



Full length article

Alcohol consumption and S-Klotho plasma levels in sedentary healthy middle-aged adults: A cross sectional study

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ABSTRACT

Introduction: Alcohol consumption is related to several diseases and injuries that accelerate the ageing process and increase the mortality and morbidity risk. The soluble form of the α -Klotho gene (called S-Klotho) is widely considered as a powerful anti-ageing biomarker.

Aim: The aim of the present study was to analyze the association between alcohol consumption and S-Klotho plasma levels in sedentary middle-aged adults.

Methods: 74 (39 women) middle-aged sedentary adults (40–65 years old) participated in the present study. Alcohol consumption was assessed with a food frequency questionnaire previously validated and with three non-consecutive 24-hours recall. The S-Klotho plasma levels were measured by ELISA using a soluble α -Klotho ELISA assay kit.

Results: Total alcoholic drinks consumption was negatively associated with S-Klotho plasma levels ($\beta = -17.031$; $R^2 = 0.096$, $P = 0.013$). Furthermore, we observed that higher consumption of total alcoholic drinks is associated with lower S-Klotho plasma levels in middle-aged sedentary adults controlling for BMI ($\beta = -16.372$; $R^2 = 0.201$, $P = 0.011$), LMI ($\beta = -31.854$; $R^2 = 0.305$, $P = 0.010$) and for FMI ($\beta = -13.337$; $R^2 = 0.075$, $P = 0.049$).

Conclusion: In conclusion, our study shows that total alcoholic drinks consumption is negatively associated with the S-Klotho plasma levels in middle-aged sedentary adults.

1. Introduction

Ageing is characterized by a progressive loss of physiological integrity, leading to impaired function and increased vulnerability to death (López-otín et al., 2013). This deterioration is the primary risk factor for major human pathologies including cardiovascular diseases, osteoporosis, cancer and/or neurodegenerative disorders (López-otín et al., 2013). These diseases represent the major cause of morbidity and mortality in developed countries, and implies a substantial increase in health costs (Kingsley, 2015; Wilmoth, 2000). Therefore, it is necessary to find strategies that can attenuate the ageing process (Mora and Valencia, 2018).

The expression of the Klotho gene is related to the ageing process. It has been identified three different Klotho-related genes: (i) The α -Klotho, expressed in distal convoluted tubules in the renal cortex, the parathyroid glands, and the choroid plexus in the brain (Kuro-o et al.,

1997); (ii) The β -Klotho, expressed in the liver, the endocrine pancreas, the adipose tissue, and the brain (Hu et al., 2010); and (iii) The γ -Klotho, which is a half-size Klotho-related gene expressed in brown adipose tissue (Kim et al., 2015). The expression of the α -Klotho gene is considered a powerful anti-ageing biomarker. An overexpression of the α -Klotho gene is related with a lifespan extension and with a low incidence of ageing-related diseases (Kuro-O, 2010). The α -Klotho secreted form (S-Klotho) shows an accurate and strong relationship with the α -Klotho gene expression (Saghiv et al., 2017). The S-Klotho has been used as an anti-ageing biomarker and as an indicator of α -Klotho gene expression (Saghiv et al., 2017).

It is well known that nutrition can take an important influence in life expectancy, reducing the risk of overall mortality and morbidity (Chrysohoou and Stefanadis, 2013). In this sense, the traditional Mediterranean diet is associated with (i) a significant decrease of overall mortality risk, (ii) a low incidence of ageing-related chronic diseases,

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and (iii) an increased probability of a healthy ageing (Ross, 2015). The Mediterranean diet is a dietary pattern rich in plant foods, fish, olive oil and a moderate alcohol consumption (Bach-Faig et al., 2011). Although a moderate alcohol consumption is frequent in adults which have high adherence to the traditional Mediterranean dietary pattern, there are a significant amount of individuals that reported an excessive alcohol intake (Galán et al., 2014; León-Muñoz et al., 2015).

In this context, the alcohol consumption is an important cause of disease burden in Europe (Lim et al., 2012). Indeed, the alcohol consumption is related to several diseases and injuries that accelerate the ageing process and increase the mortality and morbidity risk (e.g. infectious disease, cancer, diabetes, neuropsychiatric disease, cardiovascular disease, liver and pancreas disease, and unintentional and intentional injury) (Moonat et al., 2009). Moreover, a recent study suggested that alcohol consumption account for 10% of global deaths, decreasing lifespan and increasing the risk of all-cause mortality (Bill and Foundation, 2018).

However, it is still unknown whether the alcohol consumption could influence the S-Klotho plasma levels in humans. Therefore, the aim of the present study was to analyze the association between alcohol consumption and S-Klotho plasma levels in sedentary middle-aged adults.

2. Methods

2.1. Participants

A total of 74 (39 women) middle-aged sedentary adults (40–65 years old) participated in the present study. The participants were enrolled in the FIT-AGEING study (Amaro-Gahete et al., 2018) (clinical-trial.gov: ID: NCT03334357), a randomized controlled trial which aimed to quantify the effects of different training modalities on the S-Klotho plasma levels in sedentary healthy adults. All participants reported to be non-physically active (< 20 min on 3 days/week), with a body mass index between 18.5–35 kg/m², to not engage in a weight-loss program, with stable weight (weight changes < 5 kg) over the last 5 months, and normal electrocardiogram. Individuals with chronic diseases (i.e. cardiovascular disease, diabetes, cancer, chronic kidney diseases, hyperlipidemia, respiratory diseases, mental diseases) substance abuse or dependence (i.e. alcohol or drugs), pregnancy or planning to get pregnancy, taking any medication and/or unwillingness to complete the study requirements were excluded. All participants had a health story and a medical examination done prior to the study. If any participant suffered any injury or medical problem, a medical evaluation was performed and, if was necessary, they were excluded from the study. The study was approved by the Ethics Committee on Human Research at the University of Granada and Servicio Andaluz de Salud (CEI-Granada) (0838-N-2017). The study protocols and experimental design were applied in accordance with the last revised ethical guidelines of the Declaration of Helsinki. All participants signed an informed consent.

2.2. Anthropometric measurements and body composition assessment

The body weight and height measurements were performed 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 (BMI) was calculated (kg/m²). A dual-energy X-ray absorptiometry scanner (Discovery Wi, Hologic, Inc., Bedford, MA, USA) was conducted to measure the lean body mass (kg) and the fat body mass (kg). We considered the quality controls, the position of the participants, and the analysis results following the manufacturer's recommendations. An automatic delineation of the anatomic regions was performed by the software APEX 4.0.2. We acquired the spine phantom quality control scans on each study day. The lean mass index (LMI) was calculated as lean body mass (kg) /height (m²), as a height normalized body composition index. The fat mass index (FMI) was calculated as fat

body mass (kg) /height (m²).

2.3. Alcohol consumption measurement

Information of alcohol consumption was collected by using an interview-based questionnaire.

Alcoholic drinks portions (wine, beer, spirits and total alcoholic drinks) were obtained from a previously validated semi-quantitative 100-item Food Frequency Questionnaire (FFQ) (Vioque et al., 2013). A commonly used portion size was described (slices, cups, teaspoons, etc.) for each FFQ food item, and the participants were also asked how often they had consumed that unit on average over the last three months. Emphasis was added to ensure that the answers were related to long-term dietary factors and not to recent dietary changes.

Total alcohol consumption (g/day) was obtained from the three non-consecutive 24-hours recall which included 2 weekdays and 1 weekend day. The interviews were meal-sequence based and involved a detailed assessment and description of the food consumed. Colored photographs of different portion sizes of foods were provided to help estimate the quantity of food consumed (López and Martín-Lagos, 2010). The 24-hours recall recalls were entered into the EvalFINUT[®] software to analyze alcohol consumption. Alcohol average mean was obtained (calculated from the three 24-hours recall performed). All questionnaires were conducted by a qualified and experimented dietitian (LJF).

2.4. S-klotho plasma level assessment

Blood samples were collected from the antecubital vein after 12 h of nocturnal fasting. S-Klotho plasma levels were measured in EDTA plasma using a solid-phase sandwich enzyme-linked immunosorbent assay (Demeditec, Kiel, Germany). All participants were requested to abstain from drugs and/or caffeine, to eat a pre-established dinner, and not to do any physical activity at moderate intensity (24 h before) and/or vigorous intensity (48 h before). All samples were centrifuged and collected at the same time and were processed in a controlled-temperature room (22 ± 0.5 °C).

2.5. Adherence to the mediterranean diet

The adherence to the traditional Mediterranean diet was estimated by using the 14-point questionnaire of adherence to the Mediterranean diet used and validated in the PREDIMED trial (Schroder et al., 2011). The PREDIMED questionnaire includes 12 questions related to frequency intake of key foods, and 2 questions related to specific dietary habits of the Mediterranean diet (Schroder et al., 2011). Each question scores 0 or 1 point. The global score ranges from 0 to 14, being 0 points null adherence and 14 points complete adherence to the Mediterranean diet.

2.6. Statistical analysis

The sample size and power calculations are made based on the data of a pilot study (Amaro-Gahete et al., 2018). Visual check of histograms, Q-Q, and box plots were used to verify the distribution of all variables. The descriptive parameters were reported as mean and standard deviation. We conducted a simple linear regression model to examine the association of alcohol consumption (i.e. wine, beer, spirits, total alcoholic drinks, and total alcohol) with S-Klotho plasma levels. We also conducted multiple linear regression models to test these associations adjusting by LMI, FMI, age and sex. The analysis was conducted using the Statistical Package for Social Sciences (SPSS, v. 25.0, IBM SPSS Statistics, IBM Corporation), and the level of significance was set at < 0.05.

Table 1
Descriptive parameters.

	ALL (n = 74)	Men (n = 35)	Women (n = 39)
Age (years)	53.7 ± 5.1	54.4 ± 5.3	53.0 ± 5.0
Body mass index (kg/m ²)	26.7 ± 3.8	28.3 ± 3.6	25.3 ± 3.3
Lean mass index (kg/m ²)	15.2 ± 2.9	17.5 ± 2.0	13.2 ± 1.8
Fat mass index (kg/m ²)	10.7 ± 3.1	10.0 ± 3.2	11.4 ± 2.9
S-Klotho plasma levels (pg/ml)	775.3 ± 363.7	814.1 ± 452.2	741.4 ± 265.6
Wine (portions/week)	2.1 ± 2.7	2.9 ± 2.1	1.4 ± 3.0
Beer (portions/week)	3.9 ± 5.3	5.3 ± 6.6	2.5 ± 3.3
Spirits (portions/week)	0.4 ± 0.7	0.6 ± 0.7	0.3 ± 0.7
Total alcoholic drinks (portions/week)	6.4 ± 6.5	8.8 ± 7.2	4.2 ± 4.8
Total Alcohol (g/day)	11.0 ± 13.1	16.2 ± 16.1	6.4 ± 7.0

Values are expressed as mean ± standard deviation.

3. Results

The characteristics of the study sample are shown in Table 1.

Fig. 1 shows the association of alcoholic drinks portions consumption (wine, beer, spirits, and the sum of them) and total alcohol consumption (g/day) with S-Klotho plasma levels. The linear regression analysis showed that total alcoholic drinks consumption was negatively associated with S-Klotho plasma levels ($\beta = -17.031$; $R^2 = 0.096$, $P = 0.013$) (Fig. 1, panel D). Although non-statistically significant association was found, a tendency toward a negative association was between: (i) wine consumption and S-Klotho plasma levels ($\beta = -27.351$; $R^2 = 0.060$, $P = 0.052$) (Fig. 1, panel A); (ii) beer consumption and S-Klotho plasma levels ($\beta = -17.073$; $R^2 = 0.053$, $P = 0.067$) (Fig. 1, panel B); (iii) spirits consumption and S-Klotho plasma levels ($\beta = -62.870$; $R^2 = 0.020$, $P = 0.267$) (Fig. 1, panel C); (iv) total alcohol consumption (g/day) ($\beta = -4.171$; $R^2 = 0.025$, $P = 0.201$) (Fig. 1, panel E) and S-Klotho plasma levels.

Table 2 showed the multiple linear regression analysis of total

Table 2
Association of S-Klotho protein plasma levels with total alcoholic drinks.

	All (n = 74)		
	β	R^2	P
Total alcoholic drinks (portions/week)			
Model 1	-16.372	0.201	0.011
Model 2	-19.652	0.351	0.001
Model 3	-13.337	0.075	0.049

β : unstandardized regression coefficient; R^2 : Coefficient of determination; and p value were obtained from the multiple linear regression analyses. The analyses were controlled for: body mass index (Model 1), lean mass index (Model 2) and fat mass index (Model 3).

alcoholic drinks consumption with S-Klotho, controlled by BMI, LMI and FMI. Our results showed that higher total alcoholic drinks consumption was associated with lower S-Klotho plasma levels adjusting by BMI ($\beta = -16.372$; $R^2 = 0.201$, $P = 0.011$), by LMI ($\beta = -31.854$; $R^2 = 0.305$, $P = 0.010$) and by FMI ($\beta = -13.337$; $R^2 = 0.075$, $P = 0.049$). This association disappeared when age was included in the model ($\beta = -7.941$; $R^2 = 0.646$, $P = 0.391$).

We also found a negative association between wine consumption and S-Klotho plasma levels adjusting by LMI ($\beta = -31.854$; $R^2 = 0.305$, $P = 0.010$); between beer consumption and S-Klotho plasma levels adjusting by BMI ($\beta = -16.384$; $R^2 = 0.173$, $P = 0.039$) and by LMI ($\beta = -18.946$; $R^2 = 0.290$, $P = 0.021$); and between alcohol consumption (g/day) and S-Klotho plasma levels ($\beta = -6.195$; $R^2 = 0.279$, $P = 0.032$) (See supplementary material, Table S1). No association was found in other variables adjusting by BMI, LMI or FMI (See supplementary material, Table S1).

No association between alcoholic drinks portions consumption (wine, beer, spirits, and the sum of them) and total alcohol consumption (g/day) with S-Klotho plasma levels were found when sex was included as covariate. All associations also disappeared when we

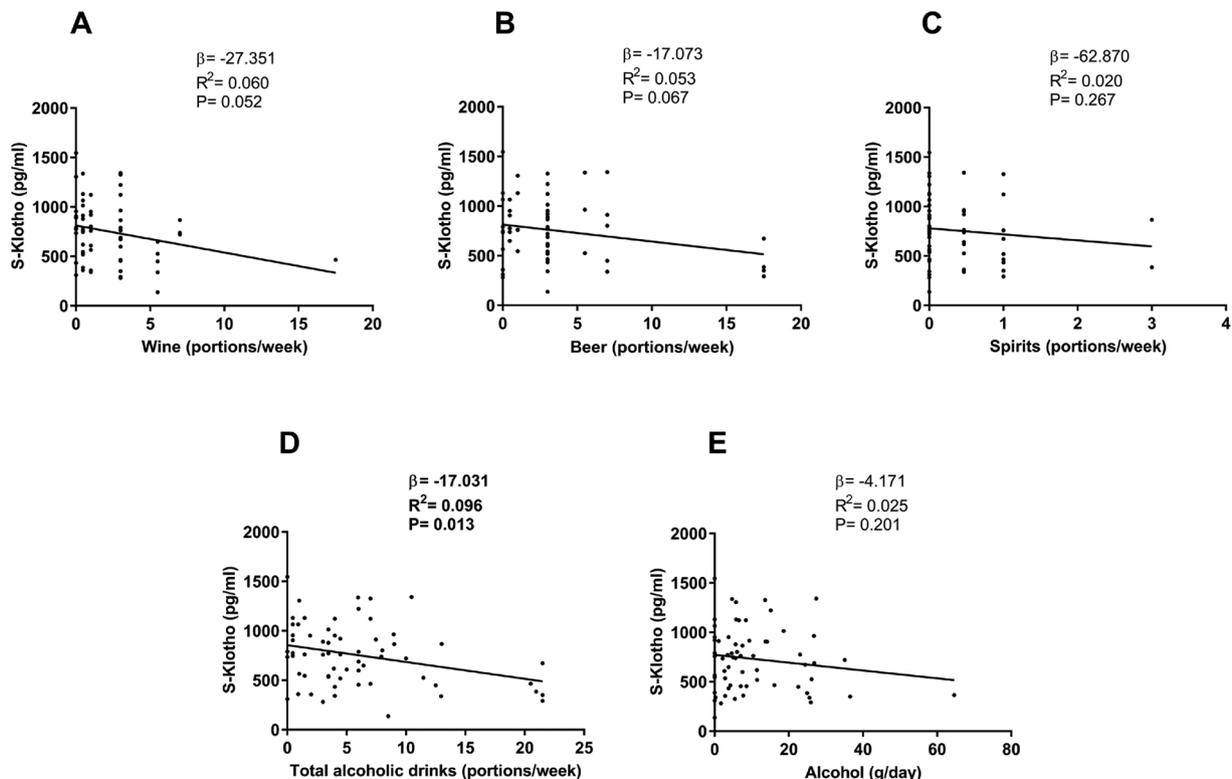


Fig. 1. Linear regression graphs between Wine consumption; 1A; Beer consumption; 1B; Spirits consumption; 1C; Total alcoholic drinks consumption; 1D; Alcohol consumption; 1E, and S-Klotho plasma levels. β : Non-Standardized linear regression coefficient; R^2 : Coefficient of determination; P: p value.

introduced the total PREDIMED score in the multiple linear regression model (all $P > 0.05$) (data not shown), except for the negative association of total alcoholic drinks and S-Klotho plasma levels ($\beta = -14.683$ $R^2 = 0.075$, $P = 0.036$). We repeated all analyses in men and women separately, and no association was found (data not shown).

4. Discussion

The present study shows that higher total alcoholic drinks consumption is associated with lower S-Klotho plasma levels in middle-aged sedentary adults. Furthermore, we observed that higher consumption of total alcoholic drinks is associated with lower S-Klotho plasma levels in middle-aged sedentary adults controlling for BMI, LMI and FMI.

High alcohol consumption has been previously associated with a lifespan reduction due to the detrimental effects on health and the higher risk of different diseases associated with its consumption (Adamson et al., 2017). Therefore, this study elucidates the negative association of high alcohol consumption in a powerful biomarker of ageing as is S-Klotho plasma levels.

To the best of our knowledge, this is the first study to show the relationship between alcohol consumption and S-Klotho plasma levels in middle-aged sedentary adults. A previous study observed higher S-Klotho plasma levels among alcoholics than in controls, but these differences were dependent on the presence of cirrhosis (Quintero-Platt et al., 2017). This higher S-Klotho plasma levels in cirrhotic patients could be due to a pathologic status, where the liver function is impaired (Quintero-Platt et al., 2017). However, since Quintero Platt et al. did not study in depth this relationship, the physiological mechanisms involved in this concern are yet uncertain. Moreover, a number of studies registered an increase of S-Klotho plasma levels in diabetics (Inci et al., 2016), patients with acromegaly (Schmid, 2012), and patients with autosomal dominant polycystic kidney disease (Sari et al., 2016). Therefore, it seems plausible that the findings of Quintero-Platt et al. (Quintero-Platt et al., 2017) are not applicable to healthy individuals, since it is previously shown that a pathological status affects S-Klotho plasma levels (Inci et al., 2016; Sari et al., 2016; Schmid, 2012).

It has been previously described that the lack of expression of β -Klotho gene in the brain could increase alcohol consumption (Schumann et al., 2016). β -Klotho is expressed in a single-pass transmembrane protein which function as an obligatory co-receptor for fibroblast growth factor 21 (FGF-21) together with fibroblast growth factor receptor 1 (Kuro-o, 2012). In this sense, β -Klotho could affect alcohol consumption through the influence of FGF-21 which is involved on alcohol consumption and sweet preference (Schumann et al., 2016). FGF-21, in turn, acts on brain to suppress sweet preference and to limit alcohol consumption (Schumann et al., 2016). However, no evidence has been previously reported about the association of alcohol consumption and S-Klotho plasma levels, neither the relationship between β -Klotho and S-Klotho plasma levels.

Several physiological mechanisms could explain the negative association between alcohol consumption and S-Klotho plasma levels obtained in our study: (i) Alcohol consumption leads to dehydration through its diuretic action (Hobson and Maughan, 2010), and a previous study revealed that dehydration leads to a marked down-regulation of α -Klotho (Tang et al., 2011). (ii) Alcohol consumption can contribute to increase the uremic toxin concentration (Barreto et al., 2014), and these uremic toxins downregulates renal α -Klotho through stimulation of oxidative pathways (Hu et al., 2013). (iii) Alcohol consumption increases oxidative stress through the reactive oxygen species production (Li et al., 2015). Oxidative stress has previously demonstrated to decrease the α -Klotho gene expression (Mitobe et al., 2005). (iv) High alcohol consumption increases proinflammatory cytokines secretion, especially tumor necrosis factor- α (TNF- α) and interferon γ (IFN- γ) (González-Reimers et al., 2014). The cytokines TNF- α and IFN- γ downregulates the α -Klotho gene expression (Hu et al., 2013). (v)

High alcohol consumption is associated with dyslipidaemia, due to the increased VLDL secretion, impaired lipolysis and increased hepatic delivery of free fatty acids from adipose tissue (Klop et al., 2013). Dyslipidaemia has previously demonstrated to downregulates the α -Klotho gene expression (Hu et al., 2013). (vi) Chronic high alcohol consumption is linked to a disruption of glucose homeostasis (Kim and Kim, 2012). In this sense, hyperglycaemia downregulates the α -Klotho gene expression (Hu et al., 2013).

A previous study evidenced that the α -Klotho expression may be modulated by skeletal muscle activity (Avin et al., 2014); for that reason, we controlled our analysis by LMI avoiding the possible interaction between them. In our study we observed that higher alcohol consumption (total alcoholic drinks) is associated with lower S-Klotho plasma levels adjusting by LMI. These results remained when we controlled our analysis by BMI and FMI in total alcoholic drinks. Therefore, the negative association between alcohol consumption (in terms of total alcoholic drinks) and S-Klotho plasma levels was independent on the LMI, BMI and FMI.

Our study has some limitations. Firstly, the cross-sectional design does not allow to identify any causal association between the variables included. Secondly, the participants were sedentary middle-aged adults (45–65 years old), and we do not know whether these results can be extended to younger or physically active populations. Finally, the difficulty of an accurate evaluation of the alcohol consumption with possible underreporting or over-reporting should be considered, as in all cross-sectional studies.

5. Conclusion

In conclusion, our study shows that total alcoholic drinks consumption is negatively associated with the S-Klotho plasma levels in middle-aged sedentary adults. Nevertheless, further studies are needed to confirm the observed association in individual with similar and different biological characteristics, since the sample size of this study was relatively small, and to establish how alcohol plays a role in the regulation of S-Klotho plasma levels. Dietary intervention studies are needed to understand how dietary factors (i.e. alcohol consumption) could change S-Klotho plasma levels. Longitudinal studies are also required to establish the causal association between alcohol consumption and S-Klotho plasma levels.

Conflict of interest

No conflict declared.

Role of funding source

Nothing declared.

Contributors

LJF, FAG, AOP, AGS and MCG conceived and designed the study; LJF, FAG, AOP acquired data; LJF, FAG, elaborated the statistical section; LJ, FAG, drafted, and MCG revised the manuscript; all authors read and approved the final manuscript.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.drugalcdep.2018.09.024>.

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