In a Nutshell: Findings from the Bruneck Study

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Keywords

Population study · Cohort · Cardiovascular disease · Stroke · Myocardial infarction · Atherosclerosis · Inflammation · Vascular remodeling · Innate immunity · Aging

Abstract

The Bruneck Study is a prospective community-based study enrolling an age- and sex-stratified random sample of 1,000 men and women. It achieved an extraordinary participation rate of 93.4% and a near-complete long-term follow-up exceeding a quarter of a century (1990–2018). High-quality ascertainment of most common human diseases enables reliable evaluation of: disease epidemiology, overlaps between age-related diseases, and risk factors, and discovery of novel biomarkers. Research priorities include atherosclerosis, cardiovascular disease, aging and longevity, neurological diseases, disorders of the bone, and cancer. This review summarizes the main scientific contributions of the Bruneck Study over the past decades, outlines recent highlights, and gives an outlook of what is planned next.

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Back to the Roots

The Bruneck Study was launched in summer 1990 (Fig. 1). Over the following quarter of a century, it has advanced to one of the most successful community-based

epidemiological cohort studies in terms of scientific output and contributions to important novel hypotheses in vascular biology and medicine.

Three researchers – Johann Willeit, Friedrich Oberholzenzer, and Georg Egger (online suppl. Fig.; see www.karger.com/doi/10.1159/000492329 for all online suppl. material) – met in the late 80s and decided to start and pursue this lifetime project – initially without any funding and only based on the researchers' enthusiasm. Based on experiences from a small preceding pilot study, they had available field-proven protocols and decided to integrate newly emerging techniques like high-resolution carotid ultrasound. The study commenced with a strong cardiovascular priority but quickly extended its focus to many age-dependent diseases. In parallel, the local initiative soon grew to a large international affair with involvement of dedicated scientists from all over Europe and the US.

Bruneck (or Brunico, Bolzano Province, Italy), the home town of the three researchers, is located in the Alps within a 100-mile distance from Innsbruck (Austria) and highly suitable for epidemiologic research in the light of low population mobility (<0.1% per year) enabling long-term follow-up. While the Bruneck Hospital served as the platform for the field work, the Medical University of Innsbruck qualified as the academic study headquarter. Later on, the Bruneck Hospital was honored as an official scientific outpost of the university.

Friedrich Oberholzenzer, now beyond 90 years, is still a driving force in the large study team full of scientific spir-

Fig. 1 The Bruneck Study with its long-term follow-up and recent extension as well as key variables and outcomes including carotid atherosclerosis. ECG, electrocardiogram; OA, osteoarthritis; IMT, intima-media thickness; ICA, internal carotid artery; CCA, common carotid artery; NMR, nuclear magnetic resonance; ABI, ankle-brachial index; FFQ, food frequency questionnaire; PWV, pulse wave velocity.

it and extraordinary research ideas. When the study started, he was the head of the Internal Department of the Bruneck Hospital and, through his ties with all segments of the population in Bruneck, took a central role in achieving high participation and follow-up rates. *Johann Willeit*, the neurologist in the team, ensured scientific rigor and quality and set new methodological standards in epidemiological research at that time. *Georg Egger*, the cardiologist, succeeded in convincing the stakeholders and politicians to pass a federal law that stipulated the conduction of the Bruneck Study as part of South Tyrol's healthcare act. This law was in place for more than a decade. Recently, the Bruneck Study was extended and integrated into the Research Center of Excellence on Vascular Ageing (VASCage) and is now financed by public funding.

Some Notes on Quality and Quantity

The Bruneck Study recruited an age- and sex-stratified random sample of overall 1,000 individuals (125 men and women of each the 4th–7th decade). A remarkable 93.4% of eligible subjects joined the study making the highest participation rates of all population- and community-based studies worldwide. This design ensured stable prevalence and incidence rates of carotid arteriosclerosis and cardiovascular disease [1, 2]. In a trade-off of quality and quantity, a sample size of 1,000 individuals is on the small side for genetic association studies (including GWAS) and epidemiological studies of rare diseases, but very well suited for biomarker discovery, testing of hypothesis on disease mechanisms, and epidemiological studies on common age-dependent diseases. The key focus of the

Table 1. Outcome and exposure variables in the Bruneck Study

Outcome/Exposure	Year of baseline/follow-up examination					
	1990	1995	2000	2005	2010	2015–18
CVD outcome variables						
Cardiovascular diseases ^a	x	x	x	x	x	x
Carotid IMT	–	x	x	x	x	x
Carotid plaque	x	x	x	x	x	x
Femoral IMT/plaque	–	–	x	x	x	x
Abdominal aorta diameter/plaque	–	–	–	–	x	x
Ankle-brachial index	x	x	x	x	x	x
ECG	x	x	x	x	x	x
Exposure variables						
Vascular risk factors ^b	x	x	x	x	x	x
Lifestyle factors	x	x	x	x	x	x
Diet	x	x	x	x	x	x
Bioimpedance	x	–	–	–	–	x
Neurological disorders						
Movement disorders ^c	–	–	x	x	x	x
Frailty/falls	–	–	x	x	x	x
Sleep disorders and quality	–	–	x	–	–	–
Cognitive functioning	x	x	x	x	x	x
Headache/migraine	x	–	x	–	–	–
Epilepsy	x	x	x	x	x	x
Rheumatological disorders						
Pain	–	–	x	x	x	x
Fractures	–	–	x	x	x	x
Osteoporosis/osteoarthritis	–	–	x	x	x	x
Others						
Demographic/socioeconomic factors	x	x	x	x	x	x
Employment/retirement	x	x	x	x	x	x
Use of care	–	–	–	–	–	x
Personal network	x	x	x	x	x	x
Quality of life	–	x	x	x	x	x
Global Health Assessment	–	x	x	x	x	x
Comorbidities	x	x	x	x	x	x
Tumors	x	x	x	x	x	x
NAFLD	–	–	–	–	–	x

^a Fatal and nonfatal myocardial infarction, fatal and nonfatal stroke, angina pectoris, transitory ischemic attack, fatal and nonfatal heart failure, atrial fibrillation, pulmonary heart disease, pulmonary or arterial embolism, peripheral artery disease, revascularization procedure, pacemaker implantation, vascular death (ascertained in the clinical examination, questionnaires, interviews, medical records of practitioners or hospitals and on death certificates). ^b Hypertension, diabetes mellitus, dyslipidemia, tobacco use, alcohol consumption, physical inactivity, obesity, family history. ^c Parkinson's disease, tremor, chorea, dystonia, myoclonus, tic, gait disorders.

Bruneck Study is on the quality side. Several thousand study characteristics were carefully recorded for each participant (Table 1). Of note, the subjects' self-report was obligatorily confirmed by study exams and a thorough review of the medical records provided by the Bruneck

Hospital and GPs. The situation in Bruneck is unique in that the whole area is served by a single hospital which houses the only wet lab and radiology facility. All medical records ever made on study subjects were available to the study investigators. All prevalent and new-onset diseases

were ascertained based on state-of-the-art diagnostic criteria. There is a remarkable 100% follow-up for main clinical endpoints and deaths including causes of death based on a stable community. This all makes an extensive database and rich resource for epidemiological research (Fig. 1).

Uniqueness and Success Criteria

Several additional characteristics of the Bruneck Study are responsible for its success in a highly competitive environment: (a) in the early 90s, cardiovascular research was largely confined to coronary artery disease in male subjects. The Bruneck Study was far ahead of its time with including identical shares of men and women. (b) The long-term follow-up of more than 25 years ensures adequate numbers of outcome events and facilitates calculation of reliable lifetime prevalences and cumulative risks. Follow-up beyond 20 years is only exceptionally achieved in cohort studies, another example being the Framingham Study. (c) Our study was the first community-based ultrasound study with a primary focus on atherosclerotic plaques (rather than IMT), serial measurements of plaque progression (up to 5 remeasurements), and a single highly experienced sonographer making all scans in all individuals over the whole study period (Johann Willeit) [1, 3]. As a unique research proposition, the study has a validated ultrasound-based plaque progression model allowing differentiation between early and advanced stages of the atherosclerosis process [1, 3, 4]. (d) High-quality assessment of many relevant human diseases (Table 1) provides a unique opportunity for targeting health broadly, studying disease overlaps, and unravelling shared risk factors and potential mechanisms of inflammatory and metabolic diseases. (e) Based on an amicable collaboration with basic science researchers, a number of innovative new laboratory techniques were first applied to this cohort and allowed for pioneer research [5–9]. (f) The very broad focus enabled research outside the mainstream and enriched our knowledge about the pathogenesis of atherosclerosis, cardiovascular disease, and aging.

Limitations of epidemiological research apply to the Bruneck Study as well. Epidemiological analyses, even if prospective in nature and of high quality, require further elaboration by experimental studies, intervention trials, and/or Mendelian randomization approaches to infer causality.

Collaborators and Meta-Analyses

A particular strength of the Bruneck Study is its large international network of collaborating researchers which are listed in the acknowledgment and exemplified when addressing the key hypotheses. Our study contributed to multiple large epidemiologic consortia and initiatives. It was one of the founding members of the *Fibrinogen Studies Collaboration (FSC)* [10] which advanced to the *Emerging Risk Factors Collaboration (ERFC)* [11–15]. This initiative was coordinated by a member of the Bruneck Study team for several years. We also supported the PROGIMT consortium, which shed light into the scientific validity and capacity of serial IMT measurements and included about 60 studies with multiple ultrasound exams in the same subjects [16]. PROGIMT was recently advanced to *Proof-ATHERO*, the headquarter of which is located in Innsbruck. Further contributions are made to genetic consortia like the QT consortium [17], the *Global Burden of Disease (GBD)* and *Non-Communicable Disease (NCD)* collaborations endorsed by the WHO [18].

Meta-analyses that rely on individual participant data are of importance for studying strength and shape of associations and the interaction thereof in a large number of populations, different ethnicities and settings, and to create a solid basis for health care planning. The prime role of high-quality individual studies, in contrast, consists in eliciting innovation (novel lab techniques, hypotheses, and epidemiological evidence of disease mechanisms) to be substantiated and elaborated in large meta-analyses in a second step. More than ever, the combination of first-row basis science work and high-quality research in individual long-term prospective community-based studies like ours with subsequent replication and translation in large research consortia represents a gold standard.

Key Hypotheses Emerging from or Supported by the Bruneck Study

Vascular Remodeling in the Carotid Arteries

In the early 90s, Glagov's concept of vessel remodeling, based on cross-sectional histopathological studies of coronary arteries, was broadly accepted. It proposed that vessel stenosis emerges when the plaque area exceeds 40% of the entire vessel cross-section – a critical limit to exhaust compensatory mechanisms. In sharp contrast, prospective data from the Bruneck Study [1, 3, 4] indicated that there are two different types of plaque growth. The most frequent type is characterized by slow and continuous

plaque extension, usually affecting several lesions and vessel segments simultaneously. This type of plaque growth is accompanied by compensatory vessel enlargement at the site of active atherosclerosis thereby preserving a normal lumen or being even overcompensatory and resulting in diffuse dilative atherosclerosis. This process is superimposed by an age-dependent dilation of the carotid arteries, which starts in the 5th decade and continuously accelerates thereafter in vessels with high intima-media thickness [3]. The early stage of atherosclerosis relies on a cumulative exposure to traditional cardiovascular risk factors and does not cause vessel stenosis even in the long run [4]. The second main type of plaque growth is characterized by occasional prominent increases in lesion size, usually at the origin of the internal carotid artery, and insufficient or even no vessel dilation at the site of plaque expansion. As a consequence, this episodic plaque growth leads to lumen compromise and manifestation or growth of vessel stenosis. Remarkably, standard risk factors are not associated with this advanced stage of atherosclerosis. Instead, determinants of plaque vulnerability and pro-coagulant risk conditions were found to be of relevance [4, 19–21]. Both diffuse dilative and focal stenotic atherosclerosis may coincide in the same individual but underlie different regulatory and pathological mechanisms.

The Iron Hypothesis

As one of the first findings of the Bruneck Study [22, 23], body iron stores reflected by serum ferritin levels exhibited strong relationships to incident carotid atherosclerosis, both in men and in women. Moreover, the substantial sex difference in carotid atherosclerosis incidence disappeared within 5 years after menopause along with the filling-up of iron stores in the very same period. These findings suggest that the relative protection of premenopausal women against atherosclerosis and cardiovascular disease may partly arise from monthly blood loss. Data from the Bruneck Study and identical observations in a Finnish study [24] stimulated the conduction of a large randomized controlled trial on lowering of body iron stores by regular blood donation and its effects on the risk of all-cause mortality and the combined endpoint of death, myocardial infarction, and stroke. This study (FeAST) [25] was overall negative but showed a highly significant and quantitatively remarkable reduction of the combined endpoint among individuals aged 65 and under, the typical target population for blood donations. This subgroup analysis was prespecified and is commonly cited to encourage blood donations in healthy individuals.

Lifestyle

The U-shaped association between alcohol consumption and cardiovascular disease was already well established when the Bruneck Study was launched. We, however, provided first population-based evidence for an association of identical shape with carotid atherosclerosis – a surrogate of and window to system atherosclerosis [26]. Low amounts of alcoholic beverages (predominantly wine) protected against manifestation and progression of carotid plaques whereas high amounts constituted a risk condition comparable to heavy smoking. Well in line with the view that alcohol is responsible for the French paradox (low risk of cardiovascular disease in France in spite of high lipid levels), protective effects of alcoholic beverages were most pronounced in individuals with high low-density lipoprotein cholesterol [27]. Overall, these data indicate that ethanol and nonalcoholic components of alcoholic beverages impact the vessel wall on top of its well-known effects on platelet function, high-density lipoprotein cholesterol, and atherothrombosis [27]. Low amounts of alcohol also improve insulin sensitivity and favorably affect metabolic risk [28].

While it was not possible to decipher potential effects of alcohol quality on vascular risk in our study, we coined a second key paradox in relation to alcohol – the South-east Asian Paradox [27]. This paradox originates from the INTERHEART Study and suggests that low-quality spirits, which form the main source of alcohol in Southeast Asia, do not confer protection against cardiovascular disease even at low dosages, potentially indicating that quality matters. Unfavorable effects of spirits were confirmed in the most recent and comprehensive meta-analysis on alcohol and health, whereas wine came off best. This meta-analysis yielded evidence that safe dosages of alcohol are lower than assumed previously and 100 g ethanol per week represents the intake related to the lowest risk of all-cause mortality [15].

Analysis within the Bruneck cohort on how smoking affects atherosclerosis revealed evidence that lifetime cumulative exposure (pack-years) mainly impacts early stages of atherosclerosis, whereas peak levels contribute to plaque destabilization and fissuring and influence atherothrombosis [29]. It takes a full decade before accelerated atherogenesis returns to normal after smoking cessation whereas effects on atherothrombosis and blood coagulation are rapidly reversible [29]. Systemic inflammation and chronic infections appear to be main mediators of unfavorable smoking effects on the vasculature [29]. Recent research on diet and cardiovascular disease revealed several intriguing findings. High intake of the

polyamine spermidine was inversely related to mortality, indicating that the lifespan-prolonging effects of spermidine, well-documented in a variety of model organisms and human cell lines [30], potentially extend to mankind [31]. Main sources of spermidine in our cohort were wholemeal, apples, pears, green salad, vegetable sprouts, potatoes, and cheese. In line, another popular concept, i.e., the extension of lifespan by caloric restriction in experimental animals, received supportive epidemiological evidence from the Bruneck Study [31].

The Inflammation Hypothesis

From a histopathological perspective, atherosclerosis represents an inflammatory disease with this hypothesis dating back to the mid of the 19th century. It was substantiated by a wealth of experimental data and direct visualization of plaque inflammation by sophisticated imaging techniques. Interestingly, T cells are among the first cells to appear in early vascular lesions followed by macrophages [32, 33]. The Bruneck Study provided broad epidemiological support to the inflammation hypothesis in that multiple blood-born markers of inflammation were linked to active atherosclerosis and cardiovascular disease alike [10, 13, 34–36]. Atherosclerosis may be considered to arise from an inflammatory innate immune response against proatherogenic factors like lipid depositions in the vessel wall. Innate immune receptors such as scavenger receptors are implicit in the formation of foam cells, a central phenomenon in early atherosclerosis, and signaling receptors are of outstanding importance as well. This is particularly true for toll-like receptors (TLRs) including TLR-2 and TLR-4 [37]. We published epidemiological data supporting that TLR-4 (and its ligands endotoxin and heat-shock proteins) fuel plaque inflammation and promote cardiovascular disease [38].

While there is no doubt that the innate immune system is key to atherosclerosis, there is also a role for adaptive immunity and effector T cells. The autoimmune hypothesis of atherosclerosis, coined by Georg Wick based on his seminal experimental work [39], received a strong epidemiological underpinning by data from the Bruneck Study [35, 40–42], ARFY and ARMY Studies [43, 44], and recent histological studies in vessels of young organ donors [33]. In brief, heat-shock protein 60 reactive T cells appear to play a role in the initiation of atherosclerosis whereas humoral immune reaction against heat-shock protein 60 emerged as strongly related to the risk of atherosclerosis, myocardial infarction, and stroke in the elderly [35, 40–42]. Continuous exposure to heat-shock protein 60 (bacterial heat-shock protein 60 in the course

of chronic infections and biochemically altered autologous heat-shock protein 60 expressed on and released by endothelial cells) triggers the immune response and results in a breakdown of immune tolerance and subsequent immune attack.

The clinical consequence of all this is a promise that anti-inflammatory therapies might stabilize atherosclerosis and fight cardiovascular disease by diminishing inflammation. Actually, Ridker et al. [45] convincingly showed in 2017 that interleukin 1 antagonism by canakinumab reduces the risk of myocardial infarction and stroke in vascular high-risk individuals without affecting lipid levels or blood pressure. This cutting-edge publication now stimulates planning and conduction of a large number of new studies on anti-inflammatory agents in the secondary prevention of myocardial infarction and stroke (for example using colchicine).

Equally important from a clinical point of view, virtually all human diseases featured by systemic inflammation are related to a heightened burden of atherosclerosis. The Bruneck Study was among the first to demonstrate associations between atherosclerosis and chronic (usually minor) infections like chronic bronchitis [46]. In line, asthma heightens the risk of cardiovascular disease [47] potentially mediated by proatherogenic and prothrombotic properties of mast cells, specific mediators like leukotrienes, and especially eosinophils [47, 48]. The prominent role of eosinophils in atherothrombosis has recently been uncovered and substantiated by epidemiological data from our cohort [48]. Another potentially important contribution to the field was an observation in the late 90s that high circulating endotoxin levels in blood, probably originating from a breakdown of the mucosa-blood barrier in the gut, confer increased cardiovascular disease risk [34]. This study pioneered the intriguing concept that systemic inflammation partly arises from the gut. Other inflammatory diseases, linked to atherosclerosis and cardiovascular disease, are rheumatoid arthritis, inflammatory bowel disease, and even cancer (unpublished data).

Lipoprotein(a)

There is no doubt now that lipoprotein(a) (Lp[a]) represents a true risk factor of cardiovascular disease [49]. Contributions from our study [50, 51] involve the demonstration of a highly significant association between Lp(a) level and atherosclerosis: as one of few risk factors, Lp(a) predicts early as well as advanced stages of atherosclerosis, probably reflecting its capacity to elicit plaque inflammation and to facilitate atherothrombosis given its structure homology with plasminogen [51]. Of note, both Lp(a)

concentration and the apo(a) phenotype contribute independently to atherogenesis [51] and measurement of the Lp(a) concentration in subjects at an intermediate cardiovascular risk improves risk prediction [52]. The European Society of Cardiology recently changed their guidelines and now recommend routine Lp(a) measurements in specific subgroups of individuals (class IIa) [53]. From a physiological perspective, Lp(a) binds and clears highly active and pro-atherogenic phospholipid species [54–56]. Lp(a) research recently attracted substantial interest owing to the development of a specific Lp(a) therapy [57].

Neuroepidemiology

From its very beginning, the Bruneck Study also had a neurological priority. As a main objective, we strived for high-quality data on the epidemiology of common neurological diseases in the general (elderly) population. One highlight was the first comprehensive evaluation of all movement disorders in the same population, applying highest standards in disease ascertainment [58]. No less than 30% of men and women aged 50 and over and even 50% of men and women aged 80 and over suffered from one or more movement disorders including tremor, dystonia, restless leg syndrome, and Parkinson's disease [58, 59]. A second example is the first all-embracing attempt to assess primary headaches in the elderly population applying the (at this time) newly released ICHD-2 diagnostic criteria [60]. Given the long-term follow-up of our study, lifetime prevalences were accurately estimated and emerged as high as 20% for migraine and 40% for tension-type headache [60]. We refuted the misconception that the heightened stroke and myocardial infarction risk in migraine patients is due to an elevated burden of atherosclerosis [61] and yielded evidence that migraine facilitates venous thromboembolism as recently replicated on a large scale [62]. Further studies in the Bruneck cohort specifically targeted sleeping disorders, depression, and cognitive decline [63, 64].

The Cross-Link between Cardiovascular and Bone Disease

The common co-incidence of cardiovascular and bone disease like osteoporosis partly arises from shared pathways and mediators. Epidemiological data from our cohort, in concert with high-quality experimental research, revealed first evidence that receptor activator of nuclear factor- κ B ligand (RANKL) is involved in the development of the hepatic insulin resistance and diabetes mel-

litus [65]. RANKL is not expressed in normal vascular tissue and early stages of atherosclerosis but is substantially upregulated in advanced vulnerable plaques [20]. It significantly contributes to plaque destabilization by boosting inflammation, promoting matrix degeneration, and facilitating microcalcification. Accordingly, it is not surprising that levels of circulating RANKL relate to cardiovascular diseases that rely on plaque fissuring or rupture but not to cardiovascular diseases based on stable vessel stenosis [20]. Osteoprotegerin (OPG), a decoy receptor of RANKL, ranks among the most powerful blood biomarkers of cardiovascular disease risk. OPG adequately reflects the activity of the RANKL-RANK-OPG network and may also exert proatherogenic properties if substantially upregulated for a prolonged period of time [36]. A recent analysis unraveled a protective role for Dickkopf-3 (DKK-3) [66], while ongoing research focuses on sclerostin and DKK-1, all regulators of Wnt signaling implicit in bone homeostasis.

Recent Highlights

Substantial interest in circulating microRNAs (miRNAs) as biomarkers of vascular diseases has recently emerged [67, 68]. Contributions from the Bruneck Study in this context included the discovery of a typical miRNA signature of diabetes and its complications [6]. Most of the miRNAs in the circulation have a platelet origin and may be suitable for estimating the function of platelet inhibitors. Development of specific assays for clinical use and widespread validation studies are currently underway. Of note, miRNA profiles for myocardial infarction are mainly composed of platelet miRNAs and the endothelial miRNA-126 [69].

miRNA-122 holds a unique position in the miRNA network because it is one of very few that is largely organ specific. It is the main miRNA of liver tissue and a promising biomarker for fatty liver disease and metabolic health [70, 71]. Currently ongoing work targets changes in miRNAs during platelet maturation (potential biomarker of platelet turnover).

Recent advances in laboratory techniques enabled high-throughput analysis of "lipid quality." This intriguing progress led to a new era in lipid research beyond quantification of low-density lipoprotein cholesterol and enabled full characterization of the plasma lipidome [7]. Actually, the Bruneck Study succeeded in deciphering a comprehensive lipid signature of cardiovascular disease risk in the general community [7]. Not surprisingly, species of vari-

ous lipid classes, including cholesterol esters and triglycerides but also sphingomyelins, phosphatidylcholines, phosphatidylethanolamines, and lysophosphatidylcholines were all associated with cardiovascular disease risk. Overall, lipid quality was superior to standard clinical measures of lipid quantity in cardiovascular disease risk prediction [7]. Most proatherogenic lipid species were characterized by a limited spectrum of medium-chain fatty acids, namely myristate, palmitate, palmitoleate, and oleate. Interestingly, these fatty acids are synthesized in the liver by *de novo* lipogenesis with sugars serving as a main fuel. Our data suggest *de novo* lipogenesis to be a promising new target for drug development and cast doubts about the traditional view that a healthy diet has low lipid but high carbohydrate content. Actually, the PURE Study confirmed that lipids are the least dangerous and carbohydrates, especially sugars, the most dangerous energy sources, prompting a redefinition of a healthy diet [72].

On top of the composition of lipids, the pattern of apolipoproteins is of importance for lipid functionality. The first comprehensive assessment of a broad spectrum of apolipoproteins in the Bruneck Study by the multiple reaction monitoring (MRM)-MS technique resulted in a variety of potentially important findings [9]: apolipoproteins CII, CIII and E, the main apolipoproteins of very-low-density lipoprotein, emerged as the apolipoproteins most consistently associated with cardiovascular disease risk even surpassing apolipoprotein B-100, the main apolipoprotein of low-density lipoprotein. This finding probably reflects a shift in atherosclerosis epidemiology, which has previously been suggested by cardiologists: plaque erosion has been proposed to replace plaque rupture as the main pathological culprit in myocardial infarction and plaque erosion relies on triglycerides more than on cholesterol [73]. These potential trends may be a consequence of the widespread use of statins and of the aging of populations as well as changes in risk factors (smoking bans, etc.) [73]. Targeting apolipoprotein CIII is probably the best way to lower very-low-density lipoprotein and respective interventions are currently being tested in ongoing randomized controlled trials [9].

VASCage and Future Directions

The Bruneck Study has recently been integrated in the VASCage (headquarter at the Medical University of Innsbruck) supported by the Austrian Research Promotion Agency. Strong synergism enabled extension of the original Bruneck population to all inhabitants aged 65 and

over. Recruitment is currently underway. If finished, this will form one of the largest unselected populations of elderly individuals. Current and future research priorities are vascular protection, longevity, platelets' contribution to vascular integrity, the microbiome and gut as drivers of cardiovascular disease, protective effects of autophagy, nutrient sensing, mechanisms of genuine aging of the vessel wall including matrix degeneration [8], vascular calcification, cell senescence [19, 74], protein aging (proteostasis), biomarkers and pathomechanisms of atrial fibrillation [75], and metabolic health and disease [14].

In spite of the journey already lasting for more than a quarter of a century, the Bruneck Study has still not reached its finishing point; on the contrary, it is currently being extended and pursued for the long run.

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