength, fat mass index, or lean mass index (see Table S2 and Table S3).

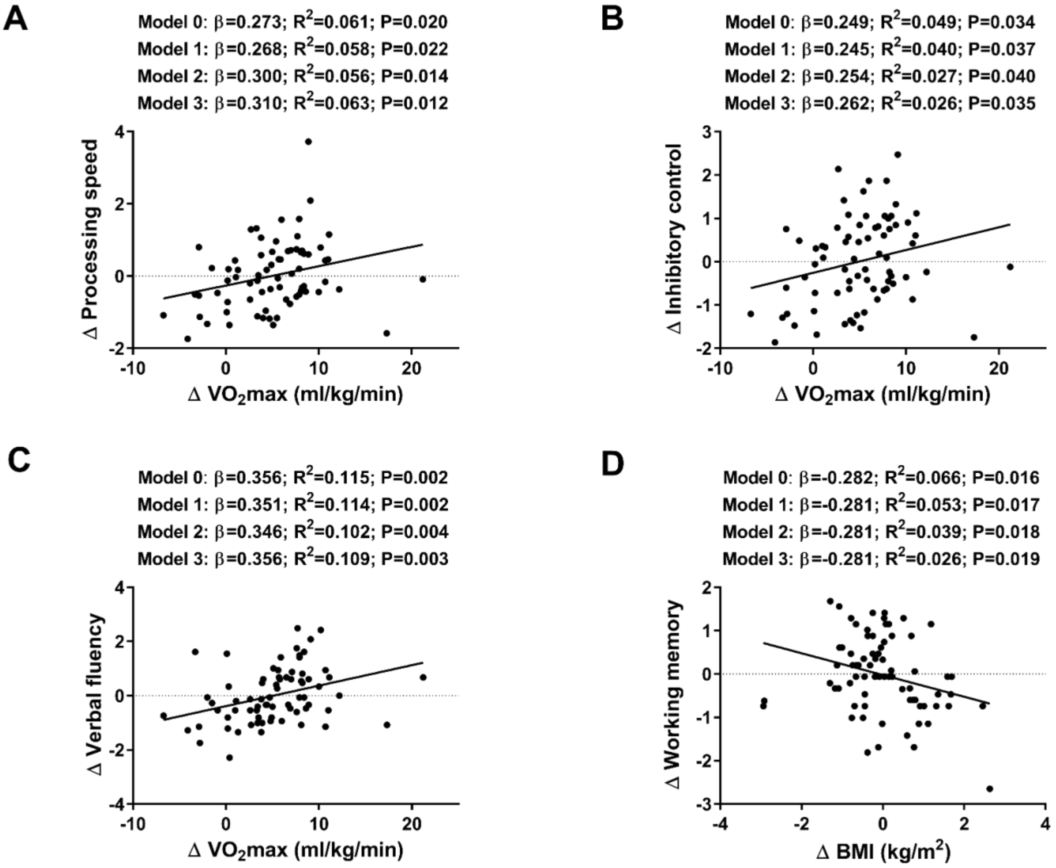

Figure 3. Association between maximal oxygen uptake (VO_2max) (Fig. 3.A) with processing speed (composite score), VO_2max (Fig. 3B) with inhibitory control (z-score), VO_2max (Fig. 3C) with verbal fluency (total z-score), and body mass index (BMI) (Fig. 3D) with working memory (composite score) in young healthy adults. The analyses were controlled for: Age (Model1); both age and sex (Model 2); age, sex, and occupational activity (Model 3). β (standardized linear regression coefficient), R^2 (coefficient of determination), and P value were obtained from the linear regression analyses.

Discussion

The purpose of the present study was to test the hypothesis that a HIIT program would improve cognitive function in healthy young adults. Our results did not completely support our hypothesis because, although improvements were found in learning process, memory, processing speed, inhibitory control, and verbal fluency, we cannot conclude that those benefits were elicited by our HIIT intervention since the Non-Training group showed similar enhancements. Interestingly, our results suggest that moderate alcohol consumption does not negatively influence those beneficial adaptations on cognitive function. Additionally, in line with our hypothesis, the current findings showed that changes in physical fitness after the HIIT intervention were positively correlated to changes in cognitive function.

Intervention effects of a HIIT program on cognitive function

Previous studies have found that short bouts of intense exercise directly improves learning in healthy male sport students.[33] These enhancements have been attributed to physiological processes that occur during exercise including an increase of catecholamines (i.e., dopamine, epinephrine, and norepinephrine) and neurotropic factors release. [33] Our results showed an improvement of learning process after the intervention program which cannot be explained by the HIIT intervention program since the participants of the control group also reached such increases. These findings are somewhat consistent with those obtained by similar investigations applying HIIT interventions [8,34]. Hence, an important unanswered question concerns why some studies find improvements in cognitive performance after exercise interventions while other studies have failed to observe such a relationship. One obvious reason for this is that cognitive health peaks during young adulthood, which suggests that there is no space for exercise-related improvement to cognitive function during this period of the lifespan.[35] Another reason to fail finding those cognitive improvements in our study could be the result of methodological factors, such as (i) the general health and fitness level of our participants at the beginning of the study, (ii) the tasks we used to measure aspects of cognition, or (vii) the nature of our Non-Training - control group.

Mekari et al. (2020) found that short-term HIIT resulted in meaningful improvements in reaction time and cognitive flexibility in older adults.[7] Indeed, a recent review has concluded that there is overall moderate evidence supporting the notion that physical activity benefits cognitive functioning during early and late periods of the life span.[1] Though these results are encouraging, there are still major gaps in our understanding of the effects of a HIIT intervention on cognition in people with different biological characteristics, particularly in young to middle-age adulthood (ages 18-50 years).[1,3] In this regard, it should be recognized that our HIIT intervention could be a light stimulus to induce changes on cognition in healthy young adults. Although cognitive improvements were found in memory, processing speed, inhibitory control, and verbal fluency we cannot conclude that those benefits were consequence of our HIIT intervention, since the Non-Training group showed similar enhancements. Further studies applying a greater training load (i.e., longer duration of training (> 10 weeks), high volume and/or high intensity) may be required to achieve a more comprehensive adaptation on cognition in participants with similar conditions than those included in the present project. Our results in working memory also concur with those obtained in previous studies, which reported no exercise-induced changes in working memory after 7-12 weeks of a HIIT intervention program in healthy middle-aged individuals.[8,9] In summary, there is still controversy regarding the effects of moderate-to vigorous-intensity physical activity on cognition in young and middle-aged adults. Therefore, further studies are needed to well-elucidated the potential benefits of exercise on cognition during young and middle adulthood.[1]

A recent systematic review has highlighted the significant heterogeneity in study designs, cognitive instruments used, lack of consistent reporting of blinding and adherence/compliance, and poor descriptions of the interventions when the effects of exercise on cognition are studied [1] Indeed, many of the instruments used to assess executive functioning are traditional neuropsychological tools primarily developed to aid in clinical diagnosis rather than to assess individual variation in normative cognitive functioning.[1] Similarly, their sensitivity to detect changes in response to an intervention (especially in the context of a normative sample) remains questionable. We found that our 10-week HIIT program did not elicited a sufficient stimulus for increasing executive functions in healthy young adults. It should be noted that the majority of exercise and cognition experiments have been designed from a quantitative approach which looks forward to modify physical fitness and/or additional health-related outcomes, ignoring brain or cognitive variables during the design process of the exercise interventions. Thus, changes in cognitive functions are only expected as a result of physical fitness enhancements.[10] However, it is unclear precisely what role cardiovascular fitness might play in instantiating these cognitive changes.[36]

Besides its role in physical health, low to moderate alcohol consumption has been suggested as a potential risk factor in the development of cognitive impairment and dementia, conditions that are highly associated with cardiovascular diseases.[18] However, some inconsistencies can be observed across studies. Firstly, Zhang et al. (2020), hypothesized that the role of alcohol drinking in cognitive function may be a balance of its beneficial and harmful effects on the cardiovascular system.[18] However, other studies have found that moderate drinkers are more likely to have hippocampal atrophy,[37] suggesting that, in line with other study, [14] that there is no safe level of drinking when referring to health. These uncertain ideas could arise from the complicated and multiple mechanisms through which alcohol consumption affects health,[14] as well as the influence of individual's consumption volume and pattern of drinking.[38] Our findings suggest that moderate alcohol consumption did not impair cognitive function after 10-week in healthy young adults. These results concur with those obtained by Zhang et al. (2020)[18], who found that low to moderate alcohol drinking was associated with a protection of cognitive functions that may decrease with age in middle-aged or older adults. Moreover, these authors identified a U-shaped relationship with cognitive function scores, with an optimal dosage of 10 to 14 drinks per week for all participants. However, the association of low to moderate drinking with cognitive function in the younger age as well as the mechanisms underlying this doubtful association warrant further investigation.

Relationships of changes in cognitive function with changes in physical fitness and body composition after the intervention

Previous studies have confirmed an enhancement of cardiovascular fitness are thought to be associated with changes in underlying cognitive performance in children and older adults.[1–3,39,40] However, as regard the effects of physical activity on cognition and brain outcomes in young a middle-adults (18-59 yr), there is a dearth of high-quality data available. Our results are partially in accordance with the above-mentioned idea since a positive relationship between changes in cardiorespiratory fitness and improvements in cognitive functions were noted in our healthy young adults' cohort. These findings suggest that cardiorespiratory fitness may have a great impact on challenging and complex cognitive processes, which has been previously demonstrated in other samples.[41] However, a previous meta-analytic review concluded that the empirical literature does not support the cardiovascular fitness hypothesis.[42] Based upon past findings in this area of research, the authors discussed that the relationship between fitness and cognition might differ depending on age, health status, data collection and analysis to measure fitness, or the cognitive test category.[42] These inconclusive results suggest alternative outcomes may play a relevant role in predicting cognitive performance independently of cardiorespiratory fitness. Future research is needed to bring to light whether exercise training (i.e., HIIT) can elicit a sufficient stimulus for optimizing cognitive function.

Limitations

Although this study had a number of strengths, it was not without limitations. Firstly, the control group was not purely sedentary since it was instructed to keep an active lifestyle. Although, physical activity levels were registered, it was performed by a subjective method (i.e., self-reported). It would have been desirable to have included the assessment of physical activity levels by objective methods (i.e., accelerometer system). Secondly, one potential reason for the lack of differences across groups on cognitive function could be young age of the included participants and their healthy-active condition as well.[43] Finally, participants were not purely randomized, basically due to ethical considerations. Thus, a real double-blind design, placebo controlled for alcohol, was not possible. Subsequently, future studies are required to determine the effects of the same training intervention in participants with different biological characteristics to identify effective public health strategies that promote a healthy lifestyle where, if wished and considered acceptable, fermented beverages in moderate amounts co-ingested with meals could be included.

Conclusions

We potentially provided evidence for understanding the protector benefits of highly intensity exercise training over moderate alcohol intake on cognition through full engagement in a 10-week HIIT program. Moreover, our results confirmed that exercise-induces changes in physical fitness are associated with changes in cognitive performance.

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Authors' contributions

CMH participated in the design of the study, contributed to data collection, data reduction/analysis, and interpretation of results, and contributed to the manuscript writing – original draft; FAG participated in the design of the study, contributed to the interpretation of results and participated in the manuscript writing – original draft; KIE contributed to interpretation of results and participated in the manuscript writing – review and editing; JP contributed to data analysis, interpretation of results, and participated in the manuscript writing – review and editing ACB participated in the manuscript writing – review and editing; MC participated in the design of the study and participated in the manuscript writing – review and editing; AC participated in to the manuscript writing – review and editing.

Competing interest

The authors declare that they have no competing interests.

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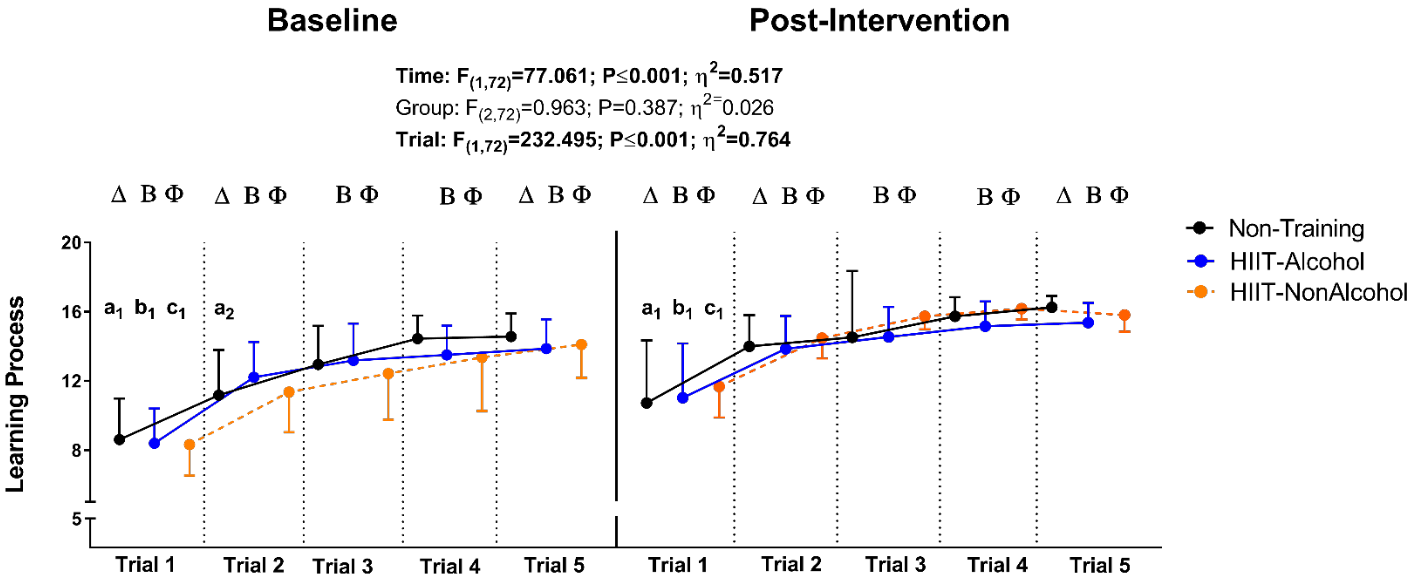

Figure S1. Changes in learning process (Trial 1 to 5) measured by Spanish-Complutense Verbal Learning Test (TAVEC), tested at Baseline and Post-Intervention study. F, p, and η of repeated-measures analysis of variance (ANOVA) for time, group, and trial. Intragroup changes obtained by repeated-measures ANOVA (group x trial x time, adjusting by trial), with a post hoc Bonferroni-corrected test, are indicated as: Trial 1 vs. Trial 2 (a_1 $p < 0.05$), Trial 2 vs. Trial 3 (a_2 $p < 0.05$) for Non-Training group; Trial 1 vs. Trial 2 (b_1 $p < 0.05$) for HIIT-Alcohol group; Trial 1 vs. Trial 2 (c_1 $p < 0.05$) for HIIT-NonAlcohol group. Intragroup changes before and post-intervention obtained by repeated-measures ANOVA (group x trial x time, adjusting by time), with a post hoc Bonferroni-corrected test, are indicated as: Δ $p < 0.05$ for Non-Training group, Β $p < 0.05$ for HIIT-Alcohol group, and Φ $p < 0.05$ for HIIT-NonAlcohol group. Raw data are presented as mean and standard deviation. Abbreviations: HIIT-Alcohol, group that performed high-intensity interval training and consumed alcoholic beverage; HIIT-NonAlcohol, group that performed high-intensity interval training and consumed non-alcoholic beverage.

Table S1. Changes in Cognitive measures adjusted by baseline values and age (Model 1), by baseline values and sex (Model 2), and by baseline values and occupational activity (Model 3).

Models	Model 1			Model 2			Model 3		
	Analysis of covariance								
	F	P value	η^2	F	P value	η^2	F	P value	η^2
Memory									
Immediate memory	0.767	0.468	0.021	0.790	0.458	0.022	0.718	0.491	0.020
Short-term memory	0.350	0.706	0.010	0.768	0.468	0.021	0.633	0.534	0.018
Delay memory	1.108	0.336	0.031	1.816	0.170	0.049	1.689	0.192	0.046
Recognition (tc)	2.438	0.095	0.065	1.887	0.160	0.051	1.724	0.186	0.047
Recognition (id)	0.837	0.437	0.023	1.653	0.199	0.045	1.335	0.270	0.037
Composite score	0.749	0.477	0.021	1.292	0.281	0.036	1.051	0.355	0.029
Working Memory									
Direct Score	0.084	0.919	0.002	0.105	0.901	0.003	0.064	0.938	0.002
Processing Score	0.498	0.610	0.014	0.501	0.608	0.014	0.430	0.652	0.012
Composite score	0.140	0.870	0.004	0.162	0.851	0.005	0.111	0.895	0.003
Processing Speed									
Total productivity	0.502	0.608	0.014	0.645	0.528	0.018	0.724	0.489	0.020
Correct work	0.304	0.739	0.009	0.570	0.569	0.016	0.630	0.536	0.018
Concentration Index	0.371	0.691	0.011	0.480	0.621	0.014	0.763	0.470	0.021
Composite score	0.413	0.663	0.012	0.637	0.532	0.018	0.783	0.461	0.022
Inhibitory Control									
Omissions	1.109	0.335	0.031	1.397	0.254	0.038	1.330	0.271	0.037
Total Effectiveness	0.406	0.668	0.011	0.643	0.529	0.018	0.603	0.550	0.017
Verbal Fluency									
Total Phonologic	0.771	0.467	0.022	1.020	0.366	0.028	0.691	0.504	0.019
Total Semantic	2.098	0.130	0.057	2.450	0.094	0.065	2.459	0.093	0.066
Total Score	1.067	0.350	0.030	1.237	0.296	0.034	0.886	0.417	0.025

Abbreviations: tc, total number of correct responses in the recognition test; id, index of discriminability from learning list in the recognition test.

Table S2. Association of VO_2max and hand grip strength/weight changes with Cognitive Composite changes. The analyses were controlled for: age (Model 1); both age and sex (Model 2); age, sex, and occupational activity (Model 3).

	VO_2max (ml/min)			VO_2max (ml/kg/min)			Hand grip strength			Hand grip strength/weight		
	B	R^2	P	B	R^2	P	B	R^2	P	B	R^2	P
Memory												
Model 0	0.135	0.004	0.256	0.102	0.004	0.390	0.018	-0.014	0.880	-0.010	-0.014	0.932
Model 1	0.133	-0.007	0.263	0.100	-0.015	0.405	0.016	-0.023	0.892	-0.015	-0.024	0.899
Model 2	0.063	0.018	0.612	0.039	0.015	0.705	0.000	0.023	0.999	-0.011	0.022	0.924
Model 3	0.055	0.015	0.662	0.031	0.013	0.708	-0.015	0.021	0.897	-0.034	0.021	0.777
Working Memory												
Model 0	-0.122	0.001	0.302	-0.021	-0.014	0.863	-0.174	0.017	0.138	-0.066	-0.010	0.579
Model 1	-0.123	-0.012	0.303	-0.022	-0.027	0.854	-0.175	0.004	0.139	-0.068	-0.024	0.571
Model 2	-0.146	-0.023	0.254	-0.029	-0.041	0.817	-0.176	-0.010	0.140	-0.069	-0.039	0.573
Model 3	-0.149	-0.036	0.249	-0.031	-0.056	0.806	-0.185	-0.020	0.127	-0.081	-0.050	0.515
Processing Speed												
Model 0	0.198	0.025	0.094	0.273	0.061	0.020	-0.034	-0.013	0.775	-0.047	-0.012	0.693
Model 1	0.195	0.023	0.098	0.268	0.058	0.022	-0.037	-0.012	0.755	-0.056	-0.012	0.641
Model 2	0.233	0.020	0.065	0.300	0.056	0.014	-0.037	-0.027	0.758	-0.056	-0.026	0.642
Model 3	0.244	0.026	0.054	0.301	0.063	0.012	-0.020	-0.025	0.871	-0.033	-0.028	0.790
Inhibitory Control												
Model 0	0.176	0.017	0.137	0.249	0.049	0.034	-0.006	-0.014	0.960	-0.004	-0.014	0.971
Model 1	0.174	0.010	0.141	0.245	0.040	0.037	-0.008	-0.019	0.944	-0.011	-0.021	0.927
Model 2	0.182	-0.004	0.153	0.254	0.027	0.040	-0.013	-0.030	0.916	-0.010	-0.033	0.933
Model 3	0.190	-0.007	0.137	0.262	0.026	0.035	0.001	-0.034	0.991	0.010	-0.038	0.936
Verbal Fluency												
Model 0	0.313	0.085	0.007	0.356	0.115	0.002	0.077	-0.008	0.514	0.072	-0.009	0.547
Model 1	0.311	0.087	0.007	0.351	0.114	0.002	0.074	-0.008	0.532	0.064	-0.011	0.595
Model 2	0.308	0.074	0.013	0.346	0.102	0.004	0.069	-0.016	0.563	0.065	-0.024	0.593
Model 3	0.319	0.080	0.010	0.356	0.109	0.003	0.084	-0.018	0.484	0.084	-0.030	0.499

B, standardized linear regression coefficient; R^2, coefficient of determination, and P value were obtained from the linear regression analyses. Bold values are values that are significant ($p < .05$). Abbreviations: tc, total number of correct responses in the recognition test; id, index of discriminability from learning list in the recognition test

Table S3. Association of BMI, FMI, and LMI changes with Cognitive Composite changes. The analyses were controlled for: age (Model 1); both age and sex (Model 2); age, sex, and occupational activity (Model 3).

	BMI			FMI			LMI		
	B	R^2	P	B	R^2	P	B	R^2	P
Memory									
Model 0	0.004	-0.014	0.970	0.136	0.005	0.251	-0.125	0.002	0.293
Model 1	0.008	-0.024	0.945	0.133	-0.006	0.263	-0.118	-0.010	0.327
Model 2	0.002	0.026	0.988	0.136	0.046	0.241	-0.130	0.044	0.266
Model 3	0.003	0.023	0.978	0.139	0.043	0.234	-0.138	0.042	0.243
Working Memory									
Model 0	-0.282	0.066	0.016	-0.213	0.032	0.070	-0.059	-0.011	0.619
Model 1	-0.281	0.053	0.017	-0.215	0.019	0.070	-0.057	-0.025	0.637
Model 2	-0.281	0.039	0.018	-0.215	0.005	0.072	-0.057	-0.040	0.639
Model 3	-0.281	0.026	0.019	-0.214	-0.008	0.075	-0.060	-0.053	0.626
Processing Speed									
Model 0	0.112	-0.001	0.344	0.058	-0.011	0.626	0.038	-0.013	0.749
Model 1	0.119	0.000	0.316	0.053	-0.012	0.657	0.054	-0.012	0.652
Model 2	0.120	-0.014	0.318	0.053	-0.026	0.660	0.055	-0.026	0.648
Model 3	0.118	-0.014	0.324	0.050	-0.026	0.677	0.063	-0.025	0.602
Inhibitory Control									
Model 0	0.065	-0.010	0.587	0.050	-0.012	0.672	0.031	-0.013	0.791
Model 1	0.070	-0.015	0.559	0.047	-0.018	0.697	0.044	-0.018	0.715
Model 2	0.069	-0.028	0.569	0.047	-0.030	0.695	0.042	-0.031	0.732
Model 3	0.067	-0.034	0.577	0.045	-0.036	0.710	0.048	-0.036	0.694
Verbal Fluency									
Model 0	0.185	0.021	0.118	0.190	0.022	0.108	-0.090	-0.006	0.450
Model 1	0.192	0.023	0.105	0.185	0.021	0.118	-0.076	-0.009	0.527
Model 2	0.190	0.012	0.109	0.186	0.011	0.118	-0.079	-0.019	0.512
Model 3	0.189	0.006	0.113	0.183	0.004	0.124	-0.073	-0.026	0.545

BMI, body mass index; FMI, fat mass index; LMI, lean mass index, standardized linear regression coefficient; R^2, coefficient of determination, and P value were obtained from the linear regression analyses. Bold values are values that are significant ($p < 0.05$).

SECTION 3

Combined effects of highly demanding training program and moderate alcohol/beer consumption on psychosocial variables and mood state

Chapter 7

Psychosocial and Mood state adaptations during a highly demanding training program in conjunction with moderate alcohol/beer intake in healthy adults. The BEER-HIIT study (Study 5)

ABSTRACT

Purpose. The main purpose of this investigation was to determine the effects of a High Intensity Interval Training (HIIT) intervention parallel to a moderate alcohol consumption on mental wellbeing and mood state in healthy young adults.

Methods. We conducted a 10-week HIIT program taking four type of beverages with/without alcohol content. A total of 74 healthy adults (18-40 years; 46% females) were randomly assigned to either a control Non-Training group or a HIIT program group (2 days/week). Using block randomization, participants in the HIIT group were further allocated to a HIIT-Alcohol group (alcohol beer or sparkling water with vodka added, 5.4%) or a HIIT-NonAlcohol group (sparkling water or non-alcohol beer, 0.0%). The control group were asked to maintain an active lifestyle, but did not complete any regular training. A set of questionnaires were used to assess psychosocial parameters, as well as mood adaptations at baseline and after 10-week HIIT program. In order to control the evolution of the mental variables during the intervention, perceived stress, positive and negative affection, emotional intelligence, and mood state, were measured every 2-week.

Results. Both intervention groups showed improvements in depression, sleep quality, affective and mood states, and emotional intelligence after 10-week HIIT program (all $p<0.05$), independently of sex and alcohol consumption, with no statistical differences between groups (all $p>0.050$). Further, changes in intrinsic motivation and in goals orientation to ego were found during the intervention program in both intervention groups (all $p<0.050$).

Conclusions. We provide evidence that a 10-week HIIT program elicits improvements in psychosocial parameters and mood adaptations in healthy young adults. No significant impairments were noted due to alcohol intake in moderate amounts.

Introduction

Recently, there has been a great deal of interest in the effects of exercise training on psychological functioning [1–3]. Mood, in particular less depressive symptomology, is one likely psychosocial mechanism of exercise [4]. In population samples of healthy individuals, higher levels of objectively measured physical activity are associated with lower levels of depressive symptoms and better cognitive function in children[5,6] and adolescents (ages 5–17 yr) [7,8], as well as in older adults [9]. Previous meta-analyses of exercise interventions have concluded that exercise is an effective additive treatment (compared with treatment as usual) to reduce symptoms of depression in adolescents, young adults, and older adults with depressive or psychiatric disorders [10,11]. Some of the mechanisms proposed as mediators of the psychological effect of exercise are primarily based on the volume and/or duration and intensity of exercise [12]. In this regard, improvements in anxiety and depression have been seen following a high-intensity interval training (HIIT) and these results seem to be consistent across a range of ages and across healthy populations [13]. HIIT may be a viable strategy for obtaining positive psychological responses and sessions of this type of training could be perceived more enjoyable than moderate-to-vigorous intensity continuous training [14]. However, despite there is a strong evidence indicating that HIIT can elicit a positive effect on depression [15] and emotional well-being [16] in a range of adult population groups (i.e., older adults, cancer patients, and cancer survivors), little is known regarding the effect of an highly demanding training program on cognitive and mental health outcomes in young and middle-aged populations. Therefore, additional studies including both psychological and neurocognitive functioning as outcome variables are needed to enhance our understanding of this level of analysis.

In contrast to exercise, alcohol consumption is not typically regarded as a health-promoting behavior. However, lifestyle habits, such as alcohol consumption, have been positively related to exercise as part of the recovery process and social relationship after practicing exercise [17,18]. Importantly, this link between physical activity and alcohol intake extends to non-athletes as well as to people who do not play team sports [19]. Alcohol and exercise both have a broad range of effects on brain chemicals and circuitry by invoking activity in the brain's mesocorticolimbic pathway and affecting the hypothalamic–pituitary–adrenal axis[19]. In this regard, both represent rewarding stimuli that evolved to respond to natural rewards that promote survival, such as sex, food, or exercise; and also both seem to help in the regulation of anxiety [19]. Actually, beer is the most popular alcoholic beverage among healthy adults being mainly consumed with meals [20–22]. Recent studies have found that happiness was a significant predictor of alcohol consumption, suggesting that could be psychosocial benefits of alcohol consumption in sport [23]. However, because of the ethanol contained in beer and its deleterious effect, this practice may be not advisable particularly when the exercise is very demanding or when the individual is training in order to achieve improvements in physical fitness or maintain overall health.

Although the multiple and negative effects of high intakes of alcohol have been widely studied and demonstrated [24,25], the effect of moderate alcohol doses after exercise is not clear and it is currently under debate [17,26,27]. To the best of our knowledge, there is no study exploring the combination of a

HIIT intervention and moderate alcohol consumption in healthy individuals, which is widely common in a social context for active people.

The primary purpose of the present study was to evaluate the effects of a 10-week HIIT intervention on psychosocial and mood variables, in healthy young adults. The secondary purpose of the study was to assess whether those potential benefits could be influenced by a moderate alcohol consumption. Finally, the third purpose of our study was to investigate the mood state adaptations while performing a highly demanding training program.

Material and methods

Trial Design

The BEER-HIIT study is a registered controlled trial (ClinicalTrials.gov ID: NCT03660579) with a follow-up of 10 weeks. A detailed study protocol description has been published elsewhere .[1] The procedures were designed accordingly with last revised Declaration of Helsinki and approved by the Ethics Committee on Human Research of the University of Granada (321/CEIH/2017).

Participants

Potential participants of the BEER-HIIT project were healthy young adults who lived in the province of Granada, Spain. The study was announced via social networks, local media, and posters. Prior to the enrolment, all individuals completed a medical examination, provided a written informed consent, and were fully informed about the study objectives, design, inclusion criteria, assessments to be undertaken, exercise program intervention, and types of beverages to be ingested. Subjects who met the inclusion criteria (i.e. (i) having a body mass index (BMI) from 18.5 to 30 kg/m^2, (ii) not being engaged in a previous structured training program or a weight-loss program (last 5 months), (iii) having a stable body weight during the last 5 months (body weight changes $<$ 3 kg), (iv) being free of disease, (v) not being pregnant or lactating, (vi) not taking any medication for chronic diseases, and (vii) not suffering pain, recent injuries, or other problems preventing strenuous physical activity) were invited to an information meeting in which the research staff gave specific information about healthy dietary patters and the physical activity recommendations provided by the World Health Organization.[2]

Randomisation and follow-up

After the baseline measurements, a total of 83 individuals were allocated to a training (i.e., HIIT) or a Non-Training group based on personal preferences. Participants in the Non-Training group were instructed to maintain their usual physical activity levels and not to be engaged in a structured exercise program. Those participants included into the training group were subsequently allocated in an ethanol-containing beverage (i.e. 5.4% alcohol) group or an alcohol-free beverage group. The participants willing to consume ethanol were randomly allocated to either a group consuming alcohol beer (HIIT-Beer) or to a group consuming sparkling water with added vodka ethanol (HIIT-Ethanol). The participants choosing non-alcoholic beverages were randomly allocated to either an alcohol-free beer group (HIIT-0.0Beer) or to a sparkling-water group (HIIT-Water). This type of non-random (i.e., based on individual preference) and random allocation of the participants was conducted following ethical considerations and advices provided by the ethical committee (321-CEIH-2017), since drinking alcohol or participating in a highly demanding training program should be a personal choice.

Intervention

The HIIT intervention consisted on 2 sessions/week performed from Monday to Friday during 10 consecutive weeks with at least 48 h of recovery between sessions. The training intervention was divided into two different phases, starting with a familiarization phase to learn the main movement patterns aiming to avoid injuries or potential drop outs. The volume and intensity of the sessions in the familiarization phase was fixed at 40 min/week and 8-9 Rating of Perceived Exertion (0-10 RPE), respectively.[3,4] Subsequent increments of both volume and intensity were established in Phase I (50 min/week and 10 RPE) and in Phase II (65 min/week and 10 RPE). Eight self-loading exercises were performed in a circuit form twice per set (i.e., frontal plank, high knees up, TRX horizontal row, squat, deadlift, side plank, push up, and burpees) with a passive rest between exercises and an active rest between sets (i.e., 6 RPE intensity, which corresponds with 60% VO_2max).[4,5] A dynamic standardized warm up and an active global-stretching cooling-down protocols were completed at the beginning and at the end of each training session, respectively. A detailed description of each exercise of the training program can be found elsewhere.[1]

During the intervention, the alcohol consumption allowed were 330 ml of the respective beverage at lunch and 330 ml at dinner for men, and 330 ml at dinner for women, from Monday to Friday: (i) HIIT-Alcohol group ingested randomly assigned alcohol beer (5.4% alcohol-Alhambra Especial®, Granada, Spain) or sparkling water with exactly the equivalent amount of distilled alcohol added (Vodka, 37.5% ethanol and 62.5% water), and (ii) HIIT-NonAlcohol group randomly assigned ingested alcohol-free beer (0.0% alcohol-Cruzcampo®, Sevilla, Spain) or sparkling water (Eliqua 2®, Font Salem, Spain). The amount of alcohol selected to ingest was based on scientific evidence (i.e. 2-3 drinks/day or 24–36 g of ethanol/day for men and 1-2 drinks/day or 12–24 g of ethanol/day for women).[6,7] During weekends, participants were requested to respect the beverage intake condition (i.e., moderate alcohol consumption and non-alcohol consumption). All beverages were coded and provided by a blinded staff member of our research laboratory at the beginning of each week. Additionally, the alcohol intakes were registered to control its consumption before and during the intervention.

Quality of life and Psychosocial measurements

Satisfaction with life - SWLS. The SWLS assesses a person's conscious evaluative judgments of his or her life as a whole, based on a comparison of the person's life with a self-imposed standard or set of standards. This scale is a 5-item self-report instrument for the measurement of global life satisfaction as a cognitive-judgmental process, by indicating their agreement on a5-point Likert scale labelled from 1 ("strongly disagree") to 5 ("strongly agree") [32,33]. Participants completed the Spanish version of the SWL [34]. **Subjective Happiness Scale - SHS.** is a 4-item self-report measure in which a single composite score for global subjective happiness is computed by averaging responses to the four items [35]. **Life Orientation Test Questionnaire - LOT-R.** we used the Spanish version of the LOT-R [36]. This test measures individual differences in generalized optimism versus pessimism. It consists of ten items referring to: (i) optimism (items 1, 4, and 10); (ii) pessimism (items 3, 7, and 9), and four filler items that were included in order to disguise (to some extent) the underlying purpose of the test. Each item presents a rating scale from 0 ("strongly disagree") to 4 ("strongly agree") [36,37]. **Perceived Stress Scale - PSS.** This scale is the most used psychological instrument for measuring the perception of stress. It is a measure of the degree to which situations in one's life are appraised as stressful. It is a 14-item self-report instrument [38,39]. **Beck Depression Inventory - BDI-II.** The BDI is a popular scale used for the assessment of the intensity of depressive symptoms within both psychiatric and normal populations. The BDI consists of 21 items that assess the intensity of depressive symptomatology, such as negative mood, pessimism, guilt, sense of failure, suicidal thoughts, fatigue, and weight loss. For each item, respondents are asked to indicate which of four statements best describes the way they were feeling during the past week. The statements are scored according to the severity of the symptomatology they reflect (0 = symptom is absent; 3 = symptom is present and severe). A total depression score is obtained by summing the rating of the 21 items, yielding scores that range from 0 to 63 [40,41]. We used the Spanish adaptation [42].

Mood state

Profile of Mood States Questionnaire – POMS. This is a self-rating scale which consisted of 65 adjectives that were rated on a 5-point scale. The POMS measures 6 identifiable mood or affective states: "tension-anxiety", "depression-dejection", "anger-hostility", "vigor-activity", "fatigue-inertia", and "confusion-bewilderment". To obtain a score for each mood factor, the sum of the responses is obtained for the adjectives defining the mood factor [43–46]. **Trait Meta-Mood Scale-24 - TMMS-24.** This is a measured of perceived emotional intelligence. Particularly, it is a self-report measure designed to assess individuals' beliefs about their own emotional abilities. The scale addresses 3 key aspects: (i) attentional conveys to what extent individuals tend to observe and think about their feelings and moods; (ii) clarity evaluates the understanding of one's emotional states; and repair refers to the individuals' beliefs about ability to regulate their feelings. Specifically, it is a 24-item Likert-type scale on which participants are required to rate the extent to which they agreed with each item [4748]. **The Positive and Negative Affect Schedule-Trait – PANAS.** The questionnaire contains 20 items on two subscales that assess a person's positive and negative trait affect using a 5-point scale from 1 (1"very slightly or not at all") to 5 ("extremely"). Both the original validation [49] and the Spanish validation[50], showed two clearly differentiated factors (positive affect and negative affect) and good psychometric properties. **Scale of Assessment of Mood - EVEA.** This scale is aimed at assessing current mood, especially in the context of the administration, although it can be used whenever there is a need to measure transitory moods at any one time. It is a 16-item self-report instrument and each item is scored between 0-10 points as function of the answer of the person being examined. After summing the score of the four items of each subscale and dividing the sum by 4, a total of four total scores are obtained, one for each subcales [51,52].

Dietary and Exercise habits

Questionnaire on Adherence to the Mediterranean Diet - MEDAS. This questionnaire consists of 12 questions on food consumption frequency and 2 questions on food intake habits considered characteristic of the Spanish Mediterranean diet [53]. **Beverage Intake Questionnaire – BEVQ.** It is a questionnaire which estimates mean daily intake of water, sugar sweet beverages, alcohol beverages, and total beverages across 19 categories [54,55].

Scale of Regulation of the Behavior in Physical Exercise - BREQ-2. The BREQ-2 is the last modification [56] of the BREQ. The BREQ-2 questionnaire is 19-item scale which measures the stages of self-determination continuum [57]. It uses a Likert-type scale of 5 points, where 0 is "not true for me" and 4 is "very true for me". **Goal Orientation in Exercise Scale – GOES.** This is a scale which assess goal perspective for exercise participation [58,59]. The Spanish version of this questionnaire consists of two second-order dimensions which measure the Perception of Task-Involving Motivational Climate and the Perception of Ego-Involving Motivational Climate. In the Spanish version, the task-involving climate factor is composed of 11 items [60].

Quality of sleep

Pittsburgh Sleep Quality Index. This is a 19 self-rated questions which assess a wide variety of factors relating to sleep quality, including estimates of sleep duration and latency and of the frequency and severity of specific sleep-related problems [61].

Statistical analyses

Considering the results of a pilot study, the sample size calculations revealed that 13 participants per group were needed to detect an effect size of 0.25 in XX scores with an α error of 0.05, and a power of 0.85.[35] However, we recruited a minimum of 16 participants per group (a total of 80) considering a maximum loss of 20% at the follow up.[36]

Descriptive parameters are reported as mean and standard deviation; 95% confidence intervals (CIs) being estimated for changes in groups over several measures (week 0-10). Standard statistical methods were used for the calculation of means and standard deviations. Normal Gaussian distribution of the data was verified by the Shapiro–Wilk test, visual check of histograms, Q-Q, and box plots. Homoscedasticity was verified by the modified Levene Test. The compound symmetry, or sphericity, was checked by the Mauchly test. When the assumption of sphericity was not met, the significance of F-ratios was adjusted according to the Greenhouse–Geisser procedure when the epsilon correction factor was < 0.75, or according to the Huyn–Feld procedure when the epsilon correction factor was > 0.75.

A recategorization of the occupational activity measure was performed into 3 levels showing a relatively high internal consistency ($\alpha = .705$). Occupational activities were classified and categorized as level 1 (unemployment, homemaker, and student), level 2 (primary sector services, retail or catering services, and administrative clerk), and level 3 (support and scientific/intellectual technicians and professionals, and business administration and management).

Age, sex, and occupational activity were used as potential confounders in the analyses. Given that we did not observe a sex interaction, we conducted the analysis including men and women together. Similarly, since no beverage interaction was observed between groups in any outcome, we conducted the analysis including both alcohol beer and sparkling water with ethanol HIIT groups in the same group (i.e. HIIT-Alcohol group) and both 0.0% alcohol beer and sparkling water HIIT groups in the same group (i.e. HIIT-NonAlcohol group).

A repeated-measured analysis of variance (2 x 2 ANOVA; time x training group; and time x beverage) was conducted to test differences between groups. Post-hoc Bonferroni corrections were conducted for multiple comparisons. The significance level was set at $p < 0.05$ for all analyses. An analysis of covariance (ANCOVA) was conducted to study the effect of the intervention (group entered as fixed factor) on psychological and mood variables, i.e., life's satisfaction, depression, positive and negative emotions, mood state, emotional intelligence, or perceived stress. Potential confounders were selected based on statistical procedures (i.e., hierarchical regressions) and theoretical bases.

All analyses were conducted using the Statistical Package for Social Sciences (SPSS, v. 25.0, IBM SPSS Statistics, IBM Corporation). Graphical presentations were prepared using GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA).

Results

A total of 74 participants (34 women) were included in the final analyses after a loss to follow up of 11% (see Fig. 1). Descriptive parameters of the study participants are listed in Table 1. Participants reported no adherence to Mediterranean diet ($p = 0.810$; see Table 1). In addition, there were no significant differences between groups at the baseline values (all $p > 0.05$), excepting for age and occupational activity ($p = 0.009$ and $p = 0.006$, respectively; see Table 1).

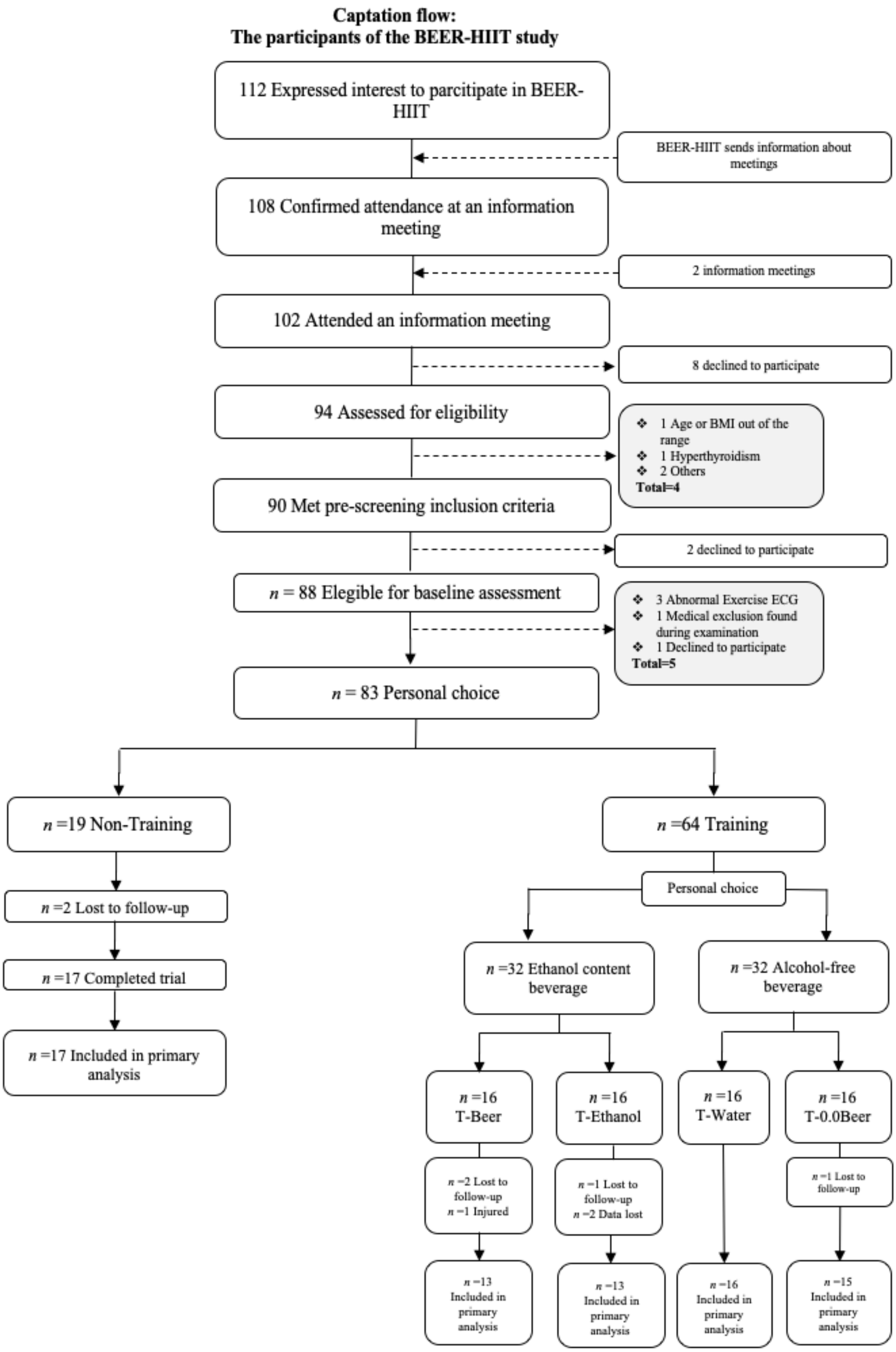

Figure 1. Flow of participants in the BEER-HIIT study.
Abbreviations: HIIT, high-intensity interval training; BMI, body mass index; ECG, electrocardiogram.

RESULTS AND DISCUSSION

Table 1. Baseline characteristics of participants.

	Non-Training (N = 17)				HIIT-Alcohol (N = 26)				
	Mean ± SD								
Sex (men% / women%)		65 %	/	35 %		46 %	/	54 %	
Age		20.0	±	2.2		25.3	±	6.2	
Alcohol Ingested (ml/week)		1144.1	±	831.9		708.1	±	872.3	1
Adherence Mediterranean diet		3.8	±	1.6		4.1	±	1.2	
Educational level (%)									
Secondary Education		47.1%				42.3%			2
Vocational Education and Training		23.5%				19.2%			2
University Degree or Certificate of Higher Education		29.5%				38.5%			5
Occupational activity (%)									
Level 1		100%†				57.7%			
Level 2		--				15.4%			
Level 3		--				26.9%			
Baseline and after 10-week measures	N	Mean ± SD			N				N
SWL	17	18.9	±	4.3	25	18.8	±	3.5	29
BDI-II	14	9.14	±	8.5	24	11.3	±	7.4	31
LOT-R	15	20.3	±	5.2	23	22.2	±	4.4	29
Pittsburgh SQI	15	5.1	±	3.2	26	5.9	±	2.1	31
SHS	14	4.4	±	0.7	21	4.9	±	0.5	30
POMS – anger	16	8.6	±	7.6	23	9.6	±	6.1	30
POMS – fatigue	16	5.9	±	5.4	23	8.7	±	5.4	30
POMS – vigor	16	17.8	±	6.2	23	18.0	±	5.4	30
POMS – tension	16	8.3	±	7.3	23	11.3	±	6.6	30
POMS – depression	16	11.3	±	12.1	23	13.0	±	9.3	30
POMS – confusion	16	5.1	±	2.8	23	5.7	±	3.3	30
Every 2 weeks measures									
PSS	16	26.3	±	3.9	25	27.0	±	4.3	30
PANAS – positive	17	53.1	±	5.9	25	54.6	±	6.5	31
PANAS – negative	17	18.3	±	7.0	25	18.0	±	5.0	31
TMMS – attention	16	25.8	±	5.8	26	25.6	±	5.9	30
TMMS – clarity	16	25.6	±	5.2	26	28.7	±	5.8	30
TMMS – repair	16	28.6	±	4.3	26	29.3	±	4.8	30

	EVEA – sadness	17	2.7	±	2.5	27	2.6	±	2.4	31
	EVEA – anger	17	1.8	±	2.0	27	1.9	±	2.0	31
	EVEA – anxiety	17	2.5	±	2.5	27	2.8	±	1.8	31
	EVEA – happiness	17	6.8	±	1.9	27	7.3	±	1.8	31

Data expressed as mean ± standard deviation. Differences in baseline characteristics between the different groups were evalu † Occupational activity: 53% unemployment, 47% student. * Boldface values indicate significance differences ($P < 0.05$). performed high-intensity interval training and consumed alcoholic beverage; HIIT-NonAlcohol, group that performed high-inter alcoholic beverage; Level 1, unemployment, homemaker, student; Level 2, agriculture, livestock and fisheries service, retail or co 3, support technicians and professionals, scientific and intellectual technicians and professionals, business administration and mo BDI-II, Beck Depression Inventory; LOT-R, Life Orientation Test Questionnaire; Pittsburgh SQI, Pittsburgh sleep quality index; SH Profile of Mood States Questionnaire; PSS, Perceived Stress Scale; PANAS, The Positive and Negative Affect Schedule; TMM Scale of Assessment of Mood.

Intervention effects of a HIIT program on psychosocial and mood variables

Table 2 shows changes in psychosocial and mood state variables tested at baseline and after 10-week HIIT program. Higher scores in life's satisfaction (measured by SWL), optimism (measured by LOT-R), and happiness (measured by EVEA) were observed in HIIT-NonAlcohol group after the HIIT program ($p = 0.017$, $p \leq 0.001$, and $p = 0.047$, respectively; see Table 2). Also, a significant decrease in depression (measured by BDI-II) was found in the HIIT-Alcohol group and HIIT-NonAlcohol group (all $p \leq 0.001$; see Table 2). Similar improvements in anger, fatigue, vigor, and depression (measured by POMS) were found in both intervention groups (all $p \leq 0.001$ for HIIT-Alcohol group, and all $p < 0.05$ for HIIT-NonAlcohol group; see Table 2). Regarding to the emotional intelligence, significant differences in the attention and clarity subscales were also noted between baseline and 10 weeks after the intervention in HIIT-Alcohol group and HIIT-NonAlcohol group (all $p < 0.05$; see Table 2). Similar improvements in sleep quality were found in the intervention groups after 10-week HIIT program ($p = 0.008$ and $p = 0.003$, respectively; see Table 2). Indeed, HIIT-NonAlcohol group showed higher scores in the repair subscale ($p = 0.038$; see Fig.2), also showed significant decreases in negative emotions (measured by PANAS), anger, and anxiety (measured by EVEA) ($p = 0.028$, $p = 0.035$, and $p = 0.044$, respectively; see Table 2).

Non-Training group showed significant decreases in anger and vigor ($p = 0.032$ and $p \leq 0.001$, respectively; see Table 2), also a significant increase was observed in tension ($p = 0.002$; all of them measured by POMS).

No significant changes were observed in subjective happiness nor in perceived stress in any group (all $p > 0.05$; see Table 2).

The ANCOVA analysis adjusting for baseline values (Model 0), is shown in Table 2. ANCOVA revealed an interaction effect on life's satisfaction ($p = 0.045$; see table 2) although no significant differences between groups were observed (all $p > 0.05$). This significant interaction persisted after adjusting by sex ($p = 0.043$; see Table S1). However, no significant between-group differences were observed in the other psychological or mood variables, such as depression, emotional intelligence, or mood state (all $p > 0.05$; see Table 2). These results persisted after controlling by potential confounders (i.e., sex, age, and occupational activity; see Table S1). Nevertheless, the vigor subscale of POMS showed significant differences between the Non-Training group and HIIT-NonAlcohol group after adjusting occupational activity (CI = 0.053 to 5.407, $p = 0.044$; see Table S1); also, the tension subscale of POMS showed significant differences between the Non-Training group and HIIT-Alcohol group, after adjusting by occupational activity (CI = 0.563 to 6.881, $p = 0.015$).

Table 2. Changes before and after the intervention study and changes over time in Cognitive Scores

		Non-Training			HIIT-Alcohol			HIIT-NonAlcohol	
		Baseline	After 10-week		Baseline	After 10-week		Baseline	After 10-week
		Mean ± SD							
				P value			P value		
	SWL	18.94 (0.78)	18.65 (0.82)	0.684	18.76 (0.82)	18.64 (0.68)	0.840	19.24 (18.05)	20.59 (19.33)
	BDI-II	9.12 (2.06)	9.57 (2.11)	0.815	11.25 (1.57)	6.33 (1.61)	**≤ 0.001**	9.77 (1.39)	6.48 (1.42)
	LOT-R	20.27 (1.19)	21.73 (1.11)	0.079	22.22 (0.96)	21.96 (0.90)	0.695	20.03 (0.85)	22.41 (0.80)
	Pittsburgh SQI	5.13 (0.68)	4.13 (0.70)	0.104	5.89 (0.52)	4.62 (0.54)	**0.008**	5.58 (0.47)	4.29 (0.49)
	SHS	4.41 (0.17)	4.71 (0.15)	0.061	4.88 (0.14)	4.82 (0.12)	0.648	4.66 (0.12)	4.70 (0.10)
POMS –	anger	8.63 (1.63)	5.25 (1.32)	**0.032**	9.61 (1.36)	4.52 (1.10)	**≤ 0.001**	6.90 (1.19)	4.60 (0.96)
POMS –	fatigue	5.87 (1.34)	6.00 (1.14)	0.917	8.65 (1.12)	4.78 (0.95)	**≤ 0.001**	7.67 (0.98)	4.27 (0.83)
POMS –	vigor	17.81 (1.41)	11.94 (0.87)	**≤ 0.001**	18.04 (1.17)	11.48 (0.73)	**≤ 0.001**	16.53 (1.03)	9.60 (0.64)
POMS –	tension	8.25 (1.16)	13.31 (0.98)	**0.002**	11.30 (1.34)	11.17 (0.82)	0.920	9.30 (1.18)	11.47 (0.72)
POMS –	depressic	11.31 (2.39)	7.31 (1.82)	0.081	13.00 (2.00)	5.09 (1.52)	**≤ 0.001**	8.67 (1.74)	5.30 (1.33)
POMS –	confusion	5.12 (2.78)	7.44 (3.20)	**0.007**	5.74 (3.33)	6.61 (2.25)	0.211	4.10 (2.47)	5.87 (3.16)
	PSS	26.31 (3.88)	25.88 (3.40)	0.711	26.96 (4.28)	25.12 (3.70)	0.055	27.03 (3.91)	25.40 (4.51)
PANAS –	positive	53.12 (5.87)	52.53 (7.36)	0.785	54.56 (6.49)	53.32 (6.49)	0.486	50.71 (6.90)	52.16 (6.90)
PANAS –	negative	18.29 (7.00)	17.59 (8.34)	0.628	18.00 (5.01)	15.32 (4.67)	**0.028**	17.13 (6.14)	16.71 (7.86)
TMMS –	attention	25.75 (5.83)	25.75 (6.88)	1.000	25.54 (5.91)	27.60 (6.59)	**0.021**	25.27 (7.02)	27.17 (7.50)
TMMS –	clarity	25.63 (5.18)	27.69 (5.87)	0.053	28.65 (5.76)	31.48 (4.52)	**≤ 0.001**	27.17 (6.35)	29.50 (6.00)
TMMS –	repair	28.56 (4.26)	29.00 (6.28)	0.718	29.27 (4.82)	30.20 (4.83)	0.409	29.43 (6.07)	31.30 (6.58)
EVEA –	sadness	2.66 (2.51)	2.03 (2.53)	0.195	2.57 (2.42)	1.75 (1.57)	**0.035**	1.73 (1.56)	1.02 (1.26)
EVEA –	anger	1.78 (2.00)	1.72 (2.00)	0.894	1.94 (1.99)	1.23 (1.48)	**0.044**	1.20 (1.67)	1.23 (1.48)
EVEA –	anxiety	2.49 (2.48)	3.12 (2.86)	0.194	2.79 (1.80)	2.20 (2.07)	0.131	2.69 (2.36)	2.29 (2.41)
EVEA –	happines	6.82 (1.94)	7.16 (1.70)	0.429	7.31 (1.75)	7.31 (2.02)	1.000	7.03 (1.75)	7.67 (1.58)

P value of intragroup changes before and post-intervention obtained by repeated-measures ANOVA.

F, p value, and η^2 of analysis of covariance adjusting by baseline values.

*Boldface values indicate significance differences ($P < 0.05$). Abbreviations: HIIT-Alcohol, group that performed high-intensity i beverage; HIIT-NonAlcohol, group that performed high-intensity interval training and consumed non-alcoholic beverage; SV Depression Inventory; LOT-R, Life Orientation Test Questionnaire; Pittsburgh SQI, Pittsburgh sleep quality index; SHS, Scale of

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Mood States Questionnaire; PSS, Perceived Stress Scale; PANAS, The Positive and Negative Affect Schedule; TMMS-24, Trait Mood Assessment.

Mood adaptations during a HIIT program

Figure 2 shows changes in perceived stress (measured by PSS; Fig. 2A), and positive and negative affect (measured by PANAS; Fig. 2B), tested every 2 weeks from the baseline to 10-week of the HIIT program. No significant changes were found during the intervention in perceived stress ($p > 0.05$; see Fig. 2); whereas significant differences over time were found in positive and negative affect ($F_{(5,310)} = 3.792$, $p = 0.003$, $\eta^2 = 0.051$, and $F_{(4,297)} = 4.436$, $p \leq 0.001$, $\eta^2 = 0.060$, respectively; see Fig. 2B and 2C). However, any group showed significant differences over time in positive and negative affect (all $p > 0.05$; see Fig. 2). Similarly, no significant differences between groups were observed in any variable ($p > 0.05$; see Fig. 2).

Figure 3 shows changes in the attention, clarity, and repair subscales of emotional intelligence (measured by TMMS-24), tested every 2 weeks from the baseline to 10-week of the HIIT program. Significant differences over time were found in the attention and clarity subscales; however, no significant time effect was found in the repair subscale ($p > 0.05$; see Fig. 3A and 3B). No significant differences over time were observed in any group (all $p > 0.05$), neither significant differences between groups were found ($p > 0.05$; see Fig. 3).

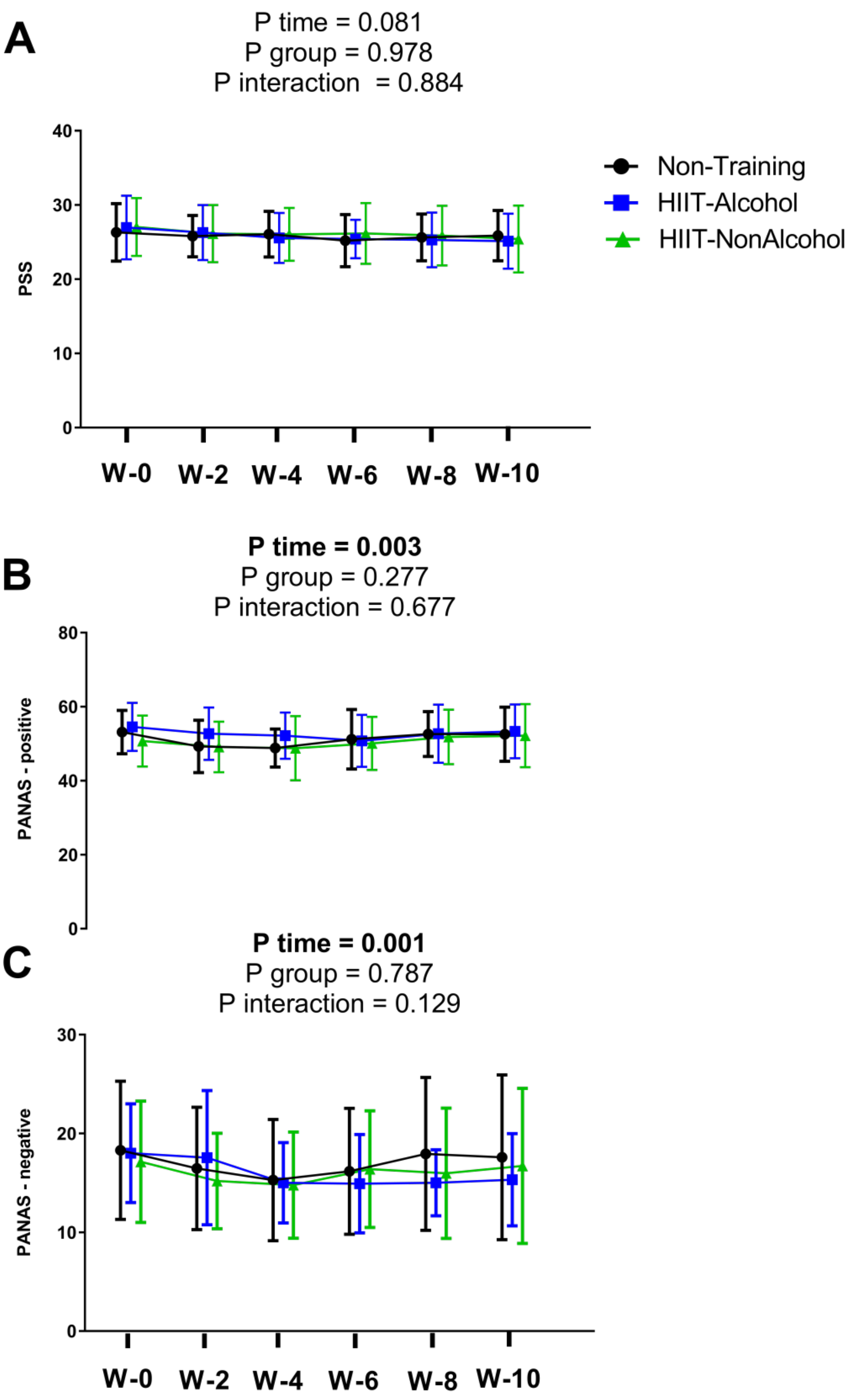

Figure 2. Changes in perceived stress (A) measured by PSS, positive (B), and negative (C) affect measured by PANAS, tested each 2 weeks (Week 0, 2, 4, 6, 8, and 10) during the intervention study. P time, P group, P interaction (time x group) of repeated measures analysis of variance (ANOVA), with a with a post hoc Bonferroni-corrected test. Data are presented as mean and standard deviation. Abbreviations: HIIT-Alcohol, group that performed high-intensity interval training and consumed alcoholic beverage; HIIT-NonAlcohol, group that performed high-intensity interval training and consumed non-alcoholic beverage; PSS, Perceived Stress Scale; PANAS, The Positive and Negative Affect Schedule.

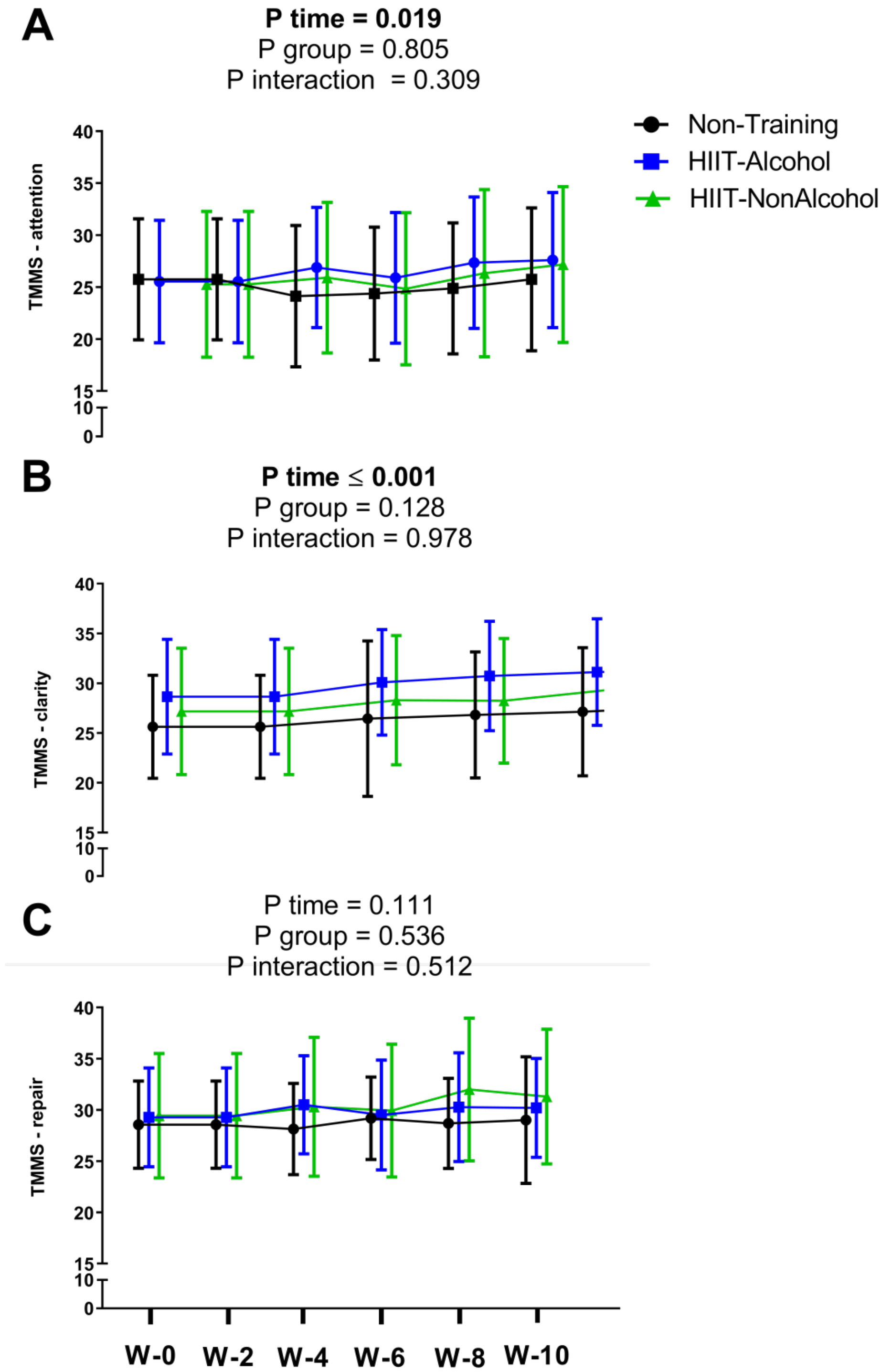

Figure 3. Changes in the attention (A), clarity (B), and repair (C) subscales of TMMS, tested each 2 weeks (Week 0, 2, 4, 6, 8, and 10) during the intervention study. P time, P group, P interaction (time x group) of repeated measures analysis of variance (ANOVA), with a with a post hoc Bonferroni-corrected test. Data are presented as mean and standard deviation. Abbreviations: HIIT-Alcohol, group that performed high-intensity interval training and consumed alcoholic beverage; HIIT-NonAlcohol, group that performed high-intensity interval training and consumed non-alcoholic beverage; TMMS, Trait Meta-Mood Scale.

Figure 4 presents changes in mood assessment (negative mood subscales: sadness-depression, anger-hostility, and anxiety; and happiness) measured by EVEA, tested every 2 weeks from the baseline to 10-week of the HIIT program. Significant differences over time were found in sadness, anger, and anxiety measures (all $p \leq 0.001$; see Fig. 4). Indeed, a significant difference in the sadness-depression subscale between week 2 and week 10 was found in HIIT-Alcohol group (CI = -2.149 to -0.147; $p = 0.013$). Moreover, significant differences over time in the anger-hostility and anxiety subscales were found in Non-Training group, HIIT-Alcohol group, and HIIT-NonAlcohol group (all $p < 0.05$).

Figure 5 presents changes in self-determined motivation in physical exercise (i.e., intrinsic, identified, introjected, external, and amotivation subscales; measured by BREQ-2) and goal orientation in exercise (i.e., goals oriented to tasks or ego; measured by GOES), tested at week 6 and week 9 of the HIIT program. A significant difference in the introjected subscale of motivation over time was observed in HIIT-NonAlcohol group (CI = -730 to -0.032, $p = 0.033$, see Fig. 3A). Indeed, significant differences between groups in the introjected subscale were found in week 6 (CI = 0.063 to 1.206, $p = 0.030$; see Fig. 5A). No significant changes over time were found in amotivation, identified, intrinsic, and external motivation (all $p > 0.05$). Further, a significant change over time in goals orientation to ego was found in the HIIT-Alcohol group (CI = 8.113 to 59.996, $p = 0.011$; see Fig. 5B). No significant changes were observed in goal orientation to tasks ($p > 0.05$).

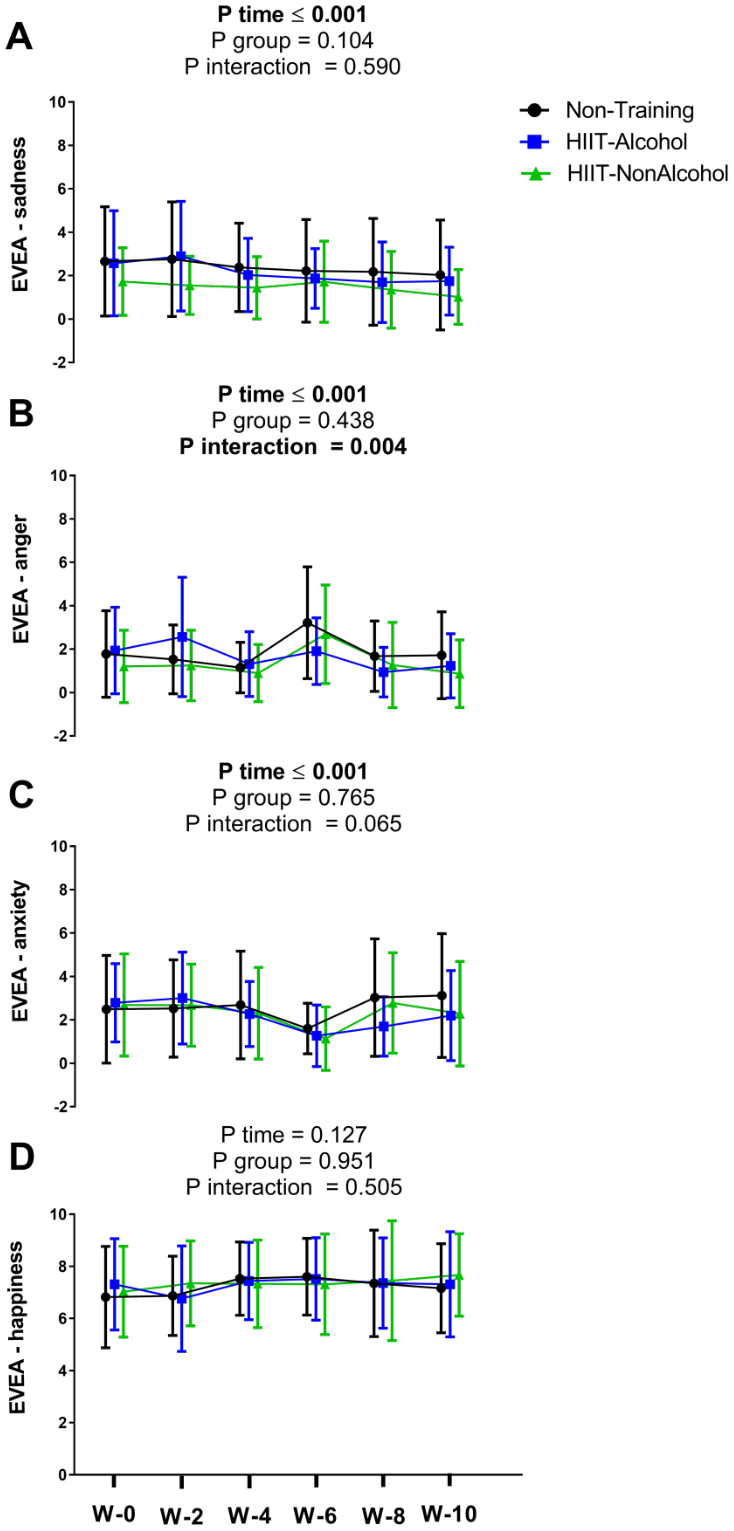

Figure 4. Changes in sadness (A), anger (B), anxiety (C), and happiness measured by EVEA, tested each 2 weeks (Week 0, 2, 4, 6, 8, and 10) during the intervention study. P time, P group, P interaction (time x group) of repeated measures analysis of variance (ANOVA), with a with a post hoc Bonferroni-corrected test. Data are presented as mean and standard deviation. Abbreviations: HIIT-Alcohol, group that performed high-intensity interval training and consumed alcoholic beverage; HIIT-NonAlcohol, group that performed high-intensity interval training and consumed non-alcoholic beverage; EVEA, Scale of Mood Assessment.

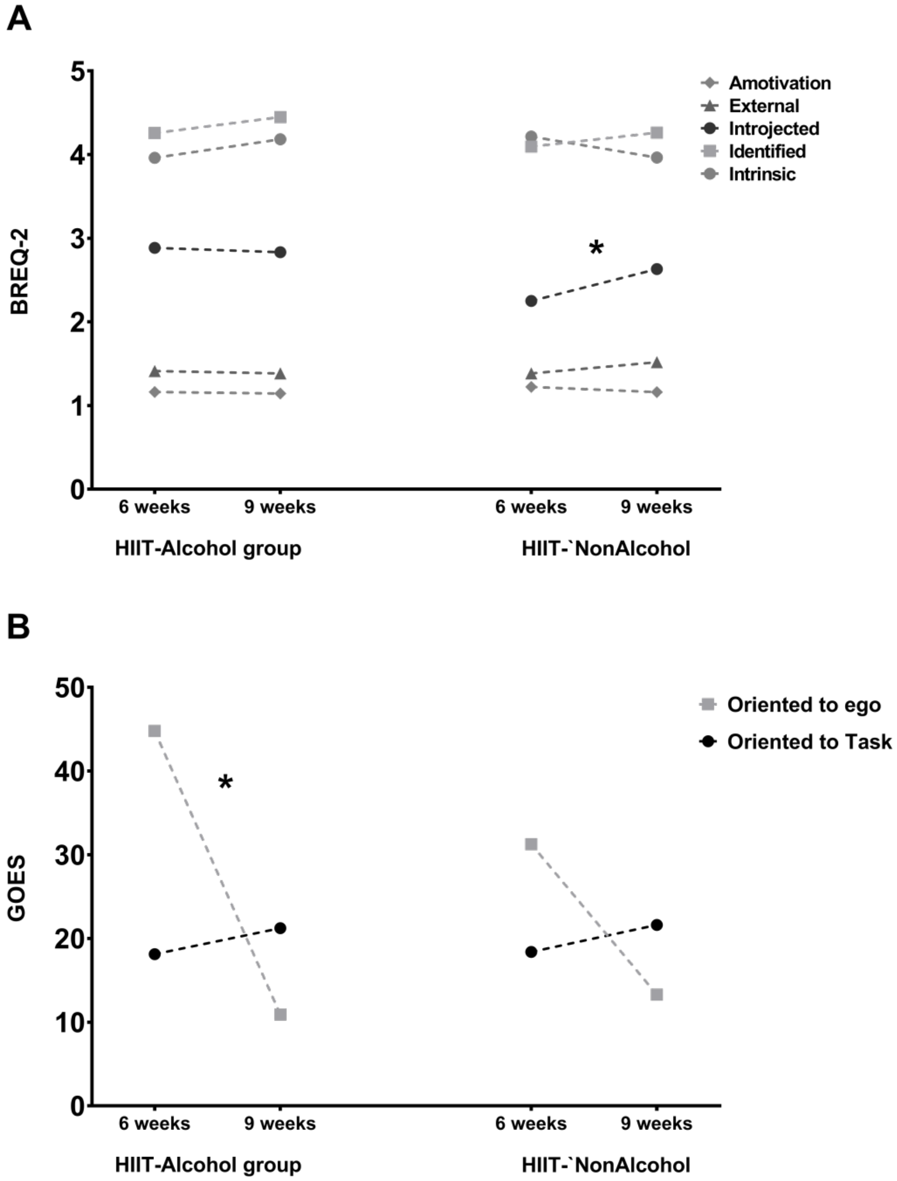

Figure 5. Changes in self-determined motivation (A) measured by BREQ-2, and goal orientation (B) measured by GOES, tested at week 6 and week 9 of the intervention study. Intragroup changes obtained by repeated-measures ANOVA (group x time, adjusting by time), with a post hoc Bonferroni-corrected test, are indicated as: * ($p < 0.05$) for HIIT-Alcohol, and å ($p < 0.05$) for HIIT-NonAlcohol group. Significant differences between groups, with post hoc Bonferroni-corrected test, are indicated as: ß HIIT-Alcohol group vs. HIIT-NonAlcohol group at week 6 ($p < 0.05$). Data are presented as mean and standard deviation. Abbreviations: HIIT-Alcohol (n=26), group that performed high-intensity interval training and consumed alcoholic beverage; HIIT-NonAlcohol, group that performed high-intensity interval training and consumed non-alcoholic beverage; BREQ, Behavioural Regulation in Exercise Questionnaire; GOES, Goal Orientation in Exercise Scale.

Discussion

Our results support the hypothesis that a highly demanding training program could elicit benefits on psychosocial variables (i.e., satisfaction with life, optimism, happiness, depression, and emotional intelligence) and mood adaptations (positive and negative affect) as consequence of exercising. Indeed, contraty to our expectations, it seems that a moderate alcohol consumption may not blunt those positive effects. Finally, our results showed increases in introjected motivation and decreases in the perception ego-involving motivation during the intervention.

Intervention effects of a HIIT program on psychosocial and mood variables

It has been found that high intensity exercise had additional benefits beyond relief of depression, including more significant improvements in health-related quality of life and sleep quality [15]. Our results agree with these findings since benefits were observed in psychosocial parameters, such as satisfaction with life, depression, and optimism in booth intervention group. Also, both training groups showed significant improvements in sleep quality after a10-week HIIT program. Indeed, HIIT has been proposed as a good strategy for obtaining positive psychological responses [14]. Regarding this, our results showed significant improvements in happiness, positive affect, optimism, and positive mood state after the 10-week HIIT intervention.

Contrary to exercise, beer consumption because of its ethanol contain and its deleterious effect, is not typically regarded as a health-promoting behavior. However, some research has suggested that alcohol consumption could have psychosocial benefits in sport and physically active people [66]. We cannot confirm that in our sample of healthy adults since no differences were found between HIIT-Alcohol group and HIIT-NonAlcohol group. Nevertheless, we did not find impairments in any psychosocial parameter because of the moderate alcohol consumption in healthy adults.

Mood adaptations during a HIIT program

Research indicates that affect experienced during the intense bursts of HIIT programs is not different from the affect experienced during continuous moderate-intensity exercise and is significantly more positive than continuous vigorous-intensity exercise [67]. We found an increasing of positive emotions during the HIIT intervention, as well as a decreasing of negative emotions. Therefore, as previous results have found in other population [16], we can confirm that HIIT can elicit a positive effect on depression emotional well-being.

Interestingly, HIIT-NonAlcohol group showed significant changes in anger-hostility and anxiety in week 6 compared to the rest of timepoints measured. It could be because week 6 was an important moment of changing timing and intensity of the HIIT program. Thus, these changes could be perceived as negative stressors for the participants, which might have affected their mood in a negative way.

Conclusions

The present study provided evidence for understanding the benefits of a highly demanding training program on psychosocial outcomes and mood state adaptations. Indeed, our results suggest that moderate alcohol consumption does not disrupt those positive effects of exercise on psychological variables. However, caution should be taken when drinking alcohol, even in moderate amounts.

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Authors' contributions

CMH participated in the design of the study, contributed to data collection, data reduction/analysis, and interpretation of results, and contributed to the manuscript writing – original draft; FAG participated in the design of the study, contributed to the interpretation of results and participated in the manuscript writing – original draft; MC participated in the design of the study and participated in the manuscript writing – review and editing; AC participated in to the manuscript writing – review and editing.

Competing interest

The authors declare that they have no competing interests.

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Table S1. Changes in Cognitive measures adjusted by baseline values and sex (Model 1), by baseline values and age (Model 2), and by baseline values and occupational activity (Model 3).

Abbreviations: SWLS, Satisfaction with Life; BDI-II, Beck Depression Inventory; LOT-R, Life Orientation Test Questionnaire; Pittsburgh SQI, Pittsburgh sleep quality index; SHS, Scale of Subjective Happiness; POMS,

Models	Model 1			Model 2			Model 3		
	Analysis of covariance								
	F	P value	η^2	F	P value	η^2	F	P value	η^2
Baseline and after 10-week measures									
SWL	3.303	0.043	0.091	3.058	0.054	0.085	2.885	0.063	0.080
BDI-II	2.521	0.088	0.073	1.311	0.277	0.039	1.436	0.245	0.043
LOT-R	2.753	0.072	0.082	2.956	0.059	0.087	2.865	0.065	0.085
Pittsburgh SQI	0.029	0.971	0.001	0.091	0.913	0.003	0.095	0.909	0.003
SHS	0.345	0.710	0.011	0.490	0.615	0.016	1.197	0.309	0.038
POMS – angry	0.415	0.662	0.013	0.285	0.753	0.009	0.905	0.410	0.027
POMS – fatigue	2.594	0.083	0.075	1.048	0.357	0.032	1.642	0.202	0.049
POMS – vigor	2.448	0.095	0.071	3.134	0.050	0.089	3.524	0.035	0.099
POMS – tension	2.889	0.063	0.083	2.768	0.070	0.080	4.423	0.016	0.121
POMS – depression	0.932	0.399	0.028	0.342	0.712	0.011	1.212	0.304	0.036
Every 2-week measures									
PSS	0.415	0.662	0.012	0.014	0.986	0.000	0.105	0.900	0.003
PANAS – positive	0.013	0.987	0.000	0.001	0.999	0.000	0.197	0.822	0.006
PANAS – negative	1.030	0.363	0.029	1.060	0.352	0.030	1.525	0.225	0.030
TMMS – attention	1.009	0.370	0.030	1.670	0.196	0.048	1.216	0.303	0.036
TMMS – clarity	1.001	0.373	0.029	1.459	0.240	0.042	1.143	0.325	0.033
TMMS – repair	0.895	0.414	0.026	0.703	0.499	0.021	0.715	0.493	0.021
EVEA – sadness	1.010	0.369	0.028	0.885	0.417	0.025	0.996	0.375	0.028
EVEA – anger	0.961	0.388	0.027	0.798	0.454	0.022	1.409	0.251	0.039
EVEA – anxiety	2.508	0.089	0.067	0.933	0.398	0.026	1.949	0.150	0.053
EVEA – happiness	0.911	0.407	0.025	0.859	0.428	0.024	0.738	0.482	0.021

Profile of Mood States Questionnaire; PSS, Perceived Stress Scale; PANAS, The Positive and Negative Affect Schedule; TMMS-24, Trait Meta-Mood Scale-24; EVEA, Scale of Assessment of Mood.

INTEGRATIVE DISCUSSION

Physical activity is an integral part of a healthy lifestyle and physical exercise is an excellent tool to preserve health, enhance well-being and improve physical and mental performance. For that, the most effective physical exercises are those leading to improvements in physical fitness and, consequently, are part of a structured training program [1]. High intensity interval training (**HIIT**) is a novel form of training particularly effective and efficient since it allows achieving results with less time investment [2,3]. Although gender differences are obvious in physical and metabolic mechanism concerning exercise, this issue has not generally received much attention [4], and data derived from men should not be directly applicable to women.[5]

Improving body composition and/or performance are two main aims of training. HIIT leads to improvements in body composition, reducing body fat levels, increasing fat free mass and bone mineral content [6,7]. HIIT also elicits improvements in aerobic capacity, resistance and muscle strength [6,8,9]. Physical activity enhances cognitive function including better processing speed, attention, and memory. Also it shows positive associations with self-perceptions, self-esteem, and mental well-being in young adults [2,10–13]. Recent studies have reported the positive effects of HIIT on cognitive function, however contradictory results have been found related to performance on the executive function tasks [12].

In order to achieve improvements associated to training, as important as exercise is the recovery process after training. Recovery requires sufficient rest, good nutrition, and the absence of toxic elements that might hinder the recovery process. Regarding to the recovery process, beer is consumed by many healthy adults to quench thirst in preference to other beverages particularly after a hard day work, as part of social relationship, or after practicing exercise [14,15]. This is particularly the case in a recreational context, where having a beer after a match is considered part of the social aspect of many sport activities [16–18]. Beer intake accompanying meals (for example, in the context of the Mediterranean diet) is common practice for many physically active individuals. Beer is more than 90% water, but it also contains around 4-7% alcohol [19,20]. This can have negative effects on the rehydration process and in the post-effort recovery process [21]. In addition to water and alcohol, other beer components, such as polyphenols, lupulins, dextrin, vitamins, or minerals can also influence the recovery process, either physically or mentally. Due to its alcohol content, beer consumption during the recovery phase, or ingested on a regular basis with meals, can be questioned particularly when training [22]. Therefore, it is important to clarify the existing controversy regarding the consumption of beer while practicing exercise or, even more, while following a highly demanding training program, such as HIIT [16,23–26]. Again, gender differences in relation to the effect of alcohol should be taken into account given the different impact that alcohol may have in the male and female body [27].

The BEER-HIIT study has been designed to shed light on the existing controversy about the moderate beer and alcohol consumption in relation to exercise or, more particularly, while training, in healthy individuals [23,25,26,28]. The primary aim of the study is to evaluate the effects of a HIIT program on body composition and physical performance, as well as in mental performance and psychosocial aspects; and subsequently, to assess whether those effects are influenced by the regular consumption of beer, or its equivalent alcohol content, in moderate amounts. Our study specifically investigates, in conditions reflecting the real-life situation of healthy young individuals, the possible interference of regular but moderate beer intake, or its alcohol equivalent, on the benefits that the practice of intensive training may have on physical and mental variables, and clarifying the role played by ethanol. We characterize the consequences of training versus non-training, and then, we studied the effects of simultaneously consuming alcohol-containing beer, alcohol-free beer, and sparkling water with alcohol added.

The findings derived from this study have a social and scientific interest as well as significant implications for our understanding of training and its effect on physical performance and mental health, also it can be of help for understanding the psychological responses during a training intervention, all of that in a real context situation. Further, this study can provide answers to a particular interest of the society about the convenience, or not, of consuming beer or alcohol beverages while being involved in a highly demanding training program.

EFFECT ON BODY COMPOSITION (STUDY 1)

HIIT has demonstrated to be effective in the improvement of body composition, by reducing fat mass [29,30] and increasing muscle mass [31], in normal-weight and also in overweight-obese individuals [32]. Actually, there is robust and growing evidence showing that HIIT may elicit greater benefits that moderate-intensity continuous training across a range of health markers [29]. Among them, HIIT has demonstrated to be effective in reducing total, abdominal, and visceral fat mass in both males and females [32]. Similarly, different HIIT protocols have resulted in modest increases in total body and trunk lean mass [33–35].

There is controversy regarding the influence of beer consumption on fat distribution and body composition [19,36–40]. Some authors have reported that moderate beer consumption is not associated with changes in body weight or body composition parameters [19,37,39,40], while others have reported that alcohol consumption is associated with increased adiposity in adults [36,38]. The intake of beer in the post-exercise recovery phase has been questioned [14,41], and alcohol intake can have influence not only in fat mass but also in lean mass [42]. It has been reported that alcohol can suppress the anabolic response to physical exercise through the reduction of muscle protein synthesis [42].

The primary findings of our study are that 10-weeks of HIIT did not influence body weight, but significantly decreased fat mass and fat mass percentage while increasing lean mass. These positive effects were not affected by the concomitant regular intake of beer, or its alcohol equivalent, in moderate amounts. Neither HIIT nor beer or alcohol intake influenced adipose tissue distribution or bone mineral density. The lack of effect on body weight or BMI was the result of the simultaneous decrease in fat mass and increase in lean mass.

EFFECT ON PHYSICAL FITNESS (STUDY 2)

Physical fitness integrates several components [43], such as cardiorespiratory fitness and muscular strength, which are widely recognized as powerful markers of sport performance, health-related outcomes and relevant determinants of current and future health status [43–45]. Emerging evidence suggests that HIIT, which involves repeated bouts of intense exercise interspersed with periods of rest or lower-intensity active recovery, is an effective strategy to get important improvements in cardiorespiratory fitness [8,9,46–48] and muscular strength [8,9,46–49].

After training, optimal resting and adequate nutrition are crucial to allow not only the recovery of energy reserves, but also the removal, regeneration, repair and protection of damaged or worn structures [50,51]. This brings the functional structures to a state of adaptation with supercompensation. These adaptations improve exercise performance, promote health as well as well-being [52–55] and represent the physiological basis of the multiple and positive benefits of moderate-to-vigorous exercise training [47]. There is a concern about the effects that beer can have on hydration, recovery, and exercise performance due to the effects of alcohol [56]. These negative effects can be associated with a reduced production of testosterone and its subsequent effects on protein synthesis and muscular adaptation/regeneration, and with an increase in the diuretic response of the body due to the inhibitory effect of ethanol on vasopressin release [26,57,58]. Even though the damages associated with a high intake of alcohol have been widely studied and are beyond doubt [26], the effects of moderate doses remain under debate [19,20]. Some authors conclude that alcohol consumed after an exercise session could interfere with some aspects of the performance, such as strength recovery [26]. However, a low dose of alcohol seems not to affect strength recovery [59], neither power recovery in men [60]. The majority of literature has been unable to establish a significant cause-effect relationship of alcohol with aerobic or anaerobic performance. While some studies have not found significant consequences of alcohol for submaximal endurance performance [61,62], other research have suggested that alcohol is detrimental to endurance performance [17,23].

Our study shows that a 10-week structured and highly demanding exercise intervention improves cardiorespiratory fitness and hand grip strength in healthy young adults. This was not influenced by the concurrent daily intake of beer or ethanol in moderate amounts. No significant improvements were found in muscular power variables. Therefore, moderate and daily beer consumption accompanying meals, like in the present study, appears not to be an issue of concern affecting physical fitness parameters such as cardiorespiratory fitness and muscular strength after a HIIT program in healthy young adults.

EFFECT ON COGNITIVE FUNCTION - PSYCHOMOTOR SPEED (STUDY 3)

The evidence about the positive relationship between exercise and improvements in brain function and cognition suggests that those benefits could be specific to some period of the lifespan, such as older adults and children [63–65]. There is converging evidence that regular physical exercise across the lifespan has beneficial effects on cognition enhancing neuroplasticity and preventing cognitive age-related decline [64,66,67]. In this regard, RT is a widely used measurement of cognitive functioning [68,69]. It has also been considered as a putative element of executive functions [70], including processing or perceptual speed, motor and sensory components, and inhibitory control [71,72]. Although little is known about the effects of exercise training on RT, it has been previously shown that regular physical exercise is effective at improving cognitive functions [65,66,73]. Similarly, there is limited scientific knowledge regarding the effect of moderate alcohol consumption on motor control and performance of skilled tasks [74]. It has been suggested that the behavioural effects of alcohol consumption on the central nervous system may be dependent of the pattern of intake and the doses ingested [75]. Although no cognitive impairments have been shown in response to an acute low dose of alcohol intake [75,76], a significant decline of cognitive function has been observed after the ingestion of high alcohol doses [77]. However, data are less clear regarding the effect of moderate alcohol consumption on cognitive function (i.e., RTs) while engaged in an exercise training intervention.

Our primary hypothesis was that a highly demanding training program might improve the RT performance, but these positive effects could be blunted by habitual alcohol intake. This hypothesis was not fully supported by the obtained results since all intervention groups decreased RT and increased accuracy, independently of the beverage intake. Along these lines, these improvements seem to be not attributable to the HIIT intervention since the Non-Training group presented similar enhancements. Our secondary hypothesis was that retesting might produce an improvement in RT task performance, and this was supported by our findings since we found improvements in RT and accuracy over the several test administrations. In this regard, our results suggest that 10-week of HIIT program could facilitate the learning process due to retesting. Finally, our findings support the "power law of practice" which assumes that retest effects decrease with the number of test administrations [71,78].

The present study suggests that exercise training can have a protector effect on cognitive function over moderate alcohol consumption. However, we cannot conclude that a HIIT intervention program elicits cognitive improvements in healthy young adults. Although prolonged periods of abusing alcohol are undoubtedly harmful to the brain impairing inhibitory control or divided attention among other negative effects on cognitive functions [19,79,80], our results show that a habitual but moderate alcohol intake does not have negative effects on the improvements of cognitive performance over time. Moreover, our study indicates that retesting produced an improvement in RT task execution (speed and accuracy) showing a loss of this practice effect when the temporal difference was greater. Additionally, we found that subjects have faster responses when the pressure becomes higher, and these effects were not influenced by regular alcohol intake.

EFFECT ON COGNITIVE FUNCTION – MEMORY, ATTENTION, AND VERBAL FLUENCY (STUDY 4)

Although previous results about the positive relationship between physical exercise and cognition are encouraging, there are still major gaps in our understanding of the effects of exercise intervention on cognition in people with different biological characteristics, particularly in young to middle-age adulthood (ages 18-50 years) [64,73]. Regarding to this population, some notorious enhancements in cognitive function (i.e., visual-spatial and short-term memory) have been found after 6 to 12 weeks of programmed exercise [81,82], although the pattern of the exercise-cognition effects has been inconsistent in this population [73]. Emerging findings have suggested that HIIT could provide additional physiological and psychological adaptations in higher-order cognitive functions and flexibility cognitive performance compared with lower intensity exercise in older adults [83]. Further, improvements in cognitive flexibility have been found after 7 week of an interval training intervention in young active individuals [84].

We intended to test the hypothesis that a HIIT program would improve cognitive function in healthy young adults but our results did not completely support our hypothesis because, although improvements were found in learning process, memory, processing speed, inhibitory control, and verbal fluency, we cannot conclude that those benefits were elicited by our HIIT intervention since the Non-Training group showed similar enhancements. These findings are somewhat consistent with those obtained by similar investigations applying HIIT interventions [12,84]. A potential reason for the lack of differences across groups on cognitive function could be young age of the included participants and their healthy-active condition as well [85].

On the other hand, although is clear the deleterious effects of excessive consumption of alcohol on general health [37], uncertain results have been reported regarding the effects of low to moderate alcohol consumption on cognitive health [86]. Interestingly, our results suggest that moderate alcohol consumption does not negatively influence those beneficial adaptations on cognitive function. Additionally, in line with our hypothesis, the current findings show that changes in physical fitness after the HIIT intervention were positively correlated to changes in cognitive function in our young adults' cohort. These findings suggest that cardiorespiratory fitness may have a great impact on challenging and complex cognitive processes.

EFFECT ON PSYCHOSOCIAL AND MOOD STATE ADAPTATIONS (STUDY 5)

The interaction of exercising with others lifestyle factors in influencing cognition and brain health has been explored for some researchers [73,87,88]. It has been emphasized the importance of how those factors could affects to the exercise-cognition relation. In previous studies, HIIT has been shown effective in reducing depression in men with pathologies [89]. Also, research has indicated positive affective responses and perceived enjoyment of HIIT [90]. There is a scholastic evidence linking regular exercise with positive mental wellbeing, as well as lower psychological reactivity to mental stress [91]. Even more, several studies have described the benefits of exercise training in the prevention of mental disorders such as depression [92]. Indeed, exercise is consistently associated with lower anxiety symptoms, and habitual exercisers experienced reduced stress reactivity [93]. It has also been noted that the largest improvements in anxiety were elicited by those exercise intervention that (i) lasted no more than 12 weeks, (ii) used exercise sessions lasting at least 30 min, and (iii) measured persistent anxiety lasting for more than 1 week [91].

The consumption of beer with food and after exercising is a common practice in physically active individuals and in amateur athletes, particularly when the level of perspiration is important [74,94,95]. Alcohol has shown to decrease sensitivity to pain, improve confidence, and modulate the rating of perceived exertion [21,96]. Indeed, the relationship between alcohol use and mood has been characterized as a J- or U-shaped curve, such that moderate drinkers tend to report better subjective well-being and fewer depressive symptoms than alcohol abstainers and excessive drinkers [97].

Our study shows that a 10-week structured and highly demanding exercise intervention improves psychosocial parameters and mood adaptations in healthy young adults. Also, those benefits seem not to be affected by the moderate consumption of alcohol. Interestingly, alcohol could elicit improvements in negative mood and affect assessments. There is a general consensus that there are many psychological and social health benefits associated with participation in sport for adults [87]. However, the mechanisms remain unknown. Some of them proposed as mediators of the psychological effect of exercise are primarily based on the volume and/or duration and intensity of exercise, but most of the theories suggested have been challenged because the placebo mechanism proposed to play a role on benefits of exercise on feeling states. Some authors suggest that the subjective aims and exercise-related expectations, in combination with the actual psychophysiological mechanisms involved in a particular exercise, jointly determine the perceived and experienced psychological effects of exercise [91]. However, despite the possibility that physical benefits on psychological wellbeing may occur via placebo effect, from a mental health perspective both aerobic (endurance) and anaerobic (strength) exercises have been shown beneficial long-term effect on psychological health [91].

GENERAL LIMITATIONS

The findings of the present International Doctoral Thesis should be considered with caution due to a number of limitations. Specific limitations of each study are presented in the discussion section of each study and an overall view of the main limitations is presented here:

- The present study presents several limitations largely because of ethical constraining pure randomization. In spite of the convenience of practicing regular physical activity, inclusion in a group to be submitted to highly demanding training (therefore susceptible to cause stress and injuries) must remain a personal option. Again, based on ethical considerations and following the advice of the ethical committee (321-CEIH-2017), it does not seem appropriate to push a healthy young adult to drink alcohol if he/she does not desire or have the habitude. Thus, a real double-blind design, placebo controlled for alcohol, was not possible. Subsequently, future studies are required to determine the effects of the same training intervention in participants with similar biological characteristics to corroborate our findings.
- The control group was not purely sedentary since it was instructed to keep an active lifestyle. Although, physical activity levels were registered after the intervention program, it was performed by a subjective method (i.e., self-reported). Thus, future research should include the assessment of physical activity levels by objective methods (i.e., accelerometer system) during a HIIT ntervention program.
- The sample size of the BEER-HIIT study was relatively small to study the influence of different alcohol beverages in moderate amounts during an exercise training intervention on physical fitness outcomes (i.e., cardiorespiratory fitness, muscular strength, and body composition) considering both sexes separately, although no interaction effects were observed. Taking into consideration that we compared a total of four different types of beverages ingested, our study could be underpowered to note statistical differences in specific physical fisness-related parameters between them. Therefore, further studies are needed to clarify the long-term effects (>10 weeks) of HIIT and other training modalities with the ingestion of a moderate dose of alcohol on physical fitness parameters, such as cardiorespiratory fitness or body composition.
- Dietary data was not collected during the intervention program; thus, we did not control the food ingestion during that tim. However, we assessed the adherence to the Mediterranean Diet by PREDIMED questionnaire before the intervention, finding that our sample had not adherence to Mediterranean diet. Therefore, our results could be extrapolated to other dietary patterns. Further studies are needed to clarify what is the role of the dietary pattern on body composition parameters during an exercise program intervention combined with moderate alcohol consumption.
- All the studies were carried out in a cohort of healthy young adults (18 to 35 years old). Therefore, these data should not be extrapolated to other populations with different

biological characteristics. Therefore, it is mandatory to replicate the present studies on different populations (i.e., older, sedentary, and/or individuals with different ethnicity and health status).

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CONCLUDING REMARKS AND FUTURE PERSPECTIVES

GENERAL CONCLUSION

Collectively, the findings from the present International Doctoral Thesis have a social and scientific interest as well as significant implications for our understanding of training and its effect on physical performance and mental health, also it could help to understand the psychological responses during a training intervention, all of that in a real context situation. Further, this study can provide answers to a particular interest of the society about the convenience, or not, of consuming beer or alcohol beverages while being involved in a highly demanding training program. Finally, this study could help to understand the role that alcohol may play into subjective perceptions, mood state, and psychosocial variables while undergoing an intensive training program.

The results of our study are both intriguing and provocative; however, they only scratch the surface in terms of understanding the manner in which different lifestyle factors, such as moderate alcohol comsuption and exercise training, interact to promote physical and cognitive improvements. Clearly, additional observational and experimental studies are needed to further explain the effects of these interactions with regards to cognition.

SPECIFIC CONCLUSIONS

The specific conclusions reached in the studies included in this International Doctoral Thesis are detailed as follow:

SECTION 1: Combined effects of a highly demanding training program and moderate alcohol/beer consumption on physical performance

- **Study 1.** In young healthy adults, a 10-weeks HIIT program determines improvement in body composition by decreasing fat mass and increasing lean mass, and these positive effects are not influenced by the concomitant intake of beer, or its alcohol equivalent, in moderate amounts. In addition, the intake of beer, or its alcohol equivalent, while exercising, does not affect body fat distribution.
- **Study 2.** In young healthy adults, a 10-weeks HIIT program determines improvement in cardiorespiratory fitness and hand grip strength. This was not influenced by the concurrent intake of beer in moderate amounts. Although we did not find deleterious effects of post-exercise alcohol consumption, the use of alcohol after strenuous exercise should be managed carefully.

SECTION 2: Combined effects of highly demanding training program and moderate alcohol/beer consumption on cognitive performance

- **Study 3.** Although exercise training can have a protector effect on cognitive function, we cannot conclude that a HIIT intervention elicits cognitive improvements in our sample of healthy young adults. Re-testing produced improvements in RT task execution (speed and accuracy) showing a loss of this practice effect when the temporal difference was greater. Additionally, the subjects have faster responses when the pressure becomes higher. A habitual but moderate alcohol intake did not interfere with these effects.
- **Study 4.** Although the improvements found in cognition cannot be attributed to the HIIT intervention, no significant impairments in cognitive functions were noted due to alcohol intake in moderate amounts. Nevertheless, training-induced changes in physical fitness are associated with changes in cognitive performance.

SECTION 3: Combined effects of highly demanding training program and moderate alcohol/beer consumption on psychosocial variables and mood state

- **Study 5**. A 10-week HIIT intervention program elicited improvements in psychological parameters, as well as mood state. Also, changes in introjected motivations and goal orientation to ego were found during the HIIT intervention program. No significant impairments in psychological wellbeing nor mood state were noted due to moderate alcohol consumption.

FUTURE PERSPECTIVES

- Future randomized controlled trials with simultaneous intervention (> 3 months) of exercise training (i.e., different modalities and intensities) and alcohol consumption (i.e., light and moderate consumption, and daily or binge drinking) should measure the effects on cognitive function in young healthy adults.
- Future work should address the gaps in our understanding of how behavioral mechanisms modulate the effects of exercise training on cognition by conducting RCTs with participants aged across the lifespan and measuring both behavioral (i.e., mood, motivation, depression, anxiety…) and cognitive outcomes at multiple time points.
- The results of the present Doctoral Thesis suggest that HIIT do not provide additive benefits in cognitive performance in young healthy adults. Therefore, further studies are needed to well-elucidated the potential benefits of exercise on cognition during young and middle adulthood. Furthermore, future studies should test using neuroimaging techniques (i.e., EEGs or fMRI) whether some changes in brain structure and function could be find, suggesting a better allocation of attentional brain resources.
- Finally, it is worth investigating whether the mechanisms that support the physical activity/exercise–cognition relationship are different in children and adults. Hence, it would help to understand the heterogeneous results found between different stages of life.

ANNEXES

PAPERS DERIVED FROM THE DOCTORAL THESIS

PUBLISHED & INCLUDED IN THIS DOCTORAL THESIS

1. Molina-Hidalgo, C., De-la-O, A., Jurado-Fasoli, L., Amaro-Gahete, F. J., & Castillo, M. J. 2019. Beer or ethanol effects on the body composition response to high-intensity interval training. The BEER-HIIT Study. Nutrients, 11(4), 909.
2. Molina-Hidalgo, C., De-la-O, A., Dote-Montero, M., Amaro-Gahete, F. J., & Castillo, M. J. (2020). Influence of daily beer or ethanol consumption on physical fitness in response to a high-intensity interval training program. The BEER-HIIT study. Journal of the International Society of Sports Nutrition, 17, 1-13. (This article was Editor's pick).

SUBMITTED & INCLUDED IN THIS DOCTORAL THESIS

1. Molina-Hidalgo, C., De-la-O, A., Jurado-Fasoli, L., Amaro-Gahete, F. J., Catena, A., & Castillo, M. J. Beer or Ethanol Effects on the Response to High-Intensity Interval Training: Description of an Intervention Study in Young Healthy Individuals. The BEER-HIIT Study. Alcohol. 2020 (under review).
2. Molina-Hidalgo, C.; Amaro-Gahete, F. J.; Carneiro-Barrera, A.; Castillo, M. J.; Catena, A. Effect of Moderate Alcohol Intake During a High-Intensity Interval Training Intervention on Choice Reaction Time Task in Healthy Adults: the BEER-HIIT Study. J Sport Heal Sci 2021 (submitted).
3. Molina-Hidalgo, C.; Amaro-Gahete, F. J.; Peven, J.; Erickson, K. I.; Castillo, M. J.; Catena, A. Does exercise have a protective effect on cognitive function over alcohol consumption? A rational research in healthy adults. J Sport Heal Sci 2021 (submitted).
4. Molina-Hidalgo, C.; Amaro-Gahete, F. J.; Carneiro-Barrera, A.; Castillo, M. J.; Catena, A. Psychosocial and Mood state adaptations during a highly demanding training program in conjunction with moderate alcohol/beer intake in healthy adults. The BEER-HIIT study. (OUTGOING).

SHORT CURRICULUM VITAE

1. Personal information

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2. Academic social networking site profiles

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4. Education

2019-2021	PhD Student in Psychology - Neuroscience of Behavior, Cognition and Affection, University of Granada, Spain.
2017-2018	Master´s degree in Neuroscience and Pain (Grade: 9.625/10), University of Granada, Spain.
2015-2016	Master´s degree in Sport Psychology and Coaching. IESPORT/Instituto de Estudios Deportivos, Barcelona, Spain.
2011-2015	Bachelor´s degree in Psychology (Grade 7.308/10), University of Granada, Spain.

5. Courses and extracurricular activities:

March – June 2020	Understanding the Brain: The Neurobiology of Everyday Life. Coursera, University of Chicago.
May 2020	Techniques for the study of bioactive compounds in preclinical experimental models. Doctoral Programmes of School of Health Sciences. University of Granada.
May 2020	Strategies to optimize the writing, publication and communication of scientific articles. Doctoral Programmes of

	School of Health Sciences. University of Granada.
April – May 2020	Systematic review of studies. Meta-analysis. Doctoral Programmes of School of Health Sciences. University of Granada.
October – November 2019	Emotional Intelligence. School of Psychology. University of Granada.
September 2019	International experiences on exercise, brain and smartphone interventions: The SmarterMove Seminar. Sport and Health University Research Institute (iMUDS), University of Granada
September 2019	Scientific disclosure. Doctoral Programmes of School of Health Sciences. University of Granada.
September – October 2018	Science of Exercise – Learning about physiology. Coursera, University of Colorado Boulder.
September 2018	Basic cognitive processes. Instituto de Altos Estudios Universitarios en Abierto.
April 2015	Emotional Intelligence and Coaching. School of Education. University of Granada.

6. Supervision

2019-2020	Professional tutor of external practices. Certificate of Higher Education in Nutrition and Dietetics, University of Granada, Spain.
2019-2020	Professional tutor of external practices. Certificate of Higher Education in Nutrition and Dietetics, University of Granada, Spain.
2019-2020	Mentor of Master's Program. Master's Degree in Research in Sport and Physical Activity, University of Granada, Spain.
2019-2020	Mentor of Master's Program. Master in Research, University of Granada, Spain.
2018-2019	Mentor of Degree's Program. Degree in Medicine, University of Granada, Spain.
2018 – 2019	Professional tutor of external practices. Degree in Psychology, University of Granada, Spain.
2017 – 2018	Professional tutor of external practices. Degree in Psychology, University of Granada, Spain.

7. Research projects

2019 - present	AGUEDA - Active gains in brain using exercise during aging.
2018 - 2019	Randomized double-blind clinical trial on the efficacy and safety of the probiotic VSL3 in the treatment of patients with fibromyalgia and associated gastrointestinal symptoms.
2017 – present	BEER-HIIT Project: Beer or Ethanol Effects on the Response to High Intensity Interval Training: A Controlled Study in Healthy Individuals.
2015 – present	FIT-AGEING Project: Exercise training as S-Klotho protein stimulator in sedentary healthy adults.
2017 - present	Effects of an exercise-based randomized controlled trial on cognition, brain structure and brain function in overweight preadolescent children. Minister of Economy and Competitiveness.
2017	Multicenter study to assess neuropsychological tests in children.

8. International Internships

2020	University of Pittsburgh, Faculty of Psychology, Pittsburgh, Pennsylvania. Prof: Kirk I. Erickson. Duration: 3 months.

9. National Internships

2018	Biomedical Research Center-CIBM. University of Granada, Granada, Spain. Prof: Cruz Miguel Cedán Martínez. Duration: 3 months.
2015	Centro de Inserción Social Matilde Campos. University of Granada, Granada, Spain. Prof: Inmaculada Valor Segura. Duration: 3 months.

10. Publications list

1. Molina-Hidalgo, C., De-la-O, A., Jurado-Fasoli, L., Amaro-Gahete, F. J., & Castillo, M. J. 2019. Beer or ethanol effects on the body composition response to high-intensity interval training. The BEER-HIIT Study. Nutrients, 11(4), 909.
2. Molina-Hidalgo, C., De-la-O, A., Dote-Montero, M., Amaro-Gahete, F. J., & Castillo, M. J. (2020). Influence of daily beer or ethanol consumption on physical fitness in response to a high-intensity interval training program. The BEER-HIIT study. Journal of the International Society of Sports Nutrition, 17, 1-13. (This article was Editor's pick).
3. Jurado-Fasoli, L., De-la-O, A., Molina-Hidalgo, C., Migueles, J. H., Castillo, M. J., & Amaro-Gahete, F. J. 2020. Exercise training improves sleep quality: a randomized controlled trial. European Journal of Clinical Investigation, 50(3), e13202.
4. Flieh SM, Moreno LA, Miguel-Berges ML, Stehle P, Marcos A, Molnár D, Widhalm K, Béghin L, De Henauw S, Kafatos A, Leclercq C, Gonzalez-Gross M, Dallongeville J, Molina-Hidalgo C, González-Gil EM. Free Sugar Consumption and Obesity in European Adolescents: The HELENA Study. Nutrients. 2020 Dec 5;12(12):3747.
5. M González-Gil E., Huybrechts, I., Aguilera, C.M., Béghin, L., Breidenassel, C., Gesteiro, E., González-Gross, M., Henauw, S., Kersting, M., Le Donne, C., Manios, Y., Marcos, A., Meirhaeghe, A., Etayo, M., Molina-Hidalgo, C., Molnár, D., Papadaki, A., Widhalm, K., Moreno, L., Bel-Serrat, S. 2021. Cardiometabolic Risk is Positively Associated with Underreporting and Inversely Associated with Overreporting of Energy Intake Among European Adolescents: The Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) Study. J Nutr. 2021 Jan 20:nxaa389.
6. Seral-Cortes M, Sabroso-Lasa S, De Miguel-Etayo P, Gonzalez-Gross M, Gesteiro E, Molina-Hidalgo C, et al. 2021. Development of a Genetic Risk Score to predict the risk of overweight and obesity in European adolescents from the HELENA study. Sci Rep. 2021 Feb 4;11(1):3067.
7. Seral-Cortes M, Sabroso-Lasa S, Bailo-Aysa A, Gonzalez-Gross M, Molnár D,

Censi L, Molina-Hidalgo C, Gottrand F, Henauw S, Manios Y, Mavrogianni C, Widhalm K, Kafatos A, Dallongeville J, Moreno LA, Esteban LM, Labayen I, De Miguel-Etayo P, The Helena Study Group OBO. 2021. Mediterranean Diet, Screen-Time-Based Sedentary Behavior and Their Interaction Effect on Adiposity in European Adolescents: The HELENA Study. Nutrients. Jan 30;13(2):474.

8. Seral-Cortes M, Sabroso-Lasa S, De Miguel-Etayo P, Gonzalez-Gross M, Gesteiro E, Molina-Hidalgo C, De Henauw S, Erhardt É, Censi L, Manios Y, Karaglani E, Widhalm K, Kafatos A, Beghin L, Meirhaeghe A, Salazar-Tortosa D, Ruiz JR, Moreno LA, Esteban LM, Labayen I. Interaction Effect of the Mediterranean Diet and an Obesity Genetic Risk Score on Adiposity and Metabolic Syndrome in Adolescents: The HELENA Study. 2020. Nutrients. Dec 16;12(12):3841.

11. Papers submitted or under review

1. Molina-Hidalgo, C., De-la-O, A., Jurado-Fasoli, L., Amaro-Gahete, F. J., Catena, A., & Castillo, M. J. Beer or Ethanol Effects on the Response to High-Intensity Interval Training: Description of an Intervention Study in Young Healthy Individuals. The BEER-HIIT Study. Alcohol. 2020. (under review).
2. Molina-Hidalgo C; Amaro-Gahete, F. J.; Carneiro-Barrera, A.; Castillo, M. J.; Catena, A. Effect of Moderate Alcohol Intake During a High-Intensity Interval Training Intervention on Choice Reaction Time Task in Healthy Adults: the BEER-HIIT Study. J Sport Heal Sci. 2021. (submitted).
3. Molina-Hidalgo, C.; Amaro-Gahete, F. J.; Peven, J., A.; Erickson, K. I.; Castillo, M. J.; Catena, A. Does exercise have a protective effect on cognitive function over alcohol consumption? A rational research in healthy adults. J Sport Heal Sci. 2021. (submitted).

12. Accepted congress communications as first author

2021	Title: " Sedentary behaviors and shapes of subcortical brain structures in children with overweight/obesity: the ActiveBrains Project". ISBNPA – Advance behavior change science - X Change 2021. Authors: Cristina Molina-Hidalgo, Irene Esteban-Cornejo, Cristina Cadenas-Sánchez, Sarah L. Aghjayan, Patricio Solís-Urra, Andrés Catena, Kirk I. Erickson, Francisco B. Ortega.
2019	Title: "Efecto aprendizaje a corto plazo en la capacidad de respuesta discriminativa a estímulos visuales y auditivos". 2019. II Congreso Nacional/IV Jornadas de Investigadores en Formación: fomentando la interdisciplinaridad (JIFFI). University of Granada, Granada, Spain. Authors: Cristina Molina-Hidalgo, Alejandro De-la-O; Lucas Jurado-Fasoli, Francisco J. Amaro-Gahete, Manuel J. Castillo.
2018	Title: "Effect of a training period of high intensity and low volume on perception and discriminative reaction time in healthy adults. 2018. Influence of beer intake". Simposio Exernet. Investigación en Ejercicio, Salud y Bienestar: "Exercise is Medicine". University of Pamplona, Navarra, Spain. Authors: Cristina Molina-Hidalgo, Alejandro De-la-O; Lucas Jurado-Fasoli, Francisco J. Amaro-Gahete, Manuel J. Castillo.

13. Other merits:

Funding applications:

2020	Programa de ayudas a la I+D+I, en régimen de concurrencia competitiva, en el ámbito del plan andaluz de investigación, desarrollo e innovación (PAIDI 2020). IP: Irene Esteban Cornejo. Equipo investigador – colaborador: Cristina Molina Hidalgo
2020	Fundación Ramón Areces 2020. 20th national competition for scientific and technical research – Ayudas a la investigación de nuevos proyectos. IP: Irene Esteban Cornejo. Equipo investigador – colaborador: Cristina Molina Hidalgo
2020	Proyectos de I+D+I por equipos de investigación en el marco del Programa Operativo FEDER de Andalucía 2014-2020. IP: Irene Esteban Cornejo. Equipo investigador – colaborador: Cristina Molina Hidalgo.
2020	Fundación Tatiana Pérez de Guzmán el Bueno - Convocatoria de Proyectos de Investigación en Neurociencia 2020. IP: Irene Esteban Cornejo. Equipo investigador – colaborador: Cristina Molina Hidalgo.
2019	Ayudas Fundación BBVA a Equipos de Investigación Científica 2019. IP: Irene Esteban Cornejo. Equipo investigador – colaborador: Cristina Molina Hidalgo.
2019	Ayudas a la Investigación Banco Sabadell 2019 – Convocatorias predoctorales. Solicitante: Cristina Molina Hidalgo.