agence d'évaluation de la recherche
et de l'enseignement supérieur

Department for the evaluation of research units

AERES report on Unit :

Prévention et traitement de la perte protéique musculaire en situation de résistance à l'anabolisme

PRETRRAM

Under the supervision of the following institutions and research bodies:

Université Paris Descartes

December 2012

agence d'évaluation de la recherche
et de l'enseignement supérieur

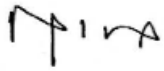
Research Units Department

President of AERES

Didier Houssin

Research Units Department

Department Head



Pierre Glaudes

The president of AERES "signs[...], the evaluation reports, [...]countersigned for each evaluation department by the relevant head of department (Article 9, paragraph 3, of french decree n°2006-1334 dated 3 november 2006, revised).

Grading

Once the visits for the 2012-2013 evaluation campaign had been completed, the chairpersons of the expert committees, who met per disciplinary group, proceeded to attribute a score to the research units in their group (and, when necessary, for these units' in-house teams).

This score (A+, A, B, C) concerned each of the six criteria defined by the AERES.

NN (not-scored) attached to a criteria indicate that this one was not applicable to the particular case of this research unit or this team.

Criterion 1 - C1: Scientific outputs and quality;
Criterion 2 - C2: Academic reputation and appeal;
Criterion 3 - C3: Interactions with the social, economic and cultural environment;
Criterion 4 - C4: Organisation and life of the institution (or of the team);
Criterion 5 - C5: Involvement in training through research;
Criterion 6 - C6: Strategy and five-year plan.

With respect to this score, the research unit concerned by this report received the following grades:

● Grading table of the unit: Prévention et traitement de la perte protéique musculaire en situation de résistance à l'anabolisme

C1	C2	C3	C4	C5	C6
A	A	A+	A+	A	B

Evaluation report

Unit name:	Prévention et traitement de la perte protéique musculaire en situation de résistance à l'anabolisme
Unit acronym:	PRETRRAM
Label demandé :	EA
Present no.:	4466
Name of Director (2012-2013) :	Mr Luc CYNOBER
Nom du porteur de projet (2014-2018) :	Mr Jean-Pascal DE BANDT

Expert Committee members

Chair :	Ms Martine LAVILLE, Université Lyon 1
Experts :	Mr Pierre FAFOURNOUX, Université de Clermont-Ferrand
	Mr Moncef GUENOUNOU, Université de Reims-Champagne-Ardenne
	Mr Gilles MITHIEUX, Université de Lyon
	Mr Francois STZARK, Université Bordeaux Segalen
	Mr Luc TAPPY, Université de Lausanne, Suisse

Scientific delegate representing the AERES:

Mr Jean GIRARD

Representative(s) of the unit's supervising institutions and bodies:

Mr Stefano MARULLO, Université Paris Descartes

1 • Introduction

EA 4466 (Stress Cellulaire : Physiopathologie, stratégies nutritionnelles et thérapeutiques innovantes**)** was previously the result of the merging of EA 2408 (Processus d'adaptation métaboliques et nouvelles stratégies nutritionnelles) and EA 3617 (Biochimie radicalaire et atteintes vasculaires et hépatiques). In the new project, only a part of the actual EA 4466 is going on with participants coming from the former EA 2498 and part of EA 3617. Mr Luc CYNOBER who was in charge of the unit for many years thought it was time for a change of head and Mr. Jean-Pascal DE BANDT has been proposed as the director for the new project.

The unit is situated at the « Faculté des Sciences Pharmaceutiques et Biologiques, Université Paris Descartes. 1020 m^2 of research surfaces are devoted to this unit. The unit has also access to platforms from the IFR and of the university.

AERES nomenclature

SVE1_LS4

Unit workforce

Unit workforce	Number as at 30/06/2012	Number as at 01/01/2014	2014-2018 Number of project producers
N1 : Permanent professors and similar positions	8 (FTE 2.76)	7 (FTE 2.34)	7
N2 : Permanent researchers from Institutions and similar positions			
N3 : Other permanent staff (without research duties)	7 (FTE 2)	7 (FTE 2)	7
N4 : Autres enseignants-chercheurs (PREM, ECC, etc.)			
N5 : Other researchers from Institutions (Emeritus Research Director, Postdoctoral students, visitors, etc.)	6 (FTE 4.43)	6 (FTE 5.2)	2
N6 :Other contractual staff (without research duties)	5 (FTE 5.0)	5(FTE 5.0)	2
TOTAL N1 à N6	**26 (FTE 14.19)**	**25 (FTE 14.55)**	**16 (+ 2 N6)**

Percentage of producers	***100 %***

Unit workforce	Number as at 30/06/2012	Number as at 01/01/2014
Doctoral students	1	
Theses defended	12	
Postdoctoral students having spent at least 12 months in the unit*	1	
Number of Research Supervisor Qualifications (HDR) taken	1	
Qualified research supervisors (with an HDR) or similar positions	8	7

2 • Assessment of the unit

Strengths and opportunities

The clinical relevance of the research question with possible applications to major human physio-pathological states such as aging, metabolic syndrome and NASH, acute injury and sepsis.

The important know-how in animal experiments in particular in multi catheters and enteral nutrition and the good access to common platforms.

The excellent valorisation of the work with numerous patents, with the creation of a start up, with large industrial grants.

The good outcome of the PhD students (8/12 got a position after their thesis).

The support of the university and the opportunity of an evolution of the faculty toward a research centre. To be part of this research centre should be a major aim of the next contract.

Weaknesses and threats

The major weakness is linked to the lack of full time permanent researchers. The project appears to be very large with regards to the number and research time of the supervisors.

Most of senior researchers have heavy teaching and hospital workload and there is some lack of focus in the unit.

The expertise in molecular and cellular biology but also in translational research should be reinforced in order to be able to conduct all the aspects of the project.

The dependency with regards to industry fund could be also viewed as a weakness and the sources of funds should be broadened with a mixture of public and private.

Recommendations

External recruitement should be favorised.

Because most members of the unit are quite experienced with mixed teaching/hospital appointments, the project should be focused on relatively important questions. The arrival of new researchers should not lead to an increase in the number of projects but should be the occasion to acquire the expertise lacking in the unit to allow better mechanistic studies.

The new clinical collaborations in the field of acute injury-sepsis and the potential of collaboration on metabolic syndrome, together with the actual involvment of the geriatric team, will reinforce the translational aspect that is not enough developed in the actual project.

3 • Detailed assessments

Assessment of scientific quality and outputs

The research questions are original with an important clinical relevance.

There are regular publications of good quality papers in the best journals of the field.

The number of publications is good with 1-2 publication/FTE/year.

No publication in general journals or in journals with high impact factors.

Assessment of the unit's academic reputation and appeal

There is an important international recognition of the teams members with numerous invited conferences both at the national and international level.

National collaborations are well established and there are international networks such as « the endocrinology and metabolism Mayo Clinic and Karolinska network ».

The members have published a large number of reviews.

They are deeply involved in the national and European Nutrition society and have organised international meetings.

Assessment of the unit's interaction with the social, economic and cultural environment

This link is very good with regards to the numerous patents, the creation of a startup and the magnitude of the industrial grants in particular the illimited Nestle Grant since numerous years and recently reconducted. The unit is a reference in the field of extra academic expertise.

Assessment of the unit's organisation and life

The entity governance is recognised as performant by staff members. This has been clearly mentionned by both researchers and engineers and technician staff.

The scientific animation is of good level. One of the researchers seems having a very important role in this scientific animation.

There is an important politic of sharing facilities. Platforms from university and IFR are also used.

The weakness is linked to lack of full time researchers the know-how relying mostly on the technical staff.

Assessment of the unit's involvement in training through research

Most of the researchers are heavily involved in teaching with participation in several Master programs at the local level including the participation in masters or other teaching modules.

The unit is involved in several master 2 programs:

- Nutrition, Metabolisme énergétique, Signalisation (Université Paris Diderot),

- Pharmacologie intégrée (Paris Decartes),

- Biologie du viellissement (Paris Descartes),

- Biologie Intégrative et Physiologie (Pierre et Marie Curie).

and in the « Ecole Doctorale Médicament Toxicologie Environnement » ED 436, Ms. Christiane GARBAY (Paris-Descartes).

The supervision of the PhD Thesis follows the rules of University Paris Decartes.

There is a significant decrease in the number of PhD students being currently working in the unit when compared to former years.

The day-to-day supervision of the PhD students is largely done by the technical staff, as most of senior researchers have heavy teaching and hospital work load.

The team is not involved in the coordination of accredited training programs and has no responsiblity in international training networks.

Most members of the teams are involved in the production of many educational papers and in the dissemination of scientific results

Assessment of the five-year plan and strategy

The research project 2014-2018 globally addresses the role of citrulline in metabolic disorders. It targets more specifically two distinct clinical conditions, that are a) sarcopenia, in the context of critical illnesses and of ageing, and b) the metabolic syndrome.

The experiments, which are proposed in this plan, correspond to two different approaches:

On one hand, they will evaluate the effects of citrulline supplementation on inflammation, hemodynamics, and muscle protein metabolism; this approach is original and highly relevant. It essentially rests on the concept that citrulline can be administered as a precursor of arginine, which escapes splanchnic sequestration, and hence increases the systemic availability of citrulline.

On the other hand, they will evaluate the regulation of citrulline production and metabolism (gut citrulline production, extra-hepatic effects of citrulline, etc).

These two approaches are fundamentally different, but are unfortunately not clearly identified in the various work packages.

WP 1: these experiments aim at assessing how gut citrulline production is regulated, and how it will impact muscle protein synthesis. The experiments rest mainly on molecular biology approaches to inactivate OCT in enterocytes, and assume that this will be a major determinant of citrulline concentrations. Then, several muscle characteristics will be assessed and the effects of citrulline supplementation will be evaluated. Particularly, anatomical characteristics of muscle will be assessed by histology, protein metabolism will be measured using tracer technique and the activation of the mTOR pathways will be assessed by western-blot.

This part of the project is highly relevant, original, and addresses the basis of citrulline physiology. The researchers have to be commended for this initiative. There are some concerns, however. The experiments fail to formulate a clear hypothesis for potential regulatory steps, and lack a systematic evaluation of the various factors possibly involved in gut citrulline synthesis (namely: arginine-glutamine intake, endogenous arginine-glutamine fluxes, possible modulation by key metabolic hormones….). There are also some concerns regarding the inactivation of OCT (efficiency of inactivation, duration of inactivation, possible effects on enterocytes viability (the latter of importance because plasma citrulline varies according to the mass of functional enterocytes in the gut). In addition, very few studies are devoted to the understanding of the molecular mechanisms involved in the regulation of muscle functions (mainly protein synthesis) by citrulline availability. For example, no experiment designed to study the effects of citrulline on the regulation of protein synthesis in a model of cultured cells has been described in the project.

WP2: this study is an attempt at obtaining translational data regarding citrulline. It will take advantage of data collected in the Su.Vi.Max studies, and will assess the relationships between plasma citrulline and various markers of nutrition, body composition and muscle functional tests. This has to be regarded as a very preliminary, exploratory step, since many factors likely to modify citrulline metabolism will co-exist in these patients… The study of citrulline concentrations in patients with OCT polymorphism may nonetheless allow the authors to obtain information on the role of this enzyme in vivo (provided that polymorphisms are associated with loss or gain of function).

WP3: This is a joined project with a German team (excellent, and one of the leader in the field of fructose metabolism), which will address the role and effects of citrulline in fructose-induced metabolic syndrome in rats. This WP mixes experiments in which citrulline and other key amino-acid fluxes will be quantified using tracer techniques in fructose-fed animals, and experiments in which the effects of citrulline supplementation will be evaluated. These experiments will have the merit of addressing the alterations of whole body and gut amino-acid metabolism in high fructose-fed animals, an area that has been largely under-investigated. The hypotheses to be tested are however poorly delineated, and sometime poorly supported (in particular the hypotheses that citrulline, which is synthesized in the gut, will be a key player in ensuring an adequate gut barrier function, and that citrulline, which is not metabolized in the liver, will directly modulate fructose-induced hepatic steatosis). Surprisingly, no mention is made that protein and some amino-acids are important modulators of diet-induced hepatic steatosis, and hence that control experiments using other amino-acids should be carefully planned… The respective roles of the French and German teams are not specified.

WP4: This is a proof-of-concept study in human patients with the metabolic syndrome, aimed at assessing the effects of a treatment with statin + citrulline. In this experiment, the rationale rests on citrulline acting as an arginine precursor to improve the endothelial function of patients. Although of interest, the targeted mechanisms appear not really in line with the rest of the project.

WP5: in this part of the project, the effects of a supplement of citrulline on catabolic responses induced by acute injury will be addressed. This is directly in line with the major research area of this team over the past 10 years. As such, it rests on solid hypothesis, solid expertise in the field, and clear questions. Major endpoints will be the effects of citrulline on muscle protein turnover and on inflammation (here, the description of the proposed experiments is not really clear, since we do not know if NF-kB activation will be assessed only in muscle, which may be only partially relevant, or also at the whole organism level and in WBC). Of interest, the effects of citrulline will be assessed in various models of acute injury (LPS administration, turpentine injection, head trauma +/-infection). This approach is quite appropriate given that different mediators may be responsible for different metabolic responses according to the type and severity of injury. Characterization of these various models in term of neuro-endocrine and overall metabolic responses is however not mentioned in the description of these experiments.

An adapted nutritional support of critically ill patients is of major importance and may decrease morbidity and mortality. The aim of this part of the project is to study the protein hypercatabolism associated to acute injury. Citrulline, at least through its antioxidative properties, could be an interesting way to counter the metabolic response to injury.

Several injury models will be tested in the rat: sepsis (LPS), inflammation (turpentine) and trauma (head injury) alone or associated to sepsis. All these models have been already used in the lab and the feasibility of the project seems very good. For a more complete clinically-relevant evaluation of the sepsis model, we suggest to test also another model like cecal ligation puncture.

The first endpoint will be the effects of citrulline on muscle protein turnover and secondly to test different signaling ways (NF-¤B, TWEAK, NRF-2). For a more translational approach, collaboration with an Intensive Care Unit team (i.e. Mr. JP MIRA, Cochin) could be very positive for the project.

Conclusion

The proposed project has an important clinical relevance. The work already done is of quality and the experts appreciated the specific expertise in particular in animal models with multi catheterism. The project has been found too large with regards to the FTE and it shoud be more focused.

The expertise in molecular and cellular biology but also in translational research should be reinforced in order to be able to conduct all the aspects of the proposed project.

4 • Conduct of the visit

Visit date:

Start : 10 decembre 2012 at 9h

End 10 decembre 2012 15h30

Visit site: Faculté des Sciences Biologiques et Pharmaceutiques
4, avenue de l'observatoire, 75006 Paris

Institution : Université Paris Descartes

Déroulement ou programme de visite :

9h00 – 9h30	Accueil des participants autour d'un café
9h30 – 10h00	Réunion du comité de visite et entretien avec le porteur du projet et l'actuel directeur de l'EA
10h00 – 11h10	Présentation du projet et discussion
11h10- 11h40	Réunion du comité de visite avec les représentants de la faculté et de l'université
11h40 – 12h10	Rencontre du comité de visite avec les chercheurs
12h10-12h15	Rencontre du comité de visite avec les personnels techniques
12h15 – 12h30	Rencontre du comité de visite avec les étudiants
12h30 - 13h30	Déjeuner sur place du comité de visite avec l'équipe
13h30 -15h30	Délibération du comité de visite
15h30	Fin de la visite

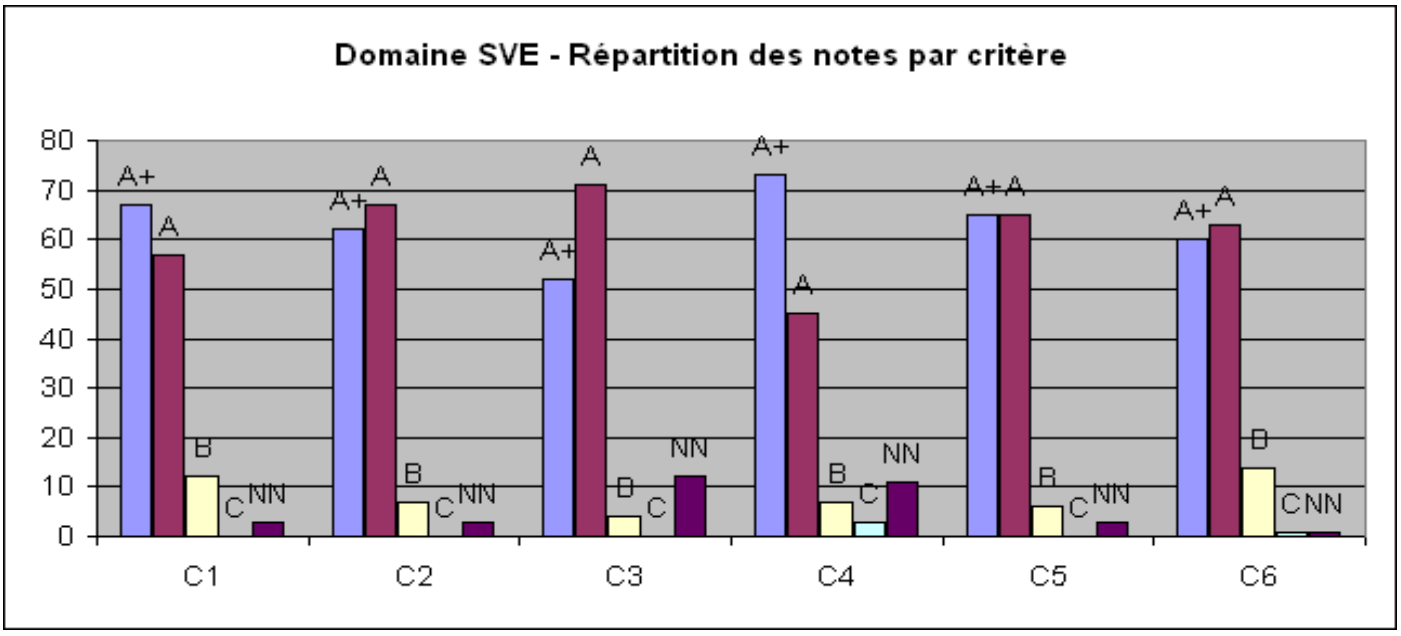
5 • Statistics by field: SVE on 10/06/2013

Notes

Critères	C1 Qualité scientifique et production	C2 Rayonnement et attractivité académiques	C3 Relations avec l'environnement social, économique et culturel	C4 Organisation et vie de l'entité	C5 Implication dans la formation par la recherche	C6 Stratégie et projet à cinq ans
A+	67	62	52	73	65	60
A	57	67	71	45	65	63
B	12	7	4	7	6	14
C	0	0	0	3	0	1
Non Noté	3	3	12	11	3	1

Pourcentages

Critères	C1 Qualité scientifique et production	C2 Rayonnement et attractivité académiques	C3 Relations avec l'environnement social, économique et culturel	C4 Organisation et vie de l'entité	C5 Implication dans la formation par la recherche	C6 Stratégie et projet à cinq ans
A+	48%	45%	37%	53%	47%	43%
A	41%	48%	51%	32%	47%	45%
B	9%	5%	3%	5%	4%	10%
C	0%	0%	0%	2%	0%	1%
Non Noté	2%	2%	9%	8%	2%	1%



6 • Supervising bodies' general comments

UNIVERSITÉ PARIS DESCARTES

Vice Président du Conseil Scientifique

Paris le 02.04.2013

Vos ref : S2PUR140006242 –
Prévention et traitement de la perte protéique musculaire en situation de résistance à l'anabolisme - 0751721N

Monsieur Pierre GLAUDES
Directeur de la section des unités de recherche
Agence d'Evaluation de la Recherche et de l'Enseignement Supérieur
20, rue Vivienne
75002 PARIS

Monsieur le Directeur

Je vous adresse mes remerciements pour la qualité du rapport d'évaluation fourni à l'issue de la visite du comité d'expertise concernant l'unité « Prévention et traitement de la perte protéique musculaire en situation de résistance à l'anabolisme »

Vous trouverez ci-joint les réponses du Directeur de l'unité, Jean-Pascal DE BANDT, auxquelles le Président et moi-même n'avons aucune remarque particulière à rajouter.

Je vous prie d'agréer, Monsieur le Directeur, l'expression de ma considération distinguée.

Le Vice Président du Conseil Scientifique

Stefano Marullo, DM, DesSci

Université Paris Descartes, Vice-présidence du conseil scientifique, 12 rue de l'école de médecine, 75170 PARIS cedex 06
Téléphone: 01 76 53 17 45; Courriel: stefano.marullo@parisdescartes.fr

EA 4466 - Prévention et traitement de la perte protéique musculaire en situation de résistance à l'anabolisme (PRETRRAM)

Université Paris 5- Paris Descartes,

Faculté des Sciences Pharmaceutiques et Biologiques

Name of Director (2010-2013) : Pr. Luc CYNOBER

Name of project leader (2014-2018) : Pr. Jean-Pascal DE BANDT

Answer to the evaluation committee report

Pr JP De Bandt

General observations

Assessment of the unit

We thank the expert committee members for their constructive comments and for the opportunity they gave us to discuss our future project. While we agree with most of the points addressed by the expert committee, we feel that some of them need clarification.

Weaknesses and threats:

"The expertise in molecular and cellular biology but also in translational research should be reinforced in order to be able to conduct all the aspects of the project. "

In fact two of the participants of our project, namely Dr M Hebert-Schuster and Dr PN Bories, have a good expertise in molecular biology; Dr P Marquet-de Rouge, who has been appointed Associated Professor in our team last September, has an expertise in lipid raft, a key component of cell signaling. Moreover collaborations are ongoing with Institut Cochin (Dr Sotiropoulos) for the development of muscle cell culture models. This is accompanied by the recruitment of a technical staff qualified in cell culture for the 2012-2013 technical staff recruitment campaign.
On the other hand, the clinicians involved in our project are at the basis of our interaction with the clinical field and of the translationality of our programs. As described in the team summary report, most of us are involved in PHRC and CRC programs in direct relationship with the project presented i.e. the search of tools enabling to counter muscle protein loss in anabolic resistance situations. Our will is indeed to pursue our effort along this line.

"The dependency with regards to industry fund could be also viewed as a weakness and the sources of funds should be broadened with a mixture of public and private".

We cannot agree on this point. Our sources of financing have been broadened in recent years in order to decrease our dependency from industry fund. As described in the 2007-2012 scientific report, during the past five years, two PHRCs, a CRC, an ANR blanc, an AFM grant … have been obtained averaging € 250,000 a year in addition to our EA 2007-2012 institutional funding (€64,000 a year); this can be compared with only one PHRC obtained by EA2498 during the preceding period. This policy of applying for public funding will be pursued.

Recommendations

"Because most members of the unit are quite experienced with mixed teaching/hospital appointments, the project should be focused on relatively important questions. The arrival of new researchers should not lead to an increase in the number of projects but should be the occasion to acquire the expertise lacking in the unit to allow better mechanistic studies."

We completely agree with the expert committee's recommendations on the importance to become more focused on the main questions of our project. In the wake of the visit of the expert committee and the discussion around our project, we have decided to drop the work package 4 (evaluation of the citrulline-statine combination).
It is our intention that the recruitment of new researchers shall be performed in close agreement with the specific requirements of our research project.

"The new clinical collaborations in the field of acute injury-sepsis and the potential of collaboration on metabolic syndrome, together with the actual involvement of the geriatric team, will reinforce the translational aspect that is not enough developed in the actual project."

We are aware of the necessity to continue developing our clinical cooperation. In fact, one study in intensive care patients in direct coherence with our project has already been published (Grimaldi D, Guivarch E, Neveux N, Fichet J, Pène F, Marx JS, Chiche JD, Cynober L, Mira JP, Cariou A. Markers of intestinal injury are associated with endotoxemia in successfully resuscitated patients. Resuscitation 2013;84:60-5). Both the publications of the team, and the PHRCs and CRC already obtained demonstrate our involvement in clinical studies in our field of expertise.

Detailed assessments.

Assessment of the unit's organization and life:

"The weakness is linked to lack of full time researchers the know-how relying mostly on the technical staff."

Maybe we have not correctly described how our team works. Experimental approaches have been divided into four platforms: one analytical, a cell culture, a molecular biology and animal experimentation. Each platform is supervised by a researcher and a technician. When a new experimental approach is developed, experiments are carried out by a researcher with the help of a technician until the complete development is achieved and technical procedures are clearly established. A special situation is that of S Le Plenier who belongs to the technical team as an engineer but whose work goes beyond that of a mere technician since she wrote her own articles

and is currently section editor for the international journal *Amino Acids*. Her contribution to the scientific activity of the team thus exceeds that of most technicians.

Assessment of the unit's involvement in training through research:

"The day-to-day supervision of the PhD students is largely done by the technical staff, as most of senior researchers have heavy teaching and hospital work load."

We disagree on this point. While most of us have indeed teaching assignments and, for some, hospital duties, most of teaching is performed on-site and hospital activities are performed next door, at Cochin Hospital. Researchers in charge of PhD students are thus able to interact on an everyday basis in the lab with their students.

Assessment of the five-year plan and strategy:

"The experiments, which are proposed in this plan, correspond to two different approaches:
On one hand, they will evaluate the effects of citrulline supplementation on inflammation, hemodynamics, and muscle protein metabolism; this approach is original and highly relevant. It essentially rests on the concept that citrulline can be administered as a precursor of arginine, which escapes splanchnic sequestration, and hence increases the systemic availability of citrulline.
On the other hand, they will evaluate the regulation of citrulline production and metabolism (gut citrulline production, extra-hepatic effects of citrulline, etc).
These two approaches are fundamentally different, but are unfortunately not clearly identified in the various work packages".

As stated in our research project, our working hypothesis is that intestinal citrulline production, which is the main endogenous source of this amino acid, is a key player in an intestine/muscle axis at the center of protein homeostasis via both direct effects of citrulline on the muscle and indirect effects as an arginine precursor (WP1 and 2). In anabolic resistance settings (WP3 to 5), citrulline supplementation enables to benefit from the regulatory properties of this amino acid and/or compensate for a defect in its intestinal synthesis.

We thank the expert committee members for these detailed comments on our project which are well taken. We just want to underscore some specific points concerning :

WP 1: *"The experiments fail to formulate a clear hypothesis for potential regulatory steps, and lack a systematic evaluation of the various factors possibly involved in gut citrulline synthesis… There are also some concerns regarding the inactivation of OCT … In addition, very few studies are devoted to the understanding of the molecular mechanisms involved in the regulation of muscle functions".*

The mechanisms of citrulline action on muscle are already under investigation as part of our 2010-2013 project. A first article has already been published (Le Plénier et al Amino Acids 2012;43:1171-8) and a second manuscript has just been accepted for publication (Faure et al, Proteomics). Stemming from a collaboration with Dr Sotiropoulos (Institut Cochin), muscle cell culture experiments have been developed in the lab and the mechanisms of citrulline action have begun to be studied in this model.
Outside alterations in the context of severe intestinal diseases such as short bowel syndrome or inflammatory bowel disease, citrulline production by the gut seems to be a fairly stable process as

shown by the relationship between plasma citrulline and gut integrity. WP1 is thus devoted at investigating whether this intestinal citrulline production is a key determinant of long term muscle function.

WP3: "*The hypotheses to be tested are however poorly delineated, and sometime poorly supported ... Surprisingly, no mention is made that protein and some amino-acids are important modulators of diet-induced hepatic steatosis … The respective roles of the French and German teams are not specified*."

In fact our ANR project is not presented in detail. The first step comparing the effect of different amino acids is already completed and the manuscript is under preparation. From this step it was shown that citrulline is the most potent amino acid for the protection against NAFLD and this has led us to go on with this study only with citrulline as described here. Only the experiments from our lab are described here. While our experiments will be performed in rats, best suited for nutritional investigation, the German team will perform similar experiments in mice in order to make profit, secondarily, of some specific KO mice strains for the mechanistic investigation.

WP4: As mentioned above, we have decided to drop this WP

WP5: "*Characterization of these various models in term of neuro-endocrine and overall metabolic responses is however not mentioned in the description of these experiments*."

All our experiments include overall assessment of metabolic, endocrine and inflammatory status as this is mandatory for the evaluation of the metabolic response to injury and the characterization of the injury severity.