

## Results report

- **A brief description of the project's objectives and background**

The objectives stated in the project proposal are as follows.

**Primary objective:** *How does physical exercise benefit the brain and how is it best administered?*

**Secondary objectives (research questions):**

1. *What is the link between exercise and enhanced brain structure and function? does the change from glucose to alternative energy substrates have a signalling role?*
2. *Is interval exercise more efficient on brain performance and structure than the same amount of exercise administered continuously?*
3. *Is there a beneficial effect of physical exercise also in individuals with genetically low physical trainability? can the training regime be optimized for this?*

- **An account of the results achieved under the project explained in the context of the project objectives**

**Primary objective:** *How does physical exercise benefit the brain and how is it best administered?*

How physical exercise exerts beneficial effects on the brain was previously unknown. The project has provided an answer to this question: We first *discovered* (Lauritzen KH et al 2014 Cereb Cortex; featured with Cover Picture in the journal, and as "Article Recommendation" by Faculty of 1000 "F1000Prime" <http://f1000.com/prime/718027781#abstract>) that the lactate receptor HCAR1 is active in brain, downregulating cAMP. Then we *discovered* (Morland C et al 2016 Nat Commun, under revision; 2016 Alfred Benzon Symposium No 62 Copenhagen [http://www.benzon-foundation.dk/?page\\_id=20](http://www.benzon-foundation.dk/?page_id=20); 2016 FENS Forum [https://ep70.eventpilot.us/web/page.php?nav=false&page=IntHtml&project=FENS16&id=abstract\\_197510](https://ep70.eventpilot.us/web/page.php?nav=false&page=IntHtml&project=FENS16&id=abstract_197510)) that HCAR1 is located at pial blood vessels supplying the brain, and that the large amounts of lactate released by exercising skeletal muscle activate HCAR1 in brain to increase (1) the hippocampal contents of vascular endothelial growth factor A (VEGFA), which is known to enhance synaptic plasticity as well as angiogenesis, and (2) the hippocampal density of capillary blood vessels. The training was administered to mice as intensive bouts of aerobic exercise, which has been convincingly shown to be the best way to administer exercise for achieving physical fitness. The effects of exercise on VEGFA and capillary density were reproduced by subcutaneous injections of lactate to achieve bouts of high blood lactate levels similar to those attained by the bouts of exercise. Absence of the effects in knockout mice lacking HCAR1 showed that the effects of exercise and lactate depend on this receptor.

This is the *first demonstration* that a substance released in large quantities from exercising skeletal muscle causes beneficial effects in the brain through an identified receptor. The findings therefore fulfil the Primary objective of the project.

**Secondary objective 1:** *What is the link between exercise and enhanced brain structure and function? does the change from glucose to alternative energy substrates have a signalling role?*

Lactate is the physiologically most abundant of energy substrates used by the brain as alternative to glucose. The brain exports lactate at rest, but imports and metabolises lactate when the blood concentration rises, such as in high intensity exercise (see, eg, review by Bergersen LH 2015 J Cereb Blood Flow Metab). During the course of the project, we *discovered* that the lactate receptor HCAR1 is present and active in the brain (Lauritzen KH et al 2014 Cereb Cortex; Morland C et al 2015 J Neurosci Res). HCAR1 had been previously identified in adipose tissue, where lactate is released as an autocrine signalling substance acting on HCAR1 to downregulate cAMP and thereby curb lipolysis (Ahmed K et al 2010 Cell Metab). We showed that the presence

of lactate or a selective HCAR1 agonist 3,5-dihydroxybenzoate downregulates cAMP in hippocampal slices (Lauritzen KH et al 2014 Cereb Cortex). This answered “yes” to the second question of Secondary objective 1.

We subsequently went on to showing (see account above under Primary objective) that activation of HCAR1 by lactate underlies part of the beneficial effect of physical exercise on the brain, ie, increased production of the combined neurotrophic and angiogenic (Licht T et al 2011 PNAS) factor VEGFA, and increased density of capillary blood vessels in hippocampus. Experiments in hippocampal slices showed that the selective HCAR1 agonist 3,5-dihydroxybenzoic acid as well as lactate stimulated activation of ERK1/2 and Akt, intracellular mediators that are known to induce angiogenesis through VEGFA. This stimulation was absent in slices from knockout mice lacking HCAR1. The enhanced angiogenesis was demonstrated in the hilus region, ie, supplying the site where new neurons are known to be formed and show enhanced synaptic plasticity in response to exercise (Farmer J et al 2004 Neuroscience). Our observations (Morland C et al 2016 Nat Commun, under revision) thereby identify one link between exercise and enhanced brain structure and function and therefore answer the Secondary objective 1.

**Secondary objective 2: *Is interval exercise more efficient on brain performance and structure than the same amount of exercise administered continuously?***

When we discovered the lactate receptor HCAR1, all available resources were focused on clarifying its function in relation to the overriding goal, the Primary objective, of the project. This resulted in the further discovery described above of a mechanism whereby physical exercise benefits the brain. This work had to be prioritized over the testing of the efficiency of interval exercise versus ‘conventional’ continuous exercise with respect to brain performance and structure. As aerobic high intensity interval training is known to increase physical performance much more efficiently than continuous moderate intensity training (Haram PM et al 2009 Cardiovasc Res), we selected the former. In agreement, during the course of the project, we have noted literature data indicating that high intensity interval exercise is more efficient than continuous exercise, also with respect to increasing cerebral levels of trophic factors (Afzalpour ME et al 2015 Physiol Behav). Further, high intensity interval training is superior to resistance training with respect to inducing adult neurogenesis in hippocampus (Nokia MS et al 2016 J Physiol). These literature data suggest that the training paradigms chosen by us are suitable and that the answer to the question of the Secondary objective 2 is “yes”.

**Secondary objective 3: *Is there a beneficial effect of physical exercise also in individuals with genetically low physical trainability? can the training regime be optimized for this?***

Genetically based variation in ability to exercise was addressed in rat strains selected during many generations (now 28) for high and low capacity for running (HCR and LCR; Koch LG, Britton SL 2001 Physiol Genomics; Wisløff U et al 2005 Science). After high intensity interval training on a treadmill for 36 weeks (5 days a week for 12 weeks, 2 days a week for 24 weeks), mitochondrial volume as percent of total cytoplasm volume in hippocampus increased significantly in LCR rats (by 20% compared to sedentary). This change did not occur in HCR rats, perhaps reflecting the fact HCR rats are already in a state adapted to performing a high level of exercise. Similarly, the amounts of two proteins protecting against damage caused by the formation (uncoupling protein 2, UCP2) of or damage (superoxide dismutase 2, SOD2) by oxygen radicals in mitochondria were concomitantly increased or maintained, respectively, after training in LCR rats. No significant changes in UCP2 or SOD2 were observed after training in HCR rats. The trained LCR rats also showed a 20% increase in aerobic capacity compared to sedentary controls (56 compared to 46 mL/min/kg VO<sub>2</sub>). Most of the time (see above), the rats exercised only twice a week, a rate that may be sustainable also in human populations. The findings indicate that a prolonged regime of high intensity exercise at moderate rate increases mitochondrial capacity in a brain region associated with memory, notably in individuals with low intrinsic aerobic fitness (LCR rats). This result suggests that a high gain may be expected in individuals that, based on genetic factors, are in the outset the least physically fit and the least active. The results were summarized in a presentation at the Federation of European Neuroscience Societies Forum in Copenhagen 2016 (Moretti L et al 2016 FENS Forum; [https://ep70.eventpilot.us/web/page.php?nav=false&page=IntHtml&project=FENS16&id=abstract\\_194461](https://ep70.eventpilot.us/web/page.php?nav=false&page=IntHtml&project=FENS16&id=abstract_194461)) and a manuscript is in preparation. The findings largely fulfil the Secondary objective 3.

**Additional results achieved:** During the project period, several results were achieved that are mutually relevant to the influence of physical exercise on brain function. Here we instance (chronologically, original research papers only):

- ✓ Monocarboxylate transport in disease (Lauritzen F et al 2012 Neurobiol Dis; Lauritzen F et al 2012 Glia; Cheng C et al 2012 Cell Oncol (Dordr); Puchades M et al 2013 J Parkinsons Dis).
- ✓ Astrocyte gliotransmission in health and disease (Bergersen LH et al 2012 Cereb Cortex ; Ormel L et al 2012 Glia; Perez EL et al 2012 Neurobiol Dis; Eid T et al 2013 Epilepsia).
- ✓ Gene damage and maintenance (Regnell CE et al 2012 Cell Rep; Lauritzen KH et al 2015 Am J Physiol Heart Circ Physiol; Lauritzen KH et al 2016 Neurobiol Aging).
- ✓ Lactate in brain function in awake humans (Vafae MS et al 2012 J Cereb Blood Flow Metab).
- ✓ High fat diets in disease and ageing (Scheibye-Knudsen M et al 2014 Cell Metab; Holm-Hansen S et al 2016 Genes Brain Behav; Lauritzen KH et al 2016 Neurobiol Aging)
- ✓ Neuronal structure and function (Soussi R et al 2015 Brain Struct Funct; Blackstad JS et al 2016 Hippocampus).
- ✓ Lactate receptor and transport in retina (Skytt DM et al 2016 Biomed Res Int; Kolko M et al 2016 Neurochem Res).
- ✓ Movement of mitochondria in oligodendrocytes (Rinholm JE et al 2016 Glia).

- **A description of the most important R&D tasks that have been carried out and the groups that have played a key role in the project implementation**

The most important R&D tasks were described above (groups with key roles as follows); papers:

- 1) Action of the lactate receptor HCAR1 in the brain (Bergersen/Storm-Mathisen UiO, Attramadal UiO); Lauritzen KH et al 2014 Cereb Cortex.
- 2) Exercise increases hippocampal VEGFA and vascularization through HCAR1 (Bergersen/Storm-Mathisen, Wisløff NTNU); Morland C et al 2016 Nat Commun (in review); 2016 FENS Forum, etc.
- 3) Long term exercise augments brain mitochondria (Bergersen/Storm-Mathisen, Wisløff NTNU); Moretti L et al 2016 FENS Forum.
- 4) Mitochondrial DNA toxicity and diet (Bergersen/Storm-Mathisen, Klungland UiO); Lauritzen KH et al 2016 Neurobiol Aging.

- **A brief assessment of the project's implementation and use of resources**

The implementation of the project and the use of resources went as expected.

- **A description of the anticipated significance/benefits of the results (e.g. for the research field, for development of expertise, for trade and industry and society as a whole)**

By identifying lactate and its receptor as an avenue for beneficial effects of exercise on the brain, the project has provided important new insight in body-brain mechanisms and opened a new field of research. Researchers trained under the project have acquired expertise in brain, cardiovascular and muscular physiology. The findings pave the way for development of an 'exercise pill', which may be important, not as a substitute for exercise (which has many effects apart from those on the brain), but as a supplement. This may prove essential in persons who for whatever reason are unable to attain an adequate level of physical exercise, which is typically the case for people at high risk for developing Alzheimer's disease and other forms of dementia. Thus the results are anticipated to benefit also industry and society as a whole.

- **A description of the plans for disseminating and utilising the results**

**DOFIs:** Based on the results on HCAR1, we have filed 2 DOFIs (Disclosure of Invention): 14041 “Non invasive imaging of lactate receptor” received by Inven2 on 13<sup>th</sup> March 2015 (Inven2 decided not to proceed), and 15236 “Brain stimulation” approved by Inven2 on 6<sup>th</sup> May 2016. Funding sought to develop ‘exercise pill’.

**Reviews & Commentaries** (for researchers and clinicians): Rinholm JE, Bergersen LH 2012 *Nature*; Bergersen LH, Gjedde A 2012 *Front Neuroenergetics*; Rodell A et al 2013 *Front Neuroenergetics*; Bergersen LH et al 2013 *Neuroscience* (412); Rasmussen LJ et al 2013 *Mech Ageing Dev*; Nagelhus EA et al 2013 *Mech Ageing Dev*; Mariga ST et al 2014 *Front Neurosci*; Bergersen LH, Eid T 2014 *N Engl J Med*; Rinholm JE, Bergersen LH 2014 *Neuroscience*; Lauritzen F et al 2015 *Brain Struct Funct*; Bergersen LH 2015 *J Cereb Blood Flow Metab*; Gundersen V, Storm-Mathisen J, Bergersen LH 2015 *Physiol Rev*; Morland C et al 2015 *J Neurosci Res*.

**Conference presentations:** Numerous, including invited talks in 2016 at: American Society for Neurochemistry (ASN) Colloquium Denver Colorado; Neuroglia Symposium University of Alabama@Birmingham; FENS Forum Copenhagen; Alfred Benzon Symposium Copenhagen; Virginia-Nordic Precision Neuroscience, Roanoke Virginia.

**Talks on exercise and brain for the public and legislators:** Stortinget Parliamentarist Group for Dementia (LHB 2016); UiO-medisin200 (LHB 2014&2015), Leva Livet Den norske Turistforening (LHB Trondheim 2014), Steffensrud Rehabiliteringssenter (JSM 2013), Kunnskapsdepts seminar Nevrovitenskap og læring (JSM 2012).

**Conferences organized for researchers and users:** Nansen Neuroscience Lectures, DNVA, *annually* since 2010; Brain Energy 2013 – Current Advances in Brain Maintenance, DNVA; Mini-symposium on Alzheimer's disease, BRAINlab, Copenhagen 2014; Network meeting by Healthy Brain Ageing Centre (HBAC) 2014.

**Popular science articles:** <http://tidsskriftet.no/2013/10/nyheter/laktat-har-signalfunksjon-i-hjernen>  
<http://tidsskriftet.no/2016/05/fra-andre-tidsskrifter/flere-mekanismer-ved-iskemisk-hjerneskaade>  
<http://forskning.no/meninger/kronikk/2012/06/kavlipris-til-hjernens-informasjonsbehandling>  
<http://forskning.no/menneskekroppen-hjernen-trening-biologi/2013/06/melkesyre-kan-trigge-nye-hjerner-celler>  
<http://forskning.no/medisinske-metoder-molekylaerbiologi/2014/02/blodprobe-varsler-dod>  
<http://forskning.no/2015/08/hjernens-tenketilstand-er-funne>

<http://forskning.no/hjertet/2016/02/skade-i-mitokondrienes-dna-gir-hjertesvikt>

<http://iform.nu/om-i-form/i-form-ekspert-linda-hildegard-bergersen>

<http://www.aftenposten.no/meninger/kronikk/Kronikk-Nobelprisen-i-medisin-kan-tiltrekke-gode-forskere-fra-hele-verden-77553b.html>

Aftenposten Vitenskap 2016 No 1, April 25: “Påviser treningseffekt på hjernen” Linda H Bergersen

**Books:** “Ditt smarte barn – slik hjelper du barnet å lære” (Hanne S Finstad 2016 Stenersens) and “Hjernen er stjernen” (Kaja Nordengen 2016 Kagge), on public brain awareness. The authors were associated with our research group (no economic contribution). These books could not have been written without a basis in the research environment, the existence of which depends on support like the present project grant.

- **A description of the results that are expected to be finalised after the completion of the project**

The following articles based on the work are to be finalized after the completion of the project period (titles and first author are tentative):

Moretti L et al 2016 "Intrinsic physical fitness level predicts effects of exercise on brain mitochondria: Hippocampal capillary density in trained and sedentary high capacity and low capacity running rats" [Collaboration with Ulrik Wisløff et al, NTN.] (See description above under Secondary objective 3)

Andersson KA et al 2016 "The role of lactate receptor HCAR1 in plaque formation in Alzheimer Disease: Amyloid deposition in pial and brain arteries in HCAR1 knockout mice and Alzheimer model mice" [Collaboration with Magnar Bjørås et al, OUS & NTNU]

Andersson KA et al 2017 "High-intensity interval exercise: relative effects on hippocampal neurogenesis and memory" [Collaboration with Ulrik Wisløff et al, NTNU]

Andersson KA et al 2017 "SOD2 expression in the brain is regulated by the lactate receptor HCAR1" [Collaboration with Ulrik Wisløff et al, NTNU]

- **References**

References cited in text: To see the complete reference, paste the string (Author YEAR Journal) into PubMed