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Review

Association of Klotho with physical performance and frailty in middle-aged and older adults: A systematic review

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ABSTRACT

Ageing is an inevitable process of physical deterioration that impairs functional autonomy and quality of life, becoming a public health issue.

Since the percentage of people over 60 years is increasing worldwide, the use of easily detectable biomarkers of ageing is a relevant tool for monitoring of the ageing process and treatment. Among them, Klotho, an ageing suppressor gene because its deficiency leads to ageing like phenotype, seems particularly promising.

This systematic review includes the last 10 years clinical studies that evaluated the association between plasma Klotho and body composition, physical performance and frailty in both sedentary and active middle-aged and older adults.

Sixteen studies have been found: nine regarding the association between Klotho and body composition, two the association of Klotho and frailty and finally five concerning the effects of physical activity on Klotho.

The results of these studies, albeit with some exceptions, point out that Klotho is positively associated with muscle strength and negatively with osteoporosis, frailty, disability and mortality while physical activity generally increases Klotho levels.

Moreover, even if there are still few clinical studies, Klotho might be positively associated with bone mineral density, muscle strength, longevity, mobility and robustness during ageing.

1. Introduction

Ageing is a process characterized by a physiological deterioration of the organ functions, induced by both genetic and environmental factors, leading to inevitable decreased function and increased mortality risk (Sen et al., 2016).

The number of people over 60 years is increasing worldwide with a current percentage of 8.5% of the world's population and it is forecasted to triple by 2050 (Yasobant, 2018).

The principal changes in body composition, observed during ageing, are the decrease in lean body mass (LBM), bone mineral density (BMD), muscle mass and an increase in fat body mass (FBM). All these changes concur to age-associated disorders, such as sarcopenia, obesity and osteoporosis (OP), with consequent reduced health, autonomy and quality of life and increase in public health costs (Pickering and Kiely, 2018; Hirschfeld et al., 2017).

Ageing is usually coupled with frailty. However, frailty is a distinct biological syndrome with high vulnerability to low-pressure stressors, decrease in functional resilience and multiorgan dysfunction (Rockwood and Bergman, 2021). Frailty is a condition associated with increased risk of hospitalization, adverse health outcomes and death. Even if not strictly related to age, frailty increases with age, from 4% to 26% from 65 to 85 years (Dent et al., 2016).

Physical performance, defined as a decline of the ability to perform physical tasks, is a relevant element for the characterization of ageing and frailty, although it can be influenced also by lifestyle and diseases (McPhee et al., 2016). Physical performance declines with ageing and it is always reduced in frail older subjects (McPhee et al., 2016). Physical performance can be improved by means of different interventions, e.g., physical activity and nutrition (Wu et al., 2021; Lai et al., 2018; LIFE Study Investigators et al., 2006), even in frail subjects (Kidd et al., 2019).

To investigate and monitor the processes of ageing and frailty, it is important to measure specific biomarkers.

Among ageing biomarkers, α-Klotho (simply Klotho) was identified for the first time in 1997 (Kuro-o et al., 1997). Klotho protein is part of

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endocrine fibroblast growth factor (FGF) receptor complexes with several different enzymatic activities, such as energy metabolism (glucose uptake, insulin sensitivity or calcium homeostasis), suppression of oxidative stress and chronic inflammation and inhibition of insulin/IGF-1 and of transforming growth factor β (TGFβ1) pathways (Kuro-o, 2018; Zhang and Liu, 2018). It is the co-receptor of FGF23, a bone-derived hormone that induces phosphate excretion into urine (Hu et al., 2013). It is considered an ageing suppressor protein because its deficiency leads to an ageing like phenotype with sarcopenia, metabolic disorders, osteoporosis, impaired cognition, gait disturbance, and atherosclerosis, as observed in mice in which Klotho gene (*KL*) was suppressed (Kuro-o et al., 1997; Kim et al., 2015; Cardoso et al., 2018). In humans, this gene expresses 3 isoforms of the Klotho protein: 1) a transmembrane glycoprotein (nearly 130 kDa) with a single-pass, mainly found in kidney tubule cells, parathyroid glands and brain; 2) the secreted form (s-Klotho), detectable in plasma (nearly 70 kDa), urine and cerebrospinal fluid; 3) the intracellular form (Saghiv et al., 2017). Plasma klotho is the most easily measurable isoform and physiologically decreases after 40 years of age.

Even if there are numerous studies that evaluated the association between plasma Klotho with chronic diseases, such as diabetes, glaucoma, atherosclerosis, or ischemic heart disease (Kuro-o, 2019), there are still few data concerning the relationship between plasma Klotho, age related conditions, e.g., sarcopenia and osteoporosis, and physical performance in middle-aged, older adults or frail patients. Furthermore, some authors evaluated the effects of physical activity in middle-aged or older adults through measurement of plasma Klotho.

The aim of the present systematic review is to summarize the last 10 years clinical studies investigating the association between plasma Klotho with ageing or frailty, considering physical performance and the effects of physical activity on klotho levels.

2. Materials and methods

The systematic search was conducted in February 2021 on 3 databases (PubMed, Scopus and Web of Science) to identify relevant papers. In PubMed database, the following Meshes were used: ("Klotho protein" [Supplementary Concept] AND "Ageing"[Mesh]) and the limits were English language and publication date from 2010/01/01 to 2021/02/28. In Scopus and Web of Science databases, "Klotho AND ageing" were used as keywords and the limits were: English language, article types and publication date from 2010 to date.

Clinical studies, with observational or interventional design, evaluating the association between plasma Klotho and ageing or frailty, considering body composition, muscle strength, mobility disability, bone metabolism, physical performance and longevity, and those concerning the effect of physical activity on Klotho levels were taken into consideration. The included clinical studies enrolled only middle-aged and older adults, both sedentary and trained.

Exclusion criteria included: reviews, in vivo, ex vivo *or* in vitro studies, case reports, articles regarding the association between plasma Klotho and diseases, such as those affecting kidney, eye, heart, brain and metabolism, diet consumption and young subjects.

The relevant articles were screened using title and abstract by one author (FV) and then, the articles were submitted to Mendeley_v.1.17.9 to eliminate duplicates.

The full-text articles were then examined by two authors (FV and VB). Any disagreement was resolved through discussion until a consensus was reached, or with the involvement of a third reviewer (MF) (Fig. 1). Finally, the reference lists of these studies were analyzed.

Quality assessment of the included studies was performed according to the instructions of https://www.nhlbi.nih.gov/health-topics/study-quality-assessment-tools website. This study was performed according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) reporting guideline (Moher et al., 2009).

3. Results

A total of 1615 studies were found in the 3 databases. After screening titles and abstracts, 273 articles were included and, after duplicates

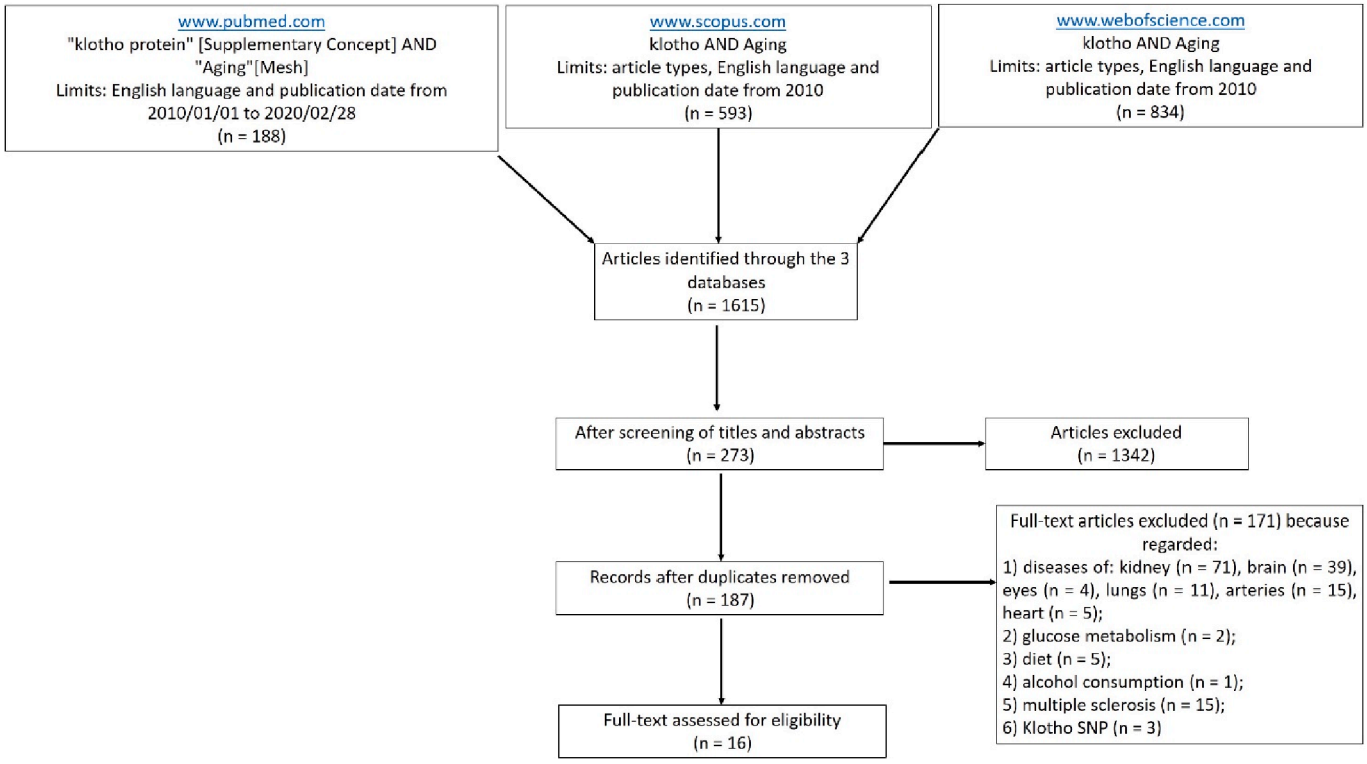

Fig. 1. Schematic representation of search strategy according to PRISMA guidelines.

removal, 187 of them were considered. After analysis of full-texts, 168 articles were excluded because they investigated diseases affecting kidneys (71/168), brain (39/168), eyes (4/168), lungs (11/168), arteries (15/168) and heart (5/168); metabolism alterations (2/168); diet or alcohol consumption (6/168); and multiple sclerosis (15/168). Finally, 16 articles were included in the present systematic review. No further studies were found by reading the reference lists of these 16 studies.

Among the included articles, nine studies investigated the association between Klotho and body composition, muscle strength, mobility disability, bone metabolism, physical performance and longevity in sedentary middle-aged or older adults (Amaro-Gahete et al., 2019a; Semba et al., 2012; Semba et al., 2016; Crasto et al., 2012; Shardell et al., 2020; Koyama et al., 2015; Chalhoub et al., 2016; Shardell et al., 2015; Semba et al., 2011); two studies evaluated the relationship between Klotho and frailty in older adults (Polat et al., 2020; Shardell et al., 2019); five studies evaluated the effect of physical activity on Klotho production in middle-aged or older adults (Matsubara et al., 2014; Amaro-Gahete et al., 2019b; Amaro-Gahete et al., 2019c; Middelbeek et al., 2021; Rosa et al., 2020). Among the latter group, only one study included postmenopausal women at baseline and after aerobic exercise training (Matsubara et al., 2014).

Observational cohort (Amaro-Gahete et al., 2019a; Shardell et al., 2020; Chalhoub et al., 2016; Shardell et al., 2015; Shardell et al., 2019; Amaro-Gahete et al., 2019b), population-based (Semba et al., 2012; Semba et al., 2016; Crasto et al., 2012; Semba et al., 2011), cross-sectional (Koyama et al., 2015; Polat et al., 2020; Amaro-Gahete et al., 2019c; Rosa et al., 2020) and controlled intervention (Matsubara et al., 2014; Middelbeek et al., 2021) studies were included. The follow-up, after which Klotho was measured, ranged between 2 (Koyama et al., 2015) and 36 months (Semba et al., 2012; Crasto et al., 2012; Shardell et al., 2015; Semba et al., 2011; Shardell et al., 2019).

Table 1 summarized the most important results of these studies. In all studies, S-Klotho was measured in plasma, but for convenience, in the manuscript and tables the term “Klotho” has been used.

3.1. Sedentary middle-aged or community-dwelling older subjects

Body composition was evaluated through weight, height, BMD, body mass index (BMI), lean mass index (LMI) and fat mass index (FMI) in 74 sedentary middle-aged (40–65 years old) adults. BMI, LMI and BMD, but not FMI, were positively associated with plasma Klotho (at a concentration of 775.3 ± 363.7 pg/ml), underling that obese people had significantly higher Klotho than normal-weight or overweight ones (Amaro-Gahete et al., 2019a).

Skeletal muscle strength was evaluated in two studies in 804 and 3075 community-dwelling older adults (70–80 years old), through the measurement of knee extensor and hand grip strength (Semba et al., 2012; Semba et al., 2016). In the first study, Klotho threshold was established at 681 pg/mL. It was observed that Klotho was positively or negatively associated with grip strength depending on the level (681 pg/ml or above, respectively). Furthermore, hand grip strength was negatively associated with ageing, physical inactivity, congestive heart failure, stroke, chronic obstructive pulmonary disease (COPD), chronic kidney disease (CKD) and cancer (Semba et al., 2012).

In the second study, plasma Klotho was divided into tertiles (< 536 pg/ml, 536–747 pg/ml, > 747 pg/ml). The highest tertile showed significantly higher knee extension strength and physical activity and lower decline over 4 years of follow-up in knee strength, C-reactive protein (CRP) and Interleukine-6 (IL6) than those of the other two tertiles (Semba et al., 2016).

In 802 and 2751 community-dwelling sedentary older subjects (≥ 65 years old), mobility disability was evaluated through assessment of activities of daily living (ADL) (Crasto et al., 2012) or through difficulty in walking or stair climbing, respectively (Shardell et al., 2020). In the first study, Klotho amount was divided into tertiles: < 575 pg/ml, 575–763 pg/ml, > 763 pg/ml (Crasto et al., 2012), while in the second one different values of Klotho were used to define tertiles, i.e. ≤535 pg/ml, 536–738 pg/ml and > 738 pg/ml (Shardell et al., 2020). Older subjects with Klotho levels below 575 pg/ml showed the highest prevalence of ADL disability and the lowest short physical performance battery (SPPB) score. No association was found between Klotho and BMI, physical activity, cholesterol and chronic diseases. In addition, ADL disability was positively associated also with ageing, coronary heart disease, heart failure, peripheral artery disease, stroke and diabetes and lower physical activity and SPPB (Crasto et al., 2012).

When the Klotho level was greater than 738 pg/ml, it was positively associated with lower walking disability rate, while when it was ≤535 pg/ml, Klotho was positively associated with walking disability. No association between Klotho and disability in climbing stairs was observed (Shardell et al., 2020).

Two studies evaluated the relationship between Klotho and bone metabolism in 52 and 3075 subjects older than 70 years old, respectively (Koyama et al., 2015; Chalhoub et al., 2016). In the first study Klotho, with a mean value of 787.6 pg/ml, positively correlated with estimated glomerular filtration rate (eGFR) and negatively with age, FGF23, inorganic phosphate and OP, while no correlation was found with CKD, BMI, parathyroid hormone (PTH), Vitamin D3 and calcium level (Koyama et al., 2015).

In the second study, Klotho concentrations were subdivided into quartiles: 397.8 (320.6–437.3); 554.5 (521.6–592.1); 709.9 (670.1–756.2); 999.5 (887.7–1186.4) pg/ml. Similar hip, femoral neck and vertebral BMD was found across quartiles and the lowest quartile was not associated with femoral neck and lumbar spine BMD, fracture risk and incidence (Chalhoub et al., 2016).

Lower extremity physical performance was evaluated using SPPB in 860 community-dwelling older adults (≥ 65 years). Klotho was categorized over or under 669 pg/ml, showing that the higher Klotho concentration grouped younger subjects with higher SPPB than those in the lower Klotho concentration group. Klotho was positively correlated with SPPB, but not with 25-hydroxyvitamin D [25(OH)D], PTH and eGFR (Shardell et al., 2015).

Finally, in the InCHIANTI study the association between mortality and Klotho levels was evaluated in 803 community-dwelling older adults (≥ 65 years old), showing an increase in all-cause mortality in subjects with Klotho <575 pg/ml and 575–763 pg/ml than in those with Klotho >763 pg/ml. In addition, when Klotho was lower than 575 pg/ml, it was positively associated with ageing, low calcium and HDL and high triglycerides (Semba et al., 2011).

3.2. Frailty in older adult

Two studies dealt with frailty, evaluated by means of the FRAIL scale or with Fried criteria, in 85 and 774 individuals over 65 years, respectively (Polat et al., 2020; Shardell et al., 2019). In one study, Klotho (540–760 pg/ml) was positively correlated with hemoglobin (Hb) and negatively with CRP. On the other hand, frailty was correlated with ageing, congestive heart failure, atrial fibrillation, COPD and CRP and negatively correlated with hand grip strength, walking speed, BMI, eGFR, albumin and Hb. However, Klotho level was similar in both frail and not frail subjects, indicating no correlation of Klotho with frailty (Polat et al., 2020).

On the contrary, in the second study, Klotho amount over 660 pg/ml was associated with younger age, and lower proability to be pre-frail or frail than klotho amount ≤ 660 pg/ml. (Shardell et al., 2019).

3.3. Physical activity in middle-aged or community-dwelling older adults

In one study, 19 postmenopausal women, who participated in an aerobic exercise consisting of cycling and walking for 12 weeks, showed an increase in Klotho amount (386–489 pg/ml), carotid artery compliance and a reduction in β stiffness index in comparison to sedentary

Table 1
Results of the clinical studies included in the review.

Type of study (follow-up)	Aim	Evaluations (klotho concentration)	Results	Ref
Sedentary middel-aged or community-dwelling older adult				
Observational cohort study (12 mo)	Evaluation of the relationship between Klotho with body composition in middle-aged sedentary adults	Weight, height, BMD, BMI, LMI, FMI. (775.3 ± 363.7 pg/ml)	Klotho positively associated with BMI, LMI, BMD. Klotho not associated with FMI. Obese people ↑ Klotho than normal-weight people	Amaro-Gahete et al., 2019a
Longitudinal population-based study (36 mo)	Evaluation of the relationship between klotho with skeletal muscle strength in older adults	BMI, PA, hand grip strength (dynamometer), hypertension, coronary artery disease, heart failure, peripheral artery disease, stroke, diabetes mellitus, cancer, CKD, COPD. (681 pg/ml)	**Klotho < 681 pg/ml:** Klotho positively associated with hand grip strength. **Klotho ≥ 681 pg/ml:** Klotho negatively associated with hand grip strength. Hand grip strength negatively associated with ageing, physical inactivity, congestive heart failure, stroke, COPD, CKD, cancer	Semba et al., 2012
Community-based prospective study (24 mo)	Evaluation of the relationship between klotho with muscle strength in older adults	Knee extensor strength, hand grip strength, serum 25[OH]D, N-tact PTHSP, CRP, IL-6, BMI, calcium, phosphorus, chronic diseases. (<536 pg/ml; 536–747 pg/ml; >747 pg/ml)	**Klotho > 747 pg/ml:** ↑ knee extension strength, PA; ↓ decline in knee strength, CRP and IL6 than **Klotho < 536, 536–747 pg/ml** **Klotho < 575 pg/ml:** ↑ ADL disability, ageing; ↓ SPPB score than **Klotho 575–763 pg/ml, > 763 pg/ml**.	Semba et al., 2016
Longitudinal population-based study (36 mo)	Evaluation of the relationship between klotho with ADL disability in community-dwelling older adults	ADL disability (need for help in getting out of a bed or chair, bathing or showering or dressing), lower extremity function (SPPB), BMI, PA, cholesterol, chronic diseases. (689 ± 238 pg/mL)	Klotho not associated with BMI, PA, total cholesterol, chronic disease prevalence. ADL disability positively associated with ageing, low PA and SPPB and high cholesterol, coronary heart disease, heart failure, peripheral artery disease, stroke and diabetes	Crasto et al., 2012
Observational cohort study (12 mo)	Evaluation of the relationship between Klotho with mobility disability in community-dwelling older adults	Mobility disability assessment (walking, stair climbing), serum FGF23, cystatin C and 25 (OH)D, eGFR, CKD, PTH, total calcium, inorganic phosphorus, UACR, PA, BMI, comorbidities. (Mean 630.4 pg/ml)	**Klotho > 738 pg/ml:** Klotho positively associated with lower walking disability rate. **Klotho ≤ 535 pg/ml:** Klotho positively associated with walking disability than **Klotho 536–738 pg/ml, > 738 pg/ml**. Klotho positively associated with CKD and high walking disability. Klotho not associated with climb disability	Shardell et al., 2020
Cross sectional study (2 mo)	Evaluation of the relationship between Klotho with mineral bone metabolism in community-dwelling older adults	Serum FGF23 and creatinine, BMI, cerebrovascular and cardiovascular diseases, hyperthension, dyslipidemia, diabetes, CKD, OP, eGFR, albumin, uric acid, albumin-corrected calcium, phosphorus, iPTH, activated vitamin D3, UACR. (Mean 787.6 pg/ml)	Klotho positively associated with eGFR. Klotho negatively associated with ageing, FGF23, serum inorganic phosphate and OP. Klotho not associated with CKD, BMI, iPTH, activated vitamin D3, albumin-corrected calcium levels	Koyama et al., 2015
Longitudinal cohort study (24 mo)	Evaluation of the relationship between Klotho with BMD loss and fractures in community-dwelling older adults	Fracture incidence, BMD, history of falls, weight, BMI, serum 25(OH)D and calcium, PA, gait speed, muscle strength, ALM, BMC. (320.6–1186.4 pg/ml)	Similar hip, femoral neck and vertebral BMD across quartiles. **Klotho 397.8 pg/ml:** Klotho not associated with femoral neck and lumbar spine BMD, fracture risk, fractures	Chalhoub et al., 2016
Prospective cohort study (36 mo)	Evaluation of the relationship between Klotho with lower-extremity physical performance in community-dwelling older adults	Lower-extremity PA (SPPB), serum 25(OH)D, intact PTH and creatinine, calcium intake, comorbidities, BMI. (Median 669 pg/mL)	Klotho and 25(OH)D positively associated with SPPB. Klotho not associated with 25(OH)D, PTH, eGFR. **Klotho > 669 pg/ml:** ↑ younger, SPPB; ↓ fallen than **Klotho ≤ 669 pg/ml**. Death positively associated with ageing, comorbidities and low 25(OH)D and Klotho	Shardell et al., 2015
Longitudinal population-based study (36 mo)	Evaluation of the relationship between Klotho with longevity in community-dwelling older adults	Plasma calcium, HDL, triglycerides, mortality, BMI, PA, mean arterial pressure, 25(OH)D, PTH, total cholesterol, LDL, chronic diseases. (697 ± 325 pg/ml)	**Klotho < 575 pg/ml:** ↑ risk of death than **Klotho > 763 pg/ml**; Klotho positively associated with ageing, low calcium and HDL and high triglycerides. **Klotho < 575 pg/ml, 575–763 pg/ml**: ↑ all-cause deaths than **Klotho > 763 pg/ml**	Semba et al., 2011
Frailty				
Cross-sectional study (6 mo)	Evaluation of the relationship between Klotho with frailty in older adults	Frailty (FRAIL scale), venus blood sedimentation, CRP, WBC, Hb, MCV, plt, albumin, total protein, BUN, creatinine, electrolytes (sodium, potassium, calcium, phosphorus), AST, ALT, GGT, alkaline phosphatase, lipid profile, TSH, 25(OH)D, vitamin B12, folate levels, fasting blood sugar, COPD, GDS, ESR, BMI, GFR. (540–760 pg/ml)	F and NF = Klotho. F: ↑ CRP; ↓ Hb than NF. Klotho positively associated with Hb. Klotho negatively associated with CRP. F positively associated with ageing, congestive heart failure, atrial fibrilation, COPD, CRP. F negatively associated with hand-grip	Polat et al., 2020

(*continued on next page*)

Table 1 (*continued*)

Type of study (follow-up)	Aim	Evaluations (klotho concentration)	Results	Ref
			strength, walking speed, BMI, GFR, albumin, Hb	
Prospective cohort study (36 mo)	Evaluation of the relationship between klotho with frailty in older adults	Frailty (Fried criteria), serum creatinine, comorbidities, BMI. (Median 660 pg/ml)	**Klotho > 660 pg/ml:** ↑ younger, NF; ↓ F and PF **than Klotho ≤ 660 pg/ml**. Death positively associated with ageing, high F and PF	Shardell et al., 2019
Physical activity in middle-aged or community-dwelling older adult				
Exercise intervention study	Evaluation of the relationship between Klotho with aerobic exercise capacity in healthy, postmenopausal women. Group 1): no exercise ($n = 8$); Group 2): 12 wk. of moderate aerobic exercise training for >3 days/wk.; cycling and walking for 30 min/day, then increased intensity ($n = 11$)	Arterial blood pressure, carotid artery compliance, blood biochemistry, PA, VO_2 at VT. (386–489 pg/ml)	Groups 1), 2): = VO_2 at VT and cholesterol levels. Group 2): ↑ Klotho, carotid artery compliance; ↓ β stiffness index	Matsubara et al., 2014
Observational cohort study (12 mo)	Evaluation of the relationship between Klotho with physical activity in middle-aged adults. (Water-based and maximum treadmill exercise)	Physical activity and sedentary time, LPA, MPA, VPA, MVPA, VO_{2max}, O_2 consumption, CO_2 production, RPE, heart rate, body muscular strength, hand grip strength, BMI. (Mean 775 pg/ml)	Klotho not associated with sedentary time and overall PA. Klotho positively associated with MVPA, VO_{2max}, extension peak torque, hand grip strength	Amaro-Gahete et al., 2019b
Cross-sectional study (12 mo)	Evaluation of the relationship between Klotho with BMR/fuel oxidation in middle-aged adults. (Water-based and maximum treadmill exercise)	BMI, LMI, FMI, MFO, MFO_{LM}, Fat_{max}, BFox, BCHox, BMR (indirect calorimetry), BMR_{LM}. (Mean 775.3 pg/ml)	Klotho positively associated with BFox and MFO. Klotho negatively associated with BCHox. Klotho not associated with BMR, BMR_{LM}, MFO_{LM}, Fat_{max}	Amaro-Gahete et al., 2019c
Randomized controlled intervention studies	Evaluation of the effects of two short-term exercise training programs on cytokines and Klotho in middle-aged sedentary man. Group 1): 2 wks of sprint interval training (6 sessions of 6 × 30 s cycle ergometer sprints with 4 min of recovery) ($n = 12$); Group 2): 2 wks of moderate intensity continuous training (6 sessions of cycle ergometer exercise at 60% VO2peak, gradually increasing from 40 to 60 min) ($n = 10$)	VO_{2peak}, body composition, abdominal subcutaneous and visceral adipose tissue masses, serum concentrations of NGF, IL-6, IL-8, Leptin, HGF, MCP-1, TNF-α. (2500–7200 pg/ml)	Groups 1), 2): ↓ IL6, Leptin, HGF, fat percentage and visceral fat; ↑ VO_{2peak}. Group 2): ↑MCP-1, Klotho	Middelbeek et al., 2021
Cross-sectional study	Comparison of redox balance, cytokine levels and biomarkers of ageing between master sprinters and endurance athletes middle-aged individuals. Group 1): 18 master runners from endurance (53 ± 8.2 yrs); Group 2): 13 master sprinters (50 ± 8.9 yrs); Group 3): 17 untrained young (22.7 ± 3.9 yrs); Group 4): 12 middle-aged untrained (45.5 ± 9.8 yrs)	Oxidative stress biomarkers (lipid peroxidation, F2-isoprostanes, protein carbonyls, 8-OHdG), Antioxidant biomarkers (total antioxidant capacity, activities of superoxide dismutase, catalase, NOx, glutathione, plasma uric acid), inflammatory markers (plasma TNFα, IL-6, IL-10, IL-15, sIL-6R, sTNF-RI), telomere length, serum ADMA and FGF23, irisin. (430–784 pg/ml)	Group 2): ↓ F2-Isoprostanes, nitrite; ↑Trolox equivalent, IL10, ADMA, Irisin, Klotho than group 1). Group 4): ↑ 8-OHdG, Uric acid, FGF23, F2-Isoprostanes, protein carbonyls, ADAMA, TNFα, sTNF-RI, sIL-6R; ↓ Catalase, nitrite, telomere length, Irisin, Klotho, SOD, IL15 than groups 1), 2), 3). Group 1): ↓Trolox equivalent, Irisin, Klotho; ↑ nitrite, TNFα, sTNF-RI, sIL-6R, F2-Isoprostanes, protein carbonyls than group 3)	Rosa et al., 2020

Abbreviations: REF = reference; BMD = bone mineral density; BMI = body mass index; LMI = lean mass index; FMI = fat mass index; PA = Physical Activity; COPD = chronic obstructive pulmonary disease; CKD = chronic kidney disease; 25[OH]D = 25-hydroxyvitamin D; PTHSP = parathyroid hormone; CRP = C-reactive protein; IL6 = Interleukin 6; ADL = activities of daily living; eGFR = estimated glomerular filtration rate; SPPB = Short Physical Performance Battery; FGF23 = fibroblast growth factor 23; UACR = urine albumin/creatinine ratio; OP = Osteoporosis; ALM = appendicular lean mass; VO2 at VT = oxygen volume at ventilatory threshold; ccIMT = Common carotid intima-media thickness; HDL = high-density lipoprotein; LDL = low-density lipoprotein; LPA = light physical activity time; MPA = moderate physical activity time; VPA = vigorous activity time; MVPA = moderate-vigorous physical activity time; RPE = rating of perceived exertion; MFO = maximal fat oxidation; MFO_{LM} = Maximal Fat Oxidation relative to Lean Mass; Fat_{max} = Intensity of exercise that elicits MFO; BFox = Basal Fat Oxidation; BCHox = Basal Carbohydrate Oxidation; BMR = Basal Metabolic Rate; BMR_{LM} = Basal Metabolic Rate relative to Lean Mass; wk. = weeks; NGF = nerve growth factor; HGF = hepatocyte growth factor; MCP-1 = monocyte chemoattractant protein-1, TNF-α = Tumor Necrosis Factor α; 8-OHdG = 8-hydroxy-2′ -deoxyguanosine; Nox = nitrite/nitrate, sIL-6R = Soluble Interleukin-6 receptor, sTNF-RI = Soluble tumor necrosis factor receptor type I, ADMA = Asymmetric Dimethylarginine; WBC = leukocyte; Hb = hemoglobin; MCV = mean erythrocyte volume, plt = platelet; BUN = blood urea nitrogen; AST = aspartate transaminase; ALT = alanine transaminase; GGT = gamma glutamyl transferase; TSH = thyroid stimulating hormone; F = frailty; PF = pre-frailty; NF = not frailty; SOD = superoxide dismutase.

postmenopausal women (50–76 years old) (Matsubara et al., 2014).

In 74 middle-aged adults (40–65 years old) with a Klotho concentration of 775 ± 364 pg/ml, Klotho was not associated with sedentary time, overall physical activity, basal metabolic rate (BMR), BMR_{LM}, maximal fat oxidation (MFO_{LM}) and intensity of exercise that elicits MFO (Fat_{max}) (Amaro-Gahete et al., 2019b; Amaro-Gahete et al., 2019c), but it was positively associated with moderate-vigorous physical activity time (MVPA), maximal oxygen uptake (VO_{2max}), extension peak torque, hand grip strength, basal fat oxidation (BFox) and MFO (Amaro-Gahete et al., 2019b; Amaro-Gahete et al., 2019c). Training consisted of water-based activities or maximum treadmill exercise (Amaro-Gahete et al., 2019b; Amaro-Gahete et al., 2019c). In addition, Klotho was negatively

associated with basal carbohydrate oxidation (BCHox) (Amaro-Gahete et al., 2019c).

Finally, two types of exercises were compared in the same study (Middelbeek et al., 2021; Rosa et al., 2020). A sprint training or moderate intensity continuous one, lasting 2 weeks, were compared in 22 middle-aged men (40–55 years old). Both types of exercise reduced IL6, Leptin, hepatocyte growth factor (HGF), visceral fat and increased VO_{2peak}. However, only moderate intensity exercise increased Klotho level (2500–7200 pg/ml) (Middelbeek et al., 2021).

Sixty middle-aged trained master runners from endurance and master sprinters were compared with each other and with untrained young or middle-aged subjects. Klotho (430–784 pg/ml) was found higher in master sprinters than in master runners, lower in master runners than in untrained younger subjects, but the lowest Klotho concentration was found in middle-aged sedentary subjects. In addition, the middle-aged sedentary group showed higher pro-oxidant activity in comparison to the other groups with the highest 8-OHdG, Uric acid, F2-Isoprostanes, protein carbonyls, Asymmetric Dimethylarginine (ADMA), TNFα, sTNF-RI, sIL-6R and lowest catalase, nitrite, telomere length, Irisin, superoxide dismutase (SOD) and IL15. Master runners showed lower antioxidant and anti-inflammatory activities than master sprinters (Rosa et al., 2020).

3.4. Quality assessment

The results of quality assessment performed for observational cohort and cross-sectional studies and controlled intervention ones are presented in Tables 2 and 3, respectively.

As observed in Table 2, all the observational cohort and cross-sectional studies had a clear research question. The exposures of interest were measured prior to the outcomes being measured, the timeframe was sufficient to see an association between exposure and outcome, they examined different levels of the exposure as related to the outcome, the exposure measures were clearly defined, valid, reliable, and implemented consistently across all study participants, the exposures were assessed more than once over time, the outcome measures were clearly defined, valid, reliable, and implemented consistently across all study participants. In addition, in all studies, the key potential confounding variables were measured and adjusted statistically for their impact on the relationship between exposures and outcomes. In all studies, it was not possible that the study participants and providers were blinded to treatment group assignment (NA, not applicable) and the outcome assessors were not blinded to the exposure status of participants in all studies. Only in one study, the study population was not clearly specified and defined (Crasto et al., 2012).

The loss to follow-up after baseline was calculated and it was ≤20% in 7 studies (Semba et al., 2012; Semba et al., 2016; Crasto et al., 2012; Shardell et al., 2020; Koyama et al., 2015; Semba et al., 2011; Rosa et al., 2020). Finally, only 2 studies clearly declared to perform a priori power analysis for the establishment of the exact number of subjects to enroll in the study (Polat et al., 2020; Rosa et al., 2020).

Globally, all the studies were considered fair, while one was good (Rosa et al., 2020).

Regarding the two controlled intervention studies (Matsubara et al., 2014; Middelbeek et al., 2021) (Table 3), they were considered not of high quality because in both articles the method of randomization was not adequate, the treatment allocation was not concealed, the people assessing the outcomes were not blinded to the participants' group assignments, other interventions were not avoided or similar in the groups, and the authors did not use an intention-to-treat analysis. On the other hand, in both studies the groups were similar at baseline on important characteristics that could affect outcomes, high adherence to

Table 2
Quality assessment tool for observational cohort and cross-sectional studies.

Reference	Criteria													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Amaro-Gahete et al., 2019a			NR	NA									CD	
Semba et al., 2012				NA										
Semba et al., 2016				NA										
Crasto et al., 2012				NA										
Shardell et al., 2020				NA										
Koyama et al., 2015			NR	NA									CD	
Chalhoub et al., 2016				NA										
Shardell et al., 2015				NA										
Semba et al., 2011				NA										
Polat et al., 2020			NR	NA									CD	
Shardell et al., 2019			NR	NA										
Amaro-Gahete et al., 2019b			NR	NA									CD	
Amaro-Gahete et al., 2019c			NR	NA									CD	

1. Was the research question or objective in this paper clearly stated? 2. Was the study population clearly specified and defined? 3. Was the participation rate of eligible persons at least 50%? 4. Were study participants and providers blinded to treatment group assignment? 5. Was a sample size justification, power description, or variance and effect estimates provided? 6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured? 7.Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed? 8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)? 9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants? 10. Was the exposure(s) assessed more than once over time? 11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants? 12. Were the outcome assessors blinded to the exposure status of participants? 13. Was loss to follow-up after baseline 20% or less? 14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?
CD, cannot determine; **NA**, not applicable; **NR**, not reported.

Table 3
Quality Assessment Tool for controlled intervention studies.

Reference	Criteria													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Matsubara et al., 2014				NA										
Middelbeek et al., 2021				NA										

1.Was the study described as randomized, a randomized trial, a randomized clinical trial, or an RCT? 2. Was the method of randomization adequate (i.e., use of randomly generated assignment)? 3. Was the treatment allocation concealed (so that assignments could not be predicted)? 4. Were study participants and providers blinded to treatment group assignment? 5. Were the people assessing the outcomes blinded to the participants' group assignments? 6. Were the groups similar at baseline on important characteristics that could affect outcomes (e.g., demographics, risk factors, co-morbid conditions)? 7. Was the overall drop-out rate from the study at endpoint 20% or lower of the number allocated to treatment? 8. Was the differential drop-out rate (between treatment groups) at endpoint 15 percentage points or lower? 9. Was there high adherence to the intervention protocols for each treatment group? 10. Were other interventions avoided or similar in the groups (e.g., similar background treatments)? 11. Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants? 12. Did the authors report that the sample size was sufficiently large to be able to detect a difference in the main outcome between groups with at least 80% power? 13. Were outcomes reported or subgroups analyzed prespecified (i.e., identified before analyses were conducted)? 14. Were all randomized participants analyzed in the group to which they were originally assigned, i.e., did they use an intention-to-treat analysis?
CD, cannot determine; **NA,** not applicable; **NR,** not reported.

the intervention protocols was observed for each treatment group, outcomes were assessed using valid and reliable measures, implemented consistently across all study participants and the outcomes reported or subgroups analyzed were prespecified. In addition, in both studies the study participants and providers couldn't be blinded to treatment group assignment (NA, not applicable). One study was not randomized, the overall drop-out rate from the study at endpoint was over 20% of the number allocated to treatment, and the authors did not report that the sample size was sufficiently large to be able to detect a difference in the main outcome between groups with at least 80% power (Matsubara et al., 2014).

4. Discussion

It is estimated that by 2035 the percentage of European population over 65 years will be nearly 25%. So, since the human body composition changes dramatically during ageing with increased risks of disease development and mortality, it is mandatory to find interventions that attenuate the public health problems associated with ageing (Foo et al., 2019).

Therefore, the identification of ageing biomarkers, that could become a reliable tool for ageing and age-related diseases monitoring, is extremely important.

In this regard, Klotho has been recently identified as an ageing suppressor protein, whose decline starts around the age of 40 (Lai et al., 2018).

The present systematic review was performed to evaluate the association between Klotho and body composition and physical performance in middle-aged and older adults as well as the effects of physical activity types on Klotho amount in studies published in the last 10 years.

Sixteen studies were found and were divided into 3 areas of interests that concerned the association of Klotho with: 1) body composition, muscle strength, mobility disability, bone metabolism, physical performance and longevity in sedentary middle-aged or older adults, 2) frailty in older subjects; 3) physical activity in middle-aged or older adults.

In all the studies Klotho was measured in venous blood through solid-phase sandwich enzyme linked immunosorbent assay (ELISA) tests. The amount of Klotho plasma levels varied in the studies included in the review with average values ranging from 386 pg/ml (Matsubara et al., 2014) to 7200 pg/ml (Middelbeek et al., 2021) while the most common value for Klotho was around 600–700 pg/ml (Amaro-Gahete et al., 2019a; Semba et al., 2012; Semba et al., 2016; Crasto et al., 2012; Shardell et al., 2020; Koyama et al., 2015; Shardell et al., 2015; Semba et al., 2011; Polat et al., 2020; Shardell et al., 2019; Matsubara et al., 2014; Amaro-Gahete et al., 2019b; Amaro-Gahete et al., 2019c; Middelbeek et al., 2021).

In sedentary middle-aged subjects, Klotho was associated with BMI, LMI and BMD, and higher Klotho was found in obese people than in normal-weight ones (Amaro-Gahete et al., 2019a). In older subjects, discordant results were found concerning muscle strength, since some authors found a correlation between Klotho and muscle strength when Klotho was lower than 681 pg/ml (Semba et al., 2012) and other ones when Klotho was higher than 747 pg/ml (Semba et al., 2016). Klotho was positively associated with walking ability and SPPB (Crasto et al., 2012; Shardell et al., 2020) and it was also protective towards OP and arterial stiffness in postmenopausal women (Matsubara et al., 2014), even if no correlation was found between Klotho and femoral or lumbar spine BMD and fracture risks (Chalhoub et al., 2016). Finally, the risk of death was higher when Klotho was at lower concentrations that at higher ones (Semba et al., 2011).

Frailty has a prevalence of around 10% in the community-dwelling older adults (Clegg et al., 2013), increasing up to 15–61% over 80 years old.

In this review conflicting results were found regarding the association between plasma Klotho and frailty. One study showed no correlation between plasma Klotho and frailty (Polat et al., 2020), while another study showed that when Klotho was >660 pg/ml there was a lower probability of pre frailty and frailty than when Klotho ≤660 pg/ml (Shardell et al., 2019).

It is well established how important physical activity is in and middle-aged and older people to improve the quality of life, and to prevent the age-related diseases (Pedersen and Saltin, 2015; Ekblom-Bak et al., 2014). Different types of exercise were evaluated in this review including swimming, daily physical activity (Amaro-Gahete et al., 2019b; Amaro-Gahete et al., 2019c), sprint interval training or moderate intensity continuous training with cycling (Middelbeek et al., 2021), master running (Rosa et al., 2020) or aerobic exercise with cycling and walking (Matsubara et al., 2014). All types of physical activities increased the amount of plasma Klotho (Middelbeek et al., 2021), and in postmenopausal women, aerobic exercise increased plasma Klotho and arterial compliance (Matsubara et al., 2014).

Another particularly important issue is the evaluation of the sex difference in the amount of Klotho in older or middle-aged subjects. In our previous review on frailty (Veronesi et al., 2021), few studies identified sex differences in frailty, with discordant results. Unfortunately, most of the studies included in this review did not take this item into account, while some studies did not find sex differences in plasma Klotho levels (Amaro-Gahete et al., 2019a; Polat et al., 2020; Amaro-Gahete et al., 2019b; Amaro-Gahete et al., 2019c). Therefore, no conclusion on the male-female difference regarding Klotho in older subjects can be drawn.

This is the first systematic review which associates a serum biomarker not only with ageing, but also with frailty in a universal way, collecting the clinical studies of the last 10 years of literature on this

topic and attempting to point out gender differences. However, the included clinical studies are still very few, especially on frailty, and are very different from each other from the point of view of the study design and the cut off of the Klotho measurement (sometimes not indicated), which makes it difficult to draw conclusions.

5. Conclusions

In conclusion, Klotho seems to be positively associated with muscle strength, BMD and lower-extremity physical performance and negatively with OP, mobility disability and all-cause mortality.

The association between Klotho and frailty is not well established, but physical activity increases Klotho levels.

Future research should investigate the role of Klotho in frailty, especially in pre-frailty. Furthermore, the evaluation of the presence of sex differences in the amount of Klotho in older subjects is important to provide a better understating of the role of klotho in ageing and age-related diseases.

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CRediT authorship contribution statement

Milena Fini and Antonio Cherubini: Conceptualization and supervision;

Francesca Veronesi, Veronica Borsari: Data curation and Formal analysis;

All the authors: Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Amaro-Gahete, F.J., De-la-O, A., Jurado-Fasoli, L., Espuch-Oliver, A., de Haro, T., Gutierrez, A., Ruiz, J.R., Castillo, M.J., 2019a. Body composition and S-klotho plasma levels in middle-aged adults: a cross-sectional study. Rejuvenation Res. 22, 478–483. https://doi.org/10.1089/rej.2018.2092.

Amaro-Gahete, F.J., De-la-O, A., Jurado-Fasoli, L., Gutiérrez, A., Ruiz, J.R., Castillo, M.J., 2019b. Association of physical activity and fitness with S-klotho plasma levels in middle-aged sedentary adults: the FIT-AGEING study. Maturitas 123, 25–31. https://doi.org/10.1016/j.maturitas.2019.02.001.

Amaro-Gahete, F.J., De-la-O, A., Jurado-Fasoli, L., Ruiz, J.R., Castillo, M.J., 2019c. Association of basal metabolic rate and fuel oxidation in basal conditions and during exercise, with plasma S-klotho: the FIT-AGEING study. Ageing (Albany NY) 11, 5319–5333. https://doi.org/10.18632/ageing.102100.

Cardoso, A.L., Fernandes, A., Aguilar-Pimentel, J.A., de Angelis, M.H., Guedes, J.R., Brito, M.A., Ortolano, S., Pani, G., Athanasopoulou, S., Gonos, E.S., Schosserer, M., Grillari, J., Peterson, P., Tuna, B.G., Dogan, S., Meyer, A., van Os, R., Trendelenburg, A.U., 2018. Towards frailty biomarkers: candidates from genes and pathways regulated in ageing and age-related diseases. Ageing Res. Rev. 47, 214–277. https://doi.org/10.1016/j.arr.2018.07.004.

Chalhoub, D., Marques, E., Meirelles, O., Semba, R.D., Ferrucci, L., Satterfield, S., Nevitt, M., Cauley, J.A., Harris, T., 2016. Association of serum klotho with loss of bone mineral density and fracture risk in older adults. J. Am. Geriatr. Soc. 64, e304–e308. https://doi.org/10.1111/jgs.14661.

Clegg, A., Young, J., Iliffe, S., Rikkert, M.O., Rockwood, K., 2013. Frailty in elderly people. Lancet 381, 752–762. https://doi.org/10.1016/S0140-6736(12)62167-9.

Crasto, C.L., Semba, R.D., Sun, K., Cappola, A.R., Bandinelli, S., Ferrucci, L., 2012. Relationship of low-circulating "Anti-ageing" klotho hormone with disability in activities of daily living among older community-dwelling adults. Rejuvenation Res. 15, 295–301. https://doi.org/10.1089/rej.2011.1268.

Dent, E., Kowal, P., Hoogendijk, E.O., 2016. Frailty measurement in research and clinical practice: a review. Eur. J. Int. Med. 31, 3–10. https://doi.org/10.1016/j.ejim.2016.03.007.

Ekblom-Bak, E., Ekblom, B., Vikström, M., De Faire, U., Hellénius, M.L., 2014. The importance of non-exercise physical activity for cardiovascular health and longevity. Br. J. Sports Med. 48, 233–238. https://doi.org/10.1136/bjsports-2012-092038.

Foo, H., Mather, K.A., Thalamuthu, A., Sachdev, P.S., 2019. The many ages of man: diverse approaches to assessing ageing-related biological and psychological measures and their relationship to chronological age. Curr. Opin. Psychiatry. 32, 130–137. https://doi.org/10.1097/YCO.0000000000000473.

Hirschfeld, H.P., Kinsella, R., Duque, G., 2017. Osteosarcopenia: where bone, muscle, and fat collide. Osteoporos. Int. 28, 2781–2790. https://doi.org/10.1007/s00198-017-4151-8.

Hu, M.C., Shiizaki, K., Kuro-o, M., Moe, O.W., 2013. Fibroblast growth factor 23 and klotho: physiology and pathophysiology of an endocrine network of mineral metabolism. Annu. Rev. Physiol. 75, 503–533. https://doi.org/10.1146/annurev-physiol-030212-183727.

Kidd, T., Mold, F., Jones, C., Ream, E., Grosvenor, W., Sund-Levander, M., Tingström, P., Carey, N., 2019. What are the most effective interventions to improve physical performance in pre-frail and frail adults? A systematic review of randomized control trials. BMC Geriatr. 19, 184. https://doi.org/10.1186/s12877-019-1196-x.

Kim, J.H., Hwang, K.H., Park, K.S., Kong, I.D., Cha, S.K., 2015. Biological role of antiageing protein klotho. J. Lifestyle. Med. 5, 1–6. https://doi.org/10.15280/jlm.2015.5.1.1.

Koyama, D., Sato, Y., Aizawa, M., Maki, T., Kurosawa, M., Kuro-o, M., Furukawa, Y., 2015. Soluble aKlotho as a candidate for the biomarker of ageing. Biochem. Biophys. Res. Comm. 467, 1019–1025. https://doi.org/10.1016/j.bbrc.2015.10.018.

Kuro-o, M., 2018. Molecular mechanisms underlying accelerated ageing by defects in the FGF23-klotho system. Int. J. Nephrol. 2018, 9679841. https://doi.org/10.1155/2018/9679841.

Kuro-o, M., 2019. The klotho proteins in health and disease. Nat. Rev. Nephrol. 15, 27–44. https://doi.org/10.1038/s41581-018-0078-3.

Kuro-o, M., Matsumura, Y., Aizawa, H., Kawaguchi, H., Suga, T., Utsugi, T., Iwasaki, H., 1997. Mutation of the mouse klotho gene leads to a syndrome resembling ageing. Nature 390, 45–51. https://doi.org/10.1038/36285.

Lai, C.C., Tu, Y.K., Wang, T.G., Huang, Y.T., Chien, K.L., 2018. Effects of resistance training, endurance training and whole-body vibration on lean body mass, muscle strength and physical performance in older people: a systematic review and network meta-analysis. Age Ageing 47, 367–373. https://doi.org/10.1093/ageing/afy009.

LIFE Study Investigators, Pahor, M., Blair, S.N., Espeland, M., Fielding, R., Gill, T.M., Guralnik, J.M., Hadley, E.C., King, A.C., Kritchevsky, S.B., Maraldi, C., Miller, M.E., Newman, A.B., Rejeski, W.J., Romashkan, S., Studenski, S., 2006. Effects of a physical activity intervention on measures of physical performance: results of the lifestyle interventions and independence for elders pilot (LIFE-P) study. J. Gerontol. A Biol. Sci. Med. Sci. 61, 1157–1165. https://doi.org/10.1093/gerona/61.11.1157.

Matsubara, T., Miyaki, A., Akazawa, N., Choi, Y., Ra, S.G., Tanahashi, K., Kumagai, H., Oikawa, S., Maeda, S., 2014. Aerobic exercise training increases plasma klotho levels and reduces arterial stiffness in postmenopausal women. Am. J. Physiol. Heart Circ. Physiol. 306, H348–H355. https://doi.org/10.1152/ajpheart.00429.2013.

McPhee, J.S., French, D.P., Jackson, D., Nazroo, J., Pendleton, N., Degens, H., 2016. Physical activity in older age: perspectives for healthy ageing and frailty. Biogerontology 17, 567–580. https://doi.org/10.1007/s10522-016-9641-0.

Middelbeek, R.J.W., Motiani, P., Brandt, N., Nigro, P., Zheng, J., Virtanen, K.A., Kalliokoski, K.K., Hannukainen, J.C., Goodyear, L.J., 2021. Exercise intensity regulates cytokine and klotho responses in men. Nutr. Diabetes. 11, 5.

Moher, D., Liberati, A., Tetzlaff, J., Altman G., D., Prisma Grroup, 2009. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLOS medicine 6 (7), e1000097. https://doi.org/10.1371/journal.pmed.1000097.

Pedersen, B., Saltin, B., 2015. Exercise as medicine - evidence for prescribing exercise as therapy in 26 different chronic diseases. Scand. J. Med. Sci. Sport. 25, 1–72. https://doi.org/10.1111/sms.12581.

Pickering, C., Kiely, J., 2018. ACTN3, morbidity, and healthy ageing. Front. Genet. 9, 1–8. https://doi.org/10.3389/fgene.2018.00015.

Polat, Y., Yalcin, A., Yazihan, N., Bahsi, R., Surmeli, D.M., Akdas, D., Aras, S., Varli, M., 2020. The relationship between frailty and serum alpha klotho levels in geriatric patients. Arch. Gerontol. Geriatr. 91, 104225 https://doi.org/10.1016/j.archger.2020.104225.

Rockwood, K., Bergman, H., 2021. FRAILTY: a report from the 3rd joint workshop of IAGG/WHO/SFGG, Athens, january 2012. Can. Geriatr. J. 15, 31–36. https://doi.org/10.5770/cgj.15.35.

Rosa, T.S., Neves, R.V.P., Deus, L.A., Sousa, C.V., da Silva Aguiar, S., de Souza, M.K., Moraes, M.R., Rosa, É.C.C.C., Andrade, R.V., Korhonen, M.T., Simões, H.G., 2020. Sprint and endurance training in relation to redox balance, inflammatory status and biomarkers of ageing in master athletes. Nitric Oxide 102, 42–51. https://doi.org/10.1016/j.niox.2020.05.004.

Saghiv, M.S., Sira, D.B., Goldhammer, E., Sagiv, M., 2017. The effects of aerobic and anaerobic exercises on circulating soluble-klotho and IGF-I in young and elderly adults and in CAD patients. J. Circ. Biomark. 6, 1–8. https://doi.org/10.1177/1849454417733388.

Semba, R.D., Cappola, A.R., Sun, K., Bandinelli, S., Dalal, M., Crasto, C., Guralnik, J.M., Ferrucci, L., 2011. Plasma klotho and mortality risk in older community-dwelling adults. J. Gerontol. A Biol. Sci. Med. Sci. 66, 794–800. https://doi.org/10.1093/gerona/glr058.

Semba, R.D., Cappola, A.R., Sun, K., Bandinelli, S., Dalal, M., Crasto, C., Guralnik, J.M., Ferrucci, L., 2012. Relationship of low plasma klotho with poor grip strength in older community-dwelling adults: the InCHIANTI study. Eur. J. Appl. Physiol. 112, 1215–1220. https://doi.org/10.1007/s00421-011-2072-3.

Semba, R.D., Ferrucci, L., Sun, K., Simonsick, E., Turner, R., Miljkovic, I., Harris, T., Schwartz, A.V., Asao, K., Kritchevsky, S., Newman, A.B., 2016. Low plasma klotho concentrations and decline of knee strength in older adults. J. Gerontol. A Biol. Sci. Med. Sci. 71, 103–108. https://doi.org/10.1093/gerona/glv077.

Sen, P., Shah, P.P., Nativio, R., Berger, S.L., 2016. Epigenetic mechanisms regulating longevity and ageing. Cell 166, 822–839. https://doi.org/10.1016/j.cell.2016.07.050.

Shardell, M., Semba, R.D., Kalyani, R.K., Hicks, G.E., Bandinelli, S., Ferrucci, L., 2015. Serum 25-hydroxyvitamin D, plasma klotho, and lower-extremity physical performance among older adults: findings from the InCHIANTI study. J. Gerontol. A Biol. Sci. Med. Sci. 70, 1156–1162. https://doi.org/10.1093/gerona/glv017.

Shardell, M., Semba, R.D., Kalyani, R.R., Bandinelli, S., Prather, A.A., Chia, C.W., Ferrucci, L., 2019. Plasma klotho and frailty in older adults: findings from the InCHIANTI study. J. Gerontol. A Biol. Sci. Med. Sci. 74, 1052–1058. https://doi.org/10.1093/gerona/glx202.

Shardell, M., Drew, D.A., Semba, R.D., Harris, T.B., Cawthon, P.M., Simonsick, E.M., Kalyani, R.R., Schwartz, A.V., Kritchevsky, S.B., Newman, A.B., 2020. Plasma soluble aKlotho, serum fibroblast growth factor 23, and mobility disability in community-dwelling older adults. J. Endocr. Soc. 4, bvz032. https://doi.org/10.1210/jendso/bvz032.

Veronesi, F., Borsari, V., Martini, L., Visani, A., Gasbarrini, A., Brodano, G.B., Fini, M., 2021. The impact of frailty on spine surgery: systematic review on 10 years clinical studies. Ageing. Dis. 12, 625–645. https://doi.org/10.14336/AD.2020.0904.

Wu, P.Y., Huang, K.S., Chen, K.M., Chou, C.P., Tu, Y.K., 2021. Exercise, nutrition, and combined exercise and nutrition in older adults with sarcopenia: a systematic review and network meta-analysis. Maturitas 145, 38–48. https://doi.org/10.1016/j.maturitas.2020.12.009.

Yasobant, S., 2018. Comprehensive public health action for our ageing world: the quintessence of public health policy. J. Int. Med. Res. 46, 555–556. https://doi.org/10.1177/0300060517718452.

Zhang, L., Liu, T., 2018. Clinical implication of alterations in serum klotho levels in patients with type 2 diabetes mellitus and its associated complications. J. Diabetes Complicat. 32, 922–930. https://doi.org/10.1016/j.jdiacomp.2018.06.002.