PART 1
Muscle Aging
CHAPTER 1
Aging of the human neuromuscular system: pathological aspects

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Introduction

This chapter discusses both our original findings and concepts, as well as some data of others from the literature. It is not able to cover all aspects of this broad topic. Selected references are presented to stimulate further exploration of the various points discussed.

Succinct introduction to the biology of the neuromuscular system, for clinicians

Aging persons often have progressive fatigability, weakness, slowness, and general frailty, accompanied by visible atrophy of limb muscles. The weakness frequently is a cause of falling, which can result in serious injury, and sometimes death. Healthy muscle is maintained by: (a) its own salutary trophic metabolic processes; (b) multifactorial trophic influences dispensed from its innervating lower motor neuron (LMN) that are received at each muscle fiber’s single neuromuscular junction; and (c) circulating trophic influences. The LMN itself is interdependent both (a) on normal trophic factors from the numerous myelin-containing Schwann cells surrounding its long axonal process like oblong beads on a string, and (b) on retrograde trophic influences acquired from its numerous muscle fibers at the neuromuscular junctions. Each LMN in the human biceps is responsible for activating about 200 muscle fibers and for the continuing trophic nurturing of good health of those muscle fibers. A motor unit refers to one LMN, its Schwann cells, and the muscle fibers it innervates. A neuromuscular disorder, or disease, is one arising from abnormality of any part of the motor unit.

The LMNs and lower sensory neurons have a vital interdependence with the Schwann cells that coat and nurture their axonal extensions: the neurons cannot survive without the Schwann cells, and vice versa. Just as trophic factors “emitting” from LMNs induce and control the special type-1 versus type-2 characteristics of the muscle fibers they innervate, the LMNs probably also induce and maintain hypothetically different sets of “type-1” and “type-2” Schwann cells, respectively, on themselves. And, probably the Schwann cells on sensory neurons are different from ones on motor neurons, because clinically there can be anti-Schwann-cell dysimmune diseases that rather preferentially affect either motor or sensory neurons, and even preferentially involve selectively large-diameter sensory nerve fibers (faster-conducting, conveying position, vibration, and touch sensations) or small-diameter sensory nerve fibers (slower-conducting, conveying pain signals).

A motor unit, with its arborizations, has been likened to a tree, the leaves being compared to the muscle fibers (I think that I shall always see, a motor unit as a tree; with apologies to Joyce Kilmer).
In regard to its loss of “leaves,” a tree in autumn, or a waning motor unit, can be affected in toto or in portio [1, 2]. In toto reflects all of the leaves becoming “malnurtured” at about the same time, and in portio is manifested as leaves on the more distal twigs being affected first, showing the first autumnal color changes (as is characteristic of maple trees).

The clinically evident muscle atrophy of elderly persons, which we call atrophy of aging muscle (AAM) (an intentionally general descriptive term), is often assumed to be strictly myogenous (defined as meaning a process involving only muscle, but not LMNs or their peripherally extending axons). However, based on our evidence, it is likely that in a number of circumstances AAM is ultimately neurogenic, i.e. caused by malfunction of the LMNs, or antecedently by impaired trophic influence of the Schwann cells on their LMNs. Because of its clinical, social, and economic importance, AAM will be discussed in regard to some facets of the known and putative malfunctions of the motor unit components, their causes, and their possible treatments.

Note that we use AAM instead of the term sarcopenia. “Sarcopenia” sounds like a definitive diagnosis but it is not. It is often erroneously interpreted as designating a singular pathogenesis. Sarcopenia simply refers, imprecisely, to muscle atrophy in aged animals; it does not indicate or imply any pathogenic mechanism, of which there are a number of possibilities. AAM is usually manifest as type-2 fiber atrophy. A further critique of “sarcopenia” is presented below.

AAM is not a definitive clinical diagnosis, no more than is anemia, or jaundice, or stroke; it is a reason to look carefully, in each individual patient, for a cause, and especially for a treatable cause. Several known causes are described below and in Chapters 2 and 3 in this volume. Whether there is also an as-yet-unidentified general pervasive cause (or causes) that eventually harms the muscles of every aging person is not known. Biochemical studies seeking a general, nearly universal cause typically do not intensively seek, in individual patients and in experimental animals, the possible presence of an identifiable and potentially treatable primary cause (such as peripheral neuropathy, nerve-root radicopathy, malnutrition, hyperparathyroidism, or a myovascular component).

Aging is a risk factor for AAM, but it is not an ultimate cause. “You’re just getting old” is not a cause of AAM, and clinically it certainly should not be used as a dismissive diagnosis of an older patient.

Cellular aging, in general
Despite a vast literature on cellular aging, the causes and mechanisms are still poorly understood, and treatment non-existent. Mature, post-mitotic muscle fibers, similarly to post-mitotic neurons, seem to be more susceptible to a chronic cellular aging than are dividing cells. Cellular aging involves abnormalities of various subcellular aspects, such as nuclei, mitochondria, endoplasmic reticulum, Golgi, and structural and aqueous components. Proteasome and lysosome degradations are especially important. Oxidative stress and endoplasmic reticulum stress are also proposed to play important roles. The “proteome” designates the large and varied family of proteins of a cell, the profile of which is cell-type-specific.

One can wonder whether the general aging changes of cells are due to effects of a still-obscure omnipotent “master vitalostat,” such as a “master gene” acting like a rheostat that gradually turns down the vitality of the cell. If there is a master vitalostat. What initiates the turning-down, what are the key steps by which it executes that turnover, and how can it be controlled? What are the underlying genetic factors, and/or important epigenetic mechanisms? (Philosophically, why are all living creatures programmed from “conception” to die?) In the atrophy process, there might be multiple stages and pathways participating, some of which, if identifiable, could become amenable to not-yet-developed treatments. Hypothetically, for skeletal muscle there might be at least two so-called master genes, Fiber2atrophin and Fiber1atrophin, that are normally inhibited, but when activated by an atrophy-promoting factor they instigate cascades of other genetic activations and inhibitions, resulting in preferential atrophy of type-2 or type-1 muscle fibers respectively. Preferential type-2 fiber atrophies are discussed below. (Preferential atrophy of type-1 fibers is seen in myotonic dystrophy type-1, a disease caused by
expansion of CTG trinucleotide repeats of the gene *DMPK*; and in preferential "congenital type-1 fiber hypotrophy with central nuclei" [3], which in some patients is attributable to a genetic mutation of myotubularin, myogenic-factor-6, or dynamin-1.)

**Some unanswered questions**

Is the muscle frailty associated with AAM universally inevitable, like the aging-related, more-visible frailty and atrophy of skin, like the failure of estrogen in menopausal women and the gradual petering-out of testosterone in aging men, like scalp follicles disappearing or producing only non-pigmented hairs, like vascular sclerosis, like accumulation of "wear-and-tear" lipofuscin pigment within lower-motor neurons, other neurons, and muscle fibers? What is the most essential mechanism that starts and perpetuates AAM? Is it something we all ingest, or do not ingest; is it the cumulative solar or cosmic irradiation, or Mother Earth’s constant radon emission; or perhaps there is something else to which we all are exposed? Is there a gradual cellular accumulation of something cumulatively more toxic than the accumulating lipofuscin – such as oxidatively damaged or otherwise-toxified misfolded proteins – that gradually "rusts" beneficent cellular functions and activates "atrophy processes"? Why can’t any of the pathogenic mechanisms putatively contributing to AAM be prevented or treated now? Much work needs to be done before we can prescribe an elixir to make the elderly intellectually brilliant and vigorous.

Indefinable are the terms “normal aged person” or “normal-control aged person.” In muscle biopsies of aging persons we nearly always have observed different combinations and various degrees of denervation atrophy and/or type-2 fiber atrophy (see below).

**Neuromuscular histology**

Normal skeletal muscle is the most abundant of human tissues. It is composed of muscle fibers that are very long cylinders. Their length is about 1000 times their typical diameter of about 45–65 μm (in the biceps). Transverse histochemical sections of muscle biopsies are diagnostically more informative than longitudinal ones. The universally used stain for general evaluation of muscle-biopsy histochemistry is the Engel trichrome [4, 5]. (It stains myofibrils green and their Z-disks red; mitochondria, t-tubules, longitudinal endoplasmic reticulum, and plasmalemma red; and DNA and RNA dark blue. It also stains the protein component of Schwann cell myelin red and neuronal axons green.) The histochemical types of human muscle fibers are most distinctively delineated by two myofibrillar ATPase reactions: (a) the regular ATPase (reg-ATPase) incubated at pH 9.4 [6], and (b) the acid-preincubated reverse-ATPase (rev-ATPase) [7]. (Some myopathologists also use antibodies against different types of myosin for fiber-type definition.)

In normal adult human and mammalian animal muscle, fibers lightly stained with reg-ATPase and reciprocally dark with rev-ATPase are arbitrarily designated type-1 fibers [4, 7–10], while the fibers oppositely stained are type-2 fibers. The type-1 fibers are high in most of the mitochondrial oxidative-enzyme activities (e.g. cytochrome oxidase (COX), succinate dehydrogenase (SDH), and hydroxybutyrate dehydrogenase), as well as myoglobin and triglyceride droplets; and they are low in the anaerobic glycolysis enzymes myophosphorylase and UDPG-glycogen transferase, in glycogen, and in the aqueous sarcoplasmic enzyme lactate dehydrogenase. The type-2 fibers are oppositely stained with those reactions. (Interestingly, the very useful mitochondrial oxidative enzyme menadione-mediated α-glycerophosphate dehydrogenase (men-αGPDH) is stronger in type-2 fibers.) Type-1 fibers have more capillaries adjacent to them and are better equipped for oxidative metabolism, and clinically are utilized for prolonged muscle activity. The type-2 fibers are better equipped for anaerobic glycolysis, and clinically are utilized for short bursts of more intense activity. In some neuromuscular disorders there is a rather selective involvement of one fiber type, and in other disorders the involvement is nonselective [11]. (A muscle biopsy, done as an outpatient procedure with local, not general anesthesia, must be from a muscle not recently needled.)
for electromyography, therapeutic injection, or "acupuncture therapy": these can produce confounding focal myopathy [12].

During normal development, each LMN induces and trophically maintains the distinctiveness and uniformity of histochemical and functional fiber type and subtype of its approximately 200 muscle fibers controlled by it as members of its motor unit. We have therefore hypothesized that there are, respectively, type-1 and type-2 LMNs, and A and B subtypes of each. (In the cat anterior horns, we were not able to histochemically distinguish the different types of LMNs from each other [13–15] but we could demonstrate that the large α-motor neurons are rich in phosphorylase and glycogen and poor in mitochondrial SDH, while the small neurons, namely the gamma efferents, rensshaw neurons and interneurons have the opposite histochemical profile.) Muscle fibers denervated from any cause gradually atrophy. If only some fibers in a muscle are denervated, they become small angular fibers when viewed in transverse sections (Figure 1.1a–e), progressing to become pyknotic nuclear clumps (Figure 1.2a). At some indefinable point, the atrophying fibers become incapable of attracting and/or accepting reinnervating nerve sprouts, but before that point of no return, they can be rescued by reinnervation. Muscle fibers can "switch" their histochemical type when denervated and then reinnervated by the type of LMN opposite to their original type of innervating LMN (i.e., foreign reinnervation) [16]. Denervated muscle fibers apparently can promiscuously accept comforting reinnervation from any type or subtype of LMN, a phenomenon commonly occurring in chronic neurogenic diseases that we have demonstrated experimentally in nerve-crush + reinnervation experiments [16–20]. In human muscle, this seemingly random foreign reinnervation results in type-grouping (Figure 1.3), which is evident as smaller or larger groups of the same histochemical fiber type replacing the normal, rather even inter-mixture of type-2 and type-1 fibers. When seen in a patient's diagnostic muscle biopsy, type-grouping is considered a manifestation of "established reinnervation" (Figure 1.3), namely previously denervated orphaned muscle fibers having been successfully reinnervated by neurite sprouts from nearby relatively healthy LMN axons of the opposite (foreign) type.

In abnormal human muscle, two situations produce muscle fibers of intermediate degree of staining with both ATPases: (a) partially converted fibers that are in the process of being “switched" due to foreign reinnervation, which is typically a neuropathic phenomenon (although in a myopathy there can sometimes be “myogenous de-innervation" due to muscle-fiber abnormality at or near the neuromuscular junction with survival of the more distal portion of the fiber thereby cut off from the innervation influence and thus able to accept foreign reinnervation); and (b) regenerating/degenerating (“regen-degen”) muscle fibers (RNA-positive, often alkaline-phosphatase-positive, and sometimes slightly acid-phosphatase-positive), which are usually evident in the setting of a myopathy, although a few of them can occur in the setting of prominent denervation (Engel, unpublished results) and in infantile spinal muscular atrophy [21].

Physiologically, type-1 fibers are considered to be slow-twitch and rather fatigue-resistant, while the type-2 fibers are fast-twitch and fast-fatiguing, as found in normal mammalian muscle [22, 23] and corroborated in human muscle [24, 25]. The designations slow-twitch and fast-twitch were introduced [4, 8, 9, 26] to distinguish the twitch properties of mammalian twitch-muscle fibers from amphibian non-twitch, extremely slow tonic fibers of the thigh adductor clasp muscles.

Relative paucity of one type of muscle fiber in a patient’s biopsy can be caused, hypothetically, by (a) preferential impairment of the corresponding type of LMNs or Schwann cells; (b) if both LMN types are equally abnormal, preferentially more successful sprouting and reinnervation ability of the opposite type of LMNs; (c) if there are large groups of both types of muscle fibers in a chronic reinnervation situation, biopsy sampling could produce a non-representative impression of paucity; or (d) preferential myopathic loss of that type of muscle fiber. (We use the term fiber-type paucity and not fiber-type predominance because it is more likely that the muscle fibers that are too few reflect the abnormal status.)

In our muscle biopsies of elderly patients, we have observed that type-1 fiber paucity (Figure 1.3d) is
Figure 1.1  (a–e) Recent denervation without innervation, evidenced by small dark angular muscle fibers; (a,b) amyotrophic lateral sclerosis; (c–e) dysimmune peripheral neuropathy. Also, moderately atrophic muscle target fibers each with a pale small central regions and often having three concentric zones of staining; (d,e) one large two-zoned muscle targetoid-core fiber (possibly a pre-target fiber); Dark dots within some normal fibers in (a) indicate esterase-positive lipofuscin collections. (a–c) Pan-esterase staining; (d,e) NADH-tetrazolium reductase staining. Magnification: (a) ×2000; (b) ×1330; (c) ×1830; (d) ×4330; (e) ×4170. Note that Figures 1.1–1.4, except Figure 1.2b, are transverse sections of fresh-frozen human biopsies. Muscle fibers are stained with various histochemical reactions.
evident more often than type-2 fiber paucity. In aging humans it is uncertain whether there is a gradual loss of spinal cord LMNs. If there is, possibly the type-1 fiber paucity could be due to an aging-associated gradual loss preferentially of type-1 LMNs.

More structurally labile are the type-2 muscle fibers, and especially the type-2B fibers. In human skeletal muscle: (a) they are more prone to selectively atrophy, which occurs in various conditions such as experimental pan-denervation [19, 20] (Figure 1.2b), glucocorticoid toxicity, disuse, cachexia, remote-effect of a neoplasm, and male castration; and (b) they are more prone to hypertrophy with work, especially in men. In normal young adult men the diameter of type-2 fibers is larger than of type-1 fibers, but in normal young adult women type-2 fibers are smaller (the gender difference has been attributed to more testosterone in men). There are two subtypes of normal type-2 fibers, 2A and 2B [27]. The 2B fibers are the more labile regarding atrophy and hypertrophy. At a less acidic acid-pre-incubation for rev-ATPase staining, the 2B fibers have properties intermediate between the 2A fibers and the type-1 fibers. In some type-2-fiber atrophies the subtype-2B fibers are the more prone to atrophy. There are also two subtypes of type-1 fibers, 1A and 1B [28].

Atrophy in aging human muscle: description and new concepts

The topic of type-2 fiber atrophy is large, multifaceted, and complex, and the subject of numerous experimental animal studies (selected references are given). Some of our personal concepts and general principles will be discussed here, but this is not a complete review of all possibly pertinent studies. The causes of type-2 atrophy are multiple, and even in an individual patient the cause can be multifactorial.

In contrast to a large body of literature regarding muscle aging in animals, there is a paucity of information regarding human muscle aging. The clinical and experimental muscle atrophies associated with cachexia, hyponutrition (starvation), remote (paraneoplastic) neoplasm, experimental (and probably human) total denervation without successful reinnervation (Figure 1.2b), glucocorticoid “myotoxicity”, and disuse all involve preferential type-2 fiber atrophy, and they have a number of their molecular degradative steps in common [29–37].

General questions include: what makes the type-2 fibers relatively susceptible to these atrophogenic processes? And what relatively protects type-1 fibers from them? Another question is why do muscle fibers (cells) have such an elaborate protein-catabolizing
Figure 1.3 (a–c) Established reinnervation, indicated by muscle fiber type-grouping, in three adult males with chronic dysimmune peripheral neuropathy. Darkly-stained type-2 fibers and lightly-stained type-1 fibers are type-grouped, in contrast to what normally would be a rather even intermixture of fiber types (not illustrated). The successfully “foreign reinnervated” fibers among the type-grouped type-1 fibers have retained, or re-achieved, their normal diameter. In (a,b) there is also rather diffuse type-2 fiber atrophy of moderate degree, because in these adult males the dark type-2 fibers normally would have been of somewhat larger diameter than the light type-1 fibers. (c) Very large type-groupings, and also a very few tiny atrophic muscle fibers. (d) Prominent paucity of type-1 fibers (dark), possibly caused by a sampling phenomenon of a very large type-grouping (or, hypothetically, by a selective loss of type-1 lower motor neurons or type-1 Schwann cells). (a–c) Regular ATPase, incubated at pH 9.4 [4–6]; (d) acid-preincubated at pH 4.35 and then the ATPase staining at pH 9.4 [7]. Magnification: (a,b) ×830; (c) ×1500; (d) ×1330.
complex, namely to unleash “atrogenses” controlling self-destructing molecular systems? Is it to provide rapid-response hypertrophy, or atrophy martyring? Most important medically, can we prevent or treat the crippling atrophy in aging persons?

There are two major categories of muscle-fiber atrophy: (a) ordinary denervation atrophy (Figures 1.1–1.3) and (b) type-2 muscle fiber atrophy (Figure 1.4), and other less frequently seen atrophies, including vacuolar atrophies. Conceptually, type-2 fiber atrophy can be either neurogenous or myogenous, or both. Determining which applies to each specific patient is essential to establishing patient-specific treatments. We propose that the putatively neurogenous type is much more common (see below).

Mechanisms of muscle-fiber atrophy involve:

- Greater catabolism than synthesis of muscle-fiber proteins, especially catabolism of myofibrillar proteins, which occurs mainly through two subsystems:

**Figure 1.4** Type-2 fiber atrophy in four males. Many of the darker-stained type-2 fibers are of smaller diameter than the lighter-stained type-1 fibers, whereas in normal men the type-2 fibers should have a larger diameter than the type-1 fibers. There is also a concurrent slight type-grouping in (a) and slight paucity of type-1 fibers in (d). The patients in (a–c) have a chronic dysimmune peripheral neuropathy, and in (d) a chronic glucocorticoid toxicity. Regular myofibrillar ATPase, pH 9.4. Magnification: (a) \( \times 830 \); (b) \( \times 2000 \); (c) \( \times 830 \); (d) \( \times 2500 \).
proteasomes involving ubiquitinated proteins and autophagy/lysosomes (see Chapter 7 for details).

- Less certain is the possibility that there might also be decreased synthesis of muscle protein in the atrophy of aging, as occurs in some forms of “cellular senescence.”

**Neurogenic atrophy**

Neurogenic changes include denervation, “dysinnervation,” and reinnervation. While these are not restricted to older persons, they are the most common pathologic changes found in the atrophying muscle of aging persons.

**Denervation**

This is a complete loss of LMN influences on its muscle fibers, and that produces weakness.

**Dysinnervation**

This is our hypothetical concept of only partially impaired, incomplete loss of neural influence, especially of molecular neurotrophic factors, some of which are still able to be produced from crippled but alive motor neurons. Our putative “dysinnervation” phenomenon can be conceptualized as having some aspects similar to a persistence of the early stages of ordinary “recent denervation,” to which it appears histochemically similar.

**Pan-denervations and pan-dysinnervations in regard to type-2 fiber atrophy**

These are postulated as adversely influencing mainly the type-2 fibers (or sub-preferentially the type-2B fibers). Hypothetically, “pan-denervations” are due to (a) abnormality of both type-2 and type-1 LMNs or of their intimately related, respectively type-2 and type-1 Schwann cells (which are nurturing the LMNs and being nurtured by them). This results in lack of trophic influence on “all” the muscle fibers, either: (i) fully (in pan-denervations) or (ii) partially deficient – quantitatively or qualitatively – (in pan-dysinnervations), to which the type-2 muscle fibers (or sub-preferentially type-2B fibers) are more susceptible; or (b) hypothetically, relatively selective abnormality at the level of the presumed type-2 LMNs, or of their closely associated Schwann cells that we designate as “type-2 (or type-2B) Schwann cells.” Whereas in *denervation* diseases the loss of each individual LMN’s trophic influence on its muscle fibers is, by this definition, total. In dysinnervations there can be a hypothetical *quantitative partial loss* impairing *all* of the LMN’s trophic influences to some degree or *qualitative loss* affecting only a fraction of the presumably several “trophic factors” originating from the affected LMNs. Denervation always produces weakness, the degree being related to the number of muscle fibers denervated.

The dysinnervations can occur in disorders of the following.

- LMNs, at the level of the soma, axon, root, proximal axon or distal axonal twigs. One putative example is “axonal hyperactivity,” which produces fasciculations, macrocramps, and multi-microcramps [38]. The discomforting and disabling multi-microcramps are presumably due to lability and persistent aberrant firings of distal axonal twigs – each twig innervating a few abnormally contracting/microcramping muscle fibers – caused by molecular abnormality essentially at (a) the axons themselves, or (b) at their enveloping Schwann cells. Dysinnervations can produce fatigue and weakness in relation to the quantity and quality of the neural impairment.

- Schwann cells: Schwann cell trophism to LMNs is vital for the normal function and survival of those LMNs. *Dysschwannian peripheral neuropathies* are the result of abnormal Schwann cells causing a secondary involvement of the proximal or distal portions of their encompassed axons, retrograde of the neuronal somas. Examples of dysschwannian neuropathies include: (a) diabetes-2 (type-2 diabetes) dysimmune neuropathies, (2) geneticodiatetoid-2 dysimmune neuropathies, (3) other dysimmune dysschwannian neuropathies, and (4) various non-dysimmune neuropathies (such as genetic and toxic ones). The first three are types of chronic immune dysschwannian polynuropathy (CIDP).

**Recent denervation without reinnervation compared to type-2 fiber atrophy**

When slightly to moderately evolved, *recent denervation without reinnervation* (Figure 1.1a–e) is manifest (in transversely cut muscle fibers) as small
angular-contoured ("angular") fibers, which are often, but not always, excessively dark with the NADH-TR and/or pan-esterase and/or the men-αGPDH reaction; sometimes those fibers are low in myophosphorylase and/or COX reactivity (Askanas and Engel, unpublished results) [4]. (The denervated type-1 fibers are more likely to be excessively dark with the NADH-TR, SDH, and pan-esterase reactions and the denervated type-2 fibers more likely excessively dark with the men-αGPDH reaction.) Slightly or moderately small "roungulated" (shape between rounded and angulated) (Figure 1.4a–c), often "pre-angular," muscle fibers can indicate either early recent denervation or type-2 fiber atrophy, the latter evidenced in its early and mid stages as more rounculated than angular atrophy. Three-zone “target fibers” (Figure 1.1d,e) in muscle are another sign of impaired innervation [4, 39], and they are often associated with an improvable dyschwannian neuropathy (Engel, unpublished results). Two-zone “targetoid fibers” (Figure 1.1e) are probably of the same neurogenic pathogenesis as target fibers but, because they are, individually, often histochemically indistinguishable from central-core disease fibers, they are called "targetoid-core fibers."

In early and mid stages of recent denervation, e.g. from amyotrophic lateral sclerosis (ALS) or peripheral neuropathy, on transverse sections typically there are scattered (not grouped) small, angular-contoured fibers, whose angularity seems to be due to their being slightly indented by the adjacent normal fibers, which apparently have greater internal hydrostatic turgidity pressure than the denervated fibers. By contrast, in early and mid stages of type-2 fiber atrophy, many of the type-2 fibers (or the 2B subset of fibers) are in about the same stage atrophy, and they are more likely to be rounculated. In nearly total recent denervation (pan-denervation), e.g. in the acute neuropathy of Guillain–Barré disease, the denervated fibers are rounculated, probably because there are no normally turgid muscle fibers to compress them. The ultrastructure of type-2 fiber atrophy resembles that of denervation atrophy [40].

(Regarding type-2 fiber atrophy, in what seem to be an advanced stage of atrophy, some fibers have become very small, dark and angular. Arbitrarily, in a setting of type-2 fiber atrophy we consider those small angular fibers and pyknotic nuclear clumps as evidence of a denervation aspect. In the advanced stage of type-2 fiber atrophy associated with small dark angular muscle fibers, the situation in that biopsy sample can be proposed to reflect (a) that all those atrophic type-2 fibers are the result of a dysinnervation process, which we favour or (b) it is “strictly a myogenous” process (if such actually exists) eventuating into atrophic fibers with denervation-like properties.) Seemingly relevant is that Goldberg et al. has reported that in rodents biochemical changes are similar between denervation atrophy and atrophy caused by cancer cachexia, starvation, disuse, and corticosteroid atrophy [29–37]. It should also be emphasized that experimental surgical pan-denervation of a muscle, plus preventing reinnervation, produces type-2 fiber atrophy, as we have shown [19, 20] (see below).

Accordingly, the neurogenic kind of type-2 fiber atrophy is proposed to be a dysinnervation evolvin into denervation.

Pan-denervation or pan-dysinnervation hypothetically can be manifest as type-2 fiber atrophy

This can be without or with manifestation of associated ordinary recent denervation and/or established reinnervation. When there is coexisting type-2 fiber atrophy and atrophic small dark angular fibers like those of recent denervation, it can be difficult to decide whether the interpretation is: (a) two separate processes consisting of type-2 fiber atrophy plus recent denervation, or (b) the small angular fibers represent the advanced state of the type-2 fiber atrophy. The latter interpretation would be especially likely if that patient’s type-2 fiber atrophy is considered to be the result of a neurogenic pan-denervation or pan-dysinnervation process. In the early and mid stages of type-2 fiber atrophy the atrophying type-2 fibers retain their normal, relative lighter-staining with NADH-TR vis-à-vis the darker type-1 fibers, for example in glucocorticoid-induced atrophy of humans, and they retain their distinctive reg-ATPase and rev-ATPase appearances throughout the type-2 atrophy [4, 9, 41–43].
Both type-2 fiber atrophy and recent denervation
Both type-2 fiber atrophy (which often seems to be due to dysinnervation) and recent denervation (with or without established reinnervation) exist concurrently in muscle biopsies of many aging patients (see details above and below).

Established reinnervation following previous denervation
This is manifest by muscle fiber type-grouping (detailed above) [4, 9, 16–18, 42, 43].

End-stage non-reinnervation following previous denervation
This is evident as “pyknotic nuclear clumps” (Figure 1.2a) of extremely atrophic muscle fibers. With the NADH-TR stain such “end-stage” atrophic fibers typically show high activity, indicating that they are still alive. These end-stage, apparently alive atrophic fibers can have long-persisting pyknotic nuclei, some of which can show certain features of apoptosis, such as DNA fragmentation by Tunel staining [44]. Because this atrophying process is extremely slow compared to the rapid cellular deterioration of ordinary apoptosis, we have called it “apoptosis lente.”

Hypoactivity (“disuse atrophy”) is manifest as type-2 fiber atrophy
This atrophy can be attributed to net reduction of overall neural activation, which triggers catabolic processes within the muscle fibers. Causes include: supra-segmental central nervous system disorders, experimental de-afferentation of LMNs, general illnesses, cast on a limb, arthritic joint pains, and psychosocial factors, such as depression or interminable television.

General neuropathic mechanisms that could cause type-2 fiber atrophy
Because the neuromuscular system is complex, there are several hypothetical neuropathic mechanisms:

1. unlimited pan-neuropathic: disorders (including supra-segmental disorders) affecting all type-2 LMNs plus all type-1 LMNs, but a disorder to which the type-2 fibers are more susceptible;
2. unlimited dysschwannian pan-neurogenic: LMN malfunction secondary to disorders affecting all type-2 plus type-1 Schwann-cells, but disorders to which the type-2 muscle fibers are more susceptible (Figure 1.2b) [19, 20];
3. limited type-2 neurogenic: disorders affecting only type-2 LMNs;
4. limited dysschwannian neurogenic: secondary to disorders affecting only type-2 Schwann-cells.

In each of these four situations, the disorder of each individual cell involved (LMN or Schwann cell) can be complete (resulting in denervation) or partial (resulting in dysinnervation).

Type-2 fiber atrophy is, after ordinary denervation and reinnervation, the second most common pathology we find in muscle atrophy of the aging. Different human conditions are associated with type-2 fiber atrophy, implying various possible pathogenetic mechanisms. In the individual patient, determining which cause of the type-2 atrophy is most influential might lead to an appropriate treatment.

In the various conditions, is there a “final common path” to the type-2 fiber atrophy? This is not certain, but several of the conditions associated with type-2 fiber atrophy have the same major players, such as: ubiquitin ligases and the ubiquitin-proteasome proteolytic system; the autophagy proteolytic system; the FoxO3 system that coordinates the two proteolytic systems [32, 34, 35, 45]; JunB [29]; and myostatin (see below). Even if there is a final common pathogenic path, finding a final common elixir must be a long and winding road.

Experimentally, certain maneuvers have been reported to allegedly prevent or retard, or even reverse, type-2 fiber-associated atrophy, such as: peroxisome proliferator-activated receptor (PPAR) co-activator 1α or 1β overexpression [32]; probably increasing puromycin-sensitive aminopeptidase [30]; decreasing insulin-like growth factor 1 (IGF-1)-phosphinositide 3-kinase (PI3K)-Akt signaling and its activation of mammalian target of rapamycin (mTOR) and FoxO3 pathways [33]; increasing peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α); by suppressing FoxO3 action and atrophy-specific
gene transcriptions [34]); enhancing JunB transcription factor [29]; and antagonizing ActRIIB [31]. If confirmed, these might provide clinical therapeutic leads.

**Type-2 fiber atrophy: further comments**

In an individual patient the cause of type-2 fiber atrophy can be multifactorial. For example, possibilities are: (a) in arthritic muscle atrophy, in which the commonly associated muscle-fiber atrophy is typically type-2 fiber atrophy [46], but whether the mechanism(s) is, speculatively, related to hypofunction/disuse, or a putative pain reflex decreasing LMN function, and/or, in rheumatoid arthritis, a concomitant dysimmune mechanism; (b) in HIV the atrophy could be dyschwannian dysimmune neuropathic, dysneuronal neuropathotoxic, cachectic, or possibly viral-myotoxic, or a combination of these.

Neuropathic mechanisms causing type-2 fiber atrophy are discussed above.

**“Myopathic” mechanisms causing type-2 fiber atrophy**

**General comments**

“Atrogenes” are a common set of genes whose expression is coordinately induced or suppressed in muscle during generalized wasting states (such as fasting, cancer cachexia, renal failure, and diabetes) [36, 45]. These can be activated by intrinsic or extrinsic muscle-fiber abnormalities.

**Atrophy: “Protectosome” Versus Atrogenes**

Hypothetically, aging changes might be considered a “wearing out” or a “weariness” of the cellular protective mechanisms. The normal muscle fiber, like all cells, has what we are calling a protectosome, i.e. a group of factors that normally inhibit expression of atrophy-producing “atrogenes;” thereby the protectosome is holding in abeyance the atrophogenic mechanisms with which the muscle fibers of all of us are normally equipped. However, those atrophogenic factors are constantly ready to be unleashed to produce self-erosion, self-catabolization, a sort of self-cannibalization; this putatively occurs when a beneficent protective system falters in an aging cellular environment, or other circumstances that cause type-2 fiber atrophy. The myofiber’s internal protective systems include control of endogenous free radicals and of misfolded proteins.

**Myostatin**

Myostatin is a negative regulator of muscle mass in normal development, and it is an important factor limiting the size of mature muscle fibers [47–53]. A normal level of myostatin is sufficient to inhibit myofibrillar synthesis rate and phosphorylation of S6K and rpS6 [54]. In normal human muscle, it is not known which fiber type expresses more myostatin, because with the antibodies used no immunoreactive myostatin was detectable in normal fibers of either type. In human type-2 muscle fiber atrophy associated with aging, myostatin protein/precursor–protein (Mstn/MstnPP), but not the mRNA, was quantitatively increased, and it was immunohistochemically increased preferentially in the atrophying type-2 muscle fibers [55]. It was also increased quantitatively in the weakening muscle of sporadic inclusion-body myositis (s-IBM), which is an aging-associated myopathy (see [55]). We propose that increased myostatin is an important pathogenic component of the type-2 fiber atrophy associated with “aging,” but of yet-undetermined mechanism. Mstn/MstnPP might play an adverse role in the pathogenic cascade of type-2 muscle fiber atrophy in various situations. Quantitative increase of myostatin and its mRNA has been reported by others in human atrophic muscle associated with arthritis, with HIV, and with glucocorticoid myotoxic type-2 fiber atrophy (see [55]). Elevated serum myostatin levels occur in end-stage liver diseases, in which patients have profound muscle wasting [56]. In normal animal muscle there seems to be a pool of extracellular pro-myostatin [57]. In animal models of chronic heart failure, skeletal muscle myostatin is increased, and treadmill exercise can mitigate that myosin protein expression [58]. The actual mechanisms by which myostatin protein is pathologically increased could be a therapeutic target. IGF-1 inhibits the effects of myostatin and tends to preserve skeletal muscle in mouse models of cachexia. Administration of...
ACVR2B-Fc inhibited myostatin and muscle wasting in two models of cancer cachexia, without affecting tumor growth [59]. Three days of lower-limb suspension in humans causes the unloaded (“disused”) muscles to increase myostatin mRNA and protein [60], and acute antibody-directed myostatin inhibition attenuated similar disuse muscle atrophy and weakness in mice [61].

In aneurally cultured muscle fibers, tumor necrosis factor alpha (TNFα)-induced expression of myostatin through a p38 mitogen-activated protein kinase (MAPK)-dependent pathway [58]. In rats, fenofibrate, a PPARα agonist, reportedly decreased atrogens and myostatin expression, and improved adjuvant-arthritis-induced muscle atrophy: not discussed was the hypothetical possibility that the adjuvant, collaterally, also has an unrecognized myotoxic effect [62]. That adjuvant-arthritis-associated muscle atrophy could also be attenuated by systemically administrated IGF-1, which also decreased atrogin-1 and insulin-like growth factor-binding protein 3 (IGFBP3) [63].

Clinical arthritis is associated with muscle atrophy, which may be multifactorial, involving disuse and perhaps myotoxic cytokines [46]. Terracciano et al. [46] found type-2 fiber atrophy in patients with osteoporosis or osteoarthritis (more prominent in the former), that was associated with increased circulating “inflammatory mediators,” namely interleukin-6, C-reactive protein, and TNFα.

Ubiquitin-proteasome System
This is considered a major site of protein catabolism in muscle-fiber atrophies, and the activity is upregulated by ubiquitin ligases: muscle RING-finger 1 (MuRF1) and MAFbx (atrogen-1). They target particular protein substrates for degradation via the ubiquitin-proteasome pathway. The growth factor IGF-1 can block that upregulation. MuRF substrates include components of the muscle sarcomeric thick filaments, especially the myosin heavy chain. In denervation or fasting muscle atrophy, there is loss of: myosin-binding protein c and myosin light chains 1 and 2 from the myofibrils before any measurable decrease of myosin heavy chain. This selective loss requires MuRF1. Myosin heavy chain (MyHC) in myofibrils is relatively protected from ubiquitination by its associated proteins. Because the targeted proteins stabilize the myosin-containing thick filaments, their selective ubiquitination may facilitate thick filament disassembly (the filament components are decreased by a mechanism not requiring MuRF) [37]. Others agree that during muscle atrophy, thick (myosin), but not thin (act), filaments are degraded by MuRF1-dependent ubiquitination.

FoxO3 Signaling
This coordinates activation of both autophagy/lysosomal and the proteasome catabolic pathways by FoxO3, a transcription factor that produces rapid loss of muscle mass with disuse, and systemically with fasting, cancer, and other disorders due to its causing overall accelerated breakdown of muscle proteins [33, 64–69]. Activation of the transcription factor FoxO3 is essential for muscle atrophy, via transcription of a set of atrophy-related genes (atrogenes) including critical ubiquitin ligases, as well as autophagy-related genes. FoxO3 coordinately activates both proteolytic systems, but especially autophagy/lysosomal proteolysis. FoxO3 is necessary and sufficient for the induction of autophagy in skeletal muscle, and FoxO3 is said to control the transcription of autophagy-related genes Bnip3 and LC3. Activated FoxO3 stimulates autophagy through, transcription-dependent mechanisms, increasing transcription of many autophagy-related genes, which are also induced in mouse muscle atrophying due to denervation or fasting. In atrophying muscle, decreased IGF-1-PI3K-Akt signaling stimulates autophagy not only through TOR, but also more slowly by FoxO3-dependent transcription, thereby coordinating regulation of proteasome and lysosome systems. Elevated PGC-1α or PGC-1β [34, 70, 71] was reportedly “therapeutic,” as manifested in several ways. It prevented the accelerated proteolysis induced by starvation or by FoxO3 transcription factors. In mouse muscle, it inhibited denervation atrophy by preventing FoxO’s induction of autophagy and atrophy-specific ubiquitin ligases, and it decreased inhibition of ubiquitin ligase’s induction of transcription by nuclear factor κB (NFκB). In myotubes, it caused increased protein content
and decreased overall protein degradation without altering protein synthesis.

Altruistic martyring
The type-2 fibers, and especially the 2B fibers, more readily atrophy in a number of different clinical situations, but the reasons are not known. In general, atrophying type-2 fibers are undergoing self-cannibalization and can be considered either victims or martyrs.

Martyring type-2 fibers can be thought of as, survivalistically, selflessly giving up their protein, especially their myofibrillar protein, to be broken down into its component amino acids, which then are utilized by other cells that are more essential for survival of the patient or animal, such as brain, liver, kidney, and blood cells. The muscle amino acids are used (a) via the alanine shunt, for synthesis by the liver into glucose that is circulated for wider utilization, or (b) for building cellular peptides and proteins. This martyring occurs in states of hyponutrition and cachexia, which are often induced by a chronic illness such as cancer or renal, pulmonary, cardiac or infectious disease. Autophagy, through bulk degradation of muscle-fiber protein and organelles by lysosomal enzymes and proteasomal proteolysis, helps other cells and the animal to survive during starvation.

Cancer cachexia (cachexia being the loss of lean body mass) impairs the patient’s quality of life and response to antineoplastic therapies, and reportedly accounts for a least 20% of the deaths in cancer patients. Cachectic atrophy is, contraversally, defined by some as muscle wasting that cannot be reversed nutritionally, while others, and ourselves, consider malnutrition muscle atrophy also as cachexia. One analytical difficulty clinically is that many cachectic patients are undernourished due to decreased food intake, which they often deny. Provoked by a cancer or other chronic disease, toxic cytokines can be released into the circulation and then probably can: (a) have a direct toxic/cachectic effect on muscle fibers; (b) acting via intermediate cells, including LMNs, have an indirect myoatrophying effect; and (c) suppress the appetite. Cachectogenic toxic cytokines include TNFα and interleukins 1β, 6, 8, 12, and 23, and these can be released by neoplastic cells, macrophages, and adipocytes. Released by adipocytes, leptin is appetite-suppressing and adiponectin is appetite-stimulating. Some of these factors in some test systems can be blocked by the following: an NFκB inhibitor SN50; anti-TNFα drugs etanercept, infliximab, and adalimumab; an anticytokine effect of thalidomide; and anti-IL12 and anti-IL23 drug ustekinumab (independently of a TNFα effect).

Treatment of cancer cachexia experimentally is to attack: the mediators, including cytokines and tumor-derived factors including TNFα and their receptors; androgen receptor inhibitors; proteolytic pathways (ubiquitin-proteasome and autophagy paths), intracellular signaling pathways NFκB, AP1, FoxO, and PKP, and the negative modulators of muscle growth/hypertrophy (myostatin, glycogen synthase kinase 3β (GSK3-β)) [72]. In tumor-bearing mice, there is marked muscle wasting and weight loss, associated with increased phospho-extracellular-signal-regulated kinase (pERK) and decreased myosin heavy chain: this is prevented by ERK inhibition and return of atrogin-1 expression to normal [73]. But despite “benefits” to some experimental animals, there is no good treatment for cachectic muscle atrophy in patients (see IGF-1, below).

Is there a hypothetical cachexia lente in many aging persons, due to a variable, additive multifactorial combination of low-grade systemic illness, hyponutrition, hypoactivity, and, possibly low-grade ischemia from anywhere in the vascular tree, including the capillaries?

Myotoxic phenomena: glucocorticoid atrophy
Muscle biopsies of glucocorticoid (corticosteroid)-treated patients having muscle weakness show what we first identified as preferential atrophy of type-2 fibers (the glycolytic, fast-twitch fibers) [4, 8, 10, 41]. In animal experiments, glucocorticoid is considered to act directly on muscle fibers to cause the now well-known type-2 fiber atrophy. (Hypothetically, perhaps with glucocorticoid toxicity there could also be a concurrent toxic neuropathic mechanism contributing directly to the atrophy, including the possibility of neuropathically susceptibilizing the muscle fiber to the glucocorticoid toxicity.)
Glucocorticoid treatment can cause insulin resistance of muscle fibers, and often aggravates diabetes-2 or makes manifest type-2 diabetes in genetically predisposed and/or obese patients.

In glucocorticoid atrophy, muscle proteolysis is especially through the ubiquitin-proteasome system, which is considered to have the major role in that catabolism. It is mediated through increased expression of several atrogenes, genes involved in muscle atrophy (such as atrogen1 and MuRF1, which are two ubiquitin ligases involved in targeting proteins to be degraded by the proteasome machinery). Glucocorticoids are, according to some investigators, also anti-anabolic, blunting muscle protein synthesis. Some aspects of the glucocorticoid atrophy may result from a demonstrated decreased production of IGF-1 and increased myostatin. IGF-1, by inhibition through the PI3K-Akt pathway, antagonizes the catabolic action of glucocorticoid. The activity of the transcription factor FoxO is a major activation switch for the stimulation of several atrogenes [74]. Glucocorticoid increases myostatin expression, and in mice myostatin gene deletion prevents glucocorticoid-induced muscle atrophy. Glucocorticoid increases mRNA of enzymes involved in proteolytic pathways (atrogen1, MuRF1, and cathepsin-L), and it increases chymotrypsin-like proteasomal activity [48, 52, 75]. Glucocorticoid- and sepsis-induced muscle wasting are associated with down-regulating the expression of the nuclear cofactor PGC-1β in skeletal muscle, suggesting that this contributes to the muscle wasting [71]. In glucocorticoid-linked muscle atrophy, the myosin heavy chain is preferentially lost.

**Other extrinsic triggering mechanisms or associations of muscle atrophy in aging persons**

- Hormonal abnormalities: high glucocorticoid; low androgen; high parathyroid hormone; low growth hormone; insulin resistance (e.g. from high glucocorticoid); high thyroid hormone; diabetes-2 dysimmune and genitico-diabetoid-2 dysimmune neuropathic mechanisms; possibly high parathyroid-related-protein (sometimes released from tumor cells).
- Abnormal immune complexes and/or toxic antibodies, as follows.

1. In dermatomyositis we have described deposition of toxic immune complexes in small blood vessels of muscle, which is typically associated with perifascicular atrophy (meaning the atrophic muscle fibers, which are often vacuolated, tend to be located at the periphery of fascicles of muscle fibers), and location of that atrophy is probably due to ischemia, caused by those vascular deposits.

2. In myasthenia gravis, which is caused by toxic antibodies against the nicotinic receptor at the post-synaptic (muscle side) part of the neuromuscular junction, we have found muscle-biopsy features of recent denervation and/or type-2 fiber atrophy in every myasthenia gravis patient [76]. This mechanism of atrophy is literally a myopathic dysreception and might be multifactorial, resulting from both hypoactivity and possibly impaired concurrent dysreception of trophic-factors from the LMN. Additionally, there is probably some binding of toxic antibody to the nicotinic receptors located at the pre-synaptic neural tips of the LMNs, evident by α-bungarotoxin binding [77], thereby causing an additional true neuropathic component. (Those pre-synaptic acetylcholine receptors are probably the mediators of pyridostigmine-provoked fasciculations in myasthenics and normals; Engel, unpublished results.) (Two other possibly pathogenically relevant phenomena are α-bungarotoxin-delineated: (a) neo-appearance of nicotinic receptors on denervated human muscle fibers [78], and (b) presence of nicotinic receptors on human thymic epithelial cells [79].)

3. Circulating toxic auto-antibodies can result in type-2 fiber atrophy in dysimmune neuropathies: these are dyschwannian more often than dysneuronal type (see above).

- Putative prion, or “prionoid”, and other misfolded, “sticky” abnormal proteins, originating intracellularly or extracellularly, could be capable of disrupting normal cellular function.

- Neurogenic “susceptibilization:” in individual persons with “elder-atrophy,” it is unknown whether or not hypothetical denervation or dysinnervation is occurring and susceptibilizing the muscle fibers to undergo atrophy from a concurrent myopathic mechanism.

- Other external hypothetical susceptibilization mechanisms for muscle atrophy. These could
include impaired supply of blood, oxygen, glucose, or other vital factors.

**The speculative likelihood of type-2 fiber atrophy “altruism” being important in different conditions**

**Likely**

Hyponutrition/starvation: the mechanism, in principle, could be increased catabolism affecting: (a) an aspect that is more active in type-2 fibers, or decrease of a normal mechanism that is more important in type-2 fibers (such as anerobic glycolysis or mitochondrial \(\alpha\)-glycerolphosphate dehydrogenase); or (b) an aspect having a narrower margin of error in type-2 fibers, meaning being closer to being insufficient (for example, mitochondrial oxidative metabolism, such as affecting COX or SDH).

**Uncertain**

In HIV there is often a complex pathogenesis of the muscle atrophy, which histochemically is type-2 fiber atrophy with or without denervation atrophy. It can have five components: (a) neuropathic, viz. dysimmune dysschwannian denervation neuropathy early in the course of the disease, and virogenic toxicity causing dysneuronal neuropathy later; (b) often cachexia; (c) hypomotility/disuse; (d) possible nerve toxicity of anti-HIV drugs; (e) infrequently, myotoxicity from viral products. With HIV, type-2 fiber atrophy in response to a hyponutritional/cachectic aspect would be altruistic, but when in response to the other causes it would not be.

**Probable not likely**

(a) Hypoactivity from lassitude, or immobilization in a cast or brace; (b) atrogenic toxins attributed to a remote neoplasm (unless it is acting via hyponutrition); (c) glucocorticoid toxicity (possibly by causing insulin resistance, more so in type 2-fibers); (d) HIV viral toxicity.

**Not likely**

(a) Myotoxins; (b) denervation/dysinnervation diseases (e.g., ALS, peripheral neuropathy, root/nerve mechanical pressure), although they secondarily cause dysphagia and hyponutrition; (c) myasthenia gravis (before hyponutrition), possibly involving both hypoactivity and insufficient neurotrophic factors; (d) pain, local or regional (arthritis, osteo- and rheumatoid, causing hypoactivity and/or a putative “dolorogenic atrophy”); (e) surgical or pharmacological castration of normal males; and (f) the “feminosity” aspect of normal women having type-2 fibers smaller than type-1 fibers. (We do not know the gender-specific diameters of muscle fibers in species of spiders in which the female is much larger and typically eats the male right after copulation.)

**Other changes in aging muscle**

**Mitochondrial abnormalities: histochemical aspects**

**Diminished COX staining**

Specifically this is the absence, or prominent decrease, of COX staining at a given transverse level of a muscle fiber and often segmentally distributed as multifocal absences along the long individual muscle fibers (Figure 1.5b) (see [80]). These foci are often increased in aging human muscle, without an identified mitochondria-related mitochondrial DNA (mtDNA) or nuclear DNA defect (Engel, unpublished results) [80]. They indicate mitochondrial abnormality, and we suggest that they might be only the tip of the iceberg, evoking the possibility of an accompanying, histochemically inapparent crippling reduction or absence of COX in a portion of the muscle fiber’s multitudinous mitochondria. Because type-2 fibers normally have considerably less COX activity (and perhaps less “COX reserve”), hypothetically they might be more likely to atrophy from a partial impairment of COX activity. The specific pathogenesis of multifocal COX deficiency and other mitochondrial abnormalities in aging muscle fibers is unknown, and treatment not established.

**Specific or not-yet-specific mitochondrial myopathies**

Primary defects of mtDNA, or nuclear DNA, affecting muscle mitochondria (with or without other cells),
Figure 1.5 Disturbed mitochondrial activity in patients over age 70. (a) “Moth-eaten” and “large central pallor” patterns in various muscle fibers stained for cytochrome-oxidase activity. At the lower right are two longitudinally cut muscle fibers showing a very elongated distribution of central pallor within those fibers; these are an occasional finding in a chronic peripheral neuropathy patient. (b,c) Many normal-diameter muscle fibers (some indicated by asterisks) have complete absence of cytochrome oxidase staining (white fibers in this photograph), intermingled with normal type-1 (very dark) and type-2 (slightly dark) fibers. (c–e) Several “ragged-red” type of muscle fibers, actually “ragged-blue” fibers in these succinate dehydrogenase stainings of mitochondria. They are excessively stained in comparison to the other surrounding normally stained fibers. Magnification: (a) ×2000; (b) ×1670; (c) ×3000; (d) ×3670; (e) ×3670.
associated with characteristic clinical syndromes, can be highlighted by muscle biopsy histochemistry, but identifying the specific defect requires special biochemical techniques (see Chapter 4 in this volume). Not-yet-specific defects presumably underlie the more commonly seen muscle fibers having either absent, diminished or, infrequently, excessive, staining with COX or SDH, and they are more abundant in aging human muscle (Figures 1.5a and Figure 1.6a–c). When unassociated with a specific clinical syndrome, aging human muscle can have increasingly abnormal mitochondrial biochemical functions, to various degrees [81, 82]. Some mitochondrial focused investigators have deemed the general aging of muscle a “mitochondrial myopathy” (see Chapter 4) [83]. Like aging humans, “otherwise normal” aging animals have more fatigable muscle associated with mitochondrial biochemical abnormalities [84]. Mitochondrial abnormalities of aging humans are not yet treatable.

Ragged-red fibers and rugged-red fibers
In both of these, the accumulations of abnormal mitochondria are bright red with the Engel trichrome stain [4, 5, 85–87], and their mitochondriality can be demonstrated histochemically by abnormally dark (Figure 1.5c–e), or sometimes concurrently absent, staining with one of these: SDH, COX, β-hydroxybutyrate dehydrogenase, or men-αGPDH: they are sometimes deficient in one mitochondrial enzyme accompanied by an increased amount of others. The overall cytoarchitecture of the ragged-red fibers (which we first described [88]) appears abnormally loose, while the rugged-red fibers have a firm, fully-packed appearance. Molecular genetic analyses in special laboratories can sometimes identify the exact mtDNA or nuclear DNA defect crippling the mitochondria (see Chapter 4). These mitochondrial accumulations are often, but not always, more evident in type-1 fibers (which normally have more mitochondria). Ragged-red fibers are more frequent in aging human muscle, and especially in s-IBM (an aging-associated muscle disease) (see Chapter 7 in this volume).

A normal variation can be the rugged-red appearance of a bright red rim of packed mitochondria in many of the type-1 fibers, especially in some persons doing very vigorous prolonged endurance exercising such as frequent long-distance cycling. In normal human muscle, histochemistry demonstrates that the mitochondria are qualitatively different between type-1 and type-2 muscle fibers: mitochondria in type-1 fibers have stronger SDH and weaker men-αGPDH activities, and type-2 fibers show the converse [4, 8–10].

“One-way streaks” and “increase-decrease fibers”
These “mitochondrial pattern abnormalities” (Figure 1.6) are based on rearrangements of the normal orderly array of mitochondria in a delicate pattern amongst the myofibrils. These pattern abnormalities probably reflect myofibrillar abnormality and/or mitochondrial abnormality.

Secondary mitonchondriopathy
This hypothetical pathogenesis might occur in an unrecognized “noxious neighborhood.” viz. an abnormal milieu within muscle fibers that affects mitochondrial function; for example, one involving a glycolytic enzyme, or another nonmitochondrial defect.

In adult-onset muscle phosphorylase deficiency there are prominent histochemically-evident mitochondrial disturbances. An example was our patient (the first identified) who had clinical onset of fatigue and weakness at age 48. Her muscle biopsy showed prominent patchy absence of mitochondrial oxidative enzyme activity histochemically (Figure 1.6d) [89]. We now hypothesize that the mitochondria were secondarily impaired due to intracellular lack of ATP consequent to defective anaerobic glycolysis caused by the myophosphorylase deficiency. By analogy, possibly other, or even many, mitochondrial abnormalities might be due to damage inflicted by a glycolytic or other nonmitochondrial biochemical abnormality (things one sees are not always what at first they seem to be). For example, possibly the patchy mitochondrial activity loss in other aging patients (Figures 1.5a,b and Figure 1.6a–c) could have an underlying aspect of insufficient glycolysis-generated ATP. Therapeutically speculating, patients like ours with late-onset myophosphorylase deficiency, and perhaps ones
Figure 1.6 Loss of mitochondrial cytochrome oxidase activity in various patterns of distribution, in patients over age 70. (a) Regions of “large central pallor” (which are much larger than the pale regions of targetoid-core fibers); this is an occasional finding, as in this chronic peripheral neuropathy patient. (Apparently-identical changes were evident in muscle 14 days following suprasegmental cordotomy [21].) (b,c) “Decrease–increase” pattern in type-1 fibers: this is a disturbed arrangement of mitochondrial staining, due probably to abnormality of both mitochondria and myofibrils, and possibly also of stabilizing desmin filaments. This looks like a “myopathic” phenomenon, but it can be accompanying recent denervation, or type-2 fiber atrophy (evidenced by the small angular lightly stained fibers in panel b). (d) Muscle fibers showing regions of complete or moth-eaten absence of mitochondrial staining, in a patient with adult-onset myophosphorylase activity deficiency (completely absent myophosphorylase was proved histochemically and biochemically). This suggests that the manifested mitochondrial abnormality may be secondary to the glycolytic defect. Magnification: (a) × 2170; (b) × 2000; (c) × 4000; (d) × 3170.
with another glycolytic defect, might benefit from a putative “mitochondrial therapy,” such as L-carnitine or CoQ10, or another that in the future will be found more beneficial.

In most cases of type-2 fiber atrophy we have observed histochemically that the atrophic type-2 fibers seem to have somewhat reduced oxidative enzyme activity, while maintaining the usual abundant myophosphorylase activity typical of type-2 fibers. Possible caveat: there could be impairment of an unstudied glycolytic or other non-mitochondrial enzyme involved in ATP production (or other important mitochondrial-supporting function).

**Intracellular amyloid-β oligomers in s-IBM:** in s-IBM, ragged-red fibers are somewhat more abundant than in similarly aged non-IBM patients, and mitochondrial functional defects occur (see Chapters 7 and 10). From Askanas’ studies, the earliest identifiable pathogenic step in s-IBM is intracellular increase of amyloid-β oligomers, which are considered to be mitotoxic because their over-expression within cultured human muscle fibers produces mitochondrial abnormalities (see Chapters 7 and 10).

**Uncontrolled free radicals** can also damage mitochondria.

**Other histochemical changes in aging muscle fibers**

**Increased acid phosphatase staining, a marker of lysosomal activity**

Histochemically, the major amount of enzymatically active acid phosphatase is associated with accumulations of lipofuscin granules, typically located beneath the plasmalemma at the periphery of muscle fibers, and especially adjacent to nuclei (Figure 1.7b,c) (including being adjacent to any internal nuclei). There is also a delicate multipunctate distribution of acid phosphatase (without histochemically discernable lipofuscin granules) throughout the muscle-fiber cytoplasm. Acid phosphatase at both of these sites increases with aging in everyone, but the amount in aging muscle varies quantitatively from person to person (Engel, unpublished results). It is not established whether the gradual increase of the lysosomal acid phosphatase is beneficial, neutral, or detrimental. It could be a signal that various invisible, possibly toxic, misfolded and indigestible molecules are gradually accumulating in the aging muscle fiber. The lipofuscin itself is thought to be *indigestible cellular detritus*.

**Triglyceride lipid droplets are sometimes increased in muscle fibers**

These droplets (Figure 1.7a), which do not seem to be aging-related, can result from excessive circulating triglycerides, or from impairment of their catabolism within the muscle fibers, including the mitochondrial phase of their utilization for ATP/energy fuel. They also seem to be more numerous in obese persons (Engel and Askanas, unpublished results).

**Vacuolar myopathies of adult onset**

These include the following: (a) s-IBM is a vacuolar myopathy with protein aggregates (inclusions), which include aggregates containing amyloid-β, phosphorylated-tau, α-synuclein, parkin, and many other Alzheimer- and Parkinson-type proteins in those accumulations (see Chapters 7 and 10). s-IBM still lacks a definitive treatment (see Chapter 7). (b) Hereditary inclusion-body myopathy is due to mutation of the GNE or VCP gene (see Chapters 12 and 15). (c) Adult-onset acid-maltase deficiency. (d) Dermatomyositis often well-treatable (see above and Chapter 3).

**Amyloid**

Abnormal aggregations of misfolded protein molecules stainable with the fluorescence Congo red method of Askanas [90], or more simply but less inclusively with crystal violet, are called “amyloid.” The amyloid in skeletal muscle tissue can be: (a) extracellular, usually in muscle connective tissue regions or in blood-vessel walls, including blood vessels of peripheral nerves; or (b) intracellular (within muscle fibers) of s-IBM (see Chapters 7 and 10). Muscle extracellular amyloid is often composed of the variable portion of an immunoglobulin light chain, or mutant transthyretin, but sometimes other proteins are involved primarily or secondarily. (Extracellular amyloid can be clinically identified, noninvasively, by Mibi radioisotope scanning [91]). We have previously postulated that cyto-disturbance.
from extracellular amyloid [92] and from intracellular amyloid (see Chapters 7 and 10) is not due to space-occupying mechanical pressure of the visible deposits, but rather a molecular cytotoxic affinity of the misfolded amyloid precursor molecules existing as toxic oligomers and monomers.

Rods
In adult-onset rod myopathy, which we now designate as “adult-onset rod myopathy syndrome” because of its association with a monoclonal gammopathy [93–95], with or without dysimmune, neuropathy [96]. Recently, we have

Figure 1.7 (a) Excessive triglyceride droplets in two normal-size muscle fibers. With this photographic exposure, the staining of normal fibers is only faintly evident in the lower part of the figure. Oil red O stain. (b,c) Excessive staining of acid phosphatase activity in the form of multiple tiny dots. The larger clumps of staining, usually peripherally located, are closely associated with lipofuscin granules. Although the acid-phosphatase staining in everyone gradually increases with aging, this amount of staining is excessive for this man’s age of 45 (it is more like that of someone age 85). Magnification: (a) ×3330; (b) ×4500; (c) ×5830.
successfully treated a patient with intravenous immunoglobulin (IVIG) [94], and even more effectively when rituximab was added [96]. Interestingly, rods in animal muscle fibers can be produced by tenotomy [97]. Perhaps in the patients the autoimmune attack is on the tendons.

**Vascular aspects of aging muscle**
These are discussed in Chapter 2 of this volume.

**Putative animal “models” of human “AAM”: biochemical studies**
Such studies are numerous. They will not be reviewed in detail, partly because it is not certain which are directly relevant to the complex human problem. Because therapy for patients is the ultimate goal, biochemical studies of aging human muscle would be relevant to understanding the pathogenesis of aging atrophy if, in the patients studied, the disorder is actually primary within their muscle fibers. But if the problem is caused by a dysinnervation or denervation phenomenon, the essential trouble needing repair is located elsewhere, farther upstream in the motor unit.

**“Sarcopenia”: critique of the term, concept, and “diagnosis”**

“Sarcopenia” simply refers to muscle atrophy in aged animals, and it is often considered to be manifest typically as type-2 fiber atrophy. However, sarcopenia is a term we never use because it is imprecise: it is not a definite pathogenic diagnosis. It is often erroneously interpreted as designating a singular pathogenesis, but it still is enigmatic. The designation “sarcopenia” is commonly employed in experimental work, in which it is sometimes used, pseudoprecisely, for the broad topic of muscle atrophy in aged animals, a phenomenon for which we prefer to use the more direct, non-specific designation AAM. For an aging patient complaining of muscle weakness and atrophy, stating a diagnosis of sarcopenia is meaningless: it adds nothing beyond the patient’s chief complaint.

**So-called “sarcopenia” seems more neuropathic than purely myopathic**
If, as we postulate, there is a significant neuropathic component in “sarcopenia,” analyses of homogenized muscle are actually looking only at the train wreck but not seeking the upstream cause of the derailment. If a denervation/dysinnervation component is indeed present, that would require moving the focus of pathogenic interest and analysis upstream to the LMNs, their Schwann cells, and possibly to their presynaptic afferent neurons.

Because muscle of “sarcopenic” animals reportedly shows, typically, type-2 fiber atrophy, the following comments are relevant.

- “Dysinnervation” is our concept of partially impaired neurotrophic influence from crippled but still-alive motor neurons. In human type-2 fiber atrophies, including those of aged persons, histochemically we often (a) identify a subtle partial- or pan-denervation or (b) suspect a dysinnervation, neither being primarily myogenous. In this respect, we have strikingly demonstrated type-2 atrophy in experimental animals by acute pan-denervation, produced by total sciatic-nerve transection without reinnervation [19, 20]. Likewise, it is quite possible that in some, perhaps many examples of AAM (so-called sarcopenia) of aging animals a neurogenic mechanism may have a major role.

- Ideally, the AAM animals whose muscles are homogenized for biochemical and molecular-genetic studies, should also:
  1. be pre-screened “clinically,” including by electrophysiology, for identifiable abnormalities that in aging humans are considered to cause muscle-fiber atrophy, especially ones causing type-2 fiber atrophy. These potential abnormalities include: overt or subtle partial or pan-denervation or pan-dysinnervation, hyponutrition/cachexia, and hormone dysregulations. Without this clinical evaluation, it is difficult to be precise about the type of pathogenesis of muscle atrophy in AAM animals;
  2. have the same experimental muscle (or the contralateral one) concurrently studied histochemically to visualize and correlate diagnostically the morphology and distribution of the presumably abnormal fibers one is homogenizing.
with an awareness that different muscles have different mixtures of muscle-fiber types.

- As “disease-controls” for biochemical/molecular-genetic studies of muscle atrophy of aged animals, the same investigative techniques should be utilized to examine in mid-adult-age animals the effects of: (a) induced acute denervation, slow denervation and dysinnervation; and (b) hyponutrition/cachexia.
- Because a relatively greater atrophy of myofibrils characterizes most muscle-fiber atrophies, including type-2 fiber atrophy, denervation atrophy, and atrophic fibers in the several myositides and other myopathies, is there an absolutely unchanging denominator that should be used in studying the various aspects of muscle atrophy in humans and animals? Is use of the “housekeeping” gene/protein actin absolutely reliable?

**A critical question**

How could one ever prove that there is not a neuropathic denervation-dysinnervation component underlying some or many examples of the type-2 fiber atrophy in aged animals, or humans?

**Treatment and prevention of human AAM**

There is no sure method. Considerations include the following.

**Specific treatments**

Carefully seek all possibly relevant treatable diseases, and treat any identified correctable causative disorder. e.g. dysimmune neuropathy or primary hyperparathyroidism.

**Non-specific treatments**

1. Correct any hyponutrition, which, in our experience, many undernourished patients deny.
2. Consider available general treatments of “muscle atrophy” discussed below.
3. Monitor the literature for new drugs from animal and human studies.
4. If in a specific situation the type-2 atrophy itself is considered to be actually beneficial – e.g. altruistic martyring via the survivalistic muscle alanine to liver gluconeogenesis pathway in starvation (see above) – one might not be able to symptomatically stop the type-2 atrophy without correcting its cause.

**Results from animal and human studies**

There are numerous studies of muscle atrophy in aging animals, but their practical relevance to understanding and treating muscle-fiber weakness and atrophy of aging humans is still being determined. They are disclosing interesting molecular mechanisms, but as yet no successful, enduring prevention or treatment has come from them; no new clinically useful drugs for aging atrophy have emerged [98–102]. Accordingly, myophysiologists and myopharmacologists consider that currently the only somewhat beneficial measure is exercise, preferably resistance exercise, if it can be performed diligently.

**Exercise**

The dictum is “maintain a physically active life” (see [103–105]). Despite a plethora of recent and ongoing pharmacologic research, “exercise is the primary therapy,” but that can be difficult. Many of the aged persons with muscle weakness and atrophy have coexisting exercise-limiting conditions such as: pain from arthritis, peripheral neuropathy, radiculopathy or another cause; obesity; general fatigue from another disorder such as cardiac, pulmonary, renal, or neoplastic disease; fatigue from various prescription medicines; or even the weakness itself produced by the atrophying muscles. Exercise-wise, the following should be done.

1. Exercise to the extent possible, and active resistance exercise is encouraged. The actual ongoing amount of exercise required to develop and sustain improved strength in aging persons is not known; in actual studies of exercise in aged humans, usually only short-term studies were done.
2. Reduce neuropathic and joint pain: this will also facilitate mobility and exercising. Exercising in a pool can be easier.
3. Reduce obesity: that will facilitate mobility and exercising and, as an extra benefit, perhaps mitigate or prevent a type-2 diabetes status.
Oral leucine, with or without isoleucine and valine (branch-chain amino acids)

These are considered anabolic and utilizable directly by the muscle fibers for their protein synthesis [106]. For patients, the precise salutary amounts and scheduling of these branch-chain amino acids are not established (see Chapter 6 in this volume). They are somewhat difficult to dissolve in liquid.

If these substances actually benefit, possible schedules are: (a) before exercise sessions, as suggested by others; or (b) multiple times during the day is our suggestion, especially for less mobile older persons. However, some investigators consider that these substances are beneficial only when combined with diligent exercise, a difficult assignment for many of the elderly.

Testosterone

Aging men without significant illness typically have a gradual decrease of free and total circulating testosterone after about age 65–70. This can be of testicular origin (associated with elevated circulating follicle-stimulating hormone (FSH) and luteinizing hormone (LH), or of pituitary origin (associated with low circulating FSH and LH). It is not known whether the degree of reduced testosterone correlates with an amount of type-2 fiber atrophy. Both LMNs and muscle fibers have androgen receptors responsive to testosterone/dihydrotestosterone; thus, testosterone-therapy benefit could be on LMNs, muscle fibers, or both. Weekly injections of depo-testosterone, a known masculinizing anaboloid, in some men with a neuromuscular disease (e.g. ALS, s-IBM, or myotonic dystrophy-1), have increased skeletal muscle endurance and strength for about 4–8 days (Engel, unpublished results). (Probably testosterone is the reason human male muscle is stronger than female muscle.) Castration experiments, in animals, suggest that androgens are not required for peak muscle performance in females but are in males, where they act through the androgen receptor to regulate multiple gene pathways that control muscle mass, strength, and resistance to fatigue [66]. In a clinical use of depo-testosterone, one must be mindful of the patient’s ongoing prostate-specific antigen (PSA) status, and also avoid giving it concurrently with a glucocorticoid (a combination that can provoke diabetes-2).

Therapeutically, and potentially prophylactically, the ideal amount, route (intramuscular or transcutaneous), and frequency of testosterone therapy are not known, and must be weighed against its recognized side effects, including but not limited to: (a) short-temper; irritability, and anger; (b) possibly increased chance of prostate cancer; (c) possibly aggravation of an existing diabetes-2 or a genetic/obesity-based diabetes-2 tendency, especially if the patient is concurrently taking a glucocorticoid or growth hormone; (d) elevation of hematocrit. Cautious ascending-dose titration is important. Oral androgen preparations can engender liver abnormalities. A normal aromatizable metabolic product of testosterone is a high level of estradiol: its feminizing and/or other possible side effects on the human male neuromuscular system are not established.

Some clinicians have considered combining an aromatase inhibitor, such as letrozole, anastrozole, or exemestane, to sustain the benefit of testosterone and block estrogenization (but one should note that aromatase inhibitors can have their own side effects). Needed is development of a water-soluble, non-estrogenic androgen preparation that can be safely given subcutaneously or orally.

In general, androgenic anabolic effects involve early downregulation of axin and induction of IGF-1, causing nuclear accumulation of β-catenin (a pro-myogenic, anti-adipogenic stem-cell regulatory factor). This is related to type-2 fiber hypertrophy and atrophy [107]. The androgen receptor is a ligand-dependent transcription factor, containing binding sequences for the Mef2 family of transcription factors, suggesting a functional interaction in skeletal muscle between the androgen receptor and Mef2c [108].

In the future, if surreptitiously developed and used androgens really do enhance athletic performance (more home-runs!), and are safe, perhaps they can be brought to light and tested for counteracting muscle weakness of aging and other enfeebling conditions.

Growth hormone (somatropin)

This drug is US Food and Drug Administration-approved for “HIV associated cachexia,” i.e. the HIV
muscle atrophy (wasting) that is typically type-2 fiber atrophy. The cause of the muscle weakness and atrophy in HIV patients can be multifactorial, due to: cachexia/hyponutrition; hypoactivity; and virogenic-autoimmune and virotoxic peripheral neuropathies (see above). For AAM, (an imprecise designation, v.s.) growth hormone has not yet been proved effective in a formal trial. Potential side effects of growth hormone therapy must be considered.

Anti-myostatins
Intended for general treatment of muscle-fiber atrophy in aging and in several other settings, anti-myostatins are being developed as a new approach, but they have not yet become established for clinical use in any human muscle atrophy.

- Follistatin is a normal, powerful inhibitor of myostatin action. Investigatively, it is being given intramuscularly by gene transfer of an alternately spliced cDNA of follistatin, and reportedly it has shown anti-atrophy benefit in mice and monkeys [109, 110]. Very limited human trials are in progress, but apparently none yet for "muscle atrophy of aging".
- ActRIIB-Fc is a myostatin/GDF-8 decoy-receptor, now in human trials. In animals, ActRIIB-Fc reportedly can reverse cancer cachexia and muscle wasting. In several cancer models, pharmacological blockade of the ActRIIB pathway prevented further muscle wasting and is said to have “completely reversed” prior loss of skeletal muscle (as well as the cancer-induced cardiac atrophy). It also “dramatically prolonged survival” even when the tumor growth was not inhibited. That blockage abolished induction of the ubiquitin ligases and activation of the ubiquitin-proteasome system [31].
- Other potential therapeutic targets in the adverse catabolic pathways of muscle fibers are also being considered.

Potentially salutary molecular components for future therapeutic consideration

IGF-1
In rats with experimental adjuvant arthritis, systemic IGF-1 reportedly counteracted the muscle atrophy and decreased the atrogen-1 and IGFBP-3 [63] (also, see above).

β-Adrenergic agonists
These are experimental anabolic agents for muscle fibers, causing increased protein synthesis and decreased catabolism. An IGF-1-independent path activates β-adrenoreceptors, enhancing skeletal muscle growth and producing hypertrophy of cultured C2C12 cells (a muscle-derived cell line) [111]. However, clinically significant side effects are of potential concern. β-Adrenergic receptors have been demonstrated autoradiographically in human muscle [112].

JunB
The JunB transcription factor allegedly maintains skeletal muscle mass and can promote hypertrophy. JunB is also a major determinant of whether adult muscles grow or atrophy. In adult muscle, decreasing JunB expression by RNA interference causes atrophy, and overexpression of JunB causes independently stimulated protein synthesis and muscle-fiber hypertrophy. JunB transfected into denervated muscle “prevents” fiber atrophy. JunB protects atrophy-targeted proteins by blocking FoxO3 binding to promoters of the ubiquitin ligases atrogin-1 and MuRF-1, and thus reducing protein breakdown in proteasomes. Thus, JunB in adult muscle is required for maintenance of muscle size: it can induce hypertrophy and block atrophy [30]. Autophagy inhibition reportedly can induce “atrophy and myopathy” in adult skeletal muscles, and does not protect skeletal muscles from atrophy during denervation and fasting [113].

Conjugated linoleic acid
Based on treatment of cultured muscle cells, this has been suggested for treatment of cancer cachexia [114].

Pharmacological/biochemical substances without established value for human muscle atrophy of aging
Many substances have been tried, including creatine and DHEA, but none has yet proved effective for AAM, partly because we do not yet understand the essential pathogenesis, or pathogeneses.
Finally, persons should be wary of nostrums advertised for “preventing or treating muscle aging.”

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