



## INDEX

<b>KEYNOTE LECTURES</b> .....	2
THEME 1 BEYOND INTEGRATIVE PHYSIOLOGY – THE PHYSIOME I .....	2
THEME 2 BEYOND INTEGRATIVE PHYSIOLOGY – THE PHYSIOME II .....	3
THEME 3 PHYSIOLOGY OF STRESS.....	4
THEME 4 BY WAY OF THE SKIN .....	5
THEME 5 PHYSIOLOGICAL INFORMATICS.....	6
<b>PODIUM PRESENTATIONS</b> .....	8
THEME 1 BEYOND INTEGRATIVE PHYSIOLOGY – THE PHYSIOME I .....	8
THEME 2 BEYOND INTEGRATIVE PHYSIOLOGY – THE PHYSIOME II .....	10
THEME 3 PHYSIOLOGY OF STRESS.....	14
THEME 4 BY WAY OF THE SKIN .....	17
THEME 5 PHYSIOLOGICAL INFORMATICS.....	20
<b>POSTERS</b> .....	24
THEMES 1 & 2 BEYOND INTEGRATIVE PHYSIOLOGY – THE PHYSIOME.....	24
THEME 3 PHYSIOLOGY OF STRESS.....	29
THEME 4 BY WAY OF THE SKIN .....	32
THEME 5 PHYSIOLOGICAL INFORMATICS.....	37
<b>AUTHORS' INDEX</b> .....	42



## KEYNOTE LECTURES

### THEME 1 BEYOND INTEGRATIVE PHYSIOLOGY – THE PHYSIOME I

#### **CENTENARIANS: AN EXAMPLE OF SUCCESSFUL PHYSIOLOGICAL ADAPTATION TO AGEING**

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Centenarians constitute an example of extraordinary ageing, not only because they reach extreme long age, but also because they enjoy an extraordinary healthspan that includes a remarkable compression of morbidity towards the end of their life.

One of the critical physiological features of ageing is that old individuals lose their capacity to maintain homeostasis, i.e. to resist the stresses of normal life. Centenarians, however, do maintain the efficient homeostatic mechanisms well into advanced age.

Centenarians usually belong to families blessed with many members who have enjoyed extraordinary ageing and made it to be a hundred or over. The proportion of centenarians is rising from one in 8,000 to approximately now 1 in 5,000 individuals in advanced societies like those in the Iberian Peninsula.

With the idea of extraordinary homeostasis in mind, we analysed the expression of miRNAs (the regulatory forms of small RNA whose main characteristic is that of controlling the expression of transcription). We observed that the expression of mRNAs in centenarians remains similar to that of young and very different from that of old individuals and that this may be due to a remarkable capacity of synthesis of mRNAs (catalysed by Drosha, Dicer, and exportin). We first analysed the mRNome of centenarians by analysing and by using big data analysis, we identified four genes that are specially upregulated in centenarians. These are Bcl-XL, FAS, FAS ligand, and CCL5. Three of these genes are associated with the control of apoptosis. The general idea being that centenarians are able to downplay the activity of intrinsic apoptosis and activate the extreme pathway for apoptosis.

Finally, we have studied families of centenarians and found that descendants of centenarians are less frail than individuals of the same age but without the genetic background of centenarians and that the RNA expression profile of centenarian descendants is similar to that of centenarians and different from that of individuals whose parents were not centenarians.

The physiological implications of this remarkable genetic background will be discussed.



*Professor Jose Viña is Full Professor in the Department of Physiology (University of Valencia, Spain) and has been working in ageing for over thirty years. He has been leading a successful research group dealing with nutritional aspects, in the first instance, with longevity and, more recently, with frailty and Alzheimer's disease. His major contributions have been: i) Experimental determination that mitochondria are key targets for ageing; ii) Identification of molecular mechanisms to explain why females live longer than males; iii) Identification of new longevity-associated genes, particularly those involved in p53 pathways, telomerase, RAS/GRF1, and antioxidants (G6PD); iv) Generation of a new experimental model for frailty in animals, and; v) Identification of biomarkers of Alzheimer's disease and frailty.*

## THEME 2 BEYOND INTEGRATIVE PHYSIOLOGY – THE PHYSIOME II

### CHRONOBIOLOGY – THE IMPORTANCE OF TIMING

Debra J. Skene

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Biological clocks are essential for the co-ordination of physiology and behaviour. Circadian clocks are endogenous autonomous oscillators conferring a near 24-h circadian rhythm on multiple processes from gene expression to behaviour. The circadian timing system consists of a central oscillator in the hypothalamic suprachiasmatic nuclei (SCN) that drives timing in multiple tissue specific clocks in the periphery (e.g., liver, pancreas, adipose tissue). This permits synchrony between different internal rhythms (e.g., metabolic and cardiovascular systems). Clocks regulate most physiological processes (e.g., cell cycle, immune function, sleep/wake regulation, metabolism, cardiovascular function). This has implications for time of day sampling, disease diagnosis (biomarker discovery), and therapeutics.

Biological clocks evolved to anticipate and adjust appropriately to environmental events, e.g., dawn/dusk, seasons, and tides. The 24 h light/dark cycle is the primary time cue entraining the SCN-clock to the 24 h day, in addition to light acutely affecting many aspects of physiology and behaviour. The advent of electricity, artificial lighting, and light-emitting electronic devices has impacted on human circadian timing and sleep/wake patterns. For example, light at night, depending on the light intensity, duration and spectral composition, is capable of delaying the timing of sleep and circadian rhythms resulting in, *inter alia*, reduced sleep duration.

Modern life with rapid travel across time zones or working rotating shifts causes a mismatch between the circadian timing system and the sleep/wake, feeding/fasting cycle with adverse health consequences. For example, shift workers have an increased risk of cancer and metabolic disease. The biochemical mechanisms underlying sleep deprivation, circadian misalignment, and disease are currently the subject of intensive study. Additional challenges include how best to minimise circadian disruption, ensuring good sleep, maintaining wakefulness/good performance during waking hours, and minimising metabolic disturbance. Optimising photic and nonphotic time cues (e.g., meals, melatonin) to synchronize/reset disturbed circadian rhythms offers promise.



*Debra J. Skene, Ph.D., is Professor of Neuroendocrinology (2001- ) and Section Lead for Chronobiology at the University of Surrey, UK. Educated in South Africa (B.Pharm, MSc, Ph.D.), she joined the University of Surrey in 1984. She has over 25 years of research experience studying the human circadian timing system and has authored over 150 refereed research publications. Her recent research has been funded by the EU FP6 and FP7 programmes, UK Cross Research Council New Dynamics of Ageing (NDA) Programme, the BBSRC and MRC (UK). She is also a past Royal Society Wolfson Research Merit Award Holder.*

*Professor Skene and her team's research is directed towards characterisation and treatment of circadian rhythm sleep/wake disorders as experienced by blind people, shift workers and older people. Her team's findings have led to the optimisation of melatonin (dose, time of administration) and light (wavelength, time of administration) to affect human circadian timing. Currently her research team is studying the links between human circadian clocks, sleep and metabolism in health, circadian rhythm disorders and metabolic diseases (shift workers, Type 2 diabetes, liver disease) using LC-MS metabolomics.*

*Professor Skene is President of the European Biological Rhythms Society (EBRS) (2015- ) and past Vice-President (Basic) of the European Sleep Research Society (ESRS) (2010-2014). She is a past Chair of a Gordon Research Conference (Pineal Cell Biology, 2012), currently an Associate Editor of the Journal of Sleep Research and on the Editorial Board of Chronobiology International.*

### THEME 3 PHYSIOLOGY OF STRESS

#### **NITRIC OXIDE SIGNALING IN THE BRAIN: TRANSLATION OF DYNAMICS INTO RESPIRATION CONTROL AND NEUROVASCULAR COUPLING**

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In a couple of decades, following its identification in the biological milieu, nitric oxide (NO), previously known as an atmospheric pollutant, emerged as a ubiquitous messenger in the cardiovascular and central nervous systems. The analysis of its peculiar physico-chemical properties provides a platform for an integrative and comprehensive understand of the diversity of biological functions in which NO acts as a core mediator. We will address the key physico-chemical properties of NO and how these can be translated in the regulation of cell functions and in the maintenance of homeostasis. In particular, we will address the critical role of NO in the regulation of two physiologic pathways: mitochondrial cellular respiration and the mediation of the neurovascular-neurometabolic axis in the brain. It will be apparent that NO emerged as a central regulator of neurometabolism with consequences for aging and neurodegeneration.



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*João Laranjinha graduated with a degree in Pharmaceutical Sciences (University of Coimbra) in 1984 and a Ph.D. in Biochemistry (University of Coimbra) in 1996. His postdoc studies were made with Enrique Cadenas in 1997-1998 in the Dept. of Molecular Pharmacology and Toxicology, University Southern California, Los Angeles, USA.*

*His research is focused on the functional impact of nitric oxide in the brain as a neurometabolic regulator and as a mediator of neurovascular coupling with impact in aging and neurodegeneration. He is also interested in dietary nitrite-driven regulatory processes, encompassing the non-enzymatic production of nitric oxide along the nitrate:nitrite:NO pathway. He has published 90+ papers ISI with 2500+ citations.*

*He has chaired the organizing and scientific committees of several international conferences, including the European (SFRRE 2007) and International (SFRRRI 2018) meetings of the Society for Free Radical Research. He is a member of the editorial board of several prestigious journals in his area of research (Free Radical Research, Free Radical Biology and Medicine, Biofactors Archives of Biochemistry and Biophysics and Redox Biology) and of the Advisory Board of Oxygen Club of California from which he has received the Science and Humanity Award at the University of California, Davies, US, in 2016. He has been a member of the Council of the European Society for Neurochemistry and the European Society for Free Radical Research for several years and President of the Portuguese Biochemical Society (2014-18). Currently, he serves as member of the Advanced Courses Committee of FEBS and as the president of the General Assembly of the Portuguese Biochemical Society.*

## THEME 4 BY WAY OF THE SKIN

### BY WAY OF THE SKIN

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The skin is the interface of the human body to the potentially harmful environment with exogenous stressors like chemicals, UV radiation and other physical stimuli. The epidermal barrier is recognized as a central key pathophysiologic element in inflammatory skin diseases such as atopic dermatitis (AD). Some bases of an impaired barrier have been elucidated on the molecular level e.g. mutation in genes encoding for filaggrin and lipid processing defects. Change in the microbiome composition and its relation to altered barrier function have been reported. The



epidermal barrier is also of interest for penetration of both irritants and allergens as well as topically applied drugs and active ingredients.

Multiple non-invasive biophysical measurement instruments are used to assess skin physiology e.g. in inflammatory skin diseases associated with an altered epidermal barrier, namely transepidermal water loss, stratum corneum hydration, surface pH, inflammatory signs and surface parameters. More sophisticated non-invasive or minimally invasive devices to assess skin physiology have been introduced over the last decade: multidimensional imaging, in vivo multiphoton spectroscopy, optical coherence tomography, atomic force microscopy, near-infrared spectroscopy (NIR), in vivo Raman micro-spectroscopy and in vivo reflectance Raman spectroscopy.

Atopic dermatitis is a prototype of translational research at the interface of epidermal barrier and inflammation. Non- or minimal-invasive methods have been used in different AD research areas. The combination of established approaches with cutting edge methods allowed to gain a deeper understanding of barrier related inflammatory skin diseases.

Molecular mechanisms and their modulation are classically studied in vitro (e.g. in cell culture models) or using invasive biopsy techniques. Recently non- or minimally-invasive methods have been introduced in different cutaneous research areas. These methods include multidimensional imaging, optical coherence tomography, atomic force microscopy and in vivo Raman Micro-Spectroscopy. Age-specific patterns and changes over life span have been elaborated.

An example of age-specific skin physiology will be presented for neonates and children. Their epidermis is physiologically and morphologically different from adult skins e.g. in water content, light sensibility, corneocyte size, percutaneous permeability, susceptibility to infections/irritants and topical treatments. The postnatal period is a time of active functional maturation and cutaneous adaptation to the dry, eventually harsh extra-uterine environment. Recent research focused on the development of functional aspects and the micro-morphological skin surface evolution of epidermal barrier e.g. with the development of the E.M.I. (Electron Microscopy Isotropy) score. Skin maturation takes approximately two years. Infant skin showed thinner epidermis and thinner stratum corneum (SC) as well as smaller corneocytes. The Stratum cohesion is a key factor in SC maturation and depends on the presence, subcellular distribution and timely coordinated degradation of the intercellular junctions.

The lecture will give an overview on established as well as new technologies to study the skin physiology under different conditions and in selected skin diseases.

## THEME 5 PHYSIOLOGICAL INFORMATICS

### PHYSIOLOGICAL INFORMATICS



Hugo Silva

*IT-IST, Universidade de Lisboa*

Physiological data has had a transforming role on multiple aspects of society, which goes beyond the health sciences domains to which they were traditionally associated with. This has been made possible through technological advances in electronics, telecommunications and informatics. Today, most people carry small pocket or wrist-worn computers, collecting a plethora of data in always on / always connected paradigm. Combined with recent advances in signal processing and machine learning, physiological data is increasingly being used in a plethora of novel areas such as human-computer interaction, entertainment, architecture, and many others.

BITalino has been revolutionising biomedical education and prototyping by providing versatile toolkits composed of low-cost hardware and software, created to enable anyone to create cool projects and applications involving physiological data. The hardware consists of modular wireless biosignal acquisition systems that can be used to acquire data in real time, interface with other devices (e.g. Arduino or Raspberry PI), or perform rapid prototyping of end-user applications. The software comprehends a set of programming APIs, a biosignal processing toolbox, and a framework for real time data acquisition and post processing. In this talk we will review how BITalino came to be and the future prospects.

Physiological data from wearable sensors and smartphone are accumulating rapidly, and this provides us the chance to collect dynamic and personalized information as phenotype to be integrated to genotype for the holistic understanding of complex diseases. This integration can be applied to early prediction and prevention of disease, therefore promoting the shifting of disease care tradition to the healthcare paradigm. In this chapter, we summarize the physiological signals which can be detected by wearable sensors, the sharing of the physiological big data, and the mining methods for the discovery of disease-associated patterns for personalized diagnosis and treatment. We discuss the challenges of physiological informatics about the storage, the standardization, the analyses, and the applications of the physiological data from the wearable sensors and smartphone. At last, we present our perspectives on the models for disentangling the complex relationship between early disease prediction and the mining of physiological phenotype data.

*Hugo received his Ph.D. in Electrical and Computer Engineering from the Instituto Superior Técnico (IST) – University of Lisbon. Since 2004 Hugo has been a researcher at the IT - Instituto de Telecomunicações ([http://www.it.pt/person\\_detail\\_p.asp?id=1293](http://www.it.pt/person_detail_p.asp?id=1293)) and a Professor at EST/IPS – Polytechnic Institute of Setúbal (<http://www.estsetubal.ips.pt/>) since 2016. In 2012 he was a visiting researcher at the Computational and NeuroEngineering Laboratory (CNEL) at the University of Florida. He is co-founder of PLUX – Wireless Biosignals (<http://www.plux.info>), established in 2007 as an innovative technology-based company operating in the field of medical devices for healthcare and quality of life, where he is currently Chief Innovation Officer.*

*More recently, Hugo has been actively working towards making the world a bit more physiological, through BITalino (<http://www.bitalino.com>), an open source software and low-cost hardware toolkit that allows anyone from students to professional app developers to create cool projects*



and applications with physiological sensors.<sup>[1][2][3][4]</sup>

*His main interest interests include biosignal research, system engineering, signal processing, and pattern recognition, and his work has been distinguished with several academic and technical awards such as the "Career Award alumniIPS" in 2018 (awarded by the Polytechnic Institute of Setúbal to former students), the "Best Industrial and Enabling Technology" at the European Commission's DG-CONNECT Innovation Radar Prize in 2017 with the project "BITalino", the 1<sup>st</sup> place at the Ordem dos Engenheiros Young Engineer Innovation Award in 2015 with the project "BIT: Biosignal Igniter Toolkit", the 1<sup>st</sup> place at the Venture Day Lisbon in 2013 with the project "Vitalidi: Your Heart (h)as a Key!", the selection as a semi-finalist to the Engadget Expand NY Insert Coin competition in 2013, the Life Sciences Award in 2010 at a yearly venture competition co-promoted by the MIT, and the "Caixa Geral de Depósitos Award" from 2003 to 2005 for recognized academic merit.*

## PODIUM PRESENTATIONS

### THEME 1 BEYOND INTEGRATIVE PHYSIOLOGY – THE PHYSIOME I

#### 1.1 PUPIL SIZE DURING AUTHENTICITY RECOGNITION IN LAUGHTER AND CRYING

Gonçalo Cosme<sup>1</sup>; Mónica Costa<sup>2</sup>; Vânia Tavares<sup>1</sup>; Katja Brodmann<sup>3</sup>; César Lima<sup>4</sup>; Pedro Rosa<sup>4</sup>; Diana Prata<sup>1</sup>

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Emotions play an important role in social interactions however some neurological pathologies manifest themselves with dysfunctional emotional recognition and/or expression, strongly motivating research on these emotional processes. Nonverbal emotional vocalizations are expressions of emotions without semantic context, can be characterized according to their category (e.g., amusement, sadness, and fear), valence (positive or negative), arousal and authenticity (whether spontaneous or voluntary). The ability to correctly recognize authenticity is an advantageous social skill that enhances group cohesion, affiliation and cooperation. Recent studies suggest that spontaneous emotions elicit different brain activation patterns compared to voluntary emotions mostly because perceivers require less mentalization (i.e. cognitive effort) to





recognize spontaneous emotions; and that spontaneous vocalizations were more arousing than voluntary.

In this work, pupil size, a well-known measure of cognitive effort and arousal, was measured during displays of nonverbal emotional vocalizations of amusement (laughter) and sadness (crying) modulated for their authenticity (spontaneous or voluntary). After sound display, 38 participants rated the sounds on their perceived authenticity in a Likert scale (1-7). Group analysis demonstrated a statistically significant main effect of valence on mean pupil size at 1 sec. of sound display; and of authenticity at 3 sec. of sound display.

These results suggest that authenticity processing manifests physiologically later than other emotion recognition processes such as valence recognition. Furthermore, they consolidate pupil size as a neurophysiological measurement by demonstrating it is sufficiently sensitive to more complex social processes as is authenticity rating in nonverbal emotional vocalizations.

**Keywords:** Pupillometry, Emotional Authenticity, Voice Processing, Emotional Arousal

#### **Presenting author profile**

*Gonçalo Cosme is a biomedical engineer and researcher in Diana Prata's team, mainly responsible for electrophysiological equipment handling and currently researching on social cognition. He is a proven researcher with one published peer-reviewed scientific publication reviewing GWAS studies of schizophrenia and bipolar disorder, and another submitted which studied emotion recognition across cultures. Currently analyzing pupillometry data collected in two separate studies, he was an invited speaker for the 3rd Encontro Nacional de Oculometria Cognitiva in ULHT and a speaker for the Methods and Research on Gaze Tracking workshop held in IST.*

## **1.2. THE LEVELS OF OSTEOCALCIN IN OBESE AND NON-OBESE HEALTHY SEDENTARY MEN AND ITS RELATION WITH ADIPOMYOKINES**

Sema Sayharman<sup>1</sup>; Muaz Belviranlı<sup>1</sup>; Nilsel Okudan<sup>1</sup>

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The aim of this study is to investigate the effect of obesity on osteocalcin, irisin, and lipid profiles in healthy sedentary men and the possible relationship between these parameters.

A total of 28 (14 normal and 14 obese) sedentary and health men aged between 20-30 were included in the study. Participants were divided into two groups as normal weight sedentary men (n = 14) and sedentary men (n = 14) in the class I obesity range, according to height, weight and body fat percentage. Lipid profile levels were measured in addition to the levels of osteocalcin and irisin in the blood taken from the participants. Weight, height, basal metabolic rate, body mass index and body fat percentage were measured by body analysis. The data obtained from the study were analyzed by comparing these two groups.



In our study, osteocalcin and irisin levels were higher in the non-obese group compared to the obese group ( $p < 0.05$ ).

**Keywords:** Irisin, osteocalcin, obesity

#### 1.4. MICROCIRCULATORY CHARACTERISATION OF THE MARCH IN PLACE ACTIVITY

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1 - Portuguese Red Cross Health School, ESSCVP – Lisboa; 2 – CBIOS, Lisboa, Portugal

Peripheral vascular dysfunction already represents the 3<sup>rd</sup> major cause of cerebral stroke, closely related to sedentary habits. However, simple exercises might act as active preventers especially in populations with mobility impairments due to their age or other limiting conditions. Our study aims to evaluate the potential impact of the march in place activity on the inferior limb microcirculation. Eleven healthy volunteers ( $25.6 \pm 5.4$  y.o.) both sexes, normal ankle-brachial index ( $1.08 \pm 0.15$ ) participated in the study after IWC. The protocol involved one minute in the standing position (Phase 1); one minute of marching in place (Phase 2) and one minute of recovery (Phase 3) again standing. Local perfusion was quantified by Photoplethysmography (PPG) and by subsurface polarisation light spectroscopy (Tissue Viability Imaging® system) by the local concentration of red blood cells (CRBC). Probes were placed as close as possible to each other, from 1 cm anterointernal region of the first metatarsus head. Descriptive and comparative statistics were applied and a 95% level of confidence adopted. Group's mean comparison between phases have shown significant differences in Phase 2 were for CBRC, not observed in Phase 3. PPG and PR have shown significant increase in Phase 3 ( $p = 0.016$  and  $p = 0.005$  respectively). Sex related differences for PPG and CBRC were not detected in Phase 1, but significant differences were found for CBRC in Phases 2 and 3 ( $p = 0.011$  and  $p = 0.006$  respectively) with higher values in men. In conclusion, the march in place activity seems to positively impact the inferior limb microcirculation, even on normal healthy individuals, suggesting an interesting potential in rehabilitation.

**Keywords:** March in place, microcirculation, PPG, TiVi, lower limb

#### **Presenting author profile**

*Physiotherapist since 1984, specialized in the area of neuromuscular conditions, with clinical practice to date. She is teaching as an assistant professor at ESSCVP-Lisboa where she also works as a Department Director. She is a Ph.D. student at the School of Health Sciences and Technologies of Lusófona University and researcher at CBIOS - Research Center for Biosciences & Health Technologies.*

## THEME 2 BEYOND INTEGRATIVE PHYSIOLOGY – THE PHYSIOME II



## 2.1. FREQUENT CONSUMPTION OF ARTIFICIALLY SWEETENED BEVERAGES IS ASSOCIATED WITH AN INCREASED RISK OF METABOLIC SYNDROME IN A MEDITERRANEAN POPULATION AT HIGH CVD RISK

Cíntia Ferreira-Pêgo<sup>1,2,15,16</sup>; Nancy Babio<sup>1,2</sup>; Maira Bes-Rastrollo<sup>2,3,4</sup>; Dolores Corella<sup>2,5</sup>; Ramon Estruch<sup>6,7</sup>; Emilio Ros<sup>2,8</sup>; Montserrat Fitó<sup>2,9</sup>; Luis Serra-Majem<sup>2,6,9</sup>; Fernando Arós<sup>6,10</sup>; Miguel Fiol<sup>2,11</sup>; José Manuel Santos-Lozano<sup>2,12</sup>; Carlos Muñoz-Bravo<sup>13</sup>; Xavier Pintó<sup>8,14</sup>; Miguel Ruiz-Canela<sup>2,3,4</sup>; Jordi Salas-Salvadó<sup>1,2,6</sup>

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This analysis evaluated the associations between intakes of artificially sweetened beverages (ASB) and the incidence of Metabolic Syndrome (MetS) in elderly individuals at high risk of cardiovascular disease and without MetS at baseline. We prospectively examined 1868 participants from the PREDIMED study. Energy and nutrients were evaluated at baseline and yearly using a validated 137-item food frequency questionnaire. Multivariable-adjusted HRs for MetS and its components were estimated from the mean intake during follow-up. We compared the two highest consumption categories (1-5 servings/week, and >5 servings/week) with the lowest category (<1 serving/week). A total of 930 incident cases of MetS were documented during a median follow-up of 3.24 years. When comparing the consumption of >5 servings/week with <1 serving/week, multivariable hazard ratios (95% CI) for MetS incidence were 1.74 (1.26-2.41) in case of ASB. As a conclusion, the occasional consumption of ASB (between 1 and 5 servings/week) was not associated with overall MetS. However, the consumptions of more than



5 servings/week of this type of beverage were associated with an increased risk of MetS and some of its components, such as abdominal obesity and hypertriglyceridemia. To date, it has been suggested that three mechanisms may explain these associations: 1) artificial sweeteners can interfere with learned responses that help to control glucose and energy homeostasis; 2) artificial sweeteners interact with sweet-taste receptors that are expressed throughout the digestive system and which may play a role in glucose absorption and trigger insulin secretion; and 3) artificial sweeteners (such as saccharin, sucralose or aspartame) can interfere with gut microbiota, thus decreasing glucose sensitivity and favoring MetS development.

**Keywords:** Artificially-sweetened beverages, Metabolic Syndrome, Metabolic Syndrome Components, PREDIMED study

**Presenting author profile**

*She graduated in Human Nutrition and Dietetics in Universitat Rovira i Virgili, and has a Master's Degree in Training and Sports Nutrition from the Universidad Europea de Madrid. She obtained his Ph.D. with International Mention in Nutrition and Metabolism from the Universitat Rovira i Virgili and University of Arkansas. Her main epidemiological research interests are the Mediterranean Diet, dairy products, hydration, and/or the consumption of beverages and its relation with health and disease.*

**2.2. THE SHORT DURATION MASSAGE OF A SINGLE LIMB IN HUMAN ALTERS CARDIOVASCULAR DYNAMICS IN BOTH LIMBS**

Clemente Rocha<sup>1</sup>; Henrique Silva<sup>1,2</sup>; Hugo Ferreira<sup>3</sup>; Luis Monteiro Rodrigues<sup>1,2</sup>

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Effleurage is surely one of the most popular massage techniques used for multiple purposes. Its physiological impact and mechanisms are still poorly understood, and sometimes inconclusive. We aim to characterize the cardiovascular impact of this technique on human in vivo microcirculation. To this end, distal blood perfusion was non-invasively quantified with both laser Doppler flowmetry (LDF) and photoplethysmography (PPG), in both feet. The massage protocol involved the application of two techniques, proximal and distal, randomly sequenced with a 15 min washout between them. 32 young healthy volunteers (mean 19.81 ± 1.55 yo) both sexes, were after informed written consent. The experimental selected a random limb for massage with the contralateral acting as control. Each protocol included a 10 minute baseline (Phase I) period, a 5 minute massage (Phase II), and a 10 minute recovery (Phase III) recordings with LDF and PPG. Blood pressure and pulse rate were also evaluated. LDF signals were decomposed in its oscillatory components with the (Morlet) WT. Results showed that massage promoted a significant perfusion increase during massage in both test and contralateral limbs. No significant differences between techniques and no sex related differences regarding response to massage



were identified. Detailed analysis of the LDF oscillatory components suggests that these procedures, significantly modify multiple components of cardiovascular integration, with cardiac, respiratory, myogenic and endothelial components playing a major role in distal microcirculatory homeostasis.

**Keywords:** Effleurage, cardiovascular effects, LDF, PPG, wavelet transform

**Presenting author profile**

*Degree in Computer Engineering from ISPGAYA and MSc in Computer Engineering (ISCTE-IUL). Has dedicated an important part of his activity to Naturopathy (General Course of Naturopathy and Holistic Traditional Sciences - IMT). Current Ph.D. student in the doctoral program in Health Sciences at U Lusófona where he concluded an advanced training in Clinical Trials.*

**2.3. TRENDS SHAPING THE FUTURE OF PHYSIOLOGY TEACHING AND PRACTICE: FINDINGS FROM A FOCUS GROUP FROM PMIG (THE PHYSIOLOGY MAJORS INTEREST GROUP) EXPERTS**

Joao Gregorio<sup>1</sup>; Erica Wehrwein<sup>2</sup>; Luis Monteiro Rodrigues<sup>1</sup>

*1 - CBIOS - Research Center for Biosciences and Health Technologies; 2 - Dept of Physiology, Michigan State University*

The future of Physiology as a critical domain in health and medicine is a crucial debate theme among experts. Various constraints modified teaching, while the status of Physiology in research has also been altered.

Our main goal was to identify the major trends that may shape physiology's future as a teaching and research domain in a 2030 horizon.

A focus group was hosted during the PMIG 2019 meeting, joining experienced physiologists, mostly from the US. An interview guide was developed following a literature review and applied to the volunteered participants.

These physiologists described physiology as a discipline with soft borders, easily permeable to the influence of other disciplines, and likewise influencing them.

It was noted how technology is changing the teacher-student relationship. E-learning/b-learning are no longer novelties and are seen as a good complement to classroom/laboratory work. The use of simulators was seen as very valuable, even if they remain "a black box". Collaboration with clinicians for teaching was also regarded as a valuable trend. The experts considered that jointly created study cases are more robust teaching tools. However, it was recognized that the clinical directive to properly diagnose, treat, and move on is of limited value to a physiology student. Moreover, educator's role switch from content provider to facilitator of critical thinking has been noted.



Diversification of funding sources was also seen as a future trend. Physiological Societies must continue to advocate the need to adequately fund physiology research through public agencies. Cooperation with industries (instrumentation, software, AI) is seen as a way to broaden the funding sources and enable access and input into new technologies. Appropriate science communication will be needed in these efforts.

Finally, the experts agreed that Physiology will maintain a proper identity in years to come. Regarding research, physiology is believed to stay in the forefront of life sciences for new knowledge, new technologies, and new skills.

**Keywords:** Research trends, teaching trends, physiologists' future

**Presenting author profile**

*João Gregório is an Assistant Researcher at CBIOS, Research Center for Biosciences & Health Technologies. He has a Ph.D. in International Health with a specialization in Health Policies and his current research interests lie in areas such as health services development, information and communication technologies applied in health service provision and capacity building of human resources for health.*

## THEME 3 PHYSIOLOGY OF STRESS

### 3.1. MENTAL AND PHYSICAL PREPARATION IMPROVES PERFORMANCE IN COGNITIVE TASKS

Carlos Moreira<sup>1</sup>; Daniel Rocha<sup>1</sup>; Paula Carvalho<sup>3</sup>; Carolina Amorim<sup>1</sup>; Ana Gouveia<sup>2</sup>; Hugo A. Ferreira<sup>1</sup>

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In this study we assessed the impact of a proper physical and mental preparation on the performance of gaming-related reaction and cognitive tasks. Ten male subjects (mean age 24.5 ± 2.2 years) participated in 5 sequential sessions, every weekday. Subjects were divided equally into 2 groups: one group followed an audio guide with a mental and physical preparation (MPP) and then completed the tasks, while the control group immediately started performing the tasks. The MPP audio guide included muscular contraction, followed by relaxation for several muscle groups, as well as a mindfulness relaxation protocol.

In total, subjects were asked to complete 2 reaction tasks simulating aspects of first-person shooter games, and 5 cognitive tasks. All subjects used a wearable headband (EMOTAI) to assess brain activity via 2 electroencephalography (EEG) prefrontal cortex channels and photoplethysmography (PPG), which informs about heart rate (HR) and its variability. After each



session, the subjects were asked to fill in the NASA-TLX questionnaire for each task, assessing the perceived workload.

Overall, the reported task load decreased over the 5 sessions for both groups but was always lower and decreased faster for the MPP group. On the last day, the MPP group perceived the tasks to be 44% easier than the control group. The performance in all tasks was consistently better in the MPP group. Reaction time was on average 7 ms faster and time to aim was 30 ms faster than the control group. Additionally, by the fifth session, the MPP group achieved a 96% accuracy in the memory tasks, versus 92% for the control group. The MPP group maintained their HR relatively constant at an average of 72.5 bpm throughout the week for all tasks. On the other hand, the control group increased the heart rate up to 83 bpm in the same period of time. By the end of the week, the two groups differed by almost 8 bpm or 10%.

**Keywords:** Performance, cognition, EEG, wearable

#### **Presenting author profile**

*Carlos Moreira is an MSc in Biomedical Engineering and Biophysics from the Faculty of Sciences of the University of Lisbon. For his dissertation, he developed a multiplayer BCI game controlled using steady-state visually-evoked potentials, to study cooperative behaviors using EEG. Now pursuing a Ph.D. in Biomedical Engineering at IBEB, in the field of cognitive and emotional assessment using physiological signals, his goal is to assess mental workload while operating critical devices or systems.*

### **3.2. REDOX REGULATION OF BREAST CANCER PROGRESSION: INSIGHTS FROM SOD MIMICS**

Nuno Saraiva<sup>1</sup>; Ana S Flórido<sup>2</sup>; João G Costa<sup>1</sup>; Ines Batinic-Haberle<sup>3</sup>; Matilde Castro<sup>2</sup>; Nuno G Oliveira<sup>2</sup>; Ana S Fernandes<sup>1</sup>

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Superoxide dismutase (SOD) levels seem to be relevant for breast carcinogenesis, but the role of these enzymes is not yet clearly understood. Contradictory reports on the influence of SOD in breast cancer can be found in the literature. SOD mimics (SODm) are synthetic compounds with drug-like properties, with the ability to mimic the functional properties of SOD. These compounds have been used in breast cancer models as mechanistic tools to explore the redox regulation of cancer development. In this perspective, SODm may also help to understand the involvement of reactive oxygen species in the mechanisms of toxicity induced by carcinogenic agents. SODm have also been suggested as redox-active drugs for breast cancer treatment. SODm may have inherent antiproliferative effects in breast cancer cells. Moreover, many studies have shown that the combination of SODm with chemotherapy or radiotherapy may be advantageous both by



boosting the efficacy of anticancer treatments and by protecting non-cancer tissues from their adverse side effects. Interestingly, we have also recently demonstrated that SODm can impact breast cancer cell adhesion, migration, and invasion, which are key features for the formation of metastases. This presentation will address the usefulness of SODm in the context of breast cancer, either as therapeutic drugs or as mechanistic tools.

**Acknowledgments:** This work was supported by Fundação para a Ciência e a Tecnologia (FCT, Portugal), through funding UID/DTP/04567/2019 to CBIOS and UID/DTP/04138/2019 to iMed.Ulisboa.

**Keywords:** breast cancer, superoxide dismutase mimics, cell migration, redox regulation

**Presenting author profile**

*Ana Sofia Fernandes graduated in Pharmaceutical Sciences (2004), has a Ph.D. in Pharmacy (specialty of Toxicology, 2010) and is a European Registered Toxicologist (2018). She is an Assistant Professor at Universidade Lusófona. She is also the Scientific Director for Innovation and the coordinator of the Laboratory of Pharmacology and Therapeutics of CBIOS Research Center. She has published, from 2007 on, over 40 scientific full papers indexed to Scopus/Pubmed. She is author/co-author of around 100 scientific communications (oral and panel) and she received four awards. Her main research interest is to explore the impact of ROS and redox modulators on cancer etiology and progression.*

**3.3. EFFECTS OF ASTAXANTHIN SUPPLEMENTATION ON OVALBUMIN-INDUCED EXPERIMENTAL ASTHMA MODEL IN RATS**

Hasan İlker Coban<sup>1</sup>; [Muaz Belviranlı](#)<sup>1</sup>; Nilsel Okudan<sup>1</sup>

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The aim of this study is to investigate the effect of astaxanthin supplementation on biochemical markers of asthma and oxidative stress and antioxidant defense markers in rats induced an experimental asthma model.

Twenty-two male Wistar albino rats were used and the rats were randomly assigned to control (n: 6), asthma (n: 8), and asthma+astaxanthin (n: 8) groups. The rats in the asthma + astaxanthin group were given 10 mg / kg astaxanthin dissolved in corn oil for 22 days via oral gavage. The rats in the asthma groups were given 200 µg ovalbumin / 10 mg Al(OH)<sub>3</sub> dissolved in 1 ml saline solution via intraperitoneally on 1, 2, 3 and 11 days of the study. On the 20th, 21th and 22nd days of the study, the rats were anesthetized with ether and administered 1.5 mg of ovalbumin dissolved in 300 µl saline solution via intranasally. Blood samples were taken under ketamine / xylazine anesthesia on 23th day of the study. Then lung tissue samples were taken after the cervical dislocation. IgE, MDA and GSH levels were analyzed in these samples.

In this study, IgE levels in the lung tissue of the asthma group were higher than the other groups. The elevation of MDA levels in both the serum and the lung tissues of the asthma group indicated





that asthma causes oxidative stress. In addition, decreased levels of MDA in the asthma + astaxanthin group indicate that astaxanthin reduced oxidative stress. The elevation of GSH levels in the lung tissue in the asthma group indicated that asthma activates the antioxidant defense system, whereas the asthma+astaxanthin group returns to the normal levels.

**Keywords:** Astaxanthin, asthma, oxidative stress, ovalbumin

## THEME 4 BY WAY OF THE SKIN

### 4.1. PRELIMINARY EVALUATION OF NON-CONVENTIONAL CONDUCTIVE MATERIALS FOR ELECTRODERMAL ACTIVITY DATA ACQUISITION

António Banganho<sup>1,2</sup>; Hugo Silva<sup>1</sup>; Marcelino Santos<sup>2,3</sup>

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**Introduction:** Electrodermal activity (EDA) signal carries useful information about the sympathetic nervous system. Normally, it is recorded using non-polarizable Ag/AgCl electrodes, on the palm of the hand or the sole of the foot, which, in turn, can impose a hard constraint. More convenient locations and sensor setups are needed. A solution may pass by the use of e-textiles or 3D printed materials as electrodes since they can be integrated in a seamless manner and present advantageous proprieties useful for long-term recordings.

**Methods:** Conductive leather (GMLC), Lycra (MedTexP-130), and PLA (3D) materials were tested as electrodes, compared with dry Ag/AgCl electrodes from Thought Technology, considered as the gold standard. We used several know resistances, each at a time, ranging from 50k $\Omega$  to 3M $\Omega$  with tolerances of 1%, and connected them to a BITalino EDA sensor and acquisition board, via the electrodes. Each electrode had a 1 cm<sup>2</sup> contact area, and exosomatic DC technique was the chosen method for EDA recording. A total of 10 measurements were taken with each resistance and each electrode material to and the Pearson correlation coefficients were computed in regard to the gold standard.

**Results:** The conductive lycra showed high correlation ( $r=0.999 \pm 0.000$ ) as well as a very low resistivity of 4.27 $\Omega$ .cm<sup>2</sup>. The 3D electrodes presented a resistivity of 3.3k $\Omega$ .cm<sup>2</sup> and high correlation ( $r=0.950 \pm 0.005$ ), although having an increased deviation under the 100k $\Omega$  mark. The conductive leather performed the worst, nonetheless, with good correlation ( $r=0.718 \pm 0.041$ ) and resistivity of 3.5k $\Omega$ .cm<sup>2</sup>, while suffering as well of an increased deviation under 0.5M $\Omega$ .

**Conclusions:** Conductive lycra is the most promising material, opening the possibility to integrate EDA sensors in a more seamless fashion. Further studies should focus on testing the different



electrode materials on live subjects to further validate our results and also study other possible locations to extract the EDA.

**Keywords:** Electrodermal activity, physiology, electrodes, e-textiles

**Presenting author profile**

*António Banganho received the B.S. degree in Biomedical Engineering from the Instituto Superior de Engenharia de Coimbra - Instituto Politécnico de Coimbra, Portugal, and currently pursuing the M.S. degree in Electrical and Computer Engineering from Instituto Superior Técnico – Universidade de Lisboa, Portugal, with the thesis “Electrodermal Activity Sensor (EDA) with Adaptive Gain Control” with the support of Instituto de Telecomunicações (IT) and INESC-ID.*

**4.2. 3D REPRESENTATION OF SKIN VISCOELASTICITY**

Henrique Silva<sup>1</sup>; Francisco Rego<sup>2</sup>; Catarina Rosado<sup>1</sup>; Luis Monteiro Rodrigues<sup>1</sup>

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The biomechanical characterization of skin is still a permanent challenge for clinical practice as for research. Recently, a new instrument, the Cutiscan<sup>®</sup> was developed for the assessment of viscoelasticity and anisotropy, allowing in vivo quantifications over a 360 angle degree range. Our objective is to explore new representation strategies for skin viscoelasticity based on the Cutiscan<sup>®</sup> principle by creating new dynamic quantitative parameters. The Cutiscan<sup>®</sup> is equipped with a combined vacuum-camera probe that creates skin suction and quantifies it in terms of displacement over time for each angle. We present data from a suction test applied to the forehead, volar forearm and leg of twenty healthy female subjects ( $37.0 \pm 18.7$  years, 10 young and 10 older), after giving informed consent. We created polar and non-polar 3D representations (time-displacement-angle), from which several dynamic parameters were calculated - stretchability, stretching speed, stretching time and rise time. Results suggest that older subjects display larger displacement heights, displacement slopes and also higher rise and stretching times, irrespective of orientation. Important differences were noted between different skin sites. Apparently these novel Cutiscan<sup>®</sup>-based 3D representations and parameters are sensitive and useful to characterize biomechanical changes occurring with skin ageing.

**Keywords:** skin viscoelasticity, skin anisotropy, Cutiscan, 3D representation

**4.3. HYDROGEL CONTAINING CHITOSAN-COATED PLGA NANOPARTICLES FOR TOPICAL DELIVERY OF INSULIN**

Patrícia Filipe<sup>1,2</sup>; Ana S. Macedo<sup>1,3</sup>; Catarina Rosado<sup>1</sup>; Pedro Fonte<sup>1,3,4</sup>



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**Purpose:** Insulin is a therapeutic protein that may be used as a growth factor in skin regeneration (1). The topical delivery of insulin is a challenge due to the harsh conditions of the wound bed. Neutrophils are the first inflammatory cells to appear in the wound, followed by lymphocytes and monocytes. These inflammatory cells produce proteinases and reactive oxygen species to protect the wound against infections (2). Our purpose was to develop a hydrogel containing nanoparticles to effectively deliver insulin on the skin by using a nanocarrier system that allows increased mucoadhesion, protein stabilization, and bioactivity.

**Methods:** Chitosan-coated PLGA nanoparticles were produced using a w/o/w double emulsion, and coated with chitosan at different concentrations (0.25%, 0.5% and 1%). The particles were further incorporated in a hydrogel containing cellulose gum, propylene glycol, and alginate. The particle and hydrogel features were evaluated.

**Results and Discussion:** The modified solvent evaporation method based on a w/o/w double emulsion technique was effective to coat the PLGA nanoparticles with divergent proportions of chitosan. Evaluation of physicochemical characteristics of uncoated and chitosan coated PLGA nanoparticles were assembled. The size of chitosan-coated PLGA nanoparticles is enhanced by the increase of chitosan concentration. Uncoated PLGA nanoparticles presents a negative zeta potential due to natural negative charge of PLGA and chitosan-coated PLGA nanoparticles retain a positive charge due to chitosan. The AE of insulin was 80-90%, and the hydrogel had good viscosity for topical administration. Finally, the developed technique enabled to yield a chitosan-coated PLGA nanoparticles hydrogel system with suitable properties. Finally, a hydrogel with good homogeneity and rheological properties was obtained.

**Conclusions:** The developed delivery system is capable to protect insulin structure and grants a moisty environment required to promote wound healing.

**Acknowledgments:** This work was financed by FEDER - Fundo Europeu de Desenvolvimento Regional funds through the COMPETE 2020 - Operational Programme for Competitiveness and Internationalization (POCI), and by Portuguese funds through FCT - Fundação para a Ciência e a Tecnologia (FCT) in the framework of the project POCI-01-0145-FEDER-032610 - PTDC/MEC-DER/32610/2017. It was also supported by FCT under the project UID/DTP/04567/2019.

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**Keywords:** insulin, wound healing, chitosan, nanoparticles, PLGA, hydrogel

**Presenting author profile**

Currently, *Patrícia Filipe* is a Ph.D. student in the Health Sciences at Research Center for Biosciences and Health Technologies (CBIOS), Escola de Ciências e Tecnologias da Saúde - Universidade Lusófona in partnership with the University of Alcalá de Henares. Her work focuses on the regeneration of human tissues and wound healing. She has several oral and poster presentations in scientific meetings.

**4.4. DEVELOPMENT OF RUTIN-LOADED HYBRID NANOPARTICLES FOR WOUND HEALING**

Ana Júlio<sup>1,2</sup>; Rita Caparica<sup>1,2</sup>; Sofia A. Costa Lima<sup>3</sup>; Ana Sofia Fernandes<sup>1</sup>; Catarina Rosado<sup>1</sup>; Duarte M. F. Prazeres<sup>4</sup>; Salette Reis<sup>3</sup>; Tânia Santos De Almeida<sup>1,5</sup>; Pedro Fonte<sup>1,3,4,5</sup>

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**THEME 5 PHYSIOLOGICAL INFORMATICS**

**5.1. AUTOMATIC CLASSIFICATION OF PHYSIOLOGICAL SIGNALS MODALITIES**

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**Introduction:** Over the last years, multimodal devices for biomedical research have greatly spread to everyone's daily lives. These enable the use of various combinations of physiological sensors, however, little attention has been paid to the problem of automatic identification of each sensor type, the knowledge of which, being of paramount importance to the adequate design of the subsequent processing and data analysis tasks.



**Methods:** In this work we propose a framework for the automatic identification of sensor data type, namely Respiration, Electrocardiography, Galvanic Skin Response, Blood Volume Pulse data, through a Random Forest (RF) classifier in two data representation spaces: (1) feature-based; (2) dissimilarity-based. The main contribution of our work consists in the recognition of physiological data both in online (evaluation per sample) and offline (evaluation per user modality file through a majority voting system) scenarios, comparing the classification results in different data spaces.

**Results:** We performed a comprehensive study of supervised learning classifiers, and for the dissimilarity-based approach of: (a) similarity metrics; (b) data space representations; and (c) sample aggregation techniques for the creation of the prototypes. A better performance was obtained for the offline setting, with the dissimilarity-based approach achieving an accuracy of  $98.5 \pm 1.5$  and  $94.1 \pm 5.0\%$  using an RF classifier, the Euclidean distance and the medoid metric. These results were surpassed by the RF in the feature-based space reaching an accuracy of  $99.7 \pm 0.8$  and  $98.4 \pm 2.5\%$ , for two datasets, respectively.

**Conclusions:** Experimental results on two databases comprising 4 signal modalities led to high accuracy results, thus paving the way to the development of systems capable of automatically identifying the sensor types solely based on the structure of the data stream itself, and subsequently apply the most appropriate data processing and analysis frameworks.

**Keywords:** Machine learning, signal classification, physiological signals

#### **Presenting author profile**

*Patrícia Bota received her MSc in Biomedical Engineering from FCT/UNL. Currently, she is a Ph.D. student at IST/UL and a researcher at the IT-Lx Pattern and Image Analysis group, where she is involved in the study of the media's impact on human emotions, cognition and behaviour through the development of machine learning algorithms for the recognition of affect based on multi-source physiological data.*

## **5.2. LIGHT-TISSUE INTERACTION AND MICROCIRCULATION PERFUSION ASSESSMENT – WHATS' NEW?**

Henrique Silva<sup>1,3</sup>; Hugo Ferreira<sup>2</sup>; Rafaela Francisco<sup>3</sup>; Alain-Pierre Gadeau<sup>4</sup>; Luis Monteiro Rodrigues<sup>1,3</sup>

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Microcirculation is often assessed by noninvasive technologies such as laser Doppler flowmetry (LDF), laser Doppler perfusion imaging (LDPI), photoplethysmography (PPG) and, more recently, polarized light spectroscopy (PLS). LDF and PPG measure perfusion in a small tissue region on a continuous mode, producing complex and multiscaled oscillatory signals, easily decomposed into their main spectral components (cardiac, respiratory, myogenic, sympathetic, endothelial NO-dependent and endothelial NO-independent) with fast Fourier (FFT) and wavelet transforms (WT), allowing the individual assessment of different perfusion regulating mechanisms. LDPI and PLS evaluate perfusion over larger tissue areas and produce maps with different color tones and textures as main outputs, even though LDPI can also be used on a single-point continuous mode. These perfusion maps can be assessed with texture analysis to obtain a clearer picture of the perfusion regulation at a regional level. A useful parameter to extract from all these signals is entropy, a measure of unpredictability, which often mirrors the competence of the biological system generating those signals. Here, the authors present data from studies from human (postural changes, hyperoxia) and animal (hind limb ischemia, hyperoxia) models, with focus on the combined use of the aforementioned tools for a more robust signal analysis and a deeper understanding of microcirculation dynamic physiology.

**Keywords:** microcirculation, laser Doppler techniques, polarized light spectroscopy, wavelet transform, light-tissue interaction

### 5.3. DETECTION OF APNEA IN RESPIRATORY SIGNALS

Mariana Abreu<sup>1</sup>; Ana Fred<sup>1,2</sup>; João Valente<sup>3</sup>; Hugo Silva<sup>1</sup>

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**Background:** Breathing plays an essential role in our life and prolonged breathing interruptions, denominated apneas, can be both life-threatening and consciously provoked. In thoracic radiotherapy, the conjugation of the dose administration with respiratory gating, which significantly improves the display of the internal structures, is essential to proper administration of the treatment. Thus, the discrimination between apneas (A) and normal (R) samples in a simple and automatic way would enable a deeper understanding both on the patients' wellbeing and the control over the radiotherapy session.

**Methods:** With pattern recognition algorithms we classified one-minute samples as A or R, according to their morphology. The respiratory signal was filtered and normalized with MinMax, and resampled to 1000 points. To detect different morphologies, an autoencoder (AE) was trained on R type samples. Then, all data was fed to the AE and the output was compared to the input using Pearson's correlation, in 10 non-overlapping segments. To reach a decision, these correlation vectors were learned by a classifier.

**Results:** The database used was acquired by João Valente, and contained 45 patients, where each performed a breathing exercise from which we can extract 10 R and 5 A. The application of



our framework achieved an accuracy of 84%7.2, with the classifier SVM. Moreover, fusion decision systems were evaluated and improved performance by 2 percentual points with SVM and RF.

**Conclusions:** In this study we developed a simple morphological framework to discriminate apneas from normal breathing in awoken patients. The system was able to detect apneas even when contaminated with normal breathing without requiring strenuous feature engineering, just by morphology comparison. In the future, we would like to go even further and contributing to the community with an anomaly detector for respiration.

**Keywords:** Deep learning, signal processing, physiological data, apnea detection

**Presenting author profile**

*Mariana Abreu completed her Master's Degree in Biomedical Engineering in 2018, from FCT-UNL. Her master thesis included the application of pattern recognition techniques to automatic recognition of human activities. During the thesis period, she worked in Fraunhofer, on the development of feature engineering and visualization tools, for time series applications. Currently she is a Ph.D. student at IST, in the scope of IT's PIA group. Her Ph.D. is on Prediction and Monitoring of Epileptic Seizures, and her areas of interest are Signal Processing and Machine Learning.*

**5.4. ANXIETY DESTROYER: A VIDEOGAME WITH NEUROFEEDBACK FOR TACKLING ANXIETY**

Pedro Pestana Da Silva<sup>1</sup>; Hugo A. Ferreira<sup>1</sup>

*1 - Instituto de Biofísica e Engenharia Biomédica, Faculdade de Ciências da Universidade de Lisboa*

Anxiety disorder is one of the most common mental illnesses, affecting 264 million people worldwide. Current treatments such as drugs and psychotherapy are limited because they cause side effects such as addiction and/or require long therapeutic periods. A more recent technique, Neurofeedback (NFB), that makes use of the patients' own brain signals to modulate brain activity via audio or visual stimuli, has been associated with the reduction of anxious symptomatology. Nonetheless, it typically uses unappealing stimuli that compromise patient adherence.

In this work a videogame was developed, Anxiety Destroyer, that makes use of electroencephalography (EEG)-based NFB, with the goal of providing an engaging and efficient treatment to be used at home. In this videogame, patients use their brain signals to drive a spacecraft from Earth to an extra-solar planet through 8 levels of increasing difficulty in order to modulate their brain activity.

Thirty subjects were studied, which performed up to 8 NFB sessions for 35 minutes. Three groups of 10 subjects were created: one group used NFB with the frontal alpha asymmetry (ALAY)



protocol (F4-F3), a reported anxiety biomarker; another with asymmetry between AF4 and AF3 channels (AF) for hairless skin usage; and the control group, with increased alpha in F4 and F3 (F+). Data was acquired using an Emotiv Epoc+ EEG headset and recorded for further analysis. The Beck Anxiety Inventory (BAI) was also applied to assess anxiety at baseline and after intervention with NFB.

The ALAY group significantly increased the asymmetry ( $p < 0.05$ ) (reduction of the anxiety biomarker), with a corresponding significant reduction ( $p < 0.01$ ) in anxiety reported by the BAI after NFB training. The AF group showed a decrease in the frontal alpha asymmetry, and the F+ a slight increase with corresponding increase and decrease of the BAI scores.

Studies with larger sample sizes are required to confirm these results, especially since some subjects in the AF and F+ groups abandoned the study because of no perceived effects. However, this study suggests the effectiveness of the videogame in the reduction of anxious symptomatology.

**Keywords:** Anxiety, electroencephalography, neurofeedback, gaming

#### **Presenting author profile**

*Pedro Pestana da Silva is a MSc in Biomedical Engineering. His research is focused on the development of physiological computing applications using neurofeedback and artificial intelligence algorithms. Finally, he is also the CEO of Neroes, a startup that provides solutions for performance improvement in sports.*

## POSTERS

### THEMES 1 & 2 BEYOND INTEGRATIVE PHYSIOLOGY – THE PHYSIOME

#### **P01 - ADHERENCE TO THE MEDITERRANEAN DIET IN PORTUGUESE UNIVERSITY STUDENTS**

Cíntia Ferreira-Pêgo<sup>1,2</sup>; Joana Rodrigues<sup>2</sup>; Adriana Costa<sup>2</sup>; Bruno Sousa<sup>1,2</sup>

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Mediterranean diet (MedDiet) is one of the most recognized healthy dietary patterns. Although the well-known beneficial effects of healthy eating habits on academic performance, the university students used to present unhealthy food choices. Cross-sectional information regarding MedDiet adherence was collected in 305 students from the *Universidade Lusófona de Humanidades e Tecnologias*, from different academic courses, related or not with health sciences. Nutrition





students presented significantly higher MedDiet adherence compared to those studying pharmaceutical sciences and also from other courses not related to health sciences. 28.90% of the total population presented poor MedDiet adherence, 58.70% presented an average adherence and only 12.50% presented a good MedDiet adherence. Nutrition sciences degree presented more students in the highest category of MedDiet adherence compared to the rest. Pharmaceutical students and other students not related to health sciences presented a significantly higher risk of poor MedDiet adherence. Nutrition students presented the highest MedDiet adherence of all the students analyzed. Pharmaceutical students, although being health professionals, showed poor adherence to the MedDiet, similar to students from courses not related to health sciences.

**Keywords:** Mediterranean diet, Mediterranean diet adherence, health sciences students, university students, Portuguese students

**Presenting author profile**

*She graduated in Human Nutrition and Dietetics in Universitat Rovira i Virgili and has a master's degree in Training and Sports Nutrition from the Universidad Europea de Madrid. She obtained her Ph.D. with International Mention in Nutrition and Metabolism from the Universitat Rovira i Virgili and University of Arkansas. Her main epidemiological research interests are the Mediterranean Diet, dairy products, hydration and/or the consumption of beverages and its relation with health and disease.*

**P02 - WHAT HAPPENS IN THE FOOT MICROCIRCULATION DURING WALKING?**

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The foot microcirculation is crucial for gait function. The aim of this study was to determine what happens in the foot microcirculation measured at different depths during walking. Nine healthy subjects (five women and four men) 28.6 ± 10 y.o., were included after informed written consent. Volunteers performed a five minutes' walk and microcirculation was assessed and compared before (Phase 1) and after walking (Phase 2). The local microcirculatory function was quantified by photoplethysmography (PPG) laser Doppler flowmetry (LDF) and Polarised Spectroscopy (TiVi) to quantify the concentration of red blood cells (CMRBC). Probes were placed as close as possible to each other, from 1 cm postero-medial of the first metatarsus head. Descriptive and comparative statistics were applied and a 95% level of confidence adopted. Our study demonstrated significant changes from Phase 1 to Phase 2 in PPG results ( $p = 0.012$  and  $0.027$ ) for left and right limbs, respectively, and also for both sides in LDFs' CMBC assessment (both  $p = 0.038$ ). A slight rise in skin temperature in both feet was registered. Perfusion units (PU) and TiVi-index presented similar results for these two phases, which shows a fast and effective capacity of the regulatory mechanisms involved in microcirculatory homeostasis.



**Keywords:** foot microcirculation, PPG, CMRBC

**Presenting author profile**

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**P03 - COMPARING LASER DOPPLER FLOWMETRY AND POLARIZED SPECTROSCOPY FOR ASSESSING PERFUSION DURING SQUATTING**

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Laser Doppler Flowmetry (LDF) is one of the most used techniques for the continuous assessment of microcirculation. However, it is highly affected by spatial variability resulting from the small measurement area and the large heterogeneity in microvascular morphology and topology. Recently, a non-contact technique based on polarization spectroscopy (PS), allowing subepidermal imaging of large skin areas - the Tissue Viability (TiVi®) System was introduced. Despite its broad interest, studies comparing it to LDF are lacking. The aim of this study was to compare the LDF and PS techniques for cutaneous perfusion assessment during a squatting exercise test. Five young healthy male ( $26.0 \pm 6.5$  y.o.) performed a squatting exercise, consisting in standing up straight for 3 minutes, then performing 15 squat repetitions for 1 minute and returning to the initial position for further 3 minutes. Both techniques were used to follow all hemodynamics at foot dorsum – LDF quantified blood perfusion, concentration of moving red blood cells (CMBC) and velocity, while PS quantified the TiVi index, a parameter related to the concentration of red blood cells (CMBC). The LDF probe was placed on the foot dorsum, on the projection of the fifth metatarsophalangeal joint, while the PS signal was obtained by placing the device at a 60cm distance from the foot on without ambient light. During the squatting exercise a significant increase in blood flow and CMBC was observed with LDF, together with a significant decrease in velocity, on both limbs. The TiVi index increased bilaterally, however, without statistical differences. These results suggest that, in the present experimental conditions, TiVi appears to be less sensitive than LDF, probably due to different penetration depths to be further clarified.

**Keywords:** Laser Doppler Flowmetry, tissue viability, microcirculation, squatting exercise test

**Presenting author profile**



*Sérgio Nuno received a degree in Physical Therapy - Portuguese Red Cross Higher Education with allocation of scholarship merit with average of 18.1 values in 2010. He earned his Master's Degree in Physical Therapy with average of 17 values. He is a Ph.D. Student (U Lusófona with U Alcalá partnership). In 4 years of higher education, he has had a merit scholarship by the Portucel Soporcel Group. He has worked at the hospital "Clínica São João de Deus" since 2010 and is currently the coordinator of the Physiotherapy Service. He is a physiotherapist responsible for the National rowing team and had achieved clearance for the Olympic Games. He is also a teacher at the Lisbon School of Health Technology of the Polytechnic Institute of Lisbon, ESTeSL-IPL.*

#### **P04 - MNTNHEX-2-PYP, A SOD MIMIC AS A MECHANISTIC TOOL TO STUDY RENAL CANCER PATHOPHYSIOLOGY**

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Cancer is a multistep process and oxidative stress has been pointed out to have critical roles on its initiation, promotion and progression. The present work addresses the redox modulation afforded by the superoxide dismutase mimic MnTnHex-2-PyP (MnP), a prototype of the Manganese(III) porphyrins (MnPs), which are catalytical polyfunctional antioxidants with the ability to modulate different cellular redox pathways. Therefore, MnPs can be used as mechanistic tools to increase our insight into renal cancer pathophysiology.

Two different renal cell models were used to address initiation and progression cellular events. First, the role of MnP on the toxicity of non-tumor renal cells exposed to the ochratoxin A (OTA), a recognized nephrotoxicant with possible carcinogenic effects, was evaluated. Afterwards, the role of the MnP in renal cancer progression was studied in vitro. Complementary endpoints of cytotoxicity and genotoxicity, as well as the intracellular reactive oxygen species (ROS) levels, and migration assays were performed. Overall, this MnP protected non-tumor cells from the toxic effects induced by OTA, while it had a beneficial effect against renal cancer cells, namely by decreasing the cell viability and migration. The results obtained herein reinforce the multiple applications of MnPs both as mechanistic tools and also for potential therapeutic use.

**Keyword:** SOD mimic, pathophysiology, renal cancer, MnTnHex-2-PyP

#### **P05 - THE BRIEF HISTORY OF NEUROSCIENCE IN TURKEY**

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### 1 - Mersin University

Turkey, which is located in the connection between Asia and Europe, has hosted a variety of nations during the centuries. Hence, all civilizations have unique effects on development of all aspects of medicine and surgery in pre-modern era. Starting with Seljuks to Ottoman Empire and by now the Republic of Turkey, beginning with İbni Sina (980-1037) to Hacı Pasha (1385-1417) and Serafeddin Sabuncuoğlu (1385-1470) many improvements have taken place regarding to medical sciences, particularly in neuroscience parallel to the modern world.

By the mid-19<sup>th</sup> century, Turkish scientists turned more in Western Medicine and the onset of modern neurosurgery was performed via Dr. Cemil Topuzlu and he has founded the first neurosurgery department in 1923. The emergence of neuroscience in Turkey may be attributed to Dr. Mazhar Osman who evaluated the reasons of many neuropsychiatric diseases and published the first Turkish neuroscience journal (1916). Then, Gazi Yasargil, who made a breakthrough in aneurysm surgeries via designing a special microscope, used microsurgery techniques in neurosurgery field and was selected as the neurosurgeon of the century. He has found new techniques in the treatment of epilepsy and brain tumors (1960s).

At the present time, there is an increasing number of publications by Turkish scientists, contributing to the total body of literature in neuroscience and follow the contemporary trends in this field. In order to mention few of them as, Ahmet Höke has significant studies about defects in peripheral neural system and the basic biology of Schwann cells and in vivo/vitro modelling of axonal degeneration and regeneration, Tarık Tihan has tremendous work about the categorization of brain tumors, Deniz Kırık who has many studies in the field of neuroscience and has discovered new cell and gene-based therapies against Parkinson and Huntington diseases, Ziya Gokaslan called as 'who define spine again' has contributed new surgery methods in spinal cord cancers in medical literature, Turgay Dalkara guides many neuroscientists concerning the working patterns of brain, Murat Emre who has found a new drug against Parkinson related dementia, Emrah Duzel who has remarkable studies to repair injured tissues or de novo tissue formation.

**Keywords:** Neuroscience, Turkey, history, physiology

#### **Presenting author profile**

*I am currently working on learning - memory behaviour in Physiology Department, Mersin University as a research assistant. I am now doing my Ph.D. studying postnatal stress-hippocampus function relations and related genes.*

#### **P06 - NON-CONTACT POLARIZED SPECTROSCOPY CAN BE USED TO EXPLORE SUPERFICIAL MICROCIRCULATORY ADAPTATION IN THE HUMAN LOWER LIMB**

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The use of non-contact systems in in vivo microcirculation studies facilitates assessment and the application of research protocols, in human beings, which means a deeper insight in microcirculatory physiology and pathophysiology. In this study, we explore the applicability of a non-contact polarized spectroscopy method to complement the conventional use of contact and well-known systems, such as Laser Doppler flowmetry (LDF) in evaluating the lower limb microcirculation in a challenge test, such as a massage manoeuvre.

Six healthy volunteers ( $44.83 \pm 12.64$  years old) both sexes, were submitted to one massage protocol (applied in the proximal direction) in one randomly selected limb, the other serving as control. The protocol included three phases, a 10 minute baseline (Phase I) period, a 5 minute massage (Phase II), and a 10 minute recovery (Phase III) recordings with Tissue Viability Imager (TiVi) and Laser Doppler flowmetry (LDF) signals being obtained from both limbs (leg and second toe, respectively). TiVi allows to detect skins' red blood cell concentration (RBCC), expressed in TiVi-index arbitrary units, while with LDF perfusion is described in terms of arbitrary Blood Perfusion Units (BPUs). A  $p < 0.05$  was adopted.

The manoeuvre evoked blood perfusion alterations detected with both systems. Both systems allowed to follow the perfusion changes in both limbs. LDF has shown a significant decrease during Phase II in the test limb and a non-significant decrease in the contralateral limb. As for TiVi, during Phase II, an increase in the RBCC contralateral limb is present, although not significant in accordance with the known responses to these manoeuvres. Also, our results suggest that TiVi does complement the instrumentation available to study microvascular adaptation in vivo.

**Keywords:** Polarized spectroscopy, LDF, microcirculation, lower limb cooperation, massage

## **P17 – ABIETANE DITERPENOIDS FROM PLECTRANTHUS SPP. AS A NEW CLASS OF PROTEIN KINASE C MODULATORS FOR CANCER THERAPY**

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Cancer is one of the highest causes of death worldwide. Protein kinase C (PKC) is a family of serine/threonine kinases divided into three groups according to their regulatory domain structure and cofactors requirement for activation: classical, novel, and atypical PKCs. Recently, PKC family isoforms have been the focus of intense research, and recognized as therapeutic targets



in anticancer drug development. Diterpenoids are commonly found in the *Plectranthus* spp., and have a widespread spectrum of biological activity, namely anticancer properties. The diterpenoid 7 $\alpha$ -acetoxy-6 $\beta$ -hydroxyroyleanone (AHR) isolated from *P. grandidentatus* displays low cytotoxicity and the basic requirements approaches for the development of pharmaceutical formulations based on AHR as a lead. These AHR features includes an extraction optimization and structural and thermal properties characterization. These features suggest that AHR can be used as a lead for drug development. Considering this, a small library of abietane derivatives was tested for their ability to activate PKC isoforms from classical (alpha,  $\alpha$ ; beta,  $\beta$ ), novel (delta,  $\delta$ ; epsilon,  $\epsilon$ ) and atypical (zeta,  $\zeta$ ) subfamilies, using a previously developed yeast-based screening assay to search for modulators of PKC isoforms. The results obtained revealed potent activators of PKC family proteins, namely: a selective activator of PKC $\delta$ , the 7 $\alpha$ -acetoxy-6 $\beta$ -benzoyloxy-12-O-benzoylroyleanone (Roy-Bz). The patented diterpenoid RoyBz was prepared using AHR as starting material. Roy-Bz potently inhibited the proliferation of colon cancer cells by inducing a PKC $\delta$ -dependent mitochondrial apoptotic pathway involving caspase-3 activation. The results indicate that Roy-Bz targets drug-resistant cancer stem cells, in HCT116 colon cancer cells, preventing tumor dissemination and recurrence. Moreover, these findings support a tumour suppressive function of PKC $\delta$  in colon cancer. Overall, these results point to promising activators of PKCs with high potency and isoform-selectivity that may emerge from the exploitation of this new family of abietane diterpenoids. Molecular docking studies are currently ongoing to further identify new selective abietane diterpenoids as new PKC modulators.

**Keywords:** Cancer, protein kinase C, diterpenoids, *Plectranthus*

**Presenting author profile**

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**P18 - EVIDENCE FOR ALTERED LIPID RAFT COMPOSITION AND SYNAPTIC PLASTICITY IN A RAT MODEL OF MTL**

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**THEME 3 PHYSIOLOGY OF STRESS**

**P07 - IS VENOARTERIAL REFLEX AGE DEPENDENT?**



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Recent studies identified a contralateral response observed in the veno arterial reflex in the test limb, both in animals and humans. This adaptative reflex is known for a long time but its mechanistic explanation is still unclear. In the present study we assessed this reflex as a function of age. 8 healthy volunteers (mean 50.13 ± 6.17 yo) from both sexes, were submitted after informed written consent, to a leg lowering manoeuvre while lying in a supine position. The leg to evoke the Venoarteriolar Reflex (VAR) was randomly selected while the other was used as control. The experiment consisted in three phases, a 10 minute baseline (Phase I) period, a 10 minute leg pending challenge (Phase II), and finally a 10 minute recovery (Phase III). Recordings were performed in both legs with laser Doppler flowmetry (LDF) and photoplethysmography (PPG) with sensors in second and first toes, respectively. Signals were decomposed in its oscillatory components with the Morlet wavelet transform (WT). A  $p < 0.05$  was chosen for statistical comparisons. Both technologies revealed that the VAR manoeuvre promoted a significant perfusion reduction in the tested limb, also in the control foot as previously observed in younger individuals. A similar evolution of LDF components is depicted in both young and old individuals although less pronounced in the later. Results might suggest that this reflex resists fairly well to ageing, at least when no significant vascular impairment (e.g diabetes or other vascular failure) is present in the lower limb.

**Keyword:** venoarteriolar reflex, laser Doppler flowmetry, photoplethysmography, wavelet transform, vascular regulation

#### **Presenting author profile**

*Clemente has a degree in Computer Engineering from ISPGAYA and MSc in Computer Engineering (ISCTE-IUL). He has dedicated an important part of his activity to Naturopathy (General Course of Naturopathy and Holistic Traditional Sciences - IMT). He is currently Ph.D. student in the doctoral program in Health Sciences at U Lusófona, where he concluded an advanced training in Clinical Trials.*

#### **P08 - PERFUSION IS SEX RELATED BUT RESPONSE TO MASSAGE EVOKES THE SAME HEMODYNAMIC ADAPTATION IN BOTH SEXES – RESULTS FROM AN EXPLORATORY FACTOR ANALYSIS**

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Microcirculatory regulation still presents many unanswered questions. Gender-based perfusion differences are known and partially attributed to different autonomic/myogenic vascular activities. Effleurage, a common massage technique, evokes similar responses in healthy volunteers from both sexes, no matter the known perfusion differences. In this study we assess the gender differences in the lower limb microcirculation using massage as the vasoactive stressor. 32 young healthy subjects ( $19.8 \pm 1.5$  y.o., 16 females, 16 males) were selected after informed consent. The protocol was applied on a randomly chosen limb, the other serving as control. Data was collected after 10 min resting (Phase I - baseline); during 5 min proximal massage (ankle to knee) (Phase II); and during recovery (10min Phase III). Perfusion was assessed by Laser Doppler Flowmetry (LDF) on the plantar side of the second toe in both limbs. LDF signals were then decomposed into their spectral components - cardiac, respiratory, myogenic, sympathetic, endothelial NO-dependent, endothelial NO-independent - with the wavelet transform (WT). Mann-Whitney and Wilcoxon signed rank tests were used to assess significance of gender differences ( $p < 0.05$ ). Spearman correlation and Exploratory Factor Analysis (EFA) were used to correlate variables. At baseline, perfusion differences were significantly higher in males ( $p = 0.015$ ). These differences seem to be mitigated during the massage protocol, reappearing in the recovery phase ( $p = 0.037$ ). Different correlations for males and females in baseline LDF components were identified (e.g. sympathetic signal only significantly correlates with the myogenic signal in females while in males it also correlates with cardiac, respiratory, and endothelial NO-dependent). EFA returned 4 factors for both genders in Phase II. In females, changes through phases seem to be induced by the interplay between myogenic, sympathetic, blood pressure and endothelial NO-dependent components. However, in males, Phase II shows heart rate and myogenic component loading in factor 1, with the cardiac and respiratory having less importance than in females. A different baseline physiology, determined by sex, seems to involve a different homeostatic adaptation when a stress factor like effleurage changes local perfusion. A deeper insight into these mechanisms, using other experimental conditions, is needed for a substantial understanding on the effect of gender on microcirculatory regulation.

**Keywords:** Microcirculatory regulation, gender differences, effleurage, laser Doppler flowmetry, Exploratory Factor Analysis

#### THEME 4 BY WAY OF THE SKIN

##### **P09 - ASSESSMENT OF THE IMPACT OF ORAL INTAKE OF THE PROBIOTIC KEFIR IN CUTANEOUS HEALTH**

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The regular intake of probiotics has been linked to beneficial effects on cutaneous health and skin barrier function, particularly in cases of atopic dermatitis. Kefir is a probiotic obtained from milk





fermentation with grains formed by a matrix of polysaccharides and proteins densely populated by acid-lactic and acid-acetic bacteria and yeast that live in symbiotic association. Its bioactivity seems to be, at least partially, linked to the action of organic, lactic, and acetic acids released during fermentation that have some anti-inflammatory activity.

This study aims to evaluate *in vivo* by non-invasive methods the cutaneous impact in healthy and atopic individuals of kefir oral intake, thus contributing to the consolidation of methodologies for studying the skin effects of probiotic foods.

During 6 weeks, a group of healthy and atopic volunteers daily drank 100ml of kefir ( $t_{\text{fermentation}}=24\text{h}$ ,  $T=20^{\circ}\text{C}$ ). A model of induction of cutaneous irritation using SLS on the forearm was used to assess its impact on skin barrier at baseline and after 6 weeks, as well as periodic skin evaluation by bioengineering methodologies (hydration, pH, and transepidermal water loss (TEWL)) on three anatomical areas- forehead, forearm and leg. The impact on the skin barrier function caused by SLS application was measured by TEWL using a Tewameter® TM300 (CK Electronics, Germany) and colorimetry. Measurements of skin colour were performed with a ChromaMeter CR300 (Minolta, Japan) using the  $L^*a^*b$  system, where skin erythema is reflected on  $a^*$ .

Results show an impact in the skin parameters and irritation capacity of SLS, especially in the atopic group. Although based on a small number of volunteers, this study confirms the interest of the methodology to assess probiotic impact in skin barrier function of both healthy and atopic subjects. The work will be continued with an increased number of volunteers to further establish the benefits of kefir consumption.

This work is financed by national funds through the FCT - Foundation for Science and Technology, I.P., under the project UID / DTP / 04567/2019.

**Keywords:** Skin, kefir, probiotic, SLS irritation model

#### **Presenting author profile**

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#### **P10 - TOWARDS THE MURINE MICROVASCULAR SPECTRAL PROFILE – EXPLORING THE EFFECTS OF ATENOLOL UNDER KETAMINE-XYLAZINE ANESTHESIA**

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Murine models are very useful for the characterization of microcirculatory function. Recently, baseline laser Doppler flowmetry (LDF) signal recordings have allowed the tentative proposal of the spectral microcirculation profile consisting of the same components (cardiac, respiratory, myogenic, sympathetic, NO-dependent and NO-independent endothelial) found in human. Still, this proposal requires confirmation using stimuli specifically targeting individual components. Our objective was the characterization of the murine spectral microcirculatory reactivity to atenolol (AT). A group of six C57/BL6 male mice under a ketamine (K, 137.5 mg/kg) and xylazine (X, 11 mg/kg) mixture received two IP doses of AT (2.5 mg/kg). Animal experiments were performed in accordance with the EU guidelines on animal welfare and complied with principles and standards for reporting animal experiments. Perfusion was assessed with LDF, collected on a random hindlimb and decomposed with the wavelet transform (WT). For each component the dominant frequency and amplitude ratio were assessed. Heart rate (HR) and respiratory rate (RR) were also assessed. Both AT doses significantly increased perfusion and HR, however no changes were noted for the RR. Both doses significantly increased the cardiac dominant frequency and decreased the myogenic dominant frequency. The cardiac ratio decreased but only significantly so after the second dose, whereas the sympathetic ratio increased with the first dose and decreased with the second. The myogenic and endothelial ratios increased with both doses, although without significance. Although no vasoactive effect is attributed to AT, its concurrent administration with KX seems to result in a perfusion increase due to myogenic-endothelial increase despite the increase in vascular sympathetic activity. These results seem to suggest the usefulness of the administration of AT under anaesthesia to better characterize the murine microcirculatory spectral profile.

**Keywords:** microcirculation, murine model, laser Doppler flowmetry, spectral organization, atenolol, ketamine-xylazine

**Presenting author profile**

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**P11 - STUDYING IRRITATION AND INFLAMMATION BY NON-INVASIVE FLOW RELATED VARIABLES**

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Alteration of skins' "barrier" efficiency begins with irritation (redness, discomfort and other symptoms) progressing to inflammation [1]. To date the physiological differences between inflammation and skin irritation are poorly characterized. This work aims to elucidate the mechanisms involved in primary skin reactions, using state-of-the-art non-invasive technologies *in vivo*. Skin "barrier" was assessed by transepidermal water loss (TEWL). Laser Doppler Flowmetry (LDF) and Tissue Viability Imager (TiVi) were used to assess skin microcirculation. Six healthy volunteers of both sexes, aged 21-32, were selected upon informed written consent. Tape-stripping (TS) and contact under occlusion with methyl nicotinate (MN, 0.1 M) were used as challengers. Measurements were taken prior and after. TEWL varied from 5-12 (g/m<sup>2</sup>/h) for the volunteers. No clear trend was able to be identified. LDF values for MN showed an increase in flow from  $9.54 \pm 0.56$  to  $30.14 \pm 1.19$  AU in males and from  $4.60 \pm 0.95$  to  $31.95 \pm 0.66$  AU in females. Values collected for TS showed an increase in flow from  $11.67 \pm 1.07$  to  $36.55 \pm 10.11$  AU in males and from  $5.06 \pm 0.70$  to  $32.17 \pm 6.80$  AU in females. Furthermore, the increase in perfusion was faster in males (12 min) compared to females (15 min).

TiVi showed an average increase in the local concentration of red blood cells from  $54.6 \pm 13.3$  to  $70.7 \pm 19.9$  UA and  $48.5 \pm 5.12$  to  $62.2 \pm 14.2$  UA. Increment in ratio was 1.3 for both TS and MN, showing evidence that the response in vasodilation is similar, even though the stimuli are different.

This work showed that inflammation and irritation have a diverging response observed by TiVi and LDF. Inflammation seems to affect the deeper blood vessels detected by LDF, but not by TiVi. This work showed imaging and non-invasive techniques combined provide are useful to provide complementary information to characterise skin irritation and inflammation.

**Acknowledgments:** The authors wish to express thanks to all the volunteers.

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**Keywords:** Inflammation, irritation, tape stripping, methyl nicotinate, laser doppler flowmetry, transepidermal water loss, tissue viability imager

#### Presenting author profile

Ana holds a Ph.D. in Pharmacy (University of South Australia, Australia). Her research interests include development and characterisation of nanoformulations for effective transdermal drug delivery, understanding the transport of molecules within the skin and nail by means of imaging and quantitative techniques.

#### P12 - SKIN COMPATIBILITY ASSESSMENT OF PLGA-NANOPARTICLE LOADED HYDROGELS FOR WOUND HEALING

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The skin protects the body from infection by microorganisms and dehydration. When the barrier is damaged, a wound healing process is started. Acute wounds heal in 12 weeks, whereas chronic wounds need months [1]. Hydrogels are hydrophilic molecular networks produced by the physical or chemical cross-linking of polymers and have been used in wound dressings. Polymer nanoparticles may carry and release growth factors in a controlled or sustained manner. In this work, we propose the use of PVA/alginate hydrogels loaded with PLGA nanoparticles as carriers for growth factors for wound healing. *Poly (lactic-co-glycolic acid)* (PLGA) was used as the matrix of the nanoparticles and PVA was the surfactant. Glycerine and urea were also included in the gels to improve flow properties, healing, and as preservatives. PLGA nanoparticles were produced following a protocol developed by our group [2]. Then, sodium alginate, glycerine, and urea were added, and the hydrogels were formed after freeze-thawing cycles.

It was observed that the hydrogel production method did not change the particle size of 300 nm and obtained a narrow Pdl of 0.25. The ATR-FTIR analysis showed an interaction between PLGA and glycerine (1075 cm<sup>-1</sup>) and urea (1650 cm<sup>-1</sup>). Skin compatibility was assessed by Laser Doppler Flowmetry, and values were similar for water and the gels containing no glycerine, 5% glycerine, and 10% glycerine (16.7, 11.0, 17.6, 13.0 AU, respectively), showing evidence of not causing erythema. In contrast, the positive control methyl nicotinate showed an increase in flowmetry to 124.8 AU.

Overall, we obtained a stable and biocompatible polymer-nanoparticle based hydrogel for wound healing purposes. Such a system is intended to load growth factors. *In vitro* and *in vivo* performance will be evaluated in the future.

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**Keywords:** Hydrogel, skin compatibility, nanoparticle, wound healing, freeze-thawing

**Presenting author profile**

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THEME 5 PHYSIOLOGICAL INFORMATICS

**P13 - OPTIMAL FEATURE EXTRACTION WITH MACHINE LEARNING FOR EMOTIONAL CLASSIFICATION**

Miguel S. Joaquim<sup>1,2</sup>; Rita Maçorano<sup>2</sup>; Francisca Canais<sup>2</sup>; Rafael Ramos<sup>2</sup>; Ana L. Fred<sup>1</sup>; Hugo A. Ferreira<sup>2</sup>

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Human emotions have always been a topic of great interest, especially due to their importance as a tool for communication between people. Nowadays, emotional analysis is also crucial regarding the way users interact with new technologies. By understanding the user's feelings, a digital interface can adapt itself to provide a more pleasant and useful experience.

In this work, 7 different emotions were studied: neutral, amusement, tenderness, sadness, disgust, anger, and fear, from 21 subjects, consisting of both males and females. Each subject was shown one commercial per emotion, in random order.

During the procedure, raw photoplethysmography and two-channel pre-frontal electroencephalography signals were collected for each individual using EmotAi's wearable headband and physiological computing platform (EmotAi, Hong Kong). Convolutional Neural Networks were implemented in Python using TensorFlow to classify the acquired signals with raw data as input. All inputs were classified according to the Positive and Negative Affect Schedule. Afterwards, correlation values between conventional features extracted from the raw inputs and the final classification were computed by adding noise to the input and checking output changes. This was done to assess the effect of each metric on the final value of the emotional classification. The most common features from the literature were considered: theta, alpha, beta and gamma band powers, asymmetry indexes, phase synchronization indexes, and statistical features.

This approach achieved average accuracies and f1-scores of 82% and 0.82 for Positive Affect, and 84% and 0.82 for Negative Affect, respectively. The features with the most significant correlations were the alpha band power, and the asymmetry and phase synchronization indexes.



The obtained results show that the extracted features seem to match the ones apprehended by the neural network, hence endorsing their validity for emotional studies.

**Keywords:** Emotions, photoplethysmography, electroencephalography, positive and negative affect schedule, machine learning, feature extraction

#### **Presenting author profile**

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#### **P14 - MAGNETIC RESONANCE IMAGING – AN INTERESTING TOOL TO QUANTITATIVELY ASSESS VASCULAR TOPOGRAPHY**

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The study of vessel topography is of great importance in vascular medicine since vascular abnormalities or dysmorphic features are present in many developmental and cardiovascular disorders, as well as in ageing, even when no clinical manifestations are present. Branching regions of any vascular tree is a common site for architectural dysmorphia and dysfunction due to well-known biophysical factors. Magnetic resonance imaging (MRI) is a powerful tool for the purpose of anatomical description, although somewhat underexplored in vascular medicine, particularly in the quantitative characterization of vascular branching topography. Our aim was to quantitatively describe the branching topography of large blood vessels in the wrist region using MRI. Four healthy subjects ( $40.5 \pm 17.2$  y.o.) participated in this study after giving informed consent. MRI scans of a randomly chosen wrist were performed for each subject in a 1.5T scanner (Magnetom Avanto, Siemens, Erlangen) with an 8-channel wrist coil. Scans included a 3D T2\*-weighted gradient-echo sequence with voxel size  $312 \times 312 \times 300$  mm<sup>3</sup>. From these MRI scans 16 bifurcating blood vessels (diameter of  $18 \pm 11$  px) were assessed using ImageJ®, which allowed the calculation of several branching parameters – branching angle, angular asymmetry, area ratio, optimality ratios, asymmetry ratio, branching coefficient, length-to-diameter ratio, junctional exponent deviation. Linear correlations between these parameters were tested ( $p < 0.05$ ). The branching coefficient was found to be positively correlated with the area and optimality ratios and negatively correlated with the junctional exponent deviation. Furthermore, the branching vessel



diameter was also positively correlated with the junctional exponent deviation parameter. These results suggest that MRI is a useful tool for the purpose of characterizing vascular topography, which adds further value to this already versatile technique.

**Keywords:** MRI, vascular architecture, topography parameter

**Presenting author profile**

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**P15 - WAVELET TRANSFORM COMPONENTS ANALYSIS OF PERFUSION OBTAINED FROM PHOTOPLETHYSMOGRAPHY SIGNALS IN HUMAN**

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The wavelet transform analysis (WT) of optical phenomena gathered from techniques such as laser Doppler flowmetry (LDF) is well known, and regarded as a useful mechanistic assessment refinement. In the present study we applied this strategy to one other technology, photoplethysmography (PPG) and compared it with equivalent signals obtained under the same conditions with LDF. Twenty-four young healthy volunteers ( $19.9 \pm 1.7$  years old) from both sexes, were submitted to a massage protocol (applied in the proximal direction) in one randomly selected limb, serving the other as control. The protocol included three phases, a 10 minute baseline (Phase I) period, a 5 minute massage (Phase II), and a 10 minute recovery (Phase III) recordings with LDF and PPG, obtained from both limbs. A  $p < 0.05$  was adopted). WT analysis of LDF and PPG oscillatory components revealed interesting differences, mostly regarding sympathetic and myogenic components, which seem to play a central role in the process. LDF and PPG were also able to detect the regional circulatory adaptation that takes place in both test and control limbs with massage, suggesting a regional circulatory adaptation. This analysis seems to confirm that these two optical technologies do not measure the same phenomena since they seem to operate at different depths. Nevertheless WT analysis confirms that in both technologies, under the present conditions, the observed myogenic activity increase, which associated to a sympathetic decreased in both signals, could contribute to the increased perfusion observed during massage, both in the tested and control feet.

**Keywords:** hind limb massage, LDF, PPG, wavelet transform, microcirculation



**Presenting author profile**

*Degree in Computer Engineering from ISPGAYA and MSc in Computer Engineering (ISCTE-IUL). He has dedicated an important part of his activity to Naturopathy (General Course of Naturopathy and Holistic Traditional Sciences - IMT). Current Ph.D. student in the doctoral program in Health Sciences at U Lusófona where he concluded an advanced training in Clinical Trials.*

**P16 - ASSESSING THE EXPERIENCE OF EATING CHOCOLATE USING PHYSIOLOGICAL SIGNALS**

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Eating is a complex behaviour, which engages not only the digestive system, but to a larger extent it influences the mental state. In this study, the emotional and cognitive responses of eating a variety of chocolates were assessed, as well as brand recognition, based on flavour and wrapping of each chocolate. The experiment included 3 types of chocolates: hazelnut, cherry liqueur and coconut.

The study enrolled 40 healthy subjects (aged  $24 \pm 7$  years old) divided into 4 gender-matched groups: 1 - blindfolded with wrapped chocolates, 2 - open eyes with wrapped chocolates, 3 - blindfolded with unwrapped chocolates and 4 - open eyes with unwrapped chocolates. Two-channel pre-frontal (Fp1 and Fp2) electroencephalography (EEG) and photoplethysmography (PPG, blood-volume pulse) signals of each subject were acquired using EmotAi's wearable headband and physiological computing platform (EmotAi, Hong Kong). These physiological signals were then processed to derive emotional valence from the EEG's alpha band interhemispheric asymmetry and heart rate from the PPG signal.

Heart rate and emotional valence were normalized to baseline values to compute indices of physiological response to different stimuli. The average HR index for all subjects increased by  $4.4 \pm 6.0\%$  when eating any chocolate, whereas the cherry liqueur chocolate caused the greatest increase in HR (30.5%).

The majority of subjects chose hazelnut as their favourite, and for each group the highest value of EV was on their favourite chocolate. Groups eating wrapped chocolates had 77% higher EV (more positive valence) after eating compared to unwrapped chocolate, as well as a longer-lasting effect in increased HR. In addition, 95% of the subjects were able to recognize each chocolate regardless of the group to which they were assigned, and 85% recognized the brand.





This study enabled to better understand the eating pattern based on different factors: the preference for the chocolate, eating with eyes closed/open, and wrapped or unwrapped chocolates.

**Keywords:** chocolate, emotional valence, heart rate, neuromarketing

***Presenting author profile***

*Justyna Zasada is a Biomedical Engineering student, currently in her last year of the bachelor's degree at Lodz University of Technology. In the previous semester, she had the chance to study at the University of Lisbon during Erasmus exchange, which gave her a new experience and a fresh view on the biomedical field. Interested in neuromarketing and computer graphics, she often combines these areas with the projects in her studies.*



## AUTHORS INDEX

- Adriana Costa, 24  
Alain-Pierre Gadeau, 21, 33  
Ana Fred, 20, 22, 37  
Ana Gouveia, 14  
Ana Júlio, 20  
Ana Macedo, 18, 34, 35  
Ana S Flórido, 15  
Ana S. Fernandes, 15, 20, 27  
António Banganho, 17  
Bruno Sousa, 24  
Carlos Moreira, 14, 40  
Carlos Muñoz-Bravo, 11  
Carolina Amorim, 14  
Catarina Rosado, 18, 20, 32, 35  
César Lima, 8  
Cíntia Ferreira-Pêgo, 11, 24  
Clemente Rocha, 12, 28, 31, 39  
Daniel Rocha, 14, 40  
Diana Prata, 8  
Dilan Deniz Koyuncu, 27  
Dolores Corella, 11  
Duarte M. F. Prazeres, 20  
Emília Alves, 32  
Emilio Ros, 11  
Erica Wehrwein, 13  
Étienne Roux, 33  
Fernando Arós, 11  
Francisca Canais, 37  
Francisco Rego, 18  
Gonçalo Cosme, 8  
Hasan İlker Coban, 16  
Henrique Silva, 12, 18, 21, 26, 28, 31, 33, 38, 39  
Hugo Ferreira, 12, 14, 21, 23, 28, 31, 37, 38, 39, 40  
Hugo Silva, 17, 20, 22  
Inês Antunes, 34  
Ines Batinic-Haberle, 15, 27  
Israa Negmeldin, 34  
Joana Rodrigues, 24  
João G. Costa, 15, 27  
João Gregório, 13, 31  
João Valente, 20, 22  
João Vieira, 35  
Jordi Salas-Salvadó, 11  
José Manuel Santos-Lozano, 11  
Justyna Zasada, 40  
Katja Brodmann, 8  
Leyla Sahin, 27  
Luis Serra-Majem, 11  
Luis Monteiro Rodrigues, 10, 12, 13, 18, 21, 26, 31, 33, 34, 38, 39  
Maira Bes-Rastrollo, 11  
Marcelino Santos, 17  
Margarida Florindo, 10, 25  
Mariana Abreu, 22  
Matilde Castro, 15  
Miguel Fiol, 11  
Miguel Ruiz-Canela, 11  
Miguel S. Joaquim, 37  
Mónica Costa, 8  
Montserrat Fitó, 11  
Muaz Belviranli, 9, 16  
Nancy Babio, 11  
Nilsel Okudan, 9, 16  
Nuno G. Oliveira, 15, 27  
Nuno Saraiva, 15, 27  
Ozge Selin Cevik, 27  
Patrícia Bota, 20  
Patrícia Filipe, 18, 35  
Patrícia Rijo, 29, 32  
Paula Carvalho, 14  
Pedro Fonte, 18, 20, 35  
Pedro Pestana Da Silva, 23  
Pedro Rosa, 8  
Rafael Ramos, 37  
Rafaela Francisco, 21, 38  
Ramon Estruch, 11  
Rita Caparica, 20  
Rita Maçorano, 37  
Salette Reis, 20  
Sema Sayharman, 9  
Sérgio Nuno, 26, 28  
Sofia A. Costa Lima, 20  
Tânia Santos De Almeida, 20  
Vânia Tavares, 8  
Xavier Pintó, 11