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European Perspectives in Cardiology

Funding: The Portuguese Foundation for Science and Technology

Funding Scientific and Technological Research in Portugal

Sixteen scientists carrying out cardiovascular research funded by the Portuguese Foundation for Science and Technology describe the funding and their research to Jennifer Taylor, BSc, MSc, MPhil.

Numerous cardiovascular science projects have been funded by the Fundação para a Ciência e a Tecnologia (FCT; Portuguese Foundation for Science and Technology). Calls for applications to fund research and development projects are evaluated by several criteria, including relevance and originality of the project proposal, scientific merit of the research team, feasibility of the work programme, expected contribution to the accumulation of knowledge and competence, and potential economic worth of the technology.

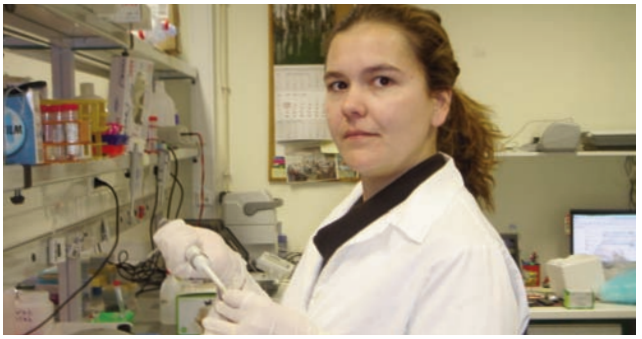
José António Belo, PhD, DSc, associate professor in biotechnology, Department of Ciências Biomédicas e Medicina, University of Algarve, Faro

Dr Belo was recently awarded 2 grants to conduct cardiovascular research: in January 2011, he received €150000 to study the role of the novel gene *Ccbe* in heart induction and organogenesis, and in 2012, he was awarded €158542 to investigate the role of the new growth factor in creating Ccbe1 cardiac tissue from embryonic stem cells. Both grants are for 3 years and will fund consumables, travel, consultants, small equipment, and staff. "These grants have been awarded solely to my lab where the research work is being conducted, although I have a priceless collaboration with Ibrahim Domian, MD, PhD, and Professor Roger Pedersen, PhD, from the Universities of Harvard [Cambridge, MA] and Cambridge, England, respectively," says Dr Belo.

Dr Belo and his colleagues have primarily studied the role of 2 genes in heart induction and organogenesis, *Cerl2* and *Ccbe1*. They have isolated and knocked-out *Cerl2* as a member of the cerberus-like gene family, which is involved in the embryonic body axis establishment mainly by their nodal inhibition activity. Besides its role in left/right body asymmetry, they have found that lack of *Cerl2* also results in severe heart defects, which they are now characterising.

The work on *Ccbe1* results from another grant received earlier for the identification and transcriptome functional analysis of heart/haemangioblast common precursor cells using a differential screening in chick embryos. "We were able to identify a number of still uncharacterised genes potentially involved in heart development, *Ccbe1* being among them," says Dr Belo. "This finding prompted us to study the role of the mouse homologue during development and now extending to its role in the generation of cardiac tissue from embryonic stem cells."

Carla Alexandra São Bento Viegas, PhD, postdoctoral researcher, Functional Biochemistry and Proteomics group headed by Professor Dina Simes, PhD, Center of Marine Sciences, University of Algarve, Faro



Dr Viegas received her first postdoctoral fellowship grant in the cardiovascular research field in October 2010 included in an FCT project grant (2010–2013) of €130000 to study the

establishment of gamma-carboxyglutamic acid-rich protein (GRP) as a biomarker for vascular calcification. This was followed by an individual postdoctoral fellowship grant in October 2011 which covers the same research area and will be renewed annually until August 2015.

After revealing the peculiar features of this new vitamin K-dependent protein during her PhD, Dr Viegas' research in the Functional Biochemistry and Proteomics group has greatly benefited from a close collaboration established with Dr Cees Vermeer, PhD, of the University of Maastricht, Maastricht, the Netherlands. They have focused on the establishment of GRP as an inhibitor of soft tissue calcification and validation of its use as a biomarker in clinical diagnostics, which is a patented system with possible industrial application.

In 2011, Dr Viegas became the principal investigator of a 3-year project funded by the FCT with €159 755 that aims to establish GRP as a soft tissue calcification inhibitor by studying its role and molecular mechanism underlying its function in vascular mineralisation using an in vitro cell model of vascular calcification. Dr Viegas says, "This research is expected to contribute new knowledge on the mechanisms leading to pathological calcification and identify novel diagnostic and therapeutic strategies for the treatment of diseases related to ectopic mineralisation of vascular tissues."

Perpétua Pinto-do-Ó, PhD, assistant investigator, Instituto de Engenharia Biomédica and affiliate professor, Instituto de Ciências Biomédicas Abel Salazar, University of Porto, Porto

Dr Pinto-do-Ó (centre) and her team at a recent meeting.

Dr Pinto-do-Ó investigates stem cells and mechanisms of regeneration. She began her cardiovascular research in 2007 in the NEWTherapies Group at INEB-Instituto de Engenharia Biomédica. Three years of FCT funding of €193 343 supported the establishment of this research line, the first in the country on the biology of heart repair and regeneration.

Dr Pinto-do-Ó's lab has since developed a mouse model of surgically induced myocardial infarction and implemented a series of tools aimed at the systematic dissection of cardiogenesis. Several young researchers have been trained and contributed to articles and the team's main output has been the creation of MIQuant, an original software tool for the assessment of myocardial infarction size in murine experimental models, and the characterisation and validation of a cell line model system for a rare population of adult derived Sca-1pos cardiac progenitor cells generated by Dr Paolo Di Nardo, MD, of the University of Rome Tor Vergata. "The cell line model is expected to contribute to the elucidation of the role of these yet elusive myocardial-resident progenitors," says Dr Pinto-do-Ó. In addition, studies performed on adult murine heart have led her lab to the current paradigm that heart repair and regeneration mechanisms might only be drawn from a thorough analysis of the developing organ.

Dr Pinto-do-Ó says, "Two distinct research lines were recently designed to investigate how the composition of the microenvironment, namely that of the heart extracellular matrix is altered during ontogeny (3 years of FCT funding of €161 203 from April 2012), and whether the developing and differentiating cardiac cell populations might contain relevant translational cues."

Henrique Girão, PhD, researcher, Faculty of Medicine, University of Coimbra, Coimbra

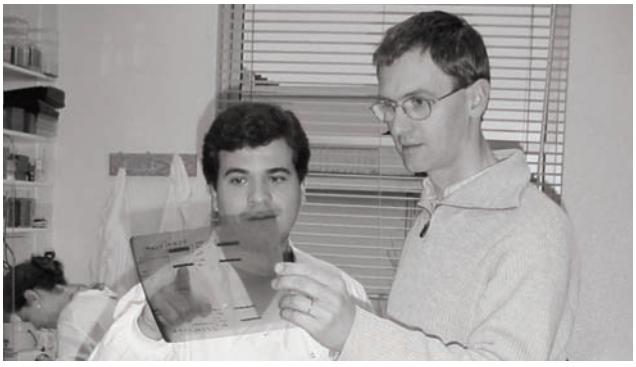
Dr Girão conducts research on the mechanisms and molecular players involved in the regulation of gap junction intercellular communication and how an impairment of these mechanisms can contribute to disease. Over the past 10 years, he and his colleagues have unveiled some of the molecular mechanisms associated with gap junction internalisation and degradation, in particular the role of ubiquitin in signalling endocytic and autophagic degradation of connexins.

In the heart, connexin 43 (the main protein forming gap junction channels in ventricular myocardium) is predominantly situated in the intercalated discs between cardiomyocytes, where it ensures efficient action potential propagation, resulting in coordinated contraction. Therefore, fine tuning and maintenance of gap junctions in cardiomyocytes is essential for normal heart function. Dysfunction of gap junction regulation has been associated with conduction block and arrhythmogenesis under various pathological conditions, such as ischaemia, infarction, and hypertrophy.

With the 3-year FCT grant of €141 793 for the project to unravel the molecular events of gap junction remodelling in the ischaemic heart, Dr Girão and his colleagues will investigate the mechanisms and machinery that regulate connexin 43 remodelling in the ischaemic heart, in particular the

molecular links between ubiquitin-mediated signal and recycling versus degradation. Dr Girão says, "We envision a new molecular mechanism whereby an orchestrated action of kinases/phosphatases and ubiquitin ligases/deubiquitinating enzymes determines the subcellular distribution of connexin 43 and its ultimate fate, thus accounting for some of the main features of heart diseases."

Peter Jordan, PhD, staff principal investigator, National Health Institute, Lisbon



Dr Jordan received a 1-year FCT pilot grant of €39 755 in March 2012 to investigate a novel signalling pathway possibly involved in renal salt handling and hypertension. The funding includes a research fellowship and expenses for consumables and small lab equipment.

The study of some rare familial diseases has shown that deregulated salt handling by the kidney is an important factor contributing to hypertension. For example, mutations in the *WNK1* and *WNK4* genes cause familial hyperkalaemic hypertension and the genes encode protein kinases that regulate various ion channels expressed in the nephron.

Dr Jordon's lab has identified a novel mechanism of ion channel regulation by the WNK4 protein. They showed that WNK4 interacts with spleen tyrosine kinase, which phosphorylates the chloride channel cystic fibrosis transmembrane conductance regulator, thereby regulating the amount of cystic fibrosis transmembrane conductance regulator expressed at the cell membrane. Bioinformatic analysis then predicted that spleen tyrosine kinase may also regulate the sodium-potassium-chloride cotransporter 2 and the potassium-chloride cotransporter 3, both involved in renal salt handling and targets for loop diuretic drugs.

With the FCT funding, Dr Jordan and his colleagues will first test in vitro whether spleen tyrosine kinase and WNK4 also regulate sodium-potassium-chloride cotransporter 2 or potassium-chloride cotransporter 3 phosphorylation, followed by studies on their cell surface abundance and ion transport activity in kidney cells. "The results will reveal whether the WNK4/spleen tyrosine kinase pathway regulates these 2 cotransporters and thus represents another mechanism through which WNK4 regulates ion homeostasis in the kidney," says Dr Jordan. "On the basis of these data, future studies can be designed to determine the contribution of this pathway to hypertension and its suitability as a drug target in the disease."

Helena Santa-Clara, PhD, auxillary professor, Exercise and Health Lab, Interdisciplinary Centre for the Study of Human Performance, Faculty of Human Kinetics, Technical University of Lisbon, Lisbon

Professor Santa-Clara received a 3-year FCT grant of €142 950 in 2012 to investigate exercise training after cardiac resynchronisation therapy in patients with chronic heart failure. She and her colleagues will carry out a stratified randomised longitudinal study to investigate the effects of 6 months of exercise training after cardiac resynchronisation therapy for NYHA stage III–IV heart failure. Professor Santa-Clara says, "The aims of the study are to determine whether a long-term exercise training programme following cardiac resynchronisation therapy provides better clinical outcomes than cardiac resynchronisation therapy alone, and to identify the mechanisms of the hypothesised improvement."

The study will use state-of-the-art methods for autonomic nervous system analysis, namely scintigraphy with ^{123}I-meta-iodobenzylguanidine, a radiolabelled analogue of norepinephrine. The team will evaluate clinical, physiological, and quality of life outcomes. The assessment of cardiac sympathetic neuronal activity with ^{123}I-meta-iodobenzylguanidine will improve understanding of the mechanisms responsible for increased sympathetic activity in heart failure and how sympathetic overactivity exerts its deleterious actions.

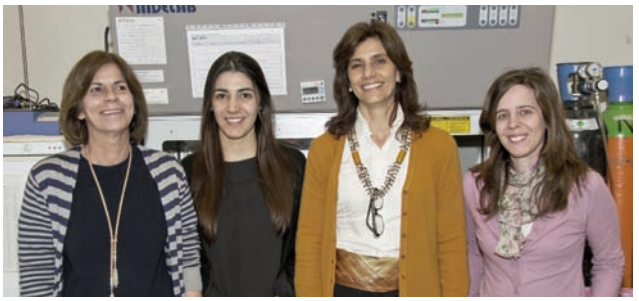
Ricardo Neves, DPhil, assistant investigator, Biomaterials and Stem Cell-based Therapeutics Lab, Centre for Neurosciences and Cell Biology, University of Coimbra, Coimbra

Dr Neves received a 3-year grant of €135 649 from the FCT in April 2011 to generate cord blood-derived induced pluripotent stem cells using a nonviral approach and their differentiation into cardiomyocytes. He has hired a technician and will use some of the funding on research consumables.

During the project, Dr Neves will develop new methods to induce reprogramming of cells to the pluripotent state and then differentiate these cells into cardiomyocytes. Since carrying out research at the University of Oxford, Oxford, England, Dr Neves has had a longstanding collaboration with the group of Professor Tariq Enver, PhD, at University College London, London, England, who is contributing expertise in stem cells. They hope to develop methodologies to deliver reprogramming agents in a tightly controlled way.

Dr Neves says, "I believe that my background articles in the fields of transcription and chromatin modulation and the high scientific quality of the project and the international partners (University of Oxford and University College London) helped in getting this competitive grant."

Maria Emília Monteiro, MD, PhD, professor of pharmacology, vice dean for education, and principal investigator, Chronic Diseases Research Centre, Faculty of Medical Sciences, New University of Lisbon, Lisbon

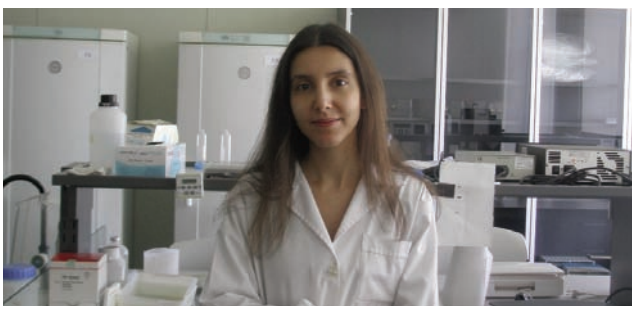


Professor Monteiro (2nd right) and her research team.

Professor Monteiro received a 3-year grant of €194 240 in 2011 to identify more effective antihypertensive drugs for the treatment of hypertension in patients with obstructive sleep apnoea and to investigate the underlying mechanisms of systemic effects associated with obstructive sleep apnoea, as well as its modulation by antihypertensive drugs. The researchers are also interested in investigating the effects of chronic intermittent hypoxia as a mechanism of brain preconditioning, as well as the effects on purinergic receptor expression, insulin sensitivity, and activation of lymphocytic subpopulations. For this they are using a rat experimental model of hypertension induced by a paradigm of chronic intermittent hypoxia that simulates obstructive sleep apnoea and also the data obtained from a large sample of patients with obstructive sleep apnoea. The financial support has been particularly helpful for establishing an animal model of hypertension caused by chronic intermittent hypoxia.

In addition to Professor Monteiro, who is principal investigator, the research team includes Lucilia Diogo (PhD student), Daniela Vasconcelos (graduate student), and Teresa Gamboa (postdoc). This translational project also involves collaboration with the groups of Professor Constancio Gonzalez, PhD, at the University of Valladolid, Valladolid, Spain, and Professors Sérgio Dias, PhD, Helena Vieira, PhD, and Cristina João, MD, in Lisbon. In the past, Professor Monteiro studied the involvement of carotid bodies in cardiorespiratory responses. She says, "This was relevant because one of the mechanisms in the genesis of hypertension in these patients included sympathetic nervous system stimulation mediated mainly by the activation of carotid body chemoreflexes."

Teresa Sousa, PharmD, PhD, assistant researcher, Department of Pharmacology and Therapeutics, Faculty of Medicine, University of Porto, Porto



Dr Sousa received a 3-year FCT grant of €102 472 in 2011 to investigate the interaction of aspirin with antihypertensive drugs and its impact on renal oxidative stress, inflammation, local renin-angiotensin system activation, and blood pressure control. She has used the funding to hire a young researcher, buy animals and lab supplies, take part in scientific meetings, and pay institutional overhead costs. The research team for this project also includes Professor António Albino Teixeira, MD, PhD, researcher Maria João Valente, MSc, and technician Joana Afonso, MSc, from the Faculty of Medicine and Professor Félix Carvalho, PharmD, PhD, and assistant professor Manuela Morato, PharmD, PhD, from the Faculty of Pharmacy.

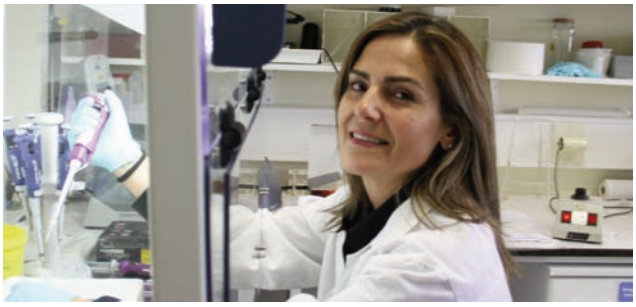
Dr Sousa has carried out in vitro and in vivo studies to evaluate the renal medullary and cortical mechanisms contributing to the dysregulation of arterial blood pressure and is studying the effects of low-dose aspirin and renin-angiotensin system blockers on renal function and blood pressure. Emphasis has been given to the study of endogenous and aspirin-triggered lipoxins, which are claimed to have potent anti-inflammatory and antioxidant effects and may contribute to the overall benefits of aspirin. Dr Sousa says, "We expect this study to unravel the putative mechanisms contributing to the protection exerted by aspirin and renin-angiotensin system blockers and provide a pharmacological rationale for using these drugs together in the treatment of severe cardiovascular and renal diseases."

Silvia Vilares Conde, PhD, professor of pharmacology, Chronic Diseases Research Centre Faculty of Medical Sciences, New University of Lisbon, Lisbon

Professor Conde received a 3-year FCT grant of €100 000 to investigate whether insulin is a stimulus for carotid body activation and a new mechanism for insulin resistance and hypertension in 2011. The grant funds a PhD student, participation in congresses, consumables, and equipment.

Professor Conde is collaborating with clinicians from the Hospital de Santa Marta and Centro Hospitalar Lisboa Central to enrol patients with metabolic syndrome and the groups of Professor Constancio Gonzalez, PhD, at the University of Valladolid, and Professor Paul Kemp, PhD, at the University of Cardiff, Cardiff, Wales. The aim is to unravel the role of peripheral chemoreceptors in the pathophysiology of arterial hypertension and insulin resistance, which are the core metabolic and haemodynamic disturbances in metabolic syndrome, type 2 diabetes mellitus, and obstructive sleep apnoea. Professor Conde says, "We hope to find that the overstimulation of carotid body chemoreceptors and the subsequent increase in sympathetic nervous system activity is the common link in the development of hypertension and insulin resistance, in both animal models of diet-induced hypertension and insulin resistance and patients with metabolic syndrome."

Rita Castro, PhD, assistant professor, Research Institute for Medicines and Pharmaceutical Sciences (iMed.UL), Faculty of Pharmacy, University of Lisbon, Lisbon



Dr Castro carries out research in the Metabolism and Genetics Group at the Research Institute for Medicines and Pharmaceutical Sciences in Lisbon. She has devoted her career to the study of homocysteine and its vascular toxicity, and lately she has focused on the impact of the homocysteine precursor, S-adenosylhomocysteine, on endothelial methylation processes. During a sabbatical from September 2009 to March 2010 funded by the FCT and the Calouste Gulbenkian Foundation, she worked with Professor Joseph Loscalzo, MD, PhD, editor-in-chief of *Circulation*, in Boston, MA. She says, "The contact with an environment of high scientific and intense research activity permitted the completion of a work plan related to the project 'Unravelling the Epigenetic Effects of In Vitro Intracellular S-Adenosylhomocysteine Accumulation on Nitric Oxide Bioavailability.' " Dr Castro was principal investigator of this FCT-funded project (€75 000), and her stay in the Loscalzo Lab resulted in 2 articles in coauthorship with Professor Joseph Loscalzo.

Working in Professor Loscalzo's lab opened up new lines of research related to scientific interests common to the labs in Lisbon and Boston. They are now working in partnership on a project for which the FCT has provided funding of €154 000 on a project titled, "S-Adenosyl Homocysteine, Non-Histone Protein Hypomethylation, and Vascular Disease: Another Brick in the Wall?" and are cosupervising an FCT-funded Portuguese PhD student.

Isabel Rocha, PharmD, PhD, associate professor and head, Cardiovascular Autonomic Lab, Institute of Physiology, Lisbon Faculty of Medicine, Lisbon

Dr Rocha has received grants as a principal investigator from different sources and particularly from the FCT. These grants since 2007 have allowed her to build and develop a research lab to study cardiovascular function with a focus on autonomic control. The multidisciplinary lab team includes medical and nonmedical scientists, technicians, and students.

"Working with animal models and in a clinical setting with patients, we focus on the autonomic nervous system in the induction, maintenance, and termination of dysrhythmias, including the role of autonomic inputs on the cellular and molecular remodelling of atrial cells and the cardiac biochemical protective systems," says Dr Rocha. "Together with the study of the physiopathology of neurogenic hypertension in animal models targeting the central sympathoexcitatory areas and their excitability, we have been developing signal processing methodologies in the time-frequency domain to study the autonomic signature on noninvasive parameters, seeking early markers of disease."

Cristina M. Sena, PhD, assistant professor, Institute of Physiology, Faculty of Medicine, University of Coimbra, Coimbra

Dr Sena received a 3-year FCT grant of €114 746 from 2011 to 2014 to investigate new therapeutic approaches for the treatment of vascular complications in type 2 diabetes mellitus and metabolic syndrome. The funding is being used for research staff, scientific meetings, overheads, reagents, antibodies, and other lab supplies. Dr Sena says, "This research project brings together a number of different scientists in

different areas of expertise, including cardiologists, medical doctors, physiologists, cell and molecular biologists, and biochemists, to better understand a major vascular dysfunction associated with obesity and diabetes mellitus."

A major contributor to diabetic complications is glycation with increased formation of advanced glycation end-products. Dr Sena and her colleagues recently showed that methylglyoxal, a glycolytic product and a strong advanced glycation end-product precursor, accumulates in tissues and leads to endothelial dysfunction. "We expect to identify new substances that may protect and repair blood vessels from the damaging effects of diabetes mellitus," says Dr Sena.

Roberto Roncon-Albuquerque, Jr, MD, PhD, invited auxiliary professor of physiology and intensive care medicine specialist, Faculty of Medicine, University of Porto, Porto

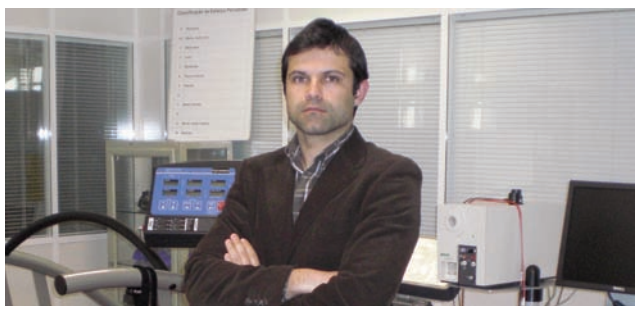
Dr Roncon-Albuquerque received a 30-month FCT grant of €64 200 starting in March 2011 to investigate the nutritional modulation of inflammation, oxidative stress, and apoptosis associated with cardiac cachexia using a rat model of cardiac cachexia complicating ascending aortic banding-induced left ventricular failure. So far Dr Roncon-Albuquerque and his colleagues have found that a Western diet attenuates pulmonary hypertension with heart failure and cardiac cachexia.

"We believe that our research project could contribute to novel paradigms in the nutritional approach to heart failure, particularly in the advanced stages of this disease, where therapeutic options are still lacking," says Dr Roncon-Albuquerque. "We expect to demonstrate, using experimental models of right and left ventricular failure, 'paradoxical' protective effects of atherogenic diets in cardiac cachexia."

Raul Agostinho Simões Martins, PhD, assistant professor, Faculty of Sport Sciences and Physical Education, University of Coimbra, Coimbra

Professor Martins received a 3-year FCT grant of €33 114 in 2010 to investigate cardiometabolic risk, physical exercise, and healthcare costs in older adults. Professor Martins and his colleagues are currently running an intervention programme for people at high risk of cardiovascular disease. The project has resulted in articles on high-sensitivity C-reactive protein, body fat, and physical exercise in older people; the effects of aerobic and strength-based training on metabolic health indicators in older adults; the effects of strength and aerobic-based training on functional fitness and mood, and the relationship between fatness and mood in older adults; new equations to determine exercise intensity using different exercise modes; and glycated hemoglobin and associated risk factors in older adults.

Fernando Ribeiro, PhD, professor of physiotherapy, Polytechnic Health Institute of the North, and investigator, Research Centre in Physical Activity, Health, and Leisure, Faculty of Sport, University of Porto, Porto



Professor Ribeiro started a 3-year FCT-funded project on the effects of exercise training on endothelial function, inflammation, and autonomic function in coronary artery disease patients in February 2011. The funding of €66 200 has contributed to research staff, lab material, and equipment.

Professor Ribeiro and his colleagues are conducting a randomised controlled trial among people with coronary artery disease to analyse the effects of an exercise training programme on endothelial function, biomarkers of inflammation, autonomic function, and arterial stiffness. They hope to discover the contribution of age and changes in traditional risk factors to the modification of endothelial dysfunction and inflammation, as well as the contribution of changes in inflammatory and endothelial function biomarkers to the modification of autonomic function and arterial stiffness.

"Over the past few years the object of our research has been therapeutic exercise and physical activity in the context of cardiovascular diseases," says Professor Ribeiro. "We became increasingly interested in the concept of exercise training as a polypill with several beneficial effects for patients with coronary heart disease and heart failure."

Jennifer Taylor is a freelance medical journalist.

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