1	A higher Mediterranean Diet adherence and exercise practice are associated with a healthier
2	drinking profile in a healthy Spanish adult population
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20	LIST OF ABBREVIATIONS
21	EFSA, European Food Safety Agency; ORs, odds ratios; IOM, Institute of Medicine; BMI, body
22	mass index; WHO, World Health Organization; CI, confidence intervals; MedDiet, Mediterranean
23	diet.
24	

25 ABSTRACT

Propose: Very few studies have examined the association between beverage intake patterns and healthy lifestyle characteristics. Most of the research that has been carried out focuses on the consumption of soft drinks or alcohol, and ignores the overall beverage pattern. To evaluate the association between consumption of different types of beverage, and physical exercise practice and MedDiet adherence.

Methods: Cross-sectional information about fluid intake from different types of beverage was collected in 1,262 men and women between 18 and 70 years old, using a 24h fluid-specific diary over 7 consecutive days. Physical exercise was evaluated with a self-reported questionnaire, and MedDiet adherence was assessed using a validated 14-item questionnaire. Both variables were classified in three categories.

36 Results: Individuals with greater adherence to the MedDiet showed a higher intake of water and wine and a lower consumption of sweet regular beverages. Participants who engaged in more 37 physical exercise consumed more water, milk and derivatives, juices and wine, and less sweet 38 regular beverages. Compared to the lowest category, the possibility of meeting the EFSA 39 40 recommendations of total fluid intake was greater in individuals with eight or more points on the MedDiet adherence questionnaire [OR:1.94;95%CI:1.25-3.01)], and in those that practice physical 41 exercise three times a week or more [OR:1.71;95%CI:1.22-2.39)]. Participants with a healthier 42 lifestyle had a lower risk of exceeding the free-sugar WHO recommendations only from beverages. 43

44 Conclusions: Participants with greater adherence to the MedDiet and who engaged in more physical
45 exercise exhibit a healthier pattern of fluid intake.

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47 Key words: fluid intake, Mediterranean diet, physical exercise, adult, beverage

49 INTRODUCTION

50 Lifestyle is one of the most important factors conditioning health [1]. Physical activity and adherence 51 to a healthy diet, including a healthy beverage pattern, are considered essential components of a 52 healthy lifestyle that reduces the risk of several non-communicable chronic diseases.

The benefits of physical exercise on health and the adverse effects of sedentary behaviour are both well recognized [2–4]. An increase in physical exercise has been shown to be associated with an increase in longevity [5, 6], and a decrease in the risk of cardiovascular and all-cause mortality [7], coronary heart disease and stroke [8], diabetes, and some types of cancer [4]. While more physically active men and women tend to have a higher daily fluid intake [9], the beverage pattern as a whole of individuals with different levels of physical exercise, has not been well ascertained in the past.

59 Similarly, one of the recognized healthy dietary patterns is the so called "Mediterranean diet" 60 (MedDiet), which has been associated with several health benefits that reduce the risk of type 2 diabetes [10–12], metabolic syndrome [13, 14] and major cardiovascular events [15]. This dietary 61 pattern is characterized by a high consumption of vegetables, legumes, grains, fruits, nuts, and virgin 62 olive oil, a moderate consumption of fish, and a low consumption of red and processed meat, 63 64 pastries, butter and creams. While the traditional MedDiet is characterized by a "healthier beverage pattern" with a moderate consumption of milk and dairy products, and red wine, mainly with meals, 65 and a low intake of sugar-sweetened beverages [16], little is known about the association between 66 MedDiet adherence and beverage pattern at a population level. In addition, as an inadequate water 67 68 intake has several consequences on health and human performance, especially in dehydration states [17, 18], it is important to evaluate determinant factors for covering the fluid intake 69 recommendations other than classical individual factors (sex, age, body surface), and environmental 70 conditions. In this sense, it is important to assess if lifestyle determines the percentage of individuals 71 meeting fluid intake recommendations established at population level. Therefore, the main aim of the 72 73 present study was to evaluate the associations between physical exercise practice or adherence to the 74 Mediterranean diet and the total fluid intake, the consumption of different types of beverage, and the

- 75 percentage of population covering fluid and free sugar recommendations in a sample of healthy
- 76 Spanish adults.

78 METHODS

79 Design and study population

The present study is a cross-sectional analysis designed to assess the sources of fluid consumption in Spain – both water and other beverages – and the associations between lifestyle and the fluid consumption pattern [19], compliance with European Food Safety Agency (EFSA) total fluid intake and the World Health Organization (WHO)'s free-sugar recommendations[20, 21].

84 The presented survey is part of an international study conducted in adults and elderly individuals 85 (≥18 years) from 13 different surveys conducted in Latin America, Europe and Asia by public and private organisations [22]. The present study took place between March and May 2012 in all regions 86 of Spain (except Ceuta, Melilla and Canary Islands) and was referred to as Liq.In⁷ [23]. The 87 participants were recruited via a systematic, non-random sample until the quotas for age, gender, 88 region, habitat and/or socioeconomic characteristics in relation to the total country population were 89 met.Participants were apparently healthy and were excluded if they were not able to read and write in 90 91 the language of the questionnaire. Participants who did not complete the full fluid intake record and 92 those that reported a mean total daily fluid intake below 0.4L/day or higher than 6L/day, were also 93 not eligible to participate in the present survey (n=240). The effective sample size for the present study was 1262 participants, all of whom provided informed consent. The present study was 94 submitted to the Ethics Committee of the University Hospital of Sant Joan de Reus (ref. C.E.I.C-95 012), was reviewed, was found to be less than minimal risk and non-invasive, and was approved 96 97 without requiring signed informed consent. Socioeconomic level was assessed using a selfadministered questionnaire and was categorized using the Market Research Society classification 98 [24, 25]. Full details of the study protocol have been published elsewhere [19]. 99

100 Assessment of fluid intake

The usual intake of fluids by participants over seven consecutive days was evaluated using a selfreported and non-validated 24h fluid-specific diary over 7 consecutive days.

This record assessed the moment of the day when the participants consumed fluids, and the type and the volumes of beverages using standard portion sizes. Sugar naturally present in beverages was calculated using a Spanish food composition table [26], and the addition (by hand) of free sugar to each beverage was also estimated by the number of teaspoons used. Hereinafter, we used the 2002 Joint WHO/FAO Expert Consultation on Diet, Nutrition and the Prevention of Chronic Diseases definition of free-sugar [21, 27].

The record items on beverages included the following EFSA used categories [28]: water (tap water, 109 110 filtered tap water, natural mineral water, sparkling natural mineral water, flavoured water, drinking-111 fountain water); hot beverages (coffee, white coffee, espresso with a drop of milk, cappuccino, tea, 112 beverages made from cereals, other infusions and hot beverages); milk and milk derivatives (milk, 113 milkshakes, milkshakes with juice, liquid yogurt, other milk drinks); juices (home-made juice, 114 bottled juice, nectars, nectar without added sugar, other fruit drinks); sweet regular beverages: carbonated soft drinks (cola, orange, lemon, bitter, tonic water, other flavours), non-carbonated soft-115 116 drinks (orange, lemon, sports drinks, energy drinks, regular iced tea, other flavours), other sugared soft drinks; sweet light beverages: diet carbonated soft drinks (cola, orange, lemon, other flavours), 117 118 diet non-carbonated soft drinks (orange, lemon, diet iced tea), other diet soft drinks; and alcoholic drinks (beer, alcohol-free beer, lemon beer, wine, wine with soda, alcoholic mixed drinks, other 119 120 alcoholic drinks). Total fluid intake is defined as the sum of all these beverages.

The percentage of individuals who did not meet the EFSA recommendations was calculated based on the EFSA recommendations for total water intake (water coming from food and beverages) set at 2.5L/day and 2L/day for men and women, respectively. EFSA takes the assumption that foods contribute about 20% to total water intake, therefore the EFSA recommendations for total fluid intake in men was set at 2L/day and 1.6L/day for women, most of which must preferably be consumed as water [29].

127 Assessment of other variables and lifestyle factors

128 Physical exercise practice of more than thirty minutes a day (active walking, swimming, cycling, 129 aerobics and team or individual sports) was evaluated with a non-validated self-reported 130 questionnaire, and categorized as: three times a week or more, between one and two times a week, 131 and once every two weeks or less. In order to assess the adherence to the Mediterranean Diet 132 (MedDiet), the participants were given a Spanish validated 14-item questionnaire [30, 31]. This 14-133 item validated screener consists of 12 questions on food consumption frequency and 2 questions on 134 food intake habits considered characteristic of the Spanish Mediterranean diet. Each question was 135 scored 0 or 1. One point was given for using olive oil as the principal source of fat for cooking, preferring white meat over red meat, or for consuming: I) 4 or more tablespoons (1 tablespoon = 136 137 13.5 g) of olive oil/d (including that used in frying, salads, meals eaten away from home, etc.); 2) 2 or more servings of vegetables/d; 3) 3 or more pieces of fruit/d; 4) <1 serving of red meat or 138 139 sausages/d; 5) <1 serving of animal fat/d; 6) <1 cup (1 cup = 100 mL) of sugar-sweetened beverages/d; 7) 7 or more servings of red wine/wk; 8) 3 or more servings of pulses/wk; 9) 3 or more 140 servings of fish/wk; 10) fewer than 2 commercial pastries/wk; 11) 3 or more servings of nuts/wk; or 141 12) 2 or more servings/wk of a dish with a traditional sauce of tomatoes, garlic, onion, or leeks 142 143 sautéed in olive oil. If the condition was not met, 0 points were recorded for the category. The final 144 score ranged from 0 to 14 points. Using this score, we have categorized MedDiet adherence into 3 145 categories: poor (< 5 points), average (between 6 and 7 points) and good adherence (> 8 points).

In addition, variables such as marital status, socioeconomic characteristics, region, habitat (urban orrural) and education level were also recorded.

The Institute of Medicine (IOM) equations were used to estimate the total energy expenditure of each individual [32], using the gender, age, weight, height and physical exercise of each individual. Therefore, the percentage of individuals consuming more than 10% of energy requirements as freesugar, as recommended by WHO, was calculated [21]. In 2015, the WHO suggested a further reduction on the free-sugars intake recommendations to 5% of total energy intake [27]. The percentage of individuals consuming more free-sugar than this suggested value was also calculated.

Weight (kg) and height (m) were evaluated with a self-reported questionnaire, and body mass index
(BMI) (kg/m²) was calculated.

156 Statistical analysis

157 Data are presented either as means and 95% confidence intervals (CI) for continuous variables or numbers and percentages for dichotomous variables. We compared the distribution of the selected 158 characteristics between groups using χ^2 tests for categorical variables or Student's t-tests or analysis 159 of variance (ANOVA), as appropriate, for continuous variables. The Bonferroni post-hoc test for 160 161 multiple comparisons was used. Logistical regression models were fitted to assess the associations 162 between meeting the EFSA recommendations for total fluid intake (dependent variable), and 163 MedDiet adherence score (three categories) or physical exercise practice (three categories) as 164 exposure. The models were adjusted for gender, age (years), habitat (5,000-30,000 inhabitants, 165 30,000-200,000 inhabitants, more than 200,000 inhabitants), socioeconomic characteristics (lower and middle-low, middle, upper-middle and high), educational level (primary education, secondary 166 167 education, higher education), physical exercise (once every 2 weeks or less, once or twice per week and ≥ 3 times per week) (except when physical exercise was the independent variable), MedDiet 168 169 score (except when MedDiet adherence was the independent variable) and body mass index (kg/m²). Linear trend tests were calculated using the median adherence to the MedDiet score of each category 170 and introducing this new value as a continuous variable in the models. All statistical tests were two-171 tailed and the significance level was set at P <0.05. All analyses were performed using the SPSS 172 173 software version 22.0 (SPSS Inc, Chicago, IL).

175 **RESULTS**

A total of 1,262 participants (630 men and 632 women) were recruited from all regions of Spain (except from Ceuta, Melilla and the Canary Islands). The baseline characteristics of participants across MedDiet adherence and physical exercise categories are summarized in Table 1. Men presented significantly lower adherence to the MedDiet and physical exercise than women. Age was higher as adherence to the MedDiet increased, and BMI was lower as physical exercise increased. Participants in the highest category of MedDiet adherence and/or physical exercise have a higher socioeconomic and educational level, respectively.

Table 2 shows the total daily fluid intake and the amount of each type of beverage consumed across the various categories of MedDiet adherence score and physical exercise practice. Individuals with the highest scores in the 14-point MedDiet adherence questionnaire consumed significantly more water and wine, and less sweet regular beverages, than individuals with the lowest scores. Consumption of milk and derivatives was significantly higher in participants with a lower MedDiet adherence score than in those with a MedDiet adherence between 6 and 7 points. No significant differences were observed in total daily fluid volume between the categories of MedDiet adherence.

190 Individuals who engaged most in physical exercise presented a higher intake of water, milk and 191 derivates and wine, and a lower consumption of sweet regular beverages than those who did little 192 physical exercise. Significant differences were observed in the total daily fluid volume intake when 193 participants who engaged in physical exercise three times a week or more were compared with 194 individuals in the lower categories.

The odds of meeting the EFSA recommendations for total fluid intake (Table 3) were significantly higher in those participants with greater adherence to the MedDiet (≥ 8 points) in the crude model. This association remained even after adjusting for potential confounders [OR: 1.94; 95% CI: 1.25-3.01 (P for trend<0.05). Women [OR: 1.83; 95% CI: 1.05-3.20] and participants with a BMI ≥ 25 kg/m² [OR: 2.83; 95% CI: 1.37-5.82] who were in the highest MedDiet adherence category showed a greater probability of meeting EFSA recommendations for total fluid intake than those who were in

the lower adherence category (P for trend<0.05). Subjects who did most physical exercise (≥ 3 times 201 202 a week) had greater odds of meeting the EFSA recommendations for total daily fluid intake [OR: 203 1.71; 95% CI: 1.22-2.39], even in the adjusted model, using the frequency of physical exercise ≤ 1 204 time every 2 weeks as reference. These results were consistent among subgroups of gender and BMI. 205 Participants in the highest category of MedDiet adherence or physical exercise practice were less 206 likely to exceed the WHO recommendations for free-sugar consumption ($\geq 10\%$ of energy from free 207 sugar) only from beverages consumption and the EFSA recommendations for total fluid intake, 208 compared with ones in the lowest categories (Figure 1. Panel A and B, respectively). Regarding the 209 MedDiet adherence, 73.6% of the participants with an adherence lower than 5 points, 55.9% of the 210 individuals presenting between 6 and 7 points and 48.4% of the participants in the higher category 211 (≥8 points) exceeded the 5% of energy from free-sugar only from beverages consumption (data not 212 shown). Regarding physical exercise, 63.5% of individuals in the lower category, 62.2% of the individuals practicing exercise between 1 and 2 times/week and 49.1% of the individuals more 213 214 physically active (≥ 3 times/week) exceeded the 5% of the total energy intake from free-sugar (data not shown). Participants in the highest category of MedDiet adherence or physical exercise were 215 216 significantly less likely to exceed the WHO suggestions for free-sugar consumption (5% of total energy from free-sugar) only from beverages consumption (data not shown). No significant 217 differences were observed in the proportion of participants meeting the EFSA recommendations for 218 total daily fluid intake across categories of MedDiet adherence or physical exercise practice. 219

Participants with a higher adherence to the MedDiet (>8 points) and physical exercise practice (\geq 3times/week) showed a greater probability of meeting EFSA recommendations in the crude model, taking into account participants in the lowest MedDiet adherence category and lower physical exercise practice as a reference. This association remained even after adjusting for potential confounders [OR: 2.84; 95% CI: 1.20-6.71] (Table 4). We have also analyzed the combined association of MedDiet adherence and physical exercise practice in relation to the WHO free-sugar recommendations (\geq 10% of energy from free sugar). Subjects with a higher adherence to the

227 MedDiet (>8points) and physical exercise practice (≥3times/ week) showed a lower probability of

228 exceeding the WHO free sugar recommendations after adjusting for potential confounders [OR:

- 229 0.53; 95% CI: 0.25-0.97], taking into account participants in the lowest MedDiet adherence category
- and lower physical exercise practice as a reference (Table 4).

232 DISCUSSION

The main objective of the present study was to evaluate the associations between MedDiet adherence or physical exercise, and the fluid consumption pattern in a sample of healthy Spanish adults. We report for the first time that healthy individuals with the highest scores for MedDiet adherence and most physical exercise practice presented the healthiest beverage patterns.

Although our results are in agreement with those reported by other investigators [33–35], to the best of our knowledge the present study is the first to have assessed not only the different types of beverage consumption but also the total daily fluid intake using a specific questionnaire designed to prospectively record beverage consumption and sugar added by hand to beverages.

241 Interestingly, in our study higher MedDiet adherence was mainly associated with being a woman and 242 age. The fact that women tend to have a healthier lifestyle, which includes a healthier dietary pattern, 243 may explain why females tend to adhere more closely to the MedDiet [36, 37]. Studies assessing dietary patterns in different populations have also found a direct association between age categories 244 245 and higher adherence to the MedDiet [37-39]. As expected, we observed that men and participants with a lower BMI were more physically active in their leisure time. The regular consumption of 246 247 wine, mainly with meals, and a lower intake of sweet regular beverages were considered key elements in defining the traditional MedDiet [15]. Although the present cross-sectional study is in 248 agreement with this definition, the increased consumption of drinking water in those individuals in 249 the highest category of MedDiet adherence suggests that it must be regarded as another key element 250 251 when defining the MedDiet in the future. This is important because the consumption of sugarsweetened beverages has mostly been associated with an increased risk of obesity and other 252 metabolic conditions [41–43]. 253

It should be pointed out that the greater consumption of wine in those individuals in the highest MedDiet category is only to be expected, because the 14-item MedDiet adherence questionnaire used in our study awarded one point for the intake of 7 glasses of wine or more per week [44].

In our study, those individuals who least followed the Mediterranean diet consumed more milk and milk products. This may be because they are younger, and several studies have demonstrated that young people tend to move away from Mediterranean pattern [37, 45, 46]. In addition, milk and other daily beverages are not regarded as typical Mediterranean foods [16, 39, 47].

To the best of our knowledge, the possible associations between physical exercise and total fluid 261 262 intake or different types of beverages consumption have not been subject to a great deal of research 263 among the general populations to date. In our study, individuals who engaged in most physical 264 exercise presented a higher total daily fluid and water consumption, probably because they had an 265 increased fluid loss and consequently a higher demand of fluid intake [48, 49]. However this study is 266 not able to conclude whether these higher intakes cover the higher intake demand. Hydration 267 biomarkers would be required to investigate this, however this was outside the scope of this survey. 268 Besides the increased water intake, individuals who engaged in most physical exercise also consume greater quantities of wine, milk and dairy products, and fewer sweet regular beverages. Although 269 270 there is no physiological explanation, a higher consumption of milk and dairy products in more active individuals has also been previously described in some populations [50, 51]. 271

In our study, compliance with the EFSA recommendations for total fluid intake was mainly associated with greater adherence to the MedDiet, mainly in women and overweight or obese individuals. Also, individuals who engaged most in physical exercise, independently of gender and BMI, tended to comply better with the EFSA total fluid intake recommendations as reported previously by our group [19].

Finally, in our study a higher percentage of individuals in the lowest category of Mediterranean diet
adherence or physical exercise show an intake of free-sugar, only from beverage, above the WHO
recommendations.

280 Several potential limitations and strengths of our study deserve comment and have, in fact, been 281 discussed elsewhere [19]. The main limitation of this pilot study is that our population is probably 282 not representative of the general Spanish population. However, the final distribution of the

283 individuals studied among age groups, gender, region and educational categories is very similar to 284 the real distribution of the population in Spain. The second limitation is inherent to its cross-sectional 285 design, which limits the potential to discern causative relationships. The third limitation is related with the assessment of the fluid intake without assessing water moisture from food; however in our 286 study we only assessed the percentage of individuals not covering the drinking water EFSA 287 288 recommendations that do not take into account water from solid foods. In addition, although fluid intake was assessed in a moderate climate temperature conditions (between March and May), we 289 290 cannot assume that total fluid and type of beverage consumed are representative of the entire year. 291 Finally, the fact that anthropometric measures and physical exercise practice were self-reported and 292 not measured may produce some systematic bias. One of the strengths of our study is that it uses a 293 24h fluid-specific diary over 7 consecutive days that makes it possible to assess the total fluid intake 294 and real fluid pattern, reducing the differences between week and weekend days.

295 This study is the first to specifically describe associations between dietary and physical activity patterns and total fluid and beverage intake in healthy Spanish adults. Participants who adhere more 296 297 closely to the MedDiet and who engage in more physical exercise have a healthier fluid intake 298 pattern, characterised by a high intake of water and wine and a low intake of sugar-sweetened beverages. Participants with a healthier lifestyle had a greater probability of complying with EFSA 299 300 total daily fluid intake and free-sugar intake WHO recommendations. These results suggest that changing the fluid intake can have beneficial effects on other lifestyle parameters. However, future 301 302 studies are warranted to confirm our results and, to establish cause-effect associations in order to 303 design future public health recommendations.

304

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- 308

309 STATEMENTS

- 310 All the participants give their consent prior the inclusion in the study. University Hospital of Sant
- Joan de Reus ethics Committee gives their approval to the study protocol (ref. C.E.I.C-012).
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313 CONFLICT OF INTERESTS

- 314 C.F.-P., reports no conflicts of interest. J.S.-S is member of the scientific advisory board on fluid
- 315 intake of Danone Research. J.S.-S. and N.B. have received consultancies from Danone S.A.

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442 FIGURE LEGENDS

- 443 Figure 1. Percentage of participants not meeting with fluid intake and exceeding the free-sugar
- 444 recommendations, according to Mediterranean diet adherence (A) and physical exercise (B).
- 445 EFSA recommendations for total fluid intake (2000ml for men and 1600ml for women), and WHO
- 446 free-sugar recommendations were used ($\geq 10\%$ of total daily energy from free-sugar) only from
- 447 beverages. *P values for comparisons between groups were tested by χ^2 .