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PEOPLE
MARIE CURIE ACTIONS

Intra-European Fellowship (IEF)

Call: FP7-PEOPLE-2011-IEF

PART B

FINAL REPORT

“SEE BAT”

STIMULATION OF ENERGY EXPENDITURE AND BROWN ADIPOSE TISSUE IN
HUMANS

Final report “SEE BAT”

1- Work progress and achievements during the period

The concise overview of the progress of the work in line with the structure of Annex I of the Grant Agreement:

*can be found below in abstract form as well as in the original grant application on` pages .8-12 and 17-19 (attached to this report). Lines on the final report are in **RED and italic.***

A summary of progress towards objectives and details for each task

is detailed within abstract and the text of the original application and in the appendix to this report.

- A summary of the progress of the researcher training activities/transfer of knowledge activities/integration activities

is detailed within the abstract and the text of the original application.

- Highlight clearly significant results as far as analysed and known,

have been highlighted concerning the cold response, AITC effect and thermal imaging. Also results have been highlighted with regard to determinants of energy expenditure in a weight losing cohort (see next bullet). The results have also been depicted in the appendix to this report.

- If applicable, explain the reasons for deviations from Annex I and their impact on other tasks as well as on available resources and planning,

These are also detailed within the text and concern the use of a an MRI based imaging modality that might prevent the need for an radiation based imaging such as FDG PET CT scan.

Additionally, we have investigated the thermogenic reponse to mustard without capsule because unfortunately the capsulated mustard did not increase energy expenditure sufficiently.

Because of this inability of mustard to increase energy expenditure we finally investigated determinants of energy expenditure in our studies subjects as well as in a weight loss cohort

- If applicable, explain the reasons for failing to achieve critical objectives and/or not being on schedule:

Because of this inability of mustard to increase energy expenditure we finally investigated determinants of energy expenditure in our studies subjects as well as in a weight losing cohort

- A statement on the use of resources, in particular highlighting and explaining deviations between actual and planned researcher-months in Annex 1 (Description of Work)

All planned research months have been carried out according to schedule in Annex 1.

- If applicable, propose corrective actions

not applicable

2- Additional information

NONE

3- Project management

Please use this section to summarise management activities during the period:

- Project planning and status – from management point of view;

As outlined in section “APPROPRIATENESS OF RESEARCH METHODOLOGY AND APPROACH” and “COMPLIMENTARY SKILLS” the management of the project so far has been uneventful. The project mile stones are being reached in time, there have been no (practical) issues that would have slowed down the project. Interaction with diverse professionals (e.g. scientists, nursing staff, supervisors) as well as allocated experiment time and space on the CRF has been smoothly. However, due to the results of objective A we have amended the objective A.

- Problems which have occurred and how they were solved or envisaged solutions;

Because of the inability of mustard to increase energy expenditure we finally investigated determinants of energy expenditure in our studies subjects as well as in a weight loss cohort

- Changes to the legal status of any of the beneficiaries, in particular, SME status;

There were no changes.

- Impact of possible deviations from the planned milestones and deliverables, if any;

The deviations (in terms of using a non radiation based imaging modality in contrast to FDG PET CT scans) will only have a positive effects on the project since a non radiation imaging method is very welcome to enable repetitive and unharmed imaging.

Because of this inability of mustard to increase energy expenditure we finally investigated determinants of energy expenditure in our studies subjects as well as in a weight loss cohort. This will surely contribute to a better understanding of energy expenditure and weight control: the primary important area addressed in this project

- Development of the project website (if applicable);

N.A.

- Gender issues; Ethical issues;

No problems have been encountered here from either the subject of staff point of view.

- Justification of subcontracting (if applicable);

N.A.

- Justification of real costs (management costs);

N.A.

- Other

N.A.

Part B – Table of Contents of Proposals

B1-Scientific and technological Quality (maximum 8 pages)

- Research and technological quality
- Appropriateness of research methodology and approach
- Originality and innovative nature of the project, relationship to the 'state of the art' of research in the field
- Timeliness and relevance of the project
- Host scientific expertise in the field
- Quality of the group/supervisors

B2-Training (maximum 2 pages)

- Clarity and quality of the research training objectives for the researcher
- Relevance and quality of additional scientific training
- Host expertise in training *experienced researchers* in the field

B3-Researcher (maximum 7 pages which includes a CV and a list of main achievements)

- Research experience
- Research results including patents, publications, teaching etc.
- Independent thinking and leadership qualities
- Match between the *fellow's* profile and project
- Potential for reaching a position of professional maturity
- Potential to acquire new knowledge

B4-Implementation (maximum 6 pages)

- Quality of infrastructures/facilities and international collaborations of host
- Practical arrangements for the implementation and management of the research project
- Feasibility and credibility of the project, including work plan
- Practical and administrative arrangements and support for the hosting of the *fellow*

B5-Impact (maximum 4 pages)

- Potential of acquiring competencies during the fellowship to improve the prospects of reaching and/or reinforcing a position of professional maturity, diversity and independence, in particular through exposure to complementary skills training with special attention to exposure to the industry sector, where appropriate
- Contribution to career development or re-establishment where relevant
- Contribution to European excellence and European competitiveness
- Benefit of the mobility to the European Research Area
- Impact of the proposed outreach activities

B6-Ethical Issues (no maximum pages)

- Informed consent
- Privacy and data protection / Use of human biological samples and data

B1 RESEARCH AND TECHNOLOGICAL QUALITY

RESEARCH AND TECHNICAL QUALITY, INCLUDING ANY INTERDISCIPLINARY AND MULTIDISCIPLINARY ASPECTS OF THE PROPOSAL.

Obesity and its associated metabolic complications, such as type II diabetes, form a major and so far unresolved global health burden. Treatment strategies for obesity aim to alter the balance between energy intake and energy expenditure in such a manner that weight loss is induced, thereby preventing the development of diabetes and its complications.

The most frequently used strategy is to decrease energy intake by diets and/or appetite decreasing medication, but these often fail. An attractive option would therefore be to induce weight loss and or prevent weight regain by increasing energy expenditure. Conversely, increased energy expenditure does not seem to result in as strong a compensatory response in terms of increased food intake, making strategies that increase energy expenditure an attractive method to combat obesity. However treatments that aimed to increase energy expenditure so far came with unwanted side effects (e.g. treatment with thyroid hormone and dinitrophenol).

Brown adipose tissue (BAT) has been known for a long time to be the only tissue with the capacity to safely generate heat at the expense of energy by uncoupling fatty acid oxidation from adenosine triphosphate (ATP) generation, thus increasing metabolic rate (1). Therefore BAT is a thermogenic tissue, that safely converts produces heat via the unique uncoupling protein (UCP) 1. Thus activation of BAT has the potential to induce weight loss. Recently there has been a re-awakened interest in brown adipose tissue (BAT) in humans due to the finding that BAT has been shown to be present and active in adult humans. Prospective studies, using fluorodeoxyglucose positron-emission tomography scans (FDG-PET CT), showed that cold exposure activates BAT in lean, healthy individuals (2, 3). Moreover, very recent biopsy studies have shown the presence of BAT in humans (4, 5). This revived attention for brown adipose tissue (BAT) in humans may lead to therapies that increase energy expenditure.

FDG-PET-scans showed BAT signal in predilection areas such as the supraclavicular region (2, 3). The chance of detecting active BAT on non-temperature controlled FDG-PET CT was shown to be predicted by multiple factors such as young age, female sex and low body mass index (BMI). The use of beta-blockers was associated with a lower chance of detecting BAT presence on FDG-PET scans, compatible with adrenergic mediated activation of BAT. Importantly, the outdoor temperature at time of scan is negatively associated with the chance of detecting active BAT, indicating the effect of cold on BAT activation.

Activation of brown adipose tissue in humans

Cold exposure activates BAT generally as follows (1): when skin and mucous membranes of the gastrointestinal tract are exposed to cold, activation of transient receptor potential channels (TRPs, i.e. TRPA1) in nerve endings follows (6). The TRP cation channel superfamily is a diverse family of 28 cation channels that have varied physiological functions, including thermal and other sensations (7). The cold signal then reaches the hypothalamus via afferent neuronal pathways. Via activation of certain cerebral nuclei, a sympathetic response is produced that reaches end organs such as BAT, skin and skeletal muscle (figure 1) (8). This may be mediated by the extreme-cold receptor transient receptor potential channel member A1 (TRPA1) that is present in the skin and mucosal lining of the gastrointestinal tract and activated, as suggested by its name, cold exposure (4). The important role of TRPA1 in human cold mediated perception and signalling is highlighted by patients with the Familial Episodic Pain Syndrome. These patients harbour a gain-of-function mutation in the TRPA1 gene (chromosome 8q12–8q13) and experience pain to various stressors including cold (9).

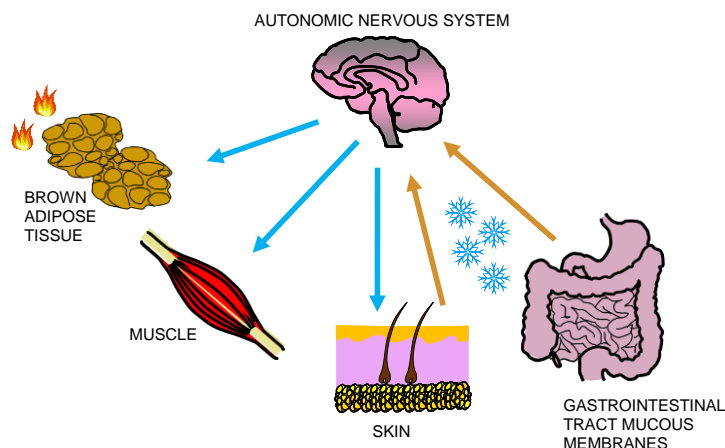


Figure 1, Cartoon showing how cold activation of the extreme-cold receptor transient receptor potential channel member A1 in skin and gastrointestinal tract activate higher centres, thereby affecting end organs such as BAT (heat), muscle (shivering in severe cold) and skin (vasoconstriction).

The transient receptor potential cation channel subfamily V member 1 (TRPV1) is another TRP (7). TRPV1 agonists have an dual effect: i.e., the stimulation of heat loss (sweating) and heat

production (thermogenesis) (10). This makes it an interesting TRP to study next to TRPA1. TRPV1 is present in the central nervous system and many other tissues. Activation of the above receptors then affects the beta 3 adrenergic receptor via the autonomic nervous system finally increasing BAT activity (12, 13).

In addition cold exposure has been shown to increase glucose and lipid availability for thermogenic purposes (14). Where as animal studies showed increased secretion and hydrolysis of triglycerides (TG), it was shown in humans that acute exposure to cold increases plasma and interstitial glycerol concentration with also enhanced non-oxidative disposal of fatty acids (i.e., TG/FFA cycling) (14). Finally, cold exposure (depending on its magnitude) induces vasoconstriction and increases blood pressure (15). In fact, mild cold exposure vasoconstriction is also a thermoregulatory mechanism to decrease heat loss (15).

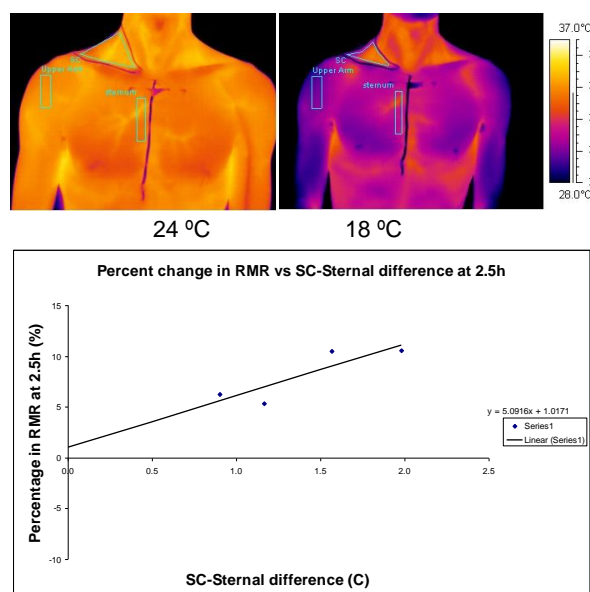
BAT may very well have an impact on energy balance. It has been suggested that the lack of BAT activation in FDG-PET CT imaging in obese subjects may be partly responsible for the weight gain in these obese individuals (2). However, it is difficult to study the contribution of BAT activity to energy expenditure in different settings due to a lack of appropriate imaging techniques. Even more, a recent biopsy study showed that BAT may be present in more individuals who do not reveal BAT on FDG-PET CT (4); this finding shows the importance of including such individuals in studies that investigate the BAT induced increase in energy expenditure.

Imaging of brown adipose tissue

Currently, imaging of BAT in humans relies on PET-CT, which has several limitations. High radiation exposure prevents sequential imaging in longitudinal studies, and rendering analyzing effect of interventions on BAT activity impossible. Also BAT needs to be activated to be imaged by PET-CT (so far done by cold exposure). However, BAT could be present, not being visible on FDG- PET CT, depending on its abundance (4).

Figure 2 Thermographic images in 18 °C and 24 °C and the correlation of energy expenditure with skin temperature.

These limitations indicate the need for an alternative way of imaging BAT and several lines of basic research show promising results. Infrared thermography is potentially a good method to image activity of BAT, since it



visualizes the primary product of brown adipose tissue: heat. Moreover, it is a simple and fast method with relatively low costs and can be used in the same individual as often as necessary (not dependent on a large scanner and allowing rapid analysis of BAT activity under different circumstances). Infrared thermography during cold exposure shows that the skin overlying the largest BAT depot in adult humans (the supraclavicular area) remains warmer compared to a mediastinal control region that does not contain BAT (16). Moreover, differences in skin temperature of the subcutaneous (overlying BAT) and sternal area correlate with the change in energy expenditure after cold exposure (pilot data, see figure 2).

Objectives

In this proposal we want to develop BAT activation protocols using different thermogenic stimuli. The research objectives of this proposal are as follows:

- A)** *To assess differential effects of BAT activating stimuli (cold, activation of TRPA1 and/or TRPV1) on thermogenic response, BAT, glucose/lipid metabolism and cardiovascular changes in healthy lean individuals.*

Final report

During the first year of the fellowship we have made great progress already in the project by fortifying our pilot work and executing large parts of Objective A (see objectives A and C under “APPROPRIATENESS OF RESEARCH METHODOLOGY AND APPROACH”. In this year we have strengthened the pilot data. Here we have included extra numbers of subjects. By doing so we were not only able to optimize the cold exposure protocol (see below), but also the thermogenic nutrient protocol (see below). Hereafter we have started the study in which healthy volunteers were studied on three occasions up to now (in thermoneutrality, cold and thermogenic nutrient exposure). See full details below in the section “APPROPRIATENESS OF RESEARCH METHODOLOGY AND APPROACH”.

In appendix A to this report the results are depicted. Since the mustard (in capsules) unfortunately was not effective enough we decided in the second year to repeat the measurements after ingestion of mustard without capsules to expose the mucosal lining of the mouth and throat to the AITC. Although this led to a mild stress response (see cortisol data in appendix 1), we found no increase in energy expenditure. Therefore we did not repeat the experiment in obese subjects. However, we investigated energy expenditure and its variability in our volunteers that were studied 4 times and in a weight loss cohort.

- B)** *To test energy expenditure inducing protocols (derived from objective A) in obese/insulin resistant subjects with respect to thermogenic response, BAT, glucose/lipid metabolism and cardiovascular changes.*

Final report

In appendix A to this report the results are depicted. Since the mustard (in capsules) unfortunately was not effective enough we decided in the second year to repeat the measurements after ingestion of mustard without capsules to expose the mucosal lining of the mouth and throat to the AITC. Although this led to a mild stress response (see cortisol data in appendix 1), we found no increase in energy expenditure. Therefore we did not repeat the experiment in obese subjects. However, we investigated energy expenditure and its variability in our volunteers that were studied 4 times and in a weight loss cohort.

- C)** *Develop infrared thermography to be used repetitively in humans for accurate measurement of the thermogenic response of BAT.*

Final report

As for objective A, we have initially strengthened the pilot data. Here we have included extra numbers of subjects. By doing so we were again able to optimize the infrared thermal imaging protocol.

Here after we have included (see details below) subjects that have underwent infrared thermal imaging in thermoneutrality, cold and thermogenic nutrient exposure. An important point is that we have embarked on a MRI based imaging technique in contrast to FDG-PET CT scans to visualize brown adipose tissue. We have started this since an MRI technique enables nonradiation visualisation that can be used under multiple stimulative conditions (repetitive) without harmful side effects for the subject. However, the noise to signal ratio for this latter technique was not strong enough. The results on thermography are depicted in appendix 1.

Interdisciplinary/multidisciplinary aspects of the proposal

The current proposal has a multidisciplinary approach with the participation of basic and clinical researchers within the Addenbrooke's Hospital (University of Cambridge). The proposal is founded on the results of BAT oriented research that has been conducted during recent years within the Institute of Metabolic Science (Addenbrooke's Hospital). The studies will be conducted in the Wellcome Trust Clinical Research Facility (WTCRF) within the Addenbrooke's Centre for Clinical Investigation (ACCI). WTCRF staff members are already familiar with techniques described in the proposals as they are also used in other ongoing projects within the Facility. The institute of metabolic science has developed a track record in the brown adipose tissue research and we aim to translate this acquired knowledge to the clinical phase combining a metabolic (patho)physiological approach and imaging.

Final report

We have been very successful in implementing the study protocol in the Welcome Trust Clinical Research Facility (CRF) of the Institute of Metabolic Science - Addenbrookes Hopspital in Cambridge. Here we work closely together with different professionals.

There has been extensive interaction with the nursing staff in the day to day clinical care for the subjects both during the afternoon/evening/night before the experiment as well as during the experiment.

Moreover the applicant has been able to work closely together with the clinical scientists of the CRF who are very experienced and involved in the collection of indirect calorimetry, heart rate variability, body composition, core body temperature data etc. This has enabled the applicant to increase his knowledge and capability to carry out the studies depicted in the protocol.

Multiple research assistants have been contributing to the projects. Additionally (see also under "B2 TRAINING"), the applicant has been able to interact with different scientist within the Institute of Metabolic Science besides Professor A. Vidal-Puig. All in all, the applicant has been well embedded and supported in both the IMS and the CRF.

APPROPRIATENESS OF RESEARCH METHODOLOGY AND APPROACH

General aim: Investigate activation of thermogenesis in BAT in humans using cold and activation of TRP activators (TRPA1/TRPV1) in vivo in humans following *three* objectives.

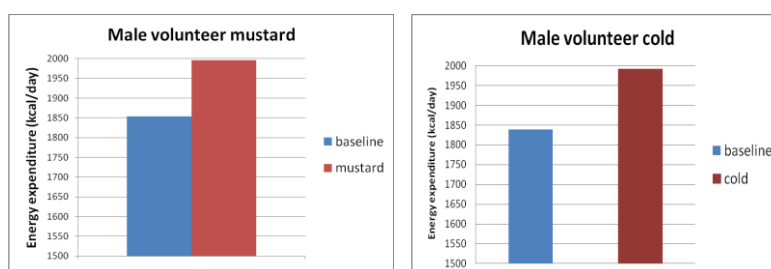
Hypothesis: Activation of BAT via stimulation of transient receptor potential channels in humans results in increased energy expenditure.

Objective A) *To assess differential effects of BAT activating stimuli (cold, activation of TRPA1 and/or TRPV1) on thermogenic response, BAT, glucose/lipid metabolism and cardiovascular changes in healthy lean individuals.*

As discussed above, cold, activation of TRPA1 and/or TRPV1 have been shown (in animal studies) to activate energy expenditure via BAT (6). Allyl-isothiocyanate (AITC) is potentially useful since it causes BAT activation via activation of the extreme-cold receptor transient receptor potential channel member A1 (TRPA1) (6). AITC mimics physiological cold and is found in common food ingredients such as mustard and wasabi, making it a safe method to stimulate brown adipose tissue. Using indirect calorimetry (figure 3) we found an increase in energy expenditure of 10.3% in response to mild cold exposure (16°C) and in increase in energy expenditure of 7.7% 30 minutes after AITC ingestion (10 grams Colmans Mustard containing 10.6 mg AITC). In objective A we will study the thermogenic response to control and AITC (10 gr. mustard) under thermoneutrality (24°C) in 8 subjects during 4,5 hours.

Figure 3, effect of mustard and cold on energy expenditure

As a proof of involvement of the autonomic nervous system, experiments will be repeated after beta-blockade (propranolol 20 mg with the highest affinity for the beta 3 receptor involved in BAT activation). To assess to what extent the effects of AITC actually mimic the cold effect, studies will be repeated under mild cold exposure (18°C).



Volunteers will be studied in the Wellcome Trust Clinical Research Facility of the Addenbrooke's Hospital. This facility has metabolic rooms that can **a-** be acclimatised to the requested temperature and **b-** analyse resting energy expenditure via indirect calorimetry. Subjects come the evening before the experiment for admission and have a standardised meal. The following morning (0800hrs) they will be acclimatised at 24°C during 2 hours. Hereafter they will have either control, AITC (10 grams Colmans Mustard), AITC plus propranolol or cold exposure and measurements for another 2,5 hours. Skin temperature will be measured using infrared thermography as described above and via direct skin thermography. Core body temperature will be continuously measured by temperature pill (encapsulated thermometer that is orally ingested) and the signal will be detected by a pocket-receiver. Blood samples will be drawn at baseline and each 30 minutes during the experiment AITC concentrations (analysed via high performance liquid chromatography (HPLC), stress hormones (e.g. catecholamines/cortisol) and substrates (glucose, free fatty acids). Glucose and lipid turnover will be assessed by state of the art stable isotope techniques (*not* radioactive) using primed, continuous infusion of [6,6-²H₂]glucose (glucose turnover and clearance rates) and [U-¹³C]palmitate (palmitate turnover and oxidation (¹³CO₂ breath analyses). Cardiovascular measurements include bloodpressure, ECG tracing and heart rate variability (Actigraph). Finally the presence of imagable BAT will be confirmed by FDG-PET CT under cold circumstances.

To further dissect the responses to cold and AITC (TRPA1 stimulation) we will investigate stimulation of the thermogenic response by activating TRPV1. Indeed TRPV1 stimulation by capsaicin or a capsinoid (nonpungent capsaicin-related substances found in all tested variants of the Capsicum genus of plants, red pepper) increases energy expenditure and weightloss in humans (11). Experiments will be repeated (same study design as described for AITC) using capsinoids. In short: Capsicum Anuum L. [Solanaceae (pepper fruit)] variety CH-19 Sweet will be used to extract capsinoid oil as described earlier (11). This will consist of capsiate, dihydrocapsiate, and nordihydrocapsiate and the respective concentrations will be analyzed a priori by HPLC (11).

Final report

-Fortification pilot data

First, we have fortified the pilot data. Here we have included extra numbers of subjects. We have paid attention to the optimal control of our cold rooms as well as the optimal placement, calibration and setup of the calorimeters. We have set out to do so to minimize variation in data obtained during indirect calorimetry.

Also, in these studies we have investigated the different ways of AITC (mustard) administration. Since it is difficult to administer mustard blinded (e.g. taste) we have investigated the differential effects of mustard in capsules or by a spoon. No differential effects seemed to be present. By carrying out these additional pilot studies we were not only able to optimize the cold exposure protocol but also to optimize the thermogenic nutrient protocol.

-Study (control/cold/mustard)

After the pilot data we have started the official study. We have included 11 subjects whom (except for one) have been studied four times under the following conditions (balanced assigned): control (thermoneutrality, 24 degrees Celsius), mild cold (18 degrees Celsius) and the addition of a thermogenic nutrient (10 grams of Colemans mustard, packed in veggi capsules or simply on a spoon, containing AITC). Under control and cold conditions, subjects did receive a placebo that was matched for the composition of macronutrients and calories.

During the study visits of the subject (three visits taking two consecutive days per study visit) we have assessed the following parameters to characterise subjects metabolically (metabolical phenotyping) in order to place the results in an adequate clinical context.

Study day 1 - First, after entrance on the CRF (and subsequent informed consent), body composition was assessed by using both DXA and BODPOD techniques. In order to measure oxidative capacity, an exercise test was done to assess VO_2max after taking an electrocardiogram.

In the early evening, subjects received a standardised (calories) meal as well as chest electrodes for the measurements of interbeat intervals (IBI, heart rate) to assess heart rate variability as a measure of para- and orthosympathetic tone.

Patients entered the metabolic room at 2000 hours in the evening (at thermoneutrality) after which the collection of calorimetric data started. Here subjects were normally active (reading, television or computer) but no exercise was permitted. A period of ~8 hours of sleep has been custom (2300-0700 hours).

Study day 2 - After being woken up early morning (when the collection of overnight whole room calorimetric data was finished), bedside indirect calorimetry was performed after the administration for infrared imaging labels, skin and core body temperature sensors. Normal observations for blood pressure, tympanic temperature, pulse rate, weight and visual analogue scores for temperature and hunger scores were also taken. Infrared thermography was used to measure skin temperature over the reference and brown adipose tissue predilection areas. Additionally blood samples were taken for different metabolites, hormones and other lab tests.

After these baseline measurements, subjects were balanced assigned to control (thermoneutrality, 24 degrees Celsius), mild cold (18 degrees Celsius), a thermogenic nutrient (10 grams of Colemans mustard in capsules in thermoneutrality) or a thermogenic nutrient (10 grams of Colemans mustard on a spoon in thermoneutrality). This "thermogenic intervention" lasted for two and a half hours during which all measurements were repeated each 30 minutes. After we finished the mustard in capsules, the thermogenic effect did not prove to be significant. Therefore we investigated the response when mustard mustard was

in direct contact with the mucosa of the mouth and throat. Direct contact of the AITC (mustard) with the mucosa might be of importance for the thermogenic response.

To assess the effects of the thermogenic interventions on appetite and satiety, subjects received an ad lib meal during which the amount and speed of eating (appetite and satiety respectively) were assessed using a universal eating monitor.

Unfortunately, we were not able in the end to find significant effects of the mustard either given as capsule or on a spoon. Although this is a negative result, the performed studies provided us with valuable biological data on the physiology of energy expenditure and cold exposure.

Analysis of study data – At this moment, all collected data during the study days have been analysed and interpreted by the applicant. All plasma samples that have been stored so far are analysed by the laboratory of clinical chemistry. We expect to publish the forthcoming results in the end of the summer or early autumn. Here we plan to publish results on the cold exposure and the thermogenic food intervention separately. For this final report, the data have been added to this final report as appendix (incorporating combined data for cold, thermogenic nutrient and the infrared thermal imaging).

Again, we were not able in the end to find significant effects of the mustard but the performed studies provided us with valuable biological data on the physiology of energy expenditure and cold exposure and hence will be published.

Objective B) *To test energy expenditure inducing protocols (derived from objective A) in obese/insulin resistant subjects with respect to thermogenic response, BAT, glucose/lipid metabolism and cardiovascular changes.*

When we have met the aims of objective A we will proceed to test the optimal protocol in 12 obese (but not diabetic) subjects (BMI 25-30 kg/m², HbA1c IFCC <48 mmol/mol, no comorbidity) and 12 glucose tolerant matched (BMI, body composition and age) controls. Lean subjects will participate in thermoneutrality experiments only but obese subjects will participate in three experiments: **a-** placebo, **b-** optimised BAT activation protocol (derived from objective A) under thermoneutrality and **c-** under cold circumstances. Experiments will be performed as described in objective A.

Final report

*Objective B would be carried out according to the results of objective A. However since these results were negative (no effect of mustard on energy expenditure) we have **amended** the objective B. Here we were able to determine the response of weight loss with respect to energy expenditure, substrate oxidation and various clinical and scientific parameters. To this end we analysed 60 obese subjects that were allocated to weight loss via either diet, exercise or sibutramine therapy. In this study we were able to identify patterns of weight loss in relation to these various parameters. This was an objective that was not originally planned for, but proved to be very fruitful.*

During this weight loss trial subjects were followed up intensively, but here we report on the visits at the start and after 4 and 12 weeks of the study. We assessed body composition, weight, energy expenditure (including substrate oxidation) and various plasma parameters such as acylcarnitines that are indicative of substrate oxidation and energy expenditure. IN this project we were primarily interested to analyse certain plasma metabolites (acylcarnitines) in relation to the clinical parameters described above and energy expenditure in particular.

Here we found that these plasma metabolites (that are thought to reflect substrate oxidation at the mitochondrial level) correlate with plasma fatty acids and not so much with rates of energy expenditure or substrate oxidation. For clarity and full description and nature of this

project we refer to the appendix where we have added the draft of the manuscript that will be offered for publication in due time.

Objective C) *Develop infrared thermography to be used repetitively in humans for accurate measurement of the thermogenic response of BAT.*

Within objectives A en B, we develop a method that permits repetitive use in short experiments/periods (i.e. no radiation exposure). During the experiments described objects A and B, infrared thermography will be performed continuously. Skin temperature will be assessed in three pre-defined areas: supraclavicular region (overlying BAT), sternal region (overlying bone, used as reference for temperature range) and skin temperature of the hand (that is prone to vasoconstriction). As shown above, our pilot data show good correlation of the difference in supraclavicular-sternal temperature with the rise in energy expenditure during cold. Finally, we will validate the infrared thermography against FDG-PET CT scanning that currently is the golden standard to asses BAT activation.

Final report

Fortification pilot data

In the first year, we have fortified the pilot data. Here we have included extra numbers of subjects. We have paid attention to the optimal control of the camera with respect to distance to the object, air humidity and temperature. Also we have assessed different confounding effects on thermal imaging (such as changing posture e.g. reclining versus standing position).

Additionally, we have performed all imaging studies in the AITC (mustard) intervention as well to assess effects on brown adipose tissue predilection areas and skin vasoconstriction.

Study (control/cold/mustard) performed in year 1 and 2.

Here, we have started the official study as outlined in objective A (and its Mid-term report). As described above, we have included 11 subjects whom all have been studied four times under control, cold and AITC conditions. Here I refer to the description that has been detailed under objective A. And the first part of the appendix added to this report.

An important point is that we have embarked on a MRI based imaging technique in contrast to FDG-PET CT scans to visualize brown adipose tissue. We have started this since an MRI technique enables nonradiation visualisation that can be used under multiple stimulative conditions (repetitive) without side effects for the subject. However, technical reasons made it difficult to detect changed in brown adipose tissue temperature and this led to termination of this project.

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11. Snitker S, Fujishima Y, Shen H, Ott S, Pi-Sunyer X, Furuhashi Y, Sato H, Takahashi M 2009 Effects of novel capsinoid treatment on fatness and energy metabolism in humans: possible pharmacogenetic implications. *Am J Clin Nutr* 89:45-50
12. Inokuma K, Okamatsu-Ogura Y, Omachi A, Matsushita Y, Kimura K, Yamashita H, Saito M 2006 Indispensable role of mitochondrial UCP1 for antiobesity effect of beta3-adrenergic stimulation. *Am J Physiol Endocrinol Metab* 290:E1014-1021
13. Mory G, Bouillaud F, Combes-George M, Ricquier D 1984 Noradrenaline controls the concentration of the uncoupling protein in brown adipose tissue. *FEBS Lett* 166:393-396
14. Vallerand AL, Zamecnik J, Jones PJ, Jacobs I 1999 Cold stress increases lipolysis, FFA Ra and TG/FFA cycling in humans. *Aviat Space Environ Med* 70:42-50
15. Savage MV, Brengelmann GL 1996 Control of skin blood flow in the neutral zone of human body temperature regulation. *J Appl Physiol* 80:1249-1257
16. Lee P, Ho KK, Greenfield JR Hot fat in a cool man: infrared thermography and brown adipose tissue. *Diabetes Obes Metab* 13:92-93
17. Yoneshiro T, Aita S, Matsushita M, Kameya T, Nakada K, Kawai Y, Saito M Brown adipose tissue, whole-body energy expenditure, and thermogenesis in healthy adult men. *Obesity (Silver Spring)* 19:13-16

ORIGINALITY AND INNOVATIVE NATURE OF THE PROJECT

Research leading to this proposal: The interest in BAT has been awakened in the recent years by the findings of BAT on FDG-PET CT scans (2, 3). More over since BAT activation might be a novel way to control weight and thus obesity, several of these reports have been published in high impact journals such as the New England Journal of Medicine (2, 3).

Although animal data had shown already that the autonomic nervous system (beta 3 adrenergic receptor in particular) mediates BAT activity, this was also suggested by Cypess et al showing an inverse correlation of the amount of BAT on FDG-PET CT with beta-blocker use (2). Very recently, data has become available on the effects on cold exposure and the imaging by FDG-PET CT showing a correlation between the BAT signal and body fat content (17). Even more, data suggests that BAT may be present in humans that are not BAT positive on FDG-PET CT (4). This implies that these subjects may still expend energy by BAT activation. Since it is known that diverse stimuli (such as cold) affect receptors (such as TRPA1 and TRPV1) and subsequently BAT, this emphasizes the need of human studies that investigate the increase in energy expenditure in response to their respective ligands mustard/AITC and capsaicin/capsinoids (6, 11). See reference list above.

In summary, we hypothesize that cold exposure and activation of transient receptor potential channels (such as TRPA1 and TRPV1) induce energy expenditure via BAT. Moreover such strategies to activate BAT are likely candidates to be used in future studies as tools for the induction of energy expenditure (via BAT) and weight control. This proposal embodies a translational physiological approach with all requirements (e.g. knowledge, metabolic

laboratory, clinical research facility) being available in the Institute of Metabolic Science to approach this important scientific question.

TIMELINES AND RELEVANCE OF THE PROJECT

This is a very timely and important project because:

1) Its contribution to the knowledge of the pathophysiology of obesity associated metabolic complications which can give direction to therapeutical approaches for this important public health problem. This project provides translation of basic knowledge to the human BAT physiology to implement **a-** strategies that are likely to be used in future studies on the role of BAT on weight control and **b-** nutritional approaches that may control weight gain and obesity.

2) The intrinsic quality, innovation and ambition of the program of research

3) The availability of powerful technologies in the WTCRF such as human metabolic chambers (permitting temperature regulation and indirect calorimetry), infrared thermal imaging and stable isotope techniques to analyse substrate kinetics.

4) The outcome of this proposal may have important value for the agricultural, nutraceutical and pharmaceutical industry, where the ability to increase energy expenditure may lead to growing crops that can be used for increasing energy expenditure and the development of nutraceutical as well as pharmacological compounds.

HOST SCIENTIFIC EXPERTISE IN THE FIELD

The University of Cambridge is one of the world's leading research academic institutions. Specifically the Addenbrooke's campus of the University of Cambridge is the fastest growing biomedical campus in Europe. Prof Vidal-Puig, as director of this programme of research, has a joint appointment in the Department of Medicine and the Department of Clinical Biochemistry. Both Departments were rated 5*, the highest rating in the last national assessment of research quality (2008). Since October 2007 the host laboratory is part of the new state of the art Metabolic Research Laboratories (MRL) within the Institute of Metabolic Science (IMS-MRL) at Cambridge University (<http://www.mrl.ims.cam.ac.uk>). The IMS-MRL is a cross-departmental institute within the University of Cambridge, School of Clinical Medicine, which is housed in the purpose-built Institute of Metabolic Sciences on the Cambridge Biomedical Campus Addenbrooke's Hospital site). The mission of the IMS-MRL is to undertake basic and translational research relevant to the understanding, prevention and treatment of diabetes, obesity and other related endocrine and metabolic disorders. This centre has close links with other elements of the IMS (MRC Epidemiology Unit and IMS Clinical Care Centre) as well as with other investigators in the University of Cambridge, local MRC units and the Sanger Institute. The IMS-MRL also hosts the MRC Centre for Obesity and Related metabolic Disease (MRC-CORD), one of the six newly established MRC Centre's for Translational Medicine in the UK.

The host of the applicant (Maarten Soeters), is Prof. Vidal-Puig, the Deputy Director of the MRC- CORD. As a training environment, the IMS –MRL provides expertise in a wide range of molecular and cellular techniques for the study of mammalian biology in health and disease. Core facilities and expertise in integrative whole-organism physiology, applicable to human and animal models, have been firmly established. As indicated above, the IMS-MRL is focused on diabetes, obesity and metabolism. The IMS-MRL investigators have developed and sustained a high international reputation, publishing in the last 10 years over hundreds of papers in high ranked journals focusing in endocrinology / metabolism. This involves several groups with different expertise interacting on a common theme. For example my host, Prof. Vidal-Puig, investigates the Molecular Mechanisms of Energy Balance, a subject that synergises with other groups that investigate the Molecular basis of obesity and insulin resistance (Prof. O'Rahilly), Mechanism of action of insulin and IGFs (Prof. Siddle), Appetite programming (Dr. Ozanne), Cytokine signalling and energy homeostasis (Prof. Sethi),

Epidemiology of Diabetes (Dr. Wareham), Genetics of Diabetes (Ines Barroso), Mitochondria biology (Dr. Brand) and NMR Metabolic Profiling (Dr. Griffin). The IMS-MRL institute also includes affiliation with Investigators from the Cambridge Institute for Medical Research including Prof. Todd, Prof. Wicker (type 1 diabetes) and Dr. Gribble (β cell biology). The IMS MRL also has strong links with the University of Oxford with Prof. Frayn. This brings not only varying approaches to common problems but also access to different specialised techniques and equipment.

Prof. Vidal-Puig's lab is associated to the Cambridge Systems Biology Centre (CSBC) which provides the mix of 'wet' (experimental) and 'dry' (computational) skills that Systems Biology demands. CSBC has particular strengths in bioinformatics and computer/mathematical modeling, transcriptomics and proteomics, and in the integration and analysis of large data sets. CSBC aims to translate state-of-the-art systems biology techniques, developed using model organisms, to applications in plant science and medicine.

Prof. Vidal-Puig, the scientist in charge of the project, is a Professor in Molecular Nutrition and Metabolism at the University of Cambridge and is an internationally recognised metabolic researcher in the field of Metabolism, Obesity and Diabetes Mellitus. Whilst at Harvard University he received the Paul Dudley White Award from the American Heart Association as an outstanding Young Physician Scientist and also NIH funding through the Boston Obesity Nutrition Career Development Award. Prof. Vidal-Puig was recruited to Cambridge University in 1999 and since then he has been the Director of the Rodent metabolic programme, funded by Wellcome Trust in 2002. More recently he has been appointed as Deputy Director of the MRCCORD center and is currently the Research Director of the Phenomics Center at the West Forvie Site within the Addenbrookes Campus, a center of excellence for murine metabolic Phenotyping.

The laboratory of Prof. Vidal-Puig is well funded through a MRC programme grant, and grants from British Heart Foundation, Wellcome Trust, Diabetes UK, BBSRC and European Union funding from Frame Work Program FP7 Etherpath and FP7 MITIN and PF7 Beta-BAT programmes which ensure the financial viability of the project. In addition, Prof. Vidal-Puig acts as consultant to large pharmaceutical and biotechnological companies including Astra Zeneca (Sweden), Tashio (Japan) and GSK (UK). Prof. Vidal-Puig's group holds formal and informal national and international collaborations.

QUALITY OF THE GROUP/SUPERVISORS

Prof Vidal-Puig will be Maarten Soeters's supervisor during the fellowship. Prof Vidal-Puig has extensive experience in supervising PhDs and post-doctoral researchers and many of them have become internationally acknowledged scientist (see paragraph 2.3). At present he is the head of a research group of 16 people consisting of 1 clinical fellow, 5 Post-Doc research scientists, 3 PhD students, 3 research assistants and 4 animal technicians. His programme of research investigates the molecular mechanisms involved in obesity and its metabolic complications including insulin resistance, lipotoxicity and fatty acid oxidation.

This laboratory specialises in the generation and characterisation of genetically modified mouse models to investigate molecular mechanism leading to obesity and diabetes. The conclusions from these studies are further investigated in humans as part of a translational strategy. In the past 6 years Prof Vidal-Puig's laboratory has focused in elucidating the molecular mechanisms involved in energy expenditure and adipogenesis using in vivo and in vitro models. In addition to genetically modified mice, this laboratory also studies the molecular and cellular properties of human adipocytes. These in vitro experiments are used as hypothesis generators to be tested with specific genetically modified animal models. Prof. Vidal-Puig's laboratory also works on transcription factors involved in adipogenesis and insulin sensitivity. Based on the information generated by in vitro experiments, new tissue specific Knock Out and transgenic mouse models are currently generated.

Relevant Publications in the last 5 years of Prof. Vidal-Puig

- 1- Prieur X et al. *Diabetes*, 2011.
- 2- Lopez M et al. *Nat Med*, 2010.
- 3- Fernandez-Real JM et al. *Diabetes*, 2010
- 4- Christodoulides et al. *Trends Endo Metab*, 2009
- 6- Medina-Gomez G et al. *Dis Model Mech*, 2009
- 6- Ishii KA et al. *Nat Med*, 2009
- 7- Lagathu et al. *Diabetes*, 2009
- 8- Liesa M et al. *PLoS One*, 2008
- 9- López M et al. *Cell Metab*, 2008.
- 10- Vazquez M et al. *Endocrinology*, 2008
- 11- Medina-Gomez G et al. *PLoS Genet*, 2007.
- 12- Chakravarthy et al. *J Clin Invest*, 2007
- 13- Lelliott CJ et al. *PLoS Biol*, 2006.
- 14- Gray SL et al. *Diabetes*, 2006.
- 15- Medina Gomez G et al. *Nat Med*, 2005

B2 TRAINING

CLARITY AND QUALITY OF THE RESEARCH TRAINING OBJECTIVES

The proposed project will provide the applicant, Maarten Soeters, with a deeper insight in human physiology and more specifically in human energy expenditure. Cold exposure and thermogenesis will be novel areas for him to cover and to expand this current knowledge of human physiology.

The host laboratory and the physiological methods in the CRF specifically analyse human thermogenesis and energy expenditure, including substrate oxidation in great detail. Prof Vidal-Puig's laboratory has an intensive collaboration with Prof. K. Chatterjee and Dr. P Murgatroyd whom are closely involved to ongoing projects in the CRF.

Through this project, Maarten Soeters will be exposed to all these technologies in human physiology. It will be expected that at the end of his training, he will have not only mastered these *in vivo* technologies, but more importantly he will have a thorough understanding of energy homeostasis. Bringing this knowledge back, will put him in an excellent position for an independent career in human physiological science as demonstrated by the careers of previous trainees in Prof. Vidal-Puig's laboratory.

Final report

As discussed above in the sections "Interdisciplinary/multidisciplinary aspects of the proposal" and "APPROPRIATENESS OF RESEARCH METHODOLOGY AND APPROACH" the applicant has succeeded to obtain deeper insight in human physiology and more specifically in human energy expenditure. Although cold exposure and thermogenesis were relative novel areas for him to cover, the first year has already resulted in collaborations within and without the Institute of Metabolic Science and the Clinical Research Facility. Also, he executes the indirect calorimetry studies by now on an autonomous base in terms of bedside work, interpretation and analysis of data.

RELEVANCE AND QUALITY OF THE ADDITIONAL SCIENTIFIC TRAINING

This broad training program should provide the scientific background and technological tools to ensure the progression of the candidate in his scientific career. Additionally, as complementary training, Maarten Soeters will participate in:

a) a weekly meeting with Prof. Vidal-Puig; b) Tuesday weekly data session of the whole group; c) Wednesday weekly journal club; d) Thursday weekly lecture from external scientists at the IMS-MR, topics related to Endocrinology, Diabetes and Metabolism; e) Dunn weekly seminars, lecture series on mitochondrial biology and bioenergetics; f) once a month interdisciplinary meeting amongst the members of Prof Vidal-Puig's (physiology), dr Lilley's (proteomics) and dr Griffin's (metabolomics) laboratories and all members of the Cambridge Systems Biology Centre (CSBC); g) an Annual Integrative Physiology Research Retreat (2 days, usually in November).

Final report

The Institute of Metabolic science is an excellent environment when it comes a broad training program as depicted in the section on "RELEVANCE AND QUALITY OF THE ADDITIONAL SCIENTIFIC TRAINING". The applicant has been able to attend virtually all meeting described here. The interaction with Professor A. Vidal-Puig has been on a regular basis. The applicant has presented his study results during the Professor A. Vidal-Puig group meetings, as well as during the Annual Integrative Physiology Research Retreat". See also the section on "COMPLIMENTARY SKILLS".

COMPLIMENTARY SKILLS

The scientific and transferable skills Maarten Soeters will acquire in Prof Vidal-Puig's laboratory as result of working on this project are:

- 1) Scientific Background on aspects related to energy expenditure, thermogenesis, substrate metabolism and adipose cell biology.
- 2) Technical skills related to intensive human physiology experiments using various *in vivo* technologies. It is expected that he will become an expert at designing and performing meaningful *in vivo* experiments humans in the field of thermogenesis and energy expenditure.
- 3) Management of a research project (time-management, adjustment of the planning of the experiments based on interim analyses of results, supervision of students/laboratory technicians)
- 4) Further development of communication/presentation skills during the work discussions and seminars.
- 5) Further knowledge of and management of the ethical issues regarding experiments with humans.
- 6) Teaching experience (preparing 3-4 seminars per year directed to undergraduates).

The experience in thermogenesis and energy expenditure will be crucial for Maarten Soeters to develop as an expert in metabolic sciences. The techniques used in the planned *in vivo* experiments will extend his repertoire and knowledge of *in vivo* skills in human experiments. Moreover, being able to observe and take part in the daily leading of a large, internationally recognized laboratory will provide him with management skills that will greatly benefit him in his future career. Continuing to develop expertise in teaching will increase his competitiveness when applying for a faculty position at a University.

Final report

Nearly all aspects of the section "COMPLIMENTARY SKILLS" have been covered in the first year and will be deepened/extended during the second year of the fellowship.

1) Scientific Background on aspects: as described in the previous sections the interaction with the basal/clinical scientists within the IMS and the clinical scientists of the Clinical Research Facility have enabled to increase the scientific and (patho)physiologic background of the applicant. Moreover external collaboration/interactions have led to new study plans and projects.

2) Technical skills related to intensive human physiology experiments: since the protocol covers many different techniques (such as indirect calorimetry, heart rate variability, infrared imaging, temperature monitoring etcetera) that the applicant uses, analyses and interprets himself before discussing them in the appropriate meetings, he has been able to learn these techniques quickly and soundly. Moreover both scientists in both IMS and CRF have been of help in on demand troubleshooting and analyses of data that were difficult when required.

3) Management of a research project. Management of the project has occurred so far on different sites (such as IMS, CRF, department of radiology, laboratory of clinical chemistry. This has enabled the applicant to interact with different professionals in a new environment when it came to implementing and planning the project. Moreover supervision of both nursing staff and research assistants on the CRF has been of great value in managing staff directly on the personal interactive level.

4) Further development of communication/presentation skills. The applicant has been presenting his data during the Professor A. Vidal-Puig group meetings, as well as during the Annual Integrative Physiology Research Retreat". Moreover, since data collections of year one are nearly complete, abstracts for (inter)national meetings and manuscripts for publication are being prepared.

5) Further knowledge of and management of the ethical issues. This has not been an important focus since the project was ethically approved when the applicant applied for the research fellowship. However we have paid attention to ethical aspects of the protocols such as the cold exposure and radiation exposure.

6) Teaching experience has not yet been fully covered in terms of undergraduate seminars.

HOST EXPERTISE IN TRAINING EXPERIENCED RESEARCHERS

As previously indicated, Prof. Vidal-Puig's laboratory is integrated in the IMS-MRL and MRCCORD. This is an ambitious programme funded by MRC to promote the training of new investigators in the field of diabetes and the Metabolic Syndrome. Training under this scheme provides a broad spectrum of technological approaches supported by some of the top laboratories in UK. As member of Prof. Vidal-Puig's laboratory, the applicant will have all the advantages derived from this status. Maarten Soeters's training will be supervised by Prof. Vidal-Puig, who has been very successful at attracting and training very talented PhD students (n=9) supported by the MRC, Wellcome Trust, and BBSRC, and 21 postdoctoral fellows from around the world supported by competitive fellowships (including Marie Curie fellowships, MRC, BHF and other international fellowship schemes from Canada, Germany, Italy, Japan, Mexico, Spain and Switzerland). His mentoring commitment includes scientific guidance, career advice, networking opportunities to facilitate access to academic positions, and technical and scientific support through the early, crucial years as an independent PI.

His trainees all have remained in science. Eleven have become independent PIs in reputed universities in Europe, Canada and Thailand, and four are working as senior scientists in pharma and biotech companies (e.g. AZ, Bayer and Illumina). The others are still in training in highly competitive labs. Prof. Vidal-Puig is strongly committed to the academic development of all his trainees by sponsoring them to obtain international awards and help raise their international profile by proposing them as speakers at reputed meetings. More importantly he continues to have strong collaborative links with previous trainees as valued colleagues (Lopez, Medina-Gomez, Gray, Prieur).

The possibility that Maarten Soeters intended to join Prof. Vidal-Puig's laboratory was raised after meeting him at a scientific meeting. This initial contact was strengthened after a short visit to the Institute of Metabolic Science. The visit was very useful since it allowed the applicant to experience the laboratory at first hand. Since this visit, the applicant has been visiting Prof. Vidal-Puig on a few occasions to discuss the proposal with him and the involved staff.

Prof. Vidal-Puig maintains close supervision of his laboratory. Having his office linked to the laboratories and near the Clinical Research Facility, he is easily accessible, which facilitates quick feedback to problems and questions. On top of this, Prof. Vidal-Puig holds weekly laboratory meetings, where lab members have the opportunity of present their most recent data and discuss them in an informal yet critical way. The atmosphere in Prof. Vidal-Puig's laboratory is very professional but friendly and all lab members are willing to help and encourage each other to excel in their work. Members of Prof. Vidal-Puig's laboratory develop a strong sense of identification and pride to be a member of this laboratory. This sense of belonging to a team is enhanced by events such as the annual Integrative Physiology Research Retreat lasting two days, where the entire group get together to discuss their work and enjoy each other's company with the other members of the MRC CORD consortium.

B3 RESEARCHER (MAXIMUM 7 PAGES WHICH INCLUDES A CV AND A LIST OF MAIN ACHIEVEMENTS)

RESEARCH EXPERIENCE

The applicant registered as MD in 2001 after which he started his training to become a consultant in internal medicine. In this period he participated in a clinical trial that investigated recombinant C1-esterase inhibitor as therapy in hereditary angioedema. The results of the project were published with the applicant as second author.

Hereafter, he joined the Metabolic Research Group of Prof. Sauerwein and started his PhD on the metabolic response to fasting. This PhD consisted of human translational studies in intermediary metabolism. Maarten acquired many techniques such as tissue biopsies and different methods to assess insulin sensitivity of various metabolic processes. Besides mastering the hyperinsulinemic euglycemic clamp to assess insulin sensitivity of liver and muscle glucose metabolism, he implemented new techniques within the Metabolic Research Group. For instance, he used a low-insulinemic pancreatic clamp (combining insulin, octreotide and glucagon) to investigate insulin sensitivity of ketone body production. To do so, he set up the stable isotope method infusing $D[2,4-^{13}C_2]$ -3-hydroxybutyrate in humans as well as the gas chromatography–mass spectrometry method to assess isotopic enrichment of the ketone bodies hydroxybutyrate and acetoacetate. His PhD project resulted in six first-author publications in well ranked journals such as the *Journal of Clinical Endocrinology and Metabolism* and the *American Journal of Clinical Nutrition* (see publications).

After obtaining his PhD he completed his training to become an endocrinologist. In the meanwhile he finished a combined project with the Universities of Leiden and Rotterdam on the relevance of deiodinase type 2 (converting inactive thyroid hormone to active thyroid hormone) in glucose metabolism during fasting. This paper (shared first authorship) was published in the *Journal of Endocrinology and Metabolism*.

RESEARCH RESULTS-MAJOR ACHIEVEMENTS

During his PhD, Maarten Soeters investigated the metabolic adaptation to fasting because investigating pathways that are involved in protecting the body from energy depletion, may provide us with answers on the metabolic adaptation to overfeeding. Three examples of findings are outlined below:

1- Women may be protected from FFA-induced insulin resistance.

This project showed that during fasting, women are relatively protected from FFA-induced insulin resistance possibly by preventing myocellular accumulation of ceramide compared to matched male volunteers. *Journal of Clinical Endocrinology and Metabolism* 2007, cited 20+ times.

2- Fasting induces physiological insulin resistance.

Maarten Soeters investigated peripheral insulin sensitivity together with muscle ceramide concentrations and protein kinase B/AKT phosphorylation after short-term fasting in male volunteers. The results showed that intramuscular ceramide concentrations tend to increase during fasting with decreased phosphorylation of protein kinase B/AKT at serine⁴⁷³. This makes insulin resistance a physiological phenomenon. In this same project he found that muscle long-chain acylcarnitines do not unconditionally reflect fatty acid oxidation. The changes in substrate metabolism (higher fatty acid oxidation under hyperinsulinemic conditions after fasting) suggest adaptation in fatty acid oxidation with a different insulin-regulated set point. *Journal of Clinical Endocrinology and Metabolism* 2008, *Clinical Science* 2009, together cited 15+ times.

3- Type 2 deiodinase is relevant for glucose metabolism in humans.

The applicant also showed that insulin levels modulate muscle type 2 deiodinase (converting inactive thyroid hormone to active thyroid hormone) expression during fasting. In the same project we found that the thyroid state does not influence 2 deiodinase. This makes type 2 deiodinase more important in glucose metabolism opposed to thyroid hormone metabolism. *Journal of Clinical Endocrinology and Metabolism* 2009, cited 17 times.

CURRICULUM VITAE

Personal information

Name: Maarten Soeters

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Telephone number: +31624660202

Marital status: married

Children: 4.

EDUCATION AND RESEARCH EXPERIENCE

2002-2010 Six year residency in Internal Medicine training (AMC) including a 2-year PhD track (PhD thesis 11-09-2008) and a 2 year fellowship in Endocrinology and Metabolism (certified internist-endocrinologist 01-04-2010)

1993-2001 Medical training, University of Amsterdam (registered MD 21-12-2001)

After his PhD, Maarten Soeters is responsible for 1 PhD student (see “awards”) who works on a research project in the field of glucose and lipid (acylcarnitine) metabolism in healthy volunteers and in patients, using stable isotope dilution in combination with molecular studies in human biopsy material and animal fatty acid oxidation models in collaboration with Dr. Sander M. Houten. The human studies are carried out in the dedicated Metabolic Research Facility (MRF) of the Academic Medical Center.

Additionally, Maarten Soeters is working on a project that will investigate the effects of bile acids on muscle, liver and incretin biology. In this project he uses mixed meal tests, indirect calorimetry, muscle biopsies and plasma measurements (including two point bile acid stable isotope technique) to assess the postprandial role of bile acids in energy metabolism. This project fits particularly well to this proposal since postprandial bile acids may stimulate BAT via TGR5 and type 2 deiodinase expression.

The MRF in the Academic Medical Center is embedded in a structural research setting including the Laboratory of Experimental Endocrinology that is renowned both for molecular studies in the metabolic field and for stable isotope studies.

SPECIALISATION COURSES

2008: Good Clinical Practice Course, Amsterdam, the Netherlands

2009: FRANK course (human clinical nutrition), Bad Homburg, Germany

2010: Intensive Stable Isotope Course, Stockholm, Sweden

TECHNICAL EXPERIENCE AND SKILLS

-stable isotope technique

-hyperinsulinemic one step/2 step clamps

-pancreatic clamps

- mixed meal tests
- muscle/adipose tissue biopsies
- designing human studies, including obtaining medical ethical permission
- indirect calorimetry

PUBLICATIONS (12 international publications of which nine original articles and one review)

- 1- Luijck YM, Hermanides J, Serlie MJ, Hoekstra JB, **Soeters MR**. The added value of oral glucose tolerance testing in pre-diabetes. *Curr Diabetes Rev* 2011 Jan;7(1):56-60.
- 2- Sommeijer DW, Ten WM, von der Thusen JH, Huidekoper HH, Van Lieshout JJ, **Soeters MR**. When nausea becomes a tricky question. *Eur J Obstet Gynecol Reprod Biol* 2011 Jan;154(1):116-8.
- 3- **Soeters MR**, Hoekstra JB, de Vries JH. [HbA1c: target value should remain 7%]. *Ned Tijdschr Geneesk* 2010;154:A2113.
- 4- **Soeters MR**, Huidekoper HH, Duran M, Ackermans MT, Endert E, Fliers E, et al. Extended metabolic evaluation of suspected symptomatic hypoglycemia: the prolonged fast and beyond. *Metabolism* 2010 Nov;59(11):1543-50.
- 5- Bolmers MD, Linthorst GE, **Soeters MR**, Nio YC, Van Lieshout JJ. Green urine, but no infection. *Lancet* 2009 Oct 31;374(9700):1566.
- 6- **Soeters MR**, Lammers NM, Dubbelhuis PF, Ackermans M, Jonkers-Schuitema CF, Fliers E, et al. Intermittent fasting does not affect whole-body glucose, lipid, or protein metabolism. *Am J Clin Nutr* 2009 Nov;90(5):1244-51.
- 7- **Soeters MR**, Serlie MJ. [Oral glucose tolerance test. Invaluable in screening of type 2 diabetes]. *Ned Tijdschr Geneesk* 2009 Apr 18;153(16):742.
- 8- **Soeters MR**, Sauerwein HP, Faas L, Smeenge M, Duran M, Wanders RJ, et al. Effects of insulin on ketogenesis following fasting in lean and obese men. *Obesity (Silver Spring)* 2009 Jul;17(7):1326-31.
- 9- Heemstra KA, **Soeters MR***, Fliers E, Serlie MJ, Burggraaf J, van Doorn MB, et al. Type 2 iodothyronine deiodinase in skeletal muscle: effects of hypothyroidism and fasting. *J Clin Endocrinol Metab* 2009 Jun;94(6):2144-50. *Equally contributed.
- 10- **Soeters MR**, Sauerwein HP, Duran M, Wanders RJ, Ackermans MT, Fliers E, et al. Muscle acylcarnitines during short-term fasting in lean healthy men. *Clin Sci (Lond)* 2009 Apr;116(7):585-92.
- 11- **Soeters MR**, Sauerwein HP, Dubbelhuis PF, Groener JE, Ackermans MT, Fliers E, et al. Muscle adaptation to short-term fasting in healthy lean humans. *J Clin Endocrinol Metab* 2008 Jul;93(7):2900-3.
- 12- **Soeters MR**, Sauerwein HP, Groener JE, Aerts JM, Ackermans MT, Glatz JF, et al. Gender-related differences in the metabolic response to fasting. *J Clin Endocrinol Metab* 2007 Sep;92(9):3646-52.
- 13- Choi G, **Soeters MR**, Farkas H, Varga L, Obtulowicz K, Bilo B, et al. Recombinant human C1-inhibitor in the treatment of acute angioedema attacks. *Transfusion* 2007 Jun;47(6):1028-32.

SCIENTIFIC MEETINGS (thirteen international presentations of which three oral).

- 1/2- Benefit of Insulin Resistance, ESPEN intensive course on NUTRITION, European Society for enteral and parenteral nutrition, oral, 2010/2011.
- 3- Intermittent fasting increases peripheral insulin sensitivity with no effects on hepatic glucose output at physiological plasma insulin levels, Endocrine Society Meeting, 2008, oral.
- 4- Gender related differences in the metabolic response to fasting, Endocrine Society, 2007, poster

- 5- Retinol Binding Protein 4 is not related to glucose metabolism during fasting, Endocrine Society, 2007, poster.
- 6- Retinol Binding Protein 4 is not related to glucose metabolism during fasting, European Association for the Study of Diabetes, 2007, poster.
- 7- Retinol Binding Protein 4 is not related to glucose metabolism during fasting, American Diabetes Association, 2007, poster.
- 8- The relation of intramyocellular 3-hydroxybutyryl-carnitine to ketone and glucose Metabolism during short-term fasting in lean and obese men. American Diabetes Association, 2008, poster.
- 9- Decreased suppression of muscle fatty acid oxidation by hyperinsulinemia during fasting, American Diabetes Association, 2008, poster.
- 10- Insulin-stimulated phosphorylation of muscle AKT/protein kinase B - serine473 is impaired in healthy fasting humans, American Diabetes Association 2008, poster.
- 11- The relation of intramyocellular 3-hydroxybutyryl-carnitine to ketone and glucose Metabolism during short-term fasting in lean and obese men, Endocrine Society, 2008, poster.
- 12- Decreased suppression of muscle fatty acid oxidation by hyperinsulinemia during fasting. Endocrine Society, 2008, poster
- 13- Insulin-stimulated phosphorylation of muscle AKT/protein kinase B - serine473 is impaired in healthy fasting humans, Endocrine Society, 2008, poster.

Over 20 oral presentations on national scientific meetings (Dutch Study Group for Diabetes (NVDO), Dutch Society of Internists (NIV)).

TEACHING EXPERIENCE

- 1- Hall lectures first year medical students on general internal medicine, Academic Medical Center, University of Amsterdam, 2009-2010 and 2010-2011.
- 2- Hall lectures first year medical students on endocrinology, Academic Medical Center, University of Amsterdam, 2011.
- 3-Small scale endocrinology lectures thyroid and pituitary, Academic Medical Center, University of Amsterdam, 2008-2011.
- 4-Clinical skills students (2006-2011), residents (2009-2011) and registrars (2010-2011).
- 5-Endocrinology Course Master Biology Students, University of Amsterdam, 2010.
- 6-Training general practitioners in diabetes, metabolism and hypoglycaemia in the Netherlands (2008-2011).
- 7-Training energy requirements dieticians (3 day training program, Fresenius-Kabi), 2011.
- 8-Head of the Module Endocrinology, Curius Study Program, first year medical students, University of Amsterdam, 2010-2011

AWARDS

Maarten Soeters received the following grants:

- 1-Grant for a project on "Acylcarnitines in the pathophysiology of insulin resistance"; 4 year PhD project.
- 2-Tjallingh Roorda Grant, 8000 euro on "Quantification of fatty acid oxidation disorders: carnitine and acylcarnitine metabolism".
- 3-Dekker Bouwman Foundation, 9900 euro on "The role of type 2 deiodinase in the regulation of insulin sensitivity of muscle and adipose tissue".
- 4-Ruitinga van Swieten Foundation, Van Leeuwen Stipendium, 10.000 euro on "post-prandial bile acid & incretin response in patients with resistance to thyroid hormone".

INDEPENDENT THINKING AND LEADERSHIP QUALITIES

During his PhD training Maarten was able to think beyond the initial design of his project. He has performed translational studies in fasting subjects for which he wrote the protocols himself. This has led to additional papers during his PhD that were not planned on forehand. During these studies he excelled at motivating both subjects and potential collaborators to participate in the invasive studies including biopsies.

The fact that Maarten did his PhD in over just 2 years emphasizes the ambition and mind to work hard to become an independent researcher with his own group focussing on topics in human energy metabolism.

During his clinical training and PhD project, Maarten Soeters has supervised many medical students. He has the ability to explain complicated matters in a clear manner, without losing sight of the level of experience of the student. Moreover he is capable to make students feel comfortable. In that way he can stimulate them to reach for the best. This brought students to stay and start projects. In doing so, he creates confidence but not without being critical. Maarten's natural leadership qualities have enabled him to instruct and supervise both the research nurses during the clinical studies as well as the medical students. He communicates clearly what the goal of a study is and what needs to be done. His style of leadership is consensual and he is always open to suggestions from those who work with him.

MATCH BETWEEN THE FELLOW'S PROFILE AND THE PROJECT

The proposed project requires an understanding of human metabolism, physiology and human experiments. As a researcher with human experience *and* a clinical background, Maarten Soeters is an ideal candidate because he has worked in this area of human translational research.

POTENTIAL FOR REACHING A POSITION OF PROFESSIONAL MATURITY

After having broadened his experience as a researcher by fulfilling the post-doctoral position such as described in this proposal, the applicant is ready to start his own line of research in human metabolism. Both Professor Eric Fliers (head of the department of endocrinology and metabolism) and Professor Hans Romijn (head of the department of general internal medicine) of the Academic Medical Center, consider Maarten Soeters to possess all the qualities, such as intelligence, perseverance, leadership abilities and creativity, to fulfil this academic career.

In due time, he is expected to become a principle investigator. Being a clinician and a scientist with thorough knowledge of human research, experienced in human metabolism and physiology, puts him in a unique position to perform translational research at its best. He is expected to acquire a position in an academic hospital, bringing science from bench to bedside.

POTENTIAL TO ACQUIRE NEW KNOWLEDGE

Maarten showed the ability to very rapidly internalize both new knowledge and skills in his PhD. The wide range in both topics and methodology in his PhD project is a demonstration of this remarkable talent. It is therefore expected that he will also familiarize himself quickly with the techniques used in Cambridge and be able to take this knowledge back to the Netherlands to be used in his future research.

B4 IMPLEMENTATION

QUALITY OF THE INFRASTRUCTURES/FACILITIES AND INTERNATIONAL COLLABORATIONS OF THE HOST

The IMS-MRL Center has state-of-the-art facilities for molecular and cellular biology studies and hosts several core facilities including mouse biochemistry, histology, genomics microarrays, proteomics, metabolomics and bioinformatics).

My sponsor, Prof. Vidal-Puig is the Scientific Director of the Phenomics Center, a state of the art murine physiology core facility supported by MRC CORD and the Wellcome Trust. The Phenomic Center consists of animal facilities for cardiometabolic phenotyping including unique facilities for thermoregulation studies, energy balance studies and studies on aspects related to the metabolic syndrome (e.g. 16 cages CLAM system for continuous monitoring of food intake, energy expenditure, activity and drinking behaviour, which has recently been put into use).

This is of particular relevance because the basic research related to this proposal on brown adipose tissue can be translated to the human situation. The laboratory of Prof. Vidal-Puig is closely connected with the Wellcome Trust Clinical Research Facility. Here, extensive phenotyping of human genetic disorders is performed by close collaborators from within the IMS such as Prof. Krishna Chatterjee (resistance to thyroid hormone), Dr. David Savage (human models of monogenetic insulin resistance) and Prof. Steve O'Rahilly (monogenetic causes of obesity). Also Prof Vidal-Puig collaborates with Dr. Peter Murgatroyd for the design and analysis of the human physiological studies.

As previously indicated, Prof. Vidal-Puig is an investigator attached to the MRC CORD and to the Consortium Cambridge-Oxford (Cam-Ox) for Integrative Physiology (<http://www.int-phys.org/>). This is an ambitious new programme funded by Wellcome Trust to facilitate the transition from basic research to in vivo models. As member of one of the laboratories of this Consortium, Maarten Soeters will have all the advantages derived from this status. As member of this consortium the fellow will have access to all the technologies available within the other consortium laboratories, for example in the biochemistry and physiology core.

From the publication record of Vidal-Puig's group it is clear that this group holds formal and informal national and international collaborations with top laboratories in Europe and overseas. These include, but are not limited to, Prof. Matej Orešič (VTT Finland), Prof. Parker MG (Imperial College London, London, UK); Cortright RN (Brody School of Medicine, East Carolina, USA); Dale Abel (University of Utah, Utah, USA); Cinti S (Institute of Anatomy, Ancona, Italy); Cannon B (Wenner-Gren Institute, Stockholm, Sweden); Prof. Johannes Klein (Lubeck, Germany); Bernard Thorens (Department of Internal Medicine and the Botnar center of Clinical Research, Lausanne, Switzerland); Prof. Urade (Osaka University, Osaka, Japan).

Prof. Vidal-Puig is involved in several European networks including Hepadip FP6 consortium for the study of adipose tissue and metabolic diseases (<http://www.hepadip.org>) that coordinates the scientific programmes of 17 groups distributed through Europe and FP7 Etherpaths and PF7 MITIN. This will allow Maarten Soeters to benefit not only from the scientific opportunities, but also from a very active programme of exchanges to learn technologies. For instance over the last two years members of Prof. Vidal-Puig's laboratory have spent time in the laboratories of Bernard Thorens (Switzerland), Barbara Cannon (Sweden), Remy Burcelin (France), Albert Emmanuel Geerts (Belgium) and Matej Orešič (Finland).

PRACTICAL ARRANGEMENTS FOR THE IMPLEMENTATION AND MANAGEMENT OF

THE PROJECT

For the management of this project, progress, results and long- and short-term strategies will be discussed with Prof. Vidal-Puig and the other members of the laboratory/WTCRF at regular weekly meetings. At each of the meetings the applicant will produce a summary of the outcomes of the discussion identifying the specific conclusions. This type of documentation has proved to be very helpful to register the progress of the project and to help to confirm that the researchers involved in the discussion have reached to similar conclusions. These documents serve as the base for subsequent discussions. Therefore these group meetings allow Maarten Soeters to present and discuss latest results in an informal manner and can be used as a report of progress. As previously indicated, a wide programme of molecular endocrinology lectures/seminars is available in the Campus to reinforce and stimulate his own research. When necessary, meetings and discussions with experts outside Prof. Vidal-Puig's laboratory will be organised, usually Thursdays mornings, to provide appropriate advice on any issue.

FEASIBILITY AND CREDIBILITY OF THE PROJECT

The feasibility of this project is supported by:

1-The fact that the project has clearly stated main objectives:

- A)** *To assess differential effects of BAT activating stimuli (cold, activation of TRPA1 and/or TRPV1) on thermogenic response, BAT, glucose/lipid metabolism and cardiovascular changes in healthy lean individuals.*
- B)** *To test energy expenditure inducing protocols (derived from objective A) in obese/insulin resistant subjects with respect to thermogenic response, BAT, glucose/lipid metabolism and cardiovascular changes.*
- C)** *Develop infrared thermography to be used repetitively in humans for accurate measurement of the thermogenic response of BAT.*

2-The fact that the techniques necessary for the translational experiments in human thermogenesis are available at the laboratory is shown by the already present pilot data. This will also positively affect the time planning of the proposal in terms of technology, design and ethical approval. This means that the human experiments and analyses will run during the entire two years of the grant. Recruitment of volunteers is executed by the staff of the WTCRF by advertisements (posters, magazines, newspapers and the internet). This recruitment has been very effective in finding suitable subjects during previous projects.

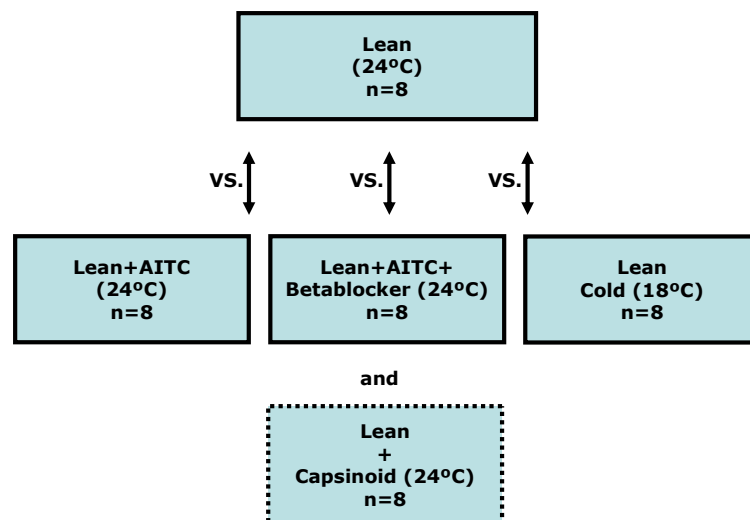
WORKPLAN

As depicted below (figure 4): the planning of the proposal follows the objectives. In objective A, human studies in healthy volunteers will start to assess the effects of cold, AITC (with and without betablockade) and capsinoids on energy expenditure and BAT. The acquired pilot data permit a swift start of the project when the applicant starts the fellowship.

Power calculation: The number of human experiments in objective A is 5 times 8 volunteers. The number of volunteers is based on paired (dependent) observations. Based on within subject variation of ~4% of the indirect calorimetry, a minimal difference to detect of 5% (from 1800kcal per day = 90 kcal/day), an alpha of 0.05 and a power of 0.80, we have calculated a power of 6 subjects, but we intend to minimally include 8 subjects per arm.

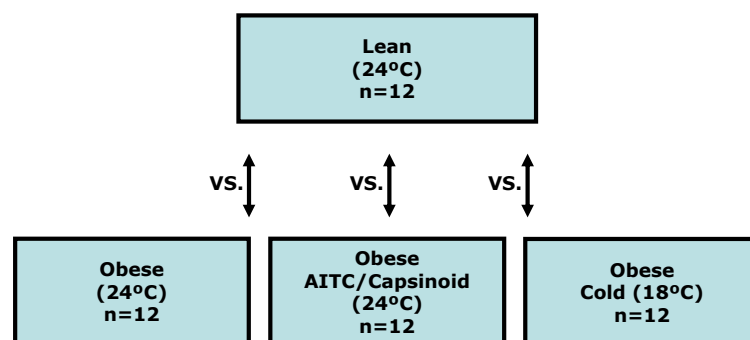
Volunteers that participate in the AITC studies also participate in the capsinoid arm: hence we expect a minimum of 40 human experiments for objective A (see figure 4a for overview).

Figure 4a, overview objective A



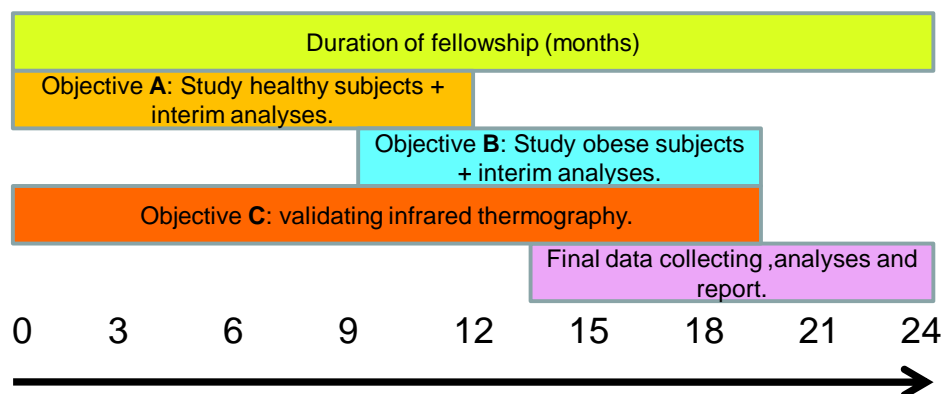
Objective B, the effects of BAT activating protocols on energy expenditure and BAT requires 3 times 12 lean and 12 obese subjects equalling 48 experiments to perform the planned amount of experiments (non-paired observations, see figure 4b for overview).

Figure 4b, overview objective B



Objective C, the validation of thermoimaging against FDG-PET CT will be performed simultaneously within objectives A and B. Interim analyses will permit a direct transition from objective A to B and the final analyses and report. The whole fellowship is depicted in Figure 4c specifying how the objectives are planned within the two years.

Figure 4c, planning fellowship.



PRACTICAL AND ADMINISTRATIVE ARRANGEMENTS

The financial management of the fellowship will be carried out by the administrative office at Cambridge University. This office is well experienced in the administration of national and European programmes including the Marie Curie Intra-European Fellowships. University of Cambridge (UCAM) can offer and facilitate all the necessary arrangements for the settlement of Maarten Soeters and his family in Cambridge. Moreover, UCAM has a wide lodging network in the Cambridge area. Furthermore, the administration services of the University will provide advice to Maarten to sort out any administrative issues that could arise during his stay at Cambridge. Excellent primary schools are situated near the Addenbrooke's hospital premises.

B5 IMPACT

POTENTIAL OF ACQUIRING COMPETENCIES

At the present stage of his research career, the applicant possesses a strong knowledge of metabolism in general and lipid metabolism especially and has performed extensive studies in human translational studies. During his post-doctoral experience he will gain specifically knowledge on thermogenesis and energy metabolism the field of human physiology through a transnational mobility of 24 months.

The proposed subject, aimed at developing protocols to stimulate thermogenesis and BAT activation will not only allow the applicant to gain new knowledge and know-how but also to use his past clinical/translational expertise in a new field. Such experience will definitely yield the applicant a recognised professional maturity and an independent way of thinking. Moreover, the increasing expertise acquired first during his PhD, clinical experience and then along a post-doctoral experience, is definitely an invaluable passport for the applicant's future career. This fellowship also will provide important transferable experimental skills as well as management skills, leadership skills, improvement of scientific writing and proficiency in English language. This will greatly contribute to his future as a independent professional research.

CONTRIBUTION TO CAREER DEVELOPMENT AND RE-ESTABLISHMENT

The applicant pursues a career in academia, where he aims to reach a position as an independent principal investigator of his own research group in the field of human metabolism, obesity and diabetes. The subject chosen is partly new and partly known, permitting deepening of his existing knowledge and experience as well as broaden his scope, specifically in the field of human physiology and in calorimetry studies.

As the host institution is one of the leading laboratories in Europe with respect to this subject, it is the applicant's opinion that this is the best choice for this specific project and the best choice in view of the development of his career.

The goal of Maarten Soeters is to apply the knowledge of and experience with novel and high-level innovative human physiological research techniques (that he will have acquired in Cambridge) in The Netherlands.

He strives after a position in which he will be able to integrate this experience in translational science with clinical science, to contribute to the development of strategies to combat the development and consequences of obesity.

His building experience in translational research, combined with the experience acquired during this fellowship, will provide is with an unique deepened research scope when it comes to well designed through human studies.

It is expected that he will attain a position at an academic hospital to establish his own line of research. The new contacts made during the project, both national and international, will contribute to building up a research group that is actively involved in this international field. The fact that he will have to integrate in the English research environment will help the applicant to understand and overcome the difficulties arising from international interactions. This experience will be invaluable to succeed in becoming a group leader with an international profile.

In view of the above, we are convinced that this project and the program of training represent an excellent value at this point in his career, and we are confident that they will greatly contribute to his further development.

CONTRIBUTION TO EUROPEAN EXCELLENCE AND EUROPEAN COMPETITIVENESS

As the incidence of obesity and its associated morbidities, such as type 2 diabetes, increases, the search for new treatment modalities to combat these conditions continues. Fundamental understanding of the metabolic processes that go amiss in obese individuals increases the possibility of finding a successful treatment for this condition. This treatment would reduce the medical costs of obesity and associated diseases that currently pose a huge and growing financial burden on national resources. The ability to increase energy expenditure resulting in net energy loss is of utmost importance for weight management. BAT, as depicted throughout this proposal, may be a promising tissue to facilitate an increase in energy expenditure.

This proposal aims mainly at translational research. The objective of the proposal is to define protocols increasing energy expenditure via cold, AITC or capsinoids may result finally in new targets for the treatment of obesity and its associated pathology with concomitant increase in fundamental knowledge.

BENEFIT TO THE EUROPEAN RESEARCH AREA

The Marie Curie fellowship will enable the previously established contact between Maarten Soeters and Prof. Vidal-Puig to develop into a research collaboration. This cooperation between the Dutch applicant and the English host institution is expected to be very fruitful, contributing to the improvement of both the applicant and the host institution and in general contributing to the European competitiveness and innovation in this research field. It can lead to close collaboration in the future, which would increase efficiency and synergy in European research programmes and contributes to the development of knowledge. Thus, this cooperation will participate to the structuring of the European Research Area (ERA). It will establish new relationships and reinforce the contact between research groups. It will lead to the spread of knowledge, especially of knowledge regarding the available tools for metabolic investigations, to other disciplines such as clinical and research medicine. This will increase the dynamism of research in the metabolic field and will benefit research in life sciences for health within the European Community. Other expected achievements of the project are its influence through the scientific publications on the molecular approach to diseases and the translation of scientific research results into practice.

IMPACT OF THE PROPOSED OUTREACH ACTIVITIES

The results of the proposal outlined in this application will be spread via non scientific and scientific communication. Non-scientific communication of results via other media (internet, journals, magazines) will be used when applicable. The host department/university as well as the department/university where the applicant originates from, have all well designed and kept to date news websites:

The University of Cambridge: <http://www.cam.ac.uk/research/category/news/>

Addenbrooke's Hospital: http://www.cuh.org.uk/research/news/news_index.html

University of Amsterdam: <http://www.english.uva.nl/news/news.cfm>

Academical Medical Center: <http://www.amc.nl/>, & www.twitter.com/AMC_Amsterdam

Data will be presented at national and international meeting such as the annual meetings of the American Diabetes Association, European Association for the Study of Diabetes and the Endocrine Society. These meetings attract large amounts of visitors (e.g. 7000-10,000 visitors per meeting) from various countries throughout the world. Publishing in peer reviewed scientific journals is an important goal. The applicant and host have both a record in high impact factor publishing.

B6 ETHICAL ISSUES

INFORMED CONSENT

As depicted above, we will perform studies in healthy volunteers will start to assess the effects of cold, AITC and capsinoids on energy expenditure and BAT. Possible volunteers will be recruited via advertisements. They will be informed orally and receive an information brochure. Participation is voluntarily and participation can be withdrawn by the participant without giving details of the reason. The consent form is added (*page 25*).

Medical ethical approval will be obtained for ALL protocols, in fact data presented above were collected under protocols that have been approved by the medical ethical committee of the Addenbrooke's Hospital.

Patients and volunteers will be offered compensation for travel or allowance expenses as applicable.

The ethical issues table is included below as applicable (*page 26*). Parts of experiments that are invasive or may cause inconvenience (e.g.: cold exposure, intravenous cannula for bloodsampling, FDG-PET CT (radiation), beta-blocker administration (dizziness, low bloodpressure)) will all be dealt with specifically during information sessions and within the information brochure.

PRIVACY AND DATA MANAGEMENT / USE OF HUMAN BIOLOGICAL SAMPLES AND DATA

Data management will be performed following direction and rules of Addenbrooke's Hospital Medical Ethical Committee and national UK guidelines as applicable. This holds also for the storage and analyses of human biological samples and data.

CONSENT FORM

LREC/COREC Reference Number: xx/xxxx/xxx

Title of project: STIMULATION OF ENERGY EXPENDITURE AND BROWN
ADIPOSE TISSUE IN HUMANS

Name of Lead Investigator: A. Vidal-Puig

Please tick box

I confirm that I have read and understood the information sheet for the
above study and have had the opportunity to ask questions

☐

I understand that my participation is voluntary and I am free to withdraw
at any time, without giving reason and without my medical care or legal
rights being affected.

☐

I agree to take part in the study

☐

I agree to my General Practitioner being informed of my participation

☐

Name of Research Subject (*please print*)

Date

Signature

Name of Witness (*please print*)
(Must **not** be member of research team)

Date

Signature

Name of research team member (*please print*)

Date

Signature

2 copies required: one copy for researcher, one copy for participant

ETHICAL ISSUES TABLE

Research on Human Embryo/ Foetus		YES	Page
*	Does the proposed research involve human Embryos?		
*	Does the proposed research involve human Foetal Tissues/ Cells?		
*	Does the proposed research involve human Embryonic Stem Cells (hESCs)?		
*	Does the proposed research on human Embryonic Stem Cells involve cells in culture?		
*	Does the proposed research on Human Embryonic Stem Cells involve the derivation of cells from Embryos?		
I CONFIRM THAT NONE OF THE ABOVE ISSUES APPLY TO MY PROPOSAL		x	

Research on Humans		YES	Page
*	Does the proposed research involve children?		
*	Does the proposed research involve patients?		
*	Does the proposed research involve persons not able to give consent?		
*	Does the proposed research involve adult healthy volunteers?	x	5-7
	Does the proposed research involve Human genetic material?		
	Does the proposed research involve Human biological samples?	x	5-7
	Does the proposed research involve Human data collection?	x	5-7
I CONFIRM THAT NONE OF THE ABOVE ISSUES APPLY TO MY PROPOSAL			

Privacy		YES	Page
	Does the proposed research involve processing of genetic information or personal data (e.g. health, sexual lifestyle, ethnicity, political opinion, religious or philosophical conviction)?		
	Does the proposed research involve tracking the location or observation of people?		
I CONFIRM THAT NONE OF THE ABOVE ISSUES APPLY TO MY PROPOSAL		x	

Research on Animals		YES	Page
	Does the proposed research involve research on animals?		
	Are those animals transgenic small laboratory animals?		
	Are those animals transgenic farm animals?		
*	Are those animals non-human primates?		
	Are those animals cloned farm animals?		
I CONFIRM THAT NONE OF THE ABOVE ISSUES APPLY TO MY PROPOSAL		x	

Research Involving Developing Countries		YES	Page
	Does the proposed research involve the use of local resources (genetic, animal, plant, etc)?		
	Is the proposed research of benefit to local communities (e.g. capacity building, access to healthcare, education, etc)?		
I CONFIRM THAT NONE OF THE ABOVE ISSUES APPLY TO MY PROPOSAL		x	

Dual Use		YES	Page
	Research having direct military use		
	Research having the potential for terrorist abuse		
I CONFIRM THAT NONE OF THE ABOVE ISSUES APPLY TO MY PROPOSAL		x	

ENDPAGE

PEOPLE

MARIE CURIE ACTIONS

Intra-European Fellowship (IEF)

Call: FP7-PEOPLE-2011-IEF

PART B

“SEE BAT”

STIMULATION OF ENERGY EXPENDITURE AND BROWN ADIPOSE TISSUE IN
HUMANS