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Teaser This review highlights routinely omitted facts on human coronary morphology and on paradoxical patterns of lipid deposition in initiation of coronary atherosclerosis. The analysis offers an alternative hypothesis on the pathogenesis of coronary atherosclerosis.

Excessive intimal hyperplasia in human coronary arteries before intimal lipid depositions is the initiation of coronary atherosclerosis and constitutes a therapeutic target

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The consensus hypothesis on coronary atherosclerosis suggests high LDL-C levels as the major cause and pursues it as the therapeutic target, explicitly assuming: (i) *tunica intima* of human coronaries consists of only one cell layer – endothelium, situated on a thin layer of scarcely cellular matrix; and (ii) subendothelial lipoprotein retention initiates the disease. Facts showed: (i) normal *tunica intima* invariably consists of multiple cellular layers; and (ii) initial lipid depositions occurred in the deepest layers of *tunica intima*. This review suggests that coronary atherosclerosis starts with pathological intimal expansion, resulting in intimal hypoxia and neovascularization from adventitial *vasa vasorum*, facilitating lipoprotein extraction by previously avascular deep intimal tissues. Until the hypothesis incorporates real knowledge, our efforts will probably be off-target.

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Introduction

The enormously costly drug discovery process today cannot predict the clinical efficacy or sustainability of prospective drugs because of our failure (or limited ability) to identify the biologic mechanisms of diseases (i.e., disease pathogenesis) [1–5]. I suggest that the same sort of failure has thwarted progress in the therapeutic treatment of coronary artery pathology. Another matter that hinders the progress in studying coronary artery pathology is that the main pathologic alterations occur only after the coronary artery wall undergoes certain age-related differentiation. For example, coronary atherosclerosis can affect the coronary wall only after its *tunica intima* differentiates from a thin, one-cell-layer compartment into a thick, multi-cell-layered compartment, in a process called normal [6] or benign [7–9] diffuse intimal thickening (DIT). This makes it tempting to designate DIT as the beginning of coronary atherosclerosis [10–13]. However, coronary DIT occurs in all human hearts without any exception, as well as in all animals with mass comparable to human body mass, which by default makes coronary DIT a normal physiologic arrangement. This misinterpretation (a phenotype occurring in all representatives without exception is perceived as a pathology, although must be perceived as the norm) could potentially jeopardize drug discovery in two ways: (i) a norm that is misguidedly suggested as pathology could be a false therapeutic target; and (ii) designation of a pathogenic status to an erroneous event could divert our efforts in search for real causation(s).

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http://dx.doi.org/10.1016/j.drudis.2016.05.017

Please cite this article in press as: Subbotin, V.M. Excessive intimal hyperplasia in human coronary arteries before intimal lipid depositions is the initiation of coronary atherosclerosis and constitutes a therapeutic target, Drug Discov Today (2016), http://dx.doi.org/10.1016/j.drudis.2016.05.017

This review puts the spotlight on selected coronary diseases and their suggested causes: (i) coronary atherosclerosis (high low-density lipoprotein cholesterol (LDL-C) levels, endothelial dysfunction and inflammation); (ii) re-stenosis following interventions and coronary bifurcation disease (mechanical injury and/or stress); (iii) transplant coronary disease (immunologic damage); (iv) Kawasaki disease (infectious etiology and genetic factors); (v) coronary pathology in Hutchinson–Gilford progeria (synthesis of progerin). The study of each disease is aimed at the particular presumed causation as the therapeutic target. However, growing evidence has showed that increased cell proliferation in the coronary *tunica intima*, transforming normal DIT into excessive intimal hyperplasia or pre-pathologic intimal thickening (pre-PIT), is the initiation of all of the above diseases. Excessive intimal hyperplasia can be itself lethal by narrowing the coronary lumen or becoming complicated by plaque formation. Accumulating evidence shows that excessive cell proliferation in the coronary *tunica intima* can be triggered by a variety of diverse, nonspecific signals. However, studying nonspecific signals in the hope of elucidating normal regulation, and finding a therapeutic target, is unproductive. This review suggests that, unless we change our approach to coronary pathology and concentrate on disease initiation (i.e., on excessive intimal hyperplasia in the coronary *tunica intima*), our research and therapeutic efforts will probably be off-target. This inquiry opens with an analysis of coronary atherosclerosis because it is the most frequent and lethal coronary malady. Because the main goal of this review is to present arguments in favor of coronary cell proliferation as the initiation of all of the above coronary diseases, the arguments on the initiation of coronary atherosclerosis are given in greater detail than those for other coronary pathologies; arguments on other coronary pathologies are grounded on the same reasoning.

Coronary atherosclerosis

Brief history of coronary morphology

Understanding the pathogenesis of any of disease always starts with a construction of undisputed knowledge on an organ's normal morphology and its variations under physiologic conditions. In the field of arterial pathology, the modern understanding of arterial design starts with the pioneering work of Richard Thoma. Over four decades, Thoma published observations and hypotheses in leading pathology journals about the resemblance between normal diffuse intimal hyperplasia (now termed DIT) and arteriosclerotic intimal thickening (now termed PIT) in different segments of the aorta, the coronary artery and other arteries. He has also published articles on similarities between the above structures and morphogenesis leading to the closure of the umbilical artery and the *ductus arteriosus*. In his publications, Thoma uses the German 'Neubildung' or 'Gewebsneubildung' to describe new tissue formation without pathologic transformation [i.e., normal hyperplasia (DIT)]. To describe a diseased hyperplasia (PIT), he adds the terms 'Angiosklerose' and 'Angiomalacie'. Thoma hypothesized that arterial intimal thickening is a physiological adaptation to changing hemodynamic demands [14–17]. In one publication (coauthored by his student N. Kaefer), Thoma suggested that the pathogenesis of arteriosclerosis begins with intimal thickening that is initially adaptive [18] (for a review see [19]). Whether one agrees or not with Thoma's 'first histomechanical laws of the bloodstream' [16], it is undisputed that he laid the foundation for coronary pathology.

Pioneering work of Kapitoline Wolkoff

The same year (1923) the final manuscript of Richard Thoma appeared in *Virchows Archiv* [17], the journal published another groundbreaking report concerning normal postnatal development of the human coronary artery, contributed by Dr Kapitoline Wolkoff (alternatively spelled Kapitolina Volkova) from St Petersburg, Russia. I believe that the significance of this report deserves special attention. For the first time, Wolkoff showed that normal human epicardial coronary arteries undergo postnatal developmental changes, gradually transforming their morphology from the after birth design to an adult architecture at an age of 15–30 years. (A personal note: I often cite works that are old. I become so preoccupied with an idea that I must find out who first discovered the facts. However, regardless of personal compulsion, I believe that if someone has overlooked crucial information published a hundred years ago they should update their knowledge). In particular, Wolkoff showed that the coronary *tunica intima* of young children (e.g., 8.5 months) consists of only 1–2 layers of cells lying on a thin amount of matrix and internal elastic lamina. With age, the number of cell layers in the *tunica intima* increases, reaching 10–15 layers at age 15. This structure then differentiates to the adult design of approximately 25–30 cell layers at age 25–30 years (Fig. 1).

From Wolkoff's descriptions, the structures of *tunica intima* above a *lamina elastica interna* (in German 'Bindegewebsschicht' and 'Elastisch-hyperplastische Schicht') correspond to DIT in the modern literature [6,21]. Wolkoff's publication came from the Department of Pathologic Anatomy of the Institute of Experimental Medicine St Petersburg, Russia, headed by N.N. Anitschkow (alternatively spelled Anichkov), who is famous for his seminal work in experimental atherosclerosis. Dr Wolkoff was a disciple of Anitschkow and a lifelong colleague. A crucial significance of Wolkoff's findings has been acknowledged by Anitschkow in the opening chapter of the prominent book: *Arteriosclerosis: A Survey of the Problem*. In his chapter, Anitschkow writes: "... in evaluating the significance of the thickening of the intima, as observed by various authors, it is important to remember that thickening of the intima also occurs in experimental animals as a purely physiological phenomenon in the process of aging. In this respect, the arteries of some animals exhibit almost the same conditions that are observed in human arteries, as may be seen from Miss Wolkoff's investigation (1924). In the view of the fact that some authors mentioned above did not pay any attention to this circumstance, the experimental results reported by them can be accepted only with very great reservations. This criticism applies only to experimental animals of considerable size, like dogs, which invariably exhibit thickening of the intima as a sign of aging..." [22]. Anitschkow referred to Wolkoff's 1923 publication [20] and the second article Wolkoff published in *Virchows Archiv* in 1924 that compared her previous observations of the human coronary artery [20] with those of the arterial morphology of animals [23]. The quotation above showed that the greatest authority in the field suggested that researchers, who were not aware of normal coronary artery intimal thickening in humans and animals, simply cannot be trusted in their results and conclusions. One might expect that such a straightforward warning by the most respectable scientist in the field would not be missed or forgotten.

The following years brought numerous publications on coronary artery design in humans and large mammals. All of them

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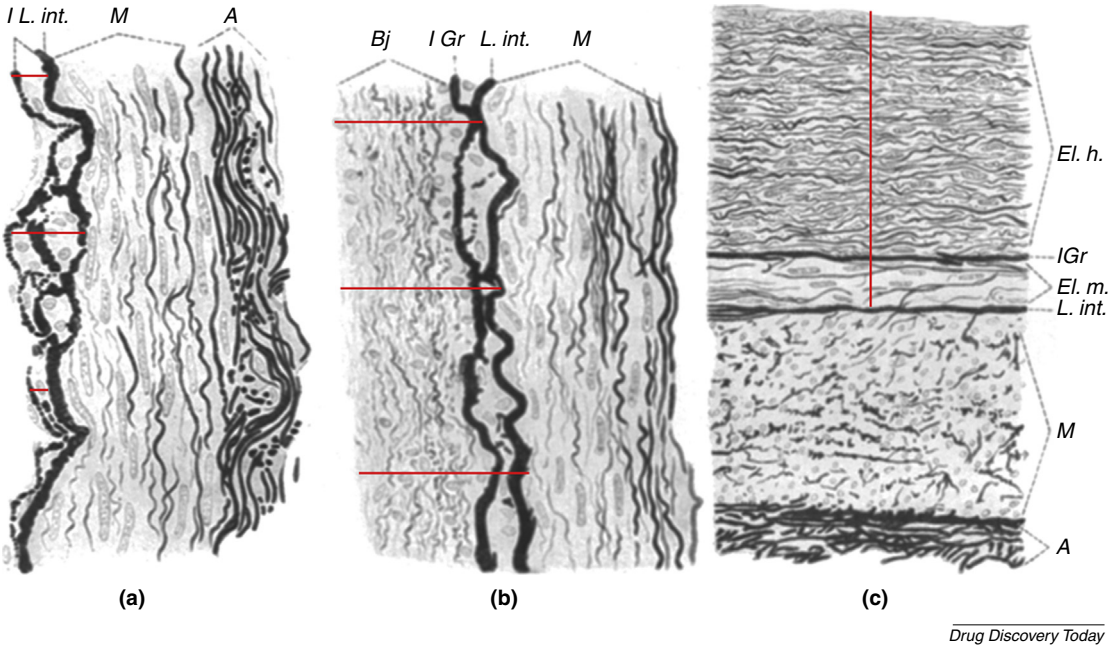

FIGURE 1

Selected drawing of human coronary arteries adapted, with permission, from [20]. **(a)** 8.5 months, **(b)** 15 years, **(c)** 32 years (all females). Red bars indicate the thickness of the *tunica intima*. The original article does not show microscopic magnifications; however they could be inferred as: (a) and (b) ×400; (c) ×200. *Source*: Reproduced, with permission, from [20].

unanimously confirmed the same facts: the *tunica intima* of coronary arteries of humans and large mammals invariably develops under physiologic conditions into normal intimal hyperplasia or DIT [24–47]. This arterial morphogenesis was particularly well described in great detail by French [25–27], Bálint [42], Velicans [28–40] and Cucu [46,47]. Therefore, has knowledge on coronary morphology prevailed and the misinterpretation been resolved?

Known facts on human coronary morphology continue to be ignored in spite of accumulated evidence

Reality is surprising: known facts on coronary artery morphology continue to be ignored in coronary research. Furthermore, in 1989, the renowned UK pathologist Collin L. Berry in the monograph *Diseases of the Arterial Wall* alerted the research community again: ''There is a considerable body of literature on the significance of what have usually been described as 'endothelial cushions', mainly in coronary arteries (see Robertson for review of early literature). Robertson concluded that the lesions, which could be found in other arteries, were not related to subsequent atherosclerosis but were normal growth phenomenon. These studies however, and the subsequent careful work of the Velicans, have been ignored in recent years''. [48].

Unfortunately, Berry's assessment in the year 1989 remains valid. Facts of normal human coronary morphology (i.e., obligatory development of DIT in human epicardial arteries) continue to be ignored (or overlooked) in scientific publications, for example [49–53], and educational material, for example [54–56]. All mainstream research and educational writings continue to be based on the incorrect assumption that the human coronary *tunica intima* comprises only one cell layer. These omissions could be caused by inertia in medical education and infrequent usage of specialized medical histology textbooks that contain correct information, including, for example, *Histology for Pathologists* [57]. I would conditionally have agreed with such an interpretation, but not after the year 2002. In 2002, *Virchows Archiv* published a detailed article by Nakashima *et al.* [21], bringing an end to any misinterpretation and doubt on normal human coronary design. This publication contains detailed information on the postnatal developmental morphology of the human coronary artery, particularly of the *tunica intima* (Figs. 2 and 3). Nakashima *et al.* performed the detailed analysis that undoubtedly documented that: (i) the normal adult coronary *tunica intima* is thicker than the *tunica media*; and (ii) the *tunica intima* comprises numerous cell layers of smooth alpha-actin-positive cells, which are arranged in a very dense manner, two-thirds of the intimal thickness, with the cell layer density progressively increasing toward the internal elastic lamina.

This morphologic study was published in *Virchows Archiv* – a most prestigious international journal in pathology – and received notable citation scores: currently 47 (Web of Science) and 81 (Google Scholar). Therefore, one should anticipate that, after this publication in 2002, all studies on coronary atherosclerosis would be grounded on well-documented facts of coronary artery morphology, and all reservations and objections expressed by Anitschkow and Berry should no longer be of concern; but, as my readers have probably already guessed, this is not the case.

To date arteriosclerosis research has taken no cognizance of fundamental facts about human coronary artery morphology

Today, mainstream analyses of the pathogenesis of coronary atherosclerosis continue to be based on the incorrect information about coronary artery design. No matter how unrealistic it sounds, the facts are stubborn and astonishing. All chief publications presenting theories on the pathogenesis of coronary atherosclerosis and possible solutions are based on the explicit assumption that

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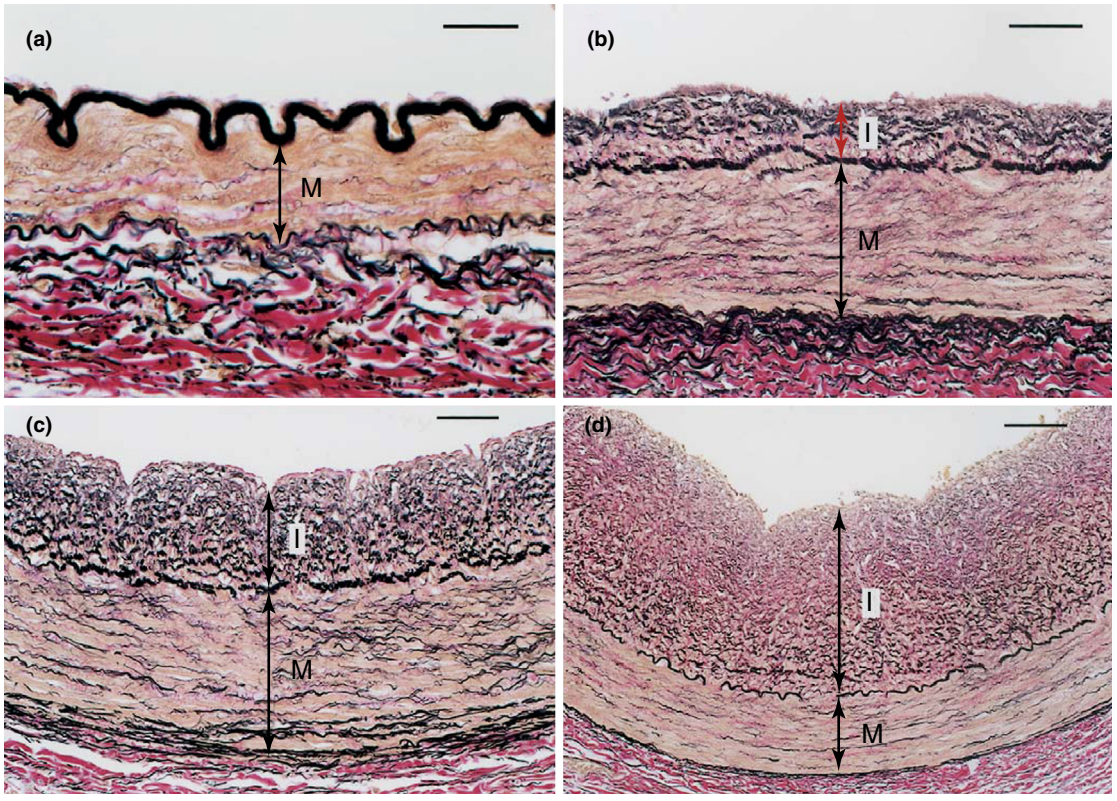

FIGURE 2

DIT in proximal coronary arteries. **(a)** Right coronary artery (RCA), 7-day-old female. **(b)** Left anterior descending artery (LAD), 5-year-old female. **(c)** LAD, 15-year-old female. **(d)** LAD, 29-year-old female. Bars in a, b, c and d represent 25 μm, 50 μm, 50 μm and 100 μm, respectively. I represents the intima and M is the media. These microscopic images represent normal morphological changes in coronary arteries from birth to adult (van Gieson stain). Please note that the *tunica intima* of a normal coronary artery is thicker than the *tunica media*.
Source: Reproduced, with permission, from [21].

the *tunica intima* of human coronary arteries consists of only one layer of cells, endothelial cells, situated on a thin layer of acellular (or scarcely cellular) matrix. This incorrect information appeared as written descriptions and as detailed schematics in analyses of the disease pathogenesis and approaches to a cure. To illustrate the above misrepresentation, I have chosen a dozen recent publications that have presented incorrect information as written descriptions and drawings and I encourage my readers to review these articles [58–70]. My conclusions on the erroneous status in this field can be demonstrated by the following figure from one the most recent publications from 2015 [69] (Fig. 4).

At this point I should like invite my readers to review again figures from Nakashima *et al.* [21], which are real microscopic images of human coronary arteries, and compare them to the depiction of the coronary *tunica intima* given here in Fig. 4. My readers do not have to be pathologists to discover a fundamental difference: numerous (25–30) compactly arranged cell layers of the real coronary *tunica intima* puzzlingly disappeared in Fig. 4 [69]. This incorrect representation of coronary morphology has been further repeated by popular medical websites (e.g., Medscape [71]) and is further amplified by science writings (e.g., [72]) and even by the most reputable informational source – *Encyclopedia Britannica* (Fig. 5).

The most recent book on coronary artery disease by J.T. Willerson [74] incorporates the same incorrect representation of coronary morphology [75]. By contrast, all real microscopic studies of human coronary arteries undoubtedly confirmed that *tunica intima* of epicardial arteries is thicker than *tunica media* and consists of multiple layers of cells [76]. Someone might ask the following question: is an adherence to the correct coronary architecture really that important for coronary atherosclerosis research? Why do I devote so much attention to incorrect writings and schematics that omit real details of coronary *tunica intima* morphology? A general response is that science cannot omit or distort facts. Although it sounds trivial and redundant, this process is important.

Why human coronary tunica intima design must be recognized as a multi-layered cellular compartment

The specific answer as to why human coronary *tunica intima* design must be recognized as a multilayered cellular compartment has been highlighted by another groundbreaking report from Nakashima's group [77]. In this detailed study, Nakashima and coauthors showed that the initiation of coronary atherosclerosis started with lipid depositions in deep layers of the *tunica intima*, which are distal to the coronary lumen and separated from the luminal blood by numerous intimal cell layers and matrix. At the same time, the subendothelial space and the region proximal to the arterial lumen of the *tunica intima* do not show any lipid accumulation. The initial lipid deposition occurred immediately above the internal

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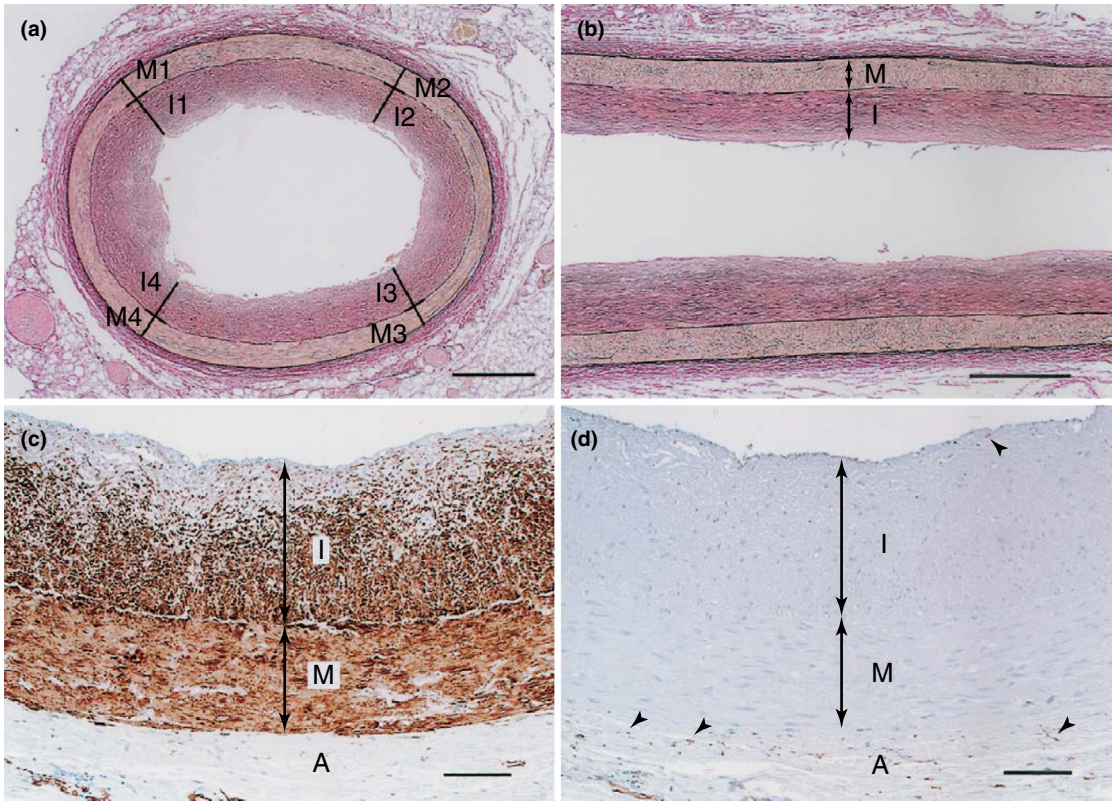

FIGURE 3

Structures and components of diffuse intimal thickening (DIT) in the proximal portion of the right coronary artery (RCA) in adults. **(a,b)** DIT was demonstrated as a uniformly thickened inner layer (van Gieson). **(c)** Immunostain for alpha smooth muscle actin. Almost all the cells in the DIT were smooth muscle cells. **(d)** Immunostain for the macrophage marker HAM56 at the same site as in (c). Only a few intimal and several adventitial cells were positive (arrowheads). I represents the intima, M is the media and A is the adventitia. These microscopic images represent a normal right adult coronary artery in two intersecting planes. Please note that the *tunica intima* of a normal coronary artery is thicker than the tunica media.
Source: Reproduced, with permission, from [21].

elastic lamina and is not accompanied by macrophage infiltration. Even with the progression of lipid deposition, lipid accumulation is always greater in deeper *tunica intima* than in surface layers [77,78]. Notably, these deep lipid deposits do not coincide with macrophage infiltration even at Grade 3 PIT with foam cells (Fig. 6).

The facts that Nakashima *et al*. reported [77,78] have placed the sequence of events in a straightforward order: at the Grade 1 fatty streak (Fig. 6d–f) lipids visibly accumulated in the deepest layers of the *tunica intima*, which are distal to the arterial lumen, whereas the subendothelial region and the outer *tunica intima* proximal to the lumen do not show any trace of lipid accumulation. At the Grade 2 fatty streak (Fig. 6g–i) lipid accumulation in the inner (distal to lumen) layers of the *tunica intima* increased, whereas the outer layers proximal to the blood region are either lipid-free or show much less lipid accumulation. At Grade 3 PIT, accumulation of lipids in the outer *tunica intima*, which is distal to the arterial lumen, dominated compared with tissues proximal to the arterial lumen (Fig. 6m–o). The same paradoxical pattern of initial deep lipid deposition in human coronary atherosclerosis was also noted in early publications by Wolkoff in 1929 [79] and others [80,81]. I wish to emphasize again that the above observations represent initiation of coronary atherosclerosis.

We must direct our attention to the initiation of coronary atherosclerosis when pathologic alterations are potentially reversible

Reviewing the current literature on the pathogenesis of coronary atherosclerosis has shown that chief analyses essentially do not discuss the disease pathogenesis. The word 'pathogenesis' comes from the Greek *pathos* (disease) and *genesis* (creation) and is interpreted as 'the origination and development of a disease' [82] or in plain language 'events from which a disease begins and then progresses'. Logically, all studies that are devoted to atherosclerotic plaque properties (i.e., plaque stability, vulnerability, rupture and thrombosis) are not about initiation or pathogenesis of coronary atherosclerosis but about inevitable complications of end-stages of the disease [83–85]. Understandably, from a lifesaving perspective, we must study atherosclerotic plaque status and plaque vulnerability, rupture and thrombosis, because these features determine morbidity and mortality. Needless to say, many studies of disease pathogenesis aim for prevention and reversal of pathology but, unfortunately, these end-stage manifestations of coronary atherosclerosis are beyond prevention and reversal and are extremely resistant to therapeutic stabilization [83–85]. Although some progress in plaque stabilization and regression has been reported, these pathologic formations are unlikely to be therapeutically curable,

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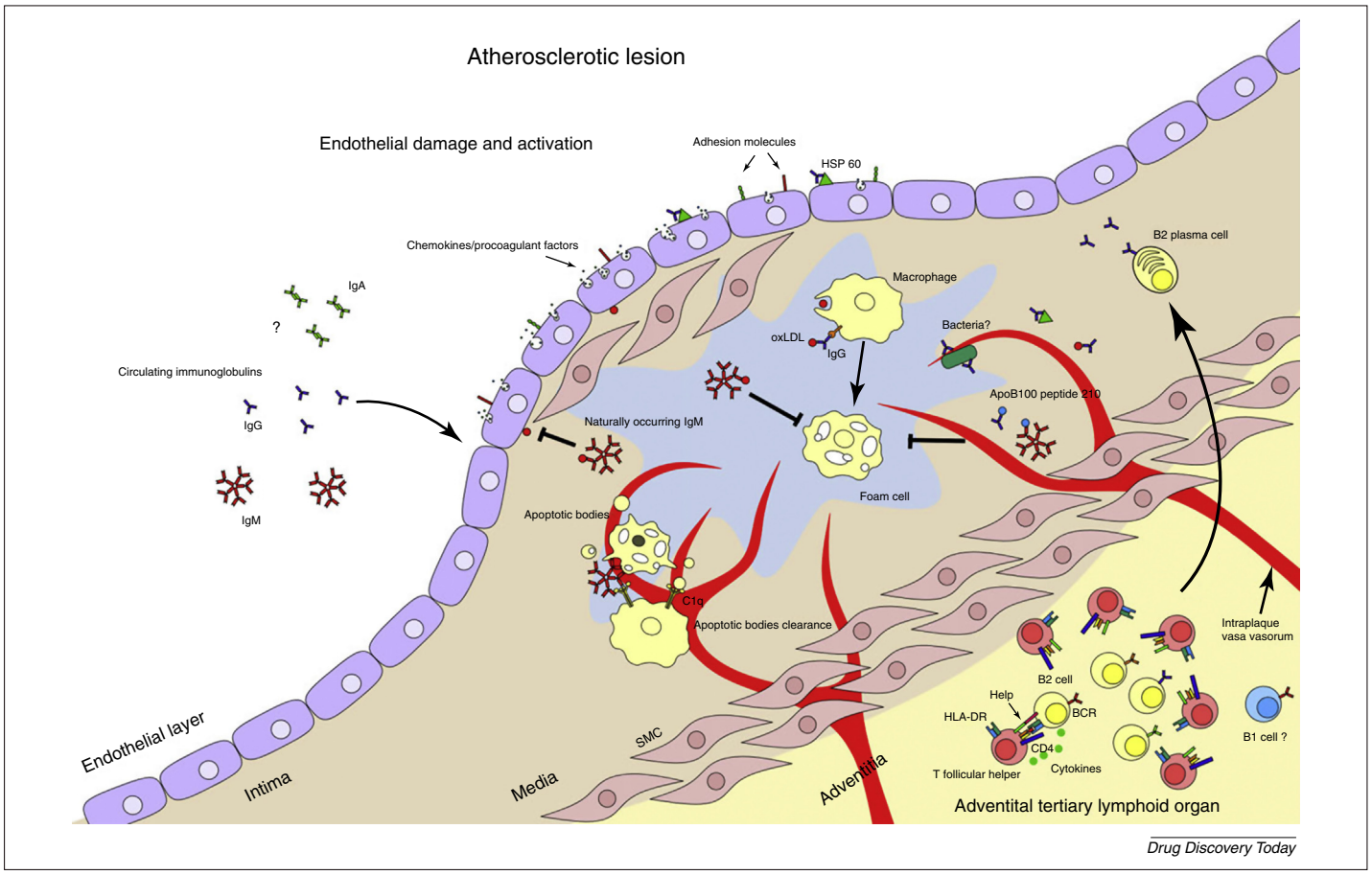

FIGURE 4

Classic incorrect presentation of coronary artery morphology. Please note that the *tunica intima* is shown as one layer of endothelial cells covering the acellular matrix. According to this schematic and the corresponding text, all other noninflammatory cells appeared in the *tunica intima* only after atherosclerotic lesion occurred.

Source: Reproduced, with permission, from [69].

with a high probability that these patients will require coronary intervention (for a review see [86]). Again, studies of advanced stages are extremely important because their results help to save lives but it is unlikely that they could bring an understanding of the disease genesis (i.e., to answer questions regarding how and why the disease occurs and how to prevent it). Therefore, we must not pretend that studying atherosclerotic plaque properties is the study of how the disease begins, because, on the contrary, it is how the disease ends. This review aims to analyze mechanisms of the initiation of coronary atherosclerosis (i.e., Grade 1–2 fatty streak and Grade 3 PIT) when lipid accumulation occurs within still viable coronary structures. Attention to the early stages is crucial because pathological alterations at these stages are potentially interruptible and reversible.

The accepted vector of lipid invasion (from coronary luminal blood into the coronary wall) is incoherent

The accepted hypothesis on the initiation of coronary atherosclerosis assumes that lipids invade the coronary wall from the arterial lumen. However, when the hypothesis is combined with undeniable facts on patterns of initial lipid deposition [77–81], it inevitably implies a self-contradictory model in which lipids from the arterial lumen have invaded and accumulated in the distal deeper layers of DIT, thereby passing through the proximal subendothelial region and the surface layers of DIT, but nevertheless leaving no trace in the proximal tissues. To rationalize this paradoxical distribution pattern, the accepted hypothesis has to be complemented by certain conditions because a parsimonious explanation for such paradoxical patterning does not exist.

From this point forward, I prefer to use to use the term 'consensus hypothesis' instead of 'accepted hypothesis' for the following reasons. First, it is not known how many scientists accept or reject the hypothesis. Second, the events surrounding the initiation of coronary atherosclerosis were the subjects of very heated and unsettled debates over the disease pathogenesis, a summary of which was termed the 'consensus statement' in 1985 [87–91]. The third reason is that I was convinced by a recent publication of Malone and Agutter [92] that the term consensus hypothesis is more appropriate wording that better defines the circumstances. In their writing, Malone and Agutter argue in favor of their hypothesis of the pathogenesis of deep vein thrombosis and appropriately use the word consensus to describe a mainstream hypothesis in their field of research that they opposed.

To reconcile the consensus hypothesis (with lipid invasion from the arterial lumen) with the discovered facts (no initial lipid deposition in tissues proximal to the lumen but visible deposition in tissues distal to the lumen), Nakashima *et al.* invoked an elegant hypothesis [77,78] that incorporates

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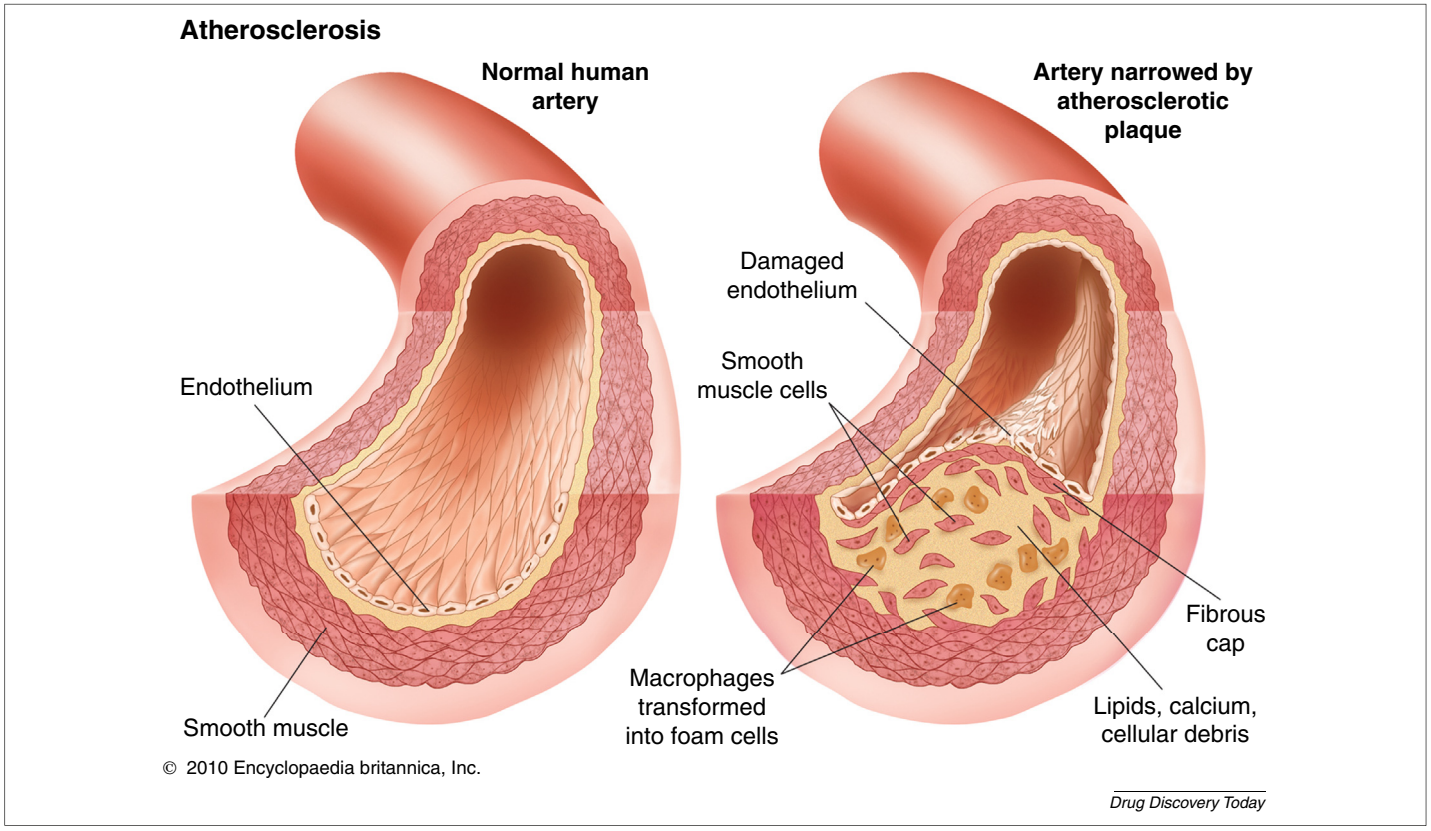

FIGURE 5

Depiction of normal human coronary artery (left) and coronary atherosclerosis (right). Please note that the *tunica intima* is presented here as one layer of endothelial cells covering the acellular matrix, which is incorrect.
Source: By courtesy of *Encyclopedia Britannica*; reproduced, with permission, from [73].

previous diffusion or 'filtration pressure' models [93,94] that led to their discovery on the co-localization of proteoglycan biglycan (which has a high binding capacity for lipoproteins [95–97]) in distal layers of coronary *tunica intima* [77,78]. However, localization of proteoglycan biglycan in the outer (distal) layers of DIT [77,78] can offer only a partial explanation: biglycan also localized in different tissues of the human body [98] including cancer tissues [99], but tissues other than the outer coronary *tunica intima* do not show pathologic lipid accumulation in regard to biglycan.

Scientists working on mathematical modeling of atherosclerosis [100,101] also favored a filtration pressure model [93] with a constant radially outward flow of plasma into the *tunica intima* caused by arterial blood pressure. In particular, Dr. Fok suggested a model with physical transport of LDL-C from the proximal parts of the intima to the distal parts, followed by washing out from the distal parts by blood; then the LDL-C accumulates near the elastic lamina because of the presence of biglycan and the permeability block by *lamina elastica interna* [102]. Although the models are well-designed, they are incongruent with known patterns of lipid accumulation resulting from the same diffusion pressure and LDL-C invasion in the *tunica intima* of nondiseased human aortas [103].

History of the presumption on lipid invasion from arterial luminal blood into coronary wall and alternative hypotheses

Therefore, the question is: why does atherosclerosis research advocate this self-contradictory model of LDL-C deposition from the coronary artery lumen to the coronary *tunica intima* if it is in conflict with known observations? There ought to be a reason why coronary atherosclerosis research is so adherent to an obviously contradictory model of disease pathogenesis. I believe that recounting how atherosclerosis research has arrived at this conclusion could help us to resolve this puzzle. Even taking into account early work Rudolph Virchow and Carl von Rokitansky, the credit for this scientific concept should be given to a series of seminal experiments by Anitschkow and Chalatow [104] and further elaborations by Anitschkow [22,105,106]. In his most famous English publication, Anitschkow stated: ''Lipids enter the arterial wall as compounds with protein fractions of blood plasma directly from arterial lumen'' [107]. Already, at that time, the extrapolations from the rabbit model (no natural cholesterol consumption, no coronary DIT) to human coronary atherosclerosis (cholesterol consumption, coronary artery with significant DIT) was criticized by experts [108,109]. Remarkably, observations of early stages of human coronary atherosclerosis from the same group had already shown initial lipid deposition not in the proximal but in the distal layers of DIT [79], thereby bearing some contradictions to the hypothesis advanced by the authors. In addition, the significance of proliferative morphogenesis in the coronary *tunica intima* forming a diffuse intimal thickening, which Anitschkow had hailed as crucial knowledge for atherosclerosis research [22], was denounced in a later report from this group [110] (the nature of the reasons behind such reversal is beyond the scope of this review).

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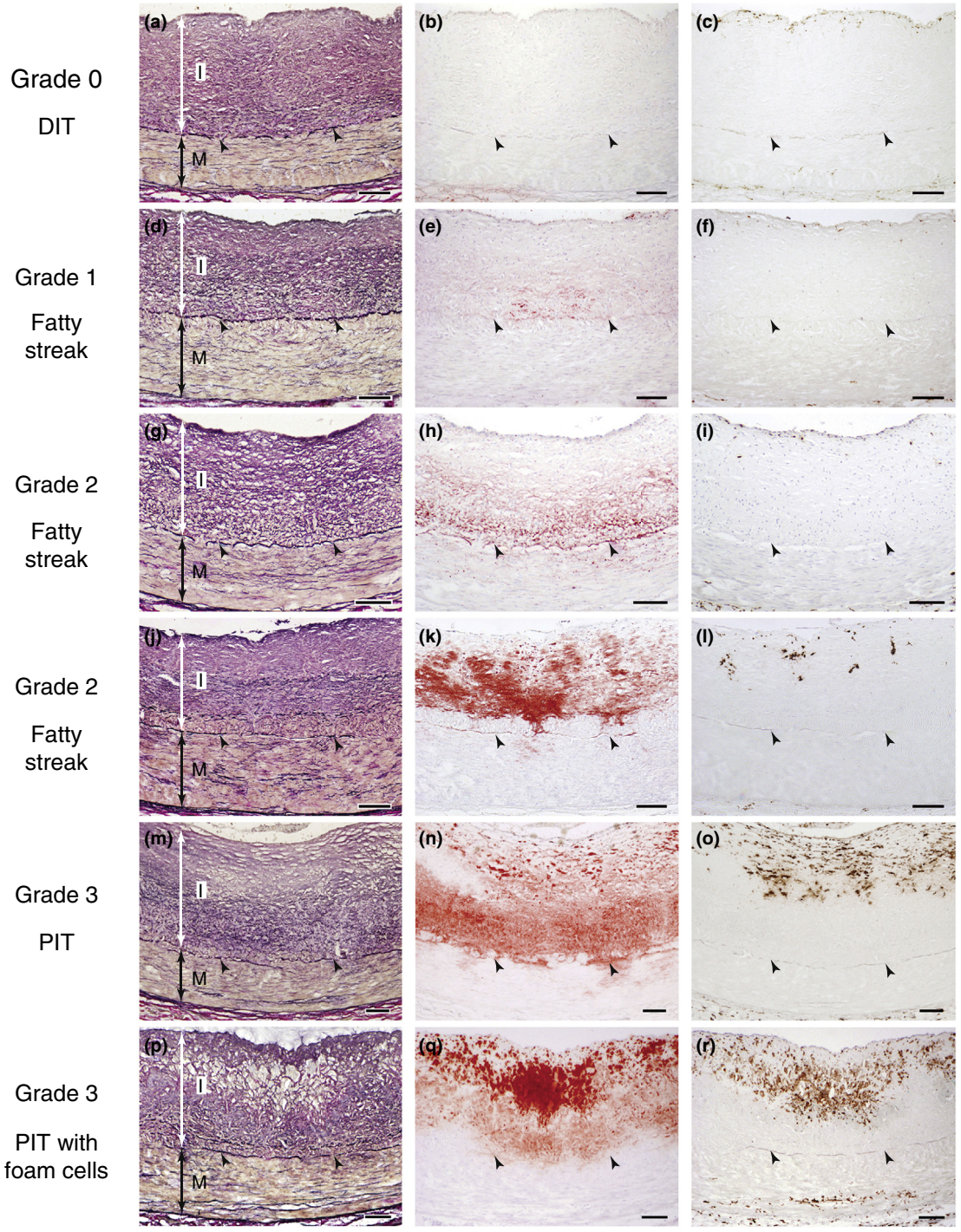

FIGURE 6

During early stages of coronary atherosclerosis, the initial lipid deposition occurs in deep layers of the *tunica intima*, which are separated from the subendothelial region by numerous cell layers and matrix. At the same time, the subendothelial region and the part of the *tunica intima* proximal to the outer endothelium do not show any lipid accumulation. The initial lipid deposition in deep layers of the *tunica intima* occurred immediately above the internal elastic lamina and is not accompanied by macrophage infiltration. Even with further accumulation of lipids in the deep *tunica intima*, lipid deposits never coincide with macrophage infiltration. I indicates the intima and M is the media. **(b,e,h,k,n,q)** Sudan IV stain. **(c,f,i,l,o,r)** Immunostaining with anti-CD68 antibody (macrophages). Arrowheads indicate internal elastic lamina. Bars represent 100 μm. DIT indicates diffuse intimal thickening and PIT is pathologic intimal thickening. Reproduced, with permission, from [77]. This high-resolution image is courtesy of Dr Nakashima.

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A validity of the consensus lipid hypothesis of pathogenesis of coronary atherosclerosis has been explicitly challenged in recent times, notably by Stehbens [111–126] and Ravnskov [127–141]. Objections highlighted incoherencies of the accepted view and offered alternative detailed pathogenetic models: hemodynamic stress and aging by Stehbens [142–147] and infection hypothesis with lipoproteins binding with microbes forming aggregates impairing arterial *vasa vasorum* by Ravnskov, this impairment was thought to be aggravated by hyperhomocysteinemia [148], see also [149,150].

The Ravnskov and McCully hypothesis [148] on development of the vulnerable plaque is of special importance because it could also explain puzzling observations of small necrotic damage of the coronary intima, occurring before atherosclerotic alterations; with frequency of such necroses increasing with aging [151]. These findings were reported by Velican and Velican (renowned experts in coronary pathology) [151], and later were attributed to vasospasm of the coronary *vasa vasorum* [152]. Although potent *vasa vasorum* contraction is possible (e.g., shown as concentration-dependent to administration of endothelin-1 and other endogenous stimuli) [153,154], the Ravnskov–McCully hypothesis [148] gives a parsimonious explanation for these early intimal necrotic alterations. However, these (and some others) well-thought alternative hypotheses have never been considered by the accepted mainstream opinion as pathogenetic models of the disease, although today's achievements in coronary atherosclerosis therapeutic treatment still are, at best, very modest.

Recently, the accepted view on coronary atherosclerosis pathogenesis has been challenged again by new interpretation (Seneff and coauthors) of the disease as the cholesterol sulfate deficiency syndrome, emphasizing increased blood viscosity [155], actually bridging to Ravnskov and McCully hypothesis [148]. It remains to be seen whether this novel hypothesis could alter traditional approaches in selection of therapeutic targets for treatment of coronary atherosclerosis. Nevertheless, the hypothesis regarding lipid invasion from the arterial lumen into the coronary wall was further advanced in a series of publications by Ross and coauthors [156–179] and formulated as 'the response-to-injury hypothesis' [160]. This hypothesis was proposed after experiments with de-endothelialization of the iliac artery of *Macaca nemestrina*. This experimental injury resulted in smooth muscle cell (SMC) proliferation and intimal thickening; the authors also suggested that the loss of endothelial integrity facilitated lipid invasion from the lumen into the arterial wall and stimulated SMC migration and proliferation [156]. Interestingly, the initial hypothesis by Ross and coauthors emphasized the proliferation of arterial SMCs in the *tunica intima* as the initiation of the pathology [156–158,180], whereas later analyses put an emphasis on the inflammatory nature of coronary atherosclerosis [174,175,178,179]. Again, although the research mentioned in one publication that the amount of intimal smooth muscle increases with age [159], the series of publications has depicted a normal coronary artery design with the *tunica intima* as a single-cell-layer compartment [159,160,180].

However, because the following studies have shown that endothelial injury is neither necessary nor sufficient for human coronary atherosclerosis initiation (e.g., [181]) and because the subendothelial space is highly accessible to lipoproteins even with intact endothelium [182], the response-to-injury hypothesis was modified. Williams and Tabas proposed the response-to-retention hypothesis to accommodate new observations [49,183]. This hypothesis proposed the subendothelial retention of atherogenic lipoproteins as the central pathogenic process in atherosclerosis [49,183,184]. Although stress-induced changes in the arterial wall were suggested as important preconditions, subendothelial lipoprotein retention was proposed as an absolute requirement for atherosclerosis initiation, which could take place even in the absence of stress-induced changes in the arterial wall [49,183,184]. The response-to-injury hypothesis continued to be adherent to the view that the human coronary *tunica intima* is a single-cell-layer compartment or is negligible in respect to the number of cell layers compared to *tunica media* [184–186]. Assumptions from this group on the initiation of human coronary atherosclerosis are based on the perception of the coronary *tunica intima* as a single cell layer or as a tissue compartment of insignificant thickness compared to *tunica media*. Notably, this depiction appeared even in the publication by Tabas *et al.*, in which the authors reprinted the microscopic images of coronary arteries from the Nakashima *et al.* publication (Fig. 4) showing that the *tunica intima* is thicker than the *tunica media* [187]. The response-to-injury hypothesis [156–179] and the response-to-retention hypothesis [184–186] assumed that SMCs migrate from media to intima and proliferate, thereby they refute initial intimal cell proliferation as the pathogenesis of the disease.

Another research development supporting lipid invasion from the arterial lumen into the coronary wall was carried out by Hansson and coauthors [188,189]. Although this research was mostly dedicated to pathology related to later events (mature plaque), it undoubtedly supports the pathway of lipoprotein invasion from luminal blood into the coronary wall [58,61]. Again, the perception of this research is that the human coronary *tunica intima* artery is a single-cell-layer compartment [58,61,63,66,190].

Undisputed facts on human coronary morphology and available information on the initial patterns of lipid deposition into the coronary wall

All of the above hypotheses are commonly based on two major notions: (i) the presentation of the coronary *tunica intima* as a thin, single-cell-layer compartment or a compartment with scarce residual cellularity; and (ii) the conjecture of lipid invasion from the luminal site into the coronary artery wall. As we can see, the first notion of coronary atherosclerosis research (the human coronary *tunica intima* is a thin single-cell-layer compartment or a compartment with scarce residual cellularity) contradicts all known facts of human coronary morphology and thereby is purely incorrect. The second notion could appear rational under the once-suggested assumption that the human coronary *tunica intima* is vascularized from the coronary lumen. This hypothesis has proposed the nutrition of the *tunica intima* from a vascular net originating from the arterial lumen [191] (republished as [192]). However, later investigations have attributed the finding of 'luminal vasculature' to an inappropriately high pressure of a dye solution injected (ten times higher than normal arterial pressure), for details see [93]. Later investigations either do not confirm luminal vasculature in the human coronary at all or, at best, give it a negligible role in intimal nutrition in normal and pathologic conditions [93,193–196].

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It has been thoroughly confirmed today that the normal coronary *tunica intima* is an avascular compartment [197]. All observations showed that the normal human coronary *tunica intima*, evolving from a single-cell-layer after birth to DIT in adults, is always an avascular compartment and remains avascular in normal hearts throughout life. Several studies have investigated this topic thoroughly and concluded that the coronary *tunica intima* receives oxygen and nutrients through diffusion from the arterial lumen and the medial *vasa vasorum* [93,198–202]. The previous suggestion that the luminal vasculature can contribute to coronary *tunica intima* nourishment [191,192] was never confirmed. Therefore, when DIT attains a thickness of several-to-ten cell layers (in humans, at approximately five years) the inner and outer compartments of the *tunica intima* are exposed to various concentrations of blood constituents because diffusion is inversely proportional to the square of the distance (i.e., DIT thickness). When this distance is increased, as happens in adult coronary DIT, it must be assumed that contact of outer (deeper) intimal layers with certain blood constituents would be significantly minimized, if not completely diminished. Therefore, for adult and normal aged-thickened coronary *tunica intima* [22,203,204], the pattern of nutrition by diffusion to the outer (deeper) intimal layers should be assumed similar to that of the arterial media in the Wolinsky and Glagov model, known as the 'critical depth' of avascular media or 'rule 29′ [205]. Therefore, the second notion (lipid invasion from a luminal site into the coronary artery wall) is not coherent either with facts of avascular design of the *tunica intima* or with the patterns of initial lipid deposition at the initiation of human coronary atherosclerosis [77,78,80,81].

Paradoxical patterns of initial lipid deposition in the human coronary artery

The facts are: (i) the initial lipid depositions have begun to appear in human coronary atherosclerosis in a region of the coronary *tunica intima* that is distal to the coronary lumen; (ii) no depositions are observed in regions of the *tunica intima* proximal to the lumen at the same time. Using common sense, such a counterintuitive pattern of initial lipid depositions constitutes a paradox.

Observations that are paradoxical to a hypothesis probably occur in all scientific studies because observations often arrive ahead of knowledge. There are two main ways paradoxical observations can be handled. One way is to supplement the initial hypothesis by conditions under which a paradox could be explained as a logical event. An alternative solution is to offer a new hypothesis in which a paradoxical observation initially appears as a logical event. Both approaches are valid and are commonly used in scientific practice. Both approaches have also been used to explain the paradoxical pattern of initial lipid accumulation in coronary atherosclerosis – complementing the initial hypothesis with new conditions [77,78,101,102] and offering a new hypothesis [206]. However, such logical approaches are extremely rare in analyses of coronary atherosclerosis.

Surprisingly, mainstream analyses of coronary atherosclerosis have chosen to pursue a third approach: presenting the consensus hypotheses grounded on lipid invasion from the arterial lumen while depicting the human coronary *tunica intima* as a thin, single-cell-layer compartment and not mentioning the counterintuitive distal lipid depositions. In such pathogenic models of coronary atherosclerosis, the paradoxical initial deep lipid depositions are simply omitted. I believe that we should not shrug off paradoxical observations of disease initiation just because they could not be explained by the consensus hypothesis, especially considering our limited achievement in the prevention and cure of coronary atherosclerosis [207,208]. I strongly believe that analysis of paradoxical patterns of the disease initiation could help us think out of the box and reveal obscure mechanisms of disease pathogenesis [206].

Could there be an alternative route for initial lipid depositions into a deep part of coronary tunica intima?

Everything eventually comes to a simple question: if at the initiation of coronary atherosclerosis lipids do not invade the coronary wall from a coronary lumen proper where do they come from? If there is no valid model of lipid invasion from the coronary lumen, is there an alternative route via which lipids can come? Yes, there is. This alternative route of lipid deposition is the vascularization of the coronary *tunica intima* from the adventitial or medial *vasa vasorum*, which has been shown by many studies to contribute to coronary plaque progression and instability (bear in mind that coronary plaques are confined to the coronary *tunica intima*) [200,209–214].

The above plaque vascularization route has been shown by numerous studies and is assumed to be the route from which additional lipoproteins and inflammatory cells invade coronary plaques and surrounding intimal tissues, significantly aggravating pathology (i.e., plaque enlargement, instability and rupture) [209,214–222]. These newly formed vessels originated from the adventitial/medial *vasa vasorum* [209,214,216–225]. However, the above research stops short from suggesting the adventitial/medial *vasa vasorum* route as the source of lipid depositions as initiation of coronary atherosclerosis. Moreno *et al*. suggested the following hypothesis: ''Adventitial-derived *vasa vasorum* neovascularization develops under the trigger of oxidized low-density lipoprotein deposits in the intima, mediated by hypoxia and Toll-like receptors'' [200], obviously designating intimal neovascularization as the event induced by pre-existed lipoprotein depositions.

Therefore, if the above route, which is the alternative route to lipid cell invasion from the coronary lumen, is undoubtedly confirmed to contribute to advanced atherosclerotic pathological formations in the coronary *tunica intima*, the inevitable question is: could the same vascular route cause not only progression but also the initiation of the disease? This question receives a positive answer: yes, this route is very credible for the initiation of lipid depositions. There are numerous observations showing that neovascularization of the coronary *tunica intima* from the adventitial *vasa vasorum* occurs under a variety of conditions before the occurrence of any pathologic features in the coronary artery [93,94,196,197,199–201,226–230].

This neovasculature extends from the *tunica media* vasculature, penetrates the internal elastic lamina and forms a capillary net in the deep layers of the coronary *tunica intima* [227,231]. This intimal neovascularization pattern of the *tunica intima* is common for all arterial DITs that begin to evolve into diseased conditions [226,229]. Adding these facts to the paradoxical initial lipid deposition in deep distal layers of the *tunica intima* (where there is no initial lipid deposition in layers proximal to the coronary lumen) has turned the paradoxical initial lipid distribution into a logical event.

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Why does the coronary tunica intima become vascularized?

But why does the coronary *tunica intima* become vascularized in the absence of any visible histopathologic alteration? What events in the coronary wall would promote this morphogenesis? It is well known that coronary atherosclerosis is an aging-related disease [232–234]. After the coronary *tunica intima* differentiates from a single-cell-layer compartment (after birth design) to a multi-layered adult *tunica intima* (adult design) this compartment remains avascular, receiving sufficient oxygen and nutrients through diffusion from the arterial lumen and the adventitial *vasa vasorum*, terminating in two-thirds of the *tunica media* [93,199–202].

However, the cells of the *tunica intima* remain the most replicating cell population in the arterial conduit including in the coronary wall [235]. It has been long known that excessive cell proliferation in coronary *tunica intima* can be triggered by a variety of diverse nonspecific signals. Friedman writes: ''...an artery responds to almost any sort of physical or chemical injury to which it may have been exposed with a hyperplasia or replication of its surviving or still-intact remnants. Thus, whether the injury is induced by transplantation, by needle puncture, by freezing, by heat, by exposure to electron radiation, by induced hypertension, by cholesterol infiltration, or by pressor amines, the typical response on the tunic affected is a hyperplastic one'' [236].

It has been confirmed by many studies that the coronary *tunica intima* (i.e., DIT) gets thicker with aging, along with the *tunica intima* of other large elastic arteries [203,237,238] and can transition from DIT to pre-PIT. However, whereas DIT occurs mostly evenly in the coronary circumference [21], pre-PIT lacks regularity and in some areas the *tunica intima* becomes initially enlarged more than in others [239,240].

What happens when the coronary *tunica intima* becomes larger owing to cell proliferation? A straightforward answer was given by Osborn: ''When the intima of the coronary artery exceeds a certain thickness parts must either die or develop secondary blood supply'' [241]. Because tissue hypoxia is a known inducer of angiogenesis and neovascularization [242–244], neovascularization of the hypoxic deep compartment of the enlarged coronary DIT from the adventitial/medial *vasa vasorum* must follow coronary DIT expansion. Therefore, the only logical deduction from the above facts is that intimal proliferation and/or thickening causing neovascularization as a result of hypoxia of deep layers of the *tunica intima* opens a door for a direct lipid extraction from the leaky neovasculature [242,243,245–247] by components of deep layers of the *tunica intima* (i.e., by biglycan).

Because the biglycan in the deep *tunica intima* of the normal adult human coronary is always separated from blood flow by many cell or matrix layers, it is never exposed to certain blood constituencies, and it is likely that blood lipoproteins are never in contact with the deep compartment. In this sense, matrix components of the deep layers of the coronary *tunica intima* can acquire some properties of privileged tissue (high affinity to LDL-C) by differentiating in a special environment, similar to the immunologic privileges defined by developmental anatomy [248,249]. As soon as newly formed capillary nets penetrate into deep layers of the coronary *tunica intima*, which never were in contact with free blood-flow before, biglycan (and possibly other matrix components that evolved in such an avascular environment) starts to bind lipoproteins and extracts them from the blood.

New pathogenetic model of the initiation of coronary atherosclerosis

This pathogenetic model of the initiation of coronary atherosclerosis, which incorporates all facts (real morphologic design of human epicardial coronary arteries, paradoxical patterns of lipid deposition caused by preceding neovascularization of deep layers of coronary *tunica intima*), was detailed in an early publication [206] and is depicted here in Fig. 7. The above hypothesis also explains why there is always a fibrous cap on top of a plaque [83,213,250–252] – because plaques are always initiated under fibromuscular *tunica intima*.

Suggested mechanism of the initiation of lipid deposition is not unique to coronary atherosclerosis

The suggested mechanism of the initiation of lipid deposition pathology is not unique. An identical mechanism, involving neovascularization of a normally avascular tissue compartment, followed by lipoprotein deposition, is well known for corneal lipid keratopathy. The central cornea is normally an avascular compartment [253–255]. More than 50 years ago, Cogan and Kuwabara described cornea lipid keratopathy, consisting of lipid deposition in the corneal *substantia propria* followed by fatty plaque formation, as occurring only in corneal areas that have been previously neovascularized [256]. The authors also pointed to morphologic similarities between cornea lipid plaques and those in atherosclerosis and suggested common pathogenesis [256]. In the following years, numerous reports reaffirmed a causal role for neovascularization in corneal lipid deposition and, hence, the main treatment modality has become the inhibition of neovascularization [253,254,257–269]. It was also noted that the contribution of inflammation to this pathogenesis is limited to the induction of angiogenesis [264]. Lipoprotein levels of the aqueous humor are thought to be close to those of blood [270–275]. It is important to note that, although the corneal *substantia propria* is separated from the aqueous humor only by a single-cell-layer of Descemet epithelium, lipid depositions were never (or extremely rarely [276]) observed before corneal neovascularization. Interestingly, another member of the proteoglycan family, a small, leucine-rich lumican, which has a vital role in embryonic development and tissue repair, is also predominantly expressed in the adult cornea and in cells of thickened coronary intima [277]. Lumican has also been implicated in lipid retention in atherosclerosis [278]. These facts strongly favor the model of direct lipid extraction from the permeable neovasculature into the cornea proper rather than a diffusion model. The fact that a similar sequence of events underlies the pathogenesis of the completely unrelated corneal disease, in terms of neovascularization and lipid deposition, reinforces the suggested new hypothesis on the mechanisms of coronary atherosclerosis [206].

Logic of scientific hypotheses

Facts showed that lipoprotein accumulation in coronary *tunica intima* is not the initiation of coronary atherosclerosis but is a consequence of enlarged intimal thickness, cell hypoxia and intimal neovascularization. I completely agree with Geiringer's assertion that ''... intimal vascularization is a function of intimal thickness and not of atherosclerosis'' [93]. Furthermore, enlarged intimal hyperplasia, or pre-PIT (i.e., cell proliferation in *tunica intima*), is the cause of coronary atherosclerosis.

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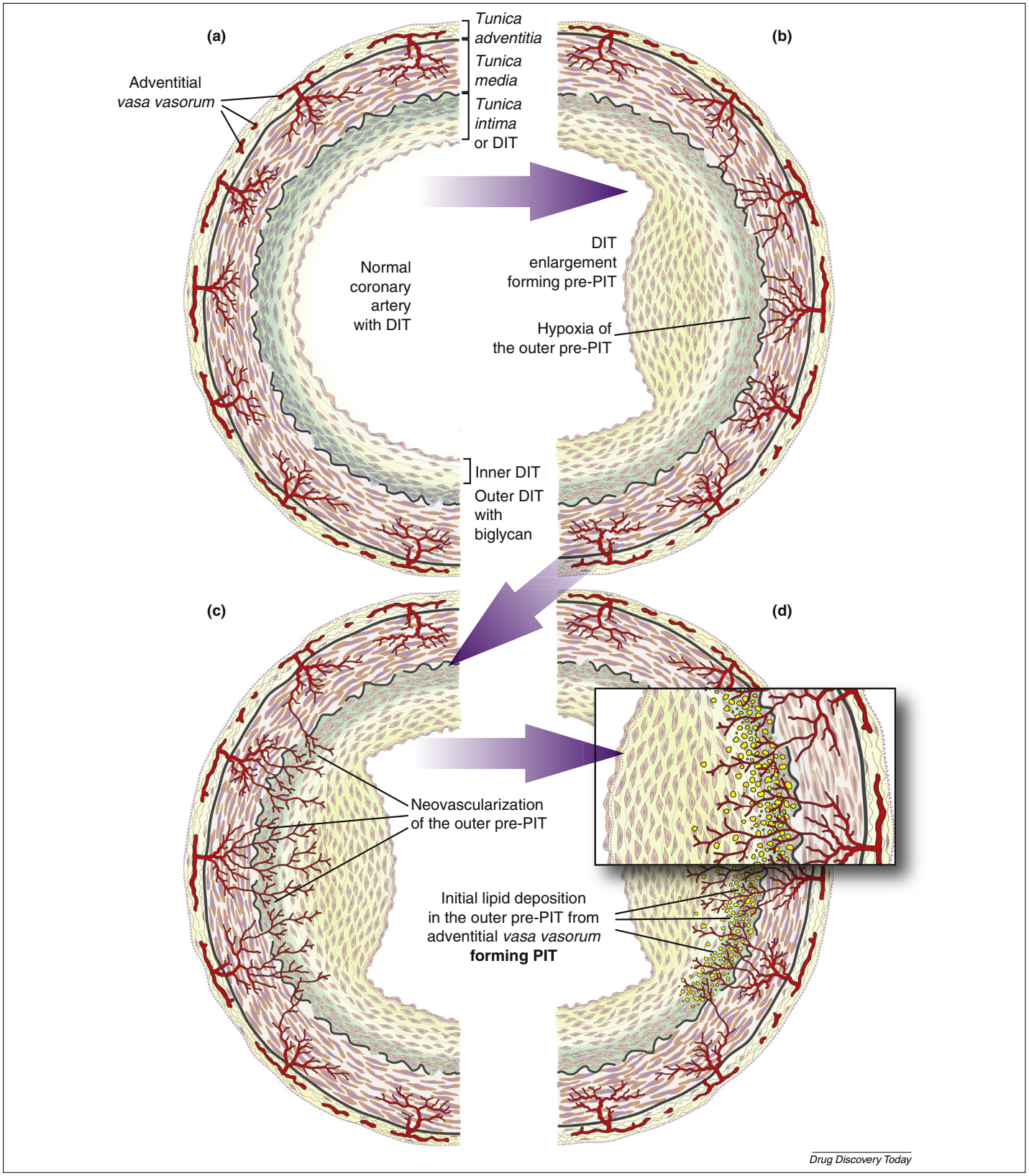

FIGURE 7

Schematic representations of the mechanism of coronary atherosclerosis. **(a)** Normal coronary artery. The coronary *tunica intima* forms diffuse intimal thickening (DIT) with biglycan accumulations in the outer DIT, which is most distant from the arterial lumen. **(b)** DIT enlarged by cell proliferation forming pre-pathologic intimal thickening (pre-PIT). Cells in the outer pre-PIT underwent hypoxia caused by increased diffusion distance. **(c)** Neovascularization of the outer pre-PIT from the adventitial *vasa vasorum*. Newly formed vessels are highly permeable. **(d)** Biglycan of the outer pre-PIT comes into direct contact with blood low-density lipoprotein cholesterol (LDL-C), which facilitates binding, retention and deposition of LDL-C in the outer PIT, whereas the inner part of PIT is free from lipoproteins. This schematic stage (d) corresponds to fatty streak Grade 1 and Grade 2 in the Nakashima *et al*. study [62].

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The current consensus hypothesis on the causation of coronary atherosclerosis (where high levels of LDL-C is the major culprit) is almost unanimously accepted, with only rarely a new hypothesis challenge [155]. The consensus hypothesis has completely superseded an earlier viewpoint that coronary atherosclerosis is initiated by proliferative intimal morphogenesis [279–281]. However, given tremendous effectiveness of current lipid-lowering therapies, how can we explain their inability to eradicate even a majority of cardiovascular events [207]? Coronary atherosclerosis is the major cause of cardiovascular events. How can we explain coronary atherosclerosis in subjects with normal cholesterol levels [282], and even in vegetarians [283,284]? It appears that the scientific and medical communities are focusing on and emphasizing biomarkers that can predict risk, without proof that these biomarkers cause the risk [63,285].

Mechanisms of diseases constitute a fast-developing, state-of-the-art scientific field, which often stretches beyond the scope of established medical concepts. Although well recognized in the past, medical concepts are not always proved to be correct; the author believes that a newer hypothesis should not contradict established concepts that have been proven to be correct so far without good reasoning. Although thoughts on a cause responsible for a phenomenon date back to Aristotle's time, applicability of the thesis to diseases was formulated by the founder of the experimental medicine Claude Bernard: ''Indeed, proof that a given condition always precedes or accompanies a phenomenon does not warrant concluding with certainty that a given condition is the immediate cause of that phenomenon. It must still be established that when this condition is removed, the phenomenon will no longer appear...'' [286].

It is well known that multiple factors participate during disease development, and can affect the progression and severity of disease. However, we have to choose one or just a few factors that are probably causative and potentially can constitute the therapeutic targets. Therefore, only through distinguishing the cause from all contributing factors can an effective therapeutic target be identified and a cure, leading to disease eradication, be achieved.

In modern medicine this thesis was formulated by William Stehbens: ''...differentiating between cause and non-causative factors is essential. Elimination of the latter only ameliorates or reduces the incidence whereas elimination of the former eradicates the disease. Swamps are not a cause of malaria. Draining swamps may reduce the incidence of malaria but it is eradication of the malarial parasites that eliminates the disease. Reduction in incidence rather than elimination of the disease precludes a causal relationship'' [287]. The notion on refutability of the consensus hypothesis on pathogenesis of coronary atherosclerosis was directly addressed by Uffe Ravnskov [134]. In this light, facts that coronary atherosclerosis occurs in subjects with normal cholesterol levels [282], even in vegetarians [283,284], and lowering LDL-C levels does not prevent cardiac events in the majority of those at risk of a cardiovascular event [207] contradicts the causative role of LDL-C.

Concluding remarks

Although this review suggested that the uncontrollable cell proliferation in coronary *tunica intima*, followed by neovascularization, is the initiation of coronary atherosclerosis and a potential therapeutic target, the analysis intends only to outline practical implementation of this suggestion. Although growing evidence suggests that targeting the hypoxia pathway and intimal neovascularization has a great therapeutic potential [288–290], even with this achievement the presence of an enlarged hypoxic intimal compartment in the coronary artery would still persist, inducing neovascularization. The latter brings us back to the Osborn notion: ''When the intima of the coronary artery exceeds a certain thickness parts must either die or develop secondary blood supply'' [241]. In regard to inhibition of intimal neovascularization, this review also perceives obvious concerns about antiangiogenesis therapy in subjects with an already jeopardized myocardial blood supply. However, even if very local antiangiogenesis actions become available, another side of such actions should be considered.

It was suggested a long time ago by Groszek and Grundy that the intimal neovasculature could be the route by which lipoprotein depositions can be extracted from coronary *tunica intima* [94]. It is worth to recall again that ''... intimal vascularization is a function of intimal thickness and not of atherosclerosis'' [93]. This review suggests that, if coronary pathologic intimal thickening and initial lipoprotein depositions are diagnosed at early stages of coronary atherosclerosis (Grade 1–2 fatty streaks, Grade 3 PIT), the disease could be reversible. If further cell proliferation and expansion of coronary *tunica intima* are prevented at a stage when lipoprotein depositions still reside in viable coronary tissues, before forming a solid lipid compartment, neovascularization of coronary *tunica intima* is a potentially crucial route for lipoprotein removal and disease reversal [94]. The implementation of the suggestions above is an extremely difficult task but, unless we start to work toward this goal, our research and therapeutic efforts will probably be off-target.

Finally, this review rests with the conclusion that uncontrolled cell proliferation in *tunica intima* of human coronary arteries is the initiation of the coronary atherosclerosis. Obviously, until an enlarged coronary DIT compartment (i.e., pre-PIT) persists, it continues causing hypoxia-inducing angiogenesis and neovascularization of deep layers of coronary *tunica intima* and lipid extraction or deposition. Logically, uncontrolled cell proliferation in coronary *tunica intima* is a cause of coronary atherosclerosis and constitutes a therapeutic target. But is a coronary atherosclerosis the only coronary disease in which intimal cell proliferation could be implicated as the initiation of pathology? Of course not – I have already mentioned that my attention to coronary atherosclerosis was determined by its status as the number one killer in the Western world. Of course, there are other coronary artery pathologies in which cell proliferation in the *tunica intima* is unanimously recognized as the major morphogenetic event. In the future we will analyze coronary pathologies that are completely unrelated to coronary atherosclerosis yet bear the same initiation hallmark – cell proliferation in the coronary *tunica intima*.

Conflicts of interest

The author has no conflict of interest to declare.

Acknowledgments

This review is dedicated to the memory of the renowned zoologist and my dear friend Rostislav A. Shilo (Ростислав Александрович Шило), October 1940 - April 2016, who introduced me to another

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world – the world of his zoo. I am lucky to be part of this world and the zoo has changed my life. Rostislav Shilo was Director (and creator) of Novosibirsk Zoo, Russia, which specializes in breeding endangered species for conservation.

The author thanks two anonymous reviewers for their constructive comments. The author is greatly indebted to a longstanding IT consultant Andrey Levochkin, Department of Physics, University of Wisconsin.

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Please cite this article in press as: Subbotin, V.M. Excessive intimal hyperplasia in human coronary arteries before intimal lipid depositions is the initiation of coronary atherosclerosis and constitutes a therapeutic target, Drug Discov Today (2016), http://dx.doi.org/10.1016/j.drudis.2016.05.017

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