

Dissertation

**NUTRITIONAL STATUS IN PATIENTS WITH PERIPHERAL
ARTERIAL DISEASE (PAD) AND CONCOMITANT PERIODONTAL
DISEASE (PD)**

**(Ernährungsstatus bei Patienten mit peripherer arterieller
Verschlusskrankheit und gleichzeitig vorliegender parodontaler
Erkrankung)**

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For the Academic Degree of

Doctor of Medical Science

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at the

Medical University Graz

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2015

Declaration

I hereby declare that this thesis is my own original work and that I have fully acknowledged by name all of those individuals and organizations that have contributed to the research for this thesis. Due acknowledgement has been made in the text to all other material used. Throughout this thesis and in all related publications I followed the guidelines of “Good Scientific Practice”.

Graz,

Acknowledgement

First of all I would like to thank my supervisor, Dr. Gerald Seinost, Associate Professor of Medicine, at the Division of Angiology, Department of Internal Medicine, Medical University of Graz, Austria, for unstinting advice, encouragement and support over the last three years. Without his patience and excellent supervision, this study would neither have been performed nor finished.

I would also like to thank the other members of my thesis committee:

Dr.in rer. nat. Sandra Johanna Wallner-Liebmann, Associate Professor, Department of Pathophysiology and Immunology, and DDr. Robert Gasser, Associate Professor of Medicine at the Division of Cardiology, Department of Internal Medicine, Medical University of Graz, for their insightful comments and assistance.

I am particularly grateful for the support of my colleagues at the outpatient clinic of the Division of Angiology, Medical University of Graz, and thank them for their help in recruiting study participants.

Many thanks also to all my colleagues who performed the DEXA measurements.

Very special thanks to Dr. Andreas Tomaschitz, Associate Professor of Medicine at the Division of Cardiology, and Dr. Stefan Pilz, Associate Professor of Medicine at the Division of Endocrinology and Metabolism, Department of Internal Medicine, Medical University of Graz, and Dr. Franz Quehenberger of the Institute of Medical Informatics, Statistics and Documentation, Medical University of Graz, who gave me valuable advice on how to conduct the statistical analysis. I would also like to thank Eugenia Lamont, BA, for her editorial support.

My sincere thanks go to my colleague, Dr. Behrouz Arefenia, of the Department of Prosthetics and Periodontology, Medical University of Graz, for performing the periodontal treatments that were so essential to this study.

In particular I want to thank all the patients for their contribution to the successful completion of this study. I am very indebted to the Medical University Graz for providing the research facilities I required for this work.

Finally, I would like to thank my family: my parents and my brother, as well as my partner, for supporting me in every possible way while I was working toward my doctoral degree.

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List of abbreviations in alphabetical order

ABI	ankle brachial index
ADMA	asymmetric dimethylarginine
BL	baseline
BMC	bone mineral content
BMD	bone mineral density
BMI	body mass index
BP	blood pressure
BW	body weight
CAD	coronary artery disease
CAL	clinical attachment loss
CBVD	cerebrovascular disease
CG	control group
CLI	critical limb ischemia
CRP	C-reactive protein
CVD	cardiovascular disease
DASH	dietary approaches to stop hypertension
DGE	Deutsche Gesellschaft für Ernährung; German nutrition society
DHA	docosahexaenoic acid
DEXA	dual energy x-ray absorptiometry
EI	energy intake
EPA	eicosapentaenoic acid
FA	fatty acids

FFI	food frequency index
FFQ	food frequency questionnaire
FMD	flow-mediated dilation
FU1	follow-up 1
FU2	follow-up 2
GFR	Glomeruläre Filtrationsrate
Hcy	homocysteine
HDL	high-density lipoprotein
IC	intermittent claudication
IL-6	interleukin-6
LBM	lean body mass
LDL	low-density lipoprotein
MMP	matrix metalloproteinase
n-3	omega-3
n-6	omega-6
NHANES	national health and nutrition examination survey
NO	nitric oxide
NO [·]	nitric oxide radical
O ₂ ^{-·}	superoxide radical
OH [·]	hydroxyl radical
PAD	peripheral arterial disease
pAVK	Periphere arterielle Verschlusskrankung
PD	periodontal disease
PE	Parodontale Erkrankung

PGU	Parodontale Grunduntersuchung
PMN	polymorphonuclear leukocytes
PT1	periodontal therapy 1 group
PT2	periodontal therapy 2 group
PUFA	polyunsaturated fatty acids
PVD	peripheral vascular disease
RDA	recommended daily allowance
RNS	reactive nitrogen species
ROS	reactive oxygen species
SFA	saturated fatty acid
sICAM	soluble intracellular adhesion molecule
sVCAM	soluble vascular adhesion molecule
TNF- α	tumour necrosis factor
WBC	white blood cell
WC	waist circumference
WHO	world health organization

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1 Zusammenfassung

HINTERGRUND:

Schlechte Ernährung wurde in der letzten Zeit vermehrt mit einem erhöhten Risiko für periphere arterielle Verschlusskrankung (pAVK) und parodontale Erkrankungen (PE) in Verbindung gebracht. PE können den Kauvorgang und damit die Nahrungsaufnahme bei Patienten stark beeinträchtigen. Der tatsächliche Einfluss einer PE auf die Nahrungsaufnahme wurde bisher nicht ausreichend untersucht. Das Ziel der vorliegenden Studie war es, den Ernährungsstatus von Patienten mit pAVK bei gleichzeitig vorliegender PE zu ermitteln. Zusätzlich wurde der Effekt einer nicht-chirurgischen Parodontitis-Therapie auf den Ernährungsstatus untersucht.

METHODIK:

Im ersten Teil der Studie wurde der Ernährungsstatus von 160 gescreenten Patienten mit pAVK und PE ermittelt. Die Patienten wurden anhand einer standardisierten parodontalen Grunduntersuchung (PGU) in 3 Schweregrade (Gingivitis, moderate Parodontitis, schwere Parodontitis) aufgeteilt. Die Ermittlung des Ernährungszustandes umfasste die Erhebung anthropometrischer Daten (Magermasse, Fettmasse gemessen mittels DEXA; Gewicht, Körpergröße, Bauchumfang), Laborparameter des Ernährungszustandes, sowie die Erhebung der Nahrungsaufnahme und Nahrungsqualität (gemessen mittels 24-h Protokoll und Verzehrshäufigkeits-Fragebogen (FFQ)).

Im zweiten Teil der Studie wurden 44 Patienten mit einer relevanten Parodontitis in 3 Behandlungsgruppen randomisiert: Parodontitistherapie mit (PT1) oder ohne (PT2) Antibiotikum und eine Kontrollgruppe ohne Behandlung für die ersten 3 Monate (CG). Der Ernährungsstatus wurde am Beginn der Studie und nach 3 (FU1) und 6 Monaten (FU2) ermittelt. Die Parodontitistherapie wurde in den ersten 3 Monaten der Studienphase durchgeführt. Kruskal-Wallis und Friedman Tests wurden für die statistische Analyse verwendet. Um Fehler durch wiederholtes Messen zu korrigieren, wurde die Bonferroni Korrektur angewendet.

ERGEBNISSE:

Die Zufuhr von Calcium, Folsäure, Vitamin A, C, D und E, Ballaststoffen und Kohlenhydraten lag deutlich unter den Empfehlungen der Deutschen Gesellschaft für Ernährung (DGE). Die tägliche Fettaufnahme unserer Patienten überschreitet die Empfehlungen der DGE um durchschnittlich 10 Prozent. Es zeigte sich kein Unterschied

im Ernährungsstatus zwischen den drei Schweregraden der PE bei den 160 Screening Patienten.

Eine signifikante Verbesserung der anthropometrischen Parameter, der Laborwerte und der Nahrungsaufnahme über den Studienzeitraum konnte nach der Parodontitis-Therapie weder mit noch ohne Antibiotikum erzielt werden. Es wurden auch keine signifikanten Veränderungen der Parameter bei der Kontrollgruppe beobachtet. Während der gesamten Studienphase blieb die Zufuhr von Calcium, Folsäure, Vitamin A, D, E und C, Ballaststoffen und Kohlenhydraten unter den Empfehlungen der DGE. Die Aufnahme von Fett lag stets über den Empfehlungen.

SCHLUSSFOLGERUNG:

Ernährungsstatus und Qualität der Ernährung zeigten bei Patienten mit pAVK und gleichzeitig vorliegender PE keinen Unterschied abhängig vom Schweregrad der PE. Die Tageszufuhr einiger wichtiger Nährstoffe lag deutlich unter den Empfehlungen der DGE. Die Ergebnisse zeigten weiters, dass eine nicht-chirurgische Behandlung der Parodontitis, mit oder ohne Antibiotikum, keine signifikante Verbesserung des Ernährungsstatus während der 6-monatigen Studienphase bewirkte.

2 Abstract

BACKGROUND:

Inadequate nutrition has been associated with an increased risk for both peripheral arterial disease (PAD) and periodontal disease (PD). PD can have profound effects on an individual's ability to chew and therefore to eat. As the influence of the extent of PD on nutrient intake in PAD patients is unknown, the primary aim of our study was to determine the nutritional status in patients with PAD and concomitant PD depending on the degree of PD. The secondary aim was to assess the effect of non-surgical periodontal treatment on nutrient intake and nutritional status.

METHODS:

In the first part of the study, the nutritional status of 160 patients suffering from PAD and PD was determined. Patients were divided into 3 groups of PD (gingivitis, moderate periodontitis, and severe periodontitis) according to a standardised basic periodontal examination (PGU). The assessment of the nutritional status included anthropometrical data (lean body and fat mass measured by DEXA; weight, height and waist circumference) and laboratory parameters of nutritional status, as well as dietary intake and quality (determined by 24-h recall and food frequency questionnaire [FFQ]).

In the second part of the study, 44 patients with periodontitis were randomised into 3 treatment groups: non-surgical periodontal treatment with (PT1) or without (PT2) antibiotics and a control group (CG), which received no treatment for 3 months. Nutritional status was assessed at baseline (BL), and after 3 (FU1) and 6 months (FU2). Periodontal therapy was administered between baseline and FU1. The Kruskal-Wallis and Friedman tests were used for statistical analysis. Bonferroni correction was applied to correct for multiple testing.

RESULTS:

Dietary intake of calcium, folic acid, vitamins A, C, D and E, fibre and carbohydrates was considerably below DGE recommendations. Average fat intake in this population was more than 10 per cent above recommendations for daily intake. There were no significant differences in nutrient intake between the PD stages in the 160 screening patients.

In the treatment groups, non-surgical periodontal therapy, either with or without antibiotics, did not produce a significant improvement in anthropometrical parameters, laboratory values or nutrient intake throughout the study period. No changes were seen in the control

group. Dietary intake of calcium, folic acid, vitamins A, D, E and C, fibre and carbohydrates was below the DGE recommendations at baseline and the follow-up visits. Fat intake continued to exceed recommendations despite periodontal therapy.

CONCLUSION:

Nutritional status and diet quality in patients with PAD and concomitant PD did not differ depending on the severity of PD. Daily intake of several important nutrients was below current recommendations. The results also indicate that non-surgical treatment of periodontitis with or without adjunctive antibiotics did not significantly alter nutritional status after a study period of 6 months.

3 Introduction

Peripheral arterial disease (PAD) has become a major contributor to the cardiovascular health care burden in recent years. It is a manifestation of atherosclerosis in the peripheral arteries and mainly affects the elderly. Its prevalence is estimated as approximately 27 million people in Europe and North America, but it is still under-diagnosed by clinicians and is often inadequately treated or even remains untreated (1). Several cardiovascular disease (CVD) risk factors can be attributed to the development of this vascular disease, which has been shown to clearly predict cerebro-vascular disease (CBVD) mortality and morbidity and to carry a high risk of vascular events (including myocardial infarction and stroke). This underscores the importance of an optimal approach to the management of PAD in clinical practice and prophylactic strategies to prevent it (2,3), including the modification of known risk factors for PAD.

Inadequate nutrient intake and nutritional behaviour have recently been associated with an increased risk and prevalence of PAD. Previous studies also suggest a role of nutrition in the development and progression of the disease (4-8). Although an adequate diet seems to have the potential to contribute to the optimal prevention of PAD, evidence of a link between the disease and nutritional status of affected individuals is still scarce. Only a small number of studies have attempted to further elucidate this matter in the past decade. Data from the National Health and Nutrition Examination Survey (NHANES) in the US, for instance, report an association between improved nutrition and a lower prevalence of PAD in the US population (6). Additionally, Gardner et al. demonstrated generally poor nutrition among PAD patients, characterized by a low intake of fibre, vitamin E and folic acid and high intakes of sodium, cholesterol and saturated fat (9). Most of the studies investigating this topic, however, only showed an association between nutrition and nutritional compounds with the incidence of PAD (4, 10), but proof of a causal relationship have yet to be provided by specific interventional trials.

Chronic PD, an inflammatory condition affecting up to 50% of the adult population (11), also seems to contribute to an increased risk of PAD. Periodontitis has already been shown to be associated with an increased risk for future cardiovascular events, with periodontal pathogens possibly involved in the pathogenesis of CVD. Chronic PD can significantly increase the inflammatory burden (11, 12). Inflammatory processes driving periodontopathogenic bacterial species might well explain the association found between PAD and periodontitis (13). The association between periodontal and CVD (CVD) is supported by recent findings, demonstrating the reversal of endothelial dysfunction, a

critical situation in the pathogenesis of atherosclerosis, with the treatment of severe periodontitis (14, 15).

The literature about the impact of nutrition on PD, however, is more conclusive. Recent findings emphasise that nutrition could modulate the level of systemic inflammation and so influence the development of PD (16). A number of studies have already shown an association between periodontitis and nutrition, and vice versa (16-18). Malnutrition and insufficient intake of essential macro- and micronutrients can significantly reduce immune function, increasing susceptibility to infection and inflammation (17, 19), and can increase the vulnerability of the periodontium to inflammatory stimuli (18). Staudte et al. showed that patients with chronic periodontitis had reduced intake of vitamin C, folic acid, magnesium and fibre as compared to healthy controls and suggested that this could be due to pain upon chewing (20).

The influence of periodontitis on nutrient intake and nutritional status is not yet clear. Tooth loss, for example, has been shown to negatively affect dietary quality and nutrient intake (21, 22). In 1994, Johansson et al. demonstrated that among older Swedes, edentulous men consumed smaller amounts of fruits and vegetables and ate less fibre (23). In a cross-sectional study of 490 community-living and institutionalized adults, vitamin levels in edentulous subjects were significantly lower (24). These findings may suggest an influence of dental condition on nutrient intake and hence nutritional status. Whether localized oral pain and discomfort per se influence nutritional status and nutrient intake in patients with PD has not yet been investigated in detail. A study by Staudte et al. showed that approximately 50% of patients with chronic periodontitis experienced discomfort in the mouth while eating (20). Reducing chronic oral pain by treating the PD could possibly contribute to a better nutritional status in affected patients. Treatment could decrease oral discomfort and patients might eat healthy foods they had previously avoided due to pain, and so benefit from a more varied diet.

The association between nutritional status and PD in patients with advanced atherosclerosis (like PAD) is unknown. PAD and periodontitis do in fact show a marked epidemiologic association (14) and are both influenced by inflammatory and immunological processes (25, 26). A reduced ingestion of essential nutrients might be a link between the pathogenesis of the two conditions.

This research project thus aims to investigate the interaction of nutritional status/nutrient intake with PAD and concomitant periodontitis. A specific research question is whether the nutritional status of PAD patients differs depending on the level of PD and if treatment of the periodontitis influences nutrient intake.

4 Peripheral arterial disease (PAD)

4.1 Definition and disease pattern

PAD is an atherosclerotic condition in the non-cardiac and non-cerebral vessels that obstructs blood flow to the limbs. The vessels in the upper extremities are less likely to be affected than those in the lower extremities. PAD is a major manifestation of atherosclerosis and its clinical symptoms range from mild extremity pain during exercise (claudication) to visible trophic changes. In the early stages, the disease remains fairly asymptomatic with clinical symptoms usually appearing only at an advanced stage of disease. (27). Other vascular complications including vasospasm, radiation fibrosis, acute arterial embolism and arterial aneurysms with thrombosis can cause non-atherosclerotic PAD, but these are only a minority of cases (28).

The most prevalent symptom of PAD is intermittent claudication (IC) with leg cramps induced by exercise, especially walking. To relieve IC, individuals with PAD need to take short rests during exercise (27). Typical claudication occurs in about one-third of all PAD patients, most commonly in the calf (29). The degree of arterial stenosis and its location define the clinical symptoms of PAD. The area of the pain or discomfort due to IC is usually located distal to the stenotic area in the artery. If the superficial femoral artery is affected, calf claudication is the consequence. Claudication symptoms can also occur in the thighs, feet, hips or buttocks, depending on the location of the stenosis (30). In a more advanced stage of the disease, when not adequately treated, critical limb ischemia (CLI) can develop, with resting pain and visible lesions including ulcers. It is a sign of severe impairment of blood flow to the limb caused by arterial stenosis and occlusion (27). CLI develops when the blood supply of the muscle is inadequate to meet metabolic demands even at rest; a typical sign is decreased skin temperature. The most severe expression of PAD manifests as trophic changes due to chronically impaired circulation in the lower extremity; untreated, it can ultimately lead to ulcers and gangrene. If ulcers or gangrene are already present, impending limb loss demands fast intervention (30).

Depending to the symptoms, PAD can be staged according to two different systems. The Fontaine and Rutherford classifications use the following categories (29) displayed in **Table 1:**

<i>Fontaine classification</i>	
Clinical	Stage
Asymptomatic	I
Mild claudication	IIa
Moderate to severe claudication	IIb
Ischemic rest pain	III
Ulceration or gangrene	IV

<i>Rutherford classification</i>		
Clinical	Grade	Category
Asymptomatic	0	0
Mild claudication	I	1
Moderate claudication	I	2
Severe claudication	I	3
Ischemic rest pain	II	4
Minor tissue loss	III	5
Major tissue loss	III	6

Table 1: Classification of peripheral arterial disease with Rutherford and Fontaine stages

4.2 Pathological processes and atherosclerosis in PAD

To understand the pathological processes involved in the development of PAD, it is necessary to start with the basic structural anatomy and history of the arteries. The adventitia, media and intima are the major layers composing the arterial wall. The adventitia is the outermost layer and is composed of connective tissue, fibroblasts, capillaries and neural fibres; it is responsible for the strength of the vessel. The media, the middle layer, is bounded by the external and internal elastic lamina; it is made of smooth muscle cells. The intima, mainly composed of endothelium, is the innermost layer of the artery that lines the luminal surface (30).

Atherosclerosis is the disease underlying the clinical manifestations of PAD. It predominantly affects medium to large arteries and causes luminal narrowing, thrombosis and occlusion, leading to ischemia. Atherosclerosis not only leads to PAD, but is also involved in many other vascular diseases, most commonly stroke, myocardial infarction and aortic aneurysm (30). It is characterized by the accumulation of lipids, cholesterol,

calcium and cellular debris in the intima as well as by endothelial dysfunction and inflammation. As a consequence of these pathological processes, plaques can form in the arteries. In addition, atherosclerosis can result in vascular remodelling, acute and chronic obstruction of the artery and alterations in blood flow that reduce the oxygen supply to target organs (31). The initial stage of atherosclerosis shows the development of the fatty streak, an early and asymptomatic foam-cell lesion that can already develop in childhood. Endothelial cells and macrophages are involved in its formation (32).

Several factors contribute to the development of atherosclerosis. One major pathogenic factor is the presence of dyslipidaemia. Lipid abnormalities have a central role in the atherosclerotic process, especially the decrease in high-density lipoprotein (HDL) and increase in low-density lipoprotein (LDL). Dyslipidaemia can impair endothelial function and vascular reactivity, damaging the vascular wall and ultimately contributing to atherosclerosis. Endothelial dysfunction has been found to be associated with PAD, and an association has also been shown between endothelial dysfunction and PAD severity, claudication symptoms, increased cardiovascular risk and cardiovascular comorbidity (33).

Atherosclerosis is a chronic inflammatory disease with altered function of endothelial cells, leukocytes and smooth muscle cells of the intima (32). It is associated with markers indicating increased systemic inflammation (28). The proliferation of smooth muscle cells and the accumulation of lipid material characterize the atherosclerotic process (30).

The functional impairment in the peripheral arteries is also an important aspect to be considered in the pathophysiology of PAD. Enhanced vasoconstriction and reduced vasomotor function contribute to the functional decline. Activation of platelet aggregation and leukocyte adhesiveness, as well as intensified release of inflammatory cytokines from tissues and the vessel endothelium also contribute to the genesis, symptomatology and progression of PAD (28).

These pathological mechanisms contribute to the development of typical symptoms such as IC, which occurs predominately during activity and can vastly limit the patient's lifestyle. During exercise, the muscle's metabolic requirements markedly change from those at rest. In healthy individuals, an increase in cardiac output and decrease in peripheral vascular resistance are enough to compensate for the muscles' increased oxygen demand. When the physiological processes are inadequate to adjust the blood flow sufficiently to the demands of the leg muscles, clinical symptoms appear during exercise.

Smaller occlusive lesions that might be unnoticed at rest can make themselves felt during exercise (28). Atherosclerotic plaques reduce the diameter of artery, limiting blood flow to the muscle and reducing the supply of oxygen and nutrients, so causing claudication. Whether an atherosclerotic plaque impairs blood flow depends on cardiac output, blood viscosity, the degree of the stenosis, and blood flow velocity (30).

4.3 Diagnosis

The diagnosis of PAD should include a physical examination, measurement of the ankle brachial index (ABI) and a treadmill or ergometer exercise test.

Physical examination comprises the palpation of the radial, ulnar, brachial, femoral, popliteal, dorsalis pedis and posterior tibial artery pulses. Another non-invasive way to diagnose PAD is the ABI, which has become a standard method to detect vascular disease. Typically, this test involves a blood pressure cuff placed just above the ankle and a Doppler device. The latter is used to measure the systolic pressure of the posterior tibial and dorsalis pedis arteries. To determine the ABI, the measured pressures need to be normalized to the higher brachial pressure of either arm. An ABI of ≤ 0.9 is considered pathological. A reduced ABI points to a hemodynamically significant occlusion somewhere between the heart and the ankle. An ABI above 1.3 reflects vascular calcification (media sclerosis), which is predominant in patients with diabetes or renal failure, whose vessels at the ankle cannot be compressed. A reduced ABI has also been found to predict the risk for cardiovascular events during follow-up (29). Still, a resting ABI can sometimes be misleading and can be in the normal range (0.91-1.30) in patients with a history of PAD or in patients with atypical extremity pain. An exercise test is then indicated.

Another way to diagnose PAD is via duplex sonography, which is a useful non-invasive and easily reproducible method to determine the degree and location of stenosis. Duplex sonography has become a widely available and is commonly used to select patients for endovascular intervention. A modern and sophisticated, but more expensive approach to determine the location of stenoses or targets for an intervention in PAD patients is magnetic resonance angiography (MRA) (34).

4.4 Epidemiology and risk factors

PAD is a worldwide disease and imposes a significant burden on the health care system. The incidence of this vascular disease increased by nearly a quarter in the past decade (35). The prevalence of PAD in the general population worldwide is between 12-14 per

cent+ and increases with age; up to 20% of patients over 75 are affected. Such individuals also show a high prevalence of coronary artery disease (CAD) and cerebrovascular disease (CVD), and symptomatic subjects with severe large-vessel PAD showed 15-fold greater mortality due to CVD (36). The number of affected individuals increases not only with age (37) but also with the number of risk factors favouring its development. These risk factors include cigarette smoking, hypertension, diabetes, hyperlipidaemia, and metabolic syndrome. All of them are also known to contribute to the development of coronary atherosclerosis (38, 39). In 2001, Hirsch et al. investigated the detection of PAD, physician awareness and intensity of risk factor treatment in primary care clinics, measuring the ABI in 6979 patients. In this population, 29% of the investigated subjects were diagnosed with PAD. Furthermore, the risk factor profiles of PAD patients with were similar to those with CVD (whereby it must be said that the risk factors for atherosclerosis are very commonly found) (39).

4.4.1 Risk factor profile

PAD patients show a broad profile of relevant risk factors. Cigarette smoking and diabetes appear to be particularly prominent in the development of PAD (37). Diabetes has been shown to be among the strongest risk factors and shows an association with higher rates of amputation and mortality.

Cigarette smoking also contributes importantly to the development of PAD and has been proven to increase endothelial dysfunction. It can alter lipid metabolism and was a strong independent predictor for repeated urgent intervention with the aim of revascularization. Unsurprisingly, the rate for complications after percutaneous interventions is higher in smokers (36) than non-smokers. Gender also appears to play a role in PAD prevalence, though gender and age-specific prevalence have not yet been fully elucidated. A higher age-related prevalence of PAD in men has been reported; however, more recent cross-sectional studies demonstrate that the total population burden of PAD may be even higher in women (40). Finally, PAD was more prevalent in African Americans than in non-Hispanic whites (38), which was not explained by higher rates of diabetes, body mass index (BMI) and hypertension (41). Data from the NHANES in the United States from 1999-2000 demonstrate a prevalence of PAD in non-Hispanic blacks of 7.9 per cent. This has been shown to be the highest among all ethnic groups (38) but remains unexplained. South Asians were also found to have a significantly lower risk for PAD than Europeans in a multi-ethnic population study (42). Other potent risk factors for the development of PAD include dyslipidaemia, hypertension, family history and genetic factors, obesity and metabolic syndrome (36) and a poor diet (39).

4.4.2 Association with CVD

Risk factors for CAD and cerebrovascular (CBVD) disease are known to resemble those for PAD (37, 43). Since the atherosclerotic disease process plays a role in all three diseases there is a considerable overlap. In the USA, 29.4 % of male PAD patients also showed the presence of CAD and CBVD, as did 21.2 % of the female individuals investigated (44). The risk of mortality in PAD patients is proportional to the severity of the disease and they are much more likely to develop CAD and CBVD than healthy individuals (37). Mortality from CVD has also increased in patients affected with PAD (45). Norman et al. in 2004 reported that the cardiovascular mortality in PAD patients was threefold to fivefold higher than in age-matched controls (46). In a prospective cohort study published by Jostens et al. in 2012, 44,985 Caucasian men with no history of CVD were followed for 25 years to study the association between four conventional cardiovascular risk factors and risk for development of PAD. It turned out that smoking, hypertension, hypercholesterolemia and diabetes were all strongly associated with risk of PAD (43).

PAD is a common disease among the elderly and is associated with an increased risk for myocardial infarction and stroke (47). Several cardiovascular risk factors contribute to the development of this vascular disease and are similar to those that increase the risk of cardiovascular events.

4.5 Treatment

4.5.1 Medical and non-surgical treatment of PAD

Non-surgical treatment of PAD aims to improve functionality of the limb and reduce progression and spread of the disease. This includes lifestyle modification, along with pharmacological and exercise therapy (48).

The elimination of risk factors and cardiovascular risk reduction are clearly the key aims and so secondary prevention is a very important issue in PAD management. To reduce morbidity and cardiovascular mortality, cessation of cigarette smoking should be considered as the primary goal, supported by pharmacological therapy (49). The reduction of cardiovascular risk also involves the use of pharmacological agents with lipid lowering (e.g. statins) and antihypertensive properties (e.g. beta-adrenergic blocking drugs among many others), as well as treatment of diabetes, including adequate foot care. Very importantly, antiplatelet therapy is indicated to reduce the risk of stroke, myocardial

infarction and vascular death. Another non-pharmacological approach involves supervised exercise training and rehabilitation for PAD patients with IC. Exercise should be done at least 3 times a week for 30-45 minutes (50).

4.5.2 Interventional treatment of PAD

When conservative treatment of PAD is not effective in reducing the life-style limitation due to IC, revascularization becomes a treatment option. Strategies for this treatment comprise surgery, angioplasty, stenting and atherectomy. In patients with CLI, revascularization is indicated to avoid amputation and improve wound healing by restoring blood flow to the limb. Several factors (including patient characteristics, severity of symptoms, likelihood of symptomatic improvement, etc.) influence the individual decision as to whether to implement interventional treatment methods or not (48). The risk-benefit ratio has to be carefully weighed before intervention (34). Through the improvement in equipment and techniques in the course of recent years, non-surgical procedures such as endovascular recanalization have become increasingly popular (48). Endovascular procedures are usually indicated in patients with life-style limiting IC without acute, critical ischemia (34).

4.6 Inflammation and oxidative stress in PAD

Several inflammatory pathways are suspected to be involved in the development of PAD. The presence of atherosclerosis is one of them and possibly the most important, and is itself considered to be an inflammatory process (26, 31). Inflammatory mechanisms and mediators are associated with CVD (51) and increased occurrence of adverse outcomes in patients with CAD (52). Their mediators are similar to those involved in the initiation and progression of PAD (53). Chronically elevated levels of inflammatory cytokines (52) and inflammatory markers seem to be associated with an increased risk of developing PAD and/or coronary events (26). These findings suggest that inflammation also plays a major role in the development of atherosclerotic processes as an underlying condition for PAD. The severity of inflammation has also been demonstrated to predict future ischemic events (53).

Another contributing factor to the progression of inflammatory and atherosclerotic processes is an increased white blood cell (WBC) count, leading to alterations of physical and chemical mechanisms. The white blood cell count, even within the normal range, has

been shown to be a major predictor for cardiovascular events such as stroke and myocardial infarction. The WBC can increase the level of oxidative stress, as has been found in patients suffering from IC. They show increased WBC aggregability, possibly due to higher levels of cellular adhesion molecules (26). An elevated level of oxidative stress markers among PAD patients has been demonstrated (54). An imbalance between NO and oxidative stress might be responsible for the reduced flow-mediated dilatation (FMD) found in PAD patients (55). An increased level of oxidative stress mediators in PAD patients may be responsible for the reduced NO generation followed by reduced vasodilatation. This may exhibit a possible role of antioxidant supplementation to counteract the oxidative stress by eliminating ROS (56).

Inflammation can also play a role in patients with claudication when it comes to exercise. A chronic inflammatory status in PAD patients might also result from the influence of exercise on inflammation. In claudicants, exercise has been shown to induce a certain systemic response characterized by neutrophil activation, increased plasma levels of soluble intracellular adhesion molecule-1 (sICAM) and soluble vascular adhesion molecule-1 (sVCAM), and by increases in endothelial dysfunction. Such responses are typical for an inflammatory reaction. The continuous inflammatory reaction induced during exercise so might contribute to the development of a chronic low-grade inflammatory state, which can then increase endothelial dysfunction (57). The post stenotic muscle ischemia induced through exercise in claudicants can produce an acute local inflammatory response with increased oxidative stress. Oxidative stress can then induce the release of inflammatory cytokines and aggravate endothelial dysfunction. These responses to physical activity in PAD patients are suggested to have adverse effects on microcirculation and skeletal muscle metabolism, thus further diminishing performance (53). On the other hand, long term exercise appears to reduce inflammation (58).

A number of recent studies have tried to further elucidate the association between inflammation and PAD. For example, in 2001 Brevetti et al. reported an elevated inflammatory status among PAD patients and the presence of endothelial dysfunction (59). The InCHIANTI study demonstrated an association of PAD with higher circulating levels of pro-inflammatory cytokines in affected individuals. After adjustment for physical activity, however, these associations were attenuated. Compared to patients without the disease levels of IL-6, IL-1 receptor antagonist, fibrinogen, and C-reactive protein (CRP) were significantly higher in PAD patients (52). These findings are supported by Silvestro et al., presenting lower FMD and higher levels of CRP and IL-6 in patients with IC than in asymptomatic individuals and controls. The degree of PAD seems to be the pivotal factor,

explaining differences in inflammatory markers and FMD between symptomatic and asymptomatic patients. The inflammatory status in each individual may also be influenced by the extent of atherosclerosis (57).

Oxidative stress has been shown to be an early trigger of atherosclerosis. It is a gradual process caused by a moderate increase in the production of pro-oxidative molecules, such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), which regulate the cellular redox status. Down-regulation of the antioxidant system can also increase oxidative stress. This increase in ROS production and subsequent elevated oxidative stress is involved in the development of endothelial dysfunction. Endothelial dysfunction is caused by the inactivation of nitric oxide (NO), oxidative damage to cellular macromolecules and activation of pro-inflammatory signalling cascades (60).

The traditional risk factors for CVD (61) and PAD (43) include hypertension, hypercholesterolemia and diabetes mellitus and are known to raise oxidative stress by increasing the production of ROS in the vascular wall (61).

Individuals affected with PAD generally have a higher level of oxidative stress than control subjects with a low burden of cardiovascular risk factors (26, 62). These patients have also been found to have a decreased antioxidant capacity, higher circulating inflammatory parameters and enhanced endothelial cell apoptosis (62). These findings emphasize the need for antioxidant therapy to improve endothelial function and reduce inflammation. Additionally, inflammatory plasma markers have a clear prognostic value for the development of vascular disease, and inflammatory mechanisms play an important role (63).

To conclude, the initiation and development of PAD is influenced by atherosclerotic processes and oxidative stress. Higher levels of inflammatory markers and oxidative stress in PAD patients indicate elevated inflammatory status.

4.7 Endothelial dysfunction in PAD patients

Endothelial dysfunction as a form of vascular impairment has become a major field of interest in cardiovascular research. It is defined as the functional decline of the endothelium (64) caused by the loss of athero-protective properties and leads to alterations in blood vessel tone (65). Characteristics illustrating a decline in endothelial function are impaired endothelium-dependent vasodilation and maintained activation of the endothelium, as characterized by increased expression of soluble adhesion molecules

in the plasma (66). The dysfunction of the endothelium induces a prothrombotic situation partly responsible for the development and progression of atherosclerosis. Recent data underline the clinical importance of maintaining a healthy endothelium (63).

Dysfunction of the endothelium has been associated with atherosclerosis and CVD (61, 67, 68). Since PAD is a major clinical consequence of atherosclerosis, a high prevalence of endothelial dysfunction among affected individuals is understandable. In fact, an association between endothelial dysfunction and PAD has been shown, as well as a correlation with disease severity, increased risk for CVD and exercise-induced claudication (33).

Endothelial dysfunction is mainly caused by reduced bioavailability or inactivation of nitric oxide (NO; a product of the endothelium itself), oxidative damage to cellular macromolecules and activation of pro-inflammatory signalling cascades (60). Apparently, it is already present in the preclinical stage of atherosclerosis (33, 67) and has been classified as a good barometer of vascular health and a predictive tool to assess cardiovascular risk (69).

Various mechanisms have been linked to endothelial dysfunction in patients with PAD. Böger et al. (70) showed increased levels of an endogenous competitive inhibitor of NO, asymmetric dimethylarginine (ADMA), in PAD patients. Increased levels of ADMA can cause a gradual decrease in NO synthesis rates, thus decreasing availability of NO. Inflammatory processes and oxidative stress also seem to play a major role in atherogenesis in patients with IC (26), since it is independently associated with PAD and contributes importantly to endothelial function (71). Reduced serum levels of nitrates and nitrites (markers of NO generation) can also be found in affected individuals (33). All of these mechanisms and pathological pathways contribute to impaired endothelial function.

Endothelial dysfunction was more pronounced with a higher degree of PAD (33). Silvestro et al. showed that asymptomatic patients had lower FMD than healthy controls, but without any difference in the inflammatory profile. In symptomatic PAD patients, however, compared to the asymptomatic group, median CRP and interleukin-6 (IL-6) were higher and FMD was lower. The ABI of asymptomatic and symptomatic PAD patients also showed a negative correlation with soluble intercellular adhesion molecule-1 and soluble vascular cell adhesion molecule-1. This means that inflammatory status and endothelial function closely reflect the severity of vascular impairment (57).

Furthermore, de Haro Miralles et al. reported that in individuals with PAD, elevated CRP appears to be an independent predictor for CVD (64) and reduced FMD has been

demonstrated to be an independent predictor for increased cardiovascular risk in patients with IC (72).

To conclude, these findings suggest that in PAD patients, several pathological pathways can contribute to endothelial dysfunction and further vascular impairment.

4.8 Influence of nutrition on cardiovascular risk and on the development and progression of PAD

Primary and secondary prevention needs to be more effectively implemented in the management of PAD. Because of the high risk for ischemic events (73), increased cardiovascular mortality (36) and higher atherosclerotic burden (74) with this disease, optimal prevention strategies should include modification of atherosclerotic and cardiovascular risk factors. Poor nutrition, as one of these risk factors, has recently gained increasing attention.

4.8.1 Nutrition and CVD

Changes in nutritional habits and diet can have a profound impact on the amelioration of traditional cardiovascular risk factors (mainly diabetes, hypertension and hyperlipidaemia) (7). The influence of nutritional intervention and dietary strategies on the prevention of CVD has already been demonstrated in several studies (75-80). For instance, high fibre intake is significantly associated with a lower risk of coronary heart disease and CVD. Insoluble fibre from vegetables and cereals is particularly important; increased fibre intake through fruits has also been associated with a reduced CVD risk (81).

The formation of specific guidelines by the World Health Organization (WHO) to decrease cardiovascular risk by modulating dietary habits underlines the importance of nutrition in CVD management. These guidelines for the prevention of cardiovascular risk recommend a diet low in total fat (<30% of calories), reduced consumption of saturated fatty acids (< 10% of calories) and trans fatty acids, reduced intake of salt (to < 5g/d) and increased consumption of fruits, vegetables and grains (82).

In 2013 Estruch et al. investigated the Mediterranean diet for the primary prevention of cardiovascular events in 7447 high-risk patients. In this study, a Mediterranean diet supplemented with either nuts or extra-virgin olive oil reduced relative risk by approximately 30% (83). The same positive effects of a Mediterranean diet were also

demonstrated by the secondary prevention Lyon Diet Heart study (84) and by Martinez-Gonzalez & Bes-Rastrollo in 2014 (85). The Mediterranean diet features high consumption of monounsaturated fatty acids, unrefined grains, nuts, fruits, vegetables, legumes and the use of olive oil for cooking and salad dressings, along with plentiful fish and little red and processed meat (7). The protective effect of this dietary pattern can most likely be attributed to its anti-inflammatory effect due to the reduction of inflammatory markers, endothelial adhesion molecules and chemokines (86, 87). Another explanation could be the high content of antioxidant polyphenols, which are found in vegetables, fruits and red wine (86), as well as the anti-atherosclerotic properties of extra-virgin olive oil. Polyphenols found in olive oil might improve the regenerative capacity of the endothelial cells and reduce endothelial damage (88). Although a low-fat diet has been promoted to prevent and control cardiovascular risk factors, current evidence favours the Mediterranean diet (89). Compliance might also be easier for patients with a diet that does not limit fat intake (olive oil) so strictly (7). The preventive effect of a Mediterranean diet in PAD patients has not yet been extensively investigated (for details on evidence see below).

The reduction of CVD risk through nutritional interventions can be explained to some extent by the modulation of typical cardiovascular risk factors. Hyperlipidaemia is a major risk factor for CVD, and nutritional interventions to improve the blood lipid profile have been demonstrated to be successful. Most significantly, a reduction in total cholesterol and LDL-cholesterol has been reported (90). A recent meta-analysis reported significant improvements of cardiovascular risk factors after dietary counselling, including reduced blood pressure (BP) and total and LDL-cholesterol levels in healthy adults (75, 91). LDL-cholesterol can be lowered with a diet that reduces the intake of total fat, cholesterol and saturated fatty acids. Plasma lipids were particularly reduced with supplements containing marine n-3 fatty acids and niacin. Furthermore, the DASH-diet (Dietary Approaches to Stop Hypertension), which is rich in fruits, vegetables, low fat dairy products and limited in added sugars and fats, produced solid evidence for positive effects on BP (92).

Adherence to high-quality dietary patterns from well-designed clinical trials such as the one that produced the DASH diet, the Mediterranean diet and the Healthy Eating Index has been associated with a substantial reduction in cardiovascular risk (7). Mechanisms accounting for this risk reduction include increased endothelial function through the actions of certain nutrients (93), reduction of low-grade inflammation and the influence of these nutrients on oxidative stress (7).

4.8.2 Nutrition and PAD

Although there is well-established evidence for an association between nutrition and CVD risk, as mentioned above, the impact of different food components on development, progression and severity of PAD remains unclear. The number of relevant studies has, however, increased in recent years and indicates that poor nutrition might contribute to the development and progression of PAD (4-10, 94-98). Primary goals of nutritional interventions to improve dietary habits are to reduce oxidative stress and enhance endothelial function, prevent disease progression and improve O₂ perfusion in muscle ischemia resulting from atherosclerosis (99).

Only a few studies have suggested an association of certain nutrients or dietary patterns with PAD. Inverse associations have been found with fibre (100), antioxidants (vitamin C and E), vitamin B6 and B12, folic acid, Vitamin D, flavonoids, dietary fats, fruits and vegetables (7).

For example, the Edinburgh Artery Study – a major cross sectional study investigating the association between ABI and dietary factors – reported a benefit from increased cereal fibre consumption resulting in a greater mean ABI in males suffering from peripheral vascular disease (PVD). A lower ABI correlated with greater consumption of meat and meat products (5).

Another cross sectional study by Lane et al., using data from the NHANES from 1999 to 2004, examined the association between the prevalence of PAD in the US and the consumption of certain nutrients. Nutrition variables were obtained from each patient through a 24-hour dietary recall interview. The results showed generally inadequate nutrition among PAD patients, with lower average caloric and nutrient intake in patients diagnosed with PAD than in participants without the disease. Consumption of antioxidants (vitamin A, C and E), polyunsaturated fatty acids (PUFA; linoleic and α -linoleic acid), total saturated fatty acids and folic acid was especially low. The intake of n-3-fatty acids, vitamins A, C, E, B₆, folic acid and fibre was associated with reduced prevalence of PAD (6). Spark et al. have also described reduced total antioxidant capacity and poor nutrition in patients with CLI (101). The consumption of specific classes of flavonoids (particularly flavonols and flavones) and flavonoids in general is also inversely associated with PAD. These plant metabolites may induce a potentially protective effect against PAD (102).

In 2011, Gardner et al. investigated the dietary intake of 46 PAD patients. The mean macronutrient composition of the participants' diet consisted of 51% carbohydrates, 30% fat and 17% protein, obtained from a seven-day food record completed by each

participant. None of them met the recommendation of the Institute of Medicine of the National Academy of Science for sodium intake. Eighty per cent of the subjects exceeded the recommendations for consumption of saturated fats. High intake of mono-unsaturated fat has also been reported to be associated with a low peak walking time. Only a few patients achieved the recommended daily fibre intake, suggesting a diet low in whole grains, fruits and vegetables. The intake of folic acid and vitamin E was low as well (9). The InCHIANTI study by Antonelli-Incalzi et al. (4) showed similar patterns: Compared to patients without PAD, affected individuals presented low consumption of folic acid, vitamins E and C, vegetables, fibre and PUFA. Gimeno et al. also confirmed a positive association of high total fat consumption with PAD and a negative association of fibre from fruits and oleic acid consumption with the disease in a Japanese population (97).

A recent cross-sectional analysis of adults in the US NHANES between 1999 and 2004, by Naqvi et al. (10), also found inverse associations between the consumption of dietary fibre, folic acid, antioxidants, vitamin B6 and the incidence of PAD. However, in contrast to earlier literature, this association did not remain after correction for total energy intake (EI) and physical activity. Naqvi et al. argue that the adjustment for EI and physical activity is necessary when assessing an association of specific nutrients with the disease. This adjustment would account for the reduced energy expenditure of PAD patients due to their physical immobility resulting in a reduced requirement for EI and subsequently a lower total EI. If the difference in EI is not accounted for, there might be a bias leading to the impression of a low consumption of essential nutrients, falsely suggesting an association with PAD (10).

Fibre

The evidence for a beneficial role of fibre in the prevention of CVD and PAD is relatively strong. There are several different types of fibre and their most important effects on the human body include increased bowel motility, suppressed carcinogenesis, lower cholesterol levels and improved glycaemic control (99). Consuming foods high in fibre such as whole grain cereals, vegetables and fruits has been associated with decreased prevalence of CVD risk factors (103). Several cohort studies have shown that of dietary fibre has protective effect with respect to myocardial infarction, stroke and development of CVD (104).

The first cardiovascular study that also showed a positive association between fibre intake and a higher ABI was the Edinburgh Artery study (5). Merchant et al. demonstrated an inverse association with PVD risk with cereal fibre consumption, emphasizing the

importance of evaluating different kinds of fibre in relation to PVD (100). This strong positive effect of fibre intake on PAD risk was confirmed by Lane et al. (6) and Gimeno et al. (97); intake of crude fibre also helped to reduce the risk of PAD (98). Fibre consumption may influence inflammatory pathways, as possibly reflected by the finding that fibre intake is negatively correlated with CRP levels (105).

The magnitude of a beneficial effect of dietary fibre intake on PAD risk is difficult to isolate, since foods containing a high amount of fibre usually also contain other beneficial nutrients, such as vitamin E and folic acid. In any case, available evidence suggests that fibre may help to prevent PAD.

Antioxidants and Vitamins

PAD patients present an increased level of oxidative stress markers, as mentioned above in 4.6 (62). A sufficient amount of antioxidants is necessary to counteract the harmful effects of free radicals originating from increased oxidative stress (106). Antioxidants are of great importance in the management of oxidative damage, since they are the initial defence against free radicals (99). The nutritional status in PAD and CLI patients is, however, often markedly impaired and it has been demonstrated that they have less total antioxidant capacity than healthy controls (62, 101). In 2000, Tornwall et al. found an inverse association of antioxidant consumption (vitamin C and α -tocopherol) and risk for PAD, suggesting a protective effect (107). This association was confirmed by Lane et al., who showed an association of antioxidants such as vitamin A, C or E with reduced odds for PAD, even in an adjusted analysis (6). Recently, the Rotterdam study, a cross-sectional study from the Netherlands, reported an inverse association between vitamin C and PAD in women; an inverse association between vitamin E and PAD in men has also been reported (96).

PAD patients have been shown to benefit from a diet that leads to higher levels of vitamin C and E (6). The association of vitamin C intake and lower risk for PAD had already been reported by Katsouyanni et al. in 1991 (98). Since the concentration of vitamin C is reduced in this patient population (4) and lower levels correlate with systemic inflammation and reduced walking distance (108), vitamin C supplementation might be beneficial. Vitamin C is suspected to be effective in the management of PAD by restoring endothelial function, though not all studies confirm this (8). Podmore et al. (109), for instance, demonstrated a pro-oxidant effect with vitamin C supplementation. Long-term supplementation with vitamin E and C did not improve endothelial function or reduce LDL oxidation in another study (110).

Vitamin E, or α -tocopherol, is a lipid soluble antioxidant and deficiencies have been associated with PAD. The deterioration of IC and impaired tolerance to ischemia are thought to be due to lower levels of this vitamin (8). Preventing cellular injury due to oxidative stress is a key function of vitamin E. It may also influence platelet aggregation and improve blood flow (99). In the InCHIANTI study, PAD patients consumed less vegetable lipids and vitamin E than patients without PAD. Although vitamin E has been found to be an antioxidant with anti-atherosclerotic and anti-inflammatory properties (4), previous studies failed to show a preventive effect on CVD (111). Since the available data are still inconsistent, vitamin E supplementation cannot at present be generally recommended (112).

Low vitamin D levels have been associated with hypertension, diabetes mellitus and other cardiovascular risk factors (113). A reduced vitamin D status has also been linked to an increased incidence of myocardial infarction and CVD in general. Vitamin D status so might also be important in PAD aetiology, partly because arterial calcification might be influenced by vitamin D hypovitaminosis (94). Melamed et al. analysed data from 4839 participants in the National Health and Nutrition survey. The aim was to evaluate the relationship between 25(OH)D and PAD. Their results demonstrated a strong association between low serum 25(OH)D-levels and the prevalence of PAD (114). This observation is in accordance with Nosova et al. (8) and Lavie et al. (115). However, although vitamin D deficiency is a critical factor in PAD patients and an independent risk factor for CVD, supplementation with vitamin D in interventional trials did not have a significant effect on cardiovascular events. Specific recommendations cannot yet be made for supplementation or screening of blood vitamin D levels and it is not yet clear whether supplementation significantly benefits CVD outcomes (8, 116).

Inadequate intake of folic acid and B-vitamins might increase the risk for PAD by impairing the regulation of homocysteine (Hcy) and disturbing its homeostasis. Increased plasma levels of Hcy are associated with inflammation, endothelial dysfunction and vasoconstriction (99). The reduced availability of folic acid can limit the remethylation of Hcy to methionine, thereby increasing its plasma concentrations. Since vitamin B6 and folic acid are cofactors and contribute to remethylation, their shortage could increase in Hcy levels even further (99). Additionally, Robinson et al. demonstrated that with a decrease in the level of Vitamin B12, vitamin B6 and folic acid, the plasma Hcy increases (117).

Among people with PAD, elevated plasma Hcy concentrations are common. Hyperhomocysteinemia is an independent risk factor for PAD (6, 118) and approximately

30% of PAD patients are affected (6). High Hcy levels are associated with an independent risk for the development of atherosclerosis and PVD (119). Low folic acid intake has been shown to correlate with a significantly higher prevalence of PAD (94). Bertoia et al. confirmed an association of higher plasma Hcy with PAD based on data from the Nurses' Health Study and the Health Professionals Follow-up Study. They included 72,348 women and 44,504 men in their analysis. Plasma Hcy values and food frequency questionnaires were used to determine the intake of B-vitamins. The results showed that Hcy levels correlated with B-vitamins in both women and men. The intakes of vitamin B6 and B12 in men were inversely associated with risk of PAD, although they were not statistically significant. Furthermore, folic acid showed an inverse, though not yet statistically significant, association with PAD risk in men, but not in women (120). Wilmink et al. also investigated the relationship between dietary folic acid and B6 intake and PAD in a population-based case-controlled study. Compared to control subjects, patients with PAD had significantly lower intake of folic acid and vitamin B6 and the consumption of these vitamins was independently predictive of PAD, even after adjustment for age, BP, cholesterol levels, diabetes, and smoking status (119).

Folic acid can be found in dark green leafy vegetables, dried beans and peas, asparagus, strawberries, peanuts and orange juice. Vitamin B6 naturally occurs in meat, fish, bread, cereals, potatoes, eggs, bananas, seeds and nuts. Vitamin B12 can be found in all animal foods (99). Since folic acid, B6 and B12 are involved in the metabolism of Hcy, supplementation with these vitamins might be beneficial. Supplementation with folic acid reduced plasma Hcy levels in PAD patients, and further supplementation with vitamins B6 and B12 might allow an even greater reduction. A strong relationship between protection from PAD and vitamin B intake has been reported (6). Lane et al. investigated the relationship between B-vitamin consumption and prevalence of PAD, demonstrating that the odds of having PAD were reduced with increased intake of folic acid. Vitamin B6 also showed a significant association with a reduced odds ratio for PAD. These findings suggest that folic acid and vitamin B help to prevent PAD (6, 99). Not all investigators, however, have reported a positive effect for folic acid supplementation and its effectiveness is not yet convincingly proven (50). More evidence is needed to form recommendations for dosage and use of these vitamins in the prevention of PAD and CVD.

Dietary fats

The consumption of the right kind of dietary fat has recently emerged as a major issue in CVD prevention. Several classes of fatty acids have been shown to influence

cardiovascular risk factors and outcomes. They comprise trans-unsaturated fatty acids, monounsaturated fatty acids, saturated fatty acids and PUFA. The latter include omega-6 (n-6) and omega-3 (n-3) PFAs (121). Supplementation with long-chain n-3 polyunsaturated fatty acids, particularly eicosapentaenoic (EPA) and docosahexaenoic (DHA) acid, has been shown to reduce systemic inflammation, as well as to protect against atherosclerosis, endothelial damage and dysfunction, and cardiac events (8). In PAD patients these fatty acids have been reported to be involved in several processes, such as increasing NO production, reducing BP, decreasing inflammatory processes, lowering triglyceride concentrations and supporting endothelial relaxation. By doing so, these fatty acids contribute to primary and secondary prevention of CVD (122). Besides EPA and DHA, another important n-3 polyunsaturated fatty acid is α -linolenic acid (8). EPA and DHA have been found to exert an anti-inflammatory effect in the vascular wall (122), and their incorporation in atherosclerotic plaques increases plaque stability and reduces macrophage infiltration (99).

People affected with PAD appear to have poor dietary habits, as mentioned in the previous section. These include inadequate intake of vegetable lipids and hence essential fatty acids compared to patients without PAD (4), while SFA and dietary cholesterol have been associated with an increased risk for PAD (98). Lane et al. demonstrated reduced consumption of PUFAs among PAD patients and suspected a protective effect of n-3 fatty acids against CAD and PAD (6). Leng et al. studied the differences in plasma fatty acid levels in PAD patients and controls. Data from the Edinburgh Artery Study were used and 113 PAD patients were compared to 122 control subjects. Particularly the intake of n-3 fatty acids (arachidonic acid, eicosapentaenoic acid, docosahexaenoic acid, and docosapentaenoic acid [DPA7n-3]) was significantly lower in PAD sufferers than in controls, but an association with the disease was only found in the case of DPA/n-3 independent of smoking habits; this fatty acid also reduced the risks associated with smoking (123).

The consumption of PUFA was associated with a reduced risk for PAD (98). A recent cross-sectional study of 6352 adults reported the association of linolenic acid with a higher ABI, as well as the association of SFA with a reduced ABI. A higher risk of PAD was found with greater dietary consumption of SFA (121). In addition, Heffron et al. showed an inverse association of nut consumption with PAD. Daily nut consumption was associated with 21% lower odds of having PAD, when compared to subjects consuming nuts less than once a week (124). Schiano et al. also investigated the effect of PUFA in patients with PAD, who received a dose of 1g b.i.d. for 3 months. A marked improvement in

endothelial function measured with FMD was reported, but there was no evidence of an anti-inflammatory effect (125).

The beneficial effect of these essential fatty acids could suggest that supplementation of n-3 fatty acids might improve clinical outcomes with PAD, but this issue is still discussed controversially. Mackay et al. (126) found no effect on platelet and endothelial activation or markers of inflammation in a 6-week supplementation trial with fish oil in patients with IC. A recent meta-analysis including 9 studies with altogether 425 participants supports this finding. The supplementation with n-3 fatty acids showed only a marginal haematological benefit in claudicants, but no differences between intervention and control groups in clinical outcomes (pain-free walking distance, ABI, blood lipids and systolic BP). N-3 fatty acids can also have unpleasant side effects: nausea, diarrhoea and flatulence; then there is also its distinctive odour (127). In any case, further interventional studies are needed to confirm whether there is any need for additional supplementation of fatty acids, especially n-3 fatty acids.

Mediterranean diet

With the Mediterranean Diet the risk for myocardial infarction and stroke can be reduced and it is an effective tool for primary and secondary prevention of CVD, as mentioned above (83), but there is little clear evidence as yet that it is beneficial for PAD patients. Ruiz-Canela & Martinez-Gonzalez demonstrated that especially olive oil has high preventive potential. This finding is consistent with an association demonstrated between antioxidant vitamins and reduced prevalence of PAD and might be attributed to the antioxidant effect of phenolic compounds found in extra-virgin olive oil (7). The adherence to the traditional Mediterranean dietary pattern showed an inverse association with the risk for developing symptomatic PAD (7, 95, 128, 129). The beneficial and protective effect of this dietary pattern is most likely due to its anti-inflammatory effect and to the reduction of adhesion molecules, as well as the high intake of polyphenols found in olive oil (7, 88). Olive oil has already been shown to be involved in the modification of immune and inflammatory responses and to reduce CVD risk factors. Consumption of extra-virgin olive oil increases the resistance of LDL to oxidation (99).

A comprehensive dietary pattern may be superior to the selection of individual food items as a form of protection against CVD and in turn PAD. To protect against atherosclerosis, the better way might be to combine different types of healthy foods to make up a balanced diet (95).

Nutritional modulation of endothelial function in PAD

Endothelial dysfunction, as mentioned above (section...), has been shown to be involved in the pathogenesis of atherosclerosis, occurring early in the atherosclerotic process (67, 130) as PAD develops (27). In the recent years, some non-pharmacological approaches to improve endothelial function, especially in high-risk patients, have been tried. These include physical activity, smoking cessation and proper diet (66). Dietary patterns and components have been shown to influence some of the essential pathways mediating endothelial function (130-132). Specific nutritional strategies can contribute to the maintenance of a healthy endothelium (93, 131, 133), also by modulating inflammatory and immune processes present in the endothelium that are involved in the development of atherosclerotic plaques (134). Recent research aiming toward improved endothelial function has particularly focused on the impact of fatty acids, antioxidants, L-arginine, folic acid and soy protein (131, 135). Antioxidants (vitamins C and E, and polyphenols) seem to play a beneficial role in reducing oxidative stress. Acute administration of folic acid appears to restore disrupted endothelial function in subjects with hyperhomocysteinemia (131).

In patients with IC, supplementation of the diet with an L-arginine enriched nutrient bar specifically designed to enhance endothelium dependent NO increases pain-free walking distance and quality of life (136).

Current dietary guidelines for PAD

Although guidelines for the nutrition-focused risk reduction for CVD in the general population are well defined, e.g. with the DASH diet, they might not be fully suitable for patients with a more advanced form of vascular disease, such as PAD. Patients with PAD have a greater systemic inflammatory burden, deficiencies in antioxidants and minerals and a more unfavourable cardiovascular risk profile (8). This patient population needs specific guidelines, with a diet that addresses particular nutritional issues associated with the disease. Emphasis should be placed on consumption of foods with anti-inflammatory and antioxidant properties, including those rich in vitamin C and E, folic acid, vitamin B₆ and B₁₂. Consumption of n-3-polyunsaturated fatty acids and dietary fibre is important, while an effort should be made to decrease intake of saturated fat and sodium (8). PAD patients should eat sufficient amounts of fresh fruits and vegetables, whole grains, soluble fibre, minimize salt intake, use high-quality vegetable oils, such as olive oil, and have at least two servings of n-3 fatty acid rich fish per week (8). The work of Gimeno et al.

supports the beneficial effect of a diet rich in oleic fatty acid and fibre and with low content of total fat on the prevalence of PAD (97).

Though all of these recommendations are incorporated in a Mediterranean dietary pattern, with its significant benefits for PAD patients, more specific recommendations cannot yet be made, given the lack of scientific evidence in this patient population.

5 Periodontal disease (PD)

Periodontal disease is one of the most common diseases worldwide and affects tissues forming the dental supporting structure, including gingiva, cementum, periodontal ligament and the alveolar bone. It comprises both gingivitis and periodontitis. These two conditions are distinguished by the presence of alveolar bone involvement. PD begins with gingivitis, which involves only the soft tissue, and progresses to periodontitis, affecting the alveolar bone. Systemic as well as local implications can result from both gingivitis and periodontitis; the main local complication is tooth loss (137).

Microorganisms located within the subgingival dental plaque are the main cause of PD. When this microbial biofilm penetrates the epithelium and initiates an inflammatory host response, periodontal tissue can be destroyed, followed by gingival recession, periodontal attachment loss and deepening of periodontal pockets (138). Ultimately there can be tooth loss. The immune response of the host to the microbiota is determined by both genetics and environmental factors (139). Chronic PD is associated with a greater risk for cardiovascular and other systemic diseases (140).

5.1 Periodontal tissue

The tissues that support and surround the tooth are defined as the periodontium. It comprises the root cementum, periodontal ligament, bone lining the tooth socket (alveolar bone) and the dentogingival junction (part of the gingiva that faces the tooth). As part of the periodontium, the latter has two layers: epithelium and connective tissue. The most important function of the epithelium is to protect the periodontal tissues (such as the cementum and alveolar bone) from the oral environment. Once the structure of the junctional epithelium is compromised, PD sets in. The hard, avascular cementum is the specialized tissue that covers the root of the tooth and attaches it to the periodontal ligament fibres. Cementum can form along the whole root of the tooth. The periodontal ligament is a soft connective tissue that covers roots of the teeth and acts as a sensory receptor to mediate the position of the jaws during mastication. A tooth is embedded in an alveolar process or socket, which is composed of an outer layer of cortical plates of compact bone followed by a central layer of spongiosa (cancellous bone) and finally the bone lining the alveolus (alveolar bone proper) (141).

5.2 Pathogenesis and predisposing factors

It is suggested that PD is an infection triggered by excessive growth of commensal microorganisms (142). The initiation of inflammation and tissue damage in PD is attributed to periodontal pathogens and other anaerobes producing a variety of enzymes, toxins and noxious waste products that harm and irritate the periodontal tissue. They are located in the biofilm that forms on the teeth (143).

Once the inflammatory process has begun, the pathogenesis of PD depends on the individual immune response (142). This response is induced by the release of virulence factors and antigens from the microbial biofilm (such as dental plaque) and the subsequent access of these organisms to the gingival tissue (144). Local tissue inflammation is usually a prolonged problem in patients with periodontitis (145). Activated cells release Inflammatory mediators including cytokines, chemokines and proteolytic enzymes, ultimately resulting in chronic destruction of periodontal tissue and bone (144). When the inflammatory process initiates the destruction of collagen structure by proteases, there will be serious damage, ultimately with tooth loss (146).

The subgingival plaques in patients with periodontitis and gingivitis harbour specific bacteria (147) including species like Veillonella, Fusobacterium, Streptococcus, Actinomyces, Treponema and possibly Bacteroides, Eikenella and Capnocytophaga (148). When gingivitis is present, the bacterial flora in the subgingival region shifts to a greater proportion of anaerobic gram-negative organisms. The micro flora of healthy periodontium mainly consists of gram-positive bacteria (149), such as Streptococcus sanguis and Fusobacterium naviforme (148). Depending on host factors (i.e. inflammatory response, oral hygiene and genetic predisposition) and environmental factors (i.e. malnutrition and smoking habits)(150), the pathological flora found in PD induces tissue destruction

The major pathological characteristics of periodontitis are (145):

- accumulation of inflammatory infiltrates in the tissues adjoining the periodontal pocket
- breakdown of connective tissue fibres anchoring the root to gingival connective tissue and alveolar bone
- apical migration of the epithelial attachment or junctional epithelium
- resorption of the marginal portion of the alveolar bone, ultimately resulting in tooth loss (145)

Page & Schroeder first described the four histopathological stages of PD in 1976: initial, early and established gingival lesions, and finally an advanced periodontal lesion (151).

The initial and early gingival lesions show the histopathological changes of an early stage of gingivitis. The initial lesion shows acute exudative vasculitis in response to a beginning microbial plaque accumulation (151). A lymphoid cell infiltrate with predominantly T-lymphocytes, as seen at sites of cell-mediated hypersensitivity reactions, is typical for the early lesion. An early lesion can then progress to an established lesion. These lesions contain B-lymphocyte and plasma cell infiltrates and manifest clinically as chronic gingivitis and periodontitis. Established lesions may remain unchanged for longer periods of time, but may also revert or progress (148).

The advanced lesion is an aggravation and is also referred to as the destructive phase, since it represents the transition from gingivitis to periodontitis (151). Clinical manifestations of gingivitis are episodic phenomena characterized by intermittent acute inflammation. The immunologic events overlap in the different stages of disease (142).

PD prevalence and severity increase with age (152), but increasing age is not necessarily a risk factor. It is not yet clear whether prevalence increases because of age per se or due to duration of disease (145). A higher prevalence of destructive periodontitis has been found in men than in women (153), but susceptibility to PD does not appear to depend on gender; presumably, the crucial factor is lifestyle (154). African Americans and Mexican Americans also showed a higher prevalence of destructive periodontitis than Caucasians (153). The negative influence of smoking on PD has been demonstrated in several cross-sectional and longitudinal studies (155-159). Other risk factors for PD include stress, genetics, systemic diseases affecting the host response (145), medical conditions such as an insufficiently controlled diabetes, osteopenia, and inadequate intake of dietary vitamin D or calcium (154). The influence of genetic factors on periodontitis is more pronounced in aggressive periodontitis than in a chronic condition (152). What is most important, however, is oral hygiene (139). Environmental, host, microbial, and genetic factors interact to produce destructive PD (145).

5.3 Gingivitis

Gingivitis is the most common form of PD, with swelling, gingival erythema and bleeding on probing (160). This first stage of PD precedes periodontitis, with loss of attachment and

alveolar bone (161). Gingivitis is an inflammatory disease affecting the soft tissues around the teeth (gingiva) (162). Pathological changes of the area surrounding the teeth are induced by microorganisms (163) through the release of bacterial toxins resulting in tissue inflammation (145) and an immune response to the microbial plaque (162). Completely normal gingival tissue free from all signs of inflammation is rarely seen and in most individuals, the gums are usually slightly inflamed due to the constant irritation by microbial plaque (145).

Certain diseases such as diabetes mellitus (162), smoking, specific drugs, hormonal changes (e.g. during puberty and pregnancy) and most importantly poor dental hygiene and increasing age (145) can all predispose to gingivitis.

Gingivitis can be classified as (164):

- 1) Dental plaque-induced gingival diseases
 - Gingival diseases mediated by systemic factors (i.e. pregnancy gingivitis)
 - Gingival diseases mediated by medications
 - Gingival diseases mediated by malnutrition
- 2) Non-plaque-induced gingival lesions
 - Gingival diseases of specific bacterial origin
 - Gingival diseases of viral origin
 - Gingival diseases of fungal origin
 - Gingival lesions of genetic origin
 - Gingival manifestations of systemic conditions
 - Traumatic lesions
 - Foreign body reactions
 - Not otherwise specified

5.4 Periodontitis

As the most common chronic inflammatory disease, periodontitis persists as a great burden and major issue in public health care (139). Severe PD affects from 15 to 20 per cent of adults aged between 35 and 44 years (165). In the UK nearly half of adults have the disease, as do 60% of those older than 65 (139). According to NHANES, from 2009 and 2010, 64.7 million adults above the age of 30 had periodontitis in the US (166).

Progression from gingivitis to periodontitis depends on the individual and can vary in duration. This disease is also influenced by the immune and inflammatory response of the host (145) and is a chronic inflammatory condition of the periodontium (144). Periodontitis is not as prevalent as gingivitis. Host susceptibility and several risk factors greatly affect the distribution of the disease and its severity (167). As with gingivitis, microbial plaques are involved in the development of periodontitis. The major difference is that in periodontitis, the supporting structures and connective tissues around the teeth are first damaged and then eventually destroyed. Gingival inflammation persists; at the same time periodontitis destroys the ligaments, soft tissues and bone, and the cementum can detach from the periodontal ligament. Clinically, the signs of periodontitis are increased probing depth, loose teeth and bleeding on probing. Ultimately, teeth are lost (145). As the many bacterial species originating from periodontal pockets spread via the bloodstream throughout the body, systemic diseases can develop (168). Not all individuals with gingivitis will develop periodontitis; it depends on their particular response to the microbial plaque (145).

Other factors that can predispose to periodontitis are smoking, diabetes mellitus and emotional stress (169). The prevalence of periodontal destruction increases with age: prevalence in adults aged 30 to 39 years is 8 per cent, and 35 per cent in those aged 60 and 69 years (153). Chronic periodontitis is the most common form of the disease (161).

Periodontitis can be classified as (164):

- 1) Chronic periodontitis
- 2) Aggressive periodontitis
- 3) Periodontitis as a manifestation of systemic diseases including
 - a haematological disorders
 - b genetic disorders
 - c not otherwise specified (NOS)

5.5 Treatment of periodontal disease

The main aetiology of both of these inflammatory diseases, gingivitis and periodontitis, is the formation of bacterial plaques that host tooth adherent microorganisms. So the primary aim of periodontal treatment is to reduce the inflammatory burden by reducing microbial plaque and controlling the accumulation of microorganisms. Gingival and periodontal tissue can only heal when inflammation has been dealt with. The most

important strategy is to explain the disease to patients and teach them effective oral hygiene (170). Regular supportive periodontal treatment should be scheduled as necessary (171).

Periodontal treatment includes both non-surgical and surgical approaches. Non-surgical periodontal therapy involves an initial deep cleaning process (scaling and root planing) to reduce inflammation and the bacterial burden. The biofilm must be removed thoroughly. The main goal of surgical periodontal treatment is to gain access to the root surface to allow cleaning (171).

Antiseptics and systemic antibiotic therapy have both proven to be successful in the treatment and control of PD by reducing the microbial burden (174). Scaling and root planing is recently sometimes combined with systemic antimicrobial treatment. The combination of antimicrobial and local therapy has been suggested to further improve periodontal parameters (i.e. probing depth and attachment loss) compared to scaling and root planing alone (173). Additional systemic antibiotic therapy may be indicated when the patient's response to mechanical periodontal therapy does not suffice. For patients with complex or mixed periodontal infection, the combination of different antibiotics and antiseptics may therefore be a treatment option (175). Systemic antimicrobial therapy is usually not necessary in simple acute gingivitis, where rinsing with chlorhexidine can suffice (176).

For most patients, a plaque control programme is sufficient to manage the inflammatory burden due to the microbial plaque; this can be supplemented as needed by non-surgical and/or surgical root debridement combined with periodontal maintenance strategies. Periodontitis treatment, however, should fight infection and restore the structures damaged by the disease. The anti-infective treatment aims to stop the progression of periodontal attachment loss by focussing on the aetiology (170).

5.6 Inflammation and oxidative stress in patients with periodontal disease

Inflammatory processes are involved in the development of PD and especially periodontitis, as mentioned above. Patients have systemic inflammation along with an increased host response and metabolic changes (172). Chronic inflammation is linked to increased production of free radicals, leading to oxidative stress. This can damage DNA

and cellular components, reduce cell repair mechanisms and ultimately result in cell death and loss of function (152).

Reactive oxygen species (ROS) have been suspected to play a crucial role in the destruction of periodontal tissue (173, 174). They emerge from molecular oxygen and if not neutralized by anti-oxidant substances, they can cause cellular and tissue damage. In chronic inflammatory conditions, ROS molecules interfere with cellular processes (174, 175); they strive to balance their unpaired electronic state and thereby damage structurally and metabolically functional macromolecules. Polymorphonuclear leukocytes (PMN) are a main source of ROS; they are suspected to be involved in the early stages of host response against bacterial pathogens in periodontitis. The term ROS covers different forms of free radicals derived from oxygen molecules; these include the superoxide radical (O_2^-), hydroxyl radical ($\cdot OH$) and nitric oxide radical ($NO\cdot$) species (174).

Oxidative stress develops due to the imbalance between anti-oxidant actions and excessive ROS formation. It has been associated with the onset of the destruction of periodontal tissue and systemic inflammation (172). A recent case-control study by D'Áiuto et al. investigated the difference in oxidative stress between 145 patients with periodontitis and 56 control subjects and found an independent association of severe periodontitis and oxidative stress as well as reduced anti-oxidant capacity in such patients (172). Other studies also found a reduced antioxidant potential and level in patients with periodontitis and gingivitis (173). Imbalances in the concentrations of antioxidants and ROS production thus seem to play a major role in the pathogenesis of inflammatory diseases and PD. There are several antioxidant mechanisms to counteract the damaging actions of ROS, including the scavenging of ROS and the direct sequestration of catalytic metal ions, which are responsible for an increased ROS formation (174).

The degradation of collagenous and non-collagenous components of the connective tissues is another problem caused by the excessive bacterial growth. This is induced by the action of bacterial toxins and antigens on circulating mononuclear cells, which are stimulated to produce pro-inflammatory cytokines (174, 176) such as IL-1, IL-2, IL-8 and TNF- α (tumour necrosis factor) (176). Inflammatory cells and resident connective tissue cells are then triggered to produce proteolytic enzymes including matrix metalloproteinases (MMP). MMP alter normal metabolism and cause degradation of components of the connective tissue; ultimately function is lost (174).

5.7 Periodontal disease and PAD

Microbial infection can spread via the bloodstream to organs and tissues distant from the actual site of infection. Chronic inflammatory processes can activate the inflammatory cascade, inducing systemic disease and initiating inflammation elsewhere in the body. PD is such an inflammatory condition and may result in increases in markers of systemic inflammation, as mentioned previously in this chapter (11). An association of periodontitis with systemic diseases, such as CVD, has already been supported by a number of observational studies (12, 177-180).

This connection between PD and CVD is confirmed by the identification of bacterial DNA in atherosclerotic plaques, emphasizing a role of periodontal pathogens in the pathogenesis of CVD (181). Inflammation, especially the systemic inflammatory response, seems to be the plausible link between atherosclerotic disease and oral infection (179, 182). Another mechanism suspected to be involved in this context is an adverse effect of systemic inflammation on endothelial function (15, 183). Endothelial dysfunction is a key pathological mechanism in CVD, and may therefore contribute to the explanation of the association between oral health and CVD (184). Treatment of periodontitis has previously been shown to ameliorate endothelial dysfunction and to improve the atherosclerotic profile (14, 15, 185). The relationship between CVD and PAD has been described in 4.4.2.

PAD is an atherosclerotic disease (186) that is induced by the same underlying pathology of atherosclerosis as other CVDs (38). Vascular inflammatory markers such as IL-6 and TNF- α have been shown to be elevated in patients with PAD. It is suggested that the level of circulating inflammatory markers correlates with the extent of atherosclerosis (187). Periodontitis as the underlying chronic inflammatory disease might be involved in the increase of serum inflammatory markers, including cytokines, IL-6 and CRP (188). As mentioned above, the inflammatory stimulus resulting from PD, especially periodontitis, is associated with CVD, but there is far less information on the association with PAD. In 1998, Mendez et al. reported a 2.27-fold greater risk for developing PAD with clinically significant periodontal diseases (189). A prospective cohort study with a 12-year follow-up period conducted by Hung and colleagues (190) demonstrated that periodontal diseases were associated with a relative risk of 1.41 for developing PAD. Another case-control study by Chen et al. suggests a possible association between periodontitis and PAD with a 5-fold greater risk for developing PAD in patients with periodontitis than in healthy controls (188). A positive relationship between PAD, determined by ABI and clinical attachment loss (CAL), as a marker for the extent of PD, has been found in a more recent

study by Soto-Barreras et al. (13). The available literature so clearly points to an association between PAD and periodontitis.

5.8 Influence of nutritional status on periodontal disease

PD is initiated by inflammatory responses and is a chronic inflammatory condition. The individual's immune system plays a key role in the development of the disease by interacting with pathogenic bacteria that stimulate immunological processes (191). One part of the host response to infection is the production of reactive oxygen species (ROS). Polymorphonuclear leukocytes (PMN) are stimulated by bacterial antigens to produce ROS during phagocytosis. These reactive molecules contribute to the oxidative damage of gingival tissue, periodontal ligament and the alveolar bone, as mentioned above in 5.6. Antioxidants, which prevent oxidation of other molecules, are needed to counteract this damage (192) and so are an important part of nutritional intake. Nutrition can influence onset and progression of periodontitis and is also involved in wound healing (193). The tissues and cells of the oral cavity are exposed to a continuing physiological process of cell regeneration (194), which requires a steady supply of essential nutrients for cell growth and differentiation.

Studies investigating the relationship of dental status with nutrition show a relationship between poor nutrition and fewer teeth in the mouth (22-24, 195), as seen with reduced vegetable, fruit and thus micronutrient intake (195). This might partly explain the association between tooth loss and CVD. Specific nutritional factors might be affected by tooth loss, particularly micronutrient and antioxidant intake. These influence the inflammatory cascade and thereby modulate both periodontal and other systemic diseases. Diet quality deteriorates when tooth loss encourages the consumption of calorie-dense and nutrient-poor foods (21). Sheiham et al. reported that patients with more teeth also had a diet richer in fat, carbohydrate, protein, fibre, calcium, iron and vitamins C and E (24).

An intact immune, system as well as local resistance at the tissue level, are important for the defence against bacterial and environmental influences. Periodontal health depends on these defences, which in turn are highly dependent on nutrition. In the following, the influence of nutrition and nutritional factors on PD will be covered.

5.9 Diet and periodontal disease

A number of studies have already shown that diet and specific nutrients play an important role in the development and progression of PD (16, 18, 21, 24, 191, 193, 196-201). This association between nutrient intake and PD can be partly explained by the influence of nutrition on the immune system and inflammatory processes (191, 196). The nutritional status of the host has a complex relationship with the inflammatory processes present in PD (196, 202). Neiva et al. also suggested an essential role of nutrition and specific nutrients in wound healing as well as an influence on periodontal status (199). Though evidence proving a direct benefit of dietary nutrients as an adjunct to periodontal therapy is still limited, it nonetheless appears crucial to prevent malnutrition or deficiency of essential nutrients in patients with PD (197).

Studies have found micronutrient depletion with reduced total antioxidant capacity in patients suffering from periodontitis (203, 204). Chapple et al. saw an association of higher serum antioxidant concentrations with a reduced risk for periodontitis (173). An association has been found between reduced micronutrient levels and periodontitis, possibly due to inadequate diet and other lifestyle factors (including smoking) (205), though it is still not clear whether periodontal patients are deficient in antioxidant vitamins (201). Administration of a multivitamin nutritional supplement improved parameters used to diagnose PD, such as periodontal pocket depth and gingival index (198, 206). Nutritional supplementation might be a valuable adjunct to periodontal treatment, but requires caution, since an overdose of supplements may also be toxic (201, 207). There is, however, no reason to give mineral and vitamin supplements to well-nourished individuals (207).

In contrast, one review by Van der Putten et al. in 2009 failed to find any consistent association between deficiencies of vitamin B complex, vitamin C, vitamin D, calcium and magnesium in non-institutionalized elderly people with PD. Since that review only covered cross-sectional studies, it could not be used to formulate any binding recommendations (208).

To summarize, an effect of nutrition and specific nutrients in PD has been demonstrated, but the literature remains inconclusive. This may be due to the lack of clarity in nutritional status assessment and of randomized controlled trials. Clear evidence for effects of nutrition on the development and progression of PD still needs to be found, as most of the literature to date only suggests associations.

5.9.1 Macronutrients

The macronutrients protein, carbohydrates and fat in our regular diet are necessary to maintain specific functions in the body and are important for regeneration, and an inadequate intake might influence PD, along with many other disorders.

Protein and its amino acids are a major component of bone, teeth, ligaments such as the periodontal ligament, and muscle. Macronutrients are also needed as components of defensive molecules to maintain host defences (including cell-mediated immunity, antibody and humoral mediated immunity, innate immunity and the complement system) (207). A deficiency in protein and especially protein energy malnutrition was observed to impair the prognosis of patients with PD by reducing the ability to produce cytokine (18). Carbohydrates are the body's primary energy source. The synthesis of the substances chondroitin, keratin and dermatan sulphates, which are important for the composition of connective tissues, also requires carbohydrates (207). But since increased intake of refined sugars has been observed to increase oxidative stress (16), a reduction in the consumption of refined carbohydrates is recommended (207).

The third macronutrient necessary for the maintenance of bodily function is fat. It is essential for providing energy and is needed to aid in the absorption of fat-soluble vitamins. Specific fatty acids such as linoleic acid and linolenic acid are essential in our diet. Dietary fat intake, however, must be controlled, particularly as regards saturated fats, since they have been reported to increase inflammation and oxidative stress when consumed to excess (16).

5.9.2 Micronutrients

Omega-3 fatty acids

N-3-fatty acids cannot be synthesized by the human body but simpler forms of these essential fatty acids can be synthesized from α -linolenic acid, which as an essential nutrient needs to be incorporated into the diet (201). Long chain fatty acids such as n-3 and omega-6 fatty acids contribute to membrane integrity and are precursors of immune system mediators such as eicosanoids that downregulate inflammation. A higher intake of n-3 polyunsaturated fatty acids is suspected to reduce the inflammatory response (209) and was shown to have anti-inflammatory and protective effects in inflammatory diseases including periodontitis (210). Due to these anti-inflammatory properties, n-3-fatty acids might help to reduce inflammation in PD. Fish oils are a particularly good source of those

fatty acids. In rats infected by *P. gingivalis*, n-3 fatty acids supported the inflammatory response and so contributed to a reduction in alveolar bone loss (207).

A 5-year longitudinal study by Iwasaki et al. demonstrated an independent inverse relationship between the intake of dietary DHA, one of the PUFAs, and the progression of PD in older people. Compared to the reference group, subjects with the lowest intake of DHA had an approximately 1.5 times greater incidence rate ratio of PD (211). Another double blind clinical study by El-Sharkawy investigated the role of dietary n-3 fatty acid supplementation for periodontal treatment. Eighty subjects with chronic advanced periodontitis were assigned to either a control group with placebo or a treatment group. The control group was treated with scaling and root planing (SRP) plus a placebo, while the test group underwent SRP followed by dietary supplementation with fish oil (900 mg EPA + DHA) and 81 mg aspirin daily. A significant reduction in pocket depth was reported, as well as a significant attachment gain after 3 and 6 months in the test group compared to baseline and control. These results suggest that dietary supplementation with n-3 PUFAs and 81 mg aspirin may enhance the effect of periodontal therapy. In other clinical trials, fish oil consumption resulted in a significant decrease in gingival index (212) and an improvement in probing depth after 12 weeks of borage oil supplementation (213). Using NHANES III data, Naqvi et al. also found a lower prevalence of periodontitis with higher intakes of DHA and EPA (214). The primary metabolites of n-3 fish oils eicosapentaenoic acid and docosahexaenoic acid are most likely responsible for improved outcomes in PD patients after n-3 fatty acid supplementation (201).

In conclusion, n-3 fatty acid supplementation and intake seem to be useful in the prevention and treatment of PD, but there is a need for further randomized controlled trials to confirm the positive effect of these fatty acids on PD.

Fibre

Low intake of dietary fibre has been shown to be associated with a poor glycaemic control, which in turn was independently related to an increased risk for PD (209), while high fibre intake improved systemic inflammation (215). Kondo et al. investigated the effect of an intervention with high-fibre and low-fat test meals consumed 3 times a day on markers of PD in high-risk subjects. The results showed significant reductions in probing depth, CAL and bleeding on probing after an eight-week intervention phase. The authors concluded that a diet high in fibre and low in fat for 8 weeks effectively improves hallmarks of PD (216). In a study of adolescent girls, a high frequency of fibre intake was associated

with a healthy gingival status. But the quantity of fibre intake did not show an association with gingival health (217).

Alcohol

Biological degradation of ethanol produces acetaldehyde, which is toxic to tissues (209), indicating that alcohol consumption might also be a risk factor for PD (218). The extent of PD in alcoholics has been shown to be greater than in non-alcoholics, but it is not yet clear to what extent light or moderate drinking affects PD risk (209). Several studies suggest a negative effect of moderate and heavy drinking on PD. Increases in the risk for CAL, gingival bleeding and probing depth have been reported (219-221), but not all observations showed a negative effect of alcohol consumption on PD. In the Copenhagen City Heart Study, there was actually an inverse association of alcohol consumption with attachment loss in men. Those findings did not agree with previous studies and did not suggest alcohol consumption as an independent risk factor for PD. Conversely, Jansson et al. (222) reported more teeth with decayed surfaces and apical lesions with a consumption of > 5cl pure alcohol per day, but the development of periodontitis and gingivitis was not increased compared to non-drinkers.

Antioxidants

Antioxidants are natural substances known to help prevent disease by counteracting free radicals. They are found as vitamins, minerals and other components of food (192). Free radicals are formed during normal metabolic processes and the amount of free radicals available is also influenced by environmental factors including smoking. A lack of antioxidants can lead to cell damage from free radicals at loose in the organism (197). As mentioned above, patients with PD have been shown to be deficient in antioxidant micronutrients (204). The key function of antioxidants is to reduce the oxidative damage due to oxidative stress that underlies the development of PD. Causes of ROS and antioxidant depletion include environmental factors and external stressors, while “antioxidant capacities” have been attributed to certain substances. In the context of PD, some such substances have been investigated including vitamins C and E, carotenoids and polyphenols. Some micronutrients not known for their antioxidant properties but investigated in relation to PD, are folic acid, the B vitamins, and n-3-PUFAS (201).

Vitamins

Vitamin E

Vitamin E is fat soluble and serves to stabilize membrane structure and terminate the free radical chain reaction (223). It is found in nuts, seeds and non-citrus fruits (224). The effectiveness of vitamin E in PD is discussed controversially. Slade et al. showed that patients with PD did not show significantly different levels of serum vitamin E than individuals without PD (223). Cohen et al. agree with this finding, reporting that a vitamin E gel for treating PD was not better than a chlorhexidine rinse and a placebo (225). A more recent study, however, demonstrated lower vitamin E levels in periodontal patients than in healthy controls. This might suggest a mitigating effect of the vitamin on inflammation of the periodontium (226). Vitamin E has further been shown to attenuate periodontal inflammation (197). In any case, the literature is not yet conclusive about the benefit of vitamin E for PD.

Vitamin D

Another vitamin possibly influencing periodontal health is vitamin D. It is important for bone development, control of inflammation, cell development and neuromuscular function (197). An adequate supply of vitamin D is necessary for optimal maintenance of bone mass and skeletal development (227). A low intake of this vitamin might play a role in PD due to its impact on inflammatory action and antimicrobial effects (228).

Chronic periodontitis improved when patients ate foods rich in vitamin D and took the vitamin as a supplement, and low dietary vitamin D intake has been suggested to have a distinct effect on related bone loss (228). Vitamin D deficiency has also been shown to result in increased inflammation, which is a common symptom of PD (229). Garcia very recently suggested that vitamin D intake might limit the progression of PD. In older men, a total intake of vitamin D ≥ 800 IU was found to be associated with lower odds of severe PD relative to an intake < 400 IU (230). For the treatment and prevention of periodontitis, adequate daily intake of vitamin D is recommended; Van der Velden et al. suggest an intake at the higher end of the daily recommended allowance (201). In 2004, Dietrich et al. demonstrated a significant inverse correlation of 25(OH)D and attachment loss in individuals aged 50 years or older (231). The fact that an association was also demonstrated between greater amounts of bleeding on probing and low serum levels of

25(OH)D in participants of the NHANES III led to the conclusion that vitamin D might decrease susceptibility to gingival inflammation due to its anti-inflammatory effect (232).

Vitamin C

Vitamin C (ascorbic acid) has been shown to have several functions such as increased polymorphonuclear lymphocyte motility (233). It is involved in the maintenance of cellular and connective tissue health (including the periodontium) and influences immunological processes and wound healing (234). This vitamin also functions as an antioxidant against ROS (173). A severe deficiency of vitamin C can lead to “scorbutic gingivitis”, a syndrome that typically results in ulcerative gingivitis, accompanied by the rapid development of periodontal pockets with tooth loss (235).

A few studies investigated the relationship of vitamin C with PD. Nishida et al. showed a weak but significant inverse relationship between vitamin C intake and PD in current and former smokers measured by clinical attachment levels. The highest level of PD was demonstrated in smokers who also had a lower intake of vitamin C (234). Reduced dietary intake of vitamin C was also associated with an increased risk for PD in the overall population upon analysis of the NHANES III data from 12,419 adults (236). Chapple et al. indicated that the risk for periodontitis was the highest in patients with serum vitamin C levels in the lowest quintile (205).

Staudte et al. investigated the effect of grapefruit consumption on vitamin C plasma levels and inflammatory parameters in periodontal patients, finding that patients with chronic periodontitis showed significantly lower plasma vitamin C levels than healthy controls. The consumption of grapefruit increased vitamin C plasma levels and improved the sulcus bleeding index (mSBI) (237). A study by Kuzmanova et al. also described a lower plasma concentration of vitamin C in patients with periodontitis than in healthy controls (238). This finding corroborates with an inverse association found between plasma vitamin C levels and the severity of periodontitis in a Japanese (239) and an Indonesian (240) population. Sheiham et al., who reported a significant association of plasma ascorbate with dental status, further supported this relationship (24). Current evidence thus indicates that vitamin C supplementation is beneficial in reducing gingival inflammation when the upper recommended limits are not exceeded (201). Yet despite extensive literature, there is no firm evidence that vitamin C supplementation as an adjunct to conventional periodontal therapy provides an additional benefit (197).

Vitamin B

Vitamins of the B-complex have also been suggested to play a role in PD by positively affecting periodontal wound healing, and a relationship to tooth loss has been observed (199, 241). In a longitudinal study from 2003, Hung et al. demonstrated a relationship between the consumption of fruits and vegetables containing essential nutrients including vitamins of the B-complex, and tooth loss in male health professionals (241). Supplementation for 60 days with a multivitamin compound (including folic acid, vitamin B12 and vitamin C, among others) in patients with early periodontitis also showed significant reductions in gingival index, bleeding index and pocked depth (198).

Another cross-sectional study using the data of 879 non-institutionalized adults from the NHANES survey reported an independent negative association of serum folic acid levels with PD (242), although, due to the cross-sectional design, a causal effect on PD could not be found (208). Esaki et al. demonstrated a significant negative correlation between dietary folic acid levels and bleeding on probing in 497 Japanese adults with 20 or more natural teeth. This suggests that dietary intake of folic acid might play an important role in preventing gingival bleeding in adults (243). Edemir & Bergstrom also reported an association of low folic acid intake levels with gingival bleeding in non-smoking adults (244).

It is reasonable to assume that deficiencies of vitamin B12 and folic acid would have an impact on the proliferation and function of immune cells, since they are involved in RNA and DNA synthesis. These two vitamins have a central role in cell growth and proliferation (196), and so are importantly involved in immunological processes and cell regeneration.

Specific vitamin supplements including vitamin B-complex seem to play a major role in wound healing by modifying key cellular events (199). In parallel, the literature suggests a possible role of vitamins of the B-complex in PD (197). Further and well-designed studies are necessary to provide conclusive data to elucidate the pathophysiology and prove the beneficial effect of vitamin B supplementation in periodontal therapy.

Mineral substances

Zinc

Antimicrobial properties has been attributed to zinc since the zinc cation glycolyzes bacterial proteases (197). The mineral has also been demonstrated to work as an

immunity booster and to be involved in wound healing (245), which is particularly important in PD (246). In 1984, Harrap et al. assumed that zinc might reduce the growth rate of plaque bacteria and decrease plaque growth. This was attributed to its effect on the metabolic activity of plaques (247). A zinc deficiency might impair immune system function, resulting in a reduced ability to counteract infection and inflammation (196), which of course would be detrimental in PD.

It is recommended that patients affected with PD should include zinc in their diet, due to its effect on immune function and plaque accumulation (197). However, dietary supplementation with zinc should be considered with caution, since excessive intake of the mineral can have adverse health effects (196).

Calcium

Calcium deficiency has been shown to result in bone loss and increased inflammation. Since these two consequences of calcium deficiency are also characteristic of PD, calcium deficiency might be a risk factor for PD (229). Optimal levels of calcium have been shown to improve periodontal health (199). Data from the NHANES III study showed that PD is more severe with low dietary intake of calcium. In young adults and in 40- to 59-year-old men there was a significant association between calcium level and CAL and Nishida et al. reported an increased risk for PD in these patients. They suggested that this finding might be linked to the association of decreased alveolar bone density with inadequate calcium intake (236). On the basis of the same database, the prevalence of periodontitis showed an inverse relationship with dairy products, known to be an essential source of calcium and other important nutrients (248). Consumption of foods containing lactic acid also showed a possible benefit for patients with PD (249).

Al-Zahrani investigated the association between intake of dairy products and prevalence of PD in the 12,764 NHANES III participants. There was a negative association between the prevalence of periodontitis and dairy product consumption and hence calcium intake. This was seen even after adjustment for major risk factors. Subjects in the highest quintile of dairy product intake compared to those in the lowest quintile were 20% less likely to be affected with periodontitis (248). This finding is in agreement with previous studies, suggesting that periodontal health and tooth loss are beneficially influenced by higher calcium intake (250). Dietary calcium intake below the recommended values also showed an association with tooth loss (249). Additionally, in the United States a higher prevalence of periodontitis and more severe forms of the disease were associated with low intake of

dietary calcium. This led the authors to the assumption that calcium might have an indirect effect on PD, since calcium intake affects both skeletal and alveolar bone density (236).

Magnesium

Magnesium consumption by pregnant Japanese women was associated with reduced tooth loss (251), and Meisel et al. observed a relationship between magnesium and PD. Increased serum magnesium levels decreased probing depth accompanied by less severe attachment loss (252). Dietary magnesium intake was shown to be suboptimal in a sample of 4257 participants in the NHANES III study. Beneficial effects of magnesium on PD have been reported, suggesting that an increase in dietary magnesium intake should be recommended. The mineral can be found in nuts, seeds, green vegetables, whole grains and meat (253).

5.9.3 Malnutrition and periodontal disease

It is important to provide adequate energy and amounts of nutrients to meet the increased nutritional requirements in patients with PD. These increased demands result from the synthesis of acute phase proteins, antioxidant defence, inflammatory mediators and the need for tissue restoration and repair (197, 254). With a deficiency in nutritional status and nutrient intake, the host cannot provide an adequate inflammatory response. A balanced diet is necessary and contributes to the successful treatment of PD (234), but most importantly, the underlying inflammatory stimulus from the dental plaque needs to be eliminated to reduce the severity of the PD (18, 234). Wound healing is another process influenced by malnutrition (199). With malnutrition, tissues are depleted of essential nutrients needed for anti-oxidative effects (255), with immunosuppression and disease progression as consequences; this in turn could exacerbate the severity of oral infections (191). Furthermore, protein energy malnutrition has been shown to reduce cytokine production (18).

Undernourished patients showed more rapid progression of PD (18). A diet low in free sugars and fat, containing high amounts of fruits, vegetables and wholegrain starchy foods may be beneficial for periodontal and oral health in general (256).

5.9.4 Dietary guidelines

It is widely accepted that good nutrition is very important for good overall health. A variety of systemic diseases and conditions are influenced by nutritional factors, such as Type 2 diabetes, hypertension, dyslipidaemia and overweight, as well as several oral diseases (257).

To date, it has neither been determined whether specific dietary recommendations for PD patients need to be formulated, nor whether common guidelines for a healthy diet are sufficient to meet the requirements for macro- and micronutrients with this particular disease. Too little evidence is available to initiate the adaptation of existing general dietary guidelines developed to support health and disease prevention, in order to promote optimal periodontal health (209).

Only a few recommendations for nutrition and the intake of specific nutrients in PD have been officially formulated:

- 1) With the consumption of simple sugars and saturated fat, oxidative stress and downstream inflammation can be elevated via the increase in superoxide generation as a side effect of ATP synthesis and receptor binding of neutrophils. Dietary intake of sugar (especially refined carbohydrates) and fat must therefore be controlled to prevent oxidative stress and downstream inflammation (16) and to decrease the oxidative burden in patients with PD.
- 2) Foods containing antioxidants such as green leafy vegetables, berries, red wine and dark chocolate with > 70% cocoa help to limit oxidative stress (16).
- 3) Patients should be advised to consume more fish oils (n-3-PUFAs), fibre, vitamin D, calcium and natural antioxidants, vegetables and fruits to aid PD prevention and treatment (72,76).

Nutritional recommendations contributing to dental and overall health include a diet rich in vegetables and fruits, nuts, fish and whole grain, as well as unsaturated fatty acids (256, 257). Additionally, as mentioned above, supplementation and the increased intake of n-3 fatty acids might have benefits for PD outcome (207, 210).

Specific recommendations for the amounts of the mentioned micronutrients have not yet been formulated. Further studies are necessary to determine the optimal recommendations for patients with PD.

6 Methods

6.1 Subjects and study design

This investigation is a sub study of the PeriPAD trial, a single-center, prospective, randomized, open trial conducted at the Department of Angiology, Medical University Graz, Austria, to investigate the influence of periodontal therapy on vascular inflammation and function in patients with peripheral arterial disease. PeriPAD was registered as a randomized controlled trial at the DRKS (Deutsches Register Klinischer Studien; https://drks-neu.uniklinik-freiburg.de/drks_web/) ID: 00004554. Ethical approval granted by the Ethics Committee of the Medical University of Graz, Austria (EK-Nr. 24-456 ex 11/12). The study was performed in full accordance with the requirements of the Declaration of Helsinki and the Good Clinical Practice Guideline. All participants included in the study gave written informed consent before participation.

In this research project, a cohort of 160 consecutive patients (120 (75%) men and 40 (25%) women) with symptomatic peripheral arterial disease was screened for the presence of a concomitant PD. They were aged between 42 and 83 years. Recruitment was from March 2013 until January 2015. Patients were recruited from the outpatient clinic and inpatient ward of the Department of Angiology as well as from the outpatient clinic of the Department of Vascular Surgery at the Medical University Graz. Communication with the participants was done either via telephone or in person and the screening was performed in the morning after an overnight fast at the outpatient clinic for preventive vascular medicine at the Department of Angiology. Patients who had recently undergone a percutaneous transluminal angioplasty were scheduled for a screening appointment at least 2 weeks after hospital discharge to avoid any influence of inflammatory processes resulting from the endovascular intervention. Patients were enrolled in the study when they met the following key inclusion criteria:

- i. Symptomatic PAD
 - a. PAD Fontaine's stages II (intermittent claudication) and documented luminal stenosis >70% on ultrasound or angiography
 - b. PAD Fontaine's stages III (critical limb ischemia) and documented luminal stenosis >70% on ultrasound or angiography
- ii. Severe periodontitis as defined by the presence of at least 12 natural teeth, including third molars, with at least two teeth with probing depth ≥ 5 mm; at least

two teeth with interproximal clinical attachment loss $\geq 5\text{mm}$; and $\geq 20\%$ of sites that bled on probing, excluding teeth scheduled for extraction

- iii. Signed informed consent form

The exclusion criteria were defined as:

- i. PAD Fontaine's stage IV (tissue damage/loss)
- ii. Life expectancy < 6 months
- iii. Unstable cerebrovascular and CVD
- iv. Clinically apparent infectious disease (e.g. pneumonia, symptomatic urinary tract infection)
- v. Systemic inflammatory disease (e.g. chronic inflammatory bowel disease, rheumatoid arthritis, vasculitis by clinical assessment)
- vi. Periodontal treatment within 6 months prior to the study
- vii. Mouth infection other than periodontitis
- viii. Uncontrolled diabetes
- ix. Pregnancy
- x. Age < 18 years
- xi. Consumption of drugs known to affect periodontal status (anticonvulsants, immunosuppressants)
- xii. Allergy to penicillin and/or metronidazole

Patients who met the inclusion criteria were eligible to be enrolled in the study and were randomized into one of three groups, two of them involving non-surgical periodontal treatment (n=44):

Group 1 (PT1): patients received periodontal therapy including the use of antibiotics.

Group 2 (PT2): patients received periodontal therapy without antibiotics.

Group 3 (CG): was designed as control group with patients receiving no specific therapy for the first 3 months of the study period.

Participants were asked to continue their regular medications and eating habits during the study phase and were free to follow any other medical care regimens. Participants who did have PAD in stages II and III, but did not fulfil the dental characteristics mentioned above were listed as "screening patients".

In the first part of this dissertation, the differences in nutritional status in patients with PAD depending on the level of PD (gingivitis, periodontitis and severe periodontitis), will be covered. The second part will highlight the effect of periodontal therapy on the nutritional status of PAD patients.

6.2 Baseline testing

After recruitment the participants were scheduled for an appointment at the outpatient clinic for preventive vascular medicine. At the first visit a careful general medical history was taken (see appendix A1). Clinical examination included ankle brachial pressure index (ABPI), BP in supine position, weight, height, waist and hip circumference (see appendix A2), pulse wave velocity and oscillometric ABI, body composition via dual energy x-ray absorptiometry as well as the assessment of dietary intake (FFQ and 24-hour recall). A fasting blood sample was taken from each patient. If patients were diagnosed with PAD and if at they had least one original tooth, they were sent to the Division of Prosthodontics and Periodontology, Dental School, Medical University of Graz, where a preliminary dental chart was compiled to determine fulfilment of the dental inclusion criteria. Patients who were diagnosed with PAD but did not fulfil the dental requirements were listed in the database as “screening patients”.

Patients who fulfilled inclusion criteria were eligible to be included in the PeriPAD study and were asked to attend another visit at the outpatient clinic, where they were randomized into one of the three patient groups described above (PT1, PT2, and CG). In the following study period, which lasted at least three months, participants allocated to one of the two treatment groups received extensive periodontal therapy either with or without antibiotics. Patients in the control group were asked not to see a dentist for periodontal treatment during the study phase. An overview of the study design is given in **Figure 1**.

6.3 Follow-up

After a 3-month study period, participants were again scheduled for a follow-up visit to the outpatient clinics of the Division of Angiology and the Division of Prosthodontics and Periodontology. All examinations done at baseline were repeated by the same operator at the follow-up visits. A second follow-up visit was scheduled 6 months after inclusion in the study.

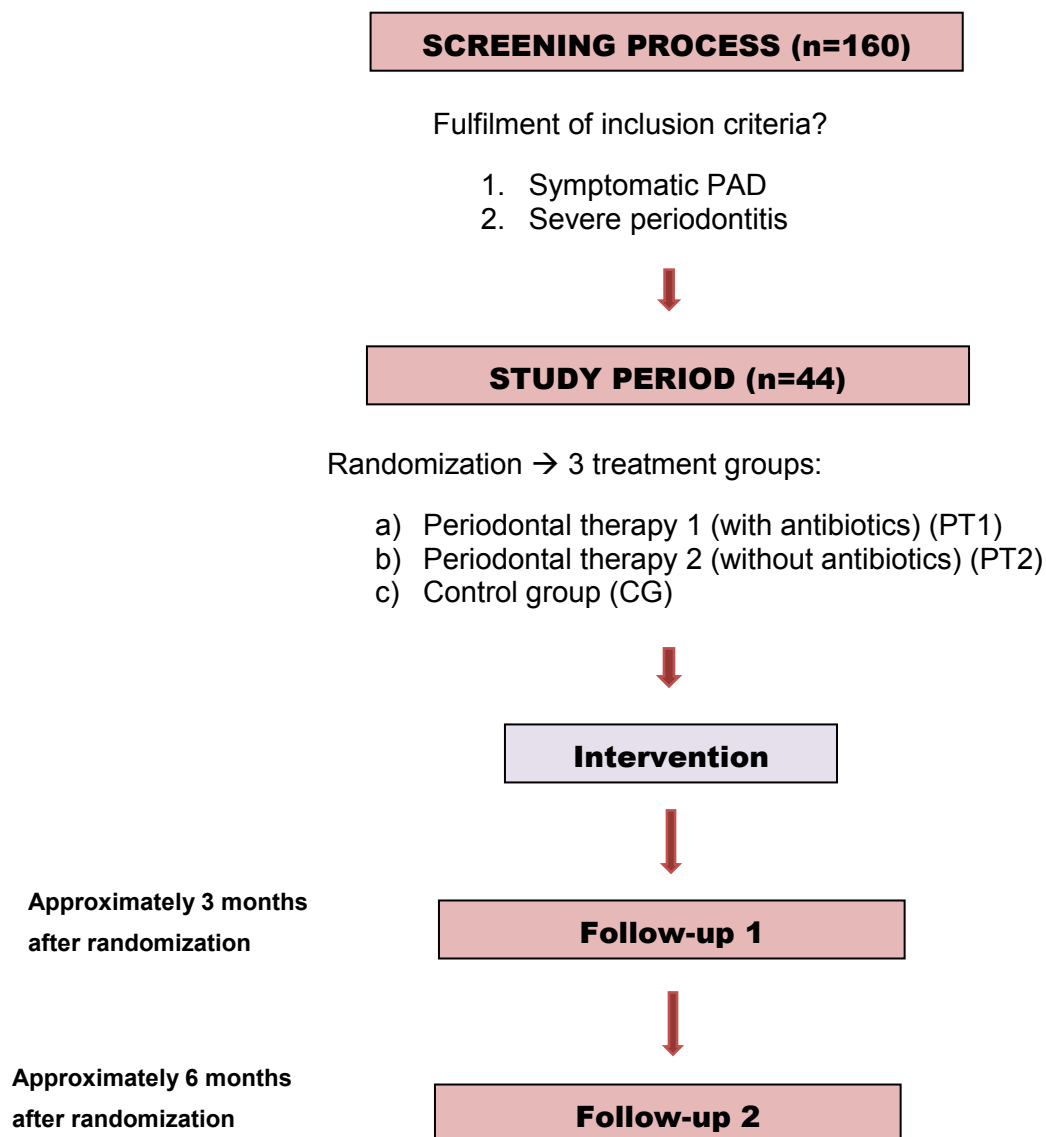


Figure 1: Study design; PAD = peripheral arterial disease

6.4 Anthropometric Measurements and blood pressure

At each visit, participants' standing height (cm) and body weight (BW, kg) were measured with a stadiometer (SECA®-220, Hamburg, Germany) and an electronic scale (WPT 100/200 OW personal scales, Radwag®, Poland). Height and weight were used to calculate the Body Mass Index (BMI kg/m²). Additionally, waist circumference (WC; at the midpoint between the anterior superior iliac crest and the lowest rib) (258) and hip

circumference (HC; at the level of maximal gluteal protrusion) was measured in a standing position using a stretch resistant measuring tape. Also, systolic and diastolic BP (mmHg) was measured in a lying position.

6.5 Body composition measurement

Body composition was measured with Dual-Energy X-ray Absorptiometry (DEXA) (Lunar iDEXA, General Electric Healthcare, USA) whole body scans at baseline and follow-up. DEXA is accurate and reproducible (259) and is a convenient and non-invasive diagnostic tool to assess body composition (260). Body composition is defined as the proportion of BW from fat in relation to BW from lean tissue (261).

The measurement of bone mineral density (BMD) and body composition by DEXA underlies the assumption that the body is a two-compartment model consisting of soft tissue (muscle, fat, skin, water) and bone (minerals) (260). DEXA with Lunar systems works by generating X-rays at two different energy levels (high and low) produced by a constant-potential X-ray generator to differentiate bone and soft tissue. Since X-rays at varied energies are attenuated differently when passing through the subject, body composition can be determined and bone mineral content (BMC) and soft tissue differentiated (262). Material with low density, such as soft tissue, attenuates the X-ray beam less than high-density material (i.e. bone) (260). The mass and composition of each pixel is mapped during the scanning process with regard to bone mineral, fat and fat-free soft tissue (263).

In all measurements the same software (Lunar iDEXA enCORE software version 14.10; GE-Company, Madison, WI, USA) and device were used according to the manufacturer's instructions. The subjects were asked to remove all objects such as watches, eyeglasses and jewellery so as not to compromise the results; during the scanning procedure they were only allowed to wear their underwear. All DEXA measurements were taken by a trained technician. A spine phantom block was used for quality control every morning. Fat mass (per cent and total), lean body mass (LBM), BMC and BMD were measured for trunk, legs (left and right) and the whole body.

6.6 Assessment of Ankle Brachial Index (ABI)

The Doppler technique (264) and the ABI calculation were used to assess the manifestations of PAD. The ABI has been widely acknowledged as a non-invasive, simple and reproducible test for the confirmation of a clinical diagnosis of PAD and its quantification. It is a measure of the BP in the leg arteries relative to brachial systolic pressure. The ABI is calculated by dividing the systolic BP measured at the ankle by the systolic BP measured in the brachial artery ABPI (265).

$$\text{ABPI} = \frac{\text{Ankle systolic blood pressure}}{\text{Brachial systolic pressure}}$$

A 5-mHz Doppler device (Dopplex[®] MD2, Huntleigh Diagnostics Ltd., UK) was used to determine the pressure at the ankle. Participants rested in a supine position for at least 5 - 10 minutes before measurement of systolic and diastolic BP in the upper extremities. BP in the lower extremities was measured at the anterior and posterior tibial arteries. Patients with an ABPI < 0.90 or less were diagnosed with PAD (265).

6.7 Periodontal examination

The periodontal screening examination included the assessment of the general oral and dental history, determination of the PGU (Parodontale Grunduntersuchung, basic periodontal exam) and an orthopantomogram. All clinical parameters were assessed by trained periodontists. The patients were categorized into 5 grades of PD:

Stage 0: healthy

Stage 1: gingivitis

Stage 2: gingivitis with calculus

Stage 3: moderate periodontitis

Stage 4: severe periodontitis

Grading was done according to the PGU, which is based on the CPINT (Community Periodontal Index of Treatment Needs), an internationally accepted method to examine the periodontium (266). Periodontal parameters were recorded at baseline and at follow-up 1 (FU1).

If advanced periodontitis was present it was scored using established criteria (267). These criteria included: the presence of at least 12 natural teeth, including third molars with at least two teeth with probing depth ≥ 5 mm; at least two teeth with interproximal clinical attachment loss ≥ 5 mm; and $\geq 20\%$ of sites having bleeding on probing, excluding teeth scheduled for extraction.

Patients who had been allocated to the periodontal therapy groups (PT1, PT2) underwent comprehensive periodontal diagnostics including clinical measurements (bleeding on probing, attachment loss, probing depth, etc.); microbiological and immunological analyses were also performed. Patients in these two groups received conservative non-surgical periodontal therapy (268, 269) (full mouth disinfection), either with oral antibiotics (amoxicillin 500 mg + clavulanic acid 125 mg and metronidazole 500 mg) or without. The disease was explained in detail and the subjects were instructed in appropriate oral hygiene. Subjects allocated to the control group were asked not to visit a dentist during the 3 months of the study and to continue their normal oral hygiene. All participants were re-evaluated 3 months after randomization (comprehensive periodontal diagnostics including clinical measurements, as well as microbiological and immunological analysis).

6.8 Assessment of dietary intake

At the baseline visit (BL), follow-up visit 1 (FU1) and follow-up visit 2 (FU2), dietary intake was assessed with a 24-hour food recall (24-h recall) and a 36-item self-administered qualitative food frequency questionnaire (FFQ). Participants were asked to report the frequency of consumption of the 36 listed food items during the previous 3 months ranging from “almost never or <1 /month” to “ >3 times/day”. A comprehensive food list of the FFQ used in this study is shown in the appendix (A3). A similarly designed self-administered FFQ has recently been validated and published and proved a good measure of dietary quality in a population of people aged 55 and older (270). The information derived from our FFQ was used to calculate the food frequency index (FFI). Development of this score has been described elsewhere (270).

However, since elderly patients sometimes had problems understanding questions or filling out the questionnaire by themselves, a trained dietician was available to explain things and help them and finally to check the FFQs for completeness. Additionally, the same dietician undertook the structured 24-h recall with the patients. Nutrient intake from 24-h recall was determined using Nut.s software (nutritional.software, dato Denkwerkzeuge, Vienna, Austria). This program was supplemented with information on

allergens, commercial foods and local foods and includes databases such as the BLS (“Bundeslebensmittelschlüssel”), USDA database and ÖNWT (“Österreichische Nährwerttabelle”). Our results were compared to the recommendations of the DGE (Deutsche Gesellschaft für Ernährung; German nutrition society).

6.9 Laboratory measurements

At baseline, FU1 and FU2 a venous blood sample was drawn from the antecubital vein of each patient in the morning after an overnight fast. Blood samples were collected in vacutainer tubes for routine blood work and clinical chemistry. Samples were also taken to determine a lipid profile and other exploratory markers. Analysis was performed in a certified in-house laboratory in close proximity to the study site to guarantee rapid processing. Laboratory methods or values were not changed during the study. Automated analyzers (Cobas® 8000, Roche, Germany; ARCHITECT®, Abbott GmbH & Co. KG, Germany; XE 5000®, Sysmex, Canada; BNA II nephelometer analyzer, Siemens, Germany; IDS-iSYS immunoassay system, IDS, Denmark) were used to measure serum concentrations of triglycerides, high-density lipoprotein, cholesterol, Lp(a), transferrin, ferritin, albumin, total protein, iron, glucose, haemoglobin, calcium, CRP, vitamin B12, folic acid, vitamin D, potassium, magnesium, prealbumin, CHOL/HDL ratio, transferrin, interleukine-6 and GFR (glomerular filtration rate). The Friedewald formula was used to determine low-density lipoprotein cholesterol (LDL-C) concentrations. The laboratories were certified according to ISO 9001:2008.

6.10 Statistical analysis

Part 1:

Statistical analyses were performed as follows. For descriptive statistics median and interquartile range (25th – 75th percentile) were calculated, due to the not normally distributed data. Normal distribution was tested by the Kolmogorov-Smirnov test. The Kruskal-Wallis test was used to determine differences in anthropometric measurements, laboratory markers and dietary intake between the three PD groups (gingivitis, moderate periodontitis, and severe periodontitis). A p-value of <0.00138 was considered statistically significant after Bonferroni correction (271) to account for multiple testing. All analyses were completed with SPSS for Windows version 22 (SSCP Inc, Chicago, Ill). Data are presented as median (25th – 75th percentile) or percentages.

Part 2:

Statistical analysis involved the testing of normal distribution with the Kolmogorov-Smirnov test. To test the overall change of laboratory markers, anthropometric measurements and dietary intake from baseline to the follow-up visits in the three treatment groups, the Friedman test was used due to the not normally distributed data. A p-value of <0.00066 was considered statistically significant after Bonferroni correction (271) to account for multiple comparisons. All analyses were completed with SPSS for Windows version 22 (SSCP Inc, Chicago, Ill). Data are presented as median (25th – 75th percentile) or percentages.

7 Part 1: Nutritional status in patients with PAD depending on the level of periodontal disease

7.1 Introduction and aim

Nutrition plays a role in both PD and PAD. The influence of nutritional factors on the pathophysiology of both diseases and their importance in disease management has been explained in the preceding chapters.

PD can have profound effects on an individual's chewing ability. When dental status and oral pain and discomfort make it difficult to eat certain kinds of food, they tend to be avoided (20, 195). This can affect nutrient intake and ultimately lead to inadequate supply of essential nutrients (22). Foods that are problematic in patients with PD because they are difficult to chew or cause pain in the mouth include apples, citrus fruits, nuts, and whole grain bread, among others (195, 202). Acute, chronic and systemic diseases with oral manifestations and oral infectious diseases can influence the functional ability to eat and so affect nutritional status. Equally, an individual's diet and nutrition can impact the progression of oral diseases and integrity of the oral cavity (200).

Inflammatory processes are involved in the pathology of PD and PAD and can be ameliorated with specific nutrients containing antioxidants (106). PAD patients have also been shown to be in a state of high oxidative stress as well (62), where nutritional antioxidants are also important (106). The severity of PD in patients with PAD might therefore have an influence on their nutritional status, with inability to consume the necessary nutrients resulting in a poor diet.

In this context, the first part of this dissertation aimed to investigate whether the degree of PD in PAD patients has an influence on the patients' nutritional status. The hypothesis was that with a more severe form of PD, oral pain and discomfort increase and nutritional status deteriorates, possibly due to inadequate intake of indispensable nutrients.

7.2 Results

7.2.1 Subjects

For this research project, 165 patients were recruited. We excluded 5 patients due to incomplete data, leaving 160 subjects for analysis (male 120 (75%), female 40 (25%), median age 62 years, range 56-70 years). Twenty-nine per cent of the patients had

diabetes, 19% were obese (BMI above 30) and 39% were currently smoking. Hyperlipidaemia and hypertension were seen in 78% and 73% of the patients, respectively. Patient's characteristics are shown in **Table 2**.

Table 2: Patient characteristics (n=160)

Age (y)	62 (56 - 70)
Weight (kg)	80.0 (69.0 - 89.8)
Height (cm)	172 (166 - 176)
BMI (kg/m²)	27.04 (24.4 - 28.7)
Waist to hip ratio	1 (0.95 - 1.04)
Body fat (%)	34.4 (29.9 - 37.7)
Lean body mass (kg)	50.5 (43.4 - 57.9)
ABI right	0.79 (0.55 - 1.00)
ABI left	0.86 (0.59 - 1.00)
Diabetes (%)	28.7
Obesity (%)	18.8
Active smokers (%)	39.4
Hyperlipidaemia (%)	78.1
Arterial hypertension (%)	72.5

*Values are given as median (25th, 75th percentile) or as otherwise indicated; BMI = Body Mass Index; ABI= Ankle Brachial Index

7.2.2 Anthropometrics and body composition

The three PD groups showed no significant differences in anthropometric measurements (weight, height, BMI, LBM, fat mass, waist to hip ratio). Median ages were 67 (58.5 – 74.5) years, 63 (57.5 - 71.3) years and 61 (55 - 67.8) years for patients with gingivitis, moderate periodontitis and severe periodontitis, respectively ($p=0.094$). BW was slightly higher in the moderate periodontitis group, with 86.5 kg (76.3 – 97.3), although this difference was not significantly different after Bonferroni correction ($p=0.013$). Median body fat per cent and LBM measured with DEXA were the highest in the moderate periodontitis group (36 [29.9 – 38.7] % and 55.5 [48.5 - 61.0] kg, respectively), yet again did not reach statistical significance after Bonferroni correction ($p=0.744$ and $p=0.012$, respectively) (**Figure 2**). Data for anthropometric measurements are shown in **Table 3**.

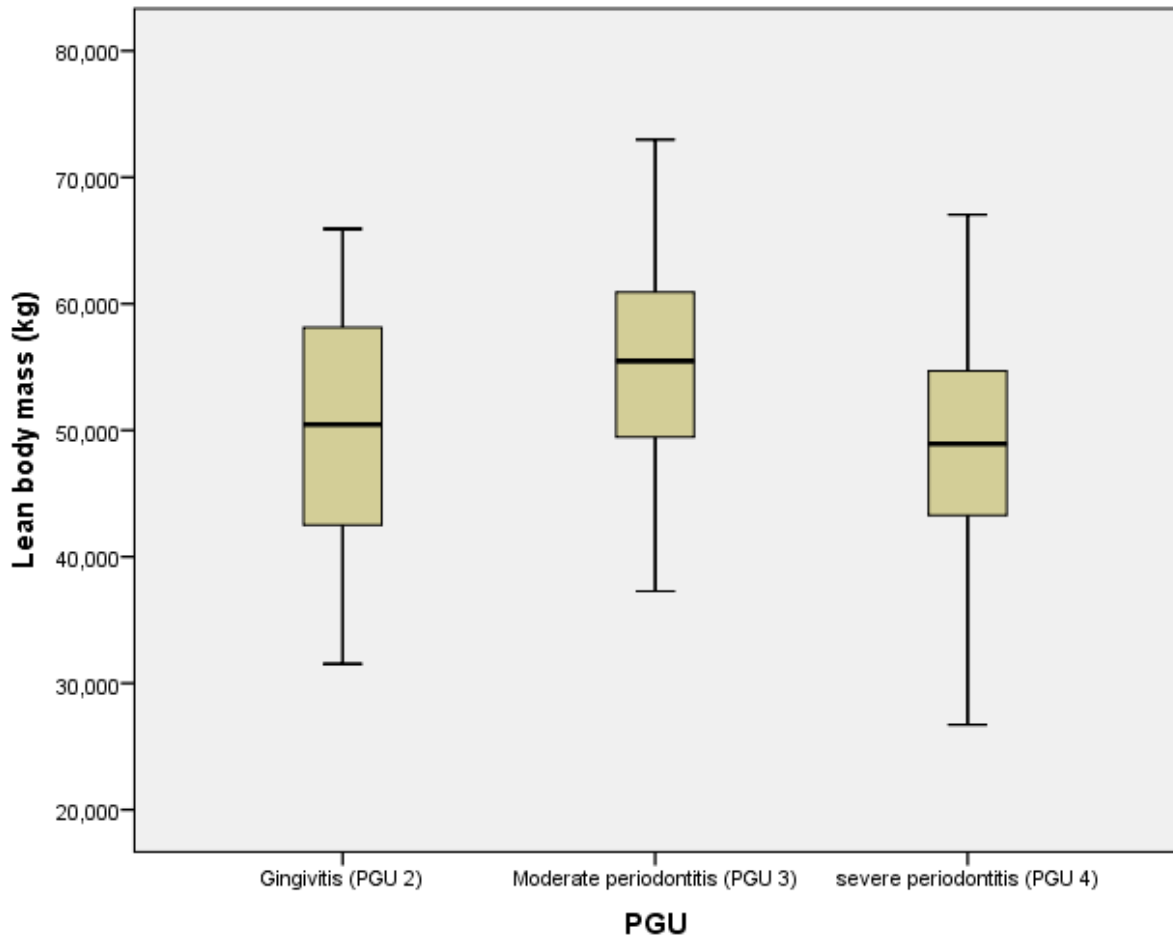


Figure 2: Median (95% CI) of lean body mass (kg) in patients with gingivitis, moderate periodontitis and severe periodontitis.

7.2.3 Laboratory parameters

Table 4 provides a summary of laboratory parameters measured in the 3 PD groups. No significant differences were detected for 25(OH)D, vitamin B12, serum calcium, serum cholesterol, serum folic acid and serum total protein concentrations between the groups. The median serum CRP value was highest in the severe periodontitis group (3 [1.53 – 6.10] mg/l, but not statistically significant ($p=0.286$). Additionally, laboratory markers of nutritional status such as serum albumin ($p=0.658$), serum prealbumin ($p=0.619$) and serum transferrin ($p=0.860$) did not vary significantly between groups. The measured laboratory markers were compared to generally used reference values (272). All median values of the parameters measured in the three PD groups were within the normal ranges.

Table 3: Anthropometric measurements in patients with gingivitis, moderate periodontitis and severe periodontitis

	Gingivitis (PGU 2)	Moderate periodontitis (PGU 3)	Severe periodontitis (PGU 4)	<i>p</i>
	<i>Median (25th -75th percentile)</i>	<i>Median (25th -75th percentile)</i>	<i>Median (25th -75th percentile)</i>	
Age (years)	67 (58 - 75)	63.0 (58 - 71)	61.0 (55 - 68)	0.094
Weight (kg)	77.5 (67.8 - 82.3)	86.5 (76.3 - 97.3)	78.0 (68.3 - 87.5)	0.013
Height (cm)	172 (168 - 176)	175 (166 - 181)	171 (166 - 176)	0.380
BMI (kg/m²)	26.9 (24.7 - 28.0)	27.7 (24.9 - 31.8)	26.8 (24.2 - 28.7)	0.107
Waist to hip ratio	0.99 (0.96 - 1.03)	1.01 (0.97 - 1.03)	0.99 (0.94 - 1.04)	0.523
Body fat (%)	35.6 (0.96 - 1.03)	36.0 (29.9 - 38.7)	34.4 (29.4 - 37.6)	0.744
Lean body mass (kg)	50.4 (42.3 - 58.6)	55.5 (48.5 - 61.0)	48.9 (43.2 - 54.8)	0.012

*Values are median (25th - 75th percentile) or as otherwise indicated
PGU= basic periodontal examination [Parodontale Grunduntersuchung]

Table 4: Laboratory parameters in patients with gingivitis, moderate periodontitis and severe periodontitis

	Reference values (272)	Gingivitis (PGU 2)	Moderate periodontitis (PGU 3)	Severe periodontitis (PGU 4)	p
		<i>Median (25th - 75th percentile)</i>	<i>Median (25th - 75th percentile)</i>	<i>Median (25th - 75th percentile)</i>	
25(OH)D (ng/ml)	25 - 45	27.3 (20.25 - 35.6)	27.9 (18.5 - 37.35)	28.35 (18.6 - 33-42)	0.812
Serum albumin (g/dl)	3.5 - 5.5	4.6 (4.4 - 4.8)	4.6 (4.3 - 4.8)	4.5 (4.3 - 4.8)	0.658
Vitamin B12 (pg/ml)	> 250	397.5 (327 - 508)	353 (305 - 428)	411 (317 - 520)	0.239
Serum calcium (mmol/l)	2.2 - 2.6	2.41 (2.36 - 2.47)	2.39 (2.26 - 2.47)	2.4 (2.27 - 2.46)	0.496
Serum cholesterol (mg/dl)	< 200	182 (148 - 201)	176 (136 - 195)	182 (156 - 256)	0.215
C-reactive protein (mg/l)	0.08 - 3.10	1.95 (0.98 - 6.38)	1.80 (0.90 - 4.60)	3.00 (1.53 - 6.10)	0.286
Ferritin (ng/ml)	30 - 300	129 (77 - 207)	154 (63 - 293)	198 (83 - 320)	0.095
Folic acid (ng/ml)	3.1 - 17.5	7.4 (4.6 - 10.2)	6.4 (4.9 - 9.5)	6.7 (5 - 11.5)	0.574
Serum total protein (g/dl)	5.5 - 8.0	7.5 (7.0 - 7.8)	7.4 (7.3 - 7.8)	7.6 (7.2 - 7.9)	0.313
Prealbumin (g/l)	0.20 - 0.40	0.30 (0.26 - 0.38)	0.31 (0.27 - 0.37)	0.32 (0.29 - 0.35)	0.619
Transferrin (g/l)	2.3 - 3.9	2.51 (2.33 - 2.83)	2.62 (2.19 - 2.94)	2.53 (2.27 - 2.82)	0.860

*Values are median (25th - 75th percentile)

7.2.4 Dietary intake

Table 5: Dietary assessment in patients with gingivitis, moderate periodontitis and severe periodontitis

	DGE- Reference values /d	Gingivitis (PGU 2)	Moderate periodontitis (PGU 3)	Severe periodontitis (PGU 4)	p
		<i>Median (25th - 75th percentile)</i>	<i>Median (25th - 75th percentile)</i>	<i>Median (25th - 75th percentile)</i>	
24-hour recall					
Kcal	2100	2182.5 (1678.1 - 2395.5)	2177.4 (1683.9 - 2769.6)	1995.3 (1621.1 - 2487.9)	0.301
Kjoule	8786.4	9137.8 (7025.7 - 10029.6)	9116.2 (7050.1 - 11595.8)	8354.0 (6787.3 - 10416.2)	0.301
Cholesterol (mg)	<300 mg	250.5 (182.7 - 445.1)	306.4 (248.9 - 493.4)	304.9 (192.5 - 446.4)	0.292
Omega-3-fatty acids (mg)	0.5 %**	1168.3 (942.8 - 1484.3)	1354.5 (1080.9 - 1861.3)	1185.6 (913.2 - 1774.4)	0.366
Calcium (mg)	1000 mg	808.3 (596.3 - 1033.5)	932.54 (569.4 - 1214.5)	585.62 (449.3 - 970.2)	0.008
Zinc (µg)	10000 µg	10214.8 (6997.9 - 13319.9)	12335.2 (8839.5 - 15073.2)	10624.9 (7804.3 - 15310.5)	0.181
Vitamin B12 (µg)	3 µg	4.15 (2.96 - 5.35)	4.52 (3.32 - 8.07)	4.58 (2.63 - 7.42)	0.485
Folic acid (µg)	300 µg	211.1 (156.5 - 291.0)	240.76 (164.8 - 294.4)	202.9 (152.4 - 258.8)	0.262
Vitamin A (µg)	800 µg	351.5 (253.5 - 503.4)	447.20 (299.5 - 615.5)	381.30 (239.0 - 575.1)	0.323

Vitamin C (ascorbic acid) (mg)	110 mg	102.00	(50.26 - 258.89)	74.18	(47.94 - 130.19)	71.68	(42.02 - 112.38)	0.156
Vitamin D (μg)	20 μ g	0.945	(0.468 - 2.340)	1.52	(0.563 - 4.533)	1.585	(0.443 - 3.338)	0.669
Vitamin E (mg)	13 mg	7.87	(4.69 - 14.51)	9.69	(6.48 - 13.05)	7.11	(4.75 - 10.31)	0.182
Alcohol (mg)	20 g	280	(0 - 4983.5)	840	(0 - 20650)	716	(0 - 20306)	0.773
Fibre (g)	>30 g	19.7	(13.6 - 25.5)	19.7	(13.6 - 23.8)	16.2	(12.5 - 19.9)	0.123
Protein (g)	0.8 g/kg BW	71.7	(59.7 - 93.1)	86.3	(68.2 - 106.8)	73.8	(58.8 - 94.6)	0.787
Fat (g)	< 30%**	92.3	(66.5 - 107.3)	97.1	(67.1 - 114.2)	82.6	(59.7 - 108.9)	0.331
Carbohydrates (g)	> 55%**	208.9	(155.8 - 271.9)	197.9	(159.0 - 319.4)	189.6	(141.3 - 245.3)	0.24
Food Frequency Index (FFI)		32	(26 - 36)	28	(24 - 34)	29	(25 - 32)	0.19

*Values are median (25th - 75th percentile)

** per cent of daily energy intake (EI)

DGE= Deutsche Gesellschaft für Ernährung (German nutrition society)

BW =body weight

Energy, macronutrient and alcohol intake

Dietary intake data from the 24-h recall and FFI, together with DGE reference values for males (273), are illustrated in **Table 5**. Reference values for males were used, since only one-fourth of the participants were female.

Total EI did not differ between the groups ($p=0.301$). EI in patients with severe periodontitis (PGU 4) (median: 8354.0 [6768.3 – 10416.2] KJ) was slightly lower than in patients with gingivitis (PGU 2; 9137.8 [7025.7 - 10029.6] KJ) and moderate periodontitis (PGU 3; 9116.2 [7050.1 - 11595.8] KJ) respectively. In addition, the consumption of

carbohydrates ($p=0.240$), fat ($p=0.331$) and protein ($p=0.787$) did not vary significantly between the three stages of PD.

Protein and fat intake were highest in the moderate periodontitis group with a median consumption of 86.3 (68.2 – 106.8) g of protein and 97.1 (70.6 – 114.2) g of fat. Similar to fat consumption, median intake of n-3 fatty acids was highest in patients with moderate periodontitis (1354.5 [1080.9 – 1861.3] mg). Patients with gingivitis had the lowest intake of dietary cholesterol (250.5 [182.7 – 445.1] mg). The lowest consumption of carbohydrates (189552.9 [141308.3 – 245307.3] mg) and fat (82604.2 [59735.7 – 108856.6] mg) was also found in the patients suffering from severe periodontitis. Median alcohol consumption was highest in patients with moderate periodontitis (716 [0 – 20306] mg). The differences in dietary intake between the groups did not, however, reach statistical significance.

The FFI indicated no difference in diet quality between the PD groups with median scores of 31.5 (26 – 36), 28 (24 – 34) and 29 (25 – 32) ($p=0.190$) for gingivitis, moderate periodontitis and severe periodontitis, respectively.

Fibre

The analysis of fibre consumption showed no statistical significant difference between the groups ($p=0.123$), although patients with severe periodontitis had the lowest intake with 16.2 (12.5 – 19.9) mg compared to the two other patient groups.

Micronutrients

The intake of vitamin B12, vitamin C and vitamin D was not significantly different between gingivitis, moderate periodontitis and severe periodontitis (**Table 5**). Patients with moderate periodontitis showed a greater, but not statistically significant consumption of folic acid (compared to gingivitis and severe periodontitis, ($p=0.262$)).

The highest intake of vitamin A and E was found in patients with moderate periodontitis, but there was no significant difference between the 3 PD stages ($p= 0.323$ and $p=0.182$, respectively). Median consumption of vitamin A was 447.20 (299.5 - 615.5) μg for patients with moderate periodontitis, 351.5 (253.5 - 503.4) μg for patients with gingivitis and 381.3 (239.0 - 575.1) μg for patients with severe periodontitis. Median vitamin E intake was 9.69 (6.48 – 13.05) μg in the moderate periodontitis group, patients with gingivitis consumed

7.87 (4.69 – 14.51) µg and the lowest intake of the vitamin was seen in patients suffering from the most severe stage of the disease (7.11 [4.74 – 10.31] µg).

Analogous to most of the vitamins, zinc ($p=0.181$) intake was also greatest in patients with moderate periodontitis compared to the other groups, but without reaching significance. Calcium consumption was highest in patients in the moderate periodontitis group (932.5 [569.4 – 1214.5] mg), without reaching statistical significance after Bonferroni correction ($p=0.008$) (**Figure 3**). The lowest calcium intake was found in patients with severe periodontitis (585.6 mg [449.3 – 970.2] mg).

Daily macronutrient intake (in %) in the three PD groups differed slightly from recommendations given by the DGE (273). Protein intake of patients with gingivitis was 13.3% (0.92 g/ kg BW), 16.1% (0.99 g/ kg BW) in patients with moderate periodontitis and 15% (0.94 g/ kg BW) of daily EI in patients suffering from severe periodontitis. Carbohydrate intake was 38.9%, 36.9% and 38.6% of daily EI for patients with gingivitis, moderate periodontitis and severe periodontitis, respectively. DGE recommendations for carbohydrate intake are > 55% of daily EI. Fat consumption was above the recommended intake of a maximum of 30% of daily EI. Patients with gingivitis consumed 39.4% fat, patients affected with moderate periodontitis consumed 41.5% and patients with severe periodontitis consumed 38.5% fat with their daily EI. The consumption of n-3-fatty acids, however, was near the recommended daily amounts of 0.5% of EI. The percentages of n-3-fatty acid intake were 0.47%, 0.54% and 0.52% in patients with gingivitis, periodontitis and severe periodontitis, respectively (**Table 6**).

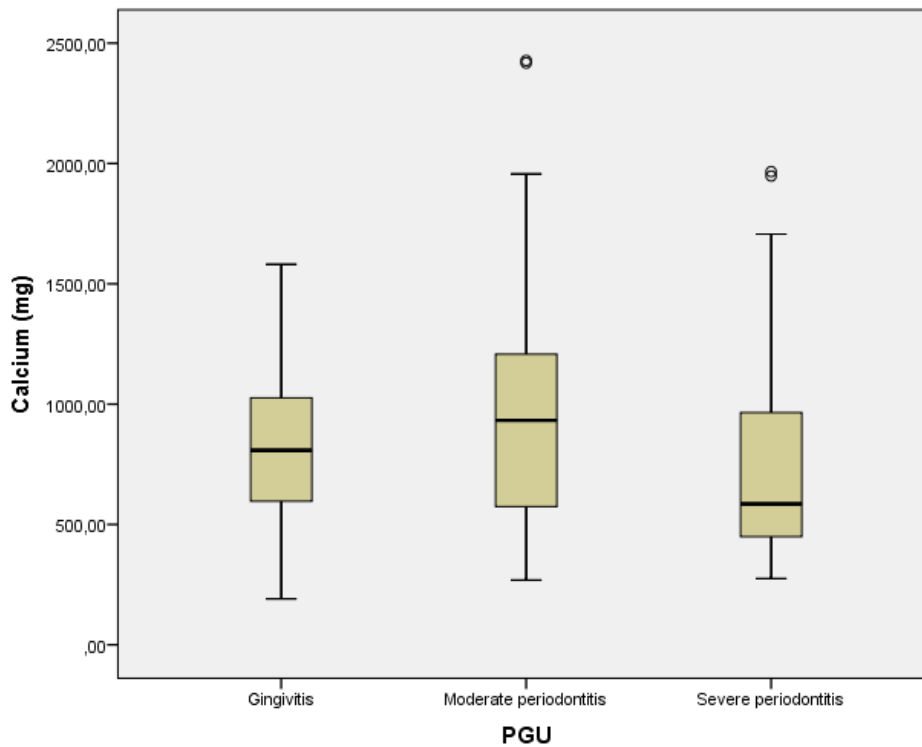


Figure 3: Median (95% CI) dietary calcium (mg) intake in patients with gingivitis, moderate periodontitis and severe periodontitis; PGU= basic periodontal examination [Parodontale Grunduntersuchung]

Table 6: Percentages of macronutrient intake in patients with gingivitis, moderate periodontitis and severe periodontitis

	<i>Reference values</i>	<i>Gingivitis</i>	<i>Moderate periodontitis</i>	<i>Severe periodontitis</i>
% Carbohydrates of daily EI	> 55%**	38.8	36.9	38.6
% Fat of daily EI	< 30%**	39.4	41.5	38.6
% Omega-3-fatty acids of daily EI	0.5 %**	0.47	0.54	0.52
% Protein of daily EI	0.8 g/kg BW (10-15%**)	13.3	16.1	15

** Per cent of daily energy intake (EI); Values are presented as median per cent of daily energy EI

8 Part 2: Effect of periodontal therapy on nutritional status and nutrient supply in PAD patients

8.1 Introduction and aim

PD has profound local and systemic effects. PD, especially when severe, affects lifestyle and quality of life. Not only are there aesthetic issues; pain and discomfort in the mouth influence eating habits and the amount and selection of foods consumed (274-276). The dietary pattern can change and the nutritional supply can deteriorate. Staudte et al. showed that approximately 50% of patients affected with chronic periodontitis experienced discomfort in the mouth while eating (20).

Dental status also influences dietary intake (195). Johansson et al. reported that edentulous middle-aged men ate less fruits, vegetables and fibre and more sweet snacks compared to controls with their natural teeth (23). In a similar vein Sheiham & Steele found that the intake of most fruits and vegetables was significantly less in edentate subjects than dentate individuals. Intake of non-starch polysaccharides, vitamin C, calcium and protein was significantly lower in the participants without natural teeth (22, 24).

The avoidance of specific food items might lead to an insufficient supply of essential nutrients. This is especially important in patients with periodontitis, since certain nutrients and especially micronutrients are involved in wound healing and the inflammatory processes linked to the disease (202).

The aim of the second part of this dissertation was to investigate the effect of periodontal therapy on nutritional status in patients with periodontitis and PAD. It was hypothesized that treatment of the PD might reduce oral discomfort and pain and so increase the ability and willingness to include food items in the diet that were difficult to chew and so were previously avoided.

8.2 Results

8.2.1 Subjects

Forty-four patients attended the three planned visits (baseline, FU1 at 3 months and FU2 at 6 months). Of these, 35 (79.5%) were male and 9 (20.5%) were female. The mean age was 59 (54 – 66) years. Participant's characteristics are presented in **Table 7**. Participants

were allocated to the three treatment groups. Fifteen patients received periodontal therapy with antibiotics (PT1), 15 were treated without antibiotics (PT2) and 14 were allocated to the control group (CG).

In the CG group 3 (21.4%) patients had diabetes and were obese (defined by a BMI > 30). Eight participants (57.1%) were former smokers and 4 (28.6%) smoked at baseline. Hyperlipidaemia was present in 85.5% of the patients and hypertension in 78.6%.

In PT1, 3 patients (20%) had diabetes and 4 were obese (26.7%); hyperlipidaemia was present in 12 (80%) and hypertension in 9 (60%). Eight (53.3%) participants were currently smoking at baseline and 7 (46.7%) had a history of smoking.

The prevalence of diabetes and obesity was slightly higher in PT2 than in the other two treatment groups, with 6 patients (40%). There was hyperlipidaemia in 10 subjects (66.7%) and hypertension in 11 (73.3%). Eight (53.3%) participants were currently smoking at baseline and 5 (33.3%) were former smokers.

Table 7: Participant's characteristics at baseline (n=44)

Age (y)	59 (54 - 66)
Weight (kg)	82 (75 - 96)
Height (cm)	172 (169 - 179)
BMI (kg/m²)	28 (26.1 - 30.7)
Waist to hip ratio	1.00 (0.93 - 1.04)
Body fat (%)	35.2 (30.7 - 37.7)
Lean body mass (kg)	54.0 (45.1 - 60.0)
ABI right	0.83 (0.56 - 1.04)
ABI left	0.86 (0.58 - 1.06)
Diabetes (%)	22.70%
Obesity (%)	29.50%

Former smokers (%)	45.50%
Smokers (%)	45.50%
Hyperlipidaemia (%)	77.30%
Hypertension (%)	70.50%

*Values are median (25th - 75th percentile) or as otherwise indicated

BMI = Body Mass Index

ABI = Ankle Brachial Index

8.2.2 Anthropometrics and body composition

Anthropometrical parameters did not significantly change over the 6 months of the study in any of the treatment groups. The percentage of body fat slightly decreased in all three-treatment groups, but this reduction did not reach statistical significance ($p=0.923$, $p=0.158$, $p=0.938$ for CG, PT1 and PT2, respectively). The CG showed a baseline body fat percentage of 35.5 (30.4 - 38.9)%, at FU1 of 33.6 (30.6 - 38.0)% and at FU2 of 34.5 (32.5 - 36.7)%. In patients allocated to PT1, the percentage of body fat decreased from 34.3 (30.6 - 37.3)% at BL to 33.6 (30.1 - 37.9)% at FU2. In the PT2 group, median the percentage of body fat changed from 35.9 (30.0 - 39.4)% at BL to 36.5 (29.1 - 38.0)% at FU2. Anthropometric data of the participants are provided in **Tables 8, 9 and 10**.

Table 8: Anthropometric measurements; group: CG

	CG						p
	Baseline		3 months (FU1)		6 months (FU2)		
	Median*	(25th-75 percentile)	Median	(25th-75 percentile)	Median	(25th-75 percentile)	
n	14						
BMI (kg/m²)	27.5	(25.7 - 30.0)	27.3	(25.4 - 29.6)	27.9	(24.5 - 29.9)	0.383
Weight (kg)	81.5	(73 - 96)	81.5	(73 - 95)	83	(72.8 - 95.5)	0.320
Waist to hip ratio	0.96	(0.92 - 1.04)	0.97	(0.93 - 1.02)	0.99	(0.95 - 1.04)	0.359
Body fat (%)	35.5	(30.4 - 38.9)	33.6	(30.6 - 38.0)	34.5	(32.5 - 36.7)	0.923
Lean body mass (kg)	54.1	(42.7 - 58.3)	52.9	(42.2 - 61.1)	54.3	(41.43 - 59.6)	1.00

*Values are median (25th - 75th percentile) unless otherwise indicated

Table 9: Anthropometric measurements; group: PT1

	PT1						<i>p</i>
	Baseline		3 months (FU1)		6 months (FU2)		
	<i>Median</i>	<i>(25th-75 percentile)</i>	<i>Median</i>	<i>(25th-75 percentile)</i>	<i>Median</i>	<i>(25th-75 percentile)</i>	
n	15						
BMI (kg/m²)	27.0	(25.6 - 30.1)	27.4	(25.3 - 31.4)	27.7	(25.6 - 30.6)	0.401
Weight (kg)	80	(75 - 91)	84	(73 - 93)	82	(74 - 93)	0.094
Waist to hip ratio	1.00	(0.95 - 1.05)	0.98	(0.96 - 1.05)	1.011	(0.9510 - 1.07)	0.105
Body fat (%)	34.3	(30.6 - 37.3)	33.8	(30.6 - 36.9)	33.6	(30.1 - 37.9)	0.158
Lean body mass (kg)	53.4	(44.9 - 59.3)	53.9	(47.3 - 58.9)	54.1	(46.35 - 59.1)	0.280

*Values are median (25th - 75th percentile) unless otherwise indicated

Table 10: Anthropometric measurements; group: PT2

	PT2						<i>p</i>
	Baseline		3 months (FU1)		6 months (FU2)		
	<i>Median</i>	<i>(25th-75 percentile)</i>	<i>Median</i>	<i>(25th-75 percentile)</i>	<i>Median</i>	<i>(25th-75 percentile)</i>	
n	15						
BMI (kg/m²)	29.0	(27.6 - 31.5)	29.6	(27.6 - 30.9)	29.9	(26.2 - 31.6)	0.587
Weight (kg)	93	(74 - 98)	91	(74 - 98)	90	(74 - 98)	0.77
Waist to hip ratio	1.00	(0.973 - 1.027)	0.99	(0.98 - 1.03)	1.00	(0.96 - 1.04)	0.876
Body fat (%)	36.5	(30.1 - 39.0)	36.5	(29.1 - 38.0)	35.9	(30.0 - 39.4)	0.983
Lean body mass (kg)	53.7	(47.5 - 60.5)	53.6	(47.0 - 60.0)	53.4	(47.5 - 59.6)	0.155

*Values are median (25th - 75th percentile) unless otherwise indicated

8.2.3 Laboratory parameters

Classical laboratory parameters (prealbumin, transferrin and albumin) used to determine nutritional status did not significantly change from baseline to FU2 within the groups. The median plasma CRP value slightly decreased in the CG from 2.5 mg/l at baseline to 1.9 mg/l after 3 months and 2.05 mg/l at FU2. There was also a decrease in the PT1 group from 3 mg/l to 2.3 mg/l at FU1 and 1.8 mg/l at FU2; however, the decrease in CRP did not reach significance ($p=0.651$ and $p=0.337$, respectively). CRP values in the PT2 group increased, without reaching significance after Bonferroni correction ($p=0.02$). **Table 11, 12 and 13** provide a summary of the laboratory markers measured in the three treatment groups.

Table 11: Laboratory parameters; group: CG

	CG					p
	<i>Reference values (272)</i>	Baseline	3 months (FU1)	6 months (FU2)		
		<i>Median* (25th-75 percentile)</i>	<i>Median (25th-75 percentile)</i>	<i>Median (25th-75 percentile)</i>		
Serum albumin (g/dl)	3.5 - 5.5	4.5 (4.3 - 4.7)	4.3 (4.2 - 4.6)	4.4 (4.3 - 4.5)		0.276
C-reactive protein (mg/l)	0.08 - 3.10	2.5 (1.28 -3.83)	1.9 (1.03 - 3.08)	2.05 (0.6 - 4.93)		0.651
Serum prealbumin (g/l)	0.20 - 0.40	0.316 (0.293 - 0.343)	0.319 (0.285 - 0.349)	0.321 (0.272 - 0.351)		0.751
Serum transferrin (g/l)	2.3 - 3.9	2.450 (2.227 – 2.638)	2.460 (2.208 – 2.733)	2.450 (2.325 – 2.848)		0.931

*Values are median (25th - 75th percentile)

Table 12: Laboratory parameters; group: PT1

	PT1				
		Baseline	3 months (FU1)	6 months (FU2)	p
	<i>Reference values (272)</i>	<i>Median (25th-75 percentile)</i>	<i>Median (25th-75 percentile)</i>	<i>Median (25th-75 percentile)</i>	
Serum albumin (g/dl)	3.5 - 5.5	4.4 (4.3 - 4.8)	4.6 (4.2 - 4.7)	4.4 (4.3 - 4.7)	
C-reactive protein (mg/l)	0.08 - 3.10	3.00 (1.3 - 5.6)	2.3 (1.6 - 3.8)	1.8 (0.9 - 3.2)	0.337
Serum prealbumin (g/l)	0.20 - 0.40	0.346 (0.307 - 0.382)	0.348 (0.278 - 0.373)	0.359 (0.318 - 0.391)	0.627
Serum transferrin (g/l)	2.3 - 3.9	2.550 (2.420 – 2.930)	2.800 (2.370 -3.040)	2.570 (2.110 – 2.740)	0.936

*Values are median (25th - 75th percentile)

Table 13: Laboratory parameters; group: PT2

	PT2				<i>p</i>
	<i>Reference values (272)</i>	Baseline <i>Median (25th-75 percentile)</i>	3 months (FU1) <i>Median (25th-75 percentile)</i>	6 months (FU2) <i>Median (25th-75 percentile)</i>	
Serum albumin (g/dl)	3.5 - 5.5	4.50 (4.4 - 4.6)	4.50 (4.3 - 4.6)	4.40 (4.2 - 4.6)	0.840
C-reactive protein (mg/l)	0.08 - 3.10	2.1 (1.5 - 7.7)	1.9 (0.8 - 3.8)	3.4 (1.5 - 6.3)	0.020
Serum prealbumin (g/l)	0.20 - 0.40	0.321 (0.291 - 0.378)	0.304 (0.283 - 0.349)	0.338 (0.263 - 0.383)	0.627
Serum transferrin (g/l)	2.3 - 3.9	2.690 (2.190 – 2.770)	2.420 (2.040 – 2.810)	2.280 (1.900 – 2.740)	0.721

*Values are median (25th - 75th percentile)

8.2.4 Dietary intake

Table 14, 15 and 16 present dietary intake data from the 24-h recall and FFI, together with DGE reference values for males (273). The dietary intake at baseline, FU1 and FU2 in the different treatment groups is displayed.

Energy, macronutrient and alcohol intake

Total EI did not differ in the control group between baseline, FU1 and FU2 ($p=0.257$). The median EI of the PT1 group increased from 9016.7 (7381.3 - 11422.3) KJ to 9386.4 (8081.4 - 11598.6) KJ after 3 months and decreased to 8776.8 (7491.4 - 11125.5) KJ at FU2, although the change was not statistically significant ($p=0.282$). Total EI in PT2 showed a slight decrease from BL to FU1 (7406.4 [5934.8 - 10152.4] KJ), then increasing again at FU2 (9429.7 [7175.7 - 2064.9] KJ) but not reaching significance after Bonferroni correction ($p=0.002$).

Carbohydrate consumption did not differ between BL, FU1 and FU2 in the PT1 group. Patients allocated to the CG group showed a slight increase in median carbohydrate intake from BL to FU1 of 49.6 g and a decrease of 45.7 g from FU1 to FU2, but this change was not statistically significant ($p=0.145$). In contrast, carbohydrate intake in patients allocated to the PT2 group increased from BL to FU2, and also did not reach significance after Bonferroni correction ($p=0.014$) (**Figure 4**). In this group the intake of carbohydrates decreased from 199.1 (143.4 - 249.1) g at baseline to 168.6 (137.1 – 238.6) g at FU1 and increased to 273.5 (184.9 – 319.8) g at FU2. No differences were found in protein, fat, omega-3-fatty acids and alcohol intake within the groups at the different time points.

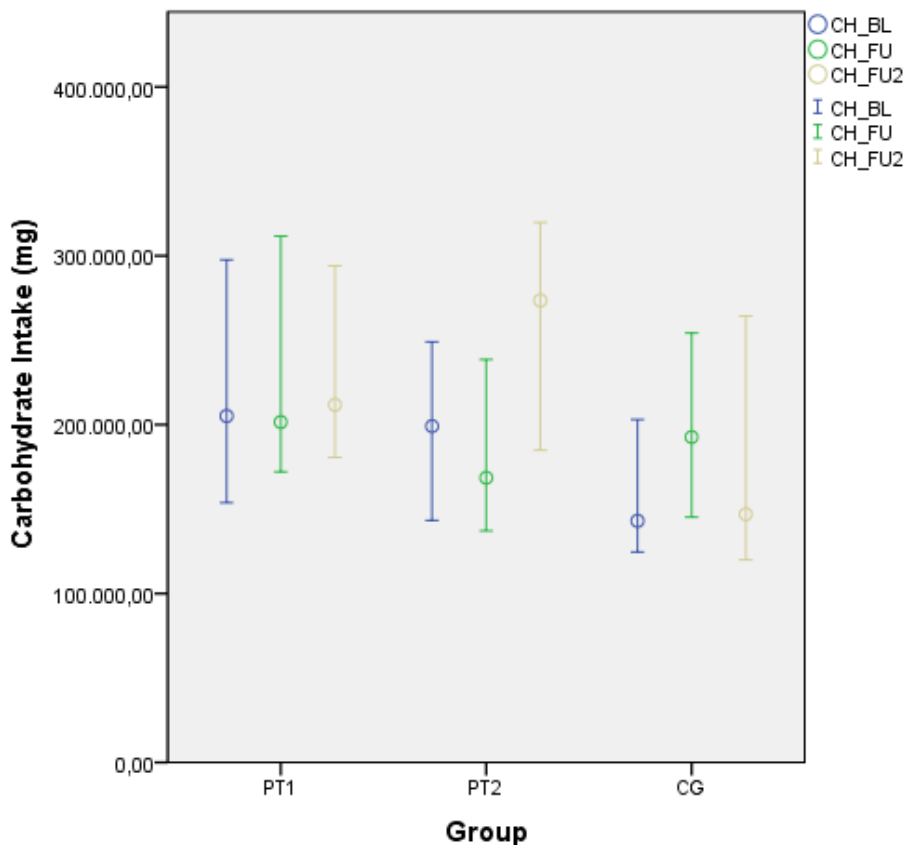


Figure 4: Median (95% CI) carbohydrate (CH) intake (mg) at baseline (BL), 3 months (FU1) and 6 months (FU2) in the three treatment groups: CG (Control group); PT1 (periodontal therapy with antibiotics); PT2 (periodontal therapy without antibiotics)

Additionally, there was no change in FFI from BL to FU1 or FU2 in any of the groups ($p=0.771$; $p=0.144$; $p=0.259$ for CG, PT1 and PT2, respectively) indicating no change in diet quality.

Macronutrient intake given in per cent of daily EI of the treatment groups differed slightly from reference values defined by the DGE (273). **Table 17** shows the median daily macronutrient intake values from baseline to FU2 in the different treatment groups. Dietary intake of fat in the three treatment groups exceeded the recommendations of less than 30% of daily EI. Fat consumption was highest in the CG group. However, consumption of omega-3-fatty acids was adequate in patients allocated to the PT1 and PT2. Patients in the CG group even exceeded the recommendation of greater than 0.5% of daily EI. Protein consumption was slightly higher than recommended in all of the three treatment groups. In the CG group, the intake met the recommended amount of 0.8g/kg BW at FU2. Consumption of carbohydrates was below the recommended amounts of more than 55% of daily EI in all of the patients.

Fibre

There was a rise in fibre consumption at both FU1 and FU2 compared to BL in all the treatment groups, but the increase was not significant ($p=0.751$; $p=0.627$; $p=0.189$ for CG, PT1 and PT2, respectively).

Micronutrients

None of the groups showed significant changes in zinc, calcium, vitamin A, C and D intake from baseline to the final visit. Furthermore, there were no changes in vitamin B12 uptake in PT1 ($p=0.936$) and PT2 ($p=0.247$). On the contrary, median vitamin B12 intake in patients of the CG group increased from 4.4 (3.1 - 5.6) μg to 5.8 (2.5 - 6.5) μg after 3 months and then decreased to 2.8 (2.5 - 7.1) μg at FU2 ($p=0.257$).

After Bonferroni correction the increase in folic acid consumption in CG and PT1 was not significant ($p=0.008$ and $p=0.017$, respectively) (**Figure 6**). In patients allocated to CG, values rose from 186 (130.7 - 230.9) μg at baseline to 252.8 (187.5 - 317.0) μg after 6 months. In the PT1 group median folic acid intake increased from 182.1 (143 - 242.8) μg at baseline to 255.2 (182.2 - 319.1) μg at FU2. No significant change was seen in the PT2 group ($p=0.189$).

The change in vitamin E intake over the three time points in the CG group remained not significant after Bonferroni correction ($p=0.017$) (**Figure 5**). After 3 months, vitamin E intake increased by 2.273 mg compared to baseline and by another 3.618 mg from FU1 to FU2 in the CG group. There was no change in PT1 and PT2 ($p=0.940$ and 0.819 , respectively).

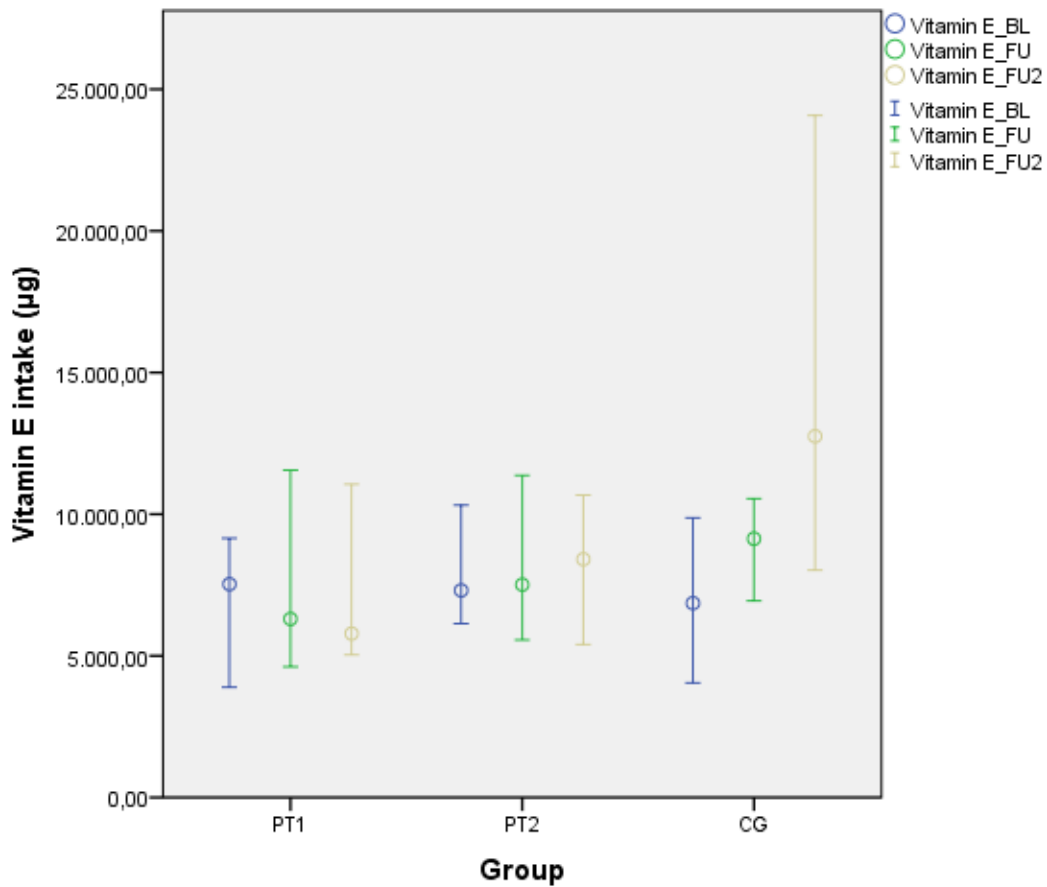


Figure 5: Median (95% CI) vitamin E intake (μg) at baseline (BL), 3 months (FU1) and 6 months (FU2) in the three treatment groups: CG (control group); PT1 (periodontal therapy with antibiotics); PT2 (periodontal therapy without antibiotics)

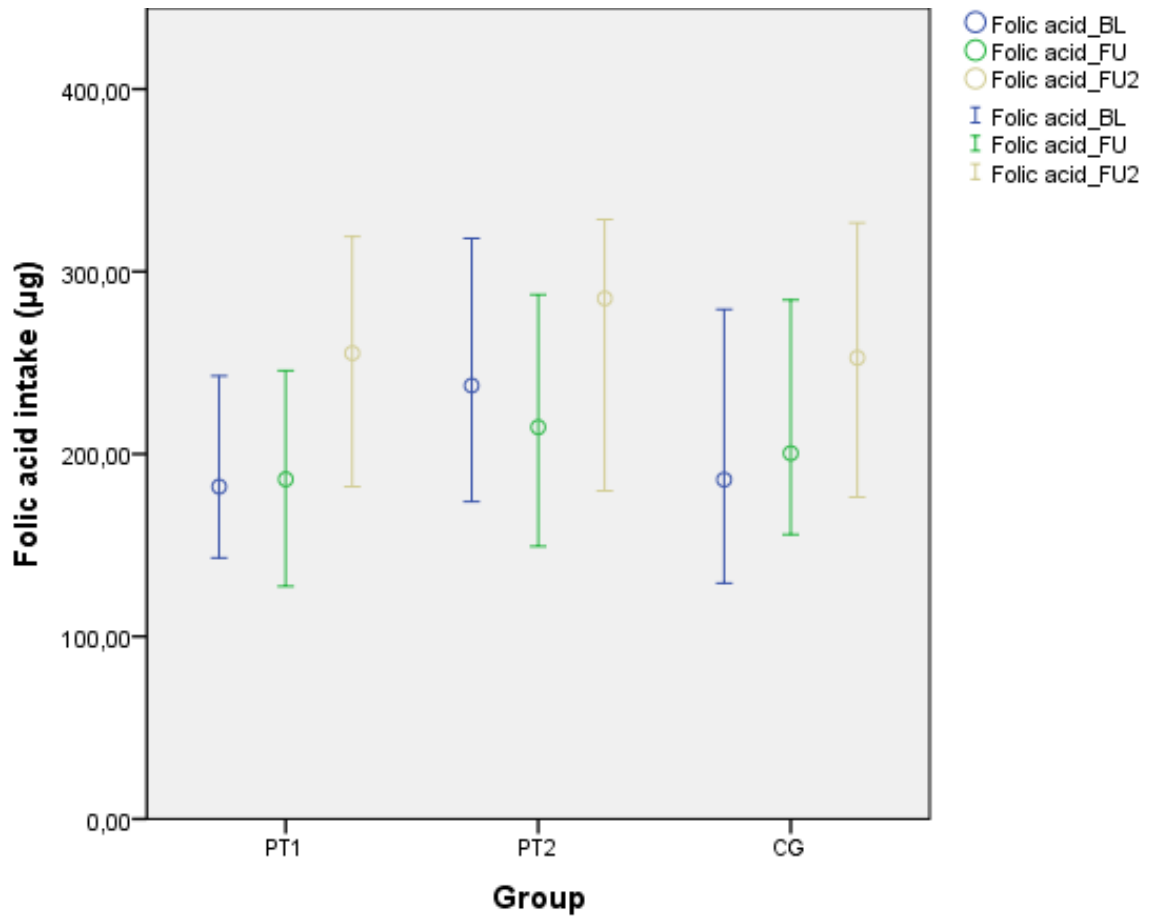


Figure 6: Median (95% CI) folic acid intake (μg) at baseline (BL), 3 months (FU1) and 6 months (FU2) in the three treatment groups: CG (Control group); PT1 (periodontal therapy with antibiotics); PT2 (periodontal therapy without antibiotics)

Table 14: Dietary assessment; group: PT1

	PT1							
	<i>DGE-Reference values /d (273)</i>	Baseline		3 months (FU1)		6 months (FU2)		<i>p</i>
		<i>Median*</i>	<i>(25th-75 percentile)</i>	<i>Median</i>	<i>(25th-75 percentile)</i>	<i>Median</i>	<i>(25th-75 percentile)</i>	
KJoule	8373.6	9016.7	(7381.3 - 11422.3)	9386.4	(8081.4 - 11598.6)	8776.8	(7491.4 - 11125.5)	0.282
Omega-3-fatty acids (mg)	0.5 %**	1253.3	(1052.6 - 1598.8)	1183	(952.4 - 1363.7)	1256.7	(1021.2 - 1666.2)	0.449
Calcium (mg)	1000 mg	597.2	(476.5 - 1036.9)	876.7	(420.1 - 1088.9)	836.7	(491.78 - 1048.9)	0.936
Zinc (mg)	10 mg	11.74	(8.29 - 18.24)	11.39	(8.66 - 15.50)	10.48	(9.00 - 14.31)	0.766
Vitamin B12 (µg)	3 µg	5.8	(2.4 - 8.8)	5.5	(3.6 - 6.6)	5.1	(2.7 - 7.3)	0.936
Folic acid (µg)	300 µg	182.1	(143 - 242.8)	186.1	(127.4 - 245.7)	255.2	(182.2 - 319.1)	0.017
Vitamin A (retinol) (µg)	800 µg	394.9	(270.5 - 814.1)	383.6	(275.2 - 559.2)	417.1	(297.1 - 573.4)	0.549
Vitamin C (ascorbic acid) (mg)	110 mg	60.75	(42.70 - 90.61)	45.99	(22.93 - 82.81)	84.84	(40.67 - 202.71)	0.282
Vitamin D (calciferol) (µg)	20 µg	1.9	(0.7 - 3.6)	1.8	(0.9 - 2.5)	1.3	(0.6 - 2.4)	0.766
Vitamin E (tocopherol equivalent)	13 mg	7.530	(3.895 - 9.149)	6.303	(4.611 - 11.559)	5.787	(5.034 - 11.059)	0.936

<i>(mg)</i>								
Alcohol (g)	20 g	0	(0 - 13.06)	13.11	(0 - 39.6)	1.5	(0 - 50.000)	0.41
Fibre (g)	30 g/d	12.52	(11.41 - 24.80)	14.45	(10.23 - 19.00)	16.32	(13.14 - 10.41)	0.627
Protein (g)	0.8 g/kg BW	89.47	(61.78 - 102.72)	81.13	(67.90 - 108.43)	87.13	(65.20 - 94.44)	0.627
Fat (g)	< 30%**	84.16	(54.75 - 115.88)	97.04	(82.15 - 108.20)	80.973	(59.10 - 97.46)	0.127
Carbohydrates (g)	> 55%**	205.12	(153.76 - 297.57)	201.54	(172.05 - 311.73)	211.71	(180.58 - 294.01)	0.819
Food Frequency Index (FFI)		29	(24 - 31)	29	(22.5 - 31.5)	25	(23.5 - 29)	0.144

*Values are median (25th - 75th percentile)
 ** per cent of daily energy intake

°DGE= Deutsche Gesellschaft für Ernährung (German nutrition society); BW =body weight"; EI = energy intake; FU = Follow-up; PT1 = periodontal therapy with antibiotics

Table 15: Dietary assessment; group: CG

	CG							
	<i>DGE-Reference values /d (273)</i>	<i>Baseline Median*</i>	<i>(25th-75 percentile)</i>	<i>3 months (FU1)</i>		<i>6 months (FU2)</i>		<i>p</i>
			<i>Median</i>	<i>(25th-75 percentile)</i>	<i>Median</i>	<i>(25th-75 percentile)</i>		
KJoule	8786.4	6989.6	(5905 - 8872.2)	7445.6	(7043.6 - 10009.9)	7011.5	(4923.9 - 9574.6)	0.257
Omega-3-fatty acids (mg)	0.5 %**	1776.1	(685.4 - 2003)	1657.6	(1278.4 - 3239)	1770.7	(1049.6 - 3318.7)	0.931
Calcium (mg)	1000 mg	642.3	(371.4 - 1132.3)	710.9	(471.9 - 1052.9)	734.6	(354.7 - 950.2)	0.931
Zinc (mg)	10 mg	11.02	(9.79 - 14.59)	10.48	(6.78 - 13.73)	10.73	(7.39 - 13.42)	0.526
Vitamin B12 (µg)	3 µg	4.4	(3.1 - 5.6)	5.8	(2.5 - 6.5)	2.8	(2.5 - 7.1)	0.257
Folic acid (µg)	300 µg	186	(130.7 - 230.9)	200.4	(158.2 - 255.1)	252.8	(187.5 - 317.0)	0.008
Vitamin A (retinol) (µg)	800 µg	393.4	(141.4 - 685.3)	382.8	(312.6 - 494.1)	269.3	(134.3 - 474)	0.109
Vitamin C (ascorbic acid) (mg)	110 mg	55.15	(24.51 - 201.16)	54.98	(44.55- 193.24)	138.69	(45.50 - 286.57)	0.607
Vitamin D (calciferol) (µg)	20 µg	1.2	(0.4 - 5.1)	1.9	(1.3 - 3.7)	1.2	(0.6 - 2.1)	0.319

Vitamin E (tocopherol equivalent) (mg)	13 mg	6.859	(4.1813 - 9.756)	9.133	(7.398 - 9.766)	12.751	(8.709 - 22.239)	0.017
Alcohol (g)	20 g	0.6	(0 - 7.24)	0	(0 - 16.16)	0.83	(0 - 19.8)	0.592
Fibre (g)	30 g/d	15.58	(13.13 - 24.52)	17.2	(1.42 - 21.39)	20.7	(12.81 - 25.69)	0.751
Protein (g)	0.8 g/kg BW	74.42	(57.31 - 82.13)	74.21	(58.42 - 98.51)	65.93	(56.26 - 73.54)	0.807
Fat (g)	< 30%**	84.8	(56.69 - 112.02)	91.59	(66.52 - 105.24)	84.56	(51.21 - 110.93)	0.931
Carbohydrates (g)	> 55%**	143.02	(125.21 - 176.77)	192.66	(145.49 - 234.71)	146.98	(124.23 - 229.01)	0.145
Food Frequency Index (FFI)		30	(25 - 34.5)	30	(27 - 36.5)	30	(29.5 - 34)	0.771

*Values are median (25th - 75th percentile)

** per cent of daily energy intake

°DGE= Deutsche Gesellschaft für Ernährung (German nutrition society)

BW =body weight"; EI = energy intake; FU = Follow-up; CG = Community dental care

Table 16: Dietary assessment; group: PT2

	<i>DGE-Reference values /d (273)</i>	PT2						<i>p</i>
		Baseline	3 months (FU1)		6 months (FU2)			
	<i>Median*</i>	<i>(25th-75 percentile)</i>	<i>Median</i>	<i>(25th-75 percentile)</i>	<i>Median</i>	<i>(25th-75 percentile)</i>		
Kjoule	8373.6	8788.0	(7018.2 - 11386.1)	7406.4	(5934.8 - 10152.4)	9429.7	(7175.7 - 12064.9)	0.002
Omega-3-fatty acids (mg)	0.5 %**	1382.1	(1258.6 - 1658.8)	1064.2	(786.3 - 1318.9)	1462.5	(1197.4 - 2111.3)	0.017
Calcium (mg)	1000 mg	574.7	(425.6 - 1070.5)	619.3	(329.5 - 872.5)	844.4	(485.5 - 1213.3)	0.627
Zinc (mg)	10 mg	10.27	(7.27 - 15.80)	9.19	(7.38 - 13.84)	12.48	(10.97 - 15.85)	0.189
Vitamin B12 (µg)	3 µg	4.8	(3.4 - 7.8)	3.9	(2.2 - 5.7)	6.9	(3.9 - 10.3)	0.247
Folic acid (µg)	300 µg	237.5	(173.9 - 318.2)	214.7	(149.4 - 287.4)	285.3	(179.9 - 328.6)	0.189
Vitamin A (retinol) (µg)	800 µg	460.1	(203.4 - 827.1)	432.7	(138 - 597.2)	353.3	(303.8 - 562.4)	0.247
Vitamin C (ascorbic acid) (mg)	110 mg	71.7	(48.79 - 124.76)	82.58	(28.49 - 125.29)	59.44	(40.54 - 143.22)	0.819
Vitamin D (calciferol) (µg)	20 µg	1.5	(0.6 - 4)	2.3	(0.8 - 3.6)	1.9	(1.4 - 3)	0.766

Vitamin E (tocopherol equivalent) (mg)	13 mg	7.309	(6.139 - 10.319)	7.509	(5.559 - 11.372)	8.408	(5.399 - 10.678)	0.819
Alcohol (g)	20 g	0	(0 - 19.800)	0.009	(0 - 25.000)	0.0002	(0 - 5.64)	0.828
Fibre (g)	30 g/d	15.5	(12.54 - 21.27)	16.644	(11.48 - 18.97)	21.16	(16.69 - 28.49)	0.189
Protein (g)	0.8 g/kg BW	82.63	(65.61 - 116.10)	83.5	(58.46 - 95.17)	101.77	(60.33 - 114.03)	0.85
Fat (g)	< 30%**	106.99	(68.38 - 122.20)	78.93	(44.22 - 95.03)	81.97	(69.60 - 98.62)	0.247
Carbohydrates (g)	> 55%**	199.09	(143.37 - 249.08)	168.56	(137.07 - 238.60)	273.51	(184.91 - 319.79)	0.014
Food Frequency Index (FFI)		26.5	(24 - 33)	27.5	(24.3 - 33)	28	(24.8 - 30.3)	0.259

*Values are median (25th - 75th percentile)

** per cent of daily energy intake

°DGE= Deutsche Gesellschaft für Ernährung (German nutrition society)
 BW =body weight"; EI = energy intake; FU = Follow-up; PT2 =
 periodontal therapy without antibiotics

Table 17: Per cent of daily macronutrient intake in the three treatment groups at baseline, FU1 and FU2

	CG			PT1			PT2		
	<i>Baseline</i>	<i>3 months (FU1)</i>	<i>6 months (FU2)</i>	<i>Baseline</i>	<i>3 months (FU1)</i>	<i>6 months (FU2)</i>	<i>Baseline</i>	<i>3 months (FU1)</i>	<i>6 months (FU2)</i>
% Protein of daily EI	18.1	16.9	16	16.9	14.7	16.8	16	19.2	18.3
% Carbohydrate of daily EI	34.8	44	35.6	38.7	36.5	41	38.5	38.7	49.3
% Fat of daily EI	44.9	45.5	44.6	34.5	38.3	34.1	45	39.4	32.2
% Omega-3-fatty acids of daily EI	0.99	0.82	0.93	0.51	0.46	0.52	0.58	0.53	0.57

Values are presented as median per cent of daily energy intake (EI); follow-up 1 (FU1) and follow-up 2 (FU2)

9 Discussion

Part 1: Nutritional status in patients with PAD depending on the level of periodontal disease

The nutritional status of an individual depends on many inter-related factors. It is commonly evaluated on the basis of anthropometric assessment, clinical examination, biochemical parameters and dietary history. No single method provides enough information to properly define an individual's nutritional status, but taken together they offer a detailed picture (277, 278). Oral diseases such as PD impair the functional ability to eat, contributing to a poor diet (20, 200) and thereby altering nutritional status.

We analysed anthropometric data, information from a 24-h recall and a FFQ for dietary assessment, and included laboratory measurements to define the nutritional status in patients with PAD and concomitant periodontitis. We then studied the relationship of nutritional status to the extent of PD.

Anthropometrical data

We assessed body composition on the basis of body weight, height, BMI and waist to hip ratio (WHR), together with iDXA data on total lean body mass and body fat percentage and found no differences in any of the anthropometrical parameters between the periodontal disease groups. Body weight, total LBM and percentage of total body fat were higher in periodontitis patients, but after Bonferroni correction these differences were not statistically significant.

AL-ZAHRANI et al. evaluated the relationship between body weight and PD using data from the 13,665 NHANES III participants. BMI and waist circumference were used as indicators of obesity. In participants aged 18-34 years, obesity was found to be associated with an increased prevalence of PD, but this association was not seen in the older participants (279), and another study by Wood et al. reported similar findings. There was a significant association between body composition (WHR, BMI and fat-free mass) and periodontitis, and the authors concluded that not only total body fat, but also its distribution might play a considerable role (280). Brostow et al. investigated the association of body composition with PAD. (94), finding that abdominal obesity was a significant predictor of a decreased ABI and adverse events in PAD patients.

Biochemical analysis

We looked closely at laboratory parameters, which are intensively discussed in the literature as fundamental to a proper determination of nutritional status. Malnutrition in general has been reported to negatively influence PD (18) while PAD has also been shown to be associated with poor dietary habits (7). Serum albumin, serum transferrin and serum prealbumin are widely used to determine malnutrition and nutritional status (281), with serum prealbumin suggested to be the most sensitive parameter (282). We assessed all these parameters but they did not differ according to the stages of PD in our patients. Median values were in the normal reference range (272).

Several other laboratory parameters also failed to show differences according to PD stage. The only exception was CRP, which was highest in the moderate periodontitis group but did not reach statistical significance. It would be reasonable to expect that our patients with both PAD and PD would differ from standardized reference values in at least some laboratory markers such as CRP (272) but the median values of the measured parameters were all in the normal range. These results show that as far as laboratory parameters are concerned, the severity of PD bears no relationship to nutritional status.

Dietary intake

An experienced dietician assisted patients with a 24-h recall to assess nutrient supply, then the patients themselves filled out a FFQ, which a dietician checked for completeness. The combination of a 24-h recall and FFQ to assess dietary intake was chosen for our elderly patients, who would have been over challenged by more elaborate methods, such as a 7-day food record as is sometimes used to assess dietary intake.

The German nutrition society (DGE) recommends an EI of 2100 kcal (8786.4 KJ) per day for men 51-65 years of age with a physical activity level (PAL) of 1.4. Their recommendations were used since 75 per cent of our subjects were male and median age was 62 years. A PAL of 1.4 was considered appropriate since patients with PAD show relatively low physical activity levels and a reduced mobility due to some degree of walking impairment (283). Analysis of their protocols showed no difference in EI among PD stages. The median EI was slightly lower in patients suffering from severe periodontitis, but did not reach significance. The DGE recommended EI was slightly exceeded by the moderate periodontitis and severe gingivitis groups; however, patients with severe periodontitis did not reach the recommended amount. This might be the result of increased pain and discomfort in the mouth due to the advanced stage of the PD.

Likewise, Sheiham et al. reported that the dental status of older people can have an impact on their dietary intake due to a reduced ability to eat, which affects food choice and amount of nutrients consumed (22). Mandel et al. (274) and Walls et al. (275) have also suggested that oral pain and discomfort can influence eating habits and the amount and selection of foods consumed.

DGE recommendations for daily intake of carbohydrate, fat and protein are 50-60%, 30% and 10-15%, respectively (273). In our study, carbohydrate intake was far below the recommended amount and total carbohydrate intake was lowest in patients with severe periodontitis, again possibly due to the discomfort of PD, especially when advanced. Periodontal inflammation and loose teeth might make it difficult to eat high-fibre foods that are hard to chew, and in fact fibre intake was far below the recommendations in our patients with all three stages of PD and lowest in patients with severe periodontitis. The fact that the patients with severe periodontitis had the lowest intake of total carbohydrates might also be related to their reduced total EI. Adequate fibre intake has previously been associated with improvements in PD markers (216) and showed an inverse association with PAD (7).

Our results show unexpectedly high daily fat consumption in all three PD stages as compared to the DGE recommendation that fat should not account for more than 30% of daily EI. Median fat intake in all groups markedly exceeded the recommended daily allowance (RDA), but without any differences among the PD groups. Fat consumption should emphasize PUFA, such as n-3 fatty acids, due to their established anti-inflammatory effects (210). Besides having the highest total fat intake, patients with moderate periodontitis (PGU = 3) consumed the most n-3 fatty acids. Along with the severe PD group, they met the recommended amount of n-3 fatty acids (0.5% of daily EI). N-3 fatty acid intake in patients with gingivitis was slightly below the recommendations.

Protein intake did not differ significantly among the PD stages. The recommended intake of 0.8 g/ kg BW was slightly exceeded in all of the groups, with the lowest intake in patients with severe gingivitis. Again, patients with moderate periodontitis consumed the most protein. Increased consumption of protein and fat might have compensated for the reduced intake of carbohydrates in all of the PD stages. These patients might find it easier to eat protein- and fat-rich foods than high-fibre and coarse foods. Individuals with PAD and periodontitis might generally have a slightly increased protein requirement due to the sustained inflammatory processes and increased demand for immunoproteins.

Our findings for macronutrient intake (shown in **Table 6**) stand in contrast to a study by Gardner et al., who reported a mean macronutrient composition of 17% protein, 51% carbohydrate, and 30% fat in patients with PAD (9).

Inflammatory processes are involved in both PD and PAD, as previously discussed. PAD patients have been shown to have an increased level of oxidative stress (62) that increases their need for nutritional antioxidants and micronutrients (106). The analysis of the micronutrient intake from the 24-h recall revealed no statistically significant difference between the three stages of PD. Only calcium intake showed a significant difference between the three stages and patients with severe periodontitis consumed the least dietary calcium, but Bonferroni correction eliminated the significance. Because we were considering several important variables to evaluate nutritional status, it was appropriate to apply Bonferroni correction to account for multiple testing.

The DGE RDA of vitamin E is 13 mg, which most of our subjects did not reach; again, there was no difference between the three PD stages. Previous studies reported low consumption of vitamin E in PAD patients (4) as well as an inverse association of the vitamin with the disease (96). The role of vitamin E in humans with PD has not yet been fully evaluated.

Vitamins of the B complex as well as zinc have been suspected to play a role in PD (199) by modifying wound healing and helping to control plaque growth (247) and we again saw no difference between the different degrees of severity of PD. Vitamin B12 has also been reported to have an inverse association with the risk for PAD (7, 120). In our study, dietary intake of zinc and vitamin B12 met the recommendations. Our patients did not reach the RDA for folic acid and vitamins A and D. Patients with moderate periodontitis consumed the most folic acid and vitamin A, but not significantly more than the other two stages of PD. Our finding of low vitamin A intake supports the findings of Lane et al. based on the NHANES III study that PAD patients have low intake of antioxidants such as vitamin A (6).

Low serum vitamin D levels have been linked to an increased prevalence of PAD (8, 114, 115). The vitamin is not only involved in vascular and bone health but also plays a role in the pathophysiology of PD, as mentioned in Chapter 5.9.2. In older men, for example, a total intake of vitamin D \geq 800 IU was found to be associated with lower odds of severe PD relative to an intake $<$ 400 IU (230). In our study, patients consumed very little vitamin D but with no differences according to PD stage. Our finding of a very low intake of vitamin D in PAD patients and concomitant PD is congruent with previous studies reporting associations between low vitamin D intake and PD (230) and between low serum 25(OH)D-levels and the prevalence of PAD (114).

We analysed folic acid, another water-soluble vitamin, since it also appears to be important for periodontal health (284). Associations between this vitamin and both PD (242, 243) and PAD (10) have been demonstrated. Lower folic acid intake correlated with a significantly higher prevalence of PAD (94). The impact of this vitamin on PD is supported by an independent negative association of serum folic acid levels with PD reported after analysis of NHANES data (242). In Japanese adults, Esaki et al. found a significant negative correlation between dietary folic acid levels and bleeding on probing (243). Folic acid is further known to be involved in the synthesis of deoxyribonucleic acid (DNA), so a lack of folic acid could negatively influence tissues with a high rate of cell regeneration, such as gingival tissue (196). Our result supports these previous findings. Although no significant difference was found between the groups, overall folic acid intake was far below recommendations, possibly influencing PD and PAD.

Vitamin C appears to be the most important vitamin in the aetiology of PD and deficiency leads to gingival and periodontal inflammation, as shown in other studies (234, 239). An association of serum vitamin C levels has now also been demonstrated in patients with PAD (108). The importance of this vitamin is particularly attributed to its antioxidant properties (6, 96, 107) and positive effects on endothelial restoration (8). In our study, dietary vitamin C intake did not differ significantly according to stage of PD. The DGE recommends 110mg of vitamin C daily. Patients with severe gingivitis (PGU 2) almost reached the RDA, but those with moderate (PGU 3) and severe periodontitis (PGU 4) did not. Our findings, although not statistically significant, support previous studies suggesting a role of vitamin C in PD (205, 234) and PAD (6, 96, 107). Patients with periodontitis and PAD might have an even greater need of adequate vitamin C levels than the normal population, since this vitamin is involved in the maintenance of connective tissue health (such as the periodontium), besides influencing immunological processes and wound healing (234).

Besides vitamins, we also looked at our patients' mineral intake. Calcium is particularly important in PD and as mentioned elsewhere, we saw significant differences in calcium intake in our patient groups before Bonferroni correction. Patients with severe periodontitis consumed the least calcium and those with moderate periodontitis the most. The DGE RDA for calcium is 1000mg, which none of our groups achieved. Nishida et al. (236) analysed NHANES III data and found that reduced dietary calcium intake was associated with an increased risk for periodontitis. A dose response relationship was demonstrated with an increased risk for PD with a dietary calcium intake below 500mg per day. Our patients' median values exceeded this, although only slightly with severe periodontitis.

Interestingly, dietary intake of most of the micronutrients was highest in the patients with moderate periodontitis (PGU = 3), but not significantly different from the other two groups. It could be hypothesized that patients in the periodontitis group (PGU = 3) have a better inflammatory status and that less periodontal inflammation makes eating easier than in the other disease stages. The inflammatory burden could be higher in patients with severe gingivitis and severe periodontitis than those with only moderate periodontitis. Our hypothesis is supported by the fact that our patients with moderate periodontitis had the lowest median level of CRP, although they did not differ significantly from the other groups.

In agreement with previous studies that reported inadequate nutrient intake among patients with PAD (4, 6) and CLI (101), our subjects deviated from the RDA for some macro- and micronutrients. Carbohydrate and fibre intake was low while fat intake exceeded recommendations in all groups. Dietary intake of vitamins A, D, E and C, folic acid and calcium in our study population did not meet DGE recommendations, which is in agreement with other studies (4, 6, 96, 230, 236). Energy intake, n-3 fatty acid, zinc and vitamin B12 met their RDAs, in contrast to other studies that reported low n-3 fatty acid consumption in PAD patients (4, 6, 123) and inverse associations with vitamin B12 intake and risk for PAD in men (120).

Taking into account all the data examined in this study, it can be concluded that the nutritional status in patients with symptomatic PAD and concomitant PD does not vary depending on the stage of PD. This is further supported by the fact that the food frequency index, as an assessment of diet quality, did not show statistically significant differences between the three stages of PD.

Part 2: Effect of periodontal therapy on nutritional status and nutrient supply in PAD patients

In this study patients with PAD and concomitant PD received either periodontal treatment alone or in combination with antibiotics. A third control group did not receive periodontal treatment during the first 3 months of the study. We aimed to evaluate the effect of periodontal treatment on nutritional status in patients affected with both PAD and PD.

As previously mentioned, it is currently recommended that nutritional status be determined by combining information from anthropometrical measurements, clinical and laboratory examination, and the dietary history and habits (277, 278). We assessed nutritional status at baseline (BL), and after 3 and 6 months of follow-up (FU1 and FU2 respectively).

Anthropometrical data

The anthropometrical data were collected as the first step in the evaluation of nutritional status; they indicated that the anthropometrical parameters did not change between baseline and the follow-up visits at 3 and 6 months in any of the groups. Our results confirm those of Altay et al., who also investigated the effect of a non-surgical periodontal treatment on systemic inflammatory, lipid, and glucose parameters in patients with chronic periodontitis. They measured BMI and waist circumference as well and also found them unchanged 3 months after periodontal therapy (285).

It may be hypothesized that anthropometrical values including muscle and fat mass do not change during this short time in well-nourished individuals in a developed country. We cannot exclude that a change in food intake and dietary behaviour after periodontal treatment might alter patients' anthropometrical data after a longer period.

Biochemical analysis

The second step in the evaluation of nutritional status is the analysis of laboratory parameters. Serum albumin, serum transferrin and serum prealbumin are specific markers commonly used to assess malnutrition and nutritional status (281), whereby serum prealbumin is suggested to be the most sensitive of them (282). We took rigorous care with our measurements, but there were no differences between baseline and the follow-up visits in any of the groups, and the median values were always in the normal reference range (272).

We also measured serum CRP, which is an acute phase protein expressed by liver cells and widely used as a marker for inflammation (286). Patients receiving periodontal therapy with antibiotics showed a slight decrease in median CRP level at the last study visit, yet always remained within the normal range. Non-surgical periodontal therapy was previously reported to reduce serum levels of CRP (287). This supports our finding of a slight, yet non-significant reduction of CRP in the patients additionally treated with antibiotics. Evidence suggests that individuals with a chronic severe PD generally have high levels of CRP (287), and a relation between CRP and the severity of PAD has been demonstrated (288, 289). In contrast, the treatment of periodontitis without antibiotics in our study revealed a significant increase in CRP after 6 months, but was no longer significant after Bonferroni correction. It should be noted, however, that after treatment these patients' CRP values only exceeded the upper level of the reference range by 0.3 mg/l.

These slightly higher CRP values found in the patients who received periodontal treatment without antibiotics cannot be clearly attributed to the lack of the medication, since our patients had both PAD and periodontitis. An additional focus of inflammation may have distorted our results, since inflammatory processes are also involved in the pathophysiology of PAD (26).

Nutrient intake

Our assessment of food intake, as the third and probably most important step in determining nutritional status, involved a 24-h recall and a standardized food frequency questionnaire at each visit. For our elderly patient population, a 24-h recall at each visit was more feasible than a 7-day food record. We carefully differentiated intake of energy, carbohydrates, fibre, vegetables, fruit, fat, n-3 fatty acids, protein, antioxidants (vitamins), and trace elements (zinc, calcium).

It turned out that non-surgical periodontal therapy combined with antibiotics did not alter overall EI in patients with PAD and concomitant PD. No change in EI was seen in control patients, although their EI was below the DGE recommendations (273) at all points of evaluation. Patients receiving non-surgical periodontal treatment without adjunctive antibiotic treatment showed a decrease in EI immediately after therapy. During the following 3 months they increased their intake by 2023 KJ (483 kcal) until the end of the study, possibly as a consequence of less oral pain. After Bonferroni correction the effect was less impressive and did not reach significance.

The current DGE guidelines for daily carbohydrate intake recommend consumption of more than 50% carbohydrates and 30g of fibre (273) but our patients consumed far smaller amounts of both. Interestingly, the patient group receiving periodontal therapy without antibiotics (PT2) increased their daily carbohydrate intake by 11% at FU2 compared to FU1 and nearly reached the lower recommended amount for this macronutrient. Again, after Bonferroni correction, this increase was no longer significant. The increase in carbohydrate intake paralleled the increase in EI seen at the end of study in the treatment group without antibiotics, as discussed above. Patients in the PT1 group also showed a slight increase in carbohydrate intake after 6 months, although their intake was still below the recommendations. Our findings stand in contrast to a study by Gardner et al., who reported consumption of 51% carbohydrates of daily EI in patients with PAD (9), which meets the recommended intake.

In general, carbohydrate foods are rich in fibre and patients with PD usually find them difficult to chew. Inflammation of the gingiva and loose teeth can compromise their ability to eat many coarse, high-fibre and whole-grain foods (24, 195, 274). We expected that patients additionally receiving antibiotics would show the greatest improvements in periodontal health and subsequently the greatest improvements in nutritional intake, but this was not in fact the case.

A major consequence of PD can be tooth loss (137). Besides pain and discomfort in the mouth due to PD (274), loss of teeth was linked to poor nutrition (23), and edentate patients tended to avoid the fibre in many vegetables and fruits (22, 23). This is supported by Javid et al., who demonstrated that the consumption of fruits and vegetables was below the recommended daily amount in patients with chronic periodontitis (290). It is notable that increased intake of fibre was associated with reduced CVD risk (81) and reduced prevalence of PAD (6), and also showed improvements in PD markers (216). High-fibre foods such as vegetables, fruit and whole grain contain a variety of different micronutrients and secondary plant products also assumed to be beneficial in inflammatory conditions (291-293). The association of increased fibre intake with benefits for PAD and PD patients might also be partly influenced by the increased consumption of fruit and vegetables containing these beneficial nutrients.

Our patients' fibre intake did not change significantly after periodontal treatment. In all treatment groups, intake was far below recommendations at baseline, but increased slightly in all the groups during the study, though still without meeting the recommendations. The periodontal treatment group without adjunctive antibiotic therapy showed the greatest increase in fibre intake, which was paralleled by the greatest

increase in total energy and carbohydrate intake. Our results are in agreement with Staudte et al. (20), who also demonstrated that patients with periodontitis ate less fibre than healthy subjects. Even though not significant, the increase in fibre intake after periodontal treatment might be explained by a better ability to chew high-fibre foods. Periodontal treatment might have reduced pain and discomfort, so that patients could again include whole-grain and fibre-rich foods in their diet.

Periodontal therapy (with or without antibiotics) did not significantly change consumption of fat, n-3 fatty acids, protein or alcohol within the patient groups during the study period. There were no changes in the control group after 3 and 6 months. Patients who had had periodontal treatment without antibiotics ate the most protein, and even exceeded current recommendations. This again might possibly be explained by the positive effect of periodontal treatment on oral comfort and in turn on nutrient intake.

As recommended by the DGE, fat intake should not exceed 30% of the daily EI (273). In our study daily fat intake markedly exceeded the recommended amount in all groups at all time-points (fat as 40% of daily intake). At the end of the study patients who had had periodontal treatment without antibiotics had the lowest value (32 %). In contrast, Gardner et al. reported fat intake of 30% in PAD patients, in accordance with recommendations (9). The consumption of PUFA, such as n-3 fatty acids, is particularly important in inflammatory diseases such as periodontitis, due to its anti-inflammatory properties (210). Lane et al. reported reduced consumption of n-3 fatty acids in patients with PAD (6). Our patients consumed enough n-3 fatty acid and even exceeded the recommended minimum of 0.5% of daily EI. This could be due to the elevated consumption of fat in general, which would also increase the proportion of PUFA consumed, including n-3 fatty acids.

As mentioned above, dental status and tooth loss have also been shown to have negative effects on dietary quality and nutrient intake (21, 22, 24). Since PD can result in tooth loss and oral pain (137, 274), the successful treatment of the disease may subsequently improve food intake and the quality of the patient's diet. In our study, however, periodontal treatment did not have a statistically significant influence on dietary quality assessed with a FFQ, but in the group of patients receiving treatment without antibiotics there was a trend toward an increase in dietary quality. Initially we had suspected that patients receiving additional antibiotic treatment would have the greatest improvements in periodontal health and consequently in nutritional status, by reducing the specific microbial burden leading to PD (294). This did not, however, prove to be the case.

In addition to macronutrient intake, we assessed changes in micronutrient consumption after periodontal therapy. Micronutrients and in particular antioxidant vitamins are important to counteract the production of ROS, which are markedly increased in higher states of oxidative stress (192) and in aggressive forms of PD (173). Periodontal patients demonstrated reduced antioxidant potential and antioxidant levels (173), as did PAD patients (101). We found that the dietary intake of the antioxidant vitamins A, C and E, did not change significantly with periodontal therapy. In the control group the consumption of vitamin E nearly doubled after 6 months and vitamin C intake increased to exceed the recommended 110mg/d (273), although the changes did not remain significant after Bonferroni correction. We do not have an explanation for this finding.

At baseline, vitamin A, C and E intake did not meet the recommendations for daily intake in any of the treatment groups. Our findings agree with those of Gardner et al., who also observed that none of their PAD patients met the recommendations for vitamin E (9). A similar finding was reported in patients with PD, who also consumed less vitamin E than healthy individuals (226). Our finding further supports Nishida et al., who found a dose response relationship between dietary vitamin C intake and PD (234). Staudte et al. (20) as well reported reduced intake of vitamin C in patients with periodontitis, possibly due to oral pain upon chewing. These problems during food intake might reduce the ability to eat crisp fruits and vegetables high in vitamin C and other antioxidants. It has also been reported that patients with periodontitis do not eat enough fruits and vegetables (290).

Vitamin D, like vitamin E a fat-soluble vitamin, appears to be influential in periodontal and peripheral arterial disease. Vitamin D intake was inversely related to the risk for PAD (7) and low serum 25(OH)vitamin D-levels were strongly associated with the prevalence of the disease (8, 114, 115). An adequate supply of vitamin D seems to be especially important in PD, since it is involved in bone metabolism, control of inflammation, cell development and neuromuscular functioning (197) as well as in the optimal maintenance of bone mass and skeletal development (227). Possibly due to its anti-inflammatory actions, vitamin D decreases susceptibility to gingival inflammation (232). Garcia et al. recently demonstrated that the intake of ≥ 800 IU (20 μg) of vitamin D was associated with lower odds of severe PD relative to an intake < 400 IU (10 μg), suggesting a protective effect of vitamin D intake against the progression of PD (230). The importance of vitamin D in PD is further supported by its interaction with calcium (295), which is an important mineral in PD. It has been shown to correlate with PD severity (236). Vitamin D promotes the absorption of calcium from the gut (296) and inadequate intake is reported to be a risk factor for PD (154). We found no change in vitamin D intake and median vitamin D intake was far below the recommended intake levels in all treatment groups at all study visits, so

that periodontal treatment had no influence on vitamin D intake in our patient population. As in the general population, vitamin D intake at baseline was insufficient in our study group. Vitamin D deficiency and inadequate vitamin D intake have been consistently observed in Europe (297).

We also assessed the dietary intake of two vitamin B-complex water-soluble vitamins, vitamin B12 and folic acid, since both have been thought to be beneficial in PAD and PD. The effect of folic acid and other B-vitamins on PAD might be a result of its influence on homocysteine metabolism (99). Reduced folic acid intake was reported to correlate with a significantly higher prevalence of PAD (94) and the intake of B-vitamins was strongly associated with protection from the disease (6); an independent negative association of serum folic acid levels with PD has been reported (242). Esaki et al. also studied dietary intake of folic acid and found a significant negative correlation between dietary folic acid levels and bleeding on probing in Japanese adults (243). Our findings corroborate these studies. Vitamin B12 intake did not change significantly during the study period in any of the groups and the median intake values were in line with DGE recommendations.

At baseline in all groups, consumption of folic acid was below the recommended amount for daily intake, but did increase in all of them over the study period. Patients receiving periodontal therapy without antibiotics (PT2 group) had the highest median folic acid intake at every assessment. At the last study visit (FU2) after periodontal treatment, patients without antibiotic treatment even reached the RDA for folic acid. But the increase in folic acid intake from baseline to the end of study was greater in patients in the control group and those receiving antibiotics (PT1-group) than in the PT2 group. Typically, this increase did not remain significant after Bonferroni correction.

We also recorded zinc and calcium intake. The DGE recommends 10 mg of zinc daily. All groups achieved this recommended amount at every visit, except for the PT2 group, whose zinc intake was slightly below the recommendations at the first follow-up visit. Zinc intake did not change throughout the study period, meaning that periodontal therapy did not alter the dietary intake of zinc in patients with PAD and PD.

Calcium is a particularly important mineral in periodontal health and inadequate intake is a risk factor for PD (154). Nishida et al. (236) found that inadequate dietary calcium intake was associated with an increased risk for periodontitis. A dose response relationship was found, with females having a 54% greater risk for PD with a dietary calcium intake below 500mg. Even with only moderately reduced calcium intake (500 to 800mg), there was a 27% greater risk for PD. In our study, the median dietary calcium intake at baseline was above 500 mg in the three treatment groups. Although not statistically significant, there

was an increase in median dietary calcium intake at the end of the study compared to baseline in all groups. Again, patients receiving periodontal treatment without adjunctive antibiotics showed the greatest increase in median calcium intake after 6 months. It increased by 270 mg over the initial visit, but the recommended amount for daily calcium intake for our patients would be 1000 mg (273), which none of the patients ever reached during the study. Our findings might support previous studies showing an association of low dietary calcium intake with an increased risk for PD (236, 248) and tooth loss (249). Sheiham et al. reported higher intake of calcium in patients with a greater number of natural teeth (24).

Unexpected findings of our investigation:

1) Those patients receiving periodontal treatment without adjunctive antibiotics showed the greatest improvements in nutritional intake at the end of the study. The consumption of energy, carbohydrate, fibre, protein, n-3 fatty acids, calcium, zinc, vitamin B12, folic acid and vitamin E increased after the 6 months of the study compared to baseline levels. Yet it has to be kept in mind that there was no statistically significant increase when exact statistical methods, i.e. Bonferroni correction, were applied.

The number of natural teeth might also play a role, since Sheiham et al. reported that patients with a greater number of natural teeth also had higher intake of energy, fat, carbohydrate, protein, fibre, calcium, iron and vitamins C and E (24). We cannot disregard this as a possible influence on nutrient intake in our patients.

2) Since Altay et al. found no changes in eating habits within a short follow-up period of 3 months after non-surgical periodontal therapy (285), we designed a 6-month trial. But, with the lack of significant changes found in our study setting, we cannot definitely exclude a beneficial effect of periodontal therapy, because the study period might still have been too short. Patients' dietary habits and overall nutrient intake might have changed to a greater extent when observed over a longer period of time.

In conclusion, we were not able to demonstrate a significant improvement of nutrient intake and nutritional status after implementation of an established treatment regimen for periodontitis. In our study, dietary intake of calcium, folic acid, vitamin A, C, E and D, fibre and carbohydrates was below the DGE recommendations in patients suffering from both PAD and PD. This may underscore the need for additional individual dietary counselling,

stressing the need to eat more fruits and vegetables and advising patients with missing teeth and chewing problems on how to prepare them. A beneficial effect of a dietary intervention focussing on increased consumption of fruit, vegetables and whole grain has already been demonstrated to be beneficial in patients with chronic periodontitis (290).

Future studies on PD should include a comprehensive oral exam for patients as well as a survey covering their oral pain and discomfort and eating habits. Long-term studies will be needed to monitor anthropometrical changes, as well as the long-term effect of periodontal treatment on nutrient intake and food selection.

Strengths and Drawbacks

In view of the literature, our study has several strengths and drawbacks that should be mentioned.

Strengths:

(1) This research project combined two different assessment methods (24-h recall and FFQ) to evaluate nutrient intake. This combination markedly increases the accuracy of nutrient intake assessment. A more elaborate method for nutrient intake determination, such as a 7-day food record as used in some studies, was not feasible in a population of multimorbid elderly outpatients.

(2) The strict inclusion and exclusion criteria were other strengths of this study (i.e. the requirement of at least 12 natural teeth for the second, interventional part of the study)

(3) To minimize a possibly negative effect of multiple testing on the final results, the Bonferroni correction, a well-established and accurate statistical method, was applied.

(4) Lean body mass and fat mass were determined with DEXA, which is the current gold standard for body composition assessment. Due to the expense, this examination is usually not included clinical studies such ours.

(5) To the best of our knowledge, this is the only study investigating a broad set of parameters important for accurate nutritional status evaluation. It included anthropometrical data, laboratory markers for inflammation and nutritional status, as well as a survey of nutrient intake (water and fat-soluble vitamins, zinc, calcium, n-3 fatty acids, macronutrients, alcohol). It was our intention to gather as much information as possible about the subjects' overall nutritional and health status in a well-defined setting.

Drawbacks:

1) A 24-h recall and a FFQ were used to determine nutrient intake. It has to be borne in mind that nutritional data for dietary assessment from self-completed questionnaires may not be completely reliable. Despite the support of a trained dietician, patients may have over- or underestimated their food intake. There could also have been a bias because participation in a nutritional study under medical supervision influenced the patients' eating behaviour.

2) A further limitation might be the relatively short duration of follow-up, although other investigations covered even shorter periods. Changes in nutritional behaviour and anthropometrical parameters following therapy might have been more prominent with longer follow-up.

3) There was no control group with patients suffering from PD without peripheral vascular disease.

4) Our results cannot be applied to a general population since PAD is mainly a disease of the elderly.

5) Finally, it has to be mentioned that serum levels of vitamins and minerals were not measured due to budgetary limitations. Future studies should consider the additional assessment of serum vitamin levels.

To assess the effect of periodontal therapy on nutrient intake more thoroughly, long-term studies are necessary to monitor anthropometrical changes, as well as the long-term effect of periodontal treatment on nutrient intake and food selection. A larger number of subjects would be desirable. Future studies need to carefully assess tooth status, oral pain and discomfort, and dietary intake.

10 Conclusion

Nutrition has not received enough attention in the past and plays only a minor role in the prevention and therapy of PAD and PD, though a number of recent studies indicate an important influence of certain nutrients on both of these health issues. This study aimed to investigate the nutritional status of patients with PAD and concomitant PD and to determine whether the treatment of the PD has an influence on nutrient intake and nutritional status.

Nutritional status in our well-defined patient population did not differ depending on the degree of PD. Dietary intake and diet quality (determined by the food frequency index) did not show significant differences between the three stages of PD at the initial visit. The consumption of calcium, folic acid, vitamin A, C, E and D, fibre and carbohydrates was considerably below the DGE recommendations. Fat intake in this population was averaged 10 per cent more than is recommended.

Non-surgical treatment of periodontitis, with or without antibiotics, did not significantly improve nutrient intake and nutritional status. Again, the dietary intake of calcium, folic acid, vitamin A, C, E and D, fibre and carbohydrates remained below DGE recommendations throughout the study, while fat consumption exceeded the recommendations. Consumption of n-3 fatty acid, zinc and vitamin B12 was adequate.

Finally, these results are most likely a consequence of PD leading to difficulties in chewing, which might have discouraged consumption of whole grain foods, fruits and vegetables. Specific dietary advice to increase the consumption of fruits and vegetables and improve diet quality might be advisable, and patients with extensive tooth loss and chewing problems should be advised on how they can prepare these foods. Whether patients with PAD and PD undergoing periodontal treatment reap an additional benefit from improved nutritional status following a detailed nutritional survey and targeted dietary counselling remains an open question.

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13 Appendix

13.1 A1: General medical history sheet

Anamneseblatt

NAME: _____

Datum: _____

Geb. Datum: _____

1) Derzeitige/aktuelle Beschwerden:

2) Herzinfarkt/ Schlaganfall/pAVK in der Verwandtschaft ersten Grades:

Ja: _____

Nein

3) Herzinfarkt/Schlaganfall bei Patient:

Ja: _____

Nein

4) Sonstige Erkrankungen:

5) OP:

6) Risikofaktoren:

Hypertonie

DM IDDM NDDM

Hyperlipidämie

Nikotin

Allergien

7) Sozialdemographische Daten

Pensionist: Ja Nein

Ausgeübter Beruf: _____

Ausbildung: Pflichtschule AHS Univ/further education

Verheiratet ledig geschieden verwitwet

13.2 A2: Form for anthropometrical measurements and blood pressure

PeriPAD-STUDIE

Messung am: _____

Name: _____

Geburtsdatum: _____

Gewicht: _____

Größe: _____

Bauchumfang / Waist: _____

Waist-to-height: _____

Hüfte: _____

Waist-to hip: _____

CW-Doppler ABI

Links

Rechts

Fuß

Hand

Blutdruck:

Links

Rechts

13.3 A3: Food list of the FFQ

List of the foods used in the food frequency questionnaire (FFQ):

Pasta, rice
Potatoes
Muesli, cereal flakes, cornflakes
White bread (buns, rolls)
Mixed-grain bread
Whole meal bread
Beef, pork, lamb
Poultry
Fish
Processed meats (sausages, ham, etc.)
Eggs
Milk, cacao and other drinks made from milk
Dairy products (milk, yoghurt, etc.)
Butter
Fruit (fresh)
Stewed/cooked fruit, fruit sauce
Vegetables (fresh or as a side dish)
Salad
Pulses (beans, lentils, etc.)
Almonds, peanuts or nuts
Vegetable oils
Cereal bars, cracker
Chocolate, confectionary
Pastries and desserts
Savoury snacks (Soletti, chips,...)
Fruit or vegetable juices
Light drinks
Energy drinks
Soft drinks
Beer, wine, sparkling wine
Liquor
Fruit or herbal tea

Coffee, black tea, green tea
Mineral water
Tap water
(Multivitamin-) supplement