Béatrice Sloth
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Ministeriet for Videnskab, Teknologi og Udvikling
Forsknings- og Innovationsstyrelsen
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Dear Ms Sloth,

This cover letter forms part of my response to the information provided by BKP and her staff and should be included within the evidence documents for the evaluation committee.

I will not burden the committee with detailed response to the issue of personal attacks, suffice to say I have suffered to several at the hands of BKP where she has used her close relationship with the Danish Press to defame me, and used her staff and friends to harass me at my UK institute.

BKP is privileged to have direct funding to run her Institute. When one reads the long and often evasive response from BKP, and her junior accomplices, one has a feeling of genuine sadness and pity. The document represents a long list of excuses for carrying out substandard work, often presented in a non-scientific manner.

The letter is interjected by dishonest technical misdirection's, several intellectual failings and, most importantly, complete silence on some of the major allegations. A further issue is the withdrawal of my access to CIM raw-data, which would no doubt have revealed scientific problems that I would have spotted.

BKP states that the advent of real-time PCR meant that the myokines were now easy to detect and thus they were "really there". This confirms she does not understand PCR. With old PCR one could run cycles until one got a band. The higher the number of cycles, the less RNA there was there in the first place. With real-time PCR, the situation is no different. She seems unable to grasp that sensitivity of detection is not relevant to the question of physiological relevance – if a gene is barely detected, it means it exists only in few cells from your biopsy, no matter the "number" you get.

I urge the committee to closely examine all the original complaints and closely examine the UVVU definition of fraud. If one respects the Danish Governments definition of scientific fraud, then there is no doubt that Dr Pedersen's center is guilty of multiple failings.

What I will do in the following few pages is address the major flaws in her rebuttal, the remainder I leave to the committee. It is very important for the sake of the reputation of research in our field and for the reputation of the University of Copenhagen, whom I continue work with on FP7 funded projects, that the highest standards of scientific pursuit are seen to be implemented.

Sincerely

Professor James A Timmons PhD

Royal Veterinary College, University of London

cc Prof Stuart Reid

Summary points reflecting the evidence BKP provided the UVVU May-Sept 2011

- BKP falsely claims to have inspired the BDNF data analysis for the Genome Medicine Paper by email in April 2009. Yet the informatics pin-pointed BDNF in 2008 and we did the BDNF qPCR in 2008 and on the 20th of Jan 2009 we submitted JCI- 38641-RG-1 which clearly states that BDNF was a relevant miRNA target in the abstract
- BKP claims she was out of the loop regarding the Genome Medicine paper – but she had copies of the main article back at the start of 2009 onwards and BKP told Camilla to coordinate the correspondence for CIM. (See "CS confirms BKP et al get a Copy of JCI version with BDNF in abstract.**PDF**)
- The clinical studies used in the Diabetologia article were falsely presented
- There was no protein biopsy sampling control in the Diabetologia article and thus we don't know what the sampling did or indeed who the subjects actually were.
- Christina Boholm confirms that someone in BDNF 'team' falsely labelled a BNDF western blot during the production of the manuscript "by accident" - is this for real?
- The critical time-course immune-blot in Diabetologia was only the best, n=1, subject
- Christina Boholm states that no loading controls were run at the time of the Western analysis but rather done a year later
- Christina Boholm states the westerns were added until significance was achieved (something called multiple testing when u add more and more samples and re-test)
- The CIM raw database management is corrupted, as they state that IL18 raw data was incorrectly labelled as IL8 in their raw database. What other samples are wrongly labelled?
- BKP highlights two English sentences that say the same basic thing in the Genome Medicine paper – claims that somehow this is wrong and insinuates wrong doing by me in her cover letter.
- BKP falsely states many times that I say muscle does not express BDNF - when in fact I state every time, that is extremely low and discretely expressed - see my main report
- CS et al suggest my cDNA for my BDNF measurements "may be degraded" yet I provided them with two plots - one for another gene measured at the same time and showing text-book profiles. Such suggestions appear disparate and dishonest.
- Muscle calculations indicate that BDNF is expressed <5% of muscle cells only - using the BKP rtQPCR values.
- BKP et al removed Raw data files when they knew that I was going to be given access - Camilla Scheele knows, as I was her PhD supervisor, that I would of course spot further flaws.
- BKP states BDNF has an unusually high number of splice variants when in fact most are UTR variants that would not impact on EXON spanning primers
- The rejection letter from FASEB J 2008 that is used in their defence actually proves they attempted to over-sell the entire story (reviewer 2's comments) so making the fraudulent Exp Physiology review even worse, as they are forewarned in 2008.

The Genome Medicine microRNA paper

BKP appears to either not know the content of the various papers done in collaboration with me and is purposefully misleading the UVVU on this point.

A first article on miRNAs and diabetes only included some selected qPCR of 3 microRNAs – a small analysis and a small paper. This had little connection with the final article and is of no relevance to the investigation. It was not published as the data were integrated into a larger project.

The miRNA work continued in my lab in Edinburgh during 2007 and 2008, and we developed new informatics techniques (the main point of the Genome Med paper). That informatics work identified BDNF as being a target in diabetes, in 2008 and work was started on BNDF protein profiling along with several other proteins (by Camilla Scheele, my former PhD student - now a post doc in BKP lab).

The version sent to JCI in Jan 2009 included the BDNF data informatics first, then protein in the revised version but that was still deemed not enough for JCI, so we sent it to Genome Medicine, almost a year after we first identified BDNF by informatics means.

BKP states that she sent the Diabetologia paper for scientific discussion in Feb 19th 2010. But in fact Camilla Scheele and I already discussed the problem that CS could not see BDNF in whole muscle and that my lab had also shown the mRNA expression was <<<low (i.e. few copies per thousands of nuclei) in 2008. In her report BKP directly misquotes me. I said it would be interesting to look at the **satellite cells** (as that is where BDNF can be found under some circumstances).

This discussion resulted in me sending BKP my concerns in Jan 2009. BKP ignored these concerns and in fact there is no evidence by email that BKP entered into any scientific discussion with me. She merely said the BDNF data that I sent her were "interesting". What you have, by way of my email is evidence of is me attempting to "get her thinking" about the fact her "global BDNF protein" data with MF and Penkowa was not consistent with 10yrs of literature or the data I was sending her.

What is also worrying is that BKP claims this was the inspiration for BDNF work in Genome Medicine – yet she sent me this email on the 10th of April 2009 (A2_7_E-mail_Cool paper_Re_ BDNF and mir133 [DOK1998627]).

This email from BKP was *~6 months* after we had the informatics data on BDNF, 3 months after she was sent the article submitted to JCI detailing BDNF and ~ 4 *months* after Camilla started working on the protein analysis. It was of course also long after we started debating the authenticity of the BDNF data that BKP and MF were so keen to push out (this is why I sent the emails in Jan and Feb 2009!). At that time Camilla Scheele fully agreed with my views.

The claim by BKP that somehow she was the source of the inspiration for the BDNF work is therefore untruthful and frankly delusional. See the new PDF I attach to the present report that confirms my version of events and the versions of the JCI submission (which you can have on request).

Did JT change the meaning of a sentence in the Genome medicine paper?

Page 7 of the BKP report states changes to the text of Genome Medicine Paper – however, all changes were sent to Camilla Scheele and in fact **the two versions say the same thing** – one is just

more precise on the details (n=24 was the endurance data that BKP was sent in Jan 2009 and chose to ignore, data from the same age range as her Diabetologia article – as samples originate from Timmons et al 2005 FASEB J).

BKP had decided during 2009 that Camilla should handle all communication with me, as BKP preferred that. I was unhappy with BKP partly due to her taking over ideas from my lab and partly because she was annoyed by me continuing to point out flaws in her CIM studies.

Camilla can be seen coordinating comments in the Jan 17th email. S**o BKP stating that she wonders why she is not getting communications about the papers is also a complete lie** (See "CS confirms BKP et al get a Copy of JCI version with BDNF in abstract.**PDF**)

Page 9 of BKP report.

I did not assume that BDNF was "not expressed" – I referred to 10yrs of literature detailing that BDNF was found at neuromuscular junctions and induced during muscle damage. This was consistent with a very low expression. Both my data and that of BKP demonstrates a very low expression and thus if the RNA for BDNF is only in a few cells

Why did BKP and MF thus claim that the protein was

a) everywhere in the muscle cross-section and

b) in their original article submitted to FASEB J in 2008 (and rejected) that the muscle "produced the BDNF into the circulation".

See BKP own 'evidence' for proof of this – (A3_5_FW_ FASEB Journal - Manuscript [DOK1998703]).

Clearly from their own email correspondence they were trying to "sell" a new Myokine story.

In Jan 2009 I write to BKP "mature muscle does not really express bndf unless damaged" – I can't think of a more direct warning that her "BDNF expressed everywhere data" was problematic. I have also reported my concerns to Diabetologia who now await the outcome of the UVVU investigation.

BDNF protein expression - F_2 [DOK1998778] – How is an unlabelled, multi-signal western profile evidence of BDNF expression? The lab is accused of fraud and one is expected to trust this n=3 blot of "something"? Now if this is really BDNF expression – why do they claim age is the reason for the difference with the Genome Med paper as now they have it in diabetics (presumably middle-aged)

RNA and cDNA – pages 12 onwards in BKP report

I sent BKP a plot of BDNF and just above the BDNF plot I show them another gene (asBDNF (a separate gene) – measured at the same time. The other gene is expressed perhaps a little higher than BDNF and demonstrates a beautiful, tight and non-varying low abundance profile **using the same cDNA that was used for the BDNF plot below.**

Thus it is clear my cDNA was OK. The reason for the variation in BDNF signal is low copy number of BDNF in human muscle....

An over-view of Cycle threshold (CT).

Because real-time qPCR is a relative procedure one typically compares all samples from a study in a single run. The value you calculate is called "CT" and it is a point on the exponential phase of the reaction above background noise (when the reagents are not limiting). The exponential phase is the 'linear' part of the log2 plot. The CT can be varied, but should be set away from evidence of back-group fluorescence. In our case (my Jan 2009 data) it could have been set to yield a CT of ~35 to 37.

Depending on the specific incorporation of florescence and the machine used this can vary slightly more. It can never take a very low abundant gene and make is moderate or high. My lab also used Applied Biosystem reagents to measure BDNF. If one doubles the RNA input into the cDNA reaction one could yield ~34 at most.

Is this an argument against my view? No. All values CIM and JT values are "very low". What else do you need to tell if a gene is really very low?

CT is not the only indicator of abundance. In fact the variance of triplicates and of related samples (e.g. all resting samples) also tells you when you are dealing with low copy number as was the case of the BDNF data (low and variable expression - as my data and the qPCR figure in the Diabetologia article also demonstrates).

How can we understand how much gene is expressed?

Well, cDNA is made using 1ug of RNA from muscle. This comes from ~5-10mg of muscle tissue. 5mg of muscle tissue contains approximately 2000 fibres when dissected out from freeze dried (i.e. an accurate estimate of the total) and each 3-5mm fibre represents hundreds of nuclear domains (*cf* cells). So the 1ug cDNA library comes from equivalent of ~200,000 cells.

10ng is put into the reaction - i.e. ~ 2000 cells worth. If you then see that you have a copy number in the real-time assay approaching 1 (unreliable, variable expression and high CT) then that tells you have 1-3 copies of BDNF mRNA for those ~2000 cells. That is 0.2% cells expression BDNF. That actually makes sense as it probably reflects the % of satellite cells activated at any one time during normal muscle turnover (Sat cell nuclei being up to 10% of total).

If you argue that my calculations are 10x out - you still get the answer that only 2% of the cells are making BDNF mRNA and thus it is impossible that an entire muscle x-section shows uniform BDNF expression.

Let's assume that BKP has really got 4ct more than me. Then BKP has ~48 copies per 2000 cells – i.e. less than 3% of cells contain BDNF.

Thus the BKP arguments about translational efficiency are mute for this reason – almost no muscle cells have any BDNF so translational efficiency is irrelevant.

"BKP states BDNF has an unusually high number of splice variants"..

The majority of genes have splice variants, many at the '3UTR. BDNF has only a small number of exons and in fact the variants are almost all 3'UTR variants (http://genome.ucsc.edu). As the PCR primers target a common exon region this 3' UTR variance is irrelevant for the real-time PCR.

Acute versus chronic exercise - a misleading tale

First BKP argues that age differences explain the difference in results between her lab and mine

But then she accepts that my exercise data is also from young people. So then it is acute versus chronic exercise that is the difference.

Is the acute versus chronic exercise argument valid? **No, it is not**. Both data sets include resting samples from the same age, and both data sets (based on BKP claims) demonstrate that BDNF mRNA is very low. Allegedly 33ct in her case and 37ct in my case.

At most she finds that BDNF increases ~1.5 fold more than her time control leg but no time point is significant.

And as stated in my report, there is literature evidence that a 6-10 fold increase in BDNF mRNA still does not yield a change in protein signal.

For sure, all we can do is evaluate the BDNF data in light of a decade of literature – and all that literature points to low levels of discrete expression at neuromuscular junctions and satellite cells in adult muscle and more induction only on muscle damage (something BKP shows doesn't happen in their "control" leg for RNA but now we know there is no control leg for the protein data).

What BKP also ignores the fact that the fraud allegations go beyond the Diabetalogia paper but include the Experimental Physiology review.

BKP has published a BDNF review in summer 2010 which I also represents scientific fraud. In that review she claims that BDNF is an important metabolic factor potentially linked to the health benefits of exercise. Yet the health benefits of exercise reflect the chronic impact of exercise.

I presented her data in Jan 2009 that BDNF is not altered with chronic training and that the expression was so low at baseline that it can only be in a sub-set of cells.

Now re-read Figure 5 and ledged in the BKP and Mark F 2010 **Experimental Physiology review**?

Without doubt the Experimental Physiology review by BKP and MF is a fraudulent piece of work based on UVVU guidelines, as based on all the information that they had at hand at the time it is not even an accurate account of their own paper never mind the Genome Medicine data.

Remaining BDNF details from BKP et al

The section which describes all the missing subject groups, false stars on figures, the incremental analysis and preliminary statistics searching for a result, the long time duration between re-blotting and the lack of real control over the protein time course data is appalling. This is supposed to be an "elite" centre? With no time control for protein, any signal may be muscle damage if multiple biopsy samples came from the same incision. As they give the impression in the article they have a perfect time control with no BDNF induction – this in its own right is a fraudulent presentation of a research study.

If they used a statistician, that person should be named and interviewed. The fact the peer reviewed failed to catch them out is no justification. They did not plot the AUC data. Why would you do an AUC calculation and then plot the non-significant data and mark it significant

If the RNA is a paired study (while the protein came from another study) why did they use an unpaired t-test for the alleged AUC plot? It is all unbelievable and demonstrates a complete lack of respect for rigorous data analysis and presentation.

Immunohistochemistry now apparently represents an n=1 example, and now it seems the RNA and the protein come from the same person on this occasion. Again, their "critical" time control experiment is used to prove no induction by biopsy is missing for protein – while we don't know how the protein data was derived.

No reasonable scientist could assemble this BDNF paper and believe that everything was rigorous and above board. If it was left to only PhD students with no input by BKP or MF then it's a failure in proper academic duty.

All roads lead to the same conclusion – this group of scientists are not fit for purpose. I strongly recommend ALL CIM publications are now evaluated as how many other times did they not declare samples, give on n=1 examples and in general disregard the ethics of the peer review process. A reviewer can only review the information they are given.

Other scientists disagree with JT regarding BDNF

This is a remarkable section – as they use their rejection letter from FASEB J to defending their position. I will come back to the fact that reviewer 2 clearly highlights that they were "over selling" the BDNF data as a "new myokine" story.

Reviewer 1 of the FASEB J also refers to literature where a **damaging** exercise protocol can induce BNDF in muscle. This I have said all along. I have also said all along that BNDF is discretely expressed in muscle – not that it is undetectable.

I have provided a detailed critique of the so called exercise articles that BKP states she put in the revised Diabetologia article after rejection from FASEB J.

Some of the other articles that the reviewer mentions do not AT ALL refer to skeletal muscle and **thus again BKP is misleading the UVVU process**.

None of the cell studies that BKP claim are "mechanistic" support an in vivo role. They include gross over-expression and work in a mouse muscle tumour cell line. It is delusional to think this *in vitro* work underpins the in vivo medical relevance of BDNF in real muscle.

Now, let's go back to the 2nd reviewer from FASEB J (who rejected their original manuscript) - **A3_5_FW_ FASEB Journal - Manuscript [DOK1998703]**

From reviewer 2's comments it is clear that BKP/MF made claims of exercise inducing BDNF protein in abundance and this entered the circulation during exercise, so contributing to the health benefits of exercise (i.e. her usual myokine story) in this 2008 FASEB J version of the paper.

That reviewer states this is not consistent with the data, and so they change it for the Diabetologia paper but keep this false story in their Exp Physiology Review.

This is fraud, based on the UVVU guidelines, as the false claims re-appear in the Experimental Physiology review. A review which ignores "her" Genome Medicine paper and the data sent to her in Jan 2009 on BDNF not changing with training in humans.

I have provided the key critique of the animal data that BKP now misrepresents in the UVVU report. A skilled independent scientist will now have to re-read these articles and look at what the authors actually show and actually claim. BKP appears to read only the abstract or highlights and not the full articles.

Page 22 – <u>Now she refers to the review – again in a dishonest manner</u>. BKP claims that both RNA and protein are markedly up and in fact he now tells us that the immuno blot comes from an n=1 exaggerated example and where it is unclear what experiment yielded this value.

It is also clear that BDNF from muscle can't act in a paracrine manner if it is not released from muscle – and even they claim it is not released. Further, its role in "peripheral metabolism" (meaning integrated in vivo inter-organ metabolism) is not at all addressed by **<u>muscle tumour line</u>** experiments in vitro (which is what she refers to!) nor addressed by artificially gross over-expression as all the evidence, including their own (claimed but not shown) RNA data, that BDNF is very poorly expressed.

<u>MiRNA fraud</u>

<u>The same level of misrepresentation continues with the responses from Camilla Scheele and BKP.</u>

CS claims to have a PhD in function genomics – but in fact she did a PhD in simple cell biology and qPCR in tissues under my supervision.

Further, CS ran the key western blots for the Genome Med paper but did not do the majority of the molecular biology (the cell work with miRNA manipulation was for example done in Edinburgh and Dundee). She extracted RNA for the study and helped write the paper.

Matt Layes version of the miRNA events demonstrates he had no idea where the ideas came from or the fact that I met with the student Soren Nielsen over 20 times to discuss miRNA research prior to his arrival, during and after ML took over as SN "boss".

This long term interaction with SN on miRNA was precisely why I was sent the manuscript and why Soren was apprehensive about what I might think. I am also clearly the person that introduced CIM to the miRNA field.

The SN miRNA data are a replication of the data generated in 2007 that I shared with the BKP group, and I also stated to Soren that I would prefer my data to be published first as that was fair. Soren sent me a manuscript with no authors listed. The project was discussed with Soren many times in person and also by email.

At no stage did I imagine that I would not be an author – even though I did wish for my labs study to be published first. It was actually under review at the same time at the same journal but mysteriously got rejected.

BKP claims not to know the reviewers - yet BKP was an editor at that very same journal (although she has now been removed from the editorial board) and the paper was reviewed by her friend Matthew Watt and she knows this. In fact, BKP was accused of irregular behaviours in peer review duties by senior editors during her time at the journal so she was asked to leave for more than one reason.

When I finally saw the version of the article that now had an author list – I was very unhappy. It was only later that I reflected on the fact that the version Soren sent me had no authors listed – a situation that now appears to me to be very odd. Most people assign the author list at the start of the process – but then I now appreciate it was to hide that Matt was senior author and I would not be on the paper because BKP insisted on this to be the case.

Myokines

I will not comment in detail on the myokine work as others will in more detail than I can.

Why Soren Nielsen presented me with IL18 data and not IL8 will have to be answered by him, but it's clear throughout he is not exactly being straight over many matters**. If indeed the explanation is that the data files were incorrectly labelled as IL8 and not IL18 then what other data from this centre has been given the wrong name? Why are raw data files not labelled properly?**

We have already seen that on close inspection the data description and continuity in the Diabetologia article on BDNF is absolutely shambolic.

If every enquiry I have made reveals a mistake in data management, coupled with the lack of scrutiny of all of the Penkowa data, how can one trust any molecular data generated from CIM now?

One needs to ask why he was even showing me this data in the first place – if we were not discussing the fact that many of the myokine stories were really poorly founded.

The arguments BKP make about the general acceptance of the Myokine work reflects the fact that she has produced an enormous number of articles and reviews over-stating their role. There are few independent data and the entire discussion around IL6 protein in the Natalie Hiscock article is completely at odds with the Penkowa data. How can these two "IL6" labs that worked so closely together get such different images for IL6 expression? If one examines the Hiscock paper you also see that IL6 induction is claimed to associate with Glycogen metabolism, yet if that were true the wrong fibres are "lighting" up. Who knows what the IL6 immuno blots actually represent.

Nobody doubts that muscle expresses IL6 **RNA**. What many doubt is that it has any significant role in human metabolism and without the protein blots from Penkowa, BKP and CIM has never shown that the protein directly comes from muscle. All the human av studies show is that a leg can produce IL6 (a leg made of many cell types).

Indeed, IL6 is not the issue for this fraud report but rather the amazing productivity of the Keller's and their relative Steensberg, during a ~6yr period.

My suspicions is that BKP and MF churned out a huge number of articles, not bothering to examine the raw data. Certainly, Pernille Keller as a post-doc working in my lab in Edinburgh was **still** not fully trained in how to apply correct statistical analysis to such data.

Personal issues

Page 1. The Report went to the University of Copenhagen, the UVVU and the editorial staff of the related journals where the fraudulent data has appeared. The report then was leaked by BKP to a press associate Poul Pilgaard Johnsen circa 18th April 2010, who did a 1-sided propaganda article for her shortly afterwards attacking me.

Thus BKP has sought to immediately ignore the confidential nature of the physical report and present a misleading story to the public and press. She has subsequently done this with parts of subsequent reports, submitted by other scientists (reporting fraud) to the UVVU this year. Once leaked to the press, where it was being misreported and including personal attacks against me, I provided the press the truth of the details being reported by making my report available.

It is correct that Pernille Keller and I share a child and his name is Arthur. He is a fabulous 2.5yr old boy and I fly to Copenhagen each month to spend a day with him and briefly meet Pernille. After I submitted my first complaints to University Copenhagen in Nov 2010 Pernille began to interact with BKP regarding impending retractions and also suddenly filed for sole custody. Two of Pernille's articles have been retracted.

It was during her interactions with BKP, in the Spring of 2010 that Pernille Keller's lawyer now attempted to black mail me into not filing an official UVVU report on the fraud case. She planned to use this against me in the child custody case. The basis for Pernille's strategy, is that under Danish family law, she would argue this was hostility to the mother. I had to give up my fight for shared custody because of this black mail, as I no longer could afford the legal fees to continue the case into the second court hearing.

During this period in 2011, I was very bitter, as Pernille was working alongside BKP using my child to try and block the report. I first complained about poor PhD student standards at CIM from the end of 2007 and about data issues from 2009 onwards.

There is clearly a time-scale disconnect between my scientific concerns and the personal matters that arose later. Also when I moved to Copenhagen in Jan 2009 that I chose to work in the department of Flemming Dela and Jorn Helge at Panum and not CIM.

The lawyer Mr Niels Eriksen (ne@bonnesen.dk) can confirm that he took the phone call from Pernille's lawyer where her lawyer attempted to "negotiate" terms around the custody on the condition that I stopped my UVVU report.