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17th April 2011

Dear Dr Knuppel,

Please find enclosed my report on the scientific behaviour of Dr Bente Klarlund Pedersen (BKP) and her staff and collaborators. I have used your guidance documents to specifically highlight matters precisely according to the DCSD criteria.

I have also provided you with background information, defining how I am aware of what I know, and my original research interactions with BKP and her staff. This back-ground information also provides a clear statement as to why I am an appropriate expert, why I had precise and first-hand knowledge of the scientific fraud and incompetence at the Centre for Metabolism and Inflammation (CIM).

In my expert opinion, the majority of the myokine work produced by CIM would not have been accepted in the field without the protein immune-blot data of Penkowa. Key molecular data, as early as 2004 indicated her data was suspect.

I made representation to BPK and her staff in January 2009 many months prior to false Penkowa protein data being published in *Diabetologia* - making them fully aware that the protein data could not be possible. This warning and the data provided was ignored and BKP published BDNF later in 2009.

In this report I am largely going to focus on two specific cases of fraud that I have first hand knowledge of. The remaining issues I will take up directly with the journals in question and are summarised at the end of the report.

I will refer to a case of scientific dishonesty that involves BDNF and I will refer to that as the "**BDNF dishonesty case**".

I will refer to a case of plagiarism and false credit given to an author, which I will refer to as the "**MicroRNA dishonesty case**".

I would like to specifically draw your attention to the fact that my original written complaint to the University of Copenhagen is dated November 2010. I also made my complaint to the Journal of Physiology regarding the MicroRNA dishonesty case first in August 2009. I made representation to the scientists in question as early as 2008.

I also asked the scientists in question to do the honourable thing, and withdraw the articles, but they refused

All email evidence from my email account can be independently verified by the DSCD if so desired.

I would be grateful if you would acknowledge receipt of this report and confirm the time scale over which I can expect a written response and update on this disgraceful case.

Sincerely

Professor James A Timmons

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This document reports activities relevant to The Consolidated Act No. 306 of 20 April 2009 and the Rules of Procedure for the Danish Committees on Scientific Dishonesty.

The following complaint falls within the jurisdiction of the Danish Committees on Scientific Dishonesty (DCSD) because of the following facts:

1. I am a qualified person to complain as I have received scientific training within the area of research that the scientific product complained about concerns:

I am a professor of physiology and systems biology (including genomics). I have been employed as a lead-scientist in the pharmaceutical sector (10yrs) as well as holding a full university professor in the United Kingdom since 2006. I have also been employed as a part-time researcher at the Panum Institute from 2009-2010. I am internationally recognised for my work on human muscle genomics and my lab has produced more data on gene-responses to exercise in muscle than any other lab in recent decades. I have been first or last author on over 40 relevant articles (proving that I have the technical expertise) and I have, for example, authored position statement reviews for the American Physiological Societies Handbook of Physiology in 2011 on the subject area of genes, genomics and muscle exercise physiology.

2. The people that have carried out the scientific dishonesty are within the jurisdiction of the DCSD because it is against a person and/or a group of people largely working in Denmark and not a company:

The primary person (but not the sole person involved) that has carried out acts of Scientific dishonesty is Dr Bente Klarlund Pedersen (BKP), employed at the University of Copenhagen during the period of time of the Scientific dishonesty was carried out. BKP obtained a grant from Danish public authorities for the preparation of the scientific product complained about.

3. The complaint against the scientific product, relating to scientific papers and plagiarism of other peoples work and writing and other serious violation of good scientific practices committed wilfully or grossly negligent on planning, performance or reporting of research results. The issues with the scientific product includes:

1. Undisclosed fabrication and construction of data or substitution with fictitious data.
2. Undisclosed selective or surreptitious discarding of a person's own undesired results.
3. Undisclosed unusual and misleading use of statistical methods.
4. Undisclosed biased or distorted interpretation of a person's own results and conclusions.
5. Plagiarism of other persons' results or publications.
6. A false credit given to the authors, misrepresentation of title or workplace.

The report and this fraud case **does not** involve the validity or truth of scientific "theories" or cases involving the research quality of a scientific product – it refers explicitly to plagiarism and the inappropriate scientific activities listed 1-6 above.

A. I will refer to a case of scientific dishonesty that involves BDNF and I will refer to that as the "**BDNF dishonesty case**".

B. I will refer to a case of plagiarism and false credit which I will refer to as the "**MicroRNA dishonesty case**".

Cont.,

C. I will finally document additional issues for the DCSD to consider - which may in turn expose additional fraudulent activities at the University of Copenhagen.

A - "BDNF dishonesty case".

This case involves undisclosed selective or surreptitious discarding of undesired results since BKP worked on BDNF that involved my lab and the Febbraio lab at the same time. Both muscle BDNF projects included work in her lab and BKP claimed authorship on both articles. Despite this, one set of data conflicted with the other prior to publication. The BDNF manuscripts (in Diabetologia and Experimental Physiology) by BKP and the Febbriao lab, also represents a distorted interpretation of a person's own results and conclusions.

1. Back-ground

In 2005 I started a research collaboration with the Centre for Inflammation and Metabolism (CIM) headed by BKP. I worked as a Group Leader at CGB, Karolinska Institutet. CIM had a capacity for large human studies, while my lab specialised in Gene Chip studies (since 1999) and qPCR (since 2003). The collaboration revolved around a large cross-sectional diabetes tissue cohort and a number of smaller studies.

In June 2006 BKP offered me a 4yr position at CIM to 'help' develop the molecular capacity of her centre. I was asked to contribute to various studies ongoing at CIM, however most were of poor design. For example, chip studies run where controls were run 6 months after treatment samples and thus the post was not attractive to me. Instead, I took a Professorship in Edinburgh. BKP then took my former PhD student, Camilla Scheele (CS), instead as a post-doc, to develop the molecular capacity of her centre.

The diabetes tissue cohort collaboration involved muscle and adipose biopsy samples (~250 samples). The samples were transferred to my Stockholm lab and from that time onwards all RNA analysis was carried out in my lab. During 2006 I became rather concerned about the lack of rigour being applied to molecular analysis at CIM and lack of supervision.

When visiting CIM I noted MD Phd students running multiple correlations of real-time qPCR (rtqPCR) data against clinical parameters, yet they had no appreciation that the 'intensity' values of the data meant the gene was not robustly expressed in the muscle cells. As CIM had published molecular studies in human muscle for >5yrs prior to this period, this represents a major concern to me (see later).

As it will transpire, unless there has been a complete failure of the Danish University sector to teach biology graduates and post-graduates the correct interpretation real-time PCR raw data, many CIM post-graduate students over the past decade must have appreciated the disconnect between their RNA data, and the ultimately implausible and in many cases, impossible, data generated by their co-worker Penkowa.

What is clear, is that BKP was warned of potential fraud, in advance of publication of BDNF, and chose to discard the data I provided.

2. Fraud scenario - BDNF

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 (MF, Baker Institute, Melbourne). MF and Mathew Watt (MW) have had several articles

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withdrawn due to fraud over the past few years [e.g. Southgate etal FASEB J 2005; Watt et al, Molecular Endocrinology 2008 etc.]

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

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].**

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]**

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].

Subsequent to the *Diabetologia* article, misleading review material referring inaccurately to the original work appeared, perpetuating the scientific misconduct [see below].

3. Details of relevant activities that alerted me of fraud - BDNF

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]**

Critically, the data I sent informed BKP and CS that BDNF mRNA was almost undetectable in whole human muscle, either at rest or 24hr after endurance training in young male subjects (n=24). In fact I showed them that the signal for BDNF appears at 37 cycles of rtPCR – consistent with expression in 3% or less of cells in the muscle tissue. A conclusion consistent with the known biology of BDNF in muscle and the *Genome Medicine* article that BKP co-authored **[PDF 4]**

The *Diabetologia* article claimed BDNF RNA was regulated by exercise in young male subjects (n=8) but did not disclose the actual level of BDNF RNA, rather presented 'relative' data, normalising to 1 (which hides the true abundance) **[PDF 5 - page 1]**. 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.

Even with the claimed ~ 1.25 fold increase over and above the comparable control sample at 5 hrs post-exercise, the RNA level would still approximate an RNA molecule in ~5% of cells (or reflect an alteration in blood contamination of the biopsy post exercise) or indeed represent technical noise and a non-significant data set.

The *Diabetologia* article also included a key immuno-blot from Penkowa showing a 'universal' and profound induction of whole muscle BDNF protein expression by 24hrs in young males after endurance exercise in all muscle fibres, but not in the time-control biopsy. Their time-control biopsy data rules out any possibility that BDNF was induced by biopsy damage by accident **[PDF 5 - page 2].**

The induction of BDNF protein across all muscle fibres is unfeasible, as there is no RNA template in most muscle cells – and without RNA you cannot make BDNF protein [PDF evidence 3 and 4].

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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 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".

4. The *Diabetologia* article and an *Experimental Physiology* article represent Undisclosed biased or distorted interpretation of a person's own results and conclusions.

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.

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].**

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"*

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".

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.

Further, BKP* appears to contradict herself across all 3 BDNF articles - all written in 2009.

*[note that BKP definitely read the BDNF *Genome Medicine* article, as she plagiarised the text I wrote to produce a grant in her own name - something she has already admitted (to Professor Niels-Henrik Holstein-Rathlou, University of Copenhagen) when she was challenged after my first complaint to the University of Copenhagen. See microRNA fraud case]

Thus, despite the fact that there should have been severe doubt about BDNF due to inconsistent results BKP's main conclusion paragraph for this review was entirely focused on muscle BDNF being a new "contraction produced protein that may regulate fat oxidation"

5. Additional information about the BDNF fraud.

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'. Since leaving CIM in 2005 she had worked in 3 other laboratories, including mine, and hence had developed a more robust sense of good scientific practice, one that appears not to be taught at CIM.

PK had been sent several draft versions of the article - an email trail can be found between CIM staff, MF and PK (at these email addresses - pnkl@novonordisk.com, pernille_keller@yahoo.dk, pernillekeller@yahoo.com or pernille.keller@googlemail.com)

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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.

PK is no longer willing to confirm these statements - nevertheless if the DCSD has the power to inspect email accounts - the manuscript record and emails will confirm all these points.

B - "MicroRNA dishonesty case".

This second case involves two types of fraud.

*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. Using access to scientific data from my laboratory CIM replicated and published the observation prior to my lab being able to publish the scientific discovery.

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

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.

1. Back-ground

As stated above, in 2005 I started a research collaboration with the Centre for Inflammation and Metabolism (CIM) headed by BKP. I worked as a Group Leader at CGB, Karolinska Institutet. CIM had a capacity for large human studies, while my lab specialised in Gene Chip studies (since 1999) and qPCR (since 2003). The collaboration revolved around a large cross-sectional diabetes tissue cohort and a number of smaller studies. Camilla Scheele carried out her PhD studies with me in Stockholm before moving to CIM in late 2006.

During 2008 my lab had made a novel discovery that we could predict cardiovascular adaptation to exercise in humans using genomics. As part of the process, we used a second clinical cohort from CIM (endurance trained young males) to carry out independent validation of our findings. In return BKP and some members of CIM would gain a co-publication. This was of interest to BKP in 2008 as it was under revision at *Nature*.

We then collaborated with Prof Claude Bouchard (USA) to extend the observation using genetics. Ultimately, the original large-scale manuscript was rejected by Nature and the article was split into two articles, reflecting two distinct themes as it was too large for publication.

The first article (on the genomic predictions) was submitted in 2009 and published in 2010 and involved CIM staff (but did not contain any microRNA data).

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The second article focused on endurance training microRNA regulation and did not feature any work from BKP, CS, SN or ML (Keller et al, J Appl Physiol. 2011 Jan;110(1):46-59) and thus they did not appear on the authorship [**PDF 8]**.

2. Fraud scenarios - microRNA

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.

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.

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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C – Other dishonest behaviours relating to the CIM myokine work

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. post-capillary white blood cells. All such data should be examined, and careful consideration given to the CT values of the qPCR data.

Examples - IL6 and IL8

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?

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.

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.

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.

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.

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).

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.

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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**.

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.

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.

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).

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.

Authorships

With reference to DCSD criteria:

1. **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).

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.