ΕΛΛΗΝΙΚΗ ΔΗΜΟΚΡΑΤΙΑ
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ΙΔΡΥΘΕΝ ΤΟ 1837

Σχολή Επιστημών Υγείας

Ιατρική Σχολή

Εργαστήριο/ Κλινική Παθολογικής Φυσιολογίας

Μονάδα Καρδιαγγειακής Πρόληψης & Έρευνας

Μελέτη της διατροφικής πρόσληψης νατρίου σε πληθυσμούς με αυξημένο καρδιαγγειακό κίνδυνο:

α. συμπεριφορική και ποσοτική ανάλυση

β. σύγκριση των μεθόδων εκτίμησής της

γ. συσχέτιση με την υποκλινική αγγειακή βλάβη

ΔΙΔΑΚΤΟΡΙΚΗ ΔΙΑΤΡΙΒΗ

Χριστιάνα Τσιριμιάγκου

Διαιτολόγος-Διατροφολόγος

ΑΘΗΝΑ

2023

HELLENIC REPUBLIC
National and Kapodistrian
University of Athens
EST. 1837

School of Health Science

Department of Clinical and Laboratory Medicine

Department of Pathophysiology

Cardiovascular Prevention & Research Unit

Dietary sodium intake in increased cardiovascular risk populations:

i. **behavioral and quantitative analysis**
ii. **comparison of evaluation methods**
iii. **association with subclinical vascular damage**

DOCTORAL DISSERTATION

Christiana Tsirimiagkou

Dietitian-Nutritionist

ATHENS

2023

Η έγκριση της διδακτορικής διατριβής από την Ιατρική Σχολή του Εθνικού και Καποδιστριακού Πανεπιστημίου Αθηνών δεν υποδηλοί την παραδοχή των γνωμών της συγγραφέως.
Ν5343/1932, άρθρο 202

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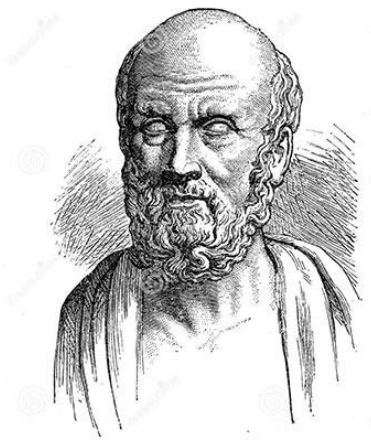

Ὄμνυμι Ἀπόλλωνα ἰητρὸν, καὶ Ἀσκληπιὸν, καὶ Ὑγείαν, καὶ Πανάκειαν, καὶ θεοὺς πάντας τε καὶ πάσας, ἵστορας ποιεύμενος, ἐπιτελέα ποιήσειν κατὰ δύναμιν καὶ κρίσιν ἐμὴν ὅρκον τόνδε καὶ ξυγγραφὴν τήνδε.

Ἡγήσασθαι μὲν τὸν διδάξαντά με τὴν τέχνην ταύτην ἴσα γενέτῃσιν ἐμοῖσι, καὶ βίου κοινώσασθαι, καὶ χρεῶν χρηίζοντι μετάδοσιν ποιήσασθαι, καὶ γένος τὸ ἐξ ωὐτέου ἀδελφοῖς ἴσον ἐπικρινέειν ἄῤῥεσι, καὶ διδάξειν τὴν τέχνην ταύτην, ἢν χρηίζωσι μανθάνειν, ἄνευ μισθοῦ καὶ ξυγγραφῆς, παραγγελίης τε καὶ ἀκροήσιος καὶ τῆς λοιπῆς ἁπάσης μαθήσιος μετάδοσιν ποιήσασθαι υἱοῖσί τε ἐμοῖσι, καὶ τοῖσι τοῦ ἐμὲ διδάξαντος, καὶ μαθηταῖσι συγγεγραμμένοισί τε καὶ ὡρκισμένοις νόμῳ ἰητρικῷ, ἄλλῳ δὲ οὐδενί.

Διαιτήμασί τε χρήσομαι ἐπ' ὠφελείῃ καμνόντων κατὰ δύναμιν καὶ κρίσιν ἐμὴν, ἐπὶ δηλήσει δὲ καὶ ἀδικίῃ εἴρξειν.

Οὐ δώσω δὲ οὐδὲ φάρμακον οὐδενὶ αἰτηθεὶς θανάσιμον, οὐδὲ ὑφηγήσομαι ξυμβουλίην τοιήνδε. Ὁμοίως δὲ οὐδὲ γυναικὶ πεσσὸν φθόριον δώσω. Ἁγνῶς δὲ καὶ ὁσίως διατηρήσω βίον τὸν ἐμὸν καὶ τέχνην τὴν ἐμήν.

Οὐ τεμέω δὲ οὐδὲ μὴν λιθιῶντας, ἐκχωρήσω δὲ ἐργάτῃσιν ἀνδράσι πρήξιος τῆσδε.

Ἐς οἰκίας δὲ ὁκόσας ἂν ἐσίω, ἐσελεύσομαι ἐπ' ὠφελείῃ καμνόντων, ἐκτὸς ἐὼν πάσης ἀδικίης ἑκουσίης καὶ φθορίης, τῆς τε ἄλλης καὶ ἀφροδισίων ἔργων ἐπί τε γυναικείων σωμάτων καὶ ἀνδρῴων, ἐλευθέρων τε καὶ δούλων.

Ἃ δ' ἂν ἐν θεραπείῃ ἢ ἴδω, ἢ ἀκούσω, ἢ καὶ ἄνευ θεραπηίης κατὰ βίον ἀνθρώπων, ἃ μὴ χρή ποτε ἐκλαλέεσθαι ἔξω, σιγήσομαι, ἄῤῥητα ἡγεύμενος εἶναι τὰ τοιαῦτα.

Ὅρκον μὲν οὖν μοι τόνδε ἐπιτελέα ποιέοντι, καὶ μὴ ξυγχέοντι, εἴη ἐπαύρασθαι καὶ βίου καὶ τέχνης δοξαζομένῳ παρὰ πᾶσιν ἀνθρώποις ἐς τὸν αἰεὶ χρόνον. παραβαίνοντι δὲ καὶ ἐπιορκοῦντι, τἀναντία τουτέων.

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To my parents

Dimitris & Aphrodite

Table of Contents

Acknowledgments

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…my supervisor, Professor Athanase D. Protogerou,

…the advisory committee members, Professor Athanasios Tzioufas and Professor Mary Yannakoulia

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Christiana

Curriculum Vitae

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EDUCATION AND TRAINING

2016 - today Ph.D. Candidate
Fellow of the Hellenic Foundation for Research and Innovation (HFRI) of the General Secretariat for Research and Technology (GSRT)

Cardiovascular Prevention and Research Unit, Department of Pathophysiology, Medical School, School of Health, National and Kapodistrian University of Athens
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2014-2016 Postgraduate Studies
Master Of Science (M.Sc) in Applied Nutrition and Dietetics
Direction: Nutrition and Exercise / Full Time Studies
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Grade: 9.7 «Excellent»
Title of dissertation: "Prevalence of Arterial Hypertension and association with obesity in schoolchildren in Greece: The Healthy Growth Study".

2009-2014 Undergraduate studies
Department of Nutrition and Dietetics
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Grade: 7.1 («Very Good»)
Title of Dissertation: "The effect of milk and dairy products consumption on vascular function in low and high grade of inflammation".

- Internship at Asklepieio Voulas and at General Hospital "Sotiria"
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WORK & RESEARCH EXPERIANCE

2021-today Dietitian-Nutritionist
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2019-today Registered Instructor, Medical School, National and Kapodistrian University of Athens

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Publications in international journals

1. Basdeki ED, Koumi K, Tsirimiagkou C, Argyris A, Chrysostomou S, Sfikakis PP, Protogerou AD, Karatzi K. Late-Night Overeating or Low-Quality Food Choices Late at Night Are Associated with Subclinical Vascular Damage in Patients at Increased Cardiovascular Risk. Nutrients. 2022 Jan 21;14(3):470. doi: 10.3390/nu14030470. PMID: 35276835; PMCID: PMC8840219.
2. Basdeki ED, Kollias A, Mitrou P, Tsirimiagkou C, Georgakis MK, Chatzigeorgiou A, Argyris A, Karatzi K, Manios Y, Sfikakis PP, Protogerou AD. Does Sodium Intake Induce Systemic Inflammatory Response? A Systematic Review and Meta-Analysis of Randomized Studies in Humans. Nutrients. 2021 Jul 30;13(8):2632. doi: 10.3390/nu13082632. PMID: 34444792; PMCID: PMC8399701.
3. Tsirimiagkou C, Karatzi K, Argyris A, Basdeki ED, Kaloudi P, Yannakoulia M, Protogerou AD. Dietary sodium and cardiovascular morbidity/mortality: a brief commentary on the 'J-shape hypothesis'. J Hypertens. 2021 Dec 1;39(12):2335-2343. doi: 10.1097/HJH.0000000000002953. PMID: 34326279.
4. Tsirimiagkou C, Argyris A, Karatzi K, Konstantina N, Sfikakis PP, Protogerou AD. Dietary sugars and subclinical vascular damage in moderate-to-high cardiovascular risk adults. Nutr Metab Cardiovasc Dis. 2022 Jan;32(1):98-108. doi: 10.1016/j.numecd.2021.09.027. Epub 2021 Oct 8. PMID: 34823975.
5. Tsirimiagkou C, Karatzi K, Argyris A, Chalkidou F, Tzelefa V, Sfikakis PP, Yannakoulia M, Protogerou AD. Reply to: "Levels of dietary sodium intake: diverging associations with arterial stiffness and atheromatosis. Concerns about the evidence review and methods". Hellenic J Cardiol. 2022 Jan-Feb;63:94-95. doi: 10.1016/j.hjc.2021.06.004. Epub 2021 Jun 19. PMID: 34157421.
6. Tsirimiagkou C, Basdeki ED, Kyriazopoulou Korovesi AA, Chairistanidou C, Ouamer DS, Argyris A, Sfikakis PP, Karatzi K, Protogerou AD. Habitual consumption of instant coffee is favorably associated with arterial stiffness but not with atheromatosis. Clin Nutr ESPEN. 2021 Oct;45:363-368. doi: 10.1016/j.clnesp.2021.07.018. Epub 2021 Aug 10. PMID: 34620341.
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8. Tzelefa V, Tsirimiagkou C, Argyris A, Moschonis G, Perogiannakis G, Yannakoulia M, Sfikakis P, Protogerou AD, Karatzi K. Associations of dietary patterns with blood pressure and markers of subclinical arterial damage in adults with risk factors for CVD. Public Health Nutr. 2021 Dec;24(18):6075-6084. doi: 10.1017/S1368980021003499. Epub 2021 Aug 16. PMID: 34392855.
9. Tsirimiagkou C, Karatzi K, Argyris A, Chalkidou F, Tzelefa V, Sfikakis PP, Yannakoulia M, Protogerou AD. Levels of dietary sodium intake: diverging associations with arterial stiffness and atheromatosis. Hellenic J Cardiol. 2021 Nov-Dec;62(6):439-446. doi: 10.1016/j.hjc.2021.02.005. Epub 2021 Feb 18. PMID: 33610752.
10. Basdeki ED, Tsirimiagkou C, Argyris A, Moschonis G, Sfikakis P, Protogerou AD, Karatzi K. Moderately increased alcohol consumption is associated with higher pressure wave reflections and blood pressure in men. Nutr Metab Cardiovasc Dis. 2021 Jan 4;31(1):85-94. doi: 10.1016/j.numecd.2020.08.013. Epub 2020 Aug 20. PMID: 33500112.
11. Tsirimiagkou C, Basdeki ED, Argyris A, Manios Y, Yannakoulia M, Protogerou AD, Karatzi K. Current Data on Dietary Sodium, Arterial Structure and Function in Humans: A Systematic Review. Nutrients. 2019 Dec 18;12(1):5. doi: 10.3390/nu12010005. PMID: 31861381; PMCID: PMC7019233.

12. Karatzi K, Protogerou AD, Moschonis G, Tsirimiagou C, Androutsos O, Chrousos GP, Lionis C, Manios Y. Reply to: "Considerations about: "Prevalence of hypertension and hypertension phenotypes by age and gender among schoolchildren in Greece: The Healthy Growth Study"". Atherosclerosis. 2017 Jun;261:167-168. doi: 10.1016/j.atherosclerosis.2017.04.006. Epub 2017 Apr 8. PMID: 28411951.
13. Manios Y, Karatzi K, Protogerou AD, Moschonis G, Tsirimiagou C, Androutsos O, Lionis C, Chrousos GP. Prevalence of childhood hypertension and hypertension phenotypes by weight status and waist circumference: the Healthy Growth Study. Eur J Nutr. 2018 Apr;57(3):1147-1155. doi: 10.1007/s00394-017-1398-y. Epub 2017 Mar 27. PMID: 28349252.
14. Karatzi K, Protogerou AD, Moschonis G, Tsirimiagou C, Androutsos O, Chrousos GP, Lionis C, Manios Y. Prevalence of hypertension and hypertension phenotypes by age and gender among schoolchildren in Greece: The Healthy Growth Study. Atherosclerosis. 2017 Apr;259:128-133. doi: 10.1016/j.atherosclerosis.2017.01.027. Epub 2017 Jan 21. PMID: 28161097.
15. Karatzi K, Aissopou EK, Tsirimiagou C, Fatmeli E, Sfikakis PP, Protogerou AD. Reply from the authors to "Comments on 'Association of consumption of dairy products and meat with retinal vessel calibers in subjects at increased cardiovascular risk'". Nutr Metab Cardiovasc Dis. 2017 Jan;27(1):89-90. doi: 10.1016/j.numecd.2016.06.002. Epub 2016 Jun 17. PMID: 27484753.
16. Karatzi K, Aissopou EK, Tsirimiagou C, Fatmeli E, Sfikakis PP, Protogerou AD. Association of consumption of dairy products and meat with retinal vessel calibers in subjects at increased cardiovascular risk. Nutr Metab Cardiovasc Dis. 2016 Aug;26(8):752-7. doi: 10.1016/j.numecd.2016.03.006. Epub 2016 Mar 19. PMID: 27139515.

Invited speaker in congresses

Effect of Salt Substitution on Cardiovascular Events and Death.
Tsirimiagkou C, 5th Congress of the Hellenic Stroke Organization, Athens 2022

Different levels of salt intake, Diabetes Mellitus and Cardiovascular disease
Tsirimiagkou C, Metabolic Seminars 2020, Athens, Greece

Salt intake: the less is better?
Tsirimiagkou C, 7th Panhellenic Conference of Hellenic Atherosclerosis Society Working Groups 2019 Athens, Greece

Posters in congresses

Development of high added value and potentially functional food products with the use of seaweed extracts from the Greek seas
Pissaridi K, Velivasaki K, Giatrakou V, Krystalli E, Antoniou J, Koutsopoulou I, Nanou A, **Tsirimiagkou C**
10th Congress of Greek Lipid Forum, Athens 2022

Development of innovative food products with anti-inflammatory function, enriched with fish oils rich in omega -3 fatty acids
Pissaridi K, Velivasaki K, Giatrakou V, Krystalli E, Spiliotis V, Antoniou J, Koutsopoulou I, Nanou,A, **Tsirimiagkou C**
10th Congress of Greek Lipid Forum, Athens 2022

Dietary habits and adherence to Meditarannean diet in patients with rheumatoid arthritis
Kaloudi P, **Tsirimiagkou C**, Basdeki ED, Argyris A, Protogerou AD, Sfikakis P, Karatzi K
16th Hellenic Congress of Nutrition and Dietetics, Athens 2021
Increased alcohol consumption is associated with higher blood pressure and augmentation index in men

Basdeki E, **Tsirimiagkou C**, Argyris A, Moschonis G, Sfikakis Protogerou A, Karatzi K
9th Hellenic Congress of Hellenic Atherosclerosis Society, Athens 2020

Habitual consumption of instant coffee is associated with increased carotid elasticity in high cardiovascular risk individuals.
Basdeki E, **Tsirimiagkou C**, Kyriazopoulou Korovesi A, Chairistanidou C, Argyris A, Karatzi K, Protogerou A
9th Hellenic Congress of Hellenic Atherosclerosis Society, Athens 2020

Oral presentations in congresses

Effect of salt substitution on cardiovascular events and death.
Neal B, Wu Y, Feng X, et al.
5ο Πανελλήνιο Συνέδριο του Ελληνικού Οργανισμού Εγκεφαλικών, Αθήνα 2022

Dietary sugars and subclinical vascular damage (Science at a Glance)
Argyris A, **Tsirimiagkou C**, Karatzi K, Ntouska K, Sfikakis P, Protogerou A
European Atherosclerosis Society (EAS) Virtual Congress 2021

The association between eating frequency, arterial pressure and subclinical vascular damage in diabetic patients
Kaloudi P, Fatmeli E, **Tsirimiagkou C**, Karatzi K, Tentolouris N, Protogerou A.
19th Panhellenic Congress on Diabetes, Athens, Greece, 2021

Dietary sodium estimation methods: comparison, reliability and accuracy, comparison and implications for further improvement
Tsirimiagkou C, Karatzi K, Basdeki E., Argyris A, Papaioannou T., Yannakoulia M., Protogerou A.
9ο Hellenic Congress of Hellenic Atherosclerosis Society, Athens 2020

Levels of dietary sodium intake: diverging associations with atheromatosis and arterial stiffness
Tsirimiagkou C, Karatzi K, Argyris A, Chalkidou F, Tzelefa V, Sfikakis P, Yannakoulia M, Protogerou A
15ο Hellenic Congress of Nutrition and Dietetics –4Hellenic Congress of Clinical Nutrition and Metabolism, Athens 2019

Prevalence of childhood hypertension and its phenotypes by weight status and waist circumference: the Healthy Growth Study
Tsirimiagkou C., Karatzi K., Protogerou A.D., Moschonis G., Androutsos O., Lionis C., Chrousos G.P., Manios Y.
14th Hellenic Congress of Nutrition and Dietetics – 3rd Hellenic Congress of Clinical Nutrition and Metabolism, Athens 2017

Prevalence or arterial hypertension and its phenotypes by age and sex in schoolchildren in Greece: The Healthy Growth Study
Tsirimiagkou C., Karatzi K., Protogerou A.D., Moschonis G., Androutsos O., Chrousos G.P., Lionis C., Manios Y.
7th Hellenic Congress of Hellenic Atherosclerosis Society, Athens 2017

Certificates

E-learning course "ICH Good Clinical Practice E6 (R2)", Global Health Training Center.

Scholarships, honors, and awards

2021: Award for the oral presentation: ""The association between eating frequency, arterial pressure and subclinical vascular damage in diabetic patients"
Kaloudi P, Fatmeli E, Tsirimiagkou C, Karatzi K, Tentolouris N, Protogerou A.

19th Panhellenic Congress on Diabetes, Athens, Greece, 2021

2020: Award of "best clinical research study" for the oral presentation: "Dietary sodium estimation methods: comparison, reliability and accuracy, comparison and implications for further improvement"

Tsirimiagkou C, Karatzi K, Basdeki E., Argyris A, Papaioannou T., Yannakoulia M., Protogerou A.

9o Hellenic Congress of Hellenic Atherosclerosis Society, Athens 2020

2020-today: Scholarship from the Operational Programme «Human Resources Development, Education and Lifelong Learning 2014-2020» in the context of the project "Dietary intake of sugars (mono- and di-saccharides) in populations with cardiovascular risk factors and/or chronic inflammatory diseases: quantitative analysis and association with subclinical vascular damage" (European Social Fund).

2017-2019: Scholarship for the Ph.D. dissertation from the Hellenic Foundation for Research and Innovation (ELIDEK) - General Secretariat for Research and Technology (GSRT)

2016: Scholarship from the Graduate Program "Applied Dietetics and Nutrition" of Harokopio University accompanied by Excellence Award (distinction: rank first in the 1st semester of study).

2015: Scholarship from the Graduate Program "Applied Dietetics and Nutrition" of Harokopio University accompanied by Excellence Award (distinction: rank first in the 2nd semester of study).

2009: Scholarship from the "Eurobank" bank in the context of the awards of the distinguished high school students of Lyceum "The great moment for Education accompanied by excellence and money prize.

Abbreviations

24DR:	24h dietary recall(s)
24UC:	24h urine collection
ABI:	Ankle-brachial index
AHA:	American Heart Association
AIx:	Augmentation Index
baPWV:	Brachial-ankle pulse wave velocity
BP:	blood pressure
cfPWV:	carotid-to-femoral pulse wave velocity
CI:	confidence interval
cIMT:	carotid intima-media thickness
Cl:	chloride
CV:	cardiovascular
CVD:	cardiovascular disease
DBP:	diastolic blood pressure
DMs:	dietary methods
DRIs:	Dietary Reference Intakes
ENaC:	epithelial Na^+ channels
FFQ:	food frequency questionnaire
FMD:	flow-mediated dilatation
GAGs:	glucose amino glycans
H:	hydrogen
ICC:	intraclass correlation coefficient
IMT:	intimal-medial thickness
K:	potassium
Na:	sodium
NaCl:	sodium chloride
NO:	nitric oxide
PWV:	pulse wave velocity
RAAS:	renin-angiotensin-aldosterone system
RCTs:	randomized clinical trials
ROS:	reactive oxygen species
SBP:	systolic blood pressure
SD:	standard deviation
SOD:	superoxide dismutase
SQ:	salt questions
SVD:	subclinical vascular damage
UMs:	urinary methods
WHO:	World Health Organization

A. THEORETICAL BACKGROUND

A1. Sodium physiology & bioavailability

Sodium (Na) is the principal ion of the extracellular fluid of the human body and contributes to a wide range of metabolic processes. These processes include the transmission of nerves impulses across cell membranes; the extracellular fluid osmolarity regulation; the acid-base balance regulation; the active transport of nutrients in the intestine cells (1, 2).

Sodium absorption by the intestinal mucosa

Four fundamental mechanisms for the intestinal absorption of Na^+ exist:

1. Cotransport with nutrients

These nutrients are usually glucose, amino acids, B-complex vitamins, etc. Na^+-coupled absorption occurs throughout jejunum and ileum of the small intestine and is mediated by distinct cotransporters (e.g., the Na^+/ glucose cotransporter SGLPT is responsible for the uptake of glucose). This mechanism is the major one for the postprandial Na^+ absorption. After the absorption in the intestine cell, Na^+ is extruded across the basolateral membrane through the Na^+-K^+ pump (**Figure 1**) (2, 3).

2. Electroneutral Na^+-H^+ exchange

This mechanism occurs in the jejunum and the duodenum of the small intestine. Luminal alkalinity -which derives from simultaneous decreases in intracellular pH and increases in luminal pH- stimulates Na^+ absorption across the apical membrane via the Na^+-H^+ exchanger (**Figure 1**) (3).

3. Parallel Na^+-H^+ and $Cl^-$$HCO_3^-$ exchange

The absorption Na^+ and Cl^- occurs in both the small and large intestine and it is the major mechanism of Na^+ absorption during the interdigestive -e.g., between meals- period. The uptake of Na^+ and Cl^- occurs in parallel with H^+ and HCO_3^- exchange. (**Figure 1**) (2, 3).

4. Electrogenic Na^+ absorption via epithelial Na^+ channels

It mainly occurs in the distal colon of the large intestine. Na^+ enters the intestinal cell through epithelial Na^+ channels (ENaC) and is extruded to extracellular fluid and blood through the Na^+-K^+ pumps. (**Figure 1**) (2, 3).

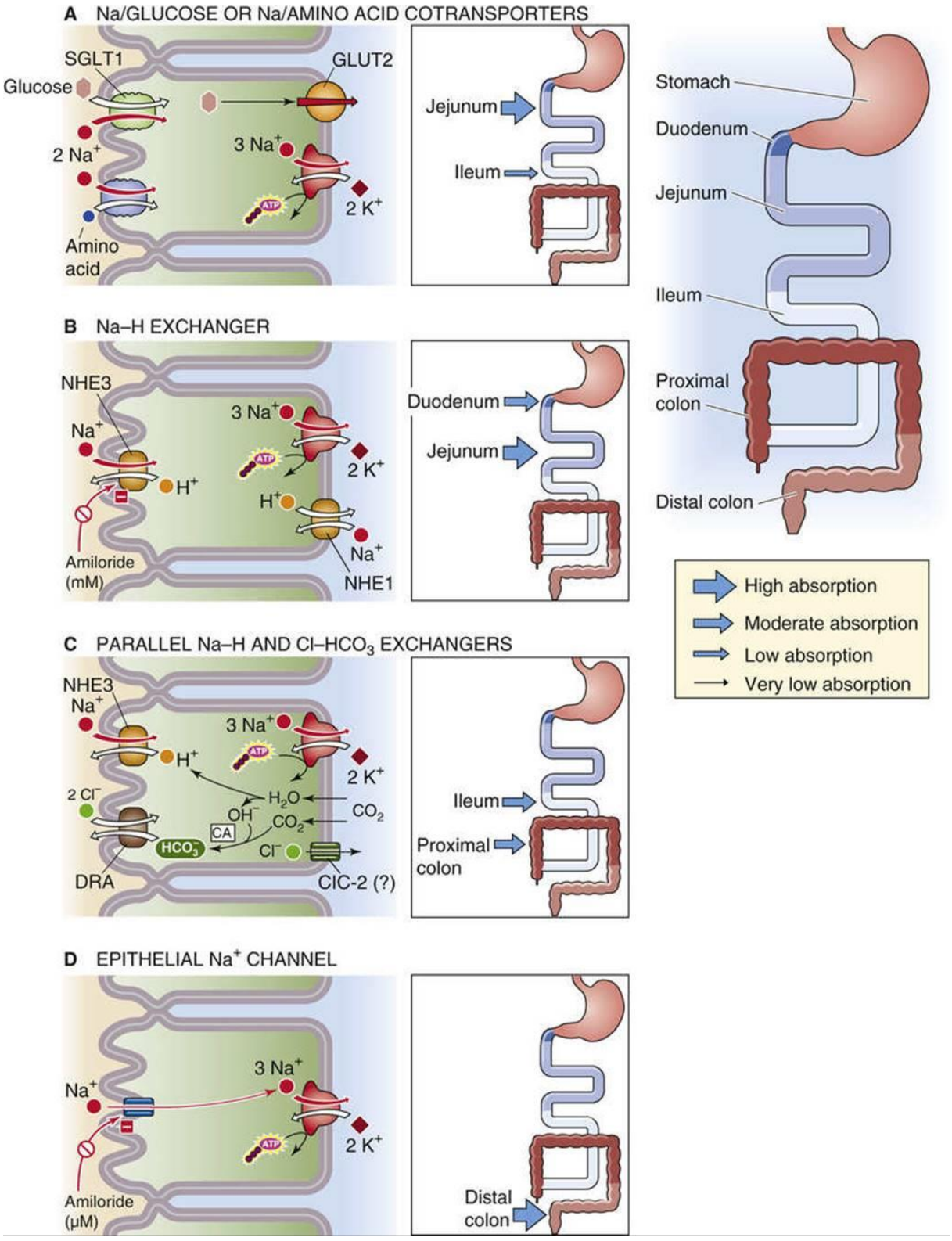

Figure 1: Modes of active Na absorption by the intestine. *Adapted from Boron WB (3).*

After the absorption in the intestinal cells, Na^+ is released into the bloodstream. Na^+ blood levels remain stable between 135-145mEq/L.

Sodium storage

About 65% of Na^+ is accumulated in extracellular fluid, while only 5-10% is found in the intracellular fluid (4). The rest of it (25%) occurs in the surface of bone crystals. Although the concept of tissue Na^+ was first suggested in the1970s (5), recent NaMRI techniques confirm the storage of significant Na quantities in skin's and muscles' interstitium, without parallel changes in plasma Na^+ concentration (6-12). Current data indicate that Na cations bond with negatively charged disaccharides biopolymers called glucose amino glycans (GAGs) (7, 13), found in interstitium of tissues. Several GAGs chains form a large proteoglycan via H^+ bonds (14). Negatively charged GAGs are bonded to Na^+, which presents the most abundant extracellular cation, therefore indicating that high Na^+ concentrations are found in this micro-environment (15). Thus, skin interstitium seems to be a major non-renal sodium balance regulator.

Sodium excretion, retention & reabsorption

Most of the ingested Na^+ is absorbed, but the excess Na^+ is excreted through urine and sweat by the kidneys and the skin respectively. In normal temperature conditions, the Na^+ losses in sweat are small. Small losses of Na^+ are also detected in feaces. Urinary Na^+ excretion is highly variable due to variability of dietary Na intake and the amount of reabsorption in the nephrons. To maintain homeostasis, renal Na excretion, retention and reabsorption are hormone-dependent processes, induced by the activation of sympathetic nervous system and stimulated by the renin-angiotensin-aldosterone system (RAAS). In low Na^+ concentrations, renin is secreted by the kidneys and converts angiotensinogen to angiotensin I, which is an inert substance that will be rapidly converted to angiotensin II by angiotensin I-converting enzyme. Angiotensin II stimulates aldosterone secretion. Aldosterone is an adrenal steroid hormone that stimulates sodium reabsorption from cortical collecting ducts. At high Na^+ concentrations aldosterone secretion is low and vice versa (16). **Figure 2** presents the RAAS system.

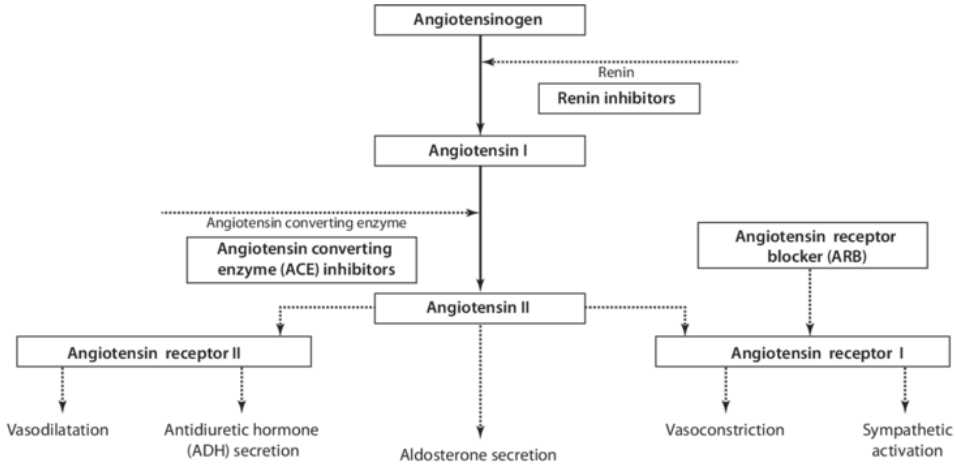

Figure 2: The renin-angiotensin-aldosterone system. *Adapted from Vivian E & Mannebach C (17).*

A2. Dietary sodium

Dietary sodium intake & food sources

The major dietary source of Na is salt, mainly in the form of sodium chloride (NaCl). The ionic compound of NaCl consist of 40% Na and 60% Cl. Food industry utilizes food additives based on Na in most food products as taste enhancers or preservatives (sodium benzoate, sodium nitrate etc.). Thus, processed food contains high amounts of Na and provides about 75% of total Na intake per day in the western diets (2). Some of the high Na food product categories include cold cuts, canned meat, sauces, ready-to-eat snacks, pickles, bakery products etc. Na also occurs naturally in some foods (meat, milk, eggs, vegetables) (2), even though it does not provide more than about 10% of the total daily dietary Na intake. Table 1 presents the Na content of certain food categories.

Table 1: Na content from different food categories. *Adapted from Kafatos A & Chasapidou M and the USDA Nanutrient database (18, 19).*

Food	Na content (mg/ 100g)
Vegetables	
Tomato	5
Lettuce	28
Cold cuts	
Bacon	1298
Ham	939
Cheeses	
Feta cheese	917
Mozzarella	650
Cereals	
Bread	450-550
Breakfast cereals	300-600
Egg	80
Margarine	500

Regardless of current recommendations, dietary Na intake globally tends to be almost double [~ 3.7 to 4.0 g/day instead of 2 g of Na per day (or 9.25 to 10.0 g of salt per day)], while some regions (e.g. Central and East Asia, high-income Asia Pacific and Eastern Europe) Na intakes are more than twice compared to recommendations (>4 g/day) (20-22). Interestingly, this trend is also observed in high-risk populations that are more often monitored by health workers and advised to limit salt intake, such as hypertensive or diabetic patients (23-25). Continent-wise distribution of dietary Na intake in most European countries is ~3.2-4.4 g/day, according to the Survey on Members States' Implementation of the EU Salt Reduction (26). Higher levels of Na intake have been detected in Asia, Europe and USA compared to Sub-Saharan Africa and Southern Latin America which present the lowest levels, but still above the recommendation (20). In eastern countries, dietary Na derives from salt added during cooking and condiments (i.e., soy sauce) which contribute to 88.4% of total Na intake, while manufactured food provides only the 11.4% of total Na intake (27). On the contrary, the manufactured food is the dominant source of dietary Na in the western diet, providing about ¾ of total daily Na intake (28-30).

Dietary sodium intake recommendations and reference values

Based on strong quality of evidence derived from metanalyses of RCTs regarding the benefits of dietary Na reduction in BP levels in hypertensive or normotensive populations (31-33), dietary guidelines recommend a limited intake of Na (**Table 2**). According to the World Health Organization (WHO) 2012 Guidelines, the European Society of Hypertension and the European Society of Cardiology 2018 guidelines as well as the Dietary guidelines for Americans, Na intake should not overpass the 2.0 or 2.3 g/day (which is equivalent to 5.0 or 5.75 g of salt respectively) for the general population (34-36). A large-scale multicenter study including data from 66 countries conducted by Mozaffarian et al., estimated that in 2010 around 1.65 million deaths from CV causes occurred due to dietary Na intake above the recommended level of 2.0 g/day. The American Heart Association and the American College of Cardiology recommend a stricter limitation of Na intake (<1.5 g/day) for adults who would benefit from BP lowering (37). Despite its harmful effects on CV health at high levels of intake, dietary Na remains an essential nutrient for human life. However, the adequacy in Na intake or the potential harmful effect of very low daily Na intake is less investigated, and, thus less described in the available guidelines. The most recently published Dietary Reference Intakes (DRIs) from the Institute of Medicine (IoM) defined the 1.5 g/day as an Adequate Intake (AI) for the general population (38). On the other hand, the European Food Safety Authority (EFSA) set the 2.0 g/day as a safe and adequate intake for the general European population including pregnant and lactating women , in the context of reducing CV risk (39).

Table 2: Dietary Na intake recommendations and reference values. *Published in Journal of Hypertension, 2021 (40).*

Organization/Institution	Na intake	Salt equivalents	Target group
Dietary recommendations			
ESC/ESH 2018 (34)	<2.0	<5.0	For the general population and all hypertensive patients.
USDA 2015-2020 (35)	<2.3	<5.75	For Americans older than 14 years of age, as part of a healthy eating pattern.
AHA/ACC 2013 (37)	• <2.4	<6.0	For adults who would benefit even from BP lowering
	• <1.5 for greater BP reduction	<3.75	For greater BP reduction
WHO 2012 (36)	<2.0	<5.0	All adults ≥16 years of age, with or without hypertension (including pregnant or lactating women), except for individuals with illnesses or taking drug therapy that may lead to hyponatremia or acute build-up of body water, or require physician-supervised diets (e.g., patients with heart failure and those with type I diabetes).
Dietary Reference Intakes/ Values			
IoM 2019 (38)	1.5	3.75	Adequate intake (AI) for the general adult population (>19 years of age)
EFSA 2019 (39)	• <2.3	<5.75	For the general population (>14 years of age) to reduce chronic disease risk (cardiovascular events and arterial hypertension).
	• 2.0	5.0	Safe and adequate intake for the general European population of adults including pregnant and lactating women.

USDA: U.S. Department of Agriculture; AHA/ACC, American Heart Association/ American College of Cardiology; EFSA, European Food Safety Authority; ESC/ESH, European Society of Cardiology/ European Society of Hypertension; IoM, Institute of Medicine; Na, sodium.

Dietary sodium assessment methods

In large-scale epidemiological studies, the accurate estimation of dietary Na intake is important for detecting actual consumption and for identifying food items, food patterns or dietary behaviors related to Na intake and their association with diseases and treatments as well. In clinical settings the assessment of Na intake is also crucial for evaluating patients' adherence to recommendations and guiding drug treatment decisions. A variety of urinary methods (UMs) and dietary methods (DMs) are available for the estimation of dietary Na intake; nevertheless its accurate and precise quantification is still elusive (41).

Spot urine samples, overnight urine collections and 24-hour urine collections (24UC) represent the UMs. Based on the knowledge that about 90% of Na consumed is excreted through urine during a 24h period, the 24UC is regarded as the gold-standard method [7-9]. However, it is a burdensome, time-consuming method and difficult to apply in large-scale studies as well as in daily clinical practice of uncomplicated arterial hypertension management. In addition, an important limitation of this method is the high rate of incomplete collections that have been observed in previous studies (41), as well as the fact that most studies do not assess the incomplete samples in order to exclude them from the analysis, leading to biased findings. Furthermore, bias may occur due to the day-to-day variability in Na excretion because of several confounding factors such as dietary intake and physical activity. Moreover, multiple collections - which are even more time consuming - have been reported to be more accurate, especially when evaluating individuals' and not a group mean dietary Na intake (42).

Spot urine samples are more convenient to estimate 24h urine Na excretion (24UNa) via specially designed equations (43-49) (**Table 3**), becoming a common alternative in research studies investigating dietary Na. Nevertheless, this method has been observed to present poor or moderate correlations with the 24UC (r=0.33-0.56) and to overestimate Na in higher levels of intake and underestimate it in lower levels of intake (50-54). Of note, the differences observed between the spot urine method and the gold standard 24UC, could be explained by a significant number of confounding factors, such as body weight, age, gender, and urinary creatinine excretion. As an example, although intra-individual creatinine excretion is considered relatively stable, it is well established that protein intake and exercise have a significant impact in creatinine excretion levels (55). Given that most of the available equations for the conversion of spot Na to 24-hour Na are based on prediction of the 24-hour creatinine excretion without taking into account parameters such as protein intake and exercise, important bias potentially occurs.

Table 3: Equations to estimate 24-hour urinary Na excretion from a single spot urine specimen. *Published in Public Health Nutrition, 2022* (56).

Kawasaki (44)	$\text{Estimated 24h Na} = 16.3 \text{ x } \sqrt{\frac{\text{Spot Na}}{Spot\ Cr}\ x\ Predicted\ 24h\ urine\ Cr}$ $Males: Predicted\ 24h\ Cr = 12.63\ x\ age + 15.12\ x\ weight + 7.39\ x\ height - 79.9$ $Females: Predicted\ 24h\ Cr = -4.72\ x\ age + 8.58\ x\ weight + 5.09\ x\ height - 74.5$	Estimated 24h Na, mmol/d spot Na, mmol/L spot Cr: mg/L Predicted 24h Cr: mg/d age, years weight, kg height, cm
Tanaka (46)	$Estimated\ 24h\ Na = 21.98\ x\ X_{Na}^{0.392}$ $X_{Na} = \frac{Spot\ Na}{Spot\ Cr\ x\ 10} x\ Predicted\ 24h\ Cr$ $Predicted\ 24\ hour\ Cr = (-2.04\ x\ age) + (14.89\ x\ weight) + (16.14\ x\ height) - 2244.45$	Estimated 24h Na, mmol/d spot Na, mmol/L spot Cr: mg/dL Predicted 24h Cr: mg/d age, years weight, kg height, cm
INTERSALT (49)	***With Spot K*** $Males: Estimated\ 24h\ Na = 25.46 + (0.46\ x\ spot\ Na) - (2.75\ x\ spot\ Cr) - (0.13\ x\ spot\ K) + (4.10\ x\ BMI) + (0.26\ x\ age)$ $Females: Estimated\ 24h\ Na = 5.07 + (0.34\ x\ spot\ Na) - (2.16\ x\ spot\ Cr) - (0.09\ x\ spot\ K) + (2.39\ x\ BMI) + (2.35\ x\ age) - (0.03\ x\ age^2)$ ***Without Spot K*** $Males: Estimated\ 24h\ Na = 23.51 + (0.45\ x\ spot\ Na) - (3.09\ x\ spot\ Cr) + (4.16\ x\ BMI) + (0.22\ x\ age)$ $Females: Estimated\ 24h\ Na = 3.74 + (0.33\ x\ spot\ Na) - (2.44\ x\ spot\ Cr) + (2.42\ x\ BMI) + (2.34\ x\ age) - (10.03\ x\ age^2)$	Estimated 24h Na, mmol/d spot Na, mmol/L spot Cr: mmol/L spot K: mmol/L BMI: kg/m^2 age, years
Toft (47)	$X_{Na} = \frac{spot\ Na}{spot\ Cr}\ x\ Predicted\ 24h\ Cr$ $Males: \ Estimated\ 24h\ Na = 33.56\ x\ X_{Na}^{0.345}$ $Predicted\ 24h\ Cr\ (males) = (-7.54\ x\ age) + (14.15\ x\ weight) + (3.48\ x\ height) + 423.15$ $Females: \ Estimated\ 24h\ Na = 52.65\ x\ X_{Na}^{0.196}$ $Predicted\ 24h\ Cr\ (females) = (-6.13\ x\ age) + (9.97\ x\ weight) + (2.45\ x\ height) + 342.73$	Estimated 24h Na, mmol/d spot Na, mmol/L spot Cr: mg/dL Predicted 24h Cr: mg/d age, years weight, kg height, cm
Mage (45)	$Estimated\ 24h\ Na = \frac{spot\ Na}{spot\ Cr}\ x\ Predicted\ 24h\ Cr$ $Males: Predicted\ Cr\ 24h = 0.00179\ x\ (140 - age)\ x\ (weight^{1.5}\ x\ height^{0.5})\ x\ (1 + 0.18\ x\ A\ x\ (1.366 - 0.0159 x BMI))$ $Females: Predicted\ Cr\ 24h = 0.00163\ x\ (140 - age)\ x\ (weight^{1.5}\ x\ height^{0.5})\ x\ (1 + 0.18\ x\ A\ x\ (1.429 - 0.0198 x BMI))$ A= African American or Black race = 1/ other race = 0	Estimated 24h Na, mmol/d spot Na, mmol/L spot Cr: mg/dL Predicted 24h Cr: mg/d BMI: kg/m^2 weight, kg height, cm

Na: sodium; Cr: creatinine; K: potassium; d: day; BMI: Body Mass Index

On the other hand, the most common DMs for Na estimation include 24h dietary recalls (24DR), food frequency questionnaires (FFQ) and diet records. These methods are commonly used in population-based studies, as they are efficient to highlight food items rich in Na; however, numerous methodological disadvantages exist (41). A major one is the inability of all these methods to quantify the discretionary use of salt (table salt or use of salt during cooking), which has been previously reported to contribute significantly to the total Na intake (29, 30, 57). They have the advantage of providing information regarding dietary Na sources and dietary habits related to salt intake, which the urinary methods cannot. However, very low correlation rates with the 24UC (r=0.15-0.50) have been reported as well as underreporting of the actual Na intake even by 39% (30, 58-60). These high levels of underreporting derive from the inability of these methods to quantify the discretionary use of salt (table salt or use of salt during cooking), which contributes significantly to total Na intake, especially in eastern countries (~75% in eastern countries, ~10-15% in western countries).

Taken altogether, this evidence suggests that the use of all these methods as alternatives of the 24UC, even though convenient, leads to inaccurate Na intake recording. Therefore, they introduce not only a major cause of heterogeneity but also bias regarding outcome estimation.

Several efforts have been made to develop an optimal diet-based tool for the estimation of mean Na intake on group level, with the majority of them focusing on short FFQs (61-64) which are brief, easily completed and estimate Na intake through larger time periods compared to other DMs. 24DR and food records are also suitable to cover a longer period if they are repeated. Nevertheless, usually FFQs are developed for particular population groups and designed according to their culture, dietary habits and traditional recipes, thus they may not be accurately applied to other populations.

Table 4 presents all the available methods for Na estimation, a brief description of them and their strengths and limitations.

Table 4: Methods for assessing sodium intake. *Adapted from Colin-Remirez E et al. (58).*

Method	Description	Strengths	Limitations
Urinary methods			
24h urine collection (65, 66)	Urine voided is collected over a 24-hour period. The urine container must be kept cool during this time until it is returned to the lab for analysis. 24-hour sodium excretion is estimated based on sodium concentration and urine volume.	In a healthy individual, urine is the major route of sodium excretion. In temperate climates, it is assumed that skin losses of sodium are insignificant. Typically, 24- hour urine sodium excretion can account for 95% to 98% of dietary sodium intake. It is not subject to reporting bias.	Participant burden is high, and rates of participation and complete collection might be low. Urine collection may interfere with daily activities. Risk of under-or over collection resulting in biased estimates. Incomplete collections need to be repeated, making the process more complex. Loop diuretics may confound the use of 24-hour urine collections in HF.
Overnight urine collection (67-69)	Urine voided overnight is collected for a timed period (usually 8 or 12 hours). The volume collected and sodium concentration are calculated so that sodium excretion may be estimated.	Moderate participant burden compared to 24-hour collection. It interferes less with daily activities. Overnight excretion correlates well (r =0.72) with 24-hour excretion in healthy individuals.	Collection must be complete and accurately timed. Assumes that the ratio of daytime to overnight excretion is constant.
Single spot urine collection (67, 70, 71)	A single voiding is collected and sodium concentration is measured in the laboratory. If time since last voiding and volume are known, then excretion rate may be calculated. Prediction equations may be used to estimate 24-hour sodium excretion.	Low participant burden compared to 24-hour or overnight sample. Voiding can be made at clinic. Can be incorporated relatively easily into population-based studies.	Concentration is dependent not only on sodium consumed, but also on fluid ingested. Depends on time of day. Highest concentrations are with first void in the morning. Formula conversion to 24hour not well validated in diseased populations. Uncertain validity in estimating individual sodium intakes.
Dietary methods			
Food record (weighed or estimated)(72, 73)	A detailed description of food and beverages consumed are recorded in a booklet. Participants describe portion size or the weight of the food and drinks eaten for a prescribed number of days. Information about the weight of food may be obtained either by weighing the food (weighted food record) or describing portions of food in terms of household measures, pictures, food models, or pack sizes. Information gathered in the food records needs to be entered into a nutritional analysis software for energy and nutrient intake estimation.	It is a prospective method. Multiple-day food record allows evaluation of within subject variation. Useful to identify the key sources of excess sodium in the diet, measure overall diet quality, and guide dietary counselling based on current patients' dietary practices.	Participants may change their behaviour when collecting dietary information. High degree of motivation required. Misreporting of foods and portion sizes consumed is common. The sodium content in processed and home-cooked foods is highly variable, and discretionary use of salt (in cooking or at the table, including salty condiments) is difficult to quantify. Subject to reporting and observer errors. Sodium intake tends to be underestimated by this method.
24-h food recall(72)	Participants are asked to report what they ate (food and drinks) the previous day. The actual foods consumed are described, and information on portion sizes or weights is obtained. Description of type of foods, cooking method and ingredients (including salt and salty condiments) needs to be provided.	It has the advantage of its speed and ease of administration. Low participant burden. The 24-hour recall is appropriate for measuring current diet in groups of subjects and is therefore well suited to studies where differences between group means will be assessed. The 24-hour recall may be administered by telephone, by face-to-face interview, or web-based.	Retrospective method. Subject to memory bias resulting in under-reporting and interpretation errors. It does not provide a reliable estimation of intake because of day-to-day variation. Interviewers must be adequately trained in the technique to obtain complete information.
Food frequency questionnaires(62, 74-76)	Questionnaires for the assessment of habitual food intake based on a predefined checklist of foods consumed over a set time period. Additional data on portion sizes may be collected by asking how often foods are used in terms of a specified unit/portion size (semiquantitative questionnaire).	Relatively easily administered. Assess habitual intake over a longer period, as compared to other dietary methods, which reduces problems associated with the high day-to-day variability of intake. Emerging sodium screening tools may provide quick estimates of sodium in clinical practice.	Precise quantification of daily intake is extremely difficult. Pre-defined questions may not capture all the significant sources of sodium and may be regionally specific, and not applicable to all dietary patterns (i.e., ethnic diets). Generally have a reduced ability to discriminate between food preparation methods or the form of foods (pre-prepared or frozen versus homemade), which may underestimate dietary sodium.

A3. Dietary sodium and cardiovascular morbidity/ mortality

Strong evidence has established a positive association between high levels of dietary Na intake and CVD and mortality (77, 78). However, in the last decade there is growing controversial evidence of a J-shaped association between Na intake and/ or excretion and CV events or deaths, suggesting that low or very low dietary Na intake may lead to increased CV risk (79-85).

Observational data on dietary Na and cardiovascular morbidity/ mortality: the J shape phenomenon

The last decades important evidence has been accumulated and established the notion that high levels of Na intake are associated with increased incidence of fatal and non-fatal CV events (21, 86-90). Nevertheless, a metanalysis of prospective cohort studies has reported non-significant associations between Na intake and incident fatal and non-fatal CVD, coronary heart disease and all-cause mortality (31). Additionally, the last decade a significant number of studies (82, 83, 85, 91-102) showed that not only high but also low levels of dietary Na intake are associated with increased CV risk. These studies (82, 83, 85, 91-102) suggested the presence of a J-shaped or inverse linear association between daily Na intake and CV mortality (82, 83, 85, 91-102), verifying early findings of the Alderman et al. study (103). The major studies that implicated low levels of dietary Na intake in CV morbidity/ mortality raising are presented in **Figure 3**. Two large meta-analyses published in 2014 and 2016 confirmed the J-shape hypothesis and suggested a safe range of Na intake between ~3.0-5.0 g/day (or 7.5-12.5 g of salt/ day), as intakes below or above this level raised the mortality level of about 1.12-1.34 times (79, 104).

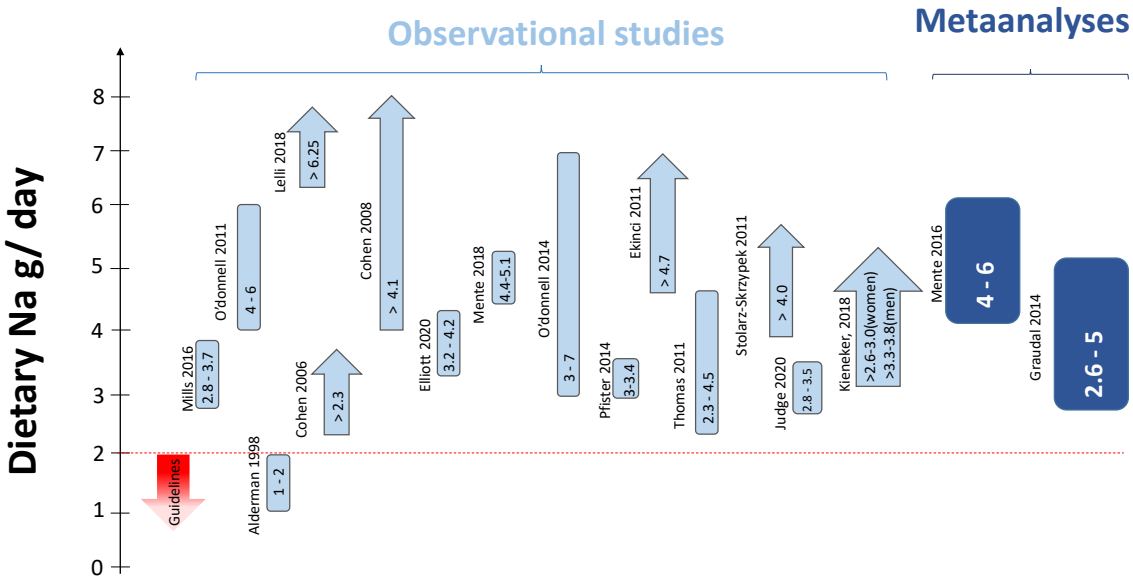

Figure 3: Major studies (light blue color) and metanalyses (dark blue color) showing J-shape or inverse association between Na intake and cardiovascular morbidity/mortality as well as all-cause mortality (79, 82, 83, 85, 91-104). Bars present the ranges showing lower risk, above and below which the risk increases. Arrows present the cut-offs above which the risk is lower. 1 gr of Na is equivalent to 2.5gr of salt. *Published in Journal of Hypertension* (40).

However, even among these studies, inconsistencies regarding the optimal range of Na intake are easily observed (**Figure 3**). All - but one (103) – of the above studies suggest that the J-shape curve starts at an unexpectedly "high" Na intake level, i.e. that CV risk increases even at daily Na intake above 3 or even 4 g/day (>7.5 or 10.0g of salt/ day). Of note, a careful dissection of several observational cohorts supporting the mainstream notion of the linear positive association between Na intake and CV mortality reveals that the range of dietary Na intake that have been compared are very high (exceeding the 5.5-6 g/day) versus moderate rather than low levels (e.g. <3-4 g/day) of Na intake (88, 90). It is also important to note that in all the available observational studies, only a very small amount of the sample present Na intake below the recommended level of 2g per day. According to the Prospective Urban Rural Epidemiology (PURE) study, which is a large epidemiological cohort that collected data from 664 communities, mean Na intake was 4.77g/ day with a range from 3.22g to 7.52 g per day, while in communities from China 80% presented intakes >5g/day (98). Finally, great heterogeneity exists between different cultures and communities since for the same e.g. low level of Na intake huge (even 3 fold) differences in CV events have been described (98), clearly suggesting that other major confounding factors (dietary or not; cultural or not) are present.

Taken all together this evidence raises several concerns about the optimal range of daily Na intake and the J-shape hypothesis cannot be disregarded.

Despite all the above presented evidence supportive of a J-shape or inverse association, current guidelines do not take them into consideration, as they only recommend a highest level of intake and not a lowest safety level for daily Na intake. This is in part due to the fact that a more careful analysis of all these studies reveals several issues regarding their methodology that they could possibly explain the inconsistencies regarding the optimal daily Na intake range. Indeed, according to the recent report of the Institute of Medicine on Na DRIs *"…the paradoxical J- and U-shaped relationships of sodium intake and cardiovascular disease and mortality are likely observed because of methodological limitations of the individual observational studies...*" (38). Studies reporting a J-shape trend between Na intake and CV events are characterized by high risk of bias according to the same organization (38). A state-of-the-art recording of all the methodological considerations regarding dietary Na and CVD has been conducted by Cobb et al. in 2014 (105). In the following paragraphs we will address some major limitations.

i) Observational findings on Na intake and CVD morbidity/ mortality derive from studies applying heterogeneous Na assessment methods, as presented in **Figure 4**. Moreover, as previously discussed the reliability of all Na assessment methods is questionable, due to low correlation rates and high levels of under- or over- recording. The analysis of He et al. in 2.974 participated in Trials of Hypertension Prevention (TOHP) could be a typical example (106). The authors evaluated the relationship between dietary Na and mortality via multiple 24UC and spot urine collections in the same sample. For the spot urine collection, they used the three most common equations (Tanaka, INTERSALT, Kawasaki) (44, 46, 49). The multiple 24UC led to a linear relationship between Na and mortality, suggesting that higher

Na intake increase risk of death. However, the use of spot urine equations altered the linear relationship to a J- or U- shaped curve, indicating that even the low intakes of Na increase the risk of death. Even though the results of this study should be confirmed by others, it strongly indicates the potential bias that is introduced in the association of mortality with Na intake due to measurement unreliability.

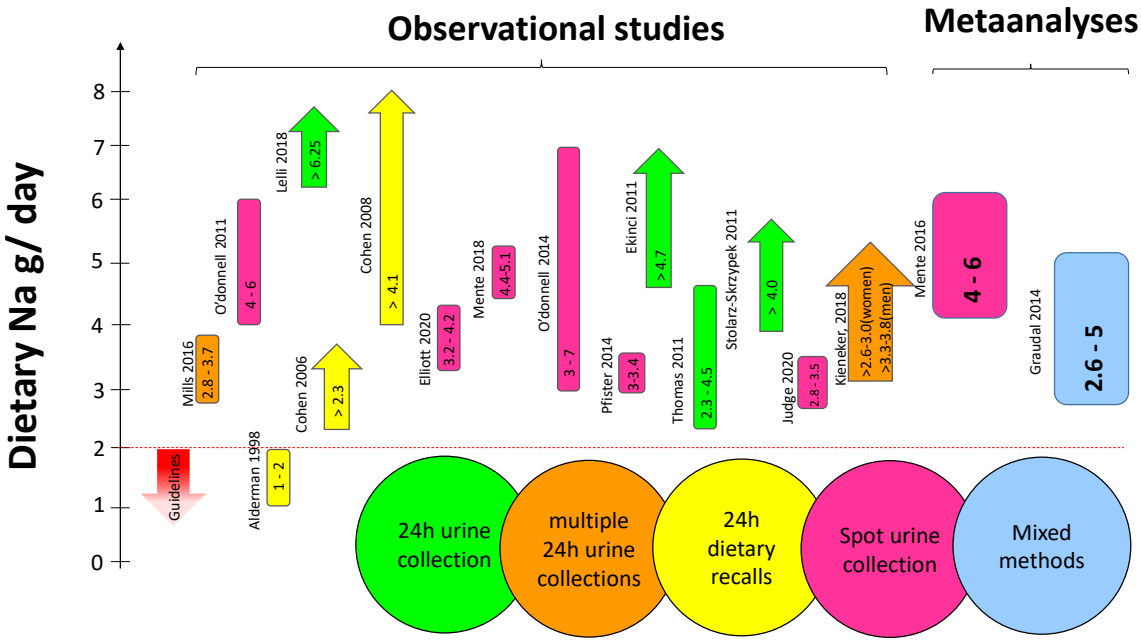

Figure 4: Methods used for the dietary Na assessment in studies showing J-shape or inverse association between Na intake and cardiovascular morbidity/mortality as well as all-cause mortality (79, 82, 83, 85, 91-104). Bars present the ranges showing lower risk, above and below which the risk increases. Arrows present the cut-offs above which the risk is lower. Green color = 24h urine collection; Orange color = multiple 24h urine collections; Yellow color = 24h dietary recall; Dark pink color = spot urine collection; light blue color = mixed methods. 1 gr of Na is equivalent to 2.5gr of salt. *Published in Journal of Hypertension (40).*

However, a close look in the schematic representation of these studies as labeled per Na methodology (**Figure 4**), reveals interesting information. At least 6 studies that applied at least 1 (85, 93, 94, 102) or even multiple (92, 101) assessments by the gold-standard 24UC methodology showed that lower Na excretion is associated with higher CV morbidity (92) and CV (85, 93) or all-cause (94, 102) mortality rates. It is therefore very likely that the bias introduction due to inaccurate Na assessment methodology cannot explain all the evidence in favor of a J-shape association.

ii) Another major limitation is related to the high heterogeneity of populations' characteristics included in the studies so far. Indeed, a significant number of those studies showing inverse or J-shape associations between Na intake and CV morbidity/mortality raising, included non-healthy volunteers (79, 82, 92-94) (**Figure 5**). Thus, a reverse-causality effect should be considered and could possibly explain the J-shaped curves. Strict medical or dietary advice in these high CV risk groups of people

could have led to reduced dietary Na intake. However, it must be highlighted that the majority of the data supporting the J-shape association derive from studies from healthy populations.

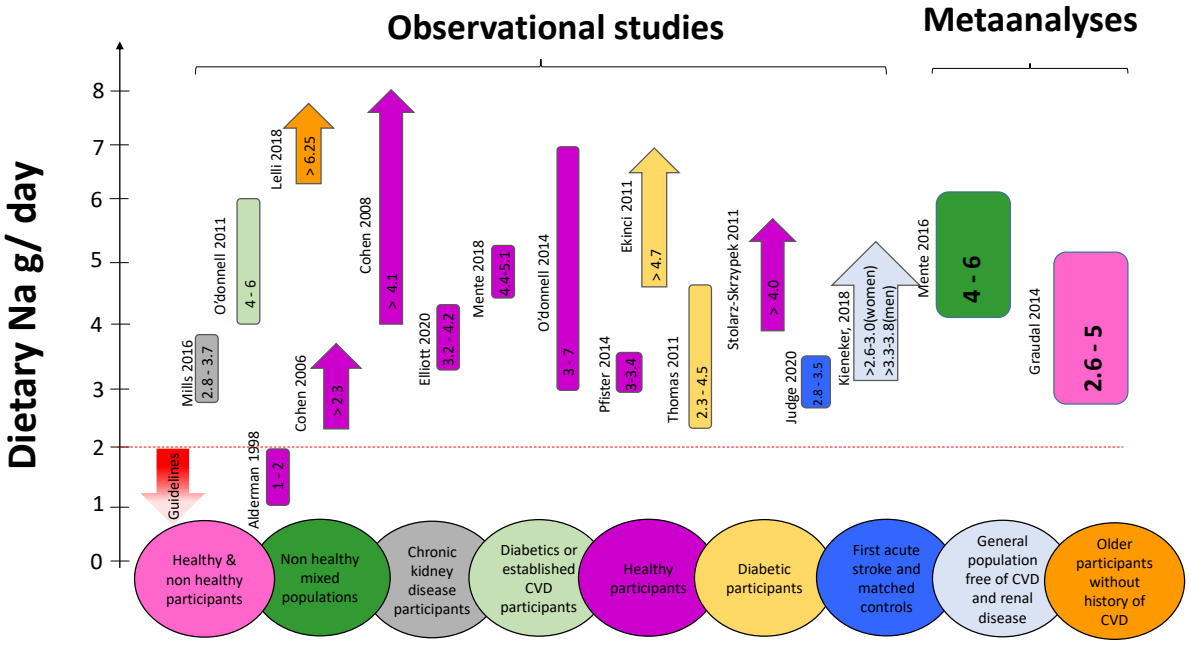

Figure 5: Population samples in studies showing J-shape or inverse association between Na intake and cardiovascular morbidity/mortality as well as all-cause mortality (79, 82, 83, 85, 91-104).Bars present the ranges showing lower risk, above and below which the risk increases. Arrows present the cut-offs above which the risk is lower. Pink color = healthy & non-healthy populations; Dark green color = non healthy mixed populations; Grey color = chronic kidney disease participants; light green color = Diabetics or established cardiovascular disease participants; Purple color = healthy participants; Yellow color = diabetic participants; Blue color = first acute stroke and matched control participants. 1 gr of Na is equivalent to 2.5gr of salt. *Published in Journal of Hypertension (40).*

Summing up the aforementioned methodological considerations, the two metanalyses supporting the J-shape curve between Na intake and CV morbidity/mortality (79, 104) - which represent the most robust evidence of the J-shape curve – present: a) heterogeneous or inaccurate Na assessment methods: Graudal et al. metanalysis, combined studies that used heterogeneous methods for dietary Na assessment to provide the J-shaped finding (104), while in Mente et al. meta-analysis (79) all studies used the spot urine method which is unreliable for dietary Na recording; b) high risk participants: both meta-analyses including high risk participants (79) or mixed healthy and non-healthy subjects (104). However, in a close review of figures 2 and 3, after excluding studies that applied methods other than the 24UC, and included non-healthy or high-risk participants, only three (85, 101, 102) remain to implicate low Na intakes in risk raising.

iii) Finally, none of the above-mentioned studies has addressed the potential confounding effect of salt sensitivity. This quite complex to define (107, 108) "*physiological trait"* (109) is accentuated in several specific population groups, including black race, hypertensives, older adults, chronic renal disease patients, women with preeclampsia history, or individuals with low weight at birth. Moreover, in a

setting of low potassium intake, even greater effect of salt on BP has been reported (110, 111). Therefore, the random inclusion of high such populations (92, 102) in observational studies, could modulate outcomes e.g. enhance the reverse-causality theory. Until now, no generally accepted method or easy to apply diagnostic tool for salt-sensitivity assessment exists, and despite its importance on studies evaluating the role of Na in health and disease, it has never been considered.

Prospective randomized clinical trials on dietary Na and cardiovascular morbidity/ mortality

Large scale well-designed prospective RCTs are known to be hierarchically more robust types of studies, having the ability to provide a state-of-the-art design and to overcome high risk of bias from which observational studies suffer. Thus, dietary guidelines are mainly based on strong evidence derived from metanalyses of prospective RCTs to avoid errors related to methods used, population selected and the reverse causality effect, although high quality prospective cohort studies are also usually included. The relationship between dietary Na and CV morbidity/mortality has been previously investigated in such studies that use gold-standard methodology for Na assessment (e.g. 24UC) and are conducted in low to medium CV risk - non acutely ill and apparently healthy - populations (e.g. older adults or hypertensive participants, without established CVD or chronic kidney disease etc.) (86, 112-115). Interventions in these studies include dietary Na reduction with long-term follow-up periods sufficient to provide fatal or non-fatal CV events (2.5-12 years) (86, 112-115).

A metanalysis of these studies conducted by the Agency for Healthcare Research and Quality for the IoM 2019 Dietary Reference Intakes for Na (38) found a 28% lower risk [95% CI: 0.59, 0.89] for incidence of CVD events after dietary Na reduction interventions, including 5 prospective RCTs (86, 112-115). The European Society of Hypertension and the European Society of Cardiology 2018 guidelines for the management of arterial hypertension (34), included two metanalyses of prospective RCTs investigating the association between dietary Na and CV morbidity, CV mortality or all-cause mortality (31, 116). The first one, by Taylor et al. (81) included 7 RCTs with a follow up period of at least 6 months; 3 conducted in normotensives (113, 117-120), 2 in hypertensives (121, 122), 1 in mixed normo- and hypertensives (114) and 1 in heart failure patients which were analyzed separately (123). When the longer-term RCTs were used for the metanalysis (113, 118, 120, 122), there was no strong evidence of benefit neither in mortality rates nor in CV morbidity in the reduced salt group relative to controls, for both normotensives (RR=0.90 for all-cause mortality, $p>0.05$; RR=0.71 for CV morbidity) and hypertensives (RR=0.96 for all-cause mortality, $p>0.05$; RR=0.84 for CV morbidity, $p>0.05$) (116). Indeed, most current guidelines and dietary reference values, agree that the relationship between dietary Na reduction and CV morbidity/mortality remains unclear because of a low or moderate evidence of benefit of such strategies to reduce CV risk or all-cause mortality (34, 36-38).

However, a major parameter to be considered is not only the effect of dietary Na reduction on CV events (fatal or non-fatal) but also the levels of Na reduction associated with the outcomes of interest.

According to the American Heart Association and the American College of Cardiology 2013 Guidelines, there is low strength evidence supporting that an approximately 1000mg reduction in Na intake per day decreases about 30% CV events (37). This evidence derives from three prospective RCTs. Chang et al. (79) studied 1981 older men; they reduced Na intake in the intervention group from 5200mg/day to 3800mg/day via substitution of Na with a potassium-enriched salt for 31 months. CV events decreased by 41% compared to the control group (114). A study conducted by Appel et al. (77) in 975 older hypertensive participants (TONE) showed a non-statistically significant reduction of CV events in the intervention versus the control group (36 versus 46 events), after reducing Na intake by 1000mg/day for 29 months (112). The observational follow-up of the prospective RCT TOHP study (3126 participants) concluded to a 30% reduction in relative risk for CV events in prehypertensive participants who reduced their dietary Na intake (~ - 800 mg/day after the initial intervention) during the 12-15 years of follow-up (113). Moreover, the WHO Guidelines for dietary Na intake in adults and children clearly addressed research questions to investigate the effects of different Na levels on adverse health outcomes. Three ranges of Na intake were evaluated: (i) less than 2g/day; (ii) 1.2-2 g/day; (iii) less than 1.2 g/day (36). However, the working group reported wide ranges of Na intake across the quartiles used in each study, with some studies with *"some consuming as little as 1.4 g/day in the lowest group and 2.6 g/day in the highest group, and others consuming as much as 4 g/day in the lowest group and 6.6 g/day in the highest group."* (36). It is important to note that very low levels of Na intake (about less than 1.5g/day) are difficult or even impossible to be achieved with the current dietary patterns and these observations of very low Na intakes possibly derive from biased methods or underreporting of Na intake. Moreover, limited data from RCTs were available and the metanalysis conducted lead to inconclusive findings (36). Indeed, despite the large body of evidence on RCTs comparing the effect of different levels of dietary Na on BP (112, 124-129), data for CV events and all-cause mortality remain limited and insufficient to address the specific question of the optimal daily Na intake range.

A4. Subclinical vascular damage

Subclinical vascular damage (SVD) -functional and structural- precedes the development of clinical symptoms and overt disease for several decades. Atheromatosis (arterial plaque formation), arteriosclerosis (arterial stiffening), endothelial dysfunction and arterial remodeling, are the major types of arterial damage leading to CVD. Although they share common risk factors, each one constitutes of a completely different pathology (130, 131). The early detection of SVD through appropriate diagnostic tools, methods, and biomarkers significantly contributes to primary and secondary CVD prevention. **Table 5** presents the most significant and widely used methods and non-invasive markers for SVD detection according to European Society of Cardiology (130).

Table 5: Usefulness of vascular biomarkers for primary and secondary cardiovascular disease prevention. *Adapted from Vlachopoulos C et al. (130).*

	Recommendation	Level of evidence	Comments
Carotid ultrasonography *cIMT* *plaque presence*	IIa	A	Moderate usefulness for risk stratification. Concomitant identification of plaque presence.
Ankle-brachial Index (ABI)	IIa	A	Useful for risk stratification, especially women.
Arterial Stiffness			
cfPWV	IIa	A	Useful for risk
baPWV	IIb	B	stratification
Central haemodynamics/ Wave reflections	IIb	B	
Endothelial function			
FMD	III	B	Requires skilled, trained operator. Reactive hyperaemia is stressful. Methodological problems are not resolved. Added value is not proven.
Endothelial peripheral arterial tonometry	III	C	Reactive hyperaemia is stressful. Added value is not proven.
Circulating biomarkers related to vascular wall biology			
High sensitivity CRP	IIb	B	

cIMT: carotid intima-media thickness; cfPWV: carotid-to-femoral pulse wave velocity; FMD: flow-mediated dilatation; CRP: C-reactive protein

Arterial remodeling

Remodeling is a complex process of the vessels in order to adapt in different pressure and flow stimuli and maintain the optimal blood flow and organ perfusion. Arterial remodeling processes include cell growth-death-migration, and the extracellular matrix synthesis and degradation as well (132). Arterial remodeling presents in distinct forms: (i) hypertrophic (increase in the cross-sectional area of the wall arteries); (ii) eutrophic (no changes in the cross-sectional area of the arteries); (iii) hypotrophic (decrease in the cross-sectional area of the arteries) (**Figure 6**).

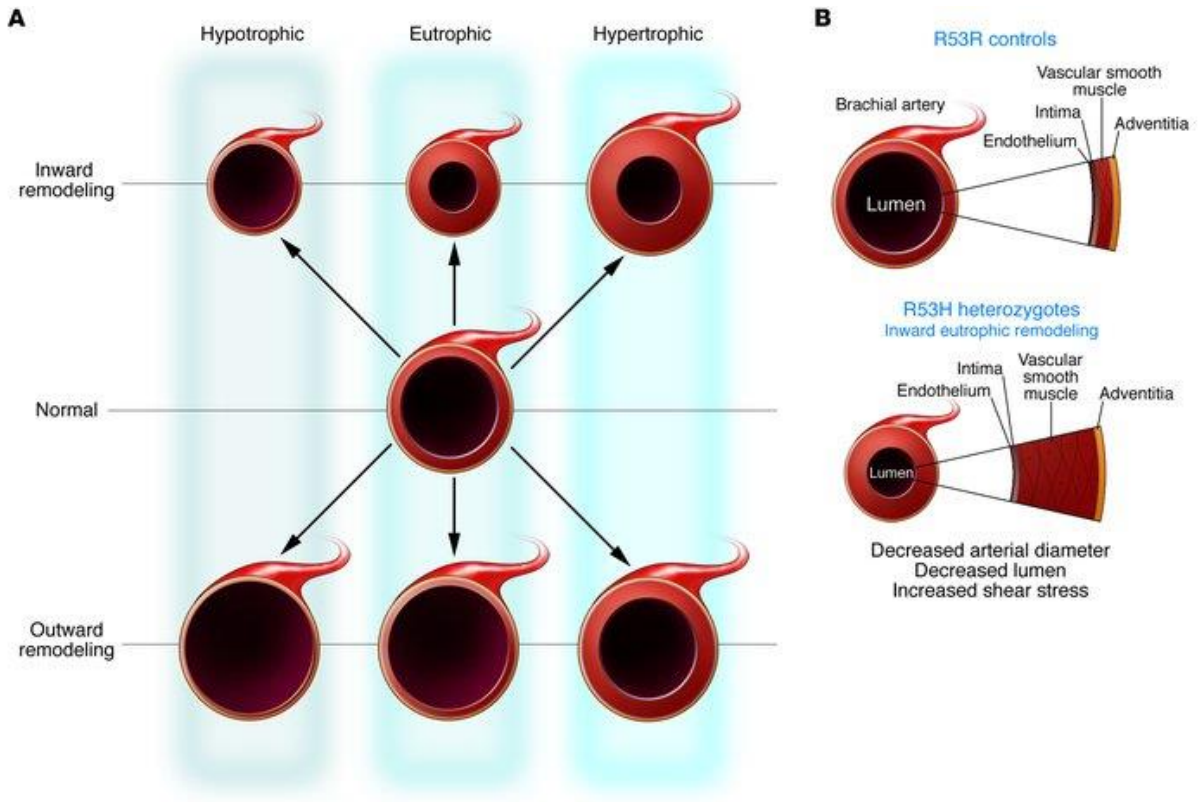

Figure 6: Arterial remodeling. *Adapted from Carretero OA (133).*

Moreover, all these changes can occur with concomitant increase or decrease of the lumen diameter respectively (134). In studies and in clinical practice as well, the alterations of the arterial wall are evaluated with intimal-medial thickness (IMT) of the common carotid artery. This biomarker is measured via ultrasonography and reflects alterations of artery wall thickness (130), while being independent risk factor for CVD.

Arterial Stiffness and pressure wave reflections

In normal conditions, the arterial wall expands and then passively returns to the initial condition in response to changes in blood pressure (BP) levels; this property is depending on arterial wall's characteristics (e.g. the elastin-to-collagen ratio) (135). Arterial stiffening is used to describe the decreased ability of the arterial wall to accommodate volume for a given BP level. It is usually assessed by the pulse wave velocity (PWV) between the carotid and the femoral artery (cfPWV) (**Figure 7**). This vascular biomarker is a useful tool for the early detection and prognosis of CVD (130) and its measurement is based usually on tonometry method, by estimating non-invasively the ratio of the distance between these two arterial sites and the time needed for the pulse wave to travel (**Figure 7**).

Except from the cfPWV, tonometry is also used for the measurement of the Augmentation Index (AIx). AIx is defined as *the difference between the first (P1) and second (P2) peaks of the central arterial waveform* (136) and it is expressed as the percentage of arterial augmentation from total pulse pressure (137) (**Figure 7**). Higher aortic AIx values are an indirect index of increased magnitude of pressure wave reflection from periphery or quicker return of the reflected pressure wave at the aortic level.

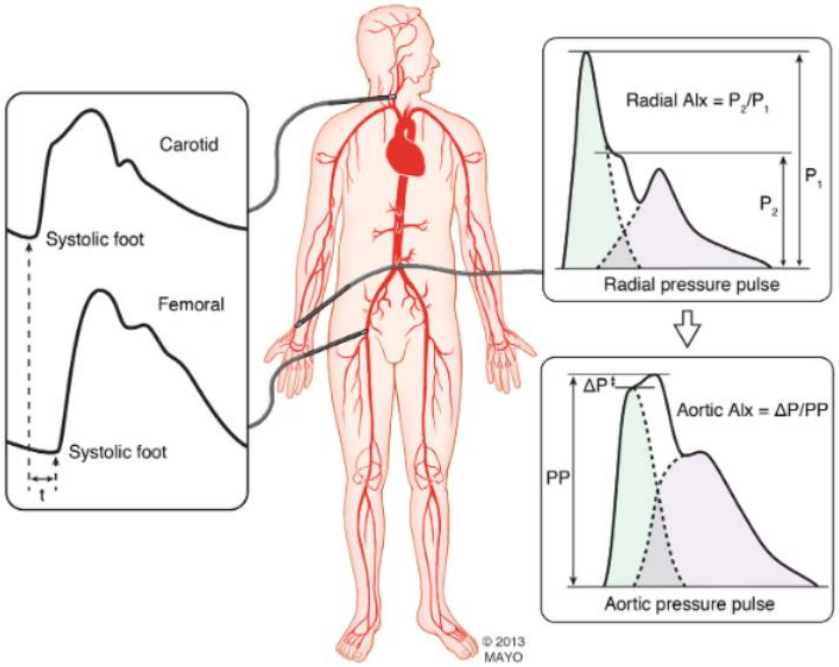

Figure 7: Carotid-femoral pulse wave velocity (cfPWV) and central arterial waveform assessment using arterial applanation tonometry. *Adapted from Coutinho T (138).*

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A5. Dietary sodium and subclinical vascular damage

It has been proposed that high Na intake impairs vascular function and induces atheromatosis (139, 140) as well as arteriosclerosis (141-147). However, there are limited yet intriguing inconsistencies indicating the presence of inverse associations between Na intake and arterial damage not only in human studies (148, 149), but particularly in animal studies, showing acceleration of atheromatosis in low salt diet (150-154). The detrimental effects of excess Na intake in CV system and specifically in vascular function and structure, occur through complex mechanisms, indirectly -e.g., as a result of increased BP- or even with direct action to the arterial structure and function. Below the relationship and possible mechanisms between major types of vascular damage and dietary Na are presented.

Several observational and/or interventional studies have tested the association of Na intake with types of SVD, but there are still many contradictory results and questions to be addressed (149, 155). Most data derive from studies investigating the relationship between Na and arterial stiffness or hypertrophy, suggesting that higher levels of Na intake are positively associated with these types of SVD (144, 156-159), although this has not been seen consistently (149, 160, 161). Studies regarding dietary Na and atheromatosis are scarce and are limited by major methodological issues (139, 140).

Sodium and endothelial dysfunction

Endothelial dysfunction has been linked to pathogenesis of atherosclerosis, being also a predictive factor of CVD (162). High dietary Na intake leads to important for the endothelium consequences, which are described below.

The high extracellular Na^+ concentrations reduce NO synthesis, leading to endothelial disjunction (163). Furthermore, high extracellular Na^+ concentrations increase ENaC, GAGs' network loses its regulatory effect, and endothelial glycocalyx is impaired. Thus, endothelial Na^+ uptake is facilitated stiffening the endothelial cytoskeleton (15, 162). Therefore, ENaC abundance determines endothelial glycocalyx's mechanical properties (**Figures 8, 9, 10**).

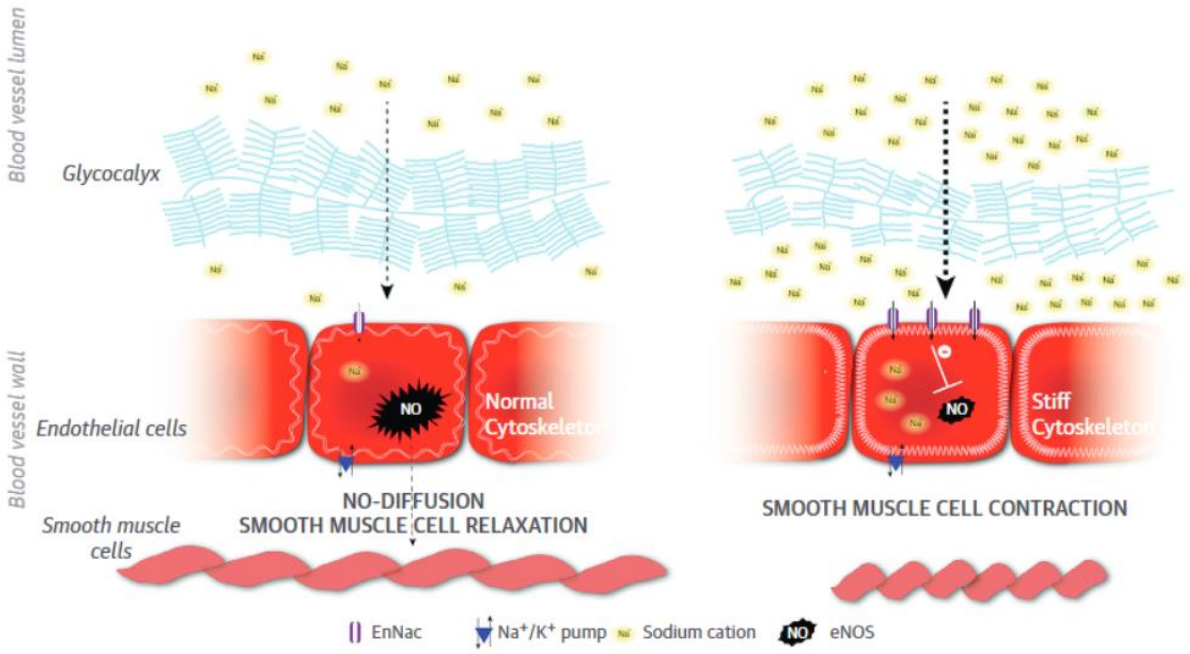

Figure 8: Endothelial dysfunction caused by high Na^+ concentration. *Adapted from Nijst P et al. (164).*
NOS: endothelial nitric oxide synthase; Na^+/K^+ pump: sodium-potassium adenosine triphosphatease pump; NO: nitric oxide

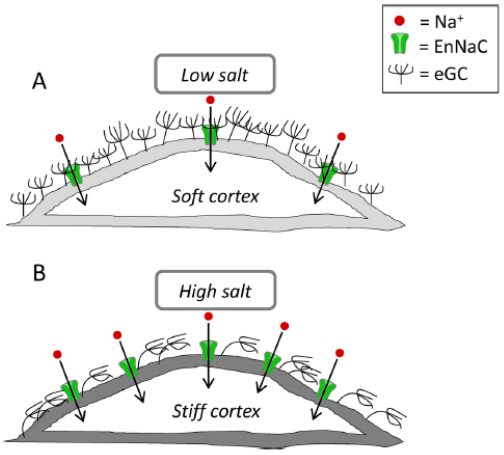

Figure 9: High salt induces increased EnNaC membrane abundance and cortical stiffness. *Adapted from Kusche-Vihrog K et al. (165).*
Na^+: sodium; EnNaC: epithelial Na^+ channels; eGC: endothelial glycocalyx.

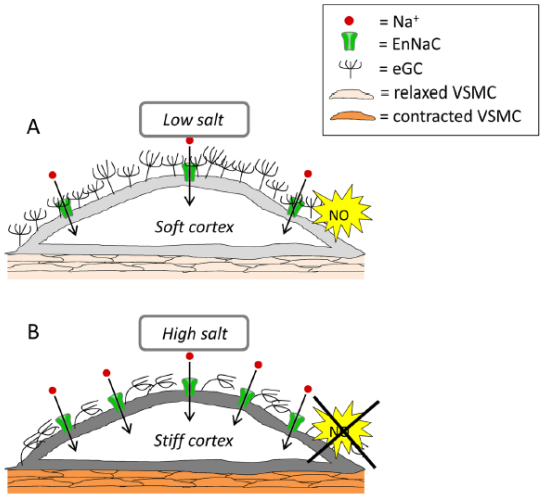

Figure 10: High salt deteriorates the endothelial glycocalyx. *Adapted from Kusche-Vihrog K et al.* (165).
Na^+: sodium; EnNaC: epithelial Na^+ channels; eGC: endothelial glycocalyx; VSMC: vascular smooth muscle cell.

Another consequence of high salt intake in endothelial function is the increased oxidative stress which contributes to endothelial dysfunction (162). Under high Na^+ concentrations, vessels lose their antioxidant capacity, due to superoxide dismutase (SOD) reduction which in normal conditions binds superoxide (166, 167). Superoxide anions are increased and reduce NO bioavailability (162, 168-170), therefore impairing endothelial function.

Regarding clinical trials assessing endothelial function, they suggest that either high dietary Na intake reduces FMD (171-174), or dietary Na restriction improves endothelial function (175-177). However, there are also a few reports indicating no association between Na intake and FMD (178) (**Table 6**).

Sodium and arterial remodeling

Evidence regarding dietary Na and arterial remodeling, as assessed by IMT, derives mostly from observational studies. A cross-sectional study conducted in 258 overweight and obese adults found that higher Na excretion through urine was associated with higher IMT values, after adjustment for age, gender, race and SBP (159). Moreover, in agreement with the previously mentioned findings, a cross-sectional analysis in 3290 subjects 40-75 years of age, showed that higher urine Na and Na/K ratio were associated with increased IMT (139). However, another study concluded to a J-shaped curve between Na (and K) intake and IMT (148), indicating that even very low Na levels could cause arterial wall remodeling. It is also important to note that findings remain conflicting, because of studies reporting no associations between dietary Na and IMT (179). More research is needed in this field to verify this relationship and explain possible mechanisms behind it.

Sodium and arterial stiffness

Arterial stiffness has been widely studied regarding dietary Na, not only in observational studies but in acute and chronic intervention studies too. Previous cross-sectional data reported that low Na intake is associated with lower PWV and therefore arterial stiffening, independent of BP (147). Most recent studies on hypertensives, found that dietary Na intake is positively associated with arterial stiffness (143, 146, 180), while an increase in Na intake for up to 6 weeks does not alter PWV on normotensives (161, 176, 181). However, in the case of normotensives, long-term effects of high levels of Na intake on PWV are not yet known (182).

Unmet Needs

Overall, the available evidence has failed to address the following issues:

(i) regarding dietary Na and SVD:

- the association between dietary Na intake and different types of SVD, especially for atheromatosis.

(ii) regarding dietary Na estimation:

- the development of an easy-to-use and simultaneously reliable tool for dietary Na estimation.
- the accurate quantification of discretionary Na intake.
- the estimation of dietary Na in a Greek population sample via different methods.

B. CLINICAL & EXPERIMENTAL DATA

B1. Objectives

The aims of this thesis are:

(i) record and quantification of dietary Na intake through all the available assessment methods;

(ii) comparison of all the examined Na assessment methods regarding their accuracy versus the gold-standard 24UC;

(iii) design and development of a new more accurate dietary tool to record Na intake in questionnaire form (NaFFQ) and improvement of the existing DMs;

(iv) investigation of the association between dietary Na intake and major types of SVD in our cohort study and

(v) investigation of the above-mentioned association through a systematic literature review.

More specifically, the aims of this thesis are to:

(i) quantify the amount of dietary Na consumed in a Greek sample consisted of medium-to-high CVD risk individuals through different DMs (e.g., multiple 24hDR and FFQ) and UMs (e.g. spot urine samples using different formulas 24UC];

(ii) correlate these methods with the gold-standard 24UC and improve the existing DMs in order to be more accurate in estimating the mean Na intake in population level;

(iii) design and develop a more accurate DM to record Na intake based on a previously validated FFQ, and to improve the existing dietary methods by trying to quantify the discretionary Na, which is not recorded in the existing methods;

(iv) test -by using very low dietary Na intake as reference group- if a moderate dietary Na intake is associated with lower prevalence of subclinical atheromatosis and decreased arterial stiffness, in a cohort of patients at increased CV risk under long-term medical guidance and free of CVD.

(v) evaluate –through a systematic literature review- data from observational and interventional studies in humans, investigating associations between dietary Na intake and SVD as well as the effect of Na intake on SVD-related changes.

B2. Methods

The present thesis consists of two distinct cohort studies, conducted in different periods. Methods are presented for each cohort study separately in the following sections.

B2.1 Methods for Cohort A

Study design and population

This is a cross-sectional analysis of data collected in a cohort study which was conducted from 2010 to 2016 at the Cardiovascular Research Laboratory, Medical School, Athens, Greece in consecutively consenting patients. The study population consisted of adults at moderate-to-high CV risk (suspected or established/ treated or untreated/ hypertension, diabetes mellitus, dyslipidemia, and/or chronic inflammatory diseases) (183). To focus on early stages of SVD, patients with established CVD were excluded (preexisting coronary artery disease, stroke, peripheral arterial disease, and/or documented arterial stenosis >50%). The study followed the World Medical Association Declaration of Helsinki and was approved by the ethical/scientific committee of "Laiko" Hospital. All participants gave written informed consent for their participation.

Assessment of anthropometric parameters

Weight was measured without shoes or heavy clothes to the nearest 0.1kg (Tanita Body Composition Analyzer, BC-418). Height was measured without shoes, with the participants standing with their shoulders relaxed, their arms hanging freely and their head in Frankfurt horizontal plane (SECA 213). Body mass index (BMI) was calculated as weight/ $(height)^2$ (kg/m^2).

Figure 11: *Tanita Body Composition Analyzer BC-418 & stadiometer 213 SECA*

Assessment of vascular and hemodynamic parameters

All participants were asked to refrain from food and any vasoactive substance or medication at least 3 hours before the vascular tests (184). Subclinical atheromatosis was defined as the presence of a plaque in either the carotid or the femoral artery in the absence of lumen stenosis >50%. High resolution B-mode ultrasound (Vivid 7 Pro, GE Healthcare, Chicago, Illinois, USA) with a 14-MHz multi-frequency linear transducer was used for the detection of plaques in 8 arterial sites (left and right common carotid artery, carotid bulb, internal carotid artery and femoral arteries). Atheromatic plaque was defined as local increase of the intima-media thickness of more than 50% compared to the adjacent vessel wall or more than 1.5 mm (130). All segments were identified in the transverse and/or longitudinal planes and scanned from multiple angles to optimize the detection of non-obstructive plaques. In the present analysis, carotid atheromatosis was defined as the presence of plaque in at least one out of the six overall measured carotid sites. Femoral atheromatosis was defined as the presence of at least one plaque either left or right.

Figure 12: High resolution B-mode ultrasound Vivid 7 Pro, GE Healthcare

Arterial stiffness was assessed by means of cfPWV using a common, validated tonometric method (Sphygmocor device; Atcor Medical, Sydney, Australia). The exact methodology has been previously described elsewhere (185). In brief, after at least 5 minutes of rest in the supine position, two different pulse waveforms were obtained, one at the right common carotid artery and the second at the right femoral artery using a high-fidelity handheld tonometer (SPT-301, Millar Instruments Inc. Houston, TX, USA). Carotid and femoral pulse wave sequences were synchronized by using the simultaneously recorded ECG. Pulse transit time from the carotid to the femoral artery was estimated by the tangential method, implemented by the SphygmoCor device. The cfPWV was calculated by the ratio of the distance traveled by the recorded pressure waves to the pulse transit time (in m/sec). The distance

was determined as the length from the suprasternal notch to femoral artery minus the length from carotid artery to the suprasternal notch. At least two repeated measurements of cfPWV were performed and their average value was used in the analysis in accordance with previous recommendations. Arteriosclerosis was defined as cfPWV values greater than 10 m/s (186).

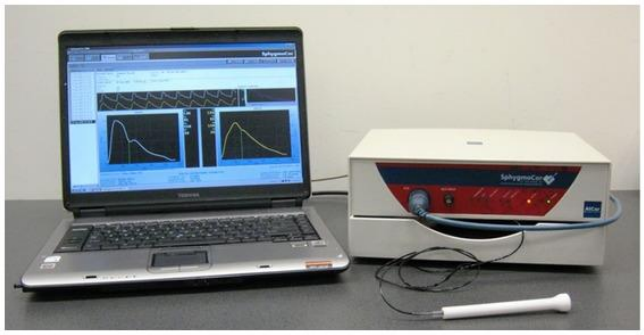

Figure 13: Sphygmocor device; Atcor Medical, Sydney, Australia

Assessment of dietary Na intake

Well-trained dietitians conducted two 24DR to all the participants by telephone interview, using the US Department of Agriculture multiple pass method (187). Participants were asked to report all the foods and beverages they consumed and their quantities in the previous 24 hours. Two 24DR were collected regarding one weekday and one weekend day, with at least 7-days interval between them. Collected dietary data were analyzed in macro- and micronutrients using appropriate software (Nutritionist Pro, version 5.2, Axxya Systems-Nutritionist Pro, Stafford, TX, USA). The Nutritionist Pro food database was expanded by adding analyses of traditional Greek foods and recipes.

Total daily Na intake was calculated using several procedures. Information from the 24DR was used to estimate Na intake from foods (processed foods and naturally occurring in unprocessed foods). Discretionary Na intake (i.e., added salt during cooking and table salt), which cannot be estimated by 24DR, was derived from literature review. In specific, major studies suggest that the discretionary salt represents around 15% of total salt intake in Europe and USA (29, 30, 57). Based on these published data we calculated the discretionary Na hypothesizing that Na from cooking and table is 15% of the total Na intake for our population. Finally, the total daily Na intake was derived as: the estimated Na intake from foods plus the discretionary Na. The overall statistical analysis was

performed using the total Na intake and then it was repeated using only the Na intake from foods (i.e., without adding the discretionary Na) to verify all the findings.

Foods from the 24DR were also grouped according to food equivalents -with further division if needed- (**Table 6**), and the servings consumed were recorded. Serving sizes derived from the food-based dietary guidelines for Greece to reflect portion sizes used by Greek population (188).

Table 6: Food groups and portion sizes.

Food groups	Food products	Portions
Dairy products		
Low fat dairy products	Milk, skimmed or semi-skimmed	1 glass (250ml)
	Evaporated milk, light	
	Soy milk	
	Yogurt, 0-2%	1 cup (180g)
Full fat dairy products	Cow milk, full fat	1 glass (250ml)
	Evaporated milk, full fat	
	Goat milk	
	Kefir	
	Yogurt, full fat	1 cup (180g)
Vegetables	Vegetables, raw	1 for medium-sized vegetables
		≥2 for smaller-sized vegetables
	Vegetables, cooked (boiled)	½ cup
Legumes	Legumes, cooked & drained	½ cup
Potatoes	Potatoes, boiled	1 small
	Potatoes, roasted	1 small (5-6 pieces)
	Potatoes, fried	4-5 pieces
	Potato purée	½ cup
Fruits		
Fruits and fresh juices	All fruits, raw & dried	Depends on fruit size (e.g., 1 cup of strawberries, ½ cup of berries)
	Fresh juices	½ cup
Processed juices	All types	½ cup
Grains		
Refined grains	Bread	1 slice, thin
	Pita bread	½ piece
	Pasta or rice, cooked	½ cup
	Cereals	½ cup
Whole meal grains	Bread, whole grain	1 slice, thin
	Pita bread, whole grain	½ piece
	Pasta or rice, whole grain, cooked	½ cup
	Breakfast cereals/oat, whole grain	½ cup

Food groups	Food products	Portions
Puff pastry	All kinds of sweet and savory puff pastry products	1 piece
Meat		
White meat	Chicken	60g
	Turkey	
	Duck	
	Rabbit	
Red meat	Beef	60g
	Pork	
	Lamb	
	Goat	
Cold cuts	All kinds of cold cuts	20-30g
Cheese		
Low fat cheese	Cottage, 0-4.5% fat	30g
	All cheeses <20% fat	30g
Full fat cheese	All cheeses >20% fat	30g
Fish		
Fish	All kinds of fresh and refrigerated fish	60g
Seafood	All kinds of seafood	60g
Egg	Egg, boiled or fried	1 small
	Omelette	½ restaurant portion
Fats		
Olive oil	Olive oil, raw	Times per day
Other unsaturated fats	Margarine	1 teaspoon
	Tahini	
	Peanut butter	
	Other vegetable butters from seeds or nuts	
Saturated fat	Butter	1 teaspoon
Olives	All kind of olives	5 large or 10 small
Sauces & Dressings		
Low fat dressings	Ketchup	1 tablespoon
	Mustard	
High fat dressings	Carbonara	1 tablespoon
	One thousand islands	
	Bechamel	
Nuts	All kinds of nuts	30g
Beverages		
Tea	All types of tea	1 cup
Coffee	All types of coffee	1 cup
Sweetened soft drinks	All types of soft drinks with sugar	1 glass (250ml)
Unsweetened soft drinks	All types of soft drinks without sugar	1 glass (250 ml)
Sweets/Desserts		
No fat sweets	Ice cream, 0%	1 piece
	Sorbet	
	Fruit jelly	1 cup
	Stewed fruit compote	½ cup
	Spoon sweets	1 teaspoon
High fat-high sugar sweets	Ice cream, full fat	1 piece
	Syrup sweets	1 small piece
	Chocolate, all kinds	6 squares
	Chocolate beverage	1 cup
	Bakery/pastry desserts	1 small piece

Food groups	Food products	Portions
Sugar	All types (white, brown, black)	1 teaspoon
Other sweeteners	Fructose Honey Sugar substitutes	1 teaspoon
Savory snacks	Potato chips Crackers Pop corn	1 cup
Alcohol		
High alcohol drinks	All kinds of high alcohol drinks	30 ml
Low alcohol drinks	Bottled low alcohol drinks	1 bottle 330ml
Wine	All types (including sparkling wine)	1 glass of wine 125ml
Fast food	Burger	1 medium-sized piece
	Hot dog Pizza	1 small piece

Assessment and definition of cardiovascular disease risk factors

Hypertension was defined as the use of antihypertensive drugs and/or office BP measurement higher than 139 and/or 89 mmHg (average of three sequential readings with 1-min interval in the supine position after at least 10 min of rest; appropriate cuff size use; Microlife WatchBP Office, Microlife AG, Widnau, Switzerland).

Figure 14: Microlife WatchBP Pro

Dyslipidemia was defined as the use of lipid-modifying drugs and/or low-density lipoprotein cholesterol level higher than 160 mg/dl. Diabetes was defined as glucose higher than 126 mg/dl or HbA1c higher than 6.5% and/or glucose-lowering treatment. Smoking was defined using at least one cigarette per day, each day of the week.

Statistical analysis

Statistical analysis was performed using the SPSS statistical package (IBM Corp. Released 2017, Armonk, NY: IBM Corp.). Distribution normality of the variables was tested using the Kolmogorov-Smirnov test and histograms. Given the fact that non-linear association between Na intake and CV mortality is suggested by the literature (79, 80, 82, 85, 93) we used a non-linear statistical approach to test our hypothesis. The possible interaction between quartiles of total Na intake and sex with atheromatosis (total plaques, carotid plaques, femoral plaques) and arterial stiffness [PWV>10m/s, based on the international guidelines (186)] was tested; due to missing values in plaques and PWV the quartiles were calculated separately for each end point. Multiple logistic regression analysis was performed to determine the relationship of total Na intake quartiles (defined separately for each sex) with atheromatosis and arterial stiffness, while adjusting for potential covariates. The adjustments for confounding factors are described in the following models: model 1: age, sex; model 2: model 1 plus BMI, presence of hypertension, smoking, diabetes, dyslipidemia; model 3a: model 2 plus energy intake (kcal); model 3b: model 2 plus potassium intake; model 4: model 2 plus energy and potassium intake. Other potential confounders such as hypertension drugs, dyslipidemia drugs, diabetes drugs, antiplatelet drugs, cortisone, and chronic inflammatory diseases duration were also tested in separate models in addition to the previous models. The results are presented as Exp. B (95% confidence intervals - CI). The level of statistical significance was set at $p<0.05$. Sensitivity analyses were performed by repeating all the above analysis: (i) using quartiles for the estimated Na intake derived from foods (processed foods and naturally occurring Na in unprocessed foods), instead of the total Na intake, (ii) for the subgroup of the population free of any chronic inflammatory disease, (iii) for the subgroup of the population who probably had not taken strict guidance for Na restriction (subjects not taking cortisone and subjects without hypertension diagnosed for more than 1 year).

B2.2 Methods for Cohort B

Study design and population

A cross-sectional study was performed from January 2017 until October 2018. The study population consisted of consecutive and consenting to participate individuals at high CVD risk due to the presence of CVD risk factors (suspected or established treated or untreated hypertension, dyslipidemia, diabetes mellitus, and/or chronic inflammatory diseases). In order to detect a minimum difference of 500 mg in daily Na intake between each Na estimation method and the 24UNa (alpha=0.05, power=0.80), the minimum sample size for each pair of methods was calculated (n=60) (189, 190). To account for attrition (non-participation, missing data or incomplete 24UC), which was estimated to be 50%, 120 individuals were invited to participate. The study was approved by the ethical/scientific committee. All participants provided informed consent and underwent dietary and urinary assessment simultaneously, which was completed within one month.

Assessment of dietary Na intake

Dietary Na intake was assessed by DMs, improved DMs, and UMs, as described below.

24h urine collection: Participants were asked to keep one 24UC following written and verbal instructions and a standardized protocol. The instructions were to carry out the collections from Sunday awakening and for the next 24 hours, discarding the first morning void without a) missing voids and b) any changes in their diet or medicine (the past 1 month). To verify completeness, sensitivity analyses were conducted after applying all available criteria for 24h urine completeness (43, 191-193) (Statistical analysis section). Na derived from the 24UC was calculated using the following equation:

24UNa (mg/day) = 24h Na concentration (mmol/L) x 24h urine volume (L) x molecular weight of Na (23 mg/mmol)

Spot urine: Participants were also asked to keep a single spot urine sample of the first morning void in a proper bottle. To estimate the 24UNa from spot urine specimens the most common conversion equations were applied (44-47, 49) (**Table 3**).

24h dietary recalls: Three 24DR using multiple-pass method were conducted (two weekdays and one weekend day with a 7-day interval) by well-trained dietitians via telephone or face-to-face interviews. Participants were asked to report all the foods and beverages they consumed and their quantities in the previous 24 hours. With the use of relevant nutrient analysis software (Nutritionist Pro, version 5.2, Axxya Systems-Nutritionist Pro, Stafford, TX, USA), food data from the 24DR were analyzed in terms of macronutrient and micronutrient intake. The average Na intake of the three days was used. If less than three 24DR were available, the average of the rest was used.

Food frequency questionnaire: The 1st week of the dietary assessment, all participants were asked to complete a semi-quantitative FFQ which is repeatable and valid for nutritional assessment regarding energy and macronutrients (194). The FFQ consisted of a list of 69 main food groups (i.e., cereals and starchy foods, fruits, vegetables, dairy products, meat, fish, legumes, added fats, sweets, alcoholic beverages) as well as questions related to dietary behaviors and habits (194). Participants were asked to report the frequency of the consumption of these food groups in the last month on a 6-grade scale (from never/rarely to more than 2 times per day) in pre-specified amounts of food expressed in grams, mL or other common measures. More details for FFQ development have been previously described (194, 195).

Daily food consumption was calculated as:

Daily food consumption = serving size x consumption frequency

, where consumption frequency was: never = 0; 1-3 times/month=0.07; 1-2 times/week=0.21; 3-6 times/week= 0.64; 1 time/day=1; ≥2 times/day=2.

The Na estimation for each food group was calculated as:

Daily consumption of food x Na content of food

derived from United States Department of Agriculture (USDA) and local food composition tables (18, 19, 196).

Improved 24h dietary recalls: 24DR plus discretionary salt questions (24DR+SQ): To estimate discretionary salt, participants were asked to answer two salt-related questions separately for breakfast, lunch and dinner for each one of the 24DR:

Question 1: How much salt did you use during the preparation of your meal?
a=none, b=a little, c=moderate, d=a lot

Question 2: Did you add extra salt on your plate (table salt)?
a=no, b=yes.

For Question 1, the following Na quantities were applied for each answer: a = none = 0 mg of Na, b = a little = 50 mg of Na per 100 gr of food, c = moderate = 350 mg of Na per 100 gr of food, d = a lot = 600 mg of Na per 100 gr of food. These estimations were based on relevant statements/assessments from the Hellenic Food Authority (EFET) (197): *"If a food contains more than 0.6g of sodium (or 1.5g of salt) per 100g, then it is high in sodium/ salt. If a food contains 0.1g of sodium or less per 100g then it is low in sodium/salt. If the amount of salt per 100g is between these values, then the food contains a medium level of salt".* Portion sizes from the 24DR were calculated in grams based on food equivalents and local food composition tables (196).

For question 2, the answer "yes" was defined as 2 dashes of salt, which are equivalent to 775mg of Na (19) and when the answer was "no", no Na (0 mg) was added. The mean Na derived from questions 1 and 2 was then added to the Na derived from the 24DR and was calculated as:

24DR+SQ = Na from the 24DR + mean Na from breakfast (question 1) + mean Na from lunch (question 1) + mean Na from dinner (question 1) + mean Na from breakfast (question 2) + mean Na from lunch (question 2) + mean Na from dinner (question 2)

The Na of the meals (breakfast, lunch, dinner) was calculated based on the estimations of Na intake from questions 1 and 2 (average from the three 24DR).

Improved 24h dietary recalls 24DR plus 15% (24DR+15%): An alternative way to estimate discretionary use of salt was applied. We calculated the discretionary Na based on the assumption that Na from cooking and table is 15% of the total Na intake for our population), as previously reported (29, 30, 57). In specific, total Na intake was then calculated as:

24DR+15% = Na from the 24DR+ (15% of Na from the 24DR)

New sodium assessment tool: Sodium Food Frequency Questionnaire (NaFFQ): In order to improve Na estimation, the food list of the previously mentioned FFQ was extended with food items rich in Na and questions regarding dietary behaviors related to discretionary use of salt (NaFFQ). The added food groups and questions are presented in **Table 7**. The foods items added were salted butter and margarine, several rich in Na cheeses (e.g. roquefort, parmesan, edam, gouda, gruyere etc.), salty crackers/biscuits, canned fish/seafood and refined tomato juice.

Table 7: Food Items and questions added in the existing FFQ. *Published in Public Health Nutrition, 2022 (56).*

Food items added	***Consumption Frequency***
In cheese food group:	
roquefort, blue cheese, 30 g	never/ rarely, 1-3 t/mo, 1-2 t/wk, 3-6 t/wk, 1 t/d, ≥2 t/d
parmesan, 30 g	never/ rarely, 1-3 t/mo, 1-2 t/wk, 3-6 t/wk, 1 t/d, ≥2 t/d
edam, gouda, 30 g	never/ rarely, 1-3 t/mo, 1-2 t/wk, 3-6 t/wk, 1 t/d, ≥2 t/d
gruyere, 30 g	never/ rarely, 1-3 t/mo, 1-2 t/wk, 3-6 t/wk, 1 t/d, ≥2 t/d
cream cheese full fat, 30 g	never/ rarely, 1-3 t/mo, 1-2 t/wk, 3-6 t/wk, 1 t/d, ≥2 t/d
feta cheese and goat cheese, 30 g	never/ rarely, 1-3 t/mo, 1-2 t/wk, 3-6 t/wk, 1 t/d, ≥2 t/d
mozzarella, 30g	never/ rarely, 1-3 t/mo, 1-2 t/wk, 3-6 t/wk, 1 t/d, ≥2 t/d
anthotiro (traditional Greek white cheese), 30 g	never/ rarely, 1-3 t/mo, 1-2 t/wk, 3-6 t/wk, 1 t/d, ≥2 t/d
In cereals and starchy foods food group:	
salty crackers and biscuits, 2 thin pieces (20 g)	never/ rarely, 1-3 t/mo, 1-2 t/wk, 3-6 t/wk, 1 t/d, ≥2 t/d
In meat food group:	
gyros pork, 150 g	never/ rarely, 1-3 t/mo, 1-2 t/wk, 3-6 t/wk, 1 t/d, ≥2 t/d
In fish food group:	
canned or corned fish, 30 g	never/ rarely, 1-3 t/mo, 1-2 t/wk, 3-6 t/wk, 1 t/d, ≥2 t/d
canned seafood, 30 g	never/ rarely, 1-3 t/mo, 1-2 t/wk, 3-6 t/wk, 1 t/d, ≥2 t/d
In nuts and seeds food group:	
salted nuts and seeds, 1 coffee cup (50 g)	never/ rarely, 1-3 t/mo, 1-2 t/wk, 3-6 t/wk, 1 t/d, ≥2 t/d
In margarine food group:	
salted margarine, 1 tsp (5 g)	never/ rarely, 1-3 t/mo, 1-2 t/wk, 3-6 t/wk, 1 t/d, ≥2 t/d
In butter food group:	
salted butter, 1 tsp (5 g)	never/ rarely, 1-3 t/mo, 1-2 t/wk, 3-6 t/wk, 1 t/d, ≥2 t/d
In cooked meals usually prepared with tomato juice,	
refined tomato juice	*added in the recipe*
4 tablespoons (60 g) for:	
pasta/ pearl barley 1 cup (140 g)	
whole meal pasta 1 cup (140 g)	
veal (150g)	
petit pois (peas), green beans, okra, artichoke (200 g)	
2 tablespoons (30 g) for:	
pastitsio/ mousakas/ papoutsakia (	
Traditional greek dishes), 1 portion (140 g)	
Dietary behaviors related to salt	
a) Which kind of salt do you use?	----------
b) How much salt do you use in your cooked meals and salads?	none, a little, moderate amount, much, very much
c) How often do you use table salt in your meals?	never, rarely, often, always
d) Do you believe that salt can cause health problems?	yes, no, I don't know
e) The amount of salt you eat in your cooked meals is:	I don consume any salt, a little, moderate, a lot
f) The amount of salt you eat in your salads is:	I don consume any salt, a little, moderate, a lot
g) Do you check food labels for salt content?	yes, no
h) Do you buy foods indicating in their package "with less salt"?	yes, no
i) Do you take any measures to control salt intake?	yes, no
j) Do you know if there are recommendations regarding upper limits of daily salt intake?	yes, no, I don't know

g: grams; t: times; mo: month; wk;: week; d: day; tsp: teaspoon

To estimate Na added in cooked meals and salads, Question b of the NaFFQ (*How much salt do you use in your cooked meals and salads?*, **Table 7**) was used, according to Hellenic Food Authority (EFET) (197) as mentioned above. Participants' answers were calculated as: none=0mg Na, a little=50mg Na/100g of food, moderate amount=350mg Na/ 100g of food, much=600mg Na/100g of food, very much=900mg Na/100g of food. Then the quantified Na derived from participants' response in Question b was added to each cooked meal and salad per 100gr of food of the NaFFQ. Na was then calculated as:

NaFFQ = Na from the existing FFQ + Na from food items added rich in Na + Na added in cooked meals and salads

Cooked meals included rice, potatoes, red and white meat, fish & seafood, legumes, traditional dishes, and home-made pies. Salads included all vegetables, raw or boiled.

Assessment of anthropometric parameters

Protocol as described for Cohort A.

Assessment and definition of CVD risk factors

Protocol as described for Cohort A.

Statistical analysis

All the analyses were conducted using SPSS version 25 (IBM Corp. Released 2017, Armonk, NY: IBM Corp.). Continuous variables are presented as mean ± one standard deviation (SD) and categorical variables as absolute frequency and percentage (%). Significance levels were set at p-value < 0.05. Distribution normality of the variables was tested using the Kolmogorov-Smirnov test and histograms. The differences between methods (bias of mean values) were calculated as 24UNa minus the Na measures of the other DMs and UMs. Paired samples t-test and Wilcoxon test, when appropriate, were used to determine the significance of differences of mean values of Na. To assess the correlation between 24UC and the other Na estimation methods, the Pearson's correlation

coefficient (for normally distributed variables) and Spearman correlation coefficient (for variables not normally distributed) were applied. Consistency between different methods of Na estimation was also assessed with the intraclass correlation coefficient (ICC) (198). It is generally accepted that there is no absolute interpretation of ICC values. However, in the present study we used the recommendation of Koo and Li (199); accordingly ICC values less than 0.5 are indicative of poor reliability, ICC between 0.5 and 0.75 indicate moderate reliability, ICC between 0.75 and 0.9 indicate good reliability, and ICC values greater than 0.90 indicate excellent reliability.

Bland-Altman plots were used to evaluate differences between Na estimation methods and the 24UC and evaluate the agreement between them (200, 201). The upper and lower limits of agreement between two different estimates of Na were calculated by the mean difference ± 1.96 x SD of differences. Linear regression analysis was used to evaluate associations in difference and mean (between 24UC and each Na estimation method). The analyses regarding correlations between 24UC and each Na estimation method and ICCs as well, were repeated after excluding all subjects having incomplete 24UC (sensitivity analysis). The exclusion criteria for incomplete 24UC were set according to international bibliography (43, 191-193) and are presented in **Table 8.**

Table 8: Exclusion criteria for incomplete 24h urine collections. *Published in Public Health Nutrition, 2022 (56).*

Incomplete 24h urine collection if:
1. Urine volume <500mL
2. Urine creatinine <6mmol/day plus 24h urine volume <1000mL or urine creatinine <5mmol/day (191)
3. $\frac{urine\ creatinine\ (\frac{mg}{day})}{body\ weight\ (kg)} < 10.8\ or > 25.2$ (193)
4. $Males$: $\frac{urine\ creatinine\ (\frac{mg}{day})}{body\ weight\ (kg)} < 14\ or > 26$, $Females$: $\frac{urine\ creatinine\ (\frac{mg}{day})}{body\ weight\ (kg)} < 11\ or > 20$ (192)
5. $Males$: $\frac{urine\ creatinine\ (\frac{mg}{day})}{24\ x\ body\ weight\ (kg)} < 0.6$, $Females$: $\frac{urine\ creatinine\ (\frac{mg}{day})}{21\ x\ body\ weight\ (kg)} < 0.6$ (43)

B2.3 Methods for the systematic literature review

The present systematic review was prepared and reported in accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (202) (**Table 9**).

Table 9: PRISMA checklist.

TITLE			*Pages*
Title	1	Identify the report as a systematic review, meta-analysis, or both.	Available in the published paper
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	Available in the published paper
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	Available in the published paper
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	41
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., web address), and, if available, provide registration information including registration number.	Not applicable
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	57, 58
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	57
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	57, 58
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	57, 58
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	57, 58
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	57, 58
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	Not applicable
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	-
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	Not applicable
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	Not applicable
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	Not applicable
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	73

Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	74-83
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Not applicable
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Not applicable
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	Not applicable
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	Not applicable
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	Not applicable
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	98
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	101
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	102, 103
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	5

Search Strategy

A systematic search of potentially relevant studies was performed through July 2019 by two separate reviewers on the PUBMED and SCOPUS databases. Search terms applied were: ((“sodium intake” or “na intake” or “na+ intake” or “sodium excretion” or “na excretion” or “na+ excretion” or “dietary sodium” or “dietary na” or “dietary na+” or “urinary sodium” or “urinary na” or “urinary na+”)) and (“arterial function” or “vascular function” or “arterial structure” or “vascular structure” or plaque or atheroma or “atheromatic plaque” or “atherosclerotic plaque” or atheromatosis or atherosclerosis or arteriosclerosis or “arterial remodeling” or “carotid plaque” or “femoral plaque” or “arterial stiffness” or “arterial stiffening” or “pulse wave velocity” or pwv or “intimal medial thickness” or “intima media thickness” or IMT or “wall to lumen ratio”). Studies were limited to the English language and human studies. Reference lists of included articles were also examined for additional relevant articles.

Inclusion and Exclusion Criteria

The following inclusion criteria were applied: relevant epidemiological studies or clinical trials, English language, human studies, males and/or females of any age regardless of diseases (chronic or acute), clearly described outcome defined as: association between Na intake and/or excretion with atheromatosis (presence of plaques), arteriosclerosis (any accepted biomarker of arterial stiffening at any arterial segment) or arterial remodeling (arterial hypertrophy (IMT) or artery lumen diameters). The following exclusion criteria were applied: epidemiological studies with a sample <100 subjects,

animal studies, reviews, systematic reviews, meta-analyses, comments/letters, studies using the assessment of Na intake and/or excretion of biomarkers other than Na (e.g., the ratio Na/K).

Selection of Studies and Data Extraction

Two reviewers screened the available titles, abstracts, and keywords of all the available articles. Discrepancies were resolved after discussion. After agreement, full text screening was carried out. Qualitative and quantitative data from all included articles were extracted by both reviewers. The extracted data included specific details for study design, population characteristics, Na estimation method and outcomes related to Na and vascular damage. All units of Na are presented as mg (converted from mmol to mg, if necessary). Predefined variables were extracted.

B3. Results

B3.1 Results from Cohort A

The following results have been published in Hellenic Journal of Cardiology, 2021 (203).

Investigation of the association between dietary Na and subclinical vascular damage

A total population of 901 adults (407 men and 494 women) with dietary and atheromatosis data was analyzed. However, data for the arterial stiffness analysis were available for 886 participants (386 men and 500 women) and therefore separate Na intake quartiles were generated for the arterial stiffness dataset.

Regarding the atheromatosis analysis, a significant interaction between sex and quartiles of total Na intake was observed ($p<0.001$ for carotid and/or femoral plaques, $p=0.002$ for carotid plaques, and $p<0.001$ for femoral plaques). Consequently, the results are presented separately for males and females. No interaction between total Na intake quartiles and sex regarding their association with arterial stiffness was revealed ($p=0.522$) and the results for this specific analysis are presented for the total population.

Descriptive characteristics of study population are presented by sex regarding the analysis for atheromatosis and for the total population regarding the analysis for arterial stiffness (**Table 10**).

Table 10: Descriptive characteristics of participants. *Published in Hellenic Journal of Cardiology, 2021 (203).*

Population with available data for atheromatosis	**MALES**					**FEMALES**				
		Quartiles of total Na intake					Quartiles of total Na intake			
	Total *(n=407)*	**1st** 1088.1±277.8 mg *(n=101)*	**2nd** 1823.5±235.1 mg *(n=104)*	**3rd** 2596.1±264.3 mg *(n=101)*	**4th** 4281.1±1074.3 mg *(n=101)*	**Total** *(n=494)*	**1st** 751.0±215.5 mg *(n=123)*	**2nd** 1239.3±128.3 mg *(n=124)*	**3rd** 1743.9±154.6 mg *(n=124)*	**4th** 2731.0±631.9 mg *(n=123)*
	Mean ± S.D.	**Mean ± S.D.**	**Mean ± S.D.**	**Mean ± S.D.**	**Mean ± S.D.**	**Mean ± S.D.**	**Mean ± S.D.**	**Mean ± S.D.**	**Mean ± S.D.**	**Mean ± S.D.**
Age, years	50.6±13.1	54.1±12.6	52.9±13.4	49.0±13.8	46.3±11.2	53.9±14.2	55.0±14.2	55.4±15.2	54.7±13.3	50.5±13.7
Weight, kg	85.6±15.4	84.2±13.4	86.5±13.5	85.3±18.4	87.7±15.7	71.7±16.4	71.8±14.6	71.8±19.0	72.6±15.7	70.6±16.2
Height, m	1.76±0.07	1.74±0.06	1.75±0.06	1.75±0.07	1.78±0.07	1.60±0.07	1.59±0.07	1.59±0.07	1.60±0.06	1.61±0.06
BMI, kg/m^2	27.9±4.7	27.8±4.1	28.3±4.0	27.8±5.8	27.7±4.7	28.0±6.1	28.3±5.6	28.3±6.8	28.4±6.0	27.1±6.1
Na intake from foods[a], mg/day	2076.2±1119.9	924.9±236.1	1550.0±199.9	2206.7±224.7	3638.9±913.2	1373.4±689.0	638.3±183.2	1053.4±109.1	1482.3±131.4	2321.4±537.1
Discretionary Na intake[b], mg/day	366.4±197.6	163.2 ± 41.7	273.5 ± 35.3	389.4±39.7	642.2±161.2	242.4±121.6	112.6±32.3	185.9±19.2	261.6±23.2	409.7±94.8
Total Na intake[c], mg/day	2442.6±1317.5	1088.1±277.8	1823.5±235.1	2596.1±264.3	4281.1±1074.3	1615.8±810.6	751.0±215.5	1239.3±128.3	1743.9±154.6	2731.0±631.9
Smoking, % (n)	42.0 (171)	38.6 (39)	38.5 (40)	45.5 (46)	45.5 (46)	30.4 (150)	28.5 (35)	30.6 (38)	28.2 (35)	34.1 (42)
Diabetes type 1, % (n)	6.1 (25)	3.0 (3)	8.7 (9)	9.9 (10)	3.0 (3)	9.3 (46)	5.7 (7)	5.6 (7)	14.5 (18)	11.4 (14)
Diabetes type 2, % (n)	10.6 (43)	16.8 (17)	12.5 (13)	5.9 (6)	6.9 (7)	12.6 (62)	13.0 (16)	13.7 (17)	13.7 (17)	9.8 (12)
Diabetes disease duration, years	12.7±10.0	11.6±8.4	13.2±9.7	14.0±13.4	12.0±8.5	12.4±9.6	11.7±9.2	11.1±9.0	11.1±9.6	16.4±10.2
Dyslipidemia, % (n)	36.1 (147)	38.6 (39)	47.1 (49)	29.7 (30)	28.7 (29)	37.4 (185)	41.5 (51)	43.5 (54)	34.7 (43)	30.1 (37)
Dyslipidemia disease duration, years	5.0±5.1	5.7±6.0	4.6±4.1	5.0±4.7	5.0±6.0	5.6±6.0	6.5±6.5	4.8±5.7	5.3±6.3	5.7±5.2
Hypertension, % (n)	45.2 (184)	53.5 (54)	53.8 (56)	41.6 (42)	31.7 (32)	44.9 (222)	48.0 (59)	49.2 (61)	45.2 (56)	37.4 (46)
Hypertension disease duration, years	6.3±7.2	6.5±6.8	7.6±7.3	5.4±7.5	4.9±7.0	7.9±7.6	7.6±7.4	8.8±9.1	6.7±6.8	8.9±6.7
Chronic inflammatory disease, % (n)	41.3 (168)	43.6 (44)	35.6 (37)	43.6 (44)	42.6 (43)	52.6 (260)	50.4 (62)	57.3 (71)	56.5 (70)	46.3 (57)
Chronic inflammatory diseases duration, years	11.7±10.3	12.6±11.6	11.7±11.2	12.5±9.8	9.9±8.8	12.1±9.3	12.2±9.1	12.4±10.3	11.3±8.6	12.8±9.4
Diabetes drugs, % (n)	15.7 (64)	19.8 (20)	18.3 (19)	15.8 (16)	8.9 (9)	20.4 (101)	17.1 (21)	18.5 (23)	25.8 (32)	20.3(25)
Dyslipidemia drugs, % (n)	29.0 (118)	32.7 (33)	38.5 (40)	22.8 (23)	21.8 (22)	27.9 (138)	33.3 (41)	33.1 (41)	21.8 (27)	23.6 (29)
Hypertension drugs, % (n)	36.6 (149)	40.6 (41)	48.1 (50)	32.7 (33)	24.8 (25)	40.3 (199)	40.7 (50)	44.4 (55)	40.3 (50)	35.8 (44)
Antiplatelet drugs, % (n)	11.5 (47)	12.9 (13)	14.4 (15)	10.9 (11)	7.9 (8)	16.6 (82)	22.8 (28)	16.9 (21)	16.9 (21)	9.8 (12)
Carotid and/or Femoral Plaques, % (n)	57.2 (233)	64.4 (65)	62.5 (65)	56.4 (57)	45.5 (46)	44.1 (218)	52.0 (64)	49.2 (61)	41.9 (52)	33.3 (41)
Carotid Plaques, % (n)	38.8 (158)	44.6 (45)	43.3 (45)	36.6 (37)	30.7 (31)	36.6 (181)	43.9 (54)	42.7 (53)	33.1 (41)	26.8 (33)
Femoral Plaques, % (n)	46.9 (191)	53.5 (54)	51.0 (53)	46.5 (47)	36.6 (37)	28.3 (140)	38.2 (47)	30.6 (38)	26.6 (33)	17.9 (22)

Population with available data for arterial stiffness	**TOTAL POPULATION**			
	Sex-specific quartiles of total Na intake			
	1st Males: 1086.4±282.8 mg Females: 751.0±216.1 mg *(n=221)*	**2nd** Males: 1826.2±237.2 mg Females: 1244.2±128.8 mg *(n=222)*	**3rd** Males: 2605.9±271.4 mg Females: 1750.1±155.6 mg *(n=222)*	**4th** Males: 4289.2±1068.1 mg Females: 2749.5±638.6 mg *(n=221)*
	Mean ± S.D.	**Mean ± S.D.**	**Mean ± S.D.**	**Mean ± S.D.**
cf-PWV[d], m/s	8.5±2.0	8.5±2.2	8.4±2.1	7.8±1.4
Increased arterial stiffness (cf-PWV>10 m/sec), % (n)	14.5 (32)	19.4 (43)	16.2 (36)	8.1 (18)

Na: sodium; BMI: body mass index; S.D: standard deviation

[a] Na derived from foods: Na in processed foods and Na naturally occurring in unprocessed foods; [b] Discretionary Na: Na derived from added salt during cooking and table salt; [c] Total Na: estimated plus discretionary Na; [d] cf-PWV: carotid to femoral pulse wave velocity

Table 11 presents the associations derived from multivariate logistic regression analysis between total Na intake quartiles and atheromatic plaques (total plaques, carotid plaques and femoral plaques) for each sex. Females at the 3rd (1743.9±154.6 mg/day) and the 4th (2731.0±631.9 mg/day) quartile of total Na intake had significantly lower probability to present plaques at the femoral arteries comparing to those at 1st quartile (751.0±215.5 mg/day) (p=0.04 and 0.008 respectively). This association remained significant after extensive adjustment for all potential confounders in all -but one- models [0.462 (0.229-0.935), p=0.032 for the 3rd quartile and 0.274 (0.118-0.638), p=0.003 for the 4th quartile in the fully adjusted model (model 4)]. Additional adjustments for hypertension drugs, dyslipidemia drugs, diabetes drugs, antiplatelet drugs, cortisone, and chronic inflammatory diseases duration did not change the mentioned findings. A similar but marginally significant trend was observed for the 2nd quartile of total Na intake in all models [0.538 (0.276-1.046), p=0.068 (model 4)] (**Table 11**). Similar findings were observed regarding carotid plaques as well as total plaques, but although marginal they did not reach statistical significance. On the contrary, no associations between total Na intake and total, carotid or femoral plaques were observed in male participants (**Table 11**).

Table 11: Multivariate logistic regression analysis between total Na intake quartiles and atheromatosis (plaques). The 1st quartile of total Na intake was used as reference. *Published in Hellenic Journal of Cardiology, 2021 (203).*

Models	Quartiles of total Na intake in males	MALES			Quartiles of total Na intake in females	FEMALES		
		Carotid and/or Femoral plaques Exp.B (95% CI)	Carotid plaques Exp.B (95% CI)	Femoral plaques Exp.B (95% CI)		Carotid and/or Femoral plaques Exp.B (95% CI)	Carotid plaques Exp.B (95% CI)	Femoral plaques Exp.B (95% CI)
Model 1 Age	2nd	1.033 (0.528-2.021)	1.022 (0.552-1.893)	0.980 (0.515-1.864)	2nd	0.790 (0.425-1.468)	0.860 (0.471-1.568)	0.572 (0.309-1.058)
	3rd	1.133 (0.578-2.221)	1.008 (0.534-1.902)	1.179 (0.612-2.269)	3rd	0.573 (0.313-1.048)	0.561 (0.309-1.020)	***0.526 (0.285-0.971)**
	4th	0.873 (0.452-1.688)	1.001 (0.523-1.915)	0.991 (0.514-1.911)	4th	0.551 (0.2977-1.023)	0.576 (0.311-1.068)	***0.412 (0.213-0.797)**
Model 2 Age Hypertension Smoking Diabetes Dyslipidemia BMI	2nd	1.019 (0.494-2.103)	1.007 (0.526-1.927)	0.836 (0.401-1.745)	2nd	0.813 (0.422-1.565)	0.901 (0.485-1.676)	0.565 (0.292-1.092)
	3rd	1.049 (0.514-2.143)	1.073 (0.555-2.074)	0.957 (0.463-1.977)	3rd	0.611 (0.321-1.164)	0.609 (0.328-1.132)	0.550 (0.284-1.064)
	4th	0.851 (0.424-1.710)	1.009 (0.515-1.976)	0.912 (0.436-1.904)	4th	0.530 (0.274-1.024)	0.571 (0.301-1.082)	***0.384 (0.190-0.778)**
Model 3a Age Hypertension Smoking Diabetes Dyslipidemia BMI Energy intake	2nd	1.111 (0.531-2.322)	1.098 (0.565-2.137)	0.908 (0.427-1.931)	2nd	0.881 (0.453-1.710)	1.000 (0.532-1.878)	0.529 (0.272-1.031)
	3rd	1.232 (0.574-2.646)	1.249 (0.615-2.536)	1.097 (0.502-2.399)	3rd	0.731 (0.371-1.440)	0.775 (0.403-1.491)	***0.460 (0.228-0.930)**
	4th	1.161 (0.487-2.767)	1.346 (0.587-3.085)	1.173 (0.472-2.911)	4th	0.758 (0.346-1.659)	0.904 (0.424-1.928)	***0.271 (0.117-0.631)**
Model 3b Age Hypertension Smoking Diabetes Dyslipidemia BMI Potassium intake	2nd	1.060 (0.512-2.197)	1.042 (0.543-2.000)	0.846 (0.404-1.773)	2nd	0.827 (0.428-1.595)	0.914 (0.490-1.704)	0.559 (0.289-1.080)
	3rd	1.128 (0.545-2.333)	1.145 (0.587-2.231)	0.974 (0.467-2.032)	3rd	0.642 (0.335-1.232)	0.638 (0.341-1.194)	***0.505 (0.258-0.989)**
	4th	0.979 (0.471-2.034)	1.156 (0.572-2.334)	0.946 (0.435-2.057)	4th	0.585 (0.296-1.158)	0.627 (0.322-1.220)	***0.326 (0.155-0.684)**
Model 4 Age Hypertension Smoking Diabetes Dyslipidemia BMI Energy intake Potassium intake	2nd	1.101 (0.525-2.306)	1.080 (0.554-2.105)	0.911 (0.428-1.938)	2nd	0.879 (0.452-1.708)	1.002 (0.533-1.882)	0.538 (0.276-1.046)
	3rd	1.209 (0.561-2.604)	1.217 (0.598-2.478)	1.108 (0.505-2.429)	3rd	0.729 (0.370-1.438)	0.777 (0.404-1.494)	***0.462 (0.229-0.935)**
	4th	1.119 (0.468-2.678)	1.294 (0.562-2.979)	1.186 (0.476-2.957)	4th	0.755 (0.345-1.653)	0.906 (0.425-1.932)	***0.274 (0.118-0.638)**

* $p<0.05$; Na: sodium; BMI: body mass index; kcal: kilocalories. Males' quartiles of total Na intake (min-max): 1st (403.2-1469.5), 2nd (1483.7-2193.1), 3rd (2202.7-3108.5), 4th (3125.2-8935.1). Females' quartiles of total Na intake (min-max): 1st (80.4-1027.1), 2nd (1029.4-1489.3), 3rd (1496.0-2043.0), 4th (2045.4-6286.9). After further adjustment for hypertension drugs, dyslipidemia drugs, diabetes drugs, antiplatelet drugs, cortisone and chronic inflammatory diseases duration no substantial changes were observed.

Table 12 presents the association between total Na intake quartiles and arterial stiffness. Subjects at 3rd quartile of total Na intake (2605.9±271.4 mg/day for males, 1750.1±155.6 mg/day for females) had significantly higher probability to have high arterial stiffness (cfPWV>10 m/sec) compared to those at 1st quartile (1086.4±282.8 mg for males, 751.0±216.1 mg for females) in models 2 and 3b [1.915(1.015-3.611), p=0.045 for model 2 and 1.991(1.047-3.785), p=0.036 for model 3b]. This association marginally lost its significance in the other models. In further adjustment for drug categories (hypertension drugs, dyslipidemia drugs, diabetes drugs, antiplatelet drugs, cortisone) and chronic inflammatory diseases duration similar results were observed. We also repeated all the arterial stiffness analysis using 1) the definition of arterial stiffness according to age reference groups and 2) the cfPWV as a continuous variable and no associations were observed (data not shown).

Table 12: Multivariate logistic regression analysis between total Na intake quartiles and arterial stiffness (pulse wave velocity). The 1st quartile of total Na intake was used as reference. *Published in Hellenic Journal of Cardiology, 2021 (203).*

Models	Sex-specific quartiles of total Na intake	**TOTAL POPULATION**
		Arterial stiffness (cfPWV>10m/s) Exp.B (95% CI)
Model 1 Age Gender	2nd	1.284 (0.730-2.257)
	3rd	1.563 (0.872-2.804)
	4th	0.863 (0.437-1.701)
Model 2 Age Gender Hypertension Smoking Diabetes Dyslipidemia BMI	2nd	1.248 (0.678-2.297)
	3rd	***1.915 (1.015-3.611)**
	4th	0.991 (0.472-2.078)
Model 3a Age Gender Hypertension Smoking Diabetes Dyslipidemia BMI Energy intake	2nd	1.246 (0.669-2.321)
	3rd	1.909 (0.959-3.801)
	4th	0.987 (0.430-2.266)
Model 3b Age Gender Hypertension Smoking Diabetes Dyslipidemia BMI Potassium intake	2nd	1.262 (0.684-2.328)
	3rd	***1.991 (1.047-3.785)**
	4th	1.063 (0.497-2.278)
Model 4 Age Gender Hypertension Smoking Diabetes Dyslipidemia BMI Energy intake Potassium intake	2nd	1.224 (0.655-2.286)
	3rd	1.869 (0.937-3.728)
	4th	0.978 (0.425-2.252)

**p*<0.05; Na: sodium; cfPWV: carotid to femoral pulse wave velocity; BMI: body mass index; kcal: kilocalories.
Males' quartiles of total Na intake (min-max): 1st (403.2-1469.5), 2nd (1481.1-3110.3), 3rd (2202.7-3108.5), 4th (3125.2-8935.1).
Females' quartiles of total Na intake (min-max): 1st (80.4-1027.1), 2nd (1029.4-1489.3), 3rd (1496.0-2043.0), 4th (2045.4-6286.9).
After further adjustment for hypertension drugs, dyslipidemia drugs, diabetes drugs, antiplatelet drugs, cortisone and chronic inflammatory diseases duration no statistical significant differences between quartiles of total Na intake were observed.

To better investigate these findings, a sensitivity analysis was conducted. All the analyses were repeated for the Na intake from foods only instead of total Na intake. Similar results were found (data not shown). Moreover, after excluding all patients with chronic inflammatory diseases (n=464) similar findings were observed. Finally, after excluding all patients who probably had taken guidance for Na restriction (subjects taking cortisone and subjects with hypertension diagnosed for more than one year) we observed similar results to those of the main analyses.

B3.2 Results from Cohort B

The following results have been published in Public Health Nutrition, 2022 (56).

Comparison of sodium assessment methods/ Design of a new tool for sodium assessment

One hundred and twenty-two (122) participants with available 24UC data were used for the analyses (56.0±12.6 years; 55.7% males) (**Table 13**). The available sample size for UMs and DMs was: Spot UMs, n=71; 24DR=119; FFQ, n=87; NaFFQ, n=60 (**Table 13**). Descriptive characteristics of study population are presented in **Table 13**. Incomplete collections represented 7.4% of participants (**Table 13**).

Table 13: Descriptive characteristics of the study population for the total sample and each Na estimation method. *Published in Public Health Nutrition, 2022 (56).*

	Urinary methods		**Dietary methods**				
	24UC *N=122* (Mean± S.D.)	**Spot Urine** *N=71* (Mean± S.D.)	**24DR** *N=119* (Mean± S.D.)			**FFQ** *N=87* (Mean± S.D.)	**NaFFQ** *N=60* (Mean± S.D.)
Age, years	56.0 ± 12.6	56.2 ± 11.9	55.9 ± 12.6			56.1 ± 13.1	56.4 ± 12.3
Weight, kg	80.7 ± 18.0	81.4 ± 18.9	80.4 ± 18.1			80.3 ± 18.7	80.1 ± 18.9
Height, cm	169.9 ± 11.3	170.7 ±11.3	169.9 ± 11.4			170.3 ± 11.6	170.8 ± 11.9
BMI, kg/m^2	27.9 ± 5.6	27.9 ± 6.0	27.9 ±5.6			27.6 ± 5.2	27.3 ± 5.1
Energy, kcal/day			1998.8±668.3			2238.7±713.7	2210.7±695.5
			Existing 24DR	***24DR+15%***	***25DR+SQ***		
Na derived from food, mg/day			1633.8±763.6	1633.8±763.6	1633.8±763.6	1704.3±800.0	1793.5±873.5
Na derived from table salt, mg/day				288.3±134.8*	58.6±90.3		1197.4±1047.2*
Na derived from cooking salt, mg/day					276.4±261.6		
	(%)	(%)	(%)			(%)	(%)
Males	55.7	56.3	55.5			57.5	60.0
Smoking,							
Current (cigarette/ e-cigarette)	40.5	43.6	41.5			40.7	38.8
Ex smoking	20.7	22.5	20.3			19.8	23.3
Never	38.8	33.8	38.1			39.5	38.3
CVD	10.7	7.1	11.0			11.5	10.0
T1DM	1.6	1.4	1.7			2.3	1.7
T2DM	8.2	11.3	8.4			8.0	10.0
DMS drugs	5.7	7.0	5.9			6.9	6.7
Hypertension	64.8	60.6	63.9			65.5	58.3
Hypertension drugs	46.7	42.3	45.4			48.3	43.3
Dyslipidemia	65.6	64.8	65.5			66.7	68.3
Dyslipidemia drugs	33.6	32.4	33.6			31.0	25.0
Autoimmune/inflammatory disease	13.2	15.5	12.7			11.5	13.3
Infectious disease	30.6	32.4	30.5			29.9	31.7
Incomplete 24UC	7.4						

24UC: 24h urine collection; 24DR: 24h dietary recalls (three 24DR were performed); 24DRNa+15%: 24h dietary recalls Na plus 15% (discretionary Na); 24hDRNa+SQ: 24h dietary recalls Na plus discretionary salt questions FFQ: food frequency questionnaire; S.D.: standard deviation; BMI: body mass index; CVD: cardiovascular disease; T1DM: type 1 diabetes mellitus; T2DM: type 2 diabetes mellitus.

* Na derived from table and cooking salt

Table 14 presents Na intake or excretion for all the available UMs and DMs applied, as well as the significance of the differences between 24UNa and each one of the other Na estimation methods. Mean 24UNa was 2810.4±1303.9 mg/day. Regarding spot urine methods, all of them overestimated 24UNa (mean bias range: -1780.9 to -492.0 mg) with the INTERSALT without spot K equation presenting the smallest bias (-492.0±1223.2 mg) (table 4). Regarding the existing DMs, both underestimated 24UNa (mean bias range: 876.6 to 1211.6 mg). From the improved DMs, 24DR+15% and 24DR+SQ underestimated 24UNa (876.6± 1342.6 and 923.3± 1345.8 mg respectively, p<0.001) but the NaFFQ marginally overestimated 24UNa showing the smallest bias from all DMs and UMs (-290.2 ± 1336.2 mg) (**Table 14**).

Table 14: Na intake/excretion for each dietary and urinary Na estimation method, bias of mean values and comparisons with the 24-hour urine collection. *Published in Public Health Nutrition, 2022 (56).*

	N	**Na intake or excretion** Mean ± S.D.	**Bias** (24UNa minus each Na estimation method) Mean ± S.D.	**p**
Urinary methods				
24UC, mg/day	122	2810.4 ± 1303.9	-	-
Kawasaki, mg/day	71	4523.0 ± 1331.0	-1780.9 ± 1235.2	<0.001
Tanaka, mg/day	71	4862.1 ± 10633.2	-894.8 ± 1154.1	<0.001
INTERSALT with spot K, mg/day	67	3209.4 ± 869.0	-599.0 ± 1140.0	<0.001
INTERSALT without spot K, mg/ day	71	3207.8 ± 843.1	-492.0 ± 1223.2	0.001
Mage, mg/day	71	3438.8 ± 2494.8	-722.6 ± 2050.6	0.016
Toft, mg/day	71	3852.8 ± 955.7	-1136.6 ± 1165.6	<0.001
Dietary methods				
Existing dietary methods				
24DR, mg/day	119	1633.8 ± 763.6	1211.6 ± 1298.8	<0.001
FFQ, mg/day	87	1704.3 ± 800.0	1058.7 ± 1335.7	<0.001
Improved dietary methods				
24DR+15%, mg/day	119	1922.2 ± 898.3	923.3 ± 1345.8	<0.001
24DR+SQ, mg/day	119	1968.9 ± 917.0	876.6 ± 1342.6	<0.001
NaFFQ, mg/day	60	2990.9 ± 1397.5	-290.2 ± 1336.2	0.098

S.D.: standard deviation; Na: sodium; 24UC: 24h urine collection; 24UNa: 24h urine Na; 24DRNa:24h dietary recalls Na; 24DRNa+15%: 24h dietary recalls Na plus 15% (discretionary Na); 24hDRNa+SQ: 24h dietary recalls Na plus discretionary salt questions; FFQ: food frequency questionnaire

Table 15 presents Pearson's and Spearman's correlation tests as well as the ICCs between 24UC and each one of the UMs and DMs. Regarding spot urine methods, Mage equation exhibited the strongest correlation with 24UC (r=0.596, p<0.001) and all other equations presented moderate reliability (ICCs range: 0.59-0.74). From the existing DMs, both weakly correlated to 24UC (r=0.232-0.263,

p<0.05). Regarding the improved DMs, 24DR+15% and 24DR+SQ were weakly correlated to 24UC (r=0.263-0.296, p≤0.01) and presented poor reliability (ICCs range: 0.42-0.44), but NaFFQ exhibited the strongest correlation with 24UC (r=0.497, p≤0.01) and was moderately reliable [ICC(95%CI): 0.66(0.43-0.80)].

Table 15: Pearson's and Spearman correlations & intraclass correlation coefficients between 24 hour urine collection and the other Na estimation methods. *Published in Public Health Nutrition, 2022 (56).*

		Na estimation methods		**Total sample**
Spot urine methods		Kawasaki	r ICC (95% CI) *N*	0.583** 0.74 (0.58-0.84) 71
		Tanaka	r ICC (95% CI) *N*	0.542** 0.66 (0.46-0.79) 71
		INTERSALT with spot K	r ICC (95% CI) *N*	0.492** 0.63 (0.40-0.77) 67
		INTERSALT without spot K	r ICC (95% CI) *N*	0.469** 0.59 (0.35-0.75) 71
		Mage	r ICC (95% CI) *N*	0.596** 0.65 (0.44-0.78) 71
		Toft	r ICC (95% CI) *N*	0.570** 0.68 (0.48-0.80) 71
Dietary methods	Existing dietary methods	24DR	r ICC (95% CI) *N*	0.263** 0.40 (0.14-0.58) 119
		FFQ	r ICC (95% CI) *N*	0.232* 0.39 (0.06-0.60) 87
	Improved dietary methods	24DR+SQ	r ICC (95% CI) *N*	0.296** 0.44 (0.20-0.61) 119
		24DR+15%	r ICC (95% CI) *N*	0.263** 0.42 (0.17-0.60) 119
		NaFFQ	r ICC (95% CI) *N*	0.497** 0.66 (0.43-0.80) 60

* p<0.05; ** p≤0.01

ICC: intraclass correlation coefficient; CI: confidence interval; Na: sodium; 24UNa: 24h urine Na; 24DRNa:24h dietary recalls Na; 24DRNa+15%: 24h dietary recalls Na plus 15% (discretionary Na); 24hDRNa+SQ: 24h dietary recalls Na plus discretionary salt questions; FFQ: food frequency questionnaire

In subgroup analysis (**Table 16**): a) 4 out of the 5 subgroups agreed that Mage equation exhibited the strongest correlation with the 24UC ($r=0.625-0.700$, $p<0.001$); b) 3 out of the 5 agreed that Kawasaki equation was the only method presenting good reliability (ICCs range: 0.76-0.80) and all the 5 subgroups agreed that regarding the existing and the improved DMs, NaFFQ was the only method presenting moderate reliability (ICCs range: 0.44-0.51), while all the other DMs presented poor reliability (ICCs range: 0.20-0.32).

Bland-Altman plots for all the spot urine methods, existing DMs and improved DMs are presented in **Figures 15, 16** and **17** respectively. Regarding spot urine methods, the use of equations of Toft, Tanaka, INTERSALT with spot K and INTERSALT without spot K resulted in underestimation at lower levels and overestimation at higher levels of Na excretion in Bland-Altman plots (**Figure 15**). On the contrary, Mage equation was the only method providing the opposite finding, presenting overestimation at low levels of Na excretion and underestimation at higher levels. Finally, the Kawasaki equation exhibited a homogeneous variation as Na excretion levels increase (**Figure 15**). All methods presented wide ranges of agreement (Kawasaki: -4201.8 to 640.0; Mage: -4741.7 to 3296.6; Toft -3421.2 to 1148.0; INTERSALT without spot K: -2889.4 to 1905.4; INTERSALT with spot K: -2833.3 to 1635.4; Tanaka: -3156.9 to 1367.3) (**Figure 15**). Linear regression analysis revealed statistically significant associations between the difference and the mean of 24UC and all the spot urine methods, except for the Kawasaki equation ($\beta=0.028$, $p=0.818$) (**Figure 15**).

Table 16: Pearson's and Spearman correlations & intraclass correlation coefficients between 24h urinary sodium excretion and the other sodium estimation methods in the subgroup of participants after applying the exclusion criteria for incomplete 24h urine collections. *Published in Public Health Nutrition, 2022 (56).*

		Na estimation methods		**Subgroups** of sample after applying the exclusion criteria for incomplete 24h urine collections (sensitivity analysis)				
				Exclusion criterion 1	**Exclusion criterion 2**	**Exclusion criterion 3**	**Exclusion criterion 4**	**Exclusion criterion 5**
Spot urine methods		Kawasaki	r	0.610**	0.613**	0.663**	0.588**	0.595**
			ICC (95% CI)	0.758 (0.603-0.852)	0.760 (0.604-0.854)	0.796 (0.641-0.884)	0.740 (0.584-0.838)	0.746 (0.507-0.869)
			N	65	64	50	71	37
		Tanaka	r	0.582**	0.581**	0.620**	0.554**	0.532**
			ICC (95% CI)	0.693 (0.497-0.813)	0.695 (0.498-0.815)	0.735 (0.534-0.850)	0.669 (0.469-0.794)	0.654 (0.329-0.822)
			N	65	64	50	71	37
		INTERSALT with spot K	r	0.496**	0.464**	0.533**	0.477**	0.629**
			ICC (95% CI)	0.633 (0.391-0.779)	0.606 (0.343-0.764)	0.677 (0.424-0.819)	0.615 (0.375-0.762)	0.736 (0.477-0.867)
			N	62	61	48	68	35
		INTERSALT without spot K	r	0.481**	0.444**	0.509**	0.466**	0.606**
			ICC (95% CI)	0.603 (0.349-0.758)	0.570 (0.293-0.739)	0.644 (0.372-0.798)	0.588 (0.339-0.743)	0.704 (0.425-0.848)
			N	65	64	50	71	37
		Mage	r	0.655**	0.649**	0.700**	0.625**	0.662**
			ICC (95% CI)	0.681 (0.476-0.805)	0.663 (0.445-0.795)	0.698 (0.468-0.829)	0.671 (0.473-0.795)	0.741 (0.498-0.867)
			N	65	64	50	71	37
		Toft	r	0.577**	0.580**	0.670**	0.554**	0.695**
			ICC (95% CI)	0.680 (0.475-0.805)	0.680 (0.474-0.806)	0.757 (0.571-0.862)	0.659 (0.454-0.788)	0.787 (0.586-0.890)
			N	65	64	50	71	37
Dietary methods	Existing dietary methods	24DR	r	0.254**	0.217*	0.293**	0.270**	0.295*
			ICC (95% CI)	0.359 (0.062-0.562)	0.345 (0.034-0.556)	0.408 (0.099-0.612)	0.389 (0.112-0.580)	0.370 (-0.029-0.614)
			N	108	104	89	112	66
		FFQ	r	0.197	0.267*	0.266*	0.267*	0.321*
			ICC (95% CI)	0.367 (0.007-0.596)	0.431 (0.096-0.642)	0.440 (0.079-0.660)	0.404 (0.076-0.616)	0.468 (0.044-0.704)
			N	78	74	64	82	47
	Improved dietary methods	24DR+SQ	r	0.296**	0.237*	0.295**	0.299**	0.283*
			ICC (95% CI)	0.403 (0.126-0.592)	0.373 (0.075-0.575)	0.431 (0.134-0.627)	0.425 (0.164-0.605)	0.376 (-0.019-0.618)
			N	108	104	89	112	66
		24DR+15%	r	0.254**	0.217*	0.293**	0.270**	0.295*
			ICC (95% CI)	0.379 (0.091-0.575)	0.363 (0.061-0.568)	0.428 (0.129-0.624)	0.411 (0.145-0.595)	0.389 (0.001-0.626)
			N	108	104	89	112	66
		NaFFQ	r	0.511**	0.442**	0.459**	0.491**	0.452**
			ICC (95% CI)	0.667 (0.429-0.806)	0.612 (0.331-0.775)	0.627 (0.334-0.791)	0.657 (0.423-0.796)	0.623 (0.260-0.808)
			N	55	54	48	59	

* p<0.05; ** p≤0.01. ICC: intraclass correlation coefficient; CI: confidence interval; Na: sodium; 24UNa: 24h urine Na; 24DRNa:24h dietary recalls Na; 24DRNa+15%: 24h dietary recalls Na plus 15% (discretionary Na); 24hDRNa+SQ: 24h dietary recalls Na plus discretionary salt questions; FFQ: food frequency questionnaire.

Exclusion criteria for incomplete 24h urine collections: Exclusion criterion 1: Urine volume <500mL; Exclusion criterion 2: Urine creatinine <6mmol/day plus 24h urine volume <1000mL or urine creatinine <5mmol/day (191); Exclusion criterion 3: (urine creatinine (mg/day))/(body weight (kg))<10.8 or>25.2 (193); Exclusion criterion 4: Males: (urine creatinine (mg/day))/(body weight (kg))<14 or>26, Females: (urine creatinine (mg/day))/(body weight (kg))<11 or>20 (192) Exclusion criterion 5: Males: (urine creatinine (mg/day))/(24 x body weight (kg))<0.6, Females: (urine creatinine (mg/day))/(21 x body weight (kg))<0.6 (43)

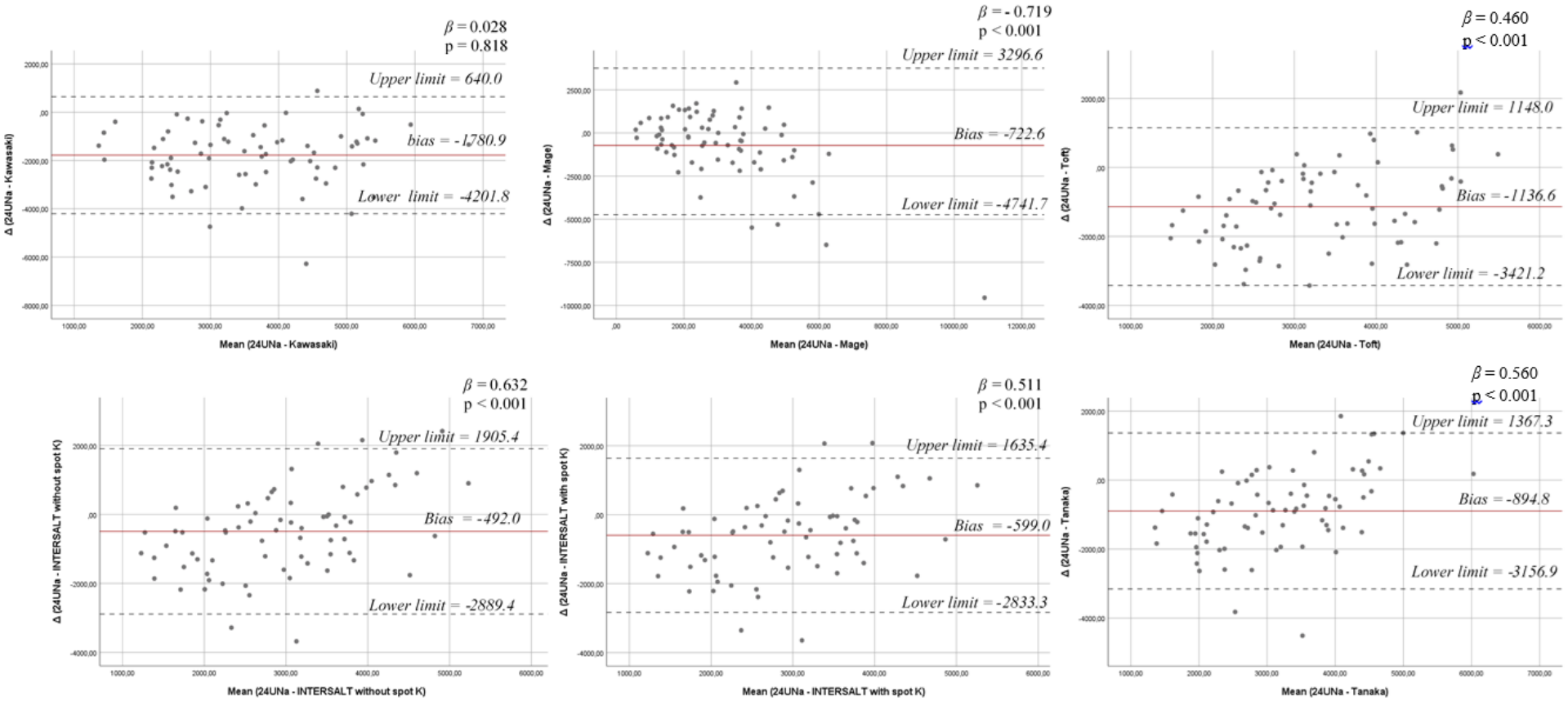

Figure 15: Bland-Altman plots comparing 24-hour urinary Na excretion with Na estimated by spot urine equations. Solid line is the mean difference between methods and dashed lines are the 95% confidence intervals of the difference between methods. Limits of agreement of the two Na assessment methods, defined as mean difference ± 1.96 × SD of differences. 24UNa: Na estimated by 24-hour urine collection. *Published in Public Health Nutrition, 2022 (56).*

Regarding the existing DMs (**Figure 16**), both of them presented consistent bias in Bland-Altman plots, underestimating the 24UNa in low levels of Na intake and overestimating in high levels of Na intake, while presenting wide ranges of agreement in Bland-Altman plots (24DR: -1334.1 to 3757.4; FFQ: -1559.2 to 3676.7) (**Figure 16**). Linear regression analysis revealed statistically significant association between the difference and the mean of 24UC and all the DMs (**Figure 16**). Regarding the improved DMs, the NaFFQ was the only one showing a) a homogeneous variation as the mean Na intake increases in Bland-Altman plots, however presenting wide ranges of agreement (-2909.2 to 2328.8) and b) not statistically significant association between the difference and the mean of 24UC and improved DMs in linear regression analysis (b=0.142, p=0.354) (**Figure 17**). The other two improved DMs (24DR+15% & 24DR+SQ) underestimated the 24UNa at low levels of Na intake and overestimated at high levels of Na intake, presenting wide ranges of agreement (24DR+SQ: -1334.1 to 3508.1; 24DR+15%: -1714.5 to 3561.2) (**Figure 17**).

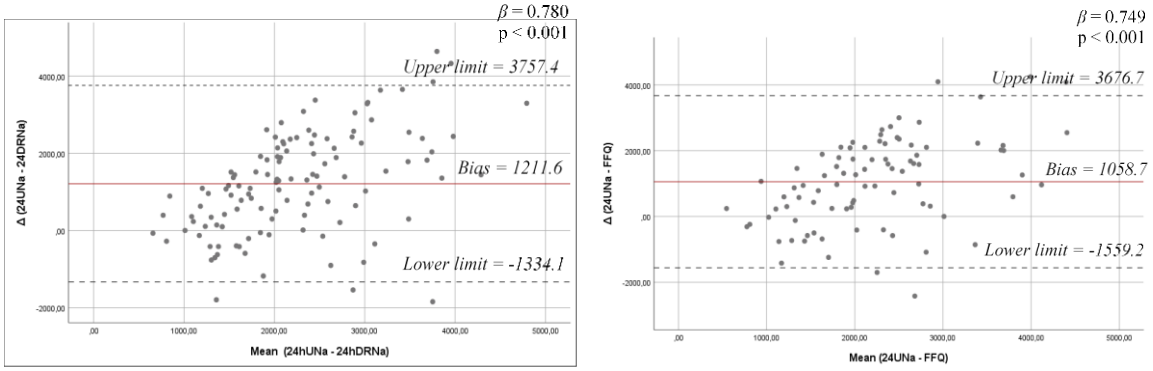

Figure 16: Bland-Altman plots comparing 24-hour urinary Na excretion with Na estimated by existing dietary methods. Solid line is the mean difference between methods and dashed lines are the 95% confidence intervals of the difference between methods. Limits of agreement of the two Na assessment methods, defined as mean difference ± 1.96 × SD of differences. 24UNa: Na estimated by 24-hour urine collection; 24hDRNa: Na estimated by 24 hour dietary recalls; FFQ: food frequency questionnaire. *Published in Public Health Nutrition, 2022 (56).*

All the analyses were repeated using 1 dash of salt instead of 2 in question *2* of the improved 24DR (24DR+SQ) and similar findings were observed (data not shown).

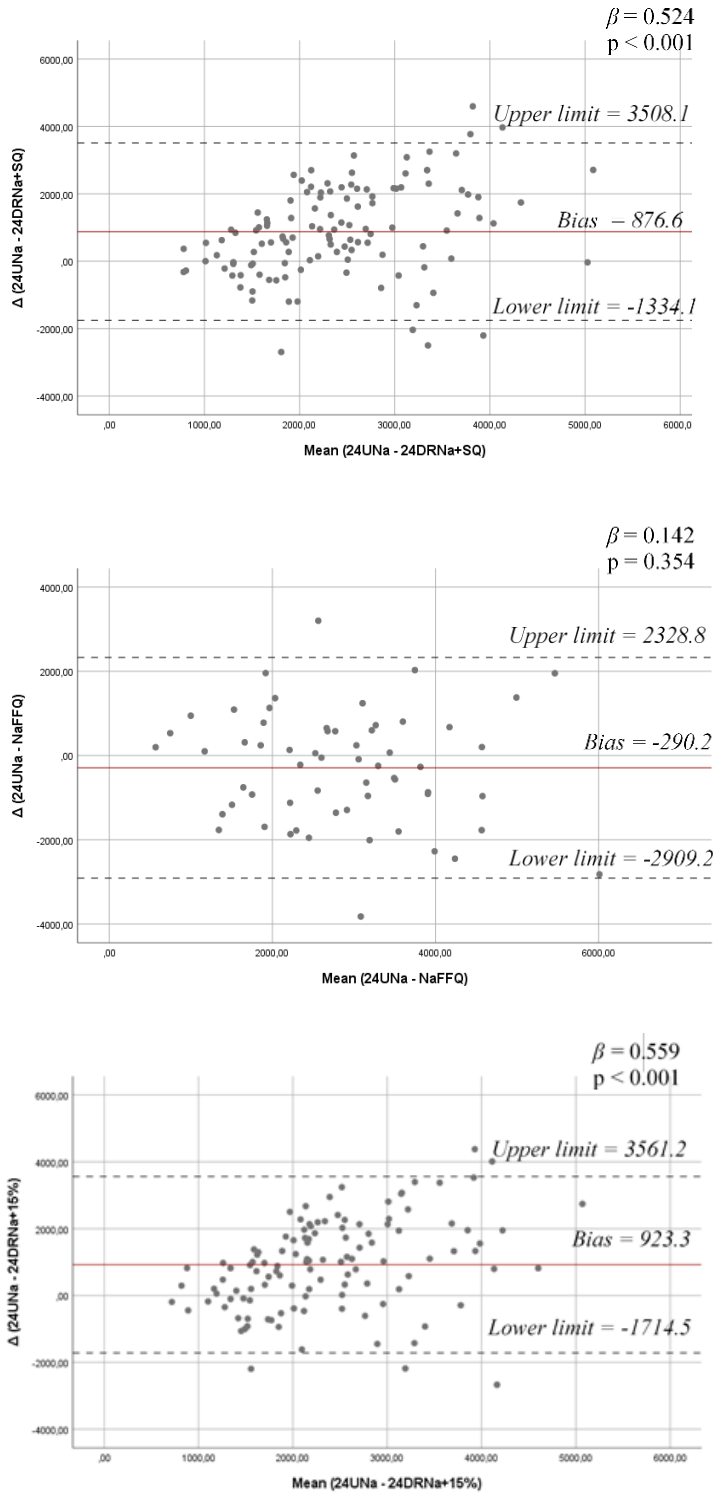

Figure 17: Bland-Altman plots comparing 24 hour urinary Na excretion with Na estimated by improved dietary methods. Solid line is the mean difference between methods and dashed lines are the 95% confidence intervals of the difference between methods. Limits of agreement of the two Na assessment methods, defined as mean difference ± 1.96 × SD of differences. 24UNa: sodium estimated by 24 hour urine collection; 24hDRNa+SQ: Na estimated by 24 hour dietary recalls plus salt-related questions; NaFFQ: Na food frequency questionnaire. *Published in Public Health Nutrition, 2022 (56).*

B3.3 Results from systematic review

The following results have been published in Nutrients, 2019 (204).

Number of studies screened and selected

Eight hundred and twenty-five (825) citations were identified through a systematic search—of which, 782 were excluded based on title/abstract. The most common exclusion criteria were: language (42), duplicates—same cohort (27), different research subject (516), not original articles (191), sample size <100 subjects for observational studies only (6). Forty-three (43) articles were then assessed for eligibility and five were excluded due to irrelevant research subject and two due to different studying parameter (Na/K ratio). As a result, the number of articles that met the inclusion criteria and were included in this study was 36 (**Figure 18**).

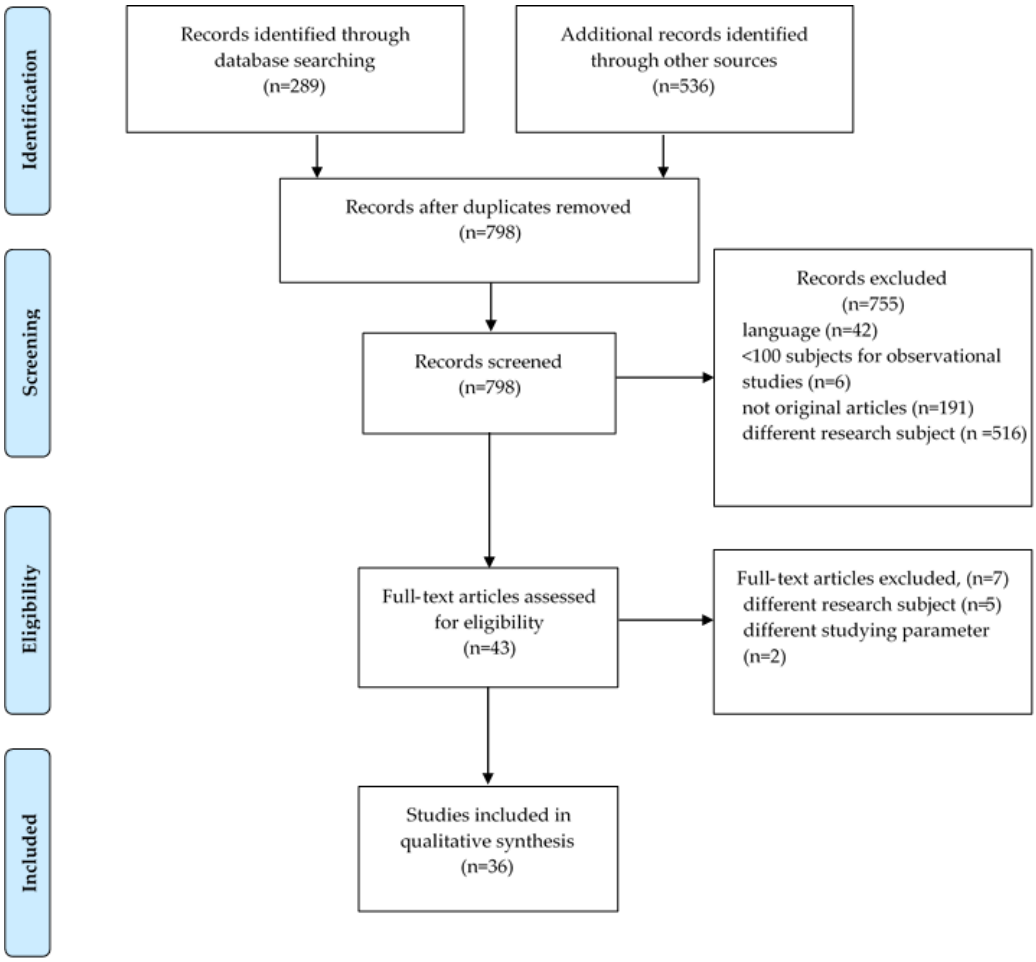

Figure 18. PRISMA flow diagram. *Published in Nutrients, 2019 (204).*

Of a total of 36 studies included, 18 were observational and 18 were interventional studies. Detailed descriptive data for both observational and interventional studies are provided in **Tables 17, 18, 19,20** and **21**.

Table 17. Descriptive characteristics of observational studies regarding arteriosclerosis. *Published in Nutrients, 2019 (204).*

ARTERIOSCLEROSIS											
cfPWV											
1. Observational Cross-Sectional Studies											
Author (Year)	**Country**	**Study Design**	**Population Description**	**FU (Years)**	**Sex**	**Race**	**N**	**Age (Years, Mean ± SD)**	**Na Estimation Method**	**Na Intake/Excretion (mg/d)**	**Vascular Assessment**	**Results**
Polónia, J. (2006) (156)	Portugal	c-sect	essential HT, recent stroke, or healthy university students	-	M/F	Mixed	426	50 ± 22	24UC	Total: 4646 ± 1472	tonometry	+ **
García-Ortiz, L. (2012) (148)	Spain	c-sect	primary care patients aged 30–80	-	M/F	N/AV	351	54.8 ± 11.7	FFQ	Total: 3180 ± 1250 Q1:1800 ± 390 Q2: 2650 ± 200 Q3: 3440 ± 270	tonometry	J-shaped curve
Kotliar, C. (2014) (205)	Argentina	c-sect	essential HT, aged 30 to 70	-	M/F	N/AV	300	48.7 ± 14.6	24UC	(a) low Na-low RAAS: 913.1 (747.5–1035)	tonometry	≠
										(b) low Na-high RAAS: 690 (602.6–740.6)		≠
										(c) High-Na-low RAAS: 2610.5 (1745.7–3604.1)		≠
										(d) high-Na-high RAAS: 2898 (2035.5–3588)		+ *
Polonia, J. (2016) (180)	Portugal	retrosp	HT adults	7.2 (0.5–11.1)	M/F	White	608	54.1 ± 14.3	24UC	4793.2 ± 1821.6	tonometry	+ *
Strauss, M. (2018) (145)	South Africa	prosp	NT adults	-	M/F	Mixed	693	24.8 ± 3.01	24UC	2967 (984.4–7613)	tonometry	total + ** black + * white ≠
Triantafyllou, A. (2018) (206)	Greece	c-sect	untreated HT—healthy individuals	-	M/F	White	197	43.7 ± 12.1	24UC	True HT: 3348.8 (2251.7–4595.4) Intermediated HT phenotypes: 3128 (1902.1–4312.5) NT: 2732.4 (1630.7–4312.5)	tonometry	≠

Table 17 *Cont.*

Author (Year)	Country	Study Design	Population Description	FU (Years)	Sex	Race	N	Age (Years, Mean ± SD)	Na Estimation Method	Na Intake/Excretion (mg/d)	Vascular Assessment	Results
2. Observational Studies with Follow Up (>1 Time Points)												
Nerbass, F.B. (2015) (207)	UK	prosp	adults in CKD stage 3	1	M/F	N/AV	1607	72.6 ± 9.0	Spot urine collections and Nerbass equation to estimate 24h Na excretion	baseline: 2599 ± 782 follow-up: 2576 ± 782	oscillometry	**cfPWV** (at both time points) Na <2300 mg + ** Na >2300 mg ≠ **ΔcfPWV** Unchanged Na ≠ Decreased Na ≠ Increased Na + *
Aortic PWV Other than cfPWV												
Siriopol, D. (2018) (208)	Romania	prosp	HT and NT Romanian adults	-	M/F	White	1599	47.3 ± 17.1	Morning spot urine sample + Kawasaki equation	4816.2 ± 1550.2	oscillometry	NT + ** HT + **
baPWV												
1. Observational Cross-Sectional Studies												
Sonoda, H. (2012) (144)	Japan	c-sect	healthy subjects	-	M/F	Asian	911	61.3 ± 8.5	24UC	720 ± 200 (mg /day/10 kg)	oscillometry	+ **
Lee, S.K. (2015) (149)	Korea	c-sect	non-HT subjects, with no use of anti-HT drugs	-	M/F	Asian	1586	tertile 1: 52.1 ± 5.5 tertile 2: 53.0 ± 6.0 tertile 3: 52.6 ± 5.5	Second morning void and Tanaka's equation to convert to 24UC	3588 ± 782	plethysmography	− **
Sun, N. (2015) (157)	China	c-sect	newly diagnosed HT, untreated HT or patients with a 1-to-5-year history of HT who had stopped taking anti-HT drugs for 1 month	-	M/F	N/AV	341	Group A: 59.3 + 13.4 Group B: 56.1 + 15.5 Group C: 57.6 + 14.2	24UC	Total: 3507.5 ± 1577.8 Group A: 1807.8 ± 411.7 Group B: 3374.1 ± 618.7 Group C: 5858.1 ± 961.4	oscillometry	+ *
Author (year)	**Country**	**Study Design**	**Population Description**	**FU (years)**	**Sex**	**Race**	**N**	**Ag (Years, Mean ± SD)**	**Na Estimation Method**	**Na Intake/Excretion (mg/d)**	**Vascular Assessment**	**Results**
Han, W. (2017) (209)	China	c-sect	HT adults	-	M/F	N/AV	431	Group A: 54.5 ± 12.9 Group B: 52.9 ± 12.6 Group C: 50.9 ± 11.4	24UC	Total: 3831.8 ± 1.610, Group A: 1768.7 ± 464.6 Group B: 3371.8 ± 650.9 Group C: 5947.8 ± 1069.5	oscillometry	+ *

Table 17 *Cont.*

2. Observational Studies with Follow Up (>1 Time Points)													
Jung, S. (2019) (155)	South Korea	prosp	adults aged >40	5.3±1.0	M/F	Mixed	2145	59.9 ± 9.1	FFQ and 3-day diet record	2538 ± 1416	oscillometry	**baPWV**	
												Na baseline	+ **
												Na average of three visits	+ **
												ΔbaPWV	
												Na baseline	+ **
												Na average of three visits	+ **
Common Carotid Arterial Elasticity (Young's Elastic Modulus, Stiffness Index, and Arterial Compliance)													
Ferreira-Sae, M.C. (2011) (158)	Brazil	c-sect	HT adults	-	M/F	N/AV	134	58 ± 1	1. FFQ 2. 24h recall 3.discretionary Na intake[1]	Na intake/d: 5520 ± 290 FFQ: 1450 ± 180 24h recall: 940 ± 70 Discretionary Na: 3130 ± 190	B-mode US	Young's elastic modulus	+ **
												stiffness index	≠
												arterial compliance	≠

The mmol of Na intake/excretion values were converted to mg. If available, results presented come from adjusted models. Abbreviations: Na: sodium; 24UC: 24h urine collection; cfPWV: carotid–femoral pulse wave velocity; baPWV: brachial–ankle pulse wave velocity; HT: hypertensives; NT: normotensives; anti-HT: antihypertensive; c-sect: cross-sectional; prosp: prospective; retrosp: retrospective; FFQ: food frequency questionnaire; FU: follow up; M/F: males and females; N/AV: not available; RAAS: renin–angiotensin–aldosterone system; CKD: chronic kidney disease; US: ultrasonography; +: positive association; -: negative association; ≠: no statistically significant association; *: $p < 0.05$; **: $p < 0.01$. [1] number of 1 kg packages of salt consumed/month/person.

Table 18: Descriptive characteristics of interventional studies regarding arteriosclerosis. *Published in Nutrients, 2019 (204).*

ARTERIOSCLEROSIS														
cfPWV														
Author	**Country**	**Study Design**	**Population Description**	**Sex**	**Race**	**N**	**Age (Years, Mean ± SD)**	**Intervention Duration (Weeks)**	**Type of Diet**	**Na Estimation Method**	**Na Intake/Excretion (mg/d)**	**Vascular Assessment**	**Results**	
													Intervention Groups	**cfPWV Change (m/s)**
Seals, D.R. (2001) (210)	USA	RCT	postmenopausal women, ≥50 years, high normal SBP or Stage 1 HTN	F	Mixed	17	65 ± 10	13	LS < 2400 mg	24UC & food records	Urinary Na excretion Preint_restr: 2852 ± 1058 Postintn_restr:1978 ± 736 Dietary Na intake Preint_restr: 2685 ± 559 Postint_restr:1421 ± 512	US	LS vs. baseline	−0.24 *
Dickinson, K.M. (2009) (176)	Australia	cross-over RCT	OW/OB, mild HT adults	M/F	N/AV	29	52.7 ± 6.0	4 (2 weeks × two diets)	Usual Na diet: 3450 mg Na/d vs. LS diet: 1150 mg Na/d	Three-day weighed food records & 24UC	Urinary method Baseline: 3553.5 ± 1568.6 Usual Na: 3594.9 ± 1304.1 LS: 1474.3 ± 949.9	oscillometry	LS vs. Usual Na	≠
He, F.J. (2009) (146)	UK	cross-over dbRCT	HT adults	M/F	Mixed	169	All: 50 ± 11 Blacks: 50 ± 9 Whites: 52 ± 12 Asians: 47 ± 10	12	9 Na tablets (×230 mg)/d & 9 placebo tablets/d. (remained on LS diet: 2000 mg Na/d)	24UC	Total: 3013 ± 1150 Blacks: 3036 ± 1058 Whites: 2921 ± 1173 Asians: 3174 ± 1311	tonometry	From Na to placebo Total Blacks Whites Asians	−0.40 ** −0.50 ** ≠ ≠
Pimenta, E. (2009) (160)	USA	cross-over RCT	resistant HT adults on a stable anti-HT drug	M/F	Mixed	12	55.5 ± 9.4	2 (1 week × two diets)	LS diet: 1495 mg Na/d vs. HS diet: suppl. >5750 mg Na/d	24UC	Baseline: 4478.1 ± 1577.8 LS diet: 1060.3 ± 616.4 vs. HS diet: 5800.6 ± 1485.8	tonometry	From HS to LS ≠	
Todd, A.S. (2010) (143)	New Zealand	cross-over sbRCT	PHT or HT, NOB adults or on anti-HT drugs	M/F	Mixed	33	51.8 ± 7.6	12	(500 mL tomato juice + LS diet/day) (A) 0 + 1380 mg (B) 2070 + 1380 mg (C) 3220 + 1380 mg	Morning spot urine samples & dietary recalls	Na intake Usual diet: 2607 ± 1289 A: 1254 ± 397 B: 1357 ± 486 C: 1306 ± 335	tonometry	B vs. A C vs. A B vs. C	+ 0.39 ** + 0.35 ** ≠

Table 18 *Cont.*

Todd, A.S. (2012) (161)	New Zealand/Australia	cross-over sbRCT	NT, NOB adults	M/F	N/AV	23	43.7 (24–61)	12	(500 mL tomato juice + LS diet/day) (A) 0 + 1380 mg (B) 2070 + 1380 mg (C) 3220 + 1380 mg	Morning spot urine samples & dietary recalls	Pre-baseline: 2410.4 After intervention (Na intake + Na tomato juice) A: 0 + 1232.8 B: 2070 + 1207.5 C: 3220 + 1140.8	tonometry	B vs. A C vs. A B vs. C	≠ ≠ ≠
McMahon, E.J. (2013) (211)	Australia	cross-over dbRCT	HT adults, with stage 3 or 4 CKD	M/F	N/AV	20	68.5 ± 11	4 (2 weeks × two diets)	HS diet: 4140-4600 mg Na/d vs. LS diet: 1380–1840 mg Na/d	24UC	LS: 1725 (1334–2576) vs. HS: 3864 (3358–5037)	tonometry	LS vs. HS	≠
Dickinson, K.M. (2014) (212)	Australia	cross-over sbRCT	OW or OB subjects	M/F	N/AV	25	N/AV	6	LS diet: 2400 mg/d vs. Usual Na diet: 3600 mg/d	24UC	baseline: 2761 ± 1031 Usual Na diet: 1729 ± 627 LS diet: 1799 ± 497	tonometry	LS vs. US	≠
Gijsbers, L. (2015) (213)	the Netherlands	cross-over RCT	untreated (P)HT, aged 40–80	M/F	White	36	65.8 (47–80)	4	Na suppl: 3000 mg/d vs. placebo	24UC	Baseline: 3535.1 Na suppl.: 4666.7 ± 1260.4 vs. Placebo: 2417.3 ± 913.1	tonometry	Na suppl. vs. placebo	≠
Suckling, F.J. (2016) (214)	United Kingdom	Cross-over dbRCT	untreated HT adults	M/F	Mixed	46	58 ± 1	12 (6 weeks × two diets)	9 Na tablets (×230 mg)/d vs. 9 placebo tablets/d.	24UC	Na diet: 3797.3 ± 207 vs. Placebo: 2681.8 ± 218.5	tonometry	Na diet vs. placebo	≠
van der Graaf, A.M. (2016) (215)	the Netherlands	cross-over RCT	women with history of preeclampsia or history of healthy former pregnancy	F	N/AV	36	36 ± 5	2	LS diet: 1150 mg/d vs. HS diet: 4600 mg/d	24UC	NT pregnancy history group: LS: 897 ± 322 HS: 5083 ± 1472 Preeclamptic pregnancy history group: LS: 1035 ± 529 HS: 5934 ± 1978	tonometry	LS vs. HS (in either group)	≠
Muth, B.J. (2017) (141)	USA	cross-over RCT	healthy, NT adults	M/F	N/AV	85	Young: 27 ± 1 Middle-aged: 52 ± 1	2	LS diet: 460 mg/d vs. HS diet:6900 mg/d	24UC	* LS diet (young & middle aged): 690 HS diet (young & middle-aged): 5405 * Approximately from diagram	tonometry	middle aged young	+0.60 ** ≠

Table 18 *Cont.*

Aortic PWV (other than cfPWV)													
Author	**Country**	**Study Design**	**Population Description**	**Sex**	**Race**	**N**	**Age (Years, Mean ± SD)**	**Intervention Duration**	**Type of Diet**	**Na Estimation Method**	**Na Intake/Excretion (mg/d)**	**Vascular Assessment**	**Results**
													Intervention groups / **PWV Change (%)**
Avolio AP. (1986) (147)	Australia	RCT	healthy NT adults and children	M/F	N/AV	114	Group 1: Control: 10.8 ± 1.9 LS: 10.4 ± 2.5 Group 2: Control: 39.4 ± 1.7 LS: 39.8 ± 1.6 Group 3: Control: 52.2 ± 3.5 LS: 54.5 ± 4.2	24.8±2.5 months (8 months to 5 years)	N/AV	24UC & diet questionnaire	Na excretion: Control group: N/AV Group 1: 1564 Group 2: 943 Group 3: 506	oscillometry	Group 1 leg Group 2 aortic arm leg Group 3 aortic leg	−11.2***** −21.8****** −10.7***** −13.3***** −22.7***** −22.3*****
hfPWV													
Rhee MY. (2016) (142)	Korea	RCT	NT and HT adults	M/F	N/AV	101	46.0 ± 16.6	2	LS DASH diet: 2320 mg Na/d vs. HS DASH diet: 7000 mg Na/d	N/AV	LS diet: 2320 vs. HS diet: 7000	US	HS vs. LS SS SR HT NT
													+4%***** ≠ ≠ ≠
baPWV													
Wang Y. (2015) (216)	China	dietary intervention study	mild HT adults	M/F	N/AV	49	49.0 ± 7.9	3 (1 week × three diets)	LS diet: 1179.9 mg/d & HS diet: 7079.4 mg/d	24UC	3999.7±1543.3	plethysmography	LS vs. HS SS vs SR Baseline After LS After HS
													≠ +2.3* +1.5* +2.0*

Table 18 *Cont.*

Arterial Elasticity (arterial compliance)														
Author	**Country**	**Study Design**	**Population Description**	**Sex**	**Race**	**N**	**Age (Years, Mean ± SD)**	**Intervention Duration**	**Type of Diet**	**Na Estimation Method**	**Na intake/Excretion (mg/d)**	**Vascular Assessment**	**Results**	
													Intervention Groups	**Vascular Change (mm/mmHg)**
Creager MA. (1991) (217)	USA	cross-over RCT	NT men	M	N/AV	17	30± 2 years	10 days	LS diet: 230 mg Na/d vs. HS diet: 4600 mg Na/d	24UC	LS: 253 ± 46 vs. HS: 4117 ± 207	diastolic blood pressure time decay method	LS vs. HS	≠
Gates PE. (2004) (218)	USA	cross-over dbRCT	stage 1 HT adults, older than 50	M/F	White	12	men: 63±1 women: 64±4	8 weeks	LS diet: 1196±92 & Normal Na diet: 1311±23	3 day dietary records & 24UC	Na excretion Baseline: 3105 LS: 1380 Normal Na: 3450	B-mode US	LS	+0.04 *
													Normal	≠

The mmol of Na intake/excretion values were converted to mg. If available, results presented come from adjusted models. Abbreviations: Na: sodium; 24UC: 24h urine collection; cfPWV: carotid–femoral pulse wave velocity; baPWV: brachial–ankle pulse wave velocity; hfPWV: heart-femoral pulse wave velocity; HT: hypertensives; NT: normotensives; PHT: pre-hypertensives; HTN: hypertension; SBP: systolic blood pressure; anti-HT: antihypertensive; OW: overweight; OB: obese; NOB: non-obese; suppl: supplementation; HS: high sodium; LS: low sodium; SS: salt sensitive; SR: salt resistant; RCT: randomized controlled trial; sbRCT: single-blind RCT; dbRCT: double-blind RCT; M/F: males & females; F: females; M: males; N/AV: not available; CKD: chronic kidney disease; US: ultrasonography; ≠: no statistically significant association; *: $p < 0.05$; **: $p < 0.01$.

Table 19. Descriptive characteristics of observational studies regarding arterial remodeling. *Published in Nutrients, 2019 (204).*

Arterial Remodeling												
cIMT												
1. Observational Cross-Sectional Studies												
Author (Year)	**Country**	**Study Design**	**Population Description**	**FU (Years)**	**Sex**	**Race**	**N**	**Age (Years, Mean ± SD)**	**Na Estimation Method**	**Na Intake/Excretion (mg/d)**	**Vascular Assessment**	**Results**
Ferreira-Sae, M.C. (2011) (158)	Brazil	c-sect	HT adults	-	M/F	N/AV	134	58 ± 1	1. FFQ 2. 24h recall 3.discretionary Na intake[1]	Na intake/d: 5520 ± 290 FFQ: 1450 ± 180 24h recall: 940 ± 70 Discretionary Na: 3130 ± 190	B-mode US	+ *
Njoroge, J.N. (2011) (159)	USA	c-sect	OW or OB, physically inactive adults	-	M/F	Mixed	258	Total: 38.5 ± 5.8 Q1: 39.3 ± 5.6 Q2: 38.7 ± 5.2 Q3: 37.6 ± 6.2 Q4: 38.2 ± 6	24UC	Total:1104–9545 Q1: 1104–3289 Q2: 3312–4117 Q3: 4140–5152 Q4: 5175–9545	B-mode US	+ *
García-Ortiz, L. (2012) (148)	Spain	c-sect	primary care patients aged 30–80	-	M/F	N/AV	351	Total: 54.8 ± 11.7 Q1: 57.6 ± 12.1 Q2: 55.9 ± 11.3 Q3: 54.7 ± 10.5	FFQ	Total: 3180 ± 1250 Q1:1800 ± 390 Q2: 2650 ± 200 Q3: 3440 ± 270	B-mode US	J-shaped
Lee SK. (2015) (149)	Korea	c-sect	non-HT subjects, with no use of anti-HT drugs	-	M/F	Asian	1586	tertile 1: 52.1 ± 5.5 tertile 2: 53.0 ± 6.0 tertile 3: 52.6 ± 5.5	second morning void & Tanaka's equation	3588±782	B-mode US	− **
Ustundag, S. (2015) (219)	Turkey	c-sect	ambulatory adult patients, in stage 2–4 CKD	-	M/F	N/AV	193	Na excretion <1955 mg/day: 47.7 ± 10.6 ≥1955 mg/day: 49.7 ± 11.0 Mean IMT <0.750 mm: 45.1 ± 12.2 ≥0.750 mm: 52.3 ± 8.3	24UC	<1955 mg/day: 3220 ± 69 ≥1955 mg/day: 3220 ± 69 Mean IMT < 0.750 mm: 3220 ± 46 Mean IMT ≥ 0.750 mm: 3220 ± 69	B-mode US	+ **
Dai, X.W. (2016) (139)	China	c-sect	Asian adults, via subject referral and community advertisement	-	M/F	Asian	3290	M: 62.1 ± 6.7 F: 59.4 ± 5.5	FFQ	Dietary Na intake: Q1: 833 ± 394 Q2: 864 ± 507 Q3: 825 ± 41 Q4: 828 ± 395	B-mode US	common cIMT ≠ carotid bifurcation IMT + *
Mazza, E. (2018) (140)	Italy	c-sect	adults aged ≥65, not suffering from any debilitating diseases	-	F	White	108	70 ± 4	24h dietary recall + 7-day food record	1476 ± 618	B-mode US	+ *

Table 19 *Cont.*

2. Observational Studies with Follow up (>1 Time Points)													
												cIMT	
Jung, S. (2019) (155)	South Korea	prosp	adults aged >40	5.4 ± 1.0	M/F	Mixed	2494	60.2 ± 9.0	FFQ + 3 day diet record	2644 ± 1573	B-mode US	Na baseline	≠
												Na average of three visits	+ **
												ΔcIMT	
												Na baseline	≠
												Na average of three visits	− *

The mmol of Na intake/excretion values were converted to mg. If available, results presented come from adjusted models. Abbreviations: Na: sodium; 24UC: 24h urine collection; cIMT: carotid Intima Media Thickness; HT: hypertensives; anti-HT: antihypertensive; OW: overweight; OB: obese; c-sect: cross-sectional; prosp: prospective; FFQ: food frequency questionnaire; FU: follow up; M/F: males & females; F: females; N/AV: not available; CKD: chronic kidney disease; US: ultrasonography; +: positive association; -: negative association; ≠: no statistically significant association; *: $p < 0.05$; **: $p < 0.01$. [1] number of 1 kg packages of salt consumed/month/person.

Table 20: Descriptive characteristics of interventional studies regarding arterial remodeling. *Published in Nutrients, 2019 (204).*

Arterial Remodeling													
Right Branchial Artery and Common Carotid Artery Diameter													
Author	**Country**	**Study Design**	**Population Description**	**Sex**	**Race**	**N**	**Age (Years, Mean ± SD)**	**Intervention Duration (Weeks)**	**Type of Diet**	**Na Estimation Method**	**Na intake/Excretion (mg/d)**	**Vascular Assessment**	**Results**
													LS diet vs. NS diet
Benetos, A. (1992) (220)	France	cross-over dbRCT	actively working, mild to moderate HT adults	M/F	N/AV	20	41.5 ± 2.4	8	Group 1 and Group 2: Normal Na diet (NS diet, Na capsules): 1400 mg and low-Na diet (LS diet), lactose capsules	24UC	Baseline: 3979 ± 299 LS diet: 1955 ± 220.8 NS diet: 3749 ± 305.9	B-mode US	Brachial artery diameter: +0.67m **
													common carotid diameter: ≠

The mmol of Na intake/excretion values were converted to mg. If available, results presented come from adjusted models. Abbreviations: sodium; 24UC: 24h urine collection; HT: hypertensives; LS: low sodium; dbRCT: double-blind RCT; M/F: males & females; N/AV: not available; US: ultrasonography; ≠: no statistically significant association; **: $p < 0.01$.

Table 21. Descriptive characteristics of observational studies regarding atheromatosis. *Published in Nutrients, 2019 (204).*

Atheromatosis											
Carotid Plaques											
Author (Year)	**Country**	**Study Design**	**Population Description**	**FU (Years)**	**Sex**	**Race**	**N**	**Age (Years, Mean ± SD)**	**Na Estimation Method**	**Na Intake/Excretion (mg/d)**	**Vascular Assessment**	**Results**
Dai, X.W. (2016) (139)	China	c-sect	Asian adults, via subject referral and community advertisement	-	M/F	Asian	3290	M: 62.1 ± 6.7 F: 59.4 ± 5.5	FFQ	Dietary Na intake: Q1: 833 ± 394 Q2: 864 ± 507 Q3: 825 ± 41 Q4: 828 ± 395	B-mode US	≠
Mazza, E. (2018) (140)	Italy	c-sect	Adults aged ≥65, not suffering from any debilitating diseases	-	F	White	108	70 ± 4	24h dietary recall + 7-day food record	1476 ± 618 Tertile I: 780–900 Tertile II: 1330–1430 Tertile III: 2050–2330	B-mode US	Tertile III vs. Tertile I + *

The mmol of Na intake/excretion values were converted to mg. If available, results presented come from adjusted models. Abbreviations: Na: sodium; c-sect: cross-sectional; FFQ: food frequency questionnaire; FU: follow up; M/F: males & females; F: females; US: ultrasonography; +: positive association; ≠: no statistically significant association; *: $p < 0.05$.

Population description and exclusion criteria are reported in **Table 22**.

Table 22: Population Description and Exclusion Criteria of Selected Studies. *Published in Nutrients, 2019 (204).*

Arteriosclerosis—Observational Studies		
cfPWV		
1. Observational Cross-Sectional Studies		
Author (year)	**Population Description**	**Exclusion Criteria**
Polónia, J. (2006)	essential hypertensives, recent stroke, or healthy university students	urine sample not meeting the required quality criteria
García-Ortiz, L. (2012)	primary care patients aged 30–80	cardiovascular and/or cerebrovascular disease
Kotliar, C. (2014)	essential hypertensives, aged 30 to 70	abnormal renal function; volume or electrolyte alterations; diabetes mellitus; history of renal disease, ischemic heart disease, stroke; loss of data; counter indication for the drug washout; use of corticoids or nonsteroidal anti-inflammatory drugs during the study
Polonia, J. (2016)	hypertensive adults	secondary hypertension, previous cardiovascular events history; estimated glomerular filtration rate >50 mL/min/1.73
Strauss, M. (2018)	normotensive adults	previously diagnosed chronic illness (self-reported); use of anti-hypertensive drugs or other chronic diseases; diabetes mellitus; HIV infected; microalbuminuria>30 mg/mL; pregnancy or lactation
Triantafyllou, A. (2018)	newly diagnosed & never-treated hypertensives—healthy individuals admitted for regular check-up	previously treated with anti-hypertensive drugs; use of any kind of drugs; other known health problems; secondary causes of hypertension; other comorbidities (e.g., diabetes mellitus, CVD)
2. Observational Studies with Follow up (>1 Time Points)		
Nerbass, F.B. (2015)	adults in CKD stage 3	solid organ transplant or terminally illness
Aortic PWV Other Than cfPWV		
Siriopol, D. (2018)	hypertensive & normotensive Romanian adults	use of diuretic treatment; CKD; missing values for the variables of interest
baPWV		
1. Observational Cross-Sectional Studies		
Sonoda, H. (2012)	healthy subjects	heart failure; valvular heart disease; atrial fibrillation; peripheral artery disease
Lee, S.K. (2015)	non-hypertensive subjects, with no use of anti-hypertensive drugs	age >70 years; preexisting CVD including significant valvular heart diseases and arrhythmia; chronic renal disease or a serum creatinine level >1.5 mg/dL; unavailable urinary Na data, inadequate data of tissue Doppler echocardiography, carotid ultrasound, or baPWV; ejection fraction of <55% after echocardiography
Sun, N. (2015)	newly diagnosed hypertensives, untreated hypertensives or patients with a 1-to-5-year history of hypertensives who had stopped taking anti-hypertensive drugs for 1 month	use of anti-hypertensive drugs; secondary hypertension; hypertensive emergency; hypertensive urgency; acute coronary syndrome; severe arrhythmias; DM; stroke; CKD
Han, W. (2017)	hypertensive adults	any secondary cause of hypertension; hypertension emergencies; serious arrhythmia; peripheral arterial disease; heart failure; impaired renal function with plasma creatinine ≥150 μmol/L; rheumatic & autoimmune diseases; malignancies
2. Observational Studies with Follow up (>1 Time Points)		
Jung, S. (2019)	adults aged >40	history of heart disease, stroke, and/or cancer; anti-hypertensive drugs; diabetes mellitus; dyslipidemia; implausible dietary intake reported (< 500 or > 4000 kcal/day); missing general characteristic data from the baseline visit
Common Carotid Arterial Elasticity (Young's Elastic Modulus, Stiffness Index, Arterial Compliance)		
Ferreira-Sae, M.C. (2011)	hypertensive adults	age <18 years; neoplastic disease; secondary hypertension

Table 22 *Cont.*

Arteriosclerosis—Interventional studies		
cfPWV		
Seals, D.R. (2001)	postmenopausal women, ≥50 years, high normal SBP or Stage 1 hypertension	anti-hypertensive drugs; other chronic disease; low-Na diet or regular exercise during the preceding 2 years; smoking
Dickinson, K.M. (2009)	overweight/obese, mild hypertensive adults	metabolic disease; CVD; SBP >160 mm Hg at screening; significant weight loss in the preceding 6 months (>2 kg); BMI < 27 or > 40; use of anti-hypertensive drugs
He, F.J. (2009)	hypertensive adults	anti-hypertensive drugs; secondary cause of hypertension; impaired renal function; previous stroke; ischemic heart disease; heart failure; diabetes mellitus; malignancy; liver disease; pregnancy or lactation or on oral contraceptive pills
Pimenta, E. (2009)	resistant hypertensive adults on stable anti-hypertensive drugs	history of atherosclerotic disease (in the previous 6 months); congestive heart failure; diabetes mellitus on insulin treatment; office blood pressure > 160/100 mm Hg
Todd, A.S. (2010)	Pre-hypertensive or hypertensive, non-obese adults or in anti-hypertensive drugs	age >65; smoking; history of CVD or diabetes mellitus or renal disease
Todd, A.S. (2012)	normotensives, non-obese adults	age >65; antihypertensive medication; smoking; history of cardiovascular disease or diabetes mellitus or renal disease
McMahon, E.J. (2013)	hypertensive adult patients with stage 3 or 4 CKD (GFR 15–59 mL/min per 1.73 m^2), non-dialyzed, non-transplanted	salt-wasting CKD, pregnant or breastfeeding, current prescription of medications providing 0.20 mmol sodium per day, life expectancy,6 months, current involvement in another intervention study, or insufficient mental or physical capacity to adhere to the study protocol.
Dickinson, K.M. (2014)	overweight or obese subjects	diabetes mellitus; dyslipidemia; inflammatory bowel disease; pulmonary disease or vasculitis
Gijsbers, L. (2015)	untreated prehypertensives, aged 40–80	smoking; diabetes mellitus, CVD; gastrointestinal, liver or renal diseases; BMI > 40; use of drugs known to affect the cardiovascular system; use of nutritional supplements, an energy-restricted or medically prescribed diet; unstable body weight in past 2 months; alcohol use over 21 (women) or 28 (men) consumptions/week; pregnancy or lactation
Suckling, F.J. (2016)	untreated hypertensive adults with diet-controlled type 2 diabetes mellitus or impaired glucose tolerance	any secondary causes of hypertension, impaired renal function (plasma creatinine >150 µmol), uncontrolled heart failure, ischemic heart disease, previous stroke, active malignancy or liver disease, pregnancy, breast feeding, or oral contraceptive drugs
van der Graaf, A.M. (2016)	women with history of preeclampsia or history of healthy former pregnancy	renal disease; diabetes mellitus or a history of gestational diabetes; obesity; use of anti-hypertensive drugs; pregnancy; lactation; postmenopausal status; use of oral contraceptives
Muth, B.J. (2017)	healthy, normotensive adults	history of hypertension; CVD; malignancy; diabetes mellitus; renal impairment; obesity; smoking
Aortic PWV (Other than cfPWV)		
Avolio, A.P. (1986)	healthy normotensive adults & children	N/AV
hfPWV		
Rhee, M.Y. (2016)	normotensive & hypertensive adults	stage 2 and 3 hypertension; secondary hypertension; angina pectoris; myocardial infarction; congestive cardiac failure; stroke; diabetes mellitus; CKD
baPWV		
Wang, Y. (2015)	mild hypertensive adults	stage 2 hypertension; history of clinical CVD; CKD; diabetes mellitus; use of anti-hypertensive drugs; high alcohol intake
Arterial Elasticity (Arterial Compliance)		
Creager, M.A. (1991)	normotensive men	hematologic, renal, or hepatic dysfunction
Gates, P.E. (2004)	hypertensive adults (stage 1), older than 50	use of anti-hypertensive drugs; abnormal blood chemistry; positive ECG-monitored exercise test; ankle–brachial index > 0.9; presence of plaque on ultrasound interrogation of the carotid and femoral arteries; smoking for previous 2 years; BMI < 35; consumption of a low-Na diet; not in postmenopausal if female (amenorrheic for at least 2 years)

Table 22 *Cont.*

Arterial Remodeling—Observational Studies		
cIMT		
1. Observational Cross-Sectional Studies		
Ferreira-Sae, M.C. (2011)	hypertensive adults	age <18 years; neoplastic disease; secondary hypertension
Njoroge, J.N. (2011)	overweight or obese, physically inactive adults	diabetes mellitus; anti-hypertensive drugs or average baseline SBP of ≥140 or DBP ≥ 90 mmHg; cholesterol lowering or anti-psychotic or vasoactive drugs; use of vasoactive devices; pregnancy or lactation
García-Ortiz, L. (2012)	primary care patients aged 30–80	cardiovascular and/or cerebrovascular disease
Lee, S.K. (2015)	non-hypertensive individuals, with no use of anti-hypertensive drugs	age >70 years; preexisting CVD including significant valvular heart diseases and arrhythmia; chronic renal disease or a serum creatinine level > 1.5 mg/dL; unavailable urinary Na data, inadequate data of tissue Doppler echocardiography, carotid ultrasound, or baPWV; ejection fraction of <55% after echocardiography
Ustundag, S. (2015)	ambulatory adult patients, in stage 2–4 CKD	BMI < 35 kg/m2; diabetes mellitus; salt-losing nephropathy or history of malignancy or cardio-cerebrovascular disease or any acute disease
Dai, X.W. (2016)	Asian adults, via subject referral and community advertisement	hospital-confirmed diabetes mellitus; CVD; renal failure; CKD; cancer
Mazza, E. (2018)	adults aged ≥65, not suffering from any debilitating diseases	history of CVD or thyroid dysfunction or excessive alcohol consumption; use of dietary supplements & psychotropic drugs
2. Observational studies with follow up (>1 time points)		
Jung, S. (2019)	adults aged >40	history of heart disease, stroke, and/or cancer; anti-hypertensive drugs; diabetes mellitus; dyslipidemia; implausible dietary intake reported (< 500 or > 4000 kcal/day); missing general characteristic data from the baseline visit
Arterial Remodeling—Interventional Studies		
Right Branchial Artery & Common Carotid Artery Diameter		
Benetos, A. (1992)	actively working, mild to moderate hypertensive adults	cardiac, neurologic or renal involvement or arteriosclerosis obliterans of the lower limbs
Atheromatosis—Observational Studies		
Carotid Plaques		
Dai, X.W. (2016)	Asian adults, via subject referral and community advertisement	hospital-confirmed diabetes mellitus; CVD; renal failure; CKD; cancer
Mazza, E. (2018)	adults aged ≥65, not suffering from any debilitating diseases	history of CVD or thyroid dysfunction or excessive alcohol consumption; use of dietary supplements & psychotropic drugs

Abbreviations: CKD: chronic kidney disease; CVD: cardiovascular disease; BMI: body mass index; Na: Sodium; N/AV: not available.

Studies Investigating Arteriosclerosis (Arterial Stiffness)

Thirty-one (31) studies examining arteriosclerosis were identified—of which, 14 were observational (144, 145, 148, 149, 155-158, 180, 205-209) and 17 were interventional (141-143, 146, 147, 160, 161, 176, 181, 210, 211, 213-218).

Observational Studies

From the observational studies investigating arteriosclerosis, 11 out of 14 found a positive association between arterial stiffness biomarkers and dietary Na (144, 145, 155-158, 180, 205, 207-209), one out of 14 found a J–shaped association (148), one out of 14 found an inverse association (149) and one out of 14 found no association (206) (**Table 17**). From the above 11

studies showing a positive association, nine of them measured vascular parameters at one time point (144, 145, 156-158, 180, 205, 208, 209) and the remaining two studies evaluated arterial stiffness at two different time points (155, 207).

Heterogeneity in the assessment of arterial stiffness existed in the above 11 studies (144, 145, 155-158, 180, 205, 207-209) due to: (a) various arterial stiffness biomarkers using different methodologies (four applanation tonometry (145, 156, 180, 205), six oscillometry (144, 155, 157, 207-209) and one b-mode ultrasonography (158)) at different arterial segments using various arterial stiffness biomarkers (five cfPWV studies (144, 160, 207-209), one aortic PWV other than cfPWV (208), four baPWV (144, 155, 157, 209) and one common carotid artery elasticity (Young's elastic modulus, stiffness index, arterial compliance) (158)) (**Table 17**); (b) various Na assessment methods (seven studies used 24UC (144, 145, 156, 157, 180, 205, 209), two spot urine collections (207, 208) and two a combination of dietary methods (155, 158)); (c) different populations (five hypertensives (157, 158, 180, 205, 209), one normotensive (145), one chronic kidney disease patient (207), three mixed populations (155, 156, 208) and one healthy subjects (144) (**Table 17**).

Moreover, one out of the 11 studies showed that high Na excretion (mean: 2898 mg/day, range 2035.5–3588) is associated with cfPWV only when high Na excretion was combined with high RAAS activity but not in the other groups (i.e., those with high Na and low RAAS, low Na and low RAAS, as well as low Na and high RAAS) (205).

Only seven out of these 11 studies adjusted the results for BP level (144, 145, 156-158, 180, 209) and only three of them persistently showed a positive association between arterial stiffness and Na after the adjustment (156, 157, 209).

In the one study that showed an inverse association between arterial stiffness and Na, the result persisted after adjustment for BP level (149).

Finally, salt sensitivity assessment was not conducted in any of the above 14 observational studies.

Interventional Studies

From the 17 interventional studies investigating the association between arteriosclerosis (141-143, 146, 147, 160, 161, 176, 181, 210, 211, 213-218), seven of them showed statistically significant changes in arterial stiffness biomarkers after Na intake intervention (141-143, 146,

147, 210, 218). On the contrary, 10 out of the 17 interventional studies found no changes in arterial stiffness biomarkers with various levels of Na intake during the intervention (160, 161, 176, 181, 211, 213-217) (**Table 18**).

In detail, three out of seven that found significant changes showed that increases in dietary Na were associated with an increase in arterial stiffness biomarkers (141-143) and four out of the seven showed that a reduction in dietary Na intake was associated with a decrease in arterial stiffness biomarkers (146, 147, 210) or even an increase in arterial elasticity biomarkers (218) (**Table 18**). Three of these seven studies found statistically significant changes only in specific intervention groups (141, 142, 146, 147) (one study found that reduced Na excretion was associated with a decrease in cfPWV only in blacks, but not in whites and Asians (146); one study found that high Na intake was associated with increased hfPWV only in salt-sensitive but not in salt-resistant participants (142); one study found that a high-salt diet was associated with increased cfPWV only in middle-aged participants and not in young participants (141).

In those seven studies finding statistically significant changes in PWV after high or low-Na diets (141-143, 146, 147, 210, 218), heterogeneity existed, regarding: (a) different methodologies used for arterial stiffness assessment (three b-mode ultrasonography (142, 210, 218), one oscillometry (147) and three tonometry (141, 143, 146) and different arterial stiffness biomarkers assessed (four cfPWV (141, 143, 146, 210), one aortic PWV other than cfPWV (147), one heart-femoral (hfPWV) (142) and one arterial compliance (218)); (b) various methodologies used for Na assessment (four combination of dietary and urinary methods (143, 147, 210, 218), two 24UC (141, 146) and one not available data (142); (c) different duration of intervention period and (d) different populations (four in hypertensives or subjects with high normal BP (143, 146, 210, 218), two in normotensives (141, 147) and one in mixed populations (hypertensives and normotensives) (142) (**Table 18**).

Of note, out of the seven studies that found statistically significant associations between Na and arterial stiffness biomarkers (141-143, 146, 147, 210, 218), only three studies adjusted the results for BP level (141, 142, 210). One out of the three studies found that the statistically significant association between high-Na diet (6900 mg/day) and cfPWV in middle-aged adults was lost after correcting for the mean BP level (141). Other two studies found that their findings were independent from mean BP level (142, 210).

In the 10 studies that found no statistically significant changes in arterial stiffness biomarkers after different levels of Na intake (160, 161, 176, 181, 211, 213-217), heterogeneity existed,

regarding: (a) different methodologies used for arterial stiffness assessment (seven tonometry (160, 161, 181, 211, 213-215), one oscillometry (176), one plethysmography (216) and one diastolic blood pressure (DBP) time decay method (217)) using similar arterial stiffness biomarkers assessed (eight cfPWV (160, 161, 176, 181, 211, 213-215), one baPWV (216) and one arterial compliance (217)); (b) various methodologies used for Na assessment (eight 24UC (160, 181, 211, 213-217) and two combination of dietary and urinary methods (161, 176)); (c) different duration of intervention period and (d) different population samples (six in hypertensives (160, 176, 211, 213, 214, 216) (one in overweight or obese hypertensives (176), one in hypertensives with chronic kidney disease patients (211), three in hypertensives (160, 214, 216), one in prehypertensives (213)), two in normotensives (161, 217), one in overweight or obese subjects (181) and one in women with preeclampsia or healthy pregnancy in the past (215) (**Table 18**).

Finally, only two out of the 17 conducted salt sensitivity assessment (142, 216). One out of the two studies revealed that the result was not statistically significant in the salt-resistant group, but only in the salt-sensitive group (142). On the contrary, in the other study no significant differences between Na interventions and PWV were revealed for both salt-sensitive and salt-resistant participants, but salt-sensitive participants had higher baPWV at each time point of the intervention (baseline, low-Na diet, high-Na diet) (216).

Studies Investigating Arterial Remodeling

Nine studies examining arterial remodeling were identified (139, 140, 148, 149, 155, 158, 159, 219, 220)—of which, eight were observational (139, 140, 148, 149, 155, 158, 159, 219) (**Table 19**) and one was interventional (220) (**Table 20**).

Observational Studies

Out of the eight observational studies, six found positive (139, 140, 155, 158, 159, 219), one inverse (149) and one J-shaped associations (148) between cIMT and Na intake or excretion (**Table 19**). Out of the eight observational studies, seven measured the outcome at one time point (cross-sectional) (139, 140, 148, 149, 158, 159, 219) and one study measured the outcome at two time points and examined the association between the change of cIMT and Na intake (prospective) as well (155) (**Table 19**). In the prospective study, although the cIMT was positively associated with Na intake, the change of cIMT during follow up was negatively associated with Na intake (155) (**Table 19**). Four out of the six studies that found positive associations between cIMT and Na adjusted their results for BP level (84, 106, 158, 159): in two of them, the result was no more statistically significant after adjustment for BP (158, 159),

in one of the studies, the result was marginally not significant after BP adjustment (219) and in the remaining one, the result was independent from BP (139). The remaining two studies did not adjust their results for BP level (140, 155). Finally, one out of the six studies that found a positive association implied a statistically significant correlation only with IMT at the carotid bifurcation but not at the common carotid artery (139).

Heterogeneity in the assessment of arterial remodeling existed in the above six studies due to: (a) different Na assessment methods (four dietary (one (139)) or a combination of dietary (three (140, 155, 158)) methods, two 24UC (159, 219)) and (b) different studied populations (chronic diseases, age, comorbidities). All studies assessed cIMT as arterial remodeling biomarker via b-mode ultrasonography excluding from the measurement arterial segments with atheromatic plaques (**Table 19**).

The only study showing an inverse association was the only one conducted in purely normotensives as well as the only one using spot urine specimens for Na assessment (149). Adjustment for BP was performed in this study and the result was independent from BP level (149). The only study which showed a J-shaped association did not adjust the results for BP level (148).

Salt sensitivity assessment was not conducted in any of the eight studies.

Interventional Studies

The only interventional study that investigated the association between Na intake and arterial remodeling (**Table 20**) used brachial and carotid artery diameter as end point (220). The brachial artery lumen increased after 8 weeks of a low-Na diet (mean ± SD: 1955 ± 220.8 mg/day) but no changes in the common carotid diameter were revealed. The findings were adjusted for BP levels (220). Salt sensitivity assessment was not conducted (220) (**Table 20**).

Studies Investigating Atheromatosis

Only two observational studies examining atheromatosis were identified, showing conflicting results (139, 140) (**Table 21**). One study showed that higher Na intake (2050–2330 mg/day vs. 780–900 mg/day) is positively associated with the prevalence off carotid plaques (140), while the other study did not find a statistically significant association (139). The two studies assessed Na via different ways (dietary and urinary) and used different populations (elderly females (140) as well as a general population (139)). Both studies examined carotid plaques via B-mode ultrasonography. One out of the two studies adjusted their results for BP levels and the result was independent from BP (139). No study assessed salt sensitivity.

B4. Discussion

B4.1 Discussion for Cohort A

Investigation of the association between dietary Na and subclinical vascular damage

We investigated for the first time in a large sample of participants free of overt CVD, the association between dietary Na intake with two different types of subclinical arterial damage that were simultaneously measured. We observed a major and novel finding. The association of dietary Na intake with each type of subclinical vascular damage was different and rather diverging, implying that very low dietary Na intake has detrimental effects in atheromatosis but not arterial stiffness.

Recent data support a J-shaped association between Na intake and cardiovascular mortality (79, 80, 82, 85, 93). This association contrasts with the current guidelines, that suggest only an upper level of intake (2 g/day) that should not be overpassed (221, 222). However, a recent update on dietary Na reference values from the European Food Safety Authority, suggested that 2 g/day is *"a safe and adequate intake for the general European population of adults".* Indeed, studies investigating the role of Na in CV morbidity/ mortality in populations with chronic CV risk factors -like our study population- support the increased CV mortality when Na intake is very low (85, 93). A review of more than 360.000 volunteers revealed a "safe" range of Na intake of around 2500 to 6000 mg/day, above and below which the CV risk raises (84). In the present analysis the reference group had Na intake below 1g, i.e. substantial lower than the thresholds of the J-shaped curves (79-85). In line with the mortality outcome data mentioned above the present results imply that very low daily Na intake may have detrimental effects on the arterial wall by accelerating atheromatosis and thus provide a potential mechanism that may lead to increased mortality.

To our knowledge there are only two studies examining the association between daily Na intake/excretion and presence of atheromatic plaques (139, 140). Dai et al. studied 3.290 apparently healthy adults, using spot urine specimens and a food frequency questionnaire to estimate the excreted and dietary Na. They found positive association between Na excretion and carotid atheromatosis (139). However, they used the Na/creatinine ratio in their analysis, therefore their data are not informative regarding the actual level of Na intake. A second very small study in just 108 but elderly (mean age 70±4 years) females by Mazza et al., showed higher prevalence of carotid plaques in those individuals with higher versus those with lower Na intake (2050-2330 VS 780-900 mg Na/day) (140). There are major differences that may

explain the inconsistency between these studies and our data, including population heterogeneity, sample and age differences, data reporting and applied methodology. Most importantly the two studies (139, 140) measured only carotid atheromatosis. On the contrary, the present study investigated -for the first time- femoral atheromatosis too. Screening of the femoral arteries provides additive value for the evaluation of subclinical atherosclerosis and prediction of CV risk (223-228). The potential effect of Na on femoral plaque formation supports previous investigations showing that specific factors like smoking and impaired metabolism accelerate atheromatosis predominately at the femoral rather than the carotid arteries (228). More investigation on the potential arterial site-specific impact of Na is necessary and, it is essential to determine pathophysiological process in different sites of the arterial wall.

Given the fact that there are major sex-specific differences in the pathophysiology of arterial function (229), we tested and found interaction between sex and Na intake, regarding their association with subclinical arterial damage. One of our major findings was that only women (but not men) with moderate Na intake had significantly lower probability to present femoral plaques compared to those with very low Na intake. Sex-specific differences in CV (229) and more specifically in vascular (230-242) function and dysfunction have been well described previously. Sex hormones lead to different stimulation of renin-angiotensin-aldosterone system, catecholamines and endothelin levels, nitric oxide production and vasodilation (243-248). Although data regarding a sex-specific effect of Na on vascular function on young humans do exist, showing that endothelial dysfunction – i.e. the origin of atheromatosis - is modulated by dietary Na intake level mainly in men but not in women (249), data on middle-aged post-menopausal women (the target population of our study) are lacking. Likewise very limited data from female animal models exist, showing that the protective effects of estrogen apart from the increase in BP were only manifested in the setting of a chronic high Na diet and suggest that the underlying Na status may have an important influence on the overall effect of reduced estrogen (250).

It was rather surprising that –even though arterial stiffness and atheromatosis share common risk factors- our results indicated a diverging association between the level of daily Na intake and each type of vascular damage, implying that very low intake has detrimental effects in atheromatosis but not arterial stiffness. However, aortic PWV and plaque burden have been previously described to be weekly correlated, suggesting that arterial stiffening and atheromatosis are *"pathologically distinct and should be considered as separate disease processes"* (131). The association between Na intake/excretion and arterial stiffness has been widely investigated in cross sectional studies (144, 145, 148, 149, 212) and randomized

controlled trials (141-143, 146, 147, 161, 213, 216) with conflicting results. As a summary of the available data, a recent meta-analysis of 11 randomized controlled trials concluded that a restriction of about 2000 mg of Na/day reduces arterial stiffness (251). In an updated systematic review from our group on this topic, a positive trend between Na intake and arterial stiffness - in agreement with the herein presented data - was verified, however the current evidence does not support a clinically meaningful and independent from BP effect of dietary Na on arterial wall (204). Therefore our findings are in accordance with the majority of the studies mentioned above (142-146); nevertheless, it still remains in controversy whether high Na intake affects arterial stiffening in non-hypertensive populations (141, 161).

Our study cannot provide insight into the underlying molecular pathways activated in the presence of low Na intake in each arterial pathology. However, it is now very well known that the two types of vascular disease have distinct pathologies and even CV risk factors (130, 131). This is particularly true in the early steps of each pathology, i.e., before the development of arterial wall calcifications which is the case for the present population free of any clinical CVD (252). Most importantly, several studies in animals have provided compelling evidence that low salt diet is associated with accelerated atheromatosis (150-153, 253-255). Most of these studies implicate (i) activation of the renin-angiotensin-aldosterone system, (ii) increased inflammation and (iii) increased intracellular calcium, in the acceleration of atheromatosis, regardless of BP lowering (150-152, 253, 256, 257). On the other hand, animal studies show that high salt diet is associated with increased BP suggesting an explanation for the acceleration of arterial stiffness (153). Whether these mechanisms can be extrapolated in humans has to be extensively investigated.

The findings of the present study should be evaluated within the context of the particularities, advantages, and disadvantages of this cohort. Firstly, this is a cross-sectional study that does not allow to establish a cause-effect relationship; therefore, we cannot totally exclude the possibility that the observed associations represent a reverse causality phenomenon, i.e. increased subclinical arterial damage in the very low sodium intake due to very strict dietary consultation (45.1% were hypertensives, 16.4% used corticosteroids). However, that fact that we investigated simultaneously both the atheromatosis and the arterial stiffness with diverging results is against the reverse causality explanation because in this case we would expect similar findings in both vascular end points. Furthermore, when we excluded the participants who probably took strict guidance for Na restriction (hypertensives and cortisone users) similar findings were observed. Similarly, to all observational studies, uncontrolled confounding factors could have affected the results even though extensive and meticulous adjustments have been performed for all known and available confounders. Another limitation of this study is

that Na intake was assessed by 24DR even though 24UC is reported to be the most accurate method to assess population's Na intake. However, the correction of estimated Na derived from processed and unprocessed foods to total Na derived from foods, table salt and added during cooking salt, approaches better true dietary Na intake. In any case similar findings were observed when total or estimated Na intake was used in each statistical analysis. Finally, this was a large observational study regarding Greek population that allowed to test multiple vascular biomarkers at the same time. However, the upper limit of Na intake in the present study (2442.6±1317.5 mg for males and 1615.8±810.6 mg for females) was not high enough to allow the detection of J-shaped curve with atheromatosis.

B4.2 Discussion for Cohort B

Comparison of sodium assessment methods/ Design of a new sodium assessment tool

This study aimed to assess and compare the most used in population studies UMs and DMs for mean Na intake and develop a new accurate and easy to use clinical tool for Na estimation in high CVD risk populations. The main findings of this study are: (i) the existing DMs tend to underestimate and spot urine methods tend to overestimate the true Na intake; (ii) all the existing DMs are weakly correlated and present poor agreement with the 24UC and all the spot urine methods are moderately correlated and present moderate agreement with the 24UC; (iii) the new NaFFQ is the only method that performed better in the analysis, having simultaneously: the smallest bias in mean differences, the strongest correlation with the 24UC regarding DMs and a homogeneous variation as the mean Na intake increases in Bland-Altman plots, but still wide limits of agreement.

Spot urine collection is an easily applicable alternative in estimating dietary Na intake. Increasing studies aim to reveal the most accurate formula for converting spot Na to 24UNa, comparing not only those commonly used (50-52, 258-260) but also those newly designed (261, 262) against the gold-standard 24UC. The most studied formulas are the INTERSALT equation, the Tanaka equation and the Kawasaki equation. Despite some controversies (262), a large number of studies support that among the existing equations, the INTERSALT performs better in estimating the 24UNa showing the least bias (258, 259, 261, 263, 264). In our findings, the INTERSALT equation presented the lowest bias among all the other equations; however, it was moderately correlated with 24UNa and presented consistent bias in Bland-Altman plots by underestimating Na intake at low levels of Na excretion and overestimating at high levels of Na excretion. However it is important to note that the studies supporting the use of

INTERSALT equation as the best alternative of 24UC for Na estimation, have all been conducted in general populations (258, 259, 261, 263, 264), which is in contrast to our high CVD risk population. Indeed, the evidence is not supportive of the use of the INTERSALT equation in high-risk patients, having chronic diseases such as chronic kidney disease or hypertension (260, 265). Dougher C.E. et al, compared commonly used equations for Na estimation in 129 chronic kidney disease patients (265). According to their findings, the authors conclude that spot urine equations do not accurately estimate dietary Na intake in this group of people. Similarly, when Ma W. et al, assessed Na intake by the INTERSALT, the Tanaka and the Kawasaki equations in 365 high risk of stroke patients, they found poor correlations (r=0.35-0.38), poor reliability (ICCs=0.31-0.38) and significant biases among all the three methods compared to the 24UC (260). These findings agree not only with our study, but with a significant number of studies, which do not recommend the use of spot equations for dietary Na estimation (50-54). It is important to note that Na excretion presents a circadian variability, which potentially could influence the estimations derived from spot urine collections. A systematic review of studies comparing the 24UC and spot urine collections for estimating salt intake, conducted by Ji C. et al. included 20 studies and 1.380.130 participants, concluded that although it is of great interest to replace the 24UC as a method for Na intake estimation, the best alternative UM remains uncertain as a wide range of correlations (r=0.17-0.94) between 24UC and the other methods presented in their work (70). Also, a systematic review and meta-analysis in 10.414 participants from 34 countries, showed that *"estimates based upon spot urine samples have excellent sensitivity (97%) and specificity (100%) at classifying mean population salt intake above or below the World Health Organization maximum target of 5 g/day but underestimate intake at high levels of consumption and overestimate at lower levels of consumption"* (22). Even more interestingly, in a recent analysis of TOHP (Trials of Hypertension Prevention) study follow-up data, conducted by He FJ et al., estimated values of Na excretion (using the Kawasaki, INTERSALT with spot K and Tanaka equations) – examining the same population sample- altered the linear association between 24UNa and mortality to J- or U- shaped (106). The authors concluded that these urinary Na estimation methods *"were systematically biased with overestimation at lower levels and underestimation at higher levels"*, indicating that estimation of Na through spot urine specimens is inaccurate (106). All these findings are consistent to a WHO/PAHO statement in the protocol for population level Na determination in 24h urine sample (266), declaring that *"the use of spot-urine is discouraged as a method to determine Na, potassium or iodine intake because of the limitations and uncertainty inherent in the method"*.

As regards the existing DMs for Na estimation, although it is useful and efficient to highlight food items rich in Na, several methodological disadvantages have been raised. The most commonly discussed include: the difficulty or even inability to assess and quantify discretionary Na; deviated estimations of Na due to high variability in Na content in recipes of homemade and manufactured food; the absence of Na derived from medicines and dietary supplements and participant-related issues (under-reporting and difficulty to recall all the food and beverages consumed; socially desired answers, dietary behavior modification) (41). A small number of studies suggest that DMs, such as food diaries or multiple 24DR can be used for Na estimation, having the ability to predict over 90% of 24UNa (30, 267). However, most of the available studies, have reported that Na estimation based on DMs tend to underestimate 24UNa (levels of underreporting 15-40%) and correlates weakly or moderately with 24UNa ($r \approx 0.15\text{-}0.50$) (30, 58, 59, 268-271). This is in line with our findings, showing weak correlations, poor reliability, and high levels of bias, suggesting that the existing DMs for Na estimation are inaccurate. In a recent meta-analysis including 28 studies, McLean et al. compared 24DR with 24UC (60). 24DR underestimated mean Na intake by 607 mg/ day, but high quality 24DR improved accuracy. The authors concluded that 24UC remains the most accurate method to assess population Na intake, however high quality 24DR (use of multiple pass methods, accurate food composition databases and quantification of discretionary salt) could be used if 24UC is not feasible (60).

To our knowledge, studies comparing different DMs and UMs simultaneously for Na intake estimation are scarce. A recent study compared the spot urine collection (using the INTERSALT equation) vs the 24DR (without quantifying the discretionary use of table salt) in a large sample of adults in New Zealand, consisting of 3321 participants (272). The authors observed poor agreement between estimated Na intake from spot urine collection and those from 24DR (272). In another study a plethora of different DMs and UMs were compared with a PABA-validated 24UC (273). The assessment of Na intake included a FFQ, a modified 24DR and three equations to convert the spot Na to 24UNa (INTERSALT, Tanaka and Kawasaki). In this study neither DMs nor UMs provided accurate estimations at individual level but for group means, the DMs and some of the UMs may be useful for Na estimation (273). However, the method for the quantification of Na intake has not been clearly described (273).

FFQs are commonly used in dietary Na assessment in population-based studies, having the ability to bypass problems related to day-to-day variability of Na intake and cover larger time periods of intake. The last four decades several FFQs have been designed for the estimation of Na (or salt) intake (61, 62, 64, 75, 274-276). However, most of them present weak correlations

with the 24UC, ranging from 0.19 to 0.35 (61, 62, 75, 275). Furthermore, the available FFQs for Na assessment have been designed for ethnic groups (62, 63, 75, 275, 276). To our knowledge only two of them have been developed for hypertensive subjects (61, 63) but until now, there was no FFQ for Na estimation in other high CV risk groups, such as patients with dyslipidemia, diabetes mellitus, infectious or autoimmune diseases. Recently, McLean et al. published a systematic review of the literature, regarding the assessment of dietary Na intake using FFQs and 24UC (59). This work revealed a poor agreement between estimates of Na from FFQ and 24UC (59), indicating that the Na FFQs until now are inadequate to estimate the true intake.

The novel NaFFQ was created to accurately estimate Na intake in high CVD risk populations, calculating not only Na derived from food content, but table and cooking Na as well. Our aim was to cover the need of an easily applicable in epidemiological studies and reliable tool for group means of Na intake, which could lead to a better management of high CVD risk populations. According to our findings, this tool presented the best correlation with- and the lowest bias from- the 24UC compared to all the existing DMs, even when attempts to further improve the accuracy of 24DR were applied. However, despite these promising findings regarding NaFFQ, it provided very wide limits of agreement in Bland-Altman plots, reaching ~3000mg/day, indicating that future improvements must be addressed. A limitation of our study is the use of a single 24UC. Due to the day-to-day variability in Na intake and excretion, multiple 24UC are recommended either for assessing accurately usual *individual* Na intake or for a more reliable record of dietary Na in studies investigating its relationship with health or disease (277, 278). In our study, our aim was to estimate Na intake in group means and not in individual level, so the use of single 24UC, which is very common in epidemiological studies, was reasonable. Indeed, the use of a single 24UC versus three to seven 24UC have been reported to provide similar mean levels of Na excretion at the population level (279). Secondly, an important limitation to be mentioned is the method used for the quantification of discretionary salt. In our study, the use of dashes of salt as well as the cut-offs which were designed for processed food, may lead to several concerns and systematic bias. However, until today, the estimation of discretionary salt in studies remains a challenge for the investigators and there is no generally accepted protocol to be applied in dietary surveys (280). Moreover, the NaFFQ is population specific and has not been externally validated in other populations. However, the methodology used here could be used to adapt other FFQs, designed for other population groups, to estimate Na intake more accurately.

B4.3 Discussion for the systematic literature review

Current data on dietary sodium, arterial structure, and function

We performed a systematic review of the literature to investigate the relationship between dietary Na intake with arterial function and structure using gold-standard non-invasive vascular biomarkers to measure arteriosclerosis, arterial remodeling and atheromatosis. The results of this systematic review indicate that: (i) although several studies have investigated the association of dietary Na with arterial stiffness, the evidence does not clearly support a clinically meaningful, direct and independent from BP effect of Na on the arterial wall to increase arterial stiffness; (ii) data regarding the association between dietary Na and arterial remodeling are limited, mostly suggesting a positive trend between dietary Na and arterial hypertrophy, but still inconclusive; (iii) data regarding the association between dietary Na and atheromatosis are scarce and the available studies present high heterogeneity.

Dietary sodium and arteriosclerosis

Although 31 human studies have investigated the association between dietary Na and arteriosclerosis, the current data are inconclusive regarding a potential direct effect of Na on the arterial wall properties that accelerate the arterial stiffening process. Indeed, the majority of the studies (observational 11/14 and interventional 7/17) do imply the presence of a harmful effect of high Na intake (141-145, 155-158, 180, 205, 207-209) or even benefits of low Na intake on arterial stiffening parameters (146, 147, 210, 218) (18 out of 31, 11 observational and seven interventional), in various populations (145, 147, 156, 208, 210), involving several different segments of the arterial tree (142, 144, 156, 158, 208, 218), independently of the applied methodology, technology used (156, 158, 208, 210). However, most of these positive studies do not take into consideration the well-known effect of Na on BP increase (143, 146, 147, 155, 205, 207, 208). Overall, only 1/3 of the studies included in our analysis, and only 10 out of the 17 positive studies adjusted their findings for BP levels (141, 142, 144, 145, 156-158, 180, 209, 210). Even more interestingly, in more than half of them (six out of 10), the association between Na and indices of arteriosclerosis was lost after correcting for BP (141, 144, 145, 158, 180, 210). Moreover, although salt sensitivity is a major factor modulating the effect of Na on BP (and therefore to arterial stiffness), only two (142, 216) out of the 31 studies evaluated this parameter and showed conflicting results. Indeed, there is evidence suggesting that a high-salt diet would increase BP in 17% of the subjects (salt sensitives), reduce BP in 11% (inverse salt sensitive) and not significantly affect BP in the remaining salt-resistant subjects (281). Finally, just one study (148) showed a J-shaped association between Na and

arteriosclerosis, mirroring the recent epidemiological data on the J-shaped association between Na and mortality.

A recent meta-analysis of randomized controlled trials, conducted by D' Elia and colleagues (251), being the first and the only one available on this topic so far, included 14 cohorts (all of them included in our work) and showed a statistically significant decrease by 2.84% in cfPWV after an average reduction of approximately 2 g (89.3 mmol) per day in Na intake independently from BP. In this meta-analysis, the authors excluded all the studies measuring other than the cfPWV, whereas we extended our systematic review to include all valid non-invasive indices of arterial stiffness including other segments of the arterial bed (such as the carotid artery and the lower limbs). Although our study is not applying a synthesis of quantitative data (as a meta-analysis) but uses only the qualitative characteristics of the selected studies, it is important to consider that the result of D' Elia et al. suggest poor, if any, clinical effects of Na on arterial stiffness. A reduction of PWV by 2.84% may not offer additional benefit in overall vascular health.

Taken all together, these data suggest that arterial stiffness can be reduced with a dietary intervention aiming at the reduction of dietary Na intake, but: (a) this reduction is modest (e.g., aortic stiffness of 10 m/s considered the high CVD risk cut-off level will be reduced to 9.8 m/s after a major reduction of Na by 2g/day) with debatable clinical effect and (b) it is not established whether this lowering effect is mediated only by BP reduction or mediated by a direct effect on the arterial wall (162, 282).

Moreover, major questions seek suitable answers, since poor data regarding the role of salt sensitivity, the RAAS, age and race exist. The hypothesis that hyperactive RAAS leads to BP elevation and consequently arterial stiffening, because of BP rising in salt-sensitive subjects, cannot yet be rejected. In a single study, Kotliar et al. indicate a significant positive association between Na and PWV only in the group of participants who had high RAAS activity. However, the group with high Na and low RAAS activity did not show a significant association with PWV (205). One of the studies suggested that only middle-aged and not young participants presented increased PWV after a high-salt diet (141). However, in the study by Avolio et al., all of the age groups (children, young adults and middle-aged adults) decreased their PWV after reducing Na intake (147). Finally, even though race has been shown to play a significant role in BP levels and salt sensitivity, indirectly affecting arterial stiffening, just one study addressed this issue and showed significant increases in PWV after Na supplementation only in black participants.

Dietary sodium and arterial remodeling

Nine studies—all of them using B-mode ultrasonography—investigating the association between dietary Na and arterial remodeling were identified (eight observational (139, 140, 148, 149, 155, 158, 159, 219, 220) and one interventional (220)). The majority of them (six out of the nine) implied a detrimental effect after high Na intake (139, 140, 155, 158, 159, 219) or even a beneficial effect after low Na intake on arterial remodeling parameters (cIMT or artery diameters) independently of different methods used for Na assessment and various population groups (different diseases and comorbidities, age groups, etc.). In most cases, higher dietary Na intake was associated with higher cIMT in plaque-free arterial segments, mostly at the common carotid, suggesting arterial hypertrophy, but also carotid bulb (139) and brachial artery (220).

However, only three out of the nine studies included large population samples (>1500 participants) (139, 149, 155) and their results were conflicting, since one of them found an inverse and BP-independent association between Na and cIMT but was the only one conducted in purely normotensives and assessed Na through spot urine specimens as well (149). Probably, the best available study so far, the only interventional study published by Benetos et al., showed that independently from BP, increased Na intake only induced arterial remodeling in a muscular artery (brachial artery) but not in an elastic one (carotid artery), suggesting a diverging effect of Na in different arterial beds (220).

Most importantly, once more, the effect of potential confounding BP on arterial remodeling was not taken into consideration in 1/3 of the studies (three out of nine) (140, 148, 155). Further, in two other studies (158, 159), the end point was mediated by BP increase. In conclusion, data on the association between dietary Na and arterial remodeling are limited, mostly suggesting a positive trend between dietary Na and arterial hypertrophy, but this is still inconclusive and conflicting. No study assessed salt sensitivity.

To our knowledge, the association between Na and arterial remodeling has not previously been subject to meta-analysis, and despite positive trends observed in most studies, there is insufficient data to conclusively establish the relationship.

Dietary sodium and atheromatosis

According to our systematic research, there is extremely limited data on the association between dietary Na and atheromatosis. Only two studies examined this association (139, 140). Mazza et al., in a very small study (140), found that high dietary Na is associated with the increased

prevalence of carotid plaques, whereas Dai et al., in a substantially larger study (139), suggested a non-significant association between dietary Na intake and carotid plaques. However, these studies presented heterogeneity in population samples (elderly females (140) and general population (139)), sample size (108 (140) and 3290 (139) participants) and Na assessment method (24DR and 7 day food record (140) and FFQ (139)). Moreover, the available studies regarding dietary Na and atheromatosis have not investigated the association between very low and very high levels of Na, and that might explain why a J-shaped trend has not been observed. Furthermore, beyond carotid arteries, plaque formation in other arterial segments that might offer an additive value in CVD prevention—such as the femoral arteries—has not been assessed in any of the available studies. In conclusion, there is not enough evidence to support a positive, negative or J-shaped association between dietary Na and arterial plaques and more studies investigating the association between larger ranges of Na intake/excretion and arterial plaques are needed.

Major strengths of our study are: (i) the novel concept of investigating the effect of dietary Na on SVD, including all the major pathogenetic mechanisms (arteriosclerosis, arterial remodeling & atheromatosis); (ii) the systematic nature of this review in order to compare and dispose all the available international literature on this specific topic; (iii) the design of our study, including clinical trials and evidence from observational studies in order to investigate the short- and long-term effects of different levels of Na intake on SVD. A limitation of our study is the absence of a quantitative analysis of the extracted data (meta-analysis), which could lead to a clearer view of the topic.

B5. Conclusions

This is the first large observational study examining simultaneously the association between dietary Na intake and two different types of arterial damage. Overall, the present data suggest that very low Na intake is associated with: a) accelerated atheromatosis, verifying findings from animal models, providing a possible explanation of the modern epidemiology, and b) lower arterial stiffness, which is in line with previous human and animal findings, therefore suggesting a diverging effect of Na in the two major arterial pathologies. However, there is not yet enough evidence to support a direct and causal association between Na and each of the major types of SVD, even in the most widely studied case of arteriosclerosis (arterial stiffening). The available data derive mostly from small, heterogeneous, not well-designed studies. Especially in the case of arterial remodeling and atheromatosis, both common and clinically relevant types of structural arterial damage have scarcely been investigated in relation to Na intake or excretion. Many studies included in our analysis do not address the cardinal effect of Na on BP and almost all of them neglect the role of salt-sensitivity. Future studies using novel diagnostic tests for individuals' salt sensitivity assessment are needed to clarify the role of dietary Na to SVD (109). One of the dominant issues is the heterogeneity of the studies in the Na assessment method. Precise quantification of Na intake is difficult and even though only the 24UC is regarded as a gold standard, based on the knowledge that approximately 90% of Na intake is excreted through urine, other dietary or spot urinary methods are commonly used in studies. Several disadvantages of the above-mentioned studies have been described, such as underreporting, equations suitable only for specific population groups, different recipes, etc., leading to inaccurate measurements. Our findings suggested that the available DMs and spot urine methods present poor accuracy compared to the gold-standard 24UC. The new FFQ -specifically designed for Na estimation- is a promising method to detect a mean Na intake at population level in high CVD risk people, even though future validation of this tool in larger populations is needed to verify its accuracy and/or provide evidence for further amelioration, making it a reliable and easy to use clinical tool for Na quantification, in population-based studies. More well-designed interventional studies are needed to resolve all the remaining controversies.

B6. Perspectives

The safe range of dietary Na intake remains unclear. There are systematic methodological errors in observational studies conducted around this topic and the "J-shape hypothesis" cannot be neither neglected nor verified. Moreover, despite the large body of evidence from RCTs, suitable data remain limited and insufficient to address the specific question of the optimal daily Na intake range which is associated with the lower CV risk. However, the evidence regarding the high Na intake and CVD risk is clear: excessive dietary Na raises the risk. Thus, future actions should focus on reduction of high levels of Na intake, especially in high CVD risk population groups. According to a recent WHO report (283), that would be the most cost-effective and life-saving strategy for the public health. Such strategies include the following actions: a) high Na food products reformulations by the food industries; b) implementation of front-of-package nutritional labelling (FOPNL) systems to inform the consumer about the Na content in food; c) population's dietary behavior improvement around salt intake through educative massive campaigns and d) national policies regarding Na content on food served or sold. Undoubtedly, there is a great need for an accurate and easily applicable dietary Na assessment tool, which would be universally applied in research studies, providing the ability to compare findings from different studies. We suggest that a hybrid approach combining the methodology of validated and easy to apply questionnaires specially designed for Na intake estimation with that of an improved spot urine equation, should be tested as an alternative option. Moreover, the incorporation of genetic or environmental factors associated with salt-sensitivity are needed to further clear this field. Finally, it is important to conduct prospective RCTs that will examine the long-term effects of dietary Na intake on CV morbidity/mortality, including at least three levels of daily Na intakes (very low, "normal", and high)· since very low dietary Na diets have been suggested to associate with subclinical vascular damage in both human (203) and animal studies (150, 151, 253, 255). Moreover, considering the large variety of potentially confounding factors related to dietary intake and cardiovascular parameters, future studies should ensure that all of them are well-controlled and reliably assessed. Given the fact that large-scale, long-term, and high quality RCTs are needed to be conducted in healthy populations, as well as the high cost of these studies and the poor adherence of participants to low salt intake for long-term follow-up periods, it is important to note that the question will not be settled soon. Therefore, surrogate non-invasive vascular biomarkers might be incorporated in such future large clinical trials (130). Future perspectives could include innovative approaches in studies' methodology to bypass the above-mentioned issues. Until then, the clinical consultation for daily Na reduction should be primarily focused on high salt consumers and the recommendations should provide ranges of safe Na intakes which can be adopted easily,

rather than a higher cut off value. Until all the afore-mentioned issues are addressed, the J-shape hypothesis will remain an open challenge.

Abstract

Subclinical vascular damage (SVD) [arteriosclerosis (arterial stiffening), arterial remodeling and atheromatosis (arterial plaques)] pre-exists decades before cardiovascular disease (CVD) onset and represents a non-invasive method to evaluate CVD risk. Worldwide, sodium (Na) intake is almost double than international recommendations and has been linked with CVD and death, although sometimes in a J-shape manner. Until today, the available data on humans regarding SVD and dietary Na remain limited, especially for particular types of SVD like atheromatosis. Moreover, accurate and easy to use methods for dietary Na assessment in population level are lacking.

The present study was conducted in medium-to-high CVD risk Greek populations (arterial hypertension, diabetes mellitus, chronic inflammatory/ autoimmune diseases, HIV) to:

(i) record and quantify dietary Na intake through all the available assessment methods [dietary methods (e.g., multiple 24h dietary recalls (24DR) and food frequency questionnaires (FFQ) and urinary methods (e.g., spot urine samples using different formulas and 24h urine collection (24UC)];

(ii) compare all the examined Na assessment methods regarding their accuracy versus the gold-standard 24UC;

(iii) design and develop a new more accurate dietary tool to record Na intake (FFQ, food items rich in Na and salt-related questions were added in a standard questionnaire (NaFFQ)) and to improve the existing dietary methods (discretionary Na quantification using salt-related questions or adding extra 15% in total Na intake);

(iv) investigate the association between dietary Na intake and major types of SVD in our cohort study, using state-of-the-art methods to record SVD biomarkers (tonometry to assess arterial stiffness using the cfPWV and b-mode ultrasonography to detect arterial plaques);

(v) as well as to investigate this association through a systematic literature review according to PRISMA criteria on 36 interventional and observational studies.

The present study led to the following findings:

(i) In 901 individuals (age: 52.4±13.8 years, 45.2% males) [Cohort A], mean dietary Na intake, recorded from two 24h dietary recalls was 2442.6±1317.5 mg/day for men and 1615.8±810.6 mg/day for women. In regard to the other Na assessment methods, in 122 high cardiovascular risk subjects (56.0±12.6 years; 55.7% males) [Cohort B], mean 24h Na excretion was 2810±1304 mg/day.

(ii) Spot urine methods overestimated the 24h Na excretion (bias range: -1781 to -492 mg) and were moderately correlated to 24UC ($r=0.469$-0.596, $p\leq0.01$). Dietary methods underestimated

the 24h Na excretion (bias range: 877 to 1212mg) and were weakly correlated with 24UC. The improved dietary methods underestimated the 24h Na excretion (bias range: 877 to 923mg).
(iii) The new NaFFQ presented the smallest bias (-290±1336mg) and the strongest correlation with 24UC ($r=0.497$, $p\leq0.01$), but wide limits of agreement in Bland-Altman plots (-2909mg; 2329mg), like all the other methods did.
(iv) Regarding the association between arterial pathologies and dietary Na, females -but not males- at 3rd and 4th quartile of total Na intake (derived from food and discretionary salt) had significantly lower probability to present femoral plaques compared to those at 1st quartile (751.0±215.5 mg/day), even in the full-adjusted model [0.462(0.229-0.935), p=0.032 3rd quartile; 0.274(0.118-0.638), p=0.003 4th quartile] (Cohort A). On the contrary, male and female individuals at 3rd quartile had significantly higher probability to present arteriosclerosis (PWV>10 m/sec) compared to those at 1st quartile [1.991(1.047-3.785), p=0.036] (Cohort A).
(v) The systematic literature review led to the following observations regarding Na and SVD: (a) Although several studies exist, the evidence does not clearly support a clinically meaningful and direct (independent from blood pressure) effect of Na on arterial wall stiffening; (b) data regarding the association of dietary Na with arterial remodeling are limited, mostly suggesting a positive trend between dietary Na and arterial hypertrophy but still inconclusive; (c) as regards to atheromatosis, data are scarce and the available studies present high heterogeneity.

Overall, the present study indicates that very low Na intake is associated with: i) accelerated atheromatosis, verifying findings from animal models, providing a possible explanation of the modern epidemiology, and ii) lower arteriosclerosis, which is in line with previous human findings, therefore suggesting a diverging effect of Na in the two major arterial pathologies. Regarding dietary Na assessment, the present analysis found out that **t**he existing methods exhibit poor accuracy. Further improvement of the newly developed NaFFQ could be promising for more accurate estimation of mean dietary Na intake in epidemiological studies. Further state-of-the-art studies must address the remaining controversies and gaps in knowledge as well.

Περίληψη

Η υποκλινική αγγειακή βλάβη [αρτηριοσκλήρυνση (αυξημένη αρτηριακή σκληρία), αρτηριακή αναδιαμόρφωση, αθηρωμάτωση (αθηρωματικές πλάκες)] προηγείται ακόμη και δεκαετίες ης εκδήλωσης καρδιαγγειακής νόσου και αξιοποιείται ως μη επεμβατικός τρόπος αξιολόγησης του καρδιαγγειακού κινδύνου. Παγκοσμίως, η ημερήσια πρόσληψη νατρίου τείνει να είναι διπλάσια της συνιστώμενης από τις διεθνείς οδηγίες. Η υψηλή πρόσληψη νατρίου έχει συνδεθεί με την καρδιαγγειακή νοσηρότητα και θνησιμότητα, αν και σε ορισμένες περιπτώσεις με τη σχέση αυτή να εμφανίζει καμπύλη σχήματος J, υποδηλώνοντας ότι εκτός από τα υψηλά επίπεδα, ακόμη και τα χαμηλά επίπεδα πρόσληψης αυξάνουν τον κίνδυνο. Έως τώρα, τα διαθέσιμα επιστημονικά δεδομένα αναφορικά με τη σχέση του προσλαμβανόμενου νατρίου με την υποκλινική αγγειακή βλάβη παραμένουν περιορισμένα, ειδικά για συγκεκριμένους τύπους αγγειακής βλάβης όπως η αθηρωμάτωση. Επιπλέον, ακόμη και σήμερα δεν υπάρχουν αξιόπιστες και συνάμα εύκολες στην εφαρμογή τους μέθοδοι για την καταγραφή του προσλαμβανόμενου ή και απεκκρινόμενου νατρίου.

Η παρούσα μελέτη διεξήχθη σε πληθυσμούς μέτριου έως αυξημένου καρδιαγγειακού κινδύνου (αρτηριακή υπέρταση, σακχαρώδης διαβήτης, χρόνια φλεγμονώδη/ αυτοάνοσα νοσήματα, HIV) με στόχο:

(i) την καταγραφή ποσοτικοποίηση του νατρίου της δίαιτας με την εφαρμογή όλων των διαθέσιμων μεθόδων [διατροφικές μέθοδοι (π.χ. πολλαπλές ανακλήσεις 24ώρου, ερωτηματολόγιο συχνότητας κατανάλωσης τροφίμων (FFQ)) και μέθοδοι ούρων (συλλογή δείγματος ούρων-σποτ με χρήση διαφορετικών εξισώσεων και 24ωρη συλλογή ούρων,

(ii) τη σύγκριση όλων των μεθόδων καταγραφής του νατρίου ως προς την αξιοπιστία τους συγκρινόμενες με την 24ωρη συλλογή ούρων,

(iii) το σχεδιασμό και την ανάπτυξη ενός νέου περισσότερο αξιόπιστου -σε σύγκριση με τις υπάρχουσες μεθόδους- εργαλείου καταγραφής του προσλαμβανόμενου νατρίου με τη μορφή ερωτηματολογίου FFQ, (NaFFQ) και τη βελτίωση των υπαρχουσών διατροφικών μεθόδων μέσω ποσοτικοποίησης του προστιθέμενου άλατος στις ανακλήσεις 24ώρου,

(iv) τη διερεύνηση της σχέσης του νατρίου της δίαιτας με τους κυριότερους τύπους υποκλινικής αγγειακής βλάβης στον πληθυσμό της παρούσας μελέτης, εφαρμόζοντας state-of-the-art μεθόδους για την αξιολόγηση των αγγειακών βιοδεικτών [τονομετρία για την αξιολόγηση της καρωτιδο-μηριαίας ταχύτητας σφυγμικού κύματος (cf-PWV) και υπερηχοτομογραφία για την καταγραφή των αθηρωματικών πλακών],

(v) καθώς και τη διερεύνηση της παραπάνω σχέσης μέσω συστηματικής ανασκόπησης 36 μελετών παρέμβασης και παρατήρησης, σύμφωνα με τα κριτήρια PRISMA.

Η παρούσα μελέτη οδήγησε στα παρακάτω ευρήματα:

(i) Σε 901 άτομα (52.4±13.8 έτη, 45.2% άνδρες), η μέση [Πληθυσμός Α], η μέση διατροφική πρόσληψη νατρίου, όπως αυτή καταγράφηκε από δύο ανακλήσεις 24ώρου ήταν 2442.6±1317.5 mg/ημέρα για τους άνδρες και 1615.8±810.6 mg/ημέρα για τις γυναίκες. Αναφορικά με τις υπόλοιπες μεθόδους αξιολόγησης του νατρίου, σε 122 συμμετέχοντες υψηλού καρδιαγγειακού κινδύνου (56.0±12.6 έτη; 55.7% άνδρες) [Πληθυσμός Β], η μέση 24ωρη απέκκριση νατρίου ήταν 2810±1304 mg/ ημέρα.

(ii) Η μέθοδος δείγματος ούρων (σποτ) με τη χρήση διαφορετικών εξισώσεων οδήγησε σε υπερεκτίμηση της 24ωρης απέκκρισης νατρίου (εύρος σφάλματος:-1781 έως -492 mg) και εμφάνισε μέτρια συσχέτιση με την 24ωρη συλλογή ούρων ($r=0.469$-0.596, $p\leq0.01$). Οι διατροφικές μέθοδοι αντίθετα, οδήγησαν σε υποεκτίμηση της 24ωρης απέκκρισης νατρίου (εύρος σφάλματος: 877 έως 1212mg) και εμφάνισαν αδύναμες συσχετίσεις με την 24ωρη συλλογή ούρων. Οι βελτιωμένες διατροφικές μέθοδοι υποεκτίμησαν την 24ωρη απέκκριση νατρίου (εύρος σφάλματος: 877 έως 923mg).

(iii) Το νέο εργαλείο που αναπτύχθηκε με τη μορφή ερωτηματολογίου (NaFFQ) παρουσίασε το μικρότερο σφάλμα (-290±1336mg) και την ισχυρότερη συσχέτιση με την 24ωρη συλλογή ούρων ($r=0.497$, $p\leq0.01$), αν και τα όρια συμφωνίας στα Bland-Altman plots ήταν διευρυμένα (-2909mg, 2329mg), όπως ακριβώς και συνέβη και με τις υπόλοιπες μεθόδους.

(iv) Σχετικά με τη διερεύνηση της συσχέτισης των αγγειακών βλαβών με το νάτριο, οι γυναίκες -όχι όμως και οι άνδρες- που ανήκαν στο 3ο και 4ο τεταρτημόριο πρόσληψης νατρίου (προερχόμενο από την τροφή και το πρόσθετο αλάτι) εμφάνισαν σημαντικά χαμηλότερη πιθανότητα να παρουσιάζουν αθηρωματικές πλάκες στις μηριαίες αρτηρίες συγκρινόμενες με τις γυναίκες του 1ου τεταρτημόριου (751.0±215.5 mg/ημέρα), ακόμη και έπειτα από διόρθωση για όλους τους πιθανούς συγχυτικούς παράγοντες [0.462(0.229-0.935), p=0.032 για το 3ο τεταρτημόριο· 0.274(0.118-0.638), p=0.003 για το 4ο τεταρτημόριο] (Πληθυσμός Α). Αντιθέτως, οι άνδρες και οι γυναίκες που ανήκαν στο 3ο τεταρτημόριο είχαν σημαντικά υψηλότερη πιθανότητα να παρουσιάζουν αρτηριοσκλήρυνση (PWV>10 m/sec) συγκριτικά με όσους ανήκαν στο 1ο τεταρτημόριο [1.991(1.047-3.785), p=0.036]. Αναφορικά με τις μεθόδους αξιολόγησης του νατρίου, σε 122 άτομα υψηλού καρδιαγγειακού κινδύνου (56.0±12.6 έτη; 55.7% άνδρες), η μέση 24ωρη απέκκριση νατρίου ήταν 2810±1304 mg/ημέρα (Πληθυσμός Α).

(v) Η συστηματική ανασκόπηση της βιβλιογραφίας οδήγησε στις παρακάτω παρατηρήσεις σχετικά με το νάτριο και την υποκλινική αγγειακή βλάβη: (α) αν και υπάρχουν αρκετές μελέτες, οι ενδείξεις δεν υποστηρίζουν με ξεκάθαρο τρόπο μία άμεση (ανεξάρτητη από την αρτηριακή πίεση) και κλινικά σημαντική επίδραση του νατρίου στη σκλήρυνση του αγγειακού τοιχώματος, (β) τα ερευνητικά δεδομένα σχετικά με το νάτριο και την αγγειακή αναδιαμόρφωση αν και είναι περιορισμένα και κάποιες φορές αντιφατικά, στην πλειοψηφία τους προτείνουν μία θετική τάση ανάμεσα στις δύο αυτές παραμέτρους, (γ) αναφορικά με την

αθηρωμάτωση, τα διαθέσιμα ερευνητικά δεδομένα είναι εξαιρετικά ελλιπή και οι μελέτες παρουσιάζουν σημαντική ετερογένεια.

Συνολικά, η παρούσα μελέτη υποδηλώνει ότι η πολύ χαμηλή πρόσληψη νατρίου συσχετίζεται με: (i) επιταχυνόμενη αθηρωμάτωση, επιβεβαιώνοντας τα ευρήματα των μελετών που έχουν διεξαχθεί σε ζωικά μοντέλα, και (ii) χαμηλότερη συχνότητα αρτηριοσκλήρυνσης, συμφωνώντας με την πλειοψηφία των διαθέσιμων μελετών σε ανθρώπους. Με αυτό τον τρόπο προτείνεται μία διαφορετική στον κάθε τύπο αγγειακής βλάβης επίδραση του προσλαμβανόμενου νατρίου. Επιπλέον, αναφορικά με τις μεθόδους αξιολόγησης του νατρίου της δίαιτας, η παρούσα ανάλυση οδήγησε στο συμπέρασμα ότι οι διαθέσιμες μέθοδοι παρουσιάζουν φτωχή αξιοπιστία συγκρινόμενες με την 24ωρη συλλογή ούρων. Με περαιτέρω βελτίωση και επικύρωση της εγκυρότητας και της επαναληψιμότητάς του σε διαφορετικές ομάδες πληθυσμών, το νέο εργαλείο που αναπτύχθηκε στα πλαίσια της παρούσας διατριβής (NaFFQ) θα μπορούσε να αποτελέσει μία υποσχόμενη μέθοδο για απλή και αξιόπιστη καταγραφή της μέσης πρόσληψης νατρίου σε επιδημιολογικές μελέτες. Περισσότερες state-of-the-art μελέτες θα πρέπει να διευθετήσουν τις ερευνητικές αντιπαραθέσεις και τα εναπομείναντα κενά στη γνώση γύρω από το θέμα.

Publications from the present thesis

1. Tsirimiagkou C, Karatzi K, Argyris A, Basdeki ED, Kaloudi P, Yannakoulia M, Protogerou AD. Dietary sodium and cardiovascular morbidity/mortality: a brief commentary on the 'J-shape hypothesis'. J Hypertens. 2021 Dec 1;39(12):2335-2343. doi: 10.1097/HJH.0000000000002953. PMID: 34326279.
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The full-text version of the above-mentioned articles is available in *Appendix*.

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Appendix

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Original Article

Levels of dietary sodium intake: diverging associations with arterial stiffness and atheromatosis

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ABSTRACT

Background: Recent epidemiological evidence suggests a J-shaped, rather than the classical linear, association between dietary sodium (Na) intake and cardiovascular (CV) disease. Numerous animal studies have shown the acceleration of atheromatosis in a low-salt diet but data in humans are scarce. Our aim was to test the hypothesis that in a cohort of patients who are CV-free, yet at increased CV risk, moderate Na intake is associated with lower prevalence of atheromatosis and arterial stiffening than those at very low Na intake.

Methods: Two 24-h dietary recalls were conducted to estimate Na intake. Atheromatosis (carotid and femoral plaques) was assessed by B-mode ultrasonography and arterial stiffness through tonometry (carotid-to-femoral pulse wave velocity, cf-PWV).

Results: In 901 individuals (age: 52.4 ± 13.8 years, 45.2% males), only females at 3rd and 4th quartile of total Na intake (derived from food and discretionary salt) had significantly lower probability to present femoral plaques than those at 1st quartile (751.0 ± 215.5 mg/day), even in the full-adjusted model [0.462(0.229-0.935) and $p = 0.032$ 3rd quartile; 0.274(0.118-0.638) and $p = 0.003$ 4th quartile]. On the contrary, male and female individuals at 3rd quartile had significantly higher probability to present arterial stiffness (PWV >10 m/s) than those at 1st quartile [1.991(1.047-3.785) and $p = 0.036$].

Conclusions: Overall, the present data suggest that very low Na intake is associated with: a) accelerated atheromatosis, verifying findings from animal models, and providing a possible explanation of the modern epidemiology and b) lower arterial stiffness, which is in line with previous human findings, therefore suggesting a diverging effect of Na in the two major arterial pathologies.

1. Introduction

High sodium (Na) intake and/or excretion has been repeatedly associated with a great risk for cardiovascular (CV) disease and/or mortality.[1–3] Based on a large number of studies supporting CV benefits from salt-lowering strategies,[4] international guidelines suggest the restriction of daily total Na intake up to 1500 mg/day for adults who would benefit from blood pressure lowering[5] or up to 2000 mg/day for the general population.[6] However, in the last decade, there was growing controversial evidence of a J-shaped association between Na intake and/or excretion and CV events or deaths, suggesting that low or very low dietary Na intake may lead to increased CV risk.[7–13] The underlying arterial pathology that might explain the association between low Na intake and increased CV risk remains obscure.

Subclinical functional and structural arterial damage precedes the development of clinical symptoms and overt disease for several

Abbreviations: Na, sodium; CV, cardiovascular; cf-PWV, carotid-to-femoral pulse wave velocity.

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decades. Atheromatosis (arterial plaque formation) and arterial stiffness are the major types of arterial damage that lead to CV disease. Although they share some common risk factors, each one constitutes a completely different pathology.[14,15] The presence of carotid artery plaque or femoral plaque and the carotid-femoral pulse wave velocity (cf-PWV) are the best available vascular biomarkers to study early steps in atheromatosis and arterial stiffening, respectively.[14,16]

High Na intake is suggested to impair vascular function and induce atheromatosis[17,18] as well as arterial stiffness.[19–25] However, there are limited yet intriguing inconsistencies indicating the presence of inverse associations between Na intake and arterial damage not only in human studies,[26,27] but particularly in animal studies, showing the acceleration of atheromatosis in a low-salt diet.[28–32] Therefore, it is evident that there are important gaps in knowledge as well as scarce data investigating simultaneously the association between dietary Na and the two types of arterial damage. Moreover, no studies have examined the aforementioned association in arterial beds beyond the carotid artery, e.g., the femoral arteries that provide additional value in the prediction of CV risk.[33–38]

Taking into consideration all the information above, our working hypothesis was that very low Na intake might be associated with impaired subclinical arterial function and structure. Therefore, the aim of the present study was to test – by using very low dietary Na intake as reference group – if a moderate dietary Na intake is associated with lower prevalence of subclinical atheromatosis (assessed by carotid and femoral plaques) and decreased arterial stiffness [assessed by cf-PWV], in a cohort of patients at increased CV risk under long-term medical guidance and free of CV disease. Given the fact that there are major sex-specific differences in the pathophysiology of arterial function,[39] we also tested for the presence of potential interaction between sex and Na intake, with regard to their association with subclinical arterial damage.

2. Methods

2.1. Study design and Population

In the present analysis, we used data from our cohort study that started in 2010 at the Cardiovascular Research Laboratory of our department, in adults who are at moderate-to-high CV risk. The study population consisted of all consecutively consenting patients (between 2010 and 2016) with CV risk factors (suspected or established, treated or untreated hypertension, diabetes mellitus, dyslipidemia, and/or chronic inflammatory diseases).[40] Patients with established CV disease (defined as preexisting coronary artery disease, stroke, peripheral arterial disease, and/or documented arterial stenosis >50%) were excluded from the analysis to focus on a population with early vascular impairment. All subjects underwent CV risk assessment as well as vascular and dietary assessment. The study was approved by the ethical/scientific committee of "Laiko" Hospital. The study was conducted according to the World Medical Association Declaration of Helsinki and all participants provided written informed consent.

2.2. Assessment of dietary Na intake

Well-trained dietitians conducted two 24-h dietary recalls to all the participants by telephone interview, using the US Department of Agriculture's multiple pass method.[41] Participants were asked to report all the foods and beverages they consumed and their quantities of the previous 24 h. Two 24-h dietary recalls were collected on one weekday and one weekend day, with at least a 7-day interval between them. Collected dietary data were analyzed in macro- and micronutrients using appropriate software (Nutritionist Pro, version 5.2, Axxya Systems-Nutritionist Pro, Stafford, TX, USA). The Nutritionist Pro food database was expanded by adding analyses of traditional Greek foods and recipes.

Total daily Na intake was calculated using several procedures. Information from the 24-h dietary recalls was used to estimate Na intake from foods (processed foods and naturally occurring in unprocessed foods). Discretionary Na intake (i.e., added salt during cooking and table salt), which cannot be estimated by dietary recalls, was derived from literature review. In specific, major studies suggest that the discretionary salt represents around 15% of total salt intake in Europe and USA.[42–44] Based on these published data, we calculated the discretionary Na hypothesizing that Na from cooking and table is 15% of the total Na intake for our population. Finally, the total daily Na intake was derived as: the estimated Na intake from foods plus the discretionary Na. The overall statistical analysis was performed using the total Na intake and then it was repeated using only the Na intake from foods (i.e., without adding the discretionary Na) to verify all the findings.

2.3. Assessment of vascular and hemodynamic parameters

All participants were asked to refrain from food and any vasoactive substance or medication at least 3 hours before the vascular tests. Subclinical atheromatosis and arterial stiffening were assessed and defined as previously discussed[14,45–47] (extensive description in the supplement).

2.4. Assessment of anthropometric parameters

Weight was measured without shoes or heavy clothes to the nearest 0.1 kg. Height was measured without shoes, with the participants standing with their shoulders relaxed, their arms hanging freely, and their head in Frankfurt horizontal plane. Body mass index (BMI) was calculated as weight/(height)^2 (kg/m^2).

2.5. Assessment and definition of CV disease risk factors

Hypertension was defined as the use of antihypertensive drugs and/or office blood pressure measurement higher than 139 and/or 89 mmHg (average of three sequential readings with 1-min interval in the supine position after at least 10 min of rest and appropriate cuff size use; Microlife WatchBP Office, Microlife AG, Widnau, Switzerland). Dyslipidemia was defined as the use of lipid-modifying drugs and/or low-density lipoprotein cholesterol level higher than 160 mg/dl. Diabetes was defined as glucose higher than 126 mg/dl or HbA1c higher than 6.5% and/or glucose-lowering treatment. Smoking was defined by the use of at least one cigarette per day, each day of the week.

2.6. Statistical analysis

Statistical analysis was performed using the SPSS statistical package (IBM Corp. Released 2017, Armonk, NY: IBM Corp.). Distribution normality of the variables was tested using the Kolmogorov-Smirnov test and histograms. Given the fact that nonlinear association between Na intake and CV mortality is suggested by the literature,[7,8,10,13,48] we used a nonlinear statistical approach to test our hypothesis. The possible interaction between quartiles of total Na intake and sex with atheromatosis (total plaques, carotid plaques, and femoral plaques) and arterial stiffness (PWV >10 m/s, based on the international guidelines[47]) was tested; because of missing values in plaques and PWV, the quartiles were calculated separately for each end point. Multiple logistic regression analysis was performed to determine the relationship of total

Na intake quartiles (defined separately for each sex) with atheromatosis and arterial stiffness, while adjusting for potential covariates. The adjustments for confounding factors are described in the following models; model 1: age, sex; model 2: model 1 plus BMI, presence of hypertension, smoking, diabetes, and dyslipidemia; model 3a: model 2 plus energy intake (kcal); model 3b: model 2 plus potassium intake; model 4: model 2 plus energy and potassium intake. Other potential confounders such as hypertension drugs, dyslipidemia drugs, diabetes drugs, antiplatelet drugs, cortisone, and chronic inflammatory diseases duration were also tested in separate models in addition to the previous models. The results are presented as Exp. B (95% confidence intervals - CI). The level of statistical significance was set at $p<0.05$. Sensitivity analyses were performed by repeating all the above analysis: (i) using quartiles for the estimated Na intake derived from foods (processed foods and naturally occurring Na in unprocessed foods) instead of the total Na intake, (ii) for the subgroup of the population free of any chronic inflammatory disease, and (iii) for the subgroup of the population who probably had not taken strict guidance for Na restriction (subjects not taking cortisone and subjects without hypertension diagnosed for more than 1 year).

3. Results

A total population of 901 adults (407 men and 494 women) with dietary and atheromatosis data was analyzed. However, data for the arterial stiffness analysis were available for 886 participants (386 men and 500 women), and therefore separate Na intake quartiles were generated for the arterial stiffness dataset.

Regarding the atheromatosis analysis, a significant interaction between sex and quartiles of total Na intake was observed ($p<0.001$ for carotid and/or femoral plaques, $p = 0.002$ for carotid plaques, and $p<0.001$ for femoral plaques). Consequently, the results are presented separately for males and females. No interaction between total Na intake quartiles and sex with regard to their association with arterial stiffness was revealed ($p = 0.522$), and the results for this specific analysis are presented for the total population.

Descriptive characteristics of the study population are presented for each sex separately with regard to the analysis of atheromatosis and for the total population with regard to the analysis of arterial stiffness (Table 1).

Table 2 presents the associations derived from multivariate logistic regression analysis between total Na intake quartiles and atheromatic plaques (total plaques, carotid plaques, and femoral plaques) for each sex. Females at the 3rd (1743.9 ± 154.6 mg/day) and the 4th (2731.0 ± 631.9 mg/day) quartiles of total Na intake had significantly lower probability to present plaques at the femoral arteries than those at the 1st quartile (751.0 ± 215.5 mg/day) ($p = 0.04$ and 0.008, respectively). This association remained significant after extensive adjustment for all potential confounders in all − but one − models [0.462 (0.229-0.935), $p = 0.032$ for the 3rd quartile and 0.274 (0.118-0.638), $p = 0.003$ for the 4th quartile in the fully adjusted model (model 4)]. Additional adjustments for hypertension drugs, dyslipidemia drugs, diabetes drugs, antiplatelet drugs, cortisone, and chronic inflammatory diseases duration did not change the mentioned findings. A similar but marginally significant trend was observed for the 2nd quartile of total Na intake in all models [0.538 (0.276-1.046) and $p = 0.068$ (model 4)] (Table 2). Similar findings were observed with regard to carotid plaques as well as total plaques, but although marginal they did not reach statistical significance. On the contrary, no associations between total Na intake and total carotid or femoral plaques were observed in male participants (Table 2).

Table 3 presents the association between total Na intake quartiles and arterial stiffness. Subjects at the 3rd quartile of total Na intake (2605.9 ± 271.4 mg/day for male and 1750.1 ± 155.6 mg/day for female patients) had significantly higher probability to have high arterial stiffness (cf-PWV>10 m/s) than those at 1st quartile (1086.4 ± 282.8 mg for male and 751.0 ± 216.1 mg for female patients) in models 2 and 3b [1.915(1.015-3.611), $p = 0.045$ for model 2 and 1.991(1.047-3.785), $p = 0.036$ for model 3b]. This association marginally lost its significance in the other models. In further adjustment for drug categories (hypertension drugs, dyslipidemia drugs, diabetes drugs, antiplatelet drugs, and cortisone) and chronic inflammatory disease duration, similar results were observed. We also repeated all the arterial stiffness analysis using 1) the definition of arterial stiffness according to age reference groups and 2) the cf-PWV as a continuous variable and no associations were observed (data not shown).

3.1. Sensitivity analyses

All the analyses were repeated for the Na intake from foods only instead of total Na intake. Similar results were found (data not shown).

After excluding all patients with chronic inflammatory diseases ($n = 464$), similar findings were observed.

After excluding all patients who probably had taken guidance for Na restriction (subjects who take cortisone and subjects with hypertension diagnosed for more than one year), we observed similar results in those of the main analyses.

4. Discussion

In the present study, we investigated for the first time in a large sample of participants free of overt CV disease, the association between dietary Na intake with two different types of subclinical arterial damage that were simultaneously measured. We observed a major and novel finding. The association of dietary Na intake with each type of subclinical vascular damage was different and rather diverging, implying that very low dietary Na intake has detrimental effects in atheromatosis but not arterial stiffness.

Recent data support a J-shaped association between Na intake and CV mortality.[7,8,10,13,48] This association contrasts with the current guidelines that suggest only an upper level of intake (2 g/day), which should not be overpassed.[49,50] However, a recent update on dietary Na reference values from the European Food Safety Authority suggested that 2 g/day is *"a safe and adequate intake for the general European population of adults."* Indeed, studies that investigate the role of Na in CV morbidity/mortality in populations with chronic CV risk factors − like our study population − support the increased CV mortality when Na intake is very low.[13,48] A review of more than 360000 volunteers revealed a "safe" range of Na intake of approximately 2500 - 6000 mg/day, above and below which the CV risk increases.[12] In the present analysis, the reference group had Na intake below 1 g, i.e., substantially lower than the thresholds of the J-shaped curves.[7–13] In line with the mortality outcome data mentioned above, the present results imply that very low daily Na intake may have detrimental effects on the arterial wall by the acceleration of atheromatosis and thus provide a potential mechanism that may lead to increased mortality.

To our knowledge, there are only two studies that examine the association between daily Na intake/excretion and the presence of atheromatic plaques.[17,18] Dai et al. studied 3290 apparently healthy adults, using spot urine specimens and a food frequency questionnaire to estimate the excreted and dietary Na. They found a positive association between Na excretion and carotid atheromatosis.[17] However, they used the Na/creatinine ratio in their analysis,

Table 1
Descriptive characteristics of participants

Population with available data for atheromatosis	MALES					FEMALES				
	Total *(n = 407)*	Quartiles of total Na intake				Total *(n = 494)*	Quartiles of total Na intake			
		1st 1088.1 ± 277.8 mg *(n = 101)*	2nd 1823.5 ± 235.1 mg *(n = 104)*	3rd 2596.1 ± 264.3 mg *(n = 101)*	4th 4281.1 ± 1074.3 mg *(n = 101)*		1st 751.0 ± 215.5 mg *(n = 123)*	2nd 1239.3 ± 128.3 mg *(n = 124)*	3rd 1743.9 ± 154.6 mg *(n = 124)*	4th 2731.0 ± 631.9 mg *(n = 123)*
	Mean ± S.D.	**Mean ± S.D.**	**Mean ± S.D.**	**Mean ± S.D.**	**Mean ± S.D.**	**Mean ± S.D.**	**Mean ± S.D.**	**Mean ± S.D.**	**Mean ± S.D.**	**Mean ± S.D.**
Age, years	50.6 ± 13.1	54.1 ± 12.6	52.9 ± 13.4	49.0 ± 13.8	46.3 ± 11.2	53.9 ± 14.2	55.0 ± 14.2	55.4 ± 15.2	54.7 ± 13.3	50.5 ± 13.7
Weight, kg	85.6 ± 15.4	84.2 ± 13.4	86.5 ± 13.5	85.3 ± 18.4	87.7 ± 15.7	71.7 ± 16.4	71.8 ± 14.6	71.8 ± 19.0	72.6 ± 15.7	70.6 ± 16.2
Height, m	1.76 ± 0.07	1.74 ± 0.06	1.75 ± 0.06	1.75 ± 0.07	1.78 ± 0.07	1.60 ± 0.07	1.59 ± 0.07	1.59 ± 0.07	1.60 ± 0.06	1.61 ± 0.06
BMI, kg/m^2	27.9 ± 4.7	27.8 ± 4.1	28.3 ± 4.0	27.8 ± 5.8	27.7 ± 4.7	28.0 ± 6.1	28.3 ± 5.6	28.3 ± 6.8	28.4 ± 6.0	27.1 ± 6.1
Na intake from foods[a], mg/day	2076.2 ± 1119.9	924.9 ± 236.1	1550.0 ± 199.9	2206.7 ± 224.7	3638.9 ± 913.2	1373.4 ± 689.0	638.3 ± 183.2	1053.4 ± 109.1	1482.3 ± 131.4	2321.4 ± 537.1
Discretionary Na intake[b], mg/day	366.4 ± 197.6	163.2 ± 41.7	273.5 ± 35.3	389.4 ± 39.7	642.2 ± 161.2	242.4 ± 121.6	112.6 ± 32.3	185.9 ± 19.2	261.6 ± 23.2	409.7 ± 94.8
Total Na intake[c], mg/day	2442.6 ± 1317.5	1088.1 ± 277.8	1823.5 ± 235.1	2596.1 ± 264.3	4281.1 ± 1074.3	1615.8 ± 810.6	751.0 ± 215.5	1239.3 ± 128.3	1743.9 ± 154.6	2731.0 ± 631.9
Smoking, % (n)	42.0 (171)	38.6 (39)	38.5 (40)	45.5 (46)	45.5 (46)	30.4 (150)	28.5 (35)	30.6 (38)	28.2 (35)	34.1 (42)
Diabetes type 1, % (n)	6.1 (25)	3.0 (3)	8.7 (9)	9.9 (10)	3.0 (3)	9.3 (46)	5.7 (7)	5.6 (7)	14.5 (18)	11.4 (14)
Diabetes type 2, % (n)	10.6 (43)	16.8 (17)	12.5 (13)	5.9 (6)	6.9 (7)	12.6 (62)	13.0 (16)	13.7 (17)	13.7 (17)	9.8 (12)
Diabetes disease duration, years	12.7 ± 10.0	11.6 ± 8.4	13.2 ± 9.7	14.0 ± 13.4	12.0 ± 8.5	12.4 ± 9.6	11.7 ± 9.2	11.1 ± 9.0	11.1 ± 9.6	16.4 ± 10.2
Dyslipidemia, % (n)	36.1 (147)	38.6 (39)	47.1 (49)	29.7 (30)	28.7 (29)	37.4 (185)	41.5 (51)	43.5 (54)	34.7 (43)	30.1 (37)
Dyslipidemia disease duration, years	5.0 ± 5.1	5.7 ± 6.0	4.6 ± 4.1	5.0 ± 4.7	5.0 ± 6.0	5.6 ± 6.0	6.5 ± 6.5	4.8 ± 5.7	5.3 ± 6.3	5.7 ± 5.2
Hypertension, % (n)	45.2 (184)	53.5 (54)	53.8 (56)	41.6 (42)	31.7 (32)	44.9 (222)	48.0 (59)	49.2 (61)	45.2 (56)	37.4 (46)
Hypertension disease duration, years	6.3 ± 7.2	6.5 ± 6.8	7.6 ± 7.3	5.4 ± 7.5	4.9 ± 7.0	7.9 ± 7.6	7.6 ± 7.4	8.8 ± 9.1	6.7 ± 6.8	8.9 ± 6.7
Chronic inflammatory disease, % (n)	41.3 (168)	43.6 (44)	35.6 (37)	43.6 (44)	42.6 (43)	52.6 (260)	50.4 (62)	57.3 (71)	56.5 (70)	46.3 (57)
Chronic inflammatory diseases duration, years	11.7 ± 10.3	12.6 ± 11.6	11.7 ± 11.2	12.5 ± 9.8	9.9 ± 8.8	12.1 ± 9.3	12.2 ± 9.1	12.4 ± 10.3	11.3 ± 8.6	12.8 ± 9.4
Diabetes drugs, % (n)	15.7 (64)	19.8 (20)	18.3 (19)	15.8 (16)	8.9 (9)	20.4 (101)	17.1 (21)	18.5 (23)	25.8 (32)	20.3(25)
Dyslipidemia drugs, % (n)	29.0 (118)	32.7 (33)	38.5 (40)	22.8 (23)	21.8 (22)	27.9 (138)	33.3 (41)	33.1 (41)	21.8 (27)	23.6 (29)
Hypertension drugs, % (n)	36.6 (149)	40.6 (41)	48.1 (50)	32.7 (33)	24.8 (25)	40.3 (199)	40.7 (50)	44.4 (55)	40.3 (50)	35.8 (44)
Antiplatelet drugs, % (n)	11.5 (47)	12.9 (13)	14.4 (15)	10.9 (11)	7.9 (8)	16.6 (82)	22.8 (28)	16.9 (21)	16.9 (21)	9.8 (12)
Carotid and/or femoral plaques, % (n)	57.2 (233)	64.4 (65)	62.5 (65)	56.4 (57)	45.5 (46)	44.1 (218)	52.0 (64)	49.2 (61)	41.9 (52)	33.3 (41)
Carotid plaques, % (n)	38.8 (158)	44.6 (45)	43.3 (45)	36.6 (37)	30.7 (31)	36.6 (181)	43.9 (54)	42.7 (53)	33.1 (41)	26.8 (33)
Femoral plaques, % (n)	46.9 (191)	53.5 (54)	51.0 (53)	46.5 (47)	36.6 (37)	28.3 (140)	38.2 (47)	30.6 (38)	26.6 (33)	17.9 (22)

Population with available data for arterial stiffness	**TOTAL POPULATION**			
	Sex-specific quartiles of total Na intake			
	1st Males: 1086.4 ± 282.8 mg Females: 751.0 ± 216.1 mg *(n = 221)*	**2nd** Males: 1826.2 ± 237.2 mg Females: 1244.2 ± 128.8 mg *(n = 222)*	**3rd** Males: 2605.9 ± 271.4 mg Females: 1750.1 ± 155.6 mg *(n = 222)*	**4th** Males: 4289.2 ± 1068.1 mg Females: 2749.5 ± 638.6 mg *(n = 221)*
	Mean ± S.D.	**Mean ± S.D.**	**Mean ± S.D.**	**Mean ± S.D.**
cf-PWV[d], m/s	8.5 ± 2.0	8.5 ± 2.2	8.4 ± 2.1	7.8 ± 1.4
Increased arterial stiffness (cf-PWV>10 m/s), % (n)	14.5 (32)	19.4 (43)	16.2 (36)	8.1 (18)

Na: sodium; BMI: body mass index; and S.D: standard deviation [a] Na derived from foods: Na in processed foods and Na naturally occurring in unprocessed foods; [b] Discretionary Na: Na derived from added salt during cooking and table salt; [c]Total Na: estimated plus discretionary Na; [d] cf-PWV: carotid to femoral pulse wave velocity.

Table 2
Multivariate logistic regression analysis between total Na intake quartiles and atheromatosis (plaques). The 1st quartile of total Na intake was used as a reference

Models	Quartiles of total Na intake in males	MALES			Quartiles of total Na intake in females	FEMALES		
		Carotid and/or Femoral plaques Exp.B (95% CI)	Carotid plaques Exp.B (95% CI)	Femoral plaques Exp.B (95% CI)		Carotid and/or Femoral plaques Exp.B (95% CI)	Carotid plaques Exp.B (95% CI)	Femoral plaques Exp.B (95% CI)
Model 1	2nd	1.033 (0.528-2.021)	1.022 (0.552-1.893)	0.980 (0.515-1.864)	2nd	0.790 (0.425-1.468)	0.860 (0.471-1.568)	0.572 (0.309-1.058)
Age	3rd	1.133 (0.578-2.221)	1.008 (0.534-1.902)	1.179 (0.612-2.269)	3rd	0.573 (0.313-1.048)	0.561 (0.309-1.020)	***0.526 (0.285-0.971)**
	4th	0.873 (0.452-1.688)	1.001 (0.523-1.915)	0.991 (0.514-1.911)	4th	0.551 (0.2977-1.023)	0.576 (0.311-1.068)	***0.412 (0.213-0.797)**
Model 2	2nd	1.019 (0.494-2.103)	1.007 (0.526-1.927)	0.836 (0.401-1.745)	2nd	0.813 (0.422-1.565)	0.901 (0.485-1.676)	0.565 (0.292-1.092)
Age								
Hypertension								
Smoking	3rd	1.049 (0.514-2.143)	1.073 (0.555-2.074)	0.957 (0.463-1.977)	3rd	0.611 (0.321-1.164)	0.609 (0.328-1.132)	0.550 (0.284-1.064)
Diabetes								
Dyslipidemia								
BMI	4th	0.851 (0.424-1.710)	1.009 (0.515-1.976)	0.912 (0.436-1.904)	4th	0.530 (0.274-1.024)	0.571 (0.301-1.082)	***0.384 (0.190-0.778)**
Model 3a	2nd	1.111 (0.531-2.322)	1.098 (0.565-2.137)	0.908 (0.427-1.931)	2nd	0.881 (0.453-1.710)	1.000 (0.532-1.878)	0.529 (0.272-1.031)
Age								
Hypertension								
Smoking	3rd	1.232 (0.574-2.646)	1.249 (0.615-2.536)	1.097 (0.502-2.399)	3rd	0.731 (0.371-1.440)	0.775 (0.403-1.491)	***0.460 (0.228-0.930)**
Diabetes								
Dyslipidemia								
BMI	4th	1.161 (0.487-2.767)	1.346 (0.587-3.085)	1.173 (0.472-2.911)	4th	0.758 (0.346-1.659)	0.904 (0.424-1.928)	***0.271 (0.117-0.631)**
Energy intake								
Model 3b	2nd	1.060 (0.512-2.197)	1.042 (0.543-2.000)	0.846 (0.404-1.773)	2nd	0.827 (0.428-1.595)	0.914 (0.490-1.704)	0.559 (0.289-1.080)
Age								
Hypertension								
Smoking	3rd	1.128 (0.545-2.333)	1.145 (0.587-2.231)	0.974 (0.467-2.032)	3rd	0.642 (0.335-1.232)	0.638 (0.341-1.194)	***0.505 (0.258-0.989)**
Diabetes								
Dyslipidemia								
BMI	4th	0.979 (0.471-2.034)	1.156 (0.572-2.334)	0.946 (0.435-2.057)	4th	0.585 (0.296-1.158)	0.627 (0.322-1.220)	***0.326 (0.155-0.684)**
Potassium intake								
Model 4	2nd	1.101 (0.525-2.306)	1.080 (0.554-2.105)	0.911 (0.428-1.938)	2nd	0.879 (0.452-1.708)	1.002 (0.533-1.882)	0.538 (0.276-1.046)
Age								
Hypertension								
Smoking	3rd	1.209 (0.561-2.604)	1.217 (0.598-2.478)	1.108 (0.505-2.429)	3rd	0.729 (0.370-1.438)	0.777 (0.404-1.494)	***0.462 (0.229-0.935)**
Diabetes								
Dyslipidemia								
BMI	4th	1.119 (0.468-2.678)	1.294 (0.562-2.979)	1.186 (0.476-2.957)	4th	0.755 (0.345-1.653)	0.906 (0.425-1.932)	***0.274 (0.118-0.638)**
Energy intake								
Potassium intake								

*$p<0.05$; Na: sodium; BMI: body mass index; and kcal: kilocalories. Male quartiles of total Na intake (min-max): 1st (403.2-1469.5), 2nd (1483.7-2193.1), 3rd (2202.7-3108.5), and 4th (3125.2-8935.1). Female quartiles of total Na intake (min-max): 1st (80.4-1027.1), 2nd (1029.4-1489.3), 3rd (1496.0-2043.0), and 4th (2045.4-6286.9). After further adjustment for hypertension drugs, dyslipidemia drugs, diabetes drugs, antiplatelet drugs, cortisone, and chronic inflammatory diseases duration no substantial changes were observed.

therefore their data are not informative with regard to the actual level of Na intake. A second very small study in just 108 but elderly (mean age 70 ± 4 years) female subjects by Mazza et al., showed a higher prevalence of carotid plaques in those individuals with higher versus those with lower Na intake (2050-2330 vs 780-900 mg Na/day).[18] There are major differences that may explain the inconsistency between these studies and our data, including population heterogeneity, sample and age differences, data reporting, and applied methodology. Most importantly, the two aforementioned studies[17,18] measured only carotid atheromatosis. On the contrary, the present study investigated – for the first time – the femoral atheromatosis too. To screen the femoral arteries provides additive value for the evaluation of subclinical atherosclerosis and prediction of CV risk.[33–38] The potential effect of Na on femoral plaque formation supports previous investigations showing that specific factors like smoking and impaired metabolism accelerate atheromatosis predominantly at the femoral rather than the carotid arteries.[38] More investigation on the potential arterial site-specific impact of Na is necessary and also, it is essential to determine the pathophysiological process in different sites of the arterial wall.

Given the fact that there are major sex-specific differences in the pathophysiology of arterial function,[39] we tested and found an interaction between sex and Na intake with regard to their association with subclinical arterial damage. One of our major findings was that only women (but not men) with moderate Na intake had significantly lower probability to present femoral plaques than those with very low Na intake. Sex-specific differences in CV[39] and more specifically in vascular[51–63] function and dysfunction have

Table 3
Multivariate logistic regression analysis between total Na intake quartiles and arterial stiffness (pulse wave velocity). The 1st quartile of total Na intake was used as a reference.

Models	Sex-specific quartiles of total Na intake	TOTAL POPULATION
		Arterial stiffness (cf-PWV >10 m/s) Exp.B (95% CI)
Model 1	2nd	1.284 (0.730-2.257)
Age	3rd	1.563 (0.872-2.804)
Gender	4th	0.863 (0.437-1.701)
Model 2	2nd	1.248 (0.678-2.297)
Age	3rd	***1.915 (1.015-3.611)**
Gender	4th	0.991 (0.472-2.078)
Hypertension		
Smoking		
Diabetes		
Dyslipidemia		
BMI		
Model 3a	2nd	1.246 (0.669-2.321)
Age	3rd	1.909 (0.959-3.801)
Gender	4th	0.987 (0.430-2.266)
Hypertension		
Smoking		
Diabetes		
Dyslipidemia		
BMI		
Energy intake		
Model 3b	2nd	1.262 (0.684-2.328)
Age	3rd	***1.991 (1.047-3.785)**
Gender	4th	1.063 (0.497-2.278)
Hypertension		
Smoking		
Diabetes		
Dyslipidemia		
BMI		
Potassium intake		
Model 4	2nd	1.224 (0.655-2.286)
Age	3rd	1.869 (0.937-3.728)
Gender	4th	0.978 (0.425-2.252)
Hypertension		
Smoking		
Diabetes		
Dyslipidemia		
BMI		
Energy intake		
Potassium intake		

*$p<0.05$.
Na: sodium; cf-PWV: carotid to femoral pulse wave velocity; BMI: body mass index; and kcal: kilocalories.
Male quartiles of total Na intake (min-max): 1st (403.2-1469.5), 2nd (1481.1-3110.3), 3rd (2202.7-3108.5), and 4th (3125.2-8935.1).
Female quartiles of total Na intake (min-max): 1st (80.4-1027.1), 2nd (1029.4-1489.3), 3rd (1496.0-2043.0), and 4th (2045.4-6286.9).
After further adjustment for hypertension drugs, dyslipidemia drugs, diabetes drugs, antiplatelet drugs, cortisone, and chronic inflammatory diseases duration no statistically significant differences between quartiles of total Na intake were observed.

been well described previously. Sex hormones lead to different stimulations of renin-angiotensin-aldosterone system, catecholamines and endothelin levels, nitric oxide production, and vasodilation.[64–69] Although data regarding a sex-specific effect of Na on vascular function of young humans do exist, showing that endothelial dysfunction – i.e., the origin of atheromatosis – is modulated by dietary Na intake level mainly in men but not in women,[70] data on middle-aged postmenopausal women (the target population of our study) are lacking. Likewise, extremely limited data from female animal models exist, showing that the protective effects of estrogen apart from the increase in blood pressure were only manifested in the setting of a chronic high Na diet and suggest that the underlying Na status may have an important influence on the overall effect of reduced estrogen.[71]

It was rather surprising that – even though arterial stiffness and atheromatosis share common risk factors – our results indicated a diverging association between the level of daily Na intake and each type of vascular damage, implying that very low intake has detrimental effects in atheromatosis but not arterial stiffness. However, aortic PWV and plaque burden have been previously described to be weekly correlated, suggesting that arterial stiffening and atheromatosis are *"pathologically distinct and should be considered as separate disease processes"*.[15] The association between Na intake/excretion and arterial stiffness has been widely investigated in cross sectional studies[22,23,26,27,72] and randomized controlled trials[19–21,24,25,73–75] with conflicting results. As a summary of the available data, a recent meta-analysis of 11 randomized controlled trials concluded that a restriction of about 2000 mg of Na/day reduces arterial stiffness.[76] In an updated systematic review from our group on this topic, a positive trend between Na intake and arterial stiffness – in agreement with the herein presented data – was verified; however, the current evidence does not support a clinically meaningful and independent from blood pressure effect of dietary Na on arterial wall.[77] Therefore, our findings are in accordance with the majority of the studies mentioned above[20–24]; nevertheless, it still remains in controversy whether high Na intake affects arterial stiffening in nonhypertensive populations.[19,74]

Our study cannot provide insight in the underlying molecular pathways activated in the presence of low Na intake in each arterial pathology. However, it is now very well known that the two types of vascular disease have distinct pathologies and even CV risk factors.[14,15] This is particularly true in the early steps of each pathology, i.e., before the development of arterial wall calcifications, which is the case for the present population free of any clinical CV disease.[78] Most importantly, several studies in animals have provided compelling evidence that a low-salt diet is associated with accelerated atheromatosis.[28–31,79–81] Most of these studies implicate (i) the activation of the renin-angiotensin-aldosterone system, (ii) increased inflammation, and (iii) increased intracellular calcium, in the acceleration of atheromatosis, regardless of blood pressure lowering.[28–30,79,82,83] On the other hand, animal studies show that a high-salt diet is associated with increased blood pressure that suggests an explanation for the acceleration of arterial stiffness.[31] Whether these mechanisms can be extrapolated in humans has to be extensively investigated.

The findings of the present study should be evaluated within the context of the particularities, advantages, and disadvantages of this cohort. First, this is a cross-sectional study that does not allow to establish a cause-effect relationship; therefore, we cannot totally exclude the possibility that the observed associations represent a reverse causality phenomenon, i.e., increased subclinical arterial damage in the very low Na intake due to very strict dietary consultation (45.1% were hypertensives and 16.4% used corticosteroids). However, the fact that we investigated simultaneously both the atheromatosis and the arterial stiffness with diverging results is against the reverse causality explanation because in this case, we would expect similar findings in both vascular end points. Furthermore, when we excluded the participants who probably took strict guidance for Na restriction (hypertensives and cortisone users), similar findings were observed. Similar to all observational studies, uncontrolled confounding factors could have affected the results, even though extensive and meticulous adjustments have been performed for all known and available confounders. Another limitation of this study is that Na intake was assessed by dietary recalls despite the fact that 24-h urine collection is reported to be the most accurate method to assess the population's Na intake. However, the correction of estimated Na derived from processed and unprocessed foods to total Na derived from foods, table salt, and added during cooking salt, approaches better true dietary Na

intake. In any case, similar findings were observed when total or estimated Na intake was used in each statistical analysis. Finally, this was a large observational study with regard to the Greek population that allowed to test multiple vascular biomarkers at the same time. However, the upper limit of Na intake in the present study (2442.6 ± 1317.5 mg for males and 1615.8 ± 810.6 mg for females) was not high enough to allow the detection of J-shaped curve with atheromatosis.

5. Conclusion

This is the first large observational study that examines simultaneously the association between dietary Na intake and two different types of arterial damage. Overall, the present data suggest that very low Na intake is associated with: a) accelerated atheromatosis, verifying findings from animal models, providing a possible explanation of the modern epidemiology, and b) lower arterial stiffness, which is in line with previous human and animal findings, therefore suggesting a diverging effect of Na in the two major arterial pathologies.

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Declarations of interest

None.

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Dietary sodium estimation methods: accuracy and limitations of old and new methods in individuals at high cardiovascular risk

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Abstract

Objective: Accurate and easy to use methods for dietary Na intake estimation in population level are lacking. We aimed at (i) estimating the mean Na intake in the group level using a variety of dietary methods (DM) and urinary methods (UM) and correlating them with 24-h urine collection (24UCol) and (ii) improving the accuracy of the existing DM.

Design: The most common DM (three 24-h dietary recalls (24DR) and FFQ) and UM (24UCol and spot urine collection using common equations) were applied. To improve the existing: (i) 24DR, discretionary Na was quantified using salt-related questions or adding extra 15 % in total Na intake and (ii) FFQ, food items rich in Na and salt-related questions were added in the standard questionnaire (NaFFQ).

Setting: National and Kapodistrian University of Athens, Greece.

Participants: Totally, 122 high cardiovascular risk subjects (56·0 ± 12·6 years; 55·7 % males).

Results: Mean 24 h Na excretion (24UNa) was 2810 ± 1304 mg/d. Spot urine methods overestimated the 24UNa (bias range: −1781 to −492 mg) and were moderately correlated to 24UCol ($r = 0{\cdot}469$–$0{\cdot}596$, $P \leq 0{\cdot}01$). DM underestimated the 24UNa (bias range: 877 to 1212 mg) and were weakly correlated with 24UCol. The improved DM underestimated the 24UNa (bias range: 877 to 923 mg). The NaFFQ presented the smallest bias (−290 ± 1336 mg) and the strongest correlation with 24UCol ($r = 0{\cdot}497$, $P \leq 0{\cdot}01$), but wide limits of agreement in Bland–Altman plots (−2909 mg; 2329 mg), like all the other methods did.

Conclusions: The existing methods exhibit poor accuracy. Further improvement of the newly developed NaFFQ could be promising for more accurate estimation of mean dietary Na intake in epidemiological studies. Additional validation studies are needed.

Keywords
Dietary Na assessment
24-h urine collection
Spot urine collection
24-h dietary recall
FFQ

High Na intake is an important contributor to elevated blood pressure[1], increasing CVD risk and mortality[2,3]. Although international organisations recommend a maximum daily Na intake of 2000 mg[4], globally it is estimated to be almost double, reaching 3950 mg/d[5]. In large-scale epidemiological studies, the accurate estimation of dietary Na intake is important for detecting actual consumption and for identifying food items, food patterns or dietary behaviours related to Na intake and their association with diseases and treatments as well. In clinical settings also the assessment of Na intake is crucial for evaluating patients' adherence to recommendations and guiding drug treatment decisions. A variety of urinary methods (UM) and dietary methods (DM) are available for the estimation of dietary Na intake; nevertheless, its accurate and precise quantification is still elusive[6].

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Spot urine samples, overnight urine collections and 24-h urine collections (24UCol) represent the UM. Based on the knowledge that about 90 % of Na consumed is excreted through urine during a 24-h period, the 24UCol is regarded as the gold-standard method[7–9]. However, it is a burdensome, time-consuming method and difficult to be applied in large-scale studies as well as in daily clinical practice of uncomplicated arterial hypertension management. Spot urine samples are more convenient to estimate 24-h urine Na excretion (24UNa) via specially designed equations[10–16] (Table 1), which have been evaluated in several population groups[17–19].

On the other hand, the most common DM for Na estimation include 24-h dietary recalls (24DR), FFQ and diet records. These methods are commonly used in population-based studies, as they are efficient to highlight food items rich in Na; however, numerous methodological disadvantages exist[6]. A major one is the inability of all these methods to quantify the discretionary use of salt (table salt or use of salt during cooking), which has been previously reported to contribute significantly to the total Na intake[20–22].

Several efforts have been made to develop an optimal diet-based tool for the estimation of mean Na intake on group level, with the majority of them focusing on short FFQ[23–26], which are brief, easily completed and estimate Na intake through larger time periods compared with other DM. 24-h dietary recalls and food records are also suitable to cover a longer time periods if they are repeated. Nevertheless, usually FFQ are developed for particular population groups and designed according to their culture, dietary habits and traditional recipes, thus they may not be accurately applied to other populations.

To our knowledge, studies evaluating simultaneously the accuracy of different UM and DM for Na estimation with the gold-standard 24UCol are scarce. Moreover, there are no accurate DM for the quantification of discretionary salt, designed specifically for high CVD risk populations, for whom the identification of Na intake is essential. Taking into consideration all these issues, the aim of the present study is to (a) estimate the mean Na intake of population using a variety of DM and UM; (b) correlate these methods with the gold-standard 24UCol and (c) improve the existing DM in order to be more accurate in estimating the mean Na intake in population level.

Methods

Study design and population

A cross-sectional study was performed from January 2017 until October 2018. The study population consisted of consecutive and consenting to participate individuals at high CVD risk due to the presence of CVD risk factors (suspected or established treated or untreated hypertension, dyslipidaemia, diabetes mellitus and/or chronic inflammatory diseases). In order to detect a minimum difference of 500 mg in daily Na intake between each Na estimation method and the 24UNa ($\alpha = 0{\cdot}05$, power = 0·80), the minimum sample size for each pair of methods was calculated (n 60)[27,28]. To account for attrition (non-participation, missing data or incomplete 24UCol), which was estimated to be 50 %, 120 individuals were invited to participate. The study was approved by the ethical/scientific committee. All participants provided informed consent and underwent dietary and urinary assessment simultaneously, which was completed within 1 month.

Assessment of dietary sodium intake using urinary methods

Twenty-four hour urine collection. Participants were asked to keep one 24UCol following written and verbal instructions and a standardised protocol. The instructions were to carry out the collections from Sunday awakening and for the next 24 h, discarding the first morning void without: (a) missing voids and (b) any changes in their diet or medicine (the past 1 month). To verify completeness, sensitivity analyses were conducted after applying all available criteria for 24-h urine completeness[10,29–31] (Statistical analysis section). Na derived from the 24UCol was calculated using the following equation:

$$\begin{aligned} 24\text{UNa (mg/d)} = {} & \text{24-h Na concentration(mmol/l)} \\ & \times \text{24-h urine volume (l)} \\ & \times \text{molecular weight of Na (23 mg/mmol)} \end{aligned}$$

Spot urine. Participants were also asked to keep a single spot urine sample of the first morning void in proper bottle. In order to estimate the 24-h Na excretion from spot urine specimens, the most common conversion equations were applied[11–13,16] (Table 1).

Assessment of dietary sodium intake using existing dietary methods

Twenty-four hour dietary recalls. Three 24DR using multiple-pass method were conducted (2 weekdays and 1 weekend day with a 7-d interval) by well-trained dietitians via telephone or face-to-face interviews. Participants were asked to report all the foods and beverages they consumed and their quantities the previous 24 h. With the use of a relevant nutrient analysis software (Nutritionist Pro, version 5.2, Axxya Systems-Nutritionist Pro, Stafford, TX, USA), food data from the 24DR were analysed in terms of macronutrient and micronutrient intake. The average of Na intake of the 3 d was used. If less than three 24DR were available, the average of the rest was used.

Food frequency questionnaire. In the first week of the dietary assessment, all participants were asked to complete a semi-quantitative FFQ, which is repeatable and valid for nutritional assessment regarding energy and macronutrients[32]. The FFQ consisted of a list of sixty-nine main

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Table 1 Equations used to estimate 24-h urinary Na excretion from a single spot urine specimen

Kawasaki[8]	$\text{Estimated 24 h Na} = 16 \cdot 3 \times \sqrt{\frac{\text{Spot Na}}{\text{Spot Cr}}} \times \text{Predicted 24 h urine Cr}$ $\text{Males : Predicted 24 h Cr} = 12 \cdot 63 \times \text{age} + 15 \cdot 12 \times \text{weight} + 7 \cdot 39 \times \text{height} - 79 \cdot 9$ $\text{Females : Predicted 24 h Cr} = -4 \cdot 72 \times \text{age} + 8 \cdot 58 \times \text{weight} + 5 \cdot 09 \times \text{height} - 74 \cdot 5$	Estimated 24 h Na, mmol/d spot Na, mmol/l spot Cr: mg/l Predicted 24 h Cr: mg/d age, years weight, kg height, cm
Tanaka[10]	$\text{Estimated 24 h} = 21 \cdot 98 \times X_{Na}^{0 \cdot 392}$ $X_{Na} = \frac{\text{Spot Na}}{\text{Spot Cr} \times 10} \times \text{Predicted 24 h Cr}$ $\text{Predicted 24 h Cr} = (-2 \cdot 04 \times \text{age}) + (14 \cdot 89 \ \text{weight}) + (16 \cdot 14 \times \text{height}) - 2244 \cdot 45$	Estimated 24 h Na, mmol/d spot Na, mmol/l spot Cr: mg/dl Predicted 24 h Cr: mg/d age, years weight, kg height, cm
INTERSALT[13]	With Spot K $\text{Males : Estimated 24 h Na} = 25 \cdot 46 + (0 \cdot 46 \times \text{spot Na}) - (2 \cdot 75 \times \text{spot Cr}) - (0 \cdot 13 \times \text{spot K}) + (4 \cdot 10 \times \text{BMI}) + (0 \cdot 26 \times \text{age})$ $\text{Females : Estimated 24 h Na} = 5 \cdot 07 + (0 \cdot 34 \times \text{spot Na}) - (2 \cdot 16 \times \text{spot Cr}) - (0 \cdot 09 \times \text{spot K}) + (2 \cdot 39 \times \text{BMI}) + (2 \cdot 35 \times \text{age}) - (0 \cdot 03 \times \text{age}^2)$ Without Spot K $\text{Males : Estimated 24 h Na} = 23 \cdot 51 + (0 \cdot 45 \times \text{spot Na}) - (3 \cdot 09 \times \text{spot Cr}) + (4 \cdot 16 \times \text{BMI}) + (0 \cdot 22 \times \text{age})$ $\text{Females : Estimated 24 h Na} = 3 \cdot 74 + (0 \cdot 33 \times \text{spot Na}) - (2 \cdot 44 \times \text{spot Cr}) + (2 \cdot 42 \times \text{BMI}) + (2 \cdot 34 \times \text{age}) - (10 \cdot 03 \times \text{age}^2)$	Estimated 24 h Na, mmol/d spot Na, mmol/l spot Cr: mmol/l spot K: mmol/l BMI: kg/m^2 age, years
Toft[11]	$X_{Na} = \frac{\text{spot Na}}{\text{spot Cr}} \times \text{Predicted 24 h Cr}$ $\text{Males : Estimated 24 h Na} = 33 \cdot 56 \times X_{Na}^{0 \cdot 345}$ Preited 24 h Cr (males) $= (-7 \cdot 54 \times \text{age}) + (14 \cdot 15 \times \text{weight}) + (3 \cdot 48 \times \text{height}) + 423 \cdot 15$ $\text{Females : Estimated 24 h Na} = 52 \cdot 65 \times X_{Na}^{0 \cdot 196}$ Predicted 24 h Cr (females) $= (-6 \cdot 13 \times \text{age}) + (9 \cdot 97 \times \text{weight}) + (2 \cdot 45 \times \text{height}) + 342 \cdot 73$	Estimated 24 h Na, mmol/d spot Na, mmol/l spot Cr: mg/dl Predicted 24 h Cr: mg/d age, years weight, kg height, cm
Mage[9]	$\text{Estimated 24 h Na} = \frac{\text{spot Na}}{\text{spot Cr}} \times \text{Predicted 24 h Cr}$ $\text{Males : Predicted Cr 24 h} = 0 \cdot 00179 \times (140 - \text{age}) \times (\text{weight}^{1 \cdot 5} \times \text{height}^{0 \cdot 5}) \times (1 + 0 \cdot 18 \times \text{A} \times (1 \cdot 366 - 0 \cdot 0159 \times \text{BMI}))$ $\text{Females : Predicted Cr 24 h} = 0 \cdot 00163 \times (140 - \text{age}) \times (\text{weight}^{1 \cdot 5} \times \text{height}^{0 \cdot 5}) \times (1 + 0 \cdot 18 \times \text{A} \times (1 \cdot 429 - 0 \cdot 0198 \times \text{BMI}))$ A = African American or Black race = 1/other race = 0	Estimated 24 h Na, mmol/d spot Na, mmol/l spot Cr: mg/dl Predicted 24 h Cr: mg/d BMI: kg/m^2 weight, kg height, cm

food groups (i.e. cereals and starchy foods, fruits, vegetables, dairy products, meat, fish, legumes, added fats, sweets and alcoholic beverages) as well as questions related to dietary behaviours and habits[32]. Participants were asked to report the frequency of the consumption of these food groups the last month on a six-grade scale (from never/rarely to more than 2 times/d) in pre-specified amounts of food expressed in grams, ml or other common measures[33]. More details for FFQ development have been previously described[32,34].

Daily food consumption was calculated as

$$\text{Daily food consumption} = \text{serving size} \times \text{consumption frequency}$$

where consumption frequency was: never = 0; 1–3 times/month = 0·07; 1–2 times/week = 0·21; 3–6 times/week = 0·64; 1 time/d = 1; ≥ 2 times/d = 2.

The Na estimation for each food group was calculated as

$$\text{Daily consumption of food} \times \text{Na content of food}$$

derived from United States Department of Agriculture (USDA) and local food composition tables[35–37].

Assessment of dietary sodium intake using improved dietary methods

24DR plus discretionary salt questions. In order to estimate discretionary salt, participants were asked to answer two salt-related questions separately for breakfast, lunch and dinner for each one of the 24DR:

Question 1: How much salt did you use during the preparation of your meal?
a = none, b = a little, c = moderate, d = a lot

Question 2: Did you add extra salt on your plate (table salt)?
a = no, b = yes.

For question 1, the following Na quantities were applied for each answer: a = none = 0 mg of Na, b = a little = 50 mg of Na per 100 g of food, c = moderate = 350 mg of Na per 100 g of food, d = a lot = 600 mg of Na per 100 g of food. These estimations were based on relevant statements/assessments from the Hellenic Food Authority (EFET)[38]: *'If a food contains more than 0·6 g of sodium (or 1·5 g of salt) per 100 g, then it is high in sodium/salt. If a food contains 0·1 g of sodium or less per 100 g then it is low in sodium/salt. If the amount of salt per 100 g is between these values, then the food contains a medium level of salt'*. Portion sizes from the 24DR were calculated in grams based on food equivalents and local food composition tables[36].

For question 2, the answer 'yes' was defined as 2 dashes of salt, which are equivalent to 775 mg of Na[35] and when the answer was 'no', no Na (0 mg) was added. The mean Na derived from questions 1 and 2 was then added to the Na derived from the 24DR and was calculated as

$$\begin{aligned}\text{24DR + SQ} = {} & \text{Na from the 24DR} \\ & + \text{mean Na from breakfast (question 1)} \\ & + \text{mean Na from lunch (question 1)} \\ & + \text{mean Na from dinner (question 1)} \\ & + \text{mean Na from breakfast (question 2)} \\ & + \text{mean Na from lunch (question 2)} \\ & + \text{mean Na from dinner (question 2)}\end{aligned}$$

The Na of the meals (breakfast, lunch and dinner) was calculated based on the estimations of Na intake from questions 1 and 2 (average from the three 24DR).

24DR plus 15 %. An alternative way to estimate discretionary use of salt was applied. We calculated the discretionary Na based on the assumption that Na from cooking and table is 15 % of the total Na intake for our population, as previously reported[20–22]. In specific, total Na intake was then calculated as

$$\begin{aligned}\text{24DR + 15\%} = {} & \text{Na from the 24DR} \\ & + (\text{15\% of Na from the 24DR})\end{aligned}$$

Sodium FFQ. In order to improve Na estimation, the food list of the previously mentioned FFQ was extended with food items rich in Na and questions regarding dietary behaviours related to discretionary use of salt (NaFFQ). The added food groups and questions are presented in the Supplement (see online Supplemental Table 1). The foods items added were salted butter and margarine, several rich in Na cheeses (e.g. roquefort, parmesan, edam, gouda, gruyere, etc.), salty crackers/biscuits, canned fish/seafood and refined tomato juice. To estimate Na added in cooked meals and salads, Question b of the NaFFQ (*How much salt do you use in your cooked meals and salads?* see online Supplemental Table 1) was used, according to Hellenic Food Authority (EFET)[38] as mentioned above. Participants' answers were calculated as none = 0 mg Na, a little = 50 mg Na/100 g of food, moderate amount = 350 mg Na/100 g of food, much = 600 mg Na/100 g of food, very much = 900 mg Na/100 g of food. Then the quantified Na derived from participants' response in Question b was added to each cooked meal and salad per 100 g of food of the NaFFQ. Na was then calculated as

$$\begin{aligned}\text{NaFFQ} = {} & \text{Na from the existing FFQ} \\ & + \text{Na from food items added rich in Na} \\ & + \text{Na added in cooked meals and salads}\end{aligned}$$

Cooked meals included rice, potatoes, red and white meat, fish & seafood, legumes, traditional dishes and home-made pies. Salads included all vegetables, raw or boiled.

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Assessment of anthropometric parameters

Participants' weight was measured without shoes or heavy clothes to the nearest 0·1 kg (Tanita Body Composition Analyzer, BC-418). Height was measured without shoes, with the participants standing with their shoulders relaxed, their arms hanging freely and their head in Frankfurt horizontal plane (SECA 213). BMI was calculated as weight/(height)^2 (kg/m^2).

Assessment and definition of CVD risk factors

Hypertension was defined as the use of antihypertensive drugs and/or office blood pressure measurement >139/89 mmHg (average of three sequential readings with 1-min interval in the supine position after at least 10 min of rest; Microlife WatchBP Office, Microlife AG, Widnau, Switzerland)[39]. Dyslipidaemia was defined as the use of lipid-lowering drugs and/or LDL-cholesterol level >160 mg/dl. Diabetes mellitus was defined as fasting glucose higher than 126 mg/dl or HbA1c ≥6·5 % and/or glucose-lowering treatment. Smoking or vaping was defined by the use of at least one cigarette/d each day of the week or the use of e-cigarette.

Statistical analysis

All the analyses were conducted using SPSS version 25 (IBM Corp. Released 2017, IBM Corp.). Continuous variables are presented as mean ± SD and categorical variables as absolute frequency and percentage (%). Significance levels were set at P-value < 0·05. Distribution normality of the variables was tested using the Kolmogorov–Smirnov test and histograms. The differences between methods (bias of mean values) were calculated as 24UNa minus the Na measures of the other DM and UM. Paired samples t-test and Wilcoxon test, when appropriate, were used to determine the significance of differences of mean values of Na. To assess the correlation between 24UCol and the other Na estimation methods, the Pearson's correlation coefficient (for normally distributed variables) and Spearman correlation coefficient (for variables not normally distributed) were applied. Consistency between different methods of Na estimation was also assessed with the intraclass correlation coefficient (ICC)[40]. It is generally accepted that there is no absolute interpretation of ICC values. However, in the present study, we used the recommendation of Koo and Li[41]; accordingly, ICC values <0·5 are indicative of poor reliability, ICC between 0·5 and 0·75 indicate moderate reliability, ICC between 0·75 and 0·9 indicate good reliability, and ICC values greater than 0·90 indicate excellent reliability.

Bland–Altman plots were used to evaluate differences between Na estimation methods and the 24UCol and evaluate the agreement between them[42,43]. The upper and lower limits of agreement between two different estimates of Na were calculated by the mean difference ± 1·96 × SD of differences. Linear regression analysis was used to evaluate associations in difference and mean (between 24UCol and each Na estimation method). The analyses regarding correlations between 24UCol and each Na estimation method and ICC as well were repeated after excluding all subjects having incomplete 24UCol (sensitivity analysis) and they are presented in the supplement. The exclusion criteria for incomplete 24UCol were set according to international bibliography[10,29–31] and are presented in the supplemental material (see online Supplemental Table 2).

Results

One hundred and twenty-two (122) participants with available 24UCol data were used for the analyses (56·0 ± 12·6 years; 55·7 % males) (Table 2). The available sample size for UM and DM was Spot UM, n 71; 24DR = 119; FFQ, n 87; NaFFQ, n 60 (Table 2). Descriptive characteristics of study population are presented in Table 2. Incomplete collections presented the 7·4 % of participants (Table 2).

Table 3 presents mean Na intake or excretion for all the available UM and DM applied, as well as the significance of the differences between 24UNa and each one of the other Na estimation methods. Mean 24UNa was 2810·4 ± 1303·9 mg/d. Regarding spot urine methods, all of them overestimated 24UNa (mean bias range: −1780·9 to −492·0 mg) with the INTERSALT without spot K equation presenting the smallest bias (−492·0 ± 1223·2 mg) (Table 4). Regarding the existing DM, both of them underestimated 24UNa (mean bias range: 876·6 to 1211·6 mg). From the improved DM, 24DR + 15 % and 24DR + SQ underestimated 24UNa (876·6 ± 1342·6 and 923·3 ± 1345·8 mg, respectively, P < 0·001), but the NaFFQ marginally overestimated 24UNa showing the smallest bias from all DM and UM (−290·2 ± 1336·2 mg) (Table 3).

Table 4 presents Pearson's and Spearman's correlation tests as well as the ICC between 24UCol and each one of the UM and DM. Regarding spot urine methods, Mage equation exhibited the strongest correlation with 24UCol (r = 0·596, P < 0·001), and all other equations presented moderate reliability (ICCs range: 0·59–0·74). From the existing DM, both of them weakly correlated to 24UCol (r = 0·232–0·263, P < 0·05). Regarding the improved DM, 24DR + 15 % and 24DR + SQ were weakly correlated to 24UCol (r = 0·263-0·296, $P \leq$ 0·01) and presented poor reliability (ICC range: 0·42–0·44), but NaFFQ exhibited the strongest correlation with 24UCol (r = 0·497, $P \leq$ 0·01) and was moderately reliable (ICC 0·66 (95 % CI 0·43, 0·80)). In subgroup analysis (data presented in the Supplement – see online Supplemental Table 3): (a) four out of the five subgroups agreed that Mage equation exhibited the strongest correlation with the 24UCol (r = 0·625–0·700, P < 0·001) and (b) three out of the five agreed that Kawasaki equation was the only method presenting good reliability (ICC range: 0·76–0·80) and all

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Table 2 Descriptive characteristics of the study population for the total sample and each Na estimation method

	Urinary methods						Dietary methods							
	24UCol *n* 122		Spot urine *n* 71				24DR *n* 119				FFQ *n* 87		NaFFQ *n* 60	
	Mean	SD	Mean	SD			Mean	SD			Mean	SD	Mean	SD
Age, years	56·0	12·6	56·2	11·9			55·9	12·6			56·1	13·1	56·4	12·3
Weight, kg	80·7	18·0	81·4	18·9			80·4	18·1			80·3	18·7	80·1	18·9
Height, cm	169·9	11·3	170·7	11·3			169·9	11·4			170·3	11·6	170·8	11·9
BMI, kg/m^2	27·9	5·6	27·9	6·0			27·9	5·6			27·6	5·2	27·3	5·1
Energy, kcal/d	–	–	–	–			1998·8	668·3			2238·7	713·7	2210·7	695·5
					Existing 24DR		*24DR + 15 %*		*24DR + SQ*					
Na derived from food, mg/d	–	–	–	–	1633·8	763·6	1633·8	763·6	1633·8	763·6	1704·3	800·0	1793·5	873·5
Na derived from table salt, mg/d	–	–	–	–	–	–	288·3	134·8*	58·6	90·3	–	–	1197·4	1047·2*
Na derived from cooking salt, mg/da	–	–	–	–	–	–			276·4	261·6	–	–		
	(%)		(%)				(%)				(%)		(%)	
Males	55·7		56·3				55·5				57·5		60·0	
Smoking														
Current (cigarette/e-cigarette)	40·5		43·6				41·5				40·7		38·8	
Ex smoking	20·7		22·5				20·3				19·8		23·3	
Never	38·8		33·8				38·1				39·5		38·3	
CVD	10·7		7·1				11·0				11·5		10·0	
T1DM	1·6		1·4				1·7				2·3		1·7	
T2DM	8·2		11·3				8·4				8·0		10·0	
DMS drugs	5·7		7·0				5·9				6·9		6·7	
Hypertension	64·8		60·6				63·9				65·5		58·3	
Hypertension drugs	46·7		42·3				45·4				48·3		43·3	
Dyslipidaemia	65·6		64·8				65·5				66·7		68·3	
Dyslipidaemia drugs	33·6		32·4				33·6				31·0		25·0	
Autoimmune/inflammatory disease	13·2		15·5				12·7				11·5		13·3	
Infectious disease	30·6		32·4				30·5				29·9		31·7	
Incomplete 24 h UCol	7·4													

24UCol, 24-h urine collection; 24DR, 24-h dietary recalls (three 24DR were performed); 24 DR + 15 %, 24-h dietary recalls Na plus 15 % (discretionary Na); 24 DR + SQ, 24-h dietary recalls Na plus discretionary salt questions; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus.
*Na derived from table and cooking salt.

Table 3 Na intake/excretion for each dietary and urinary Na estimation method, bias of mean values and comparisons with the 24-h urine collection

	n	Na intake or excretion mean	SD	Bias (24UNa minus each Na estimation method) mean	SD	*P*
Urinary methods						
24UCol, mg/d	122	2810·4	1303·9	–		–
Kawasaki, mg/d	71	4523·0	1331·0	−1780·9	1235·2	<0·001
Tanaka, mg/d	71	4862·1	10 633·2	−894·8	1154·1	<0·001
INTERSALT with spot K, mg/d	67	3209·4	869·0	−599·0	1140·0	<0·001
INTERSALT without spot K, mg/d	71	3207·8	843·1	−492·0	1223·2	0·001
Mage, mg/d	71	3438·8	2494·8	−722·6	2050·6	0·016
Toft, mg/d	71	3852·8	955·7	−1136·6	1165·6	<0·001
Dietary methods						
Existing dietary methods						
24 DR, mg/d	119	1633·8	763·6	1211·6	1298·8	<0·001
FFQ, mg/d	87	1704·3	800·0	1058·7	1335·7	<0·001
Improved dietary methods						
24 DR + 15 %, mg/d	119	1922·2	898·3	923·3	1345·8	<0·001
24 DR + SQ, mg/d	119	1968·9	917·0	876·6	1342·6	<0·001
NaFFQ, mg/d	60	2990·9	1397·5	−290·2	1336·2	0·098

24UCol, 24-h urine collection; 24UNa, 24-h urine Na; 24 DRNa, 24-h dietary recalls Na; 24 DR + 15 %, 24-h dietary recalls Na plus 15 % (discretionary Na); 24 DR + SQ, 24-h dietary recalls Na plus discretionary salt questions.

Table 4 Pearson's and spearman correlations & intraclass correlation coefficients between 24-h urine collection and the other Na estimation methods

		Na estimation methods		Total sample	95 % CI
Spot urine methods		Kawasaki	*r*	0·583**	
			ICC	0·74	0·58, 0·84
			n	71	
		Tanaka	*r*	0·542**	
			ICC	0·66	0·46, 0·79
			n	71	
		INTERSALT with spot K	*r*	0·492**	
			ICC	0·63	0·40, 0·77
			n	67	
		INTERSALT without spot K	*r*	0·469**	
			ICC	0·59	0·35, 0·75
			n	71	
		Mage	*r*	0·596**	
			ICC	0·65	0·44, 0·78
			n	71	
		Toft	*r*	0·570**	
			ICC	0·68	0·48, 0·80
			n	71	
Dietary methods	Existing dietary methods	24DR	*r*	0·263**	
			ICC	0·40	0·14, 0·58
			n	119	
		FFQ	*r*	0·232*	
			ICC	0·39	0·06, 0·60
			n	87	
	Improved dietary methods	24DR + SQ	*r*	0·296**	
			ICC	0·44	0·20, 0·61
			n	119	
		24DR + 15 %	*r*	0·263**	
			ICC	0·42	0·17, 0·60
			n	119	
		NaFFQ	*r*	0·497**	
			ICC	0·66	0·43, 0·80
			n	60	

ICC, intraclass correlation coefficient; 24UNa, 24-h urine Na; 24 DRNa, 24-h dietary recalls Na; 24DRNa + 15 %, 24-h dietary recalls Na plus 15 % (discretionary Na); 24-h DRNa + SQ, 24-h dietary recalls Na plus discretionary salt questions.
*$P < 0·05$.
**$P \leq 0·01$.

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the five subgroups agreed that regarding the existing and the improved DM, NaFFQ was the only method presenting moderate reliability (ICCs range: 0·44–0·51), while all the other DM presented poor reliability (ICC range: 0·20–0·32).

Bland–Altman plots for all the spot urine methods, existing DM, and improved DM are presented in Figs 1, 2, and 3, respectively. Regarding spot urine methods, the use of equations of Toft, INTERSALT with spot K and INTERSALT without spot K resulted in underestimation at lower levels and overestimation at higher levels of Na excretion in Bland–Altman plots (Fig. 1). On the contrary, Mage equation was the only method providing the opposite finding, presenting overestimation at low levels of Na excretion and underestimation at higher levels. Finally, the Kawasaki equation exhibited a homogeneous variation as Na excretion levels increase (Fig. 1). All methods presented wide ranges of agreement (Kawasaki: −4201·8 to 640·0; Mage: −4741·7 to 3296·6; Toft: −3421·2 to 1148·0; INTERSALT without spot K: −2889·4 to 1905·4; INTERSALT with spot K: −2833·3 to 1635·4; Tanaka: −3156·9 to 1367·3) (Fig. 1). Linear regression analysis revealed statistically significant associations between the difference and the mean of 24UCol and all the spot urine methods, except from the Kawasaki equation ($\beta = 0{\cdot}028$, $P = 0{\cdot}818$) (Fig. 1). Regarding the existing DM (Fig. 2), both of them presented consistent bias in Bland–Altman plots, underestimating the 24UNa in low levels of Na intake and overestimating in high levels of Na intake, while presenting wide ranges of agreement in Bland–Altman plots (24DR: −1334·1 to 3757·4; FFQ: −1559·2 to 3676·7)

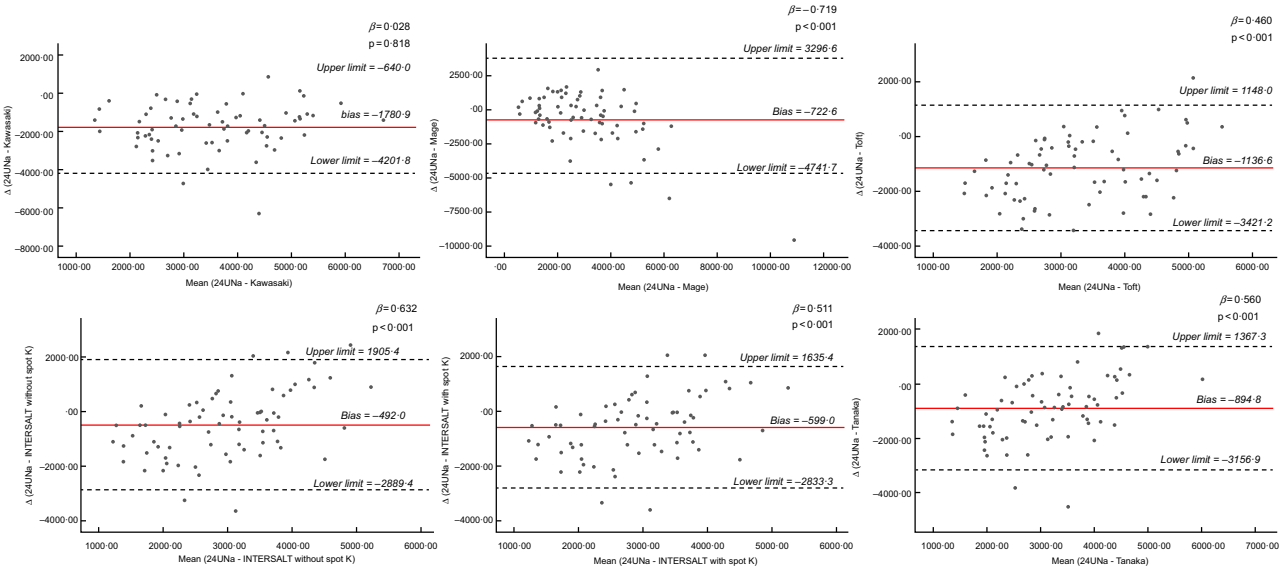

Fig. 1 (colour online) Bland–Altman plots comparing 24-h urinary Na excretion with Na estimated by spot urine equations. Solid line is the mean difference between methods and dashed lines are the 95 % CI of the difference between methods. Limits of agreement of the two Na assessment methods, defined as mean difference ± 1·96 × SD of differences. 24UNa, Na estimated by 24-h urine collection

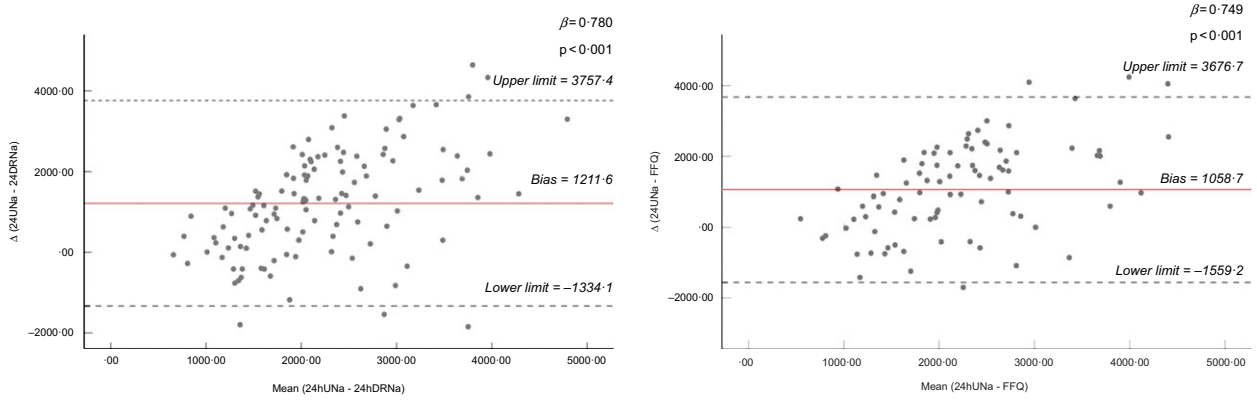

Fig. 2 (colour online) Bland–Altman plots comparing 24-h urinary Na excretion with Na estimated by existing DM. Solid line is the mean difference between methods, and dashed lines are the 95 % CI of the difference between methods. Limits of agreement of the two Na assessment methods, defined as mean difference ± 1·96 × SD of differences. 24UNa, Na estimated by 24-h urine collection; 24-h DRNa, Na estimated by 24-h dietary recalls

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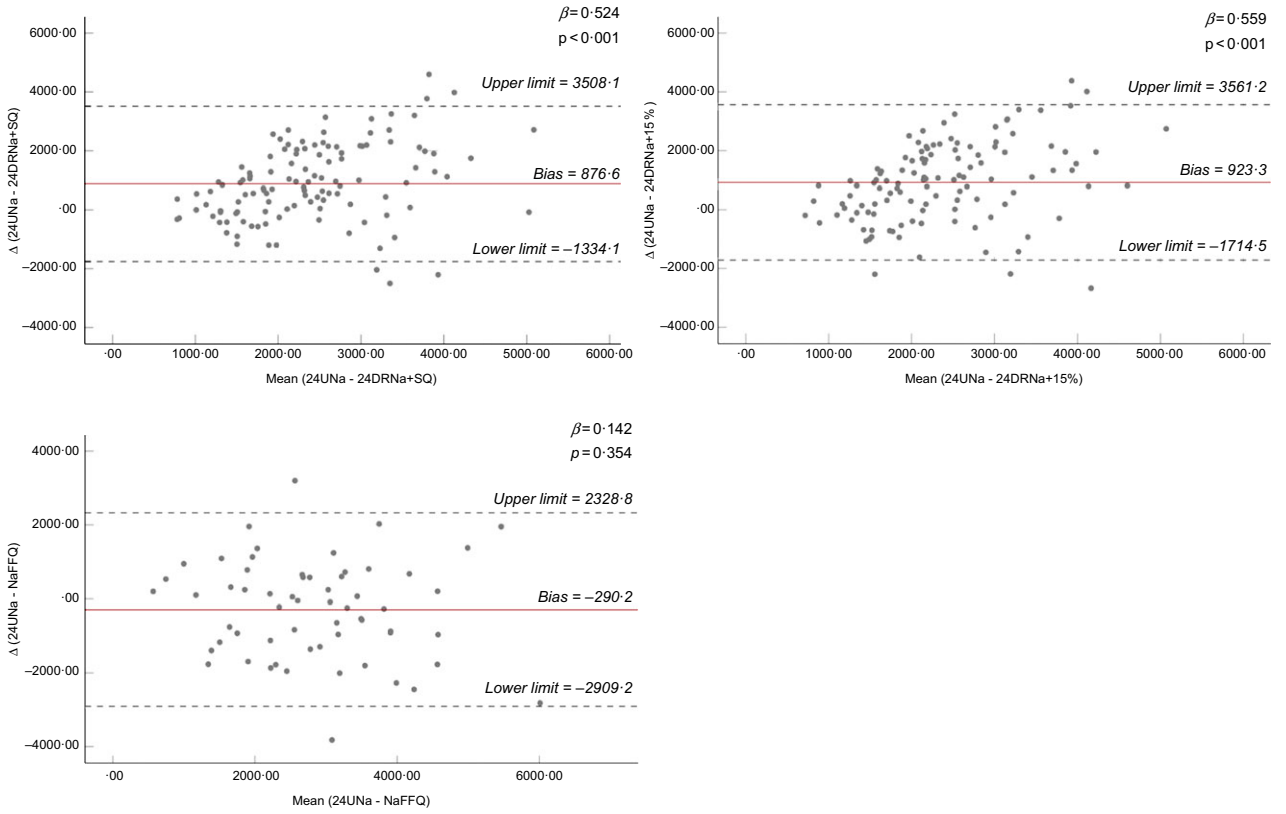

Fig. 3 (colour online) Bland–Altman plots comparing 24-h urinary Na excretion with Na estimated by improved DM. Solid line is the mean difference between methods and dashed lines are the 95 % CI of the difference between methods. Limits of agreement of the two Na assessment methods, defined as mean difference ± 1·96 × SD of differences. 24UNa, sodium estimated by 24-h urine collection; 24-h DRNa + SQ, Na estimated by 24-h dietary recalls plus salt-related questions

(Fig. 2). Linear regression analysis revealed statistically significant association between the difference and the mean of 24UCol and all the DM (Fig. 2). Regarding the improved DM, the NaFFQ was the only one showing: (a) a homogeneous variation as the mean Na intake increases in Bland–Altman plots, however, presenting wide ranges of agreement (−2909·2 to 2328·8) and (b) not statistically significant association between the difference and the mean of 24UCol and improved DM in linear regression analysis ($\beta = 0{\cdot}142$, $P = 0{\cdot}354$) (Fig. 3). The other two improved DM (24DR + 15 % & 24DR + SQ) underestimated the 24UNa at low levels of Na intake and overestimated at high levels of Na intake, presenting wide ranges of agreement (24DR + SQ: −1334·1 to 3508·1; 24DR + 15 %: −1714·5 to 3561·2) (Fig. 3).

All the analyses were repeated using 1 dash of salt instead of 2 in the *question 2* of the improved dietary recalls (24DR + SQ), and similar findings were observed (data not shown).

Discussion

This study aimed to assess and compare the most commonly used in population studies UM and DM for mean Na intake and develop a new accurate and easy to use clinical tool for Na estimation in high CVD risk populations. The main findings of this study are (i) the existing DM tend to underestimate and spot urine methods tend to overestimate the true Na intake; (ii) all the existing DM are weakly correlated and present poor agreement with the 24UCol, and all the spot urine methods are moderately correlated and present moderate agreement with the 24UCol and (iii) the new NaFFQ is the only method that performed better in the analysis, having simultaneously the smallest bias in mean differences, the strongest correlation with the 24UCol regarding DM and a homogeneous variation as the mean Na intake increases in Bland–Altman plots, but still wide limits of agreement.

Spot urine collection is an easily applicable alternative in estimating dietary Na intake. Increasing studies aim to reveal the most accurate formula for converting spot Na to 24UNa, comparing not only those commonly used[17,44–48] but also those newly designed[49,50] against the gold-standard 24UCol. The mostly studied formulas are the INTERSALT equation, the Tanaka equation and the Kawasaki equation. Despite some controversies[50], a large number of studies support that among the existing equations, the INTERSALT performs better in estimating the 24UNa showing the least bias[44,45,49,51,52]. In our findings, the INTERSALT equation presented the lowest bias among all the other equations; however, it was moderately correlated with 24UNa and also presented consistent bias in

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Bland–Altman plots by underestimating Na intake at low levels of Na excretion and overestimating at high levels of Na excretion. However, it is important to note that the studies supporting the use of INTERSALT equation as the best alternative of 24UCol for Na estimation have all been conducted in general populations[44,45,49,51,52], which is in contrast to our high CVD risk population. Indeed, the evidence is not supportive of the use of the INTERSALT equation in high-risk patients, having chronic diseases such as chronic kidney disease or hypertension[46,53]. Dougher *et al.* compared commonly used equations for Na estimation in 129 chronic kidney disease patients[53]. According to their findings, the authors conclude that spot urine equations do not estimate accurately dietary Na intake in this group of people. Similarly, when Ma *et al.* assessed Na intake by the INTERSALT, the Tanaka and the Kawasaki equations in 365 high-risk stroke patients, they found poor correlations ($r = 0{\cdot}35$–$0{\cdot}38$), poor reliability (ICCs = 0·31–0·38) and significant biases among all the three methods compared with the 24UCol[46]. These findings are in agreement not only with our study but also with a significant number of studies, which do not recommend the use of spot equations for dietary Na estimation[17,47,48,54,55]. It is important to note that Na excretion presents a circadian variability, which potentially could influence the estimations derived from spot urine collections. A systematic review of studies comparing the 24UCol and spot urine collections for estimating salt intake, conducted by Ji *et al.*, included twenty studies and 1·380·130 participants, concluded that although it is of great interest to replace the 24UCol as a method for Na intake estimation, the best alternative UM remains uncertain as a wide range of correlations ($r = 0{\cdot}17$–$0{\cdot}94$) between 24UCol and the other methods presented in their work[56]. Also, a systematic review and meta-analysis in 10·414 participants from thirty-four countries showed that *'estimates based upon spot urine samples have excellent sensitivity (97 %) and specificity (100 %) at classifying mean population salt intake above or below the World Health Organization maximum target of 5 g/d but underestimate intake at high levels of consumption and overestimate at lower levels of consumption'*[57]. Even more interestingly, in a recent analysis of TOHP (Trials of Hypertension Prevention) study follow-up data, conducted by He *et al.*, estimated values of Na excretion (using the Kawasaki, INTERSALT with spot K and Tanaka equations) – examining the same population sample – altered the linear association between 24UNa and mortality to J- or U-shaped[58]. The authors concluded that these urinary Na estimation methods *'were systematically biased with overestimation at lower levels and underestimation at higher levels'*, indicating that estimation of Na through spot urine specimens is inaccurate[58]. All these findings are consistent to a WHO/PAHO statement in the protocol for population level Na determination in 24-h urine sample, declaring that *'the use of spot-urine is discouraged as a method to determine Na, potassium or iodine intake because of the limitations and uncertainty inherent in the method'*.

As regard to the existing DM for Na estimation, although it is useful and efficient to highlight food items rich in Na, several methodological disadvantages have been raised. The most commonly discussed include the difficulty or even inability to assess and quantify discretionary Na; deviated estimations of Na due to high variability in Na content in recipes of homemade and manufactured food; the absence of Na derived from medicines and dietary supplements and participant-related issues (underreporting and difficulty to recall all the food and beverages consumed; socially desired answers and dietary behaviour modification)[6]. A small number of studies suggest that DM, such as food diaries or multiple 24DRs, can be used for Na estimation, having the ability to predict over 90 % of 24UNa[20,59]. However, the majority of the available studies have reported that Na estimation based on DM tends to underestimate 24UNa (levels of underreporting 15–40 %) and correlates weakly or moderately with 24UNa ($r \approx 0{\cdot}15$–$0{\cdot}50$)[20,60–65]. This is in line with our findings, showing weak correlations, poor reliability and high levels of bias, suggesting that the existing DM for Na estimation are inaccurate. In a recent meta-analysis including twenty-eight studies, McLean *et al.* compared 24DR with 24UCol[66]. 24DR underestimated mean Na intake by 607 mg/d, but high quality 24DR improved accuracy. The authors concluded that 24UCol remains the most accurate method to assess population Na intake; however, high-quality 24DR (use of multiple pass methods, accurate food composition databases and quantification of discretionary salt) could be used if 24UCol is not feasible[66].

To our knowledge, studies comparing different DM and UM simultaneously for Na intake estimation are scarce. A recent study compared the spot urine collection (using the INTERSALT equation) *v.* the 24DR (without quantifying the discretionary use of table salt) in a large sample of adults in New Zealand, consisting of 3321 participants[67]. The authors observed poor agreement between estimated Na intake from spot urine collection and those from 24DR[67]. In another study, a plethora of different DM and UM were compared with a PABA-validated 24UCol[16]. The assessment of Na intake included an FFQ, a modified 24DR and three equations to convert the spot Na to 24UNa (INTERSALT, Tanaka and Kawasaki). In this study neither DM nor UM provided accurate estimations at individual level, but for group means, the DM and some of the UM may be useful for Na estimation[19]. However, the method for the quantification of Na intake has not been clearly described[19].

FFQ are commonly used in dietary Na assessment in population-based studies, having the ability to bypass problems related to day-to-day variability of Na intake and cover larger time periods of intake. The last four decades several FFQ have been designed for the estimation

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of Na (or salt) intake[23,24,68–71]. However, most of them present weak correlations with the 24UCol, ranging from 0·19 to 0·35[23,24,69,71]. Furthermore, the available FFQ for Na assessment have been designed for particular ethnic groups[24,25,69–71]. To our knowledge, only two of them have been developed for hypertensive subjects[23,25] but until now, there was no FFQ for Na estimation in other high CV risk groups, such as patients with dyslipidaemia, diabetes mellitus, infectious or autoimmune diseases. Recently, McLean *et al.* published a systematic review of the literature, regarding the assessment of dietary Na intake using FFQ and 24UCol[65]. This work revealed a poor agreement between estimates of Na from FFQ and 24UCol[65], indicating that the Na FFQ until now are inadequate to estimate the true intake.

The novel NaFFQ was created to accurately estimate Na intake in high CVD risk populations, calculating not only Na derived from food content, but table and cooking Na as well. Our aim was to cover the need of an easily applicable in epidemiological studies and reliable tool for group means of Na intake, which could lead to a better management of high CVD-risk populations. According to our findings, this tool presented the best correlation with – and the lowest bias from – the 24UCol compared with all the existing DM, even when attempts to further improve the accuracy of 24DR were applied. However, despite these promising findings regarding NaFFQ, it provided very wide limits of agreement in Bland–Altman plots, reaching ~3000 mg/d, indicating that future improvements have to be addressed. A limitation of our study is the use of a single 24UCol. Due to the day-to-day variability in Na intake and excretion, multiple 24UCol are recommended either for assessing accurately usual *individual* Na intake or for a more reliable record of dietary Na in studies investigating its relationship with health or disease[72,73]. In our study, our aim was to estimate Na intake in group means and not in individual level, so the use of single 24UCol, which is very common in epidemiological studies, was reasonable. Indeed, the use of a single 24UCol *v.* three to seven 24UCol have been reported to provide similar mean levels of Na excretion at the population level[74]. Second, an important limitation to be mentioned is the method used for the quantification of discretionary salt. In our study, the use of dashes of salt, as well as the cut-offs that were designed for processed food, may lead to several concerns and systematic bias. However, until today, the estimation of discretionary salt in studies remains a challenge for the investigators, and there is no generally accepted protocol to be applied in dietary surveys[75]. Moreover, the NaFFQ is population specific and has not been externally validated in other populations. Nevertheless, the methodology used here could be used to adapt other FFQ, designed for other population groups, in order to more accurately estimate Na intake.

In conclusion, the available DM and spot urine methods present poor accuracy compared with the gold-standard 24UCol. The new FFQ – specifically designed for Na estimation – is a promising method to detect a mean Na intake at population level in high CVD-risk people. Future validation of this tool in larger populations would verify its accuracy and/or provide evidence for further amelioration, making it a reliable and easy to use clinical tool for Na quantification, in population-based studies. Similar approaches might be useful for other populations.

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Supplementary material

For supplementary material accompanying this paper visit https://doi.org/10.1017/S1368980021004390

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Review

Current Data on Dietary Sodium, Arterial Structure and Function in Humans: A Systematic Review

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Abstract: Background: Subclinical arterial damage (SAD) (arteriosclerosis, arterial remodeling and atheromatosis) pre-exists decades before cardiovascular disease (CVD) onset. Worldwide, sodium (Na) intake is almost double international recommendations and has been linked with CVD and death, although in a J-shape manner. Studies regarding dietary Na and major types of SAD may provide pathophysiological insight into the association between Na and CVD. Objectives: Systematic review of data derived from observational and interventional studies in humans, investigating the association between dietary Na with (i) atheromatosis (arterial plaques); (ii) arteriosclerosis (various biomarkers of arterial stiffness); (iii) arterial remodeling (intima–media thickening and arterial lumen diameters). Data sources: Applying the PRISMA criteria, the PubMed and Scopus databases were used. Results: 36 studies were included: 27 examining arteriosclerosis, four arteriosclerosis and arterial remodeling, three arterial remodeling, and two arterial remodeling and atheromatosis. Conclusions: (i) Although several studies exist, the evidence does not clearly support a clinically meaningful and direct (independent from blood pressure) effect of Na on arterial wall stiffening; (ii) data regarding the association of dietary Na with arterial remodeling are limited, mostly suggesting a positive trend between dietary Na and arterial hypertrophy but still inconclusive; (iii) as regards to atheromatosis, data are scarce and the available studies present high heterogeneity. Further state-of-the-art interventional studies must address the remaining controversies.

Keywords: dietary sodium; arterial structure; arterial function; arteriosclerosis; arterial stiffness; arterial remodeling; arterial hypertrophy; atheromatosis; arterial plaques

1. Introduction

Cardiovascular disease (CVD) is responsible for 31 percent of all deaths worldwide (WHO 2018). The onset of CVD is preceded for decades by subclinical vascular functional and/or structural alterations, leading to transient or permanent subclinical arterial damage (SAD). Major types of SAD include atheromatosis (arterial atheromatic plaque formation), arteriosclerosis (arterial stiffening due to loss of the arterial wall's elastic properties) and arterial remodeling (changes in arterial wall and lumen dimensions to maintain mechanical homeostasis). All the above modifications may occur simultaneously or separately.

In the last decade, a range of reliable, non-invasive vascular biomarkers have been used to detect SAD. Carotid ultrasonography is widely used to detect structural changes in the arterial wall (such as arterial plaques, indices of arterial remodeling, e.g., carotid intima–media thickness (cIMT) and arterial

lumen diameters) [1–3]. On the other hand, arterial stiffening is classically measured by applanation tonometry to obtain carotid–femoral pulse wave velocity (cfPWV), the gold standard for clinical practice, although other methods have been used [3]. The study of these vascular biomarkers provides the opportunity to not only optimize CVD risk classification but also to elucidate the pathogenesis and pathophysiology of CVD in the early clinical steps.

Globally, sodium (Na) intake is almost double (mean intake: 3.95 g/day) [4] the recommended levels by the World Health Organization (less than 2 g/day) [5]. High Na intake has been strongly correlated with CVD [6–8]. Moreover, there is strong evidence from large-scale studies of a blood pressure (BP)-lowering effect (by 3.39 mmHg for systolic BP and 1.54 mmHg for diastolic BP)—and consequently CVD-risk lowering effect—after a reduction in Na intake to less than 2 g/day compared to an intake higher than 2 g/day [8]. However, very low levels of Na intake (approximately below 1.5 g/day) have also been linked to increasing CVD risk, suggesting a J-shaped trend [9–14]. Although the effect of salt on BP is variable due to salt sensitivity subtypes, consideration of this heterogeneity has been neglected in previous meta-analyses [9,10]. Several observational and/or interventional studies have tested the association of Na intake with types of SAD, but there are still many contradictory results and questions to be addressed [15,16]. Most data derive from studies investigating the relationship between Na and arterial stiffness or hypertrophy, suggesting that higher levels of Na intake are positively associated with these types of SAD [17–21], although this has not been seen consistently [16–18]. Studies regarding dietary Na and atheromatosis are scarce and are limited by major methodological issues [19,20].

In an attempt to better understand the potential associations that link dietary Na intake and SAD, the aim of this systematic review is to evaluate—for the first time—data from observational and interventional studies in humans, investigating associations between dietary Na intake and SAD as well as the effect of Na intake on SAD-related changes. All types of SAD were considered, namely (i) atheromatosis (arterial plaques); (ii) arteriosclerosis (arterial stiffening); (iii) arterial remodeling (intima–media thickening and arterial lumen diameters).

2. Materials and Methods

This study was prepared and reported in accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [21] (Appendix A).

2.1. Search Strategy

A systematic search of potentially relevant studies was performed through July 2019 by two separate reviewers on the PUBMED and SCOPUS databases. Search terms applied were: ((“sodium intake” or “na intake” or “na+ intake” or “sodium excretion” or “na excretion” or “na+ excretion” or “dietary sodium” or “dietary na” or “dietary na+” or “urinary sodium” or “urinary na” or “urinary na+”)) and (“arterial function” or “vascular function” or “arterial structure” or “vascular structure” or plaque or atheroma or “atheromatic plaque” or “atherosclerotic plaque” or atheromatosis or atherosclerosis or arteriosclerosis or “arterial remodeling” or “carotid plaque” or “femoral plaque” or “arterial stiffness” or “arterial stiffening” or “pulse wave velocity” or pwv or “intimal medial thickness” or “intima media thickness” or IMT or “wall to lumen ratio”). Studies were limited to the English language and human studies. Reference lists of included articles were also examined for additional relevant articles.

2.2. Inclusion and Exclusion Criteria

The following inclusion criteria were applied: relevant epidemiological studies or clinical trials, English language, human studies, males and/or females of any age regardless of diseases (chronic or acute), clearly described outcome defined as: association between Na intake and/or excretion with atheromatosis (presence of plaques), arteriosclerosis (any accepted biomarker of arterial stiffening at any arterial segment) or arterial remodeling (arterial hypertrophy (IMT) or artery lumen diameters).

The following exclusion criteria were applied: epidemiological studies with a sample <100 subjects, animal studies, reviews, systematic reviews, meta-analyses, comments/letters, studies using the assessment of Na intake and/or excretion of biomarkers other than Na (e.g., the ratio Na/K).

2.3. Selection of Studies and Data Extraction

Two reviewers screened the available titles, abstracts and keywords of all the available articles. Discrepancies were resolved after discussion. After agreement, full text screening was carried out. Qualitative and quantitative data from all included articles were extracted by both reviewers. The extracted data included specific details for study design, population characteristics, Na estimation method and outcomes related to Na and vascular damage. All units of Na are presented as mg (converted from mmol to mg, if necessary). Predefined variables (shown in Tables 1–5) were extracted.

3. Results

3.1. Number of Studies Screened and Selected

Eight hundred and twenty-five (825) citations were identified through a systematic search—of which, 782 were excluded on the basis of title/abstract. The most common exclusion criteria were: language (42), duplicates—same cohort (27), different research subject (516), not original articles (191), sample size <100 subjects for observational studies only (6). Forty-three (43) articles were then assessed for eligibility and five were excluded due to irrelevant research subject and two due to different studying parameter (Na/K ratio). As a result, the number of articles that met the inclusion criteria and were included in this study were 36 (Figure 1).

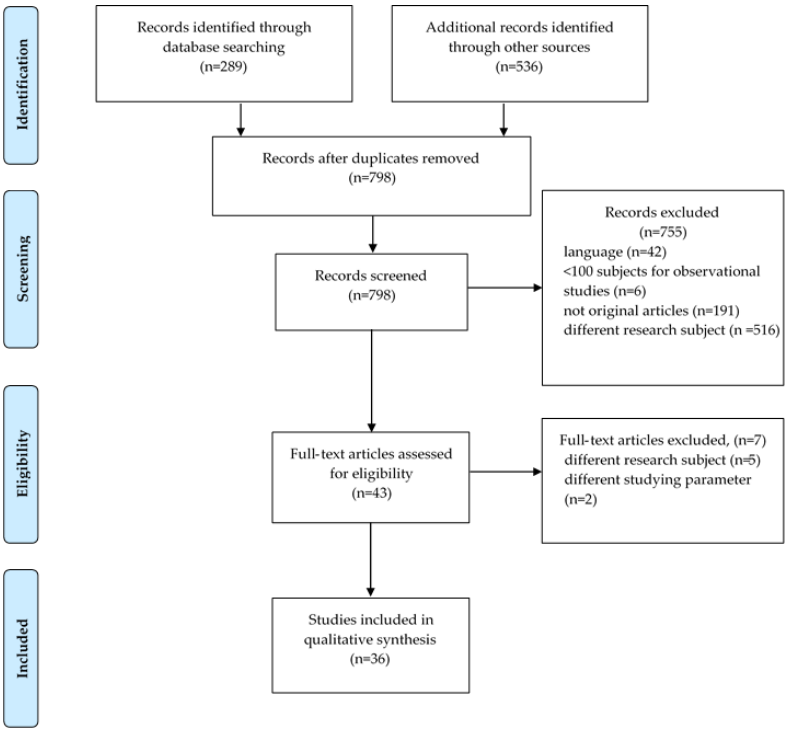

Figure 1. PRISMA flow diagram.

From a total of 36 studies included, 18 were observational and 18 were interventional studies. Detailed descriptive data for both observational and interventional studies are provided in Tables 1–5.

Table 1. Descriptive characteristics of observational studies regarding arteriosclerosis.

ARTERIOSCLEROSIS												
cfPWV												
1. Observational Cross-Sectional Studies												
Author (Year)	**Country**	**Study Design**	**Population Description**	**FU (Years)**	**Sex**	**Race**	**N**	**Age (Years, Mean ± SD)**	**Na Estimation Method**	**Na Intake/Excretion (mg/d)**	**Vascular Assessment**	**Results**
Polónia, J. (2006) [22]	Portugal	c-sect	essential HT, recent stroke or healthy university students	-	M/F	Mixed	426	50 ± 22	24hU	Total: 4646 ± 1472	tonometry	+ **
García-Ortiz, L. (2012) [23]	Spain	c-sect	primary care patients aged 30–80	-	M/F	N/AV	351	54.8 ± 11.7	FFQ	Total: 3180 ± 1250 Q1:1800 ± 390 Q2: 2650 ± 200 Q3: 3440 ± 270	tonometry	J-shaped curve
Kotliar, C. (2014) [24]	Argentina	c-sect	essential HT, aged 30 to 70	-	M/F	N/AV	300	48.7 ± 14.6	24hU	(a) low Na-low RAAS: 913.1 (747.5–1035)	tonometry	≠
										(b) low Na-high RAAS: 690 (602.6–740.6)		≠
										(c) High-Na-low RAAS: 2610.5 (1745.7–3604.1)		≠
										(d) high-Na-high RAAS: 2898 (2035.5–3588)		+ *
Polonia, J. (2016) [25]	Portugal	retrosp	HT adults	7.2 (0.5–11.1)	M/F	White	608	54.1 ± 14.3	24hU	4793.2 ± 1821.6	tonometry	+ *
Strauss, M. (2018) [26]	South Africa	prosp	NT adults	-	M/F	Mixed	693	24.8 ± 3.01	24hU	2967 (984.4–7613)	tonometry	total + **
												black + *
												white ≠
Triantafyllou, A. (2018) [27]	Greece	c-sect	untreated HT—healthy individuals	-	M/F	White	197	43.7 ± 12.1	24hU	True HT: 3348.8 (2251.7–4595.4) Intermediated HT phenotypes: 3128 (1902.1–4312.5) NT: 2732.4 (1630.7–4312.5)	tonometry	≠

Table 1. *Cont.*

ARTERIOSCLEROSIS												
2. Observational Studies with Follow Up (>1 Time Points)												
Author (Year)	**Country**	**Study Design**	**Population Description**	**FU (Years)**	**Sex**	**Race**	**N**	**Age (Years, Mean ± SD)**	**Na Estimation Method**	**Na Intake/Excretion (mg/d)**	**Vascular Assessment**	**Results**
Nerbass, F.B. (2015) [28]	UK	prosp	adults in CKD stage 3	1	M/F	N/AV	1607	72.6 ± 9.0	Spot urine collections and Nerbass equation to estimate 24h Na excretion	baseline: 2599 ± 782 follow-up: 2576 ± 782	oscillometry	**cfPWV** (at both time points) Na <2300 mg + ** Na >2300 mg ≠ **ΔcfPWV** Unchanged Na ≠ Decreased Na ≠ Increased Na + *
Aortic PWV Other than cfPWV												
Siriopol, D. (2018) [29]	Romania	prosp	HT and NT Romanian adults	-	M/F	White	1599	47.3 ± 17.1	Morning spot urine sample + Kawasaki equation	4816.2 ± 1550.2	oscillometry	NT + ** HT + **
baPWV												
1. Observational Cross-Sectional Studies												
Sonoda, H. (2012) [30]	Japan	c-sect	healthy subjects	-	M/F	Asian	911	61.3 ± 8.5	24hU	720 ± 200 (mg /day/10 kg)	oscillometry	+ **
Lee, S.K. (2015) [16]	Korea	c-sect	non-HT subjects, with no use of anti-HT drugs	-	M/F	Asian	1586	tertile 1: 52.1 ± 5.5 tertile 2: 53.0 ± 6.0 tertile 3: 52.6 ± 5.5	Second morning void and Tanaka's equation to convert to 24hU	3588 ± 782	plethysmography	– **
Sun, N. (2015) [31]	China	c-sect	newly diagnosed HT, untreated HT or patients with a 1 to 5 year history of HT who had stopped taking anti-HT drugs for 1 month	-	M/F	N/AV	341	Group A: 59.3 + 13.4 Group B: 56.1 + 15.5 Group C: 57.6 + 14.2	24hU	Total: 3507.5 ± 1577.8 Group A: 1807.8 ± 411.7 Group B: 3374.1 ± 618.7 Group C: 5858.1 ± 961.4	oscillometry	+ *
Author (year)	**Country**	**Study Design**	**Population Description**	**FU (years)**	**Sex**	**Race**	**N**	**Ag (Years, Mean ± SD)**	**Na Estimation Method**	**Na Intake/Excretion (mg/d)**	**Vascular Assessment**	**Results**
Han, W. (2017) [32]	China	c-sect	HT adults	-	M/F	N/AV	431	Group A: 54.5 ± 12.9 Group B: 52.9 ± 12.6 Group C: 50.9 ± 11.4	24hU	Total: 3831.8 ± 1.610, Group A: 1768.7 ± 464.6 Group B: 3371.8 ± 650.9 Group C: 5947.8 ± 1069.5	oscillometry	+ *

Table 1. *Cont.*

ARTERIOSCLEROSIS													
2. Observational Studies with Follow Up (>1 Time Points)													
Jung, S. (2019) [15]	South Korea	prosp	adults aged >40	5.3±1.0	M/F	Mixed	2145	59.9 ± 9.1	FFQ and 3 day diet record	2538 ± 1416	oscillometry	**baPWV**	
												Na baseline	+ **
												Na average of three visits	+ **
												ΔbaPWV	
												Na baseline	+ **
												Na average of three visits	+ **
Common Carotid Arterial Elasticity (Young's Elastic Modulus, Stiffness Index, and Arterial Compliance)													
Ferreira-Sae, M.C. (2011) [33]	Brazil	c-sect	HT adults	-	M/F	N/AV	134	58 ± 1	1. FFQ 2. 24h recall 3. discretionary Na intake [1]	Na intake/d: 5520 ± 290 FFQ: 1450 ± 180 24h recall: 940 ± 70 Discretionary Na: 3130 ± 190	B-mode US	Young's elastic modulus	+ **
												stiffness index	≠
												arterial compliance	≠

The mmol of Na intake/excretion values were converted to mg. If available, results presented come from adjusted models. Abbreviations: Na: sodium; 24hU: 24h urine collection; cfPWV: carotid–femoral pulse wave velocity; baPWV: brachial–ankle pulse wave velocity; HT: hypertensives; NT: normotensives; anti-HT: antihypertensive; c-sect: cross-sectional; prosp: prospective; retrosp: retrospective; FFQ: food frequency questionnaire; FU: follow up; M/F: males and females; N/AV: not available; RAAS: renin–angiotensin–aldosterone system; CKD: chronic kidney disease; US: ultrasonography; +: positive association; -: negative association; ≠: no statistically significant association; *: $p < 0.05$; **: $p < 0.01$. [1] number of 1 kg packages of salt consumed/month/person.

Table 2. Descriptive characteristics of interventional studies regarding arteriosclerosis.

ARTERIOSCLEROSIS														
cfPWV														
Author	**Country**	**Study Design**	**Population Description**	**Sex**	**Race**	**N**	**Age (Years, Mean ± SD)**	**Intervention Duration (Weeks)**	**Type of Diet**	**Na Estimation Method**	**Na Intake/Excretion (mg/d)**	**Vascular Assessment**	**Results**	
													Intervention Groups	**cfPWV Change (m/s)**
Seals, D.R. (2001) [34]	USA	RCT	postmenopausal women, ≥50 years, high normal SBP or Stage 1 HTN	F	Mixed	17	65 ± 10	13	LS < 2400 mg	24hU & food records	Urinary Na excretion Preint_restr: 2852 ± 1058 Postintn_restr:1978 ± 736 Dietary Na intake Preint_restr: 2685 ± 559 Postint_restr:1421 ± 512	US	LS vs. baseline	−0.24 *
Dickinson, K.M. (2009) [35]	Australia	cross-over RCT	OW/OB, mild HT adults	M/F	N/AV	29	52.7 ± 6.0	4 (2 weeks × two diets)	Usual Na diet: 3450 mg Na/d vs. LS diet: 1150 mg Na/d	Three-day weighed food records & 24hU	Urinary method Baseline: 3553.5 ± 1568.6 Usual Na: 3594.9 ± 1304.1 LS: 1474.3 ± 949.9	oscillometry	LS vs. Usual Na	≠
He, F.J. (2009) [36]	UK	cross-over dbRCT	HT adults	M/F	Mixed	169	All: 50 ± 11 Blacks: 50 ± 9 Whites: 52 ± 12 Asians: 47 ± 10	12	9 Na tablets (×230 mg)/d & 9 placebo tablets/d. (remained on LS diet: 2000 mg Na/d)	24hU	Total: 3013 ± 1150 Blacks: 3036 ± 1058 Whites: 2921 ± 1173 Asians: 3174 ± 1311	tonometry	From Na to placebo Total Blacks Whites Asians	 −0.40 ** −0.50 ** ≠ ≠
Pimenta, E. (2009) [17]	USA	cross-over RCT	resistant HT adults on a stable anti-HT drug	M/F	Mixed	12	55.5 ± 9.4	2 (1 week × two diets)	LS diet: 1495 mg Na/d vs. HS diet: suppl. >5750 mg Na/d	24hU	Baseline: 4478.1 ± 1577.8 LS diet: 1060.3 ± 616.4 vs. HS diet: 5800.6 ± 1485.8	tonometry	From HS to LS ≠	
Todd, A.S. (2010) [37]	New Zealand	cross-over sbRCT	PHT or HT, NOB adults or on anti-HT drugs	M/F	Mixed	33	51.8 ± 7.6	12	(500 mL tomato juice + LS diet/day) (A) 0 + 1380 mg (B) 2070 + 1380 mg (C) 3220 + 1380 mg	Morning spot urine samples & dietary recalls	Na intake Usual diet: 2607 ± 1289 A: 1254 ± 397 B: 1357 ± 486 C: 1306 ± 335	tonometry	B vs. A C vs. A B vs. C	+ 0.39 ** + 0.35 ** ≠
Todd, A.S. (2012) [18]	New Zealand/Australia	cross-over sbRCT	NT, NOB adults	M/F	N/AV	23	43.7 (24–61)	12	(500 mL tomato juice + LS diet/day) (A) 0 + 1380 mg (B) 2070 + 1380 mg (C) 3220 + 1380 mg	Morning spot urine samples & dietary recalls	Pre-baseline: 2410.4 After intervention (Na intake + Na tomato juice) A: 0 + 1232.8 B: 2070 + 1207.5 C: 3220 + 1140.8	tonometry	B vs. A C vs. A B vs. C	≠ ≠ ≠
McMahon, E.J. (2013) [38]	Australia	cross-over dbRCT	HT adults, with stage 3 or 4 CKD	M/F	N/AV	20	68.5 ± 11	4 (2 weeks × two diets)	HS diet: 4140–4600 mg Na/d vs. LS diet: 1380–1840 mg Na/d	24hU	LS: 1725 (1334–2576) vs. HS: 3864 (3358–5037)	tonometry	LS vs. HS	≠
Dickinson, K.M. (2014) [39]	Australia	cross-over sbRCT	OW or OB subjects	M/F	N/AV	25	N/AV	6	LS diet: 2400 mg/d vs. Usual Na diet: 3600 mg/d	24hU	baseline: 2761 ± 1031 Usual Na diet: 1729 ± 627 LS diet: 1799 ± 497	tonometry	LS vs. US	≠

Table 2. *Cont.*

ARTERIOSCLEROSIS														
Gijsbers, L. (2015) [40]	the Netherlands	cross-over RCT	untreated (P)HT, aged 40–80	M/F	White	36	65.8 (47–80)	4	Na suppl: 3000 mg/d vs. placebo	24hU	Baseline: 3535.1 Na suppl.: 4666.7 ± 1260.4 vs. Placebo: 2417.3 ± 913.1	tonometry	Na suppl. vs. placebo	≠
Suckling, F.J. (2016) [41]	United Kingdom	Cross-over dbRCT	untreated HT adults	M/F	Mixed	46	58 ± 1	12 (6 weeks × two diets)	9 Na tablets (×230 mg)/d vs. 9 placebo tablets/d.	24hU	Na diet: 3797.3 ± 207 vs. Placebo: 2681.8 ± 218.5	tonometry	Na diet vs. placebo	≠
van der Graaf, A.M. (2016) [42]	the Netherlands	cross-over RCT	women with history of preeclampsia or history of healthy former pregnancy	F	N/AV	36	36 ± 5	2	LS diet: 1150 mg/d vs. HS diet: 4600 mg/d	24hU	NT pregnancy history group: LS: 897 ± 322 HS: 5083 ± 1472 Preeclamptic pregnancy history group: LS: 1035 ± 529 HS: 5934 ± 1978	tonometry	LS vs. HS (in either group)	≠
Muth, B.J. (2017) [43]	USA	cross-over RCT	healthy, NT adults	M/F	N/AV	85	Young: 27 ± 1 Middle-aged: 52 ± 1	2	LS diet: 460 mg/d vs. HS diet:6900 mg/d	24hU	* LS diet (young & middle aged): 690 HS diet (young & middle-aged): 5405 * Approximately from diagram	tonometry	middle aged	+0.60 **
													young	≠
Aortic PWV (Other than cfPWV)														
Author	**Country**	**Study Design**	**Population Description**	**Sex**	**Race**	**N**	**Age(Years, Mean ± SD)**	**Intervention Duration**	**Type of Diet**	**Na Estimation Method**	**Na Intake/Excretion (mg/d)**	**Vascular Assessment**	**Results**	
													Intervention groups	**PWV Change (%)**
Avolio AP. (1986) [44]	Australia	RCT	healthy NT adults and children	M/F	N/AV	114	Group 1: Control: 10.8 ± 1.9 LS: 10.4 ± 2.5 Group 2: Control: 39.4 ± 1.7 LS: 39.8 ± 1.6 Group 3: Control: 52.2 ± 3.5 LS: 54.5 ± 4.2	24.8±2.5 months (8 months to 5 years)	N/AV	24hU & diet questionnaire	Na excretion: Control group: N/AV Group 1: 1564 Group 2: 943 Group 3: 506	oscillometry	Group 1 leg	−11.2 *
													Group 2 aortic arm leg	−21.8 ** −10.7 * −13.3 *
													Group 3 aortic leg	−22.7 * −22.3 *
hfPWV														
Rhee MY. (2016) [45]	Korea	RCT	NT and HT adults	M/F	N/AV	101	46.0 ± 16.6	2	LS DASH diet: 2320 mg Na/d vs. HS DASH diet: 7000 mg Na/d	N/AV	LS diet: 2320 vs. HS diet: 7000	US	HS vs. LS	
													SS	+4% *
													SR	≠
													HT	≠
													NT	≠
baPWV														
Wang Y. (2015) [46]	China	dietary intervention study	mild HT adults	M/F	N/AV	49	49.0 ± 7.9	3 (1 week × three diets)	LS diet: 1179.9 mg/d & HS diet: 7079.4 mg/d	24hU	3999.7 ± 1543.3	plethysmography	LS vs. HS	≠
													SS vs. SR	
													Baseline	+2.3 *
													After LS	+1.5 *
													After HS	+2.0 *

Table 2. *Cont.*

ARTERIOSCLEROSIS													
Arterial Elasticity (Arterial Compliance)													
Author	**Country**	**Study Design**	**Population Description**	**Sex**	**Race**	**N**	**Age (Years, Mean ± SD)**	**Intervention Duration**	**Type of Diet**	**Na Estimation Method**	**Na Intake/Excretion (mg/d)**	**Vascular Assessment**	**Results**
													Intervention Groups / **Vascular Change (mm/mmHg)**
Creager MA. (1991) [47]	USA	cross-over RCT	NT men	M	N/AV	17	30 ± 2 years	10 days	LS diet: 230 mg Na/d vs. HS diet: 4600 mg Na/d	24hU	LS: 253 ± 46 vs. HS: 4117 ± 207	diastolic blood pressure time decay method	LS vs. HS: ≠
Gates PE. (2004) [48]	USA	cross-over dbRCT	stage 1 HT adults, older than 50	M/F	White	12	men: 63 ± 1 women: 64 ± 4	8 weeks	LS diet: 1196 ± 92 & Normal Na diet: 1311 ± 23	3 day dietary records & 24hU	Na excretion Baseline: 3105 LS: 1380 Normal Na: 3450	B-mode US	LS: +0.04 *; Normal: ≠

The mmol of Na intake/excretion values were converted to mg. If available, results presented come from adjusted models. Abbreviations: Na: sodium; 24hU: 24h urine collection; cfPWV: carotid–femoral pulse wave velocity; baPWV: brachial–ankle pulse wave velocity; hfPWV: heart-femoral pulse wave velocity; HT: hypertensives; NT: normotensives; PHT: pre-hypertensives; HTN: hypertension; SBP: systolic blood pressure; anti-HT: antihypertensive; OW: overweight; OB: obese; NOB: non-obese; suppl: supplementation; HS: high sodium; LS: low sodium; SS: salt sensitive; SR: salt resistant; RCT: randomized controlled trial; sbRCT: single-blind RCT; dbRCT: double-blind RCT; M/F: males & females; F: females; M: males; N/AV: not available; CKD: chronic kidney disease; US: ultrasonography; ≠: no statistically significant association; *: $p < 0.05$; **: $p < 0.01$.

Table 3. Descriptive characteristics of observational studies regarding arterial remodeling.

Arterial Remodeling												
cIMT												
1. Observational Cross-Sectional Studies												
Author (Year)	**Country**	**Study Design**	**Population Description**	**FU (Years)**	**Sex**	**Race**	**N**	**Age (Years, Mean ± SD)**	**Na Estimation Method**	**Na Intake/Excretion (mg/d)**	**Vascular Assessment**	**Results**
Ferreira-Sae, M.C. (2011) [33]	Brazil	c-sect	HT adults	-	M/F	N/AV	134	58 ± 1	1. FFQ 2. 24h recall 3. discretionary Na intake [1]	Na intake/d: 5520 ± 290 FFQ: 1450 ± 180 24h recall: 940 ± 70 Discretionary Na: 3130 ± 190	B-mode US	+ *
Njoroge, J.N. (2011) [49]	USA	c-sect	OW or OB, physically inactive adults	-	M/F	Mixed	258	Total: 38.5 ± 5.8 Q1: 39.3 ± 5.6 Q2: 38.7 ± 5.2 Q3: 37.6 ± 6.2 Q4: 38.2 ± 6	24hU	Total:1104–9545 Q1: 1104–3289 Q2: 3312–4117 Q3: 4140–5152 Q4: 5175–9545	B-mode US	+ *
García-Ortiz, L. (2012) [23]	Spain	c-sect	primary care patients aged 30–80	-	M/F	N/AV	351	Total: 54.8 ± 11.7 Q1: 57.6 ± 12.1 Q2: 55.9 ± 11.3 Q3: 54.7 ± 10.5	FFQ	Total: 3180 ± 1250 Q1:1800 ± 390 Q2: 2650 ± 200 Q3: 3440 ± 270	B-mode US	J-shaped
Lee SK. (2015) [16]	Korea	c-sect	non-HT subjects, with no use of anti-HT drugs	-	M/F	Asian	1586	tertile 1: 52.1 ± 5.5 tertile 2: 53.0 ± 6.0 tertile 3: 52.6 ± 5.5	second morning void & Tanaka's equation	3588 ± 782	B-mode US	– **
Ustundag, S. (2015) [50]	Turkey	c-sect	ambulatory adult patients, in stage 2–4 CKD	-	M/F	N/AV	193	Na excretion <1955 mg/day: 47.7 ± 10.6 ≥1955 mg/day: 49.7 ± 11.0 Mean IMT <0.750 mm: 45.1 ± 12.2 ≥0.750 mm: 52.3 ± 8.3	24hU	<1955 mg/day: 3220 ± 69 ≥1955 mg/day: 3220 ± 69 Mean IMT < 0.750 mm: 3220 ± 46 Mean IMT ≥ 0.750 mm: 3220 ± 69	B-mode US	+ **
Dai, X.W. (2016) [19]	China	c-sect	Asian adults, via subject referral and community advertisement	-	M/F	Asian	3290	M: 62.1 ± 6.7 F: 59.4 ± 5.5	FFQ	Dietary Na intake: Q1: 833 ± 394 Q2: 864 ± 507 Q3: 825 ± 41 Q4: 828 ± 395	B-mode US	common cIMT ≠ carotid bifurcation IMT + *
Mazza, E. (2018) [20]	Italy	c-sect	adults aged ≥65, not suffering from any debilitating diseases	-	F	White	108	70 ± 4	24h dietary recall + 7 day food record	1476 ± 618	B-mode US	+ *

Table 3. *Cont.*

Arterial Remodeling												
cIMT												
2. Observational Studies with Follow up (>1 Time Points)												
Jung, S. (2019) [15]	South Korea	prosp	adults aged >40	5.4 ± 1.0	M/F	Mixed 2494	60.2 ± 9.0	FFQ + 3 day diet record	2644 ± 1573	B-mode US	**cIMT**	
											Na baseline	≠
											Na average of three visits	+ **
											ΔcIMT	
											Na baseline	≠
											Na average of three visits	− *

The mmol of Na intake/excretion values were converted to mg. If available, results presented come from adjusted models. Abbreviations: Na: sodium; 24hU: 24h urine collection; cIMT: carotid Intima Media Thickness; HT: hypertensives; anti-HT: antihypertensive; OW: overweight; OB: obese; c-sect: cross-sectional; prosp: prospective; FFQ: food frequency questionnaire; FU: follow up; M/F: males & females; F: females; N/AV: not available; CKD: chronic kidney disease; US: ultrasonography; +: positive association; -: negative association; ≠: no statistically significant association; *: $p < 0.05$; **: $p < 0.01$. [1] number of 1 kg packages of salt consumed/month/person.

Table 4. Descriptive characteristics of interventional studies regarding arterial remodeling.

Arterial Remodeling												
Right Branchial Artery and Common Carotid Artery Diameter												
Author	**Country**	**Study Design**	**Population Description**	**Sex**	**Race**	**N**	**Age (Years, Mean ± SD)**	**Intervention Duration (Weeks)**	**Type of Diet**	**Na Estimation Method**	**Na Intake/Excretion (mg/d)**	**Vascular Assessment**	**Results**	
													LS diet vs. NS diet	
Benetos, A. (1992) [51]	France	cross-over dbRCT	actively working, mild to moderate HT adults	M/F	N/AV	20	41.5 ± 2.4	8	Group 1 and Group 2: Normal Na diet (NS diet, Na capsules): 1400 mg and low-Na diet (LS diet), lactose capsules	24hU	Baseline: 3979 ± 299 LS diet: 1955 ± 220.8 NS diet: 3749 ± 305.9	B-mode US	Brachial artery diameter	+0.67m **
													common carotid diameter	≠

The mmol of Na intake/excretion values were converted to mg. If available, results presented come from adjusted models. Abbreviations: sodium; 24hU: 24h urine collection; HT: hypertensives; LS: low sodium; dbRCT: double-blind RCT; M/F: males & females; N/AV: not available; US: ultrasonography; ≠: no statistically significant association; **: $p < 0.01$.

Table 5. Descriptive characteristics of observational studies regarding atheromatosis.

Atheromatosis											
Carotid Plaques											
Author (Year)	**Country**	**Study Design**	**Population Description**	**FU (Years)**	**Sex**	**Race**	**N**	**Age (Years, Mean ± SD)**	**Na Estimation Method**	**Na Intake/Excretion (mg/d)**	**Vascular Assessment**	**Results**
Dai, X.W. (2016) [19]	China	c-sect	Asian adults, via subject referral and community advertisement	-	M/F	Asian	3290	M: 62.1 ± 6.7 F: 59.4 ± 5.5	FFQ	Dietary Na intake: Q1: 833 ± 394 Q2: 864 ± 507 Q3: 825 ± 41 Q4: 828 ± 395	B-mode US	≠
Mazza, E. (2018) [20]	Italy	c-sect	Adults aged ≥65, not suffering from any debilitating diseases	-	F	White	108	70 ± 4	24h dietary recall + 7 day food record	1476 ± 618 Tertile I: 780–900 Tertile II: 1330–1430 Tertile III: 2050–2330	B-mode US	Tertile III vs. Tertile I + *

The mmol of Na intake/excretion values were converted to mg. If available, results presented come from adjusted models. Abbreviations: Na: sodium; c-sect: cross-sectional; FFQ: food frequency questionnaire; FU: follow up; M/F: males & females; F: females; US: ultrasonography; +: positive association; ≠: no statistically significant association; *: $p < 0.05$.

3.2. Description of Studies

Population description and exclusion criteria are reported in Appendix B.

3.2.1. Studies Investigating Arteriosclerosis (Arterial Stiffness)

Thirty-one (31) studies examining arteriosclerosis were identified—of which, 14 were observational [15–20,27–34] and 17 were interventional [22,23,35–49].

Observational Studies

From the observational studies investigating arteriosclerosis, 11 out of 14 found a positive association between arterial stiffness biomarkers and dietary Na [15,17–20,28–30,32–34], one out of 14 found a J–shaped association [23], one out of 14 found an inverse association [16] and one out of 14 ound no association [27] (Table 1). From the above 11 studies showing a positive association, nine of them measured vascular parameters at one time point [17–20,28–30,33,34] and the remaining two studies evaluated arterial stiffness at two different time points [15,28].

Heterogeneity in the assessment of arterial stiffness existed in the above 11 studies [15,17–20,28–30,32–34] due to: (a) various arterial stiffness biomarkers using different methodologies (four applanation tonometry [17,28–30], six oscillometry [15,18,19,32–34] and one b-mode ultrasonography [33]) at different arterial segments using various arterial stiffness biomarkers (five cfPWV [17,28–30,32], one aortic PWV other than cfPWV [29], four baPWV [15,30–32] and one common carotid artery elasticity (Young's elastic modulus, stiffness index, arterial compliance) [33]) (Table 1); (b) various Na assessment methods (seven studies used 24h urine collection [17–19,28–30,34], two spot urine collections [28,29] and two a combination of dietary methods [15,33]); (c) different populations (five hypertensives [24,25,31–33], one normotensive [26], one chronic kidney disease patient [28], three mixed populations [15,22,29] and one healthy subjects [30]) (Table 1).

Moreover, one out of the 11 studies showed that high Na excretion (mean: 2898 mg/day, range 2035.5–3588) is associated with cfPWV only when high Na excretion was combined with high renin–angiotensin–aldosterone system (RAAS) activity but not in the other groups (i.e., those with high Na and low RAAS, low Na and low RAAS, as well as low Na and high RAAS) [24].

Only seven out of these 11 studies adjusted the results for BP level [17–20,29,30,34] and only three of them persistently showed a positive association between arterial stiffness and Na after the adjustment [22,31,32].

In the one study that showed an inverse association between arterial stiffness and Na, the result persisted after adjustment for BP level [16].

Finally, salt sensitivity assessment was not conducted in any of the above 14 observational studies.

Interventional Studies

From the 17 interventional studies investigating the association between arteriosclerosis [22,23,35–49], seven of them showed statistically significant changes in arterial stiffness biomarkers after Na intake intervention [35,37,38,44–46,49]. On the contrary, 10 out of the 17 interventional studies found no changes in arterial stiffness biomarkers with various levels of Na intake during the intervention [22,23,36,39–43,47,48] (Table 2).

In detail, three out of seven that found significant changes showed that increases in dietary Na were associated with an increase in arterial stiffness biomarkers [37,43,45] and four out of the seven showed that a reduction in dietary Na intake was associated with a decrease in arterial stiffness biomarkers [34,36,44] or even an increase in arterial elasticity biomarkers [48] (Table 2). Three of these seven studies found statistically significant changes only in specific intervention groups [36,43,45] (one study found that reduced Na excretion was associated with a decrease in cfPWV only in blacks, but not in whites and Asians [36]; one study found that high Na intake was associated with increased hfPWV

only in salt-sensitive but not in salt-resistant participants [45]; one study found that a high-salt diet was associated with increased cfPWV only in middle-aged participants and not in young participants [43]).

In those seven studies finding statistically significant changes in PWV after high or low-Na diets [35,37,38,44–46,49], heterogeneity existed, regarding: (a) different methodologies used for arterial stiffness assessment (three b-mode ultrasonography [34,45,48], one oscillometry [44] and three tonometry [36,37,43]) and different arterial stiffness biomarkers assessed (four cfPWV [34,36,37,43], one aortic PWV other than cfPWV [44], one heart-femoral (hfPWV) [45] and one arterial compliance [48]); (b) various methodologies used for Na assessment (four combination of dietary and urinary methods [34,37,44,48], two 24h urine collection [36,43] and one not available data [45]); (c) different duration of intervention period and (d) different populations (four in hypertensives or subjects with high normal BP [34,36,37,48], two in normotensives [43,44] and one in mixed populations (hypertensives and normotensives) [45]) (Table 2).

Of note, out of the seven studies that found statistically significant associations between Na and arterial stiffness biomarkers [35,37,38,44–46,49] only three studies adjusted the results for BP level [34,43,45]. One out of the three studies found that the statistically significant association between high-Na diet (6900 mg/day) and cfPWV in middle-aged adults was lost after correcting for the mean BP level [43]. Both other two studies found that their findings were independent from mean BP level [34,45].

In the 10 studies that found no statistically significant changes in arterial stiffness biomarkers after different levels of Na intake [22,23,36,39–43,47,48], heterogeneity existed, regarding: (a) different methodologies used for arterial stiffness assessment (seven tonometry [22,23,39–43], one oscillometry [35], one plethysmography [46] and one diastolic blood pressure time decay method [47]) using similar arterial stiffness biomarkers assessed (eight cfPWV [22,23,36,39–43], one baPWV [46] and one arterial compliance [47]); (b) various methodologies used for Na assessment (eight 24h urine collection [22,39–43,47,48] and two combination of dietary and urinary methods [18,35]); (c) different duration of intervention period and (d) different population samples (six in hypertensives [17,35,38,40,41,46] (one in overweight or obese hypertensives [35], one in hypertensives with chronic kidney disease patients [38], three in hypertensives [17,41,46], one in prehypertensives [40]), two in normotensives [18,47], one in overweight or obese subjects [39] and one in women with preeclampsia or healthy pregnancy in the past [42])) (Table 2).

Finally, only two out of the 17 conducted salt sensitivity assessment [45,46]. One out of the two studies revealed that the result was not statistically significant in the salt-resistant group, but only in the salt-sensitive group [45]. On the contrary, in the other study no significant differences between Na interventions and PWV were revealed for both salt-sensitive and salt-resistant participants, but salt-sensitive participants had higher baPWV at each time point of the intervention (baseline, low-Na diet, high-Na diet) [46].

3.2.2. Studies Investigating Arterial Remodeling

Nine studies examining arterial remodeling were identified [15,16,19,20,23,33,49–51]—of which, eight were observational [15,16,19,20,23,33,49,50] (Table 3) and one was interventional [51] (Table 4).

Observational Studies

Out of the eight observational studies, six found positive [15,19,20,33,49,50], one inverse [16] and one J-shaped associations [23] between cIMT and Na intake or excretion (Table 3). Out of the eight observational studies, seven measured the outcome at one time point (cross-sectional) [16,19,20,23,33,49,50] and one study measured the outcome at two time points and examined the association between the change of cIMT and Na intake (prospective) as well [15] (Table 3). In the prospective study, although the cIMT was positively associated with Na intake, the change of cIMT during follow up was negatively associated with Na intake [15] (Table 3). Four out of the six studies that found positive associations between cIMT and Na adjusted their results for BP level [19,33,49,50]: in two of them, the result was no more

statistically significant after adjustment for BP [33,49], in one of the studies, the result was marginally not significant after BP adjustment [50] and in the remaining one, the result was independent from BP [19]. The remaining two studies did not adjust their results for BP level [15,20]. Finally, one out of the six studies that found a positive association implied a statistically significant correlation only with IMT at the carotid bifurcation but not at the common carotid artery [19].

Heterogeneity in the assessment of arterial remodeling existed in the above six studies due to: (a) different Na assessment methods (four dietary (one [19]) or a combination of dietary (three [15,20,33]) methods, two 24h urine collection [49,50]) and (b) different studied populations (chronic diseases, age, comorbidities). All studies assessed cIMT as arterial remodeling biomarker via b-mode ultrasonography excluding from the measurement arterial segments with atheromatic plaques (Table 3).

The only study showing an inverse association was the only one conducted in purely normotensives as well as the only one using spot urine specimens for Na assessment [16]. Adjustment for BP was performed in this study and the result was independent from BP level [16]. The only study which showed a J-shaped association did not adjust the results for BP level [23].

Salt sensitivity assessment was not conducted in any of the eight studies.

Interventional Studies

The only interventional study that investigated the association between Na intake and arterial remodeling (Table 4) used brachial and carotid artery diameter as end point [51]. The brachial artery lumen increased after 8 weeks of a low-Na diet (mean ± SD: 1955 ± 220.8 mg/day) but no changes in the common carotid diameter were revealed. The findings were adjusted for BP levels [51]. Salt sensitivity assessment was not conducted [51] (Table 4).

3.2.3. Studies Investigating Atheromatosis

Only two observational studies examining atheromatosis were identified, showing conflicting results [19,20] (Table 5). One study showed that higher Na intake (2050–2330 mg/day vs. 780–900 mg/day) is positively associated with the prevalence off carotid plaques [20], while the other study did not find a statistically significant association [19]. The two studies assessed Na via different ways (dietary and urinary) and used different populations (elderly females [20] as well as a general population [19]). Both studies examined carotid plaques via B-mode ultrasonography. One out of the two studies adjusted their results for BP levels and the result was independent from BP [19]. No study assessed salt sensitivity.

4. Discussion

In the present study, we performed a systematic review of the literature to investigate the relationship between dietary Na intake with arterial function and structure using gold-standard non-invasive vascular biomarkers to measure arteriosclerosis, arterial remodeling and atheromatosis. The results of this systematic review indicate that: (i) although several studies have investigated the association of dietary Na with arterial stiffness, the evidence does not clearly support a clinically meaningful, direct and independent from BP effect of Na on the arterial wall to increase arterial stiffness; (ii) data regarding the association between dietary Na and arterial remodeling are limited, mostly suggesting a positive trend between dietary Na and arterial hypertrophy, but still inconclusive; (iii) data regarding the association between dietary Na and atheromatosis are scarce and the available studies present high heterogeneity.

4.1. Na and Arteriosclerosis

Although 31 human studies have investigated the association between dietary Na and arteriosclerosis, the current data are inconclusive regarding a potential direct effect of Na on the arterial wall properties that accelerate the arterial stiffening process. Indeed, the majority of the studies (observational 11/14 and interventional 7/17) do imply the presence of a harmful effect

of high Na intake [15,17–20,28–30,32–34,38,44,46] or even benefits of low Na intake on arterial stiffening parameters [34,36,44,48] (18 out of 31, 11 observational and seven interventional), in various populations [22,26,29,34,44], involving several different segments of the arterial tree [22,29,30,33,45,48], independently of the applied methodology, technology used [22,29,33,34]. However, most of these positive studies do not take into consideration the well-known effect of Na on BP increase [15,24,28,29,36,37,44]. Overall only 1/3 of the studies included in our analysis, and only 10 out of the 17 positive studies adjusted their findings for BP levels [17–20,29,30,34,35,44,46]. Even more interestingly, in more than half of them (six out of 10), the association between Na and indices of arteriosclerosis was lost after correcting for BP [25,26,30,33,34,43]. Moreover, although salt sensitivity is a major factor modulating the effect of Na on BP (and therefore to arterial stiffness), only two [45,46] out of the 31 studies evaluated this parameter and showed conflicting results. Indeed, there is evidence suggesting that a high-salt diet would increase BP in 17% of the subjects (salt sensitives), reduce BP in 11% (inverse salt sensitive) and not significantly affect BP in the remaining salt-resistant subjects [52]. Finally, just one study [23] showed a J-shaped association between Na and arteriosclerosis, mirroring the recent epidemiological data on the J-shaped association between Na and mortality.

A recent meta-analysis of randomized controlled trials, conducted by D' Elia and colleagues [53], being the first and the only one available on this topic so far, included 14 cohorts (all of them included in our work) and showed a statistically significant decrease by 2.84% in cfPWV after an average reduction of approximately 2 g (89.3 mmol) per day in Na intake independently from BP. In this meta-analysis, the authors excluded all the studies measuring other than the cfPWV, whereas we extended our systematic review to include all valid non-invasive indices of arterial stiffness including other segments of the arterial bed (such as the carotid artery and the lower limbs). Although our study is not applying a synthesis of quantitative data (as a meta-analysis), but uses only the qualitative characteristics of the selected studies, it is important to consider that the result of D' Elia et al. suggest poor, if any, clinical effects of Na on arterial stiffness. A reduction of PWV by 2.84% may not offer additional benefit in overall vascular health.

Taken all together, these data suggest that arterial stiffness can be reduced with a dietary intervention aiming at the reduction of dietary Na intake, but: (a) this reduction is modest (e.g., aortic stiffness of 10 m/s considered the high CVD risk cut-off level will be reduced to 9.8 m/s after a major reduction of Na by 2 g/day) with debatable clinical effect and (b) it is not established whether this lowering effect is mediated only by BP reduction or mediated by a direct effect on the arterial wall [54,55].

Moreover, major questions seek suitable answers, since poor data regarding the role of salt sensitivity, the RAAS, age and race exist. The hypothesis that hyperactive RAAS leads to BP elevation and consequently arterial stiffening, as a result of BP rising in salt-sensitive subjects, cannot yet be rejected. In a single study, Kotliar et al. indicate a significant positive association between Na and PWV only in the group of participants who had high RAAS activity. However, the group with high Na and low RAAS activity did not show a significant association with PWV [24]. One of the studies suggested that only middle-aged and not young participants presented increased PWV after a high-salt diet [43]. However, in the study by Avolio et al., all of the age groups (children, young adults and middle-aged adults) decreased their PWV after reducing Na intake [44]. Finally, despite the fact that race has been shown to play a significant role in BP levels and salt sensitivity, indirectly affecting arterial stiffening, just one study addressed this issue and showed significant increases in PWV after Na supplementation only in black participants.

4.2. Na and Arterial Remodeling

Nine studies—all of them using B-mode ultrasonography—investigating the association between dietary Na and arterial remodeling were identified (eight observational [15,16,19,20,23,33,49,50] and one interventional [51]). The majority of them (six out of the nine) implied a detrimental effect after high Na intake [15,19,20,33,49,50] or even a beneficial effect after low Na intake [51] on arterial remodeling

parameters (cIMT or artery diameters) independently of different methods used for Na assessment and various population groups (different diseases and comorbidities, age groups, etc.). In most cases, higher dietary Na intake was associated with higher cIMT in plaque-free arterial segments, mostly at the common carotid, suggesting arterial hypertrophy, but also carotid bulb [19] and brachial artery [51].

However, only three out of the nine studies included large population samples (>1500 participants) [15,16,19] and their results were conflicting, since one of them found an inverse and BP-independent association between Na and cIMT but was the only one conducted in purely normotensives and assessed Na through spot urine specimens as well [16]. Probably, the best available study so far, the only interventional study published by Benetos et al., showed that independently from BP, increased Na intake only induced arterial remodeling in a muscular artery (brachial artery) but not in an elastic one (carotid artery), suggesting a diverging effect of Na in different arterial beds [51].

Most importantly, once more, the effect of potential confounding BP on arterial remodeling was not taken into consideration in 1/3 of the studies (three out of nine) [15,20,23]. Further, in two other studies [33,49], the end point was actually mediated by BP increase. In conclusion, data on the association between dietary Na and arterial remodeling are limited, mostly suggesting a positive trend between dietary Na and arterial hypertrophy, but this is still inconclusive and conflicting. No study assessed salt sensitivity.

To our knowledge, the association between Na and arterial remodeling has not previously been subject to meta-analysis, and despite positive trends observed in the majority of studies, there is insufficient data to conclusively establish the relationship.

4.3. Na and Atheromatosis

According to our systematic research, there are extremely limited data on the association between dietary Na and atheromatosis. Only two studies examined this association [19,20]. Mazza et al., in a very small study [20], found that high dietary Na is associated with the increased prevalence of carotid plaques, whereas Dai et al., in a substantially larger study [19], suggested a non-significant association between dietary Na intake and carotid plaques. However, these studies presented heterogeneity in population samples (elderly females [20] and general population [19]), sample size (108 [20] and 3290 [19] participants) and Na assessment method (24h dietary recall and 7 day food record [20] and FFQ [19]). Moreover, the available studies regarding dietary Na and atheromatosis have not investigated the association between very low and very high levels of Na, and that might explain why a J-shaped trend has not been observed. Furthermore, beyond carotid arteries, plaque formation in other arterial segments that might offer an additive value in CVD prevention—such as the femoral arteries—has not been assessed in any of the available studies. In conclusion, there is not enough evidence to support a positive, negative or J-shaped association between dietary Na and arterial plaques and more studies investigating the association between larger ranges of Na intake/excretion and arterial plaques are needed.

4.4. Strengths and Limitations

Major strengths of our study are: (i) the novel concept of investigating the effect of dietary Na on SAD, including all the major pathogenetic mechanisms (arteriosclerosis, arterial remodeling & atheromatosis); (ii) the systematic nature of this review in order to compare and dispose all the available international literature on this specific topic; (iii) the design of our study, including clinical trials and evidence from observational studies in order to investigate the short- and long-term effects of different levels of Na intake on SAD. A limitation of our study is the absence of a quantitative analysis of the extracted data (meta-analysis), which could lead to a clearer view of the topic.

5. Conclusions

In conclusion, there is not yet enough evidence to support a direct and causal association between Na and each of the major types of SAD, even in the most widely studied case of arteriosclerosis (arterial

stiffening). The available data derive mostly from small, heterogeneous, not well-designed studies. Especially in the case of arterial remodeling and atheromatosis, both common and clinically relevant types of structural arterial damage have scarcely been investigated in relation to Na intake or excretion. One of the dominant issues is the heterogeneity of the studies in Na assessment method. Precise quantification of Na intake is difficult and despite the fact that only the 24h urine collection is regarded as a gold standard, based on the knowledge that approximately 90% of Na intake is excreted through urine, other dietary or spot urinary methods are commonly used in studies. Several disadvantages of the above mentioned studies have been described, such as underreporting, equations suitable only for specific population groups, different recipes, etc., leading to inaccurate measurements. Finally, many studies included in our analysis do not address the cardinal effect of Na on BP and almost all of them neglect the role of salt-sensitivity. Future studies using novel diagnostic tests for individuals' salt sensitivity assessment are needed to clarify the role of dietary Na to SAD [56]. More well-designed interventional studies are needed in order to resolve all the remaining controversies.

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Appendix A. PRISMA Checklist

TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	1
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	1,2
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	1,2
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., web address), and, if available, provide registration information including registration number.	Not applicable
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	2
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	2
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	2,3
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	3
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	2
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	2
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	Not applicable
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	-
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	Not applicable
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	Not applicable
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	Not applicable

RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	3
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	4–15
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Not applicable
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Not applicable
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	Not applicable
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	Not applicable
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	Not applicable
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	15,16
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	18,19
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	18,19
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	19

Appendix B. Population Description and Exclusion Criteria of Selected Studies

Arteriosclerosis—Observational Studies		
cfPWV		
1. Observational Cross-Sectional Studies		
Author (year)	**Population Description**	**Exclusion Criteria**
Polónia, J. (2006)	essential hypertensives, recent stroke or healthy university students	urine sample not meeting the required quality criteria
García-Ortiz, L. (2012)	primary care patients aged 30–80	cardiovascular and/or cerebrovascular disease
Kotliar, C. (2014)	essential hypertensives, aged 30 to 70	abnormal renal function; volume or electrolyte alterations; diabetes mellitus; history of renal disease, ischemic heart disease, stroke; loss of data; counter indication for the drug washout; use of corticoids or nonsteroidal anti-inflammatory drugs during the study
Polonia, J. (2016)	hypertensive adults	secondary hypertension, previous cardiovascular events history; estimated glomerular filtration rate >50 mL/min/1.73
Strauss, M. (2018)	normotensive adults	previously diagnosed chronic illness (self-reported); use of anti-hypertensive drugs or other chronic diseases; diabetes mellitus; HIV infected; microalbuminuria>30 mg/mL; pregnancy or lactation
Triantafyllou, A. (2018)	newly diagnosed & never-treated hypertensives—healthy individuals admitted for regular check-up	previously treated with anti-hypertensive drugs; use of any kind of drugs; other known health problems; secondary causes of hypertension; other comorbidities (e.g., diabetes mellitus, CVD)
2. Observational Studies with Follow up (>1 Time Points)		
Nerbass, F.B. (2015)	adults in CKD stage 3	solid organ transplant or terminally illness
Aortic PWV Other Than cfPWV		
Siriopol, D. (2018)	hypertensive & normotensive Romanian adults	use of diuretic treatment; CKD; missing values for the variables of interest
baPWV		
1. Observational Cross-Sectional Studies		
Sonoda, H. (2012)	healthy subjects	heart failure; valvular heart disease; atrial fibrillation; peripheral artery disease
Lee, S.K. (2015)	non-hypertensive subjects, with no use of anti-hypertensive drugs	age >70 years; preexisting CVD including significant valvular heart diseases and arrhythmia; chronic renal disease or a serum creatinine level >1.5 mg/dL; unavailable urinary Na data, inadequate data of tissue Doppler echocardiography, carotid ultrasound, or baPWV; ejection fraction of <55% after echocardiography
Sun, N. (2015)	newly diagnosed hypertensives, untreated hypertensives or patients with a 1 to 5 year history of hypertensives who had stopped taking anti-hypertensive drugs for 1 month	use of anti-hypertensive drugs; secondary hypertension; hypertensive emergency; hypertensive urgency; acute coronary syndrome; severe arrhythmias; DM; stroke; CKD
Han, W. (2017)	hypertensive adults	any secondary cause of hypertension; hypertension emergencies; serious arrhythmia; peripheral arterial disease; heart failure; impaired renal function with plasma creatinine ≥150 μmol/L; rheumatic & autoimmune diseases; malignancies

2. Observational Studies with Follow up (>1 Time Points)		
Jung, S. (2019)	adults aged >40	history of heart disease, stroke, and/or cancer; anti-hypertensive drugs; diabetes mellitus; dyslipidemia; implausible dietary intake reported (< 500 or > 4000 kcal/day); missing general characteristic data from the baseline visit
Common Carotid Arterial Elasticity (Young's Elastic Modulus, Stiffness Index, Arterial Compliance)		
Ferreira-Sae, M.C. (2011)	hypertensive adults	age <18 years; neoplastic disease; secondary hypertension
Arteriosclerosis—Interventional studies		
cfPWV		
Seals, D.R. (2001)	postmenopausal women, ≥50 years, high normal SBP or Stage 1 hypertension	anti-hypertensive drugs; other chronic disease; low-Na diet or regular exercise during the preceding 2 years; smoking
Dickinson, K.M. (2009)	overweight/obese, mild hypertensive adults	metabolic disease; CVD; SBP >160 mm Hg at screening; significant weight loss in the preceding 6 months (>2 kg); BMI < 27 or > 40; use of anti-hypertensive drugs
He, F.J. (2009)	hypertensive adults	anti-hypertensive drugs; secondary cause of hypertension; impaired renal function; previous stroke; ischemic heart disease; heart failure; diabetes mellitus; malignancy; liver disease; pregnancy or lactation or on oral contraceptive pills
Pimenta, E. (2009)	resistant hypertensive adults on stable anti-hypertensive drugs	history of atherosclerotic disease (in the previous 6 months); congestive heart failure; diabetes mellitus on insulin treatment; office blood pressure > 160/100 mm Hg
Todd, A.S. (2010)	Pre-hypertensive or hypertensive, non-obese adults or in anti-hypertensive drugs	age >65; smoking; history of CVD or diabetes mellitus or renal disease
Todd, A.S. (2012)	normotensives, non-obese adults	age >65; antihypertensive medication; smoking; history of cardiovascular disease or diabetes mellitus or renal disease
McMahon, E.J. (2013)	hypertensive adult patients with stage 3 or 4 CKD (GFR 15–59 mL/min per 1.73 m^2), non-dialyzed, non-transplanted	salt-wasting CKD, pregnant or breastfeeding, current prescription of medications providing 0.20 mmol sodium per day, life expectancy,6 months, current involvement in another intervention study, or insufficient mental or physical capacity to adhere to the study protocol.
Dickinson, K.M. (2014)	overweight or obese subjects	diabetes mellitus; dyslipidemia; inflammatory bowel disease; pulmonary disease or vasculitis
Gijsbers, L. (2015)	untreated prehypertensives, aged 40–80	smoking; diabetes mellitus, CVD; gastrointestinal, liver or renal diseases; BMI > 40; use of drugs known to affect the cardiovascular system; use of nutritional supplements, an energy-restricted or medically prescribed diet; unstable body weight in past 2 months; alcohol use over 21 (women) or 28 (men) consumptions/week; pregnancy or lactation
Suckling, F.J. (2016)	untreated hypertensive adults with diet-controlled type 2 diabetes mellitus or impaired glucose tolerance	any secondary causes of hypertension, impaired renal function (plasma creatinine >150 μmol), uncontrolled heart failure, ischemic heart disease, previous stroke, active malignancy or liver disease, pregnancy, breast feeding, or oral contraceptive drugs
van der Graaf, A.M. (2016)	women with history of preeclampsia or history of healthy former pregnancy	renal disease; diabetes mellitus or a history of gestational diabetes; obesity; use of anti-hypertensive drugs; pregnancy; lactation; postmenopausal status; use of oral contraceptives
Muth, B.J. (2017)	healthy, normotensive adults	history of hypertension; CVD; malignancy; diabetes mellitus; renal impairment; obesity; smoking

Aortic PWV (Other than cfPWV)		
Avolio, A.P. (1986)	healthy normotensive adults & children	N/AV
hfPWV		
Rhee, M.Y. (2016)	normotensive & hypertensive adults	stage 2 and 3 hypertension; secondary hypertension; angina pectoris; myocardial infarction; congestive cardiac failure; stroke; diabetes mellitus; CKD
baPWV		
Wang, Y. (2015)	mild hypertensive adults	stage 2 hypertension; history of clinical CVD; CKD; diabetes mellitus; use of anti-hypertensive drugs; high alcohol intake
Arterial Elasticity (Arterial Compliance)		
Creager, M.A. (1991)	normotensive men	hematologic, renal, or hepatic dysfunction
Gates, P.E. (2004)	hypertensive adults (stage 1), older than 50	use of anti-hypertensive drugs; abnormal blood chemistry; positive ECG-monitored exercise test; ankle–brachial index > 0.9; presence of plaque on ultrasound interrogation of the carotid and femoral arteries; smoking for previous 2 years; BMI < 35; consumption of a low-Na diet; not in postmenopausal if female (amenorrheic for at least 2 years)
Arterial Remodeling—Observational Studies		
cIMT		
1. Observational Cross-Sectional Studies		
Ferreira-Sae, M.C. (2011)	hypertensive adults	age <18 years; neoplastic disease; secondary hypertension
Njoroge, J.N. (2011)	overweight or obese, physically inactive adults	diabetes mellitus; anti-hypertensive drugs or average baseline SBP of ≥140 or DBP ≥ 90 mmHg; cholesterol lowering or anti-psychotic or vasoactive drugs; use of vasoactive devices; pregnancy or lactation
García-Ortiz, L. (2012)	primary care patients aged 30–80	cardiovascular and/or cerebrovascular disease
Lee, S.K. (2015)	non-hypertensive individuals, with no use of anti-hypertensive drugs	age >70 years; preexisting CVD including significant valvular heart diseases and arrhythmia; chronic renal disease or a serum creatinine level > 1.5 mg/dL; unavailable urinary Na data, inadequate data of tissue Doppler echocardiography, carotid ultrasound, or baPWV; ejection fraction of <55% after echocardiography
Ustundag, S. (2015)	ambulatory adult patients, in stage 2–4 CKD	BMI < 35 kg/m2; diabetes mellitus; salt-losing nephropathy or history of malignancy or cardio-cerebrovascular disease or any acute disease
Dai, X.W. (2016)	Asian adults, via subject referral and community advertisement	hospital-confirmed diabetes mellitus; CVD; renal failure; CKD; cancer
Mazza, E. (2018)	adults aged ≥65, not suffering from any debilitating diseases	history of CVD or thyroid dysfunction or excessive alcohol consumption; use of dietary supplements & psychotropic drugs

2. Observational studies with follow up (>1 time points)		
Jung, S. (2019)	adults aged >40	history of heart disease, stroke, and/or cancer; anti-hypertensive drugs; diabetes mellitus; dyslipidemia; implausible dietary intake reported (< 500 or > 4000 kcal/day); missing general characteristic data from the baseline visit
Arterial Remodeling—Interventional Studies		
Right Branchial Artery & Common Carotid Artery Diameter		
Benetos, A. (1992)	actively working, mild to moderate hypertensive adults	cardiac, neurologic or renal involvement or arteriosclerosis obliterans of the lower limbs
Atheromatosis—Observational Studies		
Carotid Plaques		
Dai, X.W. (2016)	Asian adults, via subject referral and community advertisement	hospital-confirmed diabetes mellitus; CVD; renal failure; CKD; cancer
Mazza, E. (2018)	adults aged ≥65, not suffering from any debilitating diseases	history of CVD or thyroid dysfunction or excessive alcohol consumption; use of dietary supplements & psychotropic drugs

Abbreviations: CKD: chronic kidney disease; CVD: cardiovascular disease; BMI: body mass index; Na: Sodium; N/AV: not available.

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Dietary sodium and cardiovascular morbidity/mortality: a brief commentary on the 'J-shape hypothesis'

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The last decade, a growing number of evidence support J-shape or inverse – instead of positive linear – associations between dietary sodium intake and cardiovascular morbidity/mortality. A careful evaluation of these studies leads to the following observations: less accurate methods for dietary sodium assessment are usually used; most studies included high-risk participants, enhancing the possibility of a 'reverse causality' phenomenon. However, these limitations do not explain all the findings. Few carefully designed randomized clinical trials comparing different levels of sodium intake that address the issue of the optimal and safe range exist; therefore, current guidelines recommend a higher cut-off instead of a safe range of intake. Given the demonstrated harmful effects of very low sodium diets leading to subclinical vascular damage in animal studies, the 'J-shape hypothesis' cannot yet be either neglected or verified. There is a great need of well-designed general population-based prospective randomized clinical trials to address the issue.

Keywords: cardiovascular morbidity, cardiovascular mortality, dietary sodium

Abbreviation: AHA/ACC, American Heart Association/American College of Cardiology; EFSA, European Food Safety Authority; ESC/ESH, European Society of Cardiology/European Society of Hypertension; IoM, Institute of Medicine; Na, sodium; NaCl, sodium chloride (salt); RCTs, randomized clinical trials

INTRODUCTION

Cardiovascular disease is the first cause of death globally [1]. Dietary sodium (Na) intake has been implicated in health conditions, such as arterial hypertension [2–5] or diabetes mellitus [6,7] and has been repeatedly linked to cardiovascular disease and mortality [8]. Moreover, it is well established from metanalyses of randomized clinical trials (RCTs) that dietary Na restriction interventions (~2.3 g/day Na restriction) lead to clinically significant decrease of both SBP and DBP (−4 mmHg/−2 mmHg, respectively) [2,9]. However, during the last decades, high levels of Na intake are reported in observational studies, which mainly derive from manufactured food in western countries [10–15] and from added salt during cooking in eastern countries [11,14,16,17]. Most importantly, there is disagreement regarding the need and quantification of a safety range of daily Na intake that would be associated with low cardiovascular disease risk and mortality. This is a consequence of gaps in knowledge, the lack of robust or even disagreement in the evidence originating from methodological issues: the optimal – but also feasible – Na assessment methods that have been applied, the lack of homogeneity in the studied population (healthy or high-risk participants), the studies' design (observational studies versus RCTs) and the lack of studies investigating simultaneously multiple levels of Na intake (e.g. very low, 'normal', very high). The aim of this study is to briefly discuss the most recent literature regarding: dietary Na recommendations; dietary Na intake around the world; the relationship between Na intake and cardiovascular morbidity/mortality as well as all-cause mortality; the appraisal of the studies showing high cardiovascular disease morbidity or mortality under low Na intake levels (~<2.0–2.3 g/day).

DIETARY SODIUM INTAKE RECOMMENDATIONS

Salt (NaCl) is the dominant source of dietary Na in human diet (Na is equivalent to 40% of NaCl). On the basis of strong quality of evidence derived from metanalyses of RCTs regarding the benefits of dietary Na reduction in BP levels

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TABLE 1. Recommendations and dietary reference values for sodium intake

Organization/institution	Na recommendation (g/day)	Salt equivalents	Target group
Dietary guidelines for Na intake			
ESC/ESH 2018 [21]	<2.0	<5.0	For the general population and all hypertensive patients.
Dietary Guidelines for Americans 2015–2020 [22]	<2.3	<5.75	For Americans older than 14 years of age, as part of a healthy eating pattern.
AHA/ACC 2013 [24]	<2.4 or *1.5 for greater BP reduction	<6.0	*For adults who would benefit even from BP lowering
WHO 2012 [23]	<2.0	<5.0	All adults ≥16 years of age, with or without hypertension (including pregnant or lactating women), except for individuals with illnesses or taking drug therapy that may lead to hyponatraemia or acute build-up of body water, or require physician-supervised diets (e.g. patients with heart failure and those with type I diabetes).
Dietary reference intakes/values (DRIs/DRVs) for Na intake			
IoM 2019 [25]	1.5	3.75	Adequate intake (AI) for the general adult population (>19 years of age)
	<2.3	<5.75	For the general population (>14 years of age) to reduce chronic disease risk (cardiovascular events and arterial hypertension).
EFSA 2019 DRVs [26]	2.0	5.0	Safe and adequate intake for the general European population of adults including pregnant and lactating women.

AHA/ACC, American Heart Association/ American College of Cardiology; EFSA, European Food Safety Authority; ESC/ESH, European Society of Cardiology/ European Society of Hypertension; IoM, Institute of Medicine; Na, sodium.

in hypertensive or normotensive populations [18–20], dietary guidelines recommend a limited intake of Na, as described in Table 1. According to the WHO 2012 Guidelines, the European Society of Hypertension and the European Society of Cardiology 2018 guidelines as well as the Dietary guidelines for Americans, Na intake should not overpass the 2.0 or 2.3 g/day (which is equivalent to 5.0 or 5.75 g of salt, respectively) for the general population [21–23]. A large-scale multicenter study including data from 66 countries conducted by Mozaffarian *et al.*, estimated that in 2010, around 1.65 million deaths from cardiovascular disease causes occurred because of dietary Na intake above the recommended level of 2.0 g/day. The American Heart Association and the American College of Cardiology recommend a stricter limitation of Na intake (<1.5 g/day) for adults who would benefit from BP-lowering [24]. Despite its harmful effects in cardiovascular health at high levels of intake, dietary Na remains an essential nutrient for human life. However, the adequacy in Na intake or the potential harmful effect of very low daily Na intake is less investigated, and, thus less described in the available guidelines. The most recently published Dietary Reference Intakes (DRIs) from the Institute of Medicine (IoM) defined the 1.5 g/day as an adequate intake for the general population [25]. On the other hand, the European Food Safety Authority (EFSA) set the 2 g/day as a safe and adequate intake for the general European population including pregnant and lactating women, in the context of reducing cardiovascular disease risk [26].

DIETARY SODIUM INTAKE AROUND THE WORLD

Regardless of current recommendations, dietary Na intake globally tends to be almost double [~ 3.7 to 4.0 g/day instead of 2 g of Na per day (or 9.25 to 10.0 g of salt per day)], whereas some regions (e.g. Central and East Asia, high-income Asia Pacific and Eastern Europe) Na intakes are more than double compared with recommendations (>4 g/day) [9,27,28]. Interestingly, this trend is also observed in high-risk populations that are more often monitored by health workers and advised to limit salt intake, such as hypertensive or diabetic patients [29–31]. Continent-wise distribution of dietary Na intake in most European countries is ~3.2–4.4 g/day, according to the Survey on Members States' Implementation of the EU Salt Reduction [32]. Higher levels of Na intake have been detected in Asia, Europe, and USA compared with sub-Saharan Africa and Southern Latin America, which present the lowest levels but still above the recommendation [27]. In eastern countries, dietary Na derives from salt added during cooking and condiments (i.e. soy sauce), which contribute to 88.4% of total Na intake, whereas manufactured food provides only the 11.4% of total Na intake [17]. On the contrary, the manufactured food is the dominant source of dietary Na in the western diet, providing about three-fourth of total daily Na intake [13,14,33].

OBSERVATIONAL DATA ON DIETARY SODIUM AND CARDIOVASCULAR MORBIDITY/MORTALITY: THE J SHAPE PHENOMENON

The last decades important evidences have been accumulated and established the notion that high levels of Na intake are associated with increased incidence of fatal and non-fatal cardiovascular events [9,34–38]. Nevertheless, a metanalysis of prospective cohort studies has reported nonsignificant associations between Na intake and incident fatal and non-fatal cardiovascular disease, coronary heart disease and all-cause mortality [18]. Additionally, in the last decade, a significant number of studies [5,39–52] showed that not only high but also low levels of

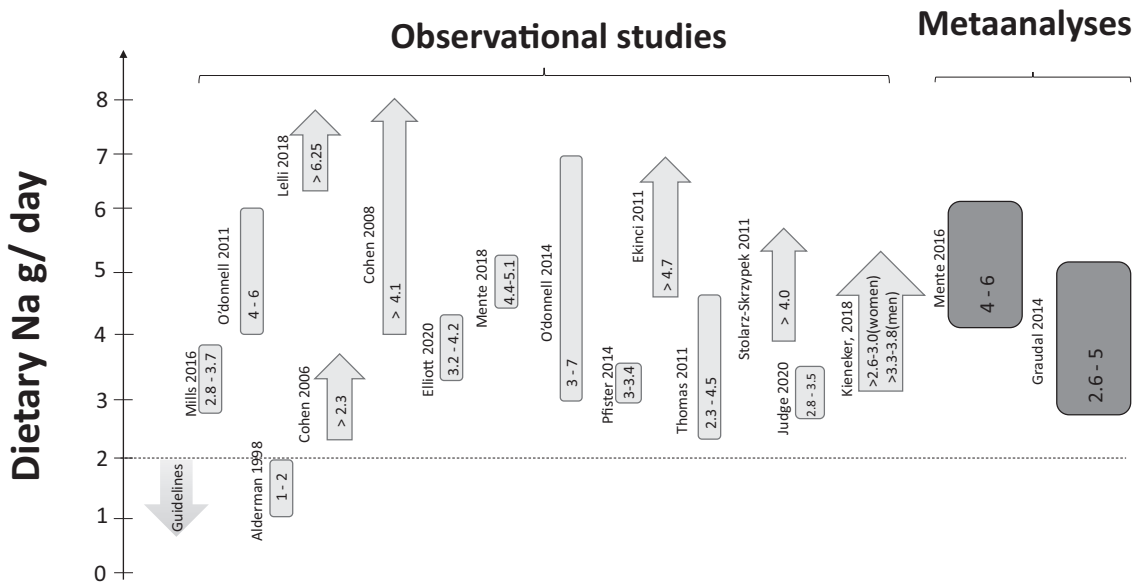

FIGURE 1 Major studies and metanalyses showing J-shape or inverse association between sodium intake and cardiovascular morbidity/mortality as well as all-cause mortality. Data from [5,39–55]. Bars present the ranges showing lower risk, above and below which the risk increases. Arrows present the cut-offs above which the risk is lower. One gram of Na is equivalent to 2.5 g of salt.

dietary Na intake are associated with increased cardiovascular risk. These studies [5,39–52] suggested the presence of a J-shaped or inverse linear association between daily Na intake and cardiovascular mortality [5,39–52], verifying early findings of the Alderman *et al*. study [53]. The major studies that implicated low levels of dietary Na intake in cardiovascular morbidity/mortality raising are presented in Fig. 1. Two large meta-analyses published in 2014 and 2016 confirmed the J-shape hypothesis and suggested a safe range of Na intake between ~3 and 5 g/day (or 7.5–12.5 g of salt/day), as intakes below or above this level raised the mortality level of about 1.12–1.34 times [54,55]. However, even among these studies, inconsistencies regarding the optimal range of Na intake are easily observed (Fig. 1). All – but one [53] – of the above studies suggest that the J-shape curve starts at an unexpectedly 'high' Na intake level, that is, that cardiovascular risk increases even at daily Na intake above 3 g/day or even 4 g/day (>7.5 or 10 g of salt/ day). Of note, a careful dissection of several observational cohorts supporting the mainstream notion of the linear positive association between Na intake and cardiovascular mortality reveals that the range of dietary Na intake that have been compared are very high (exceeding the 5.5–6 g/day) versus moderate rather than low levels (e.g. <3–4 g/day) of Na intake [36,38]. It is also important to note that in all the available observational studies, only a very small amount of the sample present Na intake below the recommended level of 2 g per day. According to the Prospective Urban Rural Epidemiology (PURE) study, which is a large epidemiological cohort that collected data from 664 communities, mean Na intake was 4.77 g/day with a range from 3.22 to 7.52 g/day, whereas in communities from China, 80% presented intakes greater than 5 g/day [47]. Finally, great heterogeneity exists between different cultures and communities since for the same, for example, low level of sodium intake huge (even three-fold) differences in cardiovascular events have been described [47], clearly suggesting that other major confounding factors (dietary or not; cultural or not) are present.

Taken all together, this evidence raises several concerns about the optimal range of daily Na intake and the J-shape hypothesis cannot be disregarded.

Despite all the above presented evidence supportive of a J-shape or inverse association, current guidelines do not take them into consideration, as they only recommend a highest level of intake and not a lowest safety level for daily Na intake. This is in part due the fact that a more careful analysis of all these studies reveals several issues regarding their methodology that they could possibly explain the inconsistencies regarding the optimal daily Na intake range. Indeed, according to the recent report of the Institute of Medicine on Na DRIs *'. . .the paradoxical J- and U-shaped relationships of sodium intake and cardiovascular disease and mortality are likely observed because of methodological limitations of the individual observational studies. . .'* [25]. Studies reporting a J-shape trend between Na intake and cardiovascular events are characterized by high risk of bias according to the same organization [25]. A state-of-the-art recording of all the methodological considerations regarding dietary Na and cardiovascular disease has been conducted by Cobb *et al*. [56] in 2014. In the following paragraphs, we will address some major limitations.

Dietary sodium assessment methods: variety and reliability

The major limitation and confounding effect in epidemiological studies is the variety of the applied methods to assess dietary Na intake or excretion. The two major categories

include dietary methods (24-h dietary recalls, food frequency questionnaires and food diaries) and the urinary methods (24-h urine collection, overnight or 12-h urine collection, spot urine collection with the use of appropriate equations converting the spot Na to 24-h Na).

Twenty-four hour urine collection: the gold-standard method to assess dietary Na intake/excretion, based on the knowledge that about 98% of Na consumed is excreted during 24-h through urine [57,58]. However, this method is burdensome and time consuming, thus it is difficult to be applied in large-scale studies. In addition, an important limitation of this method is the high rate of incomplete collections that have been observed in previous studies [59], as well as the fact that most studies do not assess the incomplete samples in order to exclude them from the analysis, leading to biased findings. Furthermore, bias may occur because of the day-to-day variability in Na excretion because of several confounding factors, such as dietary intake and physical activity. Moreover, multiple collections – which are even more time consuming – have been reported to be more accurate, especially when evaluating individuals' and not a group mean dietary Na intake [60].

Spot urine collection: spot urine equations are more convenient and easily applicable becoming a common alternative in research studies investigating dietary Na. Nevertheless, this method has been observed to present poor or moderate correlations with the 24-h urine collection ($r = 0.33–0.56$) and to overestimate Na in higher levels of intake and underestimate it in lower levels of intake [61–65]. Of note, the differences observed between the spot urine method and the gold standard 24-h urine collection, could be explained by a significant number of confounding factors, such as body weight, age, sex and urinary creatinine excretion. As an example, although intra-individual creatinine excretion is considered relatively stable, it is well established that protein intake and exercise have a significant impact in creatinine excretion levels [66]. Given that most of the available equations for the conversion of spot Na to 24-h Na are based on prediction of the 24-h creatinine excretion without taking into account parameters, such as protein intake and exercise, important bias potentially occurs. The analysis of He *et al.* [67] in 2974 participants in Trials of Hypertension Prevention (TOHP) could be a typical example. The authors evaluated the relationship between dietary Na and mortality via multiple 24-h urine collections and spot urine collections in the same sample. For the spot urine collection, they used the three most common equations (Tanaka, INTERSALT, Kawasaki) [68–70]. The multiple 24-h urine collections led to a linear relationship between Na and mortality, suggesting that higher Na intake increases risk of death. However, the use of spot urine equations altered the linear relationship to a J-shaped or U-shaped curve, indicating that even the low intakes of Na increase the risk of death. Despite the fact that the results of this study should be confirmed by others, it strongly indicates the potential bias that is introduced in the association of mortality with Na intake because of measurement unreliability.

Dietary methods: they have the advantage to provide information regarding dietary Na sources and dietary habits related to salt intake, which the urinary methods cannot. However, very low correlation rates with the 24-h urine collection ($r = 0.15–0.50$) have been reported as well as underreporting of the actual Na intake even by 39% [33,71–73]. These high levels of underreporting derive from

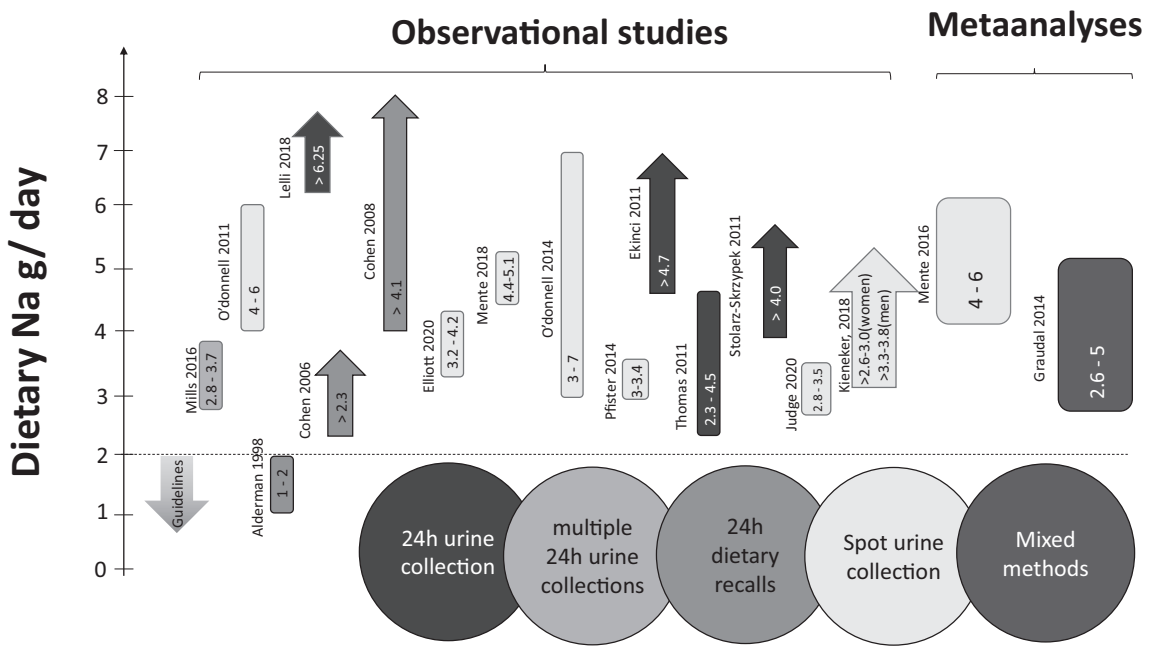

FIGURE 2 Methods used for the dietary sodium assessment in studies showing J-shape or inverse association between sodium intake and cardiovascular morbidity/mortality as well as all-cause mortality. Data from [5,39–55]. Bars present the ranges showing lower risk, above and below which the risk increases. Arrows present the cut-offs above which the risk is lower. One gram of Na is equivalent to 2.5 g of salt.

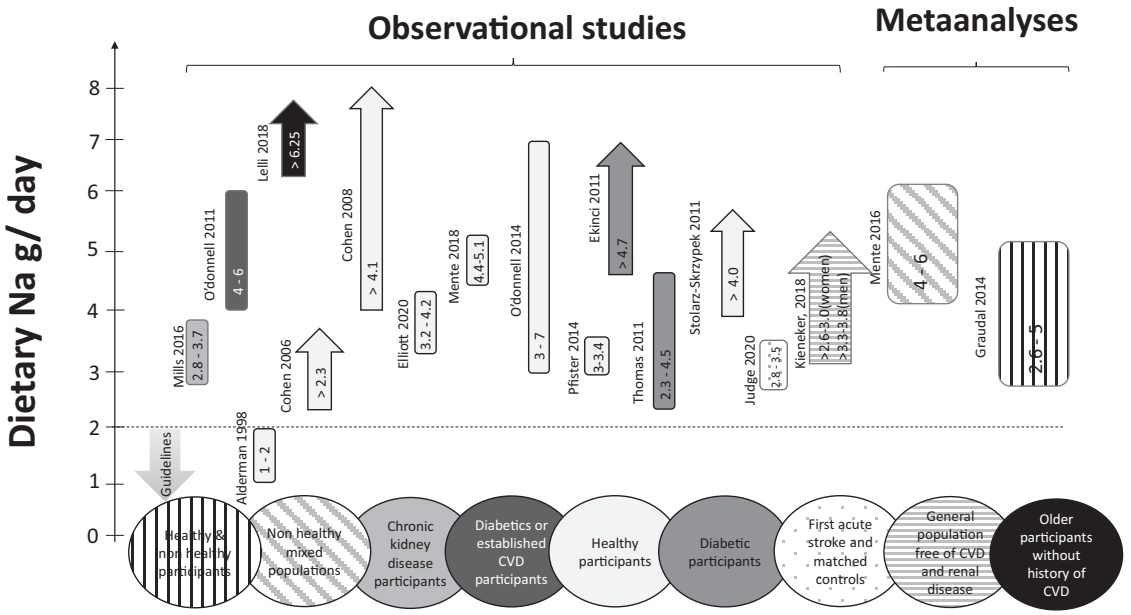

FIGURE 3 Population samples in studies showing J-shape or inverse association between sodium intake and cardiovascular morbidity/mortality as well as all-cause mortality. Data from [5,39–55]. Bars present the ranges showing lower risk, above and below which the risk increases. Arrows present the cut-offs above which the risk is lower. One gram of Na is equivalent to 2.5 g of salt.

the inability of these methods to quantify the discretionary use of salt (table salt or use of salt during cooking), which contributes significantly in total Na intake, especially in eastern countries (∼75% in eastern countries, ∼10–15% in western countries).

Taken altogether, this evidence suggests that the use of all these methods as alternatives of the 24 h urine collection, even though convenient, lead to inaccurate Na intake recording. Therefore, they introduce not only a major cause of heterogeneity but also bias regarding outcome estimation. However, a close look in the schematic representation of these studies as labeled per Na methodology (Fig. 2), reveals interesting information. At least six studies that applied at least one [5,41,43,52] or even multiple [40,51] assessments by the gold-standard 24 h urine collection methodology showed that lower Na excretion is associated with higher cadiovascular morbidity [40] and cardiovascular [5,41] or all-cause [43,52] mortality rates. It is, therefore, very likely that the bias introduction because of inaccurate Na assessment methodology cannot explain all the evidence in favor of a J-shape association.

Population characteristics

Another major limitation is related to the high heterogeneity of populations' characteristics included in the studies so far. Indeed, a significant number of those studies showing inverse or J-shape associations between Na intake and cardiovascular morbidity/mortality raising, included non-healthy volunteers [40–43,55] (Fig. 3). Thus, a reverse-causality effect should be considered and could possibly explain the J-shaped curves. Strict medical or dietary advices in these high cardiovascular risk groups of people, could have led to reduced dietary Na intake. However, it must be highlighted that the majority of the data supporting the J-shape association derive from studies from healthy populations.

Summing up the aforementioned methodological considerations, the two metanalyses supporting the J-shape curve between Na intake and cardiovascular morbidity/mortality [54,55] – which represent the most robust evidence of the J-shape curve – present:

1. heterogeneous or inaccurate Na assessment methods: Graudal *et al*. [54] metanalysis, combined studies that used heterogeneous methods for dietary Na assessment to provide the J-shaped finding, whereas in Mente *et al*. meta-analysis [55], all studies used the spot urine method, which is unreliable for dietary Na recording.
2. high-risk participants: both meta-analyses included high-risk participants [55] or mixed healthy and non-healthy subjects [54].

However, in a close review of Figs. 2 and 3, after excluding studies: that applied methods other than the 24-h urine collection, and included nonhealthy or high-risk participants, only three [5,51,52] remain to implicate low-Na intakes in risk raising.

Salt sensitivity

Finally, none of the above mentioned studies has addressed the potential confounding effect of salt sensitivity. This quite complex-to-define [74,75] 'physiological trait' [76] is accentuated in several specific population groups, including black race, hypertensive patients, older adults, chronic

renal disease patients, women with preeclampsia history, or individuals with low weight at birth. Moreover, in a setting of low potassium intake, even greater effect of salt on BP has been reported [77,78]. Therefore the random inclusion of such populations [40,52] in observational studies, could modulate outcomes, for example, enhance the reverse-causality theory. Until now, no generally accepted method or easy to apply diagnostic tool for salt-sensitivity assessment exists, and despite its importance on studies evaluating the role of Na in health and disease, it has never been taken into account.

PROSPECTIVE RANDOMIZED CLINICAL TRIALS ON DIETARY SODIUM AND CARDIOVASCULAR MORBIDITY/ MORTALITY

Large scale well-designed prospective RCTs are known to be hierarchically more robust types of studies, having the ability to provide a state-of-the-art design and to overcome high risk of bias from which observational studies suffer. Thus, dietary guidelines are mainly based on strong evidence derived from metanalyses of prospective RCTs in order to avoid errors related to methods used, population selected and the reverse causality effect, although high-quality prospective cohort studies are also usually included. The relationship between dietary Na and cardiovascular morbidity/mortality has been previously investigated in such studies that use gold-standard methodology for Na assessment (e.g. 24-h urine collections) and are conducted in low to medium cardiovascular risk – nonacutely ill and apparently healthy – populations (e.g. older adults or hypertensive participants, without established cardiovascular disease or chronic kidney disease, etc.) [34,79–82]. Interventions in these studies include dietary Na reduction with long-term follow-up periods sufficient to provide fatal or nonfatal cardiovascular events (2.5–12 years) [34,79–82].

A metanalysis of these studies conducted by the Agency for Healthcare Research and Quality for the IoM 2019 Dietary Reference Intakes for Na [25] found a 28% lower risk [95% confidence interval (CI) 0.59–0.89] for incidence of cardiovascular disease events after dietary Na reduction interventions, including five prospective RCTs [34,79–82]. The European Society of Hypertension and the European Society of Cardiology 2018 guidelines for the management of arterial hypertension [21], included two metanalyses of prospective RCTs investigating the association between dietary Na and cardiovascular morbidity, cardiovascular mortality or all-cause mortality [18,83]. The first one, by Taylor *et al.* [83] included seven RCTs with a follow-up period of at least 6 months; three conducted in normotensives [80,84–87], two in hypertensive patients [88,89], one in mixed normotensives and hypertensive patients [81] and one in heart failure patients, which were analyzed separately [90]. When the longer term RCTs were used for the metanalysis [80,85,87,89], there was no strong evidence of benefit neither in mortality rates nor in cardiovascular morbidity in the reduced salt group relative to controls, for both normotensives (RR = 0.90 for all-cause mortality, $P > 0.05$; RR = 0.71 for cardiovascular morbidity) and hypertensive patients (RR = 0.96 for all-cause mortality, $P > 0.05$; RR = 0.84 for cardiovascular morbidity, $P > 0.05$) [83]. Indeed, the majority of current guidelines and dietary reference values, agree that the relationship between dietary Na reduction and cardiovascular morbidity/mortality remains unclear because of a low or moderate evidence of benefit of such strategies to reduce cardiovascular risk or all-cause mortality [21,23–25].

However, a major parameter to be considered is not only the effect of dietary Na reduction on cardiovascular events (fatal or non-fatal) but also the levels of Na reduction associated with the outcomes of interest. According to the American Heart Association and the American College of Cardiology 2013 Guidelines, there is low strength evidence supporting that an approximately 1000 mg reduction in Na intake per day decreases about 30% cardiovascular events [24]. This evidence derives from three prospective RCTs. Chang *et al.* studied 1981 older men; they reduced Na intake in the intervention group from 5200–3800 mg/day via substitution of Na with a potassium-enriched salt for 31 months [81]. Cardiovascular events decreased by 41% compared with the control group [81]. A study conducted by Appel *et al.* [79] in 975 older hypertensive participants (TONE) showed a nonstatistically significant reduction of cardiovascular events in the intervention versus the control group (36 versus 46 events), after reducing Na intake by 1000 mg/day for 29 months [79]. The observational follow-up of the prospective RCT TOHP study (3126 participants) concluded to a 30% reduction in relative risk for cardiovascular events in prehypertensive participants who reduced their dietary Na intake (∼−800 mg/day after the initial intervention) during the 12–15 years of follow-up [80]. Moreover, the WHO Guidelines for dietary Na intake in adults and children clearly addressed research questions to investigate the effects of different Na levels on adverse health outcomes. Three ranges of Na intake were evaluated: less than 2 g/day; 1.2–2 g/day; less than 1.2 g/day [23]. However, the working group reported wide ranges of Na intake across the quartiles used in each study, with some studies with 'some consuming as little as 1.4 g/day in the lowest group and 2.6 g/day in the highest group, and others consuming as much as 4 g/day in the lowest group and 6.6 g/day in the highest group. [23]. It is important to note that very low levels of Na intake (about less than 1.5 g/day) are difficult or even impossible to be achieved with the current dietary patterns and these observations of very low Na intakes possibly derive from biased methods or under-reporting of Na intake. Moreover, limited data from RCTs were available and the metanalysis conducted lead to inconclusive findings [23]. Indeed, despite the large body of evidence on RCTs comparing the effect of different levels of dietary Na on BP [79,91–96], data for cardiovascular events and all-cause mortality remain limited and insufficient to address the specific question of the optimal daily Na intake range.

CONCLUSION

The safe range of dietary Na intake remains unclear. There are systematic methodological errors in observational studies conducted around this topic and the 'J-shape

hypothesis' cannot be neither neglected nor verified. Moreover, despite the large body of evidence from RCTs, suitable data remain limited and insufficient to address the specific question of the optimal daily Na intake range, which is associated with the lower cardiovascular risk. Undoubtedly, there is a great need of an accurate and easily applicable dietary Na assessment tool, which would be universally applied in research studies, providing the ability to compare findings from different studies. We suggest that a hybrid approach combining the methodology of validated and easy to apply questionnaires specially designed for Na intake estimation with that of an improved spot urine equation, should be tested as alternative option. Moreover, the incorporation of genetic or environmental factors associated with salt-sensitivity are needed to further clear this field. Finally, it is important to conduct prospective RCTs that will examine the long-term effects of dietary Na intake on cardiovascular morbidity/mortality, including at least three levels of daily Na intakes (very low, 'normal', and high) as very low dietary Na diets have been suggested to associate with subclinical vascular damage in both human [97,98] and animal studies [99–102]. Moreover, considering the large variety of potentially confounding factors related to dietary intake and cardiovascular parameters, future studies should secure that all of them are well-controlled and reliably assessed. Given the fact that large-scale, long-term and high-quality RCTs are needed to be conducted in healthy populations, as well as the high cost of these studies and the poor adherence of participants to low salt intake for long-term follow-up periods, it is important to note that the question will not be settled soon. Therefore, surrogate noninvasive vascular biomarkers might be incorporated in such future large clinical trials [103]. Future perspectives could include innovative approaches in studies' methodology to bypass the above-mentioned issues. Until then, the clinical consultation for daily Na reduction should be primarily focused to high salt consumers and the recommendations should provide ranges of safe Na intakes, which can be adopted easily, rather than a higher cut off value. Until all the aforementioned issues be addressed, the J-shape hypothesis will remain an open challenge.

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Conflicts of interest

There are no conflicts of interest.

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