Extension of the mitochondria dysfunction hypothesis of metabolic syndrome to atherosclerosis with emphasis on the endocrine-disrupting chemicals and biophysical laws

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ABSTRACT

Metabolic syndrome and its component phenotypes, hyperglycemia, hypertension, (abdominal) obesity and hypertriglyceridemia, are major risk factors for atherosclerosis. Recently, associations between exposure to endocrine-disrupting chemicals (EDCs), mitochondrial dysfunction, metabolic syndrome and atherosclerosis have been established, suggesting a possible common mechanism underlying these phenomena. Extending a previously proposed mitochondria dysfunction theory of metabolic syndrome and using biophysical laws, such as metabolic scaling, Murray's law and fractal geometry of the vascular branching system, we propose that atherosclerosis could be explained as an ill-adaptive change occurring in nutrient-supplying arteries in response to the decreasing tissue energy demand caused by tissue mitochondrial dysfunction. Various aspects of this new hypothesis are discussed. (J Diabetes Invest, doi: 10.1111/jdi.12048, 2013)

KEY WORDS: Atherosclerosis, Endocrine-disrupting chemicals, Mitochondrial dysfunction

INTRODUCTION

There is increasing awareness that endocrine-disrupting chemicals (EDCs) are involved in the pathogenesis of metabolic syndrome, type 2 diabetes and related conditions[1–4]. As metabolic syndrome is a constellation of risk factors for atherosclerosis, namely hyperglycemia, hypertension, (abdominal) obesity, hypertriglyceridemia and other phenotypes frequently associated with atherosclerosis, exposure to EDCs is expected to cause atherosclerosis (Figure 1).

Persistent organic pollutants (POPs) are a most important group of EDCs, which persist in our environment, bioaccumulate in the body, and exert hazardous effects on humans and animals by disrupting the endocrine system. They are key chemicals designated among EDCs by the Stockholm Convention, a United Nations organization. Among the toxic effects of EDCs, mitochondrial toxicity is well established[5,6]. We suggest here that it could explain various aspects of atherosclerosis. Central to this thesis is that mitochondrial dysfunction would decrease energy (and heat) production of the body and lower its temperature, which in turn makes the body increase its mass to maintain an optimal core temperature[7]. This mechanism is believed to be necessary, because core temperature will no longer be optimal if there is no adaptive change in heat production per mass, according to body mass change. It is a reason why there is a natural law between the body mass and its unit mitochondrial function in the animal kingdom, the metabolic scaling law; the bigger the animal, the smaller the mass-specific mitochondrial function is[8,9].

A change in body mass would also require remodeling of vasculature in an adaptive response. The heart is special in that its high energy need is primarily derived almost exclusively from oxidative phosphorylation in mitochondria, with <5% of adenosine triphosphate (ATP) production coming from the glycolytic pathway[10]. Because of this dependence, increases of cardiac activity are matched with almost instantaneous parallel increases of oxygen availability. Changes in myocardial work and, thus, in energy demand, are accompanied by proportionate changes in coronary and, thus, myocardial blood flow. Decreased metabolism of an organ (because of decreased mitochondrial function), if it persists, could lead to the remodeling or narrowing in supplying arteries; atherosclerosis in coronary arteries in the case of the heart, and carotid arteries in the case of the brain.

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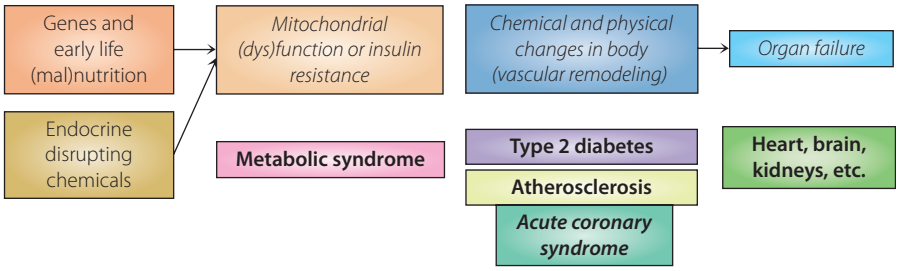

Figure 1 | General outline of mitochondria-based model. Genes, early life nutrition state and exposure to endocrine-disrupting chemicals are causes of various disease states (shown in bold), such as metabolic syndrome. Mitochondrial dysfunction changes insulin sensitivity, and chemical and physical states of the body, including vascular remodeling or atherosclerosis. In contrast to the conventional view that emphasizes the narrowing of coronary arteries by atherosclerotic plaque or coronary microvascular dysfunction causes myocardial ischemia, followed by ischemic heart failure, our model emphasizes that organ failure is the end-stage of mitochondrial dysfunction.

We will show the evidence linking (EDCs induced) mitochondrial dysfunction and many biochemical features described in atherosclerosis, and also the recent conceptual advances made around biophysics, particularly the metabolic scaling, and fractal physiology could be applied as well.

EVIDENCE SHOWING THE RELATIONSHIP BETWEEN POPS, MITOCHONDRIAL DYSFUNCTION, METABOLIC SYNDROME AND ATHEROSCLEROSIS

Evidence supporting that exposure to EDCs including POPs leads to mitochondrial dysfunction, metabolic syndrome and atherosclerosis is increasing. For example;

1. Exposure to POPs induces mitochondrial dysfunction, such as mitochondrial DNA (mtDNA) depletion and damage to mtDNA and the genome[5,6,11–13].
2. Mitochondrial dysfunction is associated with diabetes and metabolic syndrome[14–16].
3. Serum levels of POPs are positively associated with diabetes, insulin resistance, metabolic syndrome and atherosclerosis[3,4,17,18].
4. Exposure to POPs induces insulin resistance in cells and animals[5,6,12,13,19].
5. Exposure to EDCs leads to (abdominal) obesity, enhanced inflammatory response and atherosclerosis[5,12,20].
6. Mitochondrial dysfunction causes an increase in pro-inflammatory signals and reactive oxygen species (ROS), and a decrease in endothelial nitric oxide (NO) availability[21–24].
7. Exposure to dioxin leads to mtDNA depletion[25], metabolic syndrome, hypercholesterolemia and atherosclerosis in animal studies[6,12].
8. Some EDCs, such as phthalates and polychlorinated biphenyls (PCBs), are linked directly to human atherosclerosis[26,27], and lipid extract from human atheroma has been shown to exert a toxic effect on cultured monocytes[28].

However, these evidences are not included in current theories on atherosclerosis, such as the cholesterol hypothesis.

WEAKNESS IN THE CURRENT EXPLANATIONS ON THE PATHOGENESIS OF ATHEROSCLEROSIS

Does Atheromatous Plaque Narrow the Arteries and Reduce Blood Flow?

Currently, atherosclerosis is considered to be a disease in which plaque inside the arteries limits the flow of oxygen-rich blood to the organs and other parts of the body, which can lead to heart attack and stroke. Therefore, if the coronary artery is narrowed, the myocardial territory supplied by the diseased vessel will become ischemic, resulting in regional contractile dysfunction[29,30]. However, it is difficult to explain so-called coronary microvascular dysfunction (CMVD), which is diagnosed clinically. In this disease, no apparent plaques are found in a coronary artery in an imaging study. In short, there is uncertainty around the concept that atherosclerotic artery obstructs blood flow, resulting in myocardial ischemia (obstruction-ischemia theory). This chronic state should be differentiated from acute coronary syndrome, which develops when an atheroma in the coronary artery develops thrombosis and obstructs blood flow. These concepts are clearly shown in Figure 2.

Response to Injury Hypothesis

Among the hypotheses on the pathogenesis of atherosclerosis[31], the most widely held view is the 'response to injury' hypothesis of Ross[32], or its modifications. In this hypothesis, atherosclerosis is considered to be a result of a decades-long inflammatory response process to endothelial injury, which stimulates migration and proliferation of smooth-muscle cells that become intermixed with the area of inflammation. Various, possibly different, forms of insult might develop between the lining endothelium and the underlying cells of the artery wall. Ross explained that in hyperlipidemic individuals, injury inducing agents seem to be due principally to the lipids and lipoproteins associated with hyperlipidemia, whereas they might also result from molecules yet to be identified with cigarette smoking, hypertension, diabetes, or possibly even some infectious agents. EDCs fit in with the characteristics of the injury causing agent (s) Ross described very well.

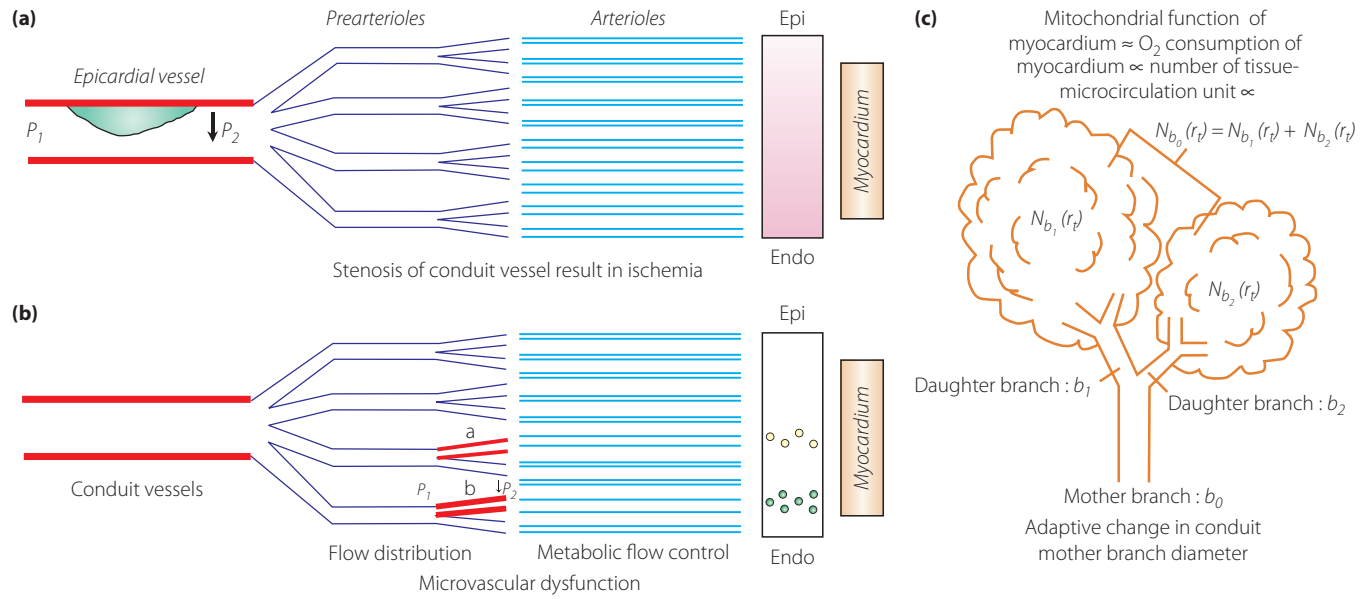

Figure 2 | Comparison of current arterial obstruction-ischemia concept of atherosclerosis, as shown in (a) and (b) (adapted from Lanza *et al.*[29] with a permission from Wolters Kluwer Health) and (c) our concept. In the stenosis of (a) epicardial (Epi) artery, the territory supplied (gray area) results in regional contractile dysfunction (P1 and P2 indicate blood pressure proximal and distal to obstructive vessels), whereas in the case of (b) microvascular alterations, myocardial ischemia is considered localized only in small myocardial areas (small circles; a and b indicate dysfunctional microvessels). In these concepts, contractile abnormalities are results. (c) Our energy demand-supply model (adapted from conceptual framework of Kamiya and Takahashi[78] with a permission from The American Physiological Society); if the sum of tissue-microcirculation units, as described here as Nb_0 (rt), decrease, the diameters of daughter branches and the mother branch have to decrease. In other words, if metabolic demand of myocardium decreases (because of mitochondrial dysfunction), its blood supply would decrease, therefore, the diameter of supplying conduit vessel decreases adaptively. Endo, endocardial.

NEED FOR A NEW HYPOTHESIS, AS ATHEROSCLEROTIC PROCESSES ARE NOT EXPLAINED WELL AND THE CHOLESTEROL HYPOTHESIS IS ONLY A PART

Injurious Agent is Unknown

Ross[32] developed his theory from the histological similarity observed between the atherosclerotic lesions and the intimal response to injury, which has been widely accepted[33]. Frink suspected that the 'injurious agent' (any agent or any process, singly or in combination) enters the wall and injures or stimulates resident vascular smooth muscle cells (VSMCs)[34]. It is part of our hypothesis that EDCs can injure the vascular wall (thus induce inflammation) and initiate fatty streaks, as shown in an animal experiment[20], as well as the whole body. Indeed, a recent study found human atherosclerotic plaque lipid extract contains substances that impair the anti-oxidant defense capacity of monocytes[28]. Those cells exposed to lipid extract showed a significant increase in the ROS level with a simultaneous rise of glutathione oxidation. A significant decrease in the intracellular anti-oxidant enzyme activity of catalase, glutathione peroxidases and thioredoxin reductases was observed, along with an increase in intracellular superoxide dismutase activity, suggesting endogenous H_2O_2 overproduction. Those kinds of responses are very similar to the responses the cells show when they are exposed to EDCs, such as dioxin, bisphenol A or phthlates[7,11,35,36]. Here, we argue the response to injury hypothesis could be a better theory if it was modified to accept EDCs as injury agents.

Endothelial Dysfunction Occurring Long Before the Appearance of Atherosclerosis is Usually not Considered

The pathophysiological changes of atherosclerosis occur long before the occurrence of structural changes, such as intimal hyperplasia. Offspring born to nutritionally deprived mothers are predisposed to metabolic syndrome, and manifest endothelial dysfunction very early in life. Diminished levels of bioavailable NO are regarded as one of the hallmarks of endothelial dysfunction, and occur through several different mechanisms, such as reduced endothelial NO synthase (eNOS) expression levels, reduced eNOS enzymatic activity and reduced NO bioavailability[24]. Endothelial dysfunction is also associated with an increase in ROS production in the vasculature. This abnormality is difficult to explain with current theories, but could be explained easily with a mitochondria-based model. We have presented evidence that the thrifty phenotype could be explained in terms of functional changes in mitochondrial unit function[37].

Vascular Remodeling is not Explained

Atheroma development is compensated for by gradual dilation of the artery, or 'remodeling', so that the lumen remains unaltered up to a point. In 1987, Glagov *et al.*[38] studied left main coronary arteries obtained at autopsy to evaluate whether atherosclerotic human coronary arteries enlarge in relation to plaque development. They found the internal elastic lamina area (representing the whole coronary artery, including the atherosclerotic area) correlates directly with the area of the atherosclerotic lesion, suggesting that coronary arteries enlarge as the lesion area increases. The lumen area does not decrease until a certain level is reached (lesion area/internal elastic lamina area $\times 100$ over 40%; Figure 3a).

Over the years, intensive studies on arterial remodeling have shown that despite comparable diffuse disease and arteriographic stenosis, functional reserve of the diseased segment might only be mildly reduced with remodeling. Readers are referred to a recent review by Kern and Samady[39] for further details on this very complex issue.

Why should gradual dilation of the artery or remodeling occur, if narrowing of the artery is the primary process? Regional myocardial blood flow studies carried out with several new techniques, including positron emission tomography, suggested alternative explanations consistent with the mitochondria-based model. This technology provided evidence that functional, rather than structural, disturbances might reflect more accurately the risks of developing coronary artery disease (Figure 3b)[40]. Furthermore, new technology showed the dissociation between the anatomical and functional measures of coronary stenosis severity as a result of diffuse atherosclerosis, and the extent of arterial remodeling. These observations are clearly against the concept that narrowing of the artery by atherosclerosis leads to functional derangement of downstream tissue; that is, myocardium. These findings are well matched with the age-dependent decline of whole-body mitochondrial function, as shown in Figure 3c.

Revascularization Therapy Does not Improve Prognosis

In a controlled trial, surgery to open a complete blockage in one of two carotid arteries did not prevent subsequent strokes[41]. Another clinical trial with a stent to open blocked arteries (by atherosclerosis) deep in the brain was also proven ineffective[42]. However, cerebral revascularization, either direct or indirect, was proven to be quite effective and sometimes recovered the lost brain function induced by ischemia in a disease called moyamoya disease, which is a chronic steno-occlusive vasculopathy involving the distal supraclinoid internal carotid arteries[43]. Why is bypass surgery ineffective in atherosclerotic brain disease, while it is effective in moyamoya disease?

For coronary heart disease, many studies have also failed to show a benefit of opening a narrowed coronary artery, except in acute coronary syndrome with significant functional reserve[44]. For example, a randomized trial involving 2,287

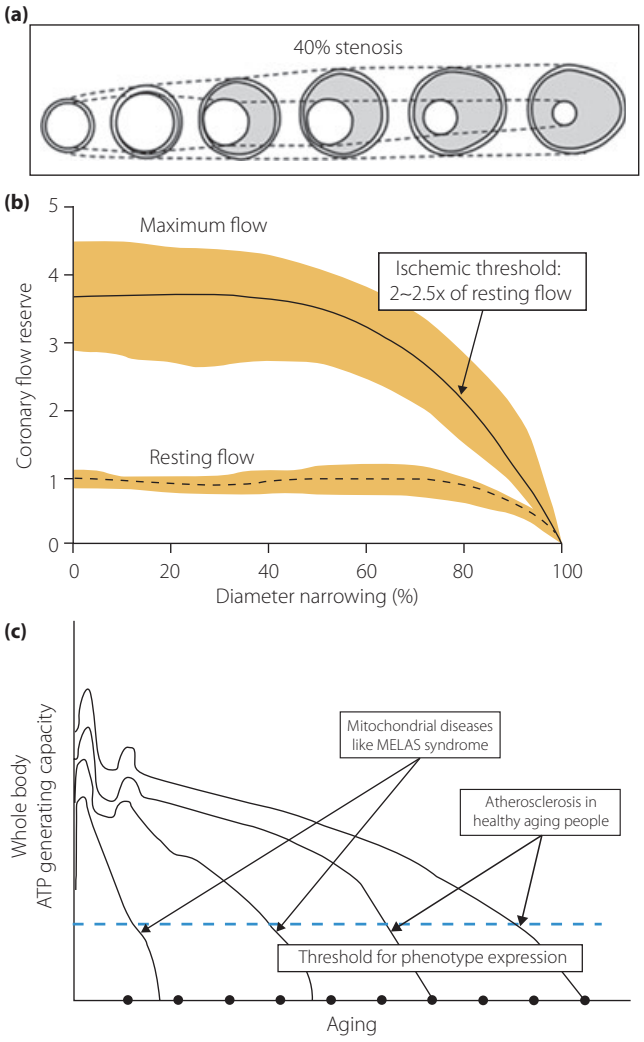

Figure 3 | (a) The sequence of adaptive change or remodeling of atherosclerotic arteries. The artery enlarges initially in association with plaque accumulation to maintain the lumen area. When the lumen reaches more than 40% stenosis, the artery is believed to no longer enlarge at a rate sufficient to prevent narrowing of the lumen (adapted from Glagov *et al.*[38] with permission from Massachusetts Medical Society). (b) However, resting coronary blood flow is maintained until arterial lumen is narrowed to approximately 80% (adapted from Gould[40] with permission from American College of Cardiology Foundation). Coronary blood flow reserve (<2–2.5 times baseline value) is believed to be more important than the degree of narrowing itself for the manifestation of ischemic symptom. Most importantly, the figure shows the relentless decline of coronary artery narrowing along the aging. (c) Adenosine triphosphate (ATP) generating capacity of body decline along with aging. If it declines below a certain threshold, clinical phenotype(s) will manifest (adapted and modified from Shoffner and Wallace[102]). In our hypothesis, ischemic heart disease occurs when coronary blood flow reaches this threshold as a result of the decreasing bioenergetic function of the myocardium, in parallel with the decline of whole-body metabolism.

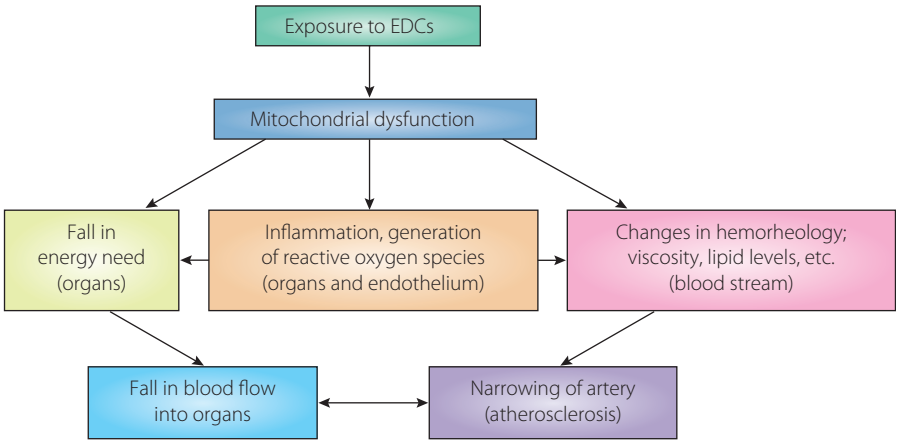

Figure 4 | Various aspects of atherosclerosis are explained by the endocrine-disrupting chemical (EDC)-induced mitochondrial dysfunction. Atherosclerosis is ill-adaptive changes (remodeling) occurring in arteries to reduce the blood supply as a response to decreased nutrient demand of organs, and blood supply and demand is in a balanced state. Exposure to EDCs, which are regarded as injurious agents, cause mitochondrial dysfunction, inflammatory response and increase reactive oxygen species production in the endothelium and tissue. Blood itself will be changed in its property; rheology, viscosity, and lipid components and levels.

patients with coronary artery disease at 50 USA and Canadian centers showed that percutaneous coronary intervention did not reduce the risk of death, myocardial infarction or other major cardiovascular events. After an average follow up of 4.5 years, the two groups were remarkably similar[45].

Simple opening of stenotic coronary arteries will not affect the natural history of atherosclerotic heart disease, which will take a downhill course relentlessly, which is similar to the decline of the whole-body bioenergetic function of the ageing process (Figure 4). Therefore, the preferred treatment for patients with stable coronary artery disease is the best available medical therapy.

ALTERNATIVE EXPLANATION FOR ATHEROSCLEROSIS: LINKING POPS EXPOSURE, MITOCHONDRIAL DAMAGE AND BIOCHEMICAL CHANGES OBSERVED DURING ATHEROSCLEROSIS

Toxin-Induced Mitochondrial Dysfunction Could Explain Vascular Inflammation

Persistent organic pollutants, such as dioxins, damage mitochondria and induce insulin resistance, metabolic syndrome and atherosclerosis. It is part of our hypothesis that these toxins can injure the vascular wall (thus induce inflammation) and initiate fatty streaks, as shown in an animal experiment[20].

The importance of the role of mitochondria in this process has become clearer in recent years. For example, Manfredi and Rovere-Querini[23] suggested that mitochondria could be the primary organelle involved in initiating inflammation. The toxic effects of dioxin and other pollutants on mitochondrion are well established. For example, Chen *et al.*[11] showed that one of most important POPs, dioxin, damages mitochondria and induces cells to release free radicals. Once mitochondria are damaged, they release additional ROS, which causes further damage in a vicious cycle. The mitochondria theory of aging is closely linked to the free radical theory of aging, with long history[21,46,47], although this issue is far from settled. West and Bergman[48] discussed the mechanism of aging from a systems biology framework, which is basically an extension of metabolic scaling law[9,49]. We want to emphasize that those points are incorporated into our mitochondria-based model[37].

Endothelial cells with damaged mitochondria release damage-associated molecular pattern molecules (including high-mobility group protein B1, heat shock proteins and S100 proteins), which recruit inflammatory cells such as macrophages. Thus, POPs could very likely induce inflammation in the endothelium, as seen in fatty streaks (a feature of atherosclerosis).

Other mitochondrial proteins activate formyl peptide receptor-1 on neutrophils, resulting in the production of a collagenase that sustains leukocyte migration in peripheral tissues. Furthermore, intravenous injection of mitochondrial proteins into mice activates circulating neutrophils, with random extravasation at peripheral organs, such as the liver and lungs[50]. These results show that mitochondrion is a critical instigator of inflammation during the atherosclerotic process.

C1C12 myoblasts that are mtDNA-depleted secrete increased amounts of interleukin-6 and other inflammation mediators, as well as being resistant to insulin stimulation[13]. Another mtDNA-depleted cell line became insulin-resistant[19]. These findings clearly suggested that mitochondrial dysfunction is at a crossroads between insulin resistance and the inflammatory response, at least at the cell level.

Mitochondrial Dysfunction can Explain Increased Endothelial Cell ROS Production in Atherosclerosis

Endothelial cells depend heavily on the glycolytic pathway for ATP production and use very little oxidative phosphorylation. However, they contain mitochondrion, which is critically

involved in maintaining the fine regulatory balance for calcium handling, ROS production and NO metabolism, which play important roles in controlling microcirculation[51].

Recently, Davidson[52] made a detailed review on this subject, and suggested how mitochondrial function in the endothelium could be linked to cardiovascular disease. He emphasized the experiment by Zhang *et al.*[53], which showed that thioredoxin (Trx2) overexpression not only prevented mitochondrial oxidative stress in hypertensive mice and preserved endothelial function, but even when Trx2 overexpression was restricted to the endothelial mitochondria, it was still able to decrease oxidative stress, improve aortic endothelia cell function and prevent atherosclerotic lesions. Davidson[54] showed evidence that the endothelium is a direct target of all major risk factors for heart disease, including diabetes, hyperlipidemia and hyperglycemia, oxidized low-density lipoprotein, inflammation, smoking, aging, and hypertension, and that the common element was ROS. However, he also acknowledged that targeting mitochondrial ROS with various agents failed to prevent the progression of atherosclerosis in humans.

One might ask if current animal models of atherosclerosis really reproduce human atherosclerotic disease, because current animal models of atherosclerosis usually do not use POPs. If POPs exposure is a major cause of atherosclerosis in humans, and if ROS overproduction is secondary to mitochondrial damage incurred by POPs exposure, one could expect that targeting mitochondrial ROS with various agents in humans might not be effective.

Disturbed NO Bioavailability of Endothelial Cells can be Explained by Mitochondrial Dysfunction

Another important functional mediator of endothelial dysfunction in atherosclerosis is NO. Disturbances in NO bioavailability have been linked to endothelial dysfunction, leading to increased susceptibility to atherosclerotic lesions, hypertension, hypercholesterolemia, diabetes mellitus, thrombosis and stroke[24]. The most compelling evidence for this conclusion is provided by transgenic mice with homozygous inactivation of the eNOS gene. These mice could not produce NO in the endothelium, and showed features of metabolic syndrome, including obesity, insulin resistance, hyperlipidemia and hypertension[55,56]; eNOS is known to play an important role in regulation of mitochondrial function and its biogenesis[57].

This role has not received much attention, however, because it is regarded as a phenomenon accompanying endothelial dysfunction caused by poor NO availability[22]. However, the realization of the ubiquitous presence of eNOS, previously thought to play a role only in the endothelial part of the vasculature, might change this view. For example, cardiac myocytes and adipocytes have eNOS, which play important roles in glucose and fatty acid metabolism, intracellular signaling, and control of mitochondrial function and its biogenesis in those cells[58,59]. Furthermore, mitochondrial dysfunction might modulate endothelial NO and superoxide generation[60]. More importantly to our discussion, Koh *et al.*[61] reported that eNOS in adipocytes plays a major role in adiponectin synthesis, which has been proposed as a central player in the pathogenesis of metabolic syndrome[62,63].

Although adiponectin and its receptors play important roles in the pathogenesis of insulin resistance, Koh *et al.*[61] clearly showed they play a secondary role to mitochondrial dysfunction. One has to appreciate a close relationship exists between eNOS function (thus NO availability and other endocrine mediators including adiponectin) and mitochondrial function.

Proliferation of VSMCs Filled with Lipids Could be Another Manifestation of Response to Endothelial (Mitochondrial) Injury

The main cell type involved in narrowing of the arteries (type 2 lesions in particular) is VSMCs filled with lipids. One of the major functions of the endothelium is controlling VSMC contraction or a tonic role[54]. Endothelium also plays a trophic role, a negative influence on smooth muscle proliferation, and controlling macrophage and leukocyte traffic through the endothelial layer. NO plays very important roles here, as discussed previously. VSMC proliferation might be another epiphenomenon of NO deficiency[24], thus mitochondrial dysfunction. Furthermore, there is evidence that mitochondrial dysfunction stimulates VSMC migration directly[64]. Note that the degree of mitochondrial dysfunction in the endothelium is somehow linked to VSMC proliferation and to narrowing of the vascular lumen, such that less blood flow is matched to the decreased metabolic demand of the myocardium it supplies. We believe that this explanation is informative; atherosclerosis could be both an inflammatory response to a toxic agent(s) and an adaptive response to the decreased tissue energy demand of its effect on mitochondrial function. Further evidence supporting this concept of adaptive response is provided later.

ATHEROSCLEROSIS IN FRACTAL PATHOPHYSIOLOGY; AN ILL-ADAPTIVE RESPONSE OF THE VASCULAR SYSTEM TO MITOCHONDRIAL DYSFUNCTION – SUPPLY AND DEMAND OF ENERGY

Glagov *et al.*[38] pointed out that the atherosclerosis process itself does not cause cardiovascular events, because blood flow is preserved through outward remodeling of the arterial wall. However, acute, occlusive luminal thrombosis does occur in a small percentage of lesions, termed 'culprit lesions', leading to ischemia or death of distal tissues. Acute coronary syndrome is a clear example. We will not discuss this rather late-stage phenomenon[65], where antiplatelet therapy and opening of the narrowed artery were proven effective[44,66].

We will be focusing on the entire stages of the atherosclerosis process and remodeling or narrowing of the artery itself. In our model, atherosclerosis is an ill-adaptive response of the supplying coronary artery to the decreased metabolic demand (in the case of coronary artery disease) of the myocardium (as a result of mitochondrial dysfunction; Figure 4).

Microvascular Rarefaction, or the Reduced Number or Combined Length of Small Vessels in a Given Volume of Tissue: Basic Abnormality

Microcirculation, including the smallest arteries, arterioles, capillaries and venules, plays a critical role in the exchange of gases, nutrients and metabolites between blood and tissues. It comprises a basic unit of physiology, and in the pathology in atherosclerosis. Abnormalities of the microvascular system are common among patients with conventional cardiovascular risk factors, including hypertension, diabetes, obesity and dyslipidemia. By quantifying the microcirculatory system, either by counting capillaries in histological sections or by a functional analysis of capillary blood flow, Levy *et al.*[67] showed that: (i) hypertensive patients have microvascular rarefaction (a reduced number or combined length of small vessels in a given volume of tissue); (ii) these microvascular changes occur very early in these conditions; and (iii) they are also hallmarks of the long-term complications of hypertension and diabetes (a result of those disease states).

It is appreciated that counting the capillary density, however, does not reflect the functional state of capillaries. Some microvessels are not perfused under the resting condition. They could be recruited during hyperemia. An estimate of the available flow reserve has been used as a useful index of microvascular function. Capillary recruitment (capacity) is often assessed in the skin by video microscopy, and functional blood flow reserve (capacity) is often measured for coronary circulation and expressed as the ratio of maximal to basal blood flow. Capillary recruitment capacity (thus functional reserve) is significantly reduced in the skin of hypertensive patients, as compared with normotensive individuals, and capillary recruitment is inversely correlated with blood pressure. This relationship extends across the normotensive and the hypertensive range, confirming impaired microcirculation in patients with hypertension[68].

Coronary flow reserve capacity is significantly lower in obese humans than in non-obese humans, and capillary recruitment is reduced compared with lean control subjects. Levy *et al.*[67] also noted that microvascular abnormalities that lead to impaired tissue perfusion appear to represent a generalized condition that affects multiple tissues and organs. They cited the following studies: (i) coronary flow reserve in hypertension is correlated with the media: lumen ratios of small arteries in biopsies of subcutaneous fat[69]; (ii) dilatation of venules in the retina independently predict progression of cerebral small-vessel disease[70]; and (iii) reduced coronary flow reserve predicts the occurrence of retinopathy in diabetes[71]. We will take these observations to the thrifty phenotype hypothesis, wherein a similar discussion is in progress.

Fetal Malnutrition and Metabolic Syndrome in Later Life: Rarefaction of Tissue-Microcirculation System Complex

The role of the intrauterine environment, particularly maternal nutrition, in influencing fetal growth and cardiovascular health in offspring in later life is known as the thrifty phenotype hypothesis. In a recent review on this subject, Clough and Norman[72] showed that suboptimal maternal nutrition and fetal growth result in reduced microvascular perfusion and functional reserve capacity, which are strongly associated with later development of obesity, type 2 diabetes and hypertension. They highlighted the fact that these conditions are linked to microvascular rarefaction and remodeling that together limit capillary recruitment, reduce exchange capacity, increased diffusion distances for metabolic substrates, and increased local and overall peripheral resistance[72]. These changes in small vessel structure and function are detectable very early, long before the onset of overt cardiovascular and metabolic disease[67,73,74].

Previously, we showed the tissue mitochondrial density is decreased in offspring of a malnutrition-afflicted dame[37]. If mitochondrial biogenesis of the offspring is not optimal as a result of malnutrition during development, resulting in decreased tissue mitochondrial density, the tissue-microcirculation system complex would also develop rather poorly.

Murrays Law, Metabolic Scaling Law and the Fractal Nature of the Vascular System

Murray's law states that the cube of the radius of a parent vessel should equal the sum of the cubes of the radii of the daughter vessels[75,76]. In Murray's optimum system, flow and vessel radius are functionally related: an optimum radius is found for a given flow. For a given metabolic coefficient, the volume of the vascular system in an organ or organism will depend on the flow required of it: vasculature optimum for high flows will have larger vessels than that for low flows, the cubes of the vessel radii being proportional to the flow required.

The overall metabolic rate in animals is generally accepted to show negative allometry, scaling mass to a power ~0.75, known as Kleiber's law, and thus the mass-specific metabolic rate is proportional to a power of approximately −0.25. This means that larger-bodied species have lower mass-specific metabolic rates than those of smaller-bodied species. This scaling relationship is applicable to different sized animals within a species and goes down to the mitochondrial function unit[8].

In 1997, West *et al.*[49] proposed a model (known now as West–Brown–Enquist or WBE model) and claimed this provided a complete analysis of scaling relationships for mammalian circulatory systems. They also claimed it predicts structural and functional properties of the vertebrate cardiovascular system, among many other distribution systems with fractal characteristics. The fractal nature of the branching systems in our body, including vascular and bronchial trees, is well appreciated[49,76–78]. The optimization of the energy dissipated in this network system to a minimum is empirically established. Recently, Huo and Kassab[77] derived scaling laws within an organ of a given species, and validated the volume-diameter and flow-length scaling laws using the minimum energy hypothesis, without using the controversial assumption of space-filling terminal branches[49], which has been debated[79]. It should be emphasized that this debate is on the 'origin' of the law, not the law itself.

Branching systems including vascular and bronchial trees increase their branch density toward terminals according to a power function with the exponent called the fractal dimension. From a stochastic model based on this feature, Kamiya and Takahashi[78] formulated the fractal-based integrals to calculate such morphological parameters as aggregated branch length, surface area and content volume for any given range of radius. Then they derived the branch number and cross-sectional area by virtue of the logarithmic sectioning of the axis of the radius, and of the branch radius–length relationship also given by a power function of the radius with an exponent. Using those derivatives, they quantified various hydrodynamic parameters of vascular and bronchial trees as fluid conduit systems, including the individual branch flow rate, mean flow velocity, wall shear rate and stress, internal pressure, and circumferential tension. The validity of these expressions was then verified by comparing the outcomes with actual data measured *in vivo* in the vascular beds. Analyses of mammalian skeletal muscles have shown that the structure of the capillary-tissue arrangement (Krogh cylinder) is most efficiently tailored for oxygen delivery to tissues during heavy muscular exercise, and that the optimum radius of the cylinder is scale-independently constant. Most importantly, they concluded the organ size proportional to body mass was mainly attributable to the accumulated number of basic units.

Basic Unit of Structure and Function: Tissue-Microcirculation System Unit

One could appreciate such units in work by Harel *et al.*,[80] who used functional magnetic resonance imaging (MRI) to map the functional units down to the level of cortical columns and lamina of the brain. They advanced a theory in which a cortical column, which is an ensemble of neurons involved in a particular neuronal computation, is spatially correlated with a specific vascular unit; that is, a cluster of an emerging principal vein surrounded by a set of diving arteries. They suspected that such a correlation between functional (neuronal) and structural (vascular) units existed as a fundamental intrinsic cortical unit, as shown in Figure 5. This theory argues that the circular nature of a regular organization of vessels within the cortex and a relatively uniform distribution of these principal veins spaced every 0.75–1 mm. From postmortem observations of humans, they have suggested such vascular organization might manifest as a 'vascular unit', which is associated with a known 'functional unit' in the cortex[81,82].

Glenny[83] suggested that the geometry of the airway and vascular trees might be considered a demonstration of emergent adaptive behavior of fractal geometry. The shapes of the bronchial and vascular trees are considered adaptive, because specific geometries are advantageous to the organism, reinforced through natural selection. As fractal geometry is conserved in branching trees throughout the plant and animal kingdoms, it is thought to confer an evolutionary advantage of efficient distribution of nutrients, suggesting that these fractal structures are

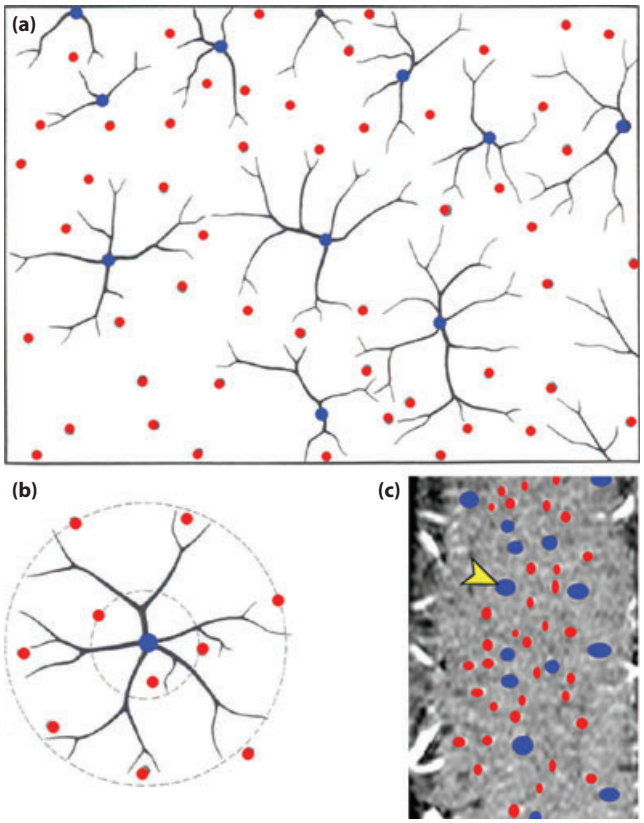

Figure 5 | Vascular unit model proposed by Harel *et al.*[80] (reproduced with permission from Frontiers in Neuroenergetics). The (a) circular nature of the vascular organization is shown in a cross-section showing the venous units and (b) their arterial rings; a principle cortical vein (blue) is surrounded by several penetrating arteries (red). (c) Vessel classification maps in a tangential section in cat visual cortex, derived from the magnetic resonance imaging data.

important to the organism. We believe that optimal organization of the tissue-microvascular system complex with tight regulatory control of vascular tree geometries is a necessity in the evolutionary process. This idea is similar to the concept of symmorphosis, which postulates a quantitative match of design and functional parameters within a defined system[84].

A tight association between the 'vascular unit' and the 'functional unit' can be also seen in muscles. Weibel and Hoppeler[79] showed that aerobic capacity is determined by two properties – body size and athletic prowess. They showed that the maximum oxygen consumption rate (VO_2max) and the morphometric characteristics of muscle are parallel. If VO_2max is plotted against the whole-body muscle mitochondrial volume (V[mt]), the two variables appear to be tightly associated (Figure 6). They also observed that mitochondria can only perform at a high rate of oxidative phosphorylation if they receive an adequate supply of O_2 from capillary blood.

Based on a model developed by Bassingthwaighte and Goresky[85], Beard[86] developed a model of oxygen transport and cellular (bio)energetics that could explain observations of

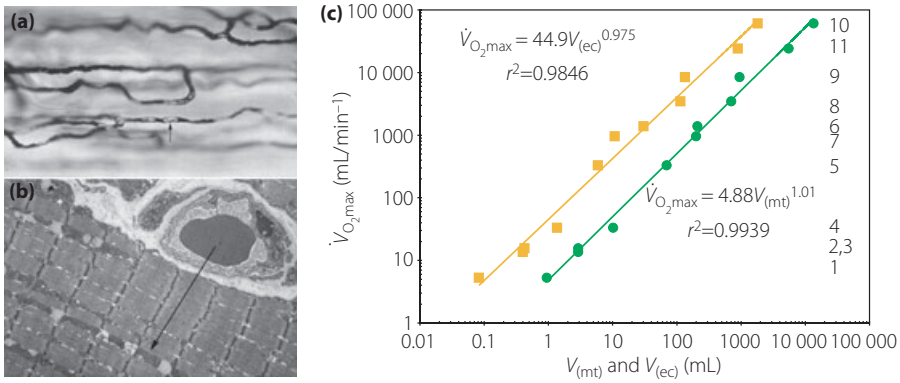

Figure 6 | (a) Light micrograph of capillary network in muscle, with an arrow pointing to an erythrocyte in stained plasma and (b) an electron micrograph shows the path for oxygen from capillary erythrocyte to mitochondria in the muscle cell, suggesting tight linkage between mitochondrial function and blood supply at the subcellular level. (c) Maximum oxygen consumption rate (VO_2max) plotted as function of total muscle mitochondrial volume (V[mt]; squares) and capillary erythrocyte volume (V[ec]; circles) in 11 species of animals show clearly the tight relations between mitochondrial function and its blood supply in quantitative terms (reproduced from Weibel and Hoppeler[79] with permission from The Company of Biologists Ltd).

in vivo cardiac energy metabolism. He assumed that within the capillary, interstitial space and cellular (myocyte) space, the concentrations of oxygen and other metabolites vary primarily along the length of the capillary (the advective transport is modeled in the capillary region): the interstitial and cellular spaces are assumed to be stagnant (non-flowing). The cellular region is further subdivided into cytoplasmic and mitochondrial compartments. He showed a 'one-dimensionally distributed blood–tissue exchange model integrating the three-regions' that is able to reproduce experimental observations on ATP, adenosine disphosphate, creatine phosphate and inorganic phosphate over a range of workloads and during coronary hypoperfusion (Figure 7). This model also explained metabolite levels observed at low to moderate workloads, and the changes in metabolite levels and tissue oxygenation detected during graded hypoperfusion.

A comparison of the model predictions with the data of Katz *et al.*[87] provides independent validation of the model of Beard. Therefore, this three region one-dimensionally distributed blood–tissue exchange model of Beard could be used to describe the integrated vascular and functional unit mathematically.

Previously, we applied the theory developed by West *et al.*[9] in explaining the metabolic scaling law to the development of obesity and/or insulin resistance[7,37]. We reasoned that if mass-specific mitochondrial function decreases, body mass must increase to compensate for a decreasing body core temperature. Earlier in the present review, we presented evidence showing that exposure to EDCs leads to mitochondrial dysfunction. The tissue-microcirculation system unit would become dysfunctional, as it is in tight correlation with unit mitochondrial function of tissue. When the function of the tissue-microcirculation system unit decreases, the blood flow into organs with less of these units will decrease, demanding remodeling of supplying arteries. This is a central tenet of our hypothesis. We concur with Harel *et al.*[80], that the tools and knowledge being developed today could be applied to metabolic syndrome and then to atherosclerosis. We also believe that the synthesis of our hypothesis could benefit further by incorporating advances made in the fractal physiology[88].

LOCALIZATION OF ATHEROMA IN ARTERIES AND THE UNDERLYING PHYSIOLOGICAL PRINCIPLE: MORE EVIDENCE FOR MITOCHONDRIAL DYSFUNCTION AS A CAUSE OF ATHEROSCLEROSIS

Decreased Blood Flow

With a color-coded and duplex Doppler sonography system, Bai *et al.*[89] found lower blood flow velocity and volume, higher resistance index in the carotid arteries, and more enlarged common carotid artery diameter in ischemic stroke patients than in controls. They also showed that common carotid end-diastolic velocity could predict future development of ischemic stroke either alone or in combination with intima-media thickness, a measure of atherosclerosis[90].

Transcranial Doppler sonography shows an excellent correlation with MRI in measuring brain blood flow and flow velocity, suggesting measuring blood flow with Doppler sonography technique is a good alternative to more valid methods using MRI[91]. Blood flow decrease was also found to be quantitatively associated with future development of stroke in that study. Thus, we could safely conclude that blood flow into the brain is decreased in atherosclerosis and precedes the development of stroke.

Boundary-Layer Separation of Blood Flow

In 1966, Fox and Hugh[92] tried to explain the focal nature of atheroma lesions, and their peculiar predilection for the curved segments and the mouths of branched vessels based on a physiological principle. They pointed out that this peculiar localization of atheroma could not be explained by a 'disorder

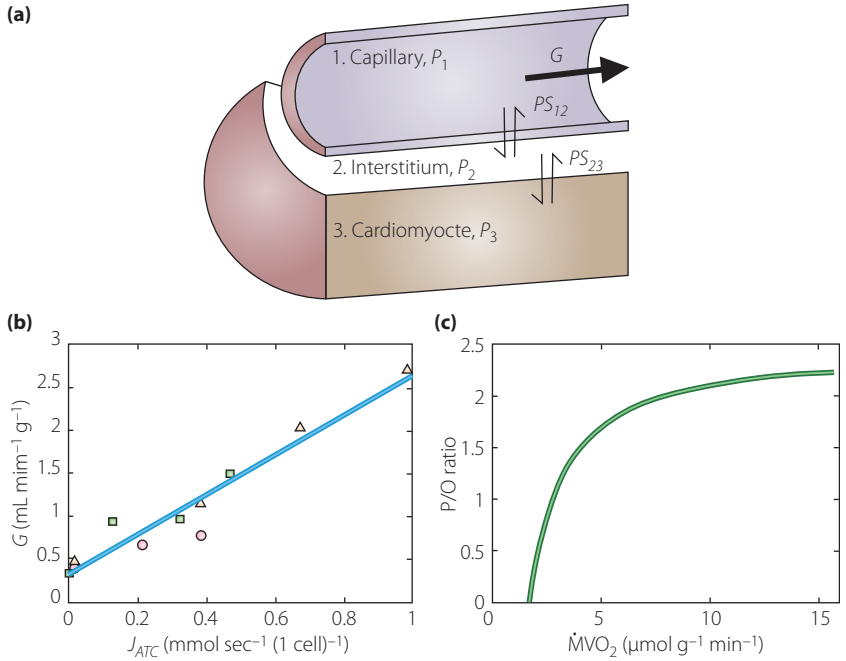

Figure 7 | (a) Three-region one dimensionally distributed blood–tissue exchange model for oxygen transport by Beard[86] (reproduced with a permission). The three regions correspond to capillary, interstitium and cell (myocyte), and oxygen is transported by passive permeation between these regions. (b) Plot of coronary flow as a function of the predicted rate of adenosine triphosphate (ATP) consumption (J_{ATC}). The model-predicted rate of oxygen consumption (MVO_2) agrees well with the reported experimentally measured estimates of MVO_2. (c) The predicted ratio between mitochondrial ATP production and rate of oxygen atom consumption (P/O ratio) is plotted for J_{ATC} values, and shows the oxygen consumption rate offsets the rate of proton leak across the inner mitochondrial membrane, which was also consistent with data. G, coronary flow.

of lipid metabolism'. They observed that in a fluid system, well-defined static zones can form despite quite high velocities nearby as a result of a process known as 'boundary-layer separation'. From the striking similarity between the regions where this occurs and the sites of predilection of atheromas, they imagined that static zones occur in the arterial system, which might allow the interaction of platelets and fibrin to form a mesh in which lipid particles become trapped, and that this becomes organized to form a plaque of atheroma as envisaged by Duguid[93].

They found the striking similarity between the areas at which atheroma is commonly found, and those where the occurrence of boundary-layer separation is predicted. They suspected a causal relationship between the two processes; the stagnant or low-velocity areas as a result of the separation of the boundary layer permit intravascular aggregation, leading to a variety of clotting; organization of the solid material or the development of a thrombus would follow, resulting in the formation of an atheromatous plaque.

Observing 12 adult human carotid bifurcations obtained at autopsy, Zarins *et al.*[94] showed pathological evidence that intimal thickening is greatest and consistently eccentric in the carotid sinus. Wall shear stress along the inner wall was higher; and along the outer wall opposite the flow divider apex, where the intima was thickest, wall shear stress was lower. Regions of moderate to high shear stress, where flow remained unidirectional and axially aligned, were relatively spared of intimal thickening. Intimal thickening and atherosclerotic plaques develop largely in regions of relatively low wall shear stress, flow separation and departure from axially aligned, unidirectional flow[94]. Using quantitative model flow studies, this group had confirmed early plaque deposition occurs in regions characterized by flow separation, stasis, increased particle residence time, low mean shear stress and oscillation in shear stress direction.

Increased Blood Viscosity and Hypercholesterolemia

One of the major predictions of the theory based on boundary-layer separation is that alteration in blood viscosity might be a contributing factor to atheroma, which was shown by Ercan *et al.*[95] They showed that plasma viscosity is positively correlated with total serum cholesterol, the atherogenic index, total cholesterol (total-C)/high-density lipoprotein cholesterol (HDL-C) ratio and low-density lipoprotein cholesterol (LDL-C)/HDL-C ratio in hypercholesterolemic patients with peripheral arterial obstructive disease (POAD). Furthermore, plasma viscosity was higher in hypercholesterolemic patients with POAD than in normocholesterolemic patients with POAD.

Total serum cholesterol is a well-known risk factor for atherosclerosis. Note that a positive linear correlation of plasma viscosity with total-C and apolipoprotein (apo) A-II and apoB

was observed in the World Health Organization sponsored multinational monitoring of trends and determinants in cardiovascular disease (MONICA) project[96]. That study also showed that blood viscosity is negatively related to HDL-C concentration, which reduces the risk of coronary heart disease.

Mitochondrial dysfunction might be the common biological reason behind all these phenomena and the relationships between them. Although there is no direct evidence linking mitochondrial dysfunction and increased blood viscosity in quantitative terms, there is indirect evidence. Analyzing longitudinal data of 12,881 initially non-diabetic adults, who participated in the Atherosclerosis Risk in Communities Study (1987–1998) for blood viscosity, Tamariz *et al.*[97] found that blood viscosity is independently associated with insulin resistance and several features of metabolic syndrome. Other studies have reported a similar association[98]. These findings suggest elevated blood viscosity and hypercholesterolemia might be different manifestations of systemic mitochondrial dysfunction. Blood flow through arteries must be lower in patients if there is systemic mitochondrial dysfunction, as the metabolic demand will be decreased. Combined with hypercholesterolemia and elevated blood viscosity, atheroma (deposition of platelets and lipid in mesh) would develop in the predilection sites, where the blood flow is stagnant and shear stress is lowest, by a process of boundary-layer separation.

Supply and Demand Mismatch: Development of Angina Pectoris and Stroke

Angina pectoris is considered a manifestation of coronary artery disease caused by atherosclerosis. A mismatch between supply and demand is given again as an explanation for this condition; for example. by Lanza *et al.*[29], which is shown in Figure 3. We suppose that blood supply to the myocardium is well adapted to the decreased metabolic needs, until lumen diameter is narrowed by approximately 80% or functional reserve is approximately 2–2.5 times the basal state, as shown in Figure 4. When myocardial energy demand is decreased for a long time, energy-supplying vasculatures are expected to narrow, in an (ill-)adaptive response. If a sudden increase in energy demand occurs, as in the case of sudden exercise, the blood supply through narrowed vessels simply cannot keep up as a result of decreased functional reserve, precipitating ischemia or angina pain.

In contrast, if myocardial metabolism is decreased well below the minimal demand to maintain cardiac function, heart failure will ensue. In this interpretation, CMVD could be regarded as a state of mitochondrial dysfunction of the myocardium, not a cause. High prevalence of small-vessel ischemic disease, lacunar infarctions and other brain abnormalities in patients undergoing coronary artery bypass graft supports this notion[99].

CONCLUSION

Extending our earlier mitochondrial dysfunction theory of metabolic syndrome, and the proposal for the necessity of research on POPs[100], we extended this hypothesis further that mitochondrial dysfunction induced by environmental toxins, such as dioxins, could be a cause of metabolic syndrome and also of atherosclerosis. We explained atherosclerosis as an adaptive change occurring in supplying arteries in response to the decreasing tissue energy demand. EDCs, such as dioxins, could induce mitochondrial dysfunction, which in turn would lead to decreased tissue metabolism, which in turn induce most of the biochemical features observed in atherosclerosis.

With systems bioenergetics and fractal physiology in mind, we showed that physiological laws, such as the metabolic scaling law, Murray's law and the fractal dimension of the vascular branching system, are useful in explaining the phenotypes of metabolic syndrome and also the pathogenesis of atherosclerosis.

These biophysical laws and biomathematics provided strong support for a concept that mitochondrial dysfunction at the level of the tissue-microvascular system complex could cause atherosclerosis in the supplying arteries. These facts suggest that mitochondrial function of the tissue-microcirculation unit could be used in the synthesis of fractal physiology. In this synthesis, therefore, EDCs-induced mitochondrial dysfunction will be seen as an environmental 'cause' of metabolic syndrome and atherosclerosis. There are mathematical tools and knowledge, such as fractal physiology, which could be developed further to describe metabolic syndrome and atherosclerosis.

In a recent essay on physics and medicine, West wrote that 'traditional medicine has typically focused on specific events at a localized level of organization and often in isolation with a narrow focus and timeframe'[101]. There might be no better example than coronary heart disease and atherosclerosis. He stressed the 'need to understand quantitatively, from underlying principles, the mechanisms that determine the baseline scale of life, and thereby develop corresponding metrics to define the average, idealized, healthy human being based on fundamental measurable parameters associated with different levels–e.g., cells, mitochondria, proteins, repair mechanisms, metabolism, environment, specific diseases, individual life history, genomic variation, and diet'.

We conclude that such an idealized model could be synthesized based on the tissue-microcirculation unit or mitochondrion and biophysical laws.

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