Short-term pre-operative dietary restriction in vascular surgery

Kip, P.

Chapter 1.

General Introduction and Thesis Outline.

Atherosclerotic Occlusive Disease.

Globally, over 200 million individuals suffer from peripheral artery disease (PAD)[1] which is frequently accompanied by coronary artery disease (CAD).[2, 3] Both CAD and PAD are occlusive arterial diseases that can lead to (partial) obstruction of blood flow, which results in local or downstream hypoxic tissue.[4] The pathophysiology of PAD and CAD is mainly driven by atherosclerosis[5], which is a multifactorial inflammatory lipid loading disease of the vascular wall.[6] Atherosclerosis is a chronic and progressive disease, and is characterized by the formation of a lipid rich core in the intimal layer of the vascular wall. This initial process is triggered by elevated low-density lipoprotein cholesterol[7], and exacerbated by risk factors such as obesity, smoking, lack of exercise and hypertension.[8] After this initial lipid-rich core is established in the vascular wall, it will then progress towards a mid-stage atherosclerotic plaque that is characterized by both a necrotic core and uncontrolled inflammation.[9] If plaque growth continues, it can eventually lead to partial obstruction (i.e. stenosis) or a complete impairment of blood flow (i.e. occlusion). More frequently, the plaque can become unstable and eventually erode or rupture. Both plaque stenosis/occlusion and erosion/rupture can lead to local and downstream ischemic tissue[10] and its clinical manifestation is dictated by the anatomical location of the diseased artery. Occurring in a coronary artery, it will give rise to a myocardial infarct and subsequent heart failure.[4] In arteries of the lower extremity, i.e. PAD lesions, the disease commonly manifests as lower extremity pain (i.e. intermittent claudication) or (chronic end-stage or acute) critical limb ischemia in severe cases.[11]

(Cardio)vascular Surgery Interventions.

Lifestyle interventions, statins and anti-platelet drugs are employed to address risk factors for the development of PAD/CAD, consequently slowing the progression of atherosclerotic lesions.[12] And although these are effective in delaying disease onset and progression, unfortunately currently no drugs exist that can mitigate severe clinically manifest coronary/peripheral atherosclerotic occlusive disease.[13] In patients presenting with an acute occlusion of a coronary artery, i.e. acute coronary syndrome/myocardial infarct, percutaneous coronary intervention (PCI) with stent (bare-metal or drug-eluting) is the gold standard.[14] In sub-acute CAD, patients will receive either PCI or coronary artery bypass grafting (CABG). Open bypass surgery is preferred generally when multi-vessel or extensive left main disease is present[15, 16], while an endovascular approach is the treatment of choice in case of single vessel disease and/or a healthy subject.[15, 17] In CABG, the internal mammary artery (IMA) is the preferred conduit compared to other possible arterial and venous grafts, with excellent results.[18, 19] However, especially in multi-vessel CAD, utilization of the greater saphenous vein can be necessary to bypass the occluded arteries.[19]

In vascular surgery practice, intermittent claudication (i.e. symptomatic PAD) is initially treated non-invasively via lifestyle interventions[20], anti-platelet drug therapy[21] and supervised exercise training.[11] In case of severe claudication or critical limb ischemia (chronic or acute), open or endovascular revascularization is necessary to restore blood flow and achieve limb salvage.[22-24] Depending on the location and size of the atherosclerotic plaque, the intervention can either be performed endovascular, open or via a hybrid combination of both.[11, 20, 24] The occlusion/stenosis can be graded according to the TASC classification system[25, 26], in which focal stenosis or occlusions over short lengths are graded TASC A-B and deemed suitable for an endovascular approach. Multiple or recurrent stenosis/occlusions over longer lengths of artery are graded TASC C- D and are candidates for open revascularization via either endarterectomy, bypass surgery or a combination.[26, 27] Endovascular interventions include balloon angioplasty, drug-coated balloons, bare-metal or drug-eluting stents, or endovascular atherectomy. While open interventions include simple endarterectomy, endarterectomy with a patch or bypass surgery employing either venous or prosthetic grafts.[20, 24, 28]

(Cardio)vascular Surgery Interventions Have High Complication and Failure Rates.

Surgical Interventions in Coronary Artery Disease.

Amongst open and endovascular cardiac interventions, PCI is commonly performed and preferred in single vessel disease.[29] Outcome and success rates depend on the type of lesion, patient population and choice of stent (drug-eluting, bare-metal). On average, stent restenosis will occur in between 4.1 - 11% of patients at one-year post-PCI.[30-34] In multi-vessel and left main coronary artery disease however, CABG results in lower 5-year all-cause mortality compared to PCI.[15] In bypass surgery, success rates will mainly depend on the type and the quality of the conduit used to bypass the artery. Graft durability after a bypass with bilateral or lateral IMA is excellent, with patency rates at around 90% 10 years post-operatively.[35, 36] When the IMA is not suited for grafting, or not of sufficient length, the greater saphenous vein is used to create a saphenous vein graft (SVG) and indeed is used more frequently in clinical practice.[37] At 1-year post-intervention, SVG patency rates are at 75%[38] while at 10-15 years post-surgery this further falls toward 50-60%.[39, 40]

Surgical interventions in Peripheral Artery Disease.

In lower extremity vascular surgery few trials have been completed that directly compared open bypass surgery with endovascular procedures in case of (chronic) limb- threatening ischemia. The BASIL trial compared bypass surgery with balloon angioplasty and found higher 2-year survival after an open bypass surgery. In that same study, one-year reintervention rates for bypass surgery and endovascular interventions were 18% and 26% respectively.[41] Since the BASIL trial was hindered by low power and a single endovascular approach (balloon angioplasty), more research is needed. Currently, several trials are underway to address this gap in knowledge regarding effectiveness and durability differences between these two approaches, including the BEST-CLI trial.[42-44] For the treatment of intermittent claudication of the upper leg, endovascular surgery is widely used and in case of short lesion-length (<15cm) with excellent results: one-year primary patency ranges between 67-85%.[45-47] For longer- and femoropopliteal obstructions, endovascular approaches may not be possible and an open bypass is warranted. Saphenous vein bypasses have a 5-year primary patency between 65-75%[48-50], while one large randomized controlled trial found a one-year primary patency as low as 61%.[51] For prosthetic conduits, patency rates are even lower and vary between 27-37%.[50, 52] Intervention failure-

rates notwithstanding, procedure success is also hampered by high incidence of peri-[53] and post-operative cardiovascular events (myocardial infarct, stroke and death)[54] and frequent poor wound healing.[55]

(Cardio)vascular Interventions Fail due to Vascular Remodeling and Vein Graft Disease.

In both open (vein graft) surgery and endovascular interventions alike, surgical injury and ischemia-reperfusion injury to the vessel wall is a common denominator of all procedures. For endovascular approaches this results in mechanical and oxidative damage to endothelial cells (EC) lining the artery, whether via stent placement or balloon angioplasty.[13, 56] In open bypass surgery, in addition to **ischemia-reperfusion damage** and **mechanical/surgical stress**, the vein is also subjected to **altered hemodynamic forces** during and after a successful procedure.[13, 57]

Early Failure and Mid-term Failure after Revascularization.

Broadly speaking, intervention failure can be divided in three temporal windows with a distinct underlying pathophysiology; early, mid- and late-term failure. Early failure, which occurs days to months after the procedure, can mostly be attributed to acute thrombosis after surgical damage to the endothelial lining, both in arteries/stents[58] and vein grafts.[57, 59] In coronary vein grafts for example, generally thrombosed vein grafts make up between 10-25% of all vein grafts that eventually fail at one-year post-operatively.[40, 60]

Mid-term failure after revascularization is generally due to **intimal hyperplasia** (IH) of the vein graft[61, 62] or artery[63-65], and is characterized by uncontrolled migration and proliferation of **vascular smooth muscle cells** (VSMC). VSMCs migrate from the adventitial and medial layers of the artery/vein into the intimal layer, ultimately restricting or occluding blood flow.[66-68] This pathological myo-fibroproliferative response is already triggered during surgery, where depending on the procedure (arterial or venous) several processes impact the vascular wall simultaneously. In vein graft surgery, greater saphenous vein harvesting activates the luminal ECs and medial/adventitial VSMCs of the conduit.[69, 70] As for arterial (endovascular) procedures the EC lining is damaged and/or activated, due to the mechanical/surgical forces exerted on the vascular wall.[71, 72] Besides this mechanical/surgical aspect, other causatives include ischemia-reperfusion injury[68] and oxidative stress.[56, 73] Specifically in bypass surgery, transplantation of the vein from a low-flow and pressure system into an arterial circulation culminates in altered flow and an increase in shear stress and circumferential diameter.[74] This sudden shift in **hemodynamic forces** on the venous wall further contributes towards EC dysfunction in vein grafts.[75]

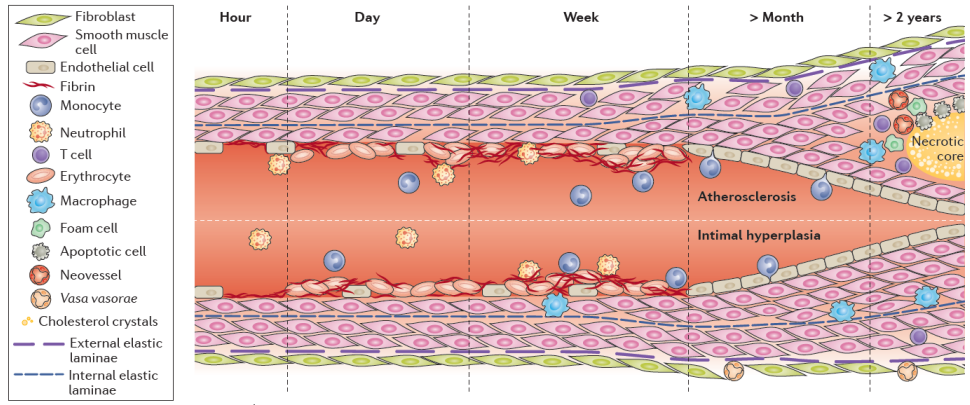

Figure 1. Mechanisms of mid- and late-term revascularization failure. *From: de Vries et al.* Nature Cardiology Reviews. 2016

Extensive surgical stress, local milieu and hemodynamic changes all lead to **dysfunctional endothelium** which upregulates various receptors, such as intracellular adhesive molecules[76] and selectins.[77] These receptors in turn facilitate the homing, attachment and subsequent **transmigration of circulating leukocytes,** while also enabling platelet attachment.[68, 70, 77, 78] Damaged endothelium is not only prone to increased leukocyte attachment and transmigration, but also enables direct inflammatory cell transmigration via dysfunctional inter-EC tight junctions.[77] What is more, the extracellular matrix (ECM) on the basal side of luminal ECs will activate and damage after ischemia-reperfusion injury and stretching of the vessel wall.[79-81] Together with damaged ECs and infiltrated leukocytes, these in turn release damage-associated-molecular patterns (DAMPs)[82] and other pro-inflammatory signaling molecules such as cytokines and chemokines. Upon the release of these molecules, both the local and systemic innate immune response will be activated.[83] Together with the surgical injury itself and subsequent ischemia-reperfusion injury, this will facilitate and enhance the local influx of circulating innate immune cells. These innate cells, which mostly include neutrophils and monocytes, will in turn activate their residential counter-parts as well as other cell types and subsets.[83-85]

This pro-**inflammatory** and pro-remodeling response to vascular injury is not only prompted via perturbations that originate from the lumen and traverse into the vascular wall, but can also stem from adventitial and perivascular tissue layers.[86-88] Local adventitial fibroblast and leukocyte subsets can activate and enhance the intimal hyperplastic signaling cascade (VSMC migration and proliferation), via paracrine signaling in response to mechanical stress and systemic inflammation.[81, 89, 90] Furthermore, most veins and arteries are enveloped by **perivascular adipose tissue (PVAT)**[91], a paracrine organ that not only maintains vascular tone and function but can also initiate and facilitate metabolic and inflammatory signaling pathways.[92] PVAT can be grouped in adipocytes and an interspersed stromal vascular fraction; which consists of residential leukocyte subsets, ECs and VSMCs. Local blood supply to the PVAT and adventitia is facilitated by the latter two cell types of this stromal vascular fraction.[91] Healthy PVAT function can be undermined by various acute and chronic stressors. For example, both obesity[93, 94] and endovascular surgical injury to the underlying blood vessel[95] will alter PVAT phenotype. **Dysfunctional PVAT** in turn will have a distinct pro-inflammatory[96, 97] and pro-atherosclerotic[98, 99] impact on the vascular wall. This "sick fat" can accelerate the intimal hyperplastic response[100] via increased oxidative stress[88, 101], and production of pro-inflammatory adipokines and cytokines.[102, 103]

The aforementioned impact of surgical and hemodynamic stress on dysfunctional ECs and ECM, followed by leukocyte transmigration, adventitial fibroblast signaling, PVAT and residential immune-cell activation; all contribute towards induction of VSMC proliferation and migration.[104] VSMC activation can occur via cell-cell, cytokine-cell and adipokine-cell interactions; but also in response to chemo-attractants.[105] Taken together these stimuli will **trigger VSMCs to undergo phenotypic switching** from a contractile towards a secretory state, which enables them to migrate to- and proliferate in the intimal layer of the vascular wall.[106] This process of medial/adventitial IH is largely responsible for mid-term failure of grafts and arteries alike[13], but if VSMC growth continues and lesion size increases eventually a atherosclerotic plaque can form.[13, 57]

Late-term Failure after Revascularization.

Post-interventional formation of atherosclerotic plaques can occur in up to 30% of arteries after an endovascular intervention[107], with similar findings in coronary vein grafts 3-years post-implantation.[108] Hypo-oxygenation of the growing VSMC lesion, accompanied by chronic inflammation and foam cell formation will contribute to the **formation of a necrotic core**.[10] This state of hypoxia and malnutrition within the intimal layer will trigger **neo-vessels** to grow from the adventitial layer inward and supply the lesion with oxygenated blood and nutrients. These vessels in turn are prone to leakage of erythrocytes which will further destabilize the lesion.[9] Eventually this atherosclerotic plaque will **either occlude the lumen, rupture or erode**, culminating in late-term revascularization failure and return of PAD/CAD symptoms.[9, 13, 109]

No Therapies Exist to Improve Success rates after Revascularization.

High peri-operative-[53, 54] and wound complication rates,[110] long in-hospital stays[111] and frequent reintervention[112, 113]; taken together highly impacts both patients, their family and the healthcare system. Despite decades of research, currently no therapies are available to counter mid- and late-term revascularization failure, whether in vein grafts[57, 114] or arteries[13], nor do strategies exist to limit peri-operative and post-operative complication risks for these patients. **In this thesis the effects of various forms of dietary restriction will be discussed as strategies to limit complication risks.**

Long-term Dietary Restriction as a Strategy to Slow Aging and other Chronic Diseases.

Over a century ago, Osborne and colleagues in 1917 subjected rats to limited access of food during the first 17 months after birth. In their follow-up analysis they found that these food-restricted rats had an extended life-span compared to their counterparts who had been on an a*d libitum* (AL) diet all their life.[115] Then in 1935 McCay and colleagues were able to repeat this finding in a significantly larger cohort of rats, and showed that long-term caloric restriction, i.e. limiting the total daily food intake (CR), was able to prolong lifespan in rats.[116] Four years earlier, in 1931, Slonaker et al. found a similar effect on the extension of life-span in rats by long-term restricting their protein intake (PR) while maintaining caloric intake (i.e. isocaloric).[117]

Subsequent research in a wide range of organisms, from worms[118] to nonhuman primates[119], built on these initial findings and established the effectiveness of long-term CR on extending lifespan, promoting healthy aging and decreasing cardiovascular disease risk.[120] And for PR, rodent data shows similar results in lifespan and metabolic studies.[121, 122] Interestingly, as an alternative to diminished intake of total calories or proteins, restriction of single amino acids in the diet can yield similar benefits. Long-term tryptophan restriction[123] but also restriction of the sulfur amino acids methionine and cysteine (MetR)[124],

again extends lifespan in rats. All these approaches to restrict certain aspect of regular dietary intake, whether this pertains total calories, total proteins, single amino acids or a combination, can be categorized as dietary restriction (DR) and appear to share common pathways towards their favorable phenotype.

Dietary Restriction Confers Benefits Beyond Lifespan Extension.
Beyond aging research, studies found that DR can lower blood pressure and circulating lipids while improving glucose homeostasis and insulin sensitivity.[125, 126] These findings indicate the potential of DR to mitigate several chronic stressors besides aging, by undercutting risk factors for cardiovascular disease and improvement of metabolic health and fitness.[120, 127-129] Interestingly, besides these chronic conditions, long-term DR is also effective in mitigating acute stressors such as ischemia-reperfusion injury to the brain[130] and heart.[131]

In those studies, however, as for most long-term DR interventions, rodents were subjected to DR (30-40% CR in both studies) from an early age. Long-term CR in humans did show improvement regarding cardiovascular risk factors, but also highlighted compliance issues in adhering to this dietary regimen.[132] It can be deducted therefore, that the potential benefits of these diets, that require restriction of calories/proteins for months to years and often from an early age onward, are not compatible with a setting of planned acute and transient stressors such as elective surgery.

Short-term Dietary Restriction and the Host Response to Surgical Injury.

It was Mitchell and colleagues[133] who in 2010 modulated this concept of long-term DR towards a brief period of preconditioning before undergoing surgery. In that seminal work, they subjected mice to either four weeks of 30% CR, a 3-day fast or an *Ad Libitum (*AL) diet and performed renal ischemia-reperfusion injury surgery. During this procedure the renal artery is clamped bilaterally for 30-35 minutes, followed by clamp-removal and subsequent reperfusion. Then immediately post-op all mice are switched back to the AL diet. About 60% of AL-fed mice died in the days following the procedure, while all animals in both the 30% CR and 3-day fasting cohorts survived the surgery. Next to absence of mortality, both diet groups were also protected from post-operative kidney dysfunction, as seen by decreased circulating levels of creatinine and urea compared to the AL group. In that same study, mice were subjected to 1-3 days of fasting followed by surgical hepatic ischemia-reperfusion. Both 1- and 3-day water only fasting groups had significantly lower serum markers of liver damage (ALAT) compared to AL mice.[133] In this first study, the authors established that DR can have rapid onset benefits, and that such short-term restriction of calories is sufficient to protect from acute stress such as renal/ hepatic IRI surgery.

Restriction of Total Proteins or Specific Amino-Acids Also Protects from Surgical Stress.
Although a short-term reduction in calories to achieve surgical stress resistance is favorable compared to any long-term CR diet, a reduction in food intake can be difficult to adhere to regardless the duration of the dietary intervention. In a follow-up study, mice were fed an isocaloric PR diet (proteins were replaced with carbohydrates while maintaining caloric intake), a diet lacking only tryptophan, or a complete diet before undergoing renal or hepatic IRI.[134] A 6-day restriction of just proteins yielded a similar protective phenotype compared to a 3-day water only fast. What is more, mice on a tryptophan restricted complete diet were equally protected from renal IRI.

Subsequent analysis showed that these effects of tryptophan restriction proved dependent on the amino acid deprivation sensor general control non depressible 2 (GCN2).[135] GNC2 in turn is upstream of a broader integrated stress response pathway (ISR) present in all mammalian cells.[136] It was previously reported that in response to a long-term MetR diet, GNC2 phosphorylates eukaryotic transcription factor–2 (eLF2) followed by activation of activating transcription factor-4 (ATF4) which then coordinates downstream metabolic signaling.[137]

Short-term Dietary Restriction Extends Protection Beyond Surgical Injury.
Next the hypothesis that short-term DR can mitigate various forms of acute stress was expanded beyond acute surgical stress. In a model of focal stroke injury, to mimic perioperative stroke, rats were subjected to 6 days of preconditioning with PR. Compared to their counterparts on a control-fed diet, PR-fed rats had limited ischemic injury and accelerated functional recovery.[138] In a preclinical model of cerebral malaria infection, mice where started on DR (40% CR) within 2 days of *P. Berghei* infection. This in turn afforded protection from neurological complications while parallelly significantly improving survival. In this study, short-term DR suppressed the leptin – mTORC1 (mechanistic target of rapamycin complex 1) – axis, which resulted in limited splenic CD8+ T-cell activation and subsequent attenuation of late-stage malaria induced neuropathology.[139]

Combining Caloric and Protein Restriction Results in Additive Protection from Surgical Stress.

Although CR and PR appear to share pathways that result in comparable phenotypes after surgical injury, it remained undetermined whether both were equally effective. Interestingly, after a side-by-side comparison it was revealed that protection was best afforded by combining a reduction in calories with a reduction in proteins. This strategy, i.e. protein-calorie restriction (PCR), was able to reduce surgical stress more effectively after renal IRI compared to either of the singular dietary interventions.140 An initial study in vascular surgery models asked the question whether diet can influence the intimal hyperplastic response to vascular injury and was performed in a focal stenosis model of the right common carotid artery (RCCA). In this model, a 9/0 suture is placed around the RCCA via which blood flow is then partially obstructed.141 Mice were first subjected to diet-induced obesity before vascular wall injury was created, which exacerbated the remodeling response compared to their lean counterparts.[142] Interestingly, in a follow-up study mice were first fed a high-fat diet and then switched to PCR before undergoing surgery. This dietary switch proved capable of decreasing intimal area along the arterial wall proximal from the stenosis, while the ratio between intima and media was not different, suggestive of a DR-induced limitation of the IH response rather the favorable overall remodeling.[143]

Short-term Dietary Restriction and Endogenous Hydrogen Sulfide Upregulation.

Hine et al. in 2015 revealed that increased production of **endogenous hydrogen sulfide (H_2S)** is an essential underlying mechanism of DR-mediated protection.[144] In their study, they supplemented mice subjected to DR with the sulfur amino acids methionine and cysteine, and this in turn failed to protect from hepatic IRI. Consequently, they found that sulfur amino acid restriction (which is part of both CR and PR) activates the transsulfuration pathway (TSP), which produces H_2S as a metabolite. This TSP pathway is an evolutionary conserved pathway consisting of several amino acids sensing enzymes, including cystathionine β-synthase (CBS), cystathionine y-lyase (**CGL**) and 3-mercaptopyruvat sulfur transferase (MST). All three can produce H_2S, but within the vasculature H_2S is mainly derived from CGL.[145]

At physiological levels, H_2S can regulate blood pressure and maintain vascular tone and function within the cardiovasculature.[146] These acute effects of H_2S, which include vasorelaxation/dilation and regulation of vessel-wall permeability, are triggered via activation or inhibition of various ion-channels

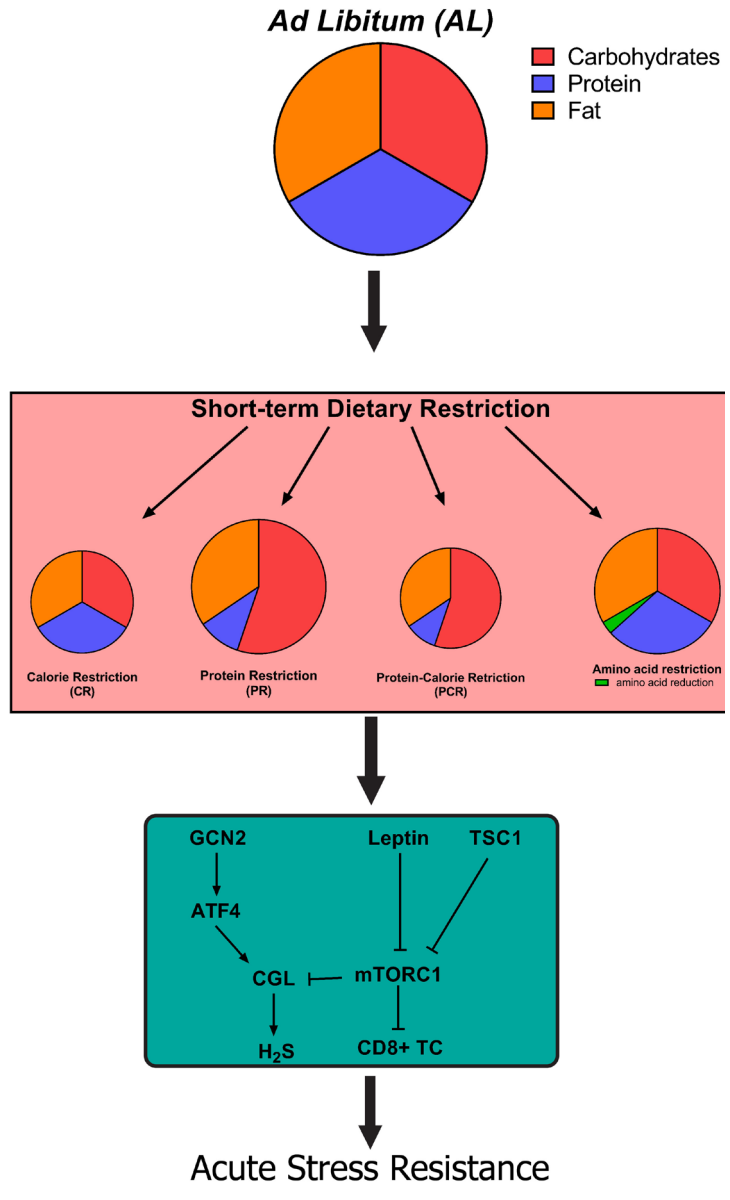

Figure 2. Types of Dietary Restriction and known mechanisms via which dietary restriction improves acute stress resistance. Size of diet charts indicates total calories.

and kinases/enzymes respectively. Vasodilation is prompted via K^+ and Ca^{2+}-channel activation, while vessel-wall permeability is increased via activation of Claudin-5 and VE-cadherin.[147] Next to this physiological role, reduced levels of H_2S have been implicated in numerous diseases and pathophysiological processess.[148] Several studies with either exogenous H_2S addition or genetic reduction of H_2S production have revealed that it possesses extensive anti-inflammatory[149], anti-atherosclerotic[150] and cytoprotective properties.[151] Recent work in a model of surgically induced critical hind limb ischemia also revealed the **pro-angiogenic** potential of endogenous H_2S upregulation (**Fig. 2**). In this study, short-term MetR promoted the post-injury growth of neo-vessels via increased expression of vascular endothelial growth factor (VEGF) and CGL activation, implicating endogenous H_2S upregulation as a novel therapy for PAD.[152]

Outline of this Thesis.

In (vascular) surgery practice during the pre-operative workup of patients there is are currently no distinct recommendations regarding their optimal nutritional state besides preventing malnutrition. Broadly, pre-, peri-, and post-operative guidelines for managing patient nutrition are described in the Enhanced Recovery After Surgery (ERAS) protocols[153], and implementation of ERAS in vascular surgery patients is linked with shorter in-hospital stays.[154] Concerning the pre-operative workup of patients scheduled for elective surgery, these guidelines recommend a "bodily state without malnutrition or malnourishment". The only other recommendation concerns ingestion of a carbohydrate drink ("carbohydrate loading") in the 24 hours before surgery to avoid a fasted state, and this is linked with improved glucose homeostasis.[155] In short, this points towards a potential gap in knowledge regarding optimal pre-operative dietary regimens, something this thesis aims to investigate. Is there room for a pre-operative dietary regimen that does not cause malnutrition, but can potentially benefit various peri- and post-operative vectors that together result in improved post-operative outcome?

Although the benefits of short-term DR have been established in several models of surgical stress and injury, whether these dietary regimens obstruct post-operative wound healing is undetermined. Therefore in **chapter 2** we tested two short-term isocaloric dietary interventions, namely protein restriction and methionine restriction, in a validated mouse model of surgical wound creation. We compared the time-to full wound closure in a McFarlane Flap wound model in both diets with an AL-fed cohort. Parallelly, we investigated the potential of these dietary interventions to improve glucose metabolism pre- and post-surgery.

Failure rates after vein graft surgery, in both coronary and lower extremity bypasses, remain high. Recently it was discovered that benefits of short-term DR seen in models of surgical IRI are derived from increased production of endogenous H_2S. But whether DR is also effective in vein graft surgery, and whether a reduction of total proteins (PR) can also upregulate endogenous H_2S is unknown. In **chapter 3**, we tested whether a short-term reduction in proteins can limit graft failure after rodent bypass surgery, and whether these benefits are also H_2S derived. We found that short-term PR attenuates graft disease, and that these benefits were CGL dependent. PR resulted in increased endothelial cell H_2S production, diminished VSMC migration and impaired neutrophil transmigration.

As a follow up of chapter 3 we study in **chapter 4** whether short-term MetR, which is feasible and doable in humans, can achieve similar benefits in rodent bypass surgery. Parallelly, there is uncertainty whether PVAT can play a protective role in VGD, or if it worsens outcomes. In the context of the interplay between adipose and DR, previous work indicated a shift towards an anti-inflammatory phenotype in inguinal adipose after MetR. However, the interaction between MetR and PVAT, and its influence on post-operative vascular remodeling remains undetermined. In **chapter 4** we not only test whether short-term MetR can protect from VGD and arterial intimal hyperplasia, but also investigate the link between MetR, PVAT and protection from VGD. Interestingly, we found that protection from VGD by MetR was PVAT-dependent, and that the interaction between MetR and PVAT also yielded a favorable cellular composition of the graft. In baseline/pre-op caval vein PVAT, short-term MetR upregulated AMPK-signaling and appeared to induce browning. Preconditioning with MetR also altered the PVAT response to surgical injury (bypass surgery), via a dampened pro-inflammatory response at post-op day 1.

Although short-term DR stands as a novel therapy to upregulate endogenous H_2S and improve remodeling after vascular injury, a preconditioning strategy via a diet may not always be feasible. Whether due to lack of diet-compliance or inadequate time available to precondition before undergoing surgery, so-called DR-mimetics are needed. In **chapter 5** we first tested the efficacy of exogenous H_2S in rodent arterial IH and VGD by supplementing drinking water with the

slow-releasing H_2S pro-drug GYY4137. Next, we dissolved GYY4137 in a Pluronic gel and enveloped arteries and vein grafts during the procedure. We elucidated the efficacy of this H_2S-gel in both arterial IH and VGD, while also interrogating its impact on the cells of the vascular wall. We developed a simple and safe H_2S-releasing gel that attenuates arterial and vein graft remodeling via a one-time, peri-procedural application. Mechanistically, we establish a link between this locally applicable DR-mimetic and VSMC migration, both *in-vivo* and *in-vitro*.

We and others have established the effectiveness of short-term DR in various preclinical models of surgical injury and stress. But whether this dietary intervention is feasible and safe in the vascular surgery patient population is unknown. In **chapter 6** we describe a first clinical trial performed in this direction. We enrolled several vascular patients scheduled for carotid endarterectomy on a 3-day PCR diet, while they resided in-hospital to closely monitor any adverse effects of the diet. Furthermore, we attempted to establish a clinical "discovery platform" regarding H_2S metabolism in humans and how the PCR diet impacts this. We report a possible link between H_2S producing gut-bacteria and short-term PCR.

This first attempt at short-term PCR in a clinical setting was performed in patients scheduled for elective carotid endarterectomy, who were required to stay in-hospital during the 3-day diet. As a follow-up towards that trial, for thesis we performed a second clinical trial. In **chapter 7** we describe the set-up and first experiences of a randomized clinical trial with short-term pre-operative PCR in elective vascular surgery patients. In this follow-up study in **chapter 7** we moved towards an out-patient setting and enrolled patients scheduled for any type of vascular surgery that involved an open wound. Furthermore, we extended the course of the diet to a 4-day diet and developed an extensive leukocyte subset flow panel to interrogate the innate and adaptive response to the PCR diet.

Both **chapter 6 and 7** highlight the very fortunate course of our work which enabled us to transition from rodent studies to vascular surgery patients, and essentially move our pre-clinical work from the lab bench towards the patient's bedside.

References.

1. Fowkes FG, Aboyans V, Fowkes FJ, et al. Peripheral artery disease: epidemiology and global perspectives. *Nat Rev Cardiol*. 2017;14:156-170.
2. Fowkes FG, Rudan D, Rudan I, et al. Comparison of global estimates of prevalence and risk factors for peripheral artery disease in 2000 and 2010: a systematic review and analysis. *Lancet*. 2013;382:1329-40.
3. Roth GA, Johnson C, Abajobir A, et al. Global, Regional, and National Burden of Cardiovascular Diseases for 10 Causes, 1990 to 2015. *J Am Coll Cardiol*. 2017;70:1-25.
4. Libby P, Buring JE, Badimon L, et al. Atherosclerosis. *Nat Rev Dis Primers*. 2019;5:56.
5. Garcia LA. Epidemiology and pathophysiology of lower extremity peripheral arterial disease. *J EndovascTher*. 2006;13 Suppl 2:II3-9.:II3-II9.
6. Ross R. Atherosclerosis — An Inflammatory Disease. *New England Journal of Medicine*. 1999;340:115-126.
7. Goldstein JL and Brown MS. A century of cholesterol and coronaries: from plaques to genes to statins. *Cell*. 2015;161:161-172.
8. Lloyd-Jones DM, Leip EP, Larson MG, et al. Prediction of lifetime risk for cardiovascular disease by risk factor burden at 50 years of age. *Circulation*. 2006;113:791-8.
9. de Vries MR and Quax PHA. Plaque angiogenesis and its relation to inflammation and atherosclerotic plaque destabilization. *Curr Opin Lipidol*. 2016;Publish Ahead of Print.
10. Hansson GK, Libby P and Tabas I. Inflammation and plaque vulnerability. *J Intern Med*. 2015;278:483-493.
11. Society for Vascular Surgery Lower Extremity Guidelines Writing G, Conte MS, Pomposelli FB, et al. Society for Vascular Surgery practice guidelines for atherosclerotic occlusive disease of the lower extremities: management of asymptomatic disease and claudication. *J Vasc Surg*. 2015;61:2S-41S.
12. Herrington W, Lacey B, Sherliker P, et al. Epidemiology of Atherosclerosis and the Potential to Reduce the Global Burden of Atherothrombotic Disease. *Circ Res*. 2016;118:535-46.
13. Yahagi K, Kolodgie FD, Otsuka F, et al. Pathophysiology of native coronary, vein graft, and in-stent atherosclerosis. *Nat Rev Cardiol*. 2016;13:79-98.
14. Fox KA, Poole-Wilson P, Clayton TC, et al. 5-year outcome of an interventional strategy in non-ST-elevation acute coronary syndrome: the British Heart Foundation RITA 3 randomised trial. *Lancet*. 2005;366:914-20.
15. Head SJ, Milojevic M, Daemen J, et al. Mortality after coronary artery bypass grafting versus percutaneous coronary intervention with stenting for coronary artery disease: a pooled analysis of individual patient data. *Lancet*. 2018;391:939-948.
16. Farkouh ME, Domanski M, Sleeper LA, et al. Strategies for multivessel revascularization in patients with diabetes. *N Engl J Med*. 2012;367:2375-84.
17. Levine GN, Bates ER, Blankenship JC, et al. 2011 ACCF/AHA/SCAI Guideline for Percutaneous Coronary Intervention: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines and the Society for Cardiovascular Angiography and Interventions. *Circulation*. 2011;124:e574-651.
18. Caliskan E, de Souza DR, Böning A, et al. Saphenous vein grafts in contemporary coronary artery bypass graft surgery. *Nat Rev Cardiol*. 2020;17:155-169.

19. Gaudino M, Taggart D, Suma H, et al. The Choice of Conduits in Coronary Artery Bypass Surgery. *J Am Coll Cardiol*. 2015;66:1729-37.
20. Firnhaber JM and Powell CS. Lower Extremity Peripheral Artery Disease: Diagnosis and Treatment. *Am Fam Physician*. 2019;99:362-369.
21. Harky A, Maskell P and Burgess M. Anti-platelet and anti-coagulant therapy in peripheral arterial disease prior to surgical intervention. *Vascular*. 2019;27:299-311.
22. Wang JC, Kim AH and Kashyap VS. Open surgical or endovascular revascularization for acute limb ischemia. *J Vasc Surg*. 2016;63:270-8.
23. Almasri J, Adusumalli J, Asi N, et al. A systematic review and meta-analysis of revascularization outcomes of infrainguinal chronic limb-threatening ischemia. *J Vasc Surg*. 2018;68:624-633.
24. Hiramoto JS, Teraa M, de Borst GJ, et al. Interventions for lower extremity peripheral artery disease. *Nat Rev Cardiol*. 2018;15:332-350.
25. Norgren L, Hiatt WR, Dormandy JA, et al. Inter-Society Consensus for the Management of Peripheral Arterial Disease (TASC II). *Eur J Vasc Endovasc Surg*. 2007;33 Suppl 1:S1-75.
26. Jaff MR, White CJ, Hiatt WR, et al. An Update on Methods for Revascularization and Expansion of the TASC Lesion Classification to Include Below-the-Knee Arteries: A Supplement to the Inter-Society Consensus for the Management of Peripheral Arterial Disease (TASC II): The TASC Steering Comittee(.). *Ann Vasc Dis*. 2015;8:343-57.
27. Norgren L, Hiatt WR, Dormandy JA, et al. Inter-Society Consensus for the Management of Peripheral Arterial Disease (TASC II). *J Vasc Surg*. 2007;45 Suppl S:S5-67.
28. Jaff MR, White CJ, Hiatt WR, et al. An update on methods for revascularization and expansion of the TASC lesion classification to include below-the-knee arteries: A supplement to the inter-society consensus for the management of peripheral arterial disease (TASC II): The TASC steering committee. *Catheter Cardiovasc Interv*. 2015;86:611-25.
29. Seung KB, Park DW, Kim YH, et al. Stents versus coronary-artery bypass grafting for left main coronary artery disease. *N Engl J Med*. 2008;358:1781-92.
30. Morice MC, Colombo A, Meier B, et al. Sirolimus- vs paclitaxel-eluting stents in de novo coronary artery lesions: the REALITY trial: a randomized controlled trial. *JAMA*. 2006;295:895-904.
31. Windecker S, Serruys PW, Wandel S, et al. Biolimus-eluting stent with biodegradable polymer versus sirolimus-eluting stent with durable polymer for coronary revascularisation (LEADERS): a randomised non-inferiority trial. *Lancet*. 2008;372:1163-73.
32. Moses JW, Leon MB, Popma JJ, et al. Sirolimus-eluting stents versus standard stents in patients with stenosis in a native coronary artery. *N Engl J Med*. 2003;349:1315-23.
33. Lemos PA, Serruys PW, van Domburg RT, et al. Unrestricted utilization of sirolimus-eluting stents compared with conventional bare stent implantation in the "real world": the Rapamycin-Eluting Stent Evaluated At Rotterdam Cardiology Hospital (RESEARCH) registry. *Circulation*. 2004;109:190-5.
34. Mauri L, Silbaugh TS, Wolf RE, et al. Long-term clinical outcomes after drug-eluting and bare-metal stenting in Massachusetts. *Circulation*. 2008;118:1817-27.
35. Fukui T, Tabata M, Manabe S, et al. Angiographic outcomes of right internal thoracic artery grafts in situ or as free grafts in coronary artery bypass grafting. *J Thorac Cardiovasc Surg*. 2010;139:868-73.
36. Tatoulis J, Buxton BF and Fuller JA. The right internal thoracic artery: the forgotten conduit--5,766 patients and 991 angiograms. *Ann Thorac Surg*. 2011;92:9-15; discussion 15-7.
37. Allen K, Cheng D, Cohn W, et al. Endoscopic Vascular Harvest in Coronary Artery Bypass Grafting Surgery: A Consensus Statement of the International Society of Minimally Invasive Cardiothoracic Surgery (ISMICS) 2005. *Innovations (Phila)*. 2005;1:51-60.
38. Hess CN, Lopes RD, Gibson CM, et al. Saphenous vein graft failure after coronary artery bypass surgery: insights from PREVENT IV. *Circulation*. 2014;130:1445-51.
39. Goldman S, Zadina K, Moritz T, et al. Long-term patency of saphenous vein and left internal mammary artery grafts after coronary artery bypass surgery: results from a Department of Veterans Affairs Cooperative Study. *J Am Coll Cardiol*. 2004;44:2149-56.
40. Fitzgibbon GM, Kafka HP, Leach AJ, et al. Coronary bypass graft fate and patient outcome: angiographic follow-up of 5,065 grafts related to survival and reoperation in 1,388 patients during 25 years. *J Am Coll Cardiol*. 1996;28:616-26.
41. Adam DJ, Beard JD, Cleveland T, et al. Bypass versus angioplasty in severe ischaemia of the leg (BASIL): multicentre, randomised controlled trial. *Lancet*. 2005;366:1925-34.
42. Farber A, Rosenfield K, Siami FS, et al. The BEST-CLI trial is nearing the finish line and promises to be worth the wait. *J Vasc Surg*. 2019;69:470-481.e2.
43. Hunt BD, Popplewell MA, Davies H, et al. BAlloon versus Stenting in severe Ischaemia of the Leg-3 (BASIL-3): study protocol for a randomised controlled trial. *Trials*. 2017;18:224.
44. Popplewell MA, Davies H, Jarrett H, et al. Bypass versus angio plasty in severe ischaemia of the leg - 2 (BASIL-2) trial: study protocol for a randomised controlled trial. *Trials*. 2016;17:11.
45. Krankenberg H, Schlüter M, Steinkamp HJ, et al. Nitinol stent implantation versus percutaneous transluminal angioplasty in superficial femoral artery lesions up to 10 cm in length: the femoral artery stenting trial (FAST). *Circulation*. 2007;116:285-92.
46. Laird JR, Katzen BT, Scheinert D, et al. Nitinol stent implantation versus balloon angioplasty for lesions in the superficial femoral artery and proximal popliteal artery: twelve-month results from the RESILIENT randomized trial. *Circ Cardiovasc Interv*. 2010;3:267-76.
47. Rastan A, Krankenberg H, Baumgartner I, et al. Stent placement versus balloon angioplasty for the treatment of obstructive lesions of the popliteal artery: a prospective, multicenter, randomized trial. *Circulation*. 2013;127:2535-41.
48. Klinkert P, Schepers A, Burger DHC, et al. Vein versus polytetrafluoroethylene in above-knee femoropopliteal bypass grafting: Five-year results of a randomized controlled trial. *Journal of Vascular Surgery*. 2003;37:149-155.
49. AbuRahma AF, Robinson PA and Holt SM. Prospective controlled study of polytetrafluoroethylene versus saphenous vein in claudicant patients with bilateral above knee femoropopliteal bypasses. *Surgery*. 1999;126:594-601; discussion 601-2.
50. Johnson WC and Lee KK. A comparative evaluation of polytetrafluoroethylene, umbilical vein, and saphenous vein bypass grafts for femoral-popliteal above-knee revascularization: a prospective randomized Department of Veterans Affairs cooperative study. *J Vasc Surg*. 2000;32:268-77.
51. Conte MS, Bandyk DF, Clowes AW, et al. Results of PREVENT III: A multicenter, randomized trial of edifoligide for the prevention of vein graft failure in lower extremity bypass surgery. *Journal of Vascular Surgery*. 2006;43:742-751.e1.
52. Rückert RI, Settmacher U, Krüger U, et al. Femorodistal PTFE bypass grafting for severe limb ischaemia: results of a prospective clinical study using a new distal anastomotic technique. *Eur J Vasc Endovasc Surg*. 2000;20:51-6.

53. Fleisher LA, Beckman JA, Brown KA, et al. ACC/AHA 2007 Guidelines on Perioperative Cardiovascular Evaluation and Care for Noncardiac Surgery: Executive Summary: A Report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Writing Committee to Revise the 2002 Guidelines on Perioperative Cardiovascular Evaluation for Noncardiac Surgery): Developed in Collaboration With the American Society of Echocardiography, American Society of Nuclear Cardiology, Heart Rhythm Society, Society of Cardiovascular Anesthesiologists, Society for Cardiovascular Angiography and Interventions, Society for Vascular Medicine and Biology, and Society for Vascular Surgery. *Circulation*. 2007;116:1971-96.
54. Baumgartner I, Norgren L, Fowkes FGR, et al. Cardiovascular Outcomes After Lower Extremity Endovascular or Surgical Revascularization: The EUCLID Trial. *J Am Coll Cardiol*. 2018;72:1563-1572.
55. Ozaki CK, Hamdan AD, Barshes NR, et al. Prospective, randomized, multi-institutional clinical trial of a silver alginate dressing to reduce lower extremity vascular surgery wound complications. *J Vasc Surg*. 2014.
56. Gallo G, Pierelli G, Forte M, et al. Role of oxidative stress in the process of vascular remodeling following coronary revascularization. *Int J Cardiol*. 2018;268:27-33.
57. de Vries MR, Simons KH, Jukema JW, et al. Vein graft failure: from pathophysiology to clinical outcomes. *Nat Rev Cardiol*. 2016;13:451-470.
58. Finn AV, Joner M, Nakazawa G, et al. Pathological correlates of late drug-eluting stent thrombosis: strut coverage as a marker of endothelialization. *Circulation*. 2007;115:2435-41.
59. Kim AY, Walinsky PL, Kolodgie FD, et al. Early loss of thrombomodulin expression impairs vein graft thromboresistance: implications for vein graft failure. *CircRes*. 2002;90:205-212.
60. Sabik JF, 3rd, Lytle BW, Blackstone EH, et al. Comparison of saphenous vein and internal thoracic artery graft patency by coronary system. *Ann Thorac Surg*. 2005;79:544-51; discussion 544-51.
61. Kim FY, Marhefka G, Ruggiero NJ, et al. Saphenous vein graft disease: review of pathophysiology, prevention, and treatment. *Cardiol Rev*. 2013;21:101-9.
62. Motwani JG and Topol EJ. Aortocoronary saphenous vein graft disease: pathogenesis, predisposition, and prevention. *Circulation*. 1998;97:916-31.
63. Weintraub WS. The pathophysiology and burden of restenosis. *Am J Cardiol*. 2007;100:3k-9k.
64. Jukema JW, Verschuren JJ, Ahmed TA, et al. Restenosis after PCI. Part 1: pathophysiology and risk factors. *Nat Rev Cardiol*. 2011;9:53-62.
65. Kipshidze N, Dangas G, Tsapenko M, et al. Role of the endothelium in modulating neointimal formation: vasculoprotective approaches to attenuate restenosis after percutaneous coronary interventions. *J Am Coll Cardiol*. 2004;44:733-9.
66. Zalewski A, Shi Y and Johnson AG. Diverse origin of intimal cells: smooth muscle cells, myofibroblasts, fibroblasts, and beyond? *CircRes*. 2002;91:652-655.
67. Geary RL, Wong JM, Rossini A, et al. Expression profiling identifies 147 genes contributing to a unique primate neointimal smooth muscle cell phenotype. *ArteriosclerThrombVascBiol*. 2002;22:2010-2016.
68. Mitra AK, Gangahar DM and Agrawal DK. Cellular, molecular and immunological mechanisms in the pathophysiology of vein graft intimal hyperplasia. *Immunol Cell Biol*. 2006;84:115-24.
69. Osgood MJ, Hocking KM, Voskresensky IV, et al. Surgical vein graft preparation promotes cellular dysfunction, oxidative stress, and intimal hyperplasia in human saphenous vein. *Journal of Vascular Surgery*. 2014;60:202-211.
70. Tseng C-N, Karlöf E, Chang Y-T, et al. Contribution of Endothelial Injury and Inflammation in Early Phase to Vein Graft Failure: The Causal Factors Impact on the Development of Intimal Hyperplasia in Murine Models. *PLoS One*. 2014;9:e98904.
71. Björkerud S and Bondjers G. Arterial repair and atherosclerosis after mechanical injury. 5. Tissue response after induction of a large superficial transverse injury. *Atherosclerosis*. 1973;18:235-55.
72. Grewe PH, Deneke T, Holt SK, et al. [Scanning electron microscopic analysis of vessel wall reactions after coronary stenting]. *Z Kardiol*. 2000;89:21-7.
73. Weaver H, Shukla N, Ellinsworth D, et al. Oxidative stress and vein graft failure: a focus on NADH oxidase, nitric oxide and eicosanoids. *Curr Opin Pharmacol*. 2012;12:160-165.
74. Owens CD, Wake N, Jacot JG, et al. Early biomechanical changes in lower extremity vein grafts—distinct temporal phases of remodeling and wall stiffness. *Journal of Vascular Surgery*. 2006;44:740-746.
75. Ward AO, Caputo M, Angelini GD, et al. Activation and inflammation of the venous endothelium in vein graft disease. *Atherosclerosis*. 2017;265:266-274.
76. Walpola PL, Gotlieb AI, Cybulsky MI, et al. Expression of ICAM-1 and VCAM-1 and Monocyte Adherence in Arteries Exposed to Altered Shear Stress. *Arteriosclerosis, thrombosis, and vascular biology*. 1995;15:2-10.
77. Schnoor M, Alcaide P, Voisin M-B, et al. Crossing the Vascular Wall: Common and Unique Mechanisms Exploited by Different Leukocyte Subsets during Extravasation. *Mediators Inflamm*. 2015;2015:23.
78. Danielsson P, Truedsson L and Norgren L. Systemic white blood and endothelial cell response after revascularization of critical limb ischemia is only influenced in case of ischemic ulcers. *Int Angiol*. 2006;25:310-5.
79. O'Brien JE, Jr., Ormont ML, Shi Y, et al. Early injury to the media after saphenous vein grafting. *AnnThoracSurg*. 1998;65:1273-1278.
80. Meng X, Mavromatis K and Galis ZS. Mechanical stretching of human saphenous vein grafts induces expression and activation of matrix-degrading enzymes associated with vascular tissue injury and repair. *ExpMolPathol*. 1999;66:227-237.
81. Kalra M and Miller VM. Early Remodeling of Saphenous Vein Grafts: Proliferation, Migration and Apoptosis of Adventitial and Medial Cells Occur Simultaneously with Changes in Graft Diameter and Blood Flow. *Journal of Vascular Research*. 2000;37:576-584.
82. Goulopoulou S, McCarthy CG and Webb RC. Toll-like Receptors in the Vascular System: Sensing the Dangers Within. *Pharmacol Rev*. 2016;68:142-167.
83. de Vries MR and Quax PHA. Inflammation in Vein Graft Disease. *Front Cardiovasc Med*. 2018;5:3.
84. Hinokiyama K, Valen G, Tokuno S, et al. Vein graft harvesting induces inflammation and impairs vessel reactivity. *Ann Thorac Surg*. 2006;82:1458-64.
85. Owens CD, Rybicki FJ, Wake N, et al. Early remodeling of lower extremity vein grafts: inflammation influences biomechanical adaptation. *J Vasc Surg*. 2008;47:1235-42.
86. Havelka GE, Hogg ME, Martinez J, et al. Adventitial contributions of the extracellular signal-regulated kinase and Akt pathways to neointimal hyperplasia. *Am J Surg*. 2011;202:515-9.

87. Havelka GE and Kibbe MR. The vascular adventitia: its role in the arterial injury response. *Vasc Endovascular Surg*. 2011;45:381-90.
88. Nosalski R and Guzik TJ. Perivascular adipose tissue inflammation in vascular disease. *Br J Pharmacol*. 2017;174:3496-3513.
89. Hu Y, Zhang Z, Torsney E, et al. Abundant progenitor cells in the adventitia contribute to atherosclerosis of vein grafts in ApoE-deficient mice. *J Clin Invest*. 2004;113:1258-65.
90. Jiang Z, Yu P, Tao M, et al. TGF-β- and CTGF-mediated fibroblast recruitment influences early outward vein graft remodeling. *American Journal of Physiology - Heart and Circulatory Physiology*. 2007;293:H482-H488.
91. Rajsheker S, Manka D, Blomkalns AL, et al. Crosstalk between perivascular adipose tissue and blood vessels. *Curr Opin Pharmacol*. 2010;10:191-6.
92. Britton KA and Fox CS. Perivascular adipose tissue and vascular disease. *Clin Lipidol*. 2011;6:79-91.
93. Bays HE. Adiposopathy is "sick fat" a cardiovascular disease? *J Am Coll Cardiol*. 2011;57:2461-73.
94. Chatterjee TK, Stoll LL, Denning GM, et al. Proinflammatory phenotype of perivascular adipocytes: influence of high-fat feeding. *Circ Res*. 2009;104:541-9.
95. Takaoka M, Suzuki H, Shioda S, et al. Endovascular injury induces rapid phenotypic changes in perivascular adipose tissue. *Arterioscler Thromb Vasc Biol*. 2010;30:1576-82.
96. Eringa EC, Bakker W and van Hinsbergh VW. Paracrine regulation of vascular tone, inflammation and insulin sensitivity by perivascular adipose tissue. *Vascul Pharmacol*. 2012;56:204-9.
97. Omar A, Chatterjee TK, Tang Y, et al. Proinflammatory phenotype of perivascular adipocytes. *Arterioscler Thromb Vasc Biol*. 2014;34:1631-6.
98. Ohman M, Luo W, Wang H, et al. Perivascular visceral adipose tissue induces atherosclerosis in ApoE-/- mice. *Atheroscl*. 2011;219:33-9.
99. Verhagen SN and Visseren FL. Perivascular adipose tissue as a cause of atherosclerosis. *Atherosclerosis*. 2011;214:3-10.
100. Manka D, Chatterjee T, Stoll L, et al. Transplanted Perivascular Adipose Tissue Accelerates Injury-Induced Neointimal Hyperplasia: Role of Monocyte Chemoattractant Protein-1. *Arterioscler Thromb Vasc Biol*. 2014;34:1723-1730.
101. Ketonen J, Shi J, Martonen E, et al. Periadventitial adipose tissue promotes endothelial dysfunction via oxidative stress in diet-induced obese C57Bl/6 mice. *Circ J*. 2010;74:1479-87.
102. Nguyen BT, Yu P, Tao M, et al. Perivascular innate immune events modulate early murine vein graft adaptations. *Journal of Vascular Surgery*. 2013;57:486-492.e2.
103. Fernández-Alfonso MS, Gil-Ortega M, Aranguez I, et al. Role of PVAT in coronary atherosclerosis and vein graft patency: friend or foe? *Br J Pharmacol*. 2017;174:3561-3572.
104. Rzucidlo EM, Martin KA and Powell RJ. Regulation of vascular smooth muscle cell differentiation. *Journal of Vascular Surgery*. 2007;45:A25-A32.
105. Wadey K, Lopes J, Bendeck M, et al. Role of smooth muscle cells in coronary artery bypass grafting failure. *Cardiovasc Res*. 2018;114:601-610.
106. Wang G, Jacquet L, Karamariti E, et al. Origin and differentiation of vascular smooth muscle cells. *J Physiol*. 2015;593:3013-30.
107. Nakazawa G, Otsuka F, Nakano M, et al. The pathology of neoatherosclerosis in human coronary implants bare-metal and drug-eluting stents. *J Am Coll Cardiol*. 2011;57:1314-22.
108. Fuchs JC, Mitchener JS, 3rd and Hagen PO. Postoperative changes in autologous vein grafts. *Ann Surg*. 1978;188:1-15.
109. de Vries MR, Niessen HWM, Löwik CWGM, et al. Plaque Rupture Complications in Murine Atherosclerotic Vein Grafts Can Be Prevented by TIMP-1 Overexpression. *PLoS One*. 2012;7:e47134.
110. Greenblatt DY, Rajamanickam V and Mell MW. Predictors of surgical site infection after open lower extremity revascularization. *J Vasc Surg*. 2011;54:433-9.
111. Malone M, Lau NS, White J, et al. The effect of diabetes mellitus on costs and length of stay in patients with peripheral arterial disease undergoing vascular surgery. *Eur J Vasc Endovasc Surg*. 2014;48:447-51.
112. Spreen MI, Martens JM, Knippenberg B, et al. Long-Term Follow-up of the PADI Trial: Percutaneous Transluminal Angioplasty Versus Drug-Eluting Stents for Infrapopliteal Lesions in Critical Limb Ischemia. *J Am Heart Assoc*. 2017;6.
113. Darling JD, Bodewes TCF, Deery SE, et al. Outcomes after first-time lower extremity revascularization for chronic limb-threatening ischemia between patients with and without diabetes. *J Vasc Surg*. 2018;67:1159-1169.
114. Collins MJ, Li X, Lv W, et al. Therapeutic strategies to combat neointimal hyperplasia in vascular grafts. *Expert Rev Cardiovasc Ther*. 2012;10:635-647.
115. Osborne TB, Mendel LB and Ferry EL. THE EFFECT OF RETARDATION OF GROWTH UPON THE BREEDING PERIOD AND DURATION OF LIFE OF RATS. *Science*. 1917;45:294-5.
116. McCay CM, Crowell MF and Maynard LA. The effect of retarded growth upon the length of life span and upon the ultimate body size. 1935. *Nutrition*. 1989;5:155-71; discussion 172.
117. Slonaker JR. THE EFFECT OF DIFFERENT PER CENTS OF PROTEIN IN THE DIET. *American Journal of Physiology-Legacy Content*. 1931;98:266-275.
118. Kapahi P, Kaeberlein M and Hansen M. Dietary restriction and lifespan: Lessons from invertebrate models. *Ageing Res Rev*. 2017;39:3-14.
119. Mattison JA, Roth GS, Beasley TM, et al. Impact of caloric restriction on health and survival in rhesus monkeys from the NIA study. *Nature*. 2012;489:318-21.
120. Fontana L and Partridge L. Promoting health and longevity through diet: from model organisms to humans. *Cell*. 2015;161:106-18.
121. Solon-Biet SM, McMahon AC, Ballard JW, et al. The ratio of macronutrients, not caloric intake, dictates cardiometabolic health, aging, and longevity in ad libitum-fed mice. *Cell Metab*. 2014;19:418-30.
122. Le Couteur DG, Solon-Biet S, Cogger VC, et al. The impact of low-protein high-carbohydrate diets on aging and lifespan. *Cell Mol Life Sci*. 2016;73:1237-52.
123. Ooka H, Segall PE and Timiras PS. Histology and survival in age-delayed low-tryptophan-fed rats. *Mech Ageing Dev*. 1988;43:79-98.
124. Orentreich N, Matias JR, DeFelice A, et al. Low methionine ingestion by rats extends life span. *J Nutr*. 1993;123:269-74.
125. Heilbronn LK, de Jonge L, Frisard MI, et al. Effect of 6-month calorie restriction on biomarkers of longevity, metabolic adaptation, and oxidative stress in overweight individuals: a randomized controlled trial. *JAMA*. 2006;295:1539-48.
126. Weiss EP, Racette SB, Villareal DT, et al. Improvements in glucose tolerance and insulin action induced by increasing energy expenditure or decreasing energy intake: a randomized controlled trial. *Am J Clin Nutr*. 2006;84:1033-42.

127. Fontana L, Partridge L and Longo VD. Extending healthy life span--from yeast to humans. *Science*. 2010;328:321-6.
128. Trepanowski JF, Canale RE, Marshall KE, et al. Impact of caloric and dietary restriction regimens on markers of health and longevity in humans and animals: a summary of available findings. *Nutr J*. 2011;10:107.
129. Speakman JR, Mitchell SE and Mazidi M. Calories or protein? The effect of dietary restriction on lifespan in rodents is explained by calories alone. *Exp Gerontol*. 2016;86:28-38.
130. Yu ZF and Mattson MP. Dietary restriction and 2-deoxyglucose administration reduce focal ischemic brain damage and improve behavioral outcome: evidence for a preconditioning mechanism. *J Neurosci Res*. 1999;57:830-9.
131. Chandrasekar B, Nelson JF, Colston JT, et al. Calorie restriction attenuates inflammatory responses to myocardial ischemia-reperfusion injury. *Am J Physiol Heart Circ Physiol*. 2001;280:H2094-102.
132. Ravussin E, Redman LM, Rochon J, et al. A 2-Year Randomized Controlled Trial of Human Caloric Restriction: Feasibility and Effects on Predictors of Health Span and Longevity. *J Gerontol A Biol Sci Med Sci*. 2015;70:1097-104.
133. Mitchell JR, Verweij M, Brand K, et al. Short-term dietary restriction and fasting precondition against ischemia reperfusion injury in mice. *Aging Cell*. 2010;9:40-53.
134. Peng W, Robertson L, Gallinetti J, et al. Surgical stress resistance induced by single amino acid deprivation requires Gcn2 in mice. *Sci Transl Med*. 2012;4:118ra11.
135. Gallinetti J, Harputlugil E and Mitchell JR. Amino acid sensing in dietary-restriction-mediated longevity: roles of signal-transducing kinases GCN2 and TOR. *Biochem J*. 2013;449:1-10.
136. Kedia-Mehta N and Finlay DK. Competition for nutrients and its role in controlling immune responses. *Nat Commun*. 2019;10:2123-2123.
137. Wanders D, Stone KP, Forney LA, et al. Role of GCN2-Independent Signaling Through a Noncanonical PERK/NRF2 Pathway in the Physiological Responses to Dietary Methionine Restriction. *Diabetes*. 2016;65:1499-510.
138. Varendi K, Airavaara M, Anttila J, et al. Short-term preoperative dietary restriction is neuroprotective in a rat focal stroke model. *PLoS One*. 2014;9:e93911.
139. Mejia P, Trevino-Villarreal JH, Hine C, et al. Dietary restriction protects against experimental cerebral malaria via leptin modulation and T-cell mTORC1 suppression. *Nat Commun*. 2015;6:6050.
140. Robertson LT, Treviño-Villarreal JH, Mejia P, et al. Protein and Calorie Restriction Contribute Additively to Protection from Renal Ischemia Reperfusion Injury Partly via Leptin Reduction in Male Mice. *The Journal of nutrition*. 2015;145:1717-1727.
141. Tao M, Mauro CR, Yu P, et al. A simplified murine intimal hyperplasia model founded on a focal carotid stenosis. *Am J Pathol*. 2013;182:277-87.
142. Yu P, Nguyen BT, Tao M, et al. Diet-Induced Obesity Drives Negative Mouse Vein Graft Wall Remodeling. *Journal of Vascular Surgery*. 2014;59:1670-1676.
143. Mauro CR, Tao M, Yu P, et al. Preoperative dietary restriction reduces intimal hyperplasia and protects from ischemia-reperfusion injury. *J Vasc Surg*. 2014.
144. Hine C, Harputlugil E, Zhang Y, et al. Endogenous Hydrogen Sulfide Production Is Essential for Dietary Restriction Benefits. *Cell*. 2015;160:132-144.
145. Wang R. Physiological Implications of Hydrogen Sulfide: A Whiff Exploration That Blossomed. *Physiol Rev*. 2012;92:791-896.

146. Polhemus DJ and Lefer DJ. Emergence of Hydrogen Sulfide as an Endogenous Gaseous Signaling Molecule in Cardiovascular Disease. *Circ Res*. 2014;114:730-737.
147. Kanagy NL, Szabo C and Papapetropoulos A. Vascular biology of hydrogen sulfide. *American Journal of Physiology - Cell Physiology*. 2017;312:C537-C549.
148. Elsey DJ, Fowkes RC and Baxter GF. Regulation of cardiovascular cell function by hydrogen sulfide (H(2)S). *Cell Biochem Funct*. 2010;28:95-106.
149. Faller S, Hausler F, Goeft A, et al. Hydrogen sulfide limits neutrophil transmigration, inflammation, and oxidative burst in lipopolysaccharide-induced acute lung injury. *Sci Rep*. 2018;8:14676.
150. Bibli SI, Hu J, Sigala F, et al. Cystathionine gamma Lyase Sulfhydrates the RNA Binding Protein HuR to Preserve Endothelial Cell Function and Delay Atherogenesis. *Circulation*. 2018.
151. King AL, Polhemus DJ, Bhushan S, et al. Hydrogen sulfide cytoprotective signaling is endothelial nitric oxide synthase-nitric oxide dependent. *Proc Natl Acad Sci U S A*. 2014;111:3182-7.
152. Longchamp A, Mirabella T, Arduini A, et al. Amino Acid Restriction Triggers Angiogenesis via GCN2/ATF4 Regulation of VEGF and H2S Production. *Cell*. 2018;173:117-129.e14.
153. Kehlet H. Multimodal approach to control postoperative pathophysiology and rehabilitation. *Br J Anaesth*. 1997;78:606-617.
154. McGinigle KL, Eldrup-Jorgensen J, McCall R, et al. A systematic review of enhanced recovery after surgery for vascular operations. *J Vasc Surg*. 2019;70:629-640.e1.
155. Weledji EP, Njong SN, Chichom A, et al. The effects of preoperative carbohydrate loading on the metabolic response to surgery in a low resource setting. *International Journal of Surgery Open*. 2017;8:18-23.