

ANTHOCYANIN AND GLUCOSINOLATE NUTRIENTS:

AN EXPLORATION OF THE

MOLECULAR BASIS AND IMPACT OF COLORFUL PHYTOCHEMICALS ON HUMAN HEALTH

Abstract: Can eating food of an assortment of colors help one stay healthy? In this study, a randomized controlled trial helped evaluate the impact of a colorful diet on 8 healthy human adults (age 20-60) with similar demographic and dietary backgrounds. One of the daily meals of the volunteers was substituted with a hand-picked ration consisting of all colors of the rainbow in the form of a Rainbow Diet Pack (RDP). Fruits and vegetables were chosen based on the exclusive molecular structure and chemical composition of the most prevalent phytonutrient(s) in each. RDP was administered daily to the intervention group (n=5) over a 10-wk intervention period. Weight loss, waist circumference, hand grip strength, and stress levels were measured. Analyses revealed that eating raspberries, oranges, carrots, broccoli, blueberries, and bananas balanced stress levels and led to weight loss, but did not impact hand-grip strength, demonstrating the healthy outcomes of a colorful diet.

BY AKSHARA SREE CHALLA¹ AND JNANA ADITYA CHALLA²

LAYOUT OUT BY ANNALISE KAMEGAWA, EDNA STEWART, CAMERON MANDLEY, JENNY KIM

INTRODUCTION

The idea that one should be eating healthy to stay healthy is not a debate. Numerous studies show how particular foods individually effect human health, but none thus far, to our knowledge, have investigated about the combined impact of a specific diet on the human body as a whole.¹⁻⁵ It is critical for us to understand which kinds of things we should eat and the ways in which their collective consumption will impact our bodies. According to Dr. Thomas J. Carlson, a distinguished pediatrician and ethnobotany researcher, choosing foods from every color in the rainbow is the key to good health.⁶ Each fruit and vegetable gets its natural color from the chemical composition of the exclusive phytonutrient(s) in it.¹⁹

Interestingly, the presence of one molecule in one fruit/vegetable does not necessarily reflect the same color in another type of fresh produce. For instance, although the rich red color in most red fruits and vegetables is naturally derived from the phytonutrient lycopene, most berries such as strawberries and raspberries do not contain lycopene. Instead, they contain brightly colored chemicals called anthocyanins, which are made in plants during ripening season through the joining of a molecule of a sugar with a molecule of their colorless “anthocyanidin”

precursors.⁷ Anthocyanins are also found in raspberries, which are high in dietary fiber and vitamin C and have a low glycemic index because they contain 6% fiber and only 4% sugar per total weight.⁸ Higher quantities of fiber in the fruit, when consumed, helps lower the levels of low-density lipoprotein (LDL) or the ‘unhealthy’ cholesterol to enhance the functionality of our heart and potentially induce weight loss.

The exact pigment that anthocyanins reflect is partly dependent on the variance in acidity or alkalinity in different plants. Because of the relatively high pH of the tissues in blueberry plants, these chemicals turn blue in color during the ripening process of the fruit.⁷ Recent research in the Journal of Nutrition suggests that the abundant antioxidant properties in wild blueberries contributes to the reduction in the development of such disorders as Alzheimer’s Dementia and cognitive loss.⁹ A type of antioxidants selectively found in yellow and orange colored foods are called cryptoxanthins. In a study conducted by Bovier et al., it is shown that the combination of the beta form of these carotenoids with other sources of nutrients such as lutein and zeaxanthin in carrots, oranges, and corn leads to improved visual processing speed with regular consumption in young healthy subjects.^{10, 18}

While green produce mainly derives its pigmentation from chlorophyll, its white counterparts get their natural color from anthoxanthins, flavonoid pigments that exhibit antioxidant properties. Among green fruits and vegetables, broccoli stands apart as the most nutritious because of the special combination in which its 3 glucosinolate phytonutrients (glucoraphanin, gluconasturtian, and glucobrassicin) are found. This “dynamic trio” makes what are called Isothiocyanates (ITCs), the detox-regulating molecules in broccoli that enhance vitamin A in the form of beta-carotene.¹¹ Many recent studies claim that the antioxidants in ITCs not only regulate metabolism and cholesterol levels when consumed but also act as cancer chemopreventive phytochemicals.¹²⁻¹³ Fruits that are on the same level as broccoli with regards to health in the white-produce family are bananas. Japanese Scientists reveal that the high amounts of vitamin B6, manganese, potassium and fiber in the ripened versions of these fruits can help prevent high blood pressure, protect against atherosclerosis, and improve immunity levels in regular eaters.¹⁴

Despite an enormous amount of scientific knowledge and evidence for the overall beneficial effect of a single fruit/vegetable and/or phytonutrient at a time on human health, no study so far, to our knowledge, has been able to conclusively link the validity of these claims to the whole human body. This offers the opportunity for one to test the combined

impact of eating a colorful diet on humans through a systematic study. The purpose of our investigation is to apply a more holistic approach to the study of how the human body is effected as a result of a diet that is composed of all the colors of the rainbow. In other words, in addition to exploring the individual food-stuff’s role in improving health, we want to analyze the outcome of the regular incorporation of a whole pack of colorful foods into one’s meals. Consequently, this study can serve to reveal the effect, if any, of a continued and rigorous diet consisting of all colors of the rainbow on the physical and mental health of a randomized sample of the adult human population in a given demographically comparable community.

METHODS

The current study is a small-scale secondary application of some of the methods used in a previously conducted study that has been reported elsewhere.¹ The primary study used a randomized controlled trial to compare the effect of daily consumption of probiotic (PY) versus low-fat (LY) conventional yogurt on weight loss in healthy obese women; the outcomes tested were changes in anthropometric measurements (waist circumference and body weight). In our study, we measured hand grip strength and stress levels in addition to some of the parameters mentioned that were tested in the primary study.

We created a Rainbow Diet Pack (RDP) that consisted of the following fruits and/or vegetables in the respective quantities: raspberry (3), orange (1), baby carrots (4), corn (1/2 cob) broccoli floret (3), blueberry (5), and banana (1). The choice of each kind of fresh produce was based on the specific nutritional facts and molecular composition of the phytonutrients in each (see introduction for details). As per the personal choice of its members, the intervention group (n=5) received daily administration of RDP during a 10-wk intervention period. Measurements were taken of both the study and the control (n=3) groups twice: at baseline and at the end of the intervention period. Our study design was in accordance with the Declaration of Helsinki. Participants



The external appearance of fruits and vegetables offer insight to the photopigments they carry.

Figure 1 Consort Diagram

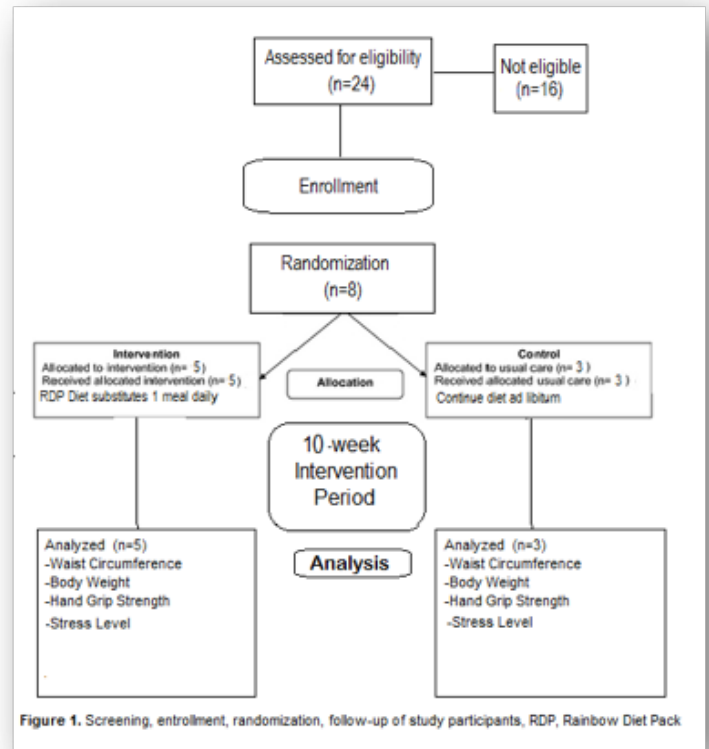


Figure 1. Screening, enrollment, randomization, follow-up of study participants, RDP, Rainbow Diet Pack

Twenty-four (24) normally healthy human adult (ages between 20 and 60) volunteers who belonged to the same demographic identity and had similar dietary backgrounds were recruited by word-of-mouth from the local community of the student investigators and screened for health. A total of eight (8) were chosen to participate. Individuals were eligible for the study if they were non-smokers, free of known disease, not allergic to items in RDP, not taking medications and were identified as being healthy according to the following criteria: body mass index (BMI) between 18.5 to 24.9 kg/m² and a self-report of no diseases/illnesses in the previous 6 months.

Randomization

A computerized random number generator was used to assign individuals chosen to participate to either the control or the intervention group. At the end of the baseline screening, a message containing the participant's number assignments was sent via email to the participants. Participants and the student investigator were aware of group assignment during the intervention phase. Participants were not aware of other participants who have agreed to be in the study. Before analysis, the primary investigator received an anonymized data set and was no longer aware of group assignment post data collection; no data can be traced back to the individual participant.

CONTROL GROUP

Participants allocated to the control group received standard advising and were allowed to continue their diet ad libitum. Participants were not asked to consume RDP. Pre- and post- intervention measurements were taken for members in the control group.

Intervention Group

Participants allocated to the intervention group received standardized nutritional support. One of the

daily meals of the volunteers in this group was substituted with a ration consisting of all colors of the rainbow in the form of a Rainbow Diet Pack (RDP). RDP consists of the following fruits and veggies in the respective quantities: raspberry (3), orange (1), baby carrots (4), corn (1/2 cob) broccoli floret (3), blueberry (5), and banana (1) will be asked to be consumed by the intervention group daily. A serving of RDP contains about 521 calories, 15 g protein, 110.6 g carbohydrate, 95 mg sodium, 38 g sugar, and 4 g fat.

Adherence

The interviews to recall daily adherence to RDP were conducted by telephone around once per 4-wk period. Participants had to keep a written record of their RDP observance.

MONITORING ADHERANCE

DIETARY INTAKE

Dietary intake was recorded daily in a diary by members of the intervention group during the 10-wk intervention period.

OUTCOME PARAMETERS

All measurements were made at baseline and 10 weeks after start of the intervention period.

ANTHROPOMETRIC MEASUREMENTS

Waist circumference and body weight were taken in traditional way using measuring tape and weighing scale and recorded at each measurement period for all partici-

About the Author

Jnana Aditya Challa

Class of 2016
Computer Science

Jnana Aditya Challa graduated from UC Berkeley in Spring 2016 with a B.A. in Computer Science. During his time at Cal, he was heavily involved with the American Red Cross and The Berkeley Project and had worked as a Computer Science T.A. His primary interests include community service, basketball, cricket, biking, hiking, app development and computer repair. He is currently working on learning more about the effects on the human brain of drugs such as Donepezil and El Dopa on Spatial Working Memory.



About the Author

Akshara Sree Challa

Class of 2018
Molecular Environmental Biology
Evolutionary Genetics Lab

Akshara Sree Challa is currently a junior at Cal from Albany, California. She is an intended Molecular Environmental Biology major with an emphasis on Human Health and plans to pursue a career in medicine. She works closely with various hospitals across the Bay Area as a volunteer and intern. She is fascinated by Nutrition and Gene Therapy and is currently involved with research at the Evolutionary Genetics Lab. In her spare time, she loves to listen to Indian music, create art and crochet.



pants. Baseline height was used for subsequent calculations. Hand Grip Strength.

Hand grip strength (kg) was measured using a hydraulic hand dynamometer (Baseline, Fabrication Enterprises, Inc., Elmsford, NY) (15). Participants were asked to perform two force trials with their non-dominant hand in a standing position and, if not possible, from a seated position at individual comfort level. The highest value was used. Participants were asked to keep track of their personalized data.

Stress Levels

Both the Social Readjustment Rating Scale (SRRS) questionnaire and Hassles and Uplifts Test were emailed to all participants to assess their stress levels.^{16,17} Participants were required to record their responses during both measurement periods. These tests include questions that the participants were comfortable to answer about personal health, relationship status, monetary commitments and other potential stressors.

RESULTS

BASELINE CHARACTERISTICS

From 24 individuals who were interested in participating in the study, 16 subjects were excluded because they did not meet the health criteria. The remaining 8 subjects gave written consent, and 5 subjects were randomly allocated to the intervention group which was required to consume the RDP and 3 to the control group, which was required to consume diet ad libitum. The RDP subjects completed the 10-wk intervention program (62.5% of the randomly assigned population, Figure 1).

After starting the intervention, no subjects dropped out. At baseline, there were no statistically significant differences in physical characteristics between the intervention groups or

between those who completed or did not complete the study once recruited (Table 1).

At baseline, 2 of 3 (66.7%) participants in the control group and 1 of 5 (20%) in the intervention group had BMI less than 20 kg/m². Ten weeks after intervention, these values slightly varied.

MONITORING ADHERENCE

Results on intake of RDP are shown in Table 2. Protein and vitamin A, C, and D intake levels were significantly higher in the intervention group than in the control group due to the contents of RDP. Adherence to RDP was 100% according to written records of each participant. All participants in the intervention group consumed RDP, with a mean intake of 1 meal per day (target 1/day). We tried contacting 62.5% of participants by telephone at least every other week, with a mean of 5.8 contacts (target 6 contacts per participant). Body Weight, BMI, and waist circumference. Body weight, BMI, and waist circumference at baseline are presented in Table 1.

Ten weeks after the program, body weight decreased to 59.43 ± 11.57 kg in the intervention group and 63 ± 13.72 kg in the control group. As shown in Table 2, there was significant weight reduction in the intervention group after 10 wk of study (mean difference in RDP was around -11.15 kg while that of control was around -2.89 kg).

BMI reduction in each group was in the expected direction with significant effects over 10 wk for both groups.

In both groups, waist circumference had decreased after 10 wk of intervention. The waist circumference decline was around -5.06 cm in the RDP group while only around -0.6 cm in the ad libitum diet group at 10 wk. This is a significant difference for the inter-

vention group when compared to the control group after 10 wk of the intervention for waist circumference (Table 2).

HAND GRIP STRENGTH

Hand grip strength has not changed significantly from baseline in the intervention or the control group. Mean increase in grip strength was around 1.0 ± 6.7 kg in both the intervention group and the control groups.

STRESS LEVELS

Stress levels changed significantly in the intervention and control group. Mean increase in SRRS score was around 1 point in the intervention group and a decrease of 2 points in the control group (Table 2). The hassles to uplifts ratio increased significantly for the RDP group, with an increase of 0.11 units. There was not a significant improvement in the ratio for hassles to uplifts in the control group.

DISCUSSION

The aim of this study was to assess the effects of eating a diet consisting of all the colors of the rainbow in the form of an RDP once a day on weight loss, stress levels, and other indexes of health in normally healthy volunteers during a 10 wk intervention program. We found that consumption of RDP as lunch may result in positive changes in waist circumference, weight loss and stress levels as measured during the program. This was despite finding no significant differences in observed hand grip strength between the study and control groups.

In spite of evidence for the beneficial effects of eating various naturally colorful produce on obesity and health, to our knowledge, this was the first randomized controlled trial that investigated the effect of consuming the RDP as a whole on weight loss and stress levels in healthy human subjects. The present study showed no significant difference in hand grip strength in this observational study with lifestyle intervention.

Overall, a decline in anthropometric measurements and cardiometabolic risk factors, including weight gain and high stress levels was observed, to a degree that would be expected with an

energy-restricted diet intervention (20). The total weight and waist circumference decreased to a significantly greater extent in the RDP group than in the control group. Nevertheless, future long-term trials are required to present evidence-based recommendation regarding the beneficial effects of RDP on further body profiles.

Finally, regarding the effects of RDP on hand-grip strength, despite similar changes in HGS in both groups, the study group presented slightly greater improvements in strength when compared with the control group over 10 wk. However, further comprehensive RCTs are necessary in order to institute a quantifiable implication of RDP consumption on carbohydrate absorption because statistical differences have been seen.

There were some draw-backs to this study. Although the sample size of eight was enough to verify the statistically significant effects on the fundamental outcomes, this number was not representative of the general population as a whole, particularly because it did not include individuals from dissimilar demographic and dietary backgrounds. Furthermore, the study was of a relatively short duration (10 wk). Longer-term studies are required to establish whether the effects can be sustained over a longer period. This would require continued consumption of the rainbow diet for a longer duration.

CONCLUSION

This study confirms and adds to the knowledge that a colorful diet can induce a positive body profile with healthy weight loss and balance stress levels in normally healthy adults. The association between nutrition and physical and mental health among humans is, as a consequence, linear. Ultimately, it is crucial to systematically maintain a colorful diet for at least one portion of daily meals to improve overall nutritional and physical status.

ACKNOWLEDGMENTS

Thanks to all the volunteers and donors without whose help this project could not have been possible. We would like to also thank Dr. Thomas Carlson for inspiring us to explore the topics of Medical Ethnobotany and Anatomy. Special thanks to Professor Kurt

Characteristics	Intervention Group (n=5)	Control Group (n=3)
Age, years	40.60 ± 11.13 [†]	37 ± 14.80
Body weight, kg	70.58 ± 11.69	65.89 ± 13.67
Height, cm	174.95 ± 13.39	176.78 ± 5.28
BMI, kg/m ²	23 ± 2.42	20.95 ± 3
Waist Circumference, cm	80.26 ± 6.38	77.05 ± 5.29
Married, %	80	67
HGS, kg	48.26 ± 10.07	44 ± 9.11
SRRS Score	299 ± 2.30	279.51 ± 5
Hassles and Uplifts Ratio	Uplifts: Hassles 0.76	Uplifts:Hassles 0.78

[†]Group Difference, P>0.05. HGS, hand grip strength, SRRS, social readjustment rating scale.

[‡]Mean ± SD (all such values).

Table 1. Baseline Characteristics of Participants[†]

	Intervention group(n=5)		Control Group(n=3)	
	Baseline	Week 10	Baseline	Week 10
<i>Body weight, kg</i>	70.58 ± 11.69	59.43 ± 11.57	65.89 ± 13.67	63 ± 13.72
<i>BMI, kg/m²</i>	23 ± 2.42	21 ± 1.93	20.95 ± 3	19.05 ± 3.11
<i>Waist Circumference, cm</i>	80.26 ± 6.38	75.20 ± 6	77.05 ± 5.29	76.46 ± 8.01
<i>HGS, kg</i>	48.26 ± 10.07	47 ± 9.88	44 ± 9.11	43 ± 8
<i>SRRS Score</i>	299 ± 2.30	300 ± 2.15	279.51 ± 5	277.50 ± 7.1
<i>Hassles and Uplifts Ratio</i>	Uplifts:Hassles	Uplifts:Hassles	Uplifts:Hassles	Uplifts:Hassles
	0.76	0.87	0.78	0.80

†Values are mean ± SDs. HGS, hand grip strength, SRRS, social readjustment rating scale.

Table 2. Anthropometric and psychological measurements in RDP group and Ad Libitum diet group before and after the 10-wk intervention†

Spreyer for his immense encouragement and help.

REFERENCES

1. Madjd, Ameneh et al. "Comparison of the Effect of Daily Consumption of Probiotic Compared with Low-fat Conventional Yogurt on Weight Loss in Healthy Obese Women following an Energy-restricted Diet: A Randomized Controlled Trial." *Am J Clin Nutr*, American Society for Nutrition (2016): n. pag. Web. 2 Jan. 2016.
2. Lam, Patrick et al. "Effects of Consuming Dietary Fructose versus Glucose on De Novo Lipogenesis in Overweight and Obese Human Subjects." *Berkeley Scientific Journal* 15.2 (2011): n. pag.
3. Neelemaat, F. et.al.(2012), Short-Term Oral Nutritional Intervention with Protein and Vitamin D Decreases Falls in Malnourished Older Adults. *Journal of the American Geriatrics Society*, 60: 691–699.
4. Russell MK. Functional Assessment of Nutrition Status. *Nutr Clin Pract*. 2015;2:211-18.
5. Leenders, M. et al. (2015), Subtypes of fruit and vegetables, variety in consumption and risk of colon and rectal cancer in the European Prospective Investigation into Cancer and Nutrition. *Int. J. Cancer*, 137: 2705–2714.
6. Carlson, T.J. (Director) (2015, November 4). *Medical Ethnobotany (IB 117) Lecture: Eye Health, Alzheimer's Dementia, Antioxidants.* .
7. "Why Are Strawberries Red." *Strawberries For Strawberry Lovers*. N.p., n.d. Web. 14 Dec. 2015.

8. "Nutrient data for raw raspberries, USDA Nutrient Database, SR-21". Conde Nast. 2014.
9. Kay, Colin D., and Bruce J. Holub. The Effect of Wild Blueberry (*Vaccinium Angustifolium*) Consumption on Postprandial Serum Antioxidant Status in Human Subjects. *Br J Nutr* 88, 389-397 88 (n.d.): 389-97. *British Journal of Nutrition*, May 2002.
10. Bovier, Emily R., and Billy R. Hammond. "A Randomized Placebo-controlled Study on the Effects of Lutein and Zeaxanthin on Visual Processing Speed in Young Healthy Subjects." *Archives of Biochemistry and Biophysics*, 15 Apr. 2015. Web. 1 Jan. 2016.
11. "Broccoli." *Broccoli*. The George Mateljan Foundation, n.d. Web. 15 Jan. 2016.
12. Zhang Y. Allyl isothiocyanate as a cancer chemopreventive phytochemical. *Mol Nutr Food Res*. 2010 Jan;54(1):127-35. 2010.
13. Thompson CA, Habermann TM, Wang AH, et al. Antioxidant intake from fruits, vegetables and other sources and risk of non-Hodgkin's lymphoma: the Iowa Women's Health Study. *Int J Cancer*. 2010 Feb 15;126(4):992-1003. 2010.
14. Iwasawa, Haruyo, and Masatoshi Yamazaki. "Differences in Biological Response Modifier-like Activities According to the Strain and Maturity of Bananas." *Food Sci. Technol. Res* 15.3 (2009): 275-82. Web. 1 Jan. 2016.
15. "Dynamometer." *AccessScience* (n.d.): n. pag. Web. 20 Dec. 2015.
16. The social readjustment rating scale, Holmes, T. H. and Rahe, R. H. 1967, *Journal of Psychosomatic research*, 11(2), 213-21.
17. DeLongis, A., Folkman, S., & Lazarus, R. (1988). The impact of daily stress on health and mood: Psychological social resources as mediators. *Journal of Personality and Social Psychology*, 54, 486–495.
18. "In Style This Summer: Color-Coded Eating." *Feathers Fringe*. N.p., 13 July 2011. Web. 5 Jan. 2016.
19. "Research.ncsu." N.p., n.d. Web. 10 Jan. 2016.
20. Kelley GA, Kelley KS, Roberts S, Haskell W. Combined effects of aerobic exercise and diet on lipids and lipoproteins in overweight and obese adults: a meta-analysis. *J Obes* 2012;2012:985902.