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Abstract ... words

Apart from major illnesses and chronic afflictions, the elderly experience lesser ailments, such as muscle weakness, cold intolerance, and transient memory lapses. Physical signs in the aged include wrinkled skin & the slow healing of skin abrasions. These ailments and signs are grouped together because they may be due in part to an age-linked waning microcirculation. A reduced capillary density (**CD**) throughout the body of aged people and animals has been reported in over 40 papers. The reduced CD is due in turn to declining levels of angiogenic growth factors (**AGFs**) throughout the body during old age, as documented in 7 reports in the literature. From this perspective, old age is a deficiency state of AGFs, much like the reduced testosterone levels in elderly males. The above data on reduced CD and AGFs are the basis for the ‘angiogenesis hypothesis of aging’, whose corollary suggests pro-angiogenesis therapy for symptoms and signs of old age. Several angiogenic growth factors are now available in recombinant forms (e.g., vascular endothelial growth factor, **VEGF**) and have been used safely in animal experiments and in short term clinical trials.

PRO-ANGIOGENESIS THERAPY AND AGING

Introduction

Elderly persons may experience a range of medical conditions: a fatal disease (cancer, stroke, etc.), chronic afflictions (diabetes, arthritis, atrial fibrillation, etc.), and troubling lesser ailments. The last is a collective term for five minor symptoms and signs of old age, which include general muscle weakness, cold intolerance, minor memory lapses, skin wrinkles, and the slow healing of bruises or abrasions in the skin [1]. The lesser ailments of aging (LAA) are the focus of this review and are grouped together here because they may have a common vascular cause and be treatable, as next explained.

It is well recognized that atherosclerosis in arteries and arterioles leads to major illnesses -- stroke, heart disease, and peripheral vascular disease. Later in life, changes occur at the terminal end of the vascular tree, where capillaries develop looping, kinking, and extensive tortuosity [2]. Not commonly appreciated is that capillaries also undergo significant regression in absolute number. Over 40 published studies have reported a reduced capillary density (CD) throughout the body of aged animals and people. These findings are discussed below with representative data being listed in Table 1.

The development and maintenance of capillaries depend on angiogenesis (AG) -- i.e., on genetically programmed levels of angiogenic growth factors (AGFs). During early growth and

maturation of the body, the development and function of various organ systems involve rising levels of AGFs and an expanding microcirculation. But during old age, people and animals show declining levels of such factors in various organ systems, paralleling the reduced CD. Data from seven such studies are discussed later and presented in [Table 2](#). Thus old age represents a deficiency condition for AG factors, much like hormone levels which are decreased in the elderly.

The idea that the lesser ailments may be due in part to age-associated diminished CD/AGFs is termed ‘the angiogenesis hypothesis of aging’. Its corollary suggests that treatment with exogenous angiogenic factors should restore reduced CD in areas experimentally depleted of capillaries and may improve function in areas of naturally impaired microcirculation. Recombinant angiogenic factors have been shown to induce new capillary formation in ischemic and normoxic tissues within days, as observed in numerous animal studies mentioned later. Thus in theory, pro-angiogenesis therapy may ease the LAA after they have appeared or delay their development. This is in contrast to the pathology in the larger blood vessels, where fatty plaques and cholesterol deposits cannot be readily eliminated once acquired but only prevented in the decades before old age by avoiding risk factors -- i.e., obesity, diabetes, hypertension, etc. [\[3\]](#).

Because of the biographical constraints of this journal, this paper has been limited to presenting studies with key data in support of the angiogenesis hypothesis and in advancing its therapeutic corollary. Not covered here are detailed discussions of specific angiogenic growth factors, therapeutic regimens with recombinant forms, and other potential treatments involving gene transfer or progenitor cells. These particular matters were treated in prior papers and in numerous primary sources cited in them [\[4,5\]](#).

Reduced Capillary Density in Old Age

The age-linked reduction of capillaries in the brain and muscle was the main focus of several earlier reviews [4,5]. Representative data from both organ systems are presented immediately below. Since the publication of these papers, additional examples of a reduced CD elsewhere in the body have been identified in other research reports and are included later and in [Table 1](#).

Brain. An age-linked reduction in capillaries was first described in the brains of older rats by E. Horne Craigie in the 1920s [6]. For example, he found CD values of 856μ and 638μ in the *regio insularis* of 2-month and 13-month old rats, respectively. Here CD values are shown as ‘data pairs’ (e.g., 856μ vs. 638μ), indicating average values found in adults versus those in the aged. Listed in [Table 1](#) are seven representative data pairs from four reports [6-9], showing reduced cerebral CD in old rats, aged people, and subjects with Alzheimer’s disease (AD).

Muscle. The reduction of CD with age has been reported also in skeletal muscles. For example, Rivard examined the CD in muscles of mice and found an average value of 710 mm^3 in 12-week old mice but only 350 mm^3 in 2-year old mice [10]. In the middle of [Table 1](#) are listed four representative data pairs from the muscles of mice, rabbits, and people [10,11].

Other organs. A similar reduction in the CD with age has been noted in other areas of the body -- skin, larynx, colon, kidneys, and lungs. For example, Helmbold et al. examined human skin and reported the average CD of 4.4 n/HPF for those age 39-40 years and only 2.3 n/HPF for those over age 70 [12]. In the lower half of [Table 1](#) are listed representative data from eight reports on various parts of the body [12-19].

Lungs. Among the LAA might be included senile exertional dyspnoea. While exertional dyspnea occurs at all ages and is generally due to cardiac insufficiency or obstructive lung

disease, during old age an additional cause may be a lowered alveolar gas exchange due to reduced alveolar CD. Butler and Kleinerman measured pulmonary CD and found a smaller capillary density to alveolar diameter ratio (CD/AD) in the lungs of older persons [20]. From Table 1 of their paper, I calculated that the CD/AD ratio averaged 0.47 for 15 measurements in five adults age 17 to 31 and 0.36 for similar set of data from older persons age 49 to 57. Georges et al. observed a decreased pulmonary capillary blood volume in subjects after 50 years of age [21]. Thurlbeck and Angus interpreted a reduction in alveolar parenchymal tissue in aged persons as reflecting a loss of pulmonary capillaries because of a widespread, age-linked reduction in CD [22].

Summary. Little mention has been made in clinical papers that a reduced CD occurs during aging throughout the body, but, as noted above, but this change has been reported in the research literature for seven different organ systems. Evidence of this reduction is the 20 representative data pairs from 14 reports presented here in Table 1. To date, a total of the 64 data pairs from 40 reports have been found, as listed above and in three previous papers [1,4,5]. The widespread reduced microcirculation is associated chronologically with the development of the symptoms and signs of old age in both higher animals and people. It seems logical to consider this reduction as one possible cause of the lesser ailments of old age.

Declining Angiogenesis in Old Age

The existence of a vasculogenic factor was first postulated in 1948 by Isaac C. Michaelson (1903-1983), a British ophthalmologist, who studied capillary growth in the developing retina of cats and people [23]. In 1971, Judah Folkman (1933-2008) isolated an angiogenic factor (later termed angiogenin) which promoted the growth of solid tumors by

developing a new capillary network nourishing them [24]. Transient local angiogenesis also occurs during the ovarian cycle and pregnancy and in areas of wound healing and other injuries involving ischemia -- e.g., cerebral infarcts.

To my knowledge, there has been no report which has measured the general development of the microcirculation throughout life relative to levels of angiogenic factors. But based on Craigie's study of capillary density in rat brains from birth to late maturity [6, its Fig.4], levels of these AG factors must follow a chronological course similar to that of CD -- i.e., they rise sharply during early life, peak with the full development of the capillary beds in organ systems, remain at a plateau level during maturity, and begin to decline in old age. Like the reduced CD during aging discussed in the previous section, waning levels of angiogenic factors in later life have been documented by many investigators in numerous organ systems (see below). As noted earlier, this age-linked decline seems analogous to the falling values of testosterone in older men and the declining levels of other hormones with age. An association between the changes in hormone level in the aged and the age-linked decline in AGFs was discussed in a prior paper [5].

Angiogenesis involves various vascular growth factors, some working in concert with others [25]. Three well-studied such agents are vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF-1 & -2), and angiopoietin (AP-1 & -2). These and other growth factors have been extensively reviewed elsewhere [26]. The aspect of angiogenesis relevant for this paper is the finding that these factors in the body decline during old age.

Organ systems affected. While there are no published reports on levels of angiogenic factors in the aging human brain, Viboolvorakul & Patumraj found reduced VEGF protein in the parietal cortex of aged rats -- e.g., values of 32.3 pg/mg protein in 4-5 month old rats vs. 20.5 pg/mg protein in 23-24 month old rats [27]. Reduced levels of VEGF protein and/or mRNA

VEGF have been recorded in other organ systems of the aged mice, rats, or humans -- i.e., in the muscles, kidney medulla, vein wall, mononuclear cells, and macrophages. In **Table 2** are listed ten representative data pairs showing diminished levels of angiogenic factors in six organs or cell systems of aged people and animals. [19, 27-32]. A general decline in AG throughout the human body would account for the reduced CD observed in many organ systems of the elderly. How such a decline may lead to aged-looking skin and long lasting bruises is explained below.

Aging Skin. The skin on the face, upper arms, and dorsum of the hands of elderly persons becomes loose and wrinkled due to loss of subcutaneous fat. Throughout adulthood, adipose tissue can expand or regress, as many weight watchers know. Body fat is highly vascularized with an extensive capillary network surrounding each adipocyte. Rupnick et al. reported that angiogenesis inhibitors “significantly decreased” adipose tissue in mice and concluded that adipose tissue mass is “regulated by its vasculature” -- i.e., via local angiogenesis [33]. Thus the age-linked decline in AG in the elderly people may cause depletion of subcutaneous fat notably in the hand dorsum, leading to the loss of a filling/smoothing effect there. Three data pairs showing a reduced CD in aged skin (likely also in its subcutaneous fat) are listed in **Table 1**. Visceral/abdominal fat is less responsive to angiogenic control, which may account for a fat belly in persons with wrinkles elsewhere [34].

Slow healing skin lesions. Bruises and small abrasions on the skin are more conspicuous in elderly people than in younger ones, perhaps because these injuries are slower to heal in the former. Various conditions account for this greater incidence, including increased capillary fragility with age. In old mice and rats slow healing has also been ascribed to a “decrease in capillary growth” and “delayed angiogenesis” [35,36].

Pro-Angiogenesis by Angiogenic Growth Factors

Support for the therapeutic corollary of the angiogenesis hypothesis comes from studies showing that recombinant angiogenic growth factors improve the local microcirculation or function not only in ischemic areas but also in normoxic organs.

Animal brain studies. See **Table 3A** [37-40]. Using an osmotic minipump, Rosenstein et al. infused recombinant VEGF to a 3 mm depth near the coronal/sagittal sutures in normal adult rats and observed “remarkable neovascularization” in the involved cortical area [37]. Another group injected basic fibroblastic growth factor into the lateral ventricle of normal rats and reported increased angiogenesis in the adjacent brain cortex [38]. Thau-Zuckner et al. administered VEGF into the later ventricle of mice following traumatized brain injury and noted increased angiogenesis in the affected area [39]. Improved motor function and memory followed intraventricular doses of VEGF in mice and rats subject to focal cerebral ischemia [40].

Animal muscle studies. An experimental model for inducing angiogenesis in muscles has involved occluding the femoral artery of a hind limb of rabbits, rats, or mice to render it ischemic. Different AGFs (VEGF, bFGF) were injected by various routes and produced an increased local CD. Three such reports are listed in **Table 3B**, where the details of therapy are abstracted in the middle column [10,41,42].

Clinical muscle trials. Persons with limited walking tolerance (ca. 5 minutes) due to intermittent claudication were given bilateral femoral artery infusions of FGF-2 according to various schedules. In some groups, the Day 90 walking time was increased by 1-2 minutes beyond the base line level [43]. The maximum tolerated dose was 30 $\mu\text{g}/\text{kg}$; higher level induced acute hypotension. Cautions concerning pro-AG therapy were discussed elsewhere [4].

Clinical myocardium trials. Patients with impaired cardiac function have received one or several injections into the myocardium of recombinant proteins of various angiogenic growth factors [44]. While initial reports suggested favorable results, later evaluations showed equivocal long-term clinical benefit in double blind, randomized placebo-control trials [44]. However, these studies demonstrated the safety of the recombinant factors. A table in the paper by Annex and Simons lists the results in nine clinical trials involving angiogenic protein therapy in subjects with myocardial insufficiency [44].

Clinical brain study. Harry S. Goldsmith performed an extraordinary surgical procedure which suggested that raising the cerebral level of AGFs would benefit persons with Alzheimer's disease. The rationale for his work rested on the extremely high levels of VEGF protein in rat omentum -- viz., 884 pg/mg compared to 8 pg/mg (sic) in the brain brain [45]. Goldsmith 'stretched' an omental pedicle flap from the transverse colon of human subjects with AD onto the surface of their brains. This was done by pulling an omentum flap from the colon through a subcutaneous pathway up the anterior abdominal wall, chest, and neck to the base of the skull. After being brought through openings in the skull, the dura mater, and arachnoid membrane, the end of the omentum flap was laid directly on the parietal-temporal area of one hemisphere -- all the while maintaining its original circulation [46]. Some AD subjects subsequently exhibited marked cognitive improvement [46]. Post mortem examination of a dozen or so AD cases given such flap-grafts showed that collateral circulation had been established in the area under the pedicle and had also increased the microcirculation in zones apart from the omental placement, such as the occipital area and contralateral cerebral hemisphere [46]. These findings indicate that local angiogenesis was induced by the growth factors released into the brain from the viable flap.

Advocates of Pro-Angiogenesis for Maintaining the Microcirculation

Pro-angiogenesis treatment of the elderly has inferential support from the comments of investigators regarding the importance of angiogenesis in maintaining the microvascular system. In 1986, Bär wrote that the capillary plexus “needs a continuous action of an angiogenic stimulus” [47: p. 223]. In 1992, Ferrara concluded that the “presence of growth factor[s] may be required to maintain the differentiated state of those vessels” [48: p. 28]. In 2005, Carmeliet stated, “Threshold levels of VEGF [are needed] for the survival and maintenance of quiescent vessels in healthy organs” [49: p. 936].

Baumgartner and Isner prefaced a 1998 article as follows: “Angiogenic growth factors constitute a potentially novel form of therapy for patients with ischemic vascular diseases” [50: p. 201]. In 2011, Carmeliet & Jain concluded that “The revascularization of ischaemic tissues would benefit millions, but therapeutic angiogenesis is an unmet medical need” [26: p. 298].

Clinical assessment

Evaluating the clinical benefit of any pro-angiogenesis therapy in the elderly would rest mainly on subjective self-appraisals, such as sensing improved strength or noting ‘a sharper memory’ -- fewer instances of lapsed recall of names or facts. Currently, the only objective measurement of an increased CD would be histologic studies on muscle biopsies or at autopsy. CD visualized under the fingernails, in the forearm skin, or in the conjunctiva have as yet insufficient background data on which to evaluate any small improvement during a therapeutic trial. Simons remarked on the absence of any useful biomarkers for CD in subjects in a clinical trial and cautioned about a placebo effect [25].

Conclusions

Countless theories have been advanced to explain aging in people, but none has led to a widely accepted treatment based on reversing an underlying cause [1,51]. Physiological aging is commonly assumed due to various causes. Indeed, if aging is the result of several enfeebling influences, then lessening any one might ease its symptoms and signs. Again, there is abundant evidence in the literature that a reduced CD and a waning AG occur during old age. It seems likely that these linked changes influence the physiological state of the aged body -- accounting for its fading functions and possibly for the lesser ailments. A reduced cerebral CD may contribute to the more profound cognitive problems of old age -- e.g., Alzheimer's disease.

Animal studies described above establish that exogenous AGFs generate new capillaries. While numerous investigators have administered recombinant angiogenic growth factors to relieve specific conditions of ischemia in the human body, to my knowledge no gerontologist has proposed pro-angiogenesis therapy for moderating or delaying the wide-spread reduced microcirculation occurring during old age. Therapeutic pro-angiogenesis seems a tenable consideration for the lesser ailments of the elderly. The 30 data pairs in **Tables 1 & 2** bring to mind George Orwell's admonition, "To see what is in front of one's nose needs a constant struggle" [52].

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Table 1: CAPILLARY DENSITY (CD)

	<u>ADULT</u>	<u>AGED (or AD)</u>
BRAIN		
-- Craigie 1925 [6]		
-- rats ... regio insularis	856 (5 mo.)	638 (13 mo.)
-- Buchweitz-Milton 1987 [7]		
-- rats ... cortex	830 (8-10 mo.)	577 (21-33 mo.)
-- rats ... 6 brain areas	837 “	592 “
-- Mann 1986 [8]		
-- people ... frontal cortex	966 (26-58 yr.)	784 (76-96 yr.)
-- Fischer 1990 ... normal vs. AD subjects [9]		
-- pre-frontal cortex	94.6 (23-90 yr.)	75.4 (AD , 76-92 yr.)
-- basal forebrain	86.8 “	42.7 (AD “)
-- hippocampus	82. “	50.2 (AD “)
MUSCLE		
-- Rivard 1999 [10]		
-- mice	710 (12 wk.)	350 (2 yr.)
-- rabbits	170 (6-8 mo.)	130 (4-5 yr.)
-- Cogan 1992 [11]		
-- male subjects	308 (av. 24 yr.)	228 (av. 64 yr.)
-- female “	338 “	248 “

SKIN-- **Helmbold** 2006 [12]-- human chest area **4.4** (15-20 & 39-40 yr.) **2.3** (>70 yr.)-- **Vollmer** 2000 [13]-- hairless rat ear **77.6** (30 wk.) **37.5** (78 wk.)-- **Vybohova** 2012 [14]-- human chest area, %/unit area **1.42** (31-50 yr.) **0.47** (81-89 yr.)**LARYNX**-- **Russell** 2008 [15]-- rat thyroarytenoid muscle **17.0** (9 mo.) **9.35** (28-30 mo.)**COLON**-- **Gabella** 2001 [16]-- guinea pig colon muscle, CD **9**/unit area (n.s.) **5**/unit area (n.s.)**KIDNEY**-- **Stefanska** 2015 [17]-- mouse kidney cortex, CD **1.40** (3 mo.) **0.93** (27 mo.)-- mouse kidney medulla, CD **1.52** “ **1.04** “-- **Urbietta-Caceres** 2012 [18]-- mouse renal cortex, CD **30** (6 mo.) **17** (18-22 mo.)-- **Kang** 2001 [19]-- rat kidney, peritubular, CD **11.3%** (3 mo.) **5.4%** (24 mo.)

NOTE: n.s. = precise ages not stated ... The units of measurements for the above data differ among the studies and are omitted to keep these tables less cluttered.

Table 2: LEVELS of ANGIOGENIC FACTORS

	<u>ADULT</u>	<u>AGED (also AD)</u>
BRAIN		
-- Viboolvorakul 2014 [27]		
-- rats ... parietal cortex, VEGF	32.3 (4-6 mo.)	20.5 (23-24 mo.)
MUSCLE		
-- Wagatsuma 2006 [28]		
-- mice ... VEGF	1.0 (av. 2.5 mo.)	0.7 (22 mo.)
-- Ryan 2006 [29]		
-- men ... VEGF	1.05 (19-25 yr.)	0.55 (60-72 yr.)
-- men ... mRNA VEGF	1.0 “	0.4 “
-- men exercise ... mRNA VEGF	3.4 “	1.8 “
KIDNEY		
-- Kang 2001 [19]		
-- rat, kidney medulla, VEGF	39.3% (3 mo.)	19.2% (24 mo.)
VEIN WALL		
-- Drubaix 1996 [30]		
-- human ... bFGF	180 (20-24 yr.)	50 (61-82 yr.)
MONONUCLEAR CELLS		
-- Solerte 2002 [31]		
-- human ... VEGF	296 (av. 32.2 yr.)	137 (av. 77 yr.)
“ “	296 “	125 (AD, av. 76.5 yr.)
MACROPHAGE, activated by IFN-γ		
-- Swift 1999 [32]		
-- mice ... secreted VEGF, pg/ml	198.9 (2-3 mo.)	124.0 (22-24 mo.)

Table 3. TREATMENT WITH AN ANGIOGENIC GROWTH FACTOR**A. BRAIN**

-- Rosenstein 1998 [37]	rat	VEGF	3 mm depth near coronal/sagittal sutures	↑ vascular tissue in infusion site
-- Puumala 1990 [38]	rat	FGF2/b	left lateral ventricle	↑ CD in left perilateral ventricular cortex
-- Thau-Zuckner 2010 [39]	mice	VEGF	lateral ventricle	↑ angiogenesis in brain area traumatized
-- Wang 2006 [40]	rat	VEGF	intraventricular doses	↑ motor/memory function after focal cerebral ischemia

B. ISCHEMIC LIMB MODEL

-- Rivard 1999 [10]	rabbits, mice	VEGF, IV bolus	x1	→ ↑ CD
-- Baffour 1992 [41]	rabbits	rh bFGF, IM	qd, x2 wks	→ ↑ CD
-- Yang 1996 [42]	rats	bFGF, subQ pump	x14 days	→ ↑ CD

NOTE: ↑ = increase ... rh = recombinant