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CHANGE IN URIC ACID LEVEL IN A DIETARY INTERVENTION TRIAL IN POST-MENOPAUSAL WOMEN

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Contents

Abstract

Background: Uric acid, which is produced when purine is broken down in the body, plays a crucial role in both health and disease. Elevated levels of uric acid have been linked to several conditions, such as gout, kidney stones, and cardiovascular disorders. Women who have gone through menopause are particularly susceptible to fluctuations in uric acid levels. As estrogen levels decline during menopause, uric acid metabolism can be affected, which may result in higher serum concentrations. Moreover, lifestyle modifications during this stage of life can also contribute to variations in uric acid levels. Therefore, examining the impact of different diets on uric acid in this population is vital for developing personalized health recommendations and preventative strategies.

Objective: The changing dietary patterns in post-menopausal women (PMW) predispose them to decreased uric acid levels. Our ongoing study aims to evaluate the impact of dietary changes on uric acid levels in PMW.

Design and Method: For 1 year, 52 PMW with BMI 24-39 Kg/m² and at least another CV risk factor were followed. Anthropometric parameters, plicometry, and blood pressure (BP) were taken. Questionnaires on medical history, physical activity, and food frequency (FFQ) were administered. Balanced low-calorie dietary plans with quantitative advice were released. Repeated measures of ANOVA at 1 year were performed.

Results: We observed at 1-year significant reductions in weight (Δ = -4.5 Kg), BMI (Δ = -1.7 Kg/m²), waist (Δ = -4.3 cm) and hips (Δ = -3.1 cm) circumferences, subscapular (Δ = -5.8 mm), suprailiac (Δ = -3.3 mm), and triceps (Δ = -5.5 mm) skinfolds, adipose area of the arm (AFA) (Δ = -7.3 cm²), and fat mass (Δ = -4.5 kg) without variation in lean mass (Δ = -0.1 kg, $p = 0.48$). All these reductions were followed by a change ($p < 0.03$) in uric acid levels (Δ = -0.38 mg/dl).

Conclusions: The study highlights the medium-term utility of a 1-year balanced low-calorie diet in postmenopausal women (PMW) with elevated cardiovascular (CV) risk factors, showing a clear reduction in CV risk, including uric acid levels.

INTRODUCTION

Uric acid

Uric acid metabolism, homeostasis, and function

Uric acid (UA) is a byproduct of the metabolic process that breaks down purines, which are the main building blocks of nucleotides. The process of generating uric acid is illustrated in Figure 1. Essentially, inosine monophosphate (IMP) is produced through both de novo purine synthesis and purine salvage. Hypoxanthine from IMP is converted to xanthine and then to uric acid by the enzyme xanthine oxidoreductase (XOR). De novo nucleotide synthesis creates IMP from ribose-5-phosphate, which is then converted to 5-phosphoribosyl-1-pyrophosphate (PRPP). The salvage pathway also generates IMP through hypoxanthine-guanine phosphoribosyl transferase (HGPRT), which inhibits the production of UA(1).

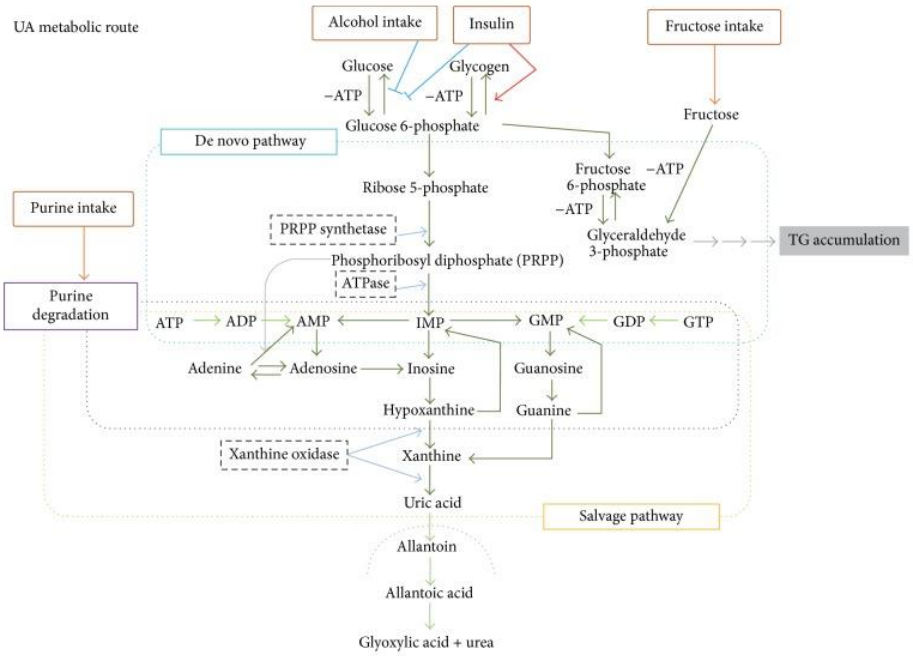

Figure 0 Metabolic pathways involving UA(1)

Due to a lack of urate oxidase or uricase, humans are unable to break down UA into the more soluble compound allantoin, resulting in a higher serum UA concentration than almost all other mammals. However, this high UA level has been considered beneficial in the presence of elevated oxidative stress. UA can neutralize prooxidant molecules such as hydroxyl radicals, hydrogen peroxide, and peroxynitrite by being oxidized to allantoin and other metabolites through nonenzymatic oxidation. UA has the highest scavenging rate constant against $O_2^{-\bullet}$, but its constants are low against $CH_3\bullet$ and t-BuOO•. In physiological environments or in conjunction with antioxidants, UA directly and preferentially eliminates nitric oxide (NO) and forms 6-aminouracil. (2)

The sources of purines include (a) de novo synthesis, (b) cellular RNA from the normal turnover of cells, and (c) exogenous, i.e., dietary sources. Under normal condition, endogenous uric acid production is quite stable at about 300–400 mg per day. The role of exogenous purines is variable though it usually contributes less than 50% of total uric acid production. The kidneys eliminate approximately two-thirds of the uric acid load, while the gastrointestinal tract eliminates the remaining one-third. The renal handling of uric acid is complex and consists of multiple steps. The proximal tubule is responsible for uric acid reabsorption and secretion, with about 90% being reabsorbed into the bloodstream. Uric acid is primarily reabsorbed in the proximal tubules of the kidneys, where transporters exchange intracellular anions for the acid. The S1 segment of the proximal tubule is responsible for almost all uric acid reabsorption, while in the S2 segment, uric acid is secreted to a greater extent than reabsorbed. About 10% of filtered uric acid appears in the urine after post-secretory reabsorption in a more distal site of the proximal tubule. Studies have identified three urate transporters - URAT1/SLC22A12, GLUT9/SLC2A9, ABCG2/BCRP - that play critical roles in regulating serum UA.(3)

UA is the most abundant aqueous antioxidant, accounting for up to 60% of plasma antioxidative capacity, and has different mechanisms of action. It scavenges free radicals, stabilizes vitamin C in serum, mostly due to its iron chelating properties, and quenches peroxynitrite, a potentially harmful oxidant, resulting in the formation of a stable nitric oxide (NO) donor in vitro. At concentrations close to physiological levels in humans, UA prevents hydrogen peroxide-induced inactivation of

extracellular superoxide-dismutase (ecSOD), an enzyme that scavenges superoxide anions (•O2−). Moreover, UA is believed to counteract oxidative damage associated with atherosclerosis and aging in humans. Taken together, these findings suggest that UA could act beneficially in preserving vascular function under physiological and pathological challenges. The potential harmful effects of UA, such as its pro-oxidant and pro-inflammatory actions, may stem from the conversion of xanthine dehydrogenase to xanthine oxidase and the subsequent accumulation of reactive oxygen species (ROS). This process occurs concurrently with UA production due to ATP degradation during ischemic conditions. The resulting ROS by-production has been linked to inflammatory reactions and arterial damage, which are often associated with elevated UA levels. However, in neurodegenerative diseases such as Parkinson's disease, multiple sclerosis, and Alzheimer's disease/dementia, hyperuricemia has been found to have a protective effect. Studies suggest that higher uric acid levels can lower the risk of PD and slow down disease progression, possibly due to the well-known antioxidant properties of uric acid.(4)

The enzyme XOR performs the crucial task of facilitating the conversion of hypoxanthine to xanthine and then to UA. This is the final process in the purine catabolism of the highest uricotelic primates. As UA is an irreversible product, XOR activity prevents the purine salvage pathway, thereby limiting the recovery of nucleotides. XOR's relatively low specificity for substrates and its diverse catalytic activities make it an effective detoxifying enzyme for both endogenous metabolites and foreign substances, including drugs. XOR is a metalloflavoprotein that plays a crucial role in the production of secondary messengers in inflammatory signaling. XOR expression is tightly regulated at both the transcriptional and post-translational levels to modulate its catalytic activities. While XOR expression is typically down-regulated in most tissues, it can be up-regulated by low oxygen tension, cytokines, growth factors, and various hormones.(5) XOR can exist in inactive forms, which retain nicotinamide adenine dinucleotide (NADH) oxidase activity at the FAD site. Additionally, XOR exists in two interconvertible forms: xanthine dehydrogenase (XDH) and xanthine oxidase (XO) (Figure 2).

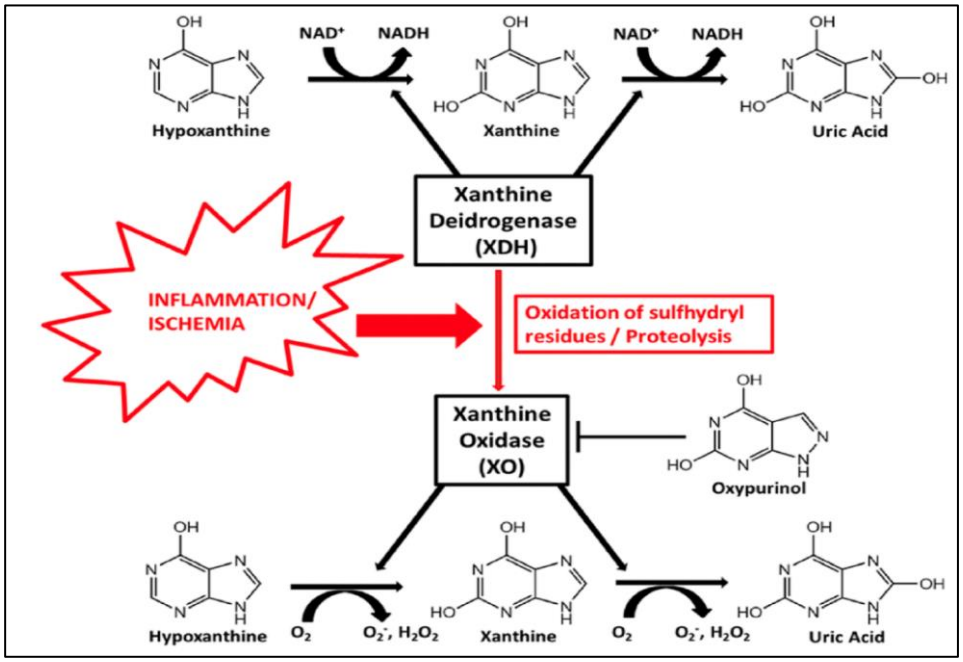

Figure 2. Uric acid homeostasis.(6)

The transition from XDH to XO occurs through limited proteolysis or oxidation of sulfhydryl groups and includes an intermediate XOR form that has both NAD+-dependent dehydrogenase and O2-dependent oxidase activities. XDH generates UA while XO produces hydrogen peroxide and superoxide anion. These substances can lead to the formation of the cytotoxic hydroxyl radical through the Haber-Weiss and Fenton reactions when transition metals are present. Under conditions of low pH and hypoxia, NADH oxidase and nitrate reductase activities of XOR can promote the formation of NO and superoxide anion. Although other enzymes may be involved in the generation of reactive oxygen and nitrogen species, XOR-derived ROS and RNS can have a cytotoxic effect that is beneficial in white blood cell defenses against bacterial illness. However, XOR products can also contribute to tissue damage in various pathological conditions, including reperfusion injury after flow restoration in previously blood-deprived organs or in ischemic tissues. In normal physiological conditions, human plasma only contains small amounts of XOR from dead cells, particularly hepatocytes, which release XOR into circulation where XDH converts to XO. XOR has been localized in the endothelium, probably due to its capacity for adhering to the endothelial surface by competing for the binding sites of heparin. The products of endothelium-linked XOR

together with those of other oxidases have the physiological role of modulating the systemic redox equilibrium by producing ROS. This indicates that XOR activity may play a role in endothelial dysfunction in cardiovascular diseases. XOR products have been found to foster a pro-inflammatory and pro-oxidant environment by regulating endothelial function. This is because ROS and RNS disrupt the vascular lining's permeability. Additionally, XOR modulates arteriolar tone, which may explain why it has implications in the development of hypertension, cardiovascular diseases, and atherosclerosis. (6)

Plasma uric acid levels

Reference ranges for UA in the blood vary according to age and gender: for children it's 2.5-5.5 mg/dL, for adult males it's 4.0-8.5 mg/dL, for adult females it's 2.7-7.3 mg/dL and for elderly individuals there may be a slight increase in values.(7). Indeed, during male puberty, serum UA levels begin to rise from childhood levels, while female levels remain low until after menopause, at which point they increase and approximate those of men. Indeed, estrogen promotes efficient renal urate clearance in premenopausal women. After menopause, this clearance is less effective, leading to increased UA levels. Therefore, postmenopausal women tend to have higher UA concentrations than premenopausal women, as reported in several studies(8). The serum concentration of UA in all remains relatively constant due to a balance between its production and excretion. However, serum UA levels can be influenced by factors such as height, blood pressure, body weight, renal function, and shifts in dietary habits with increase in meat, fructose and alcohol intake, conditions that increase UA production or decrease UA excretion leading to hyperuricemia.

Hyperuricemia and its epidemiology

UA is a weak acid with PKA of 5.75 in the blood and 5.25 in the urine. At a physiological pH of 7.4 in the extracellular compartment the reaction UA ↔urate H+ is shifted to the right; so, the majority of uric acid (about 98%) circulates in the blood as ionised urate. Because of the high concentration of sodium in the extracellular compartment, urate is largely present as monosodium urate, with a low solubility limit of about 6 mg/dL (360 µmol/L)(9). When urate concentration exceeds this limit the risk of monosodium urate crystal formation and precipitation increases. Monosodium urate deposition was demonstrated to occur in any body fluid with a urate level > 6.8 mg/dL, and monosodium urate crystals were detected in asymptomatic joints of patients with gout(10). A growing body of evidence indicates that silent deposition of monosodium urate crystals as a result of hyperuricemia may occur and lead to early destructive skeletal changes. The actual reference range of serum UA has been assessed according to its variations among healthy individuals. i.e. those without clinical evidence of gout. By this approach, serum UA values between 3.5 and 7.2 mg/dL in adult males and post-menopausal women and between 2.6 and 6.0 mg/dL in premenopausal women have been identified as normal in many countries. However, this definition of normal range of serum UA in the general population is influenced by what we consider as “normal”, since the absence of gout flares does not necessarily imply the absence of UA-related damage. A growing body of evidence demonstrates that UA might play a pathophysiological role in many “cardio-nephro-metabolic” disorders, which seems to be independent of the deposition of monosodium urate crystals since it is evident also for serum UA concentrations below the saturation point for monosodium urate. Taken together, these findings strongly suggest carefully reconsidering the concept of “asymptomaticity” for chronic hyperuricemia.

The prevalence of hyperuricemia has been growing in Western countries for the last few decades(11). An increasing prevalence of hyperuricemia (serum UA > 6 mg/dL) was observed In Italy (8.5% in 2005 vs 11.9% in 2009)(12). However, the prevalence of hyperuricemia in Italian people was lower than in other countries;

indeed, epidemiological data showed an estimated prevalence of hyperuricemia of 21% in the US general population(13). This worldwide rise in the prevalence of hyperuricemia may be related to increased consumption of foods rich in purines, alcohol, and soft drinks sweetened with fructose. As a consequence, the mean serum UA levels in United States is increased from 3.4 mg/dL in 1920s to 6.25 mg/dL in 1970s(14).

Furthermore, the incidence of hyperuricemia depends on ethnicity and it was found to be 3.11 per 1000 person-years in African-American men, compared to 1.82 in white men. The cumulative incidence was 10.9% and 5.8%, respectively(15). According to the NHANES 2007-2008 study, the prevalence of hyperuricemia was 4.0% in Whites versus 5.0% in African-Americans, reflecting a higher prevalence of hyperuricemia in African-Americans compared to whites (25.7% versus 22.1%). In a population-based biracial cohort study of individuals aged 45-65 years at baseline (1987-1989; the Atherosclerosis Risk in Communities Study), the incidence rate of hyperuricemia was higher in African-Americans compared to whites: 15.5/10,000 person-years for African-American men, 12.0/10,000 person-years for African-American women, 9.4/10,000 person-years for white men, and 5.0/10,000 person-years for white women (16). A longitudinal cohort study of a convenience sample of people with hyperuricemia in two cities (Birmingham, Los Angeles) revealed that African-Americans had worse generic and hyperuricemia-specific quality of life and functional ability compared to whites.

Uric acid levels and diseases

Gout

Gout affects 1-4% of the general population, with a higher prevalence in Western countries. It is more common in men (3-6%) than in women (1-2%), and its prevalence increases with age. It results from the buildup of monosodium urate (MSU) crystals in the body's tissues, causing inflammation and pain. In joints, MSU crystals provoke inflammation by activating synovial phagocytic cells, which then release inflammatory chemokines and enzymes(17). This activation leads to the recruitment of neutrophils and subsequent acute inflammation. Elevated levels of serum UA are required for the formation of these crystals. However, it's important to note that not everyone with high uric acid levels will develop gout; approximately 5% of individuals with levels above 9 mg/dL do so, indicating that genetics and other factors also play a role. The diagnosis of gout involves identifying MSU crystals in joint fluid or tophi (which are nodules containing urate crystals). The disease typically begins with acute joint inflammation, which can be treated with nonsteroidal anti-inflammatory drugs or colchicine. Advanced symptoms may include kidney stones and tophi. The primary treatment goal is to lower UA levels through dietary changes and medication in order to dissolve MSU crystals and prevent further attacks. Factors influencing the solubility of UA in the joints include the pH of synovial fluid, water content, and electrolyte levels. UA levels depend on the balance between production (from dietary purines or cellular turnover) and excretion (primarily by the kidneys). Poor diet, lack of exercise, obesity, and metabolic syndrome contribute to the growing incidence of gout. It's worth noting that in most cases of gout (90%), the cause can be attributed to reduced renal excretion rather than increased production(18).

Gout advances through asymptomatic hyperuricemia, acute gouty attacks, intercritical periods, and chronic tophaceous gout, each stage characterized by distinct features. Acute attacks often manifest in the big toe (podagra) but can affect other joints. Intercritical periods are symptom-free but new attacks can occur

without proper treatment. Chronic gout leads to joint destruction and tophi formation, diagnosed through clinical examination and confirmed by identifying MSU crystals (19).

Arterial Hypertension

The hypertensive response involves intracellular UA production or urate uptake into target cells, leading to oxidative stress via NADPH oxidase and mitochondria, inhibiting enzymes like aconitase and enoyl CoA hydratase, and activating stress pathways involved in inflammation and immune activation. These effects have been observed in various cell types, including monocytes, vascular endothelial and smooth muscle cells, kidney tubular cells, hepatocytes, adipocytes, and pancreatic islet cells. Hyperuricemia is common in individuals with primary hypertension. In children, hyperuricemia is indicated by serum urate levels of 5.2 to 5.5 mg/dl, and children with new-onset hypertension often exceed this range. In pregnant women, serum urate usually drops below 4 mg/dl, but those with hypertension or preeclampsia often exceed this level(20). Hyperuricemia in hypertensive individuals is associated with increased nighttime blood pressure and correlates better with central blood pressure than pulsatile pressure. These individuals also tend to have elevated plasma renin activity. The relationship between UA and blood pressure is linear within the 3 to 10 mg/dl range, and sugary beverages containing fructose are linked to increased serum urate and hypertension. Hyperuricemia is a strong predictor of hypertension, with a two-fold increased risk within 5-10 years, though this is less evident in the elderly or those with pre-existing kidney disease. Genome-wide association studies have identified genetic polymorphisms controlling serum UA levels, aiding in developing a genetic scoring system to evaluate hypertension risk. Polymorphisms in xanthine oxidase, which drives urate production, predict hypertension development.(21) However, Mendelian randomization studies suggest that while genetic scores predict gout, they do not predict hypertension,

likely due to pleiotropy. High salt diets induce intracellular fructose metabolism and hepatic urate generation, raising blood pressure and causing metabolic syndrome.

Obesity

In the last five decades, there has been a significant rise in global obesity rates. This trend is evident across diverse regions globally, except in certain areas of sub-Saharan Asia and Africa. Additionally, countries traditionally with low obesity rates, such as Sri Lanka, Indonesia, Sudan, Singapore, Djibouti, and others, are also observing an increase in obesity prevalence.(22)

The connection between serum UA levels and obesity has been widely debated, with uncertainty about whether high UA is a risk factor for obesity or merely a marker of it. While some experts propose that both conditions share a common cause, such as a high-fructose diet, research indicates that high UA levels may predict weight gain and elevated blood pressure in non-obese, normotensive men, suggesting UA may indeed be a risk factor for obesity. Lifestyle changes or medications (like SGLT-2 inhibitors or uric acid-lowering drugs) that lower UA levels may aid in preventing obesity and associated conditions like cardiovascular disease.(23) Obesity is consistently associated with hyperuricemia, as indicated by various metrics such as BMI, weight-adjusted waist index, total percentage fat or waist circumference.

Sarcopenic obesity is a condition characterized by a decrease in muscle mass and an excess of adipose tissue. Although studies have found a negative correlation between grip strength and serum UA levels in individuals between the ages of 20 and 40, this relationship appears to reverse in those aged 60 and above. While low lean mass in combination with obesity has been found to impact hyperuricemia, further research is necessary across various age groups and genders to better understand this connection (24).

Diabetes mellitus

Elevated UA levels contribute to diabetes and its complications through multiple mechanisms. It increases the expression of inflammatory markers (e.g., interleukin-1β, interleukin-6, TNF-α, and CRP), which reduces insulin sensitivity and promotes inflammation. UA also leads to oxidative stress by increasing ROS, causing lipid peroxidation, DNA damage, and reduced insulin secretion. Additionally, it causes endothelial dysfunction by decreasing NO bioavailability and inhibiting endothelial cell functions. UA directly inhibits the insulin signaling pathway, disrupting glucose homeostasis and insulin sensitivity. These factors collectively promote diabetes development. Furthermore, UA activates the renin-angiotensin-aldosterone system causing vascular dysfunction, high intraglomerular pressure, inflammation, and fibrosis, leading to cardiovascular and renal complications. It also triggers platelet adhesion and aggregation, promoting vascular thrombosis. These pathological mechanisms are closely associated with diabetic complications such as macroangiopathy, microangiopathy, and neuropathy (25).

Metabolic syndrome

Recent studies suggest that metabolic syndrome is largely a result of excessive fat storage. This excess fat is stored not only in adipose tissue but also in the liver and serum (triglycerides), often leading to insulin resistance and elevated blood pressure. While fat storage is influenced by various genetic and environmental factors, recent research indicates that nucleic acid metabolism plays a role. Specifically, the stimulation of adenosine monophosphate (AMP) deaminase promotes fat storage and insulin resistance, while the activation of AMP-activated protein kinase stimulates fat degradation and reduces gluconeogenesis. One key contributor to fat storage is the product of AMP deaminase, UA.(26) Numerous studies have suggested that hyperuricemia is a well-established risk factor for Metabolic Syndrome (MetS), although some have not found a significant relationship when comparing individuals with and without diabetes. Researchers

have also observed a higher prevalence of MetS in patients with hyperuricemia compared to healthy populations. (27)

Cardiovascular diseases

The xanthine oxidoreductase (XOR) enzyme plays a critical role in UA production, and its activation can lead to vascular dysfunction and atherosclerosis. Studies, such as the URRAH (Uric Acid Right for Heart Health) study, indicate that UA's cardiovascular impact occurs at levels below those needed for urate crystal formation. In the CARDIA study, the authors found that increasing UA levels during adulthood correlate with higher cardiovascular diseases (CVD) incidence and blood pressure later in life. These findings are consistent across other studies, which show that rising UA levels predict hypertension and CVD risk. This suggests a sequential mechanism where increased UA leads to vascular changes, elevated blood pressure, and ultimately, CVD. Tracking UA levels over time provides a more accurate cardiovascular risk assessment than single measurements. Studies show that even UA levels considered normal can increase CVD risk. Serial UA measurements can better identify at-risk individuals, supporting the need for reevaluating UA thresholds in CVD risk prediction(28).

It is worth noting that UA levels may have a potential connection to cardiovascular disease and prognosis, although their impact on general populations is not yet fully understood. The extensive community-based Framingham Heart Study has indicated that initial uric acid levels did not predict future cardiovascular events, such as coronary heart disease and death, after accounting for other clinical factors. Nevertheless, some studies have suggested that elevated serum UA levels are independently and significantly linked to an increased risk of cardiovascular mortality. Furthermore, elevated serum UA levels have been associated with a higher risk of atrial fibrillation, although the exact role of UA in AF remains unclear. A meta-analysis, which included several cross-sectional and cohort studies, affirmed an increased risk of atrial fibrillation in individuals with high serum UA levels. Various factors such as oxidative stress, inflammation, renin-angiotensin-

aldosterone system activation, and endothelial dysfunction may explain the relationship between UA and atrial fibrillation. Hyperuricemia is also frequently observed in patients with heart failure (HF). In a Japanese multicenter study, it was found that over half of the hospitalized HF patients had hyperuricemia. Additionally, the Framingham Offspring study revealed that the incidence of HF was six times higher among individuals with high serum UA levels. A meta-analysis has reported that for every 1 mg/dL increase in serum UA level, the likelihood of developing HF increased by 19%, and the risk of mortality in HF patients increased by 4%. Hyperuricemia negatively impacts exercise capacity, oxygen consumption, diastolic dysfunction, and cachexia. While the precise mechanisms are not fully understood, upregulation of XO may play a role in this process.(29)

Nutrition and uric acid levels

The levels of serum urate in an individual are determined by the balance between hepatic production of urate and intestinal or renal urate excretion pathways. This balance is influenced by both genetic and environmental factors. Studies on familial and twin populations have estimated the heritability of serum urate to be between 25% to 60%, which is consistent with findings from a genome-wide association study. This study found that common single nucleotide variants control between 25% to 40% of serum urate variability. The remaining 60% to 75% of variability is explained by genetic factors, both common and uncommon, not tagged by common variants, and non-genetic factors such as diet and other environmental exposures. Research has linked the consumption of red meat, shellfish, alcoholic beverages, sugary drinks, and tomatoes to higher levels of serum urate, while low fat milk and coffee consumption have been associated with lower levels. It's important to note that food consumption can be heritable, with coffee consumption estimated to be between 36% to 58% heritable, alcohol consumption between 43% to 53%, and sugar sweetened beverage consumption at 48%. Recent genome-wide association studies have identified genetic associations with coffee and alcohol consumption habits, suggesting that the heritable component of specific food consumption may

contribute to serum UA levels. The role of diet in the development of hyperuricemia has long been recognized as a significant modifiable factor. However, a comprehensive analysis of the role of diet in serum urate levels has yet to be conducted on a large enough dataset, and the extent to which inherited genetic variants and overall diet impact serum urate concentrations remains unclear.(4) Certain diets, such as the DASH and Mediterranean Diets, have been shown to reduce serum urate and lower the risk of gout. Traditional recommendations for preventing gout have emphasized reducing the consumption of purine-rich foods such as meat, seafood, and certain vegetables, as purines are the precursors to urate through the purine scavenger pathway. While purine-restricted diets are generally lower in protein, recent studies have suggested that high total-protein intake is not associated with elevated serum UA, and that plant-based protein consumption may even have a protective effect against hyperuricemia. However, educational efforts aimed at reducing purine intake have not been shown to effectively lower serum UA levels in patients with gout, and the potential impact of low-purine diets on CVD risk factors such as hypertension and hyperlipidemia remains unclear. Furthermore, reducing protein intake may inadvertently lead to increased consumption of refined carbohydrates and saturated fats, which can exacerbate CVD risk factors (30).

Several factors contribute to the heightened risk of gout, including the consumption of purine-rich foods, carbohydrates, proteins, fats, vitamin C, and elevated BMI. Consuming foods abundant in purines can elevate UA production, while protein intake may stimulate endogenous purine production, leading to heightened blood UA levels. Diets high in fat content can induce acidosis, hindering UA excretion by the kidneys. Conversely, vitamin C can enhance UA elimination through renal pathways. (31).

Macronutrients

For many years, adjusting macronutrient intake, such as protein, fat, and carbohydrates, has been a key tactic for weight loss and enhancing cholesterol

levels. Research has shown that certain macronutrients can also significantly decrease blood pressure, with protein having the most robust body of evidence(32).

Protein

The levels of UA in the bloodstream are often influenced by the foods we eat. Purines, which are found in many foods, are broken down into UA during metabolism. A diet that is high in protein typically contains a lot of purines. However, this type of diet may also have a uricosuric effect, which means that the body excretes more UA in urine, resulting in lower levels in the blood. Although there is some evidence to suggest that diet plays a role in the development of hyperuricemia and gout, there is limited data directly linking food intake to high levels of UA. Some studies have shown that meat and seafood can lead to higher levels of UA, while dairy intake may lower these levels. There does not appear to be any association between total protein intake and UA levels(33).

Fat

However, only a limited number of studies have explored the correlation between serum UA levels and dietary lipids. The impact of omega-3 fatty acids on metabolic health, specifically with regard to serum uric acid levels, has garnered significant interest. One intervention study involving healthy young adults found that consuming n-3 polyunsaturated fatty acids (PUFAs), specifically 2 g/day of fish oil for 8 weeks, resulted in decreased serum UA levels. Similarly, another intervention study conducted with healthy older males demonstrated that a 3-month intake of 700 mg/day of omega-3 lipid supplements led to reductions in serum UA levels. In terms of dietary patterns, a 16-week intervention employing a calorie-restricted diet where saturated fatty acids were substituted with monounsaturated and PUFAs showed promising results in inducing weight loss and lowering serum UA levels in males with gout. However, it's worth noting that all these intervention studies utilized dietary supplements or modified diets with altered lipid compositions, and none

directly investigated the relationship between dietary lipid intake and serum UA levels(34).

Recent research has investigated the potential influence of omega-3 supplementation, particularly through fish oil, on these levels. A 6-month study involving a daily 6.2-gram dose of omega-3 fish oil did not show a significant reduction in serum UA levels compared to a control group. While omega-3s are widely acknowledged for their anti-inflammatory and cardiovascular benefits, it appears that they may not directly affect serum UA levels.(35,36)

On the other hand, long-chain omega-3 PUFAs, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), can inhibit the activity of the URAT1 protein, which is responsible for UA reabsorption in the kidneys. By inhibiting URAT1, omega-3s may enhance the excretion of uric acid, potentially leading to lower serum uric acid levels(35,36). These findings indicate that while omega-3 supplementation alone may not significantly reduce serum UA levels, it may still have a supportive role in managing these levels by influencing uric acid metabolism (36).

Carbohydrate

Evidence from observational studies suggests that modifying dietary glycemic index can impact serum UA concentrations, with reductions in glycemic index associated with decreased UA levels, while reducing the proportion of dietary carbohydrates tends to increase UA concentrations. These effects appear to be independent of various metabolic parameters such as eGFR, glucose, insulin, and triglycerides. Notably, the most significant reduction in UA levels occurs when the glycemic index is decreased concurrently with an increase in low glycemic index carbohydrates, indicating the potential efficacy of a diet rich in such carbohydrates for lowering UA. However, the impact of altering the glycemic index seems to outweigh that of changing carbohydrate proportion in terms of UA reduction. Additionally, while these effects remain consistent across different baseline UA levels, they vary based on baseline alcohol consumption, suggesting that reducing glycemic index may not affect UA levels in individuals with regular alcohol intake(37).

Considering the escalating consumption of fructose, particularly from added sugars in processed foods and beverages, alongside the global rise in serum UA levels and metabolic syndrome, there's growing speculation regarding the pathogenic role of fructose-induced hyperuricemia in metabolic syndrome development. While the exact molecular and cellular mechanisms linking UA and fructose to cardiovascular risk factors are not fully elucidated, this review aims to explore and discuss the potential pathogenic mechanisms of fructose and UA in conditions associated with increased cardiovascular risk, especially in children(38)

Alcohol

The consumption of alcohol is a key dietary risk factor that contributes to elevated serum UA levels. This is primarily due to the presence of ethanol in alcoholic beverages, which can impact serum urate levels by increasing UA production and impairing the elimination of UA through the kidneys. Additionally, other ingredients found in alcoholic beverages, such as purines, can also affect serum urate levels. It's important to note that the influence of serum urate levels can vary depending on the type of alcoholic beverage consumed, such as beer, liquor, or wine(39).
Alcohol intake is thought to increase serum UA levels via two pathways: firstly, by speeding up the production of UA from adenosine, a byproduct of adenosine triphosphate breakdown to adenosine monophosphate, and secondly, by impeding urinary excretion due to heightened blood lactate levels resulting from ethanol oxidation(40).

Fiber

Dietary fiber encompasses a diverse array of indigestible plant polysaccharides abundant in fruits, legumes, vegetables, and cereals. Recognized as a crucial element of a healthy diet, dietary fiber has been linked to a reduced risk of various metabolic disorders including dyslipidemia, hypertension, obesity, and diabetes. Serum UA levels are influenced by the interplay between hepatic urate production

and the pathways of intestinal or renal urate excretion, suggesting a potential mechanistic connection with dietary fiber intake. Increased intake of dietary fiber has been associated with potential improvements in renal function, augmented gut transit rate, and increased fecal bulk. Animal studies have demonstrated that dietary fiber effectively mitigates the rise in UA levels triggered by dietary compounds such as ribonucleic acid, adenosine-50-monophosphate, adenosine, or adenine, and it has shown promise in suppressing experimental hyperuricemia in rats(22).

Micronutrients

Vitamin C

In recent years, there has been a growing interest in the potential impact of micronutrients, especially vitamin C, on UA levels. Elevated UA levels have raised concerns due to their link with various metabolic issues. Although several studies have explored the connection between vitamin C supplementation and UA levels, the results have been inconsistent. In order to clarify this relationship, a comprehensive meta-analysis of 16 randomized controlled trials involving 1,013 participants was conducted to assess the effect of oral vitamin C supplementation on UA levels. The findings of this meta-analysis suggest that vitamin C supplementation leads to a noteworthy reduction in UA levels. This effect was more evident in specific subgroups, such as younger participants, those involved in shorter-duration studies, and in trials of higher quality. These results indicate that vitamin C may be an effective approach to managing elevated UA levels, providing a potential nutritional strategy for addressing this metabolic concern(41).

Dietary patterns and uric acid levels

Mediterranean Diet

Mediterranean diet prioritizes cereals like bread, oats, wholegrain cereals and groats, as well as fruits, vegetables, nuts and legumes, which should all be consumed daily. These foods are rich in fiber and antioxidants, particularly when they're in season. Olive oil is used in place of other saturated fats, such as animal butter and margarine. Dairy products, especially yogurt and cheese, can be consumed daily in moderation, while lean animal products like fish and poultry are recommended up to twice a week. Eggs can be eaten up to 4-7 times a week. Moving up the food pyramid, red meat should only be consumed in small amounts on a monthly basis. Alcohol can accompany meals, with a safe recommended limit of 2 glasses per day for men and 1 glass per day for women, as long as there are no associated health issues. Red wine is preferred due to its flavonoid and antioxidant content. In addition to dietary choices, moderate daily physical activity is also emphasized to maintain a healthy weight, promote well-being and prevent diseases related to excess body weight(42).

The Mediterranean diet model is common in many Mediterranean countries, featuring low consumption of red meat and a preference for fish or legumes as protein sources. Extensive research has identified two main components that offer significant health benefits: Mono-unsaturated fatty acids, like oleic acid found in olive oil, and poly-unsaturated fatty acids, particularly n-3 found in oily fish and walnuts. Additionally, flavonoids in red wine (when consumed with meals) have been shown to be beneficial. Combined with the aforementioned foods, these ingredients can lead to maximum benefits for longevity and overall mortality reduction. The Mediterranean diet is high in antioxidants and vitamins, and boasts anti-inflammatory properties that reduce pro-inflammatory cytokines, increase anti-inflammatory cytokines, and reduce oxidative stress. Interestingly, diet alone has little effect on serum UA levels, but a diet low in purines can still reduce UA by 10-15%(42). However, the Dietary Intervention Randomized Controlled Trial (DIRECT) analyzed 235 obese individuals and found no significant difference in serum urate

reduction between those assigned to a low-fat restricted-calorie diet, a Mediterranean restricted-calorie diet or a low-carbohydrate non-restricted-calorie diet over two years, including those with baseline hyperuricemia. According to a meta-analysis conducted by Major et al., the Mediterranean diet does not appear to significantly lower urate when compared to other diets.(31)

The PREvención con DIeta MEDiterránea (PREDIMDED) trial, which included 4,449 older adults at high risk of CVD, those with higher Mediterranean diet scores had a lower likelihood of developing hyperuricemia.

DASH Diet

Recent research indicates that the Dietary Approaches to Stop Hypertension (DASH) diet, which is high in carbohydrates and focuses on consuming fruits, vegetables, and low-fat dairy products, not only lowers blood pressure and improves lipid levels but also decreases serum urate levels (30). The DASH model is a dietary pattern focused on natural and minimally processed foods. It emphasizes consuming abundant quantities of foods of plant origin, along with low-fat animal products and specific micronutrients. In the 1990s, the National Institute of Health conducted a study to test the effectiveness of targeted dietary interventions in treating hypertension. The results showed a significant decrease in blood pressure for both hypertensive and normotensive patients. The key elements of the DASH diet include high consumption of fruits, vegetables, whole grains, nuts, and dried fruits; adequate intake of magnesium, potassium, calcium, and fiber; use of protein sources such as low-fat dairy products, fish, poultry, eggs, and legumes; limited consumption of foods high in saturated fats, such as processed red meat and industrial foods; and reduced intake of sweets and sugary drinks.

This dietary pattern has been recommended by many health organizations as an effective nutritional strategy for the prevention and management of high blood pressure. It is a diet with a low sodium intake and high consumption of foods rich in substances such as vitamins (vitamin C and folate) and minerals, in particular Mg, K and Ca which prevent endothelial dysfunction and promote relaxation of the

endothelial smooth muscles. Furthermore, the DASH diet appears to have positive antioxidant effects, on the inflammatory response, on sympathetic activation and on gluco-insular control (31).

Low-carb Diet

Low-carbohydrate ("low-carb") diets have been a topic of considerable interest since their early promotion by William Banting in 1863 as a weight loss strategy. Over the decades, these diets have gained popularity not only for weight management and blood glucose control but also for their potential benefits related to cardiovascular health, exercise performance, inflammation, and oxidative stress. According to the International Food Information Council Foundation's 2018 Food & Health Survey, approximately 16% of adults reported following some form of low-carb eating pattern in the past year.

However, the term "low-carb" lacks a universally accepted definition, complicating efforts to assess its effects. The National Academy of Medicine (NAM) defines an Acceptable Macronutrient Distribution Range (AMDR) for carbohydrates as 45-65% of daily caloric intake, with a Recommended Dietary Allowance (RDA) of 130 grams per day. Despite these standards, many contemporary low-carb diets fall below these thresholds.(43)

The OmniCarb trial, a clinical study designed to evaluate the impact of carbohydrate intake on cardiovascular risk factors, has provided valuable insights into how dietary carbohydrates influence metabolic health. The trial demonstrated that a reduction in carbohydrate percentage led to decreased triglyceride levels, while a diet with a lower glycemic index was associated with increased LDL cholesterol and insulin resistance. Given that insulin resistance is closely linked to elevated uric acid levels, the OmniCarb trial also included an ancillary study to investigate how variations in carbohydrate type and amount affect plasma UA concentrations. This ancillary study hypothesized that a lower glycemic index would result in higher UA levels due to increased insulin levels impairing UA excretion. Additionally, it was anticipated that reducing carbohydrate intake by increasing dietary protein would similarly elevate UA concentrations(37).

Low-fat Diet

Low-fat diets, defined as those where 30% or less of calories come from fat, are widely recommended across clinical specialties to reduce the risk of CVD. This dietary approach is supported by numerous studies linking high dietary fat intake with increased cardiovascular mortality. Low-fat foods typically have 3 grams or less of fat per 100 calories and include vegetables, fruits, whole grains, egg whites, skinless poultry, beans, seafood, and low-fat dairy products. A mechanism linking fat intake and UA metabolism may involve the effects of dietary fats on insulin sensitivity. The effects of dietary fats on insulin sensitivity have been examined in metabolic syndrome with underlying insulin resistance. A systematic review of 14 observational studies and 16 clinical trials demonstrated that many of the observational studies found beneficial associations between MUFA/PUFA intake and metabolic syndrome components. Moreover, previous clinical trials support the benefits of MUFA- or PUFA-enriched diets in reducing metabolic syndrome. In addition to the abovementioned results, both the HOMA-R and blood insulin level, indices of insulin resistance, were shown to be reduced. Another clinical trial in 472 subjects with metabolic syndrome from eight European countries reported that the highest HOMA-R group, one of three groups classified by HOMA-R levels, had the greatest reduction in HOMA-R and insulin levels after consumption of a high MUFA diet or a diet supplemented with n-3 PUFA. These effects with a high MUFA diet or a diet supplemented with n-3 PUFA were also associated with the reduced plasma level of the inflammatory cytokine, IL-6. Insulin resistance disturbs glycolysis and uric acid clearance by the kidneys, leading to increased production of uric acid and decreased urinary uric acid clearance, respectively. A case-control study of nonalcoholic fatty liver disease in Caucasians indicated that dietary patterns high in unsaturated fatty acids were associated with lower serum uric acid levels and improved insulin resistance. A systematic review of insulin resistance in CKD by Spoto et al. explains that the Janus effect of adipokines stimulates fatty acid oxidation by activating AMP-activated protein kinase, thereby improving insulin sensitivity(44).

PURPOSE OF THE STUDY

Elevated levels of UA have been linked to several conditions, such as hypertension, obesity and cardiovascular disorders. Women who have gone through menopause are particularly susceptible to fluctuations in UA levels. As estrogen levels decline during menopause, UA metabolism can be affected, which may result in higher serum concentrations. Moreover, lifestyle modifications during this stage of life can also contribute to variations in UA levels. Therefore, examining the impact of different diets on UA in this specific population is vital for developing personalized health recommendations and preventative strategies.

This thesis, conducted as part of the CAR-PREDIME project (CARdiovascular PREevention with DIet in MEnopause) at the Dietetics and Clinical Nutrition Unit of the University Hospital of Padua, aims to evaluate changes in dietary pattern in post-menopausal women (PMW), which may lead to a decrease in UA levels, thus potentially reducing the risk of conditions associated with hyperuricemia, including hypertension and obesity.

MATERIALS AND METHODS

Intervention study protocol

The CAR-PREDIME project (CARdiovascular PREevention with DIet in MEnopause) aims to recruit post-menopausal women at the Dietetics and Clinical Nutrition Unit of the University Hospital of Padua. To be eligible for the study, participants must meet the following criteria:

- Physiological or surgically induced menopausal state (at least 12 months of no menstruation, FSH > 25mIU/ml, and estradiol < 50 pg/ml)
- Age between 30 and 65 years
- BMI between 24 and 39 kg/m²

Exclusion criteria include:

- Hormone replacement therapy
- Presence of chronic diseases (diabetes mellitus, moderate-advanced chronic renal failure, liver cirrhosis, advanced infectious or iatrogenic hepatic steatosis, active neoplasms, advanced cardiovascular diseases), decompensated thyroid pathologies, or pathologies difficult to control with pharmacological treatment
- Evident psychiatric pathologies and/or undergoing active therapy (depression, disorders of fueled behavior)
- Gastrointestinal pathologies or symptoms that hinder adherence to the dietary pattern

During the initial assessment, the individual's adherence to predefined characteristics is evaluated through a questionnaire gathering personal data and exploring their habits and lifestyle, including smoking, alcohol consumption, salt intake, and physical activity using the IPAQ questionnaire. Additionally, family history, past and present medical history and medication history are collected, and the individual is presented with questionnaires regarding dietary habits. Following this, anthropometric measurements, bioelectrical impedance analysis, systolic and diastolic blood pressure, heart rate measurements (lying down, standing, and seated), assessment of blood vessel elasticity, and tests for strength and physical

performance are conducted. To assess the metabolic profile, the individual undergoes blood tests.

At the end of the visit, each patient was assigned to a balanced hypocaloric dietary regimen the Mediterranean diet, DASH diet, Low-Carb diet, and Low-Fat diet. Each patient will receive a paper-based dietary plan and a food diary to complete and bring to the subsequent follow-up appointment. The study will span over 24 months, during which the participants must adhere to the assigned regimen and attend regular outpatient check-ups every 3 months. At each follow-up, anthropometric parameters, blood pressure, bioimpedance analysis, vascular stiffness assessment, and strength testing will be conducted. Dietary compliance will be evaluated using a 7-day dietary recall (conducted over the phone in the days preceding the follow-up visit), a validated 138-item food frequency questionnaire (FFQ), adherence questionnaires for the Mediterranean and low-fat diets, and a self-completed food diary for the seven days prior. Bioumoral and hematological tests, as well as physical performance assessments, will be carried out every six months.

Anthropometric parameters

- Body weight (in kg)
- Height (in m)
- Body mass index (in kg/m^2)
- Circumferences of the left arm, waist, abdomen, and hips (in cm)
- Triceps, subscapular, suprailiac, and thigh skinfold thickness (in mm)

Body weight is measured using an electronic scale while the patient is barefoot and wearing only underwear. This measurement is always taken under the same conditions. Height is measured using a stadiometer, with the patient standing upright, without shoes, head straight, arms at the sides, weight evenly distributed on both feet, knees not bent, heels together, and the tips of the toes forming a 60° angle. The body mass index (BMI) is calculated as the weight in kilograms divided by the square of the height in meters.

The circumference measurements are taken using a flexible and non-stretchable tape measure wrapped around the specific body part. During measurement, the tape measure should be positioned straight and perpendicular to the ground without any compression. For measuring the arm circumference, it is essential to first identify the reference point with the subject standing upright, the left arm close to the body, and the elbow bent at a 90° angle. The midpoint between the acromion (shoulder protrusion) and the olecranon (elbow tip) is measured, and the arm circumference is then determined with the elbow extended and the arm relaxed. The waist circumference is measured at the narrowest point of the abdomen, and the hip circumference is measured at the widest point of the buttocks, with the subject in a relaxed stance and feet together.

Skinfold measurements should be taken on the left side of the body with the muscles relaxed using a Harpenden caliper with a sensitivity of 0.2 mm and a gauge pressure of 10 g/m^2. Three measurements should be taken, and the average will be used. For the triceps fold, use the same reference point as the circumference measurement. For the subscapular fold, measure just below the lower corner of the scapula with a 45° angle. For the supra iliac fold, measure the mid-axillary line just above the iliac crest by lifting the fold obliquely. For the median fold of the thigh, measure at the midpoint between the inguinal crease and the proximal edge of the patella, with the body weight shifted to the limb opposite to the one being examined.

Skinfold measurement is a method used to assess bicompartmental body composition, specifically the percentage of fat and lean mass. It assumes that the thickness of subcutaneous adipose tissue is directly related to total body fat. The Jackson-Pollock method with three folds (tricipital, suprailiac, and anterior thigh) is utilized for these measurements. Additionally, the waist-to-hip ratio (WHR) is determined by dividing the waist circumference by the hip circumference, while the waist-to-height ratio is calculated by dividing the waist circumference by the height. The arm adipose area (AFA) in square centimeters is derived by subtracting the arm muscle area (AMA) from the total arm area (TAA). The basal metabolic rate (BMR) in kilocalories is calculated using the validated Harris-Benedict formula for women: 655.1 + (9.563 x weight in kg) + (1.850 x height in cm) - (4.676 x years)(45).

Body composition analysis

Body composition refers to the size and distribution of the different compartments that make up the body. There are different models of body composition analysis(46,47):

- Bi-compartmental model: This is the simplest model, dividing the body into two compartments - fat mass (FM) and lean mass (FFM).
- Tri-compartmental model: This model divides the body into three compartments - fat mass (FM), the extracellular mass (ECM) which includes fluids, collagen and bone tissue, and the cellular mass (BCM) which is the metabolically active mass. Impedance measurement (BIA) is a good instrument for this analysis.
- Tetra-compartmental model: This model divides the body into four parts - fat mass, water, proteins, and minerals.
- Five-compartmental model: In this measurement, the analysis includes FM, FFM, total body water (TBW), protein mass (MP), mineral mass (MM), and glycogen (G). The instruments available for these analyses are DEXA, CT scan, and MRI.

The assessment of body composition is a crucial long-term indicator of nutritional status, allowing for the evaluation of changes in the ratio between lean mass and fat mass during dietary interventions. Various methods are used to measure and evaluate body composition, all of which are indirect and provide estimates rather than direct measurements. These techniques include Bioelectrical Impedance Analysis (BIA) and Bioelectrical Impedance Vector Analysis (BIVA)(46).

While CT and MRI are considered the gold standard for body composition analysis, in clinical practice, the most commonly used methods are BIA or BIVA due to their less invasive nature, quick and easy performance, cost-effectiveness, and minimal patient preparation requirements(47).

In the CARPREDIME study, the study utilizes vector bioimpedance as a method, which correlates with measurements taken using the caliper according to the Jackson-Pollock method.

BIA is a body composition analysis method that determines fat mass (FM), fat-free mass (FFM), which includes body cell mass (BCM) and extracellular mass (ECM), and total body water (TBW), which comprises intracellular water (ICW) and

extracellular water (ECW). As an indirect method, these parameters are derived from direct measurements, including resistance (R) and reactance (Xc) as well as the subject's weight, height, sex, and age. The relationship between Xc and R is defined as the phase angle (Pa), which not only indicates intracellular and extracellular proportions but is also directly proportional to the metabolically active mass. The relationship between Xc and R is determined by the phase angle Pa, which not only indicates the intra- and extracellular proportions but also has a direct correlation with the metabolically active mass. The bioimpedance measurement is based on the differing electrical conductivity of biological tissues based on their composition. This method involves applying four skin electrodes (two injectors and two sensors) to the subject in a reclining position, in the metacarpal and metatarsal positions. A low-intensity alternating current is then passed through these electrodes. Biological tissues with high water content (lean mass) offer low resistance to the flow of alternating current, as water is an efficient electrical conductor. In contrast, adipose tissue, being a poor electrical conductor, offers high resistance.

For accurate measurements, the subject should be in a supine position with limbs slightly apart and not in contact with the torso. Moreover, the application area of the electrodes should be cleansed with alcohol or disinfectant to improve the electrical skin-electrode interface. The subject should also be fasting from food and liquids, have an empty bladder, and maintain a normal body temperature. The environmental temperature during the measurement is also crucial; incorrect temperatures (24-34 °C) can lead to vasodilation or vasoconstriction and thus affect the results significantly. It has been observed that variations in the aforementioned conditions can produce notable changes in the detected parameters(48).

The Bioelectrical Impedance Vector Analysis (BIVA) is an alternative method to the Bioelectrical Impedance Analysis (BIA) that doesn't rely on predictive equations. Instead, it presents a vector graphic representation in which the resistance (R) is plotted on the x-axis and the impedance (Xc) on the y-axis, both normalized by the subject's height. By analyzing the position of the point on the graph, the quadrant it falls into, and the vector passing through the origin, it's possible to determine the phase angle. BIVA provides a semi-quantitative assessment of hydration status and

cell mass, making it valuable for monitoring changes in these parameters over time. For instance, a shift of the vector along the reactance axis (Xc) indicates changes in cell mass, while a shift along the resistance axis (R) indicates alterations in hydration(49).

Blood pressure measurement

Blood pressure (BP) was measured using a validated electronic arm sphygmomanometer (model UA-767 Plus, A&D Company, Tokyo, Japan) endorsed by the British Hypertension Society (A/A grading). All participants were seated and after a 5-minute rest, their blood pressure was measured with an appropriate cuff. The cuff size was determined based on the participant's arm circumference. Prior to the measurement, participants were advised to avoid smoking, consuming food or caffeine, exercising for at least 30 minutes, and refraining from talking. Initial systolic and diastolic BP measurements were taken in both arms, and subsequent measurements were conducted in the arm with the higher blood pressure. Three readings were taken, and the average of the last two measurements was used for analysis. Blood pressure was also measured in reclining and orthostatic positions. Additionally, the heart rate (in beats per minute, bpm) and its rhythm at the radial pulse level were evaluated alongside the blood pressure measurement.

Evaluation of eating habits

In the CARPREDIME study protocol, data on patients' eating habits are gathered through the following methods:

- Weekly 24-hour food recall
- Weekly diary filled in by the patient
- Food intake frequency questionnaire (FFQ)

During the recall, patients are asked to detail their diet in the week leading up to their check-up. The information recorded includes the distribution and composition

of meals throughout the day, as well as the quantity and frequency of food and drink intake, along with methods of preparing dishes and cooking techniques.

The food diary is a prospective self-compiling survey that captures data on foods and drinks consumed within a specified period of time(50). Patients are required to report all food and drinks consumed, providing either measured weights (gold standard) or home measurements. It has been noted that guidance from a nutrition professional is necessary to ensure that patients accurately report the findings, including commonly omitted details such as condiments, preparation methods, and potential product brands(51).

As part of the CARPREDIME study, patients receive a pre-printed food diary template during each visit. They are required to complete this for the 7 days prior to their next check-up and return it at the visit.

The food intake frequency questionnaire (FFQ) is administered to patients during outpatient visits using the photographic atlas of food portions from the Scotti Bassani Institute. This tool includes three images for each food, illustrating three different portions, requiring the patient to visually recognize the portion they consume for each type of food. This method provides semi-quantitative information, gathering details on the frequency of food intake and approximate portions.

The various methods of evaluating eating habits have their own biases and advantages. Factors such as the method of administration (self-administered or administered by the operator) and the period of time under consideration can influence the collection of information(51,52). By integrating all these methodologies, a more comprehensive evaluation of a patient's nutrition and eating habits can be achieved.

Dietary patterns

In the interventional group of the CAR-PREDIME study, patients are assigned to one of four dietary patterns known to be potentially beneficial for cardiovascular health. These include the Mediterranean Diet, the DASH Diet, the Low-Carb diet, and the Low-Fat diet. For each dietary pattern, seven specific plans were developed using nutritional software, considering caloric intakes ranging from 1400 to 2000

kcal. The personalized plan is based on the patient's energy requirements, determined from their anthropometric data and level of physical activity. Each plan consists of five daily meals and includes food quantities, potential substitutions, and consumption frequencies. Additionally, patients receive a weekly menu outlining the characteristics of their prescribed dietary pattern, providing helpful guidance for meal composition and nutrition planning.

The dietary principles based on the Mediterranean pattern emphasize the consumption of plant-based foods such as vegetables, fruits, whole grains, legumes, and nuts. A moderate intake of animal-derived foods like lean meats, fish, eggs, and dairy products is recommended, while consumption of red and processed meats should be limited. Olive oil is the primary source of fat for seasoning. The Mediterranean pattern is characterized by a well-balanced distribution of macronutrients: proteins make up around 17% of total daily calories, lipids account for approximately 32-33%, and carbohydrates represent about 50-51%.

The DASH diet involves increasing the consumption of fruits, vegetables, whole grains, and dried and oily fruits. It also emphasizes reducing the intake of animal proteins in favor of plant-based proteins and choosing low-fat dairy products. Additionally, the diet focuses on limiting sodium intake by reducing the use of table salt and consuming fewer processed and industrial foods high in salt. In terms of nutrient distribution, the DASH diet aims for a balanced intake of approximately 18% of daily calories from proteins, 27-28% from lipids, and 54% from carbohydrates.

The Low-Carb diet involves reducing the intake of carbohydrates and increasing the percentage of daily calories from proteins and fats. This diet emphasizes consuming fruits, vegetables, and whole grains, and using olive oil as the primary source of fat. It also emphasizes alternating between plant and animal sources of protein. In a Low-Carb diet: - Proteins make up about 21% of total daily calories - Lipids make up about 40% of total daily calories - Carbohydrates make up about 39% of total daily calories.

The Low-Fat diet limits the percentage of daily calories from fats to less than 30%. In the studied diets, saturated fats accounted for 6.57%, monounsaturated fats accounted for 16.17%, polyunsaturated fats accounted for 3.87% (with an average omega-6 to omega-3 ratio of 5.15), and the average cholesterol intake was 204.57

mg. In a Low-Fat diet: - Proteins make up approximately 17-18% of total daily calories - Lipids make up approximately 27% of total daily calories - Carbohydrates make up approximately 55% of total daily calories.

Statistics

The data were analyzed using SAS version 9.4 software. Descriptive statistics are presented as mean ± standard deviation for continuous variables and as percentages for categorical variables. The normality distribution of quantitative variables was assessed using the Kolmogorov-Smirnov test. Dietary composition data (energy intake, proportion of carbohydrates, fats, and proteins) were analyzed using Metadieta 4.5 software. Statistical comparisons of continuous variables between the first and fourth follow-ups were conducted using paired Student's t-tests, while the Chi-Square test was used for categorical variables. Correlations between one-year changes in continuous variables were assessed using Spearman tests. Differences in continuous variables over time (between the first visit and the 6- and 12-month follow-ups) were evaluated using repeated measures analysis of variance (ANOVA) for single-factor studies. A p-value of less than 0.05 based on a two-tailed test was considered statistically significant for all analyses.

RESULTS

Baseline Characteristics

To date, the CAR-PREDIME study has enrolled 52 post-menopausal women with an average age of 55.6±4.6 years, who were followed for one year every 3 months to assess the impact of a targeted intervention on various health measures. The median age of menopause is 52.2±4.4 years. Drinkers were 63.5% and smokers 9.6%. Obesity (BMI ≥ 30 Kg/m^2) was present in 55.8% of the participants of whom 15.4% had truncal obesity (subscapular skinfold/triceps skinfold ≥1.32) and 96.2% abdominal obesity (waist circumference ≥80 cm). 53.9% had arterial hypertension of whom 40.4% were treated. Hypercholesterolemia (LDL cholesterol ≥ 130 mg/dL) was present in 57.7%, hypertriglyceridemia (TG ≥150 mg/dL) in 17.3%, and Glucose intolerance in 19.2%. Overall metabolic syndrome was present in 59.6% of the cohort.

Anthropometric changes at one-year follow-up

In a statistical analysis with Student's t-test for paired data, it was observed at one-year follow-up significant and clinically relevant changes in anthropometric measurements, indicating the positive impact of the intervention on body composition (Table 1).

Participants experienced a significant reduction in body weight of 6.7% (p=0.02) with a significant reduction in obesity of 21.2% (p=0.006), in waist circumference of 5.0% (p=0.02), in the waist-to-height ratio (a reliable indicator of central obesity and a predictor of cardiovascular risk) of 5.2% (p=0.02), in triceps skinfold thickness of 18.3% (p<0.0001), in subscapular skinfold thickness of 18.4% (p=0.002) and in the fat area of the arm of 18.9% (p=0.0003) without change in the muscle area of the arm (Table 1).

Table 1. Anthropometric characteristics at baseline and at one-year follow-up

Variables	Baseline (n=52)	1-year FU (n=52)	p-value
Weight (kg)	79.2±11.2	73.9±11	0.0166
Body mass index (kg/m^2)	30.4±3.5	28.4±3.8	0.0063
Arm circumference (cm)	33.8±3	32.4±3.4	0.0282
Waist circumference (cm)	94.0±10.5	89.3±10.6	0.0252
Hip circumference (cm)	110.1±7.5	106.1±8	0.0099
Waist-to-hip ratio	0.85±0.07	0.84±0.06	0.4359
Waist-to-height ratio	0.58±0.06	0.55±0.07	0.0209
Triceps skinfold (mm)	32.8±7.1	26.8±7.1	0,0001
Subscapular skinfold (mm)	33.1±9.5	27.0±10.1	0.0020
Suprailiac skinfold (mm)	21.6±6.9	19.0±8.1	0.0811
Femoral skinfold (mm)	49.9±8.1	42.2±10.9	0.0001
Muscle area of the arm (cm^2)	44.2±8.8	46.2±7.9	0.2254
Fat area of the arm (cm^2)	47.2±12.1	38.3±12.4	0.0003

Body composition and clinical changes at one-year follow-up

The intervention diet resulted in a significant reduction in absolute (kg) and relative (%) fat mass, with the average absolute fat mass dropping from 30.6 ± 6.6 kg initially to 25.6 ± 7.7 kg after 12 months (p = 0.0006). After one year of follow-up, the absolute lean mass value in kg, as determined by BIVA and plicometry, remained relatively stable. Moreover, an increase on relative lean mass measured by plicometry was observed indicating a body reshaping (table 2).

A significant reduction in systolic blood pressure by 7% (from 130.5±14.3 to 121.3±12.6 mmHg) (p=0.001) and diastolic blood pressure by 6% were observed. (from 80.3±8.3 to 75.5±7.3 mmHg) (p = 0.002) (table2).

Table 2. Body composition and clinical measurements at baseline and at one-year follow-up

Variables	Baseline (n=52)	1-year FU (n=52)	p-value
Plicometry fat mass (Kg)	30.6±6.6	25.6±7.7	0.0006
fat mass (%)	38.3±4.2	33.9±6.7	0.0001
lean mass (Kg)	48.7±5.8	48.3±4.9	0.7048
lean mass (%)	61.6±4.4	66.1±6.7	0.0001
BIVA fat mass (Kg)	29±7.7	25.6±8.1	0.0305
fat mass (%)	36.1±6.1	33.8±7.3	0.0443
lean mass (kg)	50.2±5.4	48.4±5.1	0.0836
lean mass (%)	63.9±6.1	66.1±7.5	0.1039
Sitting systolic BP (mmHg)	130.5±14.3	121.3±12.6	0.0007
Sitting diastolic BP (mmHg)	80.3±8.3	75.5±7.3	0.0023
Sitting heart rate (bpm)	64.8±8.4	63±8.1	0.2686

Dietary Intake changes at one-year follow-up

Although participants experienced significant weight loss, there was no change in daily calorie intake after 12 months, while the greatest changes were seen in macronutrient distribution. The intervention diet involved a balanced macronutrient composition with a significant reduction ($p<0.01$) in the relative (from 35.7% to 32.2%) and absolute (from 62.0±17.8 to 53.9±12.9 g/day) lipid intake, in particular a significant decrease ($p<0.001$) on animal lipids and saturated fatty acid intake by 25% and 3%, respectively. A significant ($p<0.001$) increase by 18.1% in fiber intake was observed. While the absolute and relative protein contents were unchanged, a

significant reduction on animal protein intake by 5% (p=0.005) was observed with a significant increase on plant-based protein intake by 13% (p=0.0003) (table 3).

Table 3. 24h-recall dietary intake at baseline and at one-year follow-up

Variables	Baseline (n=52)	1-year FU (n=52)	p-value
MB average sec. HB (Kcal)	1455.3±115.0	1400.9±107.6	0.0144
Caloric intake (Kcal/day)	1638.9±330.4	1577.4±238.4	0.2789
Protein (g/day)	73.1±10.5	70.5±11.1	0.2226
Animal proteins (g/day)	41.9±9	37.2±10.8	0.0177
Plant-based proteins (g/day)	29.1±6.2	32.0±7	0.0275
Protein (%)	19.1±2.8	18.9±2.6	0.7066
Animal protein (%)	57.3±7.7	52.3±10.0	0.0052
Plant-based proteins (%)	39.6±6.9	46.0±10.1	0.0003
Carbohydrates (g/day)	189.9±49.3	197.6±40.5	0.3862
Carbohydrates (%)	45.1±6.1	49.0±6.0	0.0014
Fiber (g/day)	21.5±5.2	25.4±6.2	0.0008
Soluble fiber (g/day)	3.6±1.5	4.5±1.5	0.0028
Insoluble fiber (g/day)	11.7±3.8	14.9±4.7	0.0002
Lipid (g/die)	62.0±17.8	53.9±12.9	0.0092

Lipid (%)	35.7±5.9	32.2±5.7	0.0027
Cholesterol (mg/day)	194.2±54.0	178.3±53.6	0.1349
Animal lipids (g/day)	22.4±8.3	16.8±7.6	0.0005
Plant lipids (g/day)	36.7±12.7	35.4±10.2	0.5662
Saturated fatty acids (g/day)	18.3±6.1	14.5±4.9	0.0008
Saturated Fatty Acids (% Fat)	32.0±6.9	28.5±4.6	0.0030

Metabolic changes at one-year follow-up

After one-year of follow-up, the serum uric acid levels decreased significantly (p=0.03) by 8%, as well as glycated hemoglobin (Hb1Ac) (p=0.01), while other metabolic parameters such as LDL-cholesterol and triglyceride showed no significant changes.

Table 3. Metabolic changes at baseline and at 1-year follow-up

Variables	Baseline (n=52)	1-year FU (n=52)	p-value
Uric acid (mg/dL)	5.0±1.0	4.6±0.9	0.0344
Glucose (mg/dL)	96.2±15.5	93.6±10.7	0.3219
Glycated hemoglobin (mmol/mol)	39.3±5.6	36.1±7.5	0.0154
Insulin (µUI/ml)	9.0±3.5	8.1±4.3	0.2445
Total cholesterol (mg/dL)	210.6±36.5	205.4±33.1	0.4884
HDL-cholesterol (mg/dL)	59.1±15.4	61.0±13.7	0.5077
LDL-cholesterol (mg/dL)	135.0±34.9	132.2±26.3	0.6450
Triglyceride (mg/dL)	110.9±48.3	94.0±50.7	0.0848

Discussion and Conclusions

The objective of the current study was to assess the effects of a targeted dietary and lifestyle intervention on various health indicators in postmenopausal women over a 12-month period. The findings revealed substantial enhancements in several key areas, indicating the effectiveness of the intervention in fostering improved health outcomes in this demographic, which is particularly susceptible to metabolic and cardiovascular conditions due to the hormonal changes linked to menopause.

Body composition and anthropometric Changes

Among the most significant results in the study was the noteworthy decrease in body weight, BMI, waist circumference, and skinfold thickness after 12 months with a reclassification from obesity to overweight and a significant reduction on abdominal obesity. These improvements are particularly pertinent given the heightened risk of obesity and central adiposity in postmenopausal women, both of which are major contributors to cardiovascular disease and type 2 diabetes. The reduction in waist circumference is especially significant, as central fat accumulation is closely associated with insulin resistance and other metabolic irregularities. The stability of lean mass, despite notable fat loss, suggests that the intervention successfully facilitated fat reduction without compromising muscle mass, which is crucial for preserving metabolic health and physical function in aging women.

Dietary Intake and Nutritional Quality

The study revealed that despite only minor changes in overall calorie consumption at one-year, there were notable improvements in the quality of macronutrient intake. This indicates that the participants' health enhancements were more influenced by the quality of their diet rather than the quantity of food they consumed. The intervention likely prioritized nutrient-rich, low-calorie foods that contributed to a feeling of fullness and supported metabolic health without requiring drastic calorie reduction. In particular a drastic change on protein quality from animal to plant-

based origin as well as a significant reduction on saturated fatty acid from animal origin was observed. This strategy is sustainable and can be more readily embraced by individuals, leading to long-term commitment and improved health outcomes.

Metabolic Health and Cardiovascular Risk Factors

The intervention also resulted in significant improvements in various metabolic health indicators, including blood pressure, uric acid levels, and HbA1c. The reduction in blood pressure is particularly noteworthy, given that hypertension is a significant risk factor for cardiovascular disease and is prevalent in postmenopausal women. The decrease in uric acid level could have implications for lowering the risk of conditions such as cardiovascular disease and may indicate an overall improvement in metabolic health. The substantial reduction in HbA1c levels signifies improved glucose control, which is crucial for preventing the onset of type 2 diabetes in this high-risk population.

These findings indicate that the dietary and lifestyle changes implemented in this study effectively targeted multiple risk factors associated with metabolic syndrome. The intervention's ability to enhance both anthropometric measurements and metabolic health indicators underscores its potential as a comprehensive strategy for reducing the risk of chronic diseases in postmenopausal women.

Implications for Postmenopausal Health

The results of this study have significant implications for managing the health of postmenopausal women. Considering the higher risk of obesity, cardiovascular disease, and metabolic syndrome in this demographic, it's crucial to prioritize interventions that can effectively enhance body composition and metabolic health. The successful outcomes of this intervention, achieved without extreme caloric restriction, indicate that comparable strategies could be applied in clinical settings to promote the well-being of postmenopausal women.

Future Directions

The outcomes of this study show promise, but further research is necessary to investigate the long-term effects of such interventions and to identify the most effective components of the dietary and lifestyle changes implemented. Subsequent studies could also assess the impact of similar interventions in different populations, including women of different ages and men, to establish the broader relevance of these findings.

In summary, this study presents compelling evidence that targeted dietary and lifestyle interventions can result in substantial improvements in body composition, metabolic health, and cardiovascular risk factors in postmenopausal women. These findings advocate for the use of such interventions as a crucial strategy to mitigate the risk of chronic diseases in this population, ultimately contributing to enhanced health outcomes and quality of life in aging women.

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