In focus in HCB

Douglas J. Taatjes¹ · Jürgen Roth²

Published online: 28 September 2020
 © Springer-Verlag GmbH Germany, part of Springer Nature 2020

In this month's Editorial, we highlight four Original Articles describing (1) the role of macrophages containing inducible nitric oxide synthase in muscle regeneration following injury; (2) a quantitative comparison between BODIPY and Oil-Red-O for investigating exercise-induced changes in intramuscular triglyceride and lipid droplet morphology; (3) the histochemical and immunohistochemical analyses of saphenous vein and internal thoracic artery grafts used for coronary artery bypass grafting from 1 day to up to 1 year following implantation; and (4) immunohistochemical analysis of the vitamin D receptor (VDR) and its possible use as a prognostic predictor of patient survival for ovarian cancer subtypes. We hope you enjoy these highlights, as well as the full content of this issue!

Unloading impairs muscle regeneration: the role of iNOS-expressing macrophages

The regeneration of injured skeletal muscle is accompanied by the accumulation of proinflammatory macrophages and their subsequent conversion into antiinflammatory macrophages (Arnold et al. 2007; Bencze et al. 2012; Saclier et al. 2013; Juban and Chazaud 2017), and the activation of satellite cells followed by their differentiation into muscle fibers (Yin et al. 2013). It is known that nitric oxide (NO)

synthesized by macrophages is an important regulatory molecule for proper muscle regeneration through its modulatory effect on satellite cells (Anderson 2000; Rigamonti et al. 2013). Proinflammatory macrophages present early in the injured site express inducible NO synthase (iNOS) and synthesize NO (Villalta et al. 2009). Unloading has been shown to negatively influence muscle regeneration, since it retards the migration of macrophages into the injured site (Matsuba et al. 2009; Kohno et al. 2012). Now, Kawashima et al. (2020) investigated the effect of hindlimb unloading as compared to weight-bearing control animals on the spatiotemporal behavior of iNOS-expressing macrophages (Fig. 1) and satellite cell accumulation after crush-induced muscle injury in rats.

Fig. 1 Chronological changes in the number of iNOS-expressing macrophages after muscle injury. *HU* hindlimb unloading, *WB* weight-bearing control, *UC* uncountable. From Kawashima et al. (2020)

✉ Douglas J. Taatjes
douglas.taatjes@med.uvm.edu

¹ Department of Pathology and Laboratory Medicine, Larner College of Medicine, University of Vermont, Burlington, VT 05405, USA

² University of Zurich, 8091 Zurich, Switzerland

For their quantitative investigation, they applied conventional H&E histology combined with immunohistochemistry for iNOS, CD68 (for macrophages), double labeling for Pax7 and MyoD (for activated satellite cells), and double labeling for Pax7 and dystrophin (for satellite cells in non-injured muscle areas) as well as Western blotting to quantify iNOS protein. They found that unloading attenuated and/or delayed the recruitment of iNOS⁺ macrophages in the injured muscle, which was accompanied by a reduced iNOS amount. In parallel, the numbers of activated satellite cells within the regenerating area were significantly reduced. Based on these findings, the authors concluded that unloading leads to poor muscle regeneration following mechanical injury.

Of lipid dyes and their usefulness

Adipose tissue represents the main lipid storage site in humans, and the biogenesis, structure, and function of lipid droplets is well established (Fujimoto et al. 2008). In contrast, only small amounts of lipids are stored in skeletal muscle (Hoppeler 1999; Shaw et al. 2008; van Loon et al. 2004; Morales et al. 2017) in the form of intramuscular triglyceride (IMTG). For the detection of lipid droplets and IMTG by microscopy, various dyes exist (Suzuki et al. 2012). Oil-Red-O (ORO) and BODIPY 493/503 (BODIPY) are commonly used to detect lipid droplets and IMTG in cultured cells and tissues including skeletal muscle (Koopman et al. 2001; Ohsaki et al. 2010). However, ORO seems to have certain limitations because of its broad emission spectrum and unsatisfactory specificity for IMTG, since it additionally stains all neutral lipids including plasma membrane phospholipids as well as potentially causing artificial fusion of lipid droplets when applied together with isopropanol. Several studies have indicated that BODIPY may be a superior probe for the detection and visualization of lipid droplets containing IMTG (Listenberger et al. 2016; Prats et al. 2006, 2013; Spangenburg et al. 2011; Jevons et al. 2020; Whytock et al. 2020). In their present work, Strauss et al. (2020) have systematically compared ORO and BODIPY for their use to detect and quantify IMTG and lipid droplets in human skeletal muscle. They analyzed biopsies taken from the *musculus vastus lateralis* after an > 10 h overnight fast of the participants (study group 1) and a second biopsy immediately taken after an ensuing 60 min moderate-intensity exercise (study group 2). In muscle biopsies of study group 1, the IMTG content in all muscle fiber types (type I, type IIa, and type IIx) was greater with the use of BODIPY compared to ORO, since BODIPY revealed a greater number of lipid droplets which were on average larger in size (Fig. 2).

Fig. 2 BODIPY 493/503 (BOD) detects more lipid than oil red O (ORO). **a** Intramuscular triglyceride (IMTG) content, **b** number of lipid droplets, and **c** lipid droplet size in peripheral and central subcellular regions in type I, type IIa, and type IIx muscle fibers. From Strauss et al. (2020)

Interestingly, the subcellular distribution of the lipid droplets was found to differ depending on the dye. Using BODIPY, the IMTG content of the peripheral muscle fiber

region (5 μm beneath the laminin-positive surface) was smaller compared to the remaining central region, whereas no difference was detected with the use of ORO. The latter observation was attributed to the additional detection of membrane lipids by ORO. Rightfully, the authors emphasized that BODIPY appears to reflect more accurately subcellular differences in muscle fiber IMTG content. In study group 1, the variability and photostability of ORO and BODIPY staining were additionally investigated. The coefficient of variation of BODIPY was about 6 vs. 9% for ORO, showing that labeling of lipid droplets with BODIPY was less variable. In addition, BODIPY was found to be more photostable over a time period of 13 days for measuring the IMTG content. The findings for the study group 2 can be summarized as follows: the IMTG content was reduced only in type I fibers following the 60 min exercise irrespective of the use of BODIPY or ORO. In regard to the subcellular content of IMTG following the exercise, BODIPY revealed a decrease in both the peripheral and central region of type I fibers, whereas ORO showed only a significant decrease in their peripheral region. Taken together, this meticulous analysis revealed important differences between BODIPY and ORO to detect and quantify IMTG related to fiber type and subcellular distribution. The authors propose “that BODIPY should be the dye of choice to investigate IMTG in skeletal muscle due to its lower coefficient of variation, greater photostability, and capability to specifically detect IMTG avoiding the quantification of membrane structures, compared to ORO.”

Molecular and structural properties of a CABG patch

Coronary artery bypass grafting has become a relatively common surgical procedure (approximately 400,000 surgeries per year) for the treatment of atheromatous blockages by replacing affected segments of the coronary artery with small vessel conduits of venous or arterial origin (Bachar and Manna 2020). Various anatomic locations may be used as sources for the grafted vessel sections, with the saphenous vein (SV) and internal thoracic artery (ITA) being two of the most common. Although the grafted vessel portions initially exhibit excellent patency, over time, likely due to environmental adaptation and response to fluid shear stress, they often develop luminal constrictions due to vascular remodeling, including cellular infiltration and proliferation,

concomitant with extracellular matrix deposition. These processes are similar to what occurs in injured arteries with neointimal formation (Falk 1989; Taatjes et al 2008), leading to the hypothesis that they share similar mechanisms. To test this theory, Steger et al. (2020) have performed a postmortem histochemical and immunohistochemical investigation of 150 coronary artery bypass grafts (75 each from SV and ITA grafts). These grafts were grouped according to the period of implantation as (1) baseline group without grafting (native vessels); (2) 1 day; (3) > 1 day–1 week; (4) > 1 week–1 month; and (5) > 1 month–1 year. The histochemical analyses included staining with conventional hematoxylin and eosin, van Gieson (elastic fibers), Masson's trichrome (fibrosis), and Alcian blue (mucopolysaccharides). The immunohistochemical analyses were drawn from a panel of 17 antibodies, identifying components involved in vascular remodeling, including cell type-specific markers, indicators of inflammation, growth factors and cytokines, indicators of cell proliferation and apoptosis, and matrix metalloproteinases (Fig. 3).

Fig. 3 Some differences between arterial and venous grafts in the first year after coronary artery bypass grafting. The number of smooth muscle actin (SMA) positive cells is increased in the media of internal thoracic artery (ITA) grafts (a–c) and the neointima of saphenous vein (SV) (d–f), and the intima thickness is increased in SV grafts. From Steger et al. (2020)

Their comprehensive staining results, combined with morphometric analysis comparing the SV and ITA grafts over the defined time period, revealed the following results: (1) ITA grafts displayed lesser neointima formation and a better preserved medial layer; (2) ITA grafts had higher amounts of elastic fibers providing more flexibility and stability; (3) ITA graft wall layers showed the higher percentages of the growth factors and cytokines TNF- α , PDGR- α , and VEGF-A; (4) SV grafts displayed higher amounts of MMP-7, MMP-9, EGFR, and β FGF-positive cells; and (5) implantation period also played a role in growth factor and cytokine expression for both SV and ITA grafts. Collectively, their results suggest that the properties of the vessel graft wall are mainly affected in the first days and weeks following implantation, followed by degrees of degeneration and tissue damage months and years later in SV grafts.

Vitamin D receptor (VDR) expression as a potential prognostic marker for ovarian cancer

The vitamin D receptor (VDR) is a member of the superfamily of nuclear receptors, regulating the gene expression by binding to target genes with promoters containing a vitamin D response element (Saccone et al. 2015). Vitamin D and the VDR have been shown to be involved in gynecological cancers (Deuster et al. 2017), and are increased in ovarian cancers. Epithelial ovarian cancers are classified histologically into five subtypes as follows: (1) high-grade serous; (2) low-grade serous; (3) mucinous; (4) endometrioid; and (5) clear cell histology. Given the heterogeneity of ovarian cancer subtypes, Czogalla et al. (2020) have performed an immunohistochemical analysis of VDR in these subtypes, with the aim of correlating expression with patient survival. VDR immunoreactivity was detected in both the nucleus and cytoplasm as expected. A semi-quantitative immunoreactivity score (Remmele's score) was estimated for the intensity of staining in both cellular compartments. Using this scoring system, the nuclear staining did not differ between the ovarian cancer histological subtypes, whereas the cytoplasmic staining was subtype-dependent. High cytoplasmic VDR expression was observed in serous, clear cell, and endometrioid subtypes, while a low expression was found in mucinous subtypes. Likewise, cytoplasmic VDR expression was found to correlate with clinical and pathologic data, including positive lymph-node status, higher FIGO (Fédération Internationale de Gynécologie et d'Obstétrique) stage, and with high-grade serous histology, while nuclear VDR expression showed no correlation. Moreover, high cytoplasmic VDR expression was associated with shorter overall patient survival. Finally, cytoplasmic VDR expression was shown to be a statistically independent prognostic factor,

Covariate	Coefficient	Hazard ratio	95% CI		p value
			Lower	Upper	
Histology (serous vs other)	− 0.037	0.963	0.637	1.457	0.86
Grading (low vs high)	0.472	1.604	1.158	2.138	0.000**
FIGO (I, II vs III, IV)	0.666	1.947	1.409	2.690	0.000**
Patients' age (≤ 60 vs > 60 years)	0.487	1.628	1.082	2.449	0.019*
VDR cytoplasmic	0.797	2.218	1.106	4.448	0.025*

Fig. 4 The vitamin D receptor (VDR) as an independent risk factor in ovarian cancer. Significant independent factors were identified by a multivariate Cox regression model and are indicated by asterisks (* $p < 0.05$; ** $p < 0.01$). CI confidence interval, FIGO Fédération Internationale de Gynécologie et d'Obstétrique stage. From Czogalla et al. (2020)

whereas the prognostic impact of the various ovarian cancer histological subtypes was not found to be of independent significance (Fig. 4).

Since VDR interacts with the MAPK pathway, the authors posit that cytoplasmic VDR staining level may be used as a predictive biomarker able to distinguish between ovarian tumors which are MAPK inhibitor sensitive or those which are not, allowing the possible prediction of which patients should receive MAPK inhibitor therapy.

References

- Anderson JE (2000) A role for nitric oxide in muscle repair: nitric oxide-mediated activation of muscle satellite cells. *Mol Biol Cell* 11:1859–1874. <https://doi.org/10.1091/mbc.11.5.1859>
- Arnold L, Henry A, Poron F et al (2007) Inflammatory monocytes recruited after skeletal muscle injury switch into antiinflammatory macrophages to support myogenesis. *J Exp Med* 204:1057–1069. <https://doi.org/10.1084/jem.20070075>
- Bachar BJ, Manna B (2020) Coronary artery bypass graft. In: StatPearls [Internet]. StatPearls Publishing, Treasure Island. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK507836/>
- Bencze M, Negroni E, Vallesse D et al (2012) Proinflammatory macrophages enhance the regenerative capacity of human myoblasts by modifying their kinetics of proliferation and differentiation. *Mol Ther* 20:2168–2179. <https://doi.org/10.1038/mt.2012.189>
- Czogalla B, Deuster E, Liao Y et al (2020) Cytoplasmic VDR expression as an independent risk factor for ovarian cancer. *Histochem Cell Biol*. <https://doi.org/10.1007/s00418-020-01894-6>
- Deuster E, Jeschke U, Ye Y et al (2017) Vitamin D and VDR in gynecological cancers: a systematic review. *Int J Mol Sci* 18:2328. <https://doi.org/10.3390/ijms18112328>
- Falk E (1989) Morphologic features of unstable atherothrombotic plaques underlying acute coronary syndromes. *Am J Cardiol* 63:114E–120E
- Fujimoto T, Ohsaki Y, Cheng J, Suzuki M, Shinohara Y (2008) Lipid droplets: a classic organelle with new outfits. *Histochem Cell Biol* 130(2):263–279. <https://doi.org/10.1007/s00418-008-0449-0>
- Hoppeler H (1999) Skeletal muscle substrate metabolism. *Int J Obes Relat Metab Disord* 23(Suppl 3):S7–10. <https://doi.org/10.1038/sj.jo.0800878>
- Jevons EFP, Gejl KD, Strauss JA, Ortenblad N, Shepherd SO (2020) Skeletal muscle lipid droplets are resynthesized before being coated with perilipin proteins following prolonged exercise in elite

- male triathletes. *Am J Physiol Endocrinol Metab* 318:E357–E370. <https://doi.org/10.1152/ajpendo.00399.2019>
- Juban G, Chazaud B (2017) Metabolic regulation of macrophages during tissue repair: insights from skeletal muscle regeneration. *FEBS Lett* 591:3007–3021. <https://doi.org/10.1002/1873-3468.12703>
- Kawashima M, Miyakawa M, Sugiyama M et al (2020) Unloading during skeletal muscle regeneration retards iNOS-expressing macrophage recruitment and perturbs satellite cell accumulation. *Histochem Cell Biol*. <https://doi.org/10.1007/s00418-020-01897-3>
- Kohno S, Yamashita Y, Abe T et al (2012) Unloading stress disturbs muscle regeneration through perturbed recruitment and function of macrophages. *J Appl Physiol* 112:1773–1782. <https://doi.org/10.1152/jappphysiol.00103.2012>
- Koopman R, Schaart G, Hesselink MK (2001) Optimisation of oil red O staining permits combination with immunofluorescence and automated quantification of lipids. *Histochem Cell Biol* 116:63–68
- Listenberger LL, Studer AM, Brown DA, Wolins NE (2016) Fluorescent detection of lipid droplets and associated proteins. *Curr Protoc Cell Biol* 71:4.31.1–4.31.14. <https://doi.org/10.1002/cpcb.7>
- Matsuba Y, Goto K, Morioka S et al (2009) Gravitational unloading inhibits the regenerative potential of atrophied soleus muscle in mice. *Acta Physiol* 196:329–339. <https://doi.org/10.1111/j.1748-1716.2008.01943.x>
- Morales PE, Bucarey JL, Alejandra Espinosa A (2017) Muscle lipid metabolism: role of lipid droplets and perilipins. *J Diabetes Res* 2017:1–10. <https://doi.org/10.1155/2017/1789395>
- Ohsaki Y et al (2010) A pitfall in using BODIPY dyes to label lipid droplets for fluorescence microscopy. *Histochem Cell Biol* 133:477–480
- Prats C et al (2006) Decrease in intramuscular lipid droplets and translocation of HSL in response to muscle contraction and epinephrine. *J Lipid Res* 47:2392–2399. <https://doi.org/10.1194/jlr.M600247-JLR200>
- Prats C et al (2013) An optimized histochemical method to assess skeletal muscle glycogen and lipid stores reveals two metabolically distinct populations of type I muscle fibers. *PLoS ONE* 8:e77774. <https://doi.org/10.1371/journal.pone.0077774>
- Rigamonti E, Touvier T, Clementi E et al (2013) Requirement of inducible nitric oxide synthase for skeletal muscle regeneration after acute damage. *J Immunol* 190:1767–1777. <https://doi.org/10.4049/jimmunol.1202903>
- Saccone D, Asani F, Bornman L (2015) Regulation of the vitamin D receptor gene by environment, genetics, and epigenetics. *Gene* 561:171–180. <https://doi.org/10.1016/j.gene.2015.02.024>
- Saclier M, Yacoub-Youssef H, Mackey AL et al (2013) Differentially activated macrophages orchestrate myogenic precursor cell fate during human skeletal muscle regeneration. *Stem Cells* 31:384–396. <https://doi.org/10.1002/stem.1288>
- Shaw CS, Jones DA, Wagenmakers AJ (2008) Network distribution of mitochondria and lipid droplets in human muscle fibres. *Histochem Cell Biol* 129:65–72. <https://doi.org/10.1007/s00418-007-0349-8>
- Spangenburg EE, Pratt SJP, Wohlers LM, Lovering RM (2011) Use of BODIPY (493/503) to visualize intramuscular lipid droplets in skeletal muscle. *J Biomed Biotechnol* 2011:598358. <https://doi.org/10.1155/2011/598358>
- Steger CM, Hartmann A, Rieker RJ (2020) Molecular differences between arterial and venous grafts in the first year after coronary artery bypass grafting. *Histochem Cell Biol*. <https://doi.org/10.1007/s00418-020-01896-4>
- Strauss JA, Shepherd DA, Macey M et al (2020) Divergence exists in the subcellular distribution of intramuscular triglyceride in human skeletal muscle dependent on the choice of lipid dye. *Histochem Cell Biol*. <https://doi.org/10.1007/s00418-020-01898-2>
- Suzuki M, Shinohara Y, Fujimoto T (2012) Histochemical detection of lipid droplets in cultured cells. In: Taatjes D, Roth J (eds) *Cell imaging techniques. Methods in molecular biology (methods and protocols)*, vol 931. Humana Press, Totowa. https://doi.org/10.1007/978-1-62703-056-4_25
- Taatjes DJ, Wadsworth MP, Quinn AS, Rand JH, Bovill EG, Sobel BE (2008) Imaging aspects of cardiovascular disease at the cell and molecular level. *Histochem Cell Biol* 130:235–245. <https://doi.org/10.1007/s00418-008-0444-5>
- van Loon LJ, Koopman R, Manders R, van der Weegen W, van Kranenburg GP, Keizer HA (2004) Intramyocellular lipid content in type 2 diabetes patients compared with overweight sedentary men and highly trained endurance athletes. *Am J Physiol Endocrinol Metab* 287:E558–565. <https://doi.org/10.1152/ajpendo.00464.2003>
- Villalta SA, Nguyen HX, Deng B et al (2009) Shifts in macrophage phenotypes and macrophage competition for arginine metabolism affect the severity of muscle pathology in muscular dystrophy. *Hum Mol Genet* 18:482–496. <https://doi.org/10.1093/hmg/ddn376>
- Whytock KL et al (2020) A 7-day high-fat, high-calorie diet induces fiber-specific increases in intramuscular triglyceride and perilipin protein expression in human skeletal muscle. *J Physiol* 598:1151–1167. <https://doi.org/10.1113/jp279129>
- Yin H, Price F, Rudnicki MA (2013) Satellite cells and the muscle stem cell niche. *Physiol Rev* 93:23–67. <https://doi.org/10.1152/physrev.00043.2011>

News from the Society for Histochemistry**The Robert Feulgen Prize 2020 of the Society for Histochemistry**

has been awarded ex aequo to

Dr. Christian Mühlfeld

Hannover Medical School, Hannover,
Germany

for the development of new stereological and 3D image analysis techniques and their application to the quantitative analysis of the lung alveolar capillary network.

Dr. Hari Shroff

National Institute of Biomedical Imaging
and Bioengineering, NIH,
Bethesda, MD, USA

for significant improvement of spatial resolution and speed of image acquisition for super-resolution microscopes and their application in the generation of a 4D atlas of neurodevelopment in *C. elegans* embryos.

The Editors of *Histochemistry and Cell Biology*
wish to convey their heartfelt congratulations to Drs. Mühlfeld and Shroff
on the receipt of this honor.

16TH INTERNATIONAL CONGRESS OF HISTOCHEMISTRY AND CYTOCHEMISTRY

Dear Colleagues,

In the light of the COVID-19 pandemic, the ICHC 2020 organizers and IFSHC Executive Council decided to postpone the ICHC 2020 to **5 - 8 September 2021**. The ICHC 2021 will take place as originally planned in the Cubex Centre, Prague, Czech Republic. The safety of all participants is our top priority. We are sorry for any inconvenience the postponement might have caused you.

The ICHC is held every four years under the auspices of the International Federation of Societies for Histochemistry and Cytochemistry (IFSHC), which continually strives to provide grounds for communication and cooperation among scientists all over the world in the areas of cyto- and histochemistry, cell and tissue biology, microscopy, pathology and other relevant fields.

The city of Prague, also known as the heart of Europe, provides easy access for scientists from all over the world. The congress venue, Cubex Centre Prague which offers technologically and visually unique space, promises to leave everyone with an unforgettable experience. Of course, Prague prides itself with its beautiful historical architecture, technical monuments, celebrated cafés, great food, and beer. This will be underlined by the ICHC gala dinner in the famous Art Nouveau Municipal House, and a free beer party organized in the premises of the Staropramen brewery.

We hope that you will join us in Prague to discuss together your latest achievements and that the venue will provide great opportunities for specialists at all levels of their career, bringing lots of opportunities for strengthening international collaborations. Special attention will be therefore given to the presentations of students. We also expect a rich commercial exhibition where new and emerging technologies will be presented.

We are delighted to inform you that the following speakers will present a lecture at the congress:

Stefan Hell, a Nobel Prize laureate, Max Planck Institute for Biophysical Chemistry, Germany (keynote speaker)

Alev Erisir, Department of Psychology, University of Virginia, USA

Toyoshi Fujimoto, Juntendo University, Nagoya, Japan

Hans-Joachim Gabius, Institute of Physiological Chemistry, Ludwig Maximilians University of Munich, Germany

Bożena Kamińska, Nencki Institute of Experimental Biology PAS Warszawa, Poland

Takehiko Koji, Department of Histology and Cell Biology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

Ohad Medalia, Department of Biochemistry, University of Zurich, Switzerland

See you all in Prague, September 2021!

Hinke Multhaupt, President of the IFSHC
Klara Weipoltshammer, President of the Society for Histochemistry

Pavel Hozak, Chair of the Local Organizing Committee

Contacts

We will keep the current domain:

www.ichc2020.com

If you have any questions about registration, please contact: registration@ichc2020.com

If you have any questions about abstracts, please contact: abstracts@ichc2020.com

Other inquiries and comments about the conference, please contact: info@ichc2020.com

ANNOUNCEMENT

The Society for Histochemistry

Invites scientists to apply for the 2021 Robert Feulgen Prize. The prize is awarded for an outstanding achievement in the field of histochemistry.

The contributions may be either towards the development of new histochemical and cytochemical techniques or in the application of existing technology towards solving important problems in biology and/or medicine. Addressed are scientists working in microscopical sciences (in the widest sense) as well as in biochemistry, cell biology, endocrinology, in situ molecular techniques, and neurosciences. Scientists in their mid-career (assistant or associate professor, priv. doz.) are encouraged to apply. The prize is not intended for lifetime contributions.

The Prize consists of a monetary prize of €2,000

All applications should be submitted before January 31, 2021 via the electronic submission system at: <https://www.greception.com/form-login-window/191a281d/>

The application should contain a short curriculum vitae, a 1,000 word summary of the contributions of the applicant and PDF reprints of the pertinent publications. Full description of conditions is available on the Society website: http://histochemistry.eu/description_of_conditions_.html

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.