Tuesday, 03 April 2012

Dear Pia Jørnø,

In response to your e-mail dated March 28, 2012, below please find my answers, which I have formulated to the best of my knowledge.

Sincerely

Bente Klarlund Pedersen MD DMSc
Professor of Integrative Medicine
Director of Centre of Inflammation and Metabolism (CIM)
Rigshospitalet 7641
University of Copenhagen, Faculty of Health Sciences
Phone: + 45 35 45 77 97 ; + 45 26 17 05 24 (mobile)
bkp@rh.dk; http://www.inflammation-metabolism.dk

**General comments regarding our collaboration with Milena Penkowa**

Milena Penkowa (MP) was an independent senior scientist at the Panum Institute with a research group of her own, whereas my research group has always been located at Rigshospitalet. When she and I initiated collaboration (2002), we were both associate professors. Thus, I (BKP) have never had a role as mentor to Penkowa. When the Danish Nation Research Foundation’s Centre of Inflammation and Metabolism (CIM) was founded in 2005, MP was included as a senior partner. In August 2007, I (BKP) informed Milena Penkowa that I had decided to exclude her from CIM. 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 and I even invited her to collaborate on e.g. IHC in the future, should she be interested.

MP was never involved directly in the performance of the human studies. CIM personnel obtained muscle biopsies, which were brought to the MP lab for immunohistochemistry (IHC) work. In the beginning, CIM personnel were not involved in the IHC work and we received a photo of the IHC images ready to use as a figure in the article. The last IHC data used in CIM publications, on which Penkowa had “hands-on”, are from 2005. From 2005-2006 CIM personnel used the Penkowa laboratory for IHC. In some studies MP still made the photo to be used in the figure. In other studies, CIM personnel performed all steps, including the photo(s) to be used for the figure in the article(s).

We had no doubts about the data generated by MP before the “Penkowa case” appeared in the press in 2010. In March 2011, we became aware of manipulations with the immuno-blot pictures in 4 of the Penkowa co-authored papers. I reported these four articles to the Danish Committees on Scientific Dishonesty (in Danish: UVVU) on 02.04.2011 and 03.04.2011, respectively, and informed the involved scientific journals on 04.04.2011.
A8. Associations between insulin resistance and TNF-alpha in plasma, skeletal muscle and adipose tissue in humans with and without type 2 diabetes.


MP performed the IHC work (lab work, analysis and interpretation and wrote the sections in the article on IHC) of skeletal muscle sections for TNF, Fig.3.

- How was the cooperation between you (or anyone of your group) and Milena Penkowa (MP) organised, particularly as regards how the experimental material was handled, including

- Which (type of) material did you deliver to MP?

MP received muscle biopsies from 10 controls and 10 patients with type 2 diabetes.

- Have you participated in, or have any knowledge about, MP’s handling of the material (e.g. cutting, staining and/or counting)? If yes, please describe.

No.

- Do you know where/how the cut and stained sections were stored? If yes, please describe?

We do not know where the sections for the particular article are stored.

- Have you seen or received any of the sections from MP?

We have slides with ID from the 10 controls (16774, 15552, 17007, 16443, 17164, 17122, 17182, 17001, 17197, 15655) and the 10 patients (17185, 15525, 15676, 15649, 16664, 15691, 16628, 16452, 16494, 16710).

We do, however, not know what these slides were stained for. However, it demonstrates to us that the biopsies were indeed cut and analysed.

- Have you received any photos of the sections from MP?

Yes.

- Did you receive any other material back from MP?

We received the remains of the biopsy material.

- If you have received stained sections or photos back from MP, the Panel would be very interesting in seeing these.
Peter Plomgaard received an IHC-photo from MP (attached as A8_Fig 3_REVISED-Diabetes-Figur), which was modified by Peter Plomgaard to illustrate only TNF and used as Figure 3 (attached as A8_Fig 3_Figure 3R).

In addition, we have on April 3rd 2012 for your information sent the above-mentioned slides to Ingelise Damberg at SUND/KU.

**Reflections:**

There is an agreement between Western and IHC-data with regard to muscular expression of TNF. A recent study published by our group in Diabetes 2011 (Green C. et al) shows that human primary muscle cell cultures from patients with type 2 diabetes produce more TNF than muscle cells from healthy controls, just supporting the finding that muscle cells from patients with type 2 diabetes express more TNF.

Contact info for Peter Plomgaard: plomgaard@dadlnet.dk

MP is an author on this paper because she performed a pilot experiment (IHC) indicating that BDNF was up-regulated in human skeletal muscle 24 h after exercise. This pilot experiment is not included in the article. The IHC work was performed by Maj-Britt Åstrom, who was a medical student at CIM at that time. Maj-Brit Åstrom was assisted in MP’s laboratory by a technician, Dan Sonne Pedersen, who was working for MP. According to Maj-Brit Åstrom, MP was not directly involved in supervising the IHC technique or in choosing the photos to be used in the article. As far as we can recall, MP was involved in writing the legend description to Figure 1d.

- **How was the cooperation between you (or anyone of your group) and Milena Penkowa (MP) organised, particularly as regards how the experimental material was handled, including:**

- **Which (type of) material did you deliver to MP?**

Maj-Britt Åstrom brought muscle biopsies to the MP lab.

- **Have you participated in, or have any knowledge about, MP’s handling of the material (e.g. cutting, staining and/or counting)? If yes, please describe.**

As said, the IHC was performed by Maj-Britt Aström with help from Dan Sonne Pedersen in the Penkowa laboratory. According to Maj-Brit Aström, MP was not directly involved in supervising the IHC technique or in choosing the photos to be used in the article. As far as I can recall, MP was involved in the legend description to Figure 1d.

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 pronounced mRNA response (id. CH) (attached as A16_Fig 1d_PCR-results, exercise). This person appeared also to have the most pronounced BDNF protein response (attached as A16_Fig 1d_Christian Holm, skåret i maj). The control person was Nikolai Jørgensen (attached as A16_Fig 1d_Nikolai Jørgensen, rest).

Thus, we show the IHC image that best supported the notion that the increased BDNF expression 24 h post-exercise was indeed intramyocellular, Fig 1d.

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 established a cell culture system to contract differentiated C2C12 myotubes *in vitro*.

- *Do you know where/how the cut and stained sections were stored? If yes, please describe?*

The slides are stored in CIM laboratory (attached as A16_Fig 1d_FRYSER PLACERING AF PRØVER; A16_Fig 1d_Biopsi-kasserne skåret; A16_Fig 1d_LISTE over placering af prøvemateriale).

- *Have you seen or received any of the sections from MP?*

Please see above.

- *Have you received any photos of the sections from MP?*

Maj-Britt Åstrom took the photos.

- *Did you receive any other material back from MP?*

The remains of the muscle biopsies.

- *If you have received stained sections or photos back from MP, the Panel would be very interesting in seeing these.*

We include the original photos as used for Figure 1d although MP was not involved in the generation of these (A16_Fig 1d_Christian Holm, skåret i maj; A16_Fig 1d_Nikolai Jørgensen, rest).

**Reflections:**

There is agreement between Western and IHC data in the present study with regard to BDNF protein detection in human samples. The human studies are highly supported by studies performed in vivo in mice as well as in cell cultures, including electrical stimulation of human primary cells. Penkowa’s contribution to this manuscript is minor and we find it impossible that she could have influenced the outcome of the study.

Contact info for Maj-Britt Åstrom is: aastroem.neuro@gmail.com
A33. Elevated levels of IL-18 in plasma and skeletal muscle in chronic obstructive pulmonary disease.


MP was involved in the IHC (lab work was performed by CIM technician Ruth Rousing in the MP lab). Analysis and interpretation of the IHC was performed blindly by MP. MP was involved in writing IHC sections for IL-18, TNF and IL-6, resulting in Figure 3 and 4.

- How was the cooperation between you (or anyone of your group) and Milena Penkowa (MP) organised, particularly as regards how the experimental material was handled, including:

  - Which (type of) material did you deliver to MP?

Muscle biopsies from 14 patients and controls.

- Have you participated in, or have any knowledge about, MP’s handling of the material (e.g. cutting, staining and/or counting)? If yes, please describe.

We know that CIM personnel performed the cutting and staining.

- Do you know where/how the cut and stained sections were stored? If yes, please describe?

We do not know where the stained sections, included in the present article, are stored. However, a postdoc from CIM, Remi Mounier, later went back to the MP lab and stained the sections for HIF-1-alpha. We have these slides in the CIM lab as well as sections stained for fibre types. These results were regarded as pilot experiments and they were never published.

- Have you seen or received any of the sections from MP?

Please see above.

- Have you received any photos of the sections from MP?

Anne Marie Petersen received photos, ready for publication, from MP.

- Did you receive any other material back from MP?

We believe that we received the remains of the muscle biopsies.

- If you have received stained sections or photos back from MP, the Panel would be very interesting in seeing these.

The photos are included in A33_Fig 3 and A33_Fig 4.
In addition, we have on April 3rd 2012 for your information sent the above-mentioned slides to Ingelise Damberg at SUND/KU.

**Reflections:**

With regard to IL-18, there is an agreement between the PCR and IHC data in the present study. Thus, IL-18 mRNA and IL-18 protein expression were elevated in COPD patients. Moreover, we found elevated plasma-levels of IL-18 in patients with COPD. With regard to TNF, there was an increased expression of TNF protein in type 2 fibres in muscle biopsies. However, TNF mRNA was not elevated. In another set of experiments, we find that TNF protein expression is enhanced in muscle biopsies from patients with type 2 diabetes, however, TNF mRNA is not enhanced (Plomgaard et al. Diabetologia. 2007 Dec;50(12):2562-71. Epub 2007 Oct 10.) Thus, there are some consistencies across studies. According to mail correspondance, it appears that there is agreement between the evaluation performed by Penkowa and Jesper L. Andersen.

Anne Marie Petersen may be reached on mobile 45 21 90 33 47 or e-mail: annemariewintherpetersen@hotmail.com
A38. Exercise induces interleukin-8 receptor (CXCR2) expression in human skeletal muscle.

MP performed IHC work (lab work, analysis and interpretation as well as sections in article on IHC) of skeletal muscle sections for CXCR2 resulting in Figure 2.

- How was the cooperation between you (or anyone of your group) and Milena Penkowa (MP) organised, particularly as regards how the experimental material was handled, including:

- Which (type of) material did you deliver to MP?

MP received muscle biopsies from an exercise study.

- Have you participated in, or have any knowledge about, MP’s handling of the material (e.g. cutting, staining and/or counting)? If yes, please describe.

No.

- Do you know where/how the cut and stained sections were stored? If yes, please describe?

No.

- Have you seen or received any of the sections from MP?

No.

- Have you received any photos of the sections from MP?

Lone Frydelund Larsen (LFL) tells me that she received the photos on her Rigshospital (RH) e-mail address from MP, ready to publish. LFL no longer has access to her RH e-mail account.

- Did you receive any other material back from MP?

No.

- If you have received stained sections or photos back from MP, the Panel would be very interesting in seeing these.

Please, see above.
**Reflections:**

There is an agreement between PCR and IHC data in the present study. We have confirmed that CRXCR2 gene expression is upregulated in murine muscle following exercise (data not published yet).

Lone Frydelund Larsen is at present employed by Lundbeck and may be reached on LOFL@Lundbeck.com, telephone 30 83 26 38.
MP had previously optimized the antibodies for IHC. CIMs laboratory technician (Ruth Rousing) performed the cutting of the biopsies and the staining under supervision by Plomgaard in MP’s laboratory. Fibre type staining was performed on parallel sections by Ruth Rousing in MP’s lab. Peter Plomgaard analyzed the slides and made the photos and compared these images with the fibre type stained slides from serial sections.

- How was the cooperation between you (or anyone of your group) and Milena Penkowa (MP) organised, particularly as regards how the experimental material was handled, including

- Which (type of) material did you deliver to MP?

MP did not receive any biological material from us. Peter Plomgaard took muscle biopsies from vastus lateralis (V), soleus (S) and triceps (T) on seven subjects and brought these samples to MP’s laboratory.

Do you know where/how the cut and stained sections were stored? If yes, please describe?

It appears that Plomgaard may have left the slides stained for TNF, IL-6 and IL-18 in MP’s laboratory. Each slide contained three muscles (duplicates) from one subject. In total seven subjects were investigated.

- Have you seen or received any of the sections from MP?

We have slides from the study, which include the fibre type staining.

- Have you received any photos of the sections from MP?

Peter Plomgaard made all of the photos, and thus, we have not received photos from MP.

- Did you receive any other material back from MP?

We received the remains of the biopsy material.

- If you have received stained sections or photos back from MP, the Panel would be very interesting in seeing these.

The original photos (taken by Peter Plomgaard) are attached as A40_Fig 1 with subfolders and A40_Fig 2 with subfolders.

In addition, we have on April 3rd 2012 for your information sent the above-mentioned slides to Ingelise Damberg at SUND/KU.
Reflections:

Since CIM personnel performed all experiments, we find it impossible that MP may have influenced the conclusions from this study.
**A211. Expression of interleukin-15 in human skeletal muscle effect of exercise and muscle fibre type composition.**


The biopsies used for IHC was the same as in the above-mentioned article (A40, Plomgaard et al.). Seven subjects had muscle biopsies obtained from vastus lateralis, soleus and triceps. As we recall it, MP optimised the conditions for the IL-15 antibody. We believe that MP was involved in supervising the IHC (lab work, analysis, interpretation, preparation of the final photo as well writing the sections in the article on IHC, Figure 2).

As far as we recall it, CIM laboratory technician (Ruth Rousing) performed the cutting of the biopsies. Ruth Rousing does not remember whether she or CIM postdoc Remi Mounier (the second author on the paper) performed the IL-15 staining. However, the slides with the IL-15 stainings are in CIM, suggesting to us that CIM personnel performed the staining. Ruth Rousing performed the fibre type staining on parallel sections (also present in CIM lab).

**- How was the cooperation between you (or anyone of your group) and Milena Penkowa (MP) organised, particularly as regards how the experimental material was handled, including:**

**- Which (type of) material did you deliver to MP?**

Muscle biopsies from vastus lateralis (V), soleus (S) and triceps (T) from seven subjects were brought to the MP lab by CIM personnel. Each of the seven slides contains the three muscles (duplicates) from one subject.

**- Have you participated in, or have any knowledge about, MP’s handling of the material (e.g. cutting, staining and/or counting)? If yes, please describe.**

We know that the sections were cut by CIM technician Ruth Rousing, who at least performed the fibre type staining and probably also the IL-15 staining on parallel sections. This was done in MP’s laboratory.

**- Do you know where/how the cut and stained sections were stored? If yes, please describe?**

Please see above.

**- Have you seen or received any of the sections from MP?**

All sections are in CIM lab.

**- Have you received any photos of the sections from MP?**

MP performed the photo to be used for the article and forwarded it per e-mail to the first author, Anders Rinnov Nielsen. He also received the figure on a CD-ROM.
Did you receive any other material back from MP?

The remains of the muscle biopsies.

If you have received stained sections or photos back from MP, the Panel would be very interesting in seeing these.

We have on April 3rd 2012 for your information sent the above-mentioned slides as well as the original CD ROM to Ingelise Damberg at SUND/KU.

**Reflections:**

There is good agreement between IL-15 protein detection by IHC and Western blotting. Later studies from our group have confirmed that human skeletal muscle expresses IL-15. Previous studies confirm that IL-15 is expressed by muscle (Cloning of a T cell growth factor that interacts with the beta chain of the interleukin-2 receptor. Grabstein KH, Eisenman J, Shanebeck K, Rauch C, Srinivasan S, Fung V, Beers C, Richardson J, Schoenborn MA, Ahdieh M, et al. Science. 1994 May 13;264(5161):965-8.)

Contact information for Anders Rinnov Nielsen is: rinnov@gmail.com