FACULDADE DE MEDICINA DA UNIVERSIDADE DE COIMBRA

TRABALHO FINAL DO 6º ANO MÉDICO COM VISTA À ATRIBUIÇÃO DO GRAU DE MESTRE NO ÂMBITO DO CICLO DE ESTUDOS DE MESTRADO INTEGRADO EM MEDICINA

JOANA MARGARIDA ROSMANINHO SALGADO

SLEEP APNEA SYNDROME AND OBESITY: UNRAVELING THE ROLE OF INTERMITTENT HYPOXIA ON ADIPOSE TISSUE PHYSIOLOGY

[PROJETO DE INVESTIGAÇÃO]

ÁREA CIENTÍFICA DE ENDOCRINOLOGIA

TRABALHO REALIZADO SOB A ORIENTAÇÃO DE:
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Projeto de Investigação

Área de Endocrinologia

Sleep Apnea Syndrome and obesity: unraveling the role of intermittent hypoxia on adipose tissue physiology

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Apneia Obstrutiva do Sono e Obesidade: o papel da hipóxia intermitente na fisiologia do tecido adiposo

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Março 2013

Sleep Apnea Syndrome and obesity: unraveling the role of intermittent hypoxia on adipose tissue physiology[1]

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Agradecimentos

Mais do que um trabalho final de mestrado, esta dissertação, na forma de projeto científico, é o resultado de um longo caminho percorrido durante os últimos 6 anos. Por esta razão quero expressar os meus sinceros agradecimentos a todos os que contribuíram para esta caminhada:

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Abbreviations

BAT – brown adipose tissue

$CoCl_2$ – Cobalt chloride

DMEM-F12-HG – Dulbecco's Modified Eagles Medium High Glucose

ELISA – enzyme-linked immunosorbent assay

Fabp4 – fatty acid binding protein 4

FBS – Fetal bovine serum

FCS – fetal calf serum

GLUT4 – glucose transporter type 4

HR – reoxygenation after hypoxia

IBMX – 3-isobutyl-1-methylxanthine

H - hypoxia

IH – intermittent hypoxia

IL-6 – interleukin-6

MAPK – mitogen-activated protein kinase

mtDNA – mitochondrial DNA

NK-κB - factor nuclear kappa B

NN/CNC – group of Neuroendocrinology and Neurogenesis group of the Center of Neuroscience and Cell Biology (NN/CNC)

NPY – neuropeptide Y

OSAS – obstructive Sleep Apnea Syndrome

PBS – phosphate buffered saline

PGC-1α - peroxisome proliferator-activated receptor gamma co-activator-1α

PPAR-γ - peroxisome proliferator-activated receptor gamma

PRDM16 – PR domain containing 16

ROS – reactive oxygen species

SAPK – stress-activated protein kinases

UCP- uncoupled protein

VEGF – vascular endothelial growth factor

WAT – white adipose tissue

3T3-L1 – murine pre-adipocyte cell line

Index

1. Project Description

Scientific Domain: Ciências da Vida e da Saúde

Main Area: Biomedicina: Metabolismo e Nutrição

Project Title in Portuguese: Apneia Obstrutiva do Sono e Obesidade: o papel da hipóxia intermitente na fisiologia do tecido adiposo

Project Title in English: Sleep Apnea Syndrome and obesity: unraveling the role of intermittent hypoxia on adipose tissue physiology

Requested funding: 65 040 €

Keyword 1: Obesidade (Obesity)

Keyword 2: Hipóxia Intermitente (Intermittent Hypoxia)

Keyword 3: Tecido adiposo branco (White adipose tissue)

Keyword 4: Tecido adiposo castanho (Brown adipose tissue)

Keyword 5: adipogénese (Adipogenesis)

Starting date: 01-01-2014

Duration in months: 12 months.

2. Institutions and their roles

Principal Contractor: Faculdade de Medicina da Universidade de Coimbra

Azinhaga de Santa Comba, Celas

3000-548 Coimbra

Research Unit: Serviço de Endocrinologia dos HUC- CHUC

Praceta Prof. Mota Pinto

3000-075 Coimbra

Host Institution: Centro de Neurociências e Biologia Celular

Faculdade de Medicina, Universidade de Coimbra,

Rua Larga

3004 - 504 Coimbra

3. Scientific Component

3.1. Abstract

3.1.a In Portuguese

A obesidade é definida como um estado patológico que é caracterizado por um aumento excessivo de tecido adiposo. Uma situação de obesidade pode ocorrer quando a quantidade energia que é ingerida é superior à que é despendida. Cerca de 60 a 90% das pessoas obesas tem a síndrome da apneia obstrutiva do sono (OSAS), que é caracterizada por ciclos de apneia e/ou dispneia durante o sono, e deste modo caracterizada por períodos de hipóxia intermitente (IH). Em murganhos, foi descrito que a HI conduz a uma diminuição do peso corporal dos animais, no entanto sabe-se que Homens adultos com OSAS normalmente desenvolvem excesso de peso. Apesar de existirem várias evidências que sugerem que a HI tem um papel importante na obesidade, o papel da IH na fisiologia do tecido adiposo branco (WAT) e no tecido adiposo castanho (BAT) ainda não está esclarecido. Os resultados do grupo de Neurogénese e Neuroendocrinologia do Centro de Neurociências e Biologia Celular, indicam que o cloreto de cobalto ($CoCl_2$), um agente que mimetiza uma situação de hipóxia, inibe a adipogénese e a expressão de PPAR-γ2 numa linha celular de pré-adipócitos de murganho, 3T3-L1. No entanto, sabe-se que existem diferenças fisiológicas na resposta a uma situação de hipóxia contínua, como a descrita anteriormente, comparativamente à situação de IH O papel da IH na adipogénese do WAT e do BAT ainda não é conhecido. Por outro lado, sabe-se que quando ocorre a reoxigenação depois de um período de hipóxia, esta situação funciona como um insulto para diferentes tipos de células, conduzindo ao aumento da produção de espécies reativas de oxigénio (ROS). No entanto, o papel da reoxigenação nos adipócitos do tecido adiposo ainda não é conhecido.

Com base nestes conceitos teóricos, o projeto atual pretende esclarecer o papel da HI na fisiologia do WAT e do BAT. Por outro lado, pretende-se também perceber se é a condição de

hipóxia ou a situação de reoxigenação que funciona como principal responsável pelas alterações metabólicas no WAT e no BAT. Para alcançar estes objetivos, o projeto apresenta dividido em 3 tarefas: Tarefa 1) Será que a IH é um modulador da função do WAT? Tarefa 2) Será que a IH influencia a diferenciação e a fisiologia do BAT? Tarefa 3) Qual é o papel da reoxigenação no tecido adiposo?

No final espera-se que o projeto contribua definitivamente para um melhor conhecimento do papel da IH na fisiologia do adipócito e perceber de que modo esta contribui para a formação e desregulação da formação do tecido adiposo.

3.1.b In English

Obesity is defined as a state of pathologically excessive adipose tissue mass. Obesity comes about when energy intake exceeds energy expenditure. It was described that 60-90% of obese people have Obstructive Sleep Apnea Syndrome (OSAS), which is characterized by cycles of apnea and/or hypopnea during sleep leading to intermittent hypoxia (IH). In mice, IH induces a decrease on body weight but OSAS patients usually display weight gain. Although there are strong evidences suggesting that IH may be a key factor in obesity, the role of IH on white adipose tissue (WAT) and brown adipose tissue (BAT) physiology is not yet clarified. Results from the group of Neuroendocrinology and Neurogenesis of the Center of Neuroscience and Cell Biology (NN/CNC), using a hypoxia mimetic agent, $CoCl_2$, suggest that hypoxia inhibits adipogenesis and PPAR-γ2 expression in 3T3-L1 cells. However, there are remarkable differences in the response of the physiologic systems to sustained hypoxia and IH, and the effect of IH on adipogenesis of WAT and BAT is not yet clarified. Additionally, the reoxygenation after hypoxia (HR) is a cell injury that has been described to induce reactive oxygen species (ROS) in different non-adipocyte cell models, but its effect on adipocytes is not known. With this project we aim to clarify the role of IH on WAT physiology and also on

BAT physiology. Moreover, we aim at understanding whether hypoxia or reoxygenation (HR) are responsible for the metabolic alterations observed in WAT and BAT. To achieve these aims our project will have three different tasks: **Task 1) Is IH a modulator of WAT function?; Task 2) Does IH change BAT differentiation and BAT physiology?** and **Task 3) What is the role of HR on adipose tissue?**

With this project we expect to contribute to a better knowledge of the role of IH on adipocyte physiology and how it contributes to adipose tissue formation and dysregulation.

3.2. Technical Description

3.2.1. Literature Review

Obesity is an epidemic phenomenon in both developed and developing societies. The knowledge of the adipose tissue physiology is a step forward to new putative targets of obesity therapeutics approaches. Obesity is characterized by an increase in adipose tissue mass and this adipocyte hypertrophy compromises effective O_2 supply from the vasculature, occurring hypoxia in areas within the adipose tissue. It was described that 60-90% of obese people have Obstructive Sleep Apnea Syndrome (OSAS), which is characterized by cycles of apnea and/or hypopnea during sleep leading to intermittent hypoxia (IH) [1]. In fact, there are several evidences suggesting that OSAS and obesity are associated with all components of the metabolic syndrome [2]. Thus, it appears that obesity and OSAS form a vicious cycle, worsening each other. Although OSAS are traditionally described as a consequence of obesity, the mechanism predisposing OSAS patients to weight gain is unknown, but we believe that it could be related to changes induced by IH on white adipose tissue (WAT).

There are two types of adipose tissue: white adipose tissue (WAT) that stores chemical energy as triglycerides and brown adipose tissue (BAT) that oxidizes fat in a process called thermogenesis [3]. Although these types of adipose tissue are at different locations, brown

adipocytes can also be found within the white adipose tissue of rodents [4]. Recently, using PET scanning, it was demonstrated that adult humans have several discrete areas of metabolically active brown adipose tissue (BAT) and that BAT plays a much more important role in human metabolism than previously described [5]. It was also demonstrated that experimental BAT increase in rodents is associated with lean phenotype and by contrast loss of BAT functions is linked to obesity and metabolic diseases [4, 6].

White adipocyte differentiation is controlled by a variety of hormones, growth factors and transcription factors [7]. Peroxisome proliferator-activated receptor gamma (PPAR-γ) is a well-known transcriptional regulator of white adipogenesis, and it is also essential for brown adipogenesis, and its expression has been shown to increase during the maturation of brown adipocytes [8]. Nevertheless, other transcriptional regulators such as PPAR-γ coactivator-1α (PGC-1α), PR domain containing 16 (PRDM16), play important roles in brown adipocyte-specific differentiation via modulation of thermogenic gene expression, especially uncoupling protein (UCP) [9]. However, the extracellular factors that regulate brown adipogenesis are not fully understood.

There are strong evidences suggesting that hypoxia may be a key factor in obesity [10, 11]. Adipose tissue is a highly vascularized tissue and angiogenesis is required for adipose tissue expansion. Adipocytes become hypertrophic during the development of obesity and their size increases up to 140–180 μm in diameter, while the oxygen diffusion limit is only of 100 μm [12]. Therefore, oxygen is not able to reach the cells causing local hypoxia in expanding adipocytes [13]. The group of Neuroendocrinology and Neurogenesis from the Center of Neuroscience and Cell Biology (NN/CNC) showed that hypoxia increases lipid accumulation associated with mitochondrial dysfunction (Figure 1). Additionally, it has been proposed that hypoxia may underline the endocrine response of WAT by affecting the secretion of peptides and cytokines like leptin, adiponectin, interleukin 6 (IL-6) and vascular endothelial growth

factor (VEGF) (see review [13, 14]) but no studies were performed with IH in WAT adipocyte cells. In fact, the regulation of adipose tissue (WAT and BAT) physiology is still not clear. Some authors described that IH induces a decrease on rat body weight [15] due to a decrease on brown adipose tissue (BAT) weight [16, 17] but it is well known that most of OSAS patients usually show weight gain [1, 2]. Nevertheless, it was also reported, in mice, that IH induces an increase in serum free fatty acid levels [18], serum levels of cholesterol [19] and triglyceride accumulation in the liver [20]. Finally, there is also one study showing that IH may decrease cytoplasmatic adiponectin in BAT [21].

The IH generates reactive oxygen species (ROS) during reoxygenation similar to ischemia-reperfusion by the activation of some oxidase pathways [22, 23]. After reoxygenation, ROS interact with mitogen-activated protein kinase (MAPK) and mediate proliferation or it can interact with stress-activated protein kinases (SAPK) or factor nuclear kappa B (NF-κB) and mediate apoptosis (see review, [24]). Although an adequate oxygenation is important for cellular viability, it is now clear that reoxygenation can induce an injury in different cell models, but it is not known how reoxygenation can modify the adipose tissue homeostasis. Taking into account that ROS, at physiological low levels can induce adipogenesis but at higher levels ROS inhibit differentiation (see review [25]), it can be speculated that reoxygenation can also modify the adipose tissue physiology.

In conclusion, the role of IH on adipose tissue physiology, namely on WAT and BAT, is not yet clarified, and its knowledge will contribute to a better understanding of how obstructive sleep apnea syndrome can be associated with obesity.

3.2.2. Plan and Methods

There are several evidences suggesting that Obstructive Sleep Apnea Syndrome (OSAS) promotes obesity and it has been associated with all components of the metabolic syndrome [2]. The mechanisms predisposing OSAS patients to weight gain are not known. Obesity

induces an enlargement of adipocyte size, leading to localized hypoxia and induces obstructive sleep apnea (OSAS). OSAS is a syndrome characterized by cycles of apnea and/or hypopnea during sleep leading to intermittent hypoxia (IH) and the effect of the intermittent hypoxia induced by OSA on adipose tissue function was not yet studied. Additionally, the reoxygenation after hypoxia (HR) is a cell injury that have been described to induce ROS production [22, 23] and see review [24]) but its effect on adipocytes is not known.

With this project we will have answers to the following questions: 1) *Does IH modify the WAT physiology and thereby contribute to an increase of fat tissue? 2) Can IH condition lead to changes in BAT differentiation and physiology? 3) Is hypoxia or reoxygenation (HR) responsible for metabolic alterations of adipose tissue? 4) Do adipocytes die after HR? and 5) What is the impact of adipocyte death on adipose tissue?* To answer these questions we propose 3 tasks: ***1) Is IH a modulator of WAT function?*** The role of hypoxia on lipid accumulation and adipogenesis was already described in NN/CNC group (Figure 2). However, little is known about the effect of IH on adipose tissue, namely on adipogenesis, lipolysis and secretion of peptides and hormones. So, the aim of this project is to study the effect oh IH on WAT physiology. To study WAT physiology and to better mimic the microenvironment of WAT, rat WAT explants cultures will be performed. The murine pre-adipocyte cell line, 3T3-L1 will also be used. ***2) Does IH change BAT differentiation and BAT physiology?*** It was already demonstrated that IH decreases the BAT weight [16, 17], nevertheless OSAS patients usually show weight gain [1, 2]. The aim of this task is to investigate the effect of IH on BAT differentiation and BAT physiology. It will be used BAT explants and BAT primary cell cultures to evaluate lipid accumulation, adipocyte differentiation and also the mitochondria content and viability. ***3) What is the role of HR on adipose tissue?*** The effect of reoxygenation on non-adipocyte models was already described as the main producer of ROS [22] and ROS was described to have a dual effect on

adipogenesis (see review [25]). However, nothing is known about the effect of reoxygenation on adipose tissue viability and physiology. In this task the aim is to investigate the role of hypoxia-reoxygenation (HR) on WAT adipocyte physiology, and on fatty acid release as the main inducers of lipotoxicity.

This project will be developed during a period of 12 months and the project timeline is schematically represented at the section 2.3.4. This project will be performed with the collaboration of the members of the group of Neurogenesis and Neuroendocrinology of the Center for Neuroscience and Cell Biology (CNC) of University of Coimbra.

With this project we expect to contribute to a better knowledge about the role of IH on adipocyte physiology and how it contributes to adipose tissue formation and dysregulation.

3.2.3. Tasks

Task 1

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Task denomination: Is intermittent hypoxia a modulator of WAT function?
Start time: 01-01-2014
End time: 31-08-2014
Duration (in months): 8
Person*month: 16
Task description and Expected results

Hypoxia induces severe effects on adipocyte functions: induces lipid accumulation, and alters the secretion of peptides and cytokines. However, it is not know how IH changes the WAT physiology related to lipid accumulation, lipolysis, and adipogenesis and also as an endocrine organ.

I. Aim: The aim of this task is to study the role of IH on WAT physiology

II. Methods

- **Models**

Two models will be used:

A. to study WAT physiology and to better mimic the microenvironment of WAT, rat WAT explants cultures will be used, as described by Fried and Moustaid-Mousse. Briefly, white adipose tissue is minced into 5 mg fragments, rinsed with sterile phosphate buffered saline (PBS) warmed to 37°C. Samples are then transferred to 48-well plates (20 mg/well) containing 1 ml of culture medium M199, supplemented with 100 U/ml penicillin, 100 U/ml streptomycin, and 0.25 μg/ml amphotericin B, and 1% fetal calf serum (FCS), containing insulin.

B. the murine pre-adipocyte cell line (3T3-L1) will be plated in 22.1 cm^2 flasks and maintained in a humidified atmosphere of 5% CO_2−95% air. Cells will be grown in Dulbeccos Modified Eagles Medium high glucose (DMEM-F12 - HG) with phenol red and supplemented with 2.5 mM l-glutamine, 4.5 g/L glucose, 1.5 g/L $NaHCO_3$, 10% heat-inactivated fetal bovine serum (FBS), 100 U/mL penicillin, 100 U/mL streptomycin, and 0.25 μg/mL amphotericin B.

Differentiation protocol. Pre-adipocytes are plated in 24-well plates (25000 cells/well) until they reach confluence (day 0). After 2 days, the medium is removed and replaced with DMEM supplemented with a differentiation cocktail: 3-isobutyl-1-methylxanthine (IBMX, 0.5 mM) and dexamethasone (0.25 μM) (day 2). After 3 days (day 5), the differentiation cocktail is removed and the culture medium changed to DMEM. Every 2 days the medium will be renewed until the day 9. Cell treatment with insulin is considered as the positive control of differentiation. The control conditions are cells that at day 2 are incubated with DMEM with IBMX (0.5 mM) and dexamethasone (0.25 μM) and after that, every two days, the cells are incubated with DMEM without any drug. The positive control are cells that at day 2 are incubated with DMEM with IBMX (0.5 mM), dexamethasone (0.25 μM) and insulin (1 μg/mL) and after that every two days cells are incubated only with insulin (1 μg/mL).

- **Experimental protocol**

The effect of intermittent hypoxia (IH) that occurs recurrently in obstructive sleep apnea patients will be evaluated on white adipose tissue (WAT). The recent results from NN/CNC group indicate that hypoxia induces lipid accumulation through a PPAR-γ-independent pathway and also induces changes on mitochondria function (Figure 1 and 2). However, it is not known the role of IH on WAT function. In this task, we will evaluate the effect of IH on lipid accumulation, adipogenesis, lipolysis and endocrine function of WAT. To achieve this aim we will use two models: pre-adipocyte murine cell line (3T3-L1) and WAT rat explants. IH will be achieved by using a hypoxia chamber [alternating cycles of hypoxia (1% O_2, 15 s) and normoxia (21% O_2, 3 min) at 37ºC]. Adipogenesis will be evaluated by quantifying the lipid accumulation (Oil red-O staining) and expression of PPAR-γ, a key transcription factor in adipogenesis, by immunocytochemistry and Western blotting. Lipolysis will be measured by the amount of glycerol released through an enzymatic fluorometric method. Moreover, we will evaluate the effect of IH on the expression of a lipolysis marker - the perilipin and its phosphorylated state. Furthermore, the endocrine function of WAT will be evaluated by the measurement of adipokines expression and secretion (leptin, adiponectin, resistin and neuropeptide Y (NPY)) by enzyme-linked immunosorbent assay (ELISA) as described by others [26]. Furthermore, the putative effect of IH on adipocyte cell death will be also evaluated by resazurin assay and TUNEL assay.

III. Expected Results

In the end of this task we will provide new knowledge about the effect of IH on the physiology of WAT, namely lipid accumulation, lipolysis and adipogenesis, and endocrine function. In summary, we will contribute to the understanding of the relationship between OSAS and WAT changes.

IV. Team members

Joana Salgado (JS; 100 %) – JS will perform all the experiments.

Cláudia Cavadas (CC; 50 %) – CC will give scientific support in this task.

Manuela Carvalheiro (MC, 50 %) – MC will give scientific support in this task.

Task 2

……

Task denomination: Does IH change BAT differentiation and BAT physiology
Start time: 01-06-2014
End time: 31-12-2014
Duration (in months): 7
Person*month: 14
Task description and Expected results

Obesity is characterized by local hypoxia within WAT [13] and one study suggests that hypoxia also occurs in BAT during obesity [27]. However, it is not clear whether hypoxia occurs in BAT neither the effect of IH on BAT. It was already demonstrated that IH decreases BAT weight [16, 17], nevertheless OSAS patients usually show weight gain [1, 2].

I. Aim: To investigate the effect of IH on BAT differentiation and BAT physiology

II. Methods

- **Models**

To investigate the effect of IH on BAT we will use two cell models: i) interscapular BAT explants cultures. Briefly, brown adipose tissue is minced into around 5 mg fragments, rinsed with sterile phosphate buffered saline (PBS) warmed to 37°C. Samples are then transferred to forty-eight-well plates (20 mg/well) containing 1 ml of culture medium M199, supplemented with 100 U/ml penicillin, 100 U/ml streptomycin, and 0.25 μg/ml amphotericin B, and 1% fetal calf serum (FCS), containing insulin. The duration of the cultures will be first evaluated by a time course in order to obtain the optimal culture time, which will be used in further experiments; ii) rat primary cell culture of BAT will be performed [28].

- **Experimental protocol**

BAT explants and primary cultures will be submitted to IH (as described on task 1). And the following parameters will be evaluated: A) BAT differentiation is determined by oil red-O assay (quantification of lipid accumulation) and PPAR-γ expression by Western blotting, immunohistochemistry, and mRNA by qPCR; B) the expression of BAT markers (PRDM16, PGC-1α and UCP-1) will be evaluated by qPCR and by Western blotting; C) the adipocyte selective genes common to WAT and BAT (adiponectin; fatty acid binding protein, Fabp4; glucose transporter type 4, Glut4) will be investigated by Western blotting, immunohistochemistry and mRNA will be analyzed by qPCR; D) the mitochondrial content and viability will be determined using a fluorescent mitochondrion-selective dye (mitotracker) and the mitochondrial DNA (mtDNA), as previously described [29].

III. Expected Results

In the end of this task we expect to elucidate how IH modifies the expression of brown fat selective genes and consequently BAT differentiation and function. A better knowledge of the mechanisms associated with BAT may contribute to the treatment of obesity.

IV. Team members

Joana Salgado (JS; 100 %) – JS will perform all the experiments.

Cláudia Cavadas (CC; 50 %) – CC will give scientific support in this task

Manuela Carvalheiro (MC, 50 %) – MC will give scientific support in this task

Task 3

……...

Task denomination: What is the role of reoxygenation after hypoxia (HR) on adipose tissue?
Start time: 01-07-2014
End time: 31-12-2014
Duration (in months): 6
Person*month: 12
Task description and Expected results

IH induces an increase in serum free fatty acid levels [18], and lipids are well described to be cytotoxic (see review, [25, 30]). Thereby, if fat tissue becomes dysregulated, lipotoxicity in other tissues can ensue (see review [25, 30]). It is well described in non-adipocytes cells that reoxygenation after hypoxia induces the increase of ROS in non-adipocyte cells (see review, [22, 23]). Additionally, ROS have a dual effect because at physiological low levels it can induce adipogenesis with the subsequent increase on adipocyte number and size but at higher levels ROS inhibit differentiation (see review [25]). However, in adipocytes it is not know the effect of reoxygenation on adipose tissue physiology.

I. Aim: to investigate the role of hypoxia-reoxygenation (HR) on WAT adipocyte physiology.

II. Methods

- **Models**

Two biological models will be used: rat WAT explants and the murine pre-adipocyte cell line, 3T3-L1, as described on task 1.

After 24 hours of hypoxia, the medium will be removed and replaced with a fresh, warmed and oxygenated medium. The cells will return to normoxic conditions for 2 hours.

- **Experimental protocol**

The effect of reoxygenation of adipose tissue will be determined after 24 hours of hypoxia with the subsequent oxygenation. The experimental conditions will be: 24 hours of hypoxia + 2 hours reoxygenation (HR, group A), 26 hours simple hypoxia (H, group B).

The effect of HR on PPAR-γ expression will be determined by Western blotting; the lipid accumulation will be determined by the Oil red-O assay. Moreover, the effect of HR on lipolysis will be determined by the amount of glycerol released through an enzymatic fluorometric method. Moreover, we will evaluate the effect of IH on the expression of a lipolysis marker - perilipin and its phosphorylated state.

To assess if HR affects cell viability and survival of adipocytes it will be performed the resazurin assay and TUNEL assay.

The mitochondria membrane potential will be determined by mitochondrial membrane potential assay kit with JC-1 and the mitochondria network viability through mitotracker dye. The ROS levels will be also quantified. The amount of fatty acids that are released will be also determined through an enzymatic fluorometric method as a marker of lipotoxicity.

III. Expected Results

In the end of this task we will have a better knowledge about the process that is the main inductor of metabolic changes on WAT physiology.

IV. Team members

Joana Salgado (JS; 100 %) – JS will perform all the experiments.

Cláudia Cavadas (CC; 50 %) – CC will give scientific support in this task

Manuela Carvalheiro (MC, 50 %) – MC will give scientific support in this task.

3.2.4. Project Timeline and Management

3.2.4.a Description of the Management Structure

All the team members will meet, once a month, to discuss and to define strategies. The results will be also presented in larger meetings involving other members of the Faculty of Medicine and the group of NN/CNC.

There will be presentations in national and international meetings that will give the opportunity to receive feedback from the international scientific community.

3.2.4.b Milestones List

Milestone M1
Date: 01-03-2014

Milestone denomination: Evaluation of WAT explants as cell models for the study of WAT physiology?

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Milestone M2
Date: 01-05-2014
Milestone denomination: Does IH modulate adipogenesis in WAT?

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Milestone M3
Date: 01-09-2014
Milestone denomination: Evaluation of BAT explants as models for the study of BAT physiology.

…….....

Milestone M4
Date: 01-10-2014
Milestone denomination: Does IH modulate adipogenesis in BAT?

…….....

3.2.4.c Timeline – Attachment 1

3.3 Bibliographic References

Reference	Year	Publication
1	2002	Malhotra, A. and D.P. White, *Obstructive sleep apnoea.* Lancet, 2002. 360(9328): p. 237-45.
2	2000	Phillips, B.G., et al., *Increases in leptin levels, sympathetic drive, and weight gain in obstructive sleep apnea.* Am J Physiol Heart Circ Physiol, 2000. 279(1): p. H234-7
3	2004	Cannon, B. and J. Nedergaard, *Brown adipose tissue: function and physiological significance.* Physiol Rev, 2004. 84(1): p. 277-359.
4	1998	Guerra, C., et al., *Emergence of brown adipocytes in white fat in mice is under genetic control. Effects on body weight and adiposity.* J Clin Invest, 1998. 102(2): p. 412-20.
5	2007	Nedergaard, J., T. Bengtsson, and B. Cannon, *Unexpected evidence for active brown adipose tissue in adult humans.* Am J Physiol Endocrinol Metab, 2007. 293(2): p. E444-52.
6	1997	Ghorbani, M., T.H. Claus, and J. Himms-Hagen, *Hypertrophy of brown adipocytes in brown and white adipose tissues and reversal of diet-induced obesity in rats treated with a beta3-adrenoceptor agonist.* Biochem Pharmacol, 1997. 54(1): p. 121-31.
7	2000	Rosen, E.D. and B.M. Spiegelman, *Molecular regulation of adipogenesis.* Annu Rev Cell Dev Biol, 2000. 16: p. 145-71.
8	2010	Koppen, A. and E. Kalkhoven, *Brown vs white adipocytes: the PPARgamma coregulator story.* FEBS Lett, 2010. 584(15): p. 3250-9.
9	2008	Seale, P., et al., *PRDM16 controls a brown fat/skeletal muscle switch.* Nature, 2008. 454(7207): p. 961-7.
10	2008	Rausch, M.E., et al., *Obesity in C57BL/6J mice is characterized by adipose tissue*

		hypoxia and cytotoxic T-cell infiltration. Int J Obes (Lond), 2008. 32(3): p. 451-63.
11	2009	Ye, J., *Emerging role of adipose tissue hypoxia in obesity and insulin resistance.* Int J Obes (Lond), 2009. 33(1): p. 54-66.
12	2004	Trayhurn, P. and I.S. Wood, *Adipokines: inflammation and the pleiotropic role of white adipose tissue.* Br J Nutr, 2004. 92(3): p. 347-55.
13	2007	Hosogai, N., et al., *Adipose tissue hypoxia in obesity and its impact on adipocytokine dysregulation.* Diabetes, 2007. 56(4): p. 901-11.
14	2007	Ye, J., et al., *Hypoxia is a potential risk factor for chronic inflammation and adiponectin reduction in adipose tissue of ob/ob and dietary obese mice.* Am J Physiol Endocrinol Metab, 2007. 293(4): p. E1118-28.
15	1995	Leal, T.L., et al., *Body weight loss during acute hypoxia: effects of increased convective oxygen transport or previous acclimation.* Acta Physiol Pharmacol Ther Latinoam, 1995. 45(1): p. 9-14.
16	2010	Martinez, D., et al., *Brown adipose tissue: is it affected by intermittent hypoxia?* Lipids Health Dis. 9: p. 121.
17	2008	Martinez, D., et al., *Weight loss and brown adipose tissue reduction in rat model of sleep apnea.* Lipids Health Dis, 2008. 7: p. 26.
18	2010	Jun, J., et al., *Effect of intermittent hypoxia on atherosclerosis in apolipoprotein E-deficient mice.* Atherosclerosis. 209(2): p. 381-6.
19	2005	Li, J., et al., *Intermittent hypoxia induces hyperlipidemia in lean mice.* Circ Res, 2005. 97(7): p. 698-706.
20	2012	Mirrakhimov, A.E. and V.Y. Polotsky, *Obstructive sleep apnea and non-alcoholic Fatty liver disease: is the liver another target?* Front Neurol, 2012. 3: p. 149.
21	2011	Wree, A., et al., *Adipokine Expression in Brown and White Adipocytes in Response to Hypoxia.* J Endocrinol Invest, 2011.
22	2002	Li, C. and R.M. Jackson, *Reactive species mechanisms of cellular hypoxia-reoxygenation injury.* Am J Physiol Cell Physiol, 2002. 282(2): p. C227-41.
23	2005	Zhan, G., et al., *NADPH oxidase mediates hypersomnolence and brain oxidative injury in a murine model of sleep apnea.* Am J Respir Crit Care Med, 2005. 172(7): p. 921-9.
24	2008	Jun, J., et al., *Intermittent hypoxia has organ-specific effects on oxidative stress.* Am J Physiol Regul Integr Comp Physiol, 2008. 295(4): p. R1274-81.
25	2011	Vigouroux, C., et al., *Molecular mechanisms of human lipodystrophies: from adipocyte lipid droplet to oxidative stress and lipotoxicity.* Int J Biochem Cell Biol, 2011. 43(6): p. 862-76.
26	2007	Qin, L., et al., *[Effect of intermittent hypoxia on leptin and leptin receptor expression in obesity mice].* Sheng Li Xue Bao, 2007. 59(3): p. 351-6.
27	1999	Tonello, C., et al., *Role of sympathetic activity in controlling the expression of vascular endothelial growth factor in brown fat cells of lean and genetically obese rats.* FEBS Lett, 1999. 442(2-3): p. 167-72.
28	2009	Lehr, L., et al., *Differentiation and characterization in primary culture of white adipose tissue brown adipocyte-like cells.* Int J Obes (Lond), 2009. 33(6): p. 680-6.
29	2003	Miller, F.J., et al., *Precise determination of mitochondrial DNA copy number in human skeletal and cardiac muscle by a PCR-based assay: lack of change of copy number with age.* Nucleic Acids Res, 2003. 31(11): p. e61.
30	2010	Cusi, K., *The role of adipose tissue and lipotoxicity in the pathogenesis of type 2 diabetes.* Curr Diab Rep, 2010. 10(4): p. 306-15.

3.4. Past Publications

Reference	Year	Publication
1	2012	Rosmaninho-Salgado J, Cortez V, Estrada M, Santana MM, Gonçalves A, Marques AP, Cavadas C.(2012). Intracellular mechanisms coupled to NPY Y2 and Y5 receptor activation and lipid accumulation in murine adipocytes. Neuropeptides. 2012 Dec; 46(6):359-66.

2	2012	Rosmaninho-Salgado J, Marques AP, Estrada M, Santana M, Cortez V, Grouzmann E, Cavadas C. (2012). Dipeptidyl-peptidase-IV by cleaving neuropeptide Y induces lipid accumulation and PPAR-γ expression. Peptides. 2012 Sep;37(1):49-54.
3	2010	Rosmaninho-Salgado J, Marques AP, Cortez V, Santana M, Santos J. Cavadas C. (2010). The dual effect of Hypoxia on adipogenesis. Obesity – Obesity 2010. Abstract Supplement. Volume 18 Supplement 2, November 2010. Pag 128 (415-P).
4	2011	Sousa-Ferreira L, Garrido M, Nascimento-Ferreira I, Nobrega C, Santos-Carvalho A, Alvaro AR,Rosmaninho-Salgado J, Kaster M, Kügler S, de Almeida LP, Cavadas C. Moderate long-term modulation of neuropeptide Y in hypothalamic arcuate nucleus induces energy balance alterations in adult rats. PLoS One. 2011;6(7):e22333. doi: 10.1371/journal.pone.0022333. Epub 2011 Jul 22.

3.5 Project Resubmission

Ressubmission? This project is not a ressubmission.

4. Research Team

::

4.1 Members List

Name	Role	Degree	%
Joana Salgado	Principal Investigator	Master degree	100
Cláudia Cavadas	Investigator	PhD	50
Manuela Carvalheiro	Investigator	PhD	50

4.2 Members list to hire during project's execution

No additional members will be hired

5. Other Projects

::

5.1 Funded projects

Without any funded projects

5.2 Similar applications

There is no similar application

6. Expected Indicators

Description	2014	Total
A. Publications		
Books	0	0
Papers in international journals	2	2
Papers in national journals	1	1
B. Communications		
Communications in international meetings	1	1
Communications in national meetings	2	2
C. Reports	1	1
D. Organization of seminars and conferences	0	0
E. Advanced training	0	0
PhD thesis	0	0
Master thesis	0	0
Others	0	0
F. Models	0	0
G. Software	0	0
H. Pilot plants	0	0
I. Prototypes	0	0
J. Patents	0	0
L. Others	0	0

- **Scientific activity spreading actions**

The principal investigator (PI) of the project will participate in public events by presenting the scientific importance, some techniques and results of the present project.

7. Budget

- **Principal Contractor**

Description	2014	Total
Human resources	0,00	0,00
Missions	1,700	1,700
Consultants	0,00	0,00
Service procurement and acquisitions	50,000	50,000
Patent registration	0,00	0,00
Adaptation of buildings and facilities	0,00	0,00
Overheads	10,340	10340
TOTAL CURRENT EXPENSES	62,040	62,040
Equipment	3,000	3,000
Total	65,040	65,040

- **Finance Plan**

Description	2014	Total
Request funding	65,040	65,040
Own funding	0,00	0,00
Other public-sector funding	0,00	0,00
Other private funding	0,00	0,00
Total of the Project	65,040	65,040

8. Budget rationale

8.1 Human resources rationale

No expenses with human resources

8.2 Missions rationale

Type: International meeting
Venue: USA
No. of participations: 1
Rationale for request funding: Obesity week (2014) - Boston, Massachusetts November 2 – 7 (http://www.obesityweek.com)

Type: National meeting
Venue: Portugal
No. of participations: 1

Rationale for request funding: Sociedade Portuguesa de Farmacologia; Sociedade Portuguesa de Endocrinologia, Diabetes e Metabolismo (SPEDM).

8.3 Consultants rationale

No expenses with consultants

8.4 Service procurement and acquisitions

Type: Acquisition of services and maintenance rationale
Cost (€): 50,000 €
Rationale for request funding:

Description	Amount (€)
Material for cell culture	16,000
Immunohistochemistry (including antibodies)	9,000
Western Blot material	7,500
Material for PCR	8,000
ELISA assays	5,000
Other material	4,500
TOTAL	50,000

8.6 Equipment rationale

8.6.1 Available equipment

Equipment type	Manufacturer	Model	Year
Fluorescent Microscope	Zeiss	Axiovert	2003
Laminar airflow hood	Nature	Class II	1998
Gel and Blot Imaging System	Amersham Biosciences	Storm 860	1998
ELISA reader	SLT	Spectra	2000
Multi color Real Time PCR	Biorad	iqTM5	2006
Confocal microscope	Zeiss	LSM510Meta	2006
Blotting system	Biorad	Trans-Blot Cell	2002

- **Rationale for request funding**

The hypoxia chamber is needed for the IH studies.

8.7 Patent registration

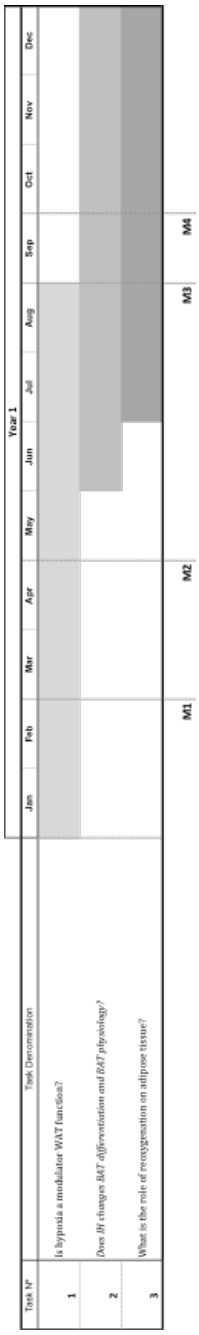
There will be no patents

8.8 Adaptation of building facilities

No adaptions will be performed

9. Attachments

Attachment 1 – Timeline



Attachment 2 – Figures

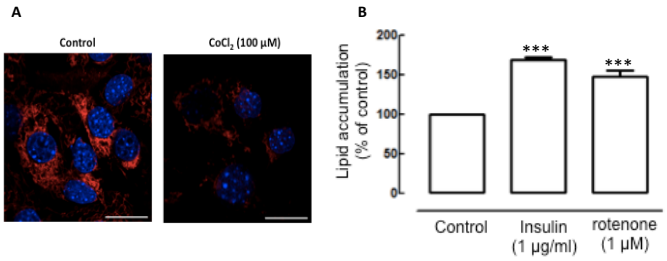


Figure 1 – Hypoxia induces mitochondrial dysfunction that is involved on lipid accumulation.

A) Adipocytes were treated with cobalt chloride ($CoCl_2$, 100 µM) or rotenone (1 µM) during 9 days. The control conditions are cells that at day 2 were incubated with DMEM with IBMX (0.5 mM) and dexamethasone (0.25 µM) and after that every two days cells were incubated with DMEM without any drug. A) Mitochondrial network was evaluated by using MitoTracker Red CMXRos stained in red. Hoescht 33342 labels the nuclei in blue. A representative image is showed in this figure. Scale bar – 20 µm. B) Lipid accumulation quantified by Oil red-O staining. Mean ± SEM, 5 different independent experiments, each condition performed in triplicate. ***p<0.01 compared to control. One-way ANOVA was used as statistical test.

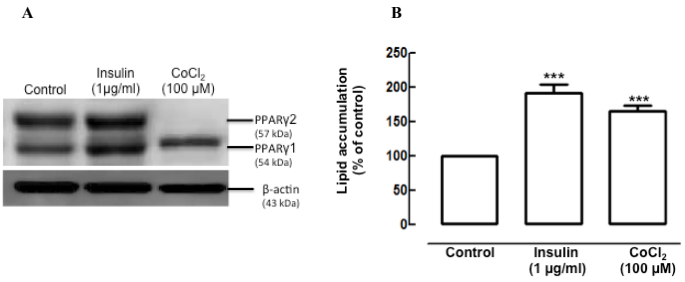


Figure 2 –Hypoxia induces lipid accumulation

Adipocytes were treated with cobalt chloride ($CoCl_2$, 100 µM) during 9 days. Pre-adipocytes are cells not treated with IBMX neither with dexamethasone. The control condition are cells that at day 2 were incubated with DMEM with IBMX (0.5 mM) and dexamethasone (0.25 µM) and after that every two days cells were incubated with DMEM without any drug. The positive controls are cells that at day 2 were incubated with DMEM with IBMX (0.5 mM), dexamethasone (0.25 µM) and insulin (1 µg/ml) after that every two days cells were incubated only with insulin (1 µg/ml). A) Western Blotting assayed for whole cell extracts was performed to evaluate the PPAR-γ2 immunoreactivity; B) Oil red-O staining to quantify lipid accumulation, as described in Material and Methods. Results were expressed as the percentage of lipid accumulation compared to control. Mean ± SEM, 5 different independent experiments, each condition performed in triplicate. All values were normalized to 100% of control **** p<0.001 compared to control One-way ANOVA was used as statistical test.

Attachment 3 – FCT MODEL

FCT Fundação para a Ciência e a Tecnologia

MINISTÉRIO DA CIÊNCIA, TECNOLOGIA E ENSINO SUPERIOR

Concursos de Projectos de I&D

Calls for R&D Projects

Voltar à descrição do projecto
Back to project description

Imprimir esta página
Print this page

Visão global da candidatura

Application overview

Ocultar todos as secções desta candidatura -
Hide all sections for this application

Referência do projeto
Project reference

PXXX/XXX-XXX/0000/2012

1. Identificação do projeto

1. Project description -

Domínio Científico
Scientific Domain

Área científica principal
Main Area

Área científica Secundária
Secondary area

(Vazio)
(Void)

Acrónimo
Acronym

(Vazio)
(Void)

Título do projeto (em português)
Project title (in portuguese)

Título do projeto (em inglês)
Project title (in english)

Financiamento solicitado
Requested funding

(Vazio)
(Void)

Palavra-chave 1	**Keyword 1**
(Vazio) *(Void)*	*(Vazio)* *(Void)*
Palavra-chave 2	**Keyword 2**
(Vazio) *(Void)*	*(Vazio)* *(Void)*
Palavra-chave 3	**Keyword 3**

(Vazio)
(Void)

(Vazio)
(Void)

Palavra-chave 4

Keyword 4

(Vazio)
(Void)

(Vazio)
(Void)

Data de início do projeto
Starting date

Duração do projeto em meses
Duration in months

00-00-0000

2. Instituições envolvidas
2. Institutions and their roles

-

Instituição Proponente
Principal Contractor

(Vazio)
(Void)

Instituição Participante
Participating Institution

(Vazio)
(Void)

Unidade de Investigação
Research Unit

(Vazio)
(Void)

Unidade de Investigação Adicional
Additional Research Unit

(Vazio)
(Void)

Instituição de Acolhimento
Host Institution

(Vazio)
(Void)

3. Componente Científica
3. Scientific Component

-

3.1. Sumário
3.1 Abstract

3.1.a Em português
3.1.a In Portuguese

(Vazio)
(Void)

3.1.b Em inglês
3.1.b In English

(Vazio)
(Void)

3.1.c Para publicação - Em português
3.1.c For publication - In Portuguese

3.1.d Para publicação - Em inglês
3.1.d For publication - In English

3.2. Descrição Técnica
3.2 Technical Description

3.2.1. Revisão da Literatura
3.2.1. Literature Review

(Vazio)
(Void)

3.2.2. Plano e Métodos
3.2.2. Plan and Methods

(Vazio)
(Void)

3.2.3. Tarefas
3.2.3. Tasks

(Não existem tarefas associadas a este projeto)
(No task has been associated to this project)

3.2.4. Calendarização e Gestão do Projeto
3.2.4. Project Timeline and Management

3.2.4.a Descrição da Estrutura de Gestão
3.2.4.a Description of the Management Structure

(Vazio)
(Void)

3.2.4.b Lista de Milestones
3.2.4.b Milestone List

(Vazio)
(Void)

3.2.4.c Cronograma
3.2.4.c Timeline

Ficheiro com a designação "timeline.pdf", no 9. Ficheiros Anexos, desta Visão Global (caso exista).
File with the name "timeline.pdf" at 9. Attachments (if exists).

3.3. Referências Bibliográficas
3.3. Bibliographic References

Referência Reference	**Ano** Year	**Publicação** Publication
(vazio) *(void)*	*(vazio)* *(void)*	*(vazio)* *(void)*
(vazio) *(void)*	*(vazio)* *(void)*	*(vazio)* *(void)*
(vazio) *(void)*	*(vazio)* *(void)*	*(vazio)* *(void)*
(vazio) *(void)*	*(vazio)* *(void)*	*(vazio)* *(void)*
(vazio) *(void)*	*(vazio)* *(void)*	*(vazio)* *(void)*

3.4. Publicações Anteriores
3.4. Past Publications

Referência Reference	**Ano** Year	**Publicação** Publication
(vazio) *(void)*	*(vazio)* *(void)*	*(vazio)* *(void)*
(vazio) *(void)*	*(vazio)* *(void)*	*(vazio)* *(void)*
(vazio) *(void)*	*(vazio)* *(void)*	*(vazio)* *(void)*
(vazio) *(void)*	*(vazio)* *(void)*	*(vazio)* *(void)*
(vazio) *(void)*	*(vazio)* *(void)*	*(vazio)* *(void)*

3.5. Ressubmissão de projectos
3.5. Project Resubmission

Ressubmissão?
Resubmission?

Estado
Status

Referência
Reference

Título do projeto (em português)
Project title (in portuguese)

Título do projeto (em inglês)
Project title (in english)

Instituição Proponente
Principal Contractor

Investigador(a) Responsável
Principal Investigator

Resposta aos Comentários do Painel
Response to Panel Comments

Alterações
Changes

4. Equipa de investigação
4. Research team

4.1 Lista de membros
4.1. Members list

Nome Name	Função Role	Grau Degree	%	CV nuclear Core CV	CV
X				✓	

(O curriculum vitae de cada membro da equipa está disponível clicando no nome correspondente)
(Curriculum vitae for each research team member is available by clicking on the corresponding name)
Total: 1

5. Outros projetos
5. Other projects

5.1. Projetos financiados
5.1. Funded projects

(Vazio)
(Void)

5.2. Candidaturas similares
5.2. Similar applications

(Vazio)
(Void)

6. Indicadores previstos
6. Expected indicators

Indicadores de realização previstos para o projeto
Expected output indicators

Descrição Description	2012	2013	2014	2015	2016	Total
A - Publicações Publications						
Livros Books	0	0	0	0	0	**0**
Artigos em revistas internacionais Papers in international journals	0	0	0	0	0	**0**
Artigos em revistas nacionais Papers in national journals	0	0	0	0	0	**0**
B - Comunicações Communications						
Comunicações em encontros científicos internacionais Communications in international meetings	0	0	0	0	0	**0**
Comunicações em encontros científicos nacionais Communications in national meetings	0	0	0	0	0	**0**

C - Relatórios Reports	0	0	0	0	0	**0**
D - Organização de seminários e conferências Organization of seminars and conferences	0	0	0	0	0	**0**
E - Formação avançada Advanced training						
Teses de Doutoramento PhD theses	0	0	0	0	0	**0**
Teses de Mestrado Master theses	0	0	0	0	0	**0**
Outras Others	0	0	0	0	0	**0**
F - Modelos Models	0	0	0	0	0	**0**
G - Aplicações computacionais Software	0	0	0	0	0	**0**
H - Instalações piloto Pilot plants	0	0	0	0	0	**0**
I - Protótipos laboratoriais Prototypes	0	0	0	0	0	**0**
J - Patentes Patents	0	0	0	0	0	**0**
L - Outros Other						
	0	0	0	0	0	**0**
	0	0	0	0	0	**0**
	0	0	0	0	0	**0**

Acções de divulgação da actividade científica
Scientific activity spreading actions

(Vazio)
(Void)

7. Orçamento
7. Budget

-

Instituição Proponente
Principal Contractor

(Não se encontra registada a Instituição Proponente para este projeto)
(The Principal Contractor is missing for this project)

Instituições Participantes
Participating Institutions

(Não se encontram registadas Instituições Participantes para este projeto)
(No Participating Institution has been registered for this project)

Orçamento Global
Global budget

Descrição Description	**2012**	**2013**	**2014**	**2015**	**2016**	**Total**
Recursos Humanos Human resources	0,00	0,00	0,00	0,00	0,00	**0,00**
Missões Missions	0,00	0,00	0,00	0,00	0,00	**0,00**
Consultores Consultants	0,00	0,00	0,00	0,00	0,00	**0,00**
Aquisição de bens e serviços Service procurement and acquisitions	0,00	0,00	0,00	0,00	0,00	**0,00**
Registo de patentes Patent registration	0,00	0,00	0,00	0,00	0,00	**0,00**
Adaptação de edifícios e instalações Adaptation of buildings and facilities	0,00	0,00	0,00	0,00	0,00	**0,00**
Gastos gerais Overheads	0,00	0,00	0,00	0,00	0,00	**0,00**
TOTAL DESPESAS CORRENTES TOTAL CURRENT EXPENSES	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**
Equipamento Equipment	0,00	0,00	0,00	0,00	0,00	**0,00**

Total	0,00	0,00	0,00	0,00	0,00	0,00

Plano de financiamento
Finance plan

Descrição Description	2012	2013	2014	2015	2016	Total
Financiamento solicitado à FCT Requested funding	0,00	0,00	0,00	0,00	0,00	**0,00**
Financiamento próprio Own funding	0,00	0,00	0,00	0,00	0,00	**0,00**
Outro financiamento público Other public-sector funding	0,00	0,00	0,00	0,00	0,00	**0,00**
Outro financiamento privado Other private funding	0,00	0,00	0,00	0,00	0,00	**0,00**
Total do Projecto Total of the project	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**

8. Justificação do orçamento
8. Budget rationale

-

8.1. Justificação dos recursos humanos
8.1. Human resources rationale

(Vazio)
(Void)

8.2. Justificação de missões
8.2. Missions rationale

(Vazio)
(Void)

8.3. Justificação de consultores
8.3. Consultants rationale

(Vazio)
(Void)

8.4. Justificação de aquisição de bens e serviços
8.4. Service procurement and acquisitions

(Vazio)
(Void)

8.6. Justificação do Equipamento
8.6. Equipment rationale

8.6.1. Equipamento já disponível para a execução do projecto
8.6.1 Available equipment

(Vazio)
(Void)

8.6.2. Discriminação do equipamento a adquirir
8.6.2. New equipment requested

(Vazio)
(Void)

8.7. Justificação de registo de patentes
8.7. Patent registration

(Vazio)
(Void)

8.8. Justificação de adaptação de edifícios e instalações
8.8. Adaptation of buildings and facilities

(Vazio)
(Void)

9. Ficheiros Anexos
9. Attachments

-

Nome Name	Tamanho Size

t

10. Possíveis conflitos de interesse
10. Possible Conflicts of Interest

-

Lista

List

Nome
Name
t

Email
Email

Instituição
Institution
t

CV

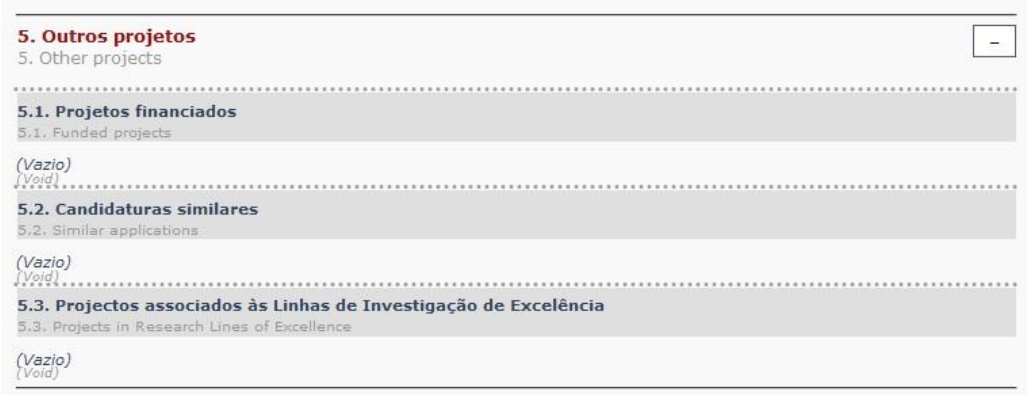
http://

Motivo
Reason
t

17-02-2012 17:11:25

Financiado por fundos estruturais da UE e fundos nacionais do MCTES

Ponto 5 do formulário de candidatura para “Projetos de IC&DT em Linhas de Investigação de Excelência” / Section 5 of the application form for the “SR&TD Projects in Research Lines of Excellence”:



Normas de candidatura à FCT e formulário

(Adaptado de: http://www.fct.pt/apoios/projectos/concursos/instrucoes)

O formulário de candidatura tem 10 secções:

1. Identificação do projeto
2. Instituições envolvidas
3. Componente científica
4. Equipa de investigação
5. Projetos financiados
6. Indicadores previstos
7. Orçamento
8. Justificação do orçamento
9. Anexos
10. Conflitos de Interesse

1. **Identificação do projeto:** Informação sobre o título e área científica (principal e secundária) do projeto, palavras chave, data de início do projeto e sua duração. O financiamento solicitado é calculado automaticamente a partir do preenchimento dos quadros do orçamento.
2. **Instituições participantes.** As instituições estão classificadas em:
    - Instituição Proponente
    - Instituições Participantes
    - Unidade de Investigação Principal
    - Unidades de Investigação Adicionais
    - Instituição de Acolhimento

As instituições participantes são inseridas uma a uma dentro do respetivo quadro. Se a instituição pretendida não se encontrar na lista deverá preencher o formulário disponibilizado para o efeito e seguir as instruções. A designação de Unidade de Investigação está reservada de acordo com critérios da FCT incluindo nomeadamente as que são objeto de financiamento plurianual.

3. Componente Científica.

A Componente Científica da candidatura organiza-se da seguinte forma:

3.1-Sumário (em português e inglês)

3.2-Descrição Técnica

3.2.1-Revisão da Literatura

3.2.2-Plano de Investigação e Métodos

3.2.3-Tarefas

3.2.4-Calendarização e Gestão do Trabalho

3.3-Referências Bibliográficas (máx. 30 referências)

3.4-Publicações Anteriores (máx. 5 referências)

3.5-Ressubmissão de Candidatura

4. Equipa de Investigação.

O quadro da equipa de investigação divide-se em 2 secções:

4.1-Lista de elementos da equipa

Nesta lista cada investigador deverá fornecer a sua chave de associação para ser adicionado como membro da equipa. Após efetuada esta operação, é apresentada para escolha a percentagem de participação no projeto.

4.2-Lista de outros elementos a contratar durante a execução do projeto

Estes elementos são automaticamente adicionados à equipa através do quadro justificação dos recursos humanos, indicando o nº de pessoas e o tipo de vínculo.

5. Outros projetos

5.1-Projetos financiados

Os projetos financiados do mesmo IR podem ser adicionados de duas formas conforme se trate de um financiamento da FCT ou de outra entidade. No caso de se tratar de um financiamento atribuído pela FCT deverá ser fornecida a referência do projeto o que aciona a importação automática de dados referentes ao projeto, alguns dos quais passíveis de correção ou de completação. Para projetos financiados por outras instituições, é necessário preencher a referência e, seguidamente, todos os elementos do projeto.

5.2-Candidaturas similares

É obrigatório referir qualquer outra candidatura similar à corrente que possa vir a configurar, se ambas forem aceites, uma situação irregular. A interface é análoga à usada para indicar projetos financiados.

5.3-Projetos a Associar à Linha de Investigação de Excelência

Tipicamente os projetos de IC&DT em linhas de investigação de excelência devem agrupar pelo menos três projetos de investigação com financiamento obtido em concursos competitivos nos últimos 5 anos.

Os projetos financiados do mesmo IR ou de outros membros da equipa de investigação podem ser adicionados de duas formas conforme se trate de um financiamento da FCT ou de outra entidade.

No caso de se tratar de um financiamento atribuído pela FCT deverá ser fornecida a referência do projeto o que aciona a importação automática de dados referentes ao projeto, alguns dos quais passíveis de correção ou de completação.

Para projetos financiados por outras instituições, é necessário preencher a referência e, seguidamente, todos os elementos do projeto.

6. Indicadores previstos

7. Orçamento

É obrigatório preencher um quadro de orçamento para a Instituição Proponente e para cada uma das Instituições Participantes. O total dos valores inscritos representa o financiamento solicitado. Adicionalmente, e quando aplicável, deve ser preenchido o quadro Plano de Financiamento. As rubricas orçamentais podem incluir (dependendo do estabelecido no edital do concurso):

-Recursos humanos: Os recursos humanos propostos nesta rubrica aparecerão automaticamente na secção de Equipa de investigação.

-Missões: O total desta rubrica deve corresponder ao total que é apresentado no quadro *Orçamento Global* na secção Orçamento.

-Consultores: O total desta rubrica deve corresponder ao total que é apresentado no quadro *Orçamento Global* na secção Orçamento. Dada a importância dos Consultores para a avaliação da execução do projeto e da equipa, as indicações de

nome e instituição devem ser não ambíguas de maneira a possibilitar a sua fácil identificação pelo painel de avaliação. Recomenda-se a existência na Internet de um pequeno currículo público atualizado e facilmente localizável.

-Aquisição bens e serviços: O total desta rubrica deve corresponder ao total que é apresentado no quadro *Orçamento Global* na secção Orçamento.

-Registo de patentes: O total desta rubrica deve corresponder ao total que é apresentado no quadro *Orçamento Global* na secção Orçamento.

-Adaptação de edifícios e instalações: As adaptações de edifícios e instalações imprescindíveis à realização do projeto, nomeadamente por razões ambientais ou de segurança.

-Equipamento: O total desta rubrica deve corresponder ao total que é apresentado no quadro *Orçamento Global* na secção Orçamento.

-Despesas Gerais

8. **Justificação do orçamento**

   A justificação do orçamento deverá ser inscrita por rubrica. O total de cada uma das rubricas deverá ser igual ao indicado no quadro *Orçamento Global*. Não existe transferência automática de verbas entre os dois quadros.

9. **Ficheiros Anexos**

   Esta secção destina-se à inserção de documentos que contendo informação não transmissível nos campos de texto: fórmulas, esquemas, diagramas, gráficos ou imagens.

10. **Conflitos de Interesse:** Esta secção destina-se a identificar os avaliadores que constituam um claro conflito de interesse na avaliação do projeto.

Limites de número de caracteres dos vários campos do formulário:

(Adaptado de: http://www.fct.pt/apoios/projectos/concursos/faq)

Campo	**Limite**
1. Identificação do projeto - Título PT	255
1. Identificação do projeto - Título EN	255
3.1. Sumário PT	5000
3.1. Sumário EN	5000
3.2.1. Revisão da Literatura	6000
3.2.2. Plano de Investigação e Métodos	10000
3.2.3. Tarefas - Descrição e resultados esperados	4000
3.2.4.a. Descrição da Estrutura de Gestão	3000
3.2.4.b. Descrição de *Milestone*	300
5. Projetos financiados – Resultados	5000
6. Indicadores previstos - Ações de divulgação da atividade científica	3000
8.1. Justificação dos Recursos Humanos	600
8.2. Justificação de Missões	600
8.3. Justificação de Consultores	600
8.4. Justificação de Aquisições de Bens e Serviços	600
8.5. Justificação de patentes	600
8.6.2. Discriminação do equipamento a adquirir - Justificação	600
8.7. Justificação da adaptação de edifícios	600