Intermittent fasting in multiple sclerosis and its animal model: starving for an answer?

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**Laura Piccio M.D. Ph.D,** has financial interests to disclose.

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  May 18th, 2016

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  - Strategic Pharma-Academic Research Consortium (SPARC)
  - National Multiple Sclerosis Society (RG-1607-25158)
  - Alector
Outline

- Multiple sclerosis (MS) and environmental factors:
  - Role of obesity in MS and mechanisms.

- Experimental autoimmune encephalomyelitis (EAE) studies:
  - Chronic calorie restriction: leptin and adiponectin.
  - Intermittent-fasting in EAE.

- Pilot clinical trial of intermittent fasting in MS.
Multiple sclerosis (MS)

- MS is an inflammatory demyelinating disease of the central nervous system (CNS) and a major non-traumatic cause of disability in the young population.

- MS is considered an autoimmune disease mediated by autoreactive lymphocytes directed against myelin components in the CNS;

- Characterized by episodic neurologic dysfunction (relapsing remitting MS-RRMS); eventually may result in a progressive course with persistent neurologic deficits (progressive forms).
MS pathology in the CNS

Focal lesions in CNS white matter are characterized by:

1) **INFLAMMATION**
   - Perivascular inflammatory infiltrates, mainly composed of T and B lymphocytes and macrophages
   - CD4+ T lymphocytes
   - CD8+ T lymphocytes
   - CD20+ B lymphocytes

2) **DEMYELINATION**
   - Damage to oligodendrocytes and myelin sheath destruction

3) **AXONAL DAMAGE**
   - Axonal loss is responsible for permanent neurological deficits

Trapp et al., NEJM 1998
MS around the world

- MS is the most common CNS demyelinating disease in **western countries**.

- MS incidence & prevalence are **related to latitude** (increase with distance from the equator).

- Changes in MS epidemiology in past 50 years (e.g. attenuation of latitude gradient, increase of the F:M ratio) suggesting an important role of environmental factors.
What does cause MS?

MS is a multifactorial disease involving the immune system and the interplay of genetic and environmental factors.

**GENETIC SUSCEPTIBILITY**
- Genes (almost all relate to the immune system)

**ENVIRONMENTAL FACTORS**
- Infections/EBV
- Vitamin D levels
- Smoking
- OBESITY

Autoimmune reaction
Studies that link obesity to risk of MS

<table>
<thead>
<tr>
<th>Findings</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nurses’ Health Studies I and II, American women; <strong>Adolescent obesity associated with 2.25X ↑ risk</strong> of MS. Age 18.</td>
<td>Munger 2009*</td>
</tr>
<tr>
<td><strong>MS /CIS risk ↑ in pediatric/adolescent girls age 11-18 (not boys).</strong> California. <strong>Multi-ethnic, BMI not retrospective.</strong> “Dose-effect” of BMI.</td>
<td>Langer-Gould 2013</td>
</tr>
<tr>
<td>Children age 7-13 born 1930-1983 (Denmark): 302,000 people. 774 MS, (501 F: 273 M) <strong>Signif ↑ BMI in children (girls) who developed MS, trend for boys.</strong></td>
<td>Munger 2013</td>
</tr>
<tr>
<td><strong>Obesity during childhood and young adulthood → MS risk factor.</strong> Population based, case-control studies in Norway and in Italy (Ages 5 to 30y). &gt;1600 MS cases. Adjusted for age, smoking, outdoor activity.</td>
<td>Wesnes K et al. MSJ 2014</td>
</tr>
</tbody>
</table>

*first two studies: No difference in current BMI in MS vs controls
The adipose tissue viewed as an endocrine organ

The white adipose tissue (WAT) functions as an endocrine organ secreting hormones, peptides, and cytokines which are collectively referred to as **ADIPOKINES**

**Normal**
- Adipocytes

**Obese**
- Adipocytes
- Macrophages
- T cells

**Adipokines**

**Metabolism**

**Immune/Inflammatory Responses**

**Pro-inflammatory:**
- LEPTIN
- RESISTIN
- TNFα
- IL-6

**Anti-inflammatory:**
- ADIPONECTIN
- IL10
Obesity exaggerates inflammation

Obesity is characterized by:

- the release by the adipose tissue of cytokines/adipokines shifting the balance to a pro-inflammatory milieu.
- a chronic low-grade inflammatory state that may promote autoimmunity.
- lower levels of vitamin D metabolites.

AUTOIMMUNITY → OBESITY

Anti-inflammatory

IL-10
Adiponectin

Pro-inflammatory

IL-6
Leptin
Resistin

Fat Tissue
The “GUT MICROBIOTA” is the population of commensal microbes in the gut.

In the gut mucosa there are numerous immune cells (lymphocytes, DCs, macrophages) with primary protective roles against pathogens.

The gut microbiota modulates the immune system in the gut:
(i) bacterial components;
(ii) bacterial metabolites from dietary components (e.g. short chain fatty acids derived from dietary fibers).

Germ-free mice do not develop EAE; colonization of the gut with bacteria confers susceptibility to EAE again. 
*Lee et al, PNAS 2011*

*Furusawa et al, Semin Immunopathol 2015*
**Link between obesity and autoimmunity**

*Role of the gut microbiota*

- Obesity is characterized by an **“unhealthy “ gut microbiota** (low diversity and more pro-inflammatory bacteria).

- **Diet** is a major determinant of the composition of the gut microbiota.

- Dietary fibers increase bacterial production of **short chain fatty acids-SCFAs** which are anti-inflammatory and promote regulatory T cell development.

- The typical “obesogenic diet” is very poor in fibers and does not promote these protective mechanisms.

*Thorburn et al. Immunity 2014*
Tipping the balance back with Calorie Restriction (CR)

- CR is defined as reduction in calorie intake below usual *ad libitum/normal* intake, without malnutrition.

- CR is associated with reduction of systemic inflammation

*Fontana, Cell, 2015; Ingram, Neuroscience 2007; Fontana, Science, 2010*
EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS (EAE)

- The most used animal model for MS.
- Induced by immunization with myelin components/peptides which trigger an autoimmune attack in the CNS (active EAE model).
- Clinically manifests as “Ascending paralysis” typically scored from 1 to 5.
- Used to study MS pathogenesis and to test new therapeutic approaches — instrumental to develop several FDA- approved MS medications.
EAE: immunopathogenesis

A) PERIPHERAL INDUCTION PHASE

T cell priming in peripheral lymph nodes

B) CNS EFFECTOR PHASE

Inflammation and demyelination in the CNS
T cells in EAE and MS

Leung et al., Cell Mol Immunol 2010
What led to our interest in the topic?

Serendipity...
EAE was inhibited in 2 mice that were smaller due to a tooth problem (malocclusion) causing more difficulties in eating

Our mice were inadvertently calorie-restricted
Calorie Restriction in EAE

➢ **Hypothesis:**
Calorie restriction (CR) can ameliorate EAE by activating multiple anti-inflammatory pathways.

➢ **Experimental Design:**
Reduced calorie intake 40% below non-restricted intake beginning at 5 weeks of age, and continued for 5 weeks until immunization to induce EAE

- **Group 1:** 40% calorie restriction
- **Group 2:** normal “ad libitum” feeding

Immunization to induce EAE
Calorie Restriction (40%) studies: body weights

<table>
<thead>
<tr>
<th></th>
<th>Mean on day of immunization:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>19.5 g</td>
</tr>
<tr>
<td>Calorie Restricted</td>
<td>15.7 g</td>
</tr>
</tbody>
</table>

* p < 0.001

<table>
<thead>
<tr>
<th></th>
<th>Weight (mean g ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40% CR started</td>
<td></td>
</tr>
<tr>
<td>Day of immunization</td>
<td></td>
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</table>

-10 0 10 20 30 40 50 60 70 80 90

Mean on day of immunization:

Control            - 19.5 g
Calorie Restricted - 15.7 g

* p < 0.001
CR beneficial effects on clinical EAE

SJL immunized w PLP

C57BL/6 immunized w MOG

Clinical score vs. days post-immunization

CR study SJL

Incidence:

Control: 11/11 (100%)

CR: 7/11 (63%)

CR study C57BL/6

Incidence:

Control: 9/9 (100%)

CR: 8/9 (88%)

Piccio et al., J Leuk Biol. 2008
Beneficial effects of CR on CNS histology in EAE

Ad lib feeding → CR →

INFLAMMATION

DEMYELINATION

AXONAL INJURY (SMI-32)

Piccio et al., J Leuk Biol. 2008
(1) Potential anti-inflammatory mechanisms of CR

Increased CORTICOSTERONE serum levels in CR mice

* $P<0.02$
** $P<0.05$

Piccio et al., J Leuk Biol. 2008
(2) Potential anti-inflammatory mechanisms of CR

Decreased LEPTIN and increased ADIPONECTIN serum levels in CR mice

* $P \leq 0.02$
** $P \leq 0.05$

Piccio et al., J Leuk Biol. 2008
Leptin in MS and EAE

- Leptin crosses the blood-brain barrier.

- Leptin KO mice and Leptin receptor KO mice are protected from EAE (De Rosa et al. JCI, 2006).

- Leptin mRNA upregulated in active MS lesions (not in chronic silent) (Lock & Steinman 2002).

- Serum and CSF levels increased in all subtypes of MS, in most but not all studies (Matarese et al. 2005, Frisullo, 2007, Kraszula 2001; Emamgholipour 2013; Chatzantoni 2004).

- Inverse correlation between leptin serum levels and circulating T regs in RRMS during a relapse (Matarese 2005).

↑ T regs in Leptin KO and leptin receptor KO mice (Matarese 2005).
Adiponectin

- Immunomodulatory / anti-inflammatory.
- Abundant protein made by adipose tissue. High plasma levels (3-30μg/ml).
- Negatively correlated with Body Mass Index and visceral fat levels.
- Several forms (full length, small globular form, oligomeric forms).
- Two signaling receptors (R1, R2).
- Doesn’t cross BBB well.

Tilg and Moschen, Nat Rev Immunol 2006
Our studies using adiponectin for immunomodulation in EAE

- Mice with genetic deletion of ADP (ADP KO)
- Treat EAE with adiponectin (globular form)
Mice lacking adiponectin have a more severe EAE

Active EAE induced with MOG$_{35-55}$ in adiponectin knock out (ADP KO) and wild-type (WT) mice

![Graph showing mean EAE clinical score over days post-immunization for ADP KO and WT mice in two experiments.]

<table>
<thead>
<tr>
<th></th>
<th>Incidence</th>
<th>Mortality</th>
<th>Day of onset</th>
<th>Mean max score</th>
<th>Cumulative clinical score</th>
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<tbody>
<tr>
<td>Exp. 1</td>
<td>WT</td>
<td>6/7</td>
<td>0/7</td>
<td>18.8 ± 5.8</td>
<td>29 ± 21.8</td>
</tr>
<tr>
<td></td>
<td>ADP KO</td>
<td>6/6</td>
<td>0/6</td>
<td>14.17 ± 1.1</td>
<td>61 ± 10.7</td>
</tr>
<tr>
<td>Exp. 2</td>
<td>WT</td>
<td>14/15</td>
<td>0/15</td>
<td>14.27 ± 5.48</td>
<td>9.1 ± 5.91</td>
</tr>
<tr>
<td></td>
<td>ADP KO</td>
<td>19/19</td>
<td>0/19</td>
<td>12.21 ± 1.8</td>
<td>23.05 ± 16.73</td>
</tr>
</tbody>
</table>

Piccio et al. Eur J Immunol 2013
Recombinant globular adiponectin ameliorates EAE

Treatment with globular adiponectin (gADP; from day 5 to 12).

RT-PCR analysis: spinal cords of mice treated with PBS vs. gADP

Piccio et al. Eur J Immunol 2013
Summary (1)

- Obesity in children and young adults is a risk factor for MS development.

- Using mouse EAE model, 40% calorie restriction was beneficial to disease course and histopathology.

- In EAE, CR reduces the pro-inflammatory adipokine leptin, and increases the anti-inflammatory adiponectin and endogenous corticosterone.

- Treatment with adiponectin reduces severity of EAE.

- Adiponectin ↑ number and function of regulatory T cells.
Outline

- Multiple sclerosis (MS) and environmental factors:
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- Experimental autoimmune encephalomyelitis (EAE) studies:
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  - Intermittent-fasting in EAE.

- Pilot clinical trial of intermittent fasting in MS.
Intermittent fasting in EAE: study design

An alternative and more feasible way in humans to do CR is intermittent fasting (IF)

C57BL/6 mice

1) IF group
2) Control group
(10 mice/group)

MOG<sub>35-55</sub> Immunization

4 weeks

EAE

1<sup>st</sup> time point
2<sup>nd</sup> time point
3<sup>rd</sup> time point

T1
T2
T3

Baseline
Before immunization
Clinical EAE
Intermittent Fasting (IF) ameliorates EAE clinical course

- IF (every other day fasting) done for one month before immunization
- Mice immunized with MOG$_{35-55}$

CR study SJL Incidence

<table>
<thead>
<tr>
<th>Group</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10/10 (100%)</td>
</tr>
<tr>
<td>IF</td>
<td>7/10 (70%)</td>
</tr>
</tbody>
</table>
IF ameliorates EAE pathology in the CNS

- IF was associated with less inflammation, demyelination and axonal damage in the CNS during EAE.
IF reduced T cell production of EAE pathogenic cytokines in draining lymph nodes

Day 6 post-immunization → Draining PLN T cells re-activated in vitro → Cytokine production profiling by flow-cytometry

- IL-17
- IFN-γ
- GM-CSF

PLN: peripheral lymph nodes
IF modulates systemic inflammation in EAE

- IF increases serum levels of corticosterone.
- IF reduces leptin and increases adiponectin serum levels.
IF increases gut microbiome diversity

- Bacterial diversity increases significantly in the IF group at T2 and T3 while this does not happen in the control group.

- Blood leptin levels (from both groups) are negatively correlated with gut microbiome diversity.

*Collaboration with Dr. Yanjiao Zhou, The Jackson Laboratory*
IF modulates gut microbiome composition

- IF changes microbial family composition with enrichment of the Lactobacillaceae and Prevotellaceae families in the IF vs. the ad libitum group.

- We identified some lactobacillus species that are over-represented in the IF group. Lactobacillus species have probiotic properties and are recognized to confer beneficial health effects (increase T regs in the gut).
IF reduced IL17-producing T cells and increased T regs in the gut lamina propria

Small intestine lamina propria

T cell cytokine production

IL-17

IFNγ

GM-CSF

T regulatory cells

*P<0.05
Are IF-induced changes in the gut microbiota contributing to EAE amelioration?

Fecal Microbiota Transplantation (FMT)

Antibiotic treatment from ad libitum mice 1 week
FMT from IF mice 1 week
FMT from IF mice 1 week

MOG35-55 Immunization
Day 7
~Day 20-25

EAE peak
Fecal microbiota transplantation from IF mice to naive recipients ameliorates EAE clinical course

- EAE severity was significantly reduced in mice that received stool from the IF mice.
- T cell activation against the myelin peptide (MOG$_{35-55}$) is reduced in mice receiving FMT from IF mice.
Summary (2)

Intermittent fasting:

- Ameliorates EAE clinical course and pathology.

- Reduces systemic inflammation by increasing corticosterone and adiponectin and decreasing leptin.

- Increases gut microbiome diversity and changes microbiome composition. This is associated with changes in the immune cells in the gut lamina propria: ↓ Th17 cells and ↑ T regs.

- Fecal microbiota transplantation from mice on IF is protective in EAE.
Outline

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  - Role of obesity in MS.

- Experimental autoimmune encephalomyelitis (EAE) studies:
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  - Intermittent-fasting in EAE.

- Pilot clinical trial of intermittent fasting in MS.
Pilot study of intermittent fasting in relapsing MS patients at Wash U

- Randomized pilot study in RRMS patients having a relapse and treated with steroids.
- Sixteen patients were enrolled.

www.clinicaltrial.gov (NCT02411838)
Dietary instructions for IF given to the study patients

- Fasting strategies include adequate hydration, reducing cues to eat, and at lunch and dinner consuming a salad with non-starchy vegetables with 1 tablespoon of olive oil or 2 tablespoon of a commercial salad dressing.

- Use fresh or roasted vegetables and combine them in mixed salads.

- Avoid starchy vegetables: potatoes, corn, kidney beans, sweet peas etc.

- The total calorie intake per day would be around 400-500.

- Done with the supervision of a nutritionist.
The study is now completed

- The study is now completed; we have reached the goal of recruiting 16 patients (8 in the fasting group and 8 in the ad libitum group) which we planned based on funding.

- All patients completed the acute IF phase (16 subjects).

- Eight patients stayed in the chronic IF phase. Among these, 2 completed until month 3 and then dropped out.

- The intervention was safe (CBC and CMP performed at each visit) and no adverse effects have been observed.
Pilot study of IF in RRMS: effects on serum adipokines

- Significant greater reduction in the % change of leptin in the IF vs. ad libitum group; no differences in adiponectin levels.

- Significant greater reduction in the % change of BMI in the IF vs. ad libitum group.

% change = Day 15 – Day 0/Day 0 * 100
In RRMS patients and mice with EAE, the abundance of *Firmicutes* is increased (around 70%) after IF while the abundance of *Bacteroidetes* is decreased.
Study just funded by the National MS society

Randomized, single-blinded controlled study to test the effects of 12 weeks IF on laboratory measures and the gut microbiome in RRMS patients.

- **n=40 eligible patients**
  - **n=20** Intermittent fasting
  - **n=20** Western diet

**Week**

**Study visits**
- Metabolic/Immunologic studies (blood)
- Microbiome studies (stool sample)
- Clinical measures
- DEXA

**Screening phase**
- 12 weeks
Summary (3)

- IF is a safe intervention (also in combination with other therapies) and no adverse effects have been observed.

- This pilot trial demonstrates that IF done in combinations with steroid treatment during a relapse reduces leptin level, which might reduce inflammation more than steroids alone (no clinical evidence for this).

- Some of the analysis for this study are still ongoing (flow cytometry data, T reg suppression assays, serum still available).

- Limitations of the pilot human study: small sample size and potential confounding factors (e.g. steroid treatment).
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Gregory Wu MD PhD
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Linda Heinrich

The Jackson Laboratory
Microbiome studies:
Yanjiao Zhou MD PhD

The Jackson Laboratory
Microbiome studies:
Yanjiao Zhou MD PhD

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National MS Society
Fondazione Italiana Sclerosi Multipla (FISM)
Adiponectin effects on a BBB model in vitro

A

![Graph A]

B

![Graph B]
Calorie restriction (CR) pilot study in relapsing MS patients at Wash U

Inclusion criteria:

1) diagnosis of RRMS (2010 McDonald criteria) and experiencing a relapse as identified by the neurologist;

2) age 18-60 years;

3) a body mass index (BMI) of 23 or higher;

4) absence of ongoing diseases in other systems. Excluded patients treated with insulin for diabetes, treated with Warfarin or Coumadin or required to follow a special diet or food restriction (diabetic, gastric bypass etc).

Patients treated with disease modifying therapies (DMT) were permitted to remain on the medication during the study.
Two phases of intermittent fasting in the study: acute and chronic

- **Acute IF phase of the study**: mandatory for everyone enrolled. All enrolled patients did IF every other day for the first 15 days.

- **Chronic IF phase**: optional. They could decide to remain in the study for 6 months and they did daily fasting 2 or 3 times per week based on their BMI.

[www.clinicaltrial.gov](http://www.clinicaltrial.gov) (NCT02411838)
Dietary Factors that may affect MS

- **Dietary fat composition**
  - Population-based epidemiological studies suggest association between incidence of MS and intake of saturated fat of animal origin.
    - Swank et al NEJM 1952 (Norway); Alter et al 1974 (multiple countries)

- **Salt NaCl content**
  - ↑Serum-glucocorticoid kinase (SGK)1 → Th17 cells
  - Worse EAE on high NaCl diet (Kleinewietfeld & Wu Nature 2013)
  - Sodium intake associated with ↑ clinical and MRI activity in MS
    - Farez et al., J Neurol Neurosurg Psychiatry 2014

- **Phytochemicals**
  - Plant polyphenols induce sirtuins, which protect from RGC loss in EAE-associated optic neuritis

- **Dietary effects on the gut microbiome**
Dietary Factors that may affect MS

- **Dietary fat composition**
  - Supported by animal studies.
  - Population-based epidemiological studies suggest association between incidence of and intake of saturated fat of animal origin. 
  - Polyunsaturated fatty acid (PUFA): no proven effects on disease progression, may reduce frequency of relapse over 2 years.
    - Farinotti et al., Cochrane Database of Syst Rev 2014

- **Salt content.**
  - Supported by EAE studies
  - Sodium intake associated with increased disease activity in MS
    - Farez et al., J Neurol neurosurg Psychiatry 2014

- **Effects on the gut microbiome.**
Roy L. Swank: “Swank Diet”

Prospective clinical observational trial in ~150 MS pts. At Montreal Neur. Inst. Diet: <20g/day saturated fat. Study duration 34 years. Those who adhered to diet fared better. No comments on weights.

1909 - 2008

## Studies that link obesity to risk of MS (2)

<table>
<thead>
<tr>
<th>Findings</th>
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<tbody>
<tr>
<td><strong>Earlier age at MS onset associated</strong> with higher BMI in early adolescence and age 25. NYMS Consortium retrospective, 237 women.</td>
<td>Kavak et al. MSJ 2014</td>
</tr>
<tr>
<td><strong>Obesity during childhood and young adulthood → MS risk factor.</strong></td>
<td>Wesnes K et al. MSJ 2014</td>
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<td>Population based, case-control studies in Norway and in Italy (Ages 5 to 30y). &gt;1600 MS cases. Adjusted for age, smoking, outdoor activity.</td>
<td></td>
</tr>
<tr>
<td>Striking <strong>interaction between adolescent obesity and 2 MS risk genes:</strong> HLADRB1<em>15 and HLA-A</em>02. Replicated in 2 case-control studies (Sweden and California).</td>
<td>Hedstrom et al. Neurology 2014; 82: 865-873</td>
</tr>
</tbody>
</table>
## Adiponectin levels in MS

(4 published reports)

<table>
<thead>
<tr>
<th>tissue</th>
<th>subjects</th>
<th>results</th>
<th>citation</th>
</tr>
</thead>
<tbody>
<tr>
<td>serum</td>
<td>57 MS all subtypes, 34 Healthy</td>
<td>Lower adiponectin in female MS, no difference in males. BMI-matched controls</td>
<td>Musabek 2011 (Turkey)</td>
</tr>
<tr>
<td>serum</td>
<td>25 acute RRMS, 25HC</td>
<td>Lower adiponectin in MS BMI matched controls</td>
<td>Kraszula 2012 (Poland)</td>
</tr>
<tr>
<td>serum</td>
<td>30 RRMS, 66 HC</td>
<td>Higher adiponectin in RRMS mean BMI sl lower in MS pts</td>
<td>Palavra 2013 (Portugal)</td>
</tr>
<tr>
<td>CSF, blood</td>
<td>4 discordant twin pairs</td>
<td>Higher CSF and trend toward ↑ plasma adiponectin in affected twins, no correlation between CSF and plasma adiponectin</td>
<td>Hietaharju 2010 (Finland)</td>
</tr>
</tbody>
</table>
What is the gut microbiota?

- Shortly after birth mucosal tissues are colonized by bacteria, viruses, fungi resulting in a complex population of microbes called “COMMENSAL MICROBIOTA”.

- Mammalian commensal microbiota constitutes over 1000 species of microbes and outnumbers host cells by 10-fold.

- The intestinal mucosa harbors the largest amount of microbes in the human body and they constitutes the “GUT MICROBIOTA”.

FUNCTIONS of gut microbiota:

- Metabolic.
- Development and regulation of the immune system.
- Defense against pathogens.
- Maintenance of intestinal structure.

Gut microbiota in healthy adults
(2) Potential anti-inflammatory mechanisms of CR

Decreased serum IL-6 levels in CR mice

- IL-6 in the presence of TGF\(\beta\), induces differentiation of Th17 cells; Th17 cells accepted as pathogenic in EAE.

- IL-6 is critical for EAE induction; EAE cannot be induced in IL-6\(-/-\) mice.

- IL-6 is secreted by T cells, B cells, macrophages, but also muscle cells and adipose tissue. Approximately 30% of circulating IL-6 comes from adipose tissue.
Adiponectin in immune responses

Most studies suggest actions on the innate immune system

- ↓ myeloid precursor → macroph development (Yokota 2000)
- ↓ TNFα production by macrophages (Yokota 2000, Thakur 2006)
- ↓ NFκB activation in macs, endoth cells (Tilg and Moschen 2006, Ouchi 2000)
- ↑ M2 phenotype and IL-10 production (mouse, human) (Ohashi 2010, Mandal 2011)
- Pro-inflammatory actions in human macs and CD4+T cells also reported, with ↑ IFNγ, IL-6 (Cheng 2012)
- Adiponectin receptors present on CNS EC and human T cells (Spranger 2006; Wilk 2011)
Enhanced T-cell proliferation/cytokine production and reduced numbers of T regs in ADPKO mice

12 days pi

MOG_{35-55} re-challenge in vitro

T cell PROLIFERATION

IFN\(\gamma\) P<0.01

IL17

TNF\(\alpha\) P<0.01

IL6 P<0.01

Spleen

CNS

EAE peak T regs
(Flow Cytometry)

Piccio et al. Eur J Immunol 2013
Compliance with the diet

- **Acute IF phase**: most of the subjects were able to complete alternate day fasting (7 total fasting days out of 15). For some of the participants, the hardest part was the concomitant steroid treatment (in some this was increasing hunger).

- **Chronic IF phase** was overall harder to follow.
  - Some subjects liked the benefits of fasting and continued it after the study (of note: one diabetic patient got off medication after the diet).
  - For some patients it was more difficult because of changes in their medications or other ongoing medical issues.
  - IF was especially difficult when traveling out of town, with vacations and during holidays.
  - Two fast days per week were better tolerated than 3 days per week.

- Mild weight loss was generally observed in subjects following the diet (around 6% compared to weight at baseline).
Baseline characteristics of RRMS patients enrolled in the IF pilot study

<table>
<thead>
<tr>
<th></th>
<th>CONTROLS</th>
<th>INTERMITTENT FASTING</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td><strong>Number</strong></td>
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<td>8</td>
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<tr>
<td><strong>Demographic characteristics</strong></td>
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<tr>
<td>Age, y, mean±SD</td>
<td>42 ± 8.2</td>
<td>40 ± 12</td>
<td>0.6</td>
</tr>
<tr>
<td>Gender, F:M</td>
<td>7:1</td>
<td>5:3</td>
<td>0.2</td>
</tr>
<tr>
<td>Race, African American : Caucasian</td>
<td>1:7</td>
<td>1:7</td>
<td></td>
</tr>
<tr>
<td><strong>Anthropometric characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m², mean±SD</td>
<td>28 ± 4.2</td>
<td>30 ± 4.7</td>
<td>0.7</td>
</tr>
<tr>
<td>Waist circumference, cm, mean±SD</td>
<td>106.6 ± 13.7</td>
<td>96.9 ± 10.2</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>Clinical characteristic</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disease duration (years; mean±SD)</td>
<td>8.5 ± 8.1</td>
<td>7.8 ± 6.4</td>
<td>0.8</td>
</tr>
<tr>
<td>EDSS (range)</td>
<td>3.3 ± 0.7</td>
<td>3.1 ± 1</td>
<td>0.5</td>
</tr>
<tr>
<td>Annual relapse rate (2y before entry)</td>
<td>0.6 (0.5)</td>
<td>0.75 (0.7)</td>
<td></td>
</tr>
<tr>
<td><strong>DMT:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interferon beta</td>
<td>1</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Fingolimod</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Tecfidera</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Aubagio</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>5</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>
Pilot study of IF in RRMS: serum outcome measures

% change = Day 15 – Day 0/Day 0 * 100
Alteration of gut microbioma in MS subjects compared to healthy controls

- **Alterations of the human gut microbiome in MS**
  - Jangi et al., *Nature Communication*, June 2016
  - Stool and blood samples from 60 MS subjects vs. 43 healthy controls;
  - Gut microbiome of treated patients (βIFN or GA) was similar to healthy controls;

- **Multiple sclerosis patients have a distinct gut microbiota compared to healthy controls**
  - Chen et al., *Scientific Report*, June 2016
  - Stool and blood samples from 31 MS subjects (remission and active) vs. 36 healthy controls;
  - Species richness was not different between RRMS and controls;
  - MS patients with active disease showed decreased richness compared to patients in remission and controls.

*Jangi et al., Nature Communication*
Leptin enhances immune responses

- Made by adipocytes mainly but also by inflammatory cells such as T cells.

- Leptin receptors are expressed by immune system cells (T cells, neutrophils, monocytes, NK cells).

- Overall enhancement of both innate and adaptive immune responses:
  - Activates monocyte/macrophages, ↑ TNFα, IL-1, IL-18, & IL-12 production;
  - Trophic for T cell development, ↑ T cell proliferation;
  - Skews toward Th1 (directly and indirectly);
  - Suppresses T regulatory cell proliferation;
  - Recent study used conditional LepR deletion in CD4+ T cells to show leptin signaling critical for Th17 cell responses.

Procaccini C., 2015