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Stories about research supported by the Novo Nordisk Foundation

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ABOUT

The Novo Nordisk Foundation owes its existence to the researchers in Canada who discovered the life-saving hormone insulin. Through a fascinating sequence of events in the early 1920s, Danish Nobel Prize laureate August Krogh and his wife Marie received the right to produce insulin in Scandinavia. With the right came an obligation to make insulin widely available and to use the profits for research and for improving care for people with diabetes.

Today, the Foundation is one of the world's largest philanthropies and a leading, long-term investor in life science companies. Within human health, our mission is to drive innovation in prevention, treatment and access to care for cardiometabolic and infectious diseases. Moreover, resistance to antibiotics and other antimicrobial medicines means that some infections are becoming increasingly difficult or impossible to treat.

Meanwhile, climate change has emerged as one of the greatest challenges to the sustainability of the planet, largely caused by the use of fossil fuels, deforestation and livestock farming. If left to continue, the consequences for people and society will be dire. The Foundation and its investment company Novo Holdings have embarked on a mission to make biotechnology a spearhead for the green transformation of industry and agriculture.

We do so by funding moonshots – fundamental research with the potential to create transformative change – and investing in and paving the way for promising biological discoveries to become large-scale solutions to major climate-related problems.

Our ScienceNews website communicates some of the groundbreaking results emerging from the research supported by the Foundation and how the researchers envision that their results can positively influence people's health and the sustainability of the planet in the near future.

The articles on ScienceNews are based on the researchers' own words, sometimes interpreted by a science writer. The researchers also have an opportunity to write about future scientific visions themselves. We call these articles ScienceViews.

The articles in this publication are just a few of the many fascinating stories on the website. Some of them have been shortened to fit the format of this publication, so visit the website, where you can also watch videos and listen to podcasts. This is all about science and how it improves the health of people and the planet on which we live.

enjoy the reading

Morten Busch
Editor-in-chief

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*This magazine is published in limited numbers and presents a wide range of research stories within **five different research chapters**. For each theme, there are six articles as well as samples of four additional articles, which you can find at **www.sciencenews.dk/en***

chapter

DISEASE AND TREATMENT

ARTICLES

How many children and adolescents in Denmark have ***been diagnosed*** with a mental disorder?

BY KRISTIAN SJØGREN

Fifteen percent of Danish children are diagnosed with a mental disorder before 18 years of age. Anxiety is the most frequent mental disorder among girls and ADHD among boys.

A new Danish study reveals that 15% of children in Denmark born between 1995 and 2016 have been diagnosed with a mental disorder before 18 years of age.

In all, 41,350 girls (14.6%) and 58,578 boys (15.5%) have been diagnosed with a mental disorder.

The types of diagnoses boys and girls receive and when they are diagnosed differ. The findings were published recently in *JAMA Psychiatry*.

"Detailed knowledge about the epidemiology of mental disorders among children and adolescents has been lacking. We simply know too little about fundamental things such as the occurrence of the individual disorders, at which ages the various disorders are first diagnosed and the gender differences in these patterns. Our study is the first to provide a detailed picture of the underlying epidemiology of mental disorders among children and adolescents," explains Søren Dalsgaard, specialist physician in child and adolescent psychiatry and Professor, Department of Economics and Business Economics, Aarhus University.

Soren Dalsgaard led the study, which was carried out in collaboration with other researchers from the National Centre for Register-based Research at Aarhus University.

Anxiety predominates among girls and ADHD among boys

The study included 1.3 million children. The researchers used data from the Danish National Patient Registry, the Danish Psychiatric Central Research Registry and other registries to examine how many children in Denmark born between 1995 and 2016 had been diagnosed with a mental disorder.

They also examined which diagnoses the children had received and when they had been diagnosed.

The study revealed the following.

• The most frequently diagnosed mental disorder among girls was anxiety: 7.9%.

• The most frequently diagnosed mental disorder among boys was ADHD (attention-deficit/ hyperactivity disorder): 5.9%.

• Neurodevelopmental disorders, such as ADHD and autism spectrum disorders, which by definition develop in early childhood, were diagnosed significantly earlier among boys than among girls. For ADHD, most boys were diagnosed at age 8 years and girls at age 17 years.

• This also applied to autism: boys were typically diagnosed when they were 4–13 years old and girls at 12–17 years old.

"Girls being diagnosed so late is noteworthy, since they must have exhibited autistic behaviour before 3 years of age. Many girls reach about 15 years of age before receiving the correct diagnosis, and this is not good enough," says Søren Dalsgaard.

Girls are not diagnosed early enough In Denmark

Søren Dalsgaard believes that Denmark is generally not good enough at detecting whether girls have ADHD or autism spectrum disorders that need to be addressed.

The problem is that girls who are diagnosed late do not receive the necessary help early enough, and this can lead to even greater difficulties later in life.

"Efforts have not targeted girls to the same degree as boys, and that is not good, because the girls may then have problems throughout their school years before being diagnosed. We have shown in several studies that girls with ADHD have higher relative risks of drug addiction, depression and psychoses than boys with ADHD. One explanation may be that they did not receive any help for their ADHD difficulties when they were younger," says Søren Dalsgaard.

Surprising discovery about schizophrenia

The researchers also found that early-onset schizophrenia, a rare disorder in which schizophrenia develops before 18 years of age, is more common among girls than boys.

This contrasts with the prevailing clinical consensus that schizophrenia is slightly more frequent among boys than girls, similar to schizophrenia in adulthood, which is by far more frequent.

In fact, the opposite is true, at least for those born between 1995 and 2016: almost twice as many girls (0.76%) as boys (0.48%) have been diagnosed with a schizophrenia spectrum disorder before 18 years of age.

"This is a completely new finding, and we do not know why. This is striking, also because some of the other mental disorders are detected late," says Søren Dalsgaard.

The proportion of young people with mental disorders is similar to that in other countries
What is the significance of 15% of children and adolescents in Denmark being diagnosed with a mental disorder?

According to Søren Dalsgaard, this percentage is similar to what has been found worldwide, including the results of a meta-analysis of 41 studies from 27 countries.

There is therefore no indication that children and adolescents are being overdiagnosed in Denmark, and Søren Dalsgaard believes that the figures mostly indicate that Denmark is good at identifying the children who have difficulties.

"This is the most important thing for me, and, for example, the fact that Denmark is good at identifying the girls with anxiety is both good and important so that we can help them further," he explains.

Søren Dalsgaard thinks that these research results should be used to focus on how mental health services can improve so that they not only identify all children with mental health problems but also discover them as early as possible. This applies especially to girls with ADHD, autism and other neurodevelopmental disorders.

"Incidence rates and cumulative incidences of the full spectrum of diagnosed mental disorders in childhood and adolescence" has been published in JAMA Psychiatry. In 2016, the Novo Nordisk Foundation awarded Søren Dalsgaard a grant for the project Polygenic Risk Scores and Early Social Adversities in Predicting the Developmental Course and Trajectory in Individuals with ADHD.

Parkinson's cure fails – but ***stem cell therapy*** is approaching

More than 6 million people have the highly debilitating Parkinson's disease. Only the symptoms of Parkinson's disease can be alleviated, and a promising cure has been unsuccessful. In contrast, stem cell transplantation offers a glimmer of hope. The first clinical trials involving patients will begin in 2021, and if these replicate the convincing results from preclinical studies in rats and pigs, stem cell treatment might be realistic in 5–7 years.

BY MORTEN BUSCH

The early signs are shaking, stiffness and slower movements. The central nervous system of people with Parkinson's disease degenerates slowly, and this often leads to difficulties in walking combined with sensory, behavioural and emotional problems. Daily medication can alleviate the symptoms, but the effect of the medicine diminishes around 7 to 15 years after disease onset, and most people with Parkinson's disease then have severely debilitating symptoms that cannot be alleviated without severe side-effects. Today, more than 6 million people worldwide have Parkinson's disease, and that number is increasing as global populations age. Researchers are therefore still struggling to understand and treat Parkinson's disease, but so far all these efforts have been in vain.

"Unfortunately, a number of recent clinical trials of a treatment using GDNF, the prime candidate for a possible cure for Parkinson's disease in the past 8 years, have failed. By contrast, developments within stem cell transplantation look very promising. We started the final preclinical trials involving rats and pigs. If these succeed, this treatment may be available to people with Parkinson's disease from 2026," says Agnete Kirkeby, Associate Professor and Group Leader, Department of Neuroscience, University of Copenhagen.

One clinical trial after another has failed

More than 30 years have elapsed since researchers seeking the cause of Parkinson's disease began investigating GDNF (glial cell–derived neurotrophic factor). The dopamine-producing neurons, a type of neurotransmitter essential for controlling human movement, degenerate in Parkinson's disease. This can be easily treated with oral dopamine in the short term, but over time this becomes less and less effective as the side-effects increase. In the 1990s, researchers discovered GDNF.

"In animals, GDNF appeared to be able to protect the dopamine-producing neurons in the brain from cell death. Clinical trials were therefore quickly initiated. The first ones probably failed because of insufficient doses of GDNF being delivered to the brain tissue. Since then, one clinical trial after another has failed. I am pretty convinced that, if GDNF really were a miracle cure, then we would have seen clear effectiveness already," says Agnete Kirkeby.

Instead, Agnete Kirkeby is counting on stem cell transplantation, another type of restorative treatment for Parkinson's disease. In 2012, Agnete Kirkeby and her colleagues at Lund University in Sweden made a major breakthrough when they produced new dopamine-producing neurons from stem cells. They showed that transplanting these cells into rats' brains completely counteracts the motor symptoms of Parkinson's disease.

"At that time, we could produce the stem cells with a purity of 60–70%, which may sound high, but when the cells need to be transplanted into a person's brain, safety is extremely important. We

then set the goal of achieving 90% purity in our production method before seriously considering transplantation into humans," explains Agnete Kirkeby.

Understanding the challenge the researchers faced requires understanding the process of producing stem cells. In principle, all stem cells can move in all directions and thus become all types of cells, but they develop in one direction or another depending on the growth factors that affect them and the environment in which they grow. The stem cell researchers therefore needed to imitate as precisely as possible what happens during fetal brain development.

"We grow the cells in small petri dishes, and if we change the concentration of a single chemical by just 10%, then the cells develop in a completely different direction than we intended. We have therefore not only had to ensure the right growth conditions for the past 12 years. We have also had to be able to replicate the same conditions again and again," says Agnete Kirkeby.

In the right direction

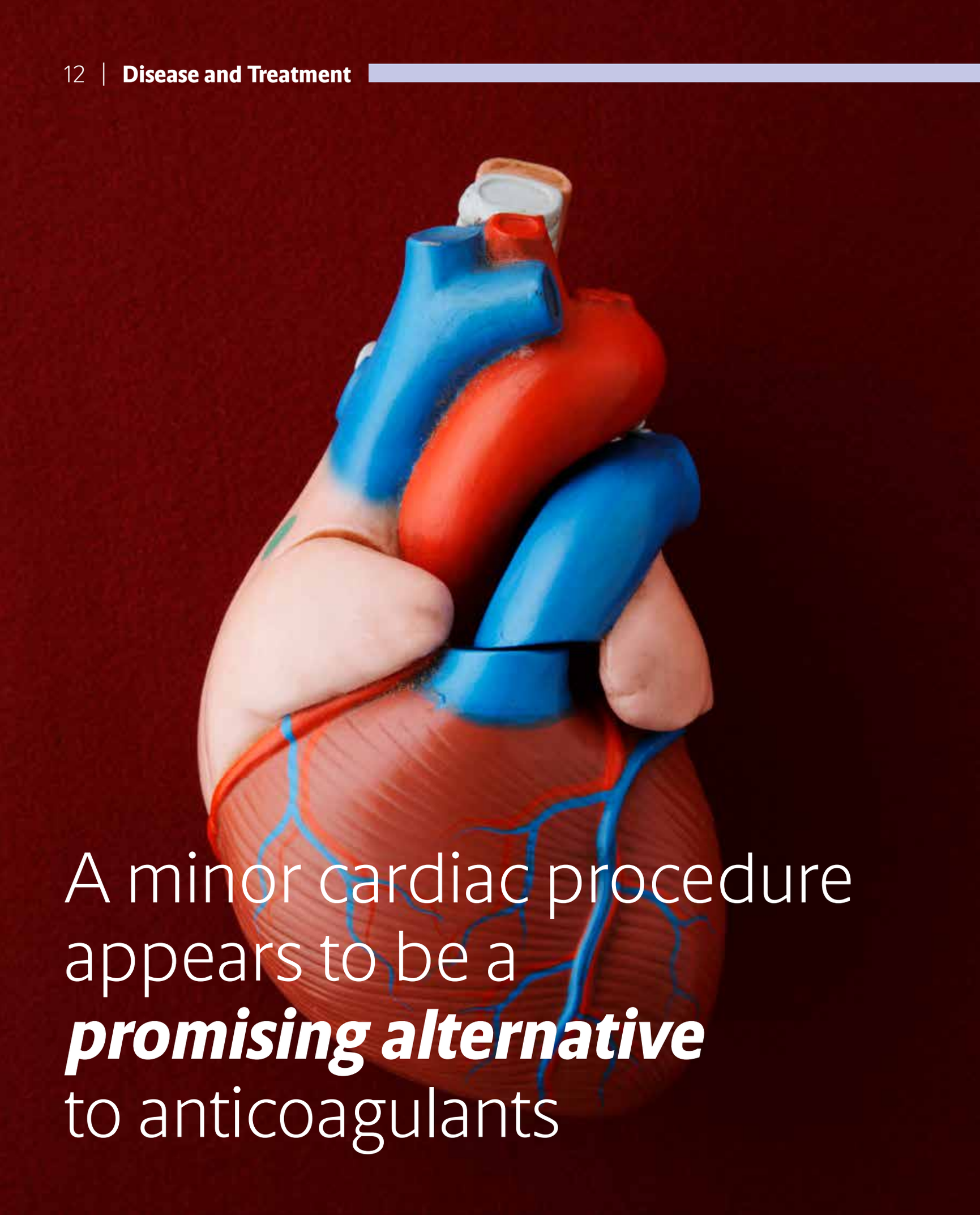
Agnete Kirkeby moved to the University of Copenhagen, and together with her former colleagues at Lund University, she succeeded in 2017 in producing the dopamine-producing stem cells with such purity and safety that the method was ready for transferring to human patients. These results were published in Nature Protocols.

"This was a massive and crucial breakthrough, because we showed that the method could potentially be used on humans and not just on rats. The reason why we still had some way to go was because we needed to be absolutely sure that we were not doing more harm than good. The stem cells must enter the brain at the right time and in the right place," explains Agnete Kirkeby.

Transplanting the cells prematurely risks them not developing in the right direction, but transplanting them too late means that they may continue to divide. This may develop tumours, but the researchers have not yet observed any tumours in any of the rats subjected to transplantation.

"Transplanting stem cells is equivalent to receiving cells from a newborn, and we therefore believe that these cells have the resilience to survive many years in the person's brain. If all goes well, we hope that a stem cell treatment can be marketed in 5–7 years, so that we can finally repair people's brains rather than alleviating the symptoms of Parkinson's," concludes Agnete Kirkeby.

"Parkinson disease and growth factors — is GDNF good enough?" has been published in Nature Reviews Neurology. "Extended treatment with glial cell line–derived neurotrophic factor in Parkinson's disease" has been published in the Journal of Parkinson's Disease. In 2018, the Novo Nordisk Foundation awarded a grant to Agnete Kirkeby for the project Mapping Human Neural Lineages in a Novel In Vitro Model of the Developing Neural Tube Built with Morphogenic Gradients



A minor cardiac procedure appears to be a ***promising alternative*** to anticoagulants

A new study suggests that selected patients with atrial fibrillation have better outcomes with left atrial appendage occlusion versus lifelong treatment with oral anticoagulants.

BY KRISTIAN SJØGREN

In Denmark, 120,000 people have atrial fibrillation, a condition in which the atrial chambers of the heart beat rapidly and irregularly. Atrial fibrillation slows the flow of blood in the atria and increases the risk of blood clots forming in the left atrial appendage, a small ear-shaped sac attached to the left atrium. Thrombotic material can detach from the left atrial appendage and be transported by the bloodstream to the brain, causing a stroke.

Lifelong oral anticoagulants are prescribed to prevent atrial fibrillation from causing stroke. This effectively prevents clots from forming but also increases the risk of severe bleeding.

A new Danish study suggests that selected patients with atrial fibrillation may benefit from left atrial appendage occlusion, a percutaneous catheter procedure in which a cardiologist inserts an implant into the heart through a catheter inserted into the femoral vein in the groin. Once in place, the implant closes and seals off (occludes) the left atrial appendage, thereby preventing blood clots from forming and detaching from the appendage. The left atrial appendage is a fetal remnant that we do not need.

The new study shows that this procedure reduces the risk of stroke to the same level as treatment with oral anticoagulants. However, left atrial appendage occlusion also substantially reduces the risk of bleeding because oral anticoagulants are no longer necessary.

"Some patients with atrial fibrillation cannot tolerate oral anticoagulants because they have already experienced severe bleeding or have an increased risk of bleeding. This procedure will be attractive for them because it reduces their risk of stroke for the rest of their lives and they do not have to remember taking oral anticoagulants daily," explains a researcher behind the study, Jens Erik Nielsen-Kudsk, Clinical Professor, Aarhus University and Senior Physician, Department of Cardiological Medicine B, Aarhus University Hospital.

The research has been published in *JACC: Cardiovascular Interventions*.

Two-year follow-up of 1,078 patients who underwent left atrial appendage occlusion

The researchers compared the risk of stroke and of severe bleeding for left atrial appendage occlusion versus the latest generation of oral anticoagulants, such as dabigatran (Pradaxa®), apixaban (Eliquis®), rivaroxaban (Xarelto®) or edoxaban (Lixiana®).

This part of the study included 1,078 patients from 61 cardiac centres in 17 countries who underwent left atrial appendage occlusion and were subsequently followed up for 2 years.

This group was compared with a similar group of patients in Denmark who had atrial fibrillation based on validated Danish registries, including the Danish National Patient Registry and the Danish National Prescription Registry. They were treated with the latest generation of oral anticoagulants and matched by propensity score based on covariates including age, heart failure, hypertension, risk of bleeding, diabetes, prior stroke or transient ischaemic attack or thromboembolism so that the groups were comparable.

Following matching, the patients who underwent left atrial appendage occlusion and those taking anticoagulants had the same expected risk of stroke and of severe bleeding at baseline.

Reduced risk of stroke and death

The results suggest that left atrial appendage occlusion may provide benefits over oral anticoagulants.

- The risk of stroke was similar in the groups.
- Left atrial appendage occlusion reduced the risk of severe bleeding by 38% versus oral coagulants. According to Jens Erik Nielsen-Kudsk, this is because anticoagulants are rendered unnecessary.
- The study also showed that left atrial appendage occlusion reduced the risk of death by 47% versus oral anticoagulants. Jens Erik Nielsen-Kudsk is surprised by the scale of this risk reduction, but fewer deaths from severe bleeding may be part of the explanation.

"These findings are interesting and thought-provoking and might seem to be too good to be true. Nevertheless, the results suggest that patients who do not have good outcomes with anticoagulants may benefit from left atrial appendage occlusion, a simple procedure that only takes 40 minutes under local anaesthesia," says Jens Erik Nielsen-Kudsk. He emphasizes, however, that this was not a randomized controlled trial and that the design limits any definitive conclusions.

More people may benefit from left atrial appendage occlusion

According to Jens Erik Nielsen-Kudsk, the results suggest that further studies should identify which people with atrial fibrillation may benefit from left atrial appendage occlusion instead of oral anticoagulants.

Many people do not have good expected outcomes with oral anticoagulants, including those likely to experience severe bleeding, those assessed as having a high risk of severe bleeding and those who might have poor adherence with oral anticoagulants and therefore have an increased risk of stroke.

"Even more people may need left atrial appendage occlusion – not simply those at very high risk but also a wider group with atrial fibrillation at moderate risk," explains Jens Erik Nielsen-Kudsk.

"Clinical outcomes associated with left atrial appendage occlusion versus direct oral anticoagulation in atrial fibrillation" has been published in JACC: Cardiovascular Interventions. In 2017, the Novo Nordisk Foundation awarded a grant to Jens Erik Nielsen-Kudsk for the project Left Atrial Appendage Occlusion Versus Novel Oral Anticoagulation for Stroke Prevention in Atrial Fibrillation: A Multicentre Randomized Clinical Trial (the Occlusion AF Trial).

Breakthrough in developing a vaccine for placental malaria

BY KRISTIAN SJØGREN

Placental malaria is the main cause of low birthweight among children in Africa. Researchers in Denmark have now discovered how to make a vaccine for pregnant women that specifically targets the malaria parasite.

The malaria parasite (*Plasmodium falciparum*) is an advanced killer that humanity has not eradicated despite enormous efforts.

One reason is that P. falciparum has developed many ways of protecting itself inside the human body. One involves hiding in a red blood cell, where it expresses a protein that mediates binding of the infected red blood cells to chondroitin sulfate in the placenta.

If the parasite is not freely circulating in the bloodstream, the immune system cannot find it and destroy it.

However, science may finally vanquish *P. falciparum* infections during pregnancy, now that researchers from Denmark and elsewhere have very accurately mapped the structure of the protein the parasite expresses to bind to the placenta.

This discovery paves the way for developing antimalarial vaccines, specifically when the parasite tries to infect a pregnant woman.

"Researchers have been trying to map the structure of this protein since 2003, a nearly impossible task, but we have succeeded now. With the modern vaccine development tools we have available, we are already developing and testing a vaccine candidate that attacks *P. falciparum* in the very place where it has been inaccessible so far," explains an author behind the study, Ali Salanti, Professor, Centre for Medical Parasitology, University of Copenhagen.

The research has been published in *Nature Communications*.

Placental malaria is a huge problem in Africa

Today, malaria remains one of the greatest problems in Africa.

In addition to killing hundreds of thousands of children every year, *P. falciparum* also results in mothers with malaria giving birth to babies weighing up to 400 grams less than the babies born to uninfected women.

This has huge consequences because low birthweight is clearly associated with many cognitive and other health problems later in life.

P. falciparum hides from a pregnant woman's immune system by expressing the VAR2CSA protein on the surface of the red blood cells. The

woman's immune response thus cannot attack the parasite, even though she has previously been infected and has therefore developed immunity.

"Pregnant women in Africa comprise a huge reservoir for malaria. Women who become pregnant for the first time have not encountered VAR2CSA before and have therefore not developed immunity to it," says Ali Salanti.

This protein does not act like other proteins – and that is the problem

Ali Salanti discovered this notorious protein in 2003 and immediately recognised the great potential for developing a vaccine that specifically teaches the immune system to recognise VAR2CSA.

However, the problem was that VAR2CSA is hypervariable and constantly mutates, so it presents differently from infection to infection. As a result, researchers in the early 2000s did not have any tools to map the protein that could help them determine how to develop a vaccine against placental malaria.

"SARS-CoV-2 has maybe three mutations in the binding domain, whereas almost half the amino acids that make up P. falciparum VAR2CSA differ between parasites. Making a vaccine that broadly covers all the variants is therefore not easy without an exact structure of the protein to map where the conserved sites are. This required comprehensively mapping VAR2CSA, which was not possible until now," explains Ali Salanti.

Cracking the code for the structure of VAR2CSA

Since 2003, many researchers have spent huge sums and resources on defining the fine structure of VAR2CSA. Now however, researchers in Denmark led by Ali Salanti have accomplished what many other researchers have been unable to do.

Ali Salanti and colleagues used a variety of scientific techniques and methods to achieve this breakthrough, including cryoelectron microscopy, plus a new generation of protein and structural chemists who approached the task in novel ways.

"Researchers from the National Institutes of Health in the United States cracked the code at the same time that we did. One month apart, two studies have been published that accurately map what VAR2CSA looks like and which amino acids interact with the chondroitin molecules in the placenta," says Ali Salanti.

Developing a vaccine using a revolutionary platform

Although the discovery is a breakthrough for developing a vaccine against placental malaria, Ali Salanti explains that obstacles still need to be overcome before a vaccine can be produced.

The main market for an antimalarial vaccine is Africa. This means that the vaccine must be stable and capable of being stored at room temperature and not –80°C or –20°C.

In addition, the vaccine must provide a long-lasting response so that doctors can vaccinate women and girls before they become pregnant, so that they are protected against malaria throughout pregnancy.

If the vaccine is given to the woman when she is pregnant, she may already have malaria, and then it is too late. Further, it does not help if the vaccine only works for 6–12 months, because no woman really knows if or when she will get pregnant.

"Cryo-EM reveals the architecture of placental malaria VAR2CSA and provides molecular insight into chondroitin sulfate binding" has been published in Nature Communications. In 2018, the Novo Nordisk Foundation awarded a grant for the project ROBUST: Resource for Biomolecular Simulations.

Cancer drug makes the body think that a ***virus is attacking***

Blood cancer affects both young and old people, but treatment options for older people are especially limited, since bone marrow transplantation is not usually carried out among older people. The only drug that prolongs life is therefore 5-azacytidine (AZA). However, only about 50% of people with blood cancer respond to this treatment. Why this is the case has not been understood, but now researchers have found that AZA activates the immune system so that it thinks a virus is attacking. This may help to make the medicine effective for more patients and may even help to combat viral infections.

BY MORTEN BUSCH

When researchers first tried 5-azacytidine (AZA) to treat people with cancer, the results were somewhat of a fiasco. It was disappointingly ineffective against most types of cancer, but in the early 2000s, the results were revisited and it turned out that, at lower doses, AZA was effective in treating leukaemia and other forms of blood cancer. Today, it is almost the only medication that can be used to treat older people with myelodysplastic syndrome, a type of blood cancer.

"AZA is very effective in keeping myelodysplastic syndrome at bay for around half the patients. However, its effectiveness decreases over time, and it is not effective at all for the other half. We could not discover why this happened because we did not really understand how AZA works. Our new trial suggests that it is effective because it activates some elements of our DNA that originate from ancient virus infections. Now we hope to find other methods to stimulate the same mechanisms among the people for whom AZA is not effective," explains Kirsten Grønbæk, Professor, Department of Haematology, Rigshospitalet, Copenhagen.

Virus alarm system

AZA has been known and used especially to treat people with blood cancer since 2009. It is similar to cytosine, one of the four main bases in DNA. The original idea for using AZA was that cancer cells, which divide faster than other cells, would incorporate the false base into their DNA to a greater extent and thus destroy their DNA. This is also the case at high doses, but lower-dose AZA has proved far more effective against cancer and less harmful to the body's other cells.

"At low doses, AZA inhibits DNA methyltransferase, which normally regulates the gene expression in cells by adding methyl groups to our DNA. And since many previous studies have shown that DNA methylation is greatly increased in cancer cells, 5-azacytidine is very effective against the cancer cells by disrupting DNA methylation," says Kirsten Grønbæk.

However, this turned out to be only part of the story and of why AZA effectively combats blood cancer. One study showed that the demethylation in the cell apparently triggered a kind of viral alarm that caused the body's innate immune system to attack foreign and abnormal cells. The researchers examined this somewhat surprising effect of AZA in their new study.

"We therefore examined 150 RNA samples from two groups of adults and a group of children with different types of blood cancer. We took samples before, during and after treatment with AZA. Because only about half normally respond positively, it was interesting to determine which genes AZA upregulated and downregulated and whether this was associated with whether the drug was effective or not," explains Kirsten Grønbæk.

These experiments confirmed the earlier laboratory experiments: the demethylation caused by AZA activates some elements of the human genome that we inherited millions of years ago from various types of viruses that have copied parts of their genome into ours. Today, these transposons comprise up to 40% of the human genome. The transposons are unique in being able to change their position within the genome.

"In response to AZA treatment, these transposons are copied into single- and double-stranded RNA, which in turn causes our immune system to think that the body is being attacked by a virus. This therefore activates the body's immune system and thus has the beneficial side-effect of eliminating cells that behave abnormally, such as cancer cells," says Kirsten Grønbæk.

New clinical trials to understand this effect more closely

The new results provide new important knowledge for the future treatment of various types of blood cancer such as leukaemia but especially myelodysplastic syndrome, which affects 250 older people each year. And since bone marrow transplantation is seldom carried out on older people, AZA treatment is the only option today. Unfortunately, treatment is effective for only half the people with myelodysplastic syndrome, and AZA gradually loses effectiveness among these.

"We have started a large international clinical trial, since our current trial only included 40 patients. If the new trial confirms the effects seen here, we hope to find other methods to kickstart the immune system similarly for those who do not respond to AZA or for whom the effectiveness has declined. We may be able to help the rest of the patients if we can achieve this," explains Kirsten Grønbæk.

The new clinical trials will also test another hypothesis the researchers want to confirm. Indeed, as many as 80% of the people with myelodysplastic syndrome appear to have severe vitamin C deficiency, and preliminary experiments indicate that combining AZA and vitamin C is very effective in activating the immune system alarm for viruses. The researchers therefore hope to be able to use the new clinical trials to understand this effect more closely at the genetic level.

"Activation of a Subset of Evolutionarily Young Transposable Elements and Innate Immunity are Linked to Clinical Responses to 5-Azacytidine" has been published in Cancer Research with support from the Stand Up to Cancer Epigenetics Dream Team and others. In 2013, the Novo Nordisk Foundation awarded a 5-year grant to Kirsten Grønbæk for the project Translational Epigenetics in Blood Cancer. Kirsten Grønbæk is one of the initiators of the Program for Translational Haematology the Foundation has supported since 2017 through the Novo Nordisk Foundation Center for Stem Cell Biology, DanStem.

Creating ***pancreatic cells*** in the laboratory to cure people with diabetes

BY KRISTIAN SJØGREN

New research shows how the Notch signalling pathway works when the pancreas forms as the fetus develops. This discovery may lead to new opportunities to cure people with diabetes and understand how pancreatic cancer develops.

Imagine doctors in the near future being able to cultivate stem cells that turn into the insulin-producing beta cells in the pancreas – and then implanting these in people with diabetes to replace their damaged beta cells and thus cure them.

This dream has just come a step closer, after researchers from the University of Copenhagen have revealed how a signalling pathway that guides the development of the pancreas works.

The discovery means that researchers now understand much better what they need to do to cultivate insulin-producing beta cells in a petri dish with the goal of curing people with diabetes.

"The interesting perspective is to take fetal stem cells and direct them to become insulin-producing cells. This requires knowing how nature does this normally, and we have come a step closer to understanding this," says Palle Serup, Professor, Novo Nordisk Foundation Center for Stem Cell Biology, DanStem, University of Copenhagen.

The research was published recently in *Developmental Cell*.

Curing people with diabetes using home-grown beta cells

Phase 1 clinical trials around the world are already trying to cure people with diabetes by inserting laboratory-grown insulin-producing beta cells into people's pancreases.

So far, the trials have been oriented towards ensuring that this procedure is safe, but the idea is to be able to cure the first people with type 1 diabetes within a few years.

The researchers from the University of Copenhagen are at the forefront of this, and leading researchers can also determine how to optimally improve the various procedures.

This applies to the procedures the researchers use to develop the insulin-producing beta cells they implant in people.

The current laboratory-grown beta cells do not respond as well to glucose as they should, and the yield of the cultivation process is also relatively low.

"One reason is that we have not yet been able to fully replicate the natural process in the laboratory," explains Palle Serup.

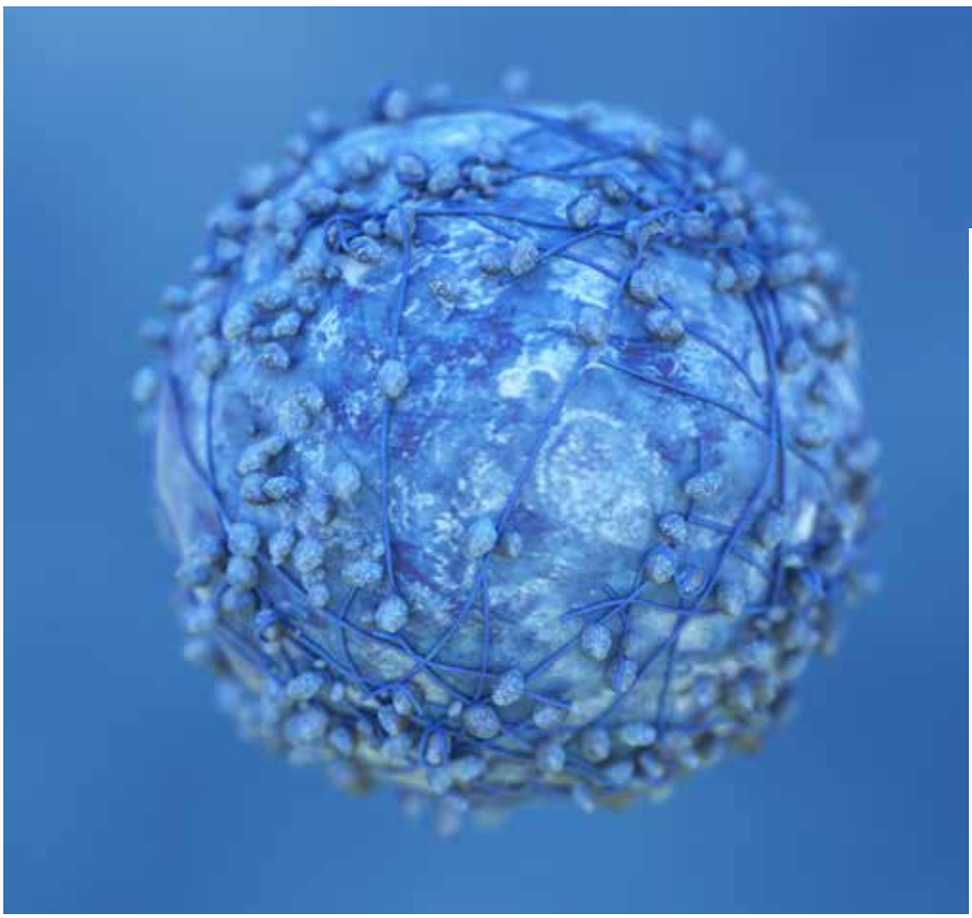
Current protocols do not exploit signalling pathways fully

Palle Serup and colleagues studied how the fetal pancreas develops.

Many signalling pathways play a role in the process of inducing the stem cells to become the various cells of a pancreas.

These signalling pathways ensure that insulin-producing beta cells, blood vessels and the ducts that secrete digestive enzymes are produced where they are needed.

The signalling pathways are communication tools between neighbouring cells, and the Notch signalling pathway that Palle Serup has now mapped is very important for the natural development of the pancreas.



"We did not know very much previously about this signalling pathway, and the protocols we use in cultivating pancreatic cells in the laboratory are therefore not very good at using the regulation of this pathway," says Palle Serup.

Signal molecules oscillate

Notch has previously been linked to pancreatic development, and the new study explains this.

The research shows that the concentration of the signal molecule DLL1 oscillates from high to low and back again, with a 45-minute interval per direction.

Similarly, the oscillation activates the HES1 gene in the neighbouring cell, so the expression of this gene also begins to oscillate.

This is complicated, but Palle Serup's research also shows that manipulating the oscillations causes the pancreas to grow more slowly.

"This gives us insight into how the cells act when the pancreas is formed, and we have to recreate that activity in the petri dishes," explains Palle Serup.

Several signal molecules guide pancreatic development

The research also shows that DLL1 is not alone in controlling pancreatic growth during fetal development. The signal molecule JAG1 also plays a role.

Both molecules target the same receptors on neighbouring cells, but DLL1 stimulates pancreatic growth by promoting cell division in neighbouring cells, whereas JAG1 inhibits growth.

JAG1 also plays a role in the paths the cells take in their development. All pancreatic cells originate from two small groups of stem cells that can develop into all the different types of pancreatic cells.

During fetal development, cells develop in one direction or another. JAG1 influences the direction in which the cells develop.

When the researchers remove JAG1, too many cells develop towards cells that secrete digestive enzymes, and too few of the other types are formed. When JAG1 is present, a more appropriate number of the cells develop into insulin-producing beta cells.

To their surprise, the researchers could change the cell types by manipulating the oscillations. Attenuating the fluctuation in HES1 concentrations was equivalent to losing JAG1, whereas the opposite happened if the interval was increased from 45 to 60 minutes.

"Our experiments showed that removing JAG1 or artificially inhibiting oscillations makes the pancreas develop almost no insulin-producing beta cells. This is important to know for growing pancreatic cells in the laboratory," says Palle Serup.

Palle Serup says that the researchers are already looking towards the next step in investigating the role of the signalling pathways in developing the pancreas. They want to confirm that these oscillations also occur in human pancreatic cells and not just in mice. Then they will investigate the extent to which they can manipulate the oscillations to control cell development.

"Jag1 modulates an oscillatory Dll1-Notch-Hes1 signaling module to coordinate growth and fate of pancreatic progenitors" has been published in Developmental Cell. Palle Serup is Professor of Development Biology at the Novo Nordisk Foundation Center for Stem Cell Biology, DanStem, University of Copenhagen. The Novo Nordisk Foundation has awarded research grants of nearly DKK 700 million to DanStem from 2010 to 2017.

Newly ***graduated nurses*** have difficulty in changing current practice

Progress requires change, and this may require new external input. Newly graduated nurses are trained to challenge current practice in the healthcare system. However, a new study shows that this is challenging because the desire to be accepted as "part of the family" and the fear of appearing incompetent often make newly graduated nurses less willing to ask questions. The researchers think that nurses lack awareness that being critical of one's own and colleagues' practice is essential for developing as nurses and ensuring high standards in nursing.

BY MORTEN BUSCH

When a daily work routine is under maximum pressure, adhering to the usual established practice is often the easiest way out. Lack of critical reflection can mean that nurses only minimally draw on knowledge from research, patients and other aspects of evidence-based practice. Studies have shown that, although new graduates are trained to work based on evidence-based practice, they have great difficulty in involving patients and research in their daily decisions. A new study focuses on why.

"Newly graduated nurses are often parachuted into a complex situation characterized by many new and unknown tasks. Nursing care requires that the nurses ask questions and enquire how they can provide optimal care. Our studies indicate that the new nurses do not ask the experienced nurses questions about patient care because they are concerned about being perceived as inadequate, insecure and not equipped for the job. Further, the experienced nurses often answer questions by saying 'if it was me I would do it like this'. Integrating new knowledge to benefit patients primarily requires a work culture with room for questions and ongoing dialogue among the nurses about whether practice can be carried out differently and better," explains Siri Lygum Voldbjerg, Postdoctoral Fellow, Clinical Nursing Research Unit, Aalborg University Hospital and Senior Lecturer, University College of Northern Denmark.

But we have always done it this way!

Healthcare systems worldwide increasingly require that clinical decision-making includes evidence-based practice that incorporates patients' experiences and preferences and the highest-quality research. This approach began in medical practice in the early 1990s and influenced nursing a few years later with the expectation that new input from research would change practice. Evidence-based practice requires a questioning approach and critical thinking, which newly graduated nurses can provide through their curiosity and lack of experience.

"Newly graduated nurses are a valuable resource. They are specifically trained to both acquire

new knowledge and put it into practice when they graduate. However, since studies have indicated that this has not been very successful, we decided to observe and interview 11 newly graduated nurses on their shifts to see how they are encouraged and hindered in incorporating new knowledge into practice and involving patients in decisions," says Siri Lygum Voldbjerg.

The researchers observed the nurses on one to three 8-hour shifts. The resulting 174 hours of observation and the interviews showed how decisions were made and how the graduates' socialization into a ward and a new working environment influenced their questioning and critical approach to practice and thus their decisions on patient care.

"The overall conclusion was that their pursuit of recognition from their new colleagues most often unintentionally suppressed their desire to explore and challenge current practice. Our observations and interviews show that they are concerned about standing out. New nurses will do anything to become part of the family and be like the others, so they are not excluded. They also fear that overly questioning behaviour will make them appear insecure or critical of the more experienced nurses," explains Siri Lygum Voldbjerg.

The way the more experienced nurses responded to the new nurses questioning existing practice were not always conducive to change.

"Rather than leading to discussion and reflection, the enquiry was often rapidly deflected by a 'this is how we do things' answer. This left little space for questions and critical reflection," says Siri Lygum Voldbjerg.

Communication is key

Instead of implementing new research knowledge, the new nurses often used the experienced nurses as the predominant knowledge source.

"In a busy work environment nurses often choose the existing practice and the source of knowledge that is most easily accessible. However, this is probably also related to how they are assessed and what documentation is required. Our study showed that the nurses primarily focus on the new knowledge aimed at the more task- and technically oriented part of nursing such as administering medication or changing dressings, whereas recommendations for the more intangible aspects related to developing therapeutic relationships are more quickly brushed aside," explains Siri Lygum Voldbjerg, emphasizing that this type of practice can be extremely important to focus on and question, since inadequate communication and relationship-building between patients and nurses can lead to many unintended consequences."

"Communication is key to nursing care. For example, a patient may previously have received a certain medication and knows that this causes a rash or more severe side-effects, but the patient may think that this is the only drug available and so does not ask about alternatives. Optimizing dialogue and close relationships is therefore essential." says Siri Lygum Voldbjerg.

"Newly graduated nurses' socialisation resulting in limiting inquiry and one-sided use of knowledge sources – an ethnographic study" has been published in the Journal of Clinical Nursing. The Novo Nordisk Foundation and the University College of Northern Denmark funded the study.

Schoolgirls' absenteeism pattern confirms the safety of ***HPV vaccination***

Although increasing numbers of studies confirm that human papillomavirus (HPV) vaccination is safe, non-specific symptoms and diagnoses continue to result in scepticism towards this vaccination. An innovative research approach was used to investigate vaccination safety by examining a possible link between vaccination and girls' absenteeism from school. The researchers conclude that HPV vaccination does not increase the risk of illness manifesting as school absenteeism.

BY JOSEFINE TOPSØE

HPV vaccination has been part of Denmark's childhood vaccination programme since 2009 and aims to protect girls from HPV infection and thereby cervical cancer later in life. Although numerous studies have found that this vaccination is very safe, many recent stories about serious side-effects of HPV vaccination have caused severe setbacks in national vaccination programmes.

"In Japan, this has had major direct effects, since they have stopped recommending this vaccination, and the United States has difficulty achieving high coverage. In Denmark, we have had a very special situation with media coverage in connection with HPV vaccination and a documentary on vaccinated girls aired by TV2 in 2015 in which several young women reported disabling symptoms after being vaccinated. Unfounded accusations are problematic, since this vaccination protects against cervical cancer, which means that some of those who are not vaccinated will get cervical cancer and may end up dying from it," explains Anders Hviid, Professor and Senior Researcher, Department of Epidemiology Research, Statens Serum Institut, Copenhagen.

Difficulty with non-specific symptoms

Diagnosing people who experience symptoms after vaccination can be difficult. The most commonly reported symptoms include fatigue, headaches and dizziness. Since the reported symptoms are rarely specific, investigating the causes of the symptoms using databases and registries is more challenging than for more classic diagnoses with well-known symptoms and guidelines.

"Many studies have investigated the safety of HPV vaccination, and many of these have focused on specific concerns, such as whether vaccination is associated with blood clots. In this case, however, addressing these concerns with specific diagnoses was difficult. We therefore tried to find other ways to address this, and our study is then based on this rather unique method of examining girls' absenteeism from school," says Anders Hviid.

Innovative investigative approaches

The researchers used a unique registry in the City of Copenhagen that records children's absenteeism from primary and lower-secondary schools. They linked sickness absenteeism data from more than 14,000 girls in grades 5–9 from 2013 to 2018 with data on HPV vaccination.

> We found that the HPV-vaccinated girls had exactly the same pattern of absenteeism as the unvaccinated girls

The study was based on the hypothesis that, if HPV vaccination is associated with an increased risk of having non-specific symptoms, then this will result in more school absenteeism from illness than among the unvaccinated girls.

No association with absenteeism

"We found that the HPV-vaccinated girls had exactly the same pattern of absenteeism as the unvaccinated girls. The lack of any difference shows that vaccination is basically really safe. This broad measure that considers all illness and not just specific disease diagnoses supports offering HPV vaccination in Denmark's vaccine programme," concludes Anders Hviid.

"Human papillomavirus vaccination and all-cause morbidity in adolescent girls: a cohort study of absence from school due to illness" has been published in the International Journal of Epidemiology. The Novo Nordisk Foundation awarded lead author Anders Hviid a Hallas-Møller Scholarship in 2014 and a Data Science Investigator grant in 2020.

Statins improve the bacterial ***gut ecosystem*** of obese people

International research with solid input from Denmark shows that obese people who take statins have both lower levels of dangerous cholesterol in their blood and a healthier composition of gut bacteria.

BY KRISTIAN SJØGREN

Researchers have known for some time that being obese is clearly associated with an imbalance in the composition and functions of gut bacteria.

Researchers have also been interested in determining how to correct this imbalance, since it can be linked to the development of a variety of diseases, including arthritis, inflammatory bowel diseases, Alzheimer's, Parkinson's and diabetes. New international research with a strong Danish contribution shows that statins may be part of the solution. In any case, the new study shows that obese people who took statins had, on average, a healthier gut microbiome than those who did not take statins.

"We must first state that statins do not cause people to lose weight, but they can help reduce the risk of developing some of the comorbidities that can be associated with obesity, such as atherosclerosis in both the brain and heart. And then our new results suggest that statins can improve the gut bacterial ecosystem among some obese people," explains a leading researcher behind the study, Oluf Borbye Pedersen, Professor and Principal Investigator at the Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen.

The research results have been published in *Nature*.

200 million people take statins daily

Researchers estimate that about 200 million people worldwide take statins daily to lower their blood cholesterol levels, especially the "dangerous" LDL cholesterol.

High levels of LDL in the blood are associated with various atherosclerotic diseases and the development of blood clots.

"Statins are a success story, and one thing we wanted to find out in our research was how statins affect the gut microbiome," says Oluf Borbye Pedersen.

Unhealthy gut microbiome is associated with many diseases

The researchers in the EU-funded MetaCardis consortium examined the composition and function of gut bacteria among 900 people from Denmark, Germany and France.

Instead of identifying all the hundreds of species of bacteria individually and examining the relationships between them, the researchers classified them into four enterotypes based on the bacterial ecosystem in the gut.

Previous research has shown that the enterotype called Bacteroides2 (Bact2) is known to be present in the intestines of some people with many chronic diseases and especially the inflammatory bowel diseases ulcerative colitis and Crohn's disease.

Bact2 is low in health-promoting bacteria.

"We have also previously seen that Bact2 is abundant in the intestines of some obese people," explains Oluf Borbye Pedersen.

Some obese people have too many unhealthy gut bacteria

The researchers analysed the gut microbiome of the participants in the trans-European study using sequencing of gut bacterial DNA. Among lean or overweight participants with a body mass index (BMI) less than 30, 4% had the harmful Bact2 enterotype in their gut.

The researchers examined the bacterial gut microbiome of obese participants with a BMI over 30 and found that 18% had the Bact2 enterotype.
However, only 6% of the obese participants who took statins to lower their cholesterol levels had the Bact2 enterotype.

A large study with 2,345 people in Belgium subsequently confirmed the result.

"This indicates that statins may help to bring the composition and function of the gut bacteria of some obese people into better balance, since they have many unhealthy gut bacteria and lack several beneficial bacteria. This is one of the first times that we have seen that a frequently used drug can positively affect the gut microbiome," says Oluf Borbye Pedersen.

Statins also reduce inflammation

Oluf Borbye Pedersen tells that statins are also known to have other positive effects on health. They lower the level of C-reactive protein in the blood, a marker of inflammation.

Inflammation results from an overactive immune system, and moderate inflammation is present in many chronic conditions, including obesity, cardiovascular diseases, arthritis and psoriasis.

C-reactive protein in the blood is elevated among people with a relatively high abundance of Bact2 in their gut, but C-reactive protein levels decrease among obese people who take statins. Oluf Borbye Pedersen says that a healthier microbial community may be one reason.

"When people take statins, the quantity of Bact2 in the gut falls, causing inflammation to decline as well. Some of the bacteria in the Bact2 enterotype have the potential for triggering inflammation. Whether they do so in real life still remains to be tested in proper and comprehensive controlled clinical trials over a long time frame. But experiments with rats suggest that statins affect the microbial community," says Oluf Borbye Pedersen.

Oluf Borbye Pedersen is excited about upcoming placebo-controlled clinical trials to clarify whether statins reduce atherosclerosis by not only lowering cholesterol but also by helping to promote enterotypes of health-promoting gut bacteria that reduce inflammation in the blood vessels and throughout the body.

"That is the real question to address, but we have to wait a few years to get the answer," he adds.
"Statin therapy is associated with lower prevalence of gut microbiota dysbiosis" has been published in Nature. Several authors are employed at the Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen.

"Statin therapy is associated with lower prevalence of gut microbiota dysbiosis" has been published in Nature. Several authors are employed at the Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen.

Type 2 diabetes ***disrupts people's*** circadian rhythms

Breakdown in communication between mitochondria and the body's biological clock may be why the cells of people with type 2 diabetes are not in synch with a normally functioning circadian rhythm.

BY KRISTIAN SJØGREN

Researchers have known for many years that circadian rhythm is clearly linked with metabolic health. The risk of developing obesity and type 2 diabetes increases if our lives are not in accordance with our circadian rhythm, such as people who work at night or have severe sleep disorders.

So far, the link between circadian rhythm and the metabolic health of people with type 2 diabetes has been less thoroughly studied.

However, a new study reveals that the dysfunctional metabolism among people with type 2 diabetes seems to be linked to a breakdown in the communication between the molecular clock that controls the circadian rhythm and the mitochondria that produce energy for the cells.

"The discovery may have implications for the treatment of type 2 diabetes including the time of day people exercise, eat or take medicine," explains an author behind the study, Juleen R. Zierath, Professor, Karolinska Institutet, Stockholm, Sweden and Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen.

The research has been published in *Science Advances*.

Genes control the circadian rhythm

Virtually all cells have a circadian rhythm that regulates various processes over a period of about 24 hours. This daily rhythm regulates the levels of various gene expressions, hormones and signalling molecules that affect all aspects of life, including metabolism, blood pressure, sleep and liver function.

A complex network of genes, proteins and molecules ensures that this circadian rhythm is constantly under relatively tight control. Several genes and associated proteins are switched on during the day, and others are switched off.

The three core-clock genes regulating the circadian rhythm are BMAL1, CLOCK and PER3, and removing them from laboratory animals renders them unable to differentiate between day and night.

Studied cells' core-clock gene expression over 54 hours

The researchers wanted to discover how the molecular-clock mechanism for regulating the circadian rhythm functions among people with type 2 diabetes and therefore obtained skeletal muscle biopsies from people with type 2 diabetes and from healthy controls.

They cultured the cells from the biopsies and examined them every 4 hours for 54 hours to determine whether the molecular-clock mechanism differed between the people with type 2 diabetes and the healthy controls.

In addition to examining the molecular-clock mechanism, the researchers also examined whether the groups differed in the regulation of 200 genes related to metabolism.

The researchers also investigated the cells' potential for oxidative capacity and oxygen metabolism to determine their functionality. The circadian rhythm regulates the cells' uptake of oxygen like all other processes in the body.

Type 2 diabetes disrupts circadian rhythm

The cells from the people with type 2 diabetes and those from the healthy controls differed significantly.

Over the 54 hours, the time of day affected fewer genes among people with type 2 diabetes, and the expression of those that were regulated was also reduced. Thus, the time of day did not have the same effect on the cell autonomous circadian rhythms of people with type 2 diabetes versus the healthy controls.

Similarly, the individuals with type 2 diabetes had reduced circadian rhythm–regulated oxidative metabolism of cells and thereby reduced muscle function.

"The oscillations of core-clock genes were reduced, and fewer genes were involved in the circadian

rhythm of people with type 2 diabetes. We are the first to show this difference in the circadian rhythm of people with type 2 diabetes and healthy controls," says Juleen R. Zierath.

Miscommunication is the problem

The researchers then took new biopsies from people with type 2 diabetes and from healthy controls and examined whether the circadian control of gene expression and metabolism is altered at the cellular level in skeletal muscle.

Using similar methods, the researchers discovered that the gene expression in the mitochondria differed between people with and without type 2 diabetes.

Specifically, the researchers found that the degree of binding of the CLOCK and BMAL1 genes to mitochondrial genes was positively associated with insulin sensitivity and thus stronger among healthy controls than among people with type 2 diabetes.

Juleen R. Zierath explains that this finding indicates that healthy metabolism requires well-functioning communication between the mitochondrial genes and the core-clock genes.

Medicine may need to be adjusted to the circadian rhythm

Juleen R. Zierath says that the discovery of a clear link between type 2 diabetes, metabolism, mitochondria and the circadian rhythm may have clinical implications.

The research suggests that the time of day may influence how effective type 2 diabetes treatment is, including the timing of sleep, meals, physical activity and taking medicine.

"Some of the most common treatments for people with type 2 diabetes affect the mitochondria. The same applies to exercise and diet, which means that the effect can differ depending on the time of day. More and more researchers are starting to incorporate chronomedicine, ensuring that medicine is administered at the right time of day to maximise the effect in synch with the cell autonomous circadian rhythm. This will be the next interesting step to take in our research," concludes Juleen R. Zierath.

"Disrupted circadian oscillations in type 2 diabetes are linked to altered rhythmic mitochondrial metabolism in skeletal muscle" has been published in Science Advances. Several authors are employed at the Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen.

Global pandemics – how do we prepare for the next one?

BY FRANK MØLLER AARESTRUP

Already now during the current COVID-19 pandemic, we should consider how we might use current experiences for the many new pandemics we will certainly experience in the years to come.

It might be provocative to write, but I have been struck with the fact that, even for a pathogen with very simple epidemiology, basically just human-to-human transmission, we have still struggled to get a global overview of the COVID-19 pandemic and to implement targeted control measures. Classical control measures such as good hygiene and physical and social distancing have been the key measures implemented.

I am also struck how we, while focusing on a single pathogen, have basically ignored that many other infectious diseases are also (if not more) important.

However, while many things certainly will become clearer in the coming years, I think several points we hopefully have learned from this wake-up call should be taken forward in preparing for the next pandemics as well as the continual control of endemic and epidemic diseases.

Utilizing the full potential of open data sharing and sequencing

During the current pandemic, we have seen an unprecedented rapid sharing of information. However, it has also become increasingly clear that we lack a common format for identifying cases and especially sharing sequencing information. Most sequencing information is not shared, and it is mainly assembled sequences and not raw data. Further, data have been shared on platforms that are not created to cover all infectious diseases and are mainly accessible to the somewhat closed virus expert communities.

We need a continuous agnostic global surveillance system that includes endemic diseases

Basing continuous surveillance on human clinical cases can be very biased over time. This is evident for COVID-19 but, perhaps more important, during the past year, many countries have given lower priority to clinical surveillance for many or all other pathogens. Further, most clinical surveillance focuses on already known pathogens, and we miss out on all novel events.

In my opinion, some type of sampling that represents the human microbiome (bacteriome and virome) needs to be collected in a comparable way over time and between countries. This material should be analysed in a way that would enable immediate reanalysis if new diseases emerge.

For me, human sewage or waste from long-distance airplanes and metagenomic analyses using next-generation sequencing seem to be the obvious choices. We are already exploring this (www.globalsurveillance.eu) and would very much like to see this continued in a long-term sustainable way. Sampling sewage relieves scientists of ethical and General Data Protection Regulation concerns because the sample is already anonymized.

Everybody wanted a piece of the pie – public health systems wanted control

When disruptive events happen, people with great ideas quite logically come forward and provide novel solutions. These innovative people need funding and, of course, access to data to develop their solutions. There have, however, been examples in which public health institutions and governments have seemed to give priority

to keeping the data under control rather than enabling other scientists to scrutinize and explore the data. This, of course, allows people to keep control, but as a society we are also missing the opportunity for novel input.

There can also be problems with giving everybody access to data. Suddenly everybody is an expert, and distinguishing between those who just want money and those who actually provide novel insight can be difficult.

We need to support neutral data-sharing platforms

Currently, most COVID-19 data, whether sequencing data or other types of data, are kept locally at individual computer servers, and I am unfortunately convinced that we will see major issues regarding patchy individual analysis and problems with comparing studies and data between countries.

The world does in fact already have an established, global, neutral data-sharing platform in place: www.insdc.org. This resource is unfortunately not fully utilized. As also seen during COVID-19, many of the sequencing data, especially the early data, were shared in an assembled format through GISAID, which might be good for a closed viral community but is not a platform supporting other infectious disease agents.

Rather than (re-)inventing different local or pathogen-specific and temporal solutions, I think we should focus on improving the existing platforms.

A nonexhaustive list of potential remedies for improvement

There is and should be a multitude of solutions for controlling infectious diseases and improving global public health. I do not have all the answers, but I do believe it is paramount to allow global creativity and thus provide systems that are, as much as possible, precompetitive and enable the involvement of as many people as possible and as many ideas as possible.

We need improved global capacity and possibilities for identifying infectious diseases

It is vital that if we want global data in the future, including those from low- and middle-income countries, then we also need to ensure that scientists in these countries have the capacity to analyse their own data and access to publish them before sharing them publicly.

Through my own research group, we have participated in initiatives and currently provide web-based analytical tools for next-generation sequencing data for bacterial species free of charge, including identification of species, subtypes, clonality and antimicrobial resistance (www.cge.cbs.dtu.dk/services).

We need to focus public resources where there is a limited private business plan

Public funding cannot pay for everything. Private industry also has a tremendous amount to offer. I find it optimal if public money and initiatives based at public research institutions and universities are those in which commercial potential is limited, and then the activities in which a profit can be made are left to private industry. Natural basic research should still take place in academia, but during a pandemic, it would perhaps have been wise to leave the development of diagnostic tests, vaccines and treatments to the primary experts: the pharmaceutical industry, which naturally also needs open access to all the data.

I personally hope that researchers, public health workers, industry, politicians, funders and all others globally will take the best lessons forward and become much better at understanding and controlling infectious diseases, both those we face every day and novel ones that will emerge.

Since we cannot predict what the next threat is going to be and we need to ensure that as many researchers as possible can rapidly come forward with novel ideas and solutions globally, I think we need to focus on some precompetitive open science solutions, such as those mentioned above, including much easier access for inexperienced scientists to perform easy analysis, especially if we want to engage with low- and middle-income countries. Doing so will save lives, time and money.

In 2016, the Novo Nordisk Foundation awarded a Challenge Programme grant of DKK 60 million to Frank Møller Aarestrup for the project Global Surveillance of Antimicrobial Resistance.

chapter

THE COVID-19 PANDEMIC

ARTICLES

Test to reveal how ***COVID-19 vaccination*** affects each person's immune system

While we wait for the vaccines that can help us escape the clutches of the COVID-19 pandemic and its resulting lockdowns and millions of deaths, a debate is in full swing worldwide. Are vaccines dangerous? Do they work? Should I get vaccinated? A new antibody test shows what types of antibodies people with COVID-19 develop, and the researchers linked this with their severity of illness. The test may also reveal which antibodies a vaccine creates in each individual and thus also whether the vaccine works or not.

BY MORTEN BUSCH

AThe scourge of the COVID-19 pandemic will not be eliminated until most of the world's population develops antibodies against SARS-CoV-2. Herd immunity can be achieved if enough people have the right antibodies at the same time, because viruses need a susceptible host. Whether you have been infected with SARS-CoV-2 or you get vaccinated, each person's immune system will develop slightly different types of antibodies and thus different ways of combatting COVID-19. A new test can reveal the types of antibodies each person has developed.

"Most existing antibody tests tell you whether you have developed antibodies but not what type. This is important knowledge, since the type of antibodies is crucial to determining whether your immune system can defend you against new virus attacks the next time. By examining antibodies among 350 people who got COVID-19 in the spring, we found that the type and number of antibodies are associated with the severity of disease. The new test enables us to test people who have had COVID-19 or have been vaccinated and is an important step in understanding how we become immune to SARS-CoV-2," explains Peter Garred, Clinical Professor, Diagnostic Center, Rigshospitalet and Department of Clinical Medicine, University of Copenhagen.

The most severely ill people appear to be better protected

The new antibody test further refines the test the researchers from Rigshospitalet in Copenhagen developed during the first wave of the pandemic. The sandwich ELISA test tracked transmission well by determining whether a person has had COVID-19 or not. The new, more advanced test is called a direct ELISA-based assay.

"The new test enables us to distinguish which of the immunoglobulin types IgM, IgA and IgG are present in the bloodstream. Different immunoglobulins provide different kinds of immunity, so while the sandwich ELISA test is ideal for tracking transmission in the community, this direct test can be used to examine the types of antibodies in the blood over time," explains Peter Garred.

The immunoglobulins are small proteins the immune system uses to recognize

microorganisms. When a person has been attacked by a bacterium or a virus or has been given a vaccine, the immune system stores information so that it can react quickly the next time the person is exposed. This means that the person has become immune.

"There are five types of immunoglobulins, and the new test can measure the three most important ones. We can measure IgM, which the immune system uses in immediately responding to external threats; IgA, which is most important on the body's surfaces and mucous membranes, where it combats intruders to prevent them from entering; and then IgG, which is present in blood serum, where it can recognize and detect threats that have entered the body," says Peter Garred.

The researchers examined 350 people who had COVID-19. They then used the new test to measure the quantities of the various types of antibodies 11 weeks after infection and determined whether the severity of COVID-19 was associated with the antibodies developed.

"The study showed that the IgM, IgA and IgG response to SARS-CoV-2 is significantly correlated with the severity of the disease. The more ill you get, the greater the antibody response, and those who had a more severe illness trajectory had especially more IgG," explains Peter Garred.

Testing whether a vaccine works

Since IgG typically stays in the body longer than IgM, the results suggest that people who have had a severe illness trajectory will also have greater protection over a longer period of time, but the researchers cannot be certain yet.

"Based on our test set-up, we have also developed a neutralization assay that specifically measures the extent to which the antibodies in the blood of each person can neutralize SARS-CoV-2. Once this is fully tested, we can then use it to measure whether a person is protected against COVID-19 in the future," says Peter Garred.

The new type of assay may also prove to be a very effective way of testing the upcoming rollout of vaccines, because each person's immune system will also react differently to a vaccine similarly to how it reacts to SARS-CoV-2 infection.

"This means that we can investigate whether vaccines against SARS-CoV-2 actually create a protective antibody response and measure how this changes over time. All this will indicate how strong each person's immunity is, so you know whether you can feel safe, and if the result is not positive, you know that you are not immune and may need another type of vaccine," concludes Peter Garred.

"SARS-CoV-2 antibody responses are correlated to disease severity in COVID-19 convalescent individuals" has been published in the Journal of Immunology. The new antibody test was developed in a collaboration between Rigshospitalet, the University of Copenhagen and Novo Nordisk A/S, with support from the Carlsberg Foundation. In 2020, the Novo Nordisk Foundation awarded a grant of DKK 4,802,750 to Peter Garred for the Copenhagen SARS-CoV-2 Antibody Testing Initiative.

COVID-19 does not affect ***pregnant mothers*** or their newborns

Many pregnant women who have contracted COVID-19 have naturally been concerned about how it would affect the risk of complications to them and their child during pregnancy or childbirth.

BY KRISTIAN SJØGREN

Since scarcely anyone had heard of COVID-19 until early 2020, physicians could not provide pregnant mothers with any reassuring answers because this had never been investigated – until now.
In two studies, researchers and physicians in Denmark showed that COVID-19 in the first trimester does not appear to increase the risk of pregnancy loss. The researchers and physicians also showed that COVID-19 does not affect the risk of complications at other times in pregnancy or associated with childbirth.

"This is a very positive conclusion, with the caveat that women of childbearing age in Denmark are generally quite healthy and that all pregnant women in Denmark have access to the relevant healthcare services and therefore do not have a pre-existing high risk of complications in connection with COVID-19. In addition, Denmark had a very low incidence of COVID-19 in the first wave. In other parts of the world, with massive COVID-19 epidemics and very different levels of healthcare services, COVID-19 increases the risk of caesarean section. We did not find this among the women in this study," explains Henriette Svarre Nielsen, Clinical Professor and specialist physician, Department of Obstetrics and Gynaecology, Copenhagen University Hospital Hvidovre, Denmark.

The research has been published in *Obstetrics and Gynecology and Human Reproduction*.

Rapidly initiated studies
When COVID-19 struck Denmark and the government and foundations rapidly awarded grants for research projects that could elucidate how COVID-19 affects both health and society, Henriette Svarre Nielsen and her colleagues rapidly applied for funding for a project to identify how COVID-19 affects pregnant women and their children.

The researchers tested for antibodies to SARS-CoV-2 in blood samples from pregnant women taken in connection with a nuchal translucency scan of their fetus at the end of the first trimester. The researchers examined blood samples from 1,019 first-trimester women and 36 women with a first-trimester pregnancy loss before the scan. In addition, the researchers examined 1,019 women at the follow-up scan in the second trimester and 1,313 women who gave birth in April–June 2020. The researchers also took blood samples from 1,188 of the women's partners at birth, and 1,206 newborns were included.

COVID-19 does not increase the risk of pregnancy loss
The researchers found that 2.6% of the women giving birth and 3.5% of their partners had COVID-19 in the latter stages of pregnancy. Of the first-trimester women, 53% with COVID-19 were asymptomatic versus 26% of the women who tested negative.

The results from the first trimester showed that having COVID-19 was not associated with the result from the fetal nuchal translucency scan or the risk of pregnancy loss in the first trimester.

Only one woman with antibodies to SARS-CoV-2 had a pregnancy loss. The physicians also obtained data from 36 women with a first-trimester pregnancy loss, but none had COVID-19.

"Other infectious diseases may negatively affect both the mother and the fetus, such as measles or severe flu, but COVID-19 does not appear to have the same harmful effect on the fetus. The conclusion of this part of the study is that COVID-19 does not increase the risk of harming the fetus during the first trimester," says Henriette Svarre Nielsen.

No additional complications among pregnant women with COVID-19
In the second part of the study, the researchers aimed to determine whether COVID-19 negatively affected obstetric complications, including the risk of caesarean section or complications in the later stages of pregnancy.

Here, too, the researchers found no difference in the risk of caesarean section, premature birth, preeclampsia, placental abruption or the baby's Apgar score, birthweight, umbilical cord pH, need for breathing support and admission to the neonatal ward.

Conversely, they found that, if the mother had COVID-19, the baby also often had antibodies to SARS-CoV-19 and was therefore probably protected against it.

"The result is not surprising, but confirming it is good, since other infectious diseases affect both the pregnant woman and the fetus. This applies to Middle East respiratory syndrome, severe acute respiratory syndrome and other diseases. Therefore, this cannot be ruled out until it has been investigated," explains Henriette Svarre Nielsen.

More results on the way
Henriette Svarre Nielsen explains that the first published results from the study are just part of the research into the possible links between COVID-19 and pregnancy.

The physicians included 5,400 active participants and 35,000 samples stored in the Copenhagen Hospital Biobank. These include samples taken in the second and third trimesters and placenta biopsies and breast-milk, which the researchers will examine.

The researchers also plan to determine how COVID-19 affects the immune systems of both the pregnant women and the newborns.

"We are conducting numerous in-depth investigations, and these are just the first results," says Henriette Svarre Nielsen.

"Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibodies at delivery in women, partners, and newborns" has been published in Obstetrics & Gynecology. "SARS-CoV-2 in first trimester pregnancy: a cohort study" has been published in Human Reproduction. Employees of the Novo Nordisk Foundation Center for Protein Research participated in the studies. In 2016, the Novo Nordisk Foundation awarded a Novo Nordisk Foundation Young Investigator Grant to co-author Eva Hoffmann.

Corona crisis stresses people with ***mental health*** problems

According to researchers, lack of contact with mental health services and relatives can stress people with mental illness so much that they end up taking their own lives. The researchers also indicate several areas in which governments should intervene to ensure that people with problems can get help.

BY KRISTIAN SJØGREN

Forty-four researchers from around the world have teamed up to write an article in Lancet Psychiatry focusing on the many people with mental health problems, who have been stressed to the limit by the COVID-19 crisis.

Physical distancing has meant that many people with mental health disorders have no contact with family members or the mental health services, and this may worsen their mental health or well-being.
For some, this may mean being stressed to the limit and ending up taking their own lives.

"The restrictions we all currently experience can have major consequences for people with mental illness. This therefore places additional demands on the mental health services, which must be especially vigilant that we do not let any of these people fall through the net as a result of the changes during the current situation," says a contributor to the article, Merete Nordentoft, Clinical Professor, Department of Clinical Medicine, University of Copenhagen.

Previous public health crises have increased suicide rates

Although the article in *Lancet Psychiatry* is not, as such, a scientific article, the researchers present some data that illustrate why the problem should be taken seriously.

Evidence indicates that deaths by suicide increased in the United States during the 1918–1919 influenza pandemic and among older people in Hong Kong during the 2003 severe acute respiratory syndrome (SARS) epidemic. Further, there is a well-known association between having a mental disorder and worsening of the disorder when it is not managed professionally. For example, if left untreated, someone with anxiety might experience worsening symptoms and find these harder to get rid of again. The pattern can then become established.

"Within my field, psychosis, the duration without treatment clearly indicates the outcome for patients in the long term," says Merete Nordentoft.

Social isolation makes life difficult for many

The social isolation associated with the COVID-19 crisis causes a whole host of unfortunate effects that the mental health services should manage both now and also after the social restrictions are eased, which hopefully will happen soon.

- People with a mental disorder often need help to take care of themselves. Although a phone call or videoconferencing suits many people who usually talk face to face with a therapist, not all feel comfortable having a potentially helpful conversation over the phone or can master the technology required to use a computer videoconferencing program.

- Unemployment can have adverse social effects for people whose everyday lives depend on having a purpose for getting out of bed every day.

- It is well known that many people drink more alcohol when they lose their job, and alcohol abuse brings its very own palette of problems related to mental health, social networks and social capital.
- For some people, the opportunity to be together with their partner 24 hours a day for 2 months is a blessing, but others find themselves trapped in a domestic violence prison.

"We face a major challenge in helping those who have been hard hit by the loss of social contact or contact with the mental health services. In addition, we have not always had the opportunity to monitor people and see how they are really coping," says Merete Nordentoft.

Vulnerable people can become superspreaders of coronavirus

Merete Nordentoft explains that positive experiences can also be harvested in the current crisis.

Many therapists have found that some conversations that previously required the therapist and patient to be in the same room can be managed through videoconferencing with the same positive effects.

However, many meetings cannot be managed over the phone, and many patients still need physical meetings and home visits and will need these in the future.

A completely independent perspective of not taking care of people with mental illness is that some may turn into superspreaders of coronavirus, since regular handwashing and physical distancing may be far down the list of priorities in a stressful daily life.

"Vulnerable people often live together in limited space, and following all the rules and guidelines issued by governments to reduce transmission can be difficult," explains Merete Nordentoft.

Everyone needs to help to minimize suicide

In the article, the researchers indicate that the current situation can affect vulnerable people in eight areas and therefore propose interventions governments should consider resourcing in their efforts to avoid increasing numbers of suicides. The eight areas are mental illness, attempted suicide, financial stressors, domestic violence, alcohol consumption, isolation, access to means and media.

"We should remember that these are unprecedented times, so we are all in the dark. But here we have come together and written about our hopes and fears and how to act to make the lives of vulnerable people better than they might otherwise be," says Merete Nordentoft.

"Suicide risk and prevention during the COVID-19 pandemic" has been published in Lancet Psychiatry. In 2020, the Novo Nordisk Foundation awarded the 2020 Novo Nordisk Prize to Merete Nordentoft, Clinical Professor, University of Copenhagen and Preben Bo Mortensen, Professor and Scientific Director, iPSYCH, Aarhus University.

Where both herpes and COVID-19 ***attack the body***

Researchers have identified the part of the immune system that both the herpes simplex virus and the COVID-19 virus need to neutralize to infect the body. In the long term, this discovery may help to prepare us for the pandemics of the future.

BY KRISTIAN SJØGREN

To infect the brain, the herpes simplex virus (HSV) must first neutralize a key part of the body's immune system. Otherwise, the virus will be eliminated immediately.

Researchers from Aarhus University and other universities recently identified the part of the immune system that HSV attacks.

The virus that causes COVID-19 (SARS-CoV-2) and probably many other viruses attack the same part of the immune system, so if researchers can develop drugs that remove this primary weapon from the arsenal that viruses use, we may be better equipped to fight potentially all viruses and pandemics in the future.

"Viruses, including influenza, HSV and SARS-CoV-2, attack the body's cells based on some general principles. Viruses need to inhibit the immune system to avoid being eliminated, and we have identified the location in the immune system that HSV attacks. Because this is also the location that SARS-CoV-2 attacks, this may be a very general strategy that may be interesting to examine in relation to developing drugs," explains a researcher behind the new study, Søren Riis Paludan, Professor, Department of Biomedicine, Aarhus University.

The research has been published in the *Journal of Experimental Medicine*.

Neutralizes part of the immune system

The immune system comprises two components.

- Innate immunity responds immediately to external threats from viruses and other threats.
- Adaptive (acquired) immunity needs to identify the threat first.

Any virus must first bypass the innate immune system to have any chance of infecting a person.

In previous studies, researchers confirmed that the part of the innate immune system that deals with virus infections is called cGAS-STING (cyclic GMP-AMP synthase–stimulator of interferon genes), which could loosely be described as a cell police officer.

cGAS-STING works by detecting the presence of cytosolic DNA in our cells. The DNA of the cells is usually isolated in the cell nucleus and, if there is DNA outside the cell nucleus, this may be a sign that a virus has penetrated the cell.

cGAS-STING localizes the cytosolic DNA and initiates an immune response, which is the function the virus must neutralize.

"A virus cannot establish an infection if it does not block the innate immune system rapidly enough, and in this study we investigated exactly how HSV does this," says Søren Riis Paludan.

Together with colleagues, Søren Riis Paludan had previously established this role of cGAS-STING in connection with an HSV infection, but the immune system's police officer has the same function in all other viral infections.

Virus deactivates the cellular immune response

The researchers investigated HSV in cell cultures and in mouse brains.

HSV is best known as the virus that causes cold sores, but in rare cases it can also infect the brain.

The researchers hypothesized that, since cGAS-STING is so important in relation to the opportunities a virus has for infecting a cell, viruses will also have developed weapons targeting cGAS-STING – and the researchers verified this in the study.

First, the researchers created many HSV mutants with one or more genes knocked out to determine which genes play a role in the virus' ability to bypass cGAS-STING.

They found that a mutant lacking the deubiquitinase activity of the VP1-2 protein activated the cell's immune system to a much greater extent, which indicated that the mutant's primary weapon had been destroyed.

"Deubiquitination works by removing ubiquitins from proteins, and in the case of cGAS-STING, the ubiquitins help to activate the immune system's police officers," explains Søren Riis Paludan.

Mechanism also applies to people's brain cells

Further experiments with mice showed that the mutant HSV were also unable to infect mice.

A final experiment with mice that had the cGAS-STING removed showed that, once that part of the immune system was neutralized, even the viruses that had had their deubiquitinase destroyed could infect the mice.

Finally, the researchers carried out an experiment with cultured immune cells from human brains, and here too they found that infection with deubiquitinase-free HSV resulted in a very strong immune response.

"The primary result is that we have found the part of the immune system that HSV needs to neutralize to infect the brain – and we have also figured out how this takes place," says Søren Riis Paludan.

Can be a universal therapeutic target

This new discovery has several perspectives.

Søren Riis Paludan indicates that both HSV and SARS-CoV-2 block cGAS-STING when they infect the body.

This shows that the attack mechanism may apply to a wide variety of viruses and is therefore a good place to start in creating medicine that can protect against more than just HSV or influenza viruses.

Further, the interaction between viruses and cGAS-STING has never been a therapeutic target.

Any medicine may potentially have two targets, but Søren Riis Paludan prefers one.

"The easiest approach would be to boost cGAS-STING, but this is too dangerous since it can activate inflammation throughout the body. The other option is to make medicine that targets the deubiquitinase that the virus uses to neutralize cGAS-STING. Fortunately, this is an enzyme, and we generally like to develop medicine against enzymes. Quite simply, one idea is to discover viral targets that are universally used to block the immune system. We may be able to benefit from these in combating future pandemics," says Søren Riis Paludan.

"HSV1 VP1-2 deubiquitinates STING to block type I interferon expression and promote brain infection" has been published in the Journal of Experimental Medicine. In 2018, the Novo Nordisk Foundation awarded a grant to Søren Riis Paludan for the project Novel Mechanisms of Early Defense against Virus Infections.

What we can learn about COVID-19 from ***autoimmune diseases***

Decades of research on autoimmune diseases may help to determine who has the highest risk of severe illness or death from COVID-19.

BY KRISTIAN SJØGREN

If two people who seem similar in all respects become infected with SARS-CoV-2, one may have no symptoms and the other may end up in the hospital with a serious lung infection.
COVID-19 has caused researchers around the world to scratch their heads because they do not fully understand why some people who seem completely healthy experience severe illness and others do not.

Now an international team of researchers has suggested that experience with autoimmune diseases may improve understanding of why people respond differently to COVID-19.
Decades of research on autoimmune diseases have provided a good sense of how the immune system works, and this might be used to determine whether people who have autoimmune diseases or are predisposed to developing them are more or less susceptible to COVID-19 and whether this knowledge can be used to treat them more effectively.

"The immune system goes haywire in both autoimmune diseases and COVID-19, and this response causes the symptoms and determines the severity of illness. We therefore believe that we can learn a lot about the differences in how the body responds to COVID-19 by examining what we already know about autoimmune diseases," explains a researcher behind a perspective article published in *Frontiers in Immunology*, Tugce Karaderi, Assistant Professor, Center for Health Data Science, University of Copenhagen.

COVID-19 and autoimmune diseases have many similarities

According to Tugce Karaderi and her colleagues, we should use our knowledge on autoimmune diseases to understand COVID-19 because of the similarities between these otherwise very different diseases.

COVID-19 is caused by the SARS coronavirus 2, whereas autoimmune diseases are caused by the body's immune system considering the body's own cells and organs to be foreign matter that needs to be eliminated.

Nevertheless, these diseases have some similarities.

The symptoms of autoimmune diseases result from an overactive immune response.

The same applies to COVID-19. The severe cases do not result from the infection itself but because the immune system creates a cytokine storm, producing many more immune cells than necessary.

The immune system thus attacks both the virus and the body.

"This similarity between autoimmune diseases and COVID-19 is interesting and supports the view that we can improve our understanding of why different individuals respond differently to COVID-19 by applying the knowledge we have gathered on autoimmune diseases over the years," says Tugce Karaderi.

Gene variants determine the risk of severe disease

Specifically, Tugce Karaderi and her colleagues focus on whether some of the same biological mechanisms apply when the immune system goes haywire and does more harm to the body than good.

They suggest that examining the biological or the genetic risk factors for developing autoimmune diseases might also enable some of these to be identified as being risk factors for severe illness or death from COVID-19.

In their article, the researchers also suggest several genes worth investigating to identify the biological link between autoimmune diseases and the immune response to SARS-CoV-2 that could potentially be used as a key to understand why

individuals respond differently to SARS-CoV-2 infection. These include toll-like receptor 7 (TLR7), a known pathogen-sensing receptor that has been shown to play a role in autoimmune diseases and is also among the initial innate immune cell receptors that sense SARS-CoV-2.

"We know of two sets of brothers from different families who had their own variant of TLR7 and all developed COVID-19. Young people rarely get severely ill, but these four brothers did," explains Tugce Karaderi.

conditions such as arthritis and that has also been shown to have a good effect in reducing mortality among people with severe or critical COVID-19 requiring ventilation or respiratory support.

"Individuals with autoimmune diseases are treated with various drugs that suppress the immune system so that it does not overreact inappropriately. There has been some discussion about whether suppressing the immune system can increase the risk of severe COVID-19 or

"We already have access to a huge quantity of data, which is waiting to be investigated to improve our understanding of COVID-19, and the link between susceptibility to autoimmune diseases and severity of COVID-19 progression," concludes Tugce Karaderi.

Drugs for autoimmune diseases may be effective against COVID-19.

In addition, greater insight into the mechanisms by which some people become severely ill from COVID-19 can also be used to identify additional targets for treatment and drug repurposing.

An example is dexamethasone, an anti-inflammatory drug used to treat autoimmune

whether these drugs keep the immune system of individuals with COVID-19 from going haywire," says Tugce Karaderi.

She elaborates that trials are already underway to investigate the effectiveness of using these drugs normally used to treat individuals with autoimmune diseases to treat the individuals with COVID-19 who do not necessarily have an autoimmune disease.

"Host genetics at the intersection of autoimmunity and COVID-19: a potential key for heterogeneous COVID-19 severity" has been published in Frontiers in Immunology. In 2020, the Novo Nordisk Foundation awarded an Emerging Investigator grant to Tugce Karaderi for the project "Data Science Approaches to Study Epidemiological and Genetic Underpinnings of Hypothyroidism to Pave the Way for Precision Medicine."

SARS-CoV-2 can be detected in the lower respiratory tract for 3 weeks – even if symptoms are mild

COVID-19 will be around for a while, and SARS-CoV-2 also lingers in the bodies of the people with COVID-19. A major study shows that traces of the virus can be detected for weeks in the upper respiratory tract and a whole week longer in the lower respiratory tract and in faeces. This is crucial knowledge, both for developing new drugs and for the researchers behind the study, who are soon starting patient trials of a drug against SARS-CoV-2, which they expect to be 40 times more effective than remdesivir.

BY MORTEN BUSCH

Do I have COVID-19, will I infect other people or am I healthy? COVID-19 often leaves more questions than answers, and the battle to understand SARS-CoV-2 has often left researchers and health authorities astonished. To understand how the virus travels through the human body, a research group has systematically screened the titles of 7,226 studies and pooled the data from 22 that could paint a credible picture of where and for how long one can find traces of SARS-CoV-2 in the body after the first symptoms occur.

"The study shows that SARS-CoV-2 can be detected in both the upper and lower respiratory tracts and in faeces, regardless of how ill a person with COVID-19 becomes. SARS-CoV-2 can be detected 1 week longer in the upper respiratory tract of people who are severely ill, but in general we can detect it in the upper respiratory tract for 2 weeks and for about 1 week longer in the lower respiratory tract and faeces. We hope that this knowledge can help the many researchers who are currently trying to develop treatment, and it has been crucial for us in attempting to develop a new treatment using inhaled niclosamide," explains Morten Sommer, Professor and Scientific Director, Novo Nordisk Foundation Center for Biosustainability at the Technical University of Denmark, Kongens Lyngby and co-founder of UNION therapeutics A/S, Hellerup, Denmark.

Surprising and somewhat worrying

The background for the study was to improve the balance between the massive activity in developing drugs and the limited knowledge about how SARS-CoV-2 spreads in the body. Researchers quickly understood how the virus infects individual cells. However, during the early stages of the pandemic there were very few studies and only a handful of small clinical trials on how SARS-CoV-2 spreads through the body over time.

"This knowledge is crucial for developing models for how SARS-CoV-2 copies itself, since a mathematical model must be tested with as much real-world data as possible. However, this

knowledge is also absolutely essential in planning clinical trials evaluating the effectiveness of various treatments aimed at reducing the viral load of people with COVID-19. Knowing how long a normal infection lasts is required to assess the effectiveness of a specific drug," explains the main author, Anne Weiss, Scientific Research Associate, UNION therapeutics.

The researchers therefore systematically reviewed small studies of how the virus spread among people with COVID-19, including determining whether the duration of viral detection differed between people with mild and moderate to severe COVID-19.

"Our review showed that SARS-CoV-2 can be detected in the upper respiratory tract, lower respiratory tract and faeces, regardless of the clinical severity of COVID-19. It also showed that the lower respiratory tract has traces of virus for 5.7 days longer for people with mild symptoms and 5.9 days longer for those with moderate to severe symptoms," says Anne Weiss.

The review also showed that SARS-CoV-2 is present longer among patients with moderately severe COVID-19, both in the upper respiratory tract and in faeces. Conversely, the various areas of the lower respiratory tract did not differ in how long the virus was present between moderate or severe versus mild COVID-19. This last result was especially surprising and somewhat worrying because the testing normally uses samples from the upper respiratory tract.

"We can detect the virus for up to 3 weeks in the lower respiratory tract, but this does not indicate whether this person can be infectious for 3 weeks. However, we can detect peak levels of SARS-CoV-2 in the upper respiratory tract within the first week of infection, whereas the viral load in the lower respiratory tract and faeces peaks within the second week of infection. Unfortunately, we do not have enough data from other places in the body, but there is no consistent indication that the virus is present in the eyes or in urine," adds Anne Weiss.

Completely free of SARS-CoV-2

This new knowledge has been essential for the researchers behind the study in their upcoming clinical trials of inhaled niclosamide for people with COVID-19. UNION therapeutics has been working with niclosamide for 5 years and was conducting a Phase 2 study of the drug involving people with atopic dermatitis. In recent years, however, UNION therapeutics has also envisioned the possibility of treating people with inflammatory and infectious diseases.

"When the COVID-19 pandemic emerged, we learned that niclosamide was a very effective inhibitor of SARS-CoV-2 with potency 30–40 times better than remdesivir," explains Morten Sommer.

Niclosamide effectively blocks SARS-CoV-2 from making new copies of itself. The extensive experience and data that UNION therapeutics has already generated with the drug enabled the company to develop an novel inhaled formulation of niclosamide for treatment of COVID-19. This product has now been advanced through a battery of animal tests and a Phase 1 study in healthy volunteers with great results.

"The new systematic review has been essential knowledge for the clinical trials of the new therapy, so that the patients are examined for a long enough time, and so that, for example, they are tested to determine whether SARS-CoV-2 is no longer present in the lower respiratory tract, where the review showed that the viral load peaks later. This will ensure that the body is completely free of the virus before stopping treatment," concludes Morten Sommer.

"Spatial and temporal dynamics of SARS-CoV-2 in COVID-19 patients: a systematic review and meta-analysis" has been published in EBioMedicine. Innovation Fund Denmark awarded a grant to the researchers for their study of niclosamide. In 2018, the Novo Nordisk Foundation awarded a Challenge Programme grant to Morten Sommer, Professor, Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, for the project Design and Engineering of Biological Molecules and Systems.

High-risk group has emerged unscathed despite the ***COVID-19 pandemic***

Many people with chronic noncommunicable diseases were anxious when the COVID-19 pandemic broke out in spring 2020. Many of these people were identified as having a high risk of severe illness and potential death, including people with inflammatory bowel disease (IBD). In addition to the increased risk from IBD, doctors did not know whether the medication increased that risk further. A new major study refutes the concern about the medication and shows that the 35,000 people with IBD in Denmark have significantly lower susceptibility to developing COVID-19 than people without IBD – probably because they have taken better care of themselves.

BY MORTEN BUSCH

What do you do if your medication might put your life at risk? Millions of people around the world had to ask themselves this question when COVID-19 changed the world we knew. People with IBD, such as Crohn's disease, take immunosuppressive drugs, and these were feared to make these people more susceptible to becoming infected with SARS-CoV-2. A new study shows no reason for concern.

"We found that significantly fewer people with IBD have been infected with and died from SARS-CoV-2. Although we know today that immunosuppressive drugs such as corticosteroids alleviate the symptoms among people already ill with COVID-19, we are very certain that the reason that people with IBD have a lower infection rate is that they have taken better care of themselves than others. Our latest figures suggest that the increased anxiety and stress these people experienced over the past 6 months because they feared an increased risk of COVID-19 infection because of their medication has been unfounded," explains Johan Burisch, a PhD and doctor at the Copenhagen Center for Inflammatory Bowel Disease in Children, Adolescents and Adults, University of Copenhagen, Hvidovre Hospital, Denmark.

No concern about IBD medication

People with immune-mediated inflammatory diseases such as IBD are especially interesting during the COVID-19 pandemic, since many receive immunosuppressive therapy, which increases the risk of infection.

"When the pandemic erupted, we were swamped by worried people who wanted to know what to do and whether to continue taking their medication. Similar to many other relevant questions during the pandemic, we had a very hard time giving a definite answer because there were no studies," says Johan Burisch.

The new investigation is the first population-based study to examine the risk of developing COVID-19 and its illness trajectory among people with IBD. The researchers investigated the general population tested for COVID-19 to determine the prevalence among people with and without IBD but also among people with other immune-mediated inflammatory diseases.

"People with IBD, including Crohn's disease and ulcerative colitis, secrete increased concentrations

of angiotensin-converting enzyme 2 receptor, which potentially enables SARS-CoV-2 to be transmitted. We had also feared that the immunosuppressive drugs would cause many more of these people to develop COVID-19, but the evidence did not suggest this," explains Johan Burisch.

Only 2.5% of the people with IBD tested positive for COVID-19 versus 3.2% for the people with immune-mediated inflammatory diseases and 3.7% for the general population.

"The evidence indicates that people with IBD do not have a significantly higher infection rate and that they should not be concerned about taking their usual medication," says Johan Burisch.

Since recent research has shown that corticosteroids can actually help people who develop COVID-19 survive, these immunosuppressive drugs may reduce the incidence of COVID-19, but the researchers do not have enough evidence to conclude this.

"We found no evidence that immunosuppressive drugs have benefits in the early stages of COVID-19, and previous studies show that adrenocorticotropic hormone especially increases the risk of infection. However, we assume instead that people with immune-mediated inflammatory diseases have been tested more often and probably also take better care of themselves," explains Johan Burisch.

No reason for greater concern

In addition to investigating the percentage of people developing COVID-19, the researchers also studied the people with IBD who developed COVID-19 to examine their illness trajectory, since people with noncommunicable diseases were identified early as having greater risk for severe illness.

"Our study found no evidence to suggest that immunosuppressive therapy or IBD affects the actual trajectory of COVID-19, either the length of illness or the incidence of severe COVID-19. Among people with IBD who developed COVID-19, 20% needed hospitalization and 5% needed intensive care," says Johan Burisch.

According to the researchers, the good news is that neither COVID-19 nor the medication should give rise to additional concern among people with IBD. The study asked people being treated for IBD to report the level of stress they experienced since the COVID-19 pandemic erupted and found that they have considerably greater risk of severe stress.

"Our patients were constantly seeking advice on COVID-19 from reliable sources. They have experienced considerable stress from seeking information that did not exist and thus from not knowing whether to take their medication and whether they had greater risk of developing COVID-19 and dying from it. Actively reaching out to the people with IBD is therefore extremely important, even those who do not have COVID-19 symptoms, so that they can get the right information, advice and guidelines and know that they do not have to worry more than anyone else," concludes Johan Burisch.

The *heart is key* to finding COVID patients at greatest risk

BY MORTEN BUSCH

COVID-19 has been called an insidious virus, in part because it affects people in vastly different ways. Some have no symptoms, but others end up requiring a ventilator in intensive care. Researchers are therefore working at full speed to understand who has the greatest risk. A new study performing ultrasound scanning of COVID patients' hearts appears to identify those at greatest risk. Whether COVID attacks the heart or these patients already have heart problems is unknown, but the new knowledge can be useful now in identifying the patients at highest risk.

The COVID-19 pandemic has resulted in hundreds of thousands of people dying globally, and many more will die. Acute respiratory failure seems to be the immediate cause of death, but new studies suggest that COVID-19 also causes complications involving the heart. A new study therefore examined whether patients hospitalized with COVID-19 who have myocardial impairment comprise a specific high-risk group.

"We tried to find a simple method to screen the people hospitalized with COVID-19 and thereby identify those who get the most serious complications. Our preliminary results show that we can do this with a simple ultrasound scan of the heart combined with blood tests. Surprisingly, most of those affected have not had heart problems previously, so the heart function could be impaired because COVID-19 attacks the heart and the combination of weak lungs and a weak heart is the reason why so many people die," explains Tor Biering-Sørensen, Department of Cardiology, Herlev & Gentofte Hospital and Associate Professor, Department of Biomedical Sciences, University of Copenhagen.

A small but important reduction

To investigate how COVID-19 patients' hearts function, the researchers commuted between the hospitals in Region Zealand with a portable ultrasound scanner. All newly admitted COVID-19 patients were offered an echocardiogram with the scanner and blood tests for the level of the heart proteins troponin and BNP (B-type natriuretic peptide). Although only 174 patients were scanned, the results are compelling.

"We found that almost four fifths of the patients had myocardial impairment, determined by ultrasound scanning or elevated cardiac biomarkers in blood. Patients who had both high infection biomarkers and impaired heart function at admission had more than 70% risk of requiring hyperbaric oxygen therapy or ventilator therapy later. However, people had less risk if they had neither high infection biomarkers nor impaired heart function at admission," says Tor Biering-Sørensen.

The researchers especially noticed the change in the volume of blood the heart pumps at each beat among COVID-19 patients. A normal heart fills up, expands and then contracts and pumps 60% of the blood out again, but this drops to about 50% for the COVID-19 patients weakened by the infection: a small but important reduction.

"The idea of scanning COVID-19 patients' hearts did not suddenly materialize, because for several years we have found that infection and heart function are very closely linked. For example, like the lungs, infections affect the heart by increasing pressure, causing the blood vessels to leak, which results in oedema, filling the lungs with fluid when the heart's pumping function is impaired," says Tor Biering-Sørensen.

Two organs fail simultaneously

Previous studies have shown that influenza can increase the risk of blood clots in the brain seven-fold, and Tor Biering-Sørensen's research team has previously shown that influenza vaccination of people with high blood pressure and people with diabetes is associated with an 18% lower risk of death during the flu season. Similarly, Tor Biering-Sørensen believes that a specific focus on myocardial impairment may help to determine who will need intensive care during hospitalization for COVID-19.

"Surprisingly, patients having heart disease before getting COVID-19 is not clearly linked with developing heart problems when they get COVID-19. The myocardial impairment among people without any history of known heart disease is just as strong as that among people with known heart disease. This again suggests that, in severe cases, the virus may weaken heart function and that these cases become really serious because the two crucial organs, the heart and lungs, fail simultaneously," explains Tor Biering-Sørensen.

The new study opens up obvious therapeutic applications and, according to Tor Biering-Sørensen, ultrasound scanning may potentially be an important supplement to other tests when COVID-19 patients are hospitalized because this knowledge can be used to assess who should be monitored and prescribe medicine to the patients who really need it. However, the research raises several unanswered questions.

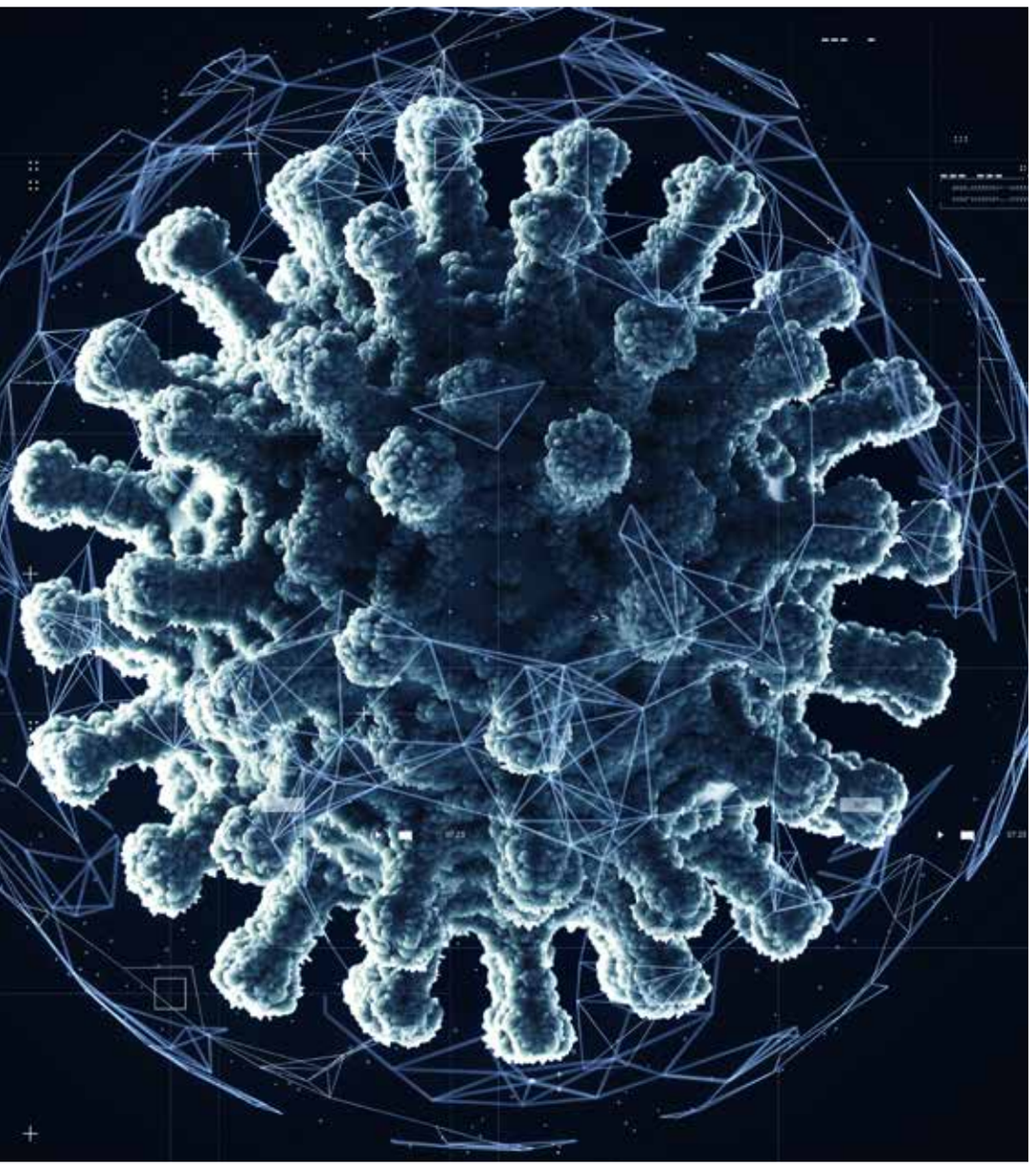
"We will try to compare our data with, for example, the Copenhagen City Heart Study, which focuses on cardiovascular diseases and their risk factors, so that we can accurately determine whether these patients become severely ill because they have a latent heart condition or because the virus weakens their heart," says Tor Biering-Sørensen.

If the researchers' theory of the virus attacking the heart is validated, the next step will be to understand the mechanism of how the virus attacks the heart.

"There are plenty of unanswered questions in addition to how this happens, whether some people are more susceptible than others and, naturally, whether anything can be done to prevent the virus from attacking the heart. In addition, we hope we can follow up the COVID-19 patients to determine whether they have lasting effects on the heart or whether the symptoms diminish over time," says Tor Biering-Sørensen.

Two medical students at the University of Copenhagen, Kristoffer Grundtvig Skaarup and Mats Højbjerg Lassen have performed ultrasound scans of coronary patients' hearts and lungs and are the main authors of a scientific article "Myocardial impairment and acute respiratory failure in hospitalized COVID-19 Study: the ECHOVID-19 Study", which has been submitted for publication. The Novo Nordisk Foundation recently awarded DKK 1.8 million to Tor Biering-Sørensen under its emergency coronavirus programme to mitigate the adverse health effects of the COVID-19 pandemic in Denmark. In 2018, the Foundation awarded Tor Biering-Sørensen a grant for the project Influenza Vaccination in Cardiovascular Disease: a Nationwide Epidemiological Study. Europcar sponsored the COVID-19 project by providing free courtesy cars for the researchers to commute between the participating hospitals with their portable ultrasound scanner. GE Healthcare sponsored the project by providing a portable ultrasound scanner.

How SARS-CoV-2 ***hijacks*** human cells



New research shows how SARS-CoV-2 binds to proteins in human cells to disable the immune system and make copies of its own genome. The researchers behind the discovery have already developed a peptide that can potentially break that bond and be used as a drug.

BY KRISTIAN SJØGREN

Thousands of people die with COVID-19 each day, but fortunately, researchers are also becoming increasingly more knowledgeable on how to prevent this from happening.

So far, research has resulted in both vaccines and antiviral drugs, and now researchers have found a new way to suppress SARS-CoV-2.

The international research team has shown how SARS-CoV-2 binds to the proteins in human cells and what it gains from this.

The same research also elucidates how a small protein can disrupt the interaction between SARS-CoV-2 and human cells and thereby defeat the virus.

The research has been published in *Nature Communications*.

"This knowledge will benefit drug developers who are trying to tame COVID-19. They can now see how SARS-CoV-2 binds to proteins in human cells and how to disrupt this interaction. Using this knowledge, they can develop a drug much more rapidly that can be used to combat the pandemic," explains a lead author behind the study, Thomas Kruse, Associate Professor, Novo Nordisk Foundation Center for Protein Research, University of Copenhagen.

Thomas Kruse carried out the research in Jakob Nilsson's laboratory in close collaboration with Matthias Mann's Clinical Proteomics Group and several research groups in Sweden.

More drugs targeting COVID-19 still required
Although good vaccines have been developed to minimise the risk of COVID-19, focusing on developing more drugs is still required.

Vaccination does not ensure full immunity for many people. For example, older people and people with weakened immune systems may not produce the necessary antibody response to keep the virus at bay and may still experience severe breakthrough infections.

Further, SARS-CoV-2 strains are probably still mutating, eliminating the certainty that current vaccines will combat future variants.

A backup plan is thus required in the form of drugs that can combat SARS-CoV-2 once it has penetrated the body.

The new study shows what happens when SARS-CoV-2 strains enter the body.

SARS-CoV-2 has only one purpose
Thomas Kruse and colleagues investigated which cellular host proteins SARS-CoV-2 strains bind to when it penetrates human cells.

All viruses that infect humans have only one purpose: to replicate themselves.

However, viruses do not have all the molecular machinery to duplicate their genomes or make proteins based on the genetic code, so they instead hijack host proteins to do it for them.

When viruses have forced cells to make more and more viruses, at some point the cells are so full of viruses that they burst and viruses erupt into the environment. Then they can enter new cells and start the whole process all over again.

"We determined which human proteins SARS-CoV-2 binds to and takes over to make copies of itself," says Thomas Kruse.

Using smart technology
The researchers used a technology called proteomic peptide phage–phage display (ProP-PD).

Phages are viruses that infect bacteria.

Using ProP-PD, the researchers cut all the proteins from the virus into small fragments and pasted each fragment onto a unique and tagged phage.

Then they tested which fragments bind to human proteins to determine very precisely how SARS-CoV-2 takes over these proteins.

"Our partners in Sweden developed this technique, which can map very precisely where on a human protein a virus binds," explains Thomas Kruse.

SARS-CoV-2 binds to specific proteins
The results show that SARS-CoV-2 binds to a protein called G3BP and also show how this interaction occurs. G3BP is usually involved in cellular stress response in connection with combating viruses.

By binding to G3BP, SARS-CoV-2 eliminates part of the cellular defence mechanism and uses the protein to amplify its own genome, a win-win situation.

Producing potential drug candidates
The researchers then set out to develop a molecule that could disrupt the interaction between G3BP and SARS-CoV-2 and thus be a possible drug candidate to treat people already infected.

Thomas Kruse says that, once the researchers know where and how viruses bind, developing peptides to disrupt the interaction between viruses and the body's proteins is not very complicated.

"This is the strength of ProP-PD, since it provides exactly the knowledge needed to carry out research that can be quickly tested in the laboratory," he says.

The researchers developed a superpeptide that binds to the same part of G3BP, thereby competing with the virus for access to this protein.

Then they tested it on cells infected with SARS-CoV-2. They found that the peptides did exactly what they were designed to do: prevent SARS-CoV-2 from binding to G3BP and making copies of itself in the cells.

"This proof-of-concept study demonstrates that we have not only identified how SARS-CoV-2 binds to proteins in human cells but also how to disrupt the interaction. We will continue to work on this," explains Thomas Kruse.

Thomas Kruse says that they are preparing the first animal experiments but would like pharmaceutical companies with greater financial resources than academia to take the discovery and use it to design drugs more quickly to benefit people with COVID-19.

"Pharmaceutical companies are probably much better geared up to develop a drug more rapidly than we are," he concludes.

"Large scale discovery of coronavirus–host factor protein interaction motifs reveals SARS-CoV-2 specific mechanisms and vulnerabilities" has been published in Nature Communications. Several authors are employed at the Novo Nordisk Foundation Center for Protein Research, University of Copenhagen.

How some ***SARS-CoV-2*** variants become more transmissible than others

When SARS-CoV-2 mutates and gives rise to new variants, they sometimes bind better to receptors in our lung cells. This may confer a fitness advantage that enables them to outcompete earlier variants and perhaps also cause more severe illness. New research shows how this happens.

BY KRISTIAN SJØGREN

SARS-CoV-2 has many names: "Wuhan" (the original SARS-CoV-2), the "British" variant (B.1.1.7, Alpha), the "South African" variant (B.1.351, Beta), the "Brazilian" variant (P.1, Gamma), the "mink" variant (Cluster 5), the "Indian" variant (B.1.617.2, Delta) and now Omicron (B.1.1.529).

The variants develop in different directions, and some become better at transmitting among people.

Now several studies show what happens biochemically when SARS-CoV-2 binds to the human target receptor, improving its transmissibility and probably also leading to more severe COVID-19.

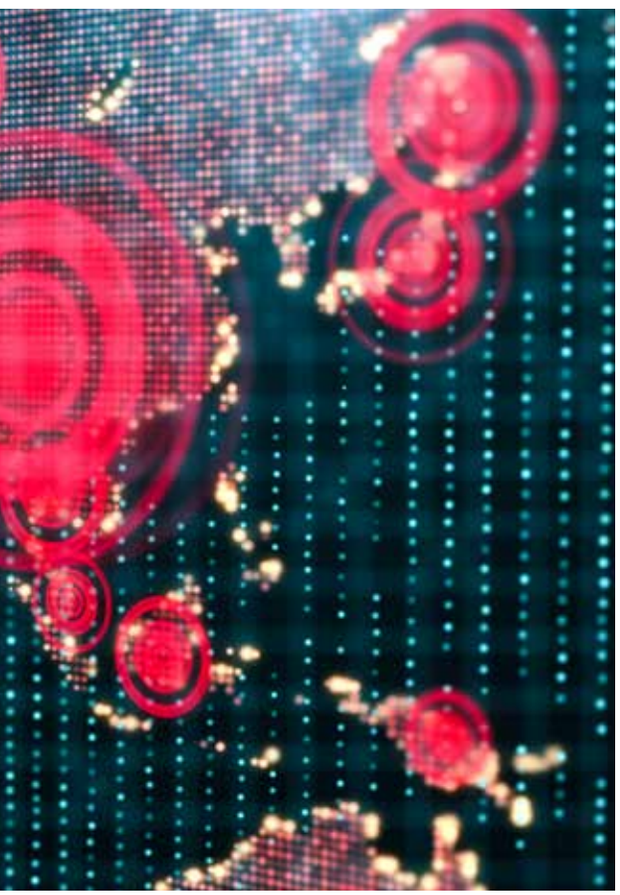
The results have been published in *Frontiers in Immunology, the Journal of Immunology, the Journal of Biological Chemistry* and *eLIFE*.

"SARS-CoV-2 is constantly trying to optimise its ability to enter our cells while evading immune

recognition at the same time. It does this by mutating, which leads to new variants. But this also means that we will probably have to decide to change the sequence of the mRNA vaccines designed to protect us from infection. The current vaccines are based on the genetic code of the original SARS-CoV-2 variant. If this changes markedly, vaccine development must also be able to keep up. In this context, knowing how SARS-CoV-2 evolves to survive in a situation in which the population has increasing immunity is important," explains a researcher behind the study, Mikkel-Ole Skjødt, Associate Professor, Department of Immunology and Microbiology, University of Copenhagen and the Laboratory of Molecular Medicine, Rigshospitalet, Copenhagen.

Part of a larger project

The research is part of several studies in which the researchers investigated functional differences of the emerging SARS-CoV-2 variants.
As a starting-point, they investigated whether people infected with the original SARS-CoV-2 variant would have difficulties in antibody neutralisation of the Cluster 5 mink variant. The results did not show this but demonstrated mutations in the spike receptor binding domain bound to the human ACE-2 (angiotensin-converting enzyme 2) target receptor four to five times stronger than that of the original variant.
The researchers also examined the link between the development of antibodies and the COVID-19 disease, finding that the level of virus-specific antibodies is directly correlated with the severity of COVID-19.



"This study indicated that treating people during a severe COVID-19 course with plasma-derived antibodies from individuals who have recovered from COVID-19 is not necessarily a good idea," says Mikkel-Ole Skjødt.

Alpha variant binds 10 times more strongly to human cells.

In two of the new studies, the researchers investigated how effective the Alpha, Beta and Gamma variants bound to the ACE2 target receptor on the surface of our lung cells.

These variants are World Health Organization variants of concern and outcompeted the existing variants shortly after they emerged because they were more transmissible.

The researchers wanted to determine how.
They specifically examined the interaction between the receptor-binding domain in the spike protein from SARS-CoV-2 and the ACE2 receptor biophysically and by studying transgenic mice with the human ACE2 gene inserted into their genome.

Mice are not normally infected by SARS-CoV-2 in the same way as humans, nor do they develop the same type of disease. However, the researchers showed that mice with the human ACE2 gene were severely infected and that the mice infected with the Alpha variant developed more severe disease than when infected with the original variant.

Furthermore, the research showed that Alpha had optimised the binding to the ACE2 receptor with 10-fold higher affinity but did not evade antibody neutralisation to any great extent. In contrast, both the Beta and Gamma variants showed intermediate binding optimisation (3–4 times stronger binding to ACE2 compared with the original variant) but markedly improved ability to evade neutralisation by our antibodies.

"This probably explains why these variants took over at some point. Biochemically, they bind better to our cells than the original variant lines, and some also evade the immune response. This enabled the gradual outcompetition of existing variants," explains Mikkel-Ole Skjødt.

SARS-CoV-2 has mutated to become a highly optimised virus

Mikkel-Ole Skjødt explains that one specific region, the receptor-binding domain, on the spike protein crucially influences the ability of SARS-CoV-2 to bind to target cells, transmit and cause illness. This region is in direct contact with the ACE2 receptor on the surface of our lung cells.

Mutations have occurred in the receptor-binding domain in the variants of SARS-CoV-2, improving its ability to bind to ACE2.

The researchers also found by examining all the variants broadly that SARS-CoV-2 might not be that prone to change.

"Although SARS-CoV-2 is constantly mutating, I would still describe it as a relatively conservative RNA virus that already is quite optimised in its ability to bind to our cells. Thus, we could expect new variants to evolve primarily to evade the immune response instead of strengthening the receptor binding. SARS-CoV-2 has evolved slowly so far, but we risk facing an ever-increasing problem," says Mikkel-Ole Skjødt.

"Functional effects of receptor-binding domain mutations of SARS-CoV-2 B.1.351 and P.1 variants" been published in Frontiers in Immunology and "The alpha/B.1.1.7 SARS-CoV-2 variant exhibits significantly higher affinity for ACE-2 and requires lower inoculation doses to cause disease in K18-hACE2 mice" has been published in eLIFE. In 2020, the Novo Nordisk Foundation awarded a grant to co-author and head of the Laboratory of Molecular Medicine Peter Garred for The Copenhagen SARS-CoV-2 Antibody Testing Initiative.

Botany is dead – *long live botany!*

We know surprisingly little about the plants around us even though plants sustain us and the agriculture of the future depends on us being able to benefit from biodiversity. This requires initiatives to make people understand how necessary knowledge about plants is – and botany should be part of the school curriculum.

BY MICHAEL BROBERG PALMGREN

Shortly before Easter, I received an e-mail from a talented and enthusiastic student who had just completed a course in basic plant biology and had become a fan of plants and had photographed them in the wild. The message was accompanied by a photo of a plant he said he could not identify. It was clear and sharp and showed a crocus.

How have we got to a point that our language has become so poor that a passionate young plant biology student in 2021 does not know the word for crocus?

Crocus is a name. It is a word in the language. The name symbolizes a plant. When we say the name, we picture the plant before us. It is no longer just a blue flower. It is a crocus.

A famous quote from Austrian philosopher Ludwig Wittgenstein translates into "The limits of my language mean the limit of my world." If we do not have a word for a crocus, then it does not exist in our consciousness. It is not part of our world.

One of the last bastions

I was thinking about this when I was standing in line with my son this Easter in a windy car park in Hyllie south of Malmö, Sweden, waiting at a COVID-19 test centre.

At my feet I noticed common whitlowgrass, Erophila verna. There was plenty of it, and it had just started to bloom. It is a small, modestly cheerful plant, and you have to bend down to be able to see how beautiful its flowers are. It was a lovely spring greeting, which I was probably the only one in the queue to receive – not shared by my son.

It was only when I once learned the words common whitlowgrass (vårgæslingeblomst in Danish) that I could see it for the first time. But then I saw it everywhere – at the roadside, between the tiles where I walked and between the roses in the flower bed, where I used to just think it was a weed.

About 1,500 species of higher plants grow in Denmark. They all have names and provide 1,500 words to the Danish language. Not long ago, the school curriculum included knowledge about plants and young students had to collect, dry, press and label the plants and remember the names.

These days, knowledge about plants is no longer mandatory in schools. No, plant diversity is not even a compulsory subject in the biology and biotechnology programmes at our largest universities. The Department of Plant and Environmental Sciences of the University of Copenhagen offers an optional course in botany for BSc students. It is one of the last bastions left in Denmark, and one requirement is that, after completing the course, a student will know the names of at least 200 plant species in Denmark.

Out of step with nature

The words crocus and common whitlowgrass are just part of what is at stake here. It is our whole ability to relate to nature, which must increasingly satiate us in an increasingly meat-free and increasingly plant-rich world in which plants help us absorb the carbon dioxide we emit.

Urbanization, not just in Denmark but all over the world, means that globally fewer and fewer people interact with nature in one way or another on a daily basis. In 1860, 52% of the labour force in Denmark worked in agriculture. In 1950, this figure had declined to 23%, and by 2010 it was as low as 3%. Our relationship with plants has become as distant as the plastic-wrapped cuts of meat on a supermarket shelf are related to the cows that must sacrifice their lives for consumers.

Professor Emeritus Dennis Woodland at Andrews University in Berrien Springs, Michigan put it very succinctly when he wrote: "As a result, never before in the history of humanity have children and teens been so 'digitally plugged-in' – and so out of touch with nature."

Richard Louv discusses this shift in our perception of nature in his 2005 book "Last child in the woods":

"Within the space of a few decades, the way children understand and experience nature has changed radically. The polarity of the relationship has reversed. Today, kids are aware of the global

threats to the environment – but their physical contact, their intimacy with nature, is fading. That's exactly the opposite of how it was when I was a child. As a boy, I was unaware that my woods were ecologically connected with any other forests. A kid today can likely tell you about the Amazon rain forest – but not about the last time he or she explored the woods in solitude, or lay in a field listening to the wind and watching the clouds move."

Erosion of knowledge, even at the highest levels

As the basis for our sustenance, plants are also the basis of future sustainable agriculture on which we depend and that also depends on being able to benefit from nature's biodiversity. This requires that some people know about plants.

The fact that very few people do this today is not just a problem in Denmark but is recognized in universities around the world.

Most botanists in Denmark, who until recently were employed at our universities, have now either retired or been laid off in austerity rounds, probably because they could not compete with molecular biologists in attracting external research funding.

The fact that children and adolescents cannot recognize the plants they encounter is one thing. But now this also applies to highly educated biologists and biotechnologists, not just MSc students but all the way up the scientific hierarchy from PhD students to postdoctoral fellows, associate professors and professors. Ignorance is prevalent even among those who make a living from being plant biologists.

Plant biology at the university level today is largely characterized by reductionism and a desire to understand the basic mechanisms that govern a plant's life. This means that plant molecular biology and plant biochemistry have come to the fore, and these disciplines can in principle be studied based on a single model plant, the world's most thoroughly studied plant, thale cress, Arabidopsis thaliana. This would be equivalent to modern zoologists only knowing about the fruit fly.

Plant of the week

So, what can we do to reverse this trend? I try to make a modest contribution to rescuing botany. Every Tuesday morning, two sections of my department have a Zoom meeting for employees and affiliated students that begins with a scientific presentation.

After that I present the Plant of the Week.

The idea is that nature is right outside your door, which in this context is the entrance to the Frederiksberg Campus, in which every little plant has its own exciting history. The criteria for becoming the Plant of the Week is that it must be wild (or naturalized), grow no more than a few hundred metres away from our department and be discoverable on the same day it is presented.

In the weeks before Christmas, we featured ivy broomrape (Orobanche hederae), red dead-nettle (Lamium purpureum), petty spurge (Euphorbia peplus) and mistletoe (Viscum album). In 2021 we have covered 16 new plants, with many more in the pipeline as long as my audience can maintain interest.

Not just lab-coat science

But professional plant biologists expanding their knowledge of plants is insufficient. To save botany, Andrew Woodland basically suggests the following:

"Begin at home. Those of you with small children or planning to have children, reevaluate how you spend time with them and what you give them for gifts. Get them involved early in the outdoors – tramp, camp, explore, swim, fish, sleep under the stars etc. If possible, let them have a pet. Get your children growing plants, planting an herb garden, using tools and containers found at home. Help them 'harvest their gardens' and savor the fruits of their labor. Teach them to be part of nature."

Learning about plants should already begin in school.

Jorge Crisci issue this call to teachers: "... give our youth a sense of the interconnectedness of life, the importance of plants for human survival, and of biodiversity as the essential tool for understanding and conserving plants and the natural communities that sustain all life."

Birgitte Nauntofte, the former CEO of the Novo Nordisk Foundation, was very foresighted when she sowed the seeds for the Foundation to invest in a non-profit educational initiative and establish the LIFE Foundation, which aims to strengthen children and adolescents' knowledge about and fascination for natural science through enquiry-based science education.

I urge the Novo Nordisk Foundation to ensure that these funds not only contribute to strengthening the fascination for lab-coat science but also for nature itself, and in so doing, please do not forget the plants!

The Botany Bill

In 2019, the Botanical Sciences and Native Plant Materials Research, Restoration, and Promotion Act (known as the Botany Bill, H.R.1572, S.2384) was introduced for the second time in the United States Congress with the summary words: "This bill requires the Department of the Interior to establish a botanical science research program, hire botanical scientists, and establish a student loan repayment program for botanical scientists."

A similar initiative should definitely be launched in other countries, including Denmark, because right now botany is dead. It needs life-saving first-aid if it is to survive and help us towards a sustainable future. Long live botany! Hopefully, the current budget models will enable the universities to take on this responsibility. Otherwise, the politicians must step in.

Michael Broberg Palmgren is Professor of Plant Physiology, Department of Plant and Environmental Science, University of Copenhagen. In 2019, the Novo Nordisk Foundation awarded a Challenge Programme grant to him for the project NovoCrops: Accelerated Domestication of Resilient Climate Change–friendly Plant Species.

chapter

BREAKING NEW GROUND

ARTICLES

Brown fat may be key to a ***healthier life***

A decade ago, scientists discovered that energy-consuming brown fat may have an important role for adults. Since then, researchers have sought to understand how it arises and how it can help to counteract obesity. Research confirms that activating brown fat among adult humans increases the metabolic rate. The focus is now on understanding the interaction between the brain and brown fat to ensure that the brain does not respond to any increased energy consumption by increasing appetite.

BY MORTEN BUSCH

The thought is captivating. Imagine if you could get your body to burn more energy – without having to lift a finger. A special type of fat cells called brown fat cells contain more of the body's power plants, mitochondria, than white fat cells. Brown fat mitochondria specialize in converting chemical energy into heat, which expends energy. This enables brown fat tissue to burn fat instead of just storing it as in regular white fat. For years, it was thought that only infants have brown fat, which regulates their body temperature. But over the past decade, researchers have learned that many adults either have this brown fat or can develop it.

"We know that certain factors can promote and activate brown fat, but we also know that activating brown fat does not immediately result in weight loss. The human body is more complex than this, and during evolution we have developed systems to maintain our body weight. In our latest review article, we discuss whether the brain responds to temporary higher energy expenditure with a feedback mechanism that makes us feel hungry in an attempt to maintain body weight," explains Camilla Schéele, Associate Professor, Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen.

This could explain the lack of weight loss in studies performed over several weeks without limiting food intake. However, several studies have shown that the amount of active brown fat is negatively correlated with obesity. In the new review, the researchers explore the idea that the endocrine capacity of brown fat could explain this discrepancy.

"In other words, in the long term, when the brown fat is better "trained", we expect it to play a more active role in regulating appetite by sending signals to the brain to increase appetite upon activation of the brown fat when we are cold and, conversely, decrease appetite when we warm up again. Adults with detectable levels of brown fat are generally healthier, but we need to understand the signals sent back and forth between the brain and the fat tissue to understand how the brown fat can promote weight loss."

Brown fat produces heat and consumes energy

Brown fat is scarcely a new concept. Scientists have known for decades that infants rely on it to regulate temperature. They cannot produce heat by moving muscles because the links between the nervous system and muscles are still developing during the first year of life.

"Brown fat has also been widely studied in rodents and other small mammals, helping to maintain body temperature in cold weather. The feeling of cold causes the nervous system to release catecholamines, such as norepinephrine, into the brown fat, and this stimulates energy consumption and heat production in brown fat. A decade ago, scientists confirmed that adults also have brown fat," explains co-author Jo B. Henningsen, a postdoctoral fellow from the Novo Nordisk Foundation Center for Basic Metabolic Research at the University of Copenhagen.

Researchers discovered that this was not a mere artefact among some adults but that most adults can recreate the brown fat and that age inactivates it among many people.

> Research has shown that the brown fat can be reactivated and that both exposure to cold and sustained pharmaceutical activation improve the thermogenic capacity of brown fat, although activation declines with age.

Brown fat communicates with the brain

Artificially activating brown fat was quickly considered as an opportunity to stimulate weight loss among obese people. Unfortunately, this turned out to be more complex because the body has natural feedback mechanisms so that it does not run out of energy.

In the short term, the increased energy consumption can therefore result in compensatory increased food intake.

"But we hypothesize that the increased active brown fat will play a greater role in the body's metabolism and result in better appetite regulation and thus counteract obesity in the long term," says Jo B. Henningsen.

Have you had a cold shock today?

Brown fat has many other positive effects, including on insulin sensitivity. Researchers do not yet completely understand how and why, but future research should try to elucidate the communication and signalling pathways between the brain, fat tissue and other metabolic organs.

The research group is currently working diligently to understand the interactions between brown fat and the brain. Camilla Schéele has just received a European Research Council Consolidator Grant, one purpose of which is to investigate this interaction in more detail.

"We hope that improved understanding of the interaction between brown fat and the brain can give us a key to reset some of the imbalances among people with obesity and type 2 diabetes. We hope that the research can reveal new mechanisms that will help us to better understand metabolic disorders and to identify new drug targets to combat obesity and type 2 diabetes," concludes Camilla Schéele.

"Brown adipose tissue: a metabolic regulator in a hypothalamic cross talk?" has been published in Annual Reviews of Physiology. The authors are employed at the Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen.

Contraceptive to ***prevent sperm*** from wriggling

The dream of developing a contraceptive pill for men is alive and well. Researchers have experimented for years to elucidate the changes in how sperm wriggle to develop ways to prevent them from reaching and merging with the egg. Researchers in the United States published experiments showing that plant triterpenoids plus testosterone and hydrocortisone slows down the penetration of sperm. A new study could not replicate this. However, the researchers think that modifying an existing drug could provide a new and safe contraceptive for men.

BY MORTEN BUSCH

Moving the fastest is not the only criteria. After a long and challenging journey through the cervix, uterus and fallopian tube, a sperm with powerful rhythmic wriggling and rolling must break through a thick protective layer around the egg itself. Researchers are attempting to prevent sperm motility and thereby enable men to take a pill to avoid getting a woman pregnant. This may sound easy, but new research shows that this journey, like the sperm's, is long and uncertain with many obstacles.

"Researchers in the United States published promising results claiming that plant triterpenoids plus testosterone and hydrocortisone can inhibit sperm motility. However, both we and researchers in Germany have tried unsuccessfully to replicate these results. But we found that an approved drug has this desired effect. By modifying it slightly, we expect to be able to amplify the effect and thus create a contraceptive that can effectively and safely make the sperm unable to fertilize the egg," explains Anders Rehfeld, Postdoctoral Fellow, Department of Biomedical Sciences and Department of Drug Design and Pharmacology, University of Copenhagen and Department of Growth and Reproduction, Rigshospitalet.

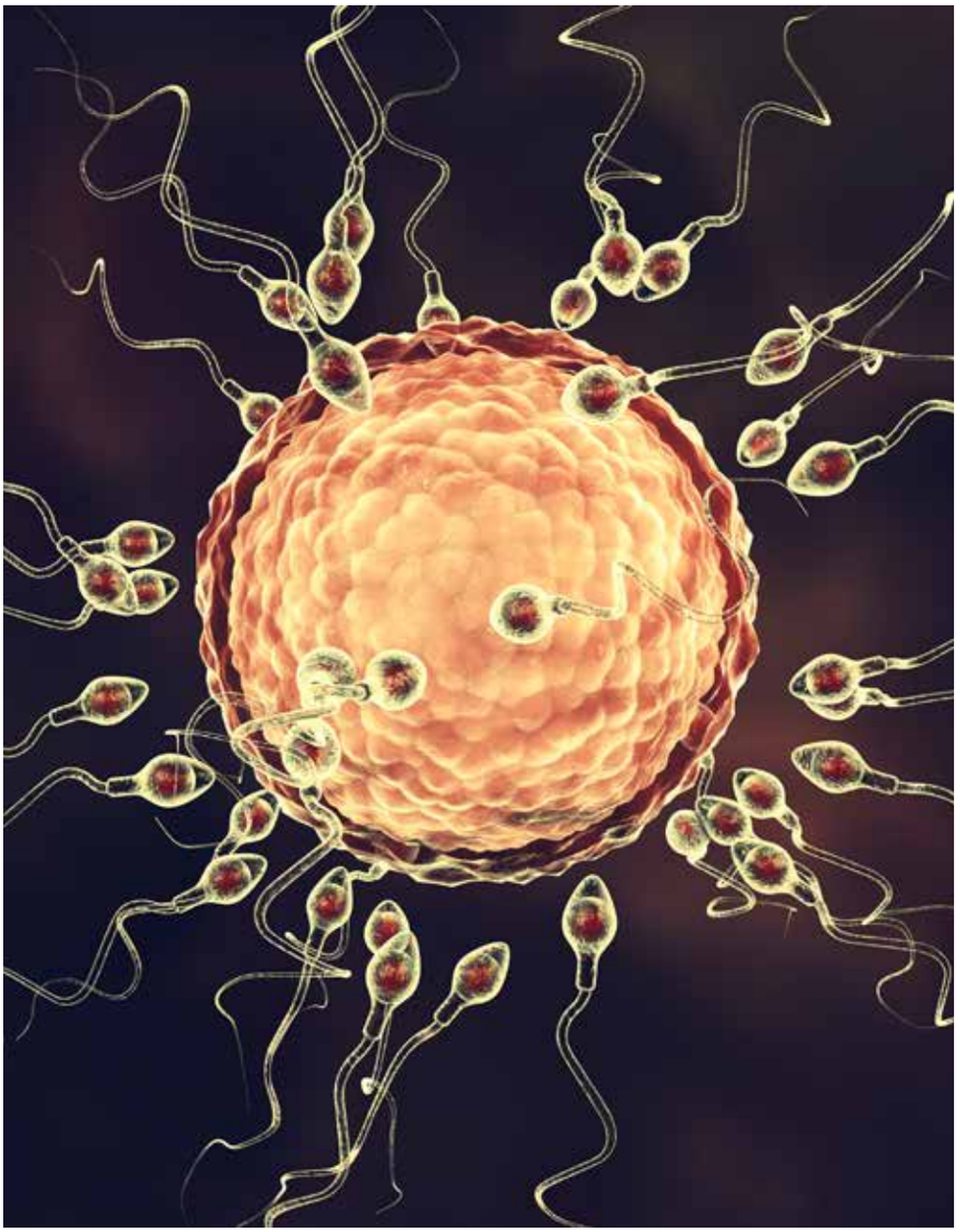
Investigating two plant triterpenoids

Several research groups have used varying strategies to prevent sperm motility by disabling the mitochondria or the transport and ion channels on the surface. The current experiments focus on the ion channels by attempting to inhibit the sperm-specific Ca2+ channel CatSper, which must be activated to enable the sperm to fertilize a woman's egg.

"CatSper is an ion channel that is activated by both high pH in the woman's reproductive tract and progesterone. Preventing this activation keeps the beating pattern of the sperm tail symmetrical. The sperm cells are thus unable to wriggle and therefore cannot penetrate the protective layer of the egg," says Anders Rehfeld.

Interest was piqued when researchers from the University of California, Berkeley claimed that they had inhibited human CatSper by using steroid-like plant triterpenoids. Cholesterol is a simple triterpenoid, but they are present in myriad variants in plants, including lupeol and pristimerin. The researchers inhibited the activation of CatSper and thus also the ability of sperm to fertilize eggs. The researchers also showed that testosterone and hydrocortisone had the same inhibitory effect on CatSper.

"We tested the effects of various compounds on CatSper and wanted to use lupeol and pristimerin as controls, but we could not replicate these researchers' results. I contacted them and they instructed me in how to change the procedures for dissolving and testing them, but this did not help. We could not replicate the effect," explains Anders Rehfeld.



Promiscuous sperm

Anders Rehfeld and colleagues, however, were not alone in being unable to replicate the results. A group from University Hospital Münster had unsuccessfully tried to replicate the effect in 2018; the triterpenoids did not inhibit CatSper. In addition, both Anders Rehfeld and the Münster group, unlike the Berkeley group, found that other known human steroids including testosterone and hydrocortisone activated CatSper just like progesterone. Although the Münster researchers performed fluorescence measurements and patch-clamp experiments to measure ion currents, Anders Rehfeld's group went further.

"I thought creating clarity was important, so we also tested the motility by measuring sperm's ability to penetrate a viscous barrier of methylcellulose, similar to the mucus in the cervix, after adding various steroids. Again, we did not find the same effect as the Berkeley group. The other steroids all seemed to mimic the effect of progesterone on CatSper and thus the motility of sperm," says Anders Rehfeld.

The fact that other natural hormones mimic the effect of progesterone on sperm is not inherently surprising. CatSper has previously been shown to be promiscuous, being activated by many chemicals. When this happens, the calcium ions flow through the channel, required for rapidly changing the motility pattern of the sperm, so they can navigate the obstacles in the woman's reproductive tract and successfully find the egg.

"But we also found another substance that, unlike the triterpenoids, can inhibit the sperm. Unfortunately, for patent-related reasons, I cannot reveal the name now, but it is an existing drug. It is not as effective as the triterpenoids reported by the Berkeley group, but there is huge potential," explains Anders Rehfeld.

In fact, great effectiveness would have been a great surprise, because then this might have been discovered previously as a side-effect that might be more or less desired. It is effective, however, and now Anders Rehfeld and colleagues hope to strengthen the drug by modifying it chemically so that they can eventually develop a new safe and effective contraceptive for men.

"We clearly still have a long way to go. We need to optimize the modifications to ensure that the drug enhances the effectiveness on CatSper without any side-effects. We have the advantage that this drug, unlike the plant triterpenoids used by the Berkeley group, is not hormone-like, but we must still develop it in pill form and in a chemical form that can be transported across the intestinal wall, through the bloodstream, and to the seminal fluid. The second option is as a suppository for women, but right now we are chasing the big dream – The Pill for men," concludes Anders Rehfeld.

"Revisiting the action of steroids and triterpenoids on the human sperm Ca2+ channel CatSper" has been published in Molecular Human Reproduction. Anders Rehfeld is part of the BRIDGE – Translational Excellence Programme at the University of Copenhagen, a 2-year postdoctoral fellowship supported by the Novo Nordisk Foundation that aims to build bridges between basic research and clinical practice. In 2020, the Programme awarded a grant for the project A Novel Nonsteroidal Male Contraceptive.

Cancer and diabetes: ***how are they linked?***

Many people with cancer have type 2 diabetes, and many people with diabetes develop cancer. Researchers now have a clearer idea of how these two diseases are linked.

BY KRISTIAN SJØGREN

People who are obese or have type 2 diabetes have an increased risk of developing cancer. But people with cancer also have a greater risk of developing type 2 diabetes.

New Danish research shows that poor regulation of lipolysis – how the body metabolizes fat – and how this affects glucose metabolism are probably what links the two diseases.

More precisely, the research shows that cancer takes over various functions of the body and induces several metabolic perturbations, including reducing insulin sensitivity and increasing the production of glucose in the liver. Both can lead to developing type 2 diabetes.

"We initially thought that cancer-induced inflammation might have caused the reduced insulin sensitivity in our study, but this turned out not to be the case. Instead, cancer appears to cause the body to secrete more fat into the blood, which can lead to insulin resistance. This benefits a cancer cell but not the person with cancer," explains author Lykke Sylow, Associate Professor, Department of Exercise, Nutrition and Sports, University of Copenhagen.

The research has been published in *Metabolism*.

Insulin and glucose fuel cancer cells

Several previous studies have suggested that insulin and glucose may promote the growth of cancer.

Insulin is a growth hormone, and cell studies have shown that high levels of insulin and glucose cause cancer cells to grow faster.

Elevated levels of insulin establish better conditions for cancer cells to grow. This applies to people with prediabetes because high blood glucose creates a need for more insulin.

In addition, the rapidly growing cancer cells need lots of glucose to grow.

"Our goal was to investigate whether there is a mechanism that enables cancer cells to affect metabolic function throughout the body," explains Lykke Sylow.

Cancer destroyed the metabolic functioning of mice

The researchers gave mice lung cancer and subsequently observed whether they developed various metabolic disorders, such as ones affecting the liver's glucose production and insulin sensitivity in the adipose tissue and skeletal muscles. These disorders are common among people with prediabetes or obesity.

The researchers did this by giving the mice insulin and investigating how it lowers blood glucose, which happens when insulin causes fat and skeletal muscles to absorb glucose.

In mice with cancer, however, the intake of glucose was markedly reduced, and the liver also produced more sugar.

"We measured the metabolism of the mice using seven parameters and found perturbations on six metabolically essential functions when the mice had cancer," says Lykke Sylow.

Cancer shuts off the blood flow to muscles

The researchers also found that some capillaries in the muscles did not open in the cancer-affected mice, which they usually do after a meal when the insulin is circulating in the blood.

The capillaries open during a meal to ensure that muscle and fat tissue have access to the nutrients in the bloodstream.

This is an important process for absorbing glucose into the muscles.

But the researchers examined the cancer-affected mice and found that this did not happen.

"This does not happen when the mice have cancer. This is very noticeable and typical for people who are very insulin resistant," explains Lykke Sylow.

Cancer regulates fat metabolism

Lykke Sylow believes that cancer probably even regulates all the metabolic processes to create as favourable conditions for itself as possible.

The more insulin and sugar that circulates throughout the body, the better the conditions for the rapidly growing cancer cells.

The question is how they do this

Both mice and people with cancer have elevated circulating fatty acids in the body, so they release more fat from adipose tissue into the blood than healthy people.

The researchers inhibited the lipolysis of adipose tissue in the mice and prevented them from becoming insulin resistant.

"We believe that this may be the mechanism the cancer cells use. They influence fat metabolism, which affects insulin sensitivity, which affects the glucose levels in the body. For the cancer cells, this is smart because the glucose bypasses the muscle and fat and the cancer cells then get more for themselves," explains Lykke Sylow, who also emphasizes that this is the first step towards understanding the link between cancer and metabolic perturbations and that further research is required.

Metabolic treatment might become cancer treatment

According to Lykke Sylow, the perspective in the new research is that the discovery can be rapidly implemented clinically, so doctors immediately screen people with cancer for metabolic syndrome, a precursor of type 2 diabetes.

"People with metabolic syndrome can be given glucose-lowering medication and be advised to exercise more. Improving their metabolic function may benefit cancer treatment," says Lykke Sylow.
She elaborates that 10–50% of women who have been treated for breast cancer experience relapse. The risk of relapse is three times greater for women who have metabolic syndrome than for women who do not have it.

"This is very relevant clinically for improving people's outcomes. People have a greater risk of dying from cancer if they also have metabolic syndrome. In the future, we might be able to test whether increasing the insulin sensitivity among people with cancer can prevent relapse after initial treatment," says Lykke Sylow.

"Cancer causes metabolic perturbations associated with reduced insulin-stimulated glucose uptake in peripheral tissues and impaired muscle microvascular perfusion" has been published in Metabolism. In 2018, the Novo Nordisk Foundation awarded a grant to Lykke Sylow for the project Identifying Key Orchestrators of Pathways in the Interactions between Metabolism, Insulin Sensitivity and Muscle Wasting.

Serotonin has an important role in migraines

A study of 150 Danish families with migraine shows that the disorder often results from variation in the genes related to how the brain manages serotonin.

BY KRISTIAN SJØGREN

Millions of people worldwide have migraine headaches, and yet researchers have not yet fully understood this disorder or its causes.

Danish researchers have now dug a little deeper to understand migraine and have found that people with migraine often have substantial variations in the genes that develop the serotonin-related receptors and signalling pathways.

Serotonin is a multifunctional neurotransmitter that regulates sleep, appetite and memory. It influences the feeling of well-being and joy and can affect the development of depression.

The discovery that serotonin is involved in migraine is new but does not come as a huge surprise to one researcher behind the new study.

“The serotonin receptor is interesting because many drugs target that receptor. We can also therefore say that our discovery is not an a-ha experience, but it makes sense in the context of what we have done,” says Thomas Folkmann Hansen, Associate Professor, Novo Nordisk Foundation Center for Protein Research, University of Copenhagen and Senior Research Manager, Danish Headache Center, Rigshospitalet, Glostrup, Denmark.

The study was recently published in *Brain*.

Different parts of the brain play a role in migraine

Two parts of the brain and the blood vessels are primarily involved when people have migraine: the trigeminal nerve, the visual cortex in the back of the head and the aorta.

The trigeminal nerve is involved in the two main types of migraine: migraine with aura and migraine without aura.

The difference between the two types of migraine is that people with migraine with aura experience various sensory disturbances, and 95% experience temporary visual disturbances that disappear again.

In the new study, the researchers investigated whether variation in the genes behind the trigeminal nerve, visual cortex and aorta are especially expressed among people with migraine.

Examined genomes from thousands of people with migraine

In the study, the researchers performed whole-genome sequencing of the DNA of 874 people from 117 families in which migraine was clearly inherited for several generations.

The researchers also collected data from an additional 1,930 people with migraine and 1,000 controls without migraine. The purpose was to determine whether they could replicate the results they had found in the families.

The researchers compared the genomes of the migraine-affected individuals with the genomes of the controls and found that migraine was often associated with high variation in the genes in the trigeminal nerve that are associated with the serotonin receptor and serotonin signalling pathways.

This means that individuals with migraine appeared to have accumulated many more mutations in precisely these genes.

“Genetic variants accumulate, and this seems to affect serotonin signalling in a direction that is related to the development of migraine,” says Thomas Folkmann Hansen.

No association with the blood vessels

Thomas Folkmann Hansen says that most of the people involved in the study had migraine with aura.

The results are therefore interesting since they contribute to the debate on the reason why people develop migraine with aura.

Researchers have generally been divided into two camps: those who claim that the brain causes migraine and those who claim the blood vessels that supply the various parts of the brain with oxygen and nutrients cause migraine.

In the study, the researchers found no accumulation of variants in the genes related to blood vessels.

“We may not be able to refute the hypothesis that the blood vessels play a role, but our results indicate that the brain is important in developing migraine with aura,” says Thomas Folkmann Hansen.

The number of variants is the smoking gun

Thomas Folkmann Hansen says that the study can help pave the way for further research into the genetic causes of migraine.

Indeed, some of the genetic variants involved appear to be common, whereas others appear to be rare.

Although the results cannot determine the individual significance of individual genetic variants for the risk of developing migraine, the research suggests which genes are especially affected when people have migraine.

“We wanted to find the migraine gene, but we did not. But we identified genes that appear to contain many mutations, which can change the functionality of the gene and thereby contribute to developing migraine. The number of variants seems to make things go wrong and not just the presence of an individual variant in an individual gene,” says Thomas Folkmann Hansen.

“Functional gene networks reveal distinct mechanisms segregating in migraine families” has been published in Brain. Co-author Thomas Folkmann Hansen is an Associate Professor at the Novo Nordisk Foundation Center for Protein Research, University of Copenhagen and Senior Research Manager, Danish Headache Center, Rigshospitalet, Glostrup, Denmark.

Newly identified mechanism ***prevents cancer*** from spreading

The risk of dying from cancer increases when it migrates from the original site (metastasis). Researchers from the University of Copenhagen have identified a physiological mechanism that can help to predict the risk of metastasis and might also be manipulated to prevent it.

BY KRISTIAN SJØGREN

Metastasis is the migration of cancer from the primary tumour to other tissues – often with fatal consequences. The new cancer sites are called metastases.

Cancer can migrate to any site in the body, forming metastases in the lungs, kidneys and liver that develop in these organs.

Danish researchers have identified a fascinating mechanism that determines how easily cancer can metastasize.

The researchers hope that this mechanism can be used diagnostically in the future to determine each person's risk of cancer metastasizing to specific organs – even before a tumour is formed.

In addition, the researchers will also investigate whether this mechanism can be used therapeutically to inhibit metastasis.

"As we develop more and more organ-specific treatments for cancer, our discovery may help to determine which treatments a person should receive to optimally protect vulnerable organs from metastasis," says a researcher behind the new study, Janine Erler, Professor, Biotech Research & Innovation Centre (BRIC), University of Copenhagen.

The research has been published in *Nature Materials*.

Softer membranes prevent metastasis

Janine Erler, Raphael Reuten and other colleagues studied the architecture of basement membranes, which surround all cells and blood vessels.

These basement membranes have varying degrees of stiffness, and new research shows that stiffer basement membranes enable cancer cells to migrate more easily across the membrane and enter the bloodstream and metastasize. Conversely, a softer barrier membrane reduces metastasis.

"Basement membranes are the frontline barrier to metastasis. We therefore knew that they are important for keeping cancer cells in check, but we did not know that their stiffness is the major determining factor," explains Janine Erler.

Stiff basement membranes increase the risk of dying from cancer

The researchers investigated basement membranes using computer models, animal models and genetic data from people with cancer.

All these studies confirmed that basement membrane stiffness affects metastasis.

"This provides insight into how easily cancer can metastasize to various organs for each individual even before tumours form," says Janine Erler.

The researchers identified a protein, netrin-4, that is associated with the stiffness of the basement membranes.

Levels of netrin-4 were a key regulator of basement membrane stiffness. More netrin-4 within the basement membrane means a softer barrier, resulting in reduced cancer cell invasion, which is associated with improved survival.

Mice that produced no netrin 4 were more prone to metastasis

The researchers used a genetic mouse model that produced no netrin-4 anymore. Tumours metastasized much more easily in these mice than in mice with normal levels of netrin-4.

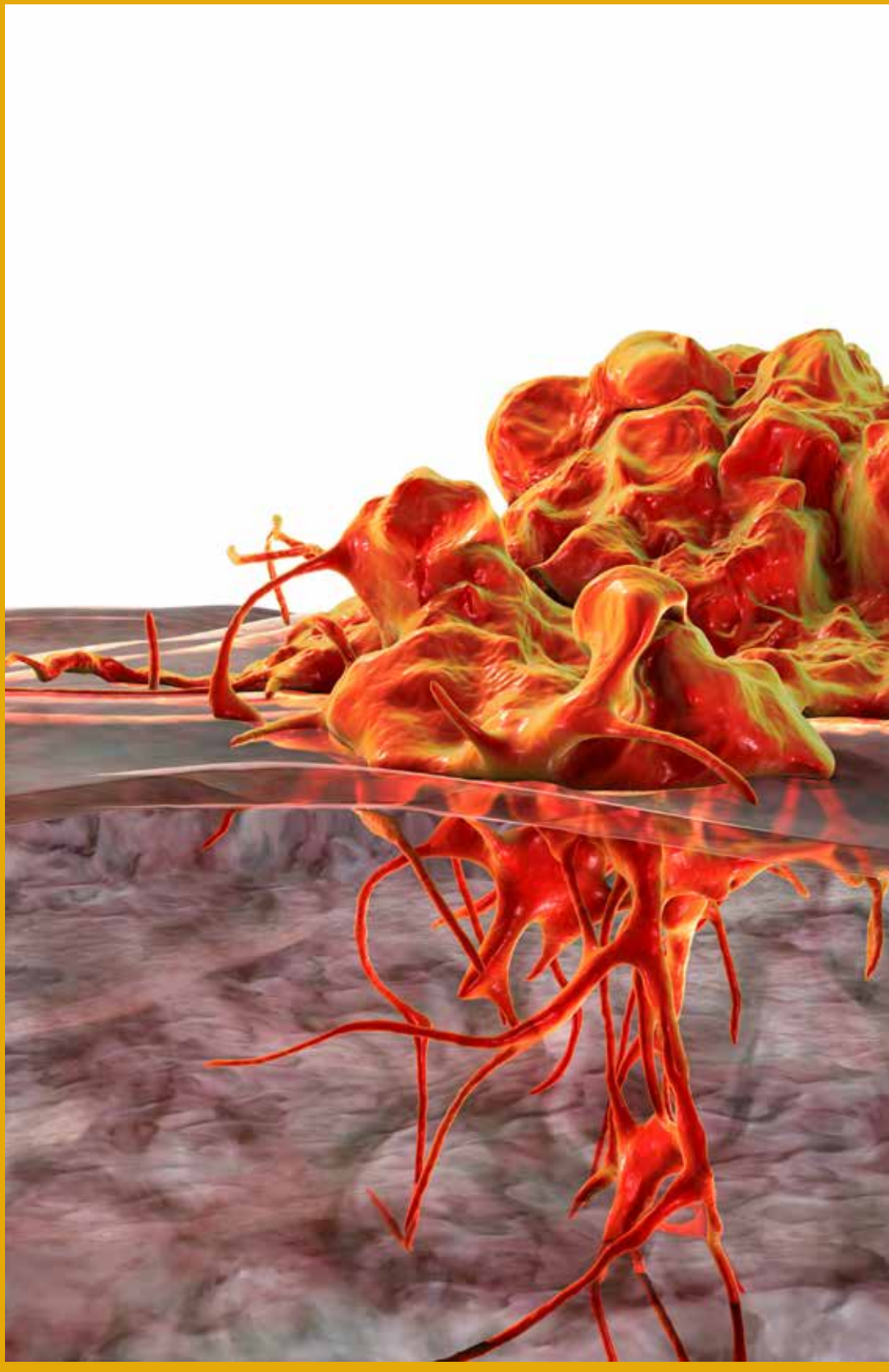
Interestingly, injecting netrin-4 into mice reduced the risk of metastasis.

"The more netrin-4 molecules, the softer the basement membrane, so cells have more difficulty in breaching the basement membrane. This keeps the cancer cells encapsulated. We are now investigating the therapeutic and diagnostic potential of our results, and we may investigate whether drugs can increase netrin-4 levels among people with cancer to minimize the risk of metastasis during cancer treatment," explains Janine Erler.

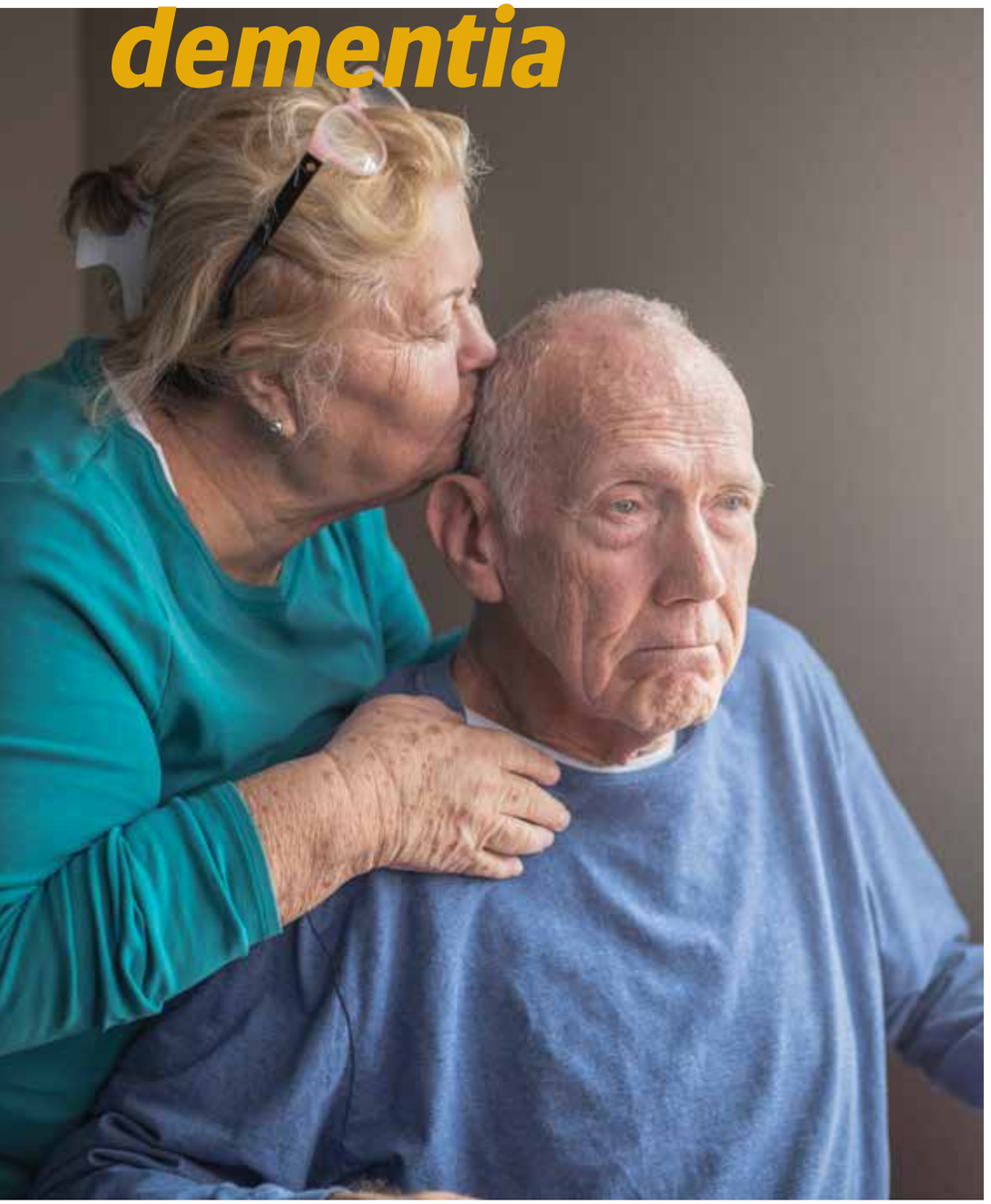
The researchers further confirmed the relationship between high netrin-4 levels and softness as well as good survival by analysing genetic data from people with cancer.

"Our findings open up a whole new way of understanding cancer and potentially paves the way for some novel therapeutic opportunities for preventing cancer from metastasizing," says Janine Erler.

"Basement membrane stiffness determines metastases formation" has been published in Nature Materials. In 2012, the Novo Nordisk Foundation awarded Janine Erler a Hallas-Møller grant.



A good night's sleep protects ***against dementia***



The glymphatic system clears protein waste products from our brain when we sleep, but impaired or insufficient sleep can lead to waste products accumulating in the brain, increasing the risk of developing dementia.

BY KRISTIAN SJØGREN

Sleep is the foundation for good physical and mental health. Less than a decade ago, Danish and other researchers discovered that one reason sleep provides benefits is a special system in the brain that removes waste products while we sleep. However, impaired or insufficient sleep enables these waste products to accumulate in the brain instead.

This mechanism Is called the glymphatic system and is now associated with the risk of developing dementia. The glymphatic system works poorly if sleep is impaired and these dementia-associated waste products in the brain are not cleared at night but accumulate instead. This leads to poor long-term outcomes.

An article on the association between sleep, the glymphatic system and dementia was recently published in Science.

"The article consolidates the consensus in this field that the glymphatic system plays an important role in the onset of dementia. So much evidence now indicates that various factors can adversely affect the glymphatic system and result in cognitive impairment, so the association is clear," explains Maiken Nedergaard, Professor, Center for Translational Neuromedicine, University of Copenhagen.

Science commissioned *Maiken Nedergaard* to write a review article on the current knowledge in this field.

The brain's fluid transport system clears waste products between brain cells

The glymphatic system clears protein waste products from the brain when we sleep.

Many waste products from various biological processes accumulate in the brain during the day, and these must be removed to reduce the burden on the brain.

The glymphatic system is highly organized for transporting and clearing cerebrospinal fluid through the glymphatic pathway. This uses perivascular spaces – open fluid-filled tunnels on the outside of the arteries – that flush the waste products away from between the brain cells.

The glymphatic system also involves the water channel protein aquaporin, which is present throughout the brain but was nevertheless first discovered in the early 21st century.

Maiken Nedergaard helped to pioneer research into this relatively recent discovery of the glymphatic system.

Sleep clears waste products from the brain

The glymphatic system works optimally and almost exclusively while we sleep.

Various factors can therefore influence both sleep and the brain's ability to remove the harmful waste: age, sleep quality, substance abuse, depression, cardiovascular disease, inactivity, sleep apnoea, obesity and disruption of the circadian rhythm.

If too many negative factors are present, waste is not removed from the brain at night and can accumulate over time.

"Almost all diseases reduce the quality of sleep, and an interesting perspective is that almost all neurodegenerative disorders such as dementia and Alzheimer's start with patients having difficulty sleeping. Sleep problems occur even before memory loss begins," explains Maiken Nedergaard.

People with Alzheimer's have impaired sleep

Maiken Nedergaard explains that the first symptom many patients with Alzheimer's have is difficulty sleeping.

Many people who end up in nursing homes do so because their sleep patterns are so misaligned with their circadian rhythm that their families have difficulty in taking care of them.

They may take long naps during the day and stay awake most of the night.

"Sixty-five percent of the people with Alzheimer's are placed in institutions because they are awake at night and their families cannot take care of them. This shows a clear link between sleep and dementia, and suggest that the the glymphatic system plays an important role," says Maiken Nedergaard.

Clearing waste products that can cause Alzheimer's and Parkinson's

Maiken Nedergaard's review article links the development of Alzheimer's and Parkinson's with the effects of glymphatic dysfunction.

Amyloid-beta and phosphorylated tau proteins accumulate in the brain of people with Alzheimer's, and people with Parkinson's accumulate alpha-synuclein.

When the glymphatic system does not clear these protein waste products from the brain and they are allowed to accumulate, the brain can develop these neurodegenerative disorders.

"The glymphatic system plays a role in several diseases in which the person has diminished cerebrospinal fluid flow. Age is associated with a significant decrease in sleep quality and decreased glymphatic flow. This reduced flow prevents the extracellular protein waste from being removed from the brain, where it accumulates, leading to local inflammation, loss of neurons and ultimately dementia," explains Maiken Nedergaard.

A pathway for early diagnosis of dementia?

According to Maiken Nedergaard, the research can be used to develop methods for diagnosing dementia at a very early stage.

Other clinical studies have shown that dementia is often diagnosed today because of memory loss, and then it is already too late to do anything about it.

However, a magnetic resonance imaging scan of the brain showing impaired function of the glymphatic system may indicate that the brain is not clearing the waste products that could lead to dementia in a few years.

"Scanning enables us to determine whether new treatments can improve the glymphatic system and thus reduce the risk of developing dementia, so we will not have to wait for 10 or 20 years to see whether these people develop dementia or not," says Maiken Nedergaard.

"Glymphatic failure as a final common pathway to dementia" has been published in Science. In 2014, the Novo Nordisk Foundation awarded a grant to Maiken Nedergaard and her co-author Steven A. Goldman to open a new neuroscience center at University of Copenhagen. In 2015, the Lundbeck Foundation added additional support to their center named Center for Translational Neuromedicine

We all carry superfluous ***Neanderthal*** DNA

We all carry Neanderthal DNA, which tells us who our extinct relatives were but has little impact on who we are. The genetic information found in all cells of the human body creates the basis for appearance, physiology and behaviour. The same information tells the history of our ancestors and the secrets of human evolution. An analysis of the genomes of 27,566 Icelanders now provides the most detailed information on the traces Neanderthals left in the human genome 50,000 years ago. This Neanderthal heritage comprises 2% of every Eurasian genome, and this study shows that it has little impact on human biology and disease. The analysis provided new interesting knowledge about the lives of Neanderthals and other unexpected ancestors and how humans met them.

BY MORTEN BUSCH

All humans are genetically unique. Most of the genetic code we carry comes from our Homo sapiens ancestors.

All human populations outside Africa trace their ancestry back to a group that travelled outside that continent 60,000 years ago and later populated the rest of the world. Although the genomes of non-African descendants come from that Homo sapiens group, a small portion of about 2% derives from archaic humans: the Neanderthals. This is because after they left Africa, modern humans and Neanderthals had descendants, probably in the Middle East. An international research team has compiled new insights into the Neanderthal nature from the fragments left in our genomes 50,000 years ago.

"We have found pieces of the Neanderthal puzzle by scanning through the genomes of more than 27,000 Icelanders. Because we find different pieces in each person, we were able to reconstruct 50% of the Neanderthal genome. Previous studies implied that these fragments were associated with an increased risk of disease (such as propensity to diabetes, depression and higher addiction to nicotine), but our new study suggests that the Neanderthal genome has had less impact on our health today than previously thought," explains Mikkel Heide Schierup, Professor, Bioinformatics Research Centre (BiRC), Aarhus University.

A better picture

Postdoctoral fellow Laurits Skov of BiRC developed this relatively new method for tracing archaic fragments of genomes from Neanderthals, and this comprised the basis for the new study. He and Moisès Coll Macià, a PhD student at BiRC, took the new method to Iceland, where the biopharmaceutical company deCODE Genetics has genetic data and health information for more than half the population.

"deCODE Genetics has the world's best and largest genomic data set. An example of this is that they know which gene copy comes from the father and which from the mother. They also know phenotypical and health information for each sample such as height, blood cholesterol or depression. By combining deCODE Genetics' data and expertise with our new method, we started a project 10 times larger than previous studies of Neanderthal DNA in the human genome," explains Laurits Skov.

The researchers tried to associate Neanderthal sequences with various physical traits or increased disease incidence from the deCODE database. Unlike previous studies, they found that the Neanderthal DNA within Icelanders has little impact on human biology.

"Many of the Neanderthal mutations previously found in the modern human genome have been thought to be associated with increased risk of disease. But our study shows that human gene

variants located right next to the Neanderthal genes explain this increased risk much better," says Moisès Coll Macià.

The traits and diseases that Neanderthal content does affect reported in this study are a slightly lower risk of prostate cancer, lower concentration of haemoglobin, shorter height and slightly faster coagulation of blood plasma.

Other archaic humans on the scene

Among all fragments, the researchers found that a non-negligible part were more associated with another prehistoric human rather than the Neanderthals: the Denisovans.

"So far, Denisovan genes have mainly been found among Australian Aborigines, people from eastern Asia and people in Papua New Guinea. To explain this Denisovan component in Iceland, we think that Neanderthals had descendants with Denisovans before they met Homo sapiens, and they transferred both Neanderthal and Denisovan DNA to the children," explains Mikkel Heide Schierup.

Another possibility is that Homo sapiens met Denisovans long before modern humans met Neanderthals.

"Both explanations are equally likely, and both explanations are a great surprise. It is well known that a group of our ancestors left Africa and met Neanderthals in Europe about 50,000 years ago and mixed. However, it is a great surprise that either of these groups also had descendants with Denisovans, who lived in a very distant area (Siberia), and this is relevant scientific news," says Mikkel Heide Schierup.

Older mothers and younger fathers

One major feature of the high quality of the deCODE data is the ability to distinguish which of the two copies of an individual's DNA come from the father and which from the mother. When one of the DNA copies contained a Neanderthal sequence and the other a human sequence – for the same genomic positions – the researchers were able to compare modern humans and Neanderthals face to face. Based on the difference in the number and type of mutations accumulating differently in modern humans and Neanderthal fragments, the study reveals new

information about the parental age of these two human groups.

"The analysis suggests that, 100,000 to 500,000 years ago, Neanderthal women became mothers on average at a later age than contemporary Homo sapiens women did in Africa. In contrast, the Neanderthal men became fathers at a younger age than their modern African cousins," says Moisès Coll Macià.

Almost a folk hero

The study was based on a total of 3 months of field studies at deCODE Genetics in Iceland by the two young researchers from Denmark, Laurits Skov and Moisès Coll Macià. The 3-month stay in Iceland was a rich learning experience for both, although they did not actually have to collect data, but only analyse and compare existing data.

"The study was far from a classic field study, and in fact we could easily have performed the analysis online – instead of going to Iceland. However, deCODE Genetics requires their investigators to be physically present to access their data, since data security is their top priority. In addition, they are very selective about who they collaborate with and for which studies," explains Moisès Coll Macià.

deCODE Genetics has genetic data from more than 160,000 adult Icelanders, or more than half the population, at their headquarters in Reykjavik. The company was founded 25 years ago by neurologist Kári Stefánsson to use genetic data to develop new methods for identifying, treating and preventing diseases. Initially, you might think that a private company would meet great resistance in being the custodian of the genetic data of an entire population.

"In the taxi to deCODE Genetics, the driver virtually referred to Kári Stefánsson as a folk hero, and it dawned on us that the Icelanders consider deCODE Genetics to be a huge benefit for the country. I think they have been incredibly good at being both open and inclusive and using public events to show how the genetic studies can benefit Icelanders by improving personalized treatment in the future. Iceland can provide many lessons for other countries planning to do the same thing," says Moisès Coll Macià.

"The nature of Neanderthal introgression revealed by 27,566 Icelandic genomes" has been published in Nature. In 2018, the Novo Nordisk Foundation awarded a grant to Mikkel Heide Schierup for the project The Extraordinary Evolution of Human Sex Chromosomes.

New cancer target: fatty acids need to be synthesized for brain metastasis

Breast cancer often metastasizes, and when this reaches the brain, the survival outlook is poor. New research shows a new way to prevent cancer from attacking the brain.

BY KRISTIAN SJØGREN

People with human epidermal growth factor receptor 2 (HER2)-positive breast cancer have few treatment options, and they have a high risk of metastasis, including into the brain. These people have an extremely poor prognosis, and the blood–brain barrier prevents drugs from reaching the brain.

However, good news may be on the way since researchers have figured out that growing cancer cells need to increase the production of fatty acids in the brain by boosting the relevant cellular machinery.

This discovery opens up a potential therapeutic target for combatting a deadly disease.

“This study aimed to understand what happens in the brain when the cancer metastasizes and what the cancer cells require to survive and spread. We found that the cancer cells upregulate the genes that control the synthesis of fatty acids in the brain, and limiting this ability to produce more fatty acids may enable us to inhibit their growth or kill them,” explains co-author Raphael Ferreira, Postdoctoral Fellow, Harvard Medical School, Boston, United States.

Gino B. Ferraro, Ahmed Ali and Alba Luengo are the first authors, and the study has been published in *Nature Cancer*.

Cancer cells boost the production of fatty acids

The researchers investigated the genetic expression of breast cancer cells, metastatic cancer cells in the brain and cells in healthy tissue by using mice. They also verified their results in samples from people. The genes controlling fatty acid synthesis were overexpressed in the metastatic cancer cells in the brain compared with breast cancer cells.

Fatty acids are an essential part of the structure of all cells, and cancer cells require lots of fatty acids since they grow much faster than normal cells.

Raphael Ferreira explains that cancer cells are extremely adaptable, and the brain has many fewer fatty acids available than breast tissue. The cancer cells therefore upregulate the relevant genes to accelerate their growth.

“Cancer cells are extremely good at utilizing the available nutrients. The brain lacks fatty acids, which causes the cancer cells to overexpress the genes for fatty acid synthesis, thereby enabling access to the fatty acids required to maintain rapid growth,” says Raphael Ferreira.

Stopping cancer from metastasizing

The discovery leads to interesting therapeutic perspectives, which the researchers also examined by genetically inhibiting the fatty acid synthesis in the brains of mice with metastasized breast cancer. This prevented the cancer cells circulating in the bloodstream from metastasizing into the brain, enabling the mice to survive longer.

In another experiment, the researchers gave the mice commercially available substances that can penetrate the blood–brain barrier and inhibit fatty acid synthesis. This reduced synthesis but not as effectively as manipulating genes.

“This proof-of-concept study shows that fatty acid synthesis can be inhibited and thus prevent HER2-positive breast cancer from metastasizing into the brain. The next step is to find substances that can more easily penetrate the blood–brain barrier and effectively inhibit synthesis,” explains Raphael Ferreira.

Aiming for the Achilles’ heel

Raphael Ferreira says that the discovery is relevant for other purposes than understanding the importance of fatty acids for HER2-positive breast cancer metastasis.

The results provide greater insight into the importance of the microenvironment for cancer and how this knowledge can be used to aim for the Achilles’ heel of different types of cancer.

“We show that fatty acid synthesis is crucial for metastatic cancer in the brain, but this may differ for other types of cancer and metastasis in other organs. The aim is to identify the weaknesses of different types of cancer and then attack these vulnerabilities,” says Raphael Ferreira.

“Fatty acid synthesis is required for breast cancer brain metastasis” has been published in Nature Cancer. Raphael Ferreira is associated with the Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Kongens Lyngby, which is supported by the Novo Nordisk Foundation.

Fewer people ***develop dementia*** – but researchers do not know why

Fewer people in Denmark are being diagnosed with dementia today than previously. The reasons remain unclear, but researchers are striving to determine how to reduce the incidence of dementia further.

BY KRISTIAN SJØGREN

Since 2004, the number of people developing dementia in Denmark has declined substantially.

A new study reveals that the incidence of dementia in Denmark declined by 23% among men and 34% among women.

Some of this may result from demographic changes, but even accounting for this, the incidence of dementia has declined by about 20%.

The study also shows that improvements in traditional risk factors for dementia have

contributed to reducing the incidence in Denmark, but even these cannot explain the decline. There must be some other explanation, but researchers have not found it yet.

"We have identified some modifiable risk factors that may prevent dementia, but they do not explain the entire recent decline. We therefore need to think outside the box to determine why fewer people develop dementia today than previously so that we can accelerate these trends and enable a further decline," explains a researcher behind the new study, Emilie Rune Hegelund, postdoctoral fellow, Section of Epidemiology, Department of Public Health, University of Copenhagen.

The research has been published in *Alzheimer's & Dementia*.

Dividing a cohort according to risk factors
Emilie Rune Hegelund and colleagues examined data from Statistics Denmark to identify 1.8 million people 65 years and older who lived in Denmark at any point from 2005 to 2018.

They then used data from the Danish National Patient Registry, the Danish Psychiatric Central Research Registry and the Danish National Prescription Registry to identify individuals who had developed dementia during the study period. The researchers also mapped these people's education level, household wealth, prescription drug use and whether they had experienced a stroke and used this to create four risk domains covering many of the well-established modifiable risk factors for dementia.

- Education. Higher educational attainment is associated with a lower risk of dementia.
- Wealth. Individuals with higher household wealth or income have a lower risk of dementia.
- Cerebrovascular health. Whether a person has had a stroke generally indicates a person's cerebrovascular health, which is negatively associated with developing dementia.
- General health. Prescription drug use indicates general health, and a person's risk of developing dementia is associated with poor general health.

"The 2020 Lancet Commission on Dementia Prevention, Intervention and Care published a life-course model of how to prevent dementia based on 12 risk factors, and we summarised these traditional modifiable risk factors in four risk domains. We then categorised the study population into low, medium and high in three of the domains, and cerebrovascular health was based on stroke versus no stroke. We did this for each year and simultaneously examined whether individuals in each year had been diagnosed with dementia," says Emilie Rune Hegelund.

Risk factors alone cannot explain the decline
The results show that the declining incidence of dementia cannot solely be attributed to the fact that the high-risk group is doing better today than before. Even the low-risk group has experienced a declining incidence of dementia since 2005.

In addition, this research shows that the four risk domains alone cannot explain the decline in the incidence of dementia, so something else must underpin this societal trend.

> Maybe people are getting better at keeping their body and brain going than they used to be. Far more older people exercise today than before.

The researchers examined the various birth cohorts and found that the decline primarily resulted from 65-year-olds and 75-year-olds developing dementia less often than 65-year-olds and 75-year-olds in the past.

The result remained valid even when the researchers considered the four risk domains.

"So something about these younger birth cohorts unrelated to the traditional risk factors has reduced their risk of dementia," explains Emilie Rune Hegelund.

Even fewer people with dementia in the next generation?
Emilie Rune Hegelund speculates that more physical activity and people taking better care of their brains may be why fewer people develop dementia.

"Maybe people are getting better at keeping their body and brain going than they used to be. Far more older people exercise today than before," says Emilie Rune Hegelund, adding that it will be interesting to see whether the decline in dementia continues.

"Today's 65-year-olds have a lower risk of developing dementia than the current 85-year-old cohort did in the past, and this is independent of the traditional risk factors. It will be exciting to see whether the present generation of 45-year-olds will also have a lower risk in 20 years, even though we may still not be able to explain why," says Emilie Rune Hegelund.

Striving to discover the causes
According to Emilie Rune Hegelund, the perspective in the new study is that the researchers have confirmed that some modifiable risk factors for developing dementia are associated with the risk of developing dementia in Denmark, but they cannot entirely explain the positive downward trend.

Researchers and doctors therefore need to continue thinking outside the box to identify the unknown factors that appear to prevent dementia.

"If we find these factors, they can potentially be used to determine how to reduce the incidence of dementia further. The problem now is that we are doing something right but do not really know what it is," concludes Emilie Rune Hegelund.

"The plasticity of late-onset dementia: A nationwide cohort study in Denmark" has been published in Alzheimer's & Dementia. In 2018, the Novo Nordisk Foundation awarded a Challenge Programme-grant to co-author Rudi Westendorp for the project Harnessing the Power of Big Data to Address the Societal Challenge of Ageing.

Interaction between ***fungi and people*** more important than previously thought

Most of the bacteria present in our gut and on our skin help us to combat disease. However, just as we were getting accustomed to how important bacteria are for people's lives, another actor has arrived on the scene. New research shows that we may have given far too little priority to studying how fungi influence our bodies. They may prove to be at least as important to our health and well-being as bacteria.

BY MORTEN BUSCH

Fungi diverged evolutionarily from plants more than a billion years ago and have since coevolved with animals as an integral part of all ecosystems – including people. Like bacteria, fungi therefore inhabit exactly the same barrier surfaces such as the skin and intestinal system. Although alterations and imbalances in the composition of fungi in the intestines have previously been associated with diseases such as inflammatory bowel disease, colorectal cancer and allergy, little has been known about the molecular mechanisms. Now, however, there has been a breakthrough.

"Ten years ago, we sought proteins that interact with one of nature's most common substances: chitin. Chitin is abundant in our food: for example, in the skeleton of crustaceans and in fungi. Back then we found that chitin can bind to fibrinogen C domain-containing 1 (FIBCD1) protein. Our newest research shows that FIBCD1 is probably one of the keys to understanding how the human body interacts with fungi," explains Jesper Bonnet Møller, Associate Professor, Department of Cancer and Inflammation Research, Institute of Molecular Medicine, University of Southern Denmark, Odense.

Less fungi means reduced inflammation

Chitin is the second most common natural biopolymer in the world – only surpassed by cellulose. Researchers are very interested in how chitin affects people because chitin is a very important component of the cell wall of fungi. However, researchers have found little about how FIBCD1 interacts with chitin inside the human body.

"In our new research, we tried to determine how FIBCD1 affects the interaction between people and especially the fungi in our gut. To study this more closely, we examined people's intestinal system to determine whether FIBCD1 is actually present there or whether it is mostly present elsewhere in the body."

The measurements produced a clear result: FIBCD1 is mostly present in the membranes of the epithelial cells on the surface of the human gut. To investigate whether and how FIBCD1 responds to the presence of microorganisms such as bacteria and fungi, the researchers decided to investigate mice. Mice, unlike people, do not naturally have FIBCD1 in their intestines. Adding FIBCD1 had substantial effects.

To investigate whether the changes in the composition of fungi actually influenced the health and well-being of the mice, the researchers subjected the mice to an inflammatory condition in the intestinal system similar to ulcerative colitis – the inflammatory bowel disease that afflicts many people.

Major implications for health and disease

The new research may provide good news to people with inflammatory bowel diseases such as Crohn's disease and ulcerative colitis – diseases affecting an ever-increasing proportion of the population in our modern society. However, the reason for this increase is unknown. Most people have chitinases that can degrade chitin and several immune receptors that recognize chitin, causing the immune system to react.

The new experiments have initially helped to identify FIBCD1 as a new and previously undescribed pattern recognition receptor that the human intestinal system uses to not only recognize but apparently also control colonization by fungi and thereby reduce intestinal inflammation.

“Although fungi only comprise about 1–2% of the human microbiome by number, the fungi are significantly larger than both bacteria and viruses. Their importance is therefore often underestimated.”

According to Jesper Bonnet Møller, the importance of fungi has not only been underestimated in connection with inflammatory bowel disease but also during antibiotic treatment, which can increase the quantity of fungi in the intestines.

“Modulation of the fungal mycobiome is regulated by the chitin-binding receptor FIBCD1” has been published in the Journal of Experimental Medicine. The Novo Nordisk Foundation awarded a grant in 2015 to Jesper Bonnet Møller for the project The Role of the Novel Chitin Receptor FIBCD1 in Inflammatory Bowel Disease and Helminth Infections and another grant in 2019 for the project FIBCD1-mediated Regulation of Intestinal Fungi in Inflammatory Bowel Disease.

Future industries can harvest ***solar energy*** and use CO_2

Why not rethink our industrial production systems completely? Instead of being based on fossil fuels and release of immense amounts of CO2 into the atmosphere, they should be based on harvesting solar energy and the consumption of CO2. It would be far better for the climate – and it is becoming a realistic option.

BY BIRGER LINDBERG MØLLER

Climate change and its effects on our everyday lives is apparent to everyone. Fortunately, renewable energy sources like solar and wind energy can now provide cost competitive electricity. Yet fossil fuels are still used to cover 80% of the total global energy demand and sold at low prices. This severely hampers international efforts to reduce the global CO2 emissions. There is an urgent need for development and implementation of new climate-friendly production systems, not for patch solutions. We need innovative thinking to address the root of the problem!

The wonder of photosynthesis

But how do we turn the production system around? As a plant biochemist through a long life, I have repeatedly realized that if we look at the right places in nature – and for me it is especially in the world of plants – we find what we seek. This is also true now and is in fact quite straight forward. Because the production process we seek to harness is taking place right in front of us.

The process is photosynthesis and it is the basis of life on Earth. Organisms such as cyanobacteria (bacteria that perform photosynthesis), green algae and plants use solar energy and ambient CO2 to be self-sufficient through photosynthesis and the uptake of mineral salts.

In essence, we need to move from the current Anthropocene epoch – where humans are our planet's paramount influencers – to the Planta-Algaecene epoch powered by plants and algae. Here, solar-driven production of biomass and natural compounds based on CO2 will become mainstream and the basis of a circular bioeconomy.

This new type of fossil-free production would produce the compounds and products that we already use in our everyday lives such as flavours (vanilla, cinnamon, hops), colours (carmine, indigo), stimulants (caffeine, cannabinoids), health promoting compounds (flavonoids, resveratrol), vitamins, and medicines (morphine, taxol). Importantly, it would also produce new high-value pharmaceutical compounds and new types of biodegradable polymers.

In addition to this, the production would provide additional amounts of biomass and biofuels to support the required industrial transition.

The real game-changer: High-value compounds

The sun is by far our largest renewable energy source. The amount of solar energy hitting the Earth´s surface is per unit of time 5000 times higher than currently consumed by humanity.

This gives an obvious window of opportunity we must seek to benefit from. We can do so by understanding the diversity and uniqueness of nature and by developing photosynthesizing organisms into "green yeast cells" that can produce both biofuels and high-value natural compounds. They can all be powered solely by solar energy and – best of all – by using CO2 rather than emitting it.

Plants naturally produce medicinal compounds, dyes, flavorings, antioxidants, texture enhancers, proteins, carbohydrates and oils. Complementary production of the high value compounds can revolutionize future agricultural and pharmaceutical production. This will be the real game-changer!

Development of efficient parallel production of high-value natural products and commodities such as oils offers the opportunity to make the transition to solar energy based production economically viable within the next decade. This can help neutralizing the immediate initial direct costs associated with switching to a green production system – and simultaneously boost the sustainable production of inexpensive oil-containing biofuels.

Like in the development of wind turbines and solar cells, the pioneering attempts to use plants and algae as biomass for commercially viable

production of biofuels have been met by severe setbacks and overselling.

The pieces of the puzzle
The good news is that many of the pieces in this puzzle have now been identified. Research has cracked many of the codes for how to do this in relevant, economically viable and environmentally sound ways in close collaboration with nature itself.

In nature, cyanobacteria, algae and higher plants have inherent abilities, that make them the world's best chemists. They use solar energy to produce everything they need themselves. From large quantities of carbohydrate, fat or protein to tiny amounts of thousands of different unique bioactive natural compounds, that the plant uses to sustain life and fend off attackers. These natural compounds often accumulate in complex mixtures, which makes them difficult and environmentally harmful to isolate.

Furthermore, it often requires an incredible large number of plants – and thus agricultural land – to provide the quantities humans need. In that sense, the plants do not really care about us humans! As a consequence, the demands are often met by chemical synthesis from fossil resources.

In our research, we specialize in elucidating how high-value natural compounds are formed in nature and utilize this knowledge to develop specific cyanobacterial and algal strains to produce the compounds in large quantities as a sustainable replacement for the currently used fossil-based chemical synthesis.

Tomorrow's photosynthetic world
Here, too, we can lean on nature. Cyanobacteria, algae and plants also produce all the raw materials, such as aromatic amino acids, isoprenoids and fatty acids, from which the many natural compounds are formed. We can use these same raw materials to synthesize the new natural compounds we want and need.

The production itself will take place in cyanobacteria and in the chloroplasts of algae and plants. This requires that we use genetic engineering to introduce the extra genes that encode the formation of the desired bioactive natural compound. In this way, we build on the inherent synthetic potential of the cyanobacteria and of the chloroplasts of algae and plants.

Moreover, a successful outcome requires careful considerations on the choice of production organisms, cultivation methods, upscaling and purification and the question of whether to use plants, algae or cyanobacteria.
Cultivating plants requires land. To avoid this, the green production may be located at sea using algae and cyanobacteria. More precisely, the production can take place in coastal waters in environmentally contained glass-covered basins constructed on floating platforms of about three hectares each.

A crucial criteria is that the selected cyanobacteria or algae can be engineered to produce large quantities of a desired high-value natural compound. The algae cultures can then be cultivated by continually draining the green algae soup at the same rate as fresh filtered seawater is added to the basin. This enables production of both biomass and high-value compounds.

Parallel production of high and low value compounds
The biomass in the drained algae soup is then isolated and used as bunker fuel or protein source. The high-value compounds are isolated using two-phase systems and large-scale flash chromatography. The entire platform would be connected to wind turbines, which would power centrifuges and other equipment for extraction as well as supply energy to modify the algae soup by hydrothermal liquefaction, if so desired.

By encapsulating the basins on the floating platforms under a tight-fitting glass cover, the production system would be environmentally contained and comply with all European Union standards for cultivating genetically modified organisms.

Depending on which high-value natural products are desired, additional mineral salts or filtered wastewater may be pumped into the basin to add nutrients and speed up biomass production.

Today, the direct costs of algae-based production of biofuels on floating platforms are higher than the current sales price of fossil fuel. However, the low fossil price does not account for the cost of their negative environmental impacts. A carbon tax needs to be implemented as an indispensable element in the global strategy to reduce CO2 emissions.

Denmark has the know-how – now investments are needed
Internationally, this development will require considerable direct investments over the next 10–15 years. For this, Denmark is well placed to participate. Based on our strong tradition in the shipbuilding industry, we have world-leading marine design competencies that can be used for constructing the offshore production platforms connected to wind turbines. This will create many jobs in Denmark outside the large cities.

Throughout the development phase, the use of advanced techno-economic model systems to fast track system optimization and de-risk the scale-up is essential to end up with robust business models. Accompanied by pilot scale-up trials, this serves to secure major investments.

Deciding to expand Denmark by constructing an energy island located 80 kilometres offshore in the North Sea is a step in the right direction.

Such an energy island could easily be surrounded by a large number of hectare-sized basins filled with highly productive cyanobacteria and algae and create the basis of a sustainable future both economically and environmentally feasible production system. We are ready!

The outlined approach will ultimately deliver economic, social and environmental benefits to Denmark. Globally it will help the international community to meet the sustainable development goals and to build circular economies that are capable of operating within our planetary boundaries.

In 2019, the Novo Nordisk Foundation awarded a grant to Birger Lindberg Møller, Professor, Plant Biochemistry Laboratory, Department of Plant and Environmental Sciences, University of Copenhagen for the research initiative "The Black Holes in the Plant Universe" addressing key issues in green production.

chapter

ENVIRONMENT AND SUSTAINABILITY

ARTICLES

New food category to create a ***real alternative*** to meat

Several researchers are collaborating to develop a new food category that can feed more people using fewer resources. With the help of microorganisms, they will create plant-based food that satisfies our taste buds in a form that can become a real alternative to meat. You can hear more about this project in the Forskningsfortællinger podcast (in Danish), which also examines what it takes to get consumers to eat more plant-based food.

BY SABINA ASKHOLM LARSEN

Imagine sinking your teeth into fermented oats or yellow peas and having an eating experience that can be a real alternative to meat in terms of both taste and nutrition.

Dennis Sandris Nielsen, Professor of Microbiology and Fermentation at the Department of Food Science, University of Copenhagen, can imagine exactly this. Together with three research colleagues from Denmark and the Netherlands, he wants to develop a completely new food category based on fermentation with microorganisms.

"We have great ambitions to basically develop the foundations of a new plant-based food category that uses microorganisms to partly transform plant-based raw materials, so that we can adjust the composition of nutrients more optimally for humans," explains Dennis Sandris Nielsen, adding:

"Our aim is to create something that will make you not miss eating meat. Something that fulfils the same role on the plate. This is not a meat substitute; it is an alternative. It is something that gives you the

same eating experience and also meets the same nutritional needs you have."

Using farmland to grow food for people instead of animals

The major challenge addressed by developing the new food category is that the Earth's resources cannot keep up with the increased demand for food resulting from increased population in the coming decades – at least not if we use meat as an essential source of protein to the same extent as now. An increasing need to feed the world's population therefore requires a fundamental change in our eating habits and thus also our food production.

Dennis Sandris Nielsen and his colleagues will therefore improve understanding of how to use and process the proteins in plants and microorganisms in food production so that they can be used as a tasty alternative to meat. They are starting with oats and yellow peas.

"Today, oats and yellow peas are mostly used for animal feed. If we can develop something that people would like to eat directly instead of first feeding it to a chicken, cow or pig, then we can feed more people using the same land we use currently to produce animal feed," says Dennis Sandris Nielsen, who adds:

"Animal feed takes up one third of the area cultivated today, and if we use that feed directly for human consumption, we could theoretically feed about 4 billion more people using the same area."

These two plants were selected because they have a fairly high natural protein content and because they represent two major plant categories, with oats representing cereals and yellow peas representing legumes. The two plants are therefore intended as model substrates about which the researchers can learn and then later apply to other raw materials.

Specifically, the researchers will ferment raw or minimally processed plants with microorganisms to form food that is worth eating, both nutritionally and in terms of taste and structure.

"We want to link all this together to create structures that provide an interesting eating experience. And we will create taste and taste precursors so that, when you fry something, for example, the precursors will react with each other and form what then becomes the final taste when you eat or smell what is on your plate," explains Dennis Sandris Nielsen.

Putting new knowledge to work

When will consumers have the prospect of sinking their teeth into this new food category? The PROFERMENT project, in which Dennis Sandris Nielsen and his colleagues will carry out the initial research for the new food category, will start in January 2022 and will run for 6 years.

Dennis Sandris Nielsen thinks that the knowledge created in the project will be applied rapidly, including in an increasing undergrowth of small start-ups, both in Denmark and elsewhere. These companies, which are exploring similar avenues, will take the knowledge generated in the project and integrate it into products.

When the project ends after 6 years, Dennis Sandris Nielsen thinks that the researchers will have a very basic understanding of how the microorganisms interact with each other and with the plant raw material and how this process can be controlled.

"We will then have the tools that will enable us to take a more application-oriented approach to how we can turn this knowledge into products. We could do this, or it could involve companies, or other universities, or a mixed collaboration. We just want to put our knowledge to work, so people can just come to us and we will be happy to collaborate," concludes Dennis Sandris Nielsen.

Dennis Sandris Nielsen has received a grant from the Novo Nordisk Foundation Challenge Programme 2021 of DKK 56.3 million for the project PROFERMENT: Solid-state Fermentations for Protein Transformations and Palatability of Plant-based Foods. Apart from Dennis Sandris Nielsen, the three other project leaders in the PROFERMENT project will each contribute their scientific expertise to developing this new food category.

Plants need special ***help to survive*** climate change

The current trajectory of global climate change will strongly affect how well plants can adapt to their environment. Contrary to what one might think, however, the plants in the hottest regions may not be those immediately hit hardest. Experiments in central and southern Europe show that especially the plants in central Europe lack the proper genes to survive a drier and warmer climate. According to Detlef Weigel, who has studied plant development and adaptation for three decades, evolution cannot keep up and therefore needs help. To recognize outstanding research or technology contributions that benefit the development of biotechnological science for innovative solutions, Detlef Weigel is receiving the 2020 Novozymes Prize.

BY MORTEN BUSCH

"Will we all die of hunger if we don't use genome editing? No, of course we won't, but if we don't use this there are things that we have to give up."

Climate change forces plant populations to adapt to changes in temperature and rainfall. For three decades, 2020 Novozymes Prize recipient professor Detlef Weigel, who is director at the Department of Molecular Biology, Max Planck Institute for Developmental Biology, Tübingen, Germany has studied what causes plants to bloom and how the future climate will affect their survival.

"The answers that we got were really quite surprising. We know that it's going to become even drier around the Mediterranean; you might think these Mediterranean Arabidopsis populations are the ones that are most at risk because the climate is going to become even more extreme. But it turns out, most at risk are the populations in central Europe, because these plants basically have no genetic toolkit to deal with extended drought."

Trees in my petri dish

Fascinated by developmental genetics, Detlef studied at the University of Bielefeld and later Cologne. Here he studied under a leading figure, José-Antonio Campos-Ortega. He moved to the United States and did a postdoc at Caltech before moving south to the Salk Institute in La Jolla.

This turned out to be a brilliant move. Soon he and his colleagues discovered a master control gene that is essential for forming flowers in thale cress, Arabidopsis thaliana. A breakthrough came with a visiting postdoc Ove Nilsson from Sweden.

"As we were sitting over lunch and looking out on the ocean, I told Ove about this discovery he said: Well, I know how to transform these poplar trees; why don't we just put this gene into trees, and I think we both agreed that this was an extremely long shot, that probably nothing would happen." Maybe trees were just too different from Arabidopsis.

"Then all of a sudden he writes to me and sends these pictures and says: You will not believe what happened. I took your LEAFY gene. I put it into my little trees here and within weeks in my petri dish I saw flowers. Normally it would take years and years and years and the plants would have to grow really substantially before you would see any flowers, and now with this LEAFY gene we can make these little trees."

Rainfall of Tübingen and Madrid

However, Detlef Weigel's work on flowering made him wonder: are there invisible differences between plants depending on where they grow. To study this, Detlef Weigel moved back to Germany in 2002 and became Director at the Max Planck Institute for Developmental Biology in Tübingen, the town where DNA was first discovered. About five years ago, a PhD student Moi Exposito came to the lab and set up an extremely ambitious experiment.

"He took hundreds of these strains that we had collected from all over the world where we had the entire genomes and then he grew them either in Tübingen or he grew them in Madrid, and we simulated the rainfall of Tübingen and Madrid. One of the most important discoveries there was that it's rare to find genes that are helpful in both conditions, so normally if a gene helps you to survive better in Tübingen, that gene is actually not good to have in Spain."

Surprisingly, the Mediterranean populations are most at risk.

"Whereas in the Mediterranean it's already not so rare that you have drought, and so it's going to become worse, yes, but they already have a lot of genes and they should be able to adapt relatively quickly, whereas in central Europe, that is not really quite the case. That means that s plants move or we help them move, they would really be at risk."

"Of course, our goal should be until you know: limit the amount of CO2 that we put in the air, but we also know that things are complicated, that things might take time, and we need to have a few other aces up our sleeve to deal with climate change."

The tomato with that extra gene

The latest report of the Intergovernmental Panel on Climate Change specifically mentioned genome editing as a valuable tool to help plants to adapt faster.

"If I put myself into shoes of a consumer who is faced with the choice of here's a tomato and here is another tomato, but this tomato has an extra gene. Then I think it's normal, it's natural that the conservative approach is: well, why should I take the tomato with that extra gene? I think I'd rather stay with the normal tomato."

But the tomato that has not been genome edited to be more sustainable comes at a cost.

"The more land we use for agriculture, the less land there is for trees, for example, to grow, and trees are still one of the best methods that we have to remove CO2 from the air. It's a method that we know really, really works. So it would be absolutely shameful if we did not use these modern tools for biotechnology."

The full-length version of this article and a video can be found on the website www.scienenews.dk. The 2020 Novozymes Prize was awarded on Friday, 27 March 2020 to Detlef Weigel, Professor, Scientific Member, and Director, Department of Molecular Biology, Max Planck Institute for Developmental Biology, Tübingen, Germany.

Brewer's ***spent grain*** can become nutritious food

Brewing beer requires malting large amounts of barley, wheat or rye to add flavour. The by-product, brewer's spent grain, accounts for up to 85% of the total residue from brewing and has traditionally been discarded. Now researchers have found out how the high protein content of the spent grains can be used as for both people and animals. The process does not even involve any environmentally harmful chemicals – only clean water.

BY MORTEN BUSCH

Beer drinkers leave about 39 million tonnes of brewer's spent grain (BSG) in their wake each year. Although BSG is packed with both sugar and protein, it often just gets discarded. The grain residue is wet and sweet after the brewing process and therefore attracts microorganisms. The sugar can be extracted from the BSG, but extracting the protein has been a greater challenge. Now researchers have found a simple and highly effective way to do this.

"We have tried various pretreatment strategies including alkaline, acid and enzymatic. They are effective but resource-intensive, non-specific and not environmentally sustainable. Now we instead tried hydrothermal treatment, using just water and heat, and we can extract 65% of the protein. In addition, the process improves the quality of the protein extracted. Since this grain has a very high protein content, the hydrothermal process has considerable potential. The large volume of protein can be used either in animal feed or for fitness products for people," explains Solange I. Mussatto, Professor and Group Leader, Department of Biotechnology and Biomedicine, Technical University of Denmark, Kongens Lyngby.

Wet and rich in protein

A growing global population combined with emerging economies in low- and middle-income countries and urbanisation have increased the demand for protein. Currently available resources for protein are mainly food crops such as wheat, soybean and maize. However, the current traditional production capacity will not be able to meet the growing anticipated demand for protein-rich foods and products.

"An alternative to cultivating more land with traditional resources is to recover protein from biomass such as agricultural wastes and by-products from industrial processes. A challenge for processing these types of resources is to develop technologies that can cost-effectively and sustainably extract protein from biomass with high yields. We have therefore focused on using BSG to obtain protein," says Solange I. Mussatto.

Depending on the type of grain mixture, BSG contains 18–30% protein and is the most abundant sidestream from brewing, accounting for 85% of all brewing residue and 20 kg of wet weight per 100 litres of beer produced. This enormous amount of protein is often discarded, mostly because BSG has a very short shelf life since it is wet and rich in protein and sugars and therefore susceptible to attack by bacteria and fungi.

"Our goal was not just to find the method that maximises the extraction yield. Acid or enzymatic treatments can recover about 90% of the protein but using acid comes at a high environmental cost in terms of pollution and energy consumption, while using enzymes is not a cheap option. Conversely, a hydrothermal pretreatment process achieved lower yields but is a clean, precise and specific method," explains Solange I. Mussatto.

Higher than many other types

Hydrothermal vents are small cracks in the Earth's surface through which scalding water flows – often associated with volcanic activity. Hydrothermal processes in the laboratory are not as dramatic. The wet spent grain is mixed with water and heated to 60°C in a closed container, creating the necessary pressure to extract the proteins.

"In contrast to acid treatment, almost all the extract is protein, and not a single chemical is used, so this is clearly preferable to the acidic process. Although hydrothermal pretreatment achieves protein extraction of 64–66% versus 90% for acid treatment at 120°C for 1 hour, the hydrothermal process clearly has environmental benefits," says Solange I. Mussatto.

The researchers think that even more can be gained from the hydrothermal pretreatment process by adjusting the temperature, but since the protein content in BSG is so much higher than in many other types of residue such as agricultural wastes and by-products of industrial processes, the potential for protein extraction is enormous despite non-optimised process conditions.

"Extraction processes for protein from this type of biomass can recover a great deal of protein to meet the growing future demand. It can enrich animal feed to increase the protein content of meat, be added directly to food products in low-income countries or become a raw material for fitness products such as protein bars. In any case, this will reduce the amount of land that needs to be cultivated, which benefits the environment," concludes Solange I. Mussatto.

"Evaluation of different pretreatment strategies for protein extraction from brewer's spent grains" has been published in Industrial Crops and Products. All authors are affiliated with the Biomass Conversion and Bioprocess Technology group, Technical University of Denmark.

CO_2 From climate burden to ***valuable resource?***

The otherwise maligned gas CO2 has great potential if we can become more proficient at capturing and recycling it before it reaches the atmosphere. With the right processing, it can replace several fossil-based raw materials such as oil and methane so that we do not have to extract them underground. This episode of the Forskningsfortællinger podcast (in Danish) takes a closer look at how to imitate a well-known process from the human body to capture CO2 and how it can be converted to a building block for the chemical industry by combining electrochemistry and microbiology.

BY SABINA ASKHOLM LARSEN

CO_2 has a massive impact on our climate when released into the atmosphere. Researchers and companies are therefore working to find methods to capture CO_2 from factories and incineration plants on a scale that can seriously help to reduce emissions of CO_2 and other greenhouse gases. However, the technology still has a long way to go before reaching the necessary scale that can significantly contribute to reducing CO_2 emissions at the rate needed to achieve the climate goals outlined by the United Nations.

"About 40 billion tonnes of CO_2 are currently emitted per year. The United Nations Intergovernmental Panel on Climate Change (IPCC) has estimated that we must remove 14% of these emissions by capturing CO_2, about 5–6 billion tonnes annually. Right now we are capturing about 40 million tonnes, so we are nowhere near achieving this goal," says Christina Lunde, Senior Science Manager, Novozymes A/S.

Similar to a well-known process in the body

Christina Lunde is involved in developing a method that uses enzymes for capturing CO_2. Enzymes are proteins that help to catalyse processes in our bodies and in other organisms. When we eat, for example, enzymes help to break down the foods we ingest. Novozymes imitates this process in its efforts to develop enzyme-enabled carbon capture.

"We are fortunate that enzymes in nature can capture CO_2. They do this in our red blood cells, and they also have a function in our lungs. For example, when we do physical work, breaking down fuel and excreting CO_2, the carbonic anhydrase family of enzymes captures the CO_2, converts it into carbonate and conveys it to the lungs, where it is again converted into CO_2 and then released. We therefore have a family of enzymes with an important biological role that we can now use for capturing CO_2," explains Christina Lunde.

An enzyme-enabled carbon capture plant has an absorber unit and a stripper unit. In the absorber unit, the CO_2 is blown through a liquid, and

the enzyme converts the CO2 into carbonate, a component of hard water. The CO_2 is then transferred with the liquid to the stripper unit, and the enzymatic CO_2 is released through heating. The product is a pure CO_2 stream, which can then be compressed to produce liquid CO_2 and then either be stored or used in another way.

Christina Lunde adds that, because the enzymes are a natural part of our biological world, the method will not leave harmful chemicals in the wastewater discharged from the carbon capture process. This is advantageous when the enzymes are used to extract CO_2 from flue gas from incineration plants.

Another benefit of the method is reduced energy consumption. The enzyme-enabled carbon process can release CO_2 at a lower temperature than conventional carbon capture solutions, which are based on chemicals such as amines.

"Releasing CO_2 requires heating the liquid and releasing the gaseous CO_2. Conventional solutions use temperatures of 100–120°C, requiring a lot of energy to heat the liquid. The advantage of the enzyme-enabled carbon capture process is that the liquid only needs to be heated to 80°C. This saves energy, and the excess heat of many processes such as producing cement or steel can be used to release the CO_2 to provide a solution that is more sustainable," adds Christina Lunde.

Microorganisms can convert CO_2 from biogas plants into methane

Capturing CO_2 to avoid harming the atmosphere is one thing. Another is to take the CO_2 collected and process it into a raw material for other products. One such example is the CO_2 formed in biogas plants. With the right processing, the CO_2 that would otherwise be emitted from these biogas plants can be converted into methane that can be reused in the chemical industry – thereby eliminating the need to extract methane from underground.

Three researchers from Aarhus University and Aalborg University have combined their knowledge of electrochemistry and microbiology in the research project Redox Mediated Microbial CO2 Reduction (ReMeSh) to develop a process using microorganisms that can rapidly convert CO_2 from biogas plants to methane.

"Biogas comprises both CO_2 and methane, and to extract the methane, the CO_2 is currently filtered out and emitted into the atmosphere. The ReMeSh project bioelectrochemically upgrades biogas, converting the CO_2 into methane, so that the gas produced by the biogas plant is nearly pure methane," says Anders Bentien, Professor and Head of Section, Department of Biological and Chemical Engineering, Aarhus University, and one of the three researchers behind the ReMeSh project.

Existing technologies use hydrogen and microorganisms to convert the CO_2 from biogas plants into methane. These are the same components the researchers behind the ReMeSh project will use, but they will use alternative technologies to speed up the process.

The Novo Nordisk Foundation has awarded an Exploratory Interdisciplinary Synergy Programme grant of DKK 4,965,291 to Anders Bentien together with Michael Kofoed, Researcher, Department of Biological and Chemical Engineering, Aarhus University and Jeppe Lund Nielsen, Professor, Department of Chemistry and Bioscience, Aalborg University for the project Redox Mediated Microbial CO_2 Reduction (ReMeSh).

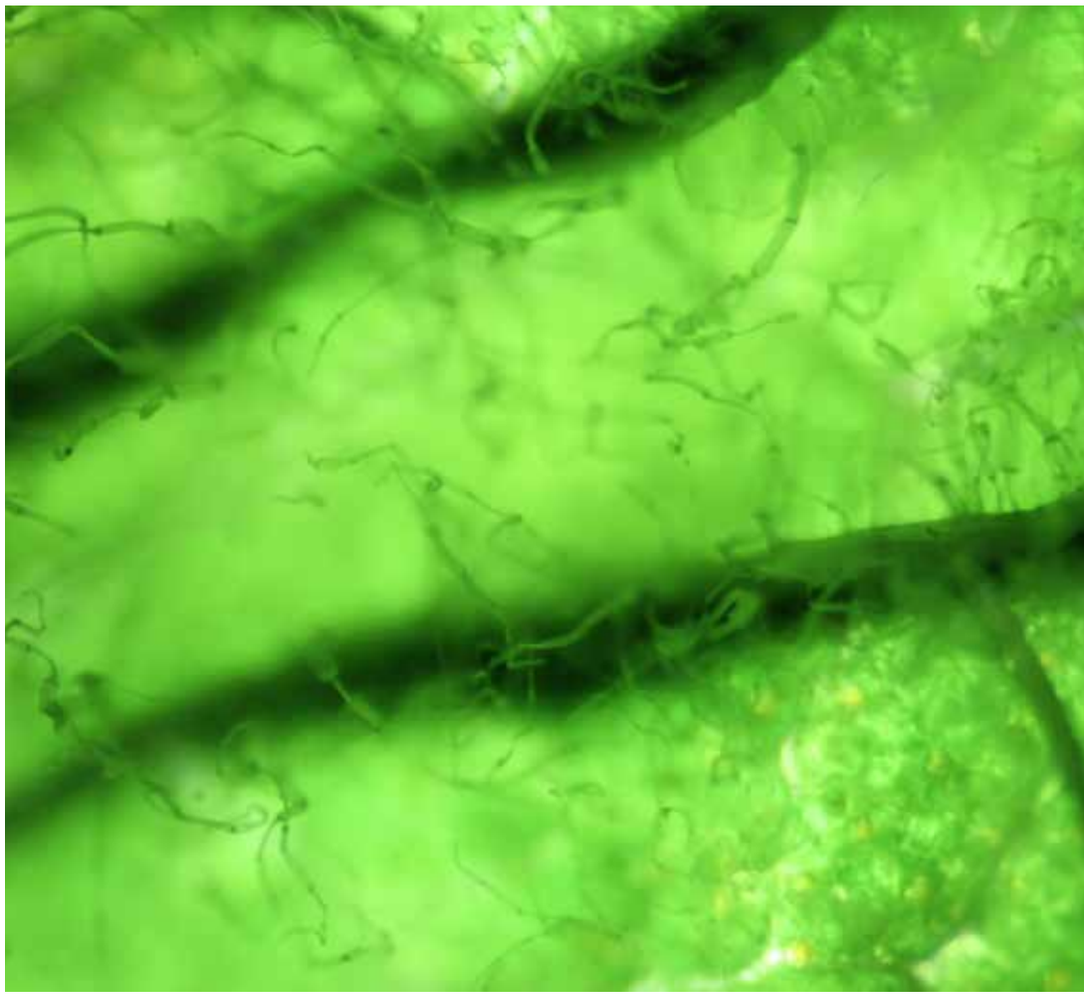
Green microalgae will reduce the ***environmental footprint*** of animal feed and food

Clearing rainforests to grow soybeans and then transporting them to the other side of the globe to be used as animal feed must be stopped. Now researchers have found an alternative feed source in the form of a microalgae with protein content exceeding 50% and high content of essential amino acids. The algae are grown in 100-metre-long plastic tubes that can be placed anywhere and do not pollute the soil or involve deforestation. This method of production is not yet economically viable, but when it becomes profitable, it can replace the current sources of animal feed and eventually also be included in food for people.

BY MORTEN BUSCH

The average person in Europe consumes more than 60 kg of soybean per year – without even realizing. Soybeans are a hidden source of protein in many people's diets because many farm animals such as chickens are fed soybeans. Unfortunately, these protein-rich beans are often shipped from South America, where the land cultivated with soybeans has increased 15-fold in the past 50 years. The environmental footprint from the soybeans and ultimately such animals as chickens is enormous. Now researchers have discovered a useful and sustainable alternative protein source.

"Microalgae have emerged as a promising alternative in recent years. We tried to grow commercially available algae strains, but these were outcompeted by another strain that was found to both grow rapidly and have very high content of



protein and essential amino acids. This is a huge benefit for sustainability, since these microalgae can be grown anywhere in long horizontal tubular reactors without polluting the environment," explains Malene Fog Lihme Olsen, Senior Specialist at the Danish Technological Institute in Taastrup, adding:

"Based on these results, we joined forces with the Department of Geosciences and Natural Resource Management at the University of Copenhagen and several industrial partners in the ReMAPP project (2018–2022), supported by Innovation Fund Denmark. The goal is to make algae cultivation economically viable, so that it can soon replace soybeans in animal feed and perhaps eventually also become a source of protein for people."

Nutritious

The discovery was made at a pig farm in Lolland. The research group had purchased several commercial strains outside Denmark to test which ones were best suited for cultivating high-protein animal feed at the northern latitudes in Denmark. Each time they tried to grow these strains on pig manure, the results were not as expected.

"We finally decided to try to determine which strain had emerged, since it was growing really well and apparently suited our latitude. Once we had identified it as the green microalgae Scenedesmus species, we then analysed the protein content, which turned out to be really high. More than half the dry matter is protein, and more importantly, 42% of the amino acids are essential," says Malene Fog Lihme Olsen.

Essential amino acids are the ones essential for people, since we can only produce 11 of the 20 amino acids that comprise protein. We therefore need to eat these essential amino acids to produce the proteins we need to keep our body healthy and well. However, the essential amino acids are not the only component that make the algae nutritious.

"Algae can also contain large quantities of polyunsaturated fatty acids, which are essential for blood pressure regulation, kidney function and the immune system. We cannot make these compounds ourselves and must therefore obtain them through food. They are a main reason why eating a lot of fish is recommended, and fish contain these essential fatty acids because they consume microalgae," explains Malene Fog Lihme Olsen.

20 times more protein than soybeans

Nutrition is not the only benefit of Scenedesmus and similar species. The microalgae can be grown in long horizontal tubular reactors and thus can be cultivated almost anywhere. They therefore do not necessarily compete with other types of agriculture and do not involve deforestation.

"In addition, we can use industrial sidestreams to supply the microalgae with nutrients, carbon dioxide and surplus heat, since we can channel manure and carbon dioxide from biogas and other substances through plastic bags to feed the microalgae. In fact, about 1.8 kg of CO2 is used to produce 1 kg of algae solids, so we can capture quite a bit," says Malene Fog Lihme Olsen.

In addition, microalgae are very adaptable to variation in light conditions, temperature, salinity and growth media. These properties can increase the potential for choosing different algae species that can be grown almost all year round, even at northern latitudes.

"With the right approach, microalgae as new protein crops will have far higher annual protein productivity than conventional protein crops, with up to 20 times more protein than soybeans and 40 times more than corn," explains Malene Fog Lihme Olsen.

The microalgae Scenedesmus can be grown in long horizontal tubular reactors and can be cultivated almost anywhere. They therefore don't necessarily compete with other types of agriculture and do not involve deforestation.

"Outdoor cultivation of a novel isolate of the microalgae Scenedesmus sp. and the evaluation of its potential as a novel protein crop" has been published in Physiologia Plantarum. In 2016, the Novo Nordisk Foundation awarded a grant to the Department of Geosciences and Natural Resource Management of the University of Copenhagen for the project Harnessing the Energy of the Sun for Biomass Conversion. The project, which also received funding from the Villum Fonden, is a collaboration between the University of Copenhagen and the Danish Technological Institute.

LITTLE NINJA microProtein is a new tool for biotechnologists

Researchers have identified a microProtein that can be used to make crops shorter and bushier and enhance the production of side shoots. This microProtein has the potential to increase crop yields.

BY KRISTIAN SJØGREN

Feeding the future population of the world requires optimally exploiting all the food resources available on the planet. This is especially true for crops such as wheat, oats, rye and all the other grass crops, which together account for a very large proportion of global food production.

Now researchers from the University of Copenhagen have identified LITTLE NINJA, a microProtein that regulates how tall grasses become and how many side shoots they produce. LITTLE NINJA thus also has the potential to regulate how productive these grasses are.

The discovery of LITTLE NINJA gives biotechnologists an extra tool for designing the crops of the future.

"We have identified a microProtein that determines the growth phenotype of plants and helps to regulate a plant's hormone signalling. LITTLE NINJA has expanded our understanding of what microProteins can do and how we can utilize them biotechnologically," explains Stephan Wenkel, Associate Professor, Copenhagen Plant Science Centre and Department of Plant and Environmental Sciences, University of Copenhagen.

The research has been published in the *Proceedings of the National Academy of Sciences of the United States of America*.

Evolution has created microProteins

Understanding the new research results requires understanding what microProteins like LITTLE NINJA are.

MicroProteins are short single-domain proteins that are sequence-related to larger, often multidomain proteins.

During the course of evolution, the size of genomes has increased by processes such as whole-genome duplications or other local amplifications. This has led to increases in the number of genes and the origin of gene families that encode similar proteins. However, in some cases, evolution has also caused some genes to be significantly shortened in the process, resulting in "gene stumps" that produce very small proteins. These microProteins can take on new roles as regulators of the original larger proteins.

This new microProtein may have functions that differ from those of the original larger protein. For example, the larger protein may have a vital function for the organism and might act by binding together in pairs. In this case, the microProtein can bind to the larger protein and modulate its effect.

"In some cases, microProteins can act on the larger proteins like brakes in a car, maintaining the effect of the proteins within optimal levels," says Stephan Wenkel.

Discovered LITTLE NINJA in a model crop plant

The researchers worked with Brachypodium, a genus of plants in the grass family that is a model organism for grasses. Brachypodium is used in biotechnological research that advances researchers' knowledge on the genetics of crops that farmers have in their fields.

The researchers set out to discover microProteins in Brachypodium and study whether these microProteins could have useful biotechnological applications in crop plants.

This was done in a computational approach in which the entire genome of Brachypodium was searched for gene sequences that could be identified as stump versions of known proteins.

This exercise resulted in the identification of LITTLE NINJA.

The researchers' further studies also identified that LITTLE NINJA is a dominant regulator of the jasmonic acid hormone signalling pathway in grasses.

More LITTLE NINJA can make plants more productive

Having identified LITTLE NINJA, the researchers could begin to manipulate the gene encoding for LITTLE NINJA in various crop plants to see how this affects plant growth and function.

The researchers made barley and rice and the model organism Arabidopsis produce more LITTLE NINJA than usual, resulting in shorter and bushier plants with enhanced production of side shoots.

"This is a desired feature from a crop science perspective. Grasses that are shorter and bushier are more wind-resistant. Enhanced production of side shoots also means that they can have more flowers and thus more fruit. Increasing the levels of LITTLE NINJA in grasses might ultimately lead to higher yields," explains Stephan Wenkel.

Stephan Wenkel further explains that LITTLE NINJA plays a role in hormone signalling in grasses and therefore plays a role in developing flowers and root systems.

An extra tool for biotechnologists

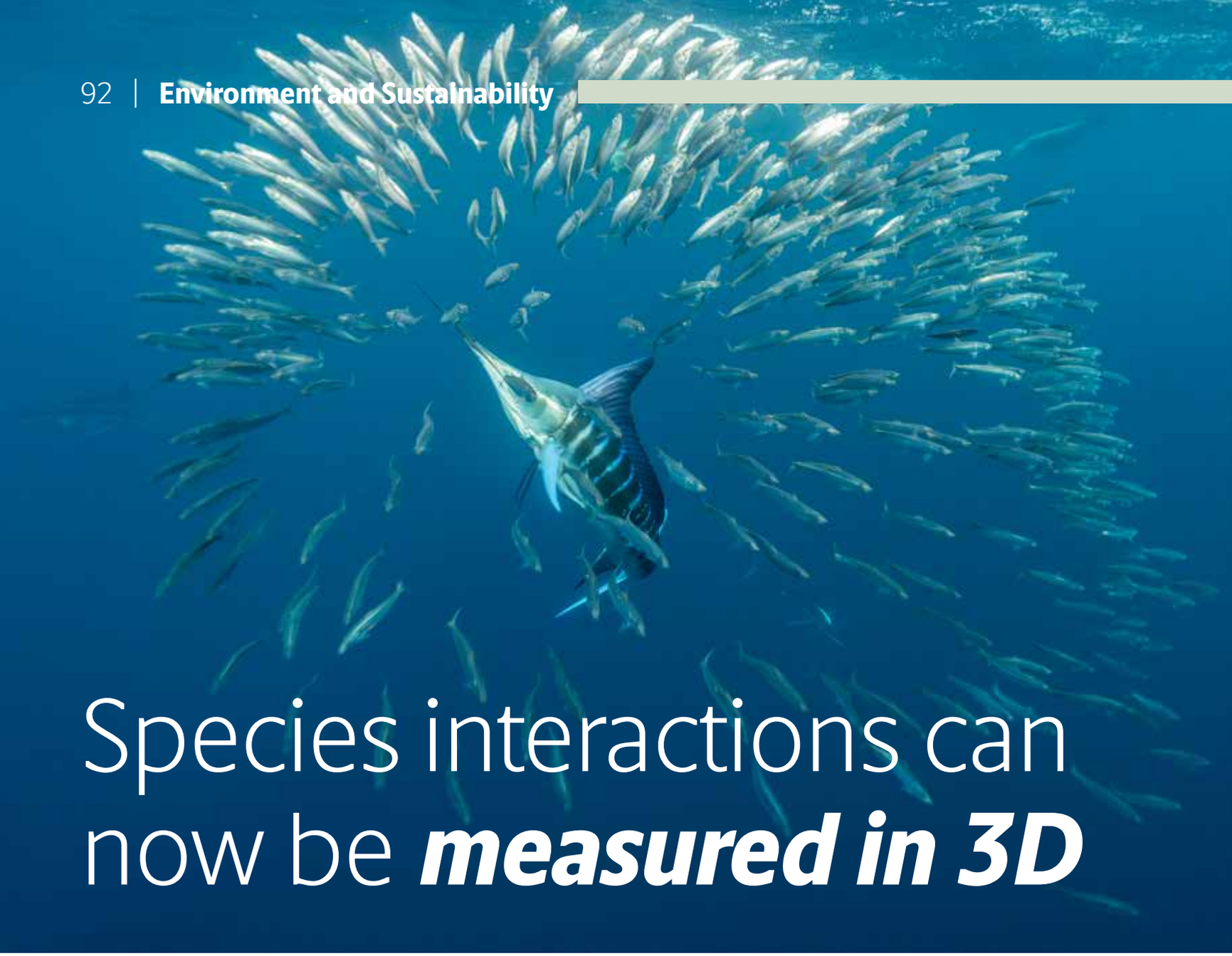
The new results also bring something new to the biotechnological table.

The researchers used CRISPR gene-editing technology to cut a piece of the gene encoding the original larger NINJA protein and transform it into LITTLE NINJA.

Many specific genes have multiple copies of the genetic code, and researchers may be able to transform the code for a larger protein into the code for a microProtein, thereby giving plants new or enhanced biological functions.

"This is the biotechnological perspective. In the past, we did not fully understand how microProteins affect plants and how we can utilize them biotechnologically. We understand that much better now," says Stephan Wenkel.

"Heterologous microProtein expression identifies LITTLE NINJA, a dominant regulator of jasmonic acid signaling" has been published in the Proceedings of the National Academy of Sciences of the United States of America. In 2018, the Novo Nordisk Foundation awarded a grant to Stephan Wenkel for the project Increasing Folate Levels in Crop Plants by MicroProtein Engineering.



Species interactions can now be ***measured in 3D***

BY MORTEN BUSCH

The oceans cover more than 70% of the Earth's surface. Nevertheless, we probably know more about the surface of the moon than we do about the ocean floor despite the oceans' enormous importance for climate change. Marine biologists are now trying to remedy this by equipping marine animals with high-resolution biologgers that measure light intensity, prey availability, and ocean temperature. A new study of elephant seals' dives through 7 months of migrating 10,000 kilometres provides unprecedented insight into the life deep in the oceans that risks being affected by global climate change.

You only see them when they are on the beach for a few months, tending to their pups or moulting. The rest of the time, elephant seals (Mirounga angustirostris) spend 7 months in the ocean travelling to and from their foraging grounds off the coast of Alaska. However, observing their behaviour first-hand is very difficult because they spend most of their time underwater. High-resolution biologgers have recently enabled researchers to obtain new insight into both what the elephant seals experience and how the oceans affect them.

"Our new study provides fascinating and important knowledge about the diving behaviour of elephant seals as they explore the open ocean. But perhaps even more importantly, the recent development and use of instruments such as biologgers provides invaluable insight into species interactions in the marine ecosystem. We hope that this knowledge will help the public and politicians make environmentally conscious decisions," explains Roxanne Beltran, Department of Ecology and Evolutionary Biology, University of California, Santa Cruz, United States.

Diving requires a lot of energy

The marine biologists aimed to study the fascinating 7-month migration of elephant seals from their breeding grounds in California 10,000 kilometres north across the North Pacific, mostly underwater – only interrupted by short minute-long trips to the surface for air before the seals again forage in the twilight zone. The researchers monitored the seals in great detail using the biologgers.

"The high-resolution biologgers enabled us to continuously measure the fat percentage and 3D movements of 71 female elephant seals during their foraging migrations. After shedding all their fur during the annual moult, seals set off on this migration – thin and debilitated – and dive continuously to nearly 1 kilometre underwater," explains Roxanne Beltran.

One reason elephant seals dive so deep and dive almost continuously throughout their 7-month trip is to avoid predators, including white sharks and killer whales, which tend to remain at the surface rather than in the dark and cold depths. As a result, the seals use a lot of energy diving into the deeper and colder water.

"Our measurements showed that seals always balance acquiring energy and ensuring safety, so they rest when it is dark and forage at depth when it is light. Throughout the migration, their fat percentage improved from 21% when they embarked on the migration to 32% at the end. As they gained more fat, they rested more than 100 metres deeper, where it was 300 times darker, so seals with a high fat percentage gave priority to safety over conserving energy," says Roxanne Beltran.

To Hell and back again

The new study is not the first study using biologgers to measure both the swimming depths and fat percentages of marine animals. The innovation is that the researchers simultaneously monitored the activity timing of the mammals with great accuracy and especially the light intensity during the drift phase, in which they rest.

"The measurement took place over a long period of time and across thousands of kilometres, and was therefore far from easy. The statistical models needed to account for the fact that time is circular, meaning that 23:00 is equally close to 01:00 as it is to 22:00," explains Roxanne Beltran.

The result was unprecedented insight into how marine mammals actively use the lightscape to determine whether to spend more or less time in the surface water, which is brighter and warmer but full of predators.

"We are not sure, but the seals are probably especially vulnerable during drift dives when they either sleep or forage for food. The female seals have just been to Hell and back again within months of suckling their pups and mating and nursing. They are at their absolute thinnest and need to regain their fat during the 7- month migration. Our results show that they constantly fine-tune their diving patterns to use the safety of darkness to rest deep below the surface," says Roxanne Beltran.

90% of world trade is seaborne

In the coming years, Roxanne Beltran and her colleagues hope to use the high-resolution biologgers for other purposes than measuring animal behaviour. By having many different marine animals carry the instruments, the researchers hope to map the oceans in terms of their noise, oxygen concentration, salinity and temperature.

"Many of my colleagues are actually quantifying global warming in 3D using temperature sensors attached to seals. We may also be able to use animal-borne video cameras to watch how species interact in the open sea. We can detect bioluminescence using light-sensitive tags. The possibilities are virtually endless, and leveraging new technologies can help us answer some really important questions about the oceans and how climate change will affect them," says Roxanne Beltran.

As much as 90% of world trade is transported on ships that emit noise in the open sea. We do not yet know whether this noise affects animals, frightening them to behave differently. It is too early to say whether this knowledge will change human behaviour, but Roxanne Beltran is optimistic.

"Convincing people to protect something they cannot see and do not understand is difficult. Bringing this to people's attention may help them to understand the possible consequences of their actions and hopefully motivate them to make different choices, whether buying things that are more sustainable or choosing to buy things that do not need to be transported quite as far," explains Roxanne Beltran.

"Lightscapes of fear: how mesopredators balance starvation and predation in the open ocean" has been published in Science Advances. In 2018, the Novo Nordisk Foundation awarded a grant to the Department of Public Health, University of Copenhagen for the project Harnessing the Power of Big Data to Address the Societal Challenge of Ageing.

Moth larvae can help in ***combatting plastic*** pollution

In recent years, all the plastic that ends up in the environment has been increasingly in focus. This ruins the environment and disseminates microplastics into land and marine food chains. However, some organisms appear to be able to break down polyethylene, including the larvae of the greater wax moth that usually eat beeswax and other parts of beehives. Now researchers have come a step closer to understanding how these larvae biodegrade polyethylene. The goal is to be able to recreate these processes so that polyethylene can be recycled rather than incinerated.

BY MORTEN BUSCH

Beekeepers curse greater wax moths (Galleria mellonella) from afar because their larvae (waxworm caterpillars) can happily chomp through the honeycombs of their bees, destroying the honey harvest. However, researchers have found that these caterpillars can consume more than honeycombs. They can also degrade certain types of plastic. Exactly how is not yet fully understood, but now researchers have come a step closer to discovering how the caterpillars biodegrade the otherwise nearly nonbiodegradable plastic.

"The big question has been whether it is the waxworm caterpillars themselves or the bacteria inside them that biodegrade the plastic. Our new research shows that the function of the caterpillars is probably to prepare the plastic for biodegrading by increasing the surface area many thousands of times. This enables the plastic to be biodegraded more quickly afterwards, although the bacteria inside the caterpillars carry out the actual biodegradation. If we can learn how to copy this symbiotic biodegradation, we may be able to build facilities that can biodegrade plastic and recycle its components," explains Jeppe Lund Nielsen, Professor with Specific Responsibilities, Department of Chemistry and Bioscience, Aalborg University.

Very fine plastic strands

Almost 3 years ago, researchers at the University of Cambridge discovered the astonishing properties of these waxworm caterpillars, which normally live on beeswax and other parts of beehives but can also biodegrade plastic bags and food wrappers made of polyethylene, nearly 100 million tonnes of which is produced annually.

"Initially, the suggestion was that these waxworm caterpillars degrade the plastic independently of their gut flora. In our experiments, we wanted to clarify that uncertainty so that we could better understand the role of both the caterpillars and the bacteria. We therefore tried to investigate how the proteins in the caterpillars' salivary glands were affected by the plastic and whether these proteins could degrade the plastic independently," says Jeppe Lund Nielsen.

The researchers therefore placed the waxworm caterpillars into small containers and fed them their favourite foods, honey and beeswax – supplemented with small pieces of polyethylene. After 10 days, the researchers removed the caterpillars and examined both the plastic and the salivary glands.

"We found that the proteins in the waxworm caterpillars' salivary glands did not degrade the polyethylene but rather the mastication by the larvae increased its surface area. So those pieces of plastic had turned into very fine strands instead. The caterpillars had increased the surface area thousands of times. We also noticed some small changes in the chemical groups on the surface," explains Jeppe Lund Nielsen.

The researchers interpret the results as a sign that the waxworm caterpillars prepare the plastic before it reaches the intestinal microbiota, where these bacteria probably help to carry out the actual degradation of the plastic. Another major question then was whether the caterpillars actually derive energy from eating the plastic.

"We therefore examined the composition of proteins in the salivary glands, and this showed that the waxworm caterpillars alter their energy metabolism when they eat the plastic: for example, the enzymes associated with digesting fatty acids were activated and hunger hormones were secreted. We interpret the results that the caterpillars do not obtain energy from eating the plastic, but instead it stresses their system, and thus it does not seem that they can metabolize it," says Jeppe Lund Nielsen.

Reusable building blocks

The new results thus suggest that the waxworm caterpillars live in symbiosis with the bacteria, with the caterpillars carrying out the initial processing and the bacteria managing the decisive biodegradation of the material from which energy is obtained from the food, which usually consists of beeswax and other parts of beehives.

"The research came about because our Iranian collaborators were looking for partners to help them investigate and understand the physiology of complex systems: how organisms convert food into energy. We usually characterize microbiomes in eukaryotic systems, such as insects, and work related to microbiology in technical systems, so this system is doubly interesting because we can reveal how nature combines initial processing by insects with biodegradation," explains Jeppe Lund Nielsen.

Polyethylene adversely affects the entire ecosystem because it is virtually nonbiodegradable, but these waxworm caterpillars have apparently cracked the code, although the natural process is very slow in nature. The researchers therefore hope in the long term to be able to discover and copy the trick of the caterpillars to degrade some of the 100 million tonnes of polyethylene used annually for plastic bags and packaging.

"If we can learn how the waxworm caterpillars use mastication in combination with their enzymes and their microbiome, we might be able to replicate these processes so that instead of incinerating plastic, we can degrade it and recycle the resulting products. These building blocks can then be turned into new plastic or used to produce other substances," says Jeppe Lund Nielsen.

"Impact of Polyethylene on Salivary Glands Proteome in Galleria Melonella" has been published in Comparative Biochemistry and Physiology. In 2016, the Novo Nordisk Foundation awarded a grant to Jeppe Lund Nielsen for the project Novel Screening Approaches for Identification of Enzymes of Biotechnological Interests Directly in Complex Microbial Consortia.

Overcoming oscillations in ***gas-to-liquid*** fuel fermentations

Bacteria have the potential to convert synthesis gas – a mixture of carbon monoxide (CO), hydrogen (H2) and often carbon dioxide (CO2) – into liquid fuel for passenger and commercial transport ranging from cars to aircraft. But first, researchers must overcome an obstacle. Danish researchers have helped to identify the mechanism behind this.

BY KRISTIAN SJØGREN

Being able to convert the residual energy from various waste streams into transport fuels is a long-held dream. Most waste streams are recalcitrant, and one option is to gasify the waste into syngas and convert this gas into fuel using one-carbon (C1) chemistry.

Chemical gas-to-liquid conversion technology, such as the Fischer–Tropsch process, has been pursued for almost 100 years, but it is relatively sensitive to gas composition and requires a very large scale. In contrast, biological conversion using gas fermentation is less sensitive to contaminants and production scale.

"We want to be able to use bacteria to convert fuel gas to liquid fuel. In this context, we have observed that the productivity of bacteria oscillates when they convert carbon monoxide and hydrogen to ethanol. We have now identified why their productivity oscillates," explains Lars Keld Nielsen, Scientific Director, Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Kongens Lyngby.

The research has been published in the *Proceedings of the National Academy of Sciences of the United States of America*.

Acetogens convert gases to fuel

The research specifically focuses on the acetogen microorganism Clostridium autoethanogenum. Acetogens are capable of converting many substrates, including various gases, into acetate, which plays an important nutritional role not only for the bacteria themselves but also for the host organisms in which the acetogens often settle.

In addition to acetate, the bacteria can also make the commercially useful ethanol, which can be further refined into aviation fuel or gasoline for cars. Researchers have evolved acetogens to make more ethanol than acetate, so they produce more of what we want and less of what we cannot really use for anything.

"Much of our research has focused on understanding the processes involved when the acetogens make ethanol. What proteins are involved? What does their metabolism look like?" explains Lars Keld Nielsen.

Bacteria have a preference

Acetogens can grow on many types of feedstocks but have some preferences. One is synthesis gas, a mixture of carbon monoxide (CO), hydrogen (H2) and often also carbon dioxide (CO2).

The acetogens can convert synthesis gas to ethanol, but long ago researchers identified an annoying obstacle to achieving commercial success.

The researchers can mix more and more synthesis gas with acetogens in their bioreactors, causing the acetogens to produce increasing amounts of ethanol, but at some point the process begins to oscillate, and instead of increasing, productivity begins to fall again.

After it declines, productivity rises again, and the process continues to vary in fixed cycles, no matter how much more synthesis gas the researchers feed into the mix.

One oscillation lasts for about 180 hours, and the difference in output from the peak to the trough may be up to twofold.

This needs to be changed to make the process commercially attractive.

"We thought we would reach a level at which production would not increase anymore but would stabilize when the amount of gas in the liquid with acetogens became the limiting factor. However, until recently, nobody knew why these oscillations occur when the system is pushed to its limit to maximize production," says Lars Keld Nielsen.
Lars Keld Nielsen says that the same thing happens with baking yeast when you push it to exceed its metabolic robustness.

Halting development

Since the obvious problem is that the system needs to be more efficient to be a commercial success, there are considerable rewards for the researchers who can crack the code to stabilize the production of ethanol by acetogens.

Lars Keld Nielsen explains that researchers need to produce more ethanol from every litre of the bioreactor mix to become an attractive alternative to extracting fossil fuels.

"This is the limiting factor, because we cannot move forward if we cannot solve this," says Lars Keld Nielsen.

Explosive growth slams on the brakes

As always, the first step in solving a problem is to identify and analyse the problem, and Lars Keld Nielsen has done exactly that with colleagues in Australia.

Lars Keld Nielsen's many experiments with acetogens in the laboratory show that bacteria start to grow by using carbon monoxide as a growth source.

When the concentration of acetogens is sufficiently high and that of carbon monoxide is low, the acetogens also start to use hydrogen to grow and then their growth increases explosively, which is actually very good.

Nevertheless, when the concentration of hydrogen declines and the acetogens have to return to growing on carbon monoxide alone, things start to go downhill because the acetogens take time to switch to solely using carbon monoxide as a growth source.

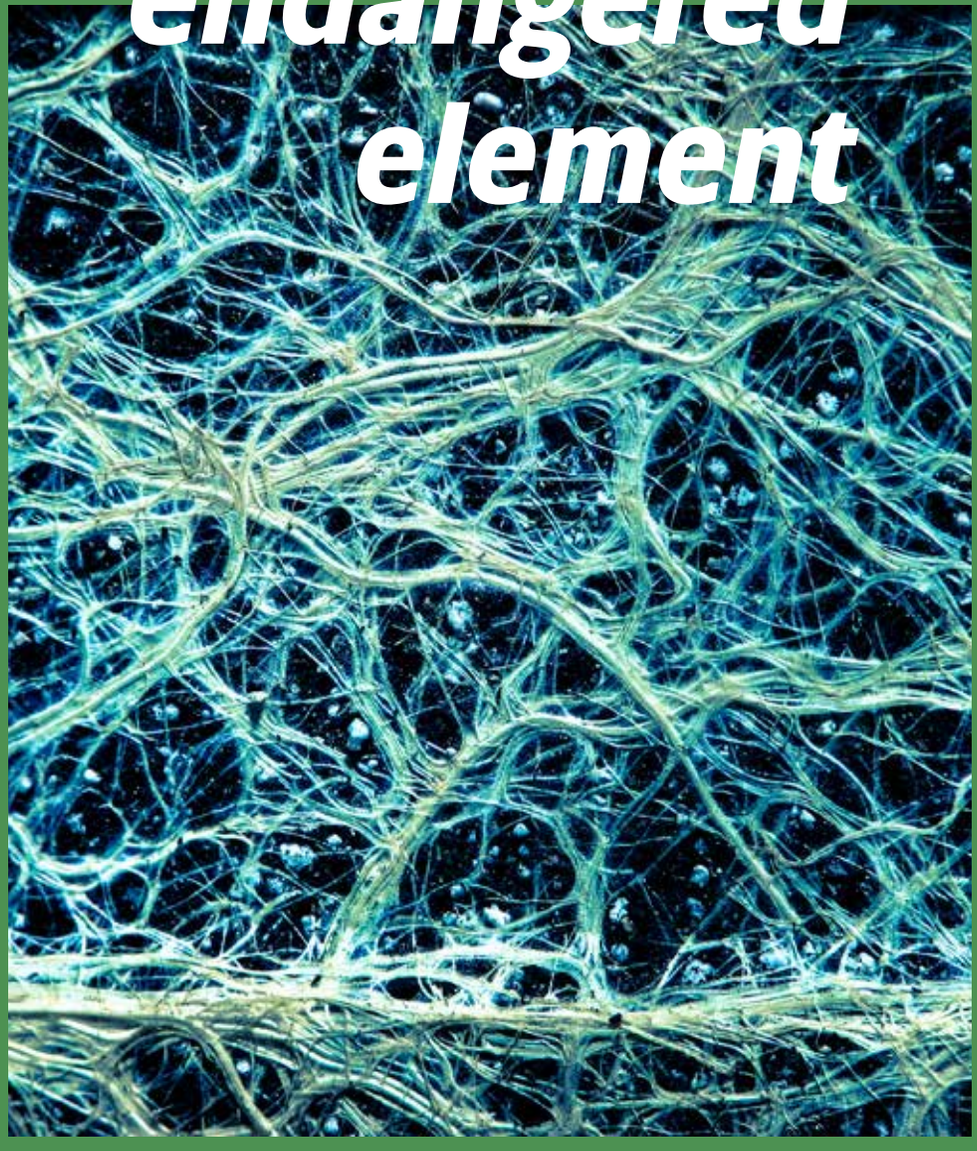
This results in decreasing metabolic robustness, which leads to lower production of ethanol.

"The way the system works is that the acetogens can accelerate their growth and metabolism quickly when they can grow on hydrogen, but then it takes a long time to return to the starting-point," explains Lars Keld Nielsen.

"Redox controls metabolic robustness in the gas-fermenting acetogen Clostridium autoethanogenum" has been published in the Proceedings of the National Academy of Sciences of the United States of America. Lars Keld Nielsen is the Scientific Director of the Novo Nordisk Foundation Center for Biosustainability.

Fungi may help to conserve ***endangered element***

Phosphorous is required for all life. However, many people think that the reserves of phosphorous may become depleted as the use of phosphates in commercial fertilizer increases. Researchers are therefore working to improve phosphorous uptake by plants, and the fungi in their root network are key to this. New results indicate a central mechanism in the symbiosis between fungi and plants that, if adjusted, may reduce the use of phosphates in fertilizer.



BY MORTEN BUSCH

Our bones, teeth, genes and the membranes of all our cells highly depend on phosphorus. However, if people continue to use phosphates in fertilizer, the reserves may become depleted. We do not know when this will happen, but fertilizer use can be drastically reduced by improving plants' ability to take up the phosphorus already present in the soil. A key factor is the symbiotic interaction between arbuscular mycorrhizal fungi (AMF) and plants that colonize the roots when the plants lack phosphorus.

"Some nutrients in fertilizer, such as phosphorus, are fixed in the soil. The AMF can transfer otherwise inaccessible phosphorus to the plant. We have found an important regulator in the interaction between plants and AMF. Strengthening this symbiosis early in the life of the plant can ensure more efficient uptake of phosphorus, reducing the need for phosphate fertilizers. This can prolong our access to phosphorus fertilizer and help avoid harmful runoff of phosphates into streams and rivers," explains Thomas Christian de Bang, Assistant Professor, Department of Plant and Environmental Sciences, University of Copenhagen.

Strengthening symbiosis

To investigate how plants regulate their interaction with AMF, the researchers used the plant Medicago truncatula (barrelclover), a small annual legume often used in basic research. The aim was to determine which genes are expressed at low versus high phosphorus levels.

"Plants use energy to interact with AMF, and they therefore only do this when it´s necessary. When phosphorus levels are low, the plant strengthens the symbiosis with AMF. Conversely, when phosphorus levels are high, the plant reduces the symbiosis to save energy," says Thomas Christian de Bang.

The researchers therefore compared which genes are activated at high versus low levels of phosphorus to find clues about which genes are important for the fungal symbiosis. After analysing a group of potential candidates, the researchers found a candidate among a group of small peptides from the CLE family of genes that specifically form in the roots of plants.

"The barrelclover genome encodes 52 CLE peptides, each with a specific function, but the results clearly showed that the MtCLE53 peptide played a special role. When we reduced phosphorus, less MtCLE53 was expressed, but at high phosphorus levels, we saw higher expression of MtCLE53. MtCLE53 levels were also high in plants colonized by AMF but with low phosphorus levels and, overall, this indicated that MtCLE53 played an important role in reducing the symbiosis with the fungus," explains Thomas Christian de Bang.

To confirm that MtCLE53 actually shuts down the symbiosis, the researchers repeated the same experiments with plant mutants lacking the SUNN receptor to which MtCLE53 likely binds and the protein RDN1, which could add sugar molecules to MtCLE53. In both cases, the symbiosis was not reduced.

"The evidence therefore suggests that the MtCLE53 peptide plays a crucial role in this symbiosis, so if we can understand exactly how and if we can easily downregulate this peptide, we might be able to strengthen the symbiosis between the plant and the fungi," says Thomas Christian de Bang.

Conserving phosphates and improving the environment

The early stages of crop growth constitute an important window for strengthening the symbiosis between plants and fungi. While the plants are small, the soil around them has plenty of phosphorus, but as they grow, they need to absorb phosphorus that is further away.

"Ample access to phosphorus in the early stages of plant growth is especially important for high yields, since phosphorus indirectly controls how many shoots a plant puts out, and thus how many seeds the plant can produce. MtCLE53 slows down the symbiosis with the fungi at high phosphorus levels, which is a paradox in modern agriculture, since this delays the plant's uptake of distant phosphorus via the AMF. Effective symbiosis with AMF will improve phosphorus uptake in agriculture, avoiding unnecessary use of this limited resource and phosphate runoff into the aquatic environment when heavy rain occurs," explains Thomas Christian de Bang.

Plants use energy to interact with AMF, and they therefore only do this when it´s necessary. When phosphorus levels are low, the plant strengthens the symbiosis with AMF.

The researchers already have a good idea of how they can change plants to regulate the production of MtCLE53. They have therefore applied for funding for proof-of-concept trials in crop plants. Using CRISPR technology, they will investigate whether the plants attain the right properties so that they can improve the effectiveness of the symbiotic interactions and thereby become more efficient at absorbing phosphorus.

"We can quantify AMF colonization using microscopy, measure gene expression levels and measure the uptake of phosphorous to determine whether the symbiosis improves. Since we are not allowed to use CRISPR or genetically engineered plants for cultivation in Europe, we will seek to recreate the changes through traditional breeding techniques, so that we ensure phosphorus retention and spare the environment from unnecessary phosphate runoff," says Thomas Christian de Bang.

"The CLE53-SUNN genetic pathway negatively regulates arbuscular mycorrhiza root colonization in Medicago truncatula" has been published in the Journal of Experimental Biology. In 2017, the Novo Nordisk Foundation awarded a grant to Thomas Christian de Bang for the project BIOPEP – Identification of Soil Microorganisms Stimulating Root Growth to Improve Phosphorus Uptake in Plants. The project also received funding from Brødrene Hartmanns Fond and the University of Copenhagen.

Carbohydrate – high-octane fuel for endurance athletes

Our understanding of the role of carbohydrate in muscle function and performance has expanded significantly in the past 120 years. Several studies have shown why carbohydrate is essential for muscle function and athletic performance. Despite the lack of evidence, many coaches and athletes still swear that endurance athletes should eliminate or reduce carbohydrate intake and instead improve the body's ability to use fat as fuel. However, the latest science underscores the importance of carbohydrate and especially muscle glycogen for endurance events.

BY KASPER DEGN GEJL

The Olympic Games are underway, and with the world's best athletes gathered in one place, small margins will determine the distribution of medals in many disciplines. Several Olympic disciplines require high work intensity, and some over a long duration. For example, the winner of the men's marathon crosses the finish line after about 2 hours of work at about 90% of the maximum heart rate, and the riders in the road cycling races are typically in the saddle for 5–6 hours, with a mix of light, moderate and very intense work periods along the way.

The composition of the athletes' diet plays a key role especially in longer events, and the wrong nutritional strategy before and during an event can decisively hurt performance. The endurance sports events naturally require a substantial amount of energy, and the athletes have two primary energy sources available – fat and carbohydrate. This has been known since about 1900, when Nathan Zuntz (1847–1920) demonstrated that fat is an energy source. Subsequently in 1920, August Krogh (1874–1949) and Johannes Lindhard (1870–1947) also observed that performance during high-intensity work deteriorated after consuming a high-fat diet. Thus, evidence established early that diet can alter the body's choice of energy source and that the transition to a high-fat diet can affect performance.

Glycogen – a valuable but limited source of energy

The body's largest carbohydrate store is the muscles' storehouse of glycogen – long-branched chains of 10.000 glucose molecules. After the muscle biopsy was introduced, Jonas Bergstrøm and colleagues conducted a landmark study in 1967 that clearly demonstrated that endurance performance depends on the availability of glycogen in the working muscles.

This presents a considerable challenge for athletes because this carbohydrate store is small and is quickly depleted during high-intensity work. However, periods of endurance training and a high-carbohydrate diet in the days leading up to competition have been shown to increase the storage of muscle glycogen, and the muscles of endurance athletes in the Olympic Games are therefore better equipped to store glycogen than the muscles of less-trained individuals.

Thus, although it has been known for more than 50 years that a lack of muscle glycogen inhibits endurance performance, the underlying mechanisms are still only partly understood. In our research unit, we therefore try to understand how glycogen influences muscle cell function.

In several studies, we have investigated how the presence of glycogen affects several of the key mechanisms that ultimately lead to contractions and force generation in the muscle fibres. We have shown how energy-intensive components in the muscle fibre, such as the sodium-potassium pump, which ensures that the electrical signals from the nervous system can be transmitted to the

muscle fibre, function optimally in the presence of glycogen.

Further, the results of other studies with well-trained endurance athletes have indicated that glycogen also promotes the non-energy-intensive and extremely important process in which muscle cells release calcium to proteins that can contract and thus initiate the mechanical contraction of muscle fibres.

Glycogen is thus more than just an energy source, and several studies have shown how abundant energy – in the form of ATP – does not optimally support the needs of muscle fibre without the presence of glycogen. Thus, great ability to store and break down glycogen seems to be important when a sport requires rapid and repeated muscle contractions.

Dietary manipulation – a future nutrition strategy for athletes?

Further, our research has shown that glycogen should not solely be considered globally in the muscle cell. The sodium-potassium pumps, the calcium-release channels and the proteins that generate force in the muscle fibres are located in different places in the muscle cell, and the same applies to the glycogen molecules.

We hypothesise that the various glycogen pools are located strategically to locally support the energy-intensive processes in different places in the muscle cell. We think that this distribution of glycogen in the cell enables ATP to be produced much closer to the site where it will be used. Therefore, if one of these specific glycogen pools is depleted, energy deficiency is likely locally and may inhibit important steps in activating the muscle cells and ultimately performance.

In connection with competitions at moderate and high intensity, the glycogen tank of athletes will continue to be emptied, and even if they consume carbohydrate during the competition, fat will become the dominant fuel at some point.

Unfortunately, fat is metabolized relatively slowly, and the ability to perform moderate- and high-intensity work therefore deteriorates significantly when the body's carbohydrate stores are reduced to a critical level and athletes metaphorically hit the wall.

But what if athletes could improve their body's ability to use fat as an energy source? Could they then conserve muscle glycogen and make it last longer, thereby performing better in the endurance disciplines?

Several inappropriate adjustments

After many years of training, Olympic endurance athletes have high capacity to metabolise fat as an energy source, but this capacity can actually be increased in just a few weeks by switching to a high-fat and low-carbohydrate diet – even among already highly trained elite athletes.

For this reason, many recreational athletes, elite athletes and coaches swear by a diet low in carbohydrate, and the discussion has sometimes been heated on social media and in scientific contexts about how relevant a high-fat diet is for endurance athletes.

Despite an improved ability to burn fat, however, identifying the literature and evidence showing that switching well-trained endurance athletes to a high-fat diet improves performance is very difficult.

In reality, the existing evidence shows that a high-fat diet does not improve endurance performance, and many studies have even shown impaired performance. The lack of effect on performance may result from several inappropriate adjustments that also come with a high-fat diet.

Possibly underestimated

As mentioned earlier, one factor is increased oxygen demand in the muscles when fat is the primary source of energy, but the ability to utilise carbohydrate and glycogen as an energy source is also reduced.

Reduced ability to store and break down glycogen should presumably impair function in essential parts of the muscle cell, which can thus help to explain the impairment of both the intensity of training and performance associated with a high-fat diet.

Reduced ability to work at high intensity is extremely critical in endurance sports competitions, with fluctuating and occasionally very-high intensity. Many people think that marathon runners and triathletes perform at relatively stable intensity, but the crucial periods in these sports, and endurance sports in general, are often high intensity.

I therefore think that some scientific contexts may even underestimate the negative effect of a high-fat diet on endurance performance, since most existing studies have evaluated endurance performance in tests with a fixed load and thus without the periodic shifts to high intensity that occur in sports such as cycling, triathlon and running.

No evidence indicates that removing carbohydrate

Periodically restricting carbohydrate intake several times a week for a longer training period does not improve performance compared with control groups training with high carbohydrate availability. This inability to convert the otherwise promising short-term effects to improved long-term performance may result from the fact that the athletes in the short-term studies were exposed to an unfamiliar training and nutrition strategy and that the positive short-term effect therefore wanes as the athletes get used to it.

Finally, the training itself may simply result in a maximal or near-maximal training response, which cannot be further enhanced by restricting carbohydrate intake. So despite the possibility of increasing the muscles' ability to use fat as an energy source with a long-term high-fat diet and despite promising short-term studies with periodic carbohydrate restriction, no evidence indicates that removing carbohydrate from the diet enhances endurance performance – neither long term nor periodically.

Thus, the endurance athletes at the Olympic Games and other athletes competing in long-distance events with high-intensity bursts should have carbohydrate both when they train and when they compete.

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chapter

DIET AND LIFESTYLE

ARTICLES

How the ***brain regulates*** how hungry and full we feel

New Danish research improves understanding of how the hypothalamus and the rest of the brain control how much we eat.

BY KRISTIAN SJØGREN

NAD+, ATP, leptin, NAMPT, SIRT1, nicotinamide, POMC and FK866.

This may sound technical, but all these molecules decisively influence how much food we eat.

A new Danish research project shows that FK866, a selective inhibitor of NAMPT (nicotinamide phosphoribosyltransferase), inhibits the NAD+ (nicotinamide adenine dinucleotide)-generating pathway in the hypothalamus, and this affects whether we feel hungry.

The research results indicate a possible target for combating obesity.

"This study is a step towards understanding how various substances influence the hypothalamus, regulating whether people feel hungry or full and influencing whether they become overweight. The more we understand this system, the better we can develop specific compounds that may be used to treat obesity by inhibiting people's hunger," says Jonas Thue Treebak, Associate Professor, Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen.

The study was recently published in *Acta Physiologica*.

Two neurons determine whether we feel hungry or full

The hypothalamus has two types of neurons, POMC and AgRP. Leptin affects POMC, and ghrelin affects AgRP.

Leptin is secreted from adipose tissue and makes us feel full; ghrelin is secreted in the stomach and makes us feel hungry.

Essentially, the balance between how leptin affects POMC and how ghrelin affects AgRP determines how hungry we feel.

The enzyme SIRT1 is involved in regulating this balance, and previous research has shown that manipulation of SIRT1 levels in the hypothalamus can either increase or decrease hunger.

NAD+ further influences SIRT1, and this is where the new study is relevant.

"Our starting-point was to look further back in the chain and examine the factors that affect the levels of NAD+ in the brain. NAMPT is an enzyme that turns vitamin B3 into NAD+, and we therefore asked what happens when we influence this enzyme, which controls the activity of SIRT1 and thereby the feeling of hunger," explains Jonas Thue Treebak.

FK866 made mice eat less

The researchers experimentally inhibited NAMPT in mouse brains by injecting FK866 very precisely through a cannula directly into the third ventricle of the brain, which is connected to the hypothalamus.

They then observed the mice and noted how much they ate. The mice ate less when FK866 was injected into their brains.

The researchers also investigated whether the mice lost their appetite because the FK866 injections caused anhedonia, but the experiments showed that the mice did quite well but were just less hungry.

Experiment confirmed hypothesis

The researchers placed a swab with the scent of female mice into the cage with male mice and then observed their behaviour.

If the mice felt unwell, they would not be interested in the scent of female mice, but the males were just as interested in this scent as control males without the FK866 injections.

The researchers also administered ghrelin to mice. This normally stimulates hunger and causes mice to eat more, but the FK866 injections inhibited the hunger that would otherwise have arisen.

"The experiment confirmed our hypothesis. We expected that inhibiting NAMPT with FK866 would reduce the levels of NAD+ and reduce food intake and we observed this in the mice," says Jonas Thue Treebak.

Overweight people cannot be treated with FK866

As one element of a larger puzzle, this new study has potentially great perspectives.

Thinking that FK866 can be used to fight the current obesity epidemic might be tempting but is unrealistic.

FK866 is toxic to the body's cells, and giving it to people who are overweight is difficult to defend.

But researchers now understand better the mechanisms they can influence to potentially reduce obesity.

"We believe that manipulating the metabolism of NAD+ in POMC and AgRP may correct any imbalance between these neurons and that this may inhibit hunger. We already know today that people who become leptin resistant have more difficulty in feeling full and that people eat more if they are unusually sensitive to ghrelin. This balance needs to be restored," says Jonas Thue Treebak.

Treating obesity with vitamin B3

Jonas Thue Treebak explains that the researchers are already examining various ways of influencing the system that determines whether we feel hungry or full.

The researchers are investigating various types of vitamin B3, the substrate NAMPT uses to make NAD+. One is nicotinamide riboside.

This can be taken orally and could become an interesting approach for combating obesity if it can restore a healthy balance of NAD+ in the neurons of the hypothalamus and thereby counteract increased feelings of hunger.

Unfortunately, experiments so far have not yielded positive results.

"We previously gave people nicotinamide riboside for 12 weeks but found no effects on the participants' weight, body composition or metabolism. We may not have given the vitamin to the participants for long enough, or it needed to be given differently to be effective. We will examine these things in the future to improve understanding of how to manipulate this system," says Jonas Thue Treebak.

"Fasting- and ghrelin-induced food intake is regulated by NAMPT in the hypothalamus" has been published in Acta Physiologica. Several authors are employed at the Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen.

Vitamin D increases ***testosterone*** production

Vitamin D deficiency and low testosterone concentrations in men can affect their fertility or lead to osteoporosis and loss of muscle mass. New Danish research shows that these two factors may be more closely related than previously thought, since vitamin D deficiency may reduce testosterone production.

BY KRISTIAN SJØGREN

We get vitamin D from sunlight exposure, food and dietary supplements. The concentration of vitamin D in the blood may affect men's testosterone production.

For most men, a higher vitamin D intake will probably not strongly influence the concentration of testosterone, but men with vitamin D deficiency might consider vitamin D supplements to boost the concentration of testosterone in the body and perhaps also fertility.

This is the conclusion of a new Danish study in which researchers linked the concentration of vitamin D in the blood and the body's production of testosterone.

"We have been researching how vitamin D affects reproduction for many years. What is new about this study, is that we can show that vitamin D influences testosterone production. There appears to be a direct link, and one may speculate whether supplementation of vitamin D for vitamin D–deficient men may give a clinically relevant increase in testosterone," says Martin Blomberg Jensen, doctor and Group Leader, Group of Skeletal, Mineral and Gonadal Endocrinology, Department of Growth and Reproduction, Rigshospitalet, Copenhagen.

The study was recently published in the *Journal of Steroid Biochemistry and Molecular Biology*.

Testosterone is important for fertility and health

Testosterone is produced in men's testicles and is strictly regulated by the pituitary hormone luteinizing hormone (LH).

The pituitary gland is sensitive to the concentration of sex hormones in the blood, and when the concentration drops, the pituitary gland secretes LH, which is the main stimulator of testosterone production.

Men who only have one testicle typically need a higher concentration of LH to achieve sufficient concentrations of testosterone, since the remaining testicle has to perform the entire task and therefore needs additional stimulation.

Lower testosterone production can result in less energy, less muscle mass, reduced sexual desire and increased risk of osteoporosis.

In addition, infertile men produce less testosterone than men with normal fertility.

Observational study of Danish men

In the new study, researchers from Rigshospitalet investigated whether they could find an association between the blood concentrations of vitamin D and testosterone among two groups of Danish men.

One group comprised 41 men who had a testicle removed because of testicular cancer and the other group comprised 300 healthy young men. The researchers took blood samples from all the men and determined the concentrations of testosterone, LH, vitamin D and the calcium-regulating hormone parathyroid hormone. Correlating parathyroid hormone and vitamin D provides researchers with a more nuanced view of an individual's vitamin D status than the vitamin D concentration alone, while testosterone and LH reveal the state of the pituitary gland and testicular function.

"The relationship between testosterone and LH indicates how much testosterone the testicles can produce compared with the amount of LH,"

explains the first author, Rune Holt, doctor and PhD student.

Vitamin D deficiency leads to reduced production of testosterone

The results of the study showed that the blood concentrations of testosterone and vitamin D were linked among the 300 healthy men with normal testosterone production.

Healthy men with low vitamin D and high parathyroid hormone concentrations, had a lower ratio of testosterone to LH in the blood suggesting that the effect of LH on testosterone production may be reduced when vitamin D is low
Forty-one men who had one testicle removed received an injection of human chorionic gonadotropin to test the ability of the remaining testicle to produce sufficient testosterone.

The researchers simultaneously determined the men's blood concentration of vitamin D and found that men with low vitamin D levels reacted less positively to the injection of human chorionic gonadotropin than men with normal or high vitamin D concentrations.

Martin Blomberg Jensen explains that this discovery is clinically relevant.

"Men who have had one testicle removed often undergo a hormone stimulation test using human chorionic gonadotropin to test whether the remaining testicle is capable of producing sufficient testosterone. Our results indicate that examining these men's vitamin D concentrations before examining their testicular function may be clinically relevant, since it may affect how well they respond to human chorionic gonadotropin stimulation," says Martin Blomberg Jensen.

Vitamin D supplements might have a doubly positive effect on bone health, since vitamin D deficiency is associated with an increased risk of osteoporosis as is testosterone deficiency. Vitamin D supplementation to ensure higher testosterone concentrations can therefore have additional positive health effects.

"Vitamin D and sex steroid production in men with normal or impaired Leydig cell function" has been published in the Journal of Steroid Biochemistry and Molecular Biology. The Novo Nordisk Foundation awarded grants to Martin Blomberg Jensen for the project Clinical Relevance of the Calcium-sensing Receptor in Reproduction in 2018 and for the project RANKL and Male Fertility, an Excellence Project for Young Researchers within Endocrinology and Metabolism, in 2017.

"Acute metabolic effects of melatonin – a randomized crossover study in healthy young men" has been published in the Journal of Pineal Research. Several authors are employed at Steno Diabetes Center Aarhus and the Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen, both of which have received funding from the Novo Nordisk Foundation.

Antioxidants can become too much of ***a good thing***

BY MORTEN BUSCH

In recent decades, many people have been taking dietary supplements to get enough antioxidants to reduce the harmful effects of free radicals and thereby stay healthy. A few years ago, researchers warned that people with cancer should not take antioxidants, and new research now questions whether these supplements benefit healthy people at all. Paradoxically, excessive intake of antioxidants may instead increase harmful free radicals in the body.

Pairs of electrons are essential for molecules to be stable. By contrast, when free electrons are created, molecules can react – by transferring an electron from or to another molecule. This creates free radicals in the body and thereby chain reactions that can potentially damage our genes or proteins. For years, the theory has been that antioxidants in our diet could stop these chain reactions. The hype has been so great that antioxidants have been available as dietary supplements, but several years of research has led to advising caution.

"If you take antioxidants briefly and in moderate doses, they probably do what they should, but taking a higher dose or for a longer period can induce reductive stress that, paradoxically, increases the production of free radicals. So whether antioxidants are beneficial or harmful really depends on the situation, but if I were to recommend anything, it would be to avoid overloading the body with this type of dietary supplement unless there is a clear indication," explains Ingrid Wernstedt Asterholm, Senior Lecturer, Department of Physiology, Institute of Neuroscience and Physiology, University of Gothenburg, Sweden.

Surprising oxidants

The results emerged from a somewhat unexpected quarter because the Swedish researchers were actually investigating something completely different: how to preserve or improve the functionality of white adipose tissue. Functional white adipose tissue can effectively store excess energy in the form of fat (triglycerides) and thereby prevent the harmful effects of excessive lipids and/or glucose in the bloodstream.

White adipose tissue can be transformed into beige adipose tissue through a process called browning that involves increasing mitochondrial biogenesis and activation. This increase in mitochondrial activity implies that the adipose tissue, besides storing fat, also can metabolize nutrients and thus become an even better sink for excess energy. Adipose tissue is normally browned when we are exposed to cold temperatures, but pharmaceutical treatment can also trigger browning. Researchers are therefore currently very interested in this browning process and whether it is a possible therapeutic target for obesity and its associated diseases.

"There are many theories about this browning process. We know that adipose tissue browning involves increased release of fatty acids from the fat cells. High levels of fatty acids can be toxic, whereas browning increases the fat cells' capacity to metabolize fatty acids. We therefore theorized that browning should be seen as an physiological adaptation to metabolic stress."

Based on previous results, the researchers thought that the molecular mechanism of this browning process involves an increase in the quantity of reactive oxygen species (free radicals). So a shifted balance towards a more oxidative state in the fat cells would activate signalling pathways that are essential for browning. This is where the antioxidants come in, because the researchers then assumed that using them as supplements would inhibit the browning process in adipose tissue, and it worked.

We were pleased that we were right. But then the problems arose.

Contradicting existing theories

The researchers tried to determine exactly which mechanisms stopped the browning process and were very surprised. Pathways that antioxidant treatment was supposed to have downregulated were, if anything, upregulated, and the antioxidant supplements (N-acetylcysteine, vitamin E or glutathione ethyl ester) led to producing more and not fewer free radicals. This result contradicted existing theories that antioxidants should reduce free radicals.

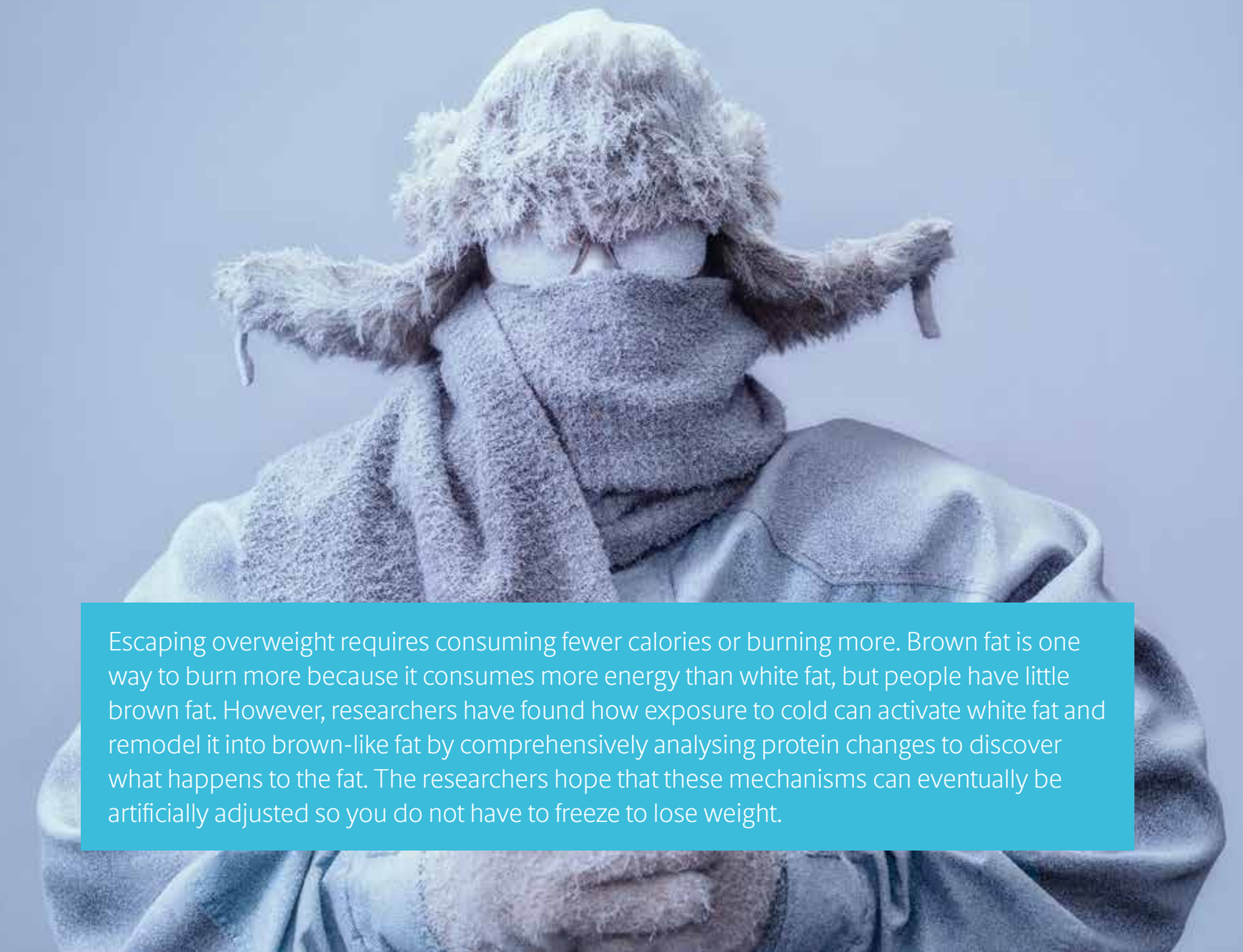
"We found the opposite, and we think the slowed-down browning process we observed results from cells or mitochondria defending themselves from excessive levels of free radicals, since increased mitochondrial metabolism will generate additional free radicals on top of those caused by the antioxidant supplements. In these experiments, we also found that the fat cells' own mitochondrial antioxidant enzymes were upregulated, and there is probably also a mechanism we have not yet identified that brakes the mitochondrial metabolism when the oxidative stress is too high."

Thus, it is still too early to speculate about the exact mechanisms behind the surprising and contradictory effect of the antioxidants. For now, the researchers hypothesize – based on preliminary results – that the cells can adapt to any antioxidants used for a long time, so that the increased presence of free radicals eventually leads to upregulation of the cells' own antioxidant-based defence and upregulation of the proteins involved in mitochondrial function.

"Antioxidant treatment induces reductive stress associated with mitochondrial dysfunction in adipocytes" has been published in the Journal of Biological Chemistry. The Novo Nordisk Foundation awarded a grant in 2012 to Ingrid Wernstedt Asterholm for the project Key Components of the Dynamic Reshaping of Adipose Tissue and a grant in 2019 for the project New Mechanisms Underlying Whole-body Metabolic Regulation.

Exposure to cold ***makes fat brown*** and burns energy

BY MORTEN BUSCH



Escaping overweight requires consuming fewer calories or burning more. Brown fat is one way to burn more because it consumes more energy than white fat, but people have little brown fat. However, researchers have found how exposure to cold can activate white fat and remodel it into brown-like fat by comprehensively analysing protein changes to discover what happens to the fat. The researchers hope that these mechanisms can eventually be artificially adjusted so you do not have to freeze to lose weight.

For decades, researchers have focused on the heat shock proteins that are activated if cells are exposed to intense heat, but YBX1 is one of the rarer cold shock proteins specifically activated by cold.

Most people know the feeling of showering in water that is slightly too cold or of jumping into even colder water. Our cells feel this shock in the same way, but instead of screaming, they initiate a series of metabolic processes. For fat cells, this increases the number of mitochondria, the cells' tiny power plants, making them consume more energy. In practice, the fat changes colour from white to brown – a remodelling process called browning, and metabolism researchers are seeking to promote this process.

"We hope that we can learn to understand these processes so well that we can also learn how to tweak the metabolism in the cells. In our new study, we found that mice express a specific cold shock protein that seems to be crucial in determining whether the fat tissue changes from white to brown. We do not yet fully understand the molecular mechanisms, but we can see that this protein is rapidly induced by cold in brown fat and in the white fat depots that have the potential to acquire brown fat characteristics. Understanding the mechanisms by which this protein contributes to promoting healthy fat tissue may help to avoid obesity and type 2 diabetes," explains Brice Emanuelli from the Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen.

Up- and downregulation

The researchers investigated what happens in fat tissue in ordinary mice when the browning of white fat is promoted. The mice lived at 29°C until the researchers suddenly lowered the temperature to 5°C. They then monitored the fat tissue to determine how the mice reacted to the cold.

"We measured changes in protein abundance in the fat tissue across a 3-week time course in which cold remodelled the white fat into brown-like fat. Exposing the mice to cold dramatically altered the protein profile of the fat tissue, and we found that one of them, YBX1 (Y box binding protein 1), was induced early and especially caught our attention," says Brice Emanuelli.

One reason why the researchers decided to focus on YBX1 was that its DNA code may be evolutionarily related to both bacteria and humans. When evolution preserves sequences and structures, this indicates that these are appropriate and essential functions. Another reason was YBX1's very remarkable activation method.

"For decades, researchers have focused on the heat shock proteins that are activated if cells are exposed to intense heat, but YBX1 is one of the rarer cold shock proteins specifically activated by cold. We therefore decided to investigate what would happen if we up- or downregulated it," explains Brice Emanuelli.

The researchers used small interfering RNAs, which can reduce or completely eliminate the expression of certain genes, and CRISPR-mediated induction of the same proteins to discover what happens if there is excess or no YBX1 protein.

"The evidence suggests that YBX1 plays a significant role in remodelling white fat into brown fat. The mice's adipocytes (fat cells) failed to turn brown if we removed YBX1, whereas they were more brown and metabolically active when YBX1 protein was present in abundance," says Brice Emanuelli.

Multifunctional protein

The new research is very exciting, since stimulating the browning of white fat is a promising way to improve metabolic health. Until now, the molecular mechanisms have been largely unknown, but with the new insight into how fat tissue responds to cold, researchers have obtained important knowledge about how to stimulate fat tissue to consume more energy – at least in mice.

"Among a core of 44 transcriptional regulators acutely regulated by cold, YBX1 is one of the key factors that we were seeking to discover, and we want to investigate how it is regulated, whether YBX1 plays a similar function in humans and especially whether YBX1, or some of the cellular processes it regulates, can be influenced in a sensible and safe way," explains Brice Emanuelli.

YBX1 may not the obvious candidate to directly regulate, since it is involved in multiple biological processes such as the repression of protein translation, RNA stabilization and splicing, DNA repair, regulation of transcription and RNA composition of extracellular exosomes. YBX1 increases tumour growth in various types of cancer and is therefore a potential drug target in anticancer therapy. But it is also involved in cellular senescence and the determination of cell fate. The researchers therefore think that understanding the molecular mechanisms underlying its mode of action in fat cells is necessary to uncover promising alternative approaches to influencing fat metabolism.

"Activating white fat has enormous potential for combatting the obesity epidemic, and thus YBX1 is key to understanding the mechanisms behind the process. We will now continue trying to understand what YBX1 does and whether we can adjust other linked mechanisms to stimulate fat browning without compromising other key mechanisms in the cells," says Brice Emanuelli.

"White adipose remodeling during browning in mice involves YBX1 to drive thermogenic commitment" has been published in Molecular Metabolism. The main authors are employed at the .Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen.

Researchers surprised – ***exercise can reduce*** insulin sensitivity

Danish research shows, very surprisingly, that exercise in some cases does not increase insulin sensitivity but reduces it. This discovery may affect how people with diabetes should exercise.

BY KRISTIAN SJØGREN

The fact that exercise increases insulin sensitivity in the body and provides accompanying beneficial health effects has long been considered an indisputable fact.

However, this may need to be modified somewhat after Danish researchers recently discovered that a single intense bout of one-legged exercise can actually reduce insulin sensitivity in the rest of the body.

This discovery may affect people with type 1 or type 2 diabetes because they may need to be advised differently on how to exercise in relation to diabetes.

"For many years, researchers have been interested in how physical activity improves health, partly because exercise increases insulin sensitivity in the muscles. Now we have shown, very surprisingly, that this may depend on the type of exercise, the intensity and/or the duration. For example, cycling increases insulin sensitivity in the whole body, whereas exercising a few muscle groups intensively in the gym can actually reduce insulin sensitivity in the rest of the body," explains Jørgen Wojtaszewski, Professor of Molecular Physiology, Department of Nutrition, Exercise and Sports, University of Copenhagen.

The study has been published in *Diabetes*.

People with diabetes might adjust their exercise routine

Commenting on the new study, Per Bendix Jeppesen, Associate Professor, Department of Clinical Medicine, Aarhus University, says: "Observing that the body increases insulin sensitivity in the working muscles while simultaneously decreasing insulin sensitivity in the resting muscles is incredibly exciting."

Per Bendix Jeppesen was not involved in the new study, but he has read the article and carries out research on how exercise affects insulin sensitivity. He has achieved great results in experiments with high-intensity interval training for people with type 2 diabetes.

"This is an exciting study that indicates some mechanisms in the body we did not previously know. This implies that we might need to recommend that people with type 2 diabetes exercise a little differently. Riding a bicycle may be fine because we know that this can increase insulin sensitivity, but exercising the arms and abdomen may further increase this effect so they do not become insulin resistant," says Per Bendix Jeppesen.

Physical activity increases insulin sensitivity
As more and more people worldwide struggle with diabetes or severe obesity, interest has increased in the body's health-promoting signalling pathways.

Health-promoting insulin sensitivity, in which the muscles are sensitive to insulin and therefore take up glucose from the blood, is one function that researchers and also the pharmaceutical industry really want to understand.

Research has repeatedly found, for example, that cycling markedly increases insulin sensitivity in the muscles that have performed the work, and this can result in the whole body showing increased insulin sensitivity for up to 2 days after each exercise session.

This is one reason why people with diabetes especially should exercise because it directly reduces their elevated blood glucose and their need for medication.

"We want to determine which mechanisms and signalling pathways are involved. The goal could be to make a pill that can have the same effect as physical activity on insulin sensitivity. After only a few days of inactivity, the insulin sensitivity of muscles and the body as a whole declines substantially," says Jørgen Wojtaszewski.

Exercise reduces insulin sensitivity in the rest of the body

In the new study, published in Diabetes, Jørgen Wojtaszewski and colleagues asked eight test subjects to perform 2.5 hours of one-legged exercise under controlled laboratory conditions. The purpose was to exercise the leg to exhaustion.

Then the researchers measured the insulin sensitivity in the muscles of both the active and the inactive legs.

After 4 hours, the insulin sensitivity of the muscles in the leg that had been active was, as expected, higher than that in the inactive leg.

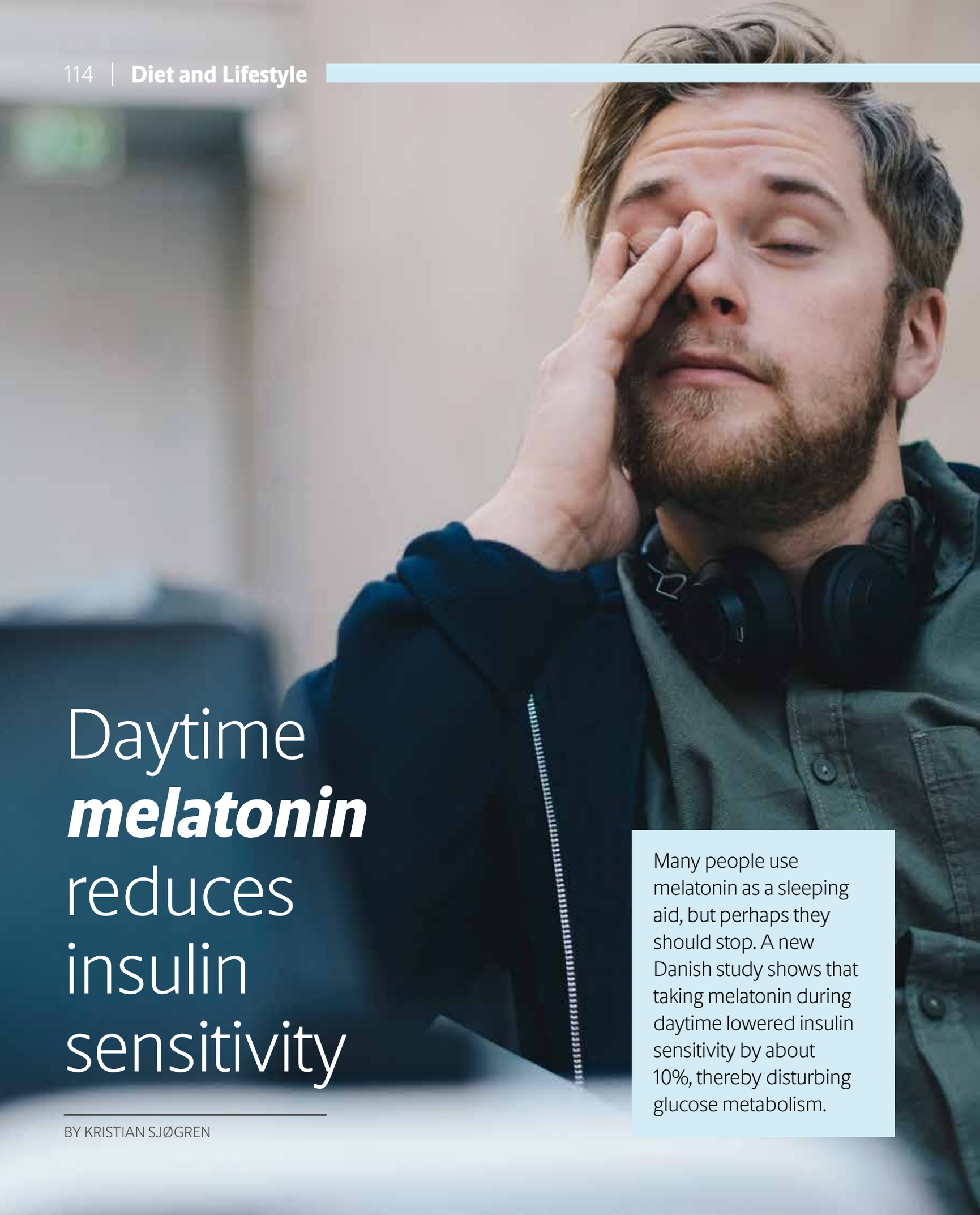
Surprisingly, however, the inactive muscles had lower insulin sensitivity than they did on a complete day of rest.

"When muscles perform physical work, they often consume large amounts of glycogen. Our body has evolved so that the muscles that need to replenish their fuel depots after a session of physical activity adapt such that they primarily benefit from the available glucose in the blood. This is achieved by increasing the insulin sensitivity of the muscles so that insulin is much more effective. We understood this intuitively, but we were greatly surprised that insulin sensitivity declined in the rest of the body. No one had observed this before, but this effect naturally only helps to increase the availability of glucose for the muscles that need to replenish their glucose reserves after physical activity," explains Jørgen Wojtaszewski.

The researchers became aware of this phenomenon in a previous study. However, the study had a different purpose and could therefore not answer the question correctly.

"In fact, some of our initial results were laughed at because they contradicted long-held beliefs. We therefore decided to set up a new study designed to answer this one question. The study showed the same result. It really surprised us that exercise may result in making the whole body insulin resistant," says Jørgen Wojtaszewski.

"A single bout of one-legged exercise to local exhaustion decreases insulin action in non-exercised muscle leading to decreased whole-body insulin action" has been published in Diabetes. In 2016, the Novo Nordisk Foundation awarded a grant to Jørgen Wojtaszewski for the project Exercising with Muscle Insulin Sensitivity.



Daytime ***melatonin*** reduces insulin sensitivity

BY KRISTIAN SJØGREN

Many people use melatonin as a sleeping aid, but perhaps they should stop. A new Danish study shows that taking melatonin during daytime lowered insulin sensitivity by about 10%, thereby disturbing glucose metabolism.

Melatonin is a natural sleep hormone secreted from the pineal gland in the brain in the absence of light. It promotes sleep, and high levels of melatonin are a starting-point for a good night's rest.

Melatonin can also be made synthetically, and many people use it as a sleeping aid when a good book, a cup of herbal tea and counting sheep are insufficient.

In Denmark, melatonin is a prescription drug, and doctors increasingly prescribe it to children and to adults older than 55 years, but it is available over the counter in the United States, where melatonin is especially popular because many people think that taking a natural hormone is safe.

However, a new Danish study shows that this conclusion may be somewhat hasty. Melatonin can reduce insulin sensitivity, which is key to developing type 2 diabetes.

"Melatonin benefits the body at the right times of the day, but our results indicate that people should not have high concentrations of melatonin in the body during daytime because it can affect glucose metabolism. People who are already predisposed to developing type 2 diabetes could consider avoiding taking melatonin regularly until more is known about its potential impact," explains a researcher behind the new study, Julie Støy, physician and PhD, Steno Diabetes Center Aarhus.

The research results have been published in the Journal of Pineal Research.

Melatonin, sleep and the risk of type 2 diabetes are associated

The fact that type 2 diabetes, sleep and melatonin are associated is not novel knowledge . Many studies have shown that disturbing the circadian rhythm increases the risk of developing type 2 diabetes. For example, this applies to people who work night shifts or are active nocturnally for other reasons. Both night work and a misaligned circadian rhythm increase the risk of developing type 2 diabetes, and melatonin could play a role.

"Poor sleep is as strong a risk factor for developing type 2 diabetes as being genetically predisposed by having family members with type 2 diabetes. In addition, genetic studies have also shown that genetic variants in the gene encoding the melatonin receptor predispose to developing type 2 diabetes. Much evidence indicates an association, but we need to find out how this all fits together," explains Julie Støy.

Gave melatonin to young men and investigated metabolic functions

Julie Støy and colleagues recruited 20 healthy men 20–40 years old for the study. They took four 10-mg capsules of melatonin at 1-hour intervals during the daytime experiments. The researchers investigated how high levels of melatonin affected the concentrations of various endocrine hormones, including corticosteroids, growth hormones and sex hormones. They also examined the body's fat metabolism, the formation of insulin in response to infusion of glucose and insulin sensitivity.

Melatonin reduced insulin sensitivity by 10%

Taking melatonin and the resulting high concentration of melatonin did not affect the production of various endocrine hormones, including insulin.

Melatonin slightly affected fat metabolism, but the most interesting result was on insulin sensitivity. Taking a large dose of melatonin during daytime reduced insulin sensitivity by about 10%.

"Reduced insulin sensitivity is absolutely key to developing type 2 diabetes, so this reduction is very interesting," says Julie Støy.

A secondary finding was that melatonin slightly lowers blood pressure, which other researchers have also observed previously.

Investigating long-term effects of melatonin

Julie Støy says that the results are exciting because they indicate a mechanism by which taking melatonin may contribute to some people developing disturbed glucose metabolism

These people may have other risk factors, and taking melatonin to enable them to fall asleep is the last straw before blood glucose rises to a level that is too high.

This finding means that the conclusions may also be clinically relevant: that people with many risk factors for developing type 2 diabetes, such as people with a family history of type 2 diabetes and obesity, should avoid taking melatonin as a regular sleeping aid.

However, Julie Støy also says that caution should be exercised in interpreting the results of a small intervention study with 20 people under controlled laboratory conditions and a dose of melatonin that is substantially higher than what people with sleep disorders use, and because melatonin was taken at a time when the blood concentration of the hormone in daytime is usually very low.

"In view of our results and the steadily increasing prevalence of type 2 diabetes, more research within this field is highly warranted. We induced an acute increase in the melatonin concentration during the day, and this may not correspond to the effect when people take more frequent but smaller doses of melatonin over many days at bedtime to fall asleep. Melatonin can improve sleep quality and thus benefit health, and this may outweigh any possible direct and negative effect of melatonin on insulin sensitivity. We are therefore investigating this in a new study, in which we give people with type 2 diabetes a 10 mg of melatonin every night at bedtime for 3 months. But overall, we currently conclude that taking high doses of melatonin during the day does not seem appropriate," says Julie Støy.

"Acute metabolic effects of melatonin – a randomized crossover study in healthy young men" has been published in the Journal of Pineal Research. Several authors are employed at Steno Diabetes Center Aarhus and the Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen, both of which have received funding from the Novo Nordisk Foundation.

Factors determining whether ***consuming alcohol*** causes liver disease

Worldwide, 75 million people have been diagnosed with an alcohol use disorder that puts them at risk of developing alcohol-related liver disease. About 1 million of these people die each year from liver disease. Although consuming alcohol is closely linked with the risk of liver disease, researchers have had difficulty understanding why some of these people develop severe liver disease and die and others do not. A new study suggests that genetics and insulin resistance determine whether these people develop liver disease. The new knowledge can be used to identify those who should definitely consume less alcohol.

BY MORTEN BUSCH

The liver is the body's processing and filtering organ, metabolizing carbohydrate, fat and protein and degrading and filtering out foreign substances. People who consume excessive alcohol develop an inflammatory condition in the liver that eventually can lead to the formation of fibrosis (scar tissue). However, people differ in the rate at which this happens. A new large study of people with a history of excessive alcohol use has enabled researchers to identify the people whose livers will be most severely affected by this excessive drinking.

"We investigated people with a history of excessive drinking to determine which risk factors most strongly affected whether liver fibrosis developed and thereby eventually caused cirrhosis. Although insulin resistance is the strongest indicator that alcohol-related fibrosis is underway, genetic factors play a significant role in developing liver disease. The new knowledge improves the opportunities to understand the development of this disease and to convince people who drink excessively to stop in time," explains Mads Bastrup Israelsen, a postdoctoral fellow at the FLASH Liver Research Centre, Odense University Hospital and University of Southern Denmark.

Indicating the stage of liver damage

The researchers examined 325 people with a history of excessive alcohol use based on the guidelines on safe alcohol consumption of the Danish Health Authority. The participants therefore did not necessarily have an alcohol use disorder or have developed symptoms of liver disease yet.

"Previous studies of this kind typically focused on people who developed cirrhosis, comparing them with a healthy control group. We wanted to examine liver disease while it is asymptomatic to understand how it develops and to determine whether we can identify which people are predisposed for alcohol-related liver disease and how to avoid it," says Mads Bastrup Israelsen.

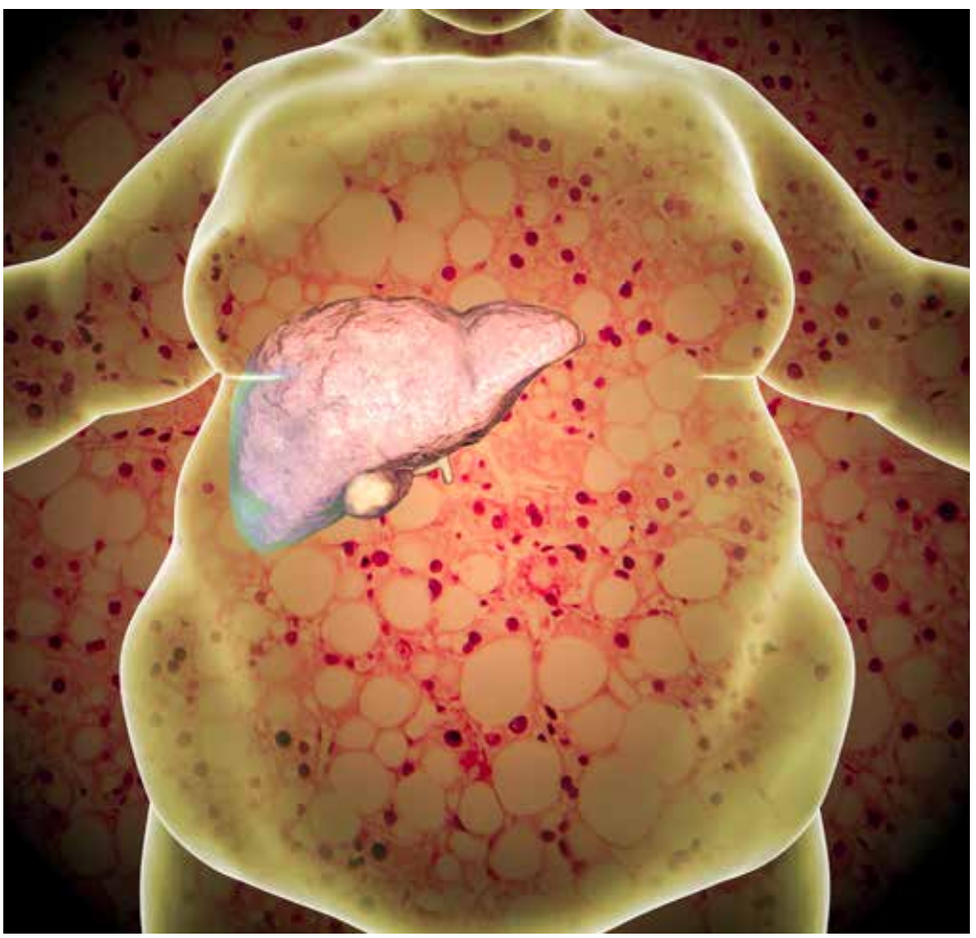
The researchers analysed participants' blood to measure their glucose and fat metabolism and to examine their genes for various genetic variants known from other liver diseases such as nonalcoholic steatohepatitis. All the participants then had a liver biopsy to determine the stage of fibrosis to indicate the extent of liver damage.

"This enabled us to compare the various risk factors with the stage of fibrosis. In particular, the homeostatic model assessment of insulin resistance was clearly linked to the stage of liver fibrosis, but the concentration of low-density lipoprotein (LDL) and a genetic variant of the PNPLA3 gene (encoding patatin-like phospholipase domain-containing protein 3) were also clearly associated," explains Mads Bastrup Israelsen.

A message that can change lifestyle

The new study cannot determine that insulin resistance and the genetic variant cause more severe alcohol-related liver disease but shows that they are associated. Future studies therefore aim to understand these associations.

"The genetic variant of PNPLA3 and insulin resistance are also associated with nonalcoholic steatohepatitis, with both increasing the risk of developing cirrhosis, so the evidence indicates that, although we currently differentiate between alcoholic and nonalcoholic liver diseases, the mechanisms that lead to these diseases probably overlap," says Mads Bastrup Israelsen.



The latest research suggests that the genetic variant of PNPLA3 also changes the bacteria in the gut, which is extremely interesting since this study is one element in two major research projects: MicrobLiver, led by Torben Hansen; and GALAXY, led by Aleksander Krag. Both projects seek to understand the interaction between the gut and the liver.

"Only 6% of the participants were metabolically healthy, which emphasizes the strong association between alcohol consumption, the liver, gut flora and people's general energy metabolism. The message is clear for preventing both alcoholic and nonalcoholic liver disease. In both cases, people need to drink less alcohol and consume fewer calories," explains Mads Bastrup Israelsen.

The new knowledge now provides an even better basis for assessing who should especially watch their alcohol consumption. In the future, people can be screened for the genetic variant of PNPLA3. In combination with a blood test for insulin resistance, this is an effective tool to assess whether an individual has higher risk.

"These people often do not have a complex alcohol use disorder but drink an extra glass of wine or beer. Therefore, dialogue often succeeds for them. The message that excessive drinking risks their liver is usually enough to make them change their lifestyle," says Mads Bastrup Israelsen.

"Metabolic and genetic risk factors are the strongest predictors of severity of alcohol-related liver fibrosis" has been published in Clinical Gastroenterology and Hepatology. In 2015, the Novo Nordisk Foundation awarded a Challenge Programme Grant to the Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen for the project MicrobLiver.

Childhood overweight associated with earlier puberty

Early puberty is a risk indicator for adult diseases such as depression, obesity, type 2 diabetes, cardiovascular diseases and cancer. There is therefore considerable interest in understanding why children trend towards beginning puberty earlier. The new study is one of the first major studies to indicate that overweight is also associated with the timing of puberty among boys. Researchers hope to learn why childhood obesity is associated with attaining puberty earlier so that those affected can avoid future negative health effects.

BY MORTEN BUSCH

Many parents want to hang on to their sweet little kids as long as possible rather than having to be dragged along by unruly teenagers. However, there are also good health reasons for children remaining in childhood until the right time. Major studies have shown that children who enter puberty too early or too late develop diseases in adulthood more often and earlier. New research has also indicated possible causes, with overweight being an important factor among girls – and now also boys.

"Unlike previously, the new study shows that overweight is not only associated with earlier puberty among girls. We found that girls who were overweight attained pubertal milestones on average 5 months earlier than normal-weight girls versus 3 months for boys who were overweight. To ensure that these trends were not caused by genetic and social factors, we examined 850 sibling pairs and observed identical trends in this group. Our theory is that being overweight shifts the balance in fat tissue by increasing the bioavailability of sex hormones, but it is still too early to say for sure," explains Cecilia Ramlau-Hansen, Professor, Department of Public Health, Aarhus University.

The association is convincing

The study is based on data from the Danish National Birth Cohort, in which 92,000 pregnant women who gave birth in 1996–2003 participated. The current study extracted data for 11,046 of the children born between 2000 and 2003. The parents reported the children's weight and height at age 7 years. Then the children completed a questionnaire every 6 months from 11 until 18 years old about their pubertal development, including pubic hair, acne, voice break and breast development.

"Based on the information on puberty the children provided, we determined when overweight children and normal-weight children reached the various stages of puberty. We were thus able to compare body mass index data with puberty and observed a definite correlation. Overweight girls attained pubertal milestones on average 5 months earlier than normal-weight girls versus 3 months for overweight boys," explains Nis Brix, PhD, Department of Public Health, Aarhus University.

The differences naturally vary enormously. The boys in the study had fully developed pubic hair (Tanner stage 5) when they turn 15 years. Boys who were overweight reached this on average 3–4 months earlier and boys with obesity 5–6 months earlier. Among the girls, the difference was even greater. Normal-weight girls usually have full pubic hair shortly before they turn 16 years, but this was 6–7 months earlier among girls with overweight and 9–10 months earlier among girls with obesity.

"These are clear associations, but to ensure that overweight is the cause rather than genetic factors, social class or something completely different, we conducted a sibling-matched analysis in which we compared children who had grown up under the same conditions and with similar DNA. The numbers naturally decrease, and so does the statistical significance, but the associations were the same, so we feel reasonably confident that the link between overweight and earlier puberty did not result from other factors associated with both overweight and early puberty," says Nis Brix.

Mechanism remains unknown

The new study indicates that body mass index and puberty are clearly linked – even after adjusting for confounders such as genetics, parents' highest educational level, maternal smoking in the first trimester, mother's body mass index during pregnancy, mother's age at menarche, birthweight, childhood diet and childhood physical activity. The researchers therefore believe that the physiological effects of childhood overweight may cause earlier puberty.

"We have considerable research ahead before we can explain the exact mechanisms that lead to the altered puberty profile. Previous studies have shown that childhood obesity is associated with elevated insulin levels and insulin resistance, which is thought to trigger earlier puberty because it disturbs the balance between the hypothalamus, pituitary gland and the body's glands," explains Nis Brix.

Much evidence suggests that the effect of insulin on the liver, adrenal glands, ovaries and fat tissue earlier in life also greatly affects the availability of steroids and sex hormones, which in turn can lead to earlier onset of puberty. In any case, the new results are worrying.

"Although previous studies suggest that earlier puberty leads to an increased risk of depression, cardiovascular diseases, diabetes and some types of cancer, such as breast cancer, it is extremely important to understand what mechanisms are affected and whether that influence can be prevented and then to try to prevent obesity in childhood," concludes Cecilia Ramlau-Hansen.

"Childhood overweight and obesity and timing of puberty in boys and girls: cohort and sibling-matched analyses" has been published in the International Journal of Epidemiology. The Novo Nordisk Foundation awarded grants to Cecilia Ramlau-Hansen in 2014 and 2019. The Danish National Birth Cohort has received grants from the Danish National Research Foundation, Denmark's administrative regions, the Novo Nordisk Foundation and others.

How the brain consolidates information while you rest

Researchers have identified a signal in the brain that appears to play an important role when the brain consolidates new information into memory so that the information can be more easily retrieved later.

BY KRISTIAN SJØGREN

he brain is an incomparable machine capable of performing absolutely amazing things.

For example, memory is the result of a combination of electrical charges and anatomical imprints, which enable you to evoke your childhood memories or the road home from the city after a pub crawl.

For many years, researchers have studied how our brains at rest spontaneously replay recently acquired information to consolidate it in memory, but how this process is orchestrated is an open question.

Now, researchers in the United Kingdom and Denmark have made a discovery that can help explain what happens in the brain when information is consolidated so that it can always be retrieved.

"In our study, we link two signals in the brain. One is related to subconscious consolidation of memory and the other to attention and cognition. Our discovery is useful because this type of memory consolidation is known to be preserved across all animals, and because errors in the same mechanisms are likely to play important roles in various brain disorders, including stroke and Alzheimer's disease," explains a researcher behind the new study, Diego Vidaurre Henche, Associate Professor, Department of Clinical Medicine, Aarhus University.

The research has been published in *Neuron*.

Memory needs to consolidate information

The brain is marvellous and excels at storing complicated information.

This applies if you want to learn to play classical music on the piano or find your way around a new city.

When the brain stores new information, it must first learn this information. The brain does this when you play music from a score or walk in the city for the first time. What you learn is therefore stored anatomically.

However, what you learn must also be consolidated in your memory, so that you do not have to actively remember all the notes in the music or the route through the city.

The brain consolidates the new information to store the information in the subconscious.

In the example of the walk in a new city, this might happen later when sitting down at a café – or when you sleep. When the brain is no longer actively engaged in learning, the information can be rapidly processed and consolidated.

"When you then sit down and relax, the brain rapidly repeats the neural sequence that was built up while walking, and eventually sends it to the cerebral cortex for final consolidation. The hippocampus is critically involved in this process, which researchers call signal replay. In experiments with mice and rats, we can find this signal in their brains as they subconsciously recapitulate what they learned," says Diego Vidaurre Henche.

Examined brain signals while trial participants rested

The researchers studied replay in more detail among participants who were asked to learn and remember a sequence of items from pictures they were shown.

The participants wore helmets that monitored the electrical activity in their brains.

The researchers studied the electrical activity of the brain when the participants actively tried to remember the images but also when they thought about other things.

The researchers used advanced statistical methods to identify signals related to consolidation.

"In a previous study, we identified signals in the brain that are likely related to paying less attention to the outside environment and more to our own thoughts. In this study, we went a step further and linked the replay signal to this "reflective" signal in the brain," explains Diego Vidaurre Henche.

In a previous study, we identified signals in the brain that are likely related to paying less attention to the outside environment and more to our own thoughts.

Associated with "doing nothing"

The second signal in the brain is the electrophysiological signature from the default mode network (DMN).

Although it has been traditionally associated with "doing nothing", the DMN is the signal the brain activates when we think or do something inside our heads. Conversely, this signal goes away when pay attention to the external world. An increase of the DMN signal can therefore be linked to the activity of the brain when we think about nothing in particular and disappear into an inner world.

Researchers have for many years studied the DMN and its signal.

"For example, we know that disruptions of the DMN are linked to mental disorders. So far, however, many aspects about this network and its significance remain enigmatic," says Diego Vidaurre Henche.

The default mode network is not simply a default setting

The evidence indicates that replay depends on the DMN.

The researchers analysed the brain scans and found that the DMN becomes more active when replay takes place.

This is the first time that researchers have discovered the link that substantiates that the DMN is related to inner attention and consolidating memories.

"Our discovery means that we should probably stop thinking of the DMN as the brain's default setting, which is initiated when nothing else happens. Our study suggests that DNM coordinates replay so that the replay signal does not conflict with ongoing cognition," concludes Diego Vidaurre Henche.

"Replay bursts coincide with activation of the default mode and parietal alpha network" has been published in Neuron. In 2019, the Novo Nordisk Foundation awarded a grant to Diego Vidaurre Henche for the project Disambiguating the Neural Threads of Perception and Cognition to Characterise Individual Behaviour.

Dual regimen targeting ***appetite and energy*** expenditure enables lasting weight loss

Losing weight is difficult enough, but maintaining weight loss is almost impossible for many people. If you lose weight, your body increases your appetite and slows down your energy expenditure. Researchers have tested a combination therapy in which an exercise programme boosts energy expenditure and a medication suppresses appetite. This results not only in significant and lasting weight loss but, even more importantly, a healthier body with reduced fat percentage, increased fitness level and improved glucose regulation.

BY MORTEN BUSCH

Everyone who has tried to lose weight has noticed how well the human body can prevent this happening. If you lose weight, your hormonal system reacts abruptly, adjusting appetite hormones so that you feel very hungry. In addition, energy expenditure decreases and you burn fewer calories. Dieting therefore often results in the same outcome: losing weight for a while, but the extra kilos often pile back on in the long term. Now people with obesity have a beacon of hope.

"People have experienced promising weight loss, but this has proved difficult to maintain. We combined an exercise programme, to increase energy expenditure, with a medication to suppress appetite, and obtained additional weight loss and beneficial health effects for up to 1 year. We hope that maintaining these effects for 1 year and preferably longer can eventually alter the body's hormone balance and by maintaining the exercise programme, creating lasting lifestyle changes," explains Signe Sørensen Torekov, Professor with Special Responsibilities, Department of Biomedical Sciences, University of Copenhagen.

Lost twice as much fat

The trial participants were 195 people with obesity and a low fitness level. All the participants followed an 8-week low-calorie diet, which resulted in losing an average of 13 kg of body weight. In addition, as expected, the participants' health improved significantly, primarily a decrease in blood glucose levels and blood pressure. Then the participants were divided into four groups.

"One carried out a moderate-to-vigorous exercise programme; one was treated with an appetite suppressant; a third group combined the appetite suppressant and the exercise programme; and the fourth group was a control group with habitual activity and placebo. The participants did not know whether they received the appetite suppressant or the placebo," says Signe Sørensen Torekov.
The trial participants were monitored for 1 year to determine whether they regained the weight

they had lost. Unsurprisingly, the people in the placebo group did, regaining 6 kg on average and, most importantly, the positive health effects that arose in the beginning disappeared. Both the exercise group and the appetite suppressant group maintained their weight loss during the trial period and reduced their body fat percentage.

"The people who both exercised and took the appetite suppressant lost another 3 kg but, even more importantly, they reduced their body fat percentage by 3.9 percentage points, about twice as much as both the exercise group and the appetite suppressant group. In addition, only the people carrying out the dual regimen had improved insulin sensitivity. Both exercise groups improved their fitness, which is an important health indicator associated with mortality from cardiovascular disease," explains Signe Sørensen Torekov.

Survival instinct for at least a full year

The dual regimen of exercise and appetite suppressant thus provided twice as many health benefits as each individual regimen and resulted in a significant loss of fat mass and either maintaining or increasing muscle mass.

"Since increasing muscle mass provides significant health benefits, not focusing solely on weight but also on the physical distribution of fat and muscle mass is important. In addition, losing weight can trigger loss of muscle mass and less energy expenditure. Obesity treatment so far has typically focused on how to lose weight and not so much on how to maintain weight loss and the long-term health benefits," says Signe Sørensen Torekov. Half of Denmark's population is overweight and almost 20% have obesity, and this can have major health effects. Although the proportion of people with overweight varies greatly between countries and continents, having obesity is definitely associated with an increased risk of premature death and comorbid conditions such as type 2 diabetes, cardiovascular disease, cancer and reduced fertility – and especially reduced quality of life.

"This is the first well-documented study of which treatment method is best for maintaining healthy weight loss. We show for the first time how people with obesity can maintain weight loss by comparing four strategies for maintaining weight loss – new knowledge that doctors, dietitians and physiotherapists can use in practice. This is the evidence we have been lacking," explains Signe Sørensen Torekov.

Thus, an exercise programme consisting of mostly vigorous-intensity exercise for about 2 hours a week combined with an appetite suppressant therefore seems to work. Obtaining the appetite suppressant, an analogue of the appetite suppressant peptide GLP-1, requires a prescription. The trial showed that the exercise programme often requires feedback from a healthcare professional.

"People who lose weight struggle against powerful biological processes, and we have previously measured the hormone-induced increase in appetite following weight loss. We hope to better understand how, whether and when the appetite hormone balance can be normalized after weight loss while maintaining the exercise programme," explains Signe Sørensen Torekov.

"Healthy weight loss maintenance with exercise, liraglutide, or both combined" has been published in The New England Journal of Medicine. In 2016, the Novo Nordisk Foundation awarded a grant to Signe Sørensen Torekov for the project Synergistic Effects of GLP-1 and Exercise on Immunometabolic Health.

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