Brief Review

Concept of Extremes in Vascular Aging

From Early Vascular Aging to Supernormal Vascular Aging

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With advancing age, changes in the arterial wall contribute to what has been called vascular aging, and in some prematurely affected subjects even early vascular aging (EVA).[1–5] Several years ago,[1] we listed various components of EVA, including arteriosclerosis, atherosclerosis, and excess vasoconstriction, with their clinical expression: arterial stiffening and increased central pulse pressure, carotid intima media thickening and endothelial dysfunction, and increased total peripheral resistance, respectively. In this review, we focus on arteriosclerosis, ie, arterial stiffening, for several reasons: this is the most characteristic clinical feature of the aging process of the arterial system,[6] its measurement has been well standardized and referenced,[7,8] and an increasing number of epidemiological studies have analyzed its independent determinants.[9–11] Increased pulse wave velocity (PWV) is the established hallmark of arterial stiffening and is suggested to be one of the best biomarkers available to calculate the prospective cardiovascular risk and mortality risk of an individual.[9,10,12,13] EVA can be diagnosed in subjects who present an abnormally high arterial stiffness for their age and sex.

In the present review, we propose the concept of supernormal vascular aging (SUPERNOVA). SUPERNOVA can be diagnosed in subjects who present an exceptionally low arterial stiffness for their age and sex. We address the issue of the metrics and definition of EVA and SUPERNOVA as 2 extremes of the distribution of vascular aging. We discuss the concept of extremes in cardiometabolic research. Further, we review the molecular basis and mechanobiology of EVA and SUPERNOVA, in parallel with their epidemiological, genetic, and epigenetic determinants. Finally, we suggest therapeutic options and insist on the need for discovering novel molecular targets for slowing arterial aging and protecting against cardiovascular complications. By choosing the wording SUPERNOVA, we referred to the life of a supernova—a large explosion that takes place at the end of a star's life cycle. The relationship between supernova and black hole is not firmly established, but physics theory states that in a black hole, time is slowed…like aging of arteries in SUPERNOVA subjects.

Concept of Extremes in Cardiometabolic Research

In cardiovascular and metabolic research, we normally focus on risk and disease but not enough on protection. In the clinical perspective, focusing on risk and disease makes sense to reduce risk and treat disease manifestation, but from another perspective, it could be worth pursuing to find protective mechanisms. The ultimate reason for this is to find biomarkers (including genes) associated with protection from clinical complications to map protective mechanism as they one day could turn into novel therapeutic targets. This is much needed as the control of conventional risk factors and early intervention aimed at reducing the progress and complications of cardiometabolic disease is not able to confer the lowest attainable risk. Although this residual risk may include duration of exposure or misclassification of the measured classical risk factors, it may also include unmeasured/unknown risk factors. By contrasting extremes, it would be possible to discover novel biomarkers, leading to new therapeutic targets, for example, based on novel understanding of protection. In addition to numerous possible targets, including adipokines such as adiponectin, leptin and resistin,[14] and vascular growth factors,[15] new targets could be defined, leading to novel therapies. Such alternatives could hopefully be combined with the well-established drugs to control hypertension, hyperlipidemia, and hyperglycemia for synergistic effects to further reduce the residual risk.

The approach using the extremes of distribution has been successfully used in genetic studies. For instance, in an extreme case-control design, Padmanabhan et al[16] selected the top 2% of the blood pressure (BP) distribution in the Swedish population and contrasted them with the lower 9.2% of the BP distribution, to show the minor allele of the uromodulin gene to be protective against hypertension and associated with lower urinary uromodulin excretion.

A few clinical models of cardiometabolic protection that used the extremes of the distribution were successful in determining the clinical characteristics of protected patients, with obvious clinical applications. For example, in long-standing type 1 diabetes mellitus of >40 to 50 years of duration and daily insulin regimen, there is a minority of patients who seem to escape major cardiovascular complications even if minor complications such as simplex retinopathy or mild microalbuminuria are present.[17–20] Previous studies have tried to map protective factors in these patients, but to date, no conclusive genetic findings have been presented. Another model is the so-called metabolically healthy obesity—a disputed condition

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normally defined by the absence of variables linked to the metabolic syndrome.[21,22] An alternative way to define these rare subjects is to find individuals with high body mass index, but escaping hospitalization during long periods of midlife, as was recently documented in subjects with body mass index >35 kg/m^2.[23] Even if metabolically healthy obesity exists, such individuals will probably not remain free of complications in the long perspective, but such events may be postponed until a higher age. A third model is represented by patients with end-stage renal disease on peritoneal dialysis for a number of years but not harmed by cardiovascular complications[24] in spite of the fact that end-stage renal disease in most patients is associated with a pronounced increased risk of atherosclerosis and media sclerosis of large arteries.[25] These protected patients are nondiabetic, not overweight, and rather young.[25]

Whether these models simply result from chance, projecting some individuals at the extreme lower end of the normal distribution of risk factors or clinical parameters, or whether those individuals really possess specific protective (genetic) causative mechanisms is still an open question that further epidemiological and mechanistic studies should try to explore.

EVA—New Findings and Developments

During the last 2 decades, a growing body of evidence has accumulated that arterial stiffness, which is an intermediate end point and an independent predictor of cardiovascular disease (CVD) and cardiovascular as well as total mortality, could be used as a proxy of vascular aging.[7,9,10,12,13,26] We also promoted the concept of EVA[1–3,5] to show that it is possible to early identify subjects with arterial damage that if undetected otherwise would lead them into premature CVD and irrecoverable residual risk despite later therapeutic interventions. The Lancet Commission on Hypertension[27] used a life course approach to better demonstrate that preventive efforts should be focused on several avoidable thresholds (elevated BP, subclinical organ damage, and cardiovascular events), with the goal to improve life course trajectory as much as possible. Figure 1 illustrates the life course concept applied to arterial stiffening. Our hypothesis is that progressive arterial wall stiffening with aging parallels incident hypertension and then subclinical target organ damage and then cardiovascular complications in a steeper way for some individuals (EVA) than in others (SUPERNOVA). EVA subjects reach each of these steps earlier than the average population. For instance, increasing blood volume, hypervolemia, and obesity are factors contributing to the upward shift from one pattern of vascular aging to another, that is, they are risk factors contributing to EVA. By contrast, SUPERNOVA subjects remain protected for a long period of time.

EVA can be diagnosed in patients who present an abnormally high arterial stiffness for their age and sex. Thus, EVA represents an altered capacity for repairing arterial damage in response to aggressors like mechanical stress and metabolic/chemical/oxidative stresses. In other words, arterial stiffening/carotid-to-femoral PWV (cfPWV) is an integrator of all damages done to the arterial wall. Moreover, aortic stiffness/cfPWV, as a marker of arterial wall damage or arteriosclerosis, integrates both the effect of risk factors and susceptibility to those risk factors (see below the discussion on the

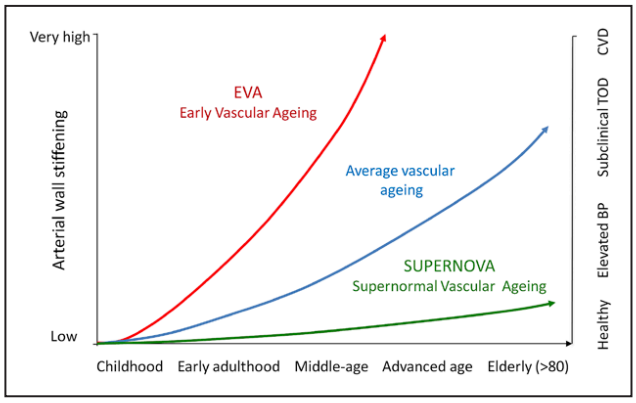

Figure 1. This figure illustrates, in a life course approach of hypertension, our hypothesis that progressive arterial wall stiffening with aging parallels incident hypertension, and then subclinical target organ damage, and then cardiovascular (CV) complications. Early vascular aging (EVA) subjects reach each of these steps earlier than the average population, whereas supernormal vascular aging subjects remain protected for a long period of time. For clarity, healthy vascular aging subjects are not represented here. BP indicates blood pressure; CVD, cardiovascular disease; and TOD, target organ damage.

epigenetic determinants of PWV) and duration of exposure. Thus, cfPWV measures not only the current arterial damage (a product of age, risk factors, and intrinsic susceptibility to them) but also its regression (when a therapeutic action is taken) or progression (when exposure continues or therapeutic actions fail). It differs from the usual snapshot that physicians get from their patients when they only measure BP, cholesterol, and glycemia. This is why EVA arterial stiffness has a higher predictive value for cardiovascular events than classical cardiovascular risk scores.[9,10,12,13]

Recent cross-sectional and longitudinal studies have extended the list of the epidemiological determinants of arterial stiffness. Most of these determinants belong to the classical risk factors, either nonmodifiable such as ethnicity, sex, chronological age, family history, and personal history or modifiable such as BP, diabetes mellitus, dyslipidemia, and smoking.[8] As arterial stiffness and elevated BP are entwined, it is difficult to separate these entities in EVA subjects. There is increasing evidence for a continuous loop (cross talk) between large and small arteries that will either produce increased wall damage through increasing levels of BP or produce increasing BP levels through arterial wall damage. The establishment of an extreme vascular phenotype (EVA or SUPERNOVA) is thus the product of the interaction between (1) the structural changes on arterial wall that are usually associated with age and (2) the mechanisms that accelerate or decelerate this process, respectively. Despite these mechanisms, high BP is not an exclusive condition for EVA. For example, EVA can be caused by chronic low-grade inflammation due to inflammatory bowel disease,[28] in patients who exhibit high PWV despite normal BP.

Additional studies underlined the role of hyperglycemia, metabolic syndrome, insulin resistance, obesity, abdominal fat, chronic kidney disease, high salt intake, chronic low-grade inflammation, oxidative stress, inadequate diet, alcohol consumption, social deprivation, perceived stress, and a number of genetic factors[1–3,5] (Table). The role of the metabolic syndrome, lack of physical activity, and social stress deserves to

Table. Putative Determinants of EVA and SUPERNOVA

Nonmodifiable Determinants	Determinants of EVA	Determinants of SUPERNOVA
Chronological age		
Ethnicity		
Sex		
Family history		
Prenatal fetal growth		
Genetics		
	Classical CV risk factors	
	High BP	Normal BP
	Hyperglycemia	Normal glycemia
	Insulin resistance	
	Diabetes mellitus	
	Obesity	Normal weight
	Abdominal fat	Low-calorie diet
	Metabolic syndrome	
	Dyslipidemia	Normal lipids
	Chronic kidney disease	
	High-salt diet	Low-salt diet
	Smoking	No smoking
	Lack of physical activity	Intense physical activity
	Additional CV risk factors	
	Oxidative stress	Insensitivity to oxidative stress
		Strong protective metabolic mechanisms
	Alcohol consumption	None of these risk factors
	Chronic low-grade inflammation	
	Gut microbiome composition	
	Social deprivation	
	High perceived stress	
	Abnormal sleep pattern	
	Thrombogenic factors	
	Hormonal status	
	…	

This list is not exhaustive. Although subjects who have those characteristics are more likely to have SUPERNOVA, SUPERNOVA subjects do not need to fulfill all the features listed in the Table (Text). BP indicates blood pressure; CV, cardiovascular; EVA, early vascular aging; and SUPERNOVA, supernormal vascular aging.

be detailed because these factors are strongly interrelated and longitudinal data are available for analyzing trajectories. The Cardiovascular Risk in Young Finns Study[29] showed that metabolic syndrome in childhood and adolescence (aged 9–18 years) predicted the level of arterial stiffness (measured as cfPWV 21 years later, then aged 30–39 years). Moreover, recovery from childhood metabolic syndrome was associated with decreased arterial stiffness in adulthood.[29] These data are important because a long-lasting increased arterial stiffness predicts incident hypertension, which in turn increases arterial stiffness. This has been first showed in middle-aged adults (60 years) participating to the Framingham Heart Study[30] and more recently in younger normotensive Finnish adults (30–45 years).[31] In both cases, cfPWV improved the prediction of incident hypertension risk prediction beyond traditional cardiovascular risk factors.

The lack of vigorous physical activity is also a major determinant of arterial stiffness trajectory. Several studies have related sedentarity to arterial stiffness in adults, but, again, trajectories are even more important from childhood to adulthood because prevention measures can be undertaken at an early stage.[32–34] Finally, a few studies showed the influence of social aspects, such as occupational status, perceived stress, and social deprivation[35,36] (Table).

Biological aging in humans is a general phenomenon affecting all organs. This is why, for example, the Whitehall II Study has reported on associations between PWV and impaired lung function, slower gait speed, and less ability for physical functioning in everyday life.[37] On a more pathophysiological aspect, all cardiovascular determinants of EVA that are listed in the Table are also possible determinants of other organs aging.

From EVA to SUPERNOVA, Definition and Metrics

Defining extremes implies having references, and one must define arbitrary thresholds on continuous variables. In Figures 1 and 2, we used the wording average vascular aging to describe the median field between the 2 extremes of arterial stiffness distribution. However, an average vascular aging may not be the desirable goal for patients and their physicians in a population where cardiovascular risk factors are prevalent, and an ideal aging[8] or at least a healthy vascular aging (HVA)[38,39] would be preferable. For sake of clarity, we will successively discuss the differences between EVA and HVA and between SUPERNOVA and HVA.

Metrics

Arterial stiffness can be determined through 3 main approaches, by decreasing levels of physical relevance and epidemiological evidence: (1) measuring the time delay between 2 arterial sites and estimating the velocity of the pulse wave from the distance between sites divided by the time delay; (2) measuring the distension of the artery and relating it to the local pulsatile pressure; and (3) estimating arterial stiffness from cuff pressure measures through models of circulation.[7]

Measurement of PWV

Since the first reports by Bramwell and Hill in 1922,[40] cfPWV methods have improved by using high precision tonometers and computing. This method has been extensively validated, standardized, and referenced.[7,8] Alternative methods have been proposed by measuring transit time between the arm and the leg (brachial-ankle PWV), the heart and the leg (heart-ankle

PWV) or between the finger and the toe (finger-toe PWV). These techniques are simpler, at the cost of imprecisions and ambiguities on the arterial path.

Measurement of Local Stiffness

Measurement of local stiffness through distension and pulse pressure corresponds to the Hook law of elasticity and thus provides a direct measure. Local (carotid) pulse pressure is necessary to calculate the stress/strain relation and derive stiffness, which induces errors due to calibration and modeling.

Single Cuff–Based Methods Are Based on Models of Circulation

One method uses overinflation of the cuff to better track the late systolic peak (reflected wave) and measures the time delay between the early and late systolic peak as the propagation time of the wave to and from a putative main reflection site. A second method uses the diastolic decay of BP, calibrated by age and BP. Both show meaningful associations with cfPWV; however, they rely on models of the circulation that are hotly discussed. Further, external calibration on age and BP questions the added value. For sake of clarity, only cfPWV will be used in the following discussion.

From EVA to HVA

cfPWV is currently the most validated marker of vascular aging.[2,5,7,12] cfPWV increases with age, and an excessive increase in PWV at any age was associated with adverse outcome.[9,10,12,13] This has been demonstrated in hospital-based hypertensive cohorts and in the general population in many different settings and locations. Thus, a value of cfPWV above a certain threshold at a given age has appeared as a true definition of EVA. However, the ability to define a threshold above which the risk increases for a given age strongly depends on the structure of the cohort database. Observational cohorts are affected by many biases. In addition, the relation of cfPWV with age is not linear but rather quadratic (ie, accelerated increase), whereas its relation with BP is linear.[8] Thus, it is difficult to propose a single definition of EVA, and attempts that do not take age or BP into account have been oversimplistic[38,41] since fixed thresholds have been used (7.6 or 10 m/s, respectively). Having a cfPWV value <10 m/s may correspond to EVA in a young subject, whereas in older people, it may correspond to SUPERNOVA.

Thus, EVA can be more appropriately diagnosed in subjects who present an abnormally high cfPWV for their age and sex. For instance, in the population of the Guimaraes study, Cunha et al[4] used a variable threshold according to age categories and used different levels expressed as percentiles (75th, 90th, and 97.5th) of the cfPWV distribution in the Reference Values Collaboration study.[8] Although this definition takes into account age, it does also account for other risk factors, especially BP. Furthermore, because the relation with age is very strong, age has a remaining effect within age categories. Multivariate models taking into account nonlinearity and the continuity of risk factors have not been published to date.

In the MARE study (Metabolic Syndrome, Arteries Research), Nilsson et al[39] defined HVA as a cfPWV value below the age quintile–specific 10th percentile of the studied population and EVA as a PWV value above the age quintile–specific 90th percentile. However, these definitions were arbitrary and only used for research purpose. As discussed above, the wording average vascular aging can be used to qualify the median field between the 2 extremes of the PWV distribution (Figure 2), but no upper and lower limits of this median field have yet been defined, above which some subjects have EVA and below which others have HVA, respectively. No consensus has been reached, and we lack epidemiological studies comparing the predictive values of EVA and HVA according to various lower and upper thresholds, respectively, along the cfPWV distribution. This is why it is preferable to consider for the present time gray zones (Figure 2) for the boundaries between EVA, average vascular aging, and HVA along the distribution of cfPWV in populations for a given age. Indeed, the present article is addressing mainly the concept of vascular aging and aims at building a framework for research, rather than establishing rigid boundaries in a field of continuous variables.

Finally, although the definition of EVA and HVA based on percentiles is scientifically valid, this information is not easy to translate to the patients. To better explain it to the patient, some investigators have proposed the notion of vascular age. Although apparently intuitive, this is complex. Basically, this is based on the comparison of the actual risk of CVD of the subject, compared with the risk of CVD of a healthy age-matched person unexposed to cardiovascular risk factors. Thus, vascular age can only be older than chronological age because of cardiovascular risk factors. Several calculators are available on the internet for calculating vascular age. Even promoters[42] of the concept acknowledge that vascular age can be better approached through cfPWV, although this is limited to centers at which cfPWV measurements are available. Vascular aging calculated on risk factors and measured through cfPWV is not similar. Calculated vascular aging (ie, more accessible) evaluates the theoretical consequences of elevated (identified) risk factors, whereas the cfPWV (needing measurement) represents the actual consequences of risk factors on the cardiovascular system. As developed above,

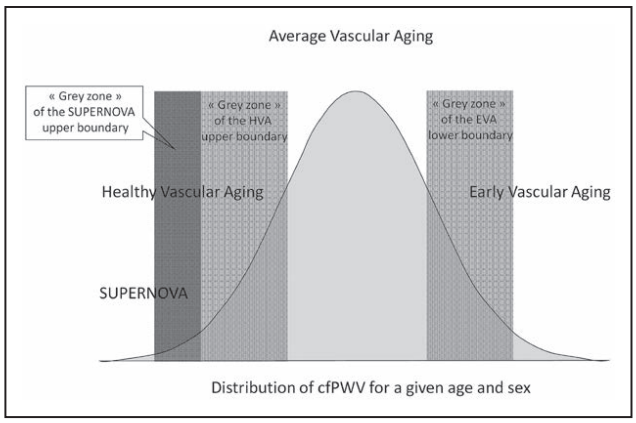

Figure 2. Schematic distribution of carotid-to-femoral pulse wave velocity (cfPWV; ie, arterial stiffness) within a given population. cfPWV values are presented for a given age and sex and according to an ideal gaussian distribution. Average vascular aging describes the median field between the 2 extremes of the pulse wave velocity distribution. The boundaries between early vascular aging (EVA), average vascular aging, healthy vascular aging (HVA), and supernormal vascular aging (SUPERNOVA) are presented as gray zones (Text).

patients are unequal against the consequences of risk factors on the cardiovascular system. Thus, vascular aging based on cardiovascular risk factor may fail at identifying people excessively sensitive (EVA) to or protected against (HVA) risk factors, whereas PWV represents the cumulative damage of all cardiovascular risk factors on the arterial wall.

Finally, we may ask whether PWV could be only calculated and not measured. Greve et al[43] showed that cfPWV calculated from age and BP conferred similar predictive values for major cardiovascular events than measured PWV. However, it does not mean that the incremental value of measuring cfPWV is null, especially because the influence of age is strong in epidemiological cohorts and vascular age aims at proposing information beyond chronological age.

From HVA to SUPERNOVA

Values of arterial stiffness are scattered at any given age for any level or category of risk factors. If attention has been focused on subjects with high cfPWV (EVA), low and very low values have drawn little attention. We propose that very low values of cfPWV, whatever the level of risk factors, define a protective phenotype, and we propose to call this phenotype SUPERNOVA. The cfPWV values of SUPERNOVA subjects are by definition lower than the cfPWV values of HVA subjects,[39] for instance, in the lower 2.5th percentile of the distribution. However, we currently prefer to consider, as discussed above, that the boundary between HVA and SUPERNOVA is rather a gray zone, as discussed above (Figure 2).

SUPERNOVA subjects are protected against the influence of cardiovascular risk factors, despite being exposed to them. The difference with EVA is that cardiovascular risk factors are not translated into subclinical organ damage and cardiovascular complications. This can be confirmed in clinical practice through simple investigations. SUPERNOVA subjects do not represent a clinical problem and should not necessarily be followed with the same intensity as EVA patients or even subjects with average vascular aging. They represent, on the contrary, a challenge for academic research to better understand pathways for vascular protection, and if any identified, consider it as a potential therapeutic target in the future.

One open analytical question is whether adjustments on cardiovascular risk factors should be extensive or not, to best select SUPERNOVA subjects among a large population. Some are easily accessible and must be taken into account (age, sex, BP, smoking), some are well known but more difficult to quantify (family history, perceived stress, socioeconomic factors for instance), and many remain unknown. In all cases (but age and genetics), the duration of exposure is difficult to quantify. The reference values that have been established for arterial stiffness according to age and BP[8] are of crucial importance because they provide the equations linking risk factors to arterial stiffness for calculating vascular age. The programming of such equations depends on coefficients obtained from reference value cohorts and must integrate nonlinearity and interaction terms, as it was done previously for calculation of cardiovascular risk through Systematic COronary Risk Evaluation equation.[44]

In conclusion, we consider that arterial stiffness (namely cfPWV) is the best proxy for the cumulative effect of known and unknown risk factors damaging the arterial wall, and we believe that it is preferable to express EVA and SUPERNOVA in term of very high or very low arterial stiffness.

Epidemiology of HVA and SUPERNOVA

In arterial research, recent observational studies have documented that some people seem to be protected from the age-associated increase in cfPWV and also from complications. This was described in the Framingham cohort, defined by low cfPWV for a given age and absence of hypertension, and called HVA.[38] It is believed that normal risk factor levels are essential to experience HVA, that is, regarding BP, glycemia, lipids, and lack of smoking, but most likely a favorable genetic profile and healthy lifestyle will contribute to this. The genetic influence could partly explain why a strong family history of cardiometabolic disease is associated with higher cfPWV in offspring, whereas the lack of such a family history seems to be protective.[45,46] Although genetic studies to map the genetic background of EVA are ongoing since at least 10 years,[15,47,48] no study on the genetic background of HVA is available based on Genome-Wide Association Study. In the Framingham Heart Study, a genetic risk score for coronary artery disease was not associated with HVA in that population.[38]

A more advanced concept than HVA could be SUPERNOVA proposed here, as tentatively defined by an exceptionally low cfPWV for age and sex. Investigating the epidemiology and characteristics of SUPERNOVA in a number of cohorts would help to identify genetic and epigenetic factors related to protective mechanism acting on the vasculature. This approach could have the potential to find new treatments, thus drug targets, for cardiovascular protection. This ambition is a reflection of the proposed strategy to modify vascular aging by lifestyle and treatment of risk factors, especially hypertension, as proposed by the Lancet Hypertension Commission 2016.[27]

The epidemiology of SUPERNOVA may benefit from observation of the few societies in the world that do not show any rise of BP with increasing age. Studies have shown that this was the case in subsistence-level populations, such as the Yanomamo Indians of Brazil, Papua New Guinean highlanders, and rural Kenyans, characterized by good physical aerobic fitness conditions, low body fat and low prevalence of obesity, low cholesterol level, low-salt diet, and high fruit and vegetable consumption.[49,50] These were indirect observations, and it was only recently that arterial stiffness was measured in representatives of these populations. Particularly, Lemogoum et al[51] measured cfPWV in Cameroon traditional pygmies on hunter-gatherer subsistence mode, contemporary pygmies who migrated to semi-urban area. They showed that hunter-gatherer lifestyle was associated with unchanged aortic stiffness with aging and attributed this finding at least partly to low weight and blunted effects of aging and BPs on arterial structure and function.

The determinants of SUPERNOVA can also be deduced from studies addressing the determinants of EVA. Although, as discussed above and below, the respective parts of genetics and epigenetics remain to be studied, SUPERNOVA determinants likely include normal BP, glycemia, weight, and lipids, low-calorie and low-salt diet, no smoking, intense physical activity, no perceived stress, no social deprivation, normal

sleep pattern, no thrombogenic factors, normal prenatal fetal growth, normal hormonal status, no chronic low-grade inflammation, insensitivity to oxidative stress, and strong protective metabolic mechanisms (Table). A word of caution should be introduced here. We do not suggest that subjects need to fulfill all the features listed in the Table to have SUPERNOVA but rather that patients who have those characteristics are more likely to have SUPERNOVA. Indeed, cumulative probabilities make the number of subjects in the general population fulfilling all criteria to be extremely low. In addition, according to the concept of protective mechanisms leading to SUPERNOVA, it is likely that SUPERNOVA subjects may fulfill only part of the criteria listed in the Table and are protected (or less responsive) against the deleterious effects of the other cardiovascular risk factors.

However, although the prevalent characteristics of subjects (in population-based studies) with both EVA and HVA have already been published in the Guimaraes and the MARE studies, respectively,[4,39] to our knowledge, no such data are currently available for SUPERNOVA subjects. The major reason is that selection of SUPERNOVA subjects in a given population is a difficult process. Selecting subjects with no cardiovascular risk factors cannot solve it because their comparison with patients with cardiovascular risk factors shows, by definition, a lower cfPWV. Solving this issue may need novel epidemiological and statistical approaches, to show that, despite several cardiovascular risk factors, SUPERNOVA subjects have a lower cfPWV than their chronological age and amount of cardiovascular risk factors would predict, thus that they are indeed protected against the deleterious effects of age and cardiovascular risk factors.

Molecular Basis and Mechanobiology of EVA and SUPERNOVA

While heritability studies and changes in the DNA sequence support a genetic predisposition to EVA,[46,52,53] accruing evidence suggests that reversible epigenetic mechanisms are master regulators of arterial stiffness, in concert with or independently of, the coding sequences. Modifications of epigenetic programming may represent the molecular cues to stave off oxidative stress and drive SUPERNOVA.

Epigenetic Determinants of PWV

Data from the Twins UK cohort reveal a new variant in the *CIB2* (calcium and integrin-binding protein-2) gene associated with hypomethylation of the promoter region, increased expression of the CIB2 protein regulating intracellular calcium and decreased PWV.[54] Increased levels of epigenetic regulators of DNA methylation states and histone modification in all vessel-wall layers appear to play a causal role in the sodium-induced increase in PWV in the stroke-prone Dahl salt-sensitive rats.[55] In nonhuman primates, the SirT1 (silent information regulator 1) activator resveratrol prevents the increase in PWV in response to Western diet. In aged mice, endothelium-specific overexpression of SirT1 epigenetically downregulates plasminogen activator inhibitor-1 transcription through decreased histone H4 lysine 16 acetylation on the promoter region, and this is accompanied by an improvement in endothelium-dependent relaxation and decreased PWV.[56] A plethora of miRNAs have been reported, either advancing or delaying the onset of arterial stiffening by targeting genes involved in TGF-β (transforming growth factor-β) or Ang II (angiotensin II) signaling, metalloproteinase activities, adhesion dynamics, vascular smooth muscle cell (VSMC) plasticity, and functions.[57]

Epigenetic Determinants of VSMC Plasticity

The contribution of VSMC to arterial stiffening is a novel aspect of mechanobiology.[15] In addition to extracellular matrix that includes proteins supporting the mechanical load, VSMC plasticity plays a major role in arterial stiffening, not only through the regulation of actomyosin interactions for contraction but also by mediating the mechanotransduction in cell-extracellular matrix homeostasis. VSMC plasticity and signaling in both conductance and resistance arteries are highly relevant to the physiology of normal and early in vascular aging.

It is tantalizing to postulate that epigenetic mechanisms are master regulators of SUPERNOVA through the regulation of VSMC phenotype. The contribution of epigenetic processes to the high degree of plasticity of VSMCs has been clearly established. The KLF4 (Krüppel-like factor 4) transcription factor and phosphorylated ETS-like transcription factor 1 cooperatively recruit HDAC (histone deacetylase) 2 to epigenetically silence the gene encoding the smooth muscle cell differentiation marker SM22α (smooth muscle specific SM22alpha protein). Migration of differentiated VSMCs from the media layer into the adventitia generates resident pluripotent progenitor cells.[58] The maintenance of the progenitor phenotype is dependent on KLF4, and progenitor cells are characterized by loss of the H4Ac chromatin mark and retention of the H3K4Me2 mark. This demonstration suggests that this physiological in situ VSMC reprogramming could be critical in SUPERNOVA.

Age-associated hypomethylation of the microRNA-203 promoter causes microRNA-203 overexpression leading to a decrease in focal adhesion signaling proteins, which, in turn, increases VSMC stiffness.[59] DNA hypermethylation of a genetic variant in the *COL15A1* (collagen, type XV, α1) gene is associated with a decrease in protein expression of COL15A1 and an increase in susceptibility to atherosclerosis.[60] The identification of COL15A1 as a molecule impacting the phenotypic state of VSMCs points to mechanisms at the intersection between genetic components and epigenetic marks that could occur in SUPERNOVA.

Epigenetic Demonstration—Aneurysms and Progeria

Several lines of evidence suggest that epigenetic scenarios regulating VSMC plasticity operate in vascular diseases. The *Uhrf1* (ubiquitin-like containing plant homeodomain and really interesting new gene finger domains 1) gene under the negative control of microRNA-145 triggers smooth muscle cell proliferation.[61] The mechanisms are the repression of cell-cycle inhibitor genes and prodifferentiation genes via the methylation of DNA and histones. The *Uhrf1* mRNA level is upregulated in abdominal aortic aneurysm tissues, and specific inhibition of UHRF1 (ubiquitin-like, containing PHD and RING finger domains, 1) in experimental models has been shown to reduce the severity of aneurysms. In thoracic aneurysmal VSMCs,

a switch from the repressive effect of Myc to p53 binding to the *SMAD2* (small worm phenotype and drosophila mothers against decapentaplegic) promoter triggers constitutive Smad2 activation.[62] The acetylation of H3K9/14 and p53 by the histone acetyl-transferases p300/CBP-associated factor plays a causal role in p53 recruitment and activation.

Nuclear defects are associated with loss of heterochromatin and premature senescence in progeria. In Apoe (apolipoprotein E)−/− mice, the induction of progerin expression specifically in VSMCs but not in macrophages is sufficient to reproduce the vascular phenotype observed in progeroid model with a disappearance of VSMCs, lipid accumulation, and intimal thickening.[63] The consequence is an elevation in cfPWV above the highest mean normal values in progeria children.[64] The vasculopathy predominates in the ascending aorta in which VSMCs are exposed to high levels of shear stress. Reprogramming fibroblasts from progeria patients into iPSCs restored normal levels of H3K4me3 and H3K27me3 associated with gene promoters.[65] Differential methylations outside of the promoters in iPSCs, which do not impact gene expression, could explain the reappearance of nuclear defects upon differentiation into VSMCs.

Silencing or negative regulation of these scenarios could at the opposite drive supernormal vascular health. Emphasis should be placed on an integrated overview of the exquisite complex mechanisms regulated by epigenetics to understand how they foster optimal mechanobiology and limit vascular diseases.

Animal Model of SUPERNOVA—the Naked Mole Rat

Insights into the mechanisms governing healthy aging or SUPERNOVA could be gleaned using the naked mole rat (NMR). NMRs appear to withstand age-related vascular changes because they display no significant increases in central BP with age and no arterial stiffening as measured by PWV.[66] Current evidence points to a crucial role of Nrf2 (nuclear factor erythroid 2–related factor)—a transcription factor driving the expression of antioxidant genes—to offer NMRs high levels of stress resistance and extended health span.[67] Nrf2 cytoprotective signaling in NMRs is thought to depend on low levels of 2 negative regulators, Kelch-like ECH-associated protein 1 and β-transducin repeat–containing protein, that promote its proteasomal degradation. The glucagon-like peptide-1 analog exendin-4 may also contribute to the redox homeostasis by reducing the nucleocytoplasmic shuttling of Nrf2 through its acetylation, thus increasing its transcriptional activity and decreasing VSMC senescence.[68] Localized disturbed blood flow has emerged as a master regulator of the association of Nrf2 with HDAC1/2/3 leading to deacetylation of Nrf2 and sensitizing the endothelium to oxidative stress.[69] Additional potential mechanisms holding off vascular aging and VSMC senescence in NMRs include increased levels of high molecular mass hyaluronan and differentially expressed long noncoding RNAs among which several are coexpressed with HA-related genes.[70]

The consensus that all epigenetic factors exert protective or deleterious regulation of VSMC functions opens up a unique approach to gain a molecular and cellular understanding of the mechanisms that halt or even reverse the onset of vascular diseases.

New Therapeutic Options

New treatment options for arteriosclerosis differ whenever we use cfPWV as a risk marker or as an intermediate end point for cardiovascular events. They are further conditioned by the presence of hypertension or diabetes mellitus or not. In hypertensive subjects, direct treatment of BP and other cardiovascular risk factors with specific drugs will have consequences on the reduction of cfPWV. Scarce evidence supports that using cfPWV reduction as a treatment target would produce added reduction of major cardiovascular events, but a growing body of evidence shows that subjects with reduced cfPWV, either untreated or after treatment for several cardiovascular risk factors, have reduced risk of developing cardiovascular events.

By contrast, for the nonhypertensive/nondiabetic subjects with high cfPWV/EVA, there is currently no evidence to support the use of traditional drugs with effect on the vascular or the metabolic system (antihypertensive/antidiabetic drugs), to reduce cfPWV. The evidence-based options are limited to changes in lifestyle (core to improvement of all arterial function measures), but new targets and strategies look promising for future aggressive and guided therapy in these patients who are at high risk of developing CVD at a younger age.[27]

The arterial system has a set of different cross talks between the microcirculation and macrocirculation on one hand and between endothelial cells, extracellular matrix, and VSMC stiffness[15] on the other hand. Any improvement on one end of the system has repercussions on the other end. Some of the evidences presented below address the benefit of interventions at a cellular level to control large artery damage.

Lifestyle and Drugs

Regular exercise practice (especially aerobic exercise) reduces arterial stiffness[71,72] through increase of endothelial NO availability and decreased vascular chronic low-grade inflammation. A meta-analysis of randomized control trials[73] shows that aerobic exercise reduces arterial stiffness in subjects with CVD.

The effect of reducing salt consumption (with short-term interventions) on arterial stiffness was reviewed in a meta-analysis of 11 randomized clinial trials with a crossover design[61] registering that an average reduction of 5.2 g of daily salt consumption would independently decrease PWV by an average 2.8%. Thus, a major reduction in salt consumption would result in a limited reduction in cfPWV and possibly cardiovascular events.

Dietary caloric restriction (CR) is another interesting strategy. Several animal models were studied with CR, showing increased life span and decreased CVD development through improvement of endothelial and cardiac functions, angiogenic capacity, and prevention of arterial stiffness.[74,75] In the absence of a uniform definition of CR, different groups used alternative settings (reduction of 10% to 40% of usual caloric intake or even intermittent fasting). The benefit of CR has been associated to the activation of cellular endogenous antioxidative and anti-inflammatory mechanisms preserving a younger phenotype at the vascular cellular level,[74] which emphasize the implications of the cross talk between endothelial cell stiffness, arterial regional stiffness, and large

artery stiffness.[15,52] Four main mechanisms mediate these effects of CR[76,77]:

1. Sirtuin activation[78]: Sirt1 has antioxidant activity, is associated with decreased circulating levels of insulin and cholesterol, and has an anti-inflammatory role. Sirtuins downregulate the expression of Ang II type 1 receptors.[79]
2. Nrf2 activation: increasing the transcription of antioxidant proteins.
3. mechanistic target of rapamycin inhibition: regulating the timing and spatial cellular growth.
4. AMPK (AMP-activated protein kinase) activation: an energy-sensing kinase, promoting increased glucose uptake and enhanced fatty acid oxidation.[80]

CR has also been studied in human[81] in the CALERIE trials (Comprehensive Assessment of Long-Term Effects of Reducing Intake of Energy). A second-phase trial enrolled 220 healthy nonobese subjects to observe the effects of a 25% reduction in caloric intake for 2 years. In the CALERIE-2 trial, obviating the difficulty to maintain compliance with a constant 25% reduction in calorie consumption, the real calorie reductions were of 19.5% in the first 6 months and 9.1% in the following 18 months.[81] Still, the results are significant. CR subjects safely tolerated a decrease in body weight, total daily energy expenditure, and inflammatory markers.[82] In addition, CR subjects had an 8-fold reduction of urinary F2-isoprostanes—an index of accumulated oxidative injury—associated to reduction of leptin concentration and improved insulin sensitivity.[83] In a smaller sample size (n=53, with a strict compliance to the 25% CR),[84] overall improvement in different risk factors was reported: decrease in adiposity measures, BP, lipid profile, and a 30% decrease in the 10-year risk of CVD. Subjects maintained aerobic capacity and strength, exercising longer on the treadmill.[85] Although the CALERIE studies did not include a direct measure of arterial function, they demonstrated for the first time in humans that the use of CR has a direct influence in the same mechanisms that influence arterial damage and could lead to a 30% reduction of cardiovascular risk at the end of the 2-year intervention.

New Antidiabetic Drugs With Effects on Arterial Function and Cardiovascular Risk

The benefit of using specific drug classes that exert an additional effect on arterial function (beyond and independently of BP reduction) is well known and documented, especially for renin-angiotensin-aldosterone blockers.[86,87] The interesting aspect of these observations is the increased knowledge of the effect of Ang II blockers at a cellular level, keeping with the abovementioned topic of large artery preservation through vascular cellular homeostasis.

Blocking of Ang II activity especially in its ACE (angiotensin converting enzyme)-Ang II-AT1R (angiotensin II receptor type 1 axis) downregulates the proliferative, pressor, and fibrotic effect of Ang II; moreover, increased knowledge of the effect of Ang II on mechanistic target of rapamycin activity (mechanistic target of rapamycin-renin angiotensin system connection), radical oxygen species generation, and mitochondrial function has been shedding light on the protective effect of renin-angiotensin-aldosterone blockers.[79]

With the publication of the EMPA-REG (Empagliflozin Cardiovascular Outcome Event Trial in Type 2 Diabetes Mellitus Patients-Removing Excess Glucose),[88] LEADER (Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome Results),[89] SUSTAIN-6 (Trial to Evaluate Cardiovascular and Other Long-term Outcomes with Semaglutide in Subjects with Type 2 Diabetes),[90] and ELIXA (Evaluation of Cardiovascular Outcomes in Patients With Type 2 Diabetes After Acute Coronary Syndrome During Treatment With AVE0010 [Lixisenatide]) studies,[91] GLP-1 (glucagon-like peptide-1) receptor antagonists and SGLT2 (sodium/glucose cotransporter 2) inhibitors have been added to the therapeutic armamentarium of diabetes mellitus, with a particular characteristic: added cardiovascular protection beyond glycemic control. The benefit could be achieved through a hemodynamic/antiatherosclerotic mechanism,[92] but other properties of these drugs could offer explanations to the reduction of CVD, through improvement of endothelial function, reduced oxidative stress, and improved large artery distensibility.[93–95] Particularly, a recent meta-analysis[96] on the effects of antidiabetic drugs on cfPWV showed that GLP-1 receptor antagonists have an effect on PWV reduction, whereas SGLT2 inhibitors reduce forearm-mediated dilation.

Finally, compound 21—a selective Ang II (AT2 [angiotensin II receptor type 2]) receptor agonist[97]—is an interesting novel drug. Compound 21 upregulates the second arm of the renin-angiotensin-aldosterone system—the Ang 1–7 (angiotensin 1–7)-ACE2-Mas axis—mediating antiproliferative, vasodilator, antifibrotic, and antithrombotic effects.[79] In animal studies, it was shown to reduce PWV (without lowering BP) and to prevent endothelial inflammation.[98]

Research Challenges

Models of extremes could be used to characterize escapers of disease risk and SUPERNOVA subjects. This could facilitate the understanding of clinical protection and identify novel drug targets. Extremes should be examined according to family and medical history, body functions, genetic mapping (Genome-Wide Association Study, exome sequencing, and epigenetics), and detailed vascular and metabolic phenotyping. If potential protective mechanisms are identified, the next step should be to intervene on these. Some genetic variants of harmful traits are nonfunctional and associated with disease protection (eg, nonfunctional variants of PCSK-9 [proprotein convertase subtilisin/kexin type 9]). If a genetic marker of vascular aging (elevated PWV) can be defined and validated, one could look for nonfunctional variants of this marker in subjects and examine their vascular function.

Developing a randomized clinical trial for demonstrating the reversal of EVA or the promotion of SUPERNOVA is challenging. An example could be CR as an intervention and arterial function measurements as outcomes. Another challenge includes developing a randomized clinical trial where CR is an intervention and arterial function measurements are outcomes, including molecular cellular pathway research. Obvious candidates to novel treatments targeting vascular aging currently under investigation (due to its core role in cellular or vascular homeostasis maintenance) are sirtuin activators (STACs). Resveratrol (a CR mimetic) is the most well-studied STAC. A recent review[99] has exposed the heterogeneity of study methodologies; the role of supplementation with resveratrol seems to

be more effective in subjects with diabetes mellitus and hypertension, rather than as a preventive measure, and associations with improvement of arterial function indexes are reported.[100] Novel strategies are proposed using pan-sirtuin activators (increasing nicotinamide adenine dinucleotide+ levels; eg, exercise). Selective mechanistic target of rapamycin inhibition, AMPK activation (metformin), Nrf2 activation, and p66Sch inhibition[101] are intervention targets that could preserve cardiovascular health in humans.

Because of the importance of radical oxygen species and oxidative stress in vascular aging and CVD development, antioxidant supplementation could be strategic; yet, contradictory results have shown reduction of radical oxygen species on one hand and no effect on cardiovascular end points and even an increase in cardiovascular mortality on the other hand.[71,94]

Conclusions

In conclusion, we have addressed in this review the concept of extremes in cardiometabolic research and discussed the molecular bases and mechanobiology of EVA and SUPERNOVA, in parallel with their epidemiological, genetic, and epigenetic determinants. We suggest that arterial stiffness should be more often measured in clinical practice to determine vascular aging and use the appropriate therapeutic options through an integrated approach for cardiovascular protection. Finally, we insist on the need for discovering novel molecular targets for slowing arterial aging and protecting against cardiovascular complications.

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