The effect of vitamin K on vascular health and physical function in older people with vascular disease

Fulton, Roberta

Award date:
2013

Awarding institution:
University of Dundee

Link to publication
The effect of vitamin K on vascular health and physical function in older people with vascular disease

Roberta Fulton

2013

University of Dundee
THE EFFECT OF VITAMIN K ON VASCULAR HEALTH AND PHYSICAL FUNCTION IN OLDER PEOPLE WITH VASCULAR DISEASE

ROBERTA LORRAINE FULTON

MSc by Research

UNIVERSITY OF DUNDEE

AUGUST 2013
# TABLE OF CONTENTS

*List of Tables* ........................................................................................................ 7

*List of Figures* ........................................................................................................ 9

*List of Appendices* ................................................................................................ 10

*Abbreviations* ........................................................................................................ 11

*Acknowledgements* ............................................................................................... 14

*Declaration* ............................................................................................................ 16

*Summary* ................................................................................................................ 17
1. LITERATURE REVIEW

1.1 The Discovery of vitamins ........................................ 19
Vitamin subgroups .................................................. 20
Action ................................................................. 20

1.2 Vitamin K .............................................................. 21
A Historical View ................................................... 21
Classification ......................................................... 22
Function ............................................................... 24
Dietary Sources ....................................................... 27
Absorption ............................................................ 28
Human requirements ................................................ 29
Toxicity ................................................................. 30
Measurement of vitamin K status ............................. 31
Role of Vitamin K in Disease Processes .................... 32
  Role of Vitamin K in Clotting ................................. 32
  Role of Vitamin K in Bone Health ......................... 34
  Role of Vitamin K in Vascular Disease ................. 36

1.3 Population Ageing .................................................. 38
Age Structure ......................................................... 39
Life Expectancy ....................................................... 41
Mortality .............................................................. 44

1.4 Cardiovascular Disease in Older Adults ..................... 45
Hypertension ......................................................... 47
2. METHODS

2.1 Study Aims

2.2 Study Design

2.3 Sample Size

2.4 Study Population

Exclusion Criteria

2.5 Investigator Training

2.6 Participant Selection and Enrolment

Recruitment

Informed Consent

Randomisation

2.7 Medication

Manufacture

Medication Adherence

2.8 Outcome Measurement

Screening Assessment

Baseline Assessment

Primary Outcome Measure

Secondary Outcome Measures

Measures of Vascular Function
3. RESULTS

3.1 Recruitment ........................................ 84

3.2 Baseline Characteristics .................................. 86

3.3 Outcome Measures ........................................ 89

Primary Outcome .......................................... 89

Secondary Outcomes ........................................ 90

Vascular Markers ........................................... 90

Measure of Physical Function .............................. 95

Blood Tests ................................................ 96

3.4 Major Recorded Adverse Events ......................... 100

Serious Adverse Events .................................. 100

Adverse Events ........................................... 101

3.5 Adherence to Medication ................................. 103

3.6 Analysis ................................................ 103
4. DISCUSSION AND CONCLUSION

4.1 Summary of Main Findings ........................................ 104
Recruitment and Retention ........................................ 104
Baseline Measurements .......................................... 106

4.2 Significance of Findings ...................................... 107
Primary Outcome ..................................................... 107
Secondary Endpoints ............................................... 109
Adherence .............................................................. 112
Adverse Events ....................................................... 113

4.3 Strengths and Weaknesses ................................. 113
Strengths .................................................................. 113
Weakness .................................................................. 114

4.4 Conclusion ......................................................... 117

5. FUTURE RESEARCH

5.1 Vitamin K and Cardiovascular Health ............... 119
5.2 Vitamin K and Neuromuscular Function and Bone Health ............................................. 120

REFERENCE LIST ..................................................... 121

APPENDIX .............................................................. 149
LIST OF TABLES

1: Name, Site & Function of Currently Identified Gla Protein .............................................................. 26

2: Trends in life expectancy and healthy life expectancy at birth, 1981 to 2001 ........................................ 42

3: Inclusion and Exclusion Criteria .................................................. 52

4: Training records for FMD healthy volunteers ......................... 54

5: Training records for PVW healthy volunteers ......................... 55

6: Baseline Characteristics of Participants ................................. 87

7: Effect of Intervention on Flow Mediated Dilatation of the Brachial Artery ........................................ 90

8: Effect of Intervention on Carotid Intima-Media Thickness .............................................................. 91

9: Effect of Intervention on Pulse Wave Velocity ...................... 92

10: Effect of Intervention on Augmentation Index ...................... 93

11: Effect of Intervention on Blood Pressure ............................. 94

12: Effect of Intervention on Grip Strength ............................... 95

13: Effect of Intervention on Short Physical Performance Battery Scores .............................................. 96

14: Effect of Intervention on B-type Natriuretic Peptide Levels .................................................................. 97

15: Effect of Intervention on C - reactive protein Levels .......................................................................... 97

16: Effect of Intervention on serum cholesterol levels ............ 99
LIST OF FIGURES

1: Structure of vitamin K₁, vitamin K₂ and vitamin K₃ --------------- 23
2: The ‘vitamin K cycle --------------------------------------------- 25
3: Diagram of the clotting cascade --------------------------------- 33
4: Population pyramids of Scotland by age group and sex
   (1911, 1951, 1991 & 2031) --------------------------------------- 40
5: The changing age structure of Scotland's population
   (2000-2010) --------------------------------------------------- 41
6: Expected healthy life years remaining for people aged 65
   throughout European countries in 2005 -------------------------- 43
7: Scotland Population - Mortality Rates -------------------------- 45
8: Positioning for measurement of brachial artery flow
   mediation -------------------------------------------------------- 64
9: Ultrasound view of carotid artery intima-media thickness ------ 67
10: Diagram of a Pulse Wave ----------------------------------------- 69
11: Diagram of applanation tonometry process ----------------------- 72
12: Schedule of Study visits ---------------------------------------- 81
13: CONSORT diagram showing participation flow through
   trial ------------------------------------------------------------- 85
LIST OF APPENDICES

1. Good clinical Practice Certificate
2. Invitation Letter
3. Patient Information Sheet
4. Patient Reply Slip
5. Consent Form
6. GP letter
7. Case Report Form
8. Short Physical Performance Battery Scoring Sheet
ABBREVIATIONS

ACEi: Angiotensin-converting-enzyme inhibitor
AF: Atrial fibrillation
Alx: Augmentation Index
ANCOVA: Analysis of covariance
ANP: Atrial Natriuretic Peptide
ARB: Angiotensin receptor blocker
BMD: Bone mineral density
BNP: B-type Natriuretic Peptide
BP: blood pressure
CABG: Coronary artery bypass graft
CHD: Coronary heart disease
CI: confidence interval
CO₂: Carbon dioxide
COPD: Chronic obstructive pulmonary disease
CRF: Case report form
CRP: C-reactive protein
CVD: Cardiovascular disease
DBP: Diastolic blood pressure
ECG: Electrocardiograph
EFSA: European Food Safety Authority
EU: European Union
FMD: Flow-mediated dilatation
Gla: gamma-carboxyglutamate acid
Glu: glutamic acid
GP: General practitioner
HDN: Haemorrhagic Disease of the Newborn
HLE: Healthy life expectancy
HDL: High density lipoprotein
HPLC: high performance liquid chromatography
IMT: Intima-media thickness
IQR: Inter quartile range
K₃: Phylloquinones
LDL: Low-density lipoprotein
MGP: Matrix Gla protein
MHRA: Medicines and Healthcare Products Regulatory Authority
MK: menaquinone
NHANES III: Third Health and Nutrition Examination Survey
NO: Nitric oxide
ONS: Office for National Statistics
PIS: Patient information sheet
PP: Pulse pressure
PT: Prothrombin Time
PTCA: Percutaneous transluminal coronary angioplasty
PVD: Peripheral vascular disease
PWA: Pulse wave analysis
PWV: Pulse wave velocity
RDI: Recommended daily intake
RF: Roberta Fulton
RH: Reactive hyperaemia
SAE: Serious adverse event
SAR: Serious adverse reaction
SCF: Scientific Committee for Food
SD: Standard deviation
SOP: Standard operating procedure
SPCRN: Scottish Primary Care Research Network
SPPB: Short Physical Performance Battery
SPPIRe: Scottish Practices and Professionals Involved in Research
SPSS: Statistical pack for social sciences
SBP: Systolic blood pressure
TIA: Transient ischaemic attack
TMF: Trial master file
U+E: Urea and electrolytes
VKD: Vitamin K dependent proteins
VKOR: Vitamin K epoxide reductase
WHO: World Health Organisation
Acknowledgements

I wish to thank everyone who has helped me with my thesis. In particular:

Professor Marion McMurdo for introducing me to research, offering me the opportunity to experience life in academia and spreading my wings beyond clinical practice. Without her encouragement and guidance I would not have made the leap and would have missed out.

Dr Miles Witham for his patience, kindness and support, without which this thesis would undoubtly remain unfinished.

Ms Valona Gouck for her timely administrative work which aided the smooth running of the study.

Mrs Lesley McFarlane and staff in the Clinical Pharmacology Laboratory and staff in the Biochemistry Laboratory who analysed the blood samples.

Dr Sandy Hill for analysis of the vitamin K samples.

Staff at Tayside Institute of Cardiovascular Research for allowing me to use their space and equipment for completion of study visits.

Mrs Marie Pitkethley and staff at the East node of the Scottish Primary Care Research Network (SPCRN) for their wonderful effort with study recruitment.
Chest Heart and Stroke Scotland for funding the study.

All the wonderful people who willingly participated in the study and gave so freely of their time.

Finally to my wonderful family for the enormous support given to me, especially my daughter Ashley who has encouraged me throughout and who never doubted me even when I doubted myself.
**Declaration**

I hereby declare that I am the author of this thesis, that I have carried out the work described have consulted all references cited. The work previously described in this thesis has not been previously accepted for a higher degree and I have defined the nature of my contribution to the work within the project described in the thesis.

The work contained within this was carried out during my appointment as a Research Fellow in the section of Ageing and Health, Department of University of Dundee between September 2011 and August 2013.

Signed ……………………………………  Dated………………………. 
Summary

“Population ageing” - the process by which older individuals make up a proportionally larger share of the total population over a period of time has thrown up new challenges such as the increasing demand and expense on health and social care. Cardiovascular disease, prevalent in older people, goes hand in hand with declining physical function and new ways of tackling the burden this places on older persons, their carers and health services are required.

Recent work has suggested that vitamin K, a known cofactor for coagulation proteins, may be required for the function of several other key proteins. In particular, Matrix Gla protein may exert beneficial effects on vascular health. I hypothesised that supplementation of the diet with vitamin K would improve vascular health and physical function in older people with established vascular disease.

In a double-blind randomised controlled clinical trial 80 participants aged ≥ 70 years with established vascular disease were randomised to receive 100mcg vitamin K or placebo daily for 6 months. The primary outcome was a between group difference in endothelial function change between baseline and 6 months. The secondary outcomes were carotid-radial pulse wave velocity, carotid intima-media thickness, Short Physical Performance Battery and grip strength.
Participant mean age was 77 years (SD 5), 44/80 (55%) were male. Only 3/80 (4%) participants failed to complete the study. Vitamin K levels rose in the intervention arm compared to placebo (+48pg/ml vs. -6pg/ml, p=0.03) at six months compared to baseline. There was no significant change in brachial artery flow mediated dilatation or indeed in any of the other markers of vascular health or physical function. This was despite excellent adherence to medication. There was however a modest 10% reduction in arterial stiffness by 6 months suggesting that changes to vascular calcification with vitamin K₂ may be possible but may require longer to achieve.
1. LITERATURE REVIEW

1.1 The Discovery of Vitamins

For centuries the belief that diseases were due to the presence of some type of organism rather than a dietary deficiency prohibited the treatment of illness such as beriberi (a deficiency of vitamin B₁), rickets (a deficiency of vitamin D) and scurvy (a deficiency of vitamin C) (1). The earliest studies by Lunin in 1881 and Pekelharing in 1905 (1) examined the response of laboratory animals to an artificial diet comprising protein, fat, carbohydrate minerals and water. In both studies there was failure to thrive and death among the mice, but milk, even when ingested in small quantities, seemed to keep the mice alive (2). By 1912 Casimir Funk had formulated the theory that certain diseases could be prevented by ingestion of a complete diet including these accessory factors, and classified these substances Vita “life” and amine a compound containing nitrogen, reflecting the fact that the first of the vitamins to be studied was vitamin B₁ (3). Two of the fat soluble vitamins were discovered in 1913 – vitamin A which was effective in preventing Xerophthalmia (dry eye syndrome) and vitamin D which was effective against rickets.

By 1920 it had become clear that not all vitamins were amines so the “e” on the end was dropped.
Vitamin subgroups

By 1915 Osborne and Mendal and McCollum and Davis had distinguished two types of accessory factors based on their solubilities – Fat soluble A which is present in butterfat and egg yolk and the previously identified water soluble B. Most vitamins were identified between 1920 and 1950 and over the past century the importance of these food factors for normal growth and metabolism has been established\(^{(4,5)}\).

In total thirteen vitamins are currently recognised in human nutrition – the four fat soluble vitamins A, D, E and K and the nine water soluble vitamins comprising of vitamin C and the eight members of the vitamin B group – thiamine (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), pyridoxine (B6), biotin (B7), folic acid (B9) and cobalamin (B12).

Action

Most vitamins act as co-enzymes, promoting catalytic functions in the body’s metabolic pathways. At least 30 vitamins and minerals (micronutrients) are required by the body. Sometimes a number of vitamins and minerals must interact to accomplish these metabolic functions: for example for optimum absorption of the mineral calcium in bone health the vitamin D must also be available, while for promotion of good vision the absorption of vitamin A improves in the presence of the mineral zinc. No single foodstuff provides all the micronutrients required.

Today, compared to the dates of their discovery, intake of these micronutrients is much improved. While some of the deficiency conditions such as beriberi and scurvy
are rare in the UK, rickets may still be evident in socially deprived groups (6) and in Asian children with darkly pigmented skin.

1.2 Vitamin K

A Historical view

The function and properties of blood were the subject of debate as long ago as the time of Hippocrates in 460BC and by AD1832 Johannes Muller described the role of fibrin in clotting (7).

Danish biochemist Henrik Dam first discovered the nutrient vitamin K while carrying out studies in the 1930s while investigating the need for cholesterol in the diet of chicks (7,8). Chicks fed a diet from which sterols had been removed developed internal bleeding and died. On examining the dead chicks, their blood was found to clot slowly. Further experiments involving the addition of different foodstuffs found that the ‘antihaemorrhagic (or clotting) factor’ was fat soluble and found in a range of different foods from a variety of cereals to animal organs. Dam named the factor Koagulations vitamin due to its apparent link with clotting. This accessory factor was later abbreviated to vitamin K and Dam was awarded the Nobel Prize in 1943 for his discovery.

Edward Doisy and his colleagues continued research into this new area and succeeded in isolating vitamin K from fish meal by the end of the 1930s. It was at this time that the first clinical trials with administration of vitamin K began (9). Along
with Carl Dam, Edward Doisy shared the Nobel Prize for his discovery of the chemical nature of vitamin K.

This extracted fishmeal product was entitled K₂ by Doisy and its availability enabled others to study the effects of administration of vitamin K to new-born babies suffering from haemorrhagic disease (7). Research in 1937 found that clotting times in normal neonates were around 30-60% of adult levels, falling to 15-30% on day two, and then gradually rising again until about 10 days following birth. This ‘evidence’ that vitamin K deficiency caused Haemorrhagic Disease of the Newborn (HDN), along with the newfound ability to synthesise vitamin K, led to various trials to ascertain which was the most effective amount and route to use in prophylaxis. By the 1950’s, most maternity units had a policy of giving infants oral vitamin K immediately after birth (10).

**Classification**

The term ‘vitamin K’ is the name given to all compounds which possess the ability to act as a cofactor for the enzyme gamma-glutamylcarboxylase. Vitamin K is classified according to its chemical structure, and two forms exist in nature, grouped as either phylloquinones (vitamin K₁) which are synthesized by green plants and which have a phytol side chain (see figure 1), or menaquinones (vitamin K₂) which are obtained from animal sources and synthesised by bacteria found in the intestine.

The menaquinone molecules have a tail composed of repeating unsaturated 5-carbon (prenyl) units. Further subdivision can be made to menaquinones. Depending on the
number of these 5 carbon repeats, they are categorised using the term menaquinone-n (MK-n), (n) relating to the number of prenyl units and currently 13 are known to exist.

A third form of vitamin K exists, Menadione, which is a synthetic form possessing a much simpler structure than the naturally occurring forms however it is unable to exert all the functions of naturally occurring vitamin K. Research has focused around Menadione’s potential antitumor properties with several in vitro studies identifying anticancer activity \(^{(11)}\).

**Figure 1 – Structure vitamin K\(_1\), vitamin K\(_2\) and vitamin K\(_3\) taken from \(^{(12)}\)**
Function

Vitamin K has been identified as the essential micronutrient needed to enable the conversion of glutamate (an amino acid) to gamma-carboxyglutamate (Gla) by post-translational carboxylation. As previously stated it acts as a cofactor for the enzyme gamma-glutamyl carboxylase which oxidizes vitamin K hydroquinone into vitamin K epoxide, while simultaneously adding CO\textsubscript{2} to protein-bound glutamic acid (Glu) to form the gamma-carboxyglutamic acid (Gla). This carboxylation reaction will only occur if the carboxylase enzyme is able to oxidize vitamin K hydroquinone to vitamin K epoxide at the same time. Finally vitamin K epoxide is reconverted back into vitamin K by vitamin K epoxide reductase (VKOR) - this process being commonly called the ‘vitamin K cycle’ (see figure 2). This recycling of vitamin K occurring within the body’s cells is one of the reasons why severe vitamin K deficiency is rare as, despite the fact that the body has a limited ability to store vitamin K, the cycle allows vitamin K to enable the gamma-carboxylation of proteins many times, decreasing the daily dietary requirement.
Figure 2: The ‘vitamin K cycle’ (modified from Stafford, 2005)

Warfarin, a commonly used anticoagulant drug, interrupts this cycle by inhibiting vitamin K epoxide reductase. If administered in large doses it causes bleeding, and is commonly used as a poison for rats due to this property, but when administered at a therapeutic dose it can be used to prevent the formation of harmful thrombi, e.g. in atrial fibrillation or to treat deep venous thrombosis.

In recent years more than a dozen vitamin K dependent proteins (VKD) have been identified (see Table 1) of which all require the presence of \( \gamma \)-carboxyglutamate (Gla) residues to function successfully. The enzyme \( \gamma \)-glutamyl carboxylase has been found not only in the liver but more recently in other tissue such as skin, lung and kidney and this has led researchers to investigate what other functions the Gla proteins may have in the body other than that of blood clotting.
**Table 1 – Name, site & function of currently identified Gla proteins**

<table>
<thead>
<tr>
<th>Gla proteins - Name</th>
<th>Abbreviation</th>
<th>Site</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prothrombin</td>
<td>FII</td>
<td>liver – plasma</td>
<td>procoagulant</td>
</tr>
<tr>
<td>Factor VII</td>
<td>FVII</td>
<td>liver – plasma</td>
<td>procoagulant</td>
</tr>
<tr>
<td>Factor IX</td>
<td>FIX</td>
<td>liver – plasma</td>
<td>procoagulant</td>
</tr>
<tr>
<td>Factor X</td>
<td>FX</td>
<td>liver – plasma</td>
<td>procoagulant</td>
</tr>
<tr>
<td>Protein C</td>
<td></td>
<td>liver – plasma</td>
<td>anticoagulant</td>
</tr>
<tr>
<td>Protein S</td>
<td></td>
<td>liver – plasma</td>
<td>anticoagulant</td>
</tr>
<tr>
<td>Protein Z</td>
<td></td>
<td>liver – plasma</td>
<td>anticoagulant</td>
</tr>
<tr>
<td>Osteocalcin</td>
<td>OC / BGP</td>
<td>bone - dentin</td>
<td>regulator of crystallization</td>
</tr>
<tr>
<td>Matrix Gla Protein</td>
<td>MGP</td>
<td>cartilage &amp; arterial vessel wall</td>
<td>inhibitor of ectopic calcification</td>
</tr>
<tr>
<td>Growth arrest specific gene-6 protein</td>
<td>gas6</td>
<td>most soft tissues</td>
<td>regulator of cell growth (may play a role in vascular &amp; ageing nervous system)</td>
</tr>
<tr>
<td>Proline-rich Gla proteins</td>
<td>PRGP</td>
<td>most soft tissues</td>
<td>Currently unknown</td>
</tr>
<tr>
<td>Transmembrane Gla proteins</td>
<td>TMG3,TMG4, PRGP1,PRGP2</td>
<td>most soft tissues</td>
<td>Currently unknown</td>
</tr>
</tbody>
</table>
Dietary Sources

With recent advances in nutritional science such as high performance liquid chromatography (HPLC), it is now possible to obtain accurate measurements of vitamin K levels in foodstuffs (15) resulting in the compilation of a national UK database for the vitamin K content in food (16). For oral intake the most abundant K vitamins appear to be the phylloquinones (K₁), which are to be found in leafy green vegetables such as cabbage and broccoli and contribute up to 40-50% of total intake.

Vitamin K₁ can also be found in some plant oils such as soybean and olive oils. The naturally occurring menaquinones (MK) collectively known as K₂ are found in small amounts in meats, eggs and also in fermented foods such as cheese. These particular food sources have limited absorption in the large intestine, and so contribute to only 10% of the total intake (10,11), the exception to this being the Japanese food natto which is made from fermented soybean and has a menaquinone content which is higher than the phylloquinones to be found in any of the green vegetables. In healthy adults the large intestine contains bacteria which synthesise many of the menaquinones, mainly MK6-MK12.

A comparative study using data gathered from the 1986-7 Dietary and Nutritional Survey of British Adults and data collected from the 2000-1 National Diet and Nutrition Survey compared daily intakes of vitamin K among British adults in both 1986-7 and 2000-1. Sixty per cent of vitamin K intake as recorded (on 7 day food diaries) came from green leafy vegetables, with higher intakes recorded in men than
in women. The intake of vitamin K was noted to be considerably lower by 2000-2001 mainly due to a reduction in the consumption of vegetables\(^{(17)}\).

**Absorption**

The efficiency of vitamin K absorption depends on the source of the vitamin and amount of fat in the diet but, in contrast to other fat soluble vitamins, vitamin K can only be stored in the body in limited quantities. Phylloquinones, which are the major source of vitamin K in humans, are absorbed in the jejunum and ileum in the presence of bile and pancreatic juices. Menaquinones are absorbed in the same fashion with the result that patients suffering from fat malabsorption syndromes such as those with coeliac disease, cystic fibrosis or Crohn’s disease may develop vitamin K deficiency\(^{(18)}\).

Once the phylloquinones have been absorbed they enter the bloodstream and are transported chemically unchanged to the liver for utilization and then distributed to other body tissues including skeletal, muscle and heart tissue. The liver has only a limited capacity for storage of vitamin K and it is the menaquinones which make up the majority (around 90%) of all stored vitamin K\(^{(8,19)}\). Compared with other fat-soluble vitamins, the total body store of vitamin K is small and the turnover of vitamin K in the liver is rapid. Although the liver contains menaquinones, their absorption and their contribution to the human vitamin K requirement has not yet been fully elucidated. Many hepatic menaquinones are untraceable in plasma.
Human requirements

The overall amount of dietary intake of vitamin K the body requires is relatively low in relation to other nutrients \(^{(20)}\). The current Adequate Intake (amount of nutrient required by the body to reduce the risk of developing associated diseases) of vitamin K, as taken from the Third Health and Nutrition Examination Survey (NHANES III) 1984 – 1994 has been set at 120µg/d for men and 90µg/d for women (equivalent to eating 3 to 4 brussel sprouts or two to three spears of broccoli per day). Debate over this recommendation exists however with 1 µg/kg/day currently taken as the standardised guideline set by the UK Department of Health in 1991 while the World Health Organisation (WHO) currently recommends a daily intake of vitamin K 60–90 µg/day as adequate for older persons \(^{(21)}\). The Framingham cohort study reported that individuals who fell into the category of high intake of vitamin K (median 254 µg/d) as recorded on food record diaries had a significantly lower risk of hip fracture than those in the lowest intake group (median 56 µg/d) \(^{(22)}\). The UK National Diet and Nutrition survey identified that the average intake of older people was between 80–120 µg/d while studies of younger populations found average intake to be around 60–70 µg/d with similar figures obtained in population based surveys in the USA \(^{(23)}\).

Experiments carried out on laboratory animals have shown that vitamin K has a short half-life of only a few days, but despite this the intake in healthy adults appears sufficient to ensure normal blood clotting; in part ensured because of the efficient way the liver extracts and accumulates the vitamin.
Current recommended figures are based purely on the requirement for vitamin K in blood clotting and do not take into consideration recent understanding of its role in both bone and vascular health, hence deficiency has to date been defined as “a state in which undercarboxylated blood coagulation factors appear in the circulation” (24).

There are currently 3 forms of supplementation available: vitamin K₁; synthetic vitamin K₂ MK-4; and natural vitamin K₂ MK-7 (menaquinone-7). Previous vitamin K comparison studies exist. One such study involving healthy volunteers assessed the pharmacokinetics of both vitamin K₁ and vitamin K₂ with both peaking at around 4 hours following ingestion but, while the vitamin K₁ serum levels displayed only a short half-life time of 1-2 hours, vitamin K₂ had a much longer half-life of 3 days resulting in more stable serum levels (25). Observational studies also suggest that it is in fact vitamin K₂ and not vitamin K₁ which is associated with a lower incidence of cardiovascular events (26) and thus vitamin K₂ was the choice of intervention for this particular study.

**Toxicity**

European legislation exists defining vitamin and mineral supplement intake and upper levels have been set by the European Food Safety Authority (EFSA) or the Scientific Committee for Food (SCF). Research carried out in 1940 on laboratory rats saw dosage of up to 25g/kg of vitamin K administered without any adverse effect (27) and there has been no known toxicity or adverse effects in healthy individuals associated with intake in excess of the recommended dose. In common with vitamin
B2, vitamin B1 and vitamin B12, no tolerable upper level of intake of vitamin K has been established \(^{(28)}\).

**Measurement of vitamin K status**

Serum vitamin K level can be measured by High Performance Liquid Chromatography (HPLC), with the normal range of plasma vitamin K in adults aged 65 to 92 years being 0.32 to 2.67ng/mL \(^{(29)}\). Levels below 0.5 ng/mL have been linked to impaired blood-clotting. The value of measuring serum vitamin K concentration to assess vitamin K status is limited because it reacts to changes in oral intake within a 24 period as noted in a study of pre and post-operative surgical patients \(^{(30)}\).

Vitamin K deficiency results in impaired blood clotting, which can be confirmed by laboratory tests which measure the concentration of the vitamin K dependent factors. Prothrombin Time (PT) specifically evaluates the presence of factors VII, V, and X, prothrombin and fibrinogen in the blood with the normal range in a healthy individual being 11 - 13.5 seconds. More recently other methods for assessing vitamin K status have been developed including the measurement of undercarboxylated prothrombin and undercarboxylated osteocalcin.
Role of Vitamin K in Disease Processes

Role of Vitamin K in Clotting

Vitamin K is required for the process of coagulation in blood clotting \(^{31,32}\), with the vitamin K dependent proteins being identified as: Prothrombin; Factor VII; Factor IX; Factor X; Protein C; Protein S and Protein Z.

Haemostasis

When damage occurs to blood vessels the homeostatic process is triggered. This consisting of 3 mechanisms which work together to stop the flow of blood. Firstly vasoconstriction of the damaged vessel slows the blood flow while the second mechanism of platelet adhesion to plug the wound occurs. Coagulation (the third mechanism in the process) then occurs as a result of activation of the ‘coagulation cascade’ – a process where a series of events, which are all interdependent, stop bleeding by the formation of a clot. Damage to tissue outside the vessel starts a release of the Gla protein factor III, which with the aid of calcium activates factor VII, which initiates the ‘extrinsic pathway’ (see below). At the same time, the Gla protein Factor XII from active platelets will activate factor XI, initiating the intrinsic pathway. Both active factor VII and active factor XI promote cascade reactions, eventually triggering the Gla protein factor X. Active factor X, along with factor III, factor V, Calcium and platelet thromboplastic factor (PF3), activate prothrombin activator which converts prothrombin to thrombin. Thrombin converts fibrinogen to fibrin which initially forms a loose web with factor XIII causing the formation of cross links; this much denser structure of fibres ensure that platelets and red blood
cells become caught in this mesh resulting in the formation of a blood clot (see Figure 3).

**Figure 3: Diagram of the clotting cascade reproduced from** (33)

![Diagram of the clotting cascade](image)

Two other vitamin K dependent proteins, protein C and protein S, play a regulatory role in clotting by inhibiting coagulation via the inactivation of Factors V and VIII. Protein C circulates in the blood as a zymogen and is activated to a serine protease
by the binding of thrombin to thrombomodulin, while protein S enhances the action of Protein C. By deactivating Factors V and VII, Proteins C and S decrease the speed of thrombin production, thereby inhibiting the cascade and acting as the regulatory mechanism of the clotting cascade. Without this mechanism, widespread thrombosis would otherwise occur. There is evidence that protein S is synthesized by several tissues including bone and blood vessels and may have other functions besides its role as an inhibitor of clotting. The other vitamin K-dependent protein (protein Z) is assumed to have a haemostatic role but as yet its function is unclear.

**Role of Vitamin K in Bone health**

Osteoporosis is one of the most common disorders associated with old age, affecting one in every three women and one in every twelve men over the age of 50 years. Reduced bone density is a major risk factor for low trauma fracture, with consequent disability, death and cost to society (34). Osteoporosis has been defined as “a progressive, systematic, skeletal disease that is characterised by low bone mass and deterioration of the small structures in the bones with subsequent increase in bone fragility and susceptibility to fracture” (35), and more recently osteoporosis has been operationally defined on the basis of bone mineral density (BMD) assessment. According to the WHO criteria, osteoporosis is defined as a BMD that lies 2.5 standard deviations or more below the average value for young healthy women (36).

Although the role of calcium in bone health has long been established, other nutritional deficiencies may also play an important part in bone loss (37). Bone matrix is formed by osteoblasts which synthesize osteocalcin, and vitamin K has been
identified as necessary to enable gamma glutanyl carboxylation of osteocalcin. Without this post translational modification osteocalcin is inactive and the body cannot mineralise bone effectively. Serum levels of under carboxylated osteocalcin levels can therefore be used to measure the effect of vitamin K intake on bone health.

Over the past few years accumulating evidence supports a role for vitamin K in bone health. In the majority of studies higher intake of phylloquinones is associated with lower serum concentrations of undercarboxylated osteocalcin. This in turn has been associated with lower bone mineral density and a higher risk of hip fracture (38;39).

The direct relationship between phylloquinone intake and bone mineral density has also been reported although this appears less consistent. The ECKO study randomised 440 postmenopausal women to receive either 5mg vitamin K\textsubscript{1} or placebo for a minimum of 2 years. Despite showing a 10 fold increase in serum K\textsubscript{1} levels along with a decrease in the proportion of undercarboxylated osteocalcin levels no improvement in age related BMD was found (40). The Framingham observational study investigated vitamin K intake in older women (mean age 75 years) with a mean vitamin K\textsubscript{1} intake of 155µg/ day. While it found no correlation between vitamin K intake and either bone mineral density or bone loss, in the Framingham offspring cohort a positive correlation was found in younger women (average age 59 years) but not men (41). The Nurses’ Health Study followed 120,000, women for a period of 10 years and a prospective analysis of this cohort discovered that the women with the lowest risk of hip fracture were those middle aged and older women who reported higher levels of vitamin K consumption on their food questionnaires (39).
An epidemiological study of Japanese premenopausal women compared those who traditionally have a high intake of vitamin K (MK7) ingested as fermented soybean with women from another Japanese area with a staple diet and British women whose diet did not include natto. Those Japanese women consuming the natto (soybean) showed significantly higher serum levels of circulating MK7 and researchers found a statistically significant inverse correlation between natto consumption and the incidence of osteoporotic hip fracture (42).

**Role of Vitamin K in Vascular Disease**

One of the cardinal signs of cardiovascular disease is the formation of atherosclerotic plaques in arterial walls. Until recently calcification of arteries was thought to be a passive and clinically irrelevant process, a result of inflammation, lipid accumulation, diabetes or high calcium. However it is now clear that vascular calcification is in fact an active, structured process which is strongly and independently associated with an increased risk of cardiovascular morbidity and mortality. Calcification results in reduced elasticity (stiffening) of the vascular walls causing a reduction of arterial compliance, which promotes development of left ventricular hypertrophy and causes decreased coronary perfusion. The end result is an increased risk of serious complications – myocardial infarction, stroke, heart failure and cardiovascular death.

In the vasculature a key inhibitor of calcification is the Gla protein Matrix Gla protein (MGP) which is synthesised by smooth muscle cells and is found in a wide variety of tissues including cartilage, heart, kidney and lung.
The role of vitamin K in vascular calcification was proposed over 30 years ago when a study of vitamin K dependent proteins in aortic valve calcification found evidence of Gla in all the calcified valves studied while those valves without calcification had no traceable Gla \(^{(43)}\). Within vascular smooth muscle cells the synthesis of MGP prevents vascular calcification as shown in studies of mice where the removal of the MGP gene resulted in calcification of the elastic lamellae of the abdominal aorta with death from rupture of the aorta occurring within two months \(^{(44)}\). Other studies found that rats who were given the vitamin K antagonist warfarin developed vascular calcification after 2 weeks \(^{(45)}\) while in humans uncarboxylated MGP (i.e. non-functional MGP) has been identified in calcified regions of the vasculature \(^{(38)}\).

The link with atherosclerosis was first considered when proteins containing Gla residues (osteocalcin and MGP) were isolated from atherosclerotic plaques. A population based study with 113 post-menopausal women found a link between low levels of vitamin K and atherosclerotic calcification in the abdominal aorta \(^{(46)}\). Apparently healthy people have shown substantial under carboxylated MGP suggesting that these healthy adults may be vitamin K deficient \(^{(47)}\). It has thus been suggested that previously identified Recommended Daily Intake levels for vitamin K, although sufficiently high to maintain a regulated clotting mechanism may not be high enough to ensure the complete carboxylation of all the other vitamin K dependent proteins required to maintain healthy bone and artery wall function. Importantly a review of current literature showed no evidence for intake of vitamin K\(_1\) but interestingly 2 cohort studies have found an association between vitamin K\(_2\) and fewer CHD events \(^{(38;48)}\), while a study of 118 postmenopausal women, assessed
the effect of supplementing the diet with vitamin K, vitamin E and calcium and found this had a beneficial effect on elasticity and compliance of the carotid artery when compared to other groups without vitamin K supplementation (49).

A recently completed randomised controlled trial confirmed that most patients requiring haemodialysis have a deficiency in vitamin K. It also demonstrated that daily supplementation with vitamin K decreases the levels of inactive MGP providing strong evidence to proceed to a full study looking at the effect on vascular calcification in this high risk group (50).

1.3 Population Ageing

Ageing, as defined by Kirkwood, is “the progressive loss of function accompanied by decreasing fertility and increasing mortality with advancing age” (51). The WHO 2008 classes those over the age of 70 years as being ‘elderly’, those 75-89 years as ‘old’ and those over the age of 90 years being ‘very old’ (52). In almost every country in the world, the proportion of people aged over 60 years is growing faster than any other age group. It has been projected that by 2035 23% of the UK population will be aged ≥65 years with 3.6 million being aged ≥85 years (19).

In the early twentieth century better hygiene, public health, nutrition and better obstetric care resulted in a rapid decline in the mortality of the population of developed countries around the world (53). This trend along with declining fertility rates has given rise to a reduction in the number of young persons under the age of 15 years but an increase in the population of those in the later stages of life. As a
result “population ageing” - the process by which older individuals make up a proportionally larger share of the total population over a period of time - has thrown up new challenges to both developed and developing countries such as the increasing demand and expense on healthcare and social care.

WHO currently records the world’s total population of those aged 60 and above at 650 million but it expects this figure to reach 2 billion by 2050. According to the Office for National Statistics (ONS) in Scotland the number of people of pensionable age is expected to rise by 3% from 1.04 million in 2010 to 1.07 million in 2020 and then reach 1.32 million in 2035, while the number of people aged 75 and over is anticipated to increase from 0.41 million in 2010 to 0.50 million in 2020 and to continue rising, reaching 0.74 million in 2035 – an increase of 82 per cent over the 25 year period.

**Age Structure**

Within Scotland there has been very little change in the total number of people over the past 50 years, but the age profile of the population has changed quite significantly within the past decade (54). One hundred years ago there were few older people and a large, young, working age population to support them; average life expectancy was only 40 years. However it is predicted that by 2031 in Scotland the number of people aged 65 years or over will increase from 0.75 million in 2000 to 1.2 million in 2031. (See figure 4.) Those who are aged ≥65 will have increased by 62% while those aged ≥85 will have increased by 144%. The population of older persons is itself ageing.
Among those aged 60 years or over, the fastest growing population is that of the oldest-old, that is, those aged 80 years or over.

Figure 4 - Population pyramids of Scotland by age group and sex (1911, 1951, 1991 and 2031) taken from National Workforce Planning Framework 2005 (55)
While population ageing is a success story for public health policies and for socioeconomic development, it brings with it many challenges for both individuals and for society as a whole. As the proportion of older people increases, so the age structure of Scotland’s population is also changing (see figure 5). By 2010 there were more people aged 65 years and over (20%) than there were under the age of 16 (17%).

**Figure 5: The changing age structure of Scotland's population, 2000-2010 taken from** *(56)*

<table>
<thead>
<tr>
<th>Age Group</th>
<th>2000</th>
<th>2010</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-15</td>
<td>800</td>
<td>700</td>
</tr>
<tr>
<td>16-29</td>
<td>1000</td>
<td>1100</td>
</tr>
<tr>
<td>30-44</td>
<td>1200</td>
<td>1300</td>
</tr>
<tr>
<td>45-59</td>
<td>1400</td>
<td>1500</td>
</tr>
<tr>
<td>60-74</td>
<td>1600</td>
<td>1700</td>
</tr>
<tr>
<td>75 &amp; Over</td>
<td>1800</td>
<td>1900</td>
</tr>
</tbody>
</table>

**Life Expectancy**

UK life expectancy at birth is 78 years for males and 82 years for females *(57)*. Old age is however associated with health problems including chronic diseases such as hypertension, diabetes mellitus, coronary artery disease, stroke, dementia and chronic obstructive pulmonary disease (COPD) with chronic disease being closely linked to a reduced quality of life *(58)*.
While life expectancy is defined as the expected number of years of life remaining at a given age, healthy life expectancy (HLE) is defined as the period of life one can expect to spend in good health and is currently calculated by the recording of self-reported overall general health status. Health expectancies can be measured in several ways, including life expectancy in good health, free from disability, or free from a specific disease. While data suggests a rise in both life expectancy and general HLE between 1981 and 2001 there has been a slight drop in the reported % of life spent in good health with women reporting more chronic ill health and disability than men (59). (See Table 2.)

Table 2: Trends in life expectancy and healthy life expectancy at birth, 1981 to 2001

(source www.statistics.gov.uk)

<table>
<thead>
<tr>
<th>Year</th>
<th>Women</th>
<th></th>
<th>Men</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Life expectancy (years)</td>
<td>76.8</td>
<td>80.4</td>
<td>70.9</td>
<td>75.7</td>
</tr>
<tr>
<td>General HLE (years)</td>
<td>66.7</td>
<td>68.8</td>
<td>64.4</td>
<td>67.0</td>
</tr>
<tr>
<td>% life in Good or fairly good health</td>
<td>86.9%</td>
<td>85.6%</td>
<td>90.0%</td>
<td>88.5%</td>
</tr>
</tbody>
</table>

HLE: - Healthy Life Expectancy

Observational data show that while for both male and females’ life expectancy at birth for the UK as a whole continues to rise and the gap between males and females is also closing, men and women in Scotland have some of the lowest life expectancy at birth in the European Union (EU). For males, life expectancy at birth is almost one year lower than the EU average and, for females; it is almost two years lower. For
both sexes, life expectancy is about four years lower than those countries with the highest life expectancy.

HLE without disability is also lower in the UK compared to the average within the EU (see figure 6. UK figures from 2005 show while men aged 65 can expect to live another 10 years without disability they should expect to spend their last 7 years with one. Women at 65 years are likely to live a further 12 years without followed by 9 years with a disability. This constitutes a total of 43% of their remaining life span at 65 years spent with some form of disability.

Figure 6: Expected healthy life years remaining for people aged 65 throughout European countries in 2005 (60).
Mortality

In previous centuries death was frequently the result of outbreaks or epidemics of infectious diseases. Improved sanitary conditions, better nutrition, antibiotics and immunisations have now left the developed countries of the world with new epidemics to deal with.

In Scotland mortality rates from the big three killers - cancer, coronary heart disease and stroke - account for more than 50% of deaths recorded in Scotland, with 15% being attributed to coronary heart disease and 9% to stroke in 2010. The past two decades however have seen these rates steadily decline (see Figure 7) partly as a result of health education, lifestyle improvements and improved health care interventions. In 2005, 60% of deaths were of people aged 75 and over, and a further 20% occurred in those who were between the ages of 65 and 74. The relative stability of the number of deaths over recent years masks significant improvements in age-specific mortality. The consequence of this is that there are ever more older people surviving with chronic cardiovascular diseases.
1.4 Cardiovascular Disease in Older adults

For many people old age is associated with ill health. Although death rates have been falling, cardiovascular disease remains the biggest cause of death in the UK accounting for 1 in 3 of all recorded deaths and currently costing the UK economy around £30 billion per year. Given that chronic disease reflects a lifetime exposure to damaging risk factors, most chronic conditions will manifest themselves in later life and are as a result of multiple disease processes.

Mortality rates in Scotland from cardiovascular disease are among the highest in the world. It is the main cause of death in the UK annually accounting for 191,000 or one in three of all deaths and, while there has been a dramatic reduction in death rates, its prevention remains high on the Scottish Government’s agenda. Once an
individual reaches the age of 65 their risk of developing cardiovascular disease increases, and cardiac-related disease accounts for around 40% of deaths in the 65 to 74 year age group (63). In 2009 of the 180,000 recorded deaths only 25,000 were under 75 years. Not only is it a major cause of mortality but it is also a major contributor to the decline in physical function in old age (64).

Current knowledge about the management of cardiovascular disease, especially in relation to drug treatments, has in the main been taken from studies carried out in younger populations (65), despite the aforementioned physiological differences and the increased likelihood of co-morbid disease. Lifestyle factors such as diet and physical activity are important contributors to the prevention and treatment of cardiovascular diseases, and there is evidence to support improvements with disease following short periods of intervention (e.g. stopping smoking and taking exercise). While much nutritional research has focused on fat and salt intake, there is growing interest in the role of micronutrients in the prevention of chronic disease.

In early life arteries are elastic and flexible, which facilitates optimal cardiac function. With advancing age however a gradual thickening of the arteries’ muscle layer (the tunica media) along with an increase in the deposition of collagen fibres and elastin becomes apparent, while other substances involved in inflammatory and atherosclerotic reactions are also more plentiful in older arteries (66). Arterial stiffness increases primarily due to a breakdown of the elastin fibrous structure of the arterial walls but also due to an increase in mean arterial pressure.
Vascular calcification is common in the older population as well as in those suffering from chronic kidney disease, diabetes and aortic stenosis. Studies using animals have shown that it is possible to reverse calcification of the arteries but two human studies using intensive lipid-lowering therapy have not proved successful in preventing the progression of coronary calcification (67;68).

**Hypertension**

Cardiovascular diseases are the leading cause of death worldwide, with high blood pressure causing 7 million deaths each year (69). Blood pressure is recorded as systolic/diastolic blood pressure measured in millimetres of mercury (mmHg). Raised blood pressure is diagnosed when either the systolic pressure exceeds 140mmHg or the diastolic exceeds 90mmHg with hypertension being the recording of raised blood pressure on three subsequent occasions (70). In Scotland one third of all the adult population has high blood pressure the prevalence of which increases with age such that more than two thirds of the Scottish population aged over 75 have high blood pressure (71;72).

Hypertension is known to accelerate artherosclerosis, increasing the risk of vascular disease, and may result in coronary heart disease, left ventricular hypertrophy, transient ischaemic attack and peripheral vascular disease (PVD) (73) with the Framington study demonstrating the link between hypertension and cardiovascular disease (CVD) as well as hypertension and left ventricular mass (74). Furthermore, in older patients there is a link between arterial stiffness and high blood pressure.
especially in those with ischemic heart disease. These patients are therefore an ideal group to target for testing the effect of vitamin K on artery function.

**Diabetes Mellitus**

Diabetes mellitus is a metabolic disorder characterised by chronic hyperglycaemia resulting from defects in insulin secretion and glucose disposal. Recent figures indicate that one person in every 25 in Scotland has been diagnosed with the condition, and that level is increasing at a rate of 4% per year (75).

Diabetes mellitus and CVD share several characteristics. Both increase in incidence with age, are associated with lifestyle factors, and both may be reduced if common risk factors are reduced (76). Diabetes has been identified as a risk factor for CVD, with coronary heart disease the most common vascular complication among people with diabetes, resulting in an increased risk of cardiovascular morbidity and mortality. The Framingham Study found that the incidence of CVD among diabetic men was double that found in non-diabetic men, while in the female population cardiovascular disease was found to be three times more common in those with a diagnosis of diabetes (77).

Diabetes is also closely linked to other risk factors for CVD; hypertension (78) and dyslipidaemia (79) are significantly more prevalent in this population, with the risk of stroke within the first 5 years of diagnosis found to be double that of the general population in a population-based cohort study (80). Medial calcification is also found
to be more prevalent in those with diabetes mellitus thus this group of patients are an ideal group to target for testing the effect of vitamin K.

**Established Vascular Disease**

For those with established vascular disease such as myocardial infarction, stroke or peripheral vascular disease, the risk of having a second event is higher than in those groups without such history.

Those with established vascular disease are more likely to have impaired endothelial function and stiff arteries, and are therefore potential targets for improvement with an intervention.

Thus vascular disease is a common, important contributor to disease in old age. There now exists a body of evidence to suggest that vitamin K may be able to modify vascular disease, but to test this hypothesis, randomised controlled trials are needed.
2. METHODS

2.1 Study Aims

The aims of the study were:

1. To determine whether supplementation with vitamin K improves markers of vascular health in older people with existing vascular disease.

2. To determine whether vitamin K supplementation improves physical function in older people with existing vascular disease.

2.2 Study Design

The study was a parallel group, double blind, placebo controlled, randomised trial.

The study was approved by Tayside Committee on Medical Research Ethics (ref number 11/009) on 12th September 2011 and was funded by Chest, Heart & Stroke Scotland (reference R11/A137).

2.3 Sample Size

The primary outcome was a change in flow-mediated dilatation of the brachial artery between baseline and 6 months. In anticipation of a 10% dropout rate (based on previous trials \((81;82)\)) a final sample size of 80 (40 per arm) would give 80% power at alpha = 0.05 to detect a 2% absolute change in FMD (given a standard deviation of change of 3% as seen in previous studies \((81)\)).
2.4 Study Population

Community dwelling people aged 70 and over with a history of hypertension, diabetes or previously diagnosed vascular disease (see Table 3 for inclusion and exclusion criteria) were recruited from the community via the Scottish Primary Care Research Network (SPCRN). This group of patients has a high risk of recurrent vascular events and therefore would have most to gain from any effective intervention.

Exclusion Criteria

A minimal exclusion criterion for the study was set to ensure the presence of as wide a range of older people with vascular disease as possible. Given that warfarin inhibits vitamin K epoxide reductase those prescribed warfarin or those with a diagnosis of atrial fibrillation and therefore potentially requiring warfarin treatment in the future, were not included in the study population or indeed those who stated that they already supplemented their diet with vitamin K supplements.

Anyone unable to give informed consent or unable to walk without physical assistance, and thus unable to participate in the physical functioning assessment, were also excluded.
# Table 3. Inclusion and exclusion criteria

## Inclusion criteria
- Age 70 and over with at least one of the following diagnosis:
  - Hypertension (based on recorded diagnosis from primary or secondary care)
  - Diabetes mellitus (based on recorded diagnosis from primary or secondary care)
  - Established Vascular disease – one or more of:
    1. Myocardial infarction (based on symptoms of ischaemia or ECG changes, plus rise in cardiac enzymes)
    2. Percutaneous transluminal coronary angioplasty
    3. Coronary artery bypass grafting
    4. Stroke/TIA (based on a recorded diagnosis established in secondary care and recorded in the medical case notes)
    5. Peripheral vascular disease (symptoms of peripheral ischaemia and either a previous ankle/brachial pressure index <0.7 OR
    6. Previous evidence of arterial stenosis on angiography or ultrasound)

## Exclusion criteria
- Atrial fibrillation
- Taking Warfarin
- Taking Vitamin K supplements
- Unable to give written informed consent
- Unable to walk without human assistance (walking aids are permitted)
- Participation in another study
2.5 Investigator Training

The investigator (RF) undertook a period of training prior to recruitment of participants to the study. Good Clinical Practice training was undertaken at the Clinical Research Centre (see appendix 1) with all other training was carried out within the department of medicine at Ninewells Hospital and encompassed both instruction in the vascular assessments and the physical function tests.

Training began with repeated demonstration of the techniques by an experienced practitioner, which gave the investigator the opportunity to observe formally the techniques being carried out successfully. All training manuals and standard operating procedures were read until RF had a full and clear understanding of the procedures.

In order to assess reproducibility, Flow Mediated Dilation (FMD), carotid intima-media thickness and pulse wave velocity (PVW) were carried out on 20 individuals initially under close supervision before being repeated at least 3 times on each volunteer. For FMD measurements are taken pre and post forearm artery occlusion with the increase expressed as percentage change from baseline. An absolute percentage change of 2% is considered to be clinically significant therefore an increase of less than 2% was considered satisfactory to demonstrate reproducibility.

Regular meetings with the experienced practitioner where held to allow results to be studied. Tables (4&5) below show FMD and PWV results on the final ten healthy
volunteers and demonstrate that techniques where being reproduced to an acceptable level and recruitment of participants could begin.

Table 4. Training records for FMD healthy volunteers

<table>
<thead>
<tr>
<th>Subject</th>
<th>Reading 1</th>
<th>Reading 2</th>
<th>Difference %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Min 4.21</td>
<td>Min 4.22</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Max 4.53</td>
<td>Max 4.56</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% Increase 7.57</td>
<td>% Increase 8.03</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Min 3.72</td>
<td>Min 3.42</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>Max 4.6</td>
<td>Max 3.69</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% Increase 7.04</td>
<td>% Increase 7.88</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Min 3.77</td>
<td>Min 3.85</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Max 3.97</td>
<td>Max 4.07</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% Increase 5.50</td>
<td>% Increase 6.02</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Min 3.0</td>
<td>Min 2.59</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>Max 3.13</td>
<td>Max 2.73</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% Increase 4.33</td>
<td>% Increase 5.16</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Min 3.98</td>
<td>Min 3.99</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>Max 4.32</td>
<td>Max 4.30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% Increase 8.52</td>
<td>% Increase 7.57</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Min 4.68</td>
<td>Min 4.60</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>Max 4.91</td>
<td>Max 4.81</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% Increase 4.91</td>
<td>% Increase 4.57</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Min 3.89</td>
<td>Min 3.81</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>Max 4.25</td>
<td>Max 4.16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% Increase 9.2</td>
<td>% Increase 9.1</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Min 5.46</td>
<td>Min 5.10</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>Max 5.60</td>
<td>Max 5.22</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% Increase 2.6</td>
<td>% Increase 2.4</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Min 4.06</td>
<td>Min 4.48</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>Max 4.27</td>
<td>Max 4.67</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% Increase 5.2</td>
<td>% Increase 4.3</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Min 4.55</td>
<td>Min 4.18</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>Max 4.88</td>
<td>Max 4.53</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% Increase 6.8</td>
<td>% Increase 7.7</td>
<td></td>
</tr>
</tbody>
</table>
Table 5. Training records PVW healthy volunteers

| Subject | Reading 1 | | Reading 2 | | Difference |
|---|---|---|---|---|
| 1 | PVW 6.9 ± 0.3 | Axl 7% | PVW 7.3 ± 0.2 | Axl 6% | 0.4 |
| 2 | PVW 9.3 ± 0.6 | Axl 13% | PVW 10.6 ± 0.9 | Axl 13% | 1.3 |
| 3 | PVW 8.6 ± 0.6 | Axl 24% | PVW 7.9 ± 0.7 | Axl 25% | 0.7 |
| 4 | PVW 8.7 ± 0.5 | Axl 9% | PVW 8.9 ± 0.6 | Axl 10% | 0.2 |
| 5 | PVW 7.5 ± 0.5 | Axl 19% | PVW 7.3 ± 0.6 | Axl 19% | 0.2 |
| 6 | PVW 8.4 ± 0.4 | Axl 35% | PVW 8.0 ± 0.5 | Axl 35% | 0.4 |
| 7 | PVW 8.1 ± 0.6 | Axl 34% | PVW 8.5 ± 0.7 | Axl 36% | 0.4 |
| 8 | PVW 8.0 ± 0.4 | Axl 12% | PVW 7.6 ± 0.3 | Axl 15% | 0.4 |
| 9 | PVW 8.0 ± 0.4 | Axl 12% | PVW 7.2 ± 0.5 | Axl 11% | 0.8 |
| 10 | PVW 8.1 ± 0.4 | Axl 31% | PVW 8.5 ± 0.6 | Axl 33% | 0.2 |

2.6 Participant Selection and Enrolment

Recruitment

Participants were recruited through SPCRN, previously known as the Scottish Practices and Professionals Involved in Research (SPPIRe). The network, which was established in 2002, aims to act as a framework to co-ordinate national research activity in primary care. It is funded by the Chief Scientist Office of the Scottish Government and is operationally managed at a regional level by four nodes based in the North, East, South East and West of Scotland.
Participants were recruited using the East of Scotland node of the SPCRN. GP practices who are members of the network were sent information regarding the study and two practices who agreed to be involved in the research project were visited by a SPCRN research officer. Using the practice’s patient computer database system and the study inclusion / exclusion criteria the research officer produced a list of participants who were potentially eligible for the study. The list was then reviewed by one of the practice GP’s to exclude any patients who they deemed unsuitable to enter the study because, for example, they had recently been diagnosed with a serious illness, or had been recently bereaved.

An invitation letter along with a Participant Information Sheet (PIS) (Appendices 2 and 3), reply slip (appendix 4) and a stamped addressed envelope were sent from the GP to the patients who met the study eligibility criteria. After reading the PIS only those individuals who wished to know more about the study were asked to respond by returning the reply slip.

Reply slips were collected by the SPCRN team and forwarded to members of the study team. Respondents were contacted by telephone by Roberta Fulton (RF). During this initial contact a brief outline was given of the purpose of the study and details of what involvement in the study would entail. Eligibility was also checked and potential participants were encouraged to ask questions at any point during the phone call.
**Informed Consent**

*Verbal consent*

All those who agreed to participate in the study and attend the screening / baseline visit gave verbal consent during the initial telephone contact for RF to review their hospital notes to further assess eligibility and document past medical history.

*Written Consent*

Based on the principles of the Declaration of Helsinki, written informed consent was obtained from each participant when they attended their screening / baseline visit (see appendix 5 for consent form)

**Randomisation**

Randomisation of study medication was carried out by Tayside Pharmaceuticals, an MHRA licensed manufacturing unit at Ninewells Hospital. Staff at Tayside Pharmaceuticals prepared and retained a computer-generated randomisation list randomised on a 1:1 ratio (active: placebo) with no stratification. Four blocks of 20 participants were randomised to ensure equal allocation across both arms of the trial, even in the event of suboptimal recruitment.

On entering the study each participant was allocated a sequential number and was dispensed the study medication bearing the same number, each bottle containing medication sufficient to last for 6 months.
Each participant was dispensed an identical looking bottle informing them that the bottle contained either vitamin K or placebo medication ensuring that the participant remained blind to the study medication. Randomisation codes were held by the pharmacy and not by the researchers themselves; however the codes were held in a sealed enveloped in the trial master file (TMF) in case emergency unblinding was required out with pharmacy times.

Researchers also remained blind to the treatment allocation which was only broken once all the data analysis was complete.

2.7 Medication

Manufacture

Study medication was manufactured by NattoPhama ASA (Høvik, Norway) and encapsulated by Legosan AB, Kumla, Sweden as vitamin K$_2$ using bacillus subtilis natto, a Japanese dish made of fermented soybeans which is currently the richest known source of natural vitamin K$_2$\(^{(83)}\). Participants were randomised to receive one capsule per day of either vitamin K$_2$ (MK7 subtype) 100mcg or placebo, with both intervention and placebo medications being over capsulated to give an identical appearance. All study medication was issued at the end of the baseline visit with instructions to take one tablet per day. Participants were instructed to contact RF with any questions concerning the medication during their time in the study.

A letter was sent to each participant’s general practitioner notifying the doctor that their patient had enrolled in the study and was therefore possibly taking vitamin K
(see appendix 6), something which they should be conscious of if they were to consider warfarin therapy over the course of the subsequent six months, as vitamin K antagonises the effect of warfarin \(^{(84)}\).

**Medication Adherence**

Medication adherence, defined by the WHO as ‘the extent to which a patient follows medical instructions’, was assessed by a tablet count at 12 weeks and 6 months following the participant’s baseline visit. The number of remaining tablets was recorded in the case report form (see appendix 7) as was the total number of days each participant had been enrolled in the study. Adherence was calculated as the proportion of tablets actually taken over the 6 month period divided by the amount they should have taken. An intake of 80% or more of the initial tablets supplied at baseline was recorded as medication adherent.

Measurements of serum vitamin K levels were also recorded at baseline and at the 6 month study visits as a further check that medication was being ingested as opposed to merely being removed from the bottle.

All unused medication was recorded on the medication accountability log before being returned to the clinical trials pharmacist at Tayside Pharmaceuticals for safe disposal.
2.8 Outcome Measurement

Screening Assessment

All visits for the study were carried out in study rooms at Tayside Institute for Cardiac Research, level 7 Ninewells Hospital. Taxi transport was provided if required. Assessment of suitability for study entry was reviewed at the screening / baseline visit. Inclusion / exclusion criteria were re-examined, and during the initial part of the screening / baseline visit a 12-lead electrocardiogram (ECG) was carried out. Any patient displaying a recording of atrial fibrillation (AF), a heart condition that causes an irregular and often abnormally fast heart rate, on the ECG was then excluded from further participation. A copy of the ECG was forwarded on to the GP for antithrombotic prophylaxis consideration as people with AF have a five-fold increase in risk of stroke and previous trials have established sound evidence to support warfarin treatment in high risk groups\(^{(85)}\). It is well documented that vitamin K alters the metabolism of warfarin\(^{(86)}\), hence patients in AF were excluded from the trial. The procedure for recording the ECG was carried out as described by Jevon\(^{(87)}\). Any person with AF on their ECG was gently informed that this is a relatively common condition affecting around 20% of the population over the age of 80 years\(^{(88)}\) which can be managed effectively, and that their GP would be informed of the findings for further consideration.

Following consent those participants eligible for inclusion continued with the baseline visit as follows:
Baseline Assessment

- Past medical history including dates of diagnosis (if known) and whether the condition was currently an on-going problem.
- Concomitant medications were recorded
- Demographic information was documented - specifically:
  (i) Current living status (own home, sheltered housing, residential or nursing home resident or living with relatives)
  (ii) Living alone at home and current home help
  (iii) Smoking history; current smoking habit
  (iv) Use of walking aids (none, 1 or 2 sticks, zimmer frame or triwheel walker).

Lying and standing blood pressure was recorded using an Omron digital blood pressure monitor model HEM-400C as per The British Hypertension Society/European Society for Hypertension guidelines (89). Participants were asked to lie in a supine position for 5 minutes prior to the blood pressure being recorded three times then asked to stand in an upright position while recordings were taken again at 30 seconds, 2 minutes and 3 minutes to test for orthostatic hypotension.

Participants were asked if they had experienced any feelings of dizziness or light-headedness during the procedure and these were also recorded in the Case Report Form (CRF). Lying and standing blood pressure recordings were documented in the CRF, then mean lying blood pressure was also calculated and recorded in the CRF.
A falls history was also taken at this time at each of the three study visits, with the number of falls occurring within the previous three month period documented along with the circumstances surrounding each event.

**Primary Outcome Measure**

The primary outcome for the trial was between group differences in endothelial function at 6 months as assessed using the technique of Flow Mediated Dilation (FMD) of the brachial artery in response to 5 minutes of forearm occlusion.

The endothelium is the largest organ in the body located between the wall of blood vessels and the blood stream. Stimuli such as pressure, shear stress, or hormonal stimuli, induce the release substances which regulate vasomotor function, activate inflammatory processes, and affect haemostasis (90).

Endothelial dysfunction was first described in human hypertension in the forearm vasculature in 1990 (91) and encompasses various abnormalities such as reduced vasodilation, altered inflammatory and anticoagulant properties as well as abnormal vascular growth remodelling. (92) It has been demonstrated to be present in patients with diabetes (93-94); hypertension (95); coronary artery disease (96); and peripheral vascular disease (97) and is associated with ageing (98).

The ability of the blood vessels to dilate in response to an increase in blood flow has been named FMD and is largely mediated by nitric oxide (NO). This increase in blood flow can be produced by triggering reactive hyperaemia (RH), that is, by
temporarily occluding an artery and then restoring the flow of blood. When blood flow through an arterial section is occluded, hypoxia causes vasodilation of downstream resistance vessels followed by an increase in blood flow upon release of the occlusion. This increase of blood flow increases the shear stress exerted on the endothelium initiating release of NO and the relaxation of vascular smooth muscle cells. The result is conduit vessel dilation. RH therefore produces a compensatory increase in blood flow inducing the release of NO from the endothelium and thus, when this technique is carried out under controlled conditions, the impact of NO bioavailability can be measured\(^{(99)}\). The size of the blood vessel, participants’ age and gender, as well therapeutic interventions are also known to influence FMD\(^{(100)}\) as are ambient temperature and food ingestion\(^{(101)}\).

FMD of the brachial artery as devised by Celermajer et al\(^{(102)}\) is a non-invasive, reproducible technique used to assess endothelial function and has been identified as a marker for future cardiovascular events in patients with diabetes mellitus\(^{(103)}\); essential hypertension\(^{(104)}\); chest pain\(^{(105)}\); peripheral vascular disease\(^{(106)}\) and chronic heart failure\(^{(107)}\). With a usual diameter of 2.5 to 5mm the brachial artery is the preferred site of measurement as arteries with smaller diameter are difficult to image accurately and reproducibly and very small changes in absolute diameter are consequently reported as large percentage changes.

Participants were asked to fast, and avoid caffeine and tobacco for 6 hours prior to the test. They were studied in a quiet, temperature-controlled room (\(23^\circ C\)) and asked to lie flat on the bed with 1 pillow under their head. The right arm was extended onto
the clamp table. Three ECG leads were placed on the chest and a sphygmomanometer cuff placed around right forearm just below the antecubital fossa (see Figure 8), as placement of the cuff more distally around the wrist has previously been shown to result in a smaller reactive hyperaemia response and lower FMD. A 2D image of the brachial artery was recorded in end-diastole, identified by the onset of the R-wave for 2 minutes just above the antecubital fossa in the longitudinal plane using an Acuson sequoia 512 ultrasound machine with an 8L5 Transducer.

**Figure 8: Positioning for measurement of brachial artery flow mediated dilation**

![Image of brachial artery measurement](image)

The cuff was then inflated using a Hokanson rapid cuff inflator (Hokanson Inc., Bellview, WA. USA) to 200mmHg or 50 mmHg more than the recorded systolic blood pressure (whichever level was higher for 5 minutes and then deflated. Previous studies have examined duration times for cuff occlusion\(^{108}\) concluding
that more than 5 min occlusion duration has been shown to prolong the duration of hyperaemia and evoke a non-NO-mediated response. A further 2 minute recording of the artery was taken and FMD calculated as the percentage change in the brachial artery diameter during hyperaemia compared to the baseline measurement using semi–automated analysis software (Brachial Tools edge detection software, Medical Imaging Applications, Iowa City, USA).

**Secondary Outcome Measures**

*Measures of Vascular Function*

Carotid intima-media thickness (IMT) of the common carotid artery has been identified as an indicator for cardiovascular disease. An increase in IMT is the earliest measurable morphological alteration of the arterial wall (109). It has been recognised as being a highly accurate, easily reproducible test, correlating well with pathophysiologic measurements (110). Hashimoto et al demonstrated direct correlation between FMD of the brachial artery and carotid IMT in men (111), while in a study of 963 participants carotid IMT was associated with atherosclerotic risk factors, increasing with the number of concurrent risk factors even after stratification for age (112).

Carotid B-mode ultrasonography enables measurement of the thickness of tunica intima and tunica media, the innermost two layers of the arterial wall. An increased cross-sectional intima-media thickness has been associated with increased risk of stroke and myocardial infarction (113,114) as well as lower extremity atherosclerosis (115). B-mode scanning of one of the carotid arteries was performed using Siemens
Acuson Sequoia 512 using an 8 MHz transducer. The participant was asked to lie in a supine position with the neck extended and the chin turned away from the side which was being examined. The participant’s carotid artery was identified initially with the transducer in a transverse plane and then studied longitudinally. The common carotid artery was examined with three measurements of the IMT being taken at a point on the far wall of the artery just below the carotid bulb (see figure 9). Although vessels at different locations throughout the circulatory system may be used to assess IMT the carotid arteries are deemed most suitable due to their position, size and limited movement with the common carotid artery positioned parallel to the skin surface.\(^{116}\)

Different techniques in the measurement of IMT currently exist with researchers in previous studies taking different approaches and carrying out measurements at different points of the carotid artery. One approach is to take multiple measurements within the three main segments of the artery – the common carotid, bifurcation and the internal carotid artery - measuring both the near and far away walls. The maximal measurements for each section are recorded and an average is taken. This approach, although widely used, is problematic when areas of the internal carotid artery have been difficult to visualise and studies using this method tend to have larger amounts of missing data.\(^{116}\) Alternatively other researchers have recorded the common carotid artery near and far wall.
Common carotid IMT was measured approximately 10mm proximal to the carotid bulb on each of the three study visits. Three measurements over a 1cm section were taken and the average calculated and recorded in the CRF.

Arterial stiffness can be increased by three key mechanisms: firstly there is a breakdown of elastin within the arterial walls, secondly damage occurs to the endothelium and thirdly there is an increase in mean arterial pressure. Several methods of measuring arterial stiffness currently exist –

*Pulse pressure* - Pulse pressure is the difference between the peak systolic blood pressure (SBP) and peak diastolic blood pressure (DBP). It is dependent on cardiac output pressure and can be easily measured using a standard sphygmomanometer. Although elevated pulse pressure has been associated with cardiovascular disease (118) both SBP and DBP usually increase with age until around the age of 60 years when there is usually no further increase in DBP and in many cases it actually declines widening the pulse pressure. Pulse pressure alone therefore is inadequate to assess arterial stiffness accurately. The ‘normal’ amplification of the pressure wave as it travels from the aorta to the periphery becomes less pronounced with increasing
age thus measurements of pulse pressure made in the periphery do not always accurately reflect the actual central pulse pressure. In fact differences of up to 20mmHg between central pressures of patients with identical brachial blood pressure readings have been recorded. Central pressure may, therefore, be a more accurate predictor of risk than peripheral blood pressure. A number of studies have shown that pulse pressure is an important predictor of risk. The Framingham study in the US demonstrated that in hypertensive patients over the age of 50 years, the pulse pressure was a better predictor of coronary heart disease risk than either systolic or diastolic pressure alone \(^{(119)}\).

*Pulse Wave Analysis (PWA)* is a method of measuring aortic pressure wave. It was first established back in the late 1800s by Frederick Akbar Mohamed who identified the difference between the carotid and radial waveform using the sphygmograph \(^{(120)}\). Following the invention of the sphygmomanometer however the recording of diastolic blood pressure became more widely used as the primary indicator of cardiovascular risk \(^{(121)}\) with studies such as the Framingham study clearly demonstrating the link between blood pressure and cardiovascular disease. \(^{(74)}\). It can be argued however that while BP has been shown to be a significant factor associated with the risk of cardiovascular disease the brachial artery routinely used to measure BP may not be as affected by atherosclerosis and thus the measurement of the ascending aortic pressure may be a better predictor \(^{(122)}\).

PWA and Pulse Wave Velocity (PWV), two techniques of recording changes in the central aorta which have arisen from the loss of arterial elasticity can be assessed easily using the Sphygmograph.
The systolic pulse wave in the central aorta is amplified by the reflection of blood from arterial bifurcation points, primarily that of the distal aorta itself. A perfectly elastic aorta will absorb the entire pulse wave produced by ventricular contraction however a completely stiff and rigid tube will reflect a large proportion of the wave. The pulse pressure waveform differs in different blood vessels in the same individual, and is reliant on several things; the viscoelastic properties of the artery; the viscosity of the blood; the wave reflection and the wave dispersion.

Wave reflection leads to amplification of the aortic pressure wave. This wave travels from the left ventricle to the periphery where it reaches vessels of greater impedance that act as a mirror, reflecting it back to the aorta. Thus the resulting pressure in the ascending aorta is the sum of the incident and reflected wave and is dependent on the heart rate, the speed of the pulse wave and the amplitude of the reflected pulse wave (see figure 10).

**Figure 10: Diagram of a pulse wave taken from** (123)
For a given heart rate, the time of arrival of the reflected wave depends on the PWV, which is in turn determined by how stiff the vasculature is. If the reflected wave arrives early in the cardiac cycle it combines with the incident wave giving a greater ascending aortic pressure against which the left ventricle has to pump; however, if it arrives later in the cardiac cycle it increases the ascending aortic pressure in diastole, leading to improved coronary circulation. The Augmentation Index (Alx), because of its correlation with PWV - which has been shown to be dependent on arterial stiffness - has been proposed as a marker for arterial stiffness.

PWV is calculated by measuring the time it takes for the arterial waveform to pass between sites (usually the carotid and radial, or carotid and femoral) two points which are a measured distance apart, and involves taking the readings simultaneously, or by gating separate recordings to a fixed point in the cardiac cycle, usually the R wave of the ECG.

The relationship between how stiff arteries are and the speed of the pulse wave was first predicted by Thomas Young in 1808 and is described as a mathematical equation - The Moens-Korteweg equation or $\text{PWV} = \sqrt{\frac{Eh}{2\rho R}}$. PWV is calculated from measurements of pulse transit time and the distance travelled by the pulse between two recording sites: $\text{PWV} = \frac{\text{Distance (metres)}}{\text{Transit Time (seconds)}}$. Various different methods are available, both invasive and non-invasive, but the development of modern tonometer systems has enabled the measurement of accurate pressure waveforms without the need for invasive catheter-based measurements.
The SphygmoCor system used in this study has a transfer function which can be used to calculate central pressure from the radial pulse waveform, using a hand-held tonometer. The SphygmoCor can also be used at other superficial arterial sites including the carotid and femoral arteries, and by ECG-gating, the time for transmission of the arterial pulse wave between sites (PWV) is calculated.

The SphygmoCor (version 7.1) is a recently-developed computerized, portable and simple-to-use device validated to assess pulse waveforms and assess arterial stiffness (124). Aortic pulse waveform, Alx, and central aortic pressure were derived at the radial artery by applanation tonometry. For this study the radial and carotid artery sites were used mainly due to ease of access.

The participant was asked to lie in a relaxed, comfortable position with their arm extended across a flat hard surface, palm of the hand facing upward. The radial and carotid artery pressure waves and amplitude were recorded non-invasively with a pencil-type probe (at the base of neck for the common carotid artery and over the right radial artery).

The probe incorporates a transducer at the tip, which has a small pressure-sensitive ceramic sensor area with a frequency response of >2 kHz that is coplanar with a longer area (7mm diameter) of flat surface in contact with the skin overlying the arterial pulse. The probe's technology is based on the principle of applanation tonometry, as used in ocular tonometry for the assessment of intraocular pressure. The probe was held on the skin over the maximal arterial pulsation by hand and
pressed down on the artery against the underlying bone (Figure 11). Recordings were taken when a reproducible signal was obtained with high amplitude excursion (usually 10 consecutive beats to cover a complete respiratory cycle are needed for subsequent analysis).

A quality control mechanism for both PVW and Alx is built into the SphygmoCor system and this was used to ensure that all data recorded was of an adequate quality for analysis. All PVW data had a SD of less than 10% of the velocity with the system highlighting clearly if the reading had not been of sufficient quality. The Alx was calculated by a computer algorithm derived from invasive pressure and flow data, and recorded as a percentage. Three successful readings where recorded for the Alx with the median value recorded in the CRF

Both Alx and PWV were recorded. It has been found in previous studies that these two measures of arterial stiffness correlate significantly with each other.

**Figure 11: Diagram of applanation tonometry process being taken.**
Research has shown that PWV is a more reliable measure of arterial stiffness in the older population than Alx. The recording of PWV is a valid and reproducible and relatively simple procedure which can be learned fairly easily. Outcome data has shown PWV to be an independent predictor of cardiovascular risk in both hypertensive patients and patients with end-stage renal disease\(^\text{(125,126)}\).

**Measures of Vascular Prognosis**

**B-type Natriuretic Peptide (BNP)**

A 10ml sample of venous blood was taken in a purple vacutainer containing potassium EDTA during both the baseline visit and the 6 month follow-up visit. On each occasion the sample was placed on ice and then centrifuged at 3000rpm at 4°C for 10 minutes. The plasma was removed from the sample and then placed in a 5ml sample bottle which was stored in a -70°C freezer until all 80 participants had completed the study at which point all samples were assayed. All 80 baselines and follow-up visit samples were assayed using the Bachem radio immune assay kit (Bachem, Peninsula Laboratories, Inc, US).

Atrial Natriuretic peptide (ANP) and Brain-type Natriuretic Peptide (BNP) belong to a family of peptide hormones and are both secreted in the cardiac ventricles in response to volume expansion and pressure overload.

The main actions of ANP and BNP include natriuresis, diuresis, and inhibition of the renin-angiotensin-aldosterone system and thereby play an important role in regulating blood pressure and blood volume\(^\text{(127)}\).
ANP is secreted as a direct response to atrial stretch while BNP is produced in the left ventricle as a direct response to elevated left ventricular pressure (128). Elevated BNP levels have been used to identify the presence of heart failure as well as a range of cardiovascular diseases such as atrial fibrillation and valvular disease in an elderly population (129), and are found to be elevated with the presence of essential hypertension (130).

A study of elderly functionally impaired adults with multiple comorbidity found elevated BNP to be an independent predictor of all cause and cardiovascular mortality, more significant than blood pressure, diabetes or age, and predictive of death in those in older functionally impaired patients with multiple comorbid disease with or without previous cardiovascular history (131).

*C-reactive protein (CRP)*

A 3ml sample of venous blood from each participant was collected in a gold vacutainer at baseline and six month follow up. The sample was then kept for a minimum of ten minutes before being centrifuged at 3000rpm for ten minutes at 4°C. Serum was extracted from the centrifuged sample, placed in a 1ml sample tube and transferred to a -80°C freezer and stored until the last participant had completed the study.

C-reactive protein (CRP) is a 224-residue protein which is found circulating in the blood where its levels are found to increase in response to inflammation, trauma and
infection only to decrease just as quickly with the resolution of the condition \(^{(128)}\). The measurement of CRP is widely used to monitor various inflammatory states such as Rheumatoid Arthritis \(^{(129)}\) and used as part of a wider assessment along with clinical and laboratory information to manage acute bacterial infections \(^{(132)}\). More recently research has shown that inflammatory processes have an active role to play in atherogenesis \(^{(133)}\).

CRP has been found to be a baseline predictor of future cardiovascular events in apparently healthy men \(^{(134)}\) while individuals with elevated levels of CRP have a risk 2 to 3 times higher than the risk of those with low levels. The American Heart Association \(^{(135)}\) classified levels obtained using “high sensitivity” CRP assays as follows: less than 1 mg/L is desirable and reflects a low overall cardiovascular risk.; levels between 1 and 3 mg/L are indicative of moderate risk, while levels of CRP in excess of 3 mg/L suggest elevated vascular risk.

CRP, which can be measured inexpensively with high sensitivity assays, has been shown to identify increased risk of future cardiovascular events in stroke \(^{(136)}\) and PVD \(^{(137)}\) as well as being associated with incident events in the elderly, especially in those with subclinical disease at baseline \(^{(138)}\).

In a study looking at the use of statins in the prevention of coronary events, CRP was an independent predictor of cardiovascular disease with statins being effective in reducing levels of CRP even in participants with relatively low lipid levels. \(^{(139)}\)
Ridker et al showed that CRP was in fact a better predictor of cardiovascular risk that low-density lipoprotein (LDL) cholesterol\textsuperscript{(140)}.

All samples were analysed in Professor Allan Struthers’ laboratory in Ninewells Hospital by Ms. Lesley McFarlane using a microplate-based EIA (ELISA) double antibody sandwich assay for C-reactive protein from Kalon Biological Ltd.

**Serum Cholesterol**

Total cholesterol, HDL cholesterol and LDL cholesterol were collected at baseline and 6 months. Patients were fasted for a minimum of 5 hours prior to collection of a 3ml sample collected in gold vacutainer. Following collection blood samples were delivered to the Biochemistry Department at Ninewells Hospital, Dundee for analysis reports returning within 48 hours to RF.

**Serum MK7 Level**

Fasting vitamin K1 reference values in healthy adults range from 0.15 to 1.0 μg/L (median 0.5 μg/L)\textsuperscript{(141)}. A 3ml sample of venous blood from each participant was obtained as for CRP collection and stored in the -80°C freezer until the last participant had completed the study.

All samples were analysed within the department laboratory by Dr Sandy Hill using high-performance liquid chromatography, using cetyl naphthoate\textsuperscript{(140)}, synthesised in house, as internal standard and based on a previously validated method for assay in human serum\textsuperscript{(141)}.  


Measures of Physical Function

Physical function along with exercise capacity decline with age and are a major source of disability in older people \(^{(142)}\). Testing physical function has been shown to be an accurate indicator of current health status, be predictive of future health and disability, and may be used to predict the likelihood of health and social care use in the future \(^{(143)}\). Function may be assessed by either self-reported questionnaires or by tests of physical performance.

Short Physical Performance Battery (SPPB)

Participants were asked to participate in a short physical function test, which was repeated at 6 months. The three-part test was explained fully and commenced only after the participant had had the opportunity to rest for 5 minutes. Each part of the test was demonstrated by the researcher and scores were obtained by the participant for each of the three parts: balance testing; gait speed testing and chair speed testing and entered into the CRF under the relevant section (see appendix 8 for SPPB scoring sheet and protocol.) The test focuses on lower limb function using tasks that mimic daily activities and includes a timed 4 metre walk to measure gait speed; one chair stand followed by 5 timed chair stands, if the first is successfully completed; and finally balance stands with the feet held in 3 different positions for 10 seconds each. These areas represent essential tasks important for independent living and are thus an important outcome measure for patients with cardiovascular disease. Any part of the test either not attempted or not completed by the participant automatically
scored a zero and was entered into the CRF while standardised encouragement was offered at various points during the test.

Standardised physical performance tests have been increasingly used in ageing research and have been found to be positively associated with health status as well as being predictive of outcomes such as hip fracture, nursing home admission and death\(^{(144)}\). Evidence also exists that these tests are suitable for use in non-disabled adults\(^{(145)}\) as well as being able to identify those who are at increased risk for the onset of functional dependence\(^{(146,147)}\).

The SPPB is an objective assessment tool for evaluating lower extremity functioning in older persons and has been designed to measure physical performance and decline over time. It was developed by the National Institute on Aging and is freely available for use without permission or the payment of royalty fees. Performance tests have increasingly been associated with measures of health status and the SPPB has indeed been found to be predictive of long term disability and of future institutionalisation\(^{(148)}\). A 4 year prospective cohort study of older, non-disabled older adults found that those with the poorest lower extremity performance at baseline spent significantly more days in hospital (17.7 v 9.7 days) when compared to those who had recorded a high performance even after adjustment for baseline chronic conditions\(^{(149)}\). In a recent study comparing the predictive value for mortality of several different performance measures, the SPPB score emerged as the strongest predictor of mortality in elderly community dwelling participants with the chair stand subtask showing highest predictive value\(^{(150)}\). The test is easily administered, takes around 10 minutes to complete and can be easily reproduced.
Hand Grip Dynamometry

Handgrip dynamometry is a simple test used to directly measure muscle strength. All participants' handgrip strength was assessed at both baseline and the 6 month follow-up visit using the T.K.K 5001 Grip – A hand held dynamometer (Takei Scientific Instruments Company Ltd, Japan). Each participant was asked to identify their dominant hand before taking the dynamometer with the non-dominant one. The grip width was adjusted so that the second joint of the pointing finger made a right angle when using the instrument. In a standing immobilised position, in order to minimise the contribution to the measured force from muscle groups in other areas of the body, with the arm hanging by their side, the participant was asked to squeeze as tightly as possible and then return the dynamometer back to the researcher who recorded the reading given in kilograms. This procedure was repeated a total of three times with the highest of the three readings recorded in the CRF for use in analysis.

Hand grip strength has been found to correlate with strength of other muscle groups and is thus a good indicator of overall strength (151). A study among healthy 45 to 68 year old men, found it to be highly predictive of functional limitations and disability 25 years later and concluded that good muscle strength in midlife may protect people from old age disability (152).

Rantanen et al examined the association between muscle strength and total and cause-specific mortality in older disabled women concluding that hand grip strength was indeed a powerful predictor of cause-specific and total mortality (153) while
Cheung et al demonstrated that handgrip strength was associated with multimorbidity, and that handgrip strength may be a more useful marker of this than chronological age in men. Furthermore, they also demonstrated that handgrip strength was associated with different chronic diseases\(^{(154)}\), while in a recent population-based cohort study based in Sweden of one million men, reduced handgrip strength was associated with increased risk of incident coronary heart disease and all strokes\(^{(155)}\).

**Follow-up visits**

Participants returned at 3 months for assessment of outcomes (FMD; PWV and Carotid IMT); lying and standing BP were also recorded. (Fig 12). Medication adherence was assessed and recorded as was any change to life circumstance or medical history. The 6 month follow-up visit repeated the assessments of handgrip strength; SPPB; serum cholesterol; BNP and CRP in addition to those carried out during the 3 month visit.
**Figure 12: Schedule of study visits**

**Screening – baseline visit (0 month)**

- ECG, consent, demographics, past medical history, concomitant medication,
- lying & standing BPs & falls history,

Baseline outcomes measured: FMD, PWV, Carotid IMT, hand grip strength,
SPPB, blood samples for CRP & BNP

**Follow-up visit - (3 month)**

Outcome measures – FMD, PVW, Carotid IMT
- Lying & standing BP & Falls history
- Medication adherence

**Follow-up visit - (6 month)**

Baseline outcomes measured: FMD, PWV, Carotid IMT, hand grip strength, SPPB,
- blood samples for CRP & BNP
- Lying & standing BP & Falls history
- Medication adherence
2.9 Data Handling

Data Entry and Management

The researcher recorded data for each participant on the CRF during each study visit. Other data such as log sheets for study medication, adverse events, serious adverse events and specimen storage were also recorded and kept securely within the department of Ageing and Health, University of Dundee.

All data were entered into an Excel spread sheet by the researcher at regular intervals during the study in preparation for data analysis. Missing data for each time point was excluded from the initial analysis of outcome measures.

Data Analysis

All data were analysed using SPSS statistical package (Version 21.0). Analyses of the primary and secondary outcomes were performed prior to breaking the treatment codes. A 2-sided p value of < 0.05 was taken to be significant for all analyses.

Differences between treatment groups for outcome measures were assessed. Baseline data were compared between groups using an independent samples t-test for all normally distributed data, and Mann Whitney test for non-normally distributed data. Categorical variables were compared at baseline using Pearson’s Chi-squared test.

All analyses of treatment effect were conducted according to the principles of intention to treat analysis. For each outcome, change scores (follow-up minus baseline) were calculated for each follow-up time point, Change scores were tested for normal distribution, and compared between groups for each follow-up time point using analysis of covariance (ANCOVA) models. Unadjusted analyses were
calculated, and adjusted analyses were performed, adjusting for a) age, b) baseline value of the outcome variable under test, and c) (for vascular outcomes only) systolic blood pressure.
3. RESULTS

3.1 Recruitment

Participants were recruited from 2 GP practices using the East of Scotland node of the SPCRN. Recruitment took place between January 1\textsuperscript{st} 2012 and September 30\textsuperscript{th} 2012.

Two GP practices approached by the SPCRN agreed to participate with a search of their databases identifying 991 potential participants. All those identified as potentially suitable were contacted by letter and of the 991 letters sent 143 (14\%) responded indicating their interest in the study. Practice A obtained 74/541 (14\%) positive replies while invitations sent to patients from Practice B resulted in 69/450 (15\%) positive replies. Recruitment was divided equally between the two practices.

A total of 97 people were contacted by telephone and screened further to assess eligibility; the remaining respondents were not contacted as the recruitment target had been reached, and these respondents were thanked for their interest. Of the 97 people, 8 felt unable to participate due to ill health or family commitments while 4 people were deemed ineligible following the telephone discussion and a more detailed check of their medical notes. One individual was found to already be enrolled in an on-going hypertension study.
In total 84 potential participants were invited to attend the detailed screening / baseline visit at Ninewells Hospital. One person was unsuitable to participate due to the presence of atrial fibrillation on their ECG recording, while 3 people agreed to take part but failed to attend the screening visit (see Figure 13 for CONSORT diagram and participant flow through the study). Thus 80 people were recruited and randomised.

**Figure 13: CONSORT diagram showing participant flow through trial**
3.2 Baseline Characteristics

Of the 80 participants who were randomised to the trial, 55% (44/80) of these were male with the participants’ mean age being 77 (SD 5) years (Table 4). More than a quarter were aged 80 years or over. Over a third lived alone and only 10% (8/80) required any formal help at home. Eighteen percent (14/80) required the use of a walking aid to mobilise, while 13% (10/80) of participants smoked despite their medical history.

Ninety percent (72/80) of all participants had been given a diagnosis of hypertension with 93% (74/80) currently on active antihypertensive treatment. Despite this, blood pressure control was suboptimal, with the mean supine blood pressure 153/82 mmHg at the baseline visit. Table 6 shows baseline characteristics of all 80 randomised participants.

Participants were generally well matched between the two groups with a few notable exceptions. Of the participants in the vitamin K supplemented group, 18/40 (45%) lived alone, significantly more than in the placebo group 10/40 (25%). The vitamin K group were on significantly more medication than the placebo group, and PWV was significantly higher in the placebo group than the vitamin K group.

Dropout during the six month follow-up period was very low; 77/80 (96%) of participants completed the final follow-up visit.
Table 6: Baseline characteristics of randomised participants

<table>
<thead>
<tr>
<th></th>
<th>Vitamin K (n=40)</th>
<th>Placebo (n=40)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Age (years) (SD)</td>
<td>76.0 (4.4)</td>
<td>77.1 (4.8)</td>
<td>0.31</td>
</tr>
<tr>
<td>Male sex (%)</td>
<td>21 (53)</td>
<td>23 (58)</td>
<td>0.37</td>
</tr>
<tr>
<td>Mean Systolic Blood Pressure (mmHg)</td>
<td>144 (17)</td>
<td>148 (20)</td>
<td>0.33</td>
</tr>
<tr>
<td>Mean Diastolic Blood Pressure (mmHg)</td>
<td>81 (11)</td>
<td>83 (10)</td>
<td>0.39</td>
</tr>
<tr>
<td><strong>Past Medical history</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>36 (90)</td>
<td>36 (90)</td>
<td>1.00</td>
</tr>
<tr>
<td>Previous stroke / TIA (%)</td>
<td>8 (20)</td>
<td>9 (23)</td>
<td>0.79</td>
</tr>
<tr>
<td>Previous Myocardial Infarction (%)</td>
<td>7 (18)</td>
<td>8 (20)</td>
<td>0.78</td>
</tr>
<tr>
<td>Angina (%)</td>
<td>11 (28)</td>
<td>7 (18)</td>
<td>0.30</td>
</tr>
<tr>
<td>CABG/PTCA (%)</td>
<td>7 (18)</td>
<td>5 (13)</td>
<td>0.76</td>
</tr>
<tr>
<td>Diabetes Mellitus (%)</td>
<td>9 (23)</td>
<td>9 (23)</td>
<td>1.00</td>
</tr>
<tr>
<td>Peripheral Vascular Disease (%)</td>
<td>5 (13)</td>
<td>3 (8)</td>
<td>0.46</td>
</tr>
<tr>
<td>Osteoarthritis (%)</td>
<td>16 (40)</td>
<td>9 (23)</td>
<td>0.15</td>
</tr>
<tr>
<td><strong>Social history</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Walking aid (%)</td>
<td>10 (25)</td>
<td>4 (10)</td>
<td>0.24</td>
</tr>
<tr>
<td>Lived alone (%)</td>
<td>18 (45)</td>
<td>10 (25)</td>
<td><strong>0.006</strong></td>
</tr>
<tr>
<td>Home help (%)</td>
<td>4 (10)</td>
<td>4 (10)</td>
<td>1.0</td>
</tr>
<tr>
<td>Current smoker (%)</td>
<td>3 (8)</td>
<td>7 (18)</td>
<td>0.26</td>
</tr>
</tbody>
</table>
### Medications

<table>
<thead>
<tr>
<th></th>
<th>%</th>
<th>%</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>On Antiplatelet medications (%)</td>
<td>25 (63)</td>
<td>18 (45)</td>
<td>0.12</td>
</tr>
<tr>
<td>On ACEi / ARB (%)</td>
<td>30 (75)</td>
<td>25 (63)</td>
<td>0.23</td>
</tr>
<tr>
<td>On Beta blocker (%)</td>
<td>8 (20)</td>
<td>10 (25)</td>
<td>0.59</td>
</tr>
<tr>
<td>On Calcium Channel Blockers (%)</td>
<td>20 (50)</td>
<td>14 (35)</td>
<td>0.18</td>
</tr>
<tr>
<td>On other antianginals (%)</td>
<td>7 (18)</td>
<td>3 (8)</td>
<td>0.81</td>
</tr>
<tr>
<td>On statins (%)</td>
<td>26 (65)</td>
<td>27 (68)</td>
<td>0.31</td>
</tr>
<tr>
<td>Median total no. of medications (IQR)*</td>
<td>8 (5)</td>
<td>5 (4)</td>
<td><strong>&lt;0.001</strong></td>
</tr>
</tbody>
</table>

### Baseline Outcome measures

<table>
<thead>
<tr>
<th>Outcome Measure</th>
<th>Mean (SD)</th>
<th>Median (IQR)*</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Flow Mediated Dilatation (%) (SD)</td>
<td>6.3 (2.7)</td>
<td>7.3 (2.4)</td>
<td>0.24</td>
</tr>
<tr>
<td>Mean Pulse Wave Velocity (m/s) (SD)</td>
<td>9.7 (2.1)</td>
<td>10.7 (2.3)</td>
<td><strong>0.05</strong></td>
</tr>
<tr>
<td>Mean Carotid IMT (cm) (SD)</td>
<td>0.077 (0.015)</td>
<td>0.080 (0.021)</td>
<td>0.50</td>
</tr>
<tr>
<td>Median Grip Strength kgs (IQR)*</td>
<td>25.8 (13.0)</td>
<td>27.3 (13.3)</td>
<td>0.20</td>
</tr>
<tr>
<td>Median SPPB score (IQR)*</td>
<td>8.8 (2.4)</td>
<td>9.3 (2.4)</td>
<td>0.25</td>
</tr>
</tbody>
</table>

### Blood Results

<table>
<thead>
<tr>
<th>Outcome Measure</th>
<th>Mean (SD)</th>
<th>Median (IQR)*</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean serum Sodium (mmol/L) (SD)</td>
<td>140 (3)</td>
<td>140 (2)</td>
<td>0.97</td>
</tr>
<tr>
<td>Mean serum Creatinine (umol/L) (SD)</td>
<td>79 (22)</td>
<td>83 (18)</td>
<td>0.16</td>
</tr>
<tr>
<td>Median Urea (mmol/L) (IQR) *</td>
<td>6.4 (3.1)</td>
<td>6.9 (2.7)</td>
<td>0.59</td>
</tr>
<tr>
<td>Median BNP level (pg/ml) (IQR)*</td>
<td>33 (29)</td>
<td>33 (22)</td>
<td>0.42</td>
</tr>
<tr>
<td>Median CRP level (mg/L) (IQR)*</td>
<td>1.8 (2.1)</td>
<td>1.8 (2.1)</td>
<td>0.34</td>
</tr>
<tr>
<td>Mean Total Cholesterol (mmol/L) (SD)</td>
<td>4.7 (1.1)</td>
<td>4.7 (1.1)</td>
<td>0.99</td>
</tr>
<tr>
<td>Median Vitamin K2 levels (pg/ml) (IQR)</td>
<td>476 (301)</td>
<td>502 (242)</td>
<td>0.43</td>
</tr>
</tbody>
</table>
CABG - coronary artery bypass graft;
PTCA - percutaneous transluminal coronary angioplasty;
ACEi – angiotensin-converting-enzyme inhibitor;
ARB - angiotensin receptor blocker;
IMT – intima-media thickness;
SPPB - short physical performance battery;
BNP - B-type natriuretic peptide;
CRP – C-reactive protein

* Data analysed using Mann-Whitney tests for non-parametric tests

### 3.3 Outcome Measures

#### Primary outcome

The primary outcome measure of change in mean FMD is given in Table 7. No significant treatment effect was seen for between-group change at either 3 months or 6 months; adjustment for baseline FMD, systolic blood pressure and age did not affect the outcome.
Table 7. Effect of Intervention on Flow Mediated Dilatation of the Brachial Artery

<table>
<thead>
<tr>
<th>Time</th>
<th>Vitamin K (%) (SD)</th>
<th>Placebo (%) (SD)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline visit</td>
<td>6.3 (2.7)</td>
<td>7.3 (2.4)</td>
<td>0.24</td>
</tr>
<tr>
<td>3 month visit</td>
<td>7.1 (2.5)</td>
<td>7.0 (3.0)</td>
<td>0.90</td>
</tr>
<tr>
<td>6 month visit</td>
<td>7.6 (2.7)</td>
<td>8.6 (2.4)</td>
<td>0.79</td>
</tr>
<tr>
<td>Treatment Effect (0 v 3 months) (%) (95% CI)</td>
<td>0.9 (-0.7 to 2.5)</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>Adjusted Treatment Effect * (0 v 3 months) (%) (95% CI)</td>
<td>0.4 (-0.9 to 1.8)</td>
<td>0.51</td>
<td></td>
</tr>
<tr>
<td>Treatment Effect (0 v 6 months) (%)</td>
<td>0.1 (-1.0 to 1.2)</td>
<td>0.87</td>
<td></td>
</tr>
<tr>
<td>Adjusted Treatment Effect * (0 v 6 months) (%) (95% CI)</td>
<td>-0.3 (-1.3 to 0.8)</td>
<td>0.62</td>
<td></td>
</tr>
</tbody>
</table>

*adjusted for baseline flow-mediated dilatation, age and systolic blood pressure

Participants at each visit: baseline n= 78; 3 month visit n= 73; 6 month visit n= 74

Secondary outcomes

Vascular Markers

No significant treatment effect was noted for carotid IMT (Table 8) and this also did not change after adjustment for baseline values. Pulse wave velocity (Table 9) and augmentation index (Table 10) also did not show any significant change with treatment, pulse wave velocity, although by six months, pulse wave velocity had fallen by 0.8 m/s in the vitamin K group relative to placebo after adjustment for...
baseline values. This was despite a significant baseline difference between the groups, with the vitamin K group starting with a lower baseline value (9.7 vs. 10.7 m/s)

Table 8. Effect of intervention on Carotid Intima-Media Thickness

<table>
<thead>
<tr>
<th>Time</th>
<th>Vitamin K</th>
<th>Placebo</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline visit (cm) (SD)</td>
<td>0.07 (0.015)</td>
<td>0.08 (0.021)</td>
<td>0.50</td>
</tr>
<tr>
<td>3 month visit (cm) (SD)</td>
<td>0.078 (0.013)</td>
<td>0.078 (0.010)</td>
<td>0.80</td>
</tr>
<tr>
<td>6 month visit (cm) (SD)</td>
<td>0.076 (0.020)</td>
<td>0.080 (0.010)</td>
<td>0.36</td>
</tr>
<tr>
<td>Treatment Effect (0 v 3 months) (cm) (95% CI)</td>
<td>0.004 (-0.003 to 0.011)</td>
<td>0.23</td>
<td></td>
</tr>
<tr>
<td>Adjusted Treatment Effect* (0 v 3 months) (cm) (95% CI)</td>
<td>0.003 (-0.002 to 0.007)</td>
<td>0.21</td>
<td></td>
</tr>
<tr>
<td>Treatment Effect (0 v 6 months) (cm) (95% CI)</td>
<td>0.001 (-0.008 to 0.009)</td>
<td>0.89</td>
<td></td>
</tr>
<tr>
<td>Adjusted Treatment Effect* (0 v 6 months) (cm) (95% CI)</td>
<td>0.003 (-0.002 to 0.007)</td>
<td>0.78</td>
<td></td>
</tr>
</tbody>
</table>

*adjusted for baseline carotid intima-media thickness and baseline age

Participants at each visit: baseline n= 79; 3 month visit n= 77; 6 month visit n= 76
### Table 9. Effect of Intervention on Pulse Wave Velocity

<table>
<thead>
<tr>
<th>Time</th>
<th>Vitamin K</th>
<th>Placebo</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline visit (m/s) (SD)</td>
<td>9.7 (2.1)</td>
<td>10.7 (2.3)</td>
<td>0.05</td>
</tr>
<tr>
<td>Visit 2 (m/s) (SD)</td>
<td>10.0 (3.1)</td>
<td>10.2 (1.7)</td>
<td>0.71</td>
</tr>
<tr>
<td>Visit 3 (m/s) (SD)</td>
<td>9.9 (1.4)</td>
<td>10.9 (3.0)</td>
<td>0.08</td>
</tr>
<tr>
<td>Treatment Effect (0 v 3 months) (m/s) (95% CI)</td>
<td>0.6 (-0.9 to 2.0)</td>
<td>0.44</td>
<td></td>
</tr>
<tr>
<td>Adjusted Treatment Effect* (0 v 3 months) (m/s) (95% CI)</td>
<td>-0.1 (-1.4 to 1.3)</td>
<td>0.91</td>
<td></td>
</tr>
<tr>
<td>Treatment Effect (0 v 6 months) (m/s) (95% CI)</td>
<td>-0.3 (-1.5 to 0.8)</td>
<td>0.58</td>
<td></td>
</tr>
<tr>
<td>Adjusted Treatment Effect* (0 to 6 months) (m/s) (95% CI)</td>
<td>-0.8 (-1.8 to 0.3)</td>
<td>0.15</td>
<td></td>
</tr>
</tbody>
</table>

*adjusted for baseline pulse wave velocity, systolic blood pressure and age

Participants at each visit: baseline n= 76; 3 month visit n= 73; 6 month visit n=76
Table 10. Effect of Intervention on Augmentation Index

<table>
<thead>
<tr>
<th>Time</th>
<th>Vitamin K</th>
<th>Placebo</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline visit (%) (SD)</td>
<td>28 (10)</td>
<td>28 (7)</td>
<td>0.92</td>
</tr>
<tr>
<td>3 month (%) (SD)</td>
<td>26 (9)</td>
<td>28 (6)</td>
<td>0.47</td>
</tr>
<tr>
<td>6 month (%) (SD)</td>
<td>25 (8)</td>
<td>27 (6)</td>
<td>0.54</td>
</tr>
<tr>
<td>Treatment Effect (0 v 3 months) (%) (95% CI)</td>
<td>-1 (-5 to 3)</td>
<td>0.55</td>
<td></td>
</tr>
<tr>
<td>Adjusted Treatment Effect* (0 v 3 months) (%) (95% CI)</td>
<td>-1 (-5 to 2)</td>
<td>0.46</td>
<td></td>
</tr>
<tr>
<td>Treatment Effect (0 v 6 months) (%) (95% CI)</td>
<td>-1 (-5 to 3)</td>
<td>0.54</td>
<td></td>
</tr>
<tr>
<td>Adjusted Treatment Effect* (0 v 6 months) (%) (95% CI)</td>
<td>-2 (-5 to 2)</td>
<td>0.32</td>
<td></td>
</tr>
</tbody>
</table>

*adjusted for baseline augmentation index, age and systolic blood pressure

Participants at each visit: baseline n= 80; 3 month visit n= 76; 6 month visit n=75

There was no significant change in either systolic or diastolic blood pressure in either group (Table 11). Mean systolic blood pressure (SBP) fell over the six month follow up period in both groups, but fell by a greater amount in the placebo group.
Table 11. Effect of Intervention on Blood Pressure

<table>
<thead>
<tr>
<th></th>
<th>Vitamin K</th>
<th>Placebo</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Systolic blood pressure</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline visit (mmHg) (SD)</td>
<td>144 (17)</td>
<td>148 (20)</td>
<td>0.33</td>
</tr>
<tr>
<td>3 month visit (mmHg) (SD)</td>
<td>141 (16)</td>
<td>144 (17)</td>
<td>0.36</td>
</tr>
<tr>
<td>6 month visit (mmHg) (SD)</td>
<td>140 (17)</td>
<td>140 (18)</td>
<td>0.86</td>
</tr>
<tr>
<td>Treatment Effect (0 v 3 months) (mmHg) (95% CI)</td>
<td>0 (-8 to 9)</td>
<td></td>
<td>0.91</td>
</tr>
<tr>
<td>Adjusted Treatment Effect* (0 v 3 months) (mmHg) (95% CI)</td>
<td>-1 (-8 to 6)</td>
<td></td>
<td>0.72</td>
</tr>
<tr>
<td>Treatment Effect (0 v 6 months) (mmHg) (95% CI)</td>
<td>3 (-5 to 11)</td>
<td></td>
<td>0.53</td>
</tr>
<tr>
<td>Adjusted Treatment Effect* (0 v 6 months) (mmHg) (95% CI)</td>
<td>1 (-6 to 8)</td>
<td></td>
<td>0.75</td>
</tr>
<tr>
<td><strong>Diastolic blood pressure</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline visit (mmHg) (SD)</td>
<td>81 (11)</td>
<td>83 (10)</td>
<td>0.39</td>
</tr>
<tr>
<td>3 month visit (mmHg) (SD)</td>
<td>82 (9)</td>
<td>82 (9)</td>
<td>0.98</td>
</tr>
<tr>
<td>6 month visit (mmHg) (SD)</td>
<td>80 (9)</td>
<td>80 (9)</td>
<td>0.98</td>
</tr>
<tr>
<td>Treatment Effect (0 v 3 months) (mmHg) (95% CI)</td>
<td>2 (-2 to 6)</td>
<td></td>
<td>0.38</td>
</tr>
<tr>
<td>Adjusted Treatment Effect* (0 v 3 months) (mmHg) (95% CI)</td>
<td>1 (-3 to 4)</td>
<td></td>
<td>0.65</td>
</tr>
<tr>
<td>Treatment Effect (0 v 6 months) (mmHg) (95% CI)</td>
<td>2 (-2 to 6)</td>
<td></td>
<td>0.39</td>
</tr>
<tr>
<td>Adjusted Treatment Effect* (0 v 6 months) (mmHg) (95% CI)</td>
<td>1 (-3 to 5)</td>
<td></td>
<td>0.65</td>
</tr>
</tbody>
</table>

*adjusted for baseline blood pressure and age
Measures of Physical Function

No change was noted in handgrip strength between baseline and follow-up with a measure of treatment effect giving a non-significant result (Table 12). The maximum possible score on the SPPB is 12. Although these data were skewed at each timepoint, change scores between timepoints were normally distributed, and thus mean values for change are presented in Table 13.

Table 12. Effect of intervention on Grip Strength

<table>
<thead>
<tr>
<th>Time</th>
<th>Vitamin K (Kg) (SD)</th>
<th>Placebo (Kg) (SD)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline visit (Kg) (SD)</td>
<td>26.2 (9.3)</td>
<td>29.1 (9.0)</td>
<td>0.16</td>
</tr>
<tr>
<td>6 month (Kg) (SD)</td>
<td>26.7 (9.8)</td>
<td>29.0 (8.8)</td>
<td>0.29</td>
</tr>
<tr>
<td>Treatment Effect (0 v 6 months) (Kg) (95% CI)</td>
<td>0.1 (-1.0 to 1.2)</td>
<td></td>
<td>0.83</td>
</tr>
<tr>
<td>Adjusted Treatment Effect* (0 v 6 months) (Kg) (95% CI)</td>
<td>-0.1 (-1.0 to 1.2)</td>
<td></td>
<td>0.87</td>
</tr>
</tbody>
</table>

*adjusted for baseline grip strength and age

Participants at each visit: baseline n= 80; 6 month visit n= 77
Table 13. Effect of Intervention on Short Physical Performance Battery Scores

<table>
<thead>
<tr>
<th>Time</th>
<th>Vitamin K</th>
<th>Placebo</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median score baseline visit (IQR)</td>
<td>9 (4)</td>
<td>10 (3)</td>
<td>0.25</td>
</tr>
<tr>
<td>Median score 6 month visit (IQR)</td>
<td>9 (3)</td>
<td>10 (4)</td>
<td>0.21</td>
</tr>
<tr>
<td>Mean treatment Effect (0 v 6 months) (95% CI)</td>
<td>-0.3 (-1.0 to 0.3)</td>
<td>0.29</td>
<td></td>
</tr>
<tr>
<td>Adjusted mean treatment Effect* (0 v 6 months)(95% CI)</td>
<td>-0.5 (-1.1 to 0.2)</td>
<td>0.14</td>
<td></td>
</tr>
</tbody>
</table>

Participants at each visit: baseline n= 80; 6 month visit n= 77

Blood Tests

B-type Natriuretic Peptide (BNP) and C-reactive protein (CRP) levels

Similarly, data at baseline and 6 month follow-up were not normally distributed for BNP and CRP thus between group comparisons were performed using Mann Whitney tests. Change scores were however normally distributed, thus mean values are given and ANOVA was used to compare change scores between groups. Results are given in Tables 14 and 15; no significant between-group differences were seen even after adjustment for baseline values.
### Table 14. Effect of intervention on B-type Natriuretic Peptide levels

<table>
<thead>
<tr>
<th>Time</th>
<th>Vitamin K</th>
<th>Placebo</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median baseline visit (pg/ml) (IQR)</td>
<td>33 (29)</td>
<td>33 (22)</td>
<td>0.83</td>
</tr>
<tr>
<td>Median 6 month visit (pg/ml) (IQR)</td>
<td>40 (24)</td>
<td>37 (29)</td>
<td>0.30</td>
</tr>
<tr>
<td>Mean treatment effect (0 v 6 months) (pg/ml) (95%) CI</td>
<td>8 (-8 to 23)</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td>Adjusted mean treatment effect* (0 v 6 months) (pg/ml) (95% CI)</td>
<td>8 (-8 to 24)</td>
<td>0.31</td>
<td></td>
</tr>
</tbody>
</table>

*adjusted for baseline B-type natriuretic peptide level, age and systolic blood pressure

### Table 15. Effect of intervention on C-reactive protein levels

<table>
<thead>
<tr>
<th>Time</th>
<th>Vitamin K</th>
<th>Placebo</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median Baseline visit (mg/L) (IQR)</td>
<td>1.8 (3.3)</td>
<td>1.4 (2.1)</td>
<td>0.76</td>
</tr>
<tr>
<td>Median 6 month visit (mg/L) (IQR)</td>
<td>1.8 (3.5)</td>
<td>1.8 (2.6)</td>
<td>0.82</td>
</tr>
<tr>
<td>Mean treatment effect (0 v 6 months) (mg/L) (95% CI)</td>
<td>1.8 (-4.7 to 8.3)</td>
<td>0.58</td>
<td></td>
</tr>
<tr>
<td>Adjusted mean treatment Effect* (0 v 6 months) (mg/L) (95% CI)</td>
<td>-0.1 (-3.8 to 3.5)</td>
<td>0.94</td>
<td></td>
</tr>
</tbody>
</table>

*adjusted for baseline C-reactive protein and age
Serum cholesterol

Baseline total cholesterol level was identical between the two groups at 4.7 mmol/L (SD 1.1). High cholesterol was a documented past medical history in a high percentage of participants with 26/40 (65%) of the vitamin K group and 27/40 (68%) of the placebo group prescribed statin therapy at baseline. Table 16 shows no significant difference in either total cholesterol, HDL cholesterol or LDL cholesterol between either group at either baseline or six months.

Serum vitamin K

Vitamin K2 levels rose in the intervention arm compared to placebo (+48 pg/ml vs -6 pg/ml, p=0.03) at six months compared to baseline, confirming that the study intervention successfully raised circulating vitamin K2 levels.
Table 16: Effect of intervention on Serum Cholesterol

<table>
<thead>
<tr>
<th>Time</th>
<th>Vitamin K</th>
<th>Placebo</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total cholesterol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline visit (mmol/L) (SD)</td>
<td>4.7 (1.1)</td>
<td>4.7 (1.1)</td>
<td>0.99</td>
</tr>
<tr>
<td>6 month visit (mmol/L) (SD)</td>
<td>4.6 (1.1)</td>
<td>4.5 (1.1)</td>
<td>0.73</td>
</tr>
<tr>
<td>Treatment Effect (0 v 6 months) (mmol/L) (95% CI)</td>
<td>0.0 (-0.3 to 0.3)</td>
<td></td>
<td>0.96</td>
</tr>
<tr>
<td>Adjusted Treatment Effect* (0 v 6 months) (mmol/L) (95% CI)</td>
<td>0.0 (-0.2 to 0.3)</td>
<td></td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>HDL cholesterol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline visit (mmol/L) (SD)</td>
<td>1.5 (0.5)</td>
<td>1.5 (0.6)</td>
<td>0.72</td>
</tr>
<tr>
<td>6 month visit (mmol/L) (SD)</td>
<td>1.4 (0.5)</td>
<td>1.4 (0.4)</td>
<td>0.47</td>
</tr>
<tr>
<td>Treatment Effect (0 v 6 months) (mmol/L) (95% CI)</td>
<td>0.0 (-0.1 to 0.1)</td>
<td></td>
<td>0.90</td>
</tr>
<tr>
<td>Adjusted Treatment Effect* (0 v 6 months) (mmol/L) (95% CI)</td>
<td>0.0 (-0.1 to 0.1)</td>
<td></td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td>LDL cholesterol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline visit (mmol/L) (SD)</td>
<td>3.3 (1.0)</td>
<td>3.3 (1.1)</td>
<td>0.89</td>
</tr>
<tr>
<td>6 month visit (mmol/L) (SD)</td>
<td>3.3 (0.9)</td>
<td>3.4 (1.0)</td>
<td>0.81</td>
</tr>
<tr>
<td>Treatment Effect (0 v 6 months) (mmol/L) (95% CI)</td>
<td>-0.1 (-0.4 to 0.3)</td>
<td></td>
<td>0.67</td>
</tr>
<tr>
<td>Adjusted Treatment Effect* (0 v 6 months) (mmol/L) (95% CI)</td>
<td>-0.1 (-0.3 to 0.2)</td>
<td></td>
<td>0.73</td>
</tr>
</tbody>
</table>

HDL: High-density lipoprotein. LDL: Low-density lipoprotein

*adjusted for baseline cholesterol and age
3.4 Major Recorded Adverse Events

Serious Adverse Events

A total of 6 serious adverse events (SAE) i.e. those resulting in hospitalisation or death were recorded, with 4 in the vitamin K group and 2 in the placebo group (see Table 17). One participant died following a myocardial infarction which occurred during participation in an exercise event prior to the final study visit while the five other hospital admissions were also unrelated to study participation.

Table 17 Record of Serious Adverse Events

<table>
<thead>
<tr>
<th>System</th>
<th>Vitamin K</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hospital admissions</td>
<td>Death</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>1 (pulmonary oedema)</td>
<td>1 (following myocardial infarction)</td>
</tr>
<tr>
<td>Musculoskeletal</td>
<td>2 (falls; one fracture)</td>
<td>0</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hospitalisation</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Death</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>
Adverse Events

Table 18 shows the total number of adverse events over the six month study period. More adverse events (42 vs. 35) were recorded by the vitamin K group than placebo, with gastrointestinal disturbance and falls mainly accounting for the excess of events.

There were 17 falls recorded in the vitamin K group, two of which required hospitalisation, and one of which had sustained a fractured wrist. Logistic regression analysis was carried with variables in the equation: group; walking aid; living alone; baseline grip strength; osteoarthritis; and medication burden at baseline. Risk of having sustaining a fall depending on group was 0.421 (95% CI 0.108 to 1.638) p=0.21 indicating that group selection did not contribute to a higher risk of falls.
Table 18. Adverse Events

<table>
<thead>
<tr>
<th>System</th>
<th>Vitamin K</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiovascular</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>(cellulitis, varicose ulcers, leg cramps &amp; poor circulation)</td>
<td>(TIA not admitted to hospital, lower limb circulatory problems)</td>
<td></td>
</tr>
<tr>
<td>Pain</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>(knee, back &amp; shoulder)</td>
<td></td>
<td>(leg, abdominal pain)</td>
</tr>
<tr>
<td>Gastro-Intestinal</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>disturbances</td>
<td></td>
<td>(weight loss, Oral thrush, gastric ulcer, dry mouth)</td>
</tr>
<tr>
<td>(nausea/vomiting, swallowing difficulties, dry throat, abnormal liver function results)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Postural symptoms</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>(Labyrinthitis &amp; dizzy spells)</td>
<td></td>
<td>(dizzy spells)</td>
</tr>
<tr>
<td>Falls / musculoskeletal</td>
<td>17</td>
<td>7</td>
</tr>
<tr>
<td>(not requiring medical attention)</td>
<td></td>
<td>(not requiring medical attention)</td>
</tr>
<tr>
<td>Infections</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>TOTAL Events:</td>
<td>42</td>
<td>27</td>
</tr>
</tbody>
</table>

TIA: transient ischaemic attack. GI: Gastrointestinal
3.5 Adherence to medication

Adherence to medication was assessed by tablet counting at each study visit with >80% of medications taken deemed as ‘adherent’. Of the 78 participants who returned for the three month visit 33/39 (83%) of the vitamin K group and 31/39 (78%) of the placebo were categorised on this basis as ‘adherent’. At the end of the study all study medication containers were returned and tablet counting categorised 35/38 (92%) of the vitamin K group and 37/39 (95%) of the placebo group adherent to medication giving a very good overall adherence of 94%.

Dropout during the six month follow-up period was very low; 77/80 (96%) of participants completed the final follow-up visit.

3.6 Analysis

All results were calculated using only data collected at each time point for each participant with missing values not included. To test if this missing data had had an effect on the results of the vascular outcome measures missing values were imputed using age; sex; baseline SBP, 6 month SBP, baseline FMD and 6 month FMD.

When results of the five sets of imputations were combined the adjusted between group difference in FMD at 6 months was -0.3% (95%CI -1.4 to 0.8), no change from the original results. It is unlikely therefore that had the data been complete it would not have resulted in a change of outcome.
4. DISCUSSION AND CONCLUSION

4.1 Summary of main findings

This randomised controlled trial found that six months of vitamin K supplementation did not improve endothelial function of the brachial artery measured by FMD when compared to placebo administration. In addition, no significant differences were seen in PWV, carotid IMT, BNP, CRP or in physical function as measured by hand grip strength and SPPB between the two groups. Arterial stiffness, as measured by PWV, was non-significantly lower in the vitamin K group at 6 months, after adjustment for baseline values.

Adherence to the study medication was high, and dropout rates were low despite the study group comprising older people with significant multimorbidity.

Missing data was low at <10% however multiple imputation analysis was carried out confirming that missing values did not have an effect on the overall results.

Recruitment and Retention

The study met its aim to recruit 80 older people with a history of vascular disease and achieved excellent retention. Several factors were likely to have contributed to this:

- All participants were recruited via their GP surgeries and it was evident from the initial telephone contact that all were aware of their vascular history and motivated to try an intervention with the potential to reduce their risk of a
vascular event. Most of the participants (90%) had a diagnosis of hypertension, and awareness of this diagnosis may have prompted people to volunteer for a study in which a simple vitamin supplement might reduce their vascular risk.

- The group of patients enrolled in this trial were relatively fit compared to those enrolled in some other trials to improve health in older people (156,157) as evidenced by their superior SPPB and grip strength measurements. They were prepared to travel to and from hospital for outcome visits, something which may have discouraged frailer, less able people from taking part. Another factor that may have improved retention in the study was that the burden of the study visits was low, with a requirement to attend three visits of between 1 and 2 hour duration over a 6 month period with a request to take only one extra medication capsule per day. Given the daily medication burden of the participants (median 5 medications per day) this did not deter the majority of volunteers from entering the study with only eleven choosing not to attend the screening visit following the initial telephone contact.

- Approximately 25% of the older population in the UK take nutritional supplements (158). Several participants acknowledged that they were motivated to participate because the study involved a vitamin rather than a medicinal product with potential of side effects; this may have made the process of recruitment easier. This factor, along with the prospect of having vascular assessments which would not be routinely offered on the NHS was also anecdotally expressed as a positive aspect of participation for many of those who consented.
Baseline Measurements

There were no significant differences at baseline between groups in the primary outcome of FMD or in any of the secondary outcome measurements except for the PWV scores. Measurement of maximum reactive hyperaemia resulted in a 1% difference between the groups at baseline, and although this did not reach statistical significance, this difference was potentially important in interpreting the analyses as calculating the change score between baseline and 6 months can be misinterpreted if correlation between these two scores is low.

Ninety per cent of participants had a diagnosis of hypertension and antihypertensive treatment was widely prescribed. This was reflected in the high mean baseline BP of 145/83 which although higher than the 140/90 target set for younger age groups, was below the 150/90 target set for patients aged 80 years and older (159).

A total of 43/80 (53%) of the participants scored > 10 on the SPPB which has a maximum score of 12, while the median score of 9 (IQR3) would indicate that the participants were located within the minimal limitation range (8 – 10) and are thus among the most independent of this population (160). The vitamin K group were noted to have a lower baseline score than placebo with 15/40 (38%) within the minimal limitations range compared to 21/40 (53%) in placebo. This was reflected in the fact that more of the vitamin group required a walking aid to mobilise and also had a lower recorded grip strength.
4.2 Significance of findings

Primary Outcome

This study found that vitamin K did not improve vascular function in older people with vascular disease as measured by endothelial function. There was no change in FMD of the brachial artery in either 3 or 6 month follow-up outcome measurements. There are several possible explanations for this finding.

I. The sample size was too small. The study was powered to detect a 2% absolute change in FMD given a standard deviation of 3% based on previous studies carried out with diabetic and stroke patients\(^ {81;161}\). The 95% CI for the 0 v 6 months change score analysis had 0.3% as the upper limit. This makes it highly unlikely that a real effect of 2% improvement in FMD would have been missed.

II. Improvements were masked by ceiling effects. The baseline measurements recorded in this study were higher than average seen in those with vascular disease in the Framingham study, and were higher than those previously measured in our laboratory in patients with isolated systolic hypertension\(^ {162}\) and stroke\(^ {81} \) using the same protocol and equipment. However, baseline FMD was considerably lower than that seen in healthy volunteers; a cut off of 10.4% has been suggested by some researchers indicating normal artery function thus it is unlikely that ceiling effects can explain the lack of improvement seen. Furthermore, both groups improved by 1 to 1.5% over the 6 month study period.
III. The dose of vitamin K was insufficient. Participants received 100 µg/L Vitamin K2 (MK7 subtype) daily. The current RDI for vitamin K is 80 µg/L but this is based purely on the role vitamin K has on the clotting mechanism. Studies examining the relationship between vitamin K and CHD have used Food Frequency Questionnaires to identify daily intake however this study supplemented the diet \(^{(163)}\). Despite the evidence linking vitamin K to vascular health it is not yet clear if it can reverse existing vascular damage or existing calcification and if so in what the optimum dosage to achieve this would be. This study administered the current recommended dose to a population regarded as having an intake well below the recommended daily intake \(^{(164)}\) and which is currently available to buy over the counter. No upper limit for vitamin K has been set and evidence pertaining to vitamin K\(_1\) has indicated that 1mg or more may be required to maximally carboxylate Osteocalcin and Matrix Gla Protein \(^{(165)}\). Thus potentially higher doses might need to be administered in order to achieve an effect as seen in post-menopausal women recruited to the ECKO study who received 5 mg of vitamin K\(_1\) \(^{(166)}\) where circulating blood vitamin K levels were ten times higher in the intervention group compared to placebo.

IV. The 6 month study duration was too short. A 6 month study duration may not have been sufficiently long to detect changes in the vascular markers selected. Calcification of blood vessels is a process which has developed over many years and intervention with vitamin K may require to be delivered over a much longer time period to alter this as shown in previous studies. FMD has shown improvement in a few weeks for several treatments such as vitamin D
and allopurinol (167). However if effects of vitamin K are not on endothelial function but solely on calcification this would fit with the results found in this study. Invasive methods such as plethysmography (81) have also been used to assess improvement in endothelial function (168;169), however Mullen et al were unable to detect a treatment effect of daily doses of enalapril after 24 weeks and concluded that brachial FMD may have been unable to detect improvement due to the complex nature of vascular disease in diabetes mellitus (170).

V. The target population was wrong. The inclusion criteria were designed to select participants diagnosed with a wide range of vascular disease. The failure of the study to improve vascular function may have been because the wrong cohort was selected. Vascular calcification develops over many years and it may be that intervention is required at a much earlier stage of the disease process i.e. before arthrosclerosis or vascular calcifications are established. This group of participants clearly had a high level of physical function for their age group and their baseline vascular measurements were reasonably good, another explanation is that they may not have had sufficient disease severity to show a significant difference in the study period.

**Secondary Endpoints**

Arterial stiffness and reflectivity: PWV and Alx were used to measure arterial stiffness and reflectivity. The vitamin K group showed a small improvement at six month follow-up while the placebo group deteriorated slightly but neither were significant. The small but gradual improvement noted in PWV over the study period
suggests there may be potential for further improvement if the intervention was to be
given over a longer period of time. This would be true especially if the improvement
was due to a reduction in calcification in the arteries which has taken many years to
develop.

Only a small, non-significant reduction in AIx was seen. However this measure is
shown to have greater validity in younger populations where changes with
interventions have been noted rather than improvements in PWV \(^{(74)}\).

No change was detected in the thickness of the intima and media of the carotid artery
over the duration of the study period. Although previous studies have used changes
in carotid IMT as a surrogate endpoint for determining the success of interventions
such as those that lower the levels of low-density lipoprotein cholesterol \(^{(171)}\) it may
have simply been the wrong tool for this study. It may also be that the 6 month time
point may have been too short for any potential changes to occur. Previous studies
have varied in duration with some taking up to 4.5 years before improvements in
IMT were noted \(^{(172)}\). Atherosclerosis is a process requiring many years to progress
and a six month follow-up may well have been insufficient to detect improvement.
This is especially likely in this study population with a mean baseline measurement
of 0.08cm when values of 0.09± 0.26 have been identified in older people without
vascular disease. Carotid IMT measurement is frequently chosen as an outcome
measure because of its quantitative value and high reproducibility. However, given
that the mean normal change over one year is between 0.010mm and 0.012mm, a 6
month study duration may have been insufficient to show any statistically significant change.

I. Markers of vascular prognosis: As with arterial stiffness and reflectivity none of the markers chosen to assess vascular prognosis changed over the study duration. BNP, blood pressure, total cholesterol, HDL cholesterol, or LDL cholesterol remained unchanged. The median BNP was relatively low in the study population therefore it may have been difficult for any intervention to have further reduced the levels.

II. Markers of inflammation: The results of the CRP blood test followed a similar pattern to the other outcome measures with no significant difference noted. Median baseline measurements of 1.8 (3.3) shown in the vitamin K group indicated that this group of participants were not identifiable as being at particularly high risk and therefore it would have been difficult for the intervention to have reduced this level. It is also possible that although vitamin K has been shown to reduce inflammation in vitro \(^{(173)}\) the mechanisms by which vitamin K influences biomarkers of inflammation are not known and it may be that vitamin K does not affect inflammation directly but by an alternative anti-inflammatory mechanism.

III. Markers of physical function: A change of 0.54 on the SPPB would have been required to identify a small change in physical function, but this did not occur in either group. Previous studies have shown that alternative measures such as the 400 metre walk test may be a better outcome measure in high functioning patients \(^{(174)}\). As with the vascular and inflammatory markers the markers of physical function were relatively good at baseline. While the
vitamin K group were identified as less physically able than the placebo group (grip strength 26.2kgs vs. 29.1kgs), the overall physical functioning of all the participants was particularly good given their age and medical history. It may be that the potential for this group of relatively fit and active individuals to improve significantly over such a short duration period was low or that again a higher dose of vitamin K was required to have an effect on muscle function. No direct link has yet been made between muscle function and vitamin K and given that no improvement in vascular health was identified in this study, positive effect on muscle was unlikely.

**Adherence**

Non-adherence to medication can be a major problem with older people often due to sensory deficits, cognitive problems and complex regimes as well as the occasional belief that the medication will not in fact improve health. It has been estimated that adherence for chronic disease averages only 50% \(^{175}\) and non-adherence to medication contributes to around 10% of hospital admissions. \(^{176}\) Adherence to medication in clinical trials of participants receiving treatment for chronic disease varies between 43 and 78% \(^{177}\).

Adherence to medication was assessed by tablet count at the two subsequent study visits. The 94% overall adherence rate found in this study was similar to that of a previous study of vitamins in older community dwelling adults \(^{178}\). Serum vitamin K levels were checked at baseline and 6 months with the rise in levels shown in the
intervention group giving some evidence that the medication was not just removed from the bottle, but was actually ingested.

**Adverse Events**

More falls were identified in the vitamin K group compared to placebo (17 vs. 7). Of note however, the vitamin K group appeared to be at higher risk of falls than the placebo group because of a higher (but non-significant) baseline incidence of osteoarthritis, walking aid usage and isolating personal home circumstance.

Also worth consideration is that although a higher incidence of adverse events occurred in the vitamin K group this may be attributable to the overall reduced functioning ability and increased dependence level of the intervention group compared to the placebo group as evidenced by the baseline physical outcome measures. The median total medications intake of the vitamin K group was also greater (8 vs. 5) suggesting a greater presence of co-morbid disease which may also have accounted for the events recorded. Given these between group differences at baseline logistic regression was carried out which concluded that group selection was not a significant indictor of falls risk.

**4.3 Strengths and Weaknesses**

**Strengths**

- The study had a number of notable strengths. It was run as a parallel group, randomised controlled trial where both the participants and investigator
remained blind to treatment. All outcome measurements, entry of data and subsequent data analysis were performed by one single trained investigator which eliminated inter-observer variation. Full analysis was performed prior to breaking the treatment code which eliminated expectation bias. The treatment period was of six months duration, considered to be reasonable time for study medication to have affected the preliminary and outcome measurements which were collected at three time points throughout the duration of the study following the protocol and as per SOPs.

- A wide range of differing vascular markers were selected as outcome measures covering vascular stiffness and calcification. All measures have been widely used in clinical research and used previously in similar trials within the department and were highly acceptable to participants as evidenced by the low drop out rate.

- The study recruited persons over the age of 70 years, a relevant group of participants as vascular disease is more prevalent in later life.

- Recruitment and retention to the study was excellent with recruitment completed within the target timescale and 77/80 (96%) participants competing all three study visits.

- Adherence to study medication was higher than usually found in clinical trials especially those targeting older people providing reassurance that the negative findings cannot be explained by participants not taking the drugs.

**Weaknesses**

- No stratification was carried out at baseline resulting in baseline differences between the groups. More of the vitamin K group required walking aids
which may have contributed to the excess of falls which occurred in this
group. The difference in primary outcome at baseline, although insignificant,
made analysis more complicated than if both groups had been ideally
matched and potentially biases the PWV and the adverse event data.

- People who volunteered were relatively fit and functionally independent. The
findings therefore apply only to this group. A further trial would be necessary
to determine whether the results would be different in a frailer group.

- Follow-up was at three and six months. Other intervention studies, e.g. the
MARS study have shown that a longer period of intervention is required in
order for any vascular calcification to regress. The MARS study found that
while lipid lowering drugs reduced progression of carotid IMT it was not
until the end of the first year of treatment that any significant reduction was
detected \(^{(179)}\). Antihypertensive drugs have also been noted to have success in
producing significant decreases in carotid IMT. These studies which included
the drugs Amlodipine \(^{(180)}\), and Ramipril \(^{(172)}\) varied in length between 48
months to 4.5 years duration before a significant reduction or decrease in
progression was identified in carotid IMT.

- Dose was set at baseline with the intervention group receiving a fixed dose of
vitamin K. Comparison with further groups receiving a higher dose of
vitamin K could have been made to determine the dose response effect but
this would have increased the size, complexity and cost of the trial.

- The choice of outcomes might not have been best suited to detecting change
within the recruited population. Many physical function tools exists however
and while the SPPB and grip strength are simple effective measures which
have been widely used in older populations, they were not sensitive to the effect of vitamin K.

- The 6 hour fast prior to study visits may have been insufficient to ensure dietary vasoactive substances did not influence results while all participants were asked to take their normal medications including any antihypertensives if prescribed on the morning of each for their assessment visits. Although these pre-visit preparation instructions may have in some way influenced results of vascular assessments, especially the FMD and PWV results, it could be argued that had participants been asked to omit regular medication or fast for a longer period this may also have influenced results thus a consistent approach was taken across the study.

- Although FMD, carotid IMT and PVW are widely used validated techniques, which once learnt, have good inter- and intra-operator reproducibility this was the first study using these techniques for the investigator, resulting in a lack of validation data. Despite training and repeated practice showing good reproducibility prior to participant recruitment this lack of experience may have affected the collection of data.

- PVW can be measured using several different artery sites with carotid-radial and carotid-femoral being the two most commonly used in clinical studies. This study measured carotid-radial PWV as it was felt that this would be less of a physical burden on the older patients being recruited. Despite this site being validated and used in other published clinical trials it does not follow the 2007 European Society of Hypertension guidelines, which set carotid-femoral as the gold standard measurement of arterial stiffness.
• It was not possible to collect data on every participant for all outcome measurements at each of the 3 study time points. This missing data, although totalling less than 10% of the overall total, may have had some affect on the results. It is however unlikely that missing data made a significant difference to the overall results.

4.4 Conclusion

This study showed that supplementation of the diet with the daily recommended dose of vitamin K did not improve vascular health or physical function in older people with established vascular disease; this despite excellent adherence to medication and a low dropout rate from the study.

Osteocalcin, Matrix Gla protein, and Gas6 have all been identified as proteins which are dependent on the presence of vitamin K. It is thus it is entirely possible that increased intake of vitamin K could exert beneficial effects on vascular health, particularly with respect to vascular calcification which is highly prevalent in older people and leads to stiff arteries and high blood pressure.

Previous studies have shown that higher intake of vitamin K is associated with lower serum concentrations of undercarboxylated osteocalcin, which in turn is associated with lower bone mineral density \(^{(38,39)}\). Animal studies have shown that the reversal of calcification of the arteries is possible, while in humans uncarboxylated MGP has been identified as being present in calcified regions of the vasculature \(^{(38)}\). A previous
study of post-menopausal women identified a link between low levels of vitamin K and atherosclerotic calcification in the abdominal aorta\(^{(181)}\) providing strong evidence to study the effect on vascular calcification in this high risk group\(^{(182)}\).

Although no improvement was noted in the primary or the majority of the secondary outcome measures a small improvement in arterial stiffness was identified at 6 months. This small but gradual reduction in PWV at 6 months may suggest that treatment with vitamin K over a longer period of time might indeed provide further benefit. These are the results which would have been expected if indeed vitamin K is acting via inhibition of calcification, rather than directly to improve endothelial function.

This was the first study testing the ability of vitamin K to improve vascular health in older people. However those who volunteered for the study were relatively fit and healthy given their age and the findings therefore apply only to this group and a further trial would be necessary to determine whether the results would be different in a frailer group.
5. **FUTURE RESEARCH**

The majority of the study population had hypertension and although vitamin K did not improve vascular calcification in this particular patient group investigation of the role of vitamin K in other populations may be worthwhile.

The dose required or time frame necessary to improve vascular function remains uncertain, with the key question of whether the *in vitro*, animal data and observational data translate into clinically important effects in humans. Further research should address the fact that this study only evaluated a single dose of vitamin K and investigate the outcome for higher vitamin K doses given over a longer period of time.

The reporting of adverse events, although higher in the vitamin K group, was very unlikely to be attributable to the supplementation in this study. However any future work in this field, particularly if higher doses are to be administered, should ensure adverse events are monitored closely.

### 5.1 Vitamin K and Cardiovascular Health

The vascular disease process has been shown to develop in arteries many years before the symptoms emerge. Giving vitamin K to a younger group of people may be a better approach.
The close links between both diabetes and CVD and between chronic kidney disease and CVD are well known but both these groups also develop medial calcification. This merits further exploration of the effect of vitamin K in future trials, as this pattern of vascular calcification differs from the atherosclerotic calcification pattern typically seen in older patients.

5.2 Vitamin K and Neuromuscular function and Bone Health

Given about a third of all people aged over 65 fall each year (equivalent to over three million people), and the available evidence reports that those with lower circulating vitamin K levels sustain higher hip fracture rates research should aim to encapsulate both these disease processes. A study of older people with a history of falls would involve participation from frailer, less independent people. This may offer the potential to exhibit change both physically as well as functionally, which was not possible within the group used for this study.
REFERENCE LIST


8. McNinch A. ‘Vitamin K deficiency bleeding: Early history and recent trends in the United Kingdom’. *Early Human Development* 2010; 86; S63-S65


   http://www.robertbarrington.net/vitamin-k.


45. Gast GCM, de Roos NM, Sluijs I, Bots ML, Beulens JWJ, Geleijnse JM et al. ‘A high menaquinone intake reduces the incidence of coronary heart disease’. Nutrition Metabolism and Cardiovascular Diseases 2009; 19; 7; 504-510.


53. The Scottish Government. ‘National Workforce Planning Framework’ 2005: 


   ‘Relationship between multimorbidity and health-related quality of life of patients in primary care’. Quality of Life Research 2006; 15; 1; 83-91.


73. Breen J. ‘An Introduction to causes, detection and management of hypertension’. 
   *Nursing Standard* 2008; 23; 14; 42-46.

74. Davies JJ, Struthers AD. ‘Pulse wave analysis and pulse wave velocity: a critical 
   review of their strengths and weaknesses’. *Journal of Hypertension* 2003; 21; 3; 
   463-472.

75. Diabetes action plan 2010 ‘Quality care for diabetics in Scotland’. Scottish 

76. Resnick HE, Howard BV. ‘Diabetes and Cardiovascular Disease’. *Annual Review 
   of Medicine* 2002; 53; 245-267.

77. Kannel WB, McGee DL. ‘Diabetes and cardiovascular risk factors: the 

78. UK Prospective Diabetes Study Group. ‘Tight blood pressure control and risk of 
   macrovascular and microvascular complications in type 2 diabetes: UKPDS 38’. 
   *British Medical Journal* 1998; 317; 7160; 703-713.

   Clinical Endocrinology & Metabolism* 2001; 86; 3; 965-971.

80. Jeerakathill T, Johnson JA, Simpson SH, Majumdar SR. ‘Short-Term Risk for 
   Stroke Is Doubled in Persons With Newly Treated Type 2 Diabetes Compared 


84. Jevon P. ‘Recording an accurate ECG’. *Practice nursing* 2010 21; 8; 404-408.


   *Heart* 2001; 86; 5; 516-521.

86. Cox B. ‘Standing up, sitting or lying down: What do these BP readings mean?’

   *The British Journal of Primary Care* 2009; 6; 3; 1-3


95. Kinlay S, Ganz P. ‘Role of Endothelial dysfunction in Coronary Artery Disease and Implications for Therapy’. *The American Journal of Cardiology* 1997; 80;(9 supplement 1); 11I-16I.


125. Hutcheon SD, Gillespie ND, Struthers AD, McMurdo MET. ‘B-type natriuretic peptide in the diagnosis of cardiac disease in elderly day hospital patients’. *Age and Ageing* 2003; 31; 4; 295-301.


129. Pincus T, Sokka T. ‘Laboratory tests to assess patients with rheumatoid arthritis: advantages and limitations’ *Rheumatic Diseases Clinics North America*. 2009 ;35; 4:731-4,

131. Blake GL, Ridker PM. C-reactive protein and other inflammatory risk markers in acute coronary syndromes. *Journal American College Cardiology* 2003; 41;4; 37S-45S.


133. Ridker PM. ‘C-Reactive Protein a simple test to help predict risk of heart attack and stroke’. *Circulation* 2003; 108; e81-e85.


139. Shearer MJ. ‘Vitamin K in parenteral nutrition’. *Gastroenterology* 2009; 137; (5S); 105-108.


168. Lonn EM, Yusuf S, Dzavik V, Doris I, Yi Q, Smith S et al. ‘Effects of ramipril and vitamin E on atherosclerosis the study to evaluate carotid ultrasound changes in patients treated with ramipril and vitamin E (SECURE)’. *Circulation* 2001; 103; 919-925.


Appendix 1 Good Clinical Practice Certificate

Clinical Research Centre Tayside

This is to certify that
ROBERTA FULTON
successfully completed the

Tayside GCP Online Assessment Update

Lesley Peebles, Clinical Research Training Committee
Date: 17/08/11

Certificate Number: 000495-OU20110817

NHS Tayside
Dear Mr/ Mrs XYZ,

**Research Project: Vitamin K to improve markers of vascular health in older people with vascular disease**  
*– a randomised controlled trial*

Our practice is helping researchers at the University of Dundee with this research study, which is funded by Chest Heart and Stroke Scotland.

People with high blood pressure, diabetes, previous heart disease or stroke often have stiff arteries, which can increase the chances of further heart and blood vessel problems. Dr Miles Witham and his team at the University of Dundee are testing whether taking a vitamin K supplement for 6 months can reduce the stiffness and improve the health of arteries.

Taking part would involve taking a single tablet of either vitamin K or a placebo (dummy) for 6 months, and visiting Ninewells Hospital on three occasions to have tests on your arteries, strength and balance.

We are writing to you because you are over the age of 70 and this study may be suitable for you to take part in.

Please complete the attached reply slip and return to me in the enclosed stamped addressed envelope. If you are interested, I will pass your name to the study team in Ageing and Health at Ninewells Hospital, Dundee, and the study team will get in touch with you to provide more information.

With best wishes

GP
Appendix 4: Patient Reply Slip

PRACTICE HEADER

*Research Project: Vitamin K to improve markers of vascular health in older people with vascular disease – a randomised controlled trial*

Please only complete and return if you would like for information about the study

Name......................................................................................................................

Address................................................................................................................

.................................................................................................................. Postcode ......................

Telephone number..............................

Best time to contact.................
PARTICIPANT INFORMATION SHEET

Can Vitamin K improve markers of vascular health and physical function in older people with vascular disease?

My name is Roberta Fulton and I am required to undertake a project as part of my MSc course at the University of Dundee. I invite you to take part in the following study however, before you decide to do so, I need to be sure that you understand firstly why I am doing it, and secondly what it would involve if you agreed. I am therefore providing you with the following information. Please read it carefully and be sure to ask any questions you might have, and, if you want, discuss it with others including your friends and family. I will do my best to explain the project to you and provide you with any further information you may ask for now or later. You do not have to make an immediate decision.

Why are we doing this study?
Vitamin K has long been known to be an important factor in maintaining good bone health but recent research has shown that it may also have a beneficial effect on our blood vessels. Unfortunately daily intake of vitamin K is below the recommended dose for the majority of adults in the UK, this low level of vitamin K has been linked to stiffening of the arteries, which in turn may cause high blood pressure, heart attacks and strokes, especially in older people.
We therefore want to test if giving a daily dose of vitamin K to people with either high blood pressure or previous cardiovascular disease (stroke, heart attack, angina, or hardening of the leg arteries) will improve the function of the blood vessels as well as general fitness.
**Why have I been chosen?**
You have been chosen to take part in the study because you are over the age of 70 years and have had a cardiovascular disease, high blood pressure or diabetes.

**Do I have to take part?**
It is up to you to decide. Participation in this study is entirely voluntary and you are free to refuse to take part or to withdraw from the study at any time without having to give a reason and without this affecting your future medical care or your relationship with medical or nursing staff looking after you.

**What will happen to me if I take part?**
The study takes 6 months in total to complete. The study is of randomized, doubled blind design. This means that you will be asked to take a capsule by mouth once a day. This will either contain vitamin K, or a placebo (dummy) medication. The one that you will be given is decided in a random way (a bit like tossing a coin, but done by a computer). Neither you nor the research team will know which tablet you are taking until after the study is finished. This means that the results of the study cannot be influenced by you or the researchers knowing what you are taking.

You will be asked to come to Ninewells Hospital in Dundee on three occasions over 6 months for study visits. We can provide a taxi to transport you to Ninewells and back for each visit or we can reimburse your travel expenses should you choose to make your own travel arrangements.

After making sure that you are suitable to participate in the study and obtaining your consent we will ask you to undergo an assessment which will last between one and two and a half hours. At each visit we will do the following:

- We will measure your blood pressure while you are lying down and then when you stand up.

- We will ask permission to take a blood test (about two tablespoons of blood will be taken). Blood samples will be
stored and analysed at the end of the study. We will also ask your permission to store the blood samples for a period of 10 years after the end of the 20 month study, so that we can run further tests on the blood in the future. We will not test any of your blood samples for genetic testing either now or in the future.

- You will be asked to do some mobility tests including standing tests, balance tests and a test to measure your hand grip strength.

- We will then measure how one of the blood vessels in your arm functions (brachial artery ultrasound), this will show any evidence of hardened (stiff) arteries. This is done using an ultrasound machine. To see how stiff the blood vessel is we need to look at how it responds to a short period of having the circulation to the forearm blocked. A blood pressure cuff will be placed below your elbow and we measure the heart tracing (ECG) at the same time. We will then take an ultrasound measurement of the artery above your elbow. This involves placing a probe gently on the skin above the elbow. We will then inflate the cuff to cut off the circulation to the forearm for a period of 5 minutes. This can be a little uncomfortable, just like if you lie on your arm when you are asleep. After 5 minutes we will release the blood pressure cuff and take another measurement of the artery above the elbow. We will also scan the artery in your neck using the ultrasound probe. This will all take about 30 minutes.

- We will test how elastic the blood vessels are in your neck, wrist and thigh by using a small pencil-like device placed over the blood vessel to measure your pulse. At the same time, the electrical activity of your heart will be monitored with a heart tracing. This will take about 10 minutes.

- We will issue your study tablets.
At 3 and 6 months we will ask you to pay us further visits where we will repeat some or all of the above tests – see the summary below.

**Summary of tests:**

Baseline visit: Sign consent form. Blood test, lying and standing blood pressure, artery tests, heart tracing, standing tests, balance tests and tests to measure your hand strength. Receive medication.

*Time: 2 hours*

3 month visit: Lying and standing blood pressure, artery tests. Receive medication.

*Time: 1 hour*

6 month visit: Blood test, lying and standing blood pressure, artery tests, heart tracing, standing tests, balance tests and tests to measure your hand strength.

*Time: 90 minutes*

**What is being tested?**

Vitamin K is a natural substance which is present in everyday foods such as leafy vegetables. Many people in Scotland don’t get as much vitamin K as recommended. We wish to test whether or not giving a daily dose of vitamin K can improve the function of blood vessels and improve general fitness.

**Will taking part in the study affect your usual care?**

We will not alter any of your medication or interfere with your treatment in any way however we would ask you not to purchase or take any vitamin K independently while on the study.

**What are the possible discomforts, risks and side effects?**

This dose of vitamin K has been used before and is known to be safe but as with all medications there is a small possibility of side effects. If you experience any side effects of sickness, diarrhoea,
dizziness or bruising, please report them to us as soon as they arise.

Having blood taken can cause some bruising. The blood pressure cuff causes mild discomfort to some people.

**What happens if something goes wrong?**
This is very unlikely, but if something does go wrong then the University of Dundee has a scheme which could provide compensation, if appropriate.
If you believe you have been harmed in any way by taking part in this research, the normal NHS complaints mechanism would also be available to you. To register a complaint against the NHS in Tayside, or to receive more information, contact:

Complaints and Advice Team
Level 7, Ninewells Hospital and Medical School
Dundee DD1 9SY.
Free-phone 0800 027 5507

**Will my GP know about this research project?**
If you agree to take part, your GP will be informed of this.

**Will my taking part in this study be kept confidential?**
All the identifiable data, samples or information collected with your consent will be retained and used in the study. They will be kept strictly confidential. The information will be kept in a locked room and held on a secure computer, with your name and other identifying details removed. Should you choose to withdraw from the study no further data or samples would be collected. Only the researchers involved in the study will have access to the information. We will keep the information for at least 15 years.

**What will happen to the results?**
The results will be examined by the researchers who have organised the study and a short report will be produced. You will not be identified in this report. The results will be shared with the funder for the study (Chest Heart and Stroke Scotland). The
results will then be published in scientific journals. Again, you will not be identified in any journal articles.

**Who is organizing and funding this research?**
The study has been organised by Dr Miles Witham and Professor Marion McMurdo (Ageing and Health, University of Dundee), Dr Faisel Khan and Dr Sandy Hill (Vascular Medicine, University of Dundee). The study is funded by Chest Heart and Stroke Scotland.

**Who has reviewed the study?**
Tayside Research Ethics Committee, which has responsibility for scrutinising proposals for medical research on humans in Tayside, has examined the proposal and has raised no objections from the point of view of medical ethics. It is a requirement that your records in this research, together with any relevant medical records, be made available for scrutiny by monitors from NHS Tayside and the University of Dundee whose role it is to check that research is properly conducted and the interests of those taking part are adequately protected.

Thank you for reading this Information Sheet and considering taking part in this study.

*For further information contact:*
**Mrs Roberta Fulton** - Research Nurse on telephone 01382 632436 or email r.l.z.fulton@dundee.ac.uk

**Dr Miles Witham** - on telephone 01382 632436 or email m.witham@dundee.ac.uk

**Dr Jacob George** - Department of Clinical Pharmacology, Ninewells Hospital. Email j.george@dundee.ac.uk
Appendix 5: Consent Form

CONSENT FORM v1.1 (05/09/2011)

Title of Project: The KIMVASC study: vitamin K to improve markers of vascular health and physical function in older people with vascular disease – a randomised controlled trial

Name of Researcher: Dr Miles D Witham

Please initial box

I confirm that I have read and understood the information sheet dated 05/09/11 (version 1.1) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.

I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without any medical care or legal rights being affected. If I withdrawn I understand any data already collected will be retained and used in the study.

I understand that relevant sections of my medical notes and data collected during the study may be looked at by representatives from NHS Tayside and University of Dundee, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.

I agree to my GP being informed of my participation in the study.

I agree to the storage of my leftover blood samples for use in future research.

I agree to take part in the above study.

________________________        _______________     __________________
Name of Participant                         Date                           Signature

________________________       ________________    ____________________
Name of Peron Taking Consent     Date

When complete, 1 for participant; 1 for researcher site file; 1 (original) to be kept in medical notes
Appendix 6: GP Letter

Vitamin K to improve markers of vascular health and physical function in older people with vascular disease - a randomised controlled trial

Dr XYZ
Surgery

Dear Dr

Re: Mr/Mrs ABC, Address
DOB/CHI: 0000000000

Your patient, Mr/Mrs ABC, has very kindly agreed to take part in the above trial.

Participants will receive 6 months of oral vitamin K₂ 100 micrograms per day or placebo. We will provide all of the trial medication while all other medications can continue as usual. Both participants and assessors will be blinded to the nature of the therapy. We will assess outcomes (endothelial function, arterial stiffness, carotid intima-media thickness, physical function, strength and balance, blood pressure, B-type natriuretic peptide and C-reactive protein) at baseline, 3 and 6 months.

Please let us know if you need to consider warfarin therapy over the course of the next six months, as vitamin K antagonises the effect of warfarin.

This project has been funded by Chest, Heart & Stroke Scotland.

The study has been approved by the Tayside Committee on Medical Ethics. If you have any questions about the study, or if there is any aspect of your patient's healthcare that we can assist you with, please do not hesitate to contact us.

Yours sincerely,

Dr Miles D Witham
Clinical Senior Lecturer and CSO Clinician Scientist in Ageing and Health
Tel: 01382 632436
CASE REPORT FORM Visit 1 – (Screening & Baseline visit)

Patient Study ID number                             Date of Study Visit

A. Inclusion Criteria

Age 70 and over [ ] And at least 1 of following:

Hypertension (as a listed diagnosis) [ ] 1
Diabetes mellitus [ ] 2        Myocardial infarction [ ] 3
Coronary artery bypass grafting [ ] 4   Coronary angioplasty [ ] 5
Stroke or TIA [ ] 6          Peripheral Vascular disease [ ] 7

*IF THE ANSWER IS NO TO ANY OF THE ABOVE, THE PATIENT IS NOT ELIGIBLE TO CONTINUE IN THE STUDY

B. Exclusion Criteria

Atrial fibrillation [ ] 1      Unable to give written informed consent [ ] 3
Taking Warfarin [ ] 2     Currently taking Vitamin k supplements [ ] 4
Unable to walk without human assistance (walking aids are permitted) [ ] 5
Enrolled in another research study [ ] 6

*IF THE ANSWER IS YES TO ANY OF THE ABOVE, THE PATIENT IS NOT ELIGIBLE TO CONTINUE IN THE STUDY

Informed Consent

Has written informed consent been obtained for study participation?  Yes [ ] / No [ ]

If yes, date consent signed

DD / MM / YYYY
Demographics

Sex: Male 1 / Female 2

Living Status: Own Home 1 / Sheltered Housing 2 / RHE 3 / NH 4 / Living with relative 5 / Other 6

If other please specify:

Living Alone: Yes 1 / No 2

Formal Help at Home: Yes 1 / No 2

Smoker: Never 1 / Ex 2 / Current 3

Approx. number of years smoked: 

Date stopped: 

Average number cigarettes per day: 

Walking Aid: None 1 / 1 Stick 2 / 2 Sticks 3 / TWW 4 / ZWF 5 / Other 6

If other please specify:

Past Medical History

<table>
<thead>
<tr>
<th>Description</th>
<th>Date of Diagnosis if known</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertension Yes 1 / No 2</td>
<td></td>
</tr>
<tr>
<td>Previous MI Yes 1 / No 2</td>
<td></td>
</tr>
<tr>
<td>Stroke / TIA Yes 1 / No 2</td>
<td></td>
</tr>
<tr>
<td>Angina Yes 1 / No 2</td>
<td></td>
</tr>
<tr>
<td>CABG / PTCA Yes 1 / No 2</td>
<td></td>
</tr>
<tr>
<td>Diabetes Mellitus Yes 1 / No 2</td>
<td></td>
</tr>
<tr>
<td>PVD Yes 1 / No 2</td>
<td></td>
</tr>
<tr>
<td>CHF Yes 1 / No 2</td>
<td></td>
</tr>
</tbody>
</table>
**Any Other relevant medical or surgical history?**

<table>
<thead>
<tr>
<th>Description</th>
<th>Date of Diagnosis if known</th>
<th>Still present</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Yes¹ / No²</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes¹ / No²</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes¹ / No²</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes / No²</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes¹ / No²</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes¹ / No²</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes¹ / No²</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes¹ / No²</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes¹ / No²</td>
</tr>
</tbody>
</table>

**Vital Signs**

Lying Supine Blood Pressure (lying for a minimum of 5 minutes prior to recording)

<table>
<thead>
<tr>
<th>Reading 1</th>
<th>Reading 2</th>
<th>Reading 3</th>
<th>Mean readings of 2 &amp; 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP</td>
<td>mmHg</td>
<td>mmHg</td>
<td>mmHg</td>
</tr>
<tr>
<td>DBP</td>
<td>mmHg</td>
<td>mmHg</td>
<td>mmHg</td>
</tr>
</tbody>
</table>

Standing 0 minute

<table>
<thead>
<tr>
<th>SBP</th>
<th>mmHg</th>
<th>Complaints of dizziness</th>
<th>Yes¹ / No²</th>
</tr>
</thead>
<tbody>
<tr>
<td>DBP</td>
<td>mmHg</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Standing 1 minute

<table>
<thead>
<tr>
<th>SBP</th>
<th>mmHg</th>
<th>Complaints of dizziness</th>
<th>Yes¹ / No²</th>
</tr>
</thead>
<tbody>
<tr>
<td>DBP</td>
<td>mmHg</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Standing 3 minutes

<table>
<thead>
<tr>
<th>SBP</th>
<th>mmHg</th>
<th>Complaints of dizziness</th>
<th>Yes¹ / No²</th>
</tr>
</thead>
<tbody>
<tr>
<td>DBP</td>
<td>mmHg</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Patient fasted for 4 hours? Yes ¹ No ²
If No, give reason

**ECG**
Has a 12 lead ECG been recorded Yes ¹ No ²
If No, give reason
If yes, does ECG show evidence of atrial fibrillation Yes ¹ No ²
If yes participant unable to continue

**Pulse Wave Velocity**
Pulse Wave velocity________m/s Augmentation index (@75%)________

**Flow Mediated Dilatation**
% change in flow following forearm occlusion ________%  

**Carotid Intima – media thickness**
Carotid Artery Scanned - Right side / Left side
Measurement 1 cm
Measurement 2 cm
Measurement 3 cm

**Blood test**
Blood tests taken for measurement of
C-reactive protein INR Plasma mk7
BNP U&E’s Cholesterol

**Strength Measures**
Grip strength (peak force) ________________ Kg (record best of 3 - using non dominant hand)
SHORT PHYSICAL PERFORMANCE BATTERY

1) BALANCE TESTS
A. Side-by-side-stand
- Held for 10 sec: 1 point
- Not held for 10 sec: 0 points
- Not attempted: 0 points

B. Semi-Tandem Stand
- Held for 10 sec: 1 point
- Not held for 10 sec: 0 points
- Not attempted: 0 points

If 0 points, end Balance Tests and go to
and go to section D

C. Tandem Stand
- Held for 10 sec: 2 points
- Held for 3 to 9.99 sec: 1 point
- Held for < 3 sec: 0 points
- Not attempted: 0 points

D. Total Balance Tests score
(sum points)

2) GAIT SPEED TEST
Time for 1\textsuperscript{st} Gait Speed Test (sec)
Time for 2\textsuperscript{nd} Gait Speed Test (sec)

Time for 4 meters: Sec
- Aid used: none, stick, other

Using the shorter of the two times:
- If the participant was unable: 0 points
- If time is 4.82 to 6.20 sec: 3 points
- If time is more than 8.70 sec: 1 point
- If time is less than 4.82 sec: 4 points
- If time is 6.21 to 8.70 sec: 2 points

3) CHAIR STAND TEST
- Unable to complete or >60 sec: 0 points
- Chair stand time 11.20 - 13.69 sec: 3 points
- Chair stand time > 16.70 sec: 1 point
- Chair stand time 13.70 to 16.69 sec: 4 points
- Chair stand time 13.70 to 16.69 sec: 2 points

Total Balance Test + Gait Speed Test + Chair Stand Test

Total Score points (sum of 3 points above)
**Falls History**

Falls recorded in past 3 months.  
Yes ¹  No ²  
If yes  Number  
Details

**Study Medication**

Medication dispensed  
Yes ¹  No ²  
Number of tablets dispensed
CASE REPORT FORM Visit 2 – (3 months)

Patient Study ID number                             Date of Study Visit

Has patient taken study medication            Yes 1 / No 2
If no state reason ____________________________

Count tablets left in bottle.       Yes 1 / No 2
If action not carried out please state why

Number of tablets left in bottle   
Number of days since last study visit   

Have there been any GP appointments since last visit
If yes how many     

Have there been any hospitalisations since last visit
If yes how many     

Have there been any changes to life circumstances since previous visit?
If yes please detail

**Medical history update**

Has the patient's medical history changed since previous visit?
Yes 1 / No 2
If yes please detail
### Study ID

#### Medication

Have there been any changes to the patient's medications since the previous visit?

Yes ¹ / No ²
(If yes please detail)

#### Adverse Events

Has the patient experienced any adverse events since the previous visit

Yes ¹ / No ²

If yes please complete a Clinical Trial Adverse Events Form.

#### Vital Signs

Lying Supine Blood Pressure (lying for a minimum of 5 minutes prior to recording)

<table>
<thead>
<tr>
<th>Reading 1</th>
<th>Reading 2</th>
<th>Reading 3</th>
<th>Mean readings 2 &amp; 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP</td>
<td>mmHg</td>
<td>mmHg</td>
<td>mmHg</td>
</tr>
<tr>
<td>DBP</td>
<td>mmHg</td>
<td>mmHg</td>
<td>mmHg</td>
</tr>
</tbody>
</table>

Standing 0 minute

<table>
<thead>
<tr>
<th>SBP</th>
<th>mmHg</th>
<th>DBP</th>
<th>mmHg</th>
<th>Complaints of dizziness</th>
<th>Yes ¹ / No ²</th>
</tr>
</thead>
</table>

Standing 1 minute

<table>
<thead>
<tr>
<th>SBP</th>
<th>mmHg</th>
<th>DBP</th>
<th>mmHg</th>
<th>Complaints of dizziness</th>
<th>Yes ¹ / No ²</th>
</tr>
</thead>
</table>

Standing 3 minutes

<table>
<thead>
<tr>
<th>SBP</th>
<th>mmHg</th>
<th>DBP</th>
<th>mmHg</th>
<th>Complaints of dizziness</th>
<th>Yes ¹ / No ²</th>
</tr>
</thead>
</table>
Study ID

Patient fasted for 4 hours?  
Yes ¹  No ²
If No, give reason

Pulse Wave Velocity
Pulse Wave velocity __________  __________ %
Augmentation index (@75%) __________

Flow Mediated Dilatation
% change in flow following forearm occlusion __________  %

Carotid Intima –media thickness
Carotid Artery Scanned -  Right side / Left side
Measurement 1 __ cm
Measurement 2 __ cm
Measurement 3 __ cm

Falls History
Falls recorded in past 3 months.  Yes ¹  No ²
If yes  Number ___
Details
CASE REPORT FORM Visit 3 – (6 months)

Patient Study ID number                             Date of Study Visit

Has patient taken study medication            Yes 1 / No 2
If no state reason ____________________________

Count tablets left in bottle.       Yes 1 / No 2
If action not carried out please state why
Number of tablets left in bottle
Number of days since last study visit

Have there been any GP appointments since last visit?       Yes 1 / No 2
If yes how many

Have there been any hospitalisations since last visit?
If yes how many

Have there been any changes to life circumstances since previous visit?
If yes please detail

Medical history update

Has the patient's medical history changed since previous visit?    Yes 1 / No 2
If yes please detail
Study ID

**Medication**

Have there been any changes to the patient’s medications since the previous visit?

Yes ¹ / No ²

(If yes please detail)

**Adverse Events**

Has the patient experienced any adverse events since the previous visit

Yes ¹ / No ²

If yes please complete a Clinical Trial Adverse Events Form.

**Vital Signs**

Lying Supine Blood Pressure (lying for a minimum of 5 minutes prior to recording)

<table>
<thead>
<tr>
<th>Reading 1</th>
<th>Reading 2</th>
<th>Reading 3</th>
<th>Mean readings 2 &amp; 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mmHg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DBP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mmHg</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Standing 0 minute

SBP       mmHg

DBP       mmHg

Complaints of dizziness   Yes ¹ / No ²

Standing 1 minute

SBP       mmHg

DBP       mmHg

Complaints of dizziness   Yes ¹ / No ²

Standing 3 minutes

SBP       mmHg

DBP       mmHg

Complaints of dizziness   Yes ¹ / No ²
Patient fasted for 4 hours?  
Yes $^1$  
No $^2$

If No, give reason

**Pulse Wave Velocity**

Pulse Wave velocity ________ ________ %

Augmentation index (@75%)_________

**Flow Mediated Dilatation**

% change in flow following forearm occlusion ________ ________ %

**Carotid Intima –media thickness**

Carotid Artery Scanned - Right side / Left side

Measurement 1 ________ cm

Measurement 2 ________ cm

Measurement 3 ________ cm

**Blood test**

Blood tests taken for measurement of

C-reactive protein □  
Cholesterol □

BNP □  
Plasma mk7 □

**Strength Measures**

Grip strength (peak force) ________________ Kg (record best of 3 - using non dominant hand)
SHORT PHYSICAL PERFORMANCE BATTERY

1) BALANCE TESTS

A. Side-by-side-stand

- Held for 10 sec: 1 point
- Not held for 10 sec: 0 points
- Not attempted: 0 points

B. Semi-Tandem Stand

- Held for 10 sec: 1 point
- Not held for 10 sec: 0 points
- Not attempted: 0 points

If 0 points, end Balance Tests and go to section D

C. Tandem Stand

- Held for 10 sec: 2 points
- Held for 3 to 9.99 sec: 1 point
- Held for < than 3 sec: 0 points
- Not attempted: 0 points

D. Total Balance Tests score

2) GAIT SPEED TEST

- Time for 1st Gait Speed Test (sec)
- Time for 2nd Gait Speed Test (sec)
- Time for 4 meters: Sec - Sec
- Aids used: none, stick, other

Using the shorter of the two times:

- If participant was unable: 0 points
- If time is 4.82 to 6.20 sec: 3 points
- If time is more than 8.70 sec: 1 point
- If time is less than 4.82 sec: 4 points
- If time is 6.21 to 8.70 sec: 2 points

3) CHAIR STAND TEST

- Unable to complete or >60 sec: 0 points
- Chair stand time 11.20 - 13.69 sec: 3 points
- Chair stand time > 16.70 sec: 1 point
- Chair stand time 11.19 sec or less: 4 points
- Chair stand time 13.70 to 16.69 sec: 2 points

Total Balance Test + Gait Speed Test + Chair Stand Test

Total Score points (sum of 3 points above)
Falls History

Falls recorded in past 3 months. Yes / No

If yes Number

Details
Appendix 8: Short Physical Performance Battery Scoring Sheet

Short Physical Performance Battery

1. **Balance Tests**

   - **Side-by-Side Stand**
     - Feet together side-by-side for 10 sec
     - 10 sec (1 pt)
     - Go to 4-Meter Gait Speed Test

   - **Semi-Tandem Stand**
     - Heel of one foot against side of big toe of the other for 10 sec
     - 10 sec (+1 pt)
     - Go to 4-Meter Gait Speed Test

   - **Tandem Stand**
     - Feet aligned heel to toe for 10 sec
     - 10 sec (+2 pt)
     - 9-9.99 sec (+1 pt)
     - <3 sec (+0 pt)

2. **Gait Speed Test**

   - Measures the time required to walk 4 meters at a normal pace (use best of 2 times)

   - 4.82 sec < 4 pt
   - 4.82-6.20 sec 3 pt
   - 6.21-8.70 sec 2 pt
   - >8.7 sec 1 pt
   - Unable 0 pt

3. **Chair Stand Test**

   - **Pre-test**
     - Participants fold their arms across their chest and try to stand up once from a chair
     - unable Scop (0 pt)

   - **5 repeats**
     - Measures the time required to perform five rises from a chair to an upright position as fast as possible without the use of the arms
     - 5.11-10 sec 4 pt
     - 11.20-13.69 sec 3 pt
     - 13.70-16.69 sec 2 pt
     - >16.7 sec 1 pt
     - >60 sec or unable 0 pt
(1) Ball GFM. Historical events leading to the establishment of vitamins. Vitamins Their Role in the Human Body. 2004: 1-7.

(2) Rosenfeld L. Clinical Chemistry 43[4], 680-685. 1997. Ref Type: Journal (Full)


(5) Carpenter K. The Nobel Prize and the Discovery of Vitamins. http://www.nobelprize.org/nobel_prizes/medicine/articles/carpenter/. 22-4-0004. 11-1-2012. Ref Type: Electronic Citation


(12) Barrington R. The nutrition blog of Robert Barrington. http://www.robertbarrington.net/vitamin-k . 2013. Ref Type: Electronic Citation

(13) KAESLER N, SCHETTGEN T, MUTUCUMARANA VP, BRADENBURG V, JAHNEN-DECHANT W, SCHURGERS LJ et al. A Fluorescent method to determine vitamin K dependent

Ref Type: Journal (Full)

Ref Type: Journal (Full)


Ref Type: Generic

(19) Washington DUNAP. DRI DIETARY INTAKES

Ref Type: Report


(21) WHO. Keep fit for life Meeting the nutritional needs of older persons. 2002.
Ref Type: Report

Ref Type: Journal (Full)


Ref Type: Journal (Full)


Ref Type: Report


Ref Type: Journal (Full)


Ref Type: Journal (Full)


Ref Type: Report


(45) Price PA, Faus SA, Williamson MK. Warfarin causes rapid calcification of the elastic lamellae in rats arteries and heart valves. American Heart Journal 18, 1400-1407. 18-3-1998. Ref Type: Journal (Full)


(49) Braam L, Hoeks APG, Brouns F, Hamulyak K, Gerichhausen MJW, Vermeer C. The elastic properties of the vessel wall in postmenopausal women: a follow up study. Thrombosis and Haemostasis 91, 373-380. 12-1-2004. Ref Type: Journal (Full)
Ref Type: Journal (Full)


Ref Type: Journal (Full)

Ref Type: Electronic Citation

Ref Type: Journal (Full)

Ref Type: Electronic Citation

Ref Type: Electronic Citation

(57) Life expectancy at birth and at age 65 by local areas in the United Kingdom. Office for National Statistics . 2012.
Ref Type: Electronic Citation


Ref Type: Electronic Citation

Ref Type: Report

Ref Type: Electronic Citation
Ref Type: Electronic Citation


Ref Type: Journal (Full)


Ref Type: Journal (Full)

Ref Type: Journal (Full)

Ref Type: Report

Ref Type: Report


(72) Scottish Public Health Observatory. high Blood Pressure Prevelence. 1-2-2012.
Ref Type: Report


Ref Type: Report


Ref Type: Journal (Full)


Ref Type: Journal (Full)


Ref Type: Pamphlet


(87) Jevon P. Recording an accurate ECG. Practice nursing 21[8], 404-408. 2010.

Ref Type: Journal (Full)

Cox B. Standing up, sitting or lying down: What do these BP readings mean? The British Journal of Primary Care, 111-113. 2012.


Corretti MC, Anderson TJ, Benjamin EJ, Celermajer DS, Charbonneau F, Creager MA et al. Guidelines for the ultrasound Assessment of Endothelial-Dependent...


Ref Type: Journal (Full)


Ref Type: Electronic Citation


Blake GL, Ridker PM. C-reactive protein and other inflammatory risk markers in acute coronary syndromes. J Am Coll Cardiol 2003; 41(4 (suppl)):37S-45S.


Tracy RP, Lemaitre RN, Psaty BN, Ives DG, Evans RW, Cushman M et al. Relationship of C-Reactive Protein to Risk of Cardiovascular Disease in the Elderly
Results From the Cardiovascular Health Study and the Rural Health Promotion Project. Arteriosclerosis, Thrombosis, and Vascular Biology 1997; 17:1121-1127.


Ref Type: Electronic Citation


Ref Type: Journal (Full)


Ref Type: Journal (Full)


(175) WHO. Adherence to Long Term therapy. 2003.
Ref Type: Report


Ref Type: Journal (Full)

Ref Type: Journal (Full)


Ref Type: Journal (Full)


Ref Type: Journal (Full)