Principles and Practice of Clinical Research

A Global Journal in Clinical Research

PPCR
ISSN: 2378-1890
In press
ppcr.org/journal/home-current

Study design

Effect of 12-week dark chocolate intake combined with low-energy diet and exercise on weight loss in obese adults: a phase II randomized controlled trial protocol

Roberta Diaz Savoldelli[1*], Catherine Pui Yin Mok[2], Wilson Luiz da Costa Junior[3], Andreia Sapienza, Fayez Bahmad Jr[4], Juraci Aparecida Rocha[5], Marcio Ferreira Marcelino, Miho Fukuda[6], Pierre Elias Piera, Renata Buccheri[7], Sara Tavarez[8], Angelica Richart Csipak, Carlos Jimenez Conde[9], Carlos Mario Palomino, Edgar Bortolini, Florian Anzengruber[10], Luis Ivan Garcia Gutierrez, Marcus Unterrainer[11], Maria Rita Dionisio, Marianne Yang Yu, Mohammed Guba[12], Nelson Murillo[13], Paulo Bernardo, Ryoji Tominaka, Wilfredo Gomez, Livia Stocco Sanches Valentin[14]

1*-**Corresponding author** - Pediatric Endocrinology Unit - Instituto da Criança – HCFMUSP – São Paulo, Brazil. Adress: Rua Lopes Trovao, 79 – Centro – Sao Bernardo do Campo – SP – Brazil - CEP 09715-110. E-mail: roberta.savoldelli@usp.br.

Received, March 16th, 2016; Revised May 30th, 2016; Accepted June 21st, 2016

Abstract

Background: Obesity is a public health problem affecting 35% of the adult population in the USA, and treatment aims at a 5-10% weight loss, which decreases the risk of cardiovascular disease and diabetes mellitus. Diet and exercise have shown to be effective, but adherence is a limitation. Dark chocolate (DC) polyphenols were proven to have beneficial effects on blood pressure, endothelial function, HDL cholesterol, lipoprotein ratios and inflammation markers. Also, it might play a role in improving fat metabolism and satiety; however, its effects on weight loss are still not clear. We propose a protocol to explore the effects of DC associated with hypocaloric diet and exercise on weight loss during a 12-week period in obese adults.

Methods and Design: The study is a phase II randomized controlled trial with two parallel arms comparing hypocaloric diet and exercise with hypocaloric diet and exercise associated with 50g dark chocolate/day during a 12 week period. A total of 408 obese adults (18 to 45 years old) will be recruited. Primary outcome is proportion of patients who had 5% or more weight loss after 12 weeks. Secondary outcomes are glucose levels and insulin resistance (HOMA-IR), lipid profile, abdominal circumference, body fat mass and blood pressure, satiety perception and drop-out rates. Primary outcome (percentage of patients achieving 5% or more weight loss) will be analyzed by chi-square test with an Intention-to-Treat approach.

Conclusions: This phase II randomized controlled trial has the aim to explore the effects of including dark chocolate as an adjuvant to classical therapies (hypocaloric diet and exercise) on weight loss in a 12-week period.

Trial registration: the trial will be registered at www.clinicaltrials.gov

Key-Words: dark chocolate; weight loss; hypocaloric diet; exercise; obesity; randomized controlled trial

Citation: Savoldelli RD, Yin Mok CP, Costa Junior WL, Sapienza A, Bahmad Jr F, Rocha JA, et al. Effect of 12-week dark chocolate intake combined with low-energy diet and exercise on weight loss in obese adults. PPCR 2016, Jan-Apr;1(1):156-161.

Introduction

Obesity is a major problem in today's society since it is associated with numerous other major health issues such as type 2 diabetes mellitus (Mokdad et al., 2003), cardiovascular diseases (Hubert, Feinleib, McNamara, & Castelli, 1983), reduced fertility (Larsen, Wagner, & Heitmann, 2007) and has even significant impact on the genesis of neoplasms (Vaughan, Davis, Kristal, & Thomas, 1995) leading to great morbidity and mortality. All in all, obesity is part of a vicious circle influencing different kinds of diseases (Grundy, 2004) and hence, having a huge impact on today's global society and health in general.

It affects almost 35% of the adult population in the United States of America (USA) (Ogden, Carroll, Kit, & Flegal, 2014)and it had an estimated annual medical cost of 147 billion dollars in 2008 (Finkelstein, Trogdon, Cohen, & Dietz, 2009). Although there are numerous strategies trying to face obesity, losing weight seems to be one of the major tasks in today's health systems. Diet and exercise have shown to be effective in obesity treatment, although low rates of adherence are a limitation (Dansinger, Gleason, Griffith, Selker, & Schaefer, 2005). Nonetheless, it is known that a weight loss of 5-10% of baseline weight decreases the risk of cardiovascular disease and diabetes mellitus (Blackburn, 1995).

Phytochemicals called polyphenols have been suggested to have a beneficial effect on type 2 diabetes (Sabu, Smitha, & Kuttan, 2002) as well as on cardiovascular diseases, providing improvements in endothelial function (Perez-Vizcaino, Duarte, & Andriantsitohaina, 2006) and blood pressure (Rostami et al., 2015). Polyphenols are content of e.g. red wine, berries, pomegranates and, most interestingly, dark chocolate (DC), where they might lead to positive effects concerning digestion and absorption of fat and carbohydrates as well (Bermudezsoto, Tomasbarberan, & Garciaconesa, 2007). In line with the literature, DC consumption indicated a significant reduction in arterial stiffness after ingestion of chocolate high in flavonoids (Pase, Grima, & Sarris, 2011); besides that, favorable effects on HDL cholesterol, lipoprotein ratios and inflammation markers have been pointed out (Mellor, Sathyapalan, Kilpatrick, Beckett, & Atkin, 2010). Thus, it is assumed that DC might have cardioprotective effect and hence, could prevent cardiovascular events (Manach, Mazur, & Scalbert, 2005), especially in case of obese patients providing a certain risk profile concerning further diseases. Moreover, it has been demonstrated in vitro and in vivo that polyphenols can inhibit adipogenesis (Min et al., 2013; Matsui et al., 2005) and clinical studies demonstrated DC effects on increasing satiety (Massolt et al., 2010) (Sørensen & Astrup, 2011), so there could also be a role for polyphenol-rich DC in reducing body weight and body fat.

However, objective data on the effect of DC on weight is still scarce, as there are only two small studies evaluating pre-menopausal women comparing ingestion of DC to other sweet snacks and no difference on weight or anthropometric measures were observed (Nickols-Richardson, Piehowski, Metzgar, Miller, & Preston, 2014; Piehowski, Preston, Miller, & Nickols-Richardson, 2011)

Therefore, we propose to conduct the first large-scale randomized trial to assess the effect of daily DC coupled with a hypocaloric diet and exercise regimen on weight loss in an obese adult population. Our hypothesis is that subjects from the intervention group will lose more weight, due to direct DC polyphenol effect on increased satiety, also an indirect effect of DC as a sweet snack that would make the perception of the diet as not restricted, improving compliance to diet.

Methods

Study Design

This study will be conducted in adult obese patients. It will be a phase II, randomized, single-rater blinding, controlled with a parallel two-arm design and a 1:1 allocation performed in a single community center in Maine, USA. It is a superiority trial in which one group will be assigned to DC intake combined with low-energy diet and exercise for 12 weeks versus the control group that will have a low-energy diet and exercise for 12 weeks. The primary outcome is percentage of patients achieving at least 5% weight loss after the 12-week intervention.

Eligibility

We will evaluate adult male and female obese patients according to the following criteria:

Inclusion Criteria

1. Patients aged 18 to 45 years old
2. Body mass index (BMI)> 30kg/m^2 at screening
3. Voluntary written consent

Exclusion Criteria

1. Weight loss or exercise program in the previous 8 weeks
2. Known hypersensitivity to polyphenol and cocoa compounds
3. Previous bariatric surgery
4. Uncontrolled diabetes mellitus at screening (glycated hemoglobin is more than 6.5 %) and patients in use of insulin.
5. Cardiovascular conditions: history of myocardial infarction, unstable symptomatic ischemic heart disease, ongoing arrhythmias, thromboembolic events, or any other cardiac condition within 6

months before treatment; electrocardiography (EKG) abnormalities (Q-wave infarction or corrected QT interval > 460 milliseconds); Uncontrolled hypertension despite appropriate medical therapy (systolic blood pressure > 160 mmHg or diastolic blood pressure >90 mmHg)

6. Chronic diseases such as renal, liver and bone diseases and inability to perform the exercise program

Recruitment

Patients will be recruited in Maine, USA, using an advertisement in the local newspaper and on radio and television. Potential subjects will be contacted by phone by a research team member for an initial screening, followed by a personal interview and exam to ensure inclusion criteria are met.

Intervention

At the first visit, the target of 5% weight loss will be reported to all patients, individually expressed in kilograms considering the baseline weight. Also, all subjects will be instructed not to reveal their assigned group to the rater responsible for taking anthropometric data, and be informed that they will be excluded from the trial if they do.

Both groups will be evaluated by a registered nutritionist, who will estimate the daily energy requirement using basal metabolic rate (calculated by Schofield BMR predictive equations) multiplied by the physical activity level (FAO, 2004) and instructed to follow a hypocaloric diet with 500kcal/day restriction in relation to the calculated value, with nutrient distributed as follows: 45%-65% carbohydrates, 10%-35% proteins and 20%-35% fat (Institute of Medicine, 2005; U.S. Department of Agriculture & Human, 2010), plus orientation about food portions and frequencies.

In the intervention group, 50g of chocolate 70% cocoa/day (commercially available Lindt® Excellence 70% Cocoa) must be ingested, with no restriction considering time during the day. Its calories and nutritional components will be considered as part of the daily total recommendations. Chocolate will be provided every visit, in 10g squares and subjects will be instructed to eat five a day and bring back all the packs (empty or not).

Also, to promote weight loss, all subjects will be instructed to perform a moderate activity (brisk walking), raising heart rate to at least 60% of maximum heart rate (estimated for age at the first visit and subjects will be taught how to check the pulse rate) for 45 minutes, 3 times a week, with the association of a daily use of a pedometer with a goal of at least 10,000 steps/day(Tudor-Locke & Bassett, 2004).

They will be instructed how to keep record in a log book of diet and exercise information, regarding time, types of foods and portions ingestion, time, frequency, duration and achieved heart rate during exercises and daily pedometer registers, and for the intervention group, also information about time and amount of chocolate consumed. The log book should be brought to every visit.

All subjects will have biweekly visits, in the morning, where at first they will be evaluated by a blind rater that will take anthropometric data, and apply the satiety questionnaire. They will also be oriented to fast in the morning until the data is taken, and then breakfast will be provided by the center. After breakfast, subjects will have individual meetings with the registered nutritionist where the log book will be reviewed together, recommendations about the diet and exercise will be reinforced, possible barriers to adherence will be checked and strategies to overcome them will be set. At the end of the visit, next visit date will be set and chocolate provided for the intervention group subjects.

Patient will be excluded from the trial if they reveal the assigned group to the blind rater responsible for anthropometric measures. Those who become pregnant or withdrawn the informed consent at any time during the study will also be excluded.

Chocolate will be discontinued if the patient develops allergic reaction, gastro intestinal intolerance, or diabetes during the study; all should be reported as an adverse event.

Assessments

Weight measures will be collected every two weeks in all subjects' visits, for secondary analysis.

Other secondary outcomes are glucose levels and insulin resistance (homeostatic model assessment - HOMA-IR), lipid profile (total cholesterol and fractions, triglycerides), abdominal circumference, body fat mass (estimated by skinfold thickness) and blood pressure, all assessed at after 6 and 12 weeks of intervention.

Also satiety perception assessed using a Satiety Labeled Magnitude Scale (SLMS) (Zalifah, Greenway, Caffin, D'Arcy, & Gidley, 2008) and drop-out rates will be analyzed as secondary outcomes.

Adherence

All subjects will have free parking and reimbursement for gas purchased to attend biweekly follow-up visits. Those who come to the study facility by public transportation will have their tickets reimbursed as well. Breakfast will be provided after assessment of anthropometric measures for all participants in every visit. Individualized meetings during visits and a phone call every week, a planned visit with a registered nutritionist to reinforce recommendations, encourage and set strategies

to achieve diet and exercise goals will also be used to enhance participation. Subjects who wish to perform the programmed exercises at the institution facility are encouraged to do so, at no extra cost.

To monitor adherence, the log books and pedometers count will be reviewed, and for the intervention group, chocolate packs will be counted. All information will be registered in appropriated forms. Common complaints about the protocol will be evaluated by a central committee and possible protocol changes discussed.

Randomization and allocation concealment

The allocation random sequence will be generated by a computer software program. Stratification for gender and baseline weight will occur. Random block sizes will be used. Participants will be randomized using a sequentially numbered, opaque, sealed envelope (SNOSE), each containing a 2-inch by 2-inch paper with a written code designating intervention or placebo, following the sequence generated by the software. There will be no detectable difference in size or weight between intervention and placebo envelops. The envelopes will be opaque and lined inside with carbon paper. The envelopes are going to be opened sequentially only after writing the subjects tracking information on the envelope so that the carbon paper serves as an audit trail.

Allocation concealment will be ensured, as the randomization list will remain with the responsible staff member for the whole duration of the study. Thus, randomization will be conducted without any influence of the principal investigators, raters or physicians.

Blinding

Since it is not possible to efficiently blind subjects to chocolate, only the investigators responsible for measurement and assessment of primary and secondary outcomes and the staff involved in statistical analysis and will be blinded.

The registered nutritionist responsible for orientation of dietary changes will also be responsible for providing the chocolate portions during visits, but they will not participate in anthropometric data collection, will not have contact with the blind rater, and data analysis.

Patients will be strictly oriented not to reveal assignment group to the rater, and will be excluded from the trial if the recommendation is violated.

Sample size calculation

Sample size was calculated based on previous literature data, to detect a difference of 15% in proportions of patients achieving the 5% weight loss target between groups (50% of patients from the control group and 65% of patients from the chocolate group), using the chi-squared test comparing two independent proportions at 12 weeks with two-sided significance level of 5% and power of 80% with equal allocation to two arms, this particular study requires 170 participants in each arm. To allow for 20% drop out, 204 subjects will be necessary per arm and 408 in total.

Statistical analysis plan

Primary outcome (percentage of patients achieving 5% or more weight loss) will be analyzed by chi-square test.

In case of clinically-significant difference of baseline characteristics between the groups, results will be adjusted through a logistic regression model.

Subgroup analysis by age, gender, baseline BMI, and patient compliance will be performed as exploratory for hypothesis generating for further studies.

Secondary outcome analysis will be performed by repeated-measures ANOVA for weight loss every two weeks (time response of weight loss) and other continuous secondary outcomes assessed at baseline, at 6 and 12 weeks. Drop-out rates comparing control and intervention groups will be analyzed by chi-square test.

Intention-to-treat approach will be used to preserve groups determined by randomization. Missing data will be handled by a multiple imputations approach.

A per-protocol analysis is also planned. Subjects must have achieved diet and exercise goals in at least 80% of days during the study (based on information contained in the log books and pedometers reviewed on every visit) and for the intervention group, at least 80% of the DC portions must have been ingested (based on log book registers and returned packages); and must have a maximum of 2 missed appointments to be included.

Data monitoring

A Data and Safety Monitoring Board (DSMB) will be instituted to check the accuracy of data, guarantee protocol adherence by the team, and safety issues.

An interim analysis for futility is planned after 60% of subjects have completed the study.

IRB submission

The study protocol will be submitted to local Institutional Review Board (IRB) for approval.

Registration

The trial will be registered at *www.clinicaltrials.gov*.

Discussion

Potential limitations

Potential limitations include patient compliance to diet and exercise, but we believe that weekly contact with trained nutritionist (biweekly phone calls and biweekly

visits) to motivate patients and financial reimbursement for transportation can minimize problems.

For subjects missing their last appointment, telephone calls to reschedule visits, allowing last visit after the ideal period, and ultimately a visit to the subject´s house if needed to obtain final weight are programmed.

Overestimating effects of intervention is also a potential problem, for that a sensitivity analysis was performed and this calculated sample size would allow at least 80% power to detect a difference 15% considering percentage of responders (at least 5% weight loss) from 30 to 45% in the control group and 45 to 60% in the intervention group.

Short study duration will not allow long-term effects of DC and maintenance of weight loss evaluation, but it was not the objective of this study.

Future perspectives

Achieving and maintaining weight loss is still a great challenge. If results in this phase II trial are promising, larger phase III trials with greater external validity and also long-term studies will still be necessary to determine the role of DC as adjuvant strategy for weight loss.

Conclusion

While obesity is a major health problem worldwide nowadays, better strategies are still needed to face it. This phase II randomized controlled trial explores the potential beneficial effects of including DC as an adjuvant to classical therapies (hypocaloric diet and exercise) that could have a role in improving satiety and fat metabolism, and also improving adherence to diet.

Conflict of interest and financial disclosure

The authors followed the International Committee or Journal of Medical Journals Editors (ICMJE) form for disclosure of potential conflicts of interest. All listed authors concur with the submission of the manuscript, the final version has been approved by all authors. The authors have no financial or personal conflicts of interest.

Acknowledgement

The authors would like to thank Maria Pia Delgado Jimenez, Jessica Paulus, Clarissa Valim, and Leslie Kalish for their important contributions in the development of this protocol.

Authors' affiliations

[1] Pediatric Endocrinology Unit, Instituto da Criança, HCFMUSP, São Paulo, Brazil

[2] Division of Cardiovascular Surgery, Department of Surgery, University of British Columbia, Vancouver, British Columbia, Canada.

[3] Núcleo de Cirurgia Abdominal, A. C. Camargo Cancer Center, São Paulo, Brazil

[4] University of Brasilia Health Sciences Faculty, Brasilia, DF, Brazil

[5] Palliative Care Unit, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, HCFMUSP, São Paulo, SP, Brazil.

[6] Clinical Development, Regience K.K., Tokyo, Japan.

[7] Instituto de Infectologia Emílio Ribas, São Paulo, Brazil.

[8] Centro de Investigaciones Biomedicas y Clinicas (CINBIOCLI), Santiago, Dominican Republic.

[9] Intensive care unit, Juan Ramón Jiménez Hospital, Huelva, Spain.

[10] Department of Dermatology, University Hospital Zurich, Switzerland

[11] Department of Nuclear Medicine, Ludwig Maximilian University of Munich, Germany.

[12] Internal Medicine Department - Woodhull Medical and Mental Health Center, Brooklyn, NY, U.S.A.

[13] Internal medicine/ Cardiology and Critical care - Angiografia de Occidente , Cali, Colombia.

[14] Discipline of Anesthesiology, LIM 8 – Laboratory of Anaesthesiology, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil.

References

Bermudez-Soto, M., Tomas-Barberan, F., & Garcia-Conesa, M. (2007). Stability of polyphenols in chokeberry (Aronia melanocarpa) subjected to in vitro gastric and pancreatic digestion. *Food Chemistry*, *102*(3), 865–874. http://doi.org/10.1016/j.foodchem.2006.06.025

Blackburn, G. (1995). Effect of degree of weight loss on health benefits. *Obesity Research*, *3 Suppl 2*(14), 211s–216s. http://doi.org/10.1002/j.1550-8528.1995.tb00466.x

Dansinger, M. L., Gleason, J. A., Griffith, J. L., Selker, H. P., & Schaefer, E. J. (2005). Comparison of the Atkins, Ornish, Weight Watchers, and Zone Diets for Weight Loss and Heart Disease Risk Reduction. *JAMA*, *293*(1), 43. http://doi.org/10.1001/jama.293.1.43

FAO. (2004). *Human energy requirements . Report of a Joint FAO/WHO/UNU Expert Consultation . FAO Food and Nutrition Technical Report Series*.

Finkelstein, E. A., Trogdon, J. G., Cohen, J. W., & Dietz, W. (2009). Annual Medical Spending Attributable To Obesity: Payer-And Service-Specific Estimates. *Health Affairs*, *28*(5), w822–w831. http://doi.org/10.1377/hlthaff.28.5.w822

Grundy, S. M. (2004). Obesity, Metabolic Syndrome, and Cardiovascular Disease. *The Journal of Clinical Endocrinology & Metabolism*, *89*(6), 2595–2600. http://doi.org/10.1210/jc.2004-0372

Hubert, H. B., Feinleib, M., McNamara, P. M., & Castelli, W. P. (1983). Obesity as an independent risk factor for cardiovascular disease: a 26- year follow-up of participants in the Framingham Heart Study. *Circulation*, *67*(5), 968–977. http://doi.org/10.1161/01.CIR.67.5.968

Institute of Medicine. (2005). *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients)*. Washington, D.C.: National Academies Press. http://doi.org/10.17226/10490

Larsen, S. H., Wagner, G., & Heitmann, B. L. (2007). Sexual function and obesity. *International Journal of Obesity*, *31*(8), 1189–1198. http://doi.org/10.1038/sj.ijo.0803604

Manach, C., Mazur, A., & Scalbert, A. (2005). Polyphenols and prevention of cardiovascular diseases. *Current Opinion in Lipidology*, *16*(1), 77–84. http://doi.org/10.1097/00041433-200502000-00013

Massolt, E. T., van Haard, P. M., Rehfeld, J. F., Posthuma, E. F., van der Veer, E., & Schweitzer, D. H. (2010). Appetite suppression through smelling of dark chocolate correlates with changes in ghrelin in young women. *Regulatory Peptides*, *161*(1-3), 81–86. http://doi.org/10.1016/j.regpep.2010.01.005

Matsui, N., Ito, R., Nishimura, E., Yoshikawa, M., Kato, M., Kamei, M., ... Hashizume, S. (2005). Ingested cocoa can prevent high-fat diet-induced obesity by regulating the expression of genes for fatty acid metabolism. *Nutrition*, *21*(5), 594–601. http://doi.org/10.1016/j.nut.2004.10.008

Mellor, D. D., Sathyapalan, T., Kilpatrick, E. S., Beckett, S., & Atkin, S. L. (2010). High-cocoa polyphenol-rich chocolate improves HDL cholesterol in Type 2 diabetes patients. *Diabetic Medicine*, *27*(11), 1318–1321. http://doi.org/10.1111/j.1464-5491.2010.03108.x

Min, S. Y., Yang, H., Seo, S. G., Shin, S. H., Chung, M.-Y., Kim, J., ... Lee, K. W. (2013). Cocoa polyphenols suppress adipogenesis in vitro and obesity in vivo by targeting insulin receptor. *International Journal of Obesity*, *37*(4), 584–592. http://doi.org/10.1038/ijo.2012.85

Mokdad, A. H., Ford, E. S., Bowman, B. A., Dietz, W. H., Vinicor, F., Bales, V. S., & Marks, J. S. (2003). Prevalence of Obesity, Diabetes, and Obesity-Related Health Risk Factors, 2001. *JAMA*, *289*(1), 76–79. http://doi.org/10.1001/jama.289.1.76

Nickols-Richardson, S. M., Piehowski, K. E., Metzgar, C. J., Miller, D. L., & Preston, A. G. (2014). Changes in body weight, blood pressure

and selected metabolic biomarkers with an energy-restricted diet including twice daily sweet snacks and once daily sugar-free beverage. *Nutrition Research and Practice*, *8*(6), 695. http://doi.org/10.4162/nrp.2014.8.6.695

Ogden, C. L., Carroll, M. D., Kit, B. K., & Flegal, K. M. (2014). Prevalence of Childhood and Adult Obesity in the United States, 2011-2012. *JAMA*, *311*(8), 806. http://doi.org/10.1001/jama.2014.732

Pase, M. P., Grima, N. A., & Sarris, J. (2011). The effects of dietary and nutrient interventions on arterial stiffness: a systematic review. *American Journal of Clinical Nutrition*, *93*(2), 446–454. http://doi.org/10.3945/ajcn.110.002725

Perez-Vizcaino, F., Duarte, J., & Andriantsitohaina, R. (2006). Endothelial function and cardiovascular disease: Effects of quercetin and wine polyphenols. *Free Radical Research*, *40*(10), 1054–1065. http://doi.org/10.1080/10715760600823128

Piehowski, K. E., Preston, A. G., Miller, D. L., & Nickols-Richardson, S. M. (2011). A Reduced-Calorie Dietary Pattern Including a Daily Sweet Snack Promotes Body Weight Reduction and Body Composition Improvements in Premenopausal Women Who Are Overweight and Obese: A Pilot Study. *Journal of the American Dietetic Association*, *111*(8), 1198–1203. http://doi.org/10.1016/j.jada.2011.05.013

Ratziu, V., Bonyhay, L., Di Martino, V., Charlotte, F., Cavallaro, L., Sayegh-Tainturier, M.-H., ... Poynard, T. (2002). Survival, liver failure, and hepatocellular carcinoma in obesity-related cryptogenic cirrhosis. *Hepatology*, *35*(6), 1485–1493. http://doi.org/10.1053/jhep.2002.33324

Rostami, A., Khalili, M., Haghighat, N., Eghtesadi, S., Shidfar, F., Heidari, I., ... Eghtesadi, M. (2015). High-cocoa polyphenol-rich chocolate improves blood pressure in patients with diabetes and hypertension. *ARYA Atheroscler*, *11*(1), 21–29.

Sabu, M., Smitha, K., & Kuttan, R. (2002). Anti-diabetic activity of green tea polyphenols and their role in reducing oxidative stress in experimental diabetes. *Journal of Ethnopharmacology*, *83*(1-2), 109–116. http://doi.org/10.1016/S0378-8741(02)00217-9

Sørensen, L. B., & Astrup, A. (2011). Eating dark and milk chocolate: a randomized crossover study of effects on appetite and energy intake. *Nutrition and Diabetes*, *1*(12), e21. http://doi.org/10.1038/nutd.2011.17

Tudor-Locke, C., & Bassett, D. R. (2004). How Many Steps/Day Are Enough? *Sports Medicine*, *34*(1), 1–8. http://doi.org/10.2165/00007256-200434010-00001

U.S. Department of Agriculture, & Human, U. S. D. of H. and. (2010). *Dietary Guidelines for Americans, 2010* (7th ed.). Washington, D.C.: U.S. Government Printing Office.

Vaughan, T. L., Davis, S., Kristal, A., & Thomas, D. B. (1995). Obesity, alcohol, and tobacco as risk factors for cancers of the esophagus and gastric cardia: adenocarcinoma versus squamous cell carcinoma. *Cancer Epidemiol Biomarkers Prev*, *4*(2), 85–92.

Zalifah, M. K., Greenway, D. R., Caffin, N. A., D'Arcy, B. R., & Gidley, M. J. (2008). Application of labelled magnitude satiety scale in a linguistically-diverse population. *Food Quality and Preference*, *19*(6), 574–578. http://doi.org/10.1016/j.foodqual.2008.03.001