

Mushroom Council Nutrition Research Update

June 2006

Updates provide Mushroom Council members, industry and the Nutrition Research Advisory Panel with the latest information on the status of Mushroom Council funded nutrition research. For additional information or clarification, contact Mary Jo Feeney, MS, RD, FADA, Nutrition Consultant, mj.feeney@earthlink.net.

Chen, Shiuang PhD. Department of Surgical Research, Beckman Research Institute of the City of Hope, Duarte, CA.

Dr. Chen has submitted a manuscript for publication on his mushroom research.

Cheskin, Lawrence J. MD Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

Does Substitution of Meat Products With White Button Mushrooms Have Potential For Weight Reduction? Studies of the Level of Short and Intermediate-Term Caloric Compensation, Satiety, and Dietary Satisfaction Among Lean and Obese Men and Women. Estimated completion summer/fall 2006 for the clinical trial: summer, 2007 for analysis.

A preliminary analysis of the dietary records of a few first participants found that the potential calorie savings on the mushroom test meal days vs. the meat meal days was 320-345 calories. The percent compensation – eating more food outside the substituted meals – to make up for the “lost” calories was on average so far about 20 percent. It is too early in the data analysis to determine if these results will hold up as the study progresses and is completed. An additional finding is that so far there is no significant difference in the palatability ratings between the mushroom and meat meals. Participants liked the meals substituted with mushrooms as much as the original meat versions. There was a trend toward reduced body weight during the week participants were on the mushroom test meals compared to the week they were on the meat test meals. The nutritional impact of the mushroom test meals compared to the meat test meals will be evaluated. Dr. Cheskin estimates that the clinical part of the study experiment will be finished by the end of the summer. He has been granted a no-cost time extension until June 30, 2007 to allow time for analysis.

Mark Kern, PhD, RD, San Diego State University, San Diego, CA.

Role Of Mushrooms Included in a Low Carbohydrate Diet on Weight Loss, Blood Lipids and Satiety. The clinical trial is completed although data continue to be analyzed.

See the January-April Update that included the titles of the three abstracts presented during Experimental Biology 2006 in San Francisco, CA in April. Dr. Kern reported that all presentations were delivered orally with good attendance. See the October-December 2005 Update that summarized previous findings to date.

Dr. Kern has submitted a fourth report on this research with includes preliminary analyses of data regarding blood pressure changes during the one month feeding periods and appetite responses to the test meals at the end of each dietary trial.

With regard to blood pressure changes, a 2 (time points) X 3 (trials) analysis of variance suggests that there was a significant main effect for decreased systolic and diastolic blood pressures for the trials (Figures 1 and 2). This response was anticipated due to the nature of the energy restriction of the diets fed to the participants. Interestingly, post-hoc examination with paired comparisons t-tests revealed that only within the trial in which subjects consumed the plant and mushroom based low carbohydrate diet were the decreases in both systolic and diastolic blood pressure from baseline statistically significant (Figure 3). The change in systolic blood pressure during the low fat diet bordered on statistical significance ($p=0.065$). It is unclear whether the more robust decreases in blood pressure after consuming the plant/mushroom-based diet is due to slightly greater loss of body weight or if a component of the diet is responsible. The striking difference, particularly for systolic blood pressure, warrants further investigation into the potential influence of mushrooms on blood pressure.

At the end of each 1-month feeding period, standardized isocaloric meals representing typical foods consumed during the respective trials were fed to fasted participants. Subjects marked a visual analog scale to determine their subjective ratings of hunger immediately before consumption of the meal as well as immediately, 1 hour, 2 hours, 3 hours and 4 hours after the meal. The responses in appetite were similar during each trial (Figure 4), despite subjects choosing to eat less energy during consumption of the 1-month plant/mushroom-based diet. Taken together, these results suggest that a low carbohydrate diet rich in mushrooms and plant foods is at least as satiating as a low carbohydrate, animal-based diet or a lower fat diet.

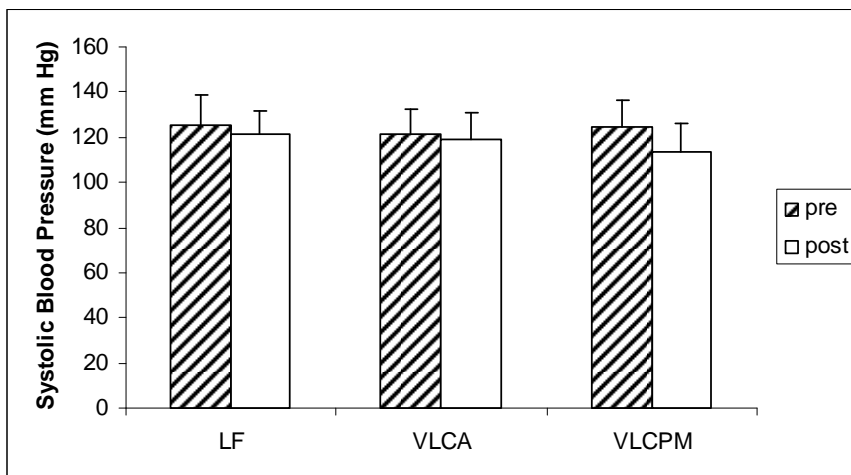


Figure 1. Systolic blood pressure responses to consuming low-fat (LF), animal-based low carbohydrate (VLCA), or plant/mushroom-based low carbohydrate (VLCPM) diets

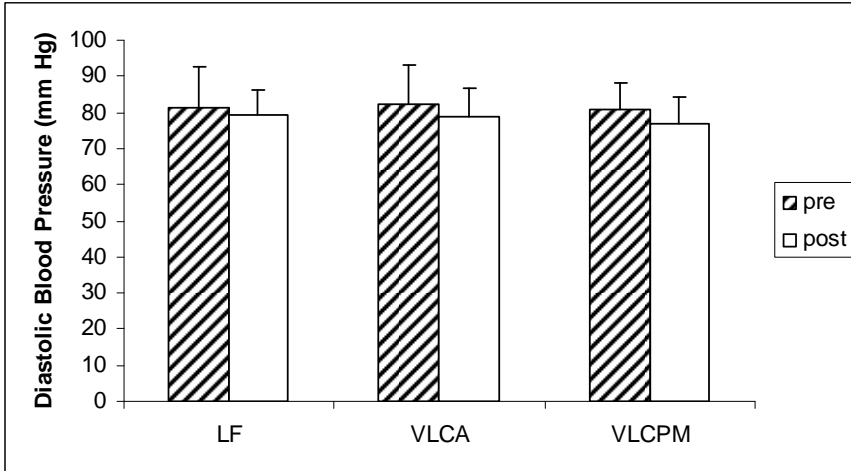


Figure 2. Diastolic blood pressure responses to consuming low-fat (LF), animal-based low carbohydrate (VLCA), or plant/mushroom-based low carbohydrate (VLCPM) diets

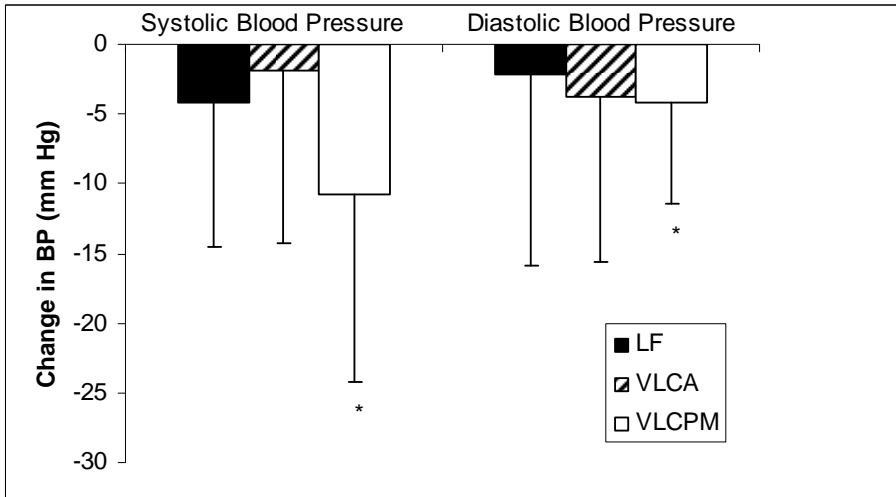


Figure 3. Blood pressure changes from baseline of subjects consuming low-fat (LF), animal-based low carbohydrate (VLCA), or plant/mushroom-based low carbohydrate (VLCPM) diets

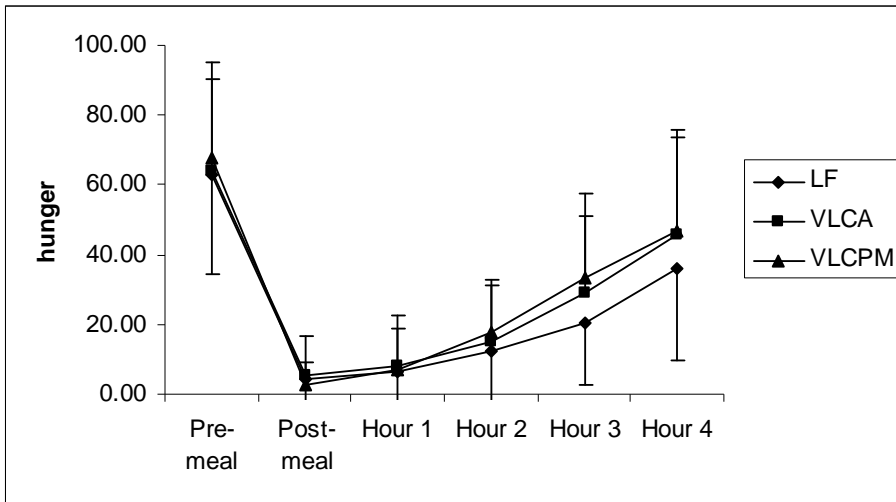


Figure 4. Hunger ratings in response to low-fat (LF), animal-based low carbohydrate (VLCA), or plant/mushroom-based low carbohydrate (VLCPM) meals fed at the end of 1-month dietary adaptations

David B. Haytowitz, U.S. Department of Agriculture, Beltsville Human Nutrition Research Center, Nutrient Data Laboratory, Beltsville, MD.

Nutrient Analysis Of Mushrooms. Analysis completed. To be included in USDA database as revisions are released.

David Haytowitz, Nutrient Data Laboratory, USDA-Agricultural Research Service, presented an abstract “Nutrient content and nutrient retention of selected mushrooms” at the annual meeting of the Institute of Food Technologists in June in Orlando. These data resulted from the Council’s nutrient analysis project. White mushrooms were analyzed stir-fried and microwaved so that retention factors could be developed and used to calculate cooked values for other mushroom varieties.

The abstract maintains that results of this study show that mushrooms provide dietary fiber, which ranges from 0.6 g/100g in oyster to 3.6 g/100g in shiitake. Potassium ranged from 200 mg/100g in maitake to 362 mg/100g in both enoki and white. Niacin ranged from 2.4 mg/100g in white to 7.4 mg/100g in enoki. Maitake contained 28.9 mcg/100g of folate, while other mushrooms contained lower amounts. All minerals and vitamins were well retained (most at 100%) during cooking. Some losses of sodium (due to leaching) and folate and vitamin B6, which are more heat labile, were observed.

These new values for mushrooms enabled USDA to update and expand the data on mushrooms in its databases. The next revision of the U.S. Dept. of Agriculture, Agricultural Research Service. 2006. USDA National Nutrient Database for Standard Reference, Release 19. Nutrient Data Laboratory Home Page, <http://www.nal.usda.gov/fnic/foodcomp> is anticipated in July.

Although USDA does not include beta-glucan and ergosterol values in its database, these values are available as a result of the complete nutrient analysis. Dr. Haytowitz will provide the Mushroom Council with these data files for use in other nutrition research.

Immunity Research

“Effect of mushroom supplementation on innate immune response,” Dayong Wu, PhD, USDA Human Nutrition Research Center on Aging at Tufts University. Immune cells from mice fed different doses of mushroom powder will be isolated for determination of innate immune responses such as macrophage function, NK cell count and activity and dendritic cell function. Dr. Wu reports that the first feeding study is about half way completed.

“Modulation of biomarkers of inflammation by mushrooms commonly consumed in the United States” Keith R. Martin, PhD, Penn State University. This study seeks to determine if mushrooms commonly consumed in the U.S (white, crimini, portabella, shiitake, oyster, king oyster, maitake and enoki). exhibit anti-inflammatory action in an *in vitro* macrophage model; beneficially alter *in vivo* pro-inflammatory status; and affect pro-inflammatory macrophage function. Please see the January-April Update for the most current information.

“Modulation of defensin production by mushroom extracts in human cell lines” Solo Kuvibidila, Ph.D, Louisiana State University. Dr. Kuvibidila submitted a very detailed and technical report that is available upon request from the Council or from the Nutrition Consultant. The following results were extracted from that report.

- DNA synthesis assessed by ^3H -thymidine uptake. Results suggest that mushroom extracts reduced ^3H -thymidine uptake by HL60 and THP1 cells by 50% - 75% at 1 and/or 10 $\mu\text{g/ml}$ extracts. However, at 0.1 $\mu\text{g/ml}$, DNA synthesis was reduced by less than 20% in HL60 cells and 6%-45% in THP1 cells. Caco2 cells were less sensitive to mushroom extracts; but nevertheless ^3H -thymidine uptake was also reduced by 38.89% to 73.3% when 10 $\mu\text{g/ml}$ of extracts were used. The inhibitory effect persisted when *E. coli* lipopolysaccharides (LPS, 2.5 $\mu\text{g/ml}$) was included in the culture medium. *No data on DNA synthesis are available in HGF1 cells because the growth rate is very slow.*
- Alpha defensin levels in the supernatant of cells incubated with and without mushroom extracts and *E. coli* lipopolysaccharides (2.5 $\mu\text{g/ml}$ LPS) for 24 h and of 48 h.

According to Dr. Kuvibidila, the data suggest that:

- White mushroom extracts increased the levels of alpha defensins in the supernatant of HL60 cells by 38.9% and 149% (maximum) in THP1 cells;
- Portabella mushroom extracts only slightly increased them in THP1 cells incubated without LPS (22%) and had very little effect in HL60 cells.
- Maitake mushroom extracts increased them by 81.8% (28.7% in the presence of LPS) in HL60 cells and 13.7% in THP1 cells;
- Shiitake mushroom extracts (in the presence of LPS) increased them by 79% and 77.6% in HL60 and THP1 cells, respectively.
- When HL60 cells were incubated for 48 h, the increase in alpha defensin secretion varied from 9% to 52%. However, in general, the rate of defensin secretion decreased with increasing concentrations of mushroom extracts.

Although more experiments must be performed, especially *in vivo*, preliminary results suggest that certain mushrooms may increase (blood) levels of alpha defensin even under baseline conditions. Additionally, since the cell lines used in these experiments are derived from human tumors, inhibition of cell proliferation by mushroom extracts being assessed in the current project might be beneficial for controlling tumor cell growth. According to Dr.

Kuvibidila, if these results are confirmed in laboratory animal studies, eating mushrooms may have several benefits:

1. In the gastrointestinal tract (one of the sites of defensin production and of daily microbial exposure/invasion), the body's capacity to kill microbes will increase leading to lower risk of microbial (bacterial) translocation. In other words, fewer bacteria will cross the mucosa, reach the blood stream, and cause septicemia.
2. The body's capacity to kill microbes that reach the blood stream will also increase because defensins, especially in high levels, "attack" and disrupt bacterial cell walls, and kill bacteria (and fungi) independently from other macromolecules secreted by neutrophils and monocytes.
3. By limiting the severity of infection, eating mushrooms and increasing baseline levels of defensins may reduce the severity of inflammation (especially chronic inflammation) usually associated with severe infection. Chronic and severe inflammation is thought to either induce or contribute to the progression of many chronic diseases in humans including rheumatoid arthritis, cancer, and type-2 diabetes.
4. In the oral cavity, increased baseline levels of defensin may reduce the risk of bacterial induced periodontal diseases. The rationale is that these defensins will kill microbes in the mouth and prevent inflammation.
5. Following infection, increased defensin secretion by mushrooms/mushroom extracts, will further increase the body's capacity to fight infection because these molecules activate and further enhance neutrophil function.

Dr. Kuvibidila also reports that according to the literature, impairment in beta-defensin activity has been implicated in chronic bacterial infections in patients with cystic fibrosis; which implies that eating mushrooms may also protect organs such as lungs from chronic infection. (Cited by Weinberg A et al, *Critical Reviews in Oral Biology & Medicine* 1998; 9(4):399-414.).

Dr. Kuvibidila assumes a new position this summer at Oklahoma Statue University where she will be able to continue this important research on mushrooms and immunity.

Pilot Study on Vitamin D2

The Council's pilot study to determine the production feasibility of exposing mushrooms to UVB light to convert ergosterol to vitamin D2 was reported at the June 11-13 Mushroom Industry Conference at Penn State University. The panel discussion entitled "Vitamin D2 Contents in Button Mushrooms" included Feeney, Guy Johnson, David Beyer, Robert Beelman, Charlee Kelly and Gary Schroeder.

Mushrooms exposed to UVB light either post harvest or on the growing bed and stored at different times at different temperatures were analyzed by MTT Agrifood Finland to discover the amount of D2 retained under these conditions. Table 1 summarizes the results which indicate a decrease in D2 with time and temperature but depending on the variables, it is still possible to obtain 100% Daily Value of D2 in a Nutrition Facts serving.

**Table 1 Analysis by Mattila, MTT Agrifood Research Finland
Calculations of D2 Per Serving and % DV by Feeney**

Sample May 2006 analysis

Monterey Mushrooms

Medium white mushrooms (harvested about 1 hour prior to experiment)

Frozen/freeze dried immediately after UV exposure

| Exposure | µg D2/g DM | µg D2/84 g fresh mushroom | % DV (10 µg) |
|---------------|------------|---------------------------|--------------|
| Control/no UV | nd | | |
| 30 seconds | 3.1 | 19.53 | 195.3% |
| 60 seconds | 5.3 | 33.39 | 333.9% |
| 5 minutes | 10.7 | 67.41 | 674.1% |

Stored +5C for 5 days after UV exposure; then frozen and freeze dried

| Exposure | µg D2/g DM | µg D2/84 g fresh mushroom | % DV (10 µg) |
|---------------|------------|---------------------------|--------------|
| Control/no UV | nd | | |
| 30 seconds | 0.96 | 6.048 | 60.5% |
| 60 seconds | 1.9 | 11.97 | 119.7% |
| 5 minutes | 5.1 | 32.13 | 321.3% |

PSU Samples

Medium white mushrooms harvested from growing beds after UV exposure for various time periods

Frozen/freeze dried immediately after exposure

| Exposure | µg D2/g DM | µg D2/84 g fresh mushroom | % DV (10 µg) |
|---------------|------------|---------------------------|--------------|
| Control/no UV | nd | | |
| 5 minutes | 0.36 | 2.268 | 22.7% |
| 15 minutes | 1.7 | 10.71 | 107.1% |
| 30 minutes | 2.2 | 13.86 | 138.6% |
| 45 minutes | 3.3 | 20.79 | 207.9% |
| 60 minutes | 4.5 | 28.35 | 283.5% |

Frozen/freeze dried after exposure and storage at +12C for 3 days

| Exposure | µg D2/g DM | µg D2/84 g fresh mushroom | % DV (10 µg) |
|---------------|------------|---------------------------|--------------|
| Control/no UV | nd | | |
| 5 minutes | 0.21 | 1.323 | 13.2% |
| 15 minutes | 0.7 | 4.41 | 44.1% |
| 30 minutes | 1.1 | 6.93 | 69.3% |
| 45 minutes | 2 | 12.6 | 126.0% |
| 60 minutes | 2.8 | 17.64 | 176.4% |

Antioxidants in Mushrooms - Ergothioneine Research

Drs. Joy Dubost, and Robert Beelman reported on antioxidant research at the June meeting of the Institute of Food Technologists (IFT). The paper at IFT was entitled “Quantification of Polyphenols and Ergothioneine in Cultivated Mushrooms and Correlation to Total Antioxidant Capacity Using the ORAC and HORAC Assays.” The work of Dubost and Dr. Beelman earlier identified mushrooms as a source of ergothioneine which functions as an antioxidant. The ORAC assay is a well known and used test of antioxidant capacity and focuses on the

peroxyl radical, the most predominant in the human body. However the ORAC assay does not identify what compounds contribute to antioxidant activity (it only measures TOTAL antioxidant activity), nor can it determine the physiological activity of the compounds in the body.

According to the study, portabella mushrooms and crimini have the highest ORAC values and even the white button mushroom has an ORAC value above some other commonly eaten vegetables.