CIM
Centre of Inflammation and Metabolism

Udvalgene vedr. Videnskabelig Uredelighed
Ministeriet for Videnskab, Teknologi og Udvikling
Forsknings- og Innovationsstyrelsen
Bredgade 40
1260 Copenhagen K

Copenhagen, May 23, 2011

In April we were informed by Jamie Timmons (JT) as well as by several of our colleagues and via the Danish media that on April 18th 2011 JT had forwarded a report to UVVU in which he accuses me and members of my research group as well as international collaborators of fraudulent behaviour in regard to several scientific publications and other matters. The receipt of the report by UVVU was later acknowledged in my telephone conversation with Lene Kløcker Knüppel.

We are convinced that JT's motives for filing his report are highly personal and that his intent is to damage our reputation and credibility. In addition to filing his report to the UVVU, JT has chosen to circulate it to the Danish and international press and to a vast number of people in the scientific community around the world. We find JT's conduct in this matter and general behaviour highly inappropriate and very damaging to the basis of the Danish National Research Foundation's Centre of Inflammation and Metabolism and its researchers.

However, we must clear ourselves, and I (BKP) have therefore prepared the attached report to respond in a point-by-point fashion to the charges put forward against us. References to accusations put forward by JT in his report to UVVU are marked in blue. The report has been approved by Mark Febbraio (MF), Matthew Laye (ML), Adam Steensberg (AS), Pernille Keller (PK), Charlotte Keller Steensberg (CKS), Camilla Scheele (CS) and Søren Nielsen (SN), who are all mentioned in JT's report.

We include documentation, e.g. original data and e-mails, which refutes the accusations put forward by JT. We are prepared to submit any other material that may be required by UVVU.

In order to conduct its internationally acknowledged cutting-edge research the Centre of Inflammation and Metabolism has secured an annual turnover of external funding of around DKK 17 mio of which approx 1/3 comes from the Danish National Research Foundation and the remaining from national and international public and private funding agencies. Following the media coverage of JT's doings a number of granting agencies have expressed their concern to fund our research before the outcome of UVVU's examination of JT's report has been made public. Thus, I must earnestly request that your investigation is initiated and concluded at your earliest convenience.

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Our account to UVVU will be submitted also for information to our authorities at the University of Copenhagen and Rigshospitalet as well as to the Danish National Research Foundation.

Sincerely

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A - BDNF

A1. Background

To disprove the criticism regarding lack of supervision, we hereby include:

a) Organizational structure of CIM showing that each PhD student belongs to a thematic research group with daily or almost daily supervision by a group leader.
b) Protocols for various laboratory analyses.
c) Our internal rules for conducting clinical studies.

(A1_1 to A1_20)

A2. BDNF

JT Report:
The diabetes collaboration with my lab led to the study of global mRNA and microRNA muscle profiles in my lab in Stockholm and then Edinburgh. In 2008 my lab identified several proteins, using informatics, including BDNF. This work was submitted for publication in 2008 and 2009 and finally published in "Genome Medicine" (submitted May and August 2009, resubmitted and accepted Sept 2009 and published Feb 2010 [PDF1.1).

Unknown to me, until early 2009, BKP had parallel collaborations on BDNF in her own lab and through a collaboration with Dr Mark Febbraio.

Reply:
I (BKP) initially presented the human BDNF data in 2006 at an international meeting in Seoul, Korea. My collaborator Mark Febbraio (MF) was present during the meeting and we decided to collaborate on examining the biological role of BDNF.

Jamie Timmons claims that he was not aware of this work until 2009. However, in his report he contradicts himself as he apparently discusses our BDNF work with PK in 2008 (p.5 bottom). Notwithstanding, although JT and I collaborated on some studies, he was of course not informed about all projects going on within CIM. From JT's report one gets the impression that the Genome Medicine paper (A2_1) and the Diabetologia paper (A2_2) are two competing studies on BDNF. This is certainly not true. The Diabetologia study focuses entirely on the muscular expression of BDNF, whereas there was no mention of BDNF at all in the first versions of the later so-called Genome Medicine-paper that focuses on microRNA.

In the Genome Medicine paper, we contributed muscle biopsy samples obtained from our diabetes cohort. The first version of this manuscript was submitted to Diabetes on January 14, 2008 (A2_3). This paper did not include any data or discussions about BDNF. The paper was rejected by Diabetes. On July 2nd 2008, JT submitted a new version of the paper (A2_4) to JCI. Indeed, this version of the manuscript also did not contain any data on BDNF. The

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manuscript was rejected. On February 19, 2009, I forwarded the BDNF Diabetologia paper to JT for information and for scientific discussion/interaction.

On February 20, 2009, JT writes:

Dear Camilla and Bente

Thanks for sharing the BDNF paper with me - its very interesting. I recall when I was in Nottingham working with Paul G, he did a collaboration looking at BDNF induction during muscle damage - Exp Neurol. 2000 Feb;161(2):597-608.
what they noted was fibre type specific and related to muscle recovering from damage. Using in situ hybs they located BDNF mRNA to the soleus muscle fibres in damaged muscle - i think the idea was some sort of signal to link msucle and nerve back together again..

Anyway, attached is the only data I have on BDNF in human muscle. I ran some analysis for Claes last year - looking at BDNF and its natural antisense before and after endurance training (n=24). Slide attached.

It is technically difficult to say that it is expressed in whole muscle - perhaps just a subtype or in the satellite cells - and it did not vary with 6wks training (samples taken 24hrs post training). We should cross compare primers to make sure that we are not measuring different parts of the gene.

I think it would be great to look at BDNF in the satelitte cells of the diabetics vs controls - western and then immuno if at all possible.

Cheers
Jamie

(A2_5)

Attachment (A2_6)

Comment:
We find it somewhat curious that JT on one hand is convinced that BDNF is not expressed in muscle biopsies, while on the other hand he says that it would be great to look at BDNF in our cohort study. I/we interpreted his e-mail as if JT was inspired to include BDNF as a possible target for the microRNAs in question. This is how fruitful scientific collaboration evolves. We interact and we inspire each other.

On april 2009, I (BKP) forwarded a Nature Medicine paper to JT regarding mir133 and BDNF of interest for the microRNA Genome Medicine paper. He finds it is "a cool paper" (A2_7). Once again, we interact and inspire each other. There was no indication that JT looked at our BDNF paper as fraud.

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It was as a consequence of the above scientific discussion that Camilla Scheele (CS) performed studies on BDNF in muscle from the diabetes cohort study. According to nucleotide based prediction analysis of differentially expressed microRNAs (in diabetics vs. controls), BDNF was one of the top-rated targets. BDNF was one out of 6 protein targets assessed by CS for the so-called Genome Medicine paper.

It was not until the June 3, 2009-revised version of the manuscript for JCI, that the BDNF data, performed at CIM by CS, were included (A2_8). The manuscript was rejected.

On September 13, 2009, I (BKP) received an e-mail from JT (A2_9) with a version of the manuscript named "Mirs_and_insulin_resistance_GM_FINAL", which included the BDNF data and the following new sentence in the discussion: "BDNF mRNA expression is not altered by endurance training status (data not shown) and is only reliably detectable in activated muscle satellite cells" (A2_10). JT informed me in the e-mail that he had been in personal contact with the editor-in-chief of Genome Medicine who would look favorably on the paper (A2_9). I then heard nothing further about the paper until JT informed me in an e-mail of 21 December 2009 that the manuscript had been accepted by Genome Medicine (see e-mail in string of mails in A2_11). On my request JT sends me on January 10, 2010 (A2_11) for our files the accepted version of the Genome Medicine paper with figures. I naturally assumed that this accepted and later printed version of the paper was identical to the one I received on September 13, 2009. It is not until recently that I discovered that in the version that JT submitted and which was accepted, he had changed the above-mentioned sentence in the discussion into the following: "Interestingly, BDNF mRNA expression is not altered by endurance training [18] and additional RT-qPCR on this material (n=24, data not shown) found it barely detectable in adult muscle tissue." (A2_12). Seeing that JT now accuses us of publishing conflicting data with regard to BDNF, we find it of interest to highlight the fact that JT chose to enter this sentence without informing or securing acceptance from BKP.

However, as we will discuss in details below, there are many explanations why there is some discrepance between the unpublished data mentioned in the sentence, which was inserted by JT, and the results presented in the Diabetologia article.

Still in the view of JT's accusations it is important to underline that the Genome Medicine paper is primarily focusing on microRNA and that BDNF was brought in as one of six target proteins, for the purpose of validating the prediction analysis.

Therefore, it is not true that we had two ongoing competing BDNF stories at the same time. If anything, JT was stimulated by our BDNF work to look at BDNF as one target for the myoMIRs in our collaborative study.

JT Report:
The BNDF muscle work with MF, which includes protein analysis work done in Copenhagen, was published in "Diabetologia" (July 2009). This was submitted on 25th February and accepted 19 days later. Nevertheless, BKP was warned that the data must be unsound prior to

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the submission date, based on directly comparable data from the other active study on BDNF (in collaboration with me) and the literature.

Reply:
We did not find any reasons to doubt our data although we, in contrast to JT, were able to demonstrate BDNF expression in human muscle. Especially when it comes to human studies, there are many examples of publications reporting contradicting findings. The samples assessed for BDNF protein expression in the Genome Medicine study (which we assume but cannot confirm from the report is the other active study at CIM to which JT is referring) were all from subjects around 60 years of age, while the subjects in the Diabetologia paper were young healthy 20-30 year-old men, and thus the subject populations from these two studies are not comparable. JT did provide us with some preliminary mRNA data from young healthy subjects (A2_6), however, this analysis does not contradict our findings; when the Ct threshold is appropriately set, there is no difference between the Ct values of the samples with the highest expression in JT's analysis, compared to our peer-reviewed and published data (possible reasons for some of JT's samples demonstrating a lower expression will be discussed further below).

It should also be noted that JT states that the BDNF manuscript was submitted on 25th February and accepted 19 days later. This is yet another inaccuracy, since the paper was submitted in December of 2008. We were asked to make revisions by the reviewers and submitted the revised manuscript on February 25th 2009 (A2_13 and A2_14).

JT Report:
Thus, on the 4th of January I alerted BKP to the interest in BDNF in our collaboration but informed her that BDNF was never expressed across all muscle fibres but rather only in a small proportion of cells in undamaged muscle. ***[PDF 1].***

Reply:
This so called "alerting" from JT is based on an assumption he makes from one qPCR analysis (and both the technical analysis and the conclusions based on this analysis can be discussed), not on peer-reviewed published data. One problem with assessing BDNF at the mRNA level is the unusually high amount of splice variants (Pruunsild P et al., Genomics, 2007 Sep;90(3):397-406. Epub 2007 Jul 12). In our study, published in Diabetologia, we therefore choose to use a pre-developed and optimized primer assay from Applied Biosystems, the leading company for quantitative real-time PCR. Basing primer design on a less expressed splice variant would of course result in a lower signal from the qPCR analysis. JT has not shared his primer sequences with us, neither has he specified which splice variant he used for his primer design.

JT Report:
On the 20th of February 2009 I followed up my 4th of January 2009 email because I was made aware by CS that BKP was ignoring the assertion that BDNF was not widely expressed in human muscle. On the 21st of Feb 2009 BKP acknowledged my email and just said the data were 'interesting' ***[PDF 2 and 3]***

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Reply:
Again, JT is merely assuming that BDNF is not expressed in muscle. However, there was and is no evidence that this is actually the case and as our data showed the opposite, why should we not proceed with the publication? In addition, as stated above the samples in the Genome Medicine study in which CS had assessed BDNF by western blot came from subjects who were much older than those in the Diabetologia study and thus were not comparable.

I regarded the information in the e-mails below of general interest. I did not then and I do not now see any of their content as being a "warning" or an indication of JT in 2009 drawing our attention to any fraudulent behaviour.

Jamie Timmons <jamie.timmons@gmail.com> 4 January 2009 18:19
To: Bente Klarlund Pedersen <bente.klarlund.pedersen@rh.regionh.dk>
Cc: Scheele <camilla.scheele@gmail.com>
OK, Same to you both - happy new year.
Attached is a slide i think you will find interesting - we found that the 60 mirs that change significantly hit the
BDNF pathway at various points.
Red means loss of mirs should up-regulate the gene in muscle, blue means gain in mirs should down-regulate
the genes in muscle.
For the BDNF changes I'm assuming we are looking at the signal from the satellite cells in muscle (where it is
known to prevents myogenic differentiation Jasmin et al 06) as the mature muscle does not really express bdnf
unless damaged..
The 5 other miRNA hit pathways we found are all strongly linked to diabetes - so it seems very validated - while
our analysis provides new candidate proteins to study in these signalling pathways
revised paper etc in a few days
Cheers
(A2_15)

JT Report:
When I challenged BKP again in November 2010 that the Diabetologia protein data was fraudulent she stated that she would leave the decision to the incoming editor, J Zierath. Zierath was a member of the scientific board of CIM and thus not independent. In addition, I have previously reported a publication of Zierath's for data manipulation in 2007, to the editorial board of J Biological Chemistry [information available on request].

Reply:
We do not retract a peer-reviewed scientific publication, just because someone tries to threaten us into doing so. When the Penkowa case came up in the media in the latter half of

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2010, Jamie Timmons took the opportunity to harass PK and her family as well as me. They and I received several e-mails in mid-November of 2010, in which JT accuses us of fraud. In addition he kept sending me threatening phone text messages 24 hours a day, such as "Penkowa gone, u next".

On November 12, 2010, I received an e-mail from JT in which he writes:

"I think the Diabetologia article should be withdrawn by your lab immediately."

On November 12, 2010, I responded:

"Dear Jamie.
I have forwarded your emails to the Dean (Ulla Wewer) and to Juleen Zierath and will await their response. Bente"
(A2_16)

I informed Ulla Wewer (Dean at the Faculty of Health Sciences - SUND) and Juleen Zierath (the editor-in-chief of Diabetologia) about the accusations and threats against me put forward by JT. In addition, Jannik Hilsted (Medical Director at Rigshospitalet) and legal counsellors at the University of Copenhagen were informed.

Juleen Zierath is the editor-in-chief of Diabetologia, in which we published the article that according to JT ought to be retracted. I formally asked JZ if I should take any actions with regard to the journal. She replied that if I stood by my data, I should not do anything in that respect and she forwarded the mail correspondence to the managing editor of the journal. She also said that if she received any formal complaint regarding this paper, the journal would immediately investigate the case. According to my information, JT has not reported us to Diabetologia.

JT Report:
Unknown to me, until early 2009, BKP had parallel collaborations on BDNF in her own lab and through a collaboration with Dr Mark Febbraio (MF, Baker Institute, Melbourne). MF and Mathew Watt (MW) have had several articles Cont., 4 withdrawn due to fraud over the past few years [e.g. Southgate etal FASEB J 2005; Watt et al, Molecular Endocrinology 2008 etc.]

Reply:
Mark Febbraio (MF) is a highly distinguished researcher. It is correct that he has retracted two papers. Once he received information that there may have been some misrepresentation of data, his organisation initiated an inquiry under the National Health and Medical Research Council of Australia Code of Research Conduct. It was found that one of MF's PhD students had inappropriately represented data and MF deemed it appropriate to retract the data. Importantly, however, neither MF nor any other author was found to be incriminated in any way. For further information, please contact professor Garry Jennings, Director of Baker IDI, Melbourne, Australia.

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A3. Details of relevant activities ……..

Timmons claim that the induction of BDNF protein is unfeasible because "without mRNA you cannot make BDNF protein". It is not entirely clear, whether he means that human skeletal muscle in general expresses very little/undetectable amount of BDNF or whether he refers to the fact that mRNA and protein do not follow. We will discuss our data in relation to the existing literature.

BDNF expression in muscle

JT Report:
In my email sent on the 20th of Feb 2009 to BKP and CS, sent after my initial warning on the 4th of January 2009, I included rtqPCR data from analysis in my lab. I relied on n=24 young male subjects before and 24hrs after endurance exercise and this data was cited in the Genome Medicine article. [PDF 3]…….

Reply:
In the Genome Medicine paper (p.15), JT states, "BDNF mRNA is not altered by endurance training (18) and additional RT-qPCR on this material (n=24, data not shown) found was barely detectable in adult muscle tissue".

It is not clear why JT found that the BDNF expression was as low as 37 Ct, while in the Diabetologia paper, we found much lower Ct values (higher mRNA levels) ranging between 30 after acute exercise and 33 before exercise, indicating a low to moderate, but robust expression of BDNF in human skeletal muscle, see below.

However, such differences in expression levels do not necessarily mean that we are facing "contradicting findings". In our view it is highly insinuating to argue that such differences make us guilty of "fraud". In fact, having a closer look at the power point slide that JT sends to me on 20/2 2009 with BDNF qPCR (A2_6), it is clear to anyone familiar with the method that the threshold can be moved down slightly and that this would actually result in a Ct value of 33 (which would be similar to our finding) for the sample with the highest expression. However, there is a large variation between JT's samples. If the variation is random (i.e., does not vary systematically with treatment or subject groups, which seems to be the case here), this could indicate that the cDNA is in bad condition, i.e degraded.

The samples that JT use for assessing BDNF in muscle, which, according to himself, was analysed in 2008, originate from RNA that CS isolated during her PhD studies at Karolinska Institutet in 2006, thus the cDNA is 2 years old (unless of course JT has produced a new batch) and the RNA/cDNA has been freeze-thawed several times, which does affect the quality and subsequently the results.

Nevertheless, the finding that training had no effect is not in conflict with the data in the Diabetologia paper, which investigated the effect of acute exercise.

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In a response to one of the several recent group e-mails in which JT accuses us for "knowing" that BDNF is undetectable in muscle, Camilla Scheele states on April 6th 2011:

"There has been a misunderstanding of my opinion in this matter. I do not believe that there is a major discrepancy between the two studies mentioned in your e-mail. The muscle samples assessed in the Genome Medicine paper were from old/middle-aged healthy or diabetic subjects, while the sample set in the BDNF Diabetologia paper consisted of young healthy men before and after exercise. As you mention, BDNF is higher expressed in satellite cells than in mature muscle. It is possible that the quality and amount of satellite cells differs between muscle biopsies from old versus young subjects. This might explain why BDNF was detected in the younger sample set but not in the older."
(A3_1)

JT replies:

"Dear Camilla,

I note that yesterday you decide to not honour your private comments about the BDNF issues. That is your choice and you will have to live with it.

You stated " I do not believe that there is a major discrepancy between the two studies mentioned in your e-mail"

You then cite an idea that you and BKP cooked up to explain away differences. But it would appear now neither of you have read the Genome Medicine paper you are supposed to be authors on.

In the Gallagher paper we refer to data from <u>both young and old subjects</u> - so your made-up explanation is flawed.
(A3_2)

Comment:
We assess BDNF at protein levels using western blots – these samples were all from old people. The only reference to data from young people is the qPCR data discussed above. This data is referred to in the discussion as "data not shown".

JT continues
"*In young people, BDNF occurs at ~37ct after endurance training - attached is the slide we sent Bente in Jan 2009 - a slide you fully endorced at the time and we discussed. Surely you don't need me to explain the amplification plots to you for a real-time PCR graph?"*
(A3_2)

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Comment:
Again, JT appears to be twisting the truth to match his purposes. As discussed above, lowering the Ct-threshold (still keeping it within the exponential phase) in the analysis of JT's samples would result in a Ct value of around 33 for the sample with the highest expression. JT detects no difference in mRNA expression following 6 weeks of endurance training, however, the Diabetologia study examines mRNA changes following acute exercise, which is a completely different biological state. Hence, the expression in JT's samples can only be compared to the resting samples in the Diabetologia study, which are around 33.

JT also writes to CS:
"At the 24hr post-exercise time point Penkowa demonstrates complete global protein induction in the Febbraio/Pedersen Diabetologia paper. I sent you and BKP that information prior to publishing the Diabetologia paper and you demonstrated no ability to detect protein other than proliferating satellite cells (in older people). If there is not RNA where would the global protein come from - the 'little people' ?"
(A3_2)

Comment:
Again, JT is mixing up the basis for his arguments as he compares studies with completely different subject populations and qPCR data (measuring mRNA levels) with western blot data (measuring protein levels).

General comments of interest for the disagreement

We also want to put forward the general statement that there are several ways that proteins may change without a (large) change in the mRNA levels. Several ways are as follows:

- Alterations in translational efficiency, without altering transcript abundance.
- Alteration of protein degradation rate, while maintaining the same translational rate.
- miRNA regulation: Some papers have shown that miRNAs have a larger role on regulating protein abundance without an effect on mRNA levels. For example, our own paper with JT shows no change in mRNA using the microarray, but the differences in miRNAs are predicted to result in a difference in protein. BDNF was in fact shown to be changed in the proliferating cells.

In regard to BDNF, an interesting paper claims that there are two distinct 3'UTR regions that result in different translational rates. So in theory one could get enhanced translation without much of an increase in mRNA: http://www.pnas.org/content/107/36/15945.long

Another article suggests (without directly showing it) how BDNF can be regulated post-transcriptional in the brain following voluntary wheel running in mice

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http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6T0F-528YXF4-5&_user=9843566&_coverDate=04%2F28%2F2011&_rdoc=1&_fmt=high&_orig=gateway&_origin=gateway&_sort=d&_docanchor=&view=c&_acct=C000034378&_version=1&_urlVersion=0&_userid=9843566&md5=7402995f2a9d48319a1553f5898c29ce&searchtype=a

Yet another article shows how high frequency stimulation in the brain increases mature BDNF protein: http://www.ncbi.nlm.nih.gov/pubmed/19147841, but again they did not measure mRNA levels.

There are also large-scale studies that have measured all mRNAs and all proteins in the same study, which show that there is a substantial difference between mRNA and protein expressions. Also, the difference is greater in eukaryotic cells versus more simple cell types suggesting that with evolution there are more post-translational modifications (De Sousa Abreu R et al., Molecular Biosystems, 2009 Dec;5(12):1512-26. Epub 2009 Oct 1).

In the Genome Medicine paper, it is shown that BDNF is expressed in human primary satellite cells (proliferating), but not in differentiated myocytes. The discrepancy between data from mature primary cultured myotubes and human muscle biopsies can easily be explained by the fact that human muscle biopsies contain muscle cells at all stages (i.e. quiescent satellite cells, proliferating myoblasts and fully differentiated myocytes (myotubes)) as well as other cell types such as fibroblasts, blood cells, endothelial cells etc, while our cultures myotubes represent a more pure population of cells. The western blot data presented in the Genome Medicine paper to which JT refers were accumulated by CS in the CIM laboratory. Unpublished data, also produced in the CIM laboratory confirm a two-fold higher expression of BDNF in human primary proliferating myoblasts compared to differentiated myotubes (Matthew Laye). Thus, there is no controversy between the findings in differentiated muscle cell cultures and in human muscle biopsies and we have been able to repeat our own findings in independent experiments.

The BDNF Diabetologia paper (A2_2)

Healthy humans performed bicycle exercise for two hours and muscle biopsies were obtained at time points 0, 2, 3, 5, 8, 24, 48 and 72 hours. A control group rested and had biopsies taken at the same time points. We have made the discovery that by a mistake, there is no description of the control group in the Diabetologia paper. The description of all of the subjects is present in folders kept at CIM. All original data are included (A3_3).

BDNF mRNA

JT Report:
Instead of applying an ANOVA as they made repeated measurements, they 'created' an AUC analysis and a single unpaired t-test. This is not conventional or valid.

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Reply:
We describe that there was no differences in BDNF mRNA levels in the exercise cohort compared with the resting cohort (BDNF paper Fig. 1a). This was likely due to the fact that the BDNF mRNA levels demonstrated a marked inter-individual variation with peak mRNA levels between 5 and 8 h for the majority of the subjects. Advised by our statistician, we calculated the area under the curve over time for both groups and showed a difference ($P<0.05$) for the AUC when comparing the two groups. This method of statistical analyses was not questioned in the peer review process of the manuscript. All of the original data are included (A3_3).

Thus, the data show a low to moderate, but robust expression of BDNF mRNA in human skeletal muscle, which is induced from app. 33 Ct values to 30 Ct values. We think we describe the data as they are without any overinterpretation.

BDNF protein

The antibody against BDNF protein recognises both the pro- and mature-form of BDNF. In all western blot experiments, rhBDNF was loaded into one lane as a positive control. In the human samples, the mature form of BDNF was recognised and the expression appeared to increase progressively following exercise. At 24 h into recovery from exercise, BDNF protein expression was increased (~50%;$P<0.05$) in the muscle homogenates (Fig. 1b).

JT Report:
PK also stated to me in late 2008 and 2009 that the original western blot in the Diabetologia article had been repeated several times, and early in the manuscript's history it was not significant. That work was done at CIM. However, in the early days, when the manuscript was sent to MF and returned, the Figure showing the western blot suddenly had a "star" annotating the change as significant.

Reply:
Christa Broholm, PhD student in CIM, performed the Westerns referred to in JT's accusation above. Following one of JT's many group e-mails she writes an e-mail to the CIM group on April 7th 2011 to clarify the situation

Dear All

Jamie Timmons has lately accused us of re-running the western blots for BDNF to achieve significance. This is absolutely false. I did these blots so I should know that. This is how the events occurred:

February 2007:
I am asked by Bente to optimise the rhBDNF antibody (R&D systems) and analyse the expression of BDNF in human muscle biopsies before and after acute bicycle exercise. To begin with I did a pilot study with n=3. The results showed a tendency towards increased BDNF expression

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after exercise. We always knew that this was not significant. At some point, a significant symbol was by mistake placed on this figure by someone in our lab. However, we quickly noticed that and I sent out an e-mail to all co-authors. Shortly after the symbol was removed.

September 2007
I increased the n-numbers to 8 and sent the results to Mark Febbraio (without loading control)

July 2008
I was asked by Mark Febbraio to run an internal control. Since the previous BDNF membranes were almost 1 year old (and thus too old to strip), I loaded the samples again. I then cut the membrane and incubated for BDNF and b-actin. These results were used in the final article and show that the BDNF protein expression is increased with acute exercise.
(A3_4)

All the original data regarding Westerns are included (A3_3)

BDNF IHC

The quantification of the BDNF protein was performed by Western. The immune-histochemistry data (Fig 1d in the publication) were accumulated to support data generated by two independent scientists (KS Krabbe and C Broholm; Fig 1a-c) in CIM and two independent scientists (VB Matthews and MHS Chan; Fig 2 d-f) in the Febbraio laboratory.

The IHC was also performed to obtain some indications of whether the BDNF expression might be increased within muscle fibres. We chose to show the IHC image from the person, who had the most mRNA response (CH). This person appeared also to have the most pronounced BDNF protein response. Thus, we show the IHC image that best supported that the increased BDNF expression 24 h post-exercise was indeed intramyocellular, Fig.1d.

The IHC was not performed by Milena Penkowa (MP), but by Maj-Brit Åstrom (second author on the paper), with the help from a student, Dan Sonne Pedersen in the Penkowa laboratory. MP was involved in supervising the IHC technique and in the performance and description of Fig.1d.

The studies presented in Fig.1 showed that there was a small increase in BDNF mRNA and protein (Western) in response to exercise. The IHC image suggested that muscle cells might express the BDNF protein. The original data are included (A3_3).

JT Report:
These events alone should have been sufficient for an honest scientist to stop, think and investigate what was going on and post-pone the review process of the Diabetologia article

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and question the protein data. BKP et al ignored this 'bad news' and pushed ahead with publication and parallel review articles to promote their "story".

Reply:
Throughout his report, JT states that we ought to have questioned the above-mentioned data. We will hereby substantiate that 1) other scientists disagree with JTs scientific views; 2) that we did not stop but added a sufficient amount of mechanistic data in an attempt to understand the origin and the role of muscle-derived BDNF.

1) Other scientists disagree with JT as they regarded our data as being not novel. When the manuscript was submitted to FASEB Journal, which did not include extensive cell and animal work that was performed and eventually published in Diabetologia, we received these comments (13-10-2007), which provide an excellent summary of the literature and highlight the fact that BDNF is expressed by skeletal muscle and can be induced by contractions. The reviewer actually expresses concern about the novelty of the data. On other terms, he/she finds that it is well accepted that contracting skeletal muscle expresses BDNF.

The reviewer states:

"There are major concerns about the novelty of the data presented here. Several studies in rodents have demonstrated that exercise, electrical stimulation (and contraction) of skeletal muscle leads to an induction of BNDF expression in muscle. (Copray et al. (2000), Experimental Neurobiology, 161: 597-608, Gomez-Pinilla et al. (2002), J. Neurophysiol, 88: 2187-2195' Park et al. (2003), Neuroscience Research Communications, 34:10-19, Seidl et al. (1998), J. Cellular Physiol, 176: 10-21). Moreover, recently in Med Sci Sports Exerc 2007 April; 39, 728-34 Ferris et al demonstrated that exercise induced BNDF levels in humans, they also showed that cognitive functions increased (although they did not correlate with BNDF changes). In the opinion of this reviewer this manuscript requires new additional functional insights into the role of BNDF function in skeletal muscle metabolism prior to publication. The authors should consider BNDF siRNA and/or ectopic overexpression experiments in the C2C12 in vitro cell culture system to ascertain the effect on several pertinent aspects of metabolism. The present version of the manuscript provides information that is largely descriptive (with incremental insights) rather than significant new insights.

For example, the authors suggest that the novelty of this data presented is due to the increased expression of BDNF from contracting skeletal muscle. Several studies have also reported these or similar findings. For example, Copray et al. (2000), Experimental Neurobiology, 161: 597-608, found intense contraction of soleus muscle in both normal and diabetic rats caused an increase in the expression of BDNF. Moreover, ultrastructural studies from these same authors found BDNF expression was localized within muscle fibers and activated satellite cells. Furthermore, no expression of BDNF was observed in Schwann cells or fibroblasts, suggesting that the localization of BDNF was defined within the muscle fibers.

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In other studies, Gomez-Pinilla et al. (2002), J. Neurophysiol, 88: 2187-2195, found BDNF mRNA and protein levels in rodents increased in soleus muscle after 3 and 7 days of exercise. Moreover, following paralysis of the soleus muscle, BDNF mRNA levels were reduced, demonstrating that active muscle contraction is important in modulating BDNF levels in muscle. Moreover, Park et al. (2003), Neuroscience Research Communications, 34:10-19, also identified electrical stimulation of rat soleus and medial gastocnemius muscles resulted in a significant induction of BDNF mRNA and protein" (A3_5).

2) While JT states that we did not "stop, think and investigate what was going on", this is not true. In fact, the human muscle biopsy data was supported by several mechanistic studies, Fig.2 to 6 in the Diabetologia paper. All original data are included (A3_3).

We state "that the kinetics of the exercise-induced elevation in serum BDNF levels failed to correlate with the increased expression of BDNF in muscle 24h post exercise suggesting that the skeletal muscle is <u>not</u> a source of the increase in serum BDNF observed early into recovery from exercise".

We state "Our IHC results suggested that the contraction-induced increase in BDNF protein expression was increased in skeletal muscle cells. Since it was possible that BDNF was produced by other cell types within the skeletal muscle bed before being taken up by muscle cells, it was necessary to move to a cell culture model to determine whether skeletal muscle cells *per se* can increase BDNF expression when contracted." Thus, we did not just conclude based on IHC that muscle cells express BDNF, but established a cell culture system to contract differentiated C2C12 myotubes *in vitro*.

The fact that the conclusion in the Diabetologia paper far from solely relies on the human muscle biopsy data is reflected in the paper's abstract.

"Aims- *Brain-derived neurotrophic factor (BDNF) is expressed in skeletal muscle, but the functional significance of this expression is unknown. We aimed to determine the signaling processes and metabolic actions of BDNF.*
Methods- *We first examined whether exercise induced BDNF expression in humans. Next, C2C12 skeletal muscle cells were electrically stimulated to mimic contraction. L6 myotubes and isolated rat extensor digitorum longus (EDL) muscles, were treated with BDNF and phosphorylation of the proteins AMPK (Thr^{172}) and ACCβ (Ser^{79}) were analyzed as was fatty acid oxidation (FAO). Finally, we electroporated a BDNF vector into the tibialis cranialis of mice.*
Results- *BDNF mRNA and protein expression were increased in human skeletal muscle after exercise, but muscle derived BDNF appeared not to be released into the circulation. BDNF mRNA and protein expression was increased in muscle cells which were electrically stimulated. BDNF increased phosphorylation of AMPK and ACCβ and enhanced FAO both in vitro and ex vivo. The effect of BDNF on FAO was AMPK dependent, since the increase in FAO was abrogated in cells infected with an AMPK dominant negative adenovirus or treated with Compound C, a specific pharmacological inhibitor of AMPK. Electroporation of a BDNF expression vector into the tibialis cranialis muscle resulted in increased BDNF protein*

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expression and TrkB ($^{Tyr706/707}$) and ERK (p44/42 Thr^{202}/Tyr^{204}) phosphorylation in these muscles. In addition, phosphorylation of ACCβ was markedly elevated in the BDNF electroporated muscles.
***Conclusions**- These data identify BDNF as contraction-inducible protein in skeletal muscle that increases lipid oxidation via activation of AMPK".*

We include the original material for Fig. 2, 3, 4, 5 and 6 (A3_3).

A4. The Diabetologia article and the Experimental Physiology article represent undisclosed biased or distorted interpretation of a person`s own results and conclusion.

JT Report:
As mentioned, the data I brought to the collaboration was consistent with published literature. The literature information, as well as the information I brought to the collaboration, was ignored.

Reply:
We did not ignore the data put forward by JT as discussed in details above, and we did not ignore the literature. In fact, the scientific literature suggests that BDNF is expressed in skeletal muscle and can be induced by contractions.

Several studies in rodents have demonstrated that exercise, electrical stimulation (and contraction) of skeletal muscle leads to an induction of BDNF expression in muscle.

JT refers to a study in which his collaborator Greenhalf is a co-author (Copray et al. (2000), Experimental Neurobiology, 161: 597-608)

Copray et al demonstrate that intense contraction of soleus muscle in both normal and diabetic rats caused an increase in the expression of BDNF. The finding that there also was a small increase in creatine kinase, indicating muscle damage, does not confirm that BDNF was stimulated by damage and does not exclude the possibility that muscle contractions per se might enhance BDNF mRNA expression. Moreover, ultrastructural studies from these same authors found BDNF expression was localized within muscle fibres and activated satellite cells. Furthermore, no expression of BDNF was observed in Schwann cells or fibroblasts, suggesting that the localization of BDNF was defined within the muscle fibres. These findings could easily be interpreted as a support for our findings.

Another study by Liem (Liem et al Histochem Cell Biol. 2001 Dec;116(6):545-51. Epub 2001 Nov 27) demonstrates that in adult rat muscle the constitutive expression of muscular BDNF is confined to the myofibres. However, they state that satellite cells, Schwann cells, endothelial cells, fibroblasts or axons do not appear to contribute to BDNF production in normal muscle. These authors state that although muscular BDNF is a neurotrophic factor for innervating motoneurons and supposedly released only at the motor endplates, the production of BDNF mRNA appears to occur along the entire length of the myofibres and is not confined

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to nuclei in the postsynaptic regions. This study further supports the idea that BDNF is expressed by myofibres.

In other studies, Gomez-Pinilla (Gomez-Pinilla et al. (2002), J. Neurophysiol, 88: 2187-2195) found that BDNF mRNA and protein levels in rodents increased in soleus muscle after 3 and 7 days of voluntary wheel running exercise. Moreover, following paralysis of the soleus muscle, BDNF mRNA levels were reduced. These results indicate that basal levels of neuromuscular activity are required to maintain normal levels of BDNF in the neuromuscular system.

Park et al (Park et al. (2003), Neuroscience Research Communications, 34:10-19) also identified that electrical stimulation of rat soleus and medial gastocnemius muscles resulted in a significant induction of both BDNF mRNA and protein.

Cuppini et al (Arch Ital Biol 2007 May;145(2):99-110) reported that BDNF mRNA increased over the second day after acute exercise. BDNF protein level progressively increased also after the mRNA went back to the basal level, so suggesting that it cumulates within the cell after acute exercise, whereas it followed the mRNA level time course after repetitive exercise. These results point to the following conclusions: BDNF mRNA is up-regulated by activity, but this response is delayed to the second day after acute exercise. The finding that the BDNF protein was not up-regulated until the second day after an acute bout of exercise is totally in agreement with our findings that the BDNF protein peaks on the second day after acute exercise.

Ogborn al (Muscle Nerve 2010 Mar;41(3):385-91) reported that BDNF expression was elevated in rat soleus following 5 days, but not 10 days of exercise. They conclude that BDNF may be a protein with an uncharacterized contribution to the acute adaptation of skeletal muscle to exercise.

Thus, there are many indications in the literature that rodent skeletal muscle expresses BDNF and that contractions stimulate an up-regulation of this expression. We find that it is more likely that human skeletal muscle would respond in a similar way. Therefore, we conclude that our data are consistent with the published literature.

JT Report:
Nevertheless in a first-author review by BKP in Experimental Physiology accepted on 9th September 2009 – BKP made bold new claims about BDNF's functions. ***[PDF 6]****. The review contained "wild claims" that BDNF was some-how a new regulator of muscle fat oxidation in human muscle - especially within the diagrams and figure* ***[PDF 7].***

Reply:
In the above-mentioned review (Exp Physiol) we wrote: *"In response to muscle contractions, BDNF mRNA and protein expression is markedly increased in human skeletal muscle after exercise; however, muscle-derived BDNF appears not to be released into the circulation. Brain-derived neurotrophic factor mRNA and protein expression was increased in muscle*

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cells that were electrically stimulated. Interestingly, BDNF increased phosphorylation of AMPK and ACCβ and enhanced fat oxidation both in vitro and ex vivo. Thus, BDNF has been identified as a novel contraction-induced muscle cell derived protein that exerts its effect either via autocrine or paracrine actions, whereby it increases fat oxidation in skeletal muscle in an AMPK-dependent fashion. Reproduced from Pedersen et al. (2009)".

One could argue that the word "markedly" may be considered an over-interpretation because the increase in protein expression was approximately 50% (see figure 1C). However, we think we presented our data in a balanced fashion (A4_1).

JT Report:
BKP wrote in the summer of 2009 - " By demonstrating that BDNF is expressed in muscle and has an impact on fat oxidation, we add a new dimension to the pleiotrophic nature of BDNF, which can now be identified as playing a role in neurobiology as well as in both central and peripheral metabolism"

Reply:
We believe that others and we have provided strong support for this statement. We refer to Fig. 3,4.5 and 6 in the Diabetologia paper (A3_3 and A2_2).

JT Report:
Yet BKP states in her July 2009 Diabetologia article that they only found effects of BDNF when they "over-expressed it artificially and that its in vivo role is unclear".

Reply:
In the Diabetologia article we write the following: *"It must be acknowledged, however, that the contraction-induced increase in BDNF both in vivo (Fig. 1) and in vitro (Fig. 3) was relatively modest, whereas we increased BDNF several fold with electroporation (Fig. 6). Therefore, whether BDNF plays an important role in vivo is unclear. In summary, we have identified BDNF as being a novel contraction-induced muscle cell-derived protein that can increase fat oxidation in skeletal muscle in an AMPK dependent fashion. Our data, therefore, raise the possibility that BDNF analogues could be used as a possible therapy to treat metabolic disease."*

It is not true that we only found effects of BDNF when we "over-expressed it artificially". In fact, we demonstrate that BDNF increases fat oxidation in an AMPK dependent manner in L6 myotubes. We further demonstrate that BDNF increases phosphorylation of AMPK and its downstream target ACC and results in enhanced fat oxidation in intact skeletal muscle ex vivo. Finally, we demonstrate that skeletal muscle overexpression of BDNF in vivo results in ERK, TrkB, and ACCβ phosphorylation, but does not increase circulating BDNF levels. Thus, based on cell cultures, isolated skeletal muscle and a murine in vivo model, we conclude that BDNF is a contraction-produced protein that may regulate fat oxidation.

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JT Report:
A story implying that BDNF from human muscles has been shown to be important for the regulation of human metabolism is a work of fiction and an exemplification of dishonest scientific writing.

Reply:
We do not agree. Based on our data and the existing litterature, we stand by our conclusion: Taken together, these data demonstrate that BDNF is a protein produced in skeletal muscle cells that is increased by contraction to enhance fat oxidation in an AMPK-dependent fashion, most probably by acting in an autocrine and/or paracrine manner within skeletal muscle. Hence, BDNF has been identified as a novel contraction-induced protein that may contribute to the multiple health benefits associated with exercise, possibly by enhancing fat oxidation in skeletal muscle. By demonstrating that BDNF is expressed in muscle and has an impact on fat oxidation, we add a new dimension to the pleiotrophic nature of BDNF, which can now be identified as playing a role in neurobiology as well as in both central and peripheral metabolism.

A5. Additional information about the BDNF

JT Report:
Dr Pernille Keller (PK, now employed by Novo Nordisk), a co-author of the Genome Medicine article, who carried out some of the Genome Medicine microRNA work in my laboratory in Scotland, was originally also an author on the Diabetologia article.
In 2008 and 2009 PK asked for her name to be removed from the Diabetologia article because she was unhappy with the authenticity of the work - and diplomatically wrote that she had "not done enough to merit authorship'.

Reply:
On April 6th 2011, JT forwarded nine e-mails with "cc" to numerous scientists all around the world in which he accuses us of fraud regarding the BDNF paper, mentioned above. PK responded as follows to one of these group e-mails:

"Dear Bente,

I am very sorry about the course of events. Jamie and I were in court two days ago, as I have requested full custody of our son. Jamie is obviously very angry, and I am afraid that he is taking his anger out on you to somehow get revenge.

Regarding Jamie's statement (copied in from email below):
"Why did Pernille Keller withdraw her name from that same paper with Mark Febbraio prior to submission, in 2008, and why was western blot repeated so often and digitised so heavily? Why did Mark put significant symbols on early drafts of the manuscript - prior to the final blot being produced - when there was actually no significant data? The paper trail on the email accounts prove this account of events."

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My contribution to the BDNF paper was to measure mRNA expression of the receptor for BDNF. Given that there was no effect of exercise on the BDNF receptor, we chose not to include the data in the paper. My scientific contributions to the paper were thus very minor, also given that I did not participate in the writing up of the article. My very modest scientific contribution to the article was the main reason for me withdrawing from the article (please see attached file of email correspondence between Bente and I in December 2007 regarding the BDNF paper authorship - in Danish!).

Regarding the data, then I have no reason to believe - then or now - that fraud is involved.

Best Regards,"
(A5_1 and A5_2)

B - "MicroRNA dishonesty case".

JT Report:
BKP plagiarised an article I wrote on microRNAs and used it in a grant application, claiming the microRNA work was done at CIM, and submitted the grant in the name of Henrietta Pilegaard (HP) and BKP. No mention of my name or my lab was made. This grant was used to then fund work that 'stole' observations from my lab.

On the 20th of July 2009 I also became aware that BKP had produced a short grant application in the name of HP and BKP to be submitted in Copenhagen. At this time I was working part-time in Copenhagen and attempting to obtain grants of my own in Denmark, on my research. The grant application represents several levels of plagiarism [See PDF15 vs PDF1.1]. Large parts of the text are cut and pasted from the article I wrote for Genome Medicine on microRNAs and diabetes. BKP did not write any of the Genome Medicine article. Secondly, it presents the picture that all microRNA work was done at CIM by CIM scientists, when in fact none of it was. It was all done in my lab in Scotland. It then goes on to talk about microRNA changes in relation to exercise - when in fact I sent them such data from my lab, in September 2008. For example it states: "Researchers within CIM have demonstrated robust changes in miRNA in muscle biopsies from patients with insulin resistance when compared to carefully matched controls". The grant application contains a number of untruths about the Genome Medicine diabetes miRNA data and several other technical flaws. Nevertheless it passes off discoveries from my lab, as work done at CIM and that is dishonest behaviour. So based on articles I wrote and unpublished raw data BKP produced 'new' myomir publications and grants - publishing before my lab could and also systematically presenting the new microRNA work as originating in concept from her laboratory.

Reply:
In 2009, JT and I were collaborators. Indeed, as late as in 2010, he invites me to participate in one of his grant applications to the Danish Council for Strategic Research (B_1).

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In 2009, I was called upon to submit an internal application to the UNIK program (Food, Fitness and Pharma) at the Faculty of Health Sciences, University of Copenhagen. I applied for 1/3 PhD scholarship for Søren Nielsen. I included associate professor Henriette Pilegaard as she is one of the supervisors for SN and seeing that she is affiliated with the UNIK project via me and CIM.

The UNIK project is supported by a 120 mio. DKK (16 mio Euro) grant from the Danish Ministry of Science, Technology and Innovation. UNIK is based on collaboration between research groups from 17 different institutes at 7 faculties. Only researchers within the UNIK project can apply for these grants.

http://foodfitnesspharma.ku.dk/researchers/

Thus, the "grant application" (1½ A4 page) represented an internal application, not a competing application (B_2)

On July 20th 2009, I wrote an e-mail to JT (B_3). The application to UNIK was attached. Given that he now accuses us of dishonest behaviour, I find it very peculiar that he did not complain until now.

JT accuses me of plagiarism, when in fact what I did was to incorporate a few lines in a background description "taken" from an article that has CS as shared first author, several people from CIM as co-authors, myself as second last and JT as last author.

In the internal application, we write *"Researchers within CIM have demonstrated robust changes in miRNA in muscle biopsies from patients with insulin resistance when compared to carefully matched controls".* All the clinical samples and clinical characterization of the diabetes cohort were obtained within CIM. A major part of the molecular work was performed by CS in CIM. The microRNA analyses were performed by JT, who were linked with CIM, when the study was initiated.
I/we find it unacceptable that JT in this way accuses me /us of plagiarism or of unfair competition with regard to funding.
Prof. Niels-Henrik Holstein-Rathlou (NHHR), head of the Dept. of Biological Sciences, Faculty of Health Sciences, Univ. of Copenhagen to which JT was until recently affiliated, has given a written statement that supports the view that we are not guilty of plagiarism. Note that NHHR believes the internal paper is with regard to CMRC, whereas it is in fact with regard to UNIK (B_4).

JT Report:
BKP also falsely promoted the Genome Medicine microRNA work as originating from her centre, by promoting CS to give a key-note at an international American congress she helped organise (ACSM, October, Miami 2010). This is a flagrant abuse of influence.

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Reply:
With regard to the meeting mentioned above: I was asked to give a 60 min key-note plenary talk. In addition, I was asked to chair and organize one of the scientific sessions and to give one of the four talks in that session. I found it inappropriate to give two plenary talks in one meeting. I therefore suggested to the organisers that either Mark Febbraio or someone from my lab should replace me. MF could not make it and thus CS was invited. She initially suggested the title:

"The effect of myokines on the maturation and function of visceral and subcutaneous adipocytes" (B_5).

However, CS later asked me if she could change the title into "The Regulation of Muscle – microRNA and Their Potential Role in Metabolism" (B_6), which I accepted without discussion.

JT was present during the Miami meeting and interacted with CS in a friendly manner. In her talk, CS gave credit to JT as the senior author of the paper and it was made very clear to the audience that JT had had a leading role in conducting the study in question. CS also highlighted the microRNA study.

On April 16th, 2011, JT writes to CS:

"Nevertheless, it has been clear that you have been prepared to take false credit for the miRNA work (I would have been ashamed to give the APS lecture in Miami, had our roles been reversed, as you did not actually contribute to a single aspect of the microRNA analysis)."
(B_7)

Comment:
CS note: At the time when I was preparing for the ACSM talk (in Miami), Jamie asked me if I could present the Genome Medicine paper, he saw it as a good opportunity to promote the study (on which he is senior author) to a large audience. I was happy to do this, as I am proud of what we demonstrate in that paper. I am a molecular biologist and I did the majority of the molecular biology work for the paper as well as contributing with my expertise when interpreting the data (I have a PhD in functional genomics). I did not do the bioinformatics, this is why Ian Gallagher is first and my name is second although it is a shared first authorship.

JT Report:
Following access to my labs confidential data and advice, CIM replicated my observations and packaged into an article and published in J Physiology (Nielsen S et al, J Physiol. 2010 Oct 15;588(Pt 20):4029-37) and gave false credit to a newly arrived post-doc from the USA called Dr Mathew Laye (ML) as the senior author. ML had only recently joined CIM from the USA and had no track record in microRNA biology or involvement in any of the J Physiology

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human studies. ML did not initiate or direct this work or write the article. In contrast my lab had provided substantial intellectual input.

On and before September 2008, I sent BKP, CS and Soren Nielsen (SN) a draft copy of the large-scale manuscript that was being considered by Nature. We had used biopsy samples to measure messenger RNA to help validate original observations from my lab [See PDF 8-10]. It can be seen in Figure 2a in PDF10, I presented BKP, CS and SN with clear data from my lab in 2008 indicating that the microRNA's (known as the 'myomirs') were regulated by endurance training in humans. At this time, no study of human muscle responses for these molecules was known.
BKP, CS, ML and SN submitted "duplicate" endurance training microRNA data to J Physiology in March 2010 plus additional information [PDF 11]. I was asked to help edit this manuscript by SN - and given all my input I just presumed that I would be an author. I made several recommendations for change in the article [PDF12-14]. However, the version of the article I was sent on the 11th of March had no cover page [See PDF 11]. I did not know that ML would be given a false senior authorship and I would be excluded as an author, until the Nielsen S et al, J Physiol. 2010 Oct 15;588(Pt 20):4029-37 article was published [PDF 11.1]. You can note in PDF 11.1, in contrast to PDF11, that my name now appears in the acknowledgements – and even the author contributions section stipulates that ML had nothing to do with the experiments, design or execution of the studies.

Reply:
Matthew Laye, PhD, is the senior author of the publication in question. His response is as follows:

I am very disappointed and upset by the ridiculous attempts at slander by Dr. Timmons. Until recently I considered myself a friend of Dr. Timmons', and as recently as September my fiancé and I were treated to dinner by him while on vacation in London. A dinner that he claimed to write-off as a business expense because of the intense discussion about science that we had over the meal.

At no point prior to the publication or submission of our article, (J Physiology (Nielsen S et al, J Physiol. 2010 Oct 15;588(Pt 20):4029-37) (B_8) did Dr. Timmons ever raise any of the issues brought forth in his report to the DCSD, I am deeply concerned that Dr. Timmons brought forth his concerns subsequent to the publication to the article in question, and that he has continued to pursue this matter beyond the realm of professional conduct by repeatedly sending threatening text messages to my colleagues at all hours of the night. Despite this, I feel the need to set the record straight regarding his false accusations over scientific misconduct.

First his claim of replication of data.

1) At no point in the paper have we used any data collected from, analyzed in, or initiated by Dr. Timmon's lab. We selected the 4 miRNAs based on our recent Genome Medicine Paper (Genome Med. 2010 Feb 1;2(2):9.) in which all 4 miRNAs changed in diabetics

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versus healthy skeletal muscle. Both Dr. Timmons and Dr. Pedersen are co-authors on this paper, with one of Dr. Pedersen's post-docs Dr. Scheele a co-first author. This information along with their well established role in muscle physiology within the published literature (Am J Physiol Endocrinol Metab. 2008 Dec;295(6):E1333-40. Epub 2008 Sep 30. and Nat Genet. 2006 Feb;38(2):228-33. Epub 2005 Dec 25) suggesting an exercise induced role for these targets. Regardless of when Dr. Timmons shared his data with other members of the lab it is not an intellectual leap to surmise that muscle specific miRNAs might be involved in exercise adaptations with the literature at our disposal at the time of initiating the miRNA measurements.

2) Dr. Timmons himself said at the time that it was okay to submit the data in a conversation with Søren Nielsen (B_9). Because we were aware that his group was doing similar work we asked Dr. Timmons to read the manuscript to ensure that the data overlap was minimal. After reading the manuscript prior to submission and Dr. Timmons concluded that the overlap in data was minimal and we should submit the manuscript. At no point during this process did he claim plagiarism or ask to be placed as an author. He spent a minimal amount of time reading the manuscript and as such we appropriately acknowledged his contributions in the acknowledgement section.
3) The data and training between the two studies in question differ. While we saw a downregulation of 4 different miRNAs (miR-133a, miR-133b, miR-206, miR-1) following endurance training Dr. Timmons only found a difference in 2 of the miRNAs (miRNA-133a and miR-1). The training protocols that Dr. Timmons used were dramatically different. Highly intense intervals in our case and lower intensity, less frequent, and for not as long a period of time in his study (J Appl Physiol. 2011 Jan;110(1):46-59. Epub 2010 Oct 7.). Of note, his lower training volume and intensity might explain why Jamie only saw two of the muscle specific microRNAs change, while we saw all 4 altered.
4) In addition to endurance exercise we performed analysis during acute exercise, and insulin clamps before and after the training protocol. In addition we measured the miRNAs after two weeks of cessation of training, which necessitated the inclusion immediately after endurance training. In his study he measured mRNA levels in high and low responders to physical activity, mRNA in a rat model, and did extensive bioinformatics. Thus his paper was dramatically different than ours.

Second his claim that senior authorship was not warranted for me. Dr. Timmons was not involved in any of the studies done, nor was he a deciding factor in what studies to conduct the tests in. He claims that, "ML did not initiate or direct this work or write the article." all of which is completely false. He does not have any firsthand knowledge of what I contributed to the study or of the amount of time that I spent working on the manuscript in all of its forms. To claim otherwise is frankly a lie and an attempt to slander my name and the science conducted at CIM.

1) As Søren Nielsen's PhD supervisor I oversaw all of the data, including the collection of the raw data. I was involved in the statistical tests used and the intensive discussions about interpretation of the data. Of the authors only Søren Nielsen put in more time on the manuscript than I did.

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2) Under my suggestion the measurements before and after the insulin clamp and after the cessation of training for two weeks were initiated, as I have extensive training in inactivity physiology. This resulted in the very novel finding that within two weeks of ceasing endurance training the miRNAs all returned to their pre-training level, a very significant finding within the paper. My contributions in the contributions section are limited to the manuscript preparation and editing because we feel conception and design is limited to the original human study, which was not originally conducted with the sole purpose of analyzing miRNAs, and not the decisions about which time points to use for the analysis. In our opinion data analysis is limited to the actual analysis of the raw data and does not include when myself would ask Søren to show me the raw data or express the data relative to the control RNA. Further it was I that recognized the value of the design in allowing us to make a 2 week post training measurement. I was also responsible for the decision about which protein targets to analyze as part of our response to the reviewers.
3) SN and I spent many hours writing and editing the article.
4) Dr. Timmons claims that I do not have experience in miRNA biology. He is correct in that I was not training miRNA biology, but I have done much of my PhD work on epigenetics, and upon learning that Søren Nielsen was interested in miRNA biology I did extensive reading of the literature regarding miRNA biology. As PhD we are trained to be able to read and critically evaluate work in many areas, it is part of the training we are given. I would not consider myself a good scientist if I was unable to learn about a new field and be confident in making relatively simple measurements in that field within a short period of time. I would also consider myself more qualified than Dr. Pedersen on the subject of miRNAs, who would have been last author had she not felt that I was deserving of that position in the paper. Everyone has their first article in a new field, as at one point Dr. Timmons did also, and there is nothing to prevent one from being a first, last or anywhere in between author on a subject that they have not published extensively in, or previously when the science is conducted in the proper manner.

JT Report:
BKP is an editor at J Physiology, and during the same period that her 'myomir' data was accepted for publication, my original data was rejected. The BKP article was surprisingly reviewed by a co-worker, Matthew Watt. The J Physiology apologised for the inappropriate handling of my manuscript.

Reply:
I (BKP) did not and do not know, who the reviewers of the above-mentioned paper were.

C – Other dishonest behaviours relating to the CIM myokine work

JT Report:
In my expert opinion, none of the CIM myokine work from ~2000 until 2011 would have been given substantial attention without the key muscle protein immune-blot data of Penkowa in 12 articles. All other protein data, particularly circulating and A-V protein production could easily arisen from the accepted cell types e.g. postcapillary white blood cells. All such data should be examined, and careful consideration given to the CT values of the qPCR data.

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The replies to JT's accusations under the "C"-heading has been formulated by AS; CKS, PK and BKP.

Reply:
We will go into details with the above general statement that none of the CIM myokine work from ~2000 until 2011 would have been given substantial attention without the key muscle protein immune-blot data of Penkowa in 12 articles.

Several of the best-cited myokine papers demonstrating that e.g. interleukin (IL-)6 mRNA is upregulated in muscle tissue and IL-6 protein is released from a working limb during and following exercise, were published without any immuno-blot data (e.g. Steensberg et al., 2000, J Physiol. Nov 15;529 Pt 1:237-42, Steensberg et al., 2001, J Physiol. Dec 1;537(Pt 2):633-9, Keller et al., 2001, FASEB J. Dec;15(14):2748-50. Epub 2001 Oct 29). Immuno-blots are important in order to attribute a release of a given substance to a specific cell type in the muscles. However, the Steensberg et al. (2000) IL-6 paper demonstrates a very high and robust release of IL-6 protein from a working limb – which would not be in accordance with low or not detectable IL-6 expression in muscles, as JT states throughout this section. We published the first immuno-blots on IL-6 protein in skeletal muscle cells following exercise in 2003. In 2004, supportive IHC as well as in situ hybridization data were published by Hiscock et al demonstrating that IL-6 mRNA expressions increased in the muscle cells following exercise (C_1). The authors found that at PRE, IL-6 protein was evenly distributed across all fibres at low levels, consistent with glycogen distribution. At POST, IL-6 protein was greater (P<0.05) in HIGH compared with LOW glycogen fibres, which coincided with type 2 fibres. IL-6 mRNA was distributed peripherally in all fibres at PRE. At POST, however, IL-6 mRNA appeared predominantly in type 2 fibres, which also had higher glycogen content (P<0.05). These data clearly demonstrated that myocytes per se are a source of IL-6 produced during contraction. Their data also suggested that type 2 fibres predominantly produce IL-6 during muscle contractile activity.

The study by Hiscock et al was in agreement with our study. The only difference was with regard to which muscle fibres would express IL-6. However, neither of our research groups looked at this as "conflicting data". The main findings were that muscle fibres could express the IL-6 in response to contraction. The difference with regard to muscle fibres could be due to a difference in exercise protocols.

Throughout section "C", JT mentions the Ct values of qPCR data and uses this as an argument for why we should have been alerted that the Penkowa data were fraudulent. This allegation seems to be based on confidential data from the CIM lab that has never been published. The qPCR data that JT mentions he has seen via SN are run in February 2006 by Thorbjörn Åkerstrom, that is, 1 year *after* the publication of the IL-8 paper (Akerstrom T, Exercise induces interleukin-8 expression in human skeletal muscle. J Physiol 563: 507-516, 2005).

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We have later come to know that the mentioned qPCR data actually represent IL-18 and not IL-8 and that the person who ran this particular assay considered IL-18 not to expressed and regulated by muscle contractions in healthy young humans (more about this later). The latter data have not been published.

JT Report:
It is very critical to note that the original article in 1998 (Ostrowski et al) from BKP, stated that IL6 was undetectable before exercise and only found in 5 from 8 samples, after 2hrs of exhausting exercise [PDF16].
Fast-forward 10 years and in a Physiological Reviews article BKP is pro-claiming that IL6 is a major and universal important factor for human skeletal muscle and exercise metabolism [PDF17]. So what happened during this 10 years?

Reply:
Real-time qPCR was introduced as a technique to measure mRNA abundance following the Ostrowski et al. paper. This provided a faster and much more sensitive system for determination of mRNA levels allowing detection of low-abundance transcripts. Therefore it was now possible to determine pre-exercise levels of cytokines. In fact, the first determination that IL-6 mRNA increased in human skeletal muscle measured by real time PCR was performed by the MF group and was the catalyst for the collaboration between BKP and MF (Starkie RL, et al J. Physiol 533: 585-591, 2001). Furthermore, as highlighted in our Physiology Reviews paper (Pedersen BK, Febbraio MA. Muscle as an endocrine organ: focus on muscle-derived interleukin-6. Physiol. Rev. 2008 Oct: 88(4): 1379-406) a large volume of work was completed to elucidate the role of IL-6 release from a working limb, including mice gene deletion models, infusion of IL-6 to healthy humans, further elaboration of the mechanistics behind the release of IL-6 from a working limb, etc.

JT Report:
During the years 2001 until 2007 the PhD students Pernille Keller and Charlotte Keller (CK) were responsible for the molecular determinations of much of the 'muscle myokine' RNA. This yielded a remarkable 37 articles for these students, between 2001 and 2007. This included RNA measurements of IL-6, IL-6 receptor, IL-8, IL-18 and TNFalpha.
The medical PhD student responsible for many of the studies was Adam Steensberg (AS), the partner of Charlotte Keller. He co-authored a remarkable 38 articles with BKP during this time. There is substantial evidence of extensive salami slicing of studies - and hence inappropriate statistical analysis across studies.

Reply:
During our work in BKP's laboratory, PK and CKS were responsible for the RNA related work in the laboratory. Furthermore, PK, AS, and CKS conducted a large number of trials run in BKP's laboratory. Therefore, the scientific yield was substantial and representative of a high productivity and responsibility of core competences. The muscle and fat samples obtained from a trial have indeed been used in different publications with different scientific goals. This is common practice when you have undertaken a large and complex invasive human study that is highly demanding to the subjects. We do not see this as "salami slicing"

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since different scientific questions and molecular analyses were applied – i.e. different hypotheses were examined, rather it would have been confusing and irrational to put such data into one paper. We see this as an appropriate and ethical way to use biological material from participating subjects, and we trust that JT complies with this procedure, given that the muscle samples from the diabetes cohort published in the 2010 Genome Medicine paper were re-used in a similar way. JT is senior author on that paper.

JT Report:
However, were the RNA measurements carried out by PK and CK from 2001 until 2005 compatible with the protein data produced by Penkowa? The short answer is NO: in many cases basic knowledge of the molecular biology of RNA detection would have indicated that the Penkowa protein data must have been suspect.

Reply:
This is a false and highly speculative statement from JT, which seems to arise from JT's inappropriate access to a data set that a) has never been published b) was run 1 year after publication of the IL-8 paper, and thus cannot be part of the published IL-8 paper, and c) actually represents an IL-18 measurement. Please see reply below where the Ct values for qPCR are discussed.

JT Report:
The key question is, when did members of the CIM research centre become aware that the Penkowa protein data was unlikely to be robust? I know from personal discussions with these CIM staff that by 2007 doubts already existed.

Reply:
We do not agree with this statement and do not recollect such discussions with JT.

JT Report:
Did doubts exist at the point of publication? Well, there is one of two possible conclusions. Conclusion 1: Neither CK nor PK understood the RNA assays they were using and did not thus appreciate that IL-6 RNA expression rose from nothing to a "high fold-change" of 'nothing' i.e. a moderate level meant that universal protein expression and large protein secretion levels from muscle were unlikely. Thus, they were failed by the Danish education system. Against this idea, is the fact that CK stated in 2001 (FASEB J. 2001 Dec;15(14):2748-50) that responses were variable following 2hrs of exercise but did not present how many subjects actually produced IL-6 in a normal bout of 2hrs of exercise [PDF18 and PDF19], unlike the Ostrowski 1998 article [PDF16]Conclusion 2: Inconsistencies in RNA measurements were systematically misrepresented by CK, PK and AS during the years 2001 until 2007 so that the obvious disconnect between RNA and Penkowa protein data were not obvious to reviewers.

Reply:
We strongly disagree with the speculations that JT presents as "Conclusion 1" and "Conclusion 2". From a time-perspective, we could not foresee a publication with Penkowa to

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appear in late 2003, and thus could not, as JT states, systematically have misrepresented RNA data from 2001 and onwards to mask the future publication with Penkowa. It is clear from several papers that 1) exercise duration and intensity, 2) the glycogen level within the muscle and 3) how fit a person is, are all factors that influence the expression of IL-6. Furthermore, it is important to understand that in the years from 2000 and onwards there was a technology breakthrough with the introduction of real-time PCR, which provided a much more sensitive system for determination of mRNA levels allowing detection of low-abundance transcripts. Therefore it was now possible to determine pre-exercise levels of cytokines.

JT Report:
The 2005 IL-8 paper [PDF21] presents a study that provides a scenario that suggests Conclusion 2 is correct.
In this study, the raw data stored within the CIM centre shows that IL-8 appears at around 38 to 40 cycles during real-time qPCR. However, the authors presented the data normalised to 1 so that it was impossible to know that the real levels were extraordinarily low unless you had seen the raw data at CIM (as I have via SN).

Reply:
The IL-8 Ct values for qPCR that SN recalls to have discussed with JT was run on 14. February 2006, that is, 1 year after the IL-8 article had been published. Neither CK nor PK worked at CIM at that time. We have been in contact with Thorbjörn Åkerstrom who ran this assay, and he informs us that these data represent unpublished IL-18 qPCR data (i.e. a different cytokine). The title of the file has a typo and thus says IL-8, while the detector in the assay run says IL-18. SN was not an author on the 2005 IL-8 paper and had not been involved in the analyses of the IL-8 mRNA in the muscle samples. Moreover, JT has jumped to wrong conclusions and puts forward very serious allegations based on a dataset, the nature and background of which he does not understand, and which now turns out to not even be IL-8 data.

In the IL-8 paper we normalised the pre-exercise Ct values to 1, which is common practice for presenting mRNA levels following a stimuli, especially so when comparing expression of a gene within a subject, i.e. repeated measured. It can be discussed whether the actual Ct values of the mRNA levels should have been mentioned - the practice on this varied from paper to paper at the time of publication. It is important to acknowledge that the real-time PCR technique was novel and the ways of reporting results were evolving at this time point. For the sake of clarifying this matter, the IL8 Ct values that underlie figure 1 and figure 4 in the IL-8 article are presented (mean ±SD). The figures below show the raw Ct values from the two exercise studies included in the IL-8 article.

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Figure 1 in the IL-8 article:

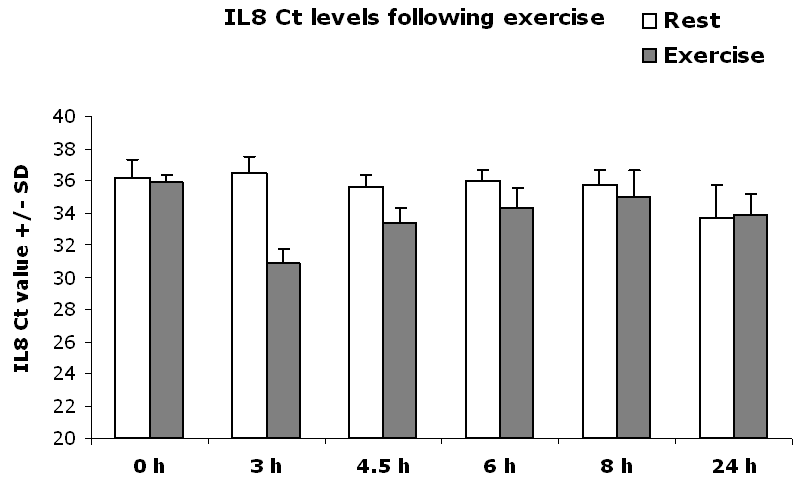
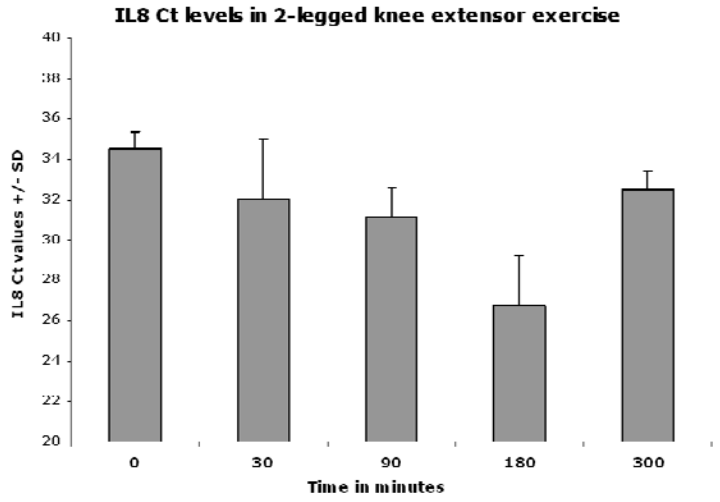


Figure 4 in the IL-8 article:



The figures demonstrate that the IL-8 Ct levels clearly decrease following exercise in both of the exercise models presented in the IL-8 article; Ct values decreasing from 36 to 31 in the 3-h cycling protocol, and from 34.5 to 27.5 for the 2-legged knee extensor exercise model (numbers that are far from the 38-40 Ct cycles that are the basis for JTs fraud allegations). It is correct that IL-8 is only expressed at low levels in muscle at rest (34-36 Ct cycles), but following 3-h of exercise, there is a robust increase in IL-8 mRNA, as shown in two independent exercise studies.

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JT Report:
Again, Penkowa produced evidence of remarkable protein induction at 6 hrs [PDF20 - Fig 2d] - with an immuno-blot figure that looks so unusual and so artificial it is implausible that AS, CK, PK and BKP would not consider it suspect.
With the combination of almost non-existent RNA and such a blot, one can only conclude that these researchers must have suspected that the Penkowa data was suspect in October 2004.

Reply:
JT's main argument is that he has seen the raw qPCR data from the IL-8 article, showing Ct values of 38-40 that would suggest that IL-8 is not expressed or with a maximum of one cDNA copy per cell. As can be seen from the figures above, the actual and published Ct values are much lower than the numbers JT refers to – and in concordance with an abundant - and not least – detectable IL-8 mRNA expression. Therefore, one can only conclude that we had no reason to suspect data fraud in October 2004.

JT Report:
Why then did they not object? Well, put simply, BKP had a government centre funded for 5 years and such a scandal would have removed any chance of a 2nd 5yr funding period.
One just needs to examine when BKP asked Penkowa to leave her official collaborative position with CIM and when the last Penkowa/BKP myokine publication was submitted. You will find that the former preceded the latter by some time and hence CIM continued to use Penkowa data long after doubt existed.

Reply:
We had no doubts about the Penkowa data before the Penkowa case appeared in the press. In April 2011, we became aware of manipulation with the immuno-blot pictures in 4 of 12 Penkowa co-authored papers. BKP has reported these four articles to UVVU on 02.04 2011 and 03.04. 2011, respectively, and informed the respective scientific journals on 04.04.2011.

When CIM opened in 2005, we had one (1) publication in collaboration with Penkowa and it is highly unlikely that our 5-year grant totalling DKK 25.000.000 from the Danish National Research Foundation (DNRF) should rely on a single publication with Penkowa.

In the winter of 2009 the research of CIM was evaluated by an international expert panel with regard to a five-year prolongation of our DNRF grant. The evaluation was based on a new research plan, our list of publications from 2005-2009 encompassing 10 PhD theses and 127 original and review articles, and a site visit. We received a satisfying evaluation and an extension of our grant of DKK 30.000.000.

The last IHC data used in CIM publications, in which Penkowa had "hands on" are from 2005. From 2005-2006 CIM personnel used the Penkowa laboratory for IHC. The last IHC experiments were carried out in September 2006 (Diabetologia paper, published 2009) by CIM personnel.

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In August 2007, I (BKP) tell Penkowa that I have decided to exclude her from CIM. The arguments for this decision are given in the attached letter (C_2 and C_3). My decision was based on the fact that she did not demonstrate any interest in the research plan of CIM. I/we did not suspect fraud.

JT Report:
Secondly, CK, AS and PK were all expecting to obtain a PhD from CIM, using the incompetent RNA data and the clearly fraudulent protein data from Penkowa. They had a choice, scientific honesty or career progression and they chose the latter.

Reply:
The only correct statement above is that we all (CKS, PK, AS) obtained PhDs while working in BKP's laboratory. Neither CK's nor AS's thesis' includes any of the Penkowa data as articles and only one of four articles in PK's thesis includes an article to which Penkowa contributed with immuno-blots. Therefore, none of our PhDs are dependent on Penkowa data.

JT Report:
Pernille Keller is the only member of the group that I am aware of that has pro-actively distanced herself from the CIM work by removing herself from the BDNF publication. In my personal experience with PK, she is not a strong person and would not have been able to stand up to BKP or deal with the overt-confidence of AS or her sister CK. PK has since leaving CIM worked with my group and been involved in producing 7 articles of good quality. I therefore do not believe that PK is guilty of fraud, but rather has been subject to adverse peer-pressure by CK, AS and BKP and inappropriate and incompetent guidance (BKP).

Reply:
We do not agree to JT's perception of incompetent PhD guidance in CIM. Further BKP has clearly presented Lab manuals for the CIM lab and instructions on how PhD students are supervised (please also see A1_17-A1_20).

JT Report:
Now that the 12 "myokine" articles with BKP and Penkowa are to be considered fraudulent and its clear these scientists must have had doubts by 2005, then the flagrant and entirely distasteful over-selling of the myokine work in a large number of review articles, must also now be considered unsound, unscientific and hence withdrawn.

Reply:
The fraud allegations in "C" seem to be based entirely on an incorrect "IL-18" mRNA dataset. The Ct values from the IL-8 publication that we present here are clearly in concordance with global IL-8 mRNA expression following exercise. We must stress that we have not had any evidence on potential fraud with the immuno-blots conducted by Penkowa until very recently – and the mRNA data on which JT has based his case does not support his so-called "Conclusions".

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It is correct that BKP has co-authored 12 papers with Penkowa which all included immuno-blots. On three of these papers, the immunoblots were performed by PhD students or Master students from the BKP laboratory in the Penkowa laboratory. Penkowa did not have "hands on" and we have no reason to believe that these data are fake. On two of the articles CIM personnel performed the IHC in collaboration with Penkowa and we know for sure that the correct muscle samples were cut and stained correctly. On another four papers, we now have strong reasons to believe that the immunoblot data made by Penkowa are fraudulent (and as mentioned above this have already been communicated to UVVU in separate reports). We are still examining the data for the remaining three publications. Regardless, all 12 publications are part of the investigation by the Independent Research Council of all Penkowas's publications.

We are of course deeply concerned about the apparent misconduct performed by Penkowa on immunoblot data in four CIM papers Therefore, we have decided to initiate a new exercise study, with multiple muscle biopsies, for the measurement of protein levels of specific cytokines in order to determine whether protein level of these cytokines increase in skeletal muscle cells following exercise.

JT Report:
In my expert opinion, none of the CIM myokine work from ~2000 until 2011 would have been given substantial attention without the key muscle protein immune-blot data of Penkowa in 12 articles.

Please, see our comments with regard to the 12 publications in question (C_4). Among these publications, only two studies can be considered key-myokine papers. Although the IHC data may be flawed, later work from our group or another group did find support for the idea that IL-6 (C_1) and IL-8 (C_5) are expressed in and released from contracting muscle cells.

The hypothesis that skeletal muscle is an endocrine organ is supported by numerous other studies including proteomics studies showing that C2C12 muscle cells have the capacity to secrete more than 600 myokines (Henningsen et al., Mol Biosyst 2011; 7(2):311-21, Henningsen et al., Mol Cell Proteomics 2010; 9(11):2482-96).

Furthermore, other researchers, e.g. Kenneth Walsh at Boston University School of medicine, have identified other myokines.

Authorships

JT Report:
A false credit given to the authors, misrepresentation of title or workplace.
There is gross inflation of publication authorship at CIM. As mentioned above, 3 PhD students each "produced" ~38 articles in the space of 2001 to 2008 (they stopped working at CIM in 2006)

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Reply:
PhD students in our lab, who are involved in human invasive studies, typically have 3 first author publications in 3 years and several co-author publications. However, many of our PhD students initiate their research career as pre-graduate students and continue to work as post-docs or (if MDs) during their clinical training and thereby gain several publications. All PhD students are heavily involved in the scientific writing, especially with regard to their first author publications. We give high priority to supervising PhD students and to teach them how to write a scientific article. The publication records of the present CIM PhD students may be viewed at:
http://www.inflammation-metabolism.dk

JT Report:
I personally have been forced to include Anders R. Nielsen (ARN) on articles where he provided no intellectual input to the analysis or the interpretation or the writing. Rather he simply took a muscle biopsy/blood. This latter activity resulted in him gaining 21 articles as a PhD student in 4years.With respect to my co-publications with ARN, I eventually refused to allow him to have multiple authorships for the same "muscle biopsy". BKP creates these "false" CV's for her student. Often, they write none of the manuscript.

Anders Rinnov Nielsen started in CIM in 2004:
http://www.inflammation-metabolism.dk/index.php?pageid=111&username=rinnov

He has been a PhD student/research assistant since 2004 and he has spent much of his free time, including weekends within CIM. He was responsible for establishing the so-called diabetes cohort, including approx. 200 individuals, who underwent clinical examination and muscle- and adipose tissue biopsies. He has also been involved in several other demanding clinical studies.

ARN has since 2004 gained 21 publications, including 5 first author publications. I (BKP) can guarantee that ARN has made a substantial contribution to these publications and deserves every single credit as author.

For your information, JT recently published an article (BMC Endocr Disord. 2011 Mar 22;11:7) including adipose biopsies from the before-mentioned CIM diabetes cohort without giving any authorship credit to CIM.

We acknowledge that we are facing different cultures with regard to authorship.

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