

# The hibernation in brown bears: A model for medical and space research

**Etienne LEFAI**

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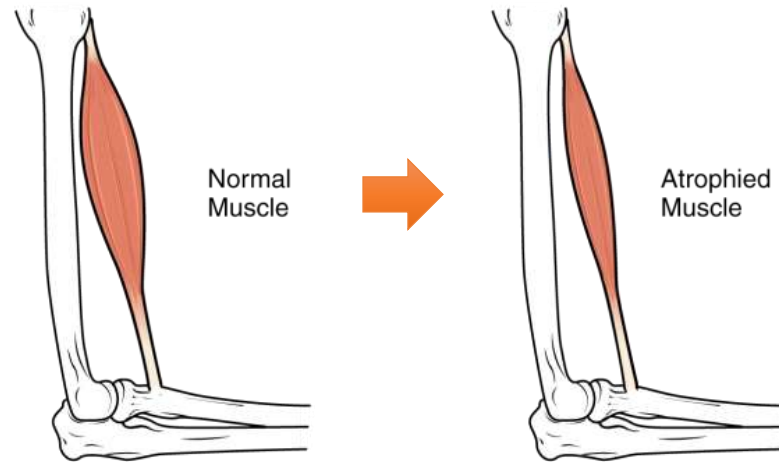
INRAE, Université Clermont Auvergne  
France



# The hibernation in brown bears: A model for medical and space research

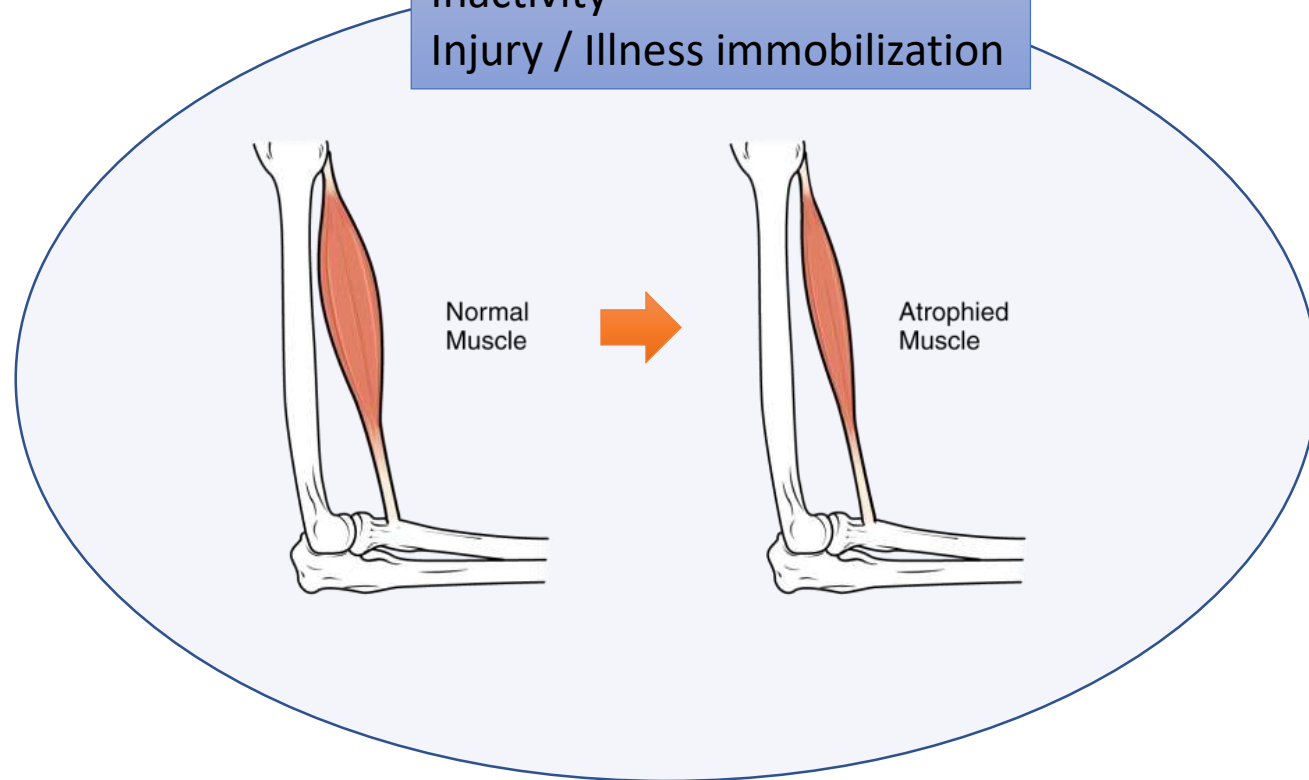


## Muscle atrophy

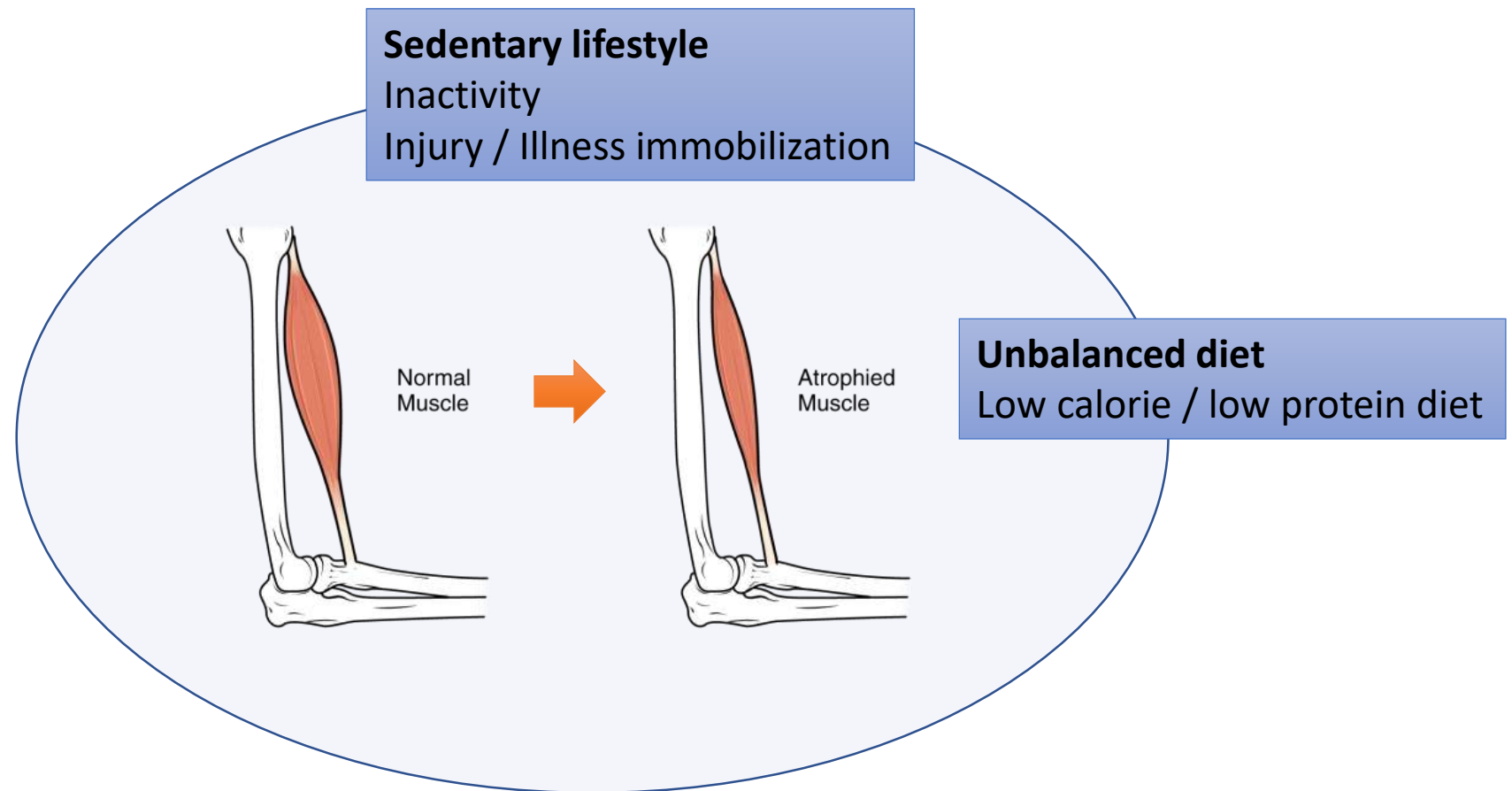


## Muscle atrophy

Sedentary lifestyle  
Inactivity  
Injury / Illness immobilization

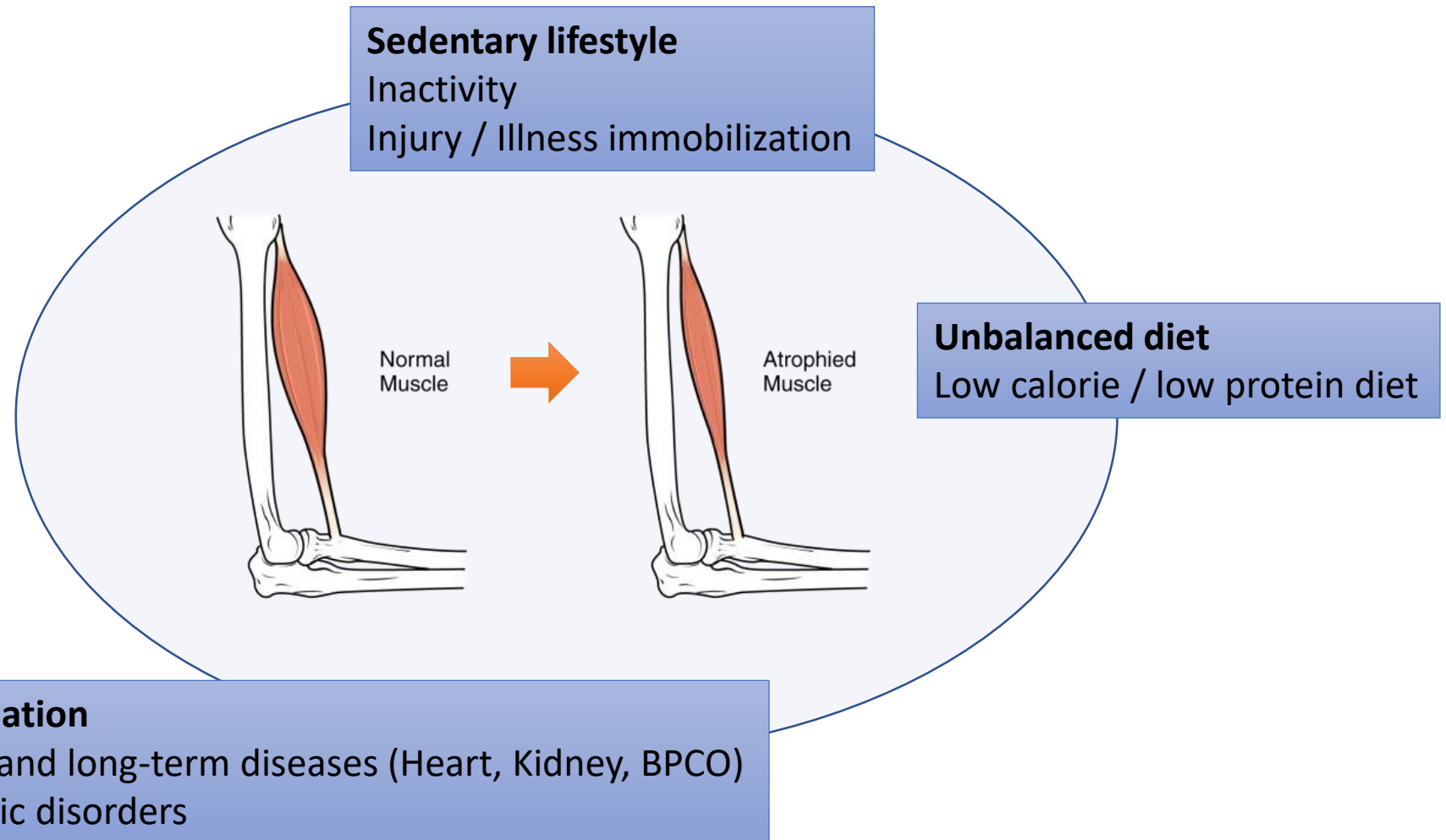


## Muscle atrophy





## Muscle atrophy

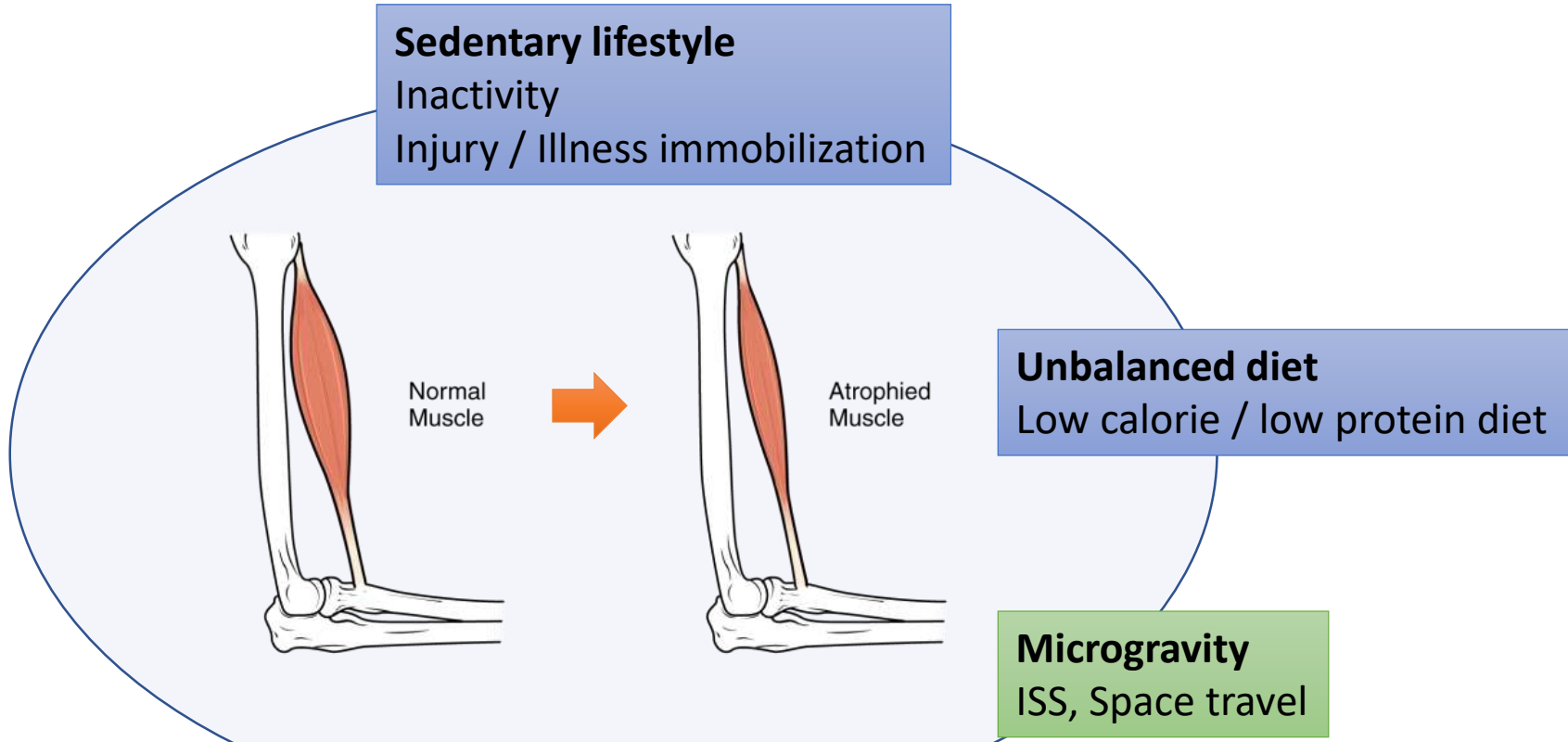


# Muscle atrophy

PHYSICAL INACTIVITY

NUTRITION

Sedentary lifestyle  
Inactivity  
Injury / Illness immobilization



Unbalanced diet  
Low calorie / low protein diet

Microgravity  
ISS, Space travel

Inflammation  
Chronic and long-term diseases (Heart, Kidney, BPCO)  
Metabolic disorders

## Experimental models to study muscle atrophy

Human



Bed rest, Dry immersion,...

Animal

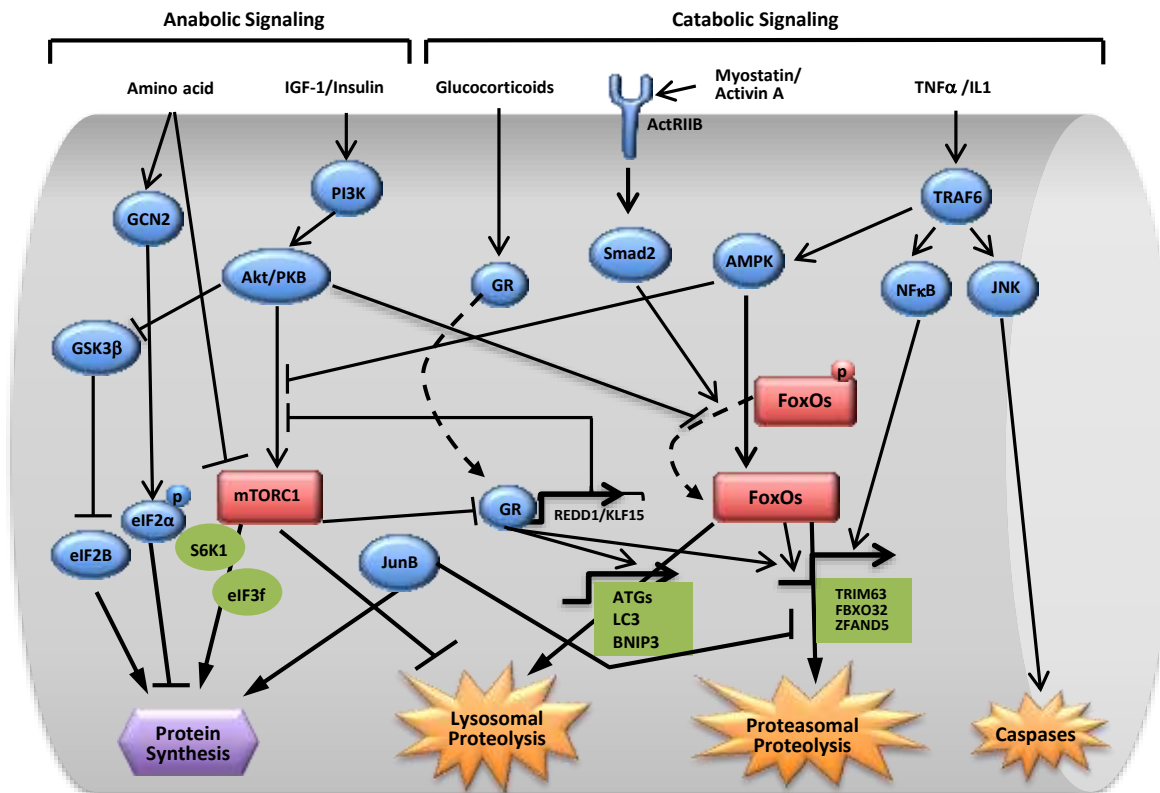


Suspension, denervation, starvation...





# Muscle protein balance



From Polge et al., 2013;6:25-39



More than 35 transgenic and KO mice for genes involved in anabolic and catabolic pathways :

- Ubiquitin proteasome
- Autophagy lysosome
- IGF1-Akt signaling pathway
- TGFβ-myostatin pathway
- Inflammatory cytokines and NFκB signaling
- Acetylating enzyme and MRFs

Efficient preventive or therapeutic treatments are still needed !!

Review in Bonaldo P , and Sandri M  
Dis. Model. Mech. 2013;6:25-39

## Biomimicry : looking for new biological models

*“For such a large number of problems there will be some animal of choice or a few such animals on which it can be most conveniently studied”*

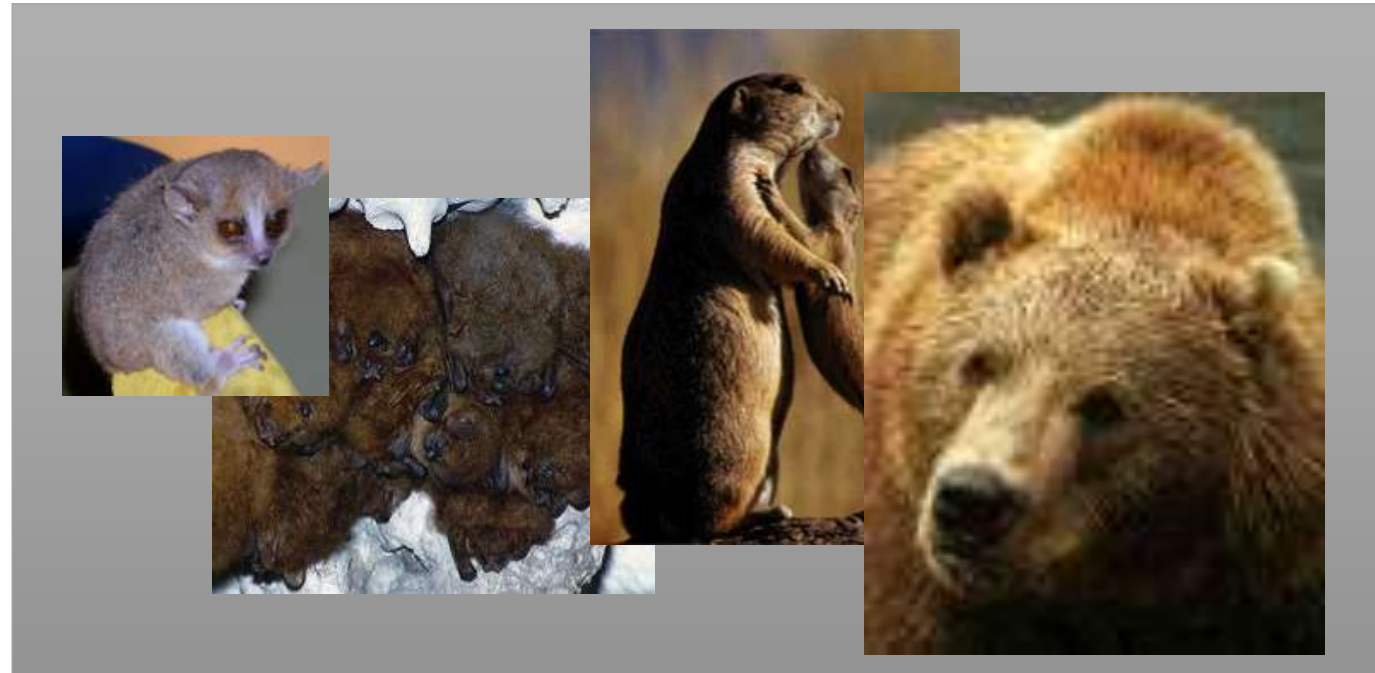
**Krogh, A. (1929). The progress of physiology. *Science* 70, 200–204**

## Biomimicry : looking for new biological models

### HIBERNATION

PHYSICAL INACTIVITY

STARVATION



Groundhog, Squirrel, Bat, Bear,...

Bears : Natural resistance to muscle loss in atrophic conditions

## Bear as a biological model

### Active period



Summer

#### Main features :

- Adult : 80/+250kg
- Hibernation : 4-7 months

### Hibernation

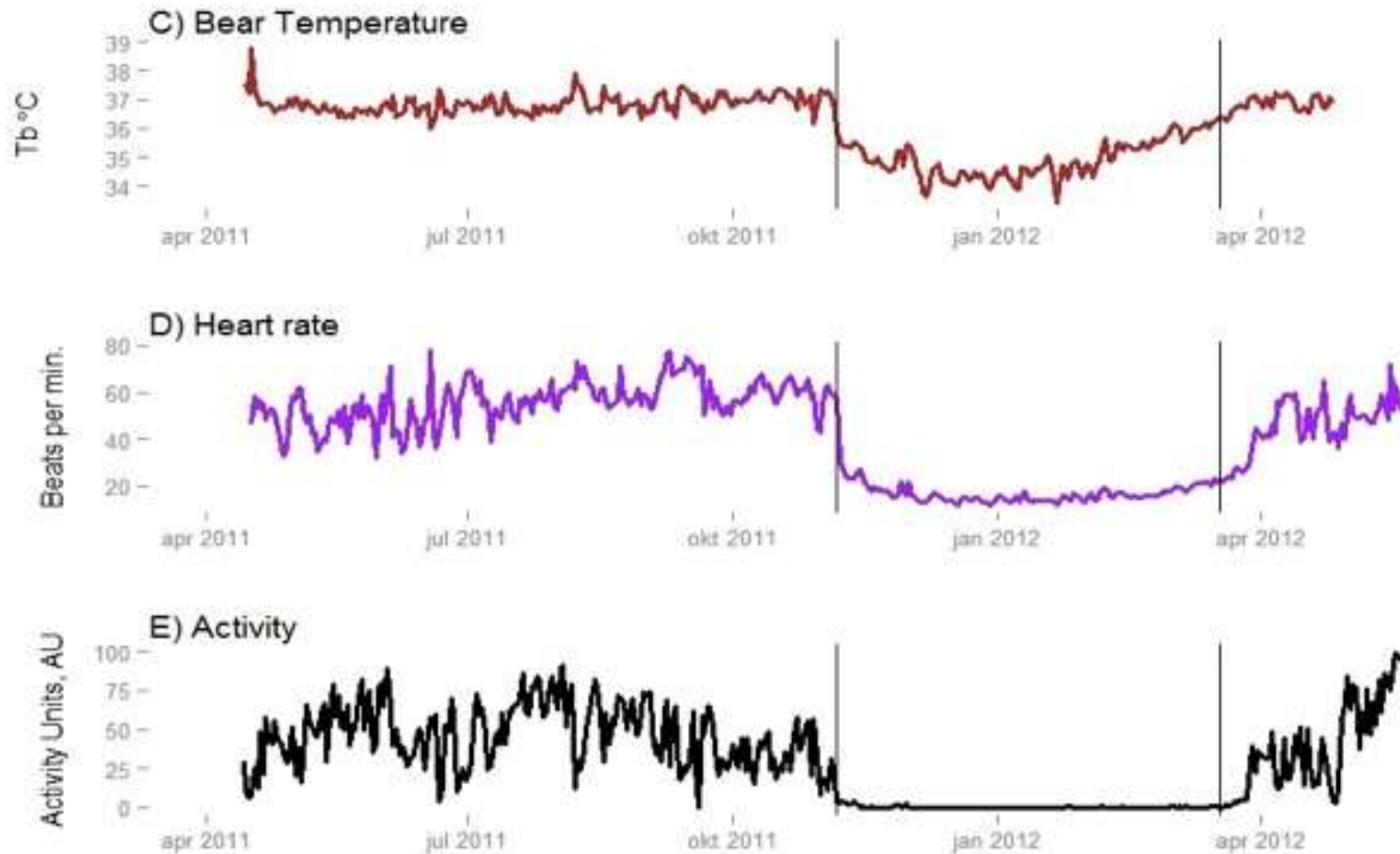


Winter

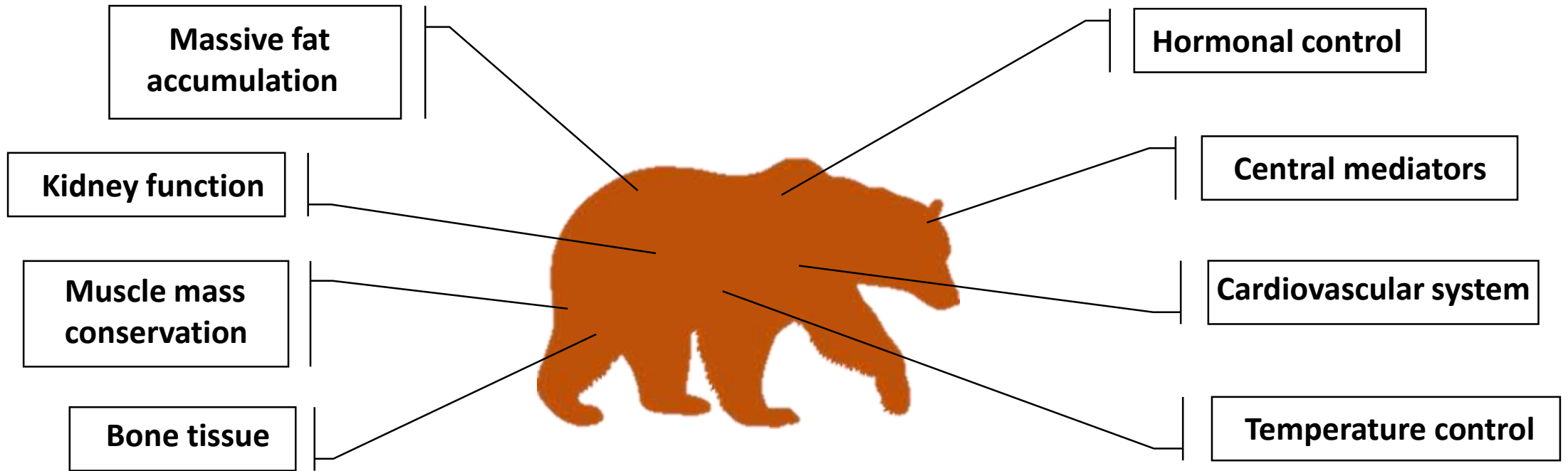
#### Hibernation physiology:

- No food or water intake
- Total inactivity
- Near normal body temperature

## European brown bears (SBBRG)

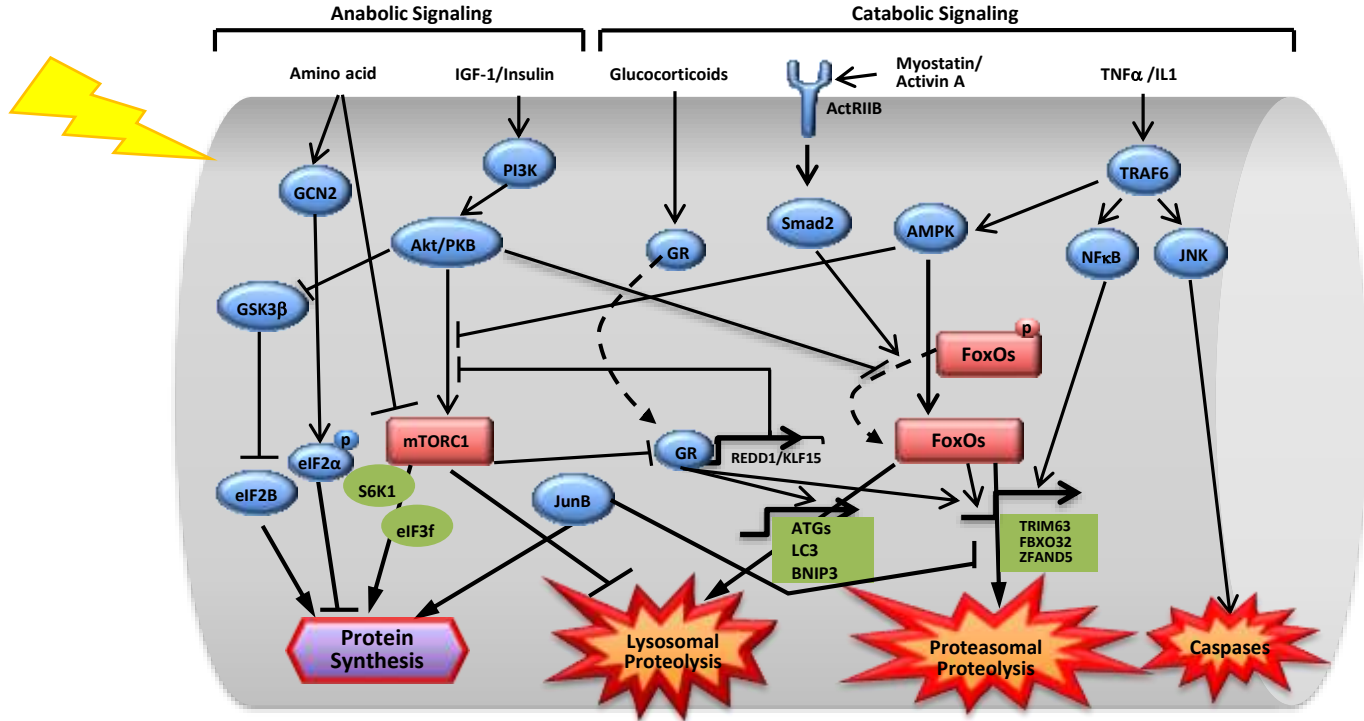






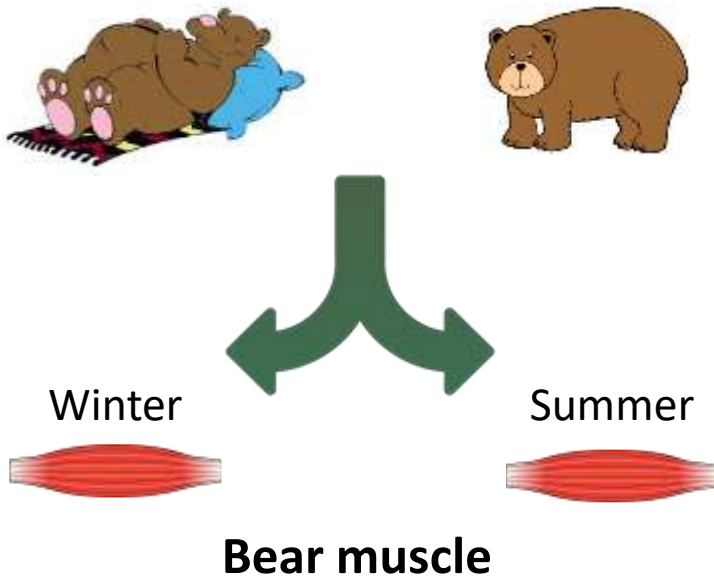
# How hibernating bear muscle differs from atrophy induced models ?

Muscle disuse,  
Fasting,  
...



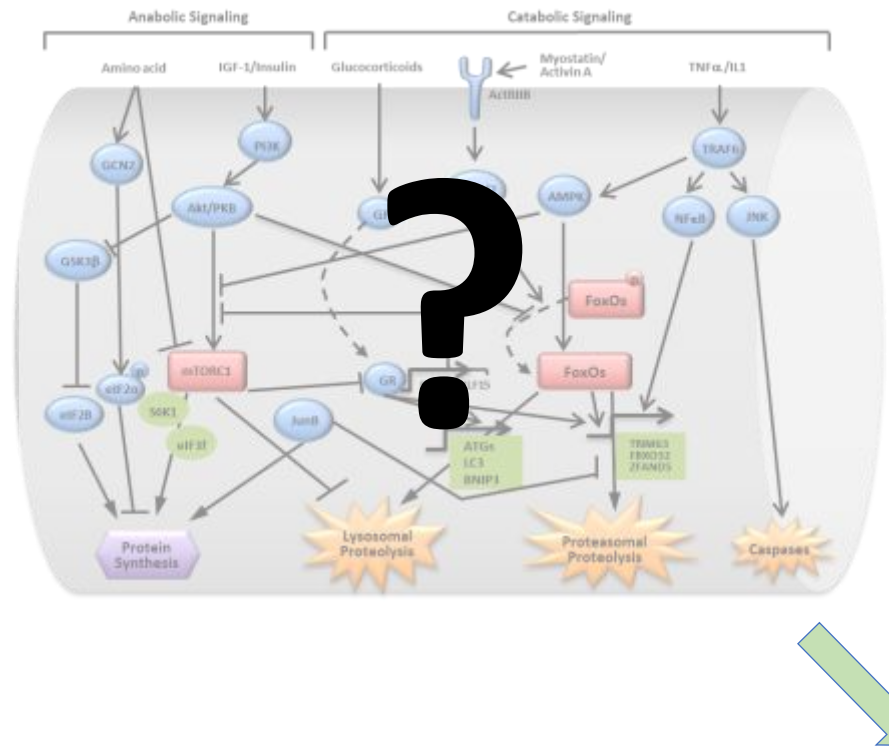
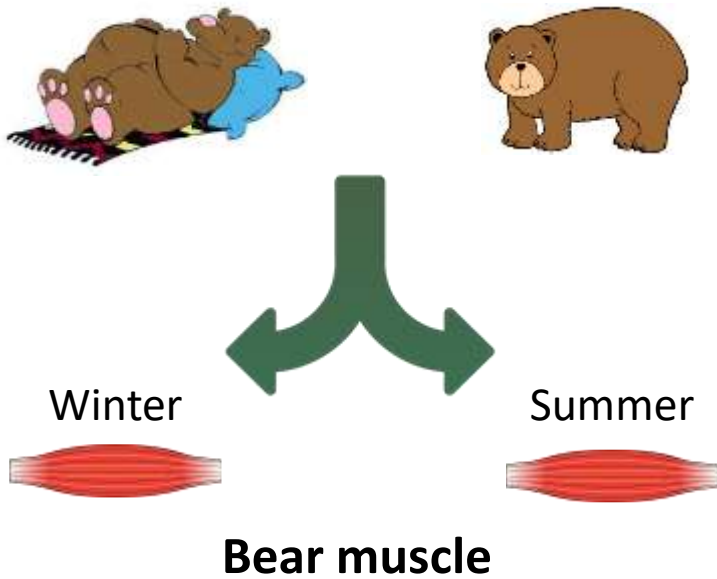
## How hibernating bear muscle differs from atrophy induced models ?

Bear tissue sample collection



# How hibernating bear muscle differs from atrophy induced models ?

Bear tissue sample collection



- Transcriptome
- Proteome
- Histology
- Metabolism
- Protein balance



## European brown bears (*Ursus arctos*)



- Free ranging animal : capture twice a year : February and June
- Young animals : 2- 3 years old (35-80 kg)
- Records for body temperature, heart rate and activity

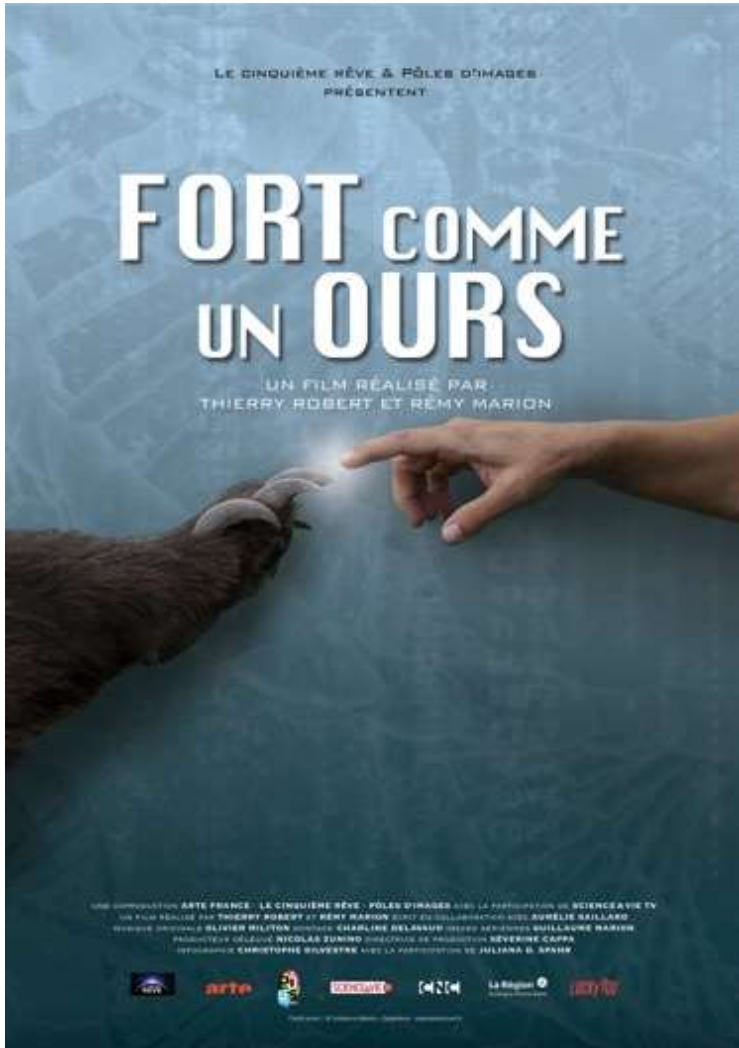


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Björnprojektet

<http://bearproject.info/>



Winter and summer captures

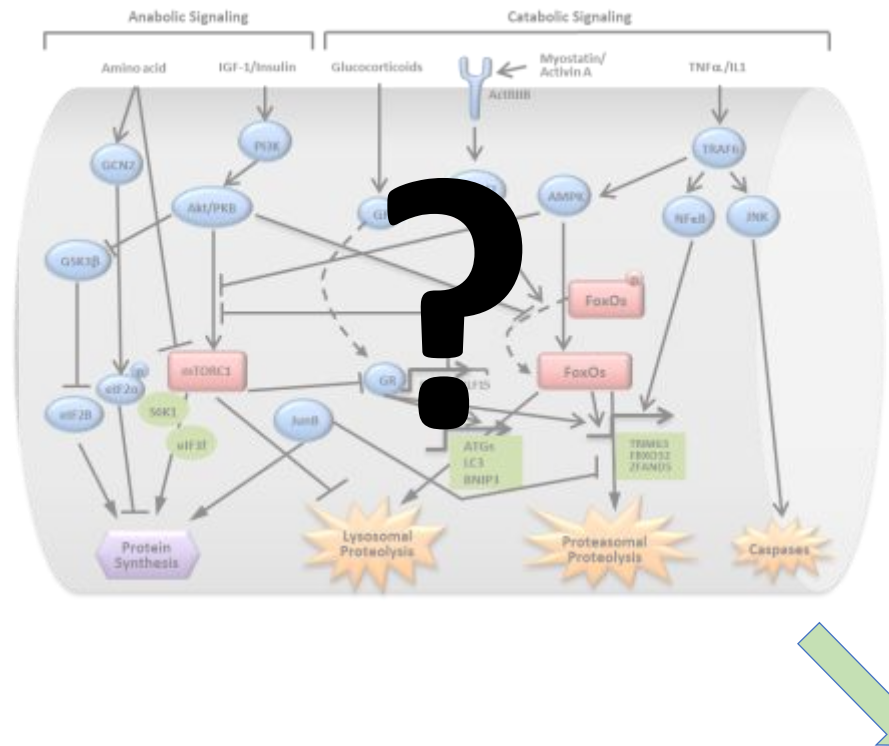
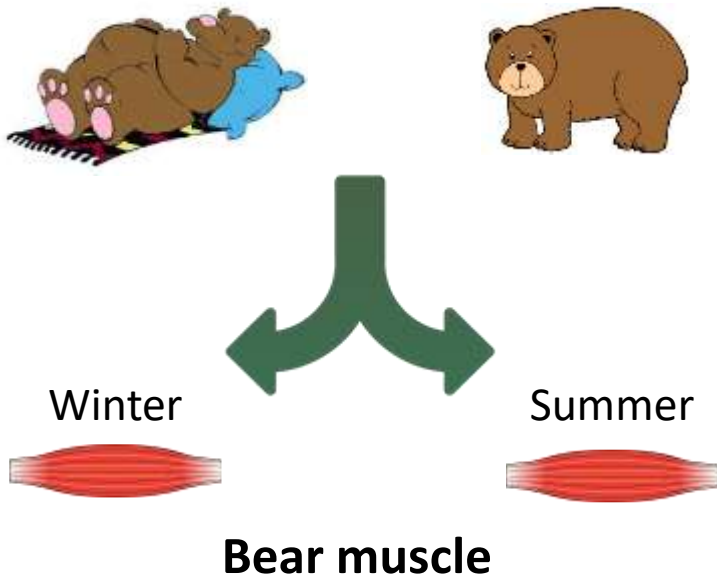


Réalisateurs : R. MARION  
T. ROBERT

Production : Le 5<sup>ème</sup> Rève  
ARTE

# How hibernating bear muscle differs from atrophy induced models ?

Bear tissue sample collection



- Transcriptome
- Proteome
- Histology
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# How hibernating bear muscle differs from atrophy induced models ?

## Bear muscle transcriptome

Brown bear (*Ursus arctos ssp horribilis*) genome assembly release (Oct. 2018)

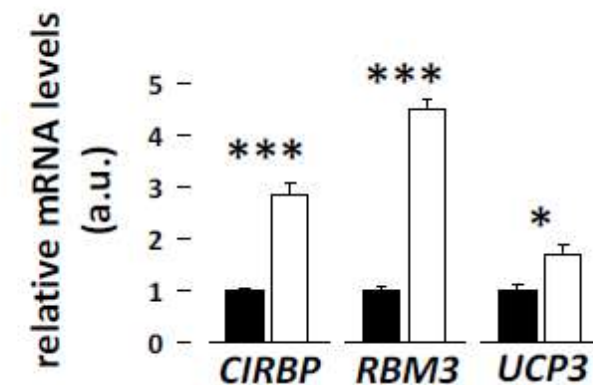
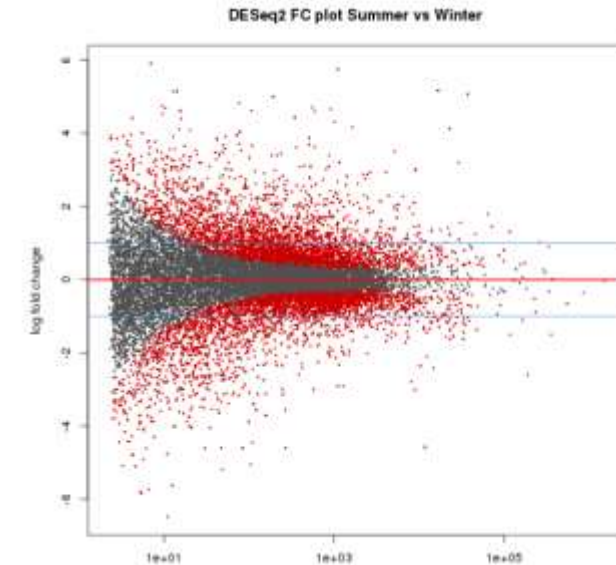
**RNA-Seq experiment** on paired summer and winter bear muscles

- Almost 18 000 individuals mRNA identified
- Nearly 6 000 display up or down regulation

Protein synthesis, ECM, mitochondria, metabolism,...

Protein balance signaling pathways

**Cold response genes**  
(Chazarin et al. *Antioxidants* 2019)



# How hibernating bear muscle differs from atrophy induced models ?

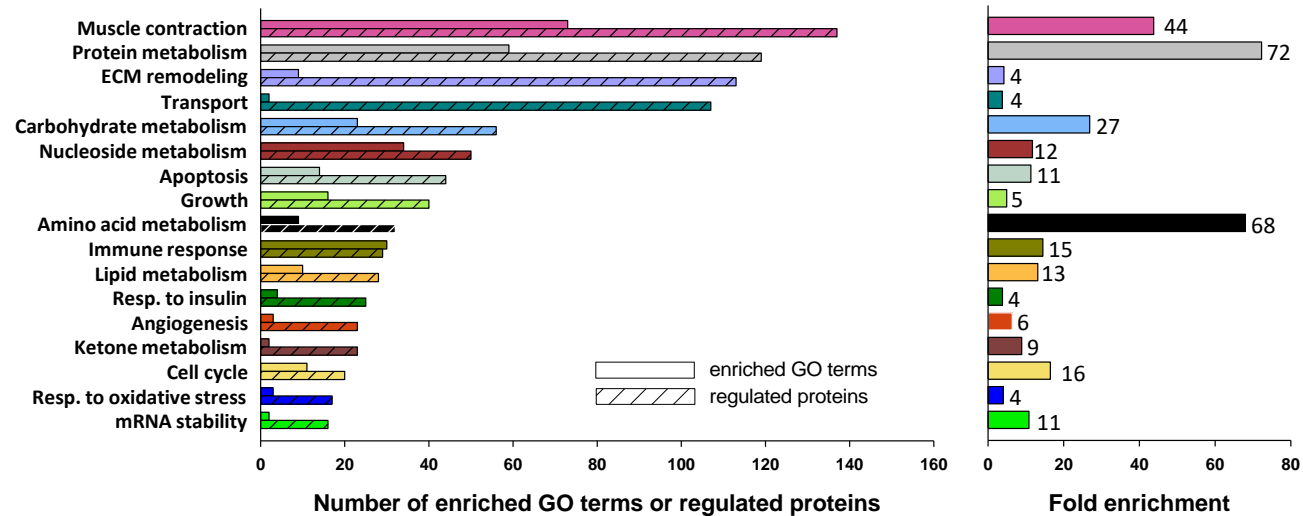
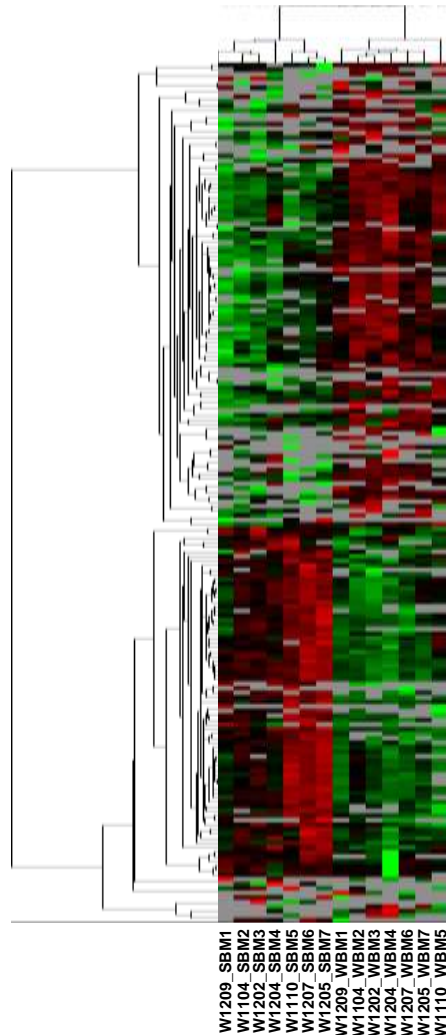
## Bear muscle proteome



### Proteomic analysis (Mass Spec intensity based label-free method) on bear muscle

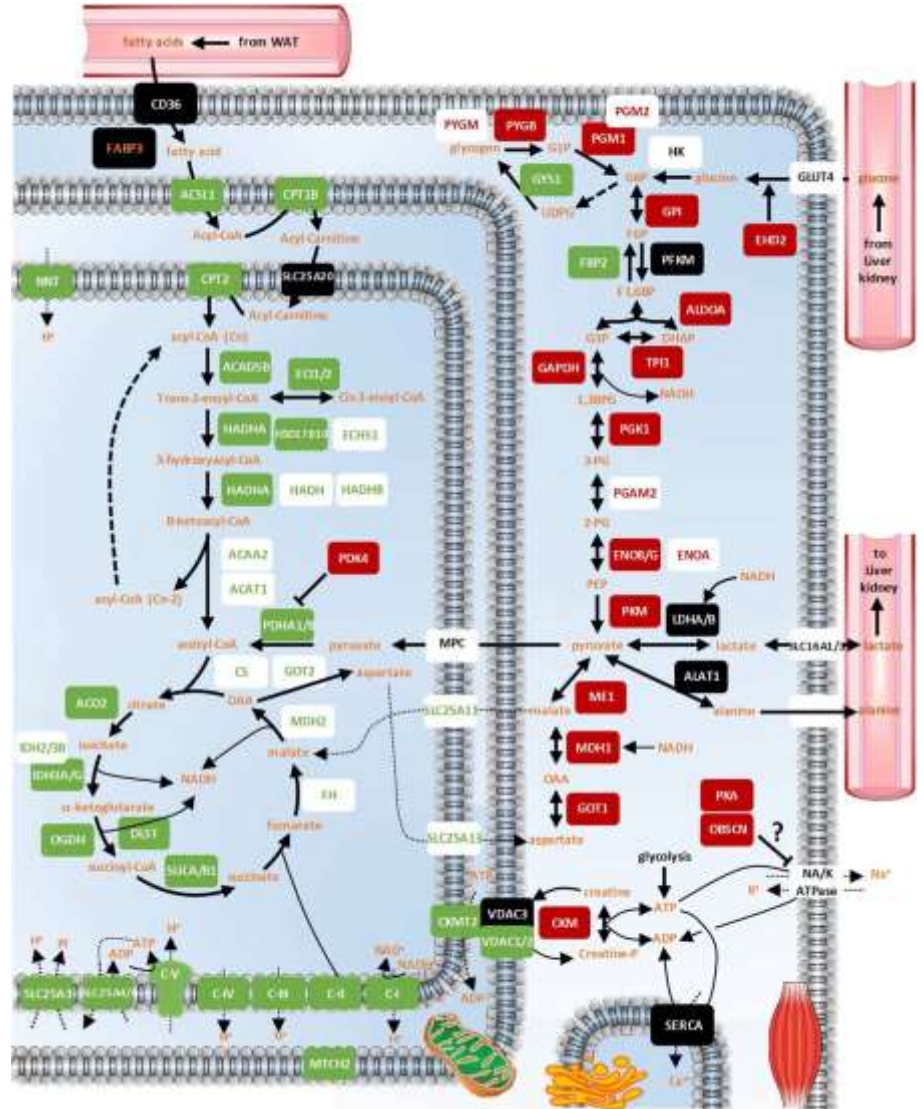
- Almost 1000 proteins quantified
- 146 proteins with differential expression between summer and winter tissues

Chazarin et al, *Front Zool* 2019





# How hibernating bear muscle differs from atrophy induced models ?



## Bear muscle metabolism

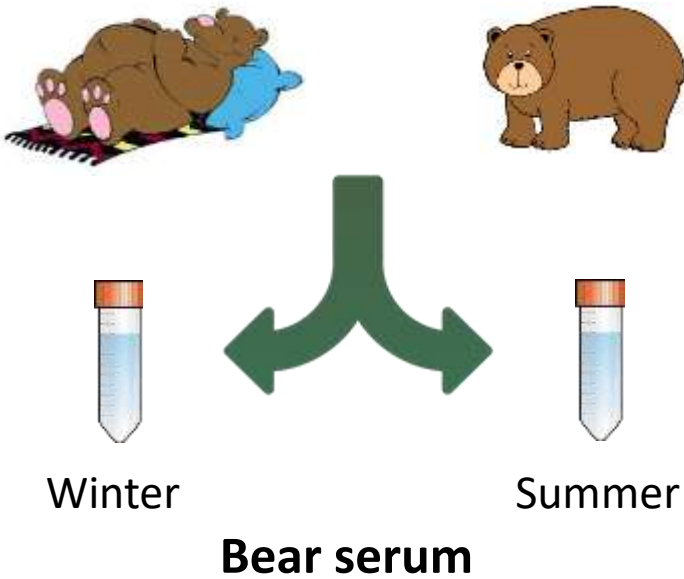
- Metabolic reprogramming**
- Glycolysis maintenance
  - Mitochondrial down regulation
- Oxidative stress reduction**
- NRF2 activation
  - Protein carbonylation

Chazarin et al, *Front Zool* 2019  
 Chazarin et al, *Antioxidants* 2019



## Is there a circulating factor involved in protein sparing ?

Bear serum sample collection

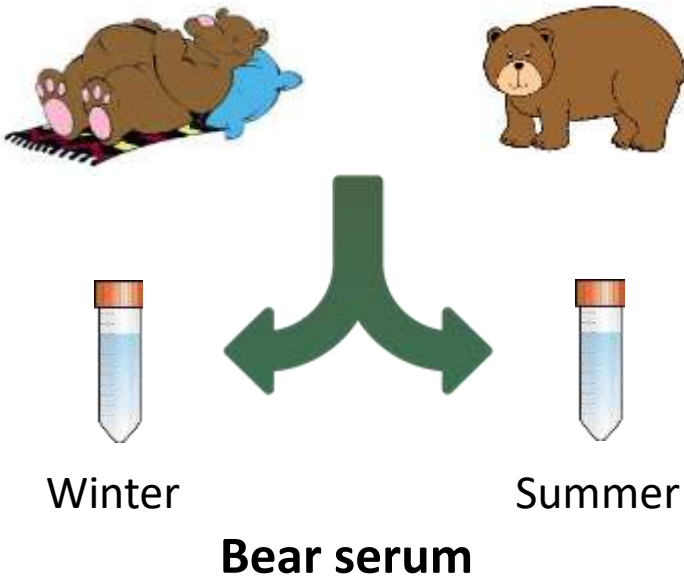


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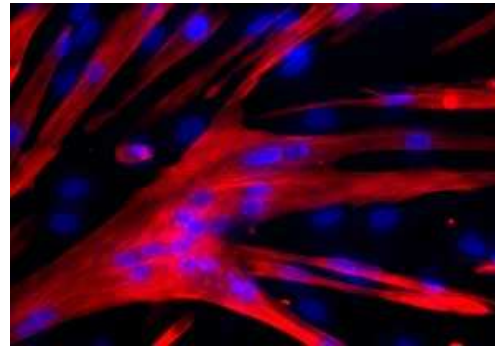
<http://bearproject.info/>

# Is there a circulating factor involved in protein sparing ?

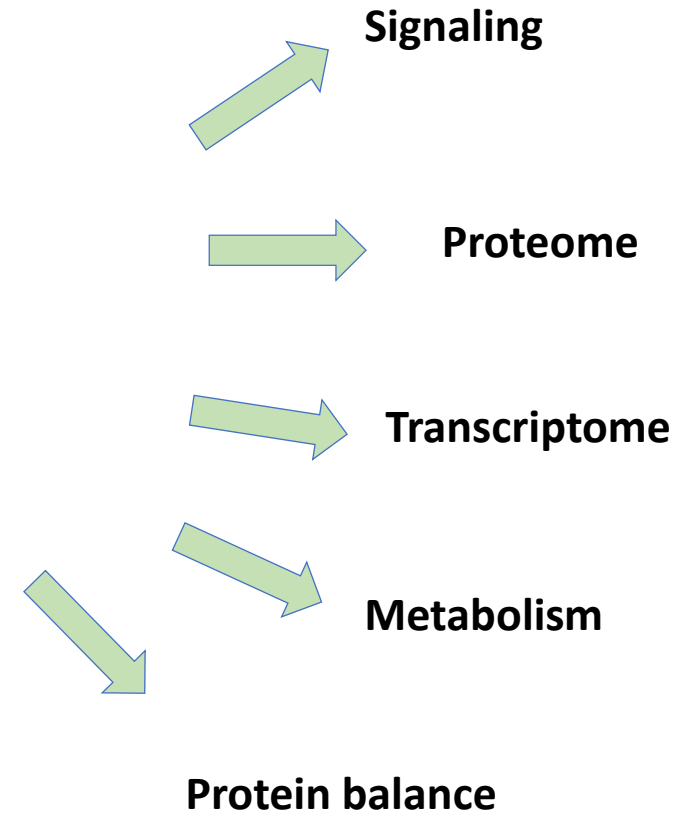
Bear serum sample collection



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Björnprojektet  
<http://bearproject.info/>

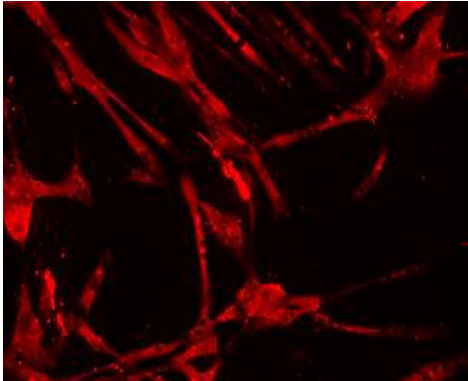


Human primary myotubes

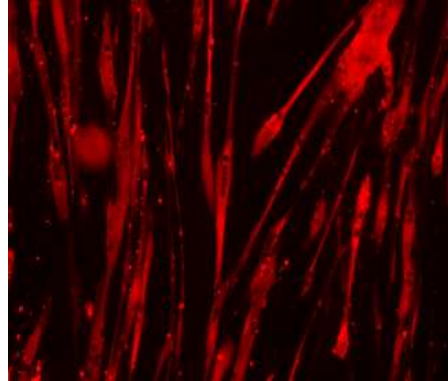


## Cell protein content : Myosin quantification

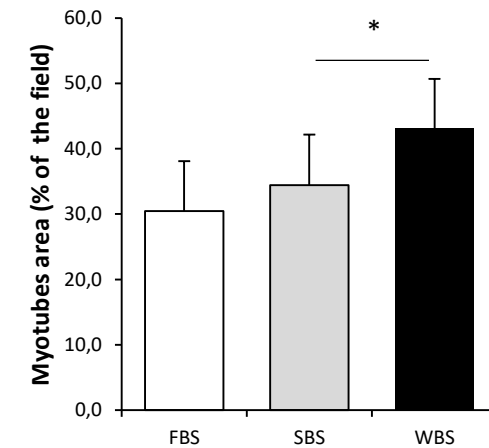
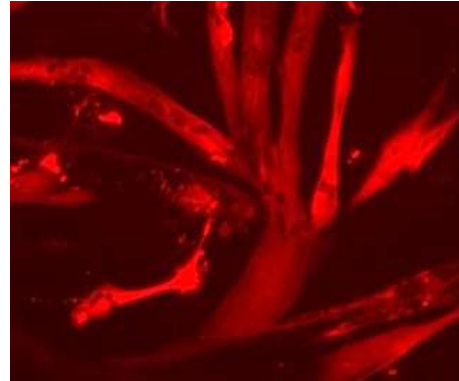
FBS



SBS



WBS



Protein content increase in WBS condition

Decreased in both protein synthesis and protein degradation rates

Chanon et al., *Sci Rep* 2018

In bears

In human muscle cells

Muscle tissue

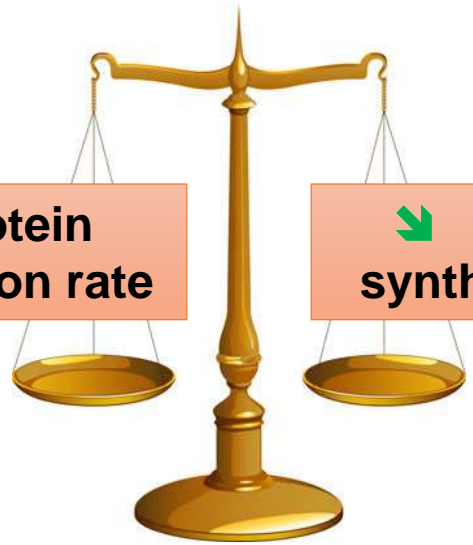


Bear serum  
Winter vs Summer



↓ Protein  
degradation rate

↓ Protein  
synthesis rate

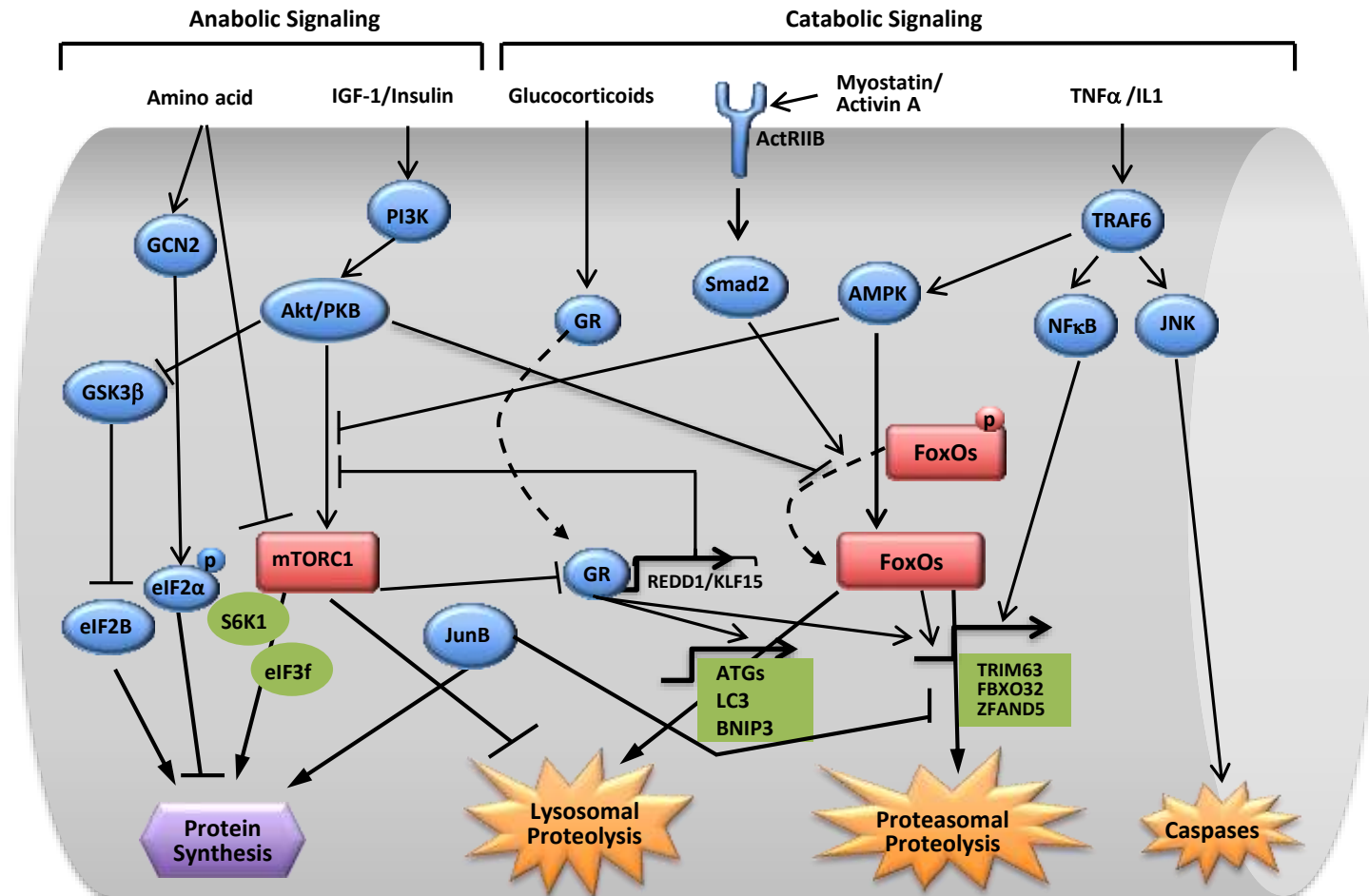


Protein turnover ↓  
Slightly negative balance

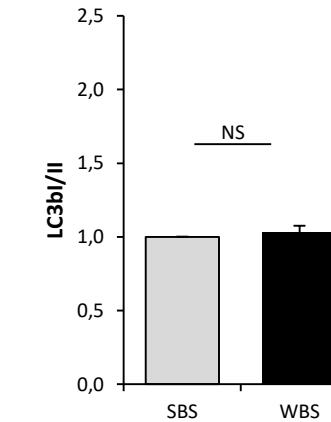
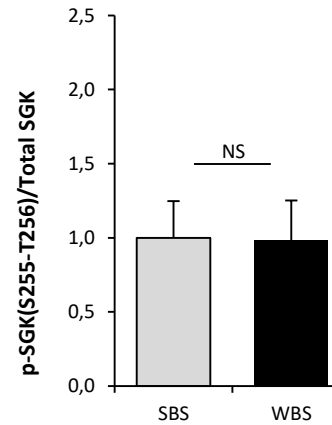
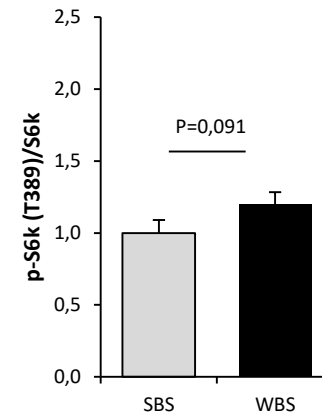
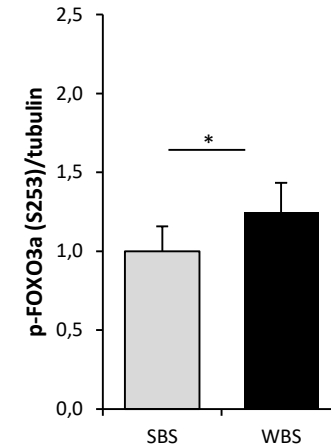
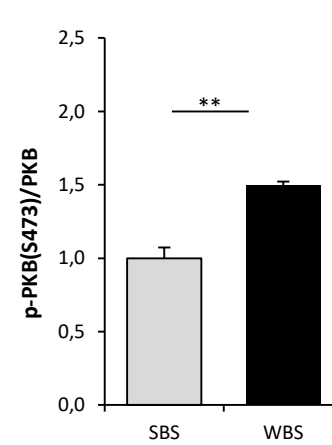
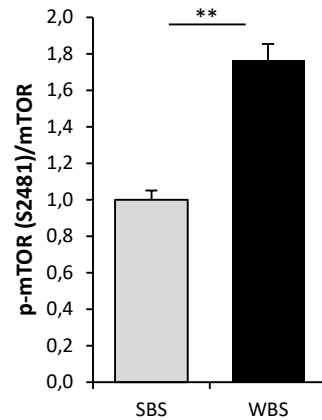
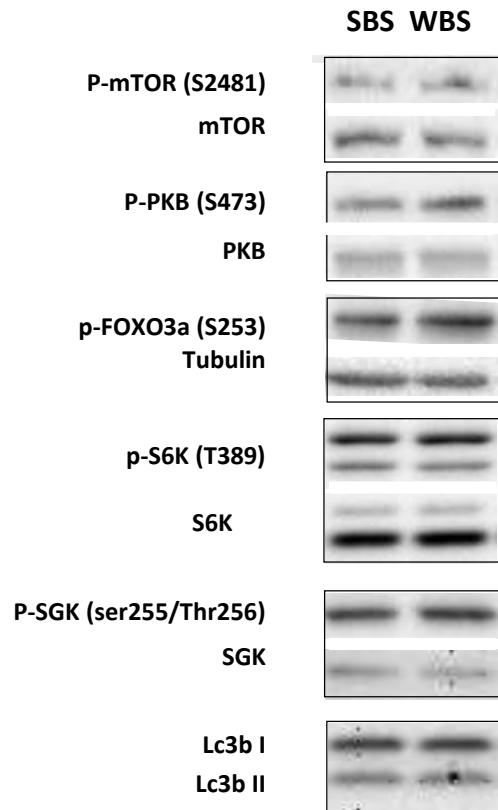
Protein turnover ↓  
Slightly positive balance



# How winter bear serum modifies human myotube protein turnover ?



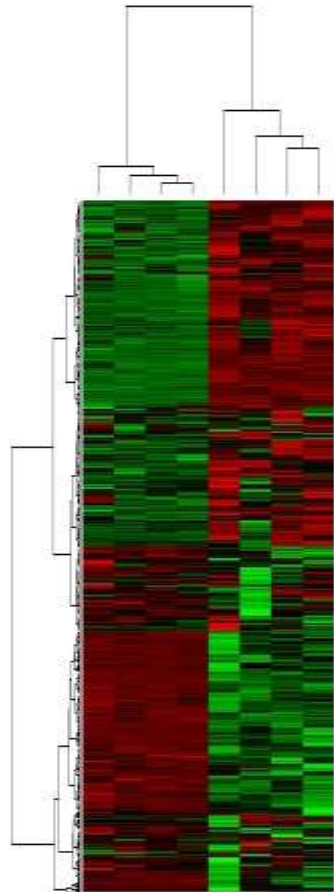
# Signaling pathways



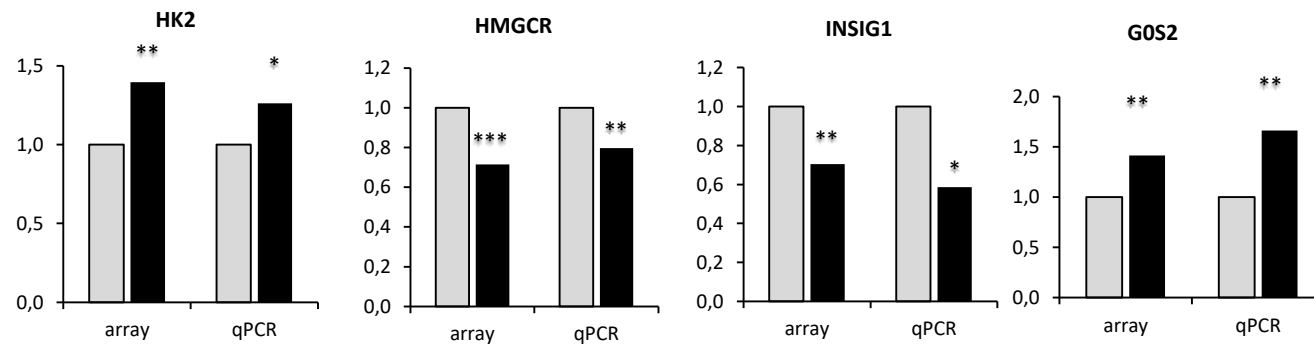
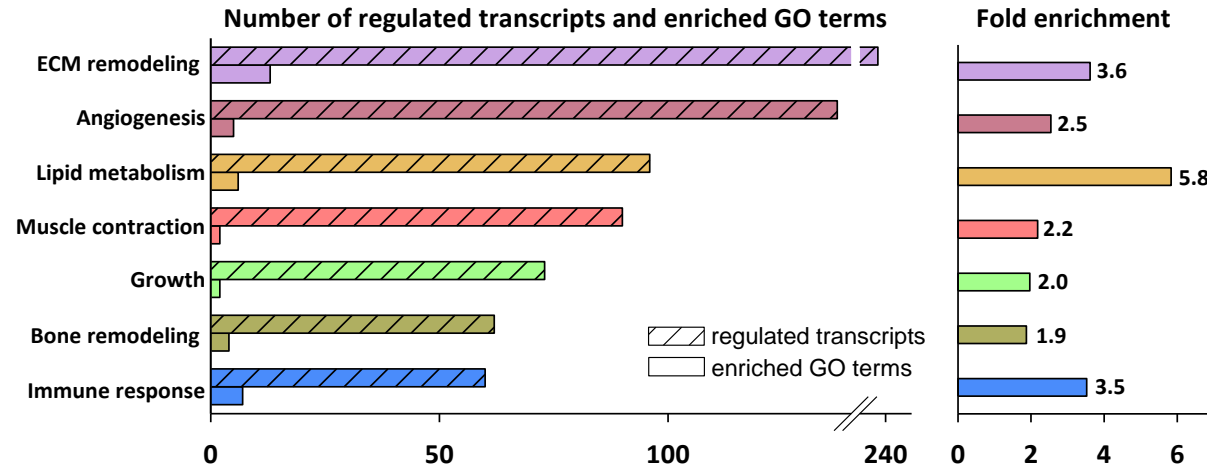
↗ mTOR/PKB

↘ Protein degradation

# Global transcriptomic analysis

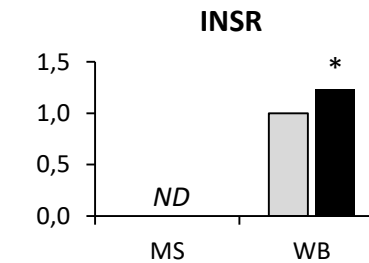
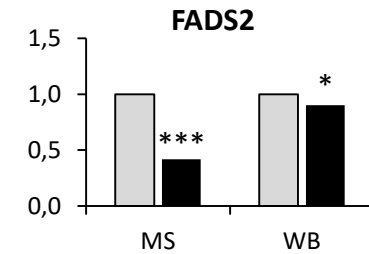
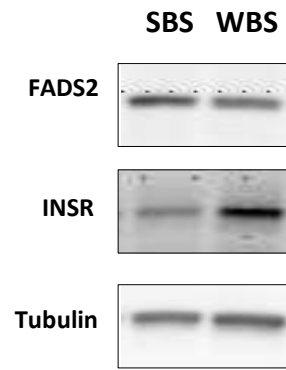
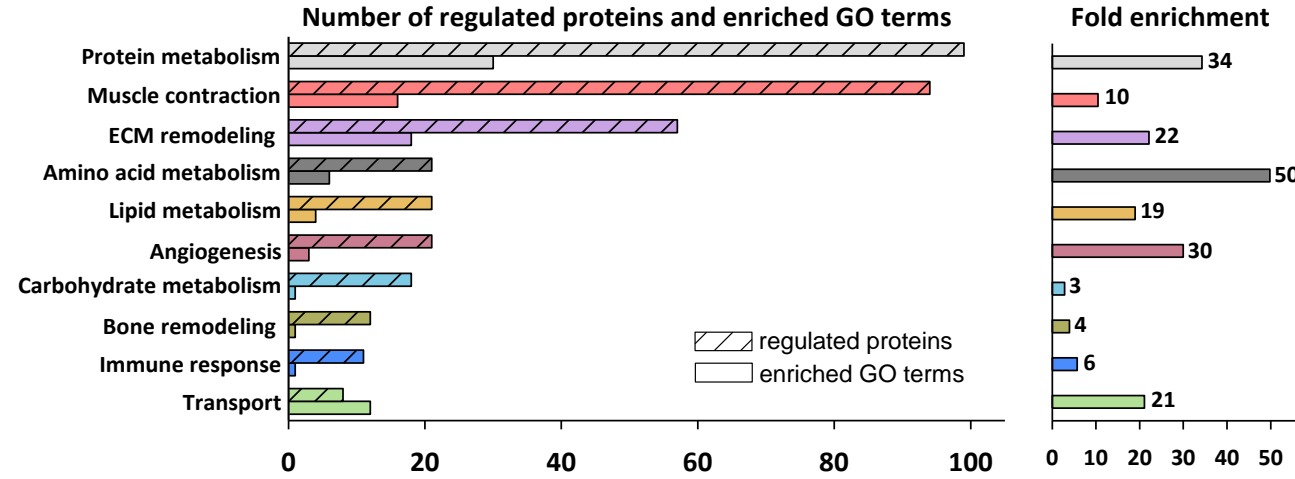
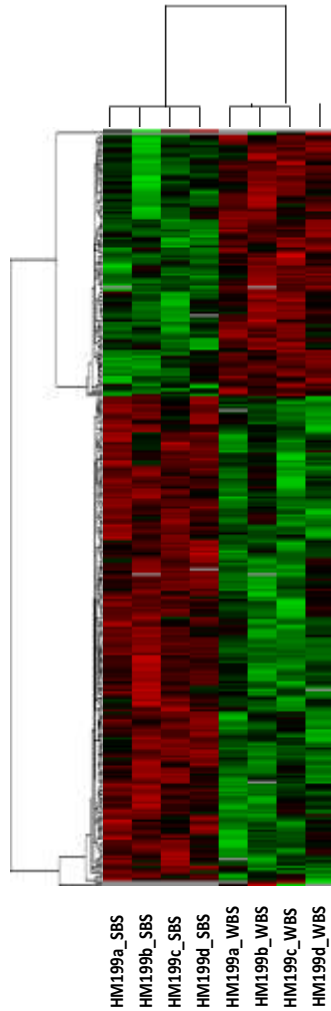


HM199\_WBS  
HM258\_WBS  
HM305a\_WBS  
HM305b\_WBS  
HM258\_SBS  
HM199\_SBS  
HM305a\_SBS  
HM305b\_SBS



Metabolic related genes : ➡ muscle lipid synthesis and storage

# Global proteomic analysis



**Next steps : toward the identification of active compounds**



## Next steps : toward the identification of active compounds

Unbiased approach

- Serum fractionation and screening for active compounds (Physical and /or chemical preparations)
- Top-down and Bottom-up approaches



Identification circulating active fraction

## Next steps : toward the identification of active compounds

### Unbiased approach

- Serum fractionation and screening for active compounds (Physical and /or chemical preparations)
- Top-down and Bottom-up approaches



**Identification circulating active fraction**

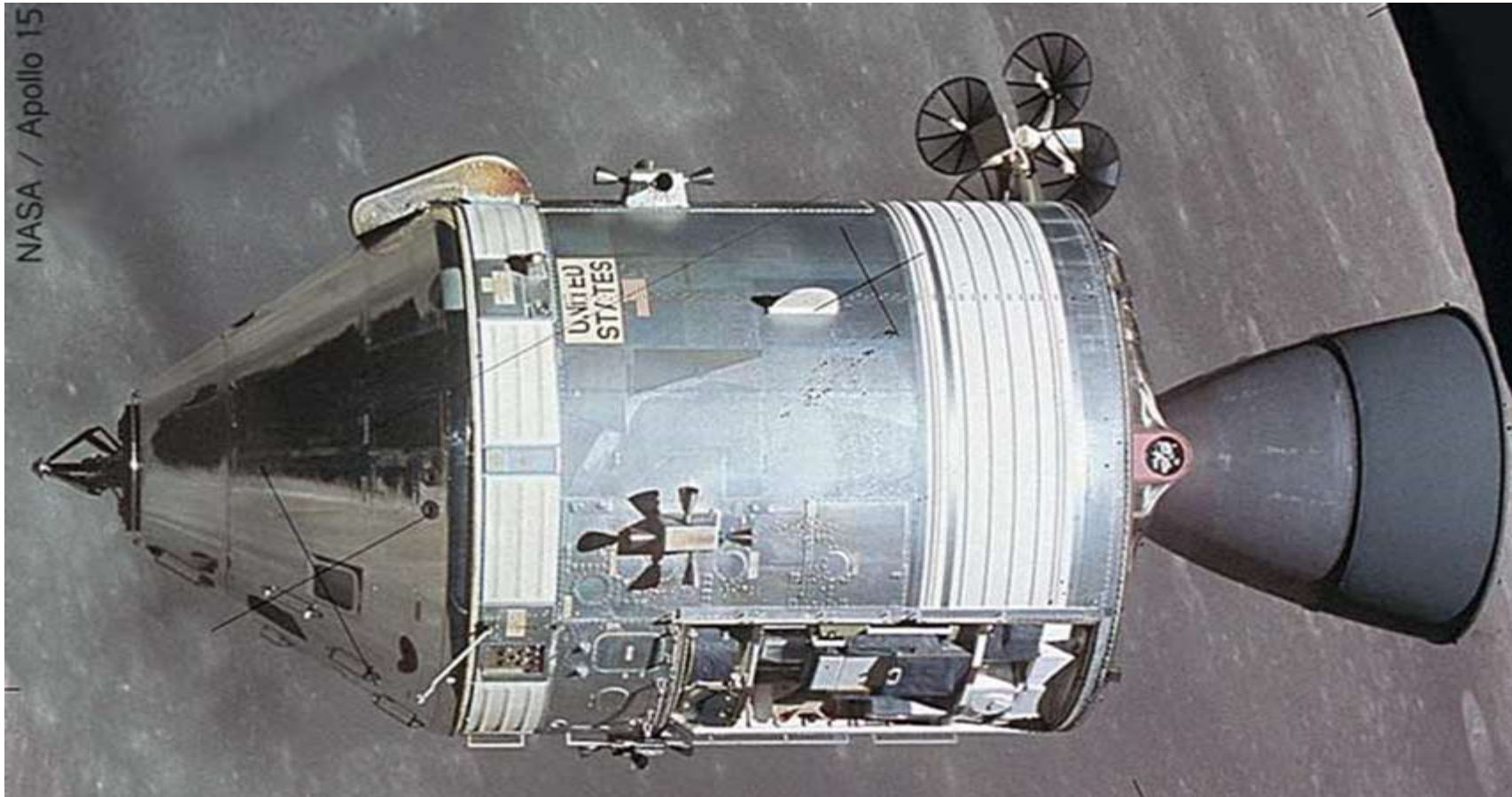
### Targeted approach

- Differences in summer and winter serum composition (Fatty acids, Ketone bodies,...)
- Involvement in human muscle cell responses



**Identification of circulating active compound**

## Beyond the protein balance...





**Permanent settlement on the Moon**



**Human mission to Mars**

- Low or no gravity (0.17 on the Moon ; 0.38 on Mars)
- Long time confinement
- Food and energy supply
- Exposition to cosmic radiation

## Cosmic radiation and Radioprotection



### Origin :

- Sun
- Deep space : Supernovae,...

### Composition :

- Photons
- Neutinos
- Electrons
- Protons
- Alpha particules

### Exposure to radiation (1 mSv):

- 1 year and half in Paris
- 9 months in Limousin
- 7 days in the ISS

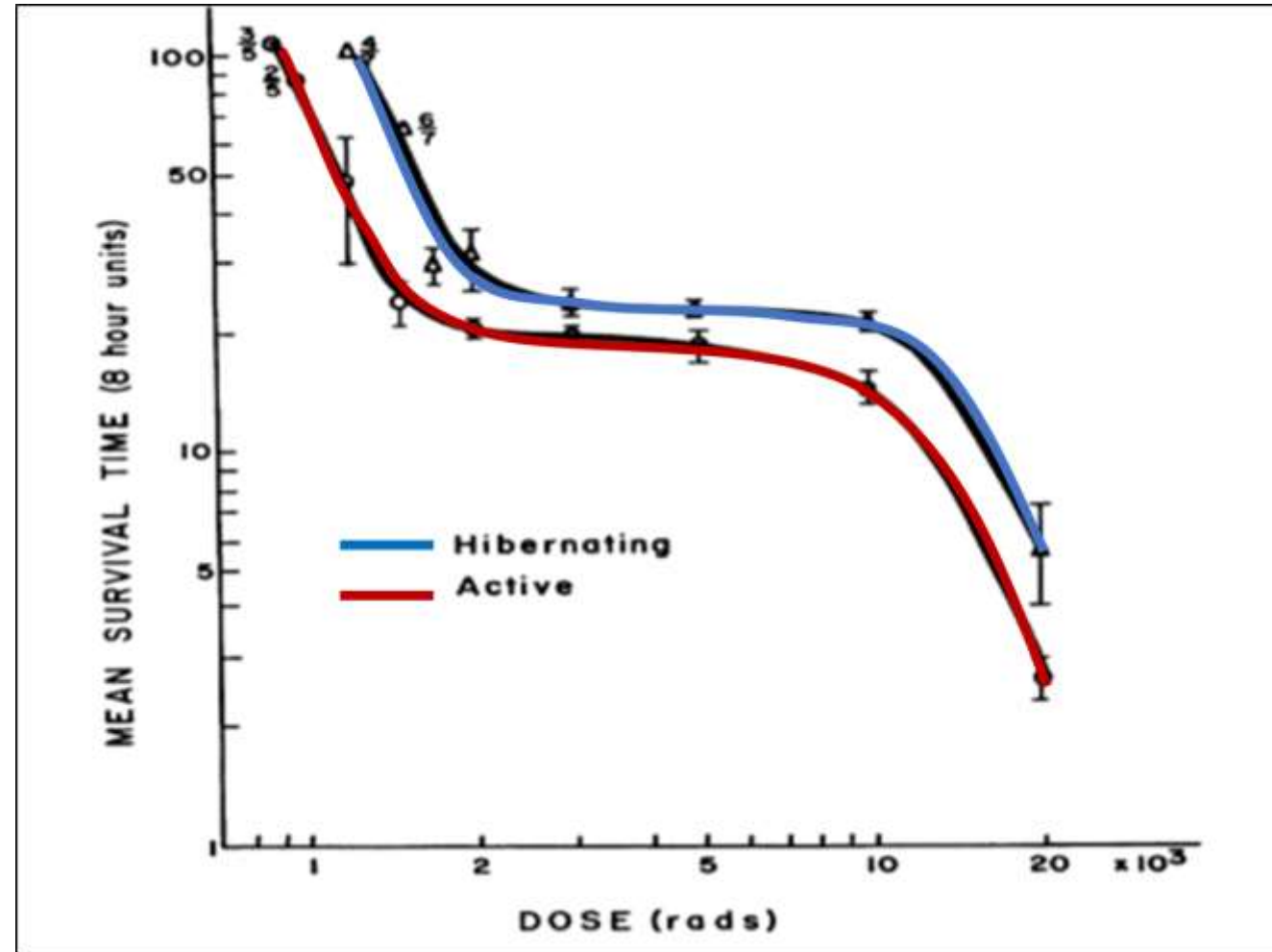


## Cosmic radiation and Radioprotection

### Irradiation of small hibernators

Hibernation and radioprotection :

- 50's to 70's
- National energy agencies
- Animal (and human ??) irradiation
- Low temperature and Hypometabolism

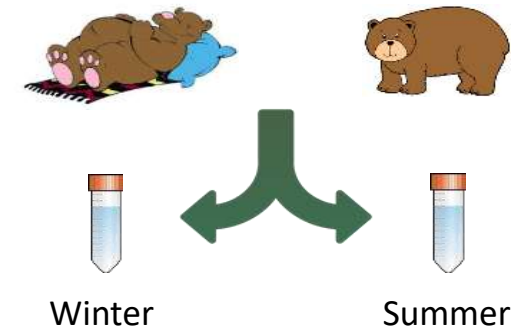


## Cosmic radiation and Radioprotection

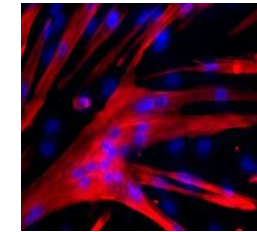
### Irradiation of small hibernators

Hibernation and radioprotection :

- 50's to 70's
- National energy agencies
- Animal (and human ??) irradiation
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### Bear serum



### Human myotubes

Indication of :

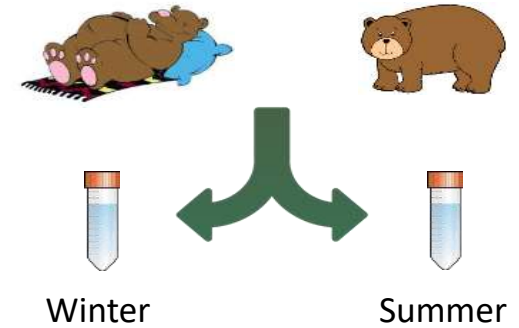
- Chromatin rearrangements
- Nuclear architecture
- DNA repair genes expression
- Oxidative stress

# Do exposition to winter bear serum protects human cells from radiation ?



Ongoing project:

- Treated human cells exposed to ionizing radiation
- DNA damage, DNA repair
- Enzymatic activities



Bear serum

Human cells

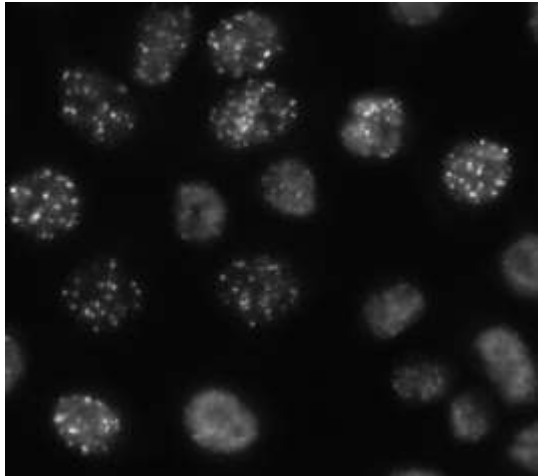


Do exposition to winter bear serum protects human cells from radiation ?



CYRCé Cyclotron (24MeV): Proton beam

IF : Anti 53BP



DNA dammage





For human health...





For human health...



...and beyond !!!

## SPACE

# HIBERNATION FOR DEEP SPACE

Space trips to the other planets would require months of travel through the vacuum of space. Maintaining the crew's health is a vital concern. If the crew could be induced to hibernate, the problems of survival become easier to solve.

### HIBERNATION, NOT FREEZING

Hibernation is a type of torpor, or reduced metabolism caused by hypothermia. Unlike in cryogenics, the body does not actually freeze.

A 30 degree drop in body temperature reduces metabolic rate by 98 to 99 percent.

*Pringle's Mouse hibernates during the colder half of the year. (CRSST/ U.S. Fish and Wildlife Service)*

*Astronaut Dawn Bennett monitors hibernating crew members on the voyage to Jupiter in "2201: A Space Odyssey" (1988)*

### MAKING THE TRIP

Hibernation solves a variety of problems connected to deep space travel.

#### HIBERNATION CAPSULE

Different astronauts would be immobilized in sleep capsules, and would not need pressurized living space or artificially generated gravity.

#### NUTRIENTS

In their comatose state, the astronauts would be immobilized in sleep capsules, and would not need pressurized living space or artificially generated gravity.

#### HEAVY RADIATION SHIELDING

Radiation is a prime concern in deep space travel. Heavy radiation shielding could be provided only to the crew's sleep capsules instead of the ship's large living areas, saving a lot of weight and fuel.

*NASA spacecraft concept for a 5-year mission to Jupiter*

### PSYCHOLOGICAL HEALTH

Hibernating astronauts would not have to remain mentally active in order to maintain their sanity on the long voyage.

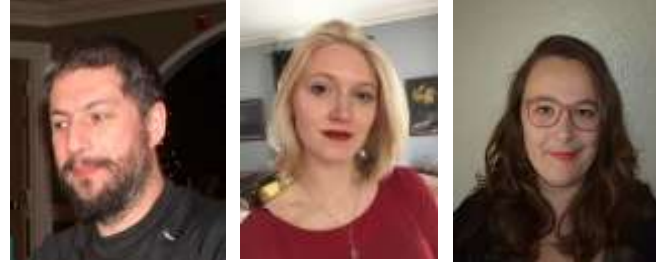
### PREVENTING MUSCLE ATROPHY

Astronauts would need some sort of therapy to avoid muscle loss during the trip. Animals such as black bears suffer very little muscle atrophy during their long annual hibernation. Further scientific research could lead to breakthroughs in this area.

SOURCES: NASA, SPACEWORKS ENGINEERING, ASHL TATE / © SPACE.com



« Hibernatus » Clermont



« Hibernatus » Strasbourg



Former Bear Team Lyon



Team « Proteostasis » Clermont-Fd





**Thank you for  
your attention**

**Tackåsen  
(Sweden)**