Mushrooms & Health Summit
Session 4

Exploring Some Mechanisms Linking Mushrooms & Health

- Moderator: Gabriela Riscuta, MD, CNS, National Institutes of Health (NIH), National Cancer Institute (NCI)
- Margherita Cantorna, PhD, Professor Molecular Immunology, Penn State University
- Dayong Wu, MD, PhD, Scientist I and the Associate Director at the Nutritional Immunology Laboratory, Jean Mayer USDA Human Nutrition Research on Aging at Tufts University
- Sue Percival, PhD, Professor and Chair, Food Science and Human Nutrition Department, University of Florida
- Lawrence Cheskin, MD, FACP, Associate Professor, Department of Health, Behavior and Society at Johns Hopkins Bloomberg School of Public Health
Sesion 4
Exploring Some Mechanisms Linking Mushrooms and Health

Gabriela Riscuta MD, CNS
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National Cancer Institute
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Mushrooms Health Benefits

- Inhibit tumor growth through modulation of immune system function;
- Antiviral, antibacterial, antiparasitic effect through microbiota modulation;
- Weight management
- Increase vitamin D levels through diet
- Anti-inflammatory properties
- Antidiabetic properties
Objectives

• Identify and evaluate the biological processes and mechanisms of mushrooms that yield health benefits

• Discuss the current body of research on mushrooms and immune response
Distinguished Speakers

• Margherita Cantorna, PhD. Prof of Molecular Immunology, Penn State.
  – White Button Mushrooms alter the Microbiome to Protect Against Gastrointestinal Injury.

• Dayong Wu MD, PhD. Scientist I and the Assoc. Director at the Nutritional Immunology Laboratory, Jean Mayer USDA Human Nutrition Research on Aging at Tufts Univ.
  – Mushrooms, Immune Functions and Host Resistance to Microbial Infection.
Distinguished Speakers, cont.

• Sue Percival, Prof, Nutritional Sciences, Univ of FL.
  – Immunity Improves after Consuming Shiitake Mushrooms Daily.

• Lawrence Cheskin, MD, FACP. Assoc Prof Dept of Health Behav and Society at J.Hopkins Bloomberg School of Public Health.
  – Mushrooms for Weight Control and Health
White button mushrooms alter the microbiome to protect against gastrointestinal injury.

Collaborators
Dr. Keith Martin, Arizona State University
Dr. Andrew Patterson, Penn State University
Dr. Istvan Albert, Penn State University

Past members:
Sanhong Yu*
Jot Ooi Hui*
Jyotika Varshney*

Funding:
Mushroom Council
NIH

The Cantorna Laboratory
Veronika Weaver*
Jing Chen
Amanda Waddell*
Jamaal James
Stephanie Bora
Kaitlin McDaniel
Lindsay Snyder
Yang-Ding Lin*
Brenita Jenkins
There are billions of micro-organisms in the gastrointestinal tract.
Interactions between the host and the microbiota are critical for the maintenance of health.

Barrier, protection from infection, immune system development and tolerance to food antigens and harmless microbes.
Environmental signals that alter gut microbial flora.

Diseases where the gut microbial flora has been implicated in outcome: Obesity, asthma, inflammatory bowel disease, multiple sclerosis, arthritis (other immune mediated diseases), gastrointestinal infections, cancer.

Signals that alter the gut:
Age, diet, stress, antibiotics, probiotics, mushrooms?
Dextransodium sulfate induced colitis

The effect of diet on the microbiome and colitis?

Disease development TD>CD>PD
diet
14 days

Day 0

DSS
water for recovery

5 days

Fecal sample
weigh daily

Hui et. al unpublished
Antibiotic disruption of the microflora partially eliminates the diet mediated effects. There are no diet mediated effects in germfree mice.

Hui, and Lin et. al unpublished
1g mushrooms/100 g PD (1%) diet

DGGE a technique useful for visualizing changes in microbial communities.

Diet affects the microbial communities.
WB fed mice were more similar to each other than to control fed or mice fed the standard chow diets (Baseline).

Varshney and Hui et. al J Nutr 2013
Sequencing confirms the diet mediated effects on the microbiome.

<table>
<thead>
<tr>
<th>CONTROL</th>
<th>CONTROL</th>
<th>MUSHROOM</th>
<th>MUSHROOM</th>
</tr>
</thead>
<tbody>
<tr>
<td>BACTERIAL Phylum</td>
<td>PERCENTAGE</td>
<td>BACTERIAL Phylum</td>
<td>PERCENTAGE</td>
</tr>
<tr>
<td>Bacteroidetes</td>
<td>80.3</td>
<td>Bacteroidetes</td>
<td>84.3</td>
</tr>
<tr>
<td>Firmicutes</td>
<td>14.7</td>
<td>Firmicutes</td>
<td>8.4</td>
</tr>
<tr>
<td>Tenericutes</td>
<td>4.2</td>
<td>Tenericutes</td>
<td>5.5</td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>0.5</td>
<td>Proteobacteria</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Verrucomicrobia</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Shifts in the microbial community as a result of 1% WB mushroom feeding.

Varshney and Hui et. al J Nutr 2013
DSS colitis and WB mushrooms

1g mushrooms/100 g PD (1%) diet

WB but not Oyster mushrooms protected from weight loss and colonic shortening following DSS induced injury.
Infection:
Mouse model of human *Escherichia coli* infection
Fecal shedding over time.
Histopathology changes.

**C. rodentium** infection

Diet
14 days

Days: 0
8-10 peak resolution
21

Fecal shedding

Mundy et. al Cell Micro 2005
Citrobacter rodentium infection

Varshney and Hui et. al J Nutr 2013
How do mushrooms affect healing in the gastrointestinal tract?

1) The polysaccharides in mushrooms may mimic bacterial polysaccharides and bind to Toll-like receptors (TLR, expressed in the host). Immune regulation? Less inflammation or more inflammation?
WB feeding results in higher inflammatory cytokines

DSS

*Col*on

\[ p<0.05 \]

\[ \text{TNF-}\alpha \text{ (pg/ml)} \]

\[ \text{Control} \quad \text{WB} \]

\[ \text{Yu et. al BMC Immunol 2009} \]

*C* _rodentium_ _infection_

\[ \text{Diet effect: } P=0.009 \]
\[ \text{Time effect: } P=0.014 \]
\[ \text{Diet x Time: } P=0.40 \]

\[ \text{Ifn-}\gamma \text{ relative to Gapdh} \]

\[ d10 \quad d14 \]

\[ \text{Diet effect: } P=0.008 \]
\[ \text{Time effect: } P=0.59 \]
\[ \text{Diet x Time: } P=0.80 \]

\[ \text{Il-17 relative to Gapdh} \]

\[ d10 \quad d14 \]

\[ \text{Varshney and Hui et. al J Nutr 2013} \]
How do mushrooms affect healing in the gastrointestinal tract?

**Prebiotics** are non-digestible food ingredients that stimulate the growth and/or activity of bacteria in the digestive system in ways claimed to be beneficial to health.

1) Competition of the microbiota for nutrients present in the WB mushrooms? It may be different than what’s in Oyster mushrooms. Or the microbiota makes bioactives from the polysaccarides in mushrooms.

2) Direct effects of the mushrooms on the host that has nothing to do with the microbiome. I don’t think so but….haven’t ruled it out. Experiments in germfree mice would be necessary to determine this.
How do changes in inflammation alter the microflora?

Inflammation oxidizes endogenous sulfur compounds to generate a respiratory electron acceptor - tetrathionate. Bacteria that express tetrathionate reductase can use this pathway to out-compete other microbes.

A. J. Baumler, UC Davis

Gut Microbes 2011

To compete with the gut microbiota, Salmonella induces the host to produce an electron acceptor.
Summary: Role of Mushrooms on Gut Microbiota

- Consuming WB mushrooms improved gastrointestinal health.
- WB mushrooms alter the microbial microbiota.
- Oyster mushroom consumption failed to show the same protective effects as WB mushrooms.
- The basal diet consumed affects the microbiota and susceptibility to injury.
- The effects of consuming WB mushrooms might depend on what diets are consumed.
Research questions:
1) How often and in what quantities do mushrooms need to be given for the effects on the microbiome?
2) Metabolomics experiments to identify the effects of WB feeding on bacterial metabolites. Compare germfree mice with conventional mice fed WB mushrooms.
3) Look at other varieties of edible mushrooms on the microbiome.
Mushrooms, immune function, and host resistance to microbial infection

Dayong Wu

Nutritional Immunology Laboratory

Mushrooms and Health Summit
Washington DC, September 9-10, 2013
Mushrooms and Immune Function

- Mushroom’s anti-tumor, anti-viral, and anti-bacterial properties are suggested to be due to their ability to modulate immune cell functions.

- Majority of these studies evaluated the effect of administering extracts of exotic mushrooms through parenteral routes.

- In contrast, little is known whether white button mushrooms (90% of mushrooms consumed in the US) would affect immune function and if so, whether it can be translated to an altered resistance to infection.

- We have conducted a series of studies to determine the effect of white button mushrooms on host’s immune response and resistance to infection.
Dietary Supplementation with White Button Mushroom Enhances Natural Killer Cell Activity in C57BL/6 Mice

Dayong Wu, Munkyong Pae, Zhihong Ren, Zuyan Guo, Donald Smith, and Simin Nikbin Meydani

3Nutritional Immunology Laboratory, 4Comparative Biology Unit, Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA 02111; and 5State Key Laboratory for Infectious Disease Prevention and Control, National Institute of Communicable Disease Control and Prevention, China CDC, 102206 Beijing, China
Study Design

- Male C57BL/6 mice (4 mo)
- 14 mice/group
- Pair-feeding
  - Control
    - 2% WBM
  - 10% WBM
  - 10 wk
- Outcome measurements
  - Immune cell phenotype
  - NK activity
  - T cell proliferation
  - Cytokine production
    - Mφ function: PGE$_2$, NO, H$_2$O$_2$ production

Body weight recording

Wu et al., J Nutr. 2007
Dietary supplementation with white button mushrooms enhanced NK activity of splenocytes

Mean±SE, n=14/group.
Means at a ratio without a common letter significantly differ at p<0.05.
Effect of white button mushroom consumption on cytokine production by splenocytes of C57BL/6 mice

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>Control</th>
<th>2% mushroom</th>
<th>10% mushroom</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IFN-(\gamma) (units/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con A</td>
<td>16.5 ± 1.9</td>
<td>22.9 ± 2.9#</td>
<td>25.9 ± 4.2*</td>
</tr>
<tr>
<td>PHA</td>
<td>6.3 ± 1.7</td>
<td>9.1 ± 2.1</td>
<td>16.5 ± 5.2#</td>
</tr>
<tr>
<td>IL-2 (pg/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con A</td>
<td>244 ± 43</td>
<td>429 ±100#</td>
<td>363 ± 70</td>
</tr>
<tr>
<td>PHA</td>
<td>24.3 ± 2.6</td>
<td>29.1 ± 3.7</td>
<td>27.9 ± 4.6</td>
</tr>
<tr>
<td>TNF-(\alpha) (pg/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con A</td>
<td>30 ± 5</td>
<td>38 ± 8</td>
<td>65 ± 18*</td>
</tr>
</tbody>
</table>

Mean ± SEM, \(n=14\). *\(p<0.05\) and #\(p<0.1\) compared to control using Student’s t test with Bonferroni adjustment.

Wu et al., J Nutr. 2007
Correlations between mouse splenocyte NK activity and Con A-stimulated IFN-γ or TNF-α production after mushroom supplementation

Mean±SE, n=14/group.

Wu et al., J Nutr. 2007
Summary of Study 1

- Dietary supplementation with white button mushrooms increases NK activity in mice.

- Increased NK activity may be mediated through increased production of IFN-γ and TNF-α.

- Mushrooms may cause a shift toward Th 1 response.

- There is a trend of higher IL-2 production and lymphocyte proliferation in mushroom-fed mice, which needs to be further confirmed in the future.

- Mushrooms do not affect macrophage secretion of inflammatory mediators (IL-6, TNF-α, PGE₂), H₂O₂, and NO.
White Button Mushroom Enhances Maturation of Bone Marrow-Derived Dendritic Cells and Their Antigen Presenting Function in Mice$^{1,2}$

Zhihong Ren, Zhuyan Guo, Simin Nikbin Meydani, and Dayong Wu*

Nutritional Immunology Laboratory, Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA 02111
Study design

- **BMC** -> IL-4/GM-CSF
  - Mushroom (0, 50, 100, 200 μg/ml)
  - 7 days

- **DC**
  - DC maturation phenotypic markers (CD40, CD80, CD86, MHC-II)
  - DC endocytosis (FITC-dextran uptake)
  - DC Functional makers (IL-12 production)
  - DC antigen-presenting function (antigen-specific T cell proliferation)

Ren et al., J Nutr. 2008
Effect of in vitro WBM supplementation on phenotypic makers of DC maturation

**TABLE 1** Effect of WBM on expression of DC maturation markers in C57BL/6 mice

<table>
<thead>
<tr>
<th>Maturation marker</th>
<th>0</th>
<th>50</th>
<th>100</th>
<th>200</th>
<th>LPS&lt;sup&gt;3&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHC class II, % positive cells</td>
<td>69.2 ± 9.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>71.9 ± 9.7&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>73.1 ± 9.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>74.2 ± 9.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>88.5 ± 5.6&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>MFI</td>
<td>201 ± 25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>210 ± 28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>214 ± 20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>247 ± 25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>414 ± 170&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>CD 40, % positive cells</td>
<td>46.9 ± 4.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>54.4 ± 7.4&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>60.3 ± 7.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>68.9 ± 5.3&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>76.9 ± 8.2&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>MFI</td>
<td>236 ± 22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>268 ± 25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>311 ± 42&lt;sup&gt;b&lt;/sup&gt;</td>
<td>399 ± 65&lt;sup&gt;c&lt;/sup&gt;</td>
<td>320 ± 56&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>CD 80, % positive cells</td>
<td>63.1 ± 7.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>68.2 ± 7.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>72.2 ± 6.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>76.7 ± 5.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>86.5 ± 3.7&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>MFI</td>
<td>523 ± 85&lt;sup&gt;a&lt;/sup&gt;</td>
<td>566 ± 82&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>615 ± 88&lt;sup&gt;b&lt;/sup&gt;</td>
<td>744 ± 96&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>837 ± 97&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>CD 86, % positive cells</td>
<td>45.9 ± 3.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>49.9 ± 7.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>53.4 ± 5.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>63.4 ± 4.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>74.1 ± 4.1&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>MFI</td>
<td>1162 ± 141&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1290 ± 172&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1463 ± 248&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1680 ± 238&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1618 ± 182&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

1. Data are means ± SEM, n = 4–6.
2. The values are percentages of DC (CD11c<sup>+</sup> cells) expressing indicated markers and mean fluorescence intensity (MFI). Means in a row without a common letter differ, P < 0.05.
3. LPS, 100 µg/L.
Effect of in vitro WBM supplementation on functional markers of DC maturation

Ren et al., J Nutr. 2008
Effect of in vitro WBM supplementation
DC antigen-presenting function

BALB/c → BMC

IL-4/GM-CSF
WBM (0, 50, 100, 200 μg/ml) → DC

MACS purification
7 days

OVA pulse

CD4+ T cells

MACS purification

CFSE labeling

Co-culture

T cell proliferation
IL-2 production

Ren et al., J Nutr. 2008
Antigen-specific CD4+ T cell proliferation and IL-2 production induced by OVA-pulsed DC

Ren et al., J Nutr. 2008
Summary of Study 2

- In vitro supplementation with WBM promotes DC maturation as indicated by phenotypic change.

- Enhanced DC maturation by WBM is further evidenced by the functional change including increased IL-12 production and reduced phagocytosis.

- WBM treatment enhances DC’s antigen-presenting function resulting in an elevated T cell response to the specific antigen.

- These results suggest that WBM may have a potential in improving the development of adaptive immunity after initial exposure to an antigen/pathogen, which requires validation in an in vivo study.
Dietary supplementation with white button mushrooms augments the protective effect of *Salmonella* vaccination in mice (unpublished)

Junpeng Wang, Xinli Niu, Xiaogang Du, Donald Smith, Simin Nikbin. Meydani, Dayong Wu
Study design

Exp. 1: Vaccine protection against *Salmonella* infection

- **Vaccination**: Attenuated strain SL1479 ∆aro A
- **Challenge**: Virulent strain SL1344
- Daily observation: BW and survival
- Control: 2% WBM
- Vaccination: 5% WBM

Exp. 2: Evaluation of immune response to vaccine

- +/- vaccination
- Control: 2% WBM
- Serum Ab levels
- Fecal Ab levels
- Immune response of spleen cells
- Wang et al., unpublished data
WBM supplementation further enhances protective effect of vaccination against *Salmonella* infection

* Different from non-vaccinated mice
$ Different from control+vaccine

Wang et al., unpublished data
WBM supplementation enhances antibody production after *Salmonella* vaccination

A. **Total IgG**
- Unimmunized: Control, WBM
- Immunized: Control, WBM
  - Diet: p=0.28
  - Vaccine: p<0.0001
  - Interaction: p=0.06

B. **Total IgA**
- Unimmunized: Control, WBM
- Immunized: Control, WBM
  - Diet: p<0.05
  - Vaccine: p<0.0001
  - Interaction: p<0.01

C. **HKST-specific IgG**
- Unimmunized: Control, WBM
- Immunized: Control, WBM
  - Diet: p<0.05
  - Vaccine: p<0.0001
  - Interaction: p<0.05

D. **HKST-specific IgA**
- Unimmunized: Control, WBM
- Immunized: Control, WBM
  - Diet: p<0.05
  - Vaccine: p<0.0001
  - Interaction: p<0.05

*Wang et al., unpublished data*
WBM-induces more IgG2a but unaltered IgG1 indicating an Th1 predominant response

A. HKST-specific IgG2a

B. HKST-specific IgG1

C. IgG2a/IgG1 Ratio

Diet: p<0.05
Vaccine: p<0.0001
Interaction: p<0.05

Wang et al., unpublished data
WBM supplementation promotes Th1 and Th17 responses induced by anti-CD3/CD28 stimulation

Wang et al., unpublished data
WBM supplementation promotes Th1 and Th17 responses induced by antigen-specific stimulation

Wang et al., unpublished data
WBM supplementation increases frequency and maturation of dendritic cells

Wang et al., unpublished data
Effect of WBM supplementation on pro-inflammatory cytokines in LPS-stimulated splenocytes

Wang et al., unpublished data
Summary of Study 3

- Dietary WBM potentiates protective effect of immunization in the subsequent *Salmonella typhimurium* infection.

- This effect of WBM is mediated through both humoral and cell-mediated adaptive immune responses.

- WBM-induced changes in IgG type and cytokine pattern suggest a Th1 predominant response, and Th17 may also be involved.

- Upregulated DC number and function may contribute to WBM’s effect in improving *Salmonella* vaccine efficacy.

- We speculate that Salmonella exposure-induced development of adaptive immunity might be enhanced by WBM consumption, which implies a potentially reduced risk of future infection.
Acknowledgment

Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University

Nutritional Immunology Lab
Simin Meydani
Zhihone Ren, Munkyong Pae
Junpeng Wang, Xinli Niu
Xiaogang Du, Zhuyan Guo

Comparative Biology Unit
Donald Smith

Funding:
USDA/ARS
The Mushroom Council

Mushroom gift:
Country Fresh Mushroom Co.

http://hnrc.tufts.edu
Mushrooms: For Weight Control and Health

Mushrooms & Health Summit
September 10, 2013
Washington, DC

Lawrence J. Cheskin, M.D.
Director, Johns Hopkins Weight Management Center
Department of Health, Behavior & Society
Johns Hopkins Bloomberg School of Public Health
Joint Appt: Medicine (GI); International Health (Human Nutrition)
**Obesity Trends Among US Adults**

1990

2008

1999

*BMI ≥30, or about 30 lbs overweight for 5’4” person*

BRFSS=Behavioral Risk Factor Surveillance System
Severe and Morbid Obesity Is Increasing More Rapidly Than Mild Obesity

Normal: 18.5-24.9
Class 1 Obesity: 25-29.9 (overweight)
Class 2 Obesity: 30.0-39.9 (severe)
Class 3 Obesity: 40 or more (morbid/extreme)

Approximately 25% of children and adolescents are overweight

- more than any other known time in history
- life expectancy may decline as a result
Life Expectancy and Obesity

- Two 2009 meta-analyses determined:
  1. 30-35 kg/m², median survival is reduced by 2-4 years
  2. 40-45 kg/m² medium survival is reduced by 8-10 years

# How Might Obesity Shorten Lifespan?

**Leading Causes of Death, North America**

<table>
<thead>
<tr>
<th>Cause</th>
<th>Rate/100,000</th>
<th>Obesity-Related?</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. CHD</td>
<td>175</td>
<td>Yes</td>
</tr>
<tr>
<td>2. Cancer</td>
<td>133</td>
<td>Yes</td>
</tr>
<tr>
<td>3. Accidents</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>4. Stroke</td>
<td>31</td>
<td>Yes</td>
</tr>
<tr>
<td>5. COPD</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>6. Diabetes</td>
<td>16</td>
<td>Yes</td>
</tr>
<tr>
<td>7. Pneumonia</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>8. Suicide</td>
<td>12</td>
<td></td>
</tr>
</tbody>
</table>
BMI and Relative Risk of Type 2 Diabetes

Medical Complications of Obesity

- Phlebitis
  - Venous Stasis

- Pulmonary disease
  - Abnormal function
  - Obstructive sleep apnea
  - Hypoventilation syndrome

- Coronary heart disease

- Pulmonary disease
  - Abnormal function
  - Obstructive sleep apnea
  - Hypoventilation syndrome

- Gastrointestinal disease
  - Nonalcoholic fatty liver disease
    - Steatosis
    - Steatohepatitis
    - Cirrhosis

- Gall bladder disease

- Cancer
  - Breast
  - Uterus
  - Cervix
  - Prostate
  - Kidney
  - Colon
  - Esophagus
  - Pancreas
  - Liver

- Skin

- Gout

- Diabetes

- Dyslipidemia

- Hypertension

- Cataracts

- Idiopathic intracranial hypertension

- Gynecologic abnormalities
  - Abnormal menses
  - Infertility
  - Polycystic ovarian syndrome

- Osteoarthritis

- Phlebitis
  - Venous Stasis
Regulation of Body Weight

Calories consumed in 1 year: 980,000

Weight gained / year* (kcals fat): 1/2 lb (1,700)

*Average over 20 yrs (30-50 yrs of age, Framingham study)
Diet Composition and Satiety

Hierarchy of satiety (per kcal):
- Fiber
- Protein
- Complex carbohydrates
- Simple carbohydrates
- Fat (unsaturated > saturated)
- Ethanol

Ethanol may even stimulate further food intake

Liquids are less satiating than solids
Dietary Fat and Obesity

- Epidemiologic evidence of a direct link
- Calorically dense; 9 kcal/gram
- Highly palatable
- Efficiently stored
- Virtually unlimited storage
Treatments for Obesity

- **Lifestyle modification**
  - Diet
  - Physical activity
  - Behavior modification

- **Pharmacotherapy**

- **Surgery**
Dietary Control of Obesity

The substitution of low calorie foods for high-calorie foods has been proposed as a means of preventing, or reversing obesity...
Why Mushrooms?

- Nutritional value
- Nutritional composition
- Satiety value
- Substitution potential for high fat, high energy density foods

The Evidence...
But Will it Work?

What is compensation?

An increase in caloric intake following a meal that is of lower energy.

(“making up for lost calories”)
Sometimes there is compensation –
e.g., after drinking diet soda vs regular

Sometimes not –
Only partial compensation eating a less-dense meal (low-fat cream cheese on bagels) following a week of energy-rich meals (regular cream cheese)

Differences in % compensation by age, gender, BMI:
• Young males exhibit more complete compensation than females and older adults (Rolls 1998)
• Obese individuals generally have been found to compensate more poorly than lean (Rolls 1994, Roe 1999)
Our Recent Work on Mushrooms & Weight Control: Mushroom Council Sponsored

- The first study to examine whether replacing one food for an entirely different one (meat vs. mushrooms) would lower overall calorie intake, and be as filling as higher calorie foods.
Methods

- Controlled intervention study, crossover design with each subject serving as his or her own control.
- Healthy men and women, aged 18-65
- 18 men and 36 women
  - Normal weight (43%),
  - Overweight (33%),
  - Obese (24%)
  - Received 4 days of lunchtime meat entrées,
  - Followed by 4 days of the same entrées substituted with mushrooms.
Methods

Daily Measures:

- Pre-meal hunger was recorded.
- Food was weighed before and after the meal was eaten.
- Satiety every hour for 5 hours after lunch
- Recording of all daily food, beverages and physical activity.
**Methods**

**First Group Schedule:**

<table>
<thead>
<tr>
<th>Day: 1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mushroom</td>
<td>Mushroom</td>
<td>Mushroom</td>
<td>Mushroom</td>
<td>Washout</td>
<td>Washout</td>
<td>Washout</td>
<td>Burger</td>
<td>Burger</td>
<td>Burger</td>
<td>Burger</td>
</tr>
</tbody>
</table>

**Second Group Schedule:**

<table>
<thead>
<tr>
<th>Day: 1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burger</td>
<td>Burger</td>
<td>Burger</td>
<td>Burger</td>
<td>Washout</td>
<td>Washout</td>
<td>Washout</td>
<td>Mushroom</td>
<td>Mushroom</td>
<td>Mushroom</td>
<td>Mushroom</td>
</tr>
</tbody>
</table>
Calories: 783 meat, 339 mushroom dishes; Potential savings = 444 calories/day
MUSHROOM LASAGNE

INGREDIENTS

- 8 strips Rippled Edged Lasagne Noodles
- 4 tbs. Butter or Margarine
- 8 oz. Mozzarella Cheese, thinly sliced
- 1/2 tsp. Salt
- 1/2 cup Parmesan Cheese, grated
- 2 lbs. Fresh White Mushrooms
- 3 - 8oz. cans Tomato Sauce
- 12 oz. Ricotta or Cottage Cheese
- 1/8 tsp. Ground Black Pepper

DIRECTIONS

Cook noodles following package directions; drain and set aside. Rinse, pat dry and slice mushrooms (makes about 10 cups). In large skillet heat butter; add mushrooms and sauté for 5 minutes. Drain off any mushroom liquid. Butter a 12 x 9-x 2-inch casserole. Cover bottom with 1 can of the tomato sauce. Top with 4 strips of the cooked noodles, overlapping edges slightly. Arrange half of the Mozzarella over the noodles and spoon half of the mushrooms over the cheese. Combine 1 can of the tomato sauce with Ricotta Cheese, salt and black pepper. Spoon half of the cheese and tomato mixture over the mushrooms. Sprinkle with half of the Parmesan cheese. Repeat. Place in a preheated moderate over (350 deg.) for 30 minutes or until bubbly. Let stand 10 minutes before serving.

YIELD: 6 Portions
Results

- There was no change in ratings of *hunger*, *satiety*, or *palatability* between meat and mushroom entrees

Hunger
Post-meal Fullness
General Sense of Fullness
Ease of Control Over Eating
Cravings For Foods
Urgency to Eat
## Average Daily Consumption, Meat vs. Mushroom Weeks

### (n=54 adults)

<table>
<thead>
<tr>
<th></th>
<th>Meat Week</th>
<th>Mushroom Wk</th>
<th>Difference</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories</td>
<td>2014</td>
<td>1635</td>
<td>385/444</td>
<td>0.000</td>
</tr>
<tr>
<td>Fat (grams; % of total intake)</td>
<td>88.4 (40%)</td>
<td>60.0 (33%)</td>
<td>28.4</td>
<td>0.000</td>
</tr>
<tr>
<td>Saturated Fat (grams)</td>
<td>21.5</td>
<td>18.7</td>
<td>2.8</td>
<td>0.099</td>
</tr>
<tr>
<td>Carbohydrates (grams)</td>
<td>201</td>
<td>206</td>
<td>5</td>
<td>Not significant</td>
</tr>
<tr>
<td>Fiber (grams)</td>
<td>16 (3.195%)</td>
<td>18 (4.37%)</td>
<td>1.77</td>
<td>0.207</td>
</tr>
<tr>
<td>Protein (grams; % of total intake)</td>
<td>105 (21%)</td>
<td>68 (17%)</td>
<td>37</td>
<td>0.000</td>
</tr>
</tbody>
</table>
**Overall Results**

*Total daily energy intake was significantly greater in the meat condition (2014 kcal) than in the mushrooms (1635 kcal)*

**Undercompensation for Fat and Calories:**
- Calorie compensation was 14%
- Fat compensation was 7%
Benefit for Weight Control?

- Lean individuals compensated for the calories saved during the mushroom week more than overweight and obese individuals.
- When adjusted for exercise, lean people compensate even more, but overweight and obese do not (thus lean people will not lose significant weight from eating mushrooms).
- Therefore, overweight or obese people will benefit the most from substituting mushrooms for meat.

<table>
<thead>
<tr>
<th></th>
<th>Lean</th>
<th>Overweight</th>
<th>Obese</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>n = 23</td>
<td>n = 18</td>
<td>n = 13</td>
</tr>
<tr>
<td>23% Caloric Compensation</td>
<td>6.3% Caloric Compensation</td>
<td>1.1% Caloric Compensation</td>
<td></td>
</tr>
</tbody>
</table>
Implications:

- Do these savings in calories persist beyond a week?
  (Encouraging in this was that the degree of energy compensation did not appear to increase from day 1 to day 4 of the mushroom substitution, nor was there compensation over the weekend between mushroom and meat weeks).

- Based on the above findings, the effect of consuming a single mushroom-substituted meal 4x/week would be to lose 20 lbs in a year.

- These projections were tested in a long-term study.
Follow-up Study Design

- **Study Design:** Randomized, controlled, parallel group, 1-year clinical weight-control trial.

- **Population:** Mushroom-eating adult men and women, overweight or obese (BMI 25-40), seeking weight loss

- n = 80 adults (based on previous weight loss studies showing that wt loss was 3 x as great on low ED (low fat, high fiber) diets.
Follow-up Study Design

- After *baseline measurements and biochemical tests*...
- Both groups (mushroom and control) were prescribed a 500 kcal/d energy-deficit diet *designed to achieve ~20 lbs weight loss over the first 6 months.*
- For the *second 6 months of the 1-yr intervention*, all participants were prescribed a *weight-maintenance* diet.
- The mushroom group *continued using mushrooms.*
Follow-up Study: Intervention

**INTERVENTION**

**Control (Meat Group)**
- Ways to eat healthy & make healthy choices without changing meat intake
- $6/wk voucher to purchase meat

**Intervention (Mushroom Group)**
- Incorporate 3 servings/wk mushrooms by substituting meat (8oz = 1 serving)
- $6/wk voucher to purchase mushroom

The *mushroom group only* was instructed and monitored in the preparation and use of mushroom substitutes for meat and other high ED foods, *provided with recipes to incorporate mushrooms in their diet*
Follow-up Study: Phases

**STUDY PHASES**

**WEIGHT LOSS**
- 0 -6 months biweekly visits (Visit 1 -15)
- Diet Counseling
- Food Vouchers for 2 weeks

**WEIGHT MAINTENANCE**
- 6-12 months monthly visits (Visit 16 -22)
- No Diet Counseling
- Food Vouchers for 2 weeks

**Weight loss phase** :- Both groups (mushroom and control) were prescribed a 500 kcal/d energy-deficit diet over the first 6 months of the intervention.

**Weight maintenance phase**:- For the second 6 months of the 1-yr intervention, all participants were prescribed a weight-maintenance diet in the mushroom group. The mushroom group continued using mushrooms in their diet.
Follow-up Study: Data Collection and Analysis

After baseline measurements and biochemical tests at Visit 1...

All the measures & biochemical tests were repeated at Visits 8, 15 & 22.

Food and physical activity logs were completed at these visits to describe changes in consumption patterns.

In addition to this, at every visit participants were asked to complete a questionnaire to assess their compliance with mushroom intake.

DATA COLLECTION & ANALYSIS

- BIA (%TBF), waist circumference, body and BMI at visit 1, 8, 15 & 22
- Multivariate Analysis, Paired Sample t test
Positive effect of white button mushrooms when substituted for meat on body weight and composition changes during weight loss and weight maintenance – A one-year randomized clinical trial.

Kavita H. Poddar, PhD, RD1; Meghan Ames, MSPH, RD1; Hsin-Jen Chen2; Mary Jo Feeney, MS, RD3; Youfa Wang, MD, PhD2; Lawrence J. Cheskin, MD1

1Department of Health Behavior and Society, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 21205
2Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 21205
3Consultant to Food and Agricultural Industries, Los Altos, CA, 94024 USA

INTRODUCTION

In the United States, weight gain and subsequent development of obesity is reaching epidemic proportions across race, gender and age groups and can be attributed to positive energy balance (1, 2).

American diets are high in fat and energy density making possible overconsumption of energy inevitable, leading to weight gain and obesity (3, 4).

Concomitantly, the consumption of red and processed meats has risen significantly in the US, are associated with high saturated fat and cholesterol and making it one of the major contributors of high density fat (5).

Substituting high density foods with low density foods is one obvious strategy to lose weight and improve body composition while providing high nutrient quality & satiety(6).

One study showed that substituting edible mushrooms for meat in the diets of participants lowered total short-term energy intake by nearly 20% in normal, overweight and obese individuals (7).

Current study assesses weight loss and weight maintenance efficacy with white button mushrooms as a substitute for meat over one year & builds on the experimental results of the short-term study described above.

OBJECTIVES

1. To assess body weight changes at the end of weight loss and weight maintenance phase
2. To assess BMI, total percent body fat & waist circumference changes at the end of weight loss and weight maintenance phase
3. To assess macronutrient intake of the participants in the mushroom versus meat diet group at the end of weight loss and weight maintenance phases of the study

SUBJECTS AND METHODS

The study protocol was approved by the Institutional Review Board at the Johns Hopkins University, Bloomberg School of Public Health.

This was a single blinded randomized clinical trial.

The study enrolled 209 overweight/obese (BMI 25-40) adult male & female participants who reported a desire to lose weight. Final analysis included 74 participants, for a drop-out rate of 65% after 12 months.

RECRUITMENT

Advertisements through the local newspaper (Baltimore Sun, City Paper), flyers posted around the campus and at local sites (supermarket bulletin boards)

METHODS

Screening and Randomization

INTERVENTION, STUDY PHASES & DATA COLLECTION

INTERVENTION

STUDY PHASES

DATA COLLECTION & ANALYSIS

MACRONUTRIENT INTAKE

RESULTS

CONCLUSION

The study design was a single-blinded randomized clinical trial. The study enrolled 209 overweight/obese (BMI 25-40) adult male & female participants who reported a desire to lose weight. Final analysis included 74 participants, for a drop-out rate of 65% after 12 months.

Treatment with mushrooms was associated with high satiety without compromising palatability (7) and current results support the potential utility of mushrooms as a low caloric substitute for red meat in overweight or obese individuals.

This substitution can help to reduce total energy and fat intake.

The more general strategy of substituting low energy-density foods for energy-dense foods will prove to be an effective method for controlling weight loss and body.

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The more general strategy of substituting low energy-density foods for energy-dense foods will prove to be an effective method for controlling weight loss and body.
Results of Long-Term Study

- Those on the mushroom diet lost an average of 7.0 pounds, or 3.6% of their starting weight; those on the standard diet lost an average of 2.2 pounds, or 1.1%.

- Waist circumference decreased on the mushroom diet -- a clinically significant mean of 2 inches during the 6-month weight loss phase (1 inch on the standard diet).

- Following initial weight loss, those who followed the mushroom-rich diet maintained their weight loss (the standard diet gained).

- At the end of 12-month study, those on the mushroom-rich diet weighed a mean of 7 lbs less, had lowered mean BMI by 1.5 kg/m², lowered mean waist circumference by 2.5 inches, and lowered mean percent body fat by 0.8% compared to baseline.

- Individuals who followed the mushroom-substituted diet pattern had lower energy & fat intake versus those who followed the standard diet.
Results of Long-Term Study

- **Meat Intake (oz/2wks)**
  - Weight-loss Phase (0 - 6 months): 66.2 oz
  - Weight-maintainance Phase (6 - 12 months): 74.8 oz

- **Mushroom Intake (oz/2wks)**
  - Weight-loss Phase (0 - 6 months): 38 oz
  - Weight-maintainance Phase (6 - 12 months): 37.9 oz
## Baseline Macronutrient Content

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline intake (Meat)</th>
<th>Baseline intake (Mushroom)</th>
<th>P Value (NS = not significant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total average calorie intake (kcals/day) a</td>
<td>1398.44±107.36</td>
<td>1513.34±107.36</td>
<td>NS</td>
</tr>
<tr>
<td>Total average carbohydrate intake (g/day) a</td>
<td>152.25±12.71</td>
<td>172.40±12.71</td>
<td>NS</td>
</tr>
<tr>
<td>Total average protein intake (g/day) a</td>
<td>69.00±5.40</td>
<td>69.03±6.12</td>
<td>NS</td>
</tr>
<tr>
<td>Total average fat intake (g/day) a</td>
<td>59.09±5.77</td>
<td>62.82±5.77</td>
<td>NS</td>
</tr>
</tbody>
</table>

aMultivariate analysis of variance; means expressed as estimated marginal mean ± standard error (SE)
## Changes in Dietary Macronutrients

### Mean change in macronutrient and fiber intake from baseline to endpoint for participants in the Meat Vs. Mushroom group

<table>
<thead>
<tr>
<th>Variable</th>
<th>Meat Diet (n=17)</th>
<th>Mushroom Diet (n=17)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline to the end of weight maintenance (0 - 12 months)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total average <strong>calorie intake</strong> (kcals/day)(^a)</td>
<td>132.48 ± 94.99</td>
<td>-122.69 ± 112.87</td>
<td>0.092</td>
</tr>
<tr>
<td>Total average <strong>carbohydrate intake</strong> (g/day)(^a)</td>
<td>36.03 ± 11.27</td>
<td>-7.65 ± 13.39</td>
<td>0.017*</td>
</tr>
<tr>
<td>Total average <strong>protein intake</strong> (g/day)(^a)</td>
<td>-8.01 ± 5.24</td>
<td>-13.09 ± 6.22</td>
<td>0.535</td>
</tr>
<tr>
<td>Total average <strong>fat intake</strong> (g/day)(^a)</td>
<td>2.17 ± 5.79</td>
<td>-4.25 ± 6.88</td>
<td>0.480</td>
</tr>
<tr>
<td>Total average <strong>fiber intake</strong> (g/day)(^a)</td>
<td>0.16 ± 1.10</td>
<td>-1.09 ± 1.30</td>
<td>0.468</td>
</tr>
</tbody>
</table>

\(^a\) Multivariate analysis of variance; \(P<0.05\); means expressed as estimated marginal mean±standard error (SE); Significant at \(P<0.05\)*.
**Changes in Body Composition**

Between group (meat vs. mushroom) differences in weight, waist circumference, % body fat and BMI at the end of weight loss and weight maintenance phases a,b,c.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Meat Diet (n=37)</th>
<th>Mushroom Diet (n=36)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weight loss phase (0 – 6 months)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body Weight (lbs) a</td>
<td>-2.21±3.11</td>
<td>-7.02±3.15</td>
<td>0.281</td>
</tr>
<tr>
<td>BMI (kg/m²) a</td>
<td>-1.04±0.76</td>
<td>-2.33±0.77</td>
<td>0.233</td>
</tr>
<tr>
<td>Waist circ. (inch) a</td>
<td>-1.41±0.36</td>
<td>-2.04±0.37</td>
<td>0.226</td>
</tr>
<tr>
<td>Percent total body fat (%) a</td>
<td>-0.49±0.99</td>
<td>-1.65±1.01</td>
<td>0.416</td>
</tr>
<tr>
<td><strong>Weight-loss from baseline to the end of 1 yr (0 - 12 months)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight (lbs) c</td>
<td>-2.20±3.29</td>
<td>-7.03±3.34</td>
<td>0.307</td>
</tr>
<tr>
<td>BMI (kg/m²) c</td>
<td>-1.00±0.36</td>
<td>-1.53±0.36</td>
<td>0.308</td>
</tr>
<tr>
<td>Waist circ. (inch) c</td>
<td>3.32±3.42</td>
<td>-2.58±3.47</td>
<td>0.230</td>
</tr>
<tr>
<td>Percent total body fat c</td>
<td>-1.01±0.52</td>
<td>-0.85±0.53</td>
<td>0.823</td>
</tr>
</tbody>
</table>
Conclusions

- Participants following a mushroom-rich diet lost an average of 7.0 pounds, or 3.6% of their starting weight, compared to the standard diet where average loss was 2.2 pounds, or 1.1% of starting weight.

- Waist circumference decreased in individuals on the mushroom diet -- a clinically significant mean of 2 inches during the 6-month weight loss phase, compared to 1 inch on the standard diet.

- Following initial weight loss, those who followed the mushroom-rich diet maintained their weight loss, and lost an additional 0.5 in waist circumference; those on the standard diet gained an average of almost 5 inches in waist circumference.

- At the end of our 12-month study, those on the mushroom rich diet weighed a mean of 7 lbs less, had lowered mean BMI by 1.5 kg/m², lowered mean waist circumference by 2.5 inches, and lowered mean percent body fat by 0.8% compared to baseline.

- It was known that consumption of energy-dense foods high in fat is associated with overweight. The current study also finds poorer results when attempting weight loss.

- Individuals who followed the mushroom-substituted diet pattern had lower energy & fat intake, and better success at losing weight and maintaining that loss.
Summary Points: Mushrooms and Weight Control

- There are now both short-term data, and data from up to 1 years’ duration, supporting the potential utility of mushrooms in assisting weight control in obese adults.

- The calories and fat saved by replacing meat with mushrooms is not fully “compensated” by other foods.

- The mechanism of this effect is likely imprecision in energy regulation, modulated by behavioral factors.

- Further testing in real-life settings is warranted.
Thank you!