Research Article

Translational Mobility Medicine and Ugo Carraro: A Life of Significant Scientific Contributions Reviewed in Celebration

Barbara Ravara[1], Walter P Giuriati[1], Amber L Pond[2]

1. Department of Biomedical Sciences (DSB), University of Padua, Italy; 2. Southern Illinois University-Carbondale, United States

Prof. Ugo Carraro will reach 80 years of age next 23 February 2023, and we wish to celebrate him and his work by reviewing his lifetime of scientific achievements. Prof. Carraro, currently is a Senior Scholar with the University of Padua, Italy, where, as a tenured faculty member, he founded the *Interdepartmental Research Center of Myology (CIR-MYO)*. Prof. Carraro is a pioneer in skeletal muscle research and a world-class expert in structural and molecular investigations of skeletal muscle biology, physiopathology and care. He is an expert in applied bidimensional gel electrophoresis for myosin light chains and was the first to separate mammalian muscle myosin heavy chain isoforms by SDS-gel electrophoresis, particularly the embryonic isoforms. He has demonstrated the ability of long-term denervated muscle to survive denervation by myofiber regeneration, as well as the beneficial impacts of an athletic lifestyle on muscle reinnervation. He has utilized his expertise in translational myology by working to develop and validate rehabilitative treatments of denervated and ageing skeletal muscle. He has authored more than 160 PubMed listed papers (several in Neurological Research, together with guest editing two issues of the journal) and numerous scholarly books. He founded and serves as Editor-in-Chief of the European Journal of Translational Myology and Mobility Medicine. He has organized more than 50 Padua Muscle Days Meetings and continues to do this, encouraging students and young scientists to participate. As his endless dreams, he is currently validating non-invasive analyses on saliva and sweat, a promising approach that will allow increased frequency sampling to analyze systemic factors (e.g., cytokenes and myokines) during the transient effects of training and rehabilitation by his proposed Full-Body in-Bed Gym for bed-ridden elderly.

Corresponding author: Amber L Pond, apond@siumed.edu

Introductory Comments

Professor Ugo Carraro (Figure 1) will reach 80 years of age on February 23, 2023. If you work in skeletal muscle and/or rehabilitation research, then you are already familiar with Prof. Carraro and his accomplishments. If you do not know him, then you should! You or a family member has likely already benefitted from some application of his research. Prof. Carraro is the son of hoteliers who operated their business in Thermae of the Euganean Hills near Padua, Italy. He earned his medical degree from the University of Padua in 1968 with the goal of becoming a Rehabilitation Specialist. Despite this attractive option, he was inspired by his association with Prof. Massimilano Aloisi, a General Pathologist, to venture into scientific research. Indeed, Prof. Carraro's strong curiosity and desire to explore unknown scientific and medical realms, led him to choose a career in University research and teaching. Impressively, Prof. Carraro's research has included investigations of the molecular and structural analyses of skeletal muscle as well as medical rehabilitation practices and related imaging techniques. From 1970 to 2013 he performed this research while also serving as a teacher of General Pathology at the University of Padova, where he founded the Interdepartmental Research Center of Myology of the University of Padua (CIR-Myo). Prof. Carraro currently serves as a Senior Scholar with the University of Padua, Italy, where he continues his research and collaborations, advises students and younger scientists, coordinates the annual Padua Muscle Days Conference, and serves as founder and Editor-in-Chief of the European Journal of Translational Myology and Mobility Medicine (EJTM). EJTM publications are listed in PubMed, SCOPU (Cite Score = 3.6, last updated on 05 May, 2022) and ESCI. Clarivate will release the 2022 Impact Factor of EJTM next June 2023, which will hopefully be a nice present for Prof. Carraro's 80th birthday.

Some of the highlights of Prof. Carraro's research include investigations of: 1) the sequential degenerative processes which occur in long term denervated skeletal muscle in rodents; 2) application of his results to animal models of muscle pathologies related to human diseases, in particular hypertension and heart failure; 3) validation of strategies to improve the condition of denervated human muscles (in collaboration with Helmut Kern, Vienna, Austria) by home-based Functional Electrical Stimulation (hbFES); and 4) development of imaging techniques (in collaboration with Prof. Paolo Gargiulo, Reikjavik, Iceland) to allow improved diagnosis and treatment of injured and rehabilitating skeletal muscle. The body of Prof. Carraro's work has contributed to the expansion of knowledge concerning muscle regeneration and apoptosis, spinal cord injuries and ageing. The methods and results of Prof. Carraro's work are well documented by original publications in leading

journals (164 PUBMED Titles; >3000 Citations of his work; and a Hirsch Index of 44). Indeed, Prof. Ugo Carraro's pioneering work in skeletal muscle structural and molecular analyses and muscle rehabilitation has earned him renown as a world class scholar in the field of skeletal muscle research. The authors of this paper are proud collaborators of Prof. Carraro and here they wish to acknowledge the contributions of this man to the world's basic scientific knowledge and medical practice by application of Translational Mobility Medicine.

Professor Ugo Carraro in 2006

Figure 1. Professor Ugo Carraro in 2006.

The Early Years

Medical School.

While still a medical student, Prof. Carraro was fascinated with the General Pathology courses he took during his second and third years at the University of Padua Medical School. During this time, he collected personal notes from the "socratic teaching" of Prof. Massimiliano Aloisi, who usually spent more time posing questions to the audience than lecturing. After graduation, Prof. Carraro remained at the Institute of General Pathology initially as a volunteer lecturer; however, it was decided that he would be retained as a paid teaching assistant, particularly to help Prof. Aloisi's students learn through practical exercise with pathological microscopy specimens and with examinations. Indeed, Prof. Carraro maintains that this experience was invaluable because those long hours of listening to

the questions and replies of both brilliant and poor students, along with the countless observations of microscopic pathology slides, aided him greatly when writing and publishing (as Associate Professor of General Pathology in the Faculty of Medicine of the University of Padua, Italy) both an atlas for practical exercises with pathologic microscopic slides (ATLANTE per le ESERCITAZIONI DI PATOLOGIA GENERALE" published by Lint, Trieste, Italy, [1A]) and three editions of the book Principles of General Pathology (PRINCIPI DI PATOLOGIA GENERALE, published by UNIPRESS, Padua, Italy) [2A; Figure 2].

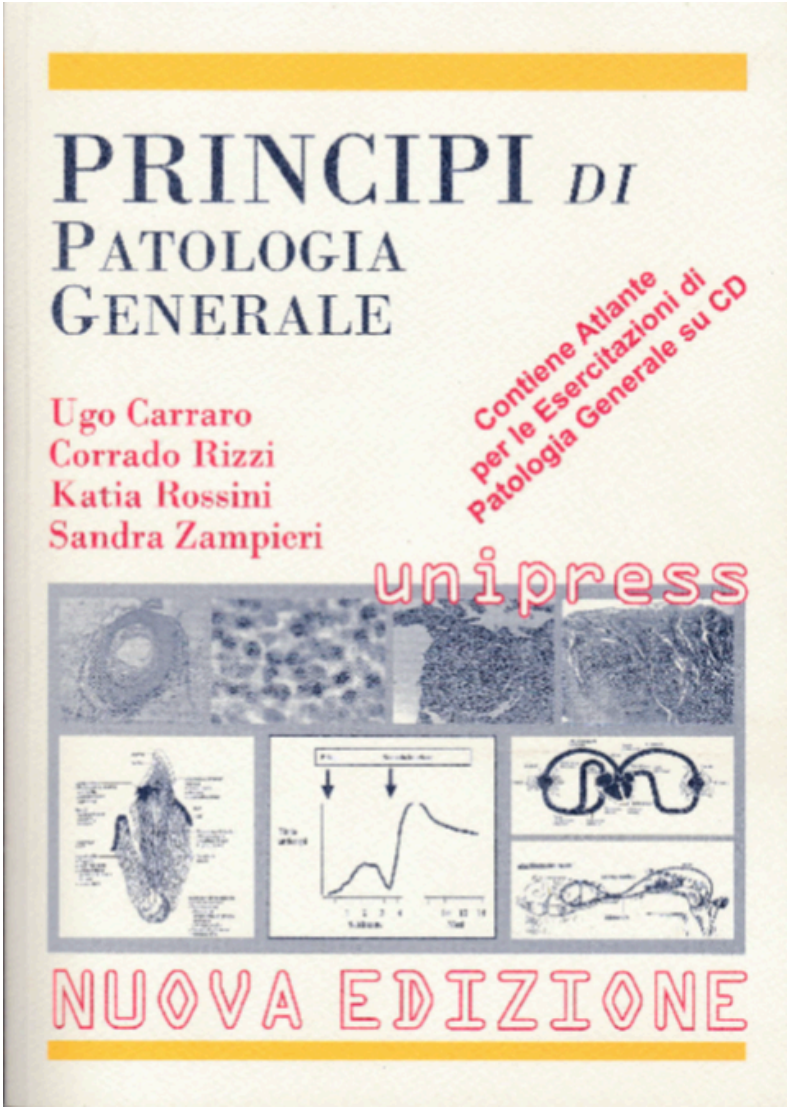

Figure 2. A text book co-authored by Professor Carraro for students of pathology: Ugo Carraro, Corrado Rizzi, Katia Rossini, Sandra Zampieri. PRINCIPI DI PATOLOGIA GENERALE [Principles of General Pathology]. 2007 Pag. 1-355. Published by UNIPRESS, Padua, Italy 3rd edition. ISBN 978-88-8098-194-7. Contains the e-Atlas for the practical examinations of pathologic microscopic slides.

Ten Years after Graduation from Medical School.

After graduating from medical school, young Dr. Ugo Carraro decided to serve as soon as possible Italy as a military physician, spending September to November 1968 in Florence at the Military Academy.

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When he returned home, he had to wait several months to be certified as a medical practitioner. Thus, during the winter of 1968-69, he worked at the Institute of General Pathology within the University of Padua. It was during a University of Padua dancing event on February 8, 1969 that Dr. Carraro met a young girl studying Mathematics, Annalisa Bossi and they fell in love. In July 1969, he was called to serve as a military doctor to the "Italian *Lagunari* Squadron" at the sea side, east of Venice. It was a wonderful summer, but in late autumn 1969, while returning to the *Lagunari* barracks at Ca' Vio, Venice from Padua (where he had spent an evening with Annalisa), he had a serious car accident. The consequences of this event lasted up to the late autumn of 1970. During his recovery from the car accident, Dr. Carraro worked at the Institute of General Pathology with Alfredo Margreth, one of the adjutants of Professor Aloisi, with whom he continued to collaborate for the following 10 years. He and Annalisa were married in 1973.

First Research Efforts.

Dr. Carraro worked as an assistant to Professor Aloisi for 10 years and was included as a coauthor on two interesting publications concerning neural control of the calcium-transport system in the sarcoplasmic reticulum of rat skeletal muscle [1][2]. Unfortunately, as does happen in research, he then began work on a topic in a rabbit model, which never yielded consistent results; and, thus, assays and analyses were repeated many times. The results were never publishable. This unfortunate waste of time was aggravated by the political turbulence occurring in Italy at the time as a result of the abuses committed by the Red Brigades (*Brigate Rosse* in Italian**)** when the prime minister Aldo Moro was murdered. The situation was further exacerbated by local events that made it difficult to teach and perform research at the University of Padua through 1977.

In the meantime, techniques for molecular studies of protein synthesis abruptly exploded and, in collaboration with Paola Arslan, Prof. Carraro moved to a new research topic with the help of an English colleague, who was on sabbatical at the University of Padua. This colleague taught Prof. Carraro how to denervate the rat left hemidiaphragm while the contralateral right hemi-muscle remained innervated (and supported pulmonary ventilation in the experimental animals). Prof. Carraro also learned how to prepare skeletal muscle ribosomes from this three-day denervated and the contralateral right innervated hemidiaphragm. The results of those studies yielded data for a single original research publication in 1980, the second with him as first author [3].

Interestingly, however, a group of denervated animals, which had been forgotten for six-months in the animal house, offered an opportunity to study the effects of long-term denervation on skeletal muscle and this work resulted in Prof. Carraro's first first-author publication in a top neurology journal [4]. Now having established an original research program, Prof. Carraro has been able to publish at least one good paper per year (even until today) on skeletal muscle biology, physiopathology and care using both experimental animals and in cases of human diseases, thus representing the two stages of biomedical research that have been defined as Translational Medicine - or in his case Translational Mobility Medicine.

An Epic Research Career.

The interests of Prof. Carraro are eclectic and may broadly be divided into the following main topics and subtopics listed chronologically as they occur in his list of publications:

Topic 1. Fiber types of skeletal muscles and their regulation by motor neurons.

Studies of long-term denervation of skeletal muscle include the hemidiaphragm model in rodents [4][5][6][7][8] *and various skeletal muscles in other animal and human models* [9][10][11][12][13][14][15]. It was generally believed that muscle fibers did not survive long after denervation; however, Prof. Carraro and collaborators demonstrated that muscle fibers survive more than two years post-denervation of the 3-5 year life-span of rats with the contribution of regenerating muscle fibers.

1.2 *The early phases of post-damage muscle fiber regeneration are independent of motor neuron innervation* [16][17][18][8][19][20][21][22]. Prof. Carraro and collaborators used light and electron microscopy, molecular analyses by SDS PAGE, and single myofiber analyses to show that muscle regeneration after severe myotoxic damage occurs in the denervated limbs of rats and continues for many cycles of damage-regeneration. Thus, they also demonstrated that satellite cells (myogenic stem cells) regenerate after revascularization and necrotic tissue removal by leucocytes.

1.3 *Muscle fiber type modulation by electrical stimulation in rodents and other mammals* [23][24][25][26][27][28][29]. The extensor digitorum longus (EDL) muscle is an almost pure fast-contracting muscle in rats. Prof. Carraro's laboratory implanted electrodes on the surface of denervated rat EDL muscles and demonstrated that a pattern of stimulation, mimicking the discharge patterns of slow motoneurons, induces the expression of slow light and heavy myosin chains. This important work strongly supports

the hypothesis that motoneurons induce and maintain the contractile characteristics of the innervated motor units, according to the pattern of activity they impose on the muscle fibers.

Topic 2. Gel electrophoresis of muscle proteins separates protein markers of different muscle fibers types: The case of myosin light and heavy chains.

The definitive evidence to demarcate and explain observed muscle changes that occur in response to various disease states, treatments, stimuli, etc. are obtained by study of the molecular characteristics of muscle fibers. As an invaluable contribution to these efforts, Carraro and collaborators developed and optimized the application of two-dimensional Sodium Dodecyl Sulfate-Poly Acrylamide Gel Electrophoresis (2D-SDS-PAGE) to skeletal muscle samples to separate the various light and heavy myosin protein "chains." They were the first in Italy, if not in the world, to perform and continuously improve this technique [4][5][30][31][32][33][34][35][36][37][38].

Topic 3. Translational Medicine: Application of gel electrophoresis of isomyosins to study the effects of arterial hypertension on animal and related human cardiac hypertrophy.

As soon as the new electrophoretic analyses were implemented and proven in animal experiments, colleagues of the University of Padua asked Prof. Carraro's team to collaborate and combine their unique specialties. This happened firstly in 1983 through a collaboration with Luciano Dalla Libera and the group of experts on hypertension and related cardiac hypertrophy. The collaboration resulted in numerous publications which collectively demonstrated that isomyosin analyses may provide biomarkers of change in cardiac and skeletal muscle adaptation/damage secondary to hypertension and related cardiac failure in animals models (rodents and turkey) and human patients [39][40][41][42][43][44][45][46][47][48][49][50][51][52]. The collaboration lasted up to 2002.

Topic 4. Studies of muscle regeneration in vitro: Leukocytes-myoblasts interactions.

After Marcello Cantini joined Prof. Carraro's group in 1985, the research activities were extended to *in vitro* cell culture. Indeed, Prof. Carraro insisted that muscle culture *in vitro* studies provides final evidence that muscle regeneration is possible in the complete absence of neural input and/or factors. With the support of Telethon Italy, it was possible for the laboratory to study the interactions between leukocytes and myoblasts. The resulting ground-breaking publication revealed that, in addition to the well-known scavenger activity of leukocytes, macrophages (in particular the ED2+ type) release

factors that induce myoblast replication, followed by their fusion and improved myotube growth [19][20][53][54][55][56][57].

Topic 5. Muscle apoptosis: A debated issue.

Prof. Carraro prefers to go against the tide, and this characteristic (in addition to having gotten him into trouble at times) led to a change in direction toward an exciting area of research: muscle apoptosis. Negated by eminent myologists and neurologists, Prof. Carraro's group used electron microscopy and molecular analyses to prove that the accepted markers of apoptosis are present in mouse muscle at two days after a night of volitional running (up to 5 km during the first night). This observation was extended to other experimental models *in vitro* and *in vivo* and in human cases of muscular dystrophies [58][59][60][61][62][63][64][65][66][67][68][69].

Topic 6. The hopes and frustrations of the Demand Dynamic Cardiomyoplasty: Twenty years of translational science research in collaboration with plastic and cardiac surgeons.

Prof. Carraro joined Giorgio Arpesella, a cardiac surgeon with the Bologna University, in a project proposed by an eminent University of Pisa Cardiologist. The idea was to produce an animal model in Italy, which mimicked the then famous Dynamic Cardiomyoplasty surgical approach proposed by the French cardiac surgeons Alain Carpentier and Juan C. Chachques. Prof. Carraro proposed that the group implement a daily resting period from on-demand stimulation of the transposed latissimus dorsi (LD). This was tested in a simplified sheep model. The results of a year-long experiment in six sheep were more than encouraging. Then, cardiac surgeons of Padua University asked Prof. Carraro to implement his Demand Dynamic Cardiomyoplasty model in a group of Italian patients, including new patients operated upon in Padua. The results were interesting in the opinions of the physicians treating these patients. Specifically, Dr Gianluca Rigatelli collected Doppler flow wire data producing direct evidence of support by the transposed LD tetanic contraction to cardiac systole [70][71][72][73][74][75][76][77][78][79][80][81][82][83][84][85]. However, despite all of the evidence, the Company decided to discontinue the production of the pacemaker necessary for this procedure because sales were not covering the costs.

A dream to alleviate suffering and promote life-saving techniques ended in a major frustration because of lost profits!

Topic 7. Helmut Kern and his requests for collaborations.

7.1. *Home-based Functional Electrical Stimulation of paraplegic human skeletal muscles:*

The EU Program RISE Project. Luckily, Dr. Helmut Kern, a Vienna (Austria)-based Rehabilitation Specialist, visited Padua to meet Prof. Carraro and asked for his expertise concerning long-term denervated muscle. Dr. Kern was interested in translation of Prof. Carraro's work to human cases of complete spinal cord injury (SCI) which resulted in permanent leg muscles denervation. Dr. Kern was treating these patients with home based functional electrical stimulation (hbFES) of the denervated muscles. The analysis of the first muscle biopsy (from a patient who had performed hbFES for five years, begun from one year after SCI) provided exciting preliminary evidence and started the very successful European Project RISE. RISE was approved with substantial funding for a group of excellent young myologists (electron microscopists and molecular analyses experts) whom Prof. Carraro introduced to Dr. Kern. The RISE project has provided the final evidence that, even three years after SCI denervation, severely atrophic human muscles can be rescued and regain strength enough to allow patients to stand and even to train by step-in-place exercise when supported by their own electrostimulated quadriceps muscle [86][87][88][89][90][91][92][52][93][94][95][96][97][98][99][100][101][102][103][104][105][106].

Finally, a dream that did not ended in frustration!

7.2. *Development and implementation of Color 2D and 3D Computer Tomography to assess skeletal muscle volume and quality: A collaboration with Paolo Gargiulo, a Naples-born Biomedical Engineer based in Reykjavik, Iceland.* Among other non-Vienna collaborators of Dr. Kern, Prof. Carraro met a young Naples-born Biomedical Engineer working in Reykjavik, Iceland. Prof. Carraro relates that, in his opinion, just this was strong evidence that Prof. Paolo Gargiulo was a brilliant man! In fact, during a half-hour drive from Reykjavik to the airport, when Prof. Carraro suggested to Paolo that they could translate the cardiologists' work (specifically, applications of Color CT Scan for cardiac muscle analysis) to skeletal muscle, he showed his best. In a few months another naive dream of Prof. Carraro's became a reality.

To date, the collaboration is still producing top-journal publications and clinically-relevant applications [107][108][109][110][111][112][113][114][115].

7.3. *Aging Muscle Decay.* After the RISE Project ended, Dr. Kern went to Padua to seek support for his new project: to extend to normally aging people the benefits of hbFES, in this case by neuromuscular

stimulation. The subsequent preliminary observations allowed him to propose and collect funds for additional years up to today. The work demonstrated that, although neuromuscular stimulation could not restore youth, it could indeed improve the condition of aging muscle, resulting in some regained muscle size and strength [116][117][118][119].

7.4. *Studying life-long active seniors: The benefits of exercise.* Muscle biopsies from young sportsmen, sedentary seniors and life-long active seniors provide strong structural, functional and molecular evidence of the benefits of an active life. Interestingly, a life-long active life-style increases also the reinnervation of skeletal muscles as humans age [120][121][122].

7.5. *Performance Decay of Master Athletes.* Aging is an inevitable process that can be delayed, but not reversed - even in the best trained master athletes, i.e., the world record men in their class of age. Paolo Gava, (an Engineer who had been a Master Athlete turned amateur scientist) and colleagues have analyzed the world records of Master Athletes, studying the behaviors of these superbly trained persons, and have shown that decay of athletic prowess proceeds linearly towards an end point at 110 years [123][124][125][126].

7.6. *Countermeasures by FES and the Full-Body in-Bed Gym in Aging and Early Aging.* This group of publications provides evidence that humans can slow (but not halt) the relentless process of ageing. Because the majority of older persons have a sedentary life-style, it is relatively easy to help them improve the functional and structural characteristics of the majority of their 700 skeletal muscles using home-base Functional Electrical Stimulation or volitional exercises. Even people who are bedridden or are very old and have difficulty being active can exercise using Prof. Carraro's Full-body in-Bed Gym [127][128][129][118][130][105][131][132][133][134][135][136][137][138][139][140][141][142][143][144][145][146].

Current Scientific Contributions.

Amazing! Prof. Carraro is about to be *80-years young* and is still working to further scientific knowledge. He continues to collaborate with other scientists, also acting as mentor/adviser to students and younger scientists all over the world. However, Prof. Carraro's real hobbies now are editing scientific journals [147][148] and organizing scientific conferences in his native Thermae of the Euganean Hills near Padua, Italy. Most impressively, Prof. Carraro continues to edit the journal he founded in 1991 and originally titled **Basic Applied Myology (BAM)**. In 2010, the journal was

renamed **European Journal of Translational Myology (EJTM)** and then, to keep up with advances in science and expansion of the journal content, was again renamed in 2018: **European Journal of Translational Myology and Mobility Medicine (EJTM3)** [51][149][150][151][152][153][154][155][156][157][158][159][160][161][162][163][164][165][166][167][168][169][170][171][172][173][174][175][176][177][178][179][180][181][182][183][184][185][186][187][188][189][190][191][192][193].

In 1985, Prof. Carraro discovered he has a talent for organizing meetings, when he and Dr. Corrado Angelini, a neurologist with the Padua University, began organizing the *First Abano Terme Meetings on Rehabilitation*, an International Symposium held in Abano Terme (Padua), Italy on August 28-30, 1985, as a Satellite Meeting of the XIII World Congress of Neurology, Hamburg 1985. After this success, Prof. Carraro began organizing the Padua Muscle Days (PMD), a conference that has been held in the Thermae one or two times a year since 1991. He continues to organize this conference yearly and, in 2018, renamed the conference as Padua Days of Muscle and Mobility Medicine (PDM3) [194][195][196][197][198][199][200][201][202][203][204][205][206][207], to be consistent with the name of the journal he founded. Prof. Ugo Carraro: A life of seeming constant motion well lived. It is not difficult to understand why he seems lazy when replying to emails: he is always very busy!

Afterword from the Authors

Sadly, we do not have enough space here to discuss in detail the numerous and impressive contributions Prof. Ugo Carraro has made to the sciences of muscle biology and mobility medicine. He has contributed by producing excellent research and by teaching and training both scientists and those in professional fields. Indeed, Prof. Carraro taught General Pathology at the University of Padova for fifty years, teaching first students of Medicine and Surgery and then students of Dentistry and of Nursing. Although not one of the authors of this piece was a student of Prof. Carraro, we each have learned a great deal from working with him. At this point, we know that we have been fortunate to work with this world-class scientist. One of us, Dr. Barbara Ravara, has strictly collaborated starting from Topic 3 and, after a break, from Topic 7.3 to date. The second of us, Dr. Walter Giuriati, has only recently collaborated with Prof. Carraro's team. The third author, Dr. Amber Pond, is proud to have collaborated with (and been mentored by) Prof. Carraro since 2009. Although now retired, Prof. Carraro continues to provide his expert knowledge to the University of Padua School of Rehabilitation by discussing histopathology and his research interests, specifically the chapters he wrote in the Springer Book: **Rehabilitation Medicine for Elderly Patients**, Stefano Masiero and Ugo Carraro, Eds,

2018 ([3A,[208]]), a top selling book with 100,000 readers and 10,000 (paid) downloads of Chapters (Figure 3). Indeed, Prof. Ugo Carraro is a most impressive individual and each of us hopes to follow his example and remain productive up to retirement and beyond.

May he continue in his fight against age for many years to come!

Figure 3. A book co-authored by Prof. Carraro to provide approaches for the care of elderly people: Stefano Masiero and Ugo Carraro, Eds, Rehabilitation Medicine for Elderly Patients, 2018ISSN 2509-6060 ISSN 2509-6079 (electronic).

I. Books

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