Fasting Reduces Inflammation

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When fasting gets tough, the tough immune cells get going—or die

Collins et al., 2019, Cell 178

Jordan et al., 2019, Cell 178

Nagai et al., 2019, Cell 178
Outline

• Background
• Results
• Summary
• Discussion
Corresponding author

Prof. Miriam Merad, MD/PhD

- an Algerian professor in cancer immunology
- the director of the Precision Immunology Institute at the Icahn School of Medicine at Mount Sinai in New York City, NY
- a member of the United States National Academy of Sciences

Research interest:

- The role dendritic cells and macrophages play within the tumor microenvironment and on how tumors prevent the normal anti-tumor functions of these cells

https://en.wikipedia.org/wiki/Miriam_Merad
https://labs.icahn.mssm.edu/meradlab/miriam-merad/
Hypocaloric diets or fasting are associated with improved outcomes of metabolic, autoimmune, and inflammatory diseases in humans:

- NAFLD (non-alcoholic fatty liver diseases)
- T2DM (type 2 diabetes mellitus)
- CVD (atherosclerosis, cardiovascular disease)
- multiple sclerosis 多发性硬化症
- rheumatoid arthritis 风湿性关节炎
- asthma 哮喘
- psoriasis 牛皮癣
- ...

Hypocaloric diets or fasting regimens have been shown to prolong lifespan

Individuals undergoing intermittent or religious fasting have reduced basal levels of circulating pro-inflammatory cytokines including TNFα, IL-6 and IL-1β

...
How fasting modulate systemic inflammation?
The design of fasting experiments

- The authors profiled the composition of blood circulating immune cells of 12 healthy normal weight volunteers (mean age = 30 ± 5 years, BMI = 22 ± 2 kg/m²) 3 h after food intake (fed state) and after 19 h of fasting (fasting state) using cytometry by time-of-flight spectrometry (CyTOF)
- To control for circadian variations, all blood samples were drawn at the same time of the day (3 pm)
Fasting led to significant reduction of subsets of circulating monocytes and circulating dendritic cells

- Cells decreased in fasting state (Human)
  - CD14+ monocytes
  - CD16+ monocytes
  - CD141+ DCs (dendritic cells)
Monocyte

- Monocytes are a type of leukocyte, or white blood cell
  - the largest type of leukocyte and can differentiate into macrophages and myeloid lineage dendritic cells
  - as a part of the vertebrate innate immune system, monocytes also influence the process of adaptive immunity

- Function:
  - replenishing resident macrophages under normal conditions
  - migration within approximately 8–12 hours in response to inflammation signals from sites of infection in the tissues

https://en.wikipedia.org/wiki/Monocyte
How about the effect of fasting in mice?
Fasting also reduced circulating pro-inflammatory Ly-6C<sup>high</sup> monocytes in healthy mice

- 4 h short-term fasting protocol during the light period (Zeitgeber [ZT]2-6)
  - ZT: 自然授时光照时间, 是由实验室所设定的环境时间, ZT 0 是灯亮的时间点
  - comparable to overnight fasting for humans and is the least stressful fasting strategy in animals

Note: Every dot represents one individual animal. Horizontal bar = mean. Vertical bar = SD. Statistical significance is indicated by *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. ns = not significant.
Fasting led to significant reduction of pro-inflammatory Ly-6C\textsuperscript{high} monocytes in peripheral tissues

- Fast: 20 h
- PC: peritoneal cavity, 腹膜腔
- AT: adipose tissues
No significant reduction of Ly-6C\textsuperscript{low} monocytes was observed in peripheral tissues.

- Fast: 20 h
- PC: peritoneal cavity, 腹膜腔
- AT: adipose tissues
What regulates the reduction of circulating monocytes in fasting mice?
Potential mechanisms for decreased numbers of circulating monocytes

1. increased monocyte cell death
2. reduced bone marrow (BM) myelopoiesis
3. reduced BM egress to the periphery
Reduction of blood monocytes is due to reduced monocyte egress from the bone marrow to the blood circulation.

(a) No increased number of activated caspase-positive monocytes in blood was observed.

(b) No increased number of CXCR4\(^+\) monocyte precursors in Bone Marrow (BM) was observed.

(c) Accumulation of Ly-6C\(^{\text{high}}\) monocytes in the BM of fasting mice was observed.
Fasting-induced inhibition of Bone Marrow egress is revoked upon food intake

- Re-feeding mice for 4 h after an overnight fast restored monocyte numbers in the periphery
The size of the monocyte pool in the blood circulation depended on the amount of carbohydrate ingested

- Absolute numbers of Ly-6C<sup>high</sup> monocytes in the blood of mice fasted for 16 h and gavaged with glucose solutions at the indicated concentrations
Hypothesis

Carbohydrates might modulate peripheral monocyte numbers by altering cellular energy levels.
Cellular energy levels controlled the blood circulating monocyte pool

- Two different inhibitors of hexokinase (己糖激酶), 2-deoxyglucose (DOG), and D-mannoheptulose, in order to block the first step in glycolysis, i.e., cellular energy production.
Clue

AMPK is a key cellular energy sensor triggered by an increase in the cellular AMP/ATP ratio that reflects low energy levels.

Next: test whether activation of AMPK is sufficient to inhibit BM monocyte egress to the blood circulation.
AMPK activation led to reduction of blood peripheral monocytes

- Phenformin 苯⼄双胍 is known to elevate the cellular AMP/ATP ratio which results in AMPK activation
- A-769662 is a small molecule activator of AMPK
Reduction of circulating monocytes in fasting mice

Reduced monocyte egress from the bone marrow to the blood circulation

Cellular energy levels controlled the blood circulating monocyte pool

AMPK activation led to reduction of blood peripheral monocytes
Clue

PPARα is a target of AMPK and is a master transcriptional regulator in the adaptive response to fasting.

Next: test whether hepatic PPARα controls peripheral blood monocyte numbers.
Activation of PPARα contributed to the regulation of monocyte homeostasis during fasting

- The number of Ly-6C<sup>high</sup> monocytes in the wt fast group was significantly smaller than that in the Ppara<sup>-/-</sup> fast group
AMPK-mediated reduction in peripheral monocyte numbers in fasting mice was in part mediated through PPARα

- Phenformin is known to elevate the cellular AMP/ATP ratio which results in AMPK activation
Question

Because in the \textit{Ppara}-/- mice, the gene was not uniquely deleted in specific tissue or organ, so we need to investigate whether PPAR\(\alpha\) functions directly in the monocytes or elsewhere.
The control of bone marrow monocyte egress required PPARα expression in cells besides monocytes

- The authors generated bone marrow chimeric animals in which wild-type or Ppara-/- bone marrow cells were injected into lethally irradiated hosts.
- The mark, \( \downarrow \), represents Ppara-/- mice reconstituted with wild-type bone marrow cells.

No significant reduction of blood monocytes in fasting Ppara-/- mice reconstituted with wild-type BM.
PPARα is expressed at higher levels in the liver and acts mainly in hepatocytes.
**Ppara^ΔHep** mice lost their ability to modulate bone marrow monocyte egress upon fasting

- Alb^{cre/cre} mice were crossed to Ppara^{fl/fl} mice to delete PPARα from hepatocytes (Ppara^ΔHep) in cre+ mice
- Ppara^ΔHep mice: PPARα was deleted uniquely in hepatocytes
Deletion of AMPK specifically in hepatocytes abrogated the reduction of circulating monocytes upon gavage with A-769662

- Alb^cre/cre^ mice were crossed to Prkaa1^{fl/fl} mice to delete AMPK from hepatocytes (AMPK^{ΔHep}) in cre+ mice
- AMPK^{ΔHep} mice: AMPK was deleted uniquely in hepatocytes
- A-769662 is a small molecule activator of AMPK
- This result demonstrated the importance of the liver
A brief summary

Energy-sensing by the liver AMPK-PPARα pathway controls the blood monocyte pool in response to caloric intake.
Reduction of circulating monocytes in fasting mice

- Reduced monocyte egress from the bone marrow to the blood circulation
- Cellular energy levels controlled the blood circulating monocyte pool
- AMPK activation led to reduction of blood peripheral monocytes

What’s next?

- The liver AMPK-PPARα pathway controlled the blood circulating monocyte pool in response to caloric intake
the liver AMPK-PPARα pathway

How? Reduced monocyte egress from the Bone Marrow to the blood circulation
Multiplex analysis for metabolic hormones in the blood of human and mice

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CCL2 binds to CCR2, a chemotactic receptor highly expressed on monocytes and shown to mediate monocyte bone marrow egress.

Strong reductions of CCL2 were observed in fasting mice as well as upon AMPK activation.

- **phen** (phenformin) is known to elevate the cellular AMP/ATP ratio which results in AMPK activation.
How to validate whether CCL2 is important or not?
What will happen if we restore plasma CCL2 levels in fasting mice?
The critical role of CCL2 in monocyte homeostasis

- Use administration of recombinant protein to restore plasma CCL2 levels
Intermittent fasting led to a strong reduction of monocyte accumulation in the EAE mice

- AL: mice were fed *ad libitum*; IF: mice were subjected to intermittent fasting
- EAE: experimental autoimmune encephalomyelitis, the main preclinical model for multiple sclerosis

**Proportion of IBA1+ myeloid cells in spinal cords**

**EAE clinical course**

- AL: solid black circles
- IF: blue circles with open circles indicating means and vertical lines indicating standard deviations
Intermittent fasting did not affect monocyte emergency mobilization upon *Listeria monocytogenes* infection

- **Clue:** tissue restoration after injury and therapeutic immunity against *Listeria monocytogenes* critically depend on monocytes
- **AL:** mice were fed *ad libitum* 随意；**IF:** mice were subjected to intermittent fasting

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**Blood**

- *** p-value: *mock* vs *L. monocytogenes* (AL)
- ns: *mock* vs *L. monocytogenes* (IF)

**BM**

- **** p-value: *mock* vs *L. monocytogenes* (AL)
- ns: *mock* vs *L. monocytogenes* (IF)

**Spleen**

- **** p-value: *mock* vs *L. monocytogenes* (AL)
- ns: *mock* vs *L. monocytogenes* (IF)

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**Legend:**

- AL mock
- AL *L. monocytogenes*
- IF mock
- IF *L. monocytogenes*
Intermittent fasting also did not affect wound repair potential compared to *ad libitum* fed mice

- Clue: mobilization of monocytes to the site of injury is critical for wound repair
- AL: mice were fed *ad libitum* 随意；IF: mice were subjected to intermittent fasting

![Skin injury](image)  

Note: Every dot represents one individual animal. Horizontal bar = mean. Vertical bar = SD.
Reduction of circulating monocytes in fasting mice → Egress from the bone marrow to the blood circulation → Cellular energy levels controlled the blood circulating monocyte pool

AMPK activation led to reduction of blood peripheral monocytes → The AMPK-PPARα-CCL2 axis

Fasting improves chronic inflammation without compromising monocyte emergency mobilization during acute inflammation

What’s next?
• **The Authors selected AMPK** to study because AMPK is a key cellular energy sensor triggered by an increase in the cellular AMP/ATP ratio that reflects low energy levels

  • How about **SIRT1 and other sirtuins**?
    
    • “SIRT1 is activated in response to changes in the energy status to promote transcription of genes that mediate the metabolic response to stress, starvation or calorie restriction.”

  • What are the effects of fasting on ageing?
  
    • Other nutrient sensors, e.g., insulin, mTOR, …

• It is about cellular energy, so what’s **the role of mitochondria in fasting**?

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Discussion

• **Metformin activates AMPK pathway**, so will the use of metformin also lead to reduced monocyte egress from the bone marrow to the blood circulation?
  • the authors used phenformin in this study

• The influence of fasting on the **microbiome** as well as the interaction between microbiome and immunity

• Is there any “**epigenetic memory**” after fasting, e.g., regulation of CCL2’s expression?
Thank you!