CIM
Centre of
Inflammation
and Metabolism

Udvalgene vedr. Videnskabelig
Uredelighed (UVVU)

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Professor Henrik Galbo has submitted his "replik" to UVVU, dated November 14, which we have received from UVVU on November 18, 2011.

In my response of August 12, 2011 to Dr. Galbo's report, my collaborators and I summarise Dr. Galbo's main points of critique in the cover letter. I take the liberty to add the summary of my "duplik" in *italic* to the previous summary in order to provide an overview of the main points in our rebuttal of Dr. Galbo's accusations against us.

1. We think it would be unethical not to take advantage of all information that can be extracted from human samples. However, Dr. Galbo raises serious accusations against us with regard to reuse of biological material, although he, himself, on several occasions has followed the same tradition regarding the optimal use of human material (tables 2-4). Moreover, Dr. Galbo has extensively reused his data.

   *In essence, Dr Galbo does not provide any new comments on the above point and we therefore assume that he agrees that it would be unethical not to "reuse" biological material. We further assume that he agrees that it has been common practise, as documented by us, to either give a cross reference or to provide a comprehensive description of the study population, just as Dr. Galbo is doing it himself as is many other researchers in our field.*

2. Throughout his report, Dr. Galbo does not only criticize the fact that there is not always parallelism between mRNA and protein levels, but he also insinuates that by neglecting such findings, we are guilty of fraud. We show that his criticism is based on an outdated and wrong perception of the "Central Dogma" of molecular biology: that DNA is transcribed into RNA, which is translated into protein. Dr.Galbo is apparently not up-dated on new era phenomenon such as microRNA, etc.

   *Dr. Galbo`s "replik" does not provide any new arguments with regard to this point, and thus we find that we have already responded to his criticism.*

3. Dr. Galbo criticizes the fact that we express our data as arbitrary units or fold-changes, although this is the common praxis when evaluating the effects of an intervention, and although he, himself, has co-authored at least 10 publications in which mRNA levels are expressed in the same way; http://www.ncbi.nlm.nih.gov/pubmed?term=galbo%20h%20mRNA.



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*Dr. Galbo argues that it is acceptable that he expresses data in arbitrary units or fold-changes, but that it is not acceptable that we do the same because we are researching expression of different genes. A PubMed search supports the fact that the way, in which we express our data, has been "common practise" in research environments, also when investigating changes in muscular mRNA levels of various cytokines in response to exercise.*

4. Dr. Galbo is confused with regard to basic endocrinological terms and the limitations of various techniques, which has made it sometimes difficult to provide a response to his criticism.

   *Unfortunately Dr. Galbo adds nothing new in his "replik", which could make us change this statement.*

5. We demonstrate that our procedures are or were at the time of publication of the articles in question commonly used in the research field of integrative human physiology. When applying the same main criteria that Dr. Galbo uses when reviewing our papers to another muscle physiology article coauthored by Penkowa (with Flemming Dela as senior author), it appears that the Dela group has used exactly the same procedures as we have.

   *We have no further comments except that Galbo in his replik provides further support for our statement (Galbo replik 2.1.g).*

6. Dr. Galbo has voluntarily decided to evaluate our papers. Although all papers have undergone previous peer review and several have been included in PhD theses, we acknowledge that Dr. Galbo identifies mistakes in two of the papers and points to some issues that could have been dealt with differently.

   *With regard to the two mistakes that were identified by Dr. Galbo, the two scientific journals in question have, without hesitation, published an erratum on our request, please see Appendices J_1_1, J_1_2_1 and J_1_2_2.*

   However, not once in his lengthy report *nor in his replik*, is he able to give a specific example of potential scientific dishonesty other than those related to the work performed by Milena Penkowa, which we ourselves have previously reported to UVVU and the scientific journals in question. In consequence, we do not find that Dr. Galbo is able to provide any valid support for his serious and potentially very damaging accusations against us.

   *We wish to elaborate further on this statement and to highlight a couple of issues of importance for the rebuttal of Dr. Galbo's accusations.*

   - *Dr. Penkowa was an independent senior scientist situated at the Panum Institute with a research group of her own. She and I were both associate professors and formally equal. I have never had a role as mentor for Penkowa.*

   - *We collaborated with Penkowa solely with regard to the IHC technique as we did not ourselves have the expertise and as we did not want to establish the IHC technique within our own laboratory. This was partly because it would be resource demanding*



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*with regard to equipment and time and partly because this technique as such only played a limited role in our research.*

- *Successful research is often interdisciplinary and includes both national and international collaborations. It is not unusual to collaborate with people you have not even met in person. Samples are shipped between countries and results are exchanged via the internet. Scientific collaboration between independent research groups is based on trust. It is therefore common practise in the international scientific world that the person to blame for fraud is the person who performed the fraud.*

- *Penkowa has collaborated with more than 200 people. We are not aware that any of these collaborators have reported Penkowa to UVVU before her case appeared in the press. This tells us that in general Penkowa's collaborators had faith in her and her work.*

- *Today, the public opinion is that Penkowa's work is fraudulent. When we collaborated with her, we did not think that she would be able to perform or even consider performing what today appears to be active manipulation with some pictures. We knew that Penkowa had had severe troubles with her doctoral thesis, but we were convinced that she had been cleared, when she was finally invited to defend her thesis. We further understood that the UC Faculty of Health Sciences had faith in her, as she was not only nominated for the Elite Researcher Prize, but also promoted to professor.*

- *In order to identify the kind of picture manipulation that Penkowa has performed, we would need to suspect that manipulation had taken place. It would mean that we proactively should have identified that a picture from one article had been reused in another article, but over a considerable span of time and at another exposure, so that e.g. a picture appeared red in one article and white in another. The fact is that we did not expect any kind of manipulation at that time. When we became aware that fraud might have occurred, we and others looked at the figures with new eyes, and it was not until then that we identified the picture manipulation.*

- *It is important to report scientific misconduct or potential fraud, when facing it. Therefore, when we became aware of possible fraud with regard to the manipulated IHC-pictures, we reported this to UVVU and the scientific journals in question and subsequently retracted the papers.*

- *Dr. Galbo keeps arguing that I had been "warned" by him during a CMRC seminar in 2003, in which I presented data on IL-6. He says that he gave this warning before the IL-6-IHC data in question was accepted for publication. This is not true. According to Dr. Galbo's own argumentation, he is referring to a CMRC-seminar, which was held not in 2003, but in April 2004, whereas the paper in question was published in 2003. Below, I shall give a more detailed comment on this issue (Galbo replik 2.1.d).*

- *While I do not personally remember the specific incident to which Dr. Galbo is referring, I am absolutely sure that he never once accused me of fraud before sending*


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*his report to UVVU in July this year. It is interesting that the Penkowa case suddenly makes him remember an episode from 2003, which in fact appears to be in 2004! Regardless of the date, I do <u>not</u> agree with Dr. Galbo that our results spoke against "common sense". Below I give a more detailed comment (Galbo replik 2.1.d).*

- *It is well known that there has existed a decade-long antagonism between Dr. Galbo and myself and that I have been exposed to his arrogant behaviour and unpleasant out-bursts at almost every occasion, when we have met. I specifically remember that Dr. Galbo at some occasion claimed that Penkowa, and not I, ought to present the story about muscle expression of IL-6 as she was the expert. Soon thereafter Galbo's protégé Flemming Dela established collaboration with Penkowa and published a paper in Diabetes. Thus, it is obvious that no one within the CMRC environment suspected fraud at that time.*

- *We do not find that Dr. Galbo is able to provide any valid support for his serious and potentially very damaging accusations against us.*

Finally, it is well known that Dr.Galbo was for many years unsuccessfully searching for "the exercise-factor". He and I have been collaborators. We have published 17 articles together, the last one in 2003. The bad tone between us accelerated in parallel with the identification by me and my collaborators of factors secreted from skeletal muscle and hence muscle cells.

It is very sad for me to realise that Dr. Galbo, a researcher I once admired, stands behind the documents that he has not only sent to UVVU, but also circulated to a vast number of colleagues, and directly or via others even circulated to the media and to Milena Penkowa.

Below I shall respond in more details to the comments raised in Dr. Galbo's replik.

The present report will be submitted also for information to the authorities at the University of Copenhagen and Rigshospitalet as well as to the Danish National Research Foundation.

Sincerely,

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**Dr. Galbo's replik p.2, para 2, line 5–7:**

It is uncertain to me if Dr.Galbo backs his statement with information he has from BT, or from his interaction with Jamie Timmons or from other sources.

**In the following, I refer to the numbering in Dr. Galbo's replik.**

2.1.a.

Personnel from the CIM laboratory had hands on, while Penkowa had optimised the antibodies and supervised the IHC method.

The procedure and the involvement of personnel are as described in my report. It should be obvious that the report was written in agreement with all of the co-authors, except Penkowa.

Penkowa was informed by e-mail when we contacted the journals in question with regard to retraction of papers. She never responded.

2.1.b.

It is impossible to publish all IHC images from all individuals. It is my impression that in consequence any researcher would choose to present the picture that is best suited to communicate the combined results to the readers.

2.1.c.

I have no additional comment.

2.1.d.

With all due respect, I do not consider Dr. Galbo's word to be law. I clearly remember a number of occasions at which Dr. Galbo has expressed his scientific views in an aggressive, rude and destructive fashion, not the least against me and my research group. My general approach has been to try to ignore and forget his personnel attacks against me and to avoid taking action in order to not provoke aggression.

Although I do not remember the specific incidence to which he is referring, I know that I do not agree with Galbo's critique. In our report dated August 12, 2011, we have given an in-depth account of the fact that there are many pieces of information, which together demonstrate that IL-6 is produced and released from muscle cells. The fact that IL-6 appears to accumulate within muscle cells are in full agreement with the fact that although muscle-derived IL-6 is likely to have endocrine effects, it certainly also has local effects, either autocrine or paracrine.

To illustrate that this fact was well known in the research environment, in which muscle-derived factors were studied and discussed, I put forward the following example:



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Together with e.g. professor Michael Kjær, Lars Rosendal has published a study in which they used the microdialysis technique to study cytokine release from working muscle (low intensity, limited muscle mass). They write

*"We conclude that upper extremity, low-intensity exercise results in a substantial increase in IL-6 in the interstitium of the stabilizing trapezius muscle, whereas no change is seen for plasma IL-6" (1).*

On May 23, 2003, Lars Rosendal asks me by e-mail for a meeting to discuss the data that was later published in the above paper, Appendix J_2_1. Our meeting took place shortly before the meeting Dr. Galbo refers to, which according to his first report was on June 19, 2003.

However, Dr. Galbo says in his initial report (July 18, 2011) that he received a letter from a PhD-student after the CMRC- meeting (i.e. shortly after June 19, 2003). This letter was from one of my former PhD students, Thorbjørn Åkerstrøm. I believe that Dr. Galbo is confused with regard to the date of the meeting, to which he is referring. According to my files Thorbjørn Åkerstrøm wrote his letter after the CMRC-seminar held on April 15, 2004, Appendix J_2_2. Thus, it is not true, when Dr. Galbo claims, that he expressed his critical view before acceptance of the paper, which included the IL-6 IHC data. The paper in question (paper 1 in Dr. Galbo's initial report) was published already in 2003. The Febbraio group had generated supporting IHC as well as in situ hybridization data, demonstrating that the IL-6 mRNA and protein expressions increased in muscle human fibres after exercise. I had in-depth knowledge about these data already on September 24, 2003, when I received the data from Mark Febbraio – data that was later published in FASEB Journal, June 2004 (2) and which, with permission from Mark Febbraio, was included in my talk on April 15, 2004.

Thus, although Dr. Galbo according to his statements expressed the view that he found it strange that IL-6 was accumulated within muscle cells following exercise, it was clear already then that the finding that IL-6 accumulates locally is in agreement with other studies, e.g. the study by Rosendal et al. and the study by the Febbraio group. I hope that it is now completely clear that already at that time there were data from other groups within the field, which supported the idea that IL-6 was accumulated locally in the muscle, in the interstitium and/or within the muscle cells. Therefore, we had substantiated reason to believe in our data.

Other studies from that period and later have supported the initial findings that muscle cells were likely to express IL-6 during exercise and that muscle-derived IL-6 has both autocrine/paracrine as well as endocrine effects.

Indeed, it was demonstrated and published already in 2001 and 2002 that the nuclear transcription rate for IL-6 and the IL-6mRNA levels increase rapidly and markedly after the onset of exercise (3;4) suggesting that a factor associated with contraction increases IL-6 transcriptional rate within the nuclei from myocytes. Further evidence that contracting muscle fibres themselves are a source of IL-6 mRNA and protein was achieved by analysis of biopsies from the human vastus lateralis using in situ hybridization and immunohistochemistry techniques (2).

The study by Rosendal et al, using microdialysis, indicated that the concentration of IL-6 within the contracting skeletal muscle may be 5- to 100-fold higher than the levels found in the circulation and


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that IL-6 appears to accumulate within the contracting muscle fibres as well as in the interstitium during exercise (1). Studies using simultaneous measurement of arteriovenous IL-6 concentrations and blood flow across the leg demonstrated that large amounts of IL-6 were released into the circulation from the exercising leg (5).

Today, IL-6 has been shown to be expressed by human myoblasts (6;7) and by human cultured myotubes (8). Moreover, IL-6 is locally and transiently produced by growing murine myofibres and associated muscle stem cells (satellite cells) (9). In addition, IL-6 is released from human primary muscle cell cultures from healthy individuals (10;11) and from patients with type 2 diabetes (11).

Thus, the idea that muscle cells produce and release IL-6 has been substantiated by numerous reports.

2.1.e.

We have no additional comments.

2.1.f.

"If this is true?", Please, see Appendix J_3.

2.1.g.

I certainly do not blame Dr. Galbo for not including the Dela study. However, I found it appropriate to notice it. Dr. Galbo argues in his report dated July 18, 2011: "I have chosen to review the 12 scientific papers, she (Penkowa) has co-authored about muscle physiology and pathophysiology." A PubMed search on "Penkowa AND muscle" clearly identifies the article by the Dela-group. Frankly speaking, I find it highly surprising that Dr. Galbo was not aware of the study.

The reason for introducing the Dela-study in our reply was to illustrate that when we apply the same main criteria as employed by Dr. Galbo in his report dated July 18, on the latter study, we are able demonstrate that our procedures are or were at the time of publication of the articles in question commonly used in the research field of integrative human physiology, as exemplified by the Dela study. Whether IHC was evaluated blinded or not was not a major part of Dr. Galbo's initial critique. When looking at the data from the Dela study, it is obvious that while he finds that there is no difference between patients and controls with regard to MT-mRNA, there appears to be a massive difference with regard to MT-protein expression. If these data were evaluated blinded, it provides further support for the finding that differential expression of protein may not correspond to differential expression of mRNA.

2.2.

Dr. Galbo says "… as regards co-variation its absence or inconsistencies were often overlooked". With all respect, I still stand by my initial statement that Dr. Galbo has an outdated view on the Central Dogma, when he states that one should always question lack of parallelism between mRNA and protein.


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In our report, we have given an in-depth description of measurements of mRNA and its relationship to protein. We further want to emphasize that it is impossible for Dr. Galbo or anyone else to know how many mRNA transcripts that is needed to make an expression "physiologically relevant". The focus on CT-values is of limited relevance, when it comes to determining whether a certain tissue expresses a gene in a physiological relevant way. In addition, different mRNA transcripts have different stabilities (depending on the composition of the 3'UTR), and a stable mRNA transcript produces more protein (i.e. can be translated several times) than a less stable mRNA transcript.

As an example, Dr. Galbo criticizes the low BDNF – mRNA level in paper 12 with a more pronounced protein expression. The BDNF mRNA values are induced from 33 to 30 CT-values. Such levels are robust, but not high. However, others find that the BDNF protein is highly expressed by human muscle. Prof. Mathias Uhlén from the Royal Institute of Technology, Sweden is developing a protein atlas. This is a very impressive project in which all human proteins are being mapped in human tissues and cells. In support of our findings with regard to BDNF, he finds that BDNF is highly expressed in skeletal muscle at the protein level:

*"Staining summary: Weak to moderate cytoplasmic and occasional membranous or nuclear positivity was observed in most normal tissues. Neuronal cells, appendix and skeletal muscle (normal) displayed strong positivity."*

http://www.proteinatlas.org/ENSG00000176697/normal/skeletal+muscle

We trust that this information will put an end to the accusations against us for trusting data demonstrating that normal human skeletal muscle has the capacity to express BDNF.

Throughout his report, Dr. Galbo does not only criticize the fact that there is not always parallelism between mRNA and protein levels, but he also insinuates that by neglecting such findings, we are guilty of fraudulent behaviour. We take the liberty to refer to our initial comments in our report dated August 12, 2011, pp 3 and 4.

p.6 second para:

With regard to IL-8 and CXCR2, The CT-values for CXCR-2 were around 30. We have nothing more to add.

MTII mRNA data in Figure 1 have been calculated and presented as MTII/GAPDH ratios, as the pre-exercise levels were hardly detectable. However, for the ease of the reader, an approximate fold change was presented in the results section. The reader was fully able to independently judge the fold change given from the ratios presented in Figure 1. There was no misleading of the reader. The CT values were 34 with exercise peak. We have no further comments.



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2.3.

I refer to Table 1 in our report dated August 12, 2011.

2.4.a.

We find that we have given a comprehensive and in-depth response to the main and relevant points of Dr. Galbo's criticism in our 72-page long report dated August 12, 2011.

2.4.b1.

We have no further comments to add.

2.4.b2.

Please, see our response under 2.1.d

2.4.b3.

We simply do not understand the question or the relevance regarding differences between papers with regard to relationships between mRNA and protein. Maybe Dr. Galbo seems to overlook the fact that the time points for measurement of plasma-IL-6 protein differ between the two independent studies. Thus, it is 0, 3, 4.5, 6, 9 and 24 h in paper 1 and 0, 0.5, 1, 2, 3, 3.5, 4, 5 and 6 h in paper 2. Although the two modes of exercise are different in the two studies, it seems that the IL-6 plasma level kinetics are similar. In paper 2, the samplings are more frequent in the immediate post-exercise period allowing the identification of a peak-IL-6 value at 4 h, which is decreased to the 3 h point already at 5 h. In paper 1, the peak value is seen at 3 h and the value at 4.5 h is slightly lower. Thus, there is no contrast between studies with regard to plasma-IL-6 protein. With regard to IL-6 expression in muscle, it appears that there is a more intense staining for the IL-6 protein for a longer period in paper 1 than 2. The modes of exercise employed in the two studies were quite different, which we assumed could be an explanation. This was discussed in details in the discussion in paper 2.

2.4.b4.

Please, see our response under 2.2.

We presented as MTII/GAPDH ratios in the figure. The only other paper available on MT measurements in muscle is in the Dela/Penkowa study in which the Dela group also expresses MT mRNA values as ratios. We do not believe that data could have been presented in a better way.

2.4.b5

We have nothing to add.

2.4.b6 and b7.



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We have provided an in-depth description of the use of biological materials in Table 1 of our response to Dr. Galbo's initial report. I do not understand this persistent critique about use of study material as Dr. Galbo, himself, on several occasions has followed the same tradition regarding the optimal use of human material (Tables 2-4 in our response dated August 12) without cross references and (in contrast to us) even with extensive reuse of data.

2.4.b8.

We have no further comments.

2.4.b9.

We do not find it "fraudulent" to provide thorough information about the procedures applied to the patients.

2.4.b10.

We have no further comments.

2.4.b11.

We have already commented on this.

2.4.b12.

It is obviously possible that a certain stimulus, e.g. exercise, can induce small increases in mRNA without a significant increase in protein. It is also obvious that a late increase in IL-15mRNA is likely to be involved in training adaptation (which has also been confirmed later), whereas a rapid increase during exercise, in e.g. muscle IL-6mRNA, suggests that the IL-6 plays a role during the exercise as such.

We have no more comments.

2.4.b13

We have nothing further to add.

2.4.b14.

We have no further comments.

2.4.b15.

We apologize if we have misunderstood the critique. We did not find a discrepancy between the findings in one rat study and our study in humans to be relevant. However, on page 55 of our response dated August 12, 2011, we provided a comprehensive summary of the rodent muscle-BDNF literature and concluded that our data are in agreement with the published literature.



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2.4.b16.

We have no further comments.

2.4.c1.

We have already commented on this issue.

2.4.c2.

We have given an in-depth response to this question under 2.1.d.

2.4.c3.

We have provided a detailed answer to this question. We have no further comments.

2.4.c4.

We have no further comments.

2.4.c5.

Dr. Galbo is criticizing the focus of the study, which was made in agreement with some of the leading experts in the field. We have no further comments.

2.4.c6.

We have no further comments with regard to lack of parallelism between mRNA and protein.

2.4.c7.

We write "this is not correct". Thereafter, we provide a detailed description as to why we disagree: A multivariate analysis was performed and five different models were presented. Plasma-TNF is significantly correlated with a measure of insulin sensitivity in all models. The control group did not include individuals with impaired glucose tolerance test and thus represents a homogenous group with regard to insulin sensitivity. Therefore, the finding of a correlational relationship between plasma-TNF and measures of insulin sensitivity cannot be expected. We have no further comments.

2.4.c8.

We have no further comments.

2.5.

We do not want to comment upon Dr. Galbo's professionalism.

With regard to BDNF, microRNA, mRNA and protein – we have commented on these issues in our response to UVVU regarding the report from Jamie Timmons dated April, 17, 2011.



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2.6.

As regards Jamie Timmons, I wrote in my accompanying letter to UVVU (August 12, 2011):

"Finally, I cannot help noticing the coincidence that this particular criticism should come from Dr. Galbo when bearing in mind the decade-long and well known antagonism between Dr. Galbo and the undersigned and indeed also the positive relationship that exists between Dr. Galbo and Professor Jamie Timmons. Thus, one might suspect that Dr. Galbo's motives for bringing forward his accusations could be personal rather than scientific. However, as this is irrelevant in relation to the scientific evaluation to be performed by UVVU, I shall refrain from elaborating further on the subject."

I still find that this information is in principle irrelevant. However, I find it timely to provide the readers of this document with some information that speaks for itself.

On the evening of April 5th 2011 I received the following phone text message from professor Timmons:

*"I see you are now lying to the press. But me and Henrik Galbo know different and can prove it ... Hope you burn for your fraud".*

Until that point I was not aware that Dr. Galbo was in what later appeared to be quite close contact with Jamie Timmons. On the same evening I e-mailed Henrik Galbo to inform him (as a colleague) that Jamie Timmons was involving him in fraud accusations against me (Appendix J_4_1).

In his reply (April 6th, 10:06) (Appendix J_4_2), Dr. Galbo did not comment on his relationship with Professor Timmons, but responded rather cryptically that he thought the wording in the text message was "untimely", however he had faith in the work by UVVU and the Independent Research Council. Subsequently when the administrator of CIM, Inge Holm, asked Dr. Galbo whether he wanted to be informed in more detail about the matter, he responded to her by e-mail on April 11th 2011, that he had never met professor Timmons and only recently heard of him and that he had no desire to get involved in a dispute between Jamie Timmons and myself. On April 6th, 11:34, I received an e-mail from Jamie Timmons, demonstrating that he had been communicating with HG (Appendix J_4_3).

It is evident from the attached e-mail of 2nd April 2011 from Dr. Galbo to Professor Timmons, which I later received from my postdoc Camilla Scheele, that Dr. Galbo and Jamie Timmons were indeed coordinating their efforts to produce reports regarding fraud accusations against me (Appendix D_3).

Upon advice from my medical director Jannik Hilsted, I forwarded the e-mail in question to Dr. Galbo as we were both convinced that the e-mail from Dr. Galbo to Prof. Timmons represented a fake e-mail correspondence. We found it impossible to believe that Dr. Galbo could stand behind such wording. However, Dr. Galbo did not deny that the e-mail was indeed written by him (Appendix D_3).



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2.7.

The HSL story was given as an example of how I actually presented work from my group on a CMRC meeting in order to stimulate discussions of a matter of mutual interest. However, I was met by suspicion and aggression from Dr. Galbo.

It is absurd to claim that I should have stated that I am "the discover of IL-6". However, for more than a decade my group and collaborators have contributed extensively to unravel the links between muscle, exercise and IL-6 production. Moreover, we have contributed extensively to identify muscle as an endocrine organ. In 2003, we suggested that cytokines and other peptides that are produced, expressed and released by muscle fibres and exert autocrine, paracrine or endocrine effects should be classified as 'myokines' (12). Based on our contributions with regard to IL-6 and other myokines, I was in 2005 awarded a 5-year grant of dkr. 25 mio from the Danish National Research Foundation (DNRF). Following an evaluation and site-visit performed by an independent foreign expert panel, DNRF has awarded us a 5-year prolongation from 2010-15 with a grant of dkr. 30 mio.

In the before-mentioned review article, in other review articles and in multiple talks on myokines, CIM associates and I often highlight that the idea about muscle as an endocrine factor has been around for nearly half a century. In an invited article, which has been accepted for Nature Reviews Endocrinology (Appendix J_5) we write:

*The idea that muscle cells might produce and release a humoral factor dates back many years before the identification of adipose tissue as an endocrine organ. For nearly half a century, researchers had hypothesized that skeletal muscle cells possessed a 'humoral' factor that was released in response to increased glucose demand during contraction (13). Due to lack of more precise knowledge, the unidentified contraction-induced factor has been named "the work stimulus", "the work factor" or the "exercise factor" (12).*

*In our view, the pluralis form "exercise factors" would be more applicable given the fact that multiple metabolic and physiologic changes are induced by exercise. The early view on the exercise factor concept was predicated on the fact that contracting skeletal muscle mediates metabolic and physiologic responses in other organs, which are not mediated via the nervous system.*
*This has been demonstrated by the fact that electrical stimulation of paralysed muscles in spinal cord injured patients with no afferent or efferent impulses, induces many of the same physiological changes as in intact human beings (14;15). Therefore, it was clear that contracting skeletal muscles were able to communicate to other organs via humoral factors, which are released into the circulation during physical activity. Such factors might directly or indirectly influence the function of other organs such as the adipose tissue, the liver, the cardiovascular system and the brain. During the past decade, myocytes have been identified as cells with a high secretory capacity in parallel with the concept of adipocytes being major endocrine cells. It appears that muscle cells, here defined as myoblasts or myocytes, have the capacity to produce several hundred secreted factors (16-18).*


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Of note, in the before-mentioned article to be published in Nature Reviews Endocrinology, there are no articles included, on which Penkowa is an author. Our main contribution to the identification of muscle as an endocrine organ does not rely on work that she has co-authored.

**Concluding remark**

I still do not find that Dr. Galbo is able to provide any valid support – whatsoever - for his serious and potentially very damaging accusations against my research group and me.



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Reference List

(1) Rosendal L, Sogaard K, Kjaer M, Sjogaard G, Langberg H, Kristiansen J. Increase in interstitial interleukin-6 of human skeletal muscle with repetitive low-force exercise. J Appl Physiol 2005 Feb;98(2):477-81.

(2) Hiscock N, Chan MH, Bisucci T, Darby IA, Febbraio MA. Skeletal myocytes are a source of interleukin-6 mRNA expression and protein release during contraction: evidence of fiber type specificity. FASEB J 2004 Jun;18(9):992-4.

(3) Keller C, Steensberg A, Pilegaard H, Osada T, Saltin B, Pedersen BK, et al. Transcriptional activation of the IL-6 gene in human contracting skeletal muscle: influence of muscle glycogen content. FASEB J 2001 Dec;15(14):2748-50.

(4) Steensberg A, Keller C, Starkie RL, Osada T, Febbraio MA, Pedersen BK. IL-6 and TNF-alpha expression in, and release from, contracting human skeletal muscle. Am J Physiol Endocrinol Metab 2002 Dec;283(6):E1272-E1278.

(5) Steensberg A, van HG, Osada T, Sacchetti M, Saltin B, Klarlund PB. Production of interleukin-6 in contracting human skeletal muscles can account for the exercise-induced increase in plasma interleukin-6. J Physiol 2000 Nov 15;529 Pt 1:237-42.:237-42.

(6) De Rossi M, Bernasconi P, Baggi F, de Waal MR, Mantegazza R. Cytokines and chemokines are both expressed by human myoblasts: possible relevance for the immune pathogenesis of muscle inflammation. Int Immunol 2000 Sep;12(9):1329-35.

(7) Bartoccioni E, Michaelis D, Hohlfeld R. Constitutive and cytokine-induced production of interleukin-6 by human myoblasts. Immunol Lett 1994;42(3):135-8.

(8) Keller C, Hellsten Y, Steensberg A, Pedersen BK. Differential regulation of IL-6 and TNF-alpha via calcineurin in human skeletal muscle cells. Cytokine 2006;36(3-4):141-7.

(9) Serrano AL, Baeza-Raja B, Perdiguero E, Jardi M, Munoz-Canoves P. Interleukin-6 is an essential regulator of satellite cell-mediated skeletal muscle hypertrophy. Cell Metab 2008 Jan;7(1):33-44.

(10) Haugen F, Norheim F, Lian H, Wensaas AJ, Dueland S, Berg O, et al. IL-7 is expressed and secreted by human skeletal muscle cells. Am J Physiol Cell Physiol 2010 Apr;298(4):C807-C816.

(11) Green CJ, Pedersen M, Pedersen BK, Scheele C. Elevated NF-{kappa}B Activation Is Conserved in Human Myocytes Cultured From Obese Type 2 Diabetic Patients and Attenuated by AMP-Activated Protein Kinase. Diabetes 2011 Nov;60(11):2810-9.

(12) Pedersen BK, Steensberg A, Fischer C, Keller C, Keller P, Plomgaard P, et al. Searching for the exercise factor: is IL-6 a candidate? J Muscle Res Cell Motil 2003;24(2-3):113-9.



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(13) Goldstein MS. Humoral nature of the hypoglycemic factor of muscular work. Diabetes 1961 May;10:232-4.:232-4.

(14) Kjaer M, Secher NH, Bangsbo J, Perko G, Horn A, Mohr T, et al. Hormonal and metabolic responses to electrically induced cycling during epidural anesthesia in humans. J Appl Physiol 1996 Jun;80(6):2156-62.

(15) Mohr T, Andersen JL, Biering-Sorensen F, Galbo H, Bangsbo J, Wagner A, et al. Long-term adaptation to electrically induced cycle training in severe spinal cord injured individuals. Spinal Cord 1997 Jan;35(1):1-16.

(16) Bortoluzzi S, Scannapieco P, Cestaro A, Danieli GA, Schiaffino S. Computational reconstruction of the human skeletal muscle secretome. Proteins 2006 Mar 15;62(3):776-92.

(17) Yoon JH, Yea K, Kim J, Choi YS, Park S, Lee H, et al. Comparative proteomic analysis of the insulin-induced L6 myotube secretome. Proteomics 2009 Jan;9(1):51-60.

(18) Henningsen J, Rigbolt KT, Blagoev B, Pedersen BK, Kratchmarova I. Dynamics of the skeletal muscle secretome during myoblast differentiation. Mol Cell Proteomics 2010;9(11):2482-96.



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