The WELL Study (Wellness Education for Liver Health Study): Reducing liver disease in genetically predisposed adults

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Methodology:
We will examine the feasibility and acceptability of our four-month, online, dietary intervention among 40 adults with NAFLD from a high-risk subpopulation (rs738409-GG and rs738409-CG).

Participant recruitment, liver clinic evaluation, and inclusion and exclusion criteria. We have already identified candidates from the Michigan Genomics Initiative using their electronic medical records including outpatient laboratory values that are homozygous for *PNPLA3* rs738409 and have elevated liver-function tests (median alanine aminotransferase above 35 international U/L). They have already consented to be re-contacted and are motivated participants interested in further research. The clinical characteristics of eligible participants are given in **Table 2**.

Table 2. Clinical Characteristics of Michigan Genomics Initiative rs738409-GGs with elevated ALT

Characteristic	rs738409 GG
Participants	363
Male Sex	162 (45%)
Age	48 [27–65]
BMI	32 [27–37]
ALT	49 [42–65]

Values are given as either median and interquartile range or as N (%).

We will contact the pool of potentially eligible participants by sending them a postcard describing the study, either in an envelope or with mailing information directly on the postcard. We may also send a follow up letter or flyer describing the study, in an envelope with either a handwritten or a typed address. Potential participants may also receive a phone call. Interested participants will fill out a screening survey; study team members will review their medical records for exclusions and elevated liver function tests. Participants that appear to meet inclusion and do not have exclusion criteria, based on their survey and medical chart, (**Table 3**) and agree to participate will be referred to our liver clinic to be evaluated to rule out other causes of elevated liver function tests (such as medications or high levels of alcohol intake). Participants who are taking blood glucose-lowering medications will be considered eligible for the trial. However, participants taking insulin will be excluded from participation. If the study team finds that recruitment is too slow and excluding insulin is too restrictive, participants taking insulin will be considered for enrollment. These participants will receive extra attention by study physicians in terms of medication management. See **Safety and medication management** section below.

Table 3. Inclusion and exclusion criteria.

Inclusion	Exclusion
Age > 18	Non NAFLD causes of elevated liver function tests
MRI with liver steatosis but not cirrhosis	Pregnant
English speaking and access to internet	Recent decompensation/hospitalization
Able to consent and follow directions	Weight loss medication or procedures planned
No physical limitations	Type 1 diabetes, cancer, heart failure, kidney failure, Cushing's syndrome
Physician approval to participate	Vegan, vegetarian, or other dietary restrictions
Rs738409 homozygote	Decompensated liver disease
Able to attend in-person appointments in Ann Arbor	Metal implants/inability to receive MRI

Potentially eligible participants will complete a screening consent survey via Qualtrics. After consenting to screening activities, they will complete an online baseline survey concerning their general health and well-being, watch a short, informational video about the trial and answer several comprehension questions, and set up an appointment for a phone call with the study coordinator. Prior to the phone appointment, the study coordinator will send participats the full study consent via SignNow. During this appointment, the study coordinator will go over the full consent with participants, having them sign on SignNow when all questions are answered. After consenting, participants will schedule two appointments with the study coordinator. The first appointment will be a virtual visit with Dr. Speliotes at Michigan Medicine's Taubman Center liver clinic and the second will be a baseline liver MRI; participants will receive instructions to call and schedule their baseline MRI to ensure the schedulers have accurate safety information when scheduling the appointment. The virtual visit will be scheduled within one month of the phone call, the second will be scheduled two to four weeks after the first. Participants who elect to participate in the optional induced pluripotent stem (iPS) cell research will be asked to informally schedule a time to get their blood drawn at the Taubman blood draw clinic. This blood draw must take place at the hospital to ensure sample viability. Participants will choose a time on a weekday, ideally in the morning, within two weeks of their appointment with Dr. Speliotes. At the virtual visit, Dr. Speliotes will evaluate the full medical history and physical health. History will be evaluated for cardiovascular disease (angina, myocardial infarction, stroke, heart failure). If participants are not getting blood drawn for iPSC research, then they will proceed to a fasting blood draw at any MM Labs location (no appointment needed) within one to two weeks following this virtual visit. If participants are getting blood drawn for iPSC research and scheduled a time for their blood draw during their consent visit, then they will go to the Taubman Center blood draw clinic at the time of their appointment. A member of Dr. Speliotes' team will either meet with participants when they arrive at the clinic to provide resarch tubes to the phlebotomist for the iPSC samples or will arrive immediately following the participant's blood draw to collect the samples. Blood tests will be drawn to assess (1) the cause of elevated liver function tests (complete blood count with differential; comprehensive metabolic panel; prothrombin time; C-reactive protein; erythrocyte sedimentation rate; hepatitis A, B, and C serologies; iron studies (iron level, iron binding capacity, percent transferrin saturation, ferritin); antinuclear antibodies; anti-LKM-1 antibodies, antimitochondrial antibodies; antismooth muscle antibodies; and alpha-1-antitrypsin levels), (2) diabetes (fasting glucose, fasting insulin, and hemoglobin A1c), and (3) dyslipidemia (serum lipid panel). Additionally, participants will have excess whole blood and serum drawn during their appointments. This serum (SST tube) will be transported to a lab in the University of Michigan's School of Public Health and tested for levels of an appetite suppressing hormone (growth differentiation factor: GDF15). For participants contributing blood for iPSC research, two tubes of whole blood (ACD solution A tubes or two CPT with sodium citrate tubes) will be drawn and transported to a lab within Medical Sciences Building II (Speliotes Lab). Participants may opt out of this step in the main study consent form. Also in the week following their virtual visit, participants must fill out an additional survey providing personal information, including their address (for mailed materials) and their primary care provider's name and phone number. Participants will also track their food for three days on the MyFitnessPal website or complete two 24-hour dietary recalls over the phone. The study team will reach out to the primary care providers of participants who have eligible blood tests. Primary care providers must approve their patient's participation before the subject is enrolled, within one month of first contact attempt. Primary care providers of participants who are taking blood glucose-lowering medications other than metformin will be advised that their patient may stop their medications as they begin the program to avoid risk of hypoglycemia. All primary care providers will be informed that their patient may reach out to them if they're experiencing symptoms or may need further medication adjustments.

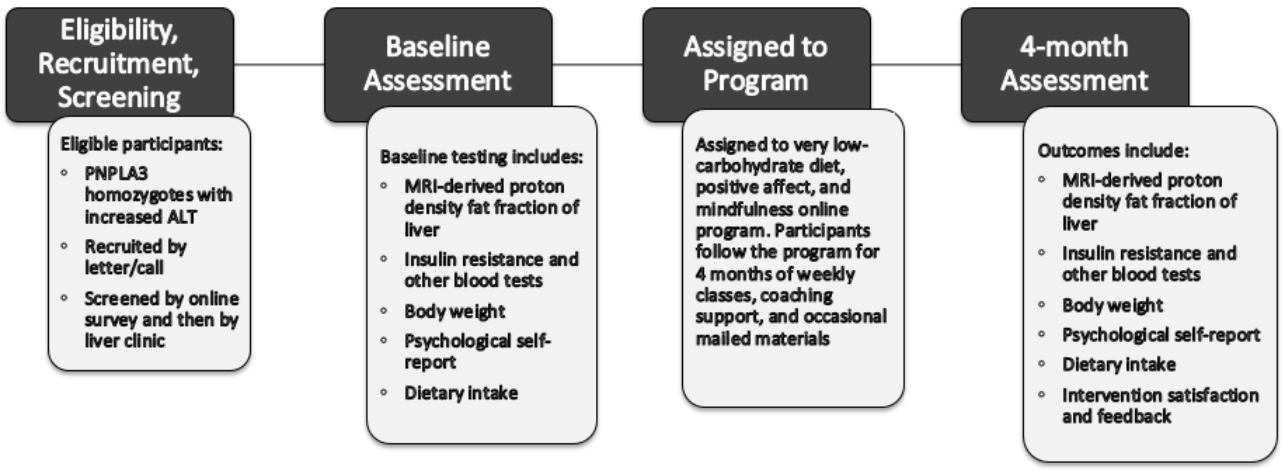
Participants may be ineligible after their blood tests and virtual visit. If no other cause of elevated liver function tests is found except for NAFLD and the patient meets inclusion with no exclusion criteria, the patient will attend their second appointment at the main Michigan Medicine hospital to receive a baseline standard-of-care, clinical liver MRI-derived proton density fat fraction test, the gold standard for non-invasive measurement of both hepatic steatosis, fibrosis, and cirrhosis. Prior to this appointment, participants of childbearing potential will be mailed a urine pregnancy test. They'll be instructed to take this test the day of their MRI and to send a photo of the result to the study email (they can write their result if they're uncomfortable with a photo).

Participants with a positive test will be asked not to come in for their appointment and will not be eligible for the trial. At the second appointment, participants will complete an MRI screening with the technicians and may be ineligible if they are unfit for an MRI. Those whose MRI shows steatosis, mild fibrosis, and early cirrhosis (F1-4), will be invited into the trial, as these are the patients for whom we may be able to reverse fibrosis with intervention. (See **Figure 1** for the full flowchart of the study.) If participants are ineligible after completing their MRI, they will be compensated $20 via Amazon gift card. The "psychological self-report" listed in Figure 1 for baseline and 4-month assessments includes questions concerning health-related quality of life,[1] dietary intake, physical activity,[2, 3] food cravings,[4] stress eating,[5, 6] dietary-based social support,[7] and hunger.[8]

Participants who are eligible after their MRI and have completed all other baseline measurements will be mailed a digital scale. Participants must weigh themselves on this scale within two weeks of receipt, before being enrolled in the trial.

Many of these measurements are standard of care for non-alcoholic fatty liver disease and will be charged to the participant's insurance. Baseline and follow up virtual visits, the baseline clinical liver MRI, and all baseline and follow up blood tests (with the exception of the excess serum, excess whole blood, and the fasting insulin tests) will be charged to the participant's insurance. Participants will be informed of this during the consent process and will be encouraged to check their plans to ensure they have coverage. The study will cover the costs of all other measurements and materials.

Figure 1. Flowchart of intervention study.



NAFLD standard of care. All participants will receive care at our NAFLD liver clinic; we will provide state-of-the-art care of and resources about NAFLD to all participants. An information packet will be provided to all participants at their baseline office visit that includes: 1) NAFLD disease information including diagnosis, clinical manifestations, natural history, and treatments; 2) NAFLD physical activity recommendations including walking programs and physical activity logs; 3) weight tracking logs; and 4) resources for exercise programs. Dr. Speliotes, with gastroenterology fellows working with her, will provide all medical care. Adverse events will be routinely queried at each clinic visit and the study physicians will be on call in the case of an adverse event. All participants will be taught a VLCD diets using our validated online program.

Participants will have a baseline virtual visit with Dr. Speliotes in the liver clinic and, if they appear eligible, they will come back for an MRI. After all baseline measurements are completed and if participants are still eligible, they will be enrolled in the 4-month online program. This program will include weekly lessons, mailed materials, and the support of a dietary coach. Details are outlined below:

VLC Diet. Participants will be encouraged to eat a VLCD, the same one Dr. Saslow has found effective in her previous research,[9] and to reduce carbohydrate intake to between 20-35 non-fiber grams of carbohydrates a day with the goal of achieving nutritional ketosis, defined as a positive urine dipstick (Bayer Ketostix), which measures the ketone acetoacetate. A VLCD restricts carbohydrate intake to a point that induces a low level of ketone production. Nutritional ketosis may indicate that insulin levels are reduced to a level that the body is using fat as the key source of energy, turning on fat burning (lipolysis).[15] When this occurs, some fats are turned into ketones, which serve as a readily used fuel.[16] This is a commonly used approach,[10, 11] and results from our pilot[9] and other studies[12-15] provide empirical support for this type of diet.[16]

Support. As changing a diet can be complex, several sessions are necessary to comprehensively and effectively teach a new way of eating. All participants will be e-mailed links every week for 16 weeks that connect them to a) a short survey to assess intervention-related behavioral adherence and health concerns, b) a short video to teach the topics, c) downloadable handouts distributed online to accompany the videos, and d) links to external resources on the web pertaining to the week's information. As some participants prefer not to watch videos, the transcripts of the embedded videos will be provided as well, in an easy-to-read downloadable format. The lessons can be watched and read whenever is convenient and take about 10 to 30 minutes to complete. As coaches have been found to be effective additions to behavioral interventions,[17] if participants in the groups have questions, they will be able to contact a coach in order to receive prompt replies. Coaches will be trained, and coaching quality will be monitored for fidelity to our coaching manual.

All participants will be provided with extra support tools. These will be taught with a weekly short video, downloadable handouts, and links to external resources. Below, we describe the justification for the behavioral support we include.

a) Training in positive affect and mindful eating. To adhere to a new diet, participants need to cope effectively with inevitable stressors. According to the revised Stress and Coping Theory[18, 19] positive emotions serve as a psychological “time-out” from the upset caused by stress and improve the motivation to adaptively cope with stress.[20] Moreover, our intervention aims to help participants better enjoy and savor their food. Hedonic theories of behavior propose that people will be more likely to continue doing behaviors they enjoy,[21] and previous research shows a connection between greater eating plan satisfaction and better eating plan adherence.[22, 23] Those who eat as a way to cope with their emotions tend to regain more weight after successful weight loss.[24, 25] The psychological causes of emotional eating may involve poor awareness of internal physiological states and differentiation between hunger cues and emotional arousal.[26-28] To counteract the tendency to eat when stressed, we will draw on Mindfulness-Based Eating Awareness Training (MB-EAT),[29, 30] which includes exercises to increase the awareness of the triggers of overeating, internal cues that signal hunger and fullness, and ways to cultivate healthier alternative behavior. We will teach these diet- and health-focused skills; how they are expected to help; research supporting them; and suggestions for practicing them.

b) Dietary self-monitoring using a free online or mobile application. We will ask participants to track their diet using a free online and mobile application, MyFitnessPal (which our participants have found easy to use, has a wide variety of foods in its database, and has over 150 million users). Previous participants have nearly unanimously enjoyed using this application. We will encourage dietary tracking daily for the first few weeks of the program, tapering to at least a few days a week by about week 4. Participants will first be asked to track their regular eating pattern with MyFitnessPal before they begin the program (during their enrollment process). Participants will be encouraged but not required track their eating throughout the intervention to support them in making dietary changes and follow the very low carb diet. Finally, participants will be asked to track for three days near the end of the intervention for assessment of dietary adherence.

c) Body weight self-monitoring using a digital scale. At the start of their 4-month program, participants will be mailed a scale. We will ask participants to track their body weight weekly with this scale, which is easy

to use and connects via a cellular network. Because it connects via its own cell network, participants do not have to set up any passwords, simplifying ease of use. We will use this information to monitor participant success and tailor coaching support.

d) Mailed materials. At week 1, week 6 and week 11, we will mail participants lay-press cookbooks to help with increasing variety in their diet. This approach has been popular with our previous participants. Although meal replacements have been studied, and are generally found to be helpful for weight loss,[31, 32] this cookbook approach is novel. Also at week 1, participants who are taking glucose-lowering medication (other than metformin) will be sent a glucose-monitoring device via Amazon, so that they may self-monitor their glucose.

e) Text messages. Reminders about targeted behaviors are tied to greater behavioral adherence.[33] Thus, to encourage the adoption and maintenance of the new intervention-related behaviors, participants will be sent text messages to give them feedback as well as to motivate, educate, and remind them about the targeted behaviors and skills.[34] Thus, as Dr. Saslow has done in her previous research, we will send automatic, motivational, and educational skill-relevant messages about 5 times a week to participants.[35, 36] Although most previous participants enjoyed the messages, participants have the option to opt out of text messages.

Safety and medication management. Participants will be asked in a weekly survey about health symptoms or adverse events they've experienced and to report any medication changes they've made. Participants who report symptoms will be reached by email as soon as possible by the study coordinator or diet coach. Participants may be called if the health event seems urgent or severe. For severe or long lasting symptoms, participants will be encouraged to reach out to their primary care physician. Participants who are taking some blood glucose-lowering medications (metformin, DPP-4s, GLP-1 agonists, and Thiazolidinediones (TZDs)) will continue their medications as normal. Participants taking all other glucose-lowering medications will be asked to stop taking these medications at the start of the trial (PCPs will be informed of this change when participants begin the trial). All participants will be asked in the aforementioned weekly survey about their highest and lowest fasted and postprandial (2 hours after eating) blood glucose levels over the previous week. If participants report low glucose (fasting below 70 mg/dL or postprandial below 110 mg/dL), they should reach out to their physician and follow self-management guidelines (sent to them as part of week 1's intervention materials). If participants report more than two weeks of high blood glucose (180 mg/dL), Dr. Dina Hafez Griauzde will reach out to participants via email or phone about management and reach out to participant PCPs.

Outcomes and analyses. After 16 weeks of the VLCD program, participants will complete a series of follow up measurements. Participants will complete a virtual follow up office visit, a follow up self-report survey, track their food, weigh themselves on the scale we sent them, take an at-home urine pregnancy test (if of childbearing potential), and return for a MRI-derived proton density fat fraction test. All final measurements should be completed within 4 weeks of completion of the program. Additionally, the second MRI will be conducted on research machines and charged to the study. Participants will also have a fasting blood draw. Follow up blood tests will include: complete blood count with differential, comprehensive metabolic panel, prothrombin time, fasting glucose, hemoglobin A1c, fasting insulin, and a serum lipid panel.

Statistical Design:

Based on a previous, small, short-term trial without genetic biomarkers,[1] we expect patients enrolled in this program to lose 12% of their liver fat with a standard deviation of 6%. With a one-sample Wilcoxon test, this sample is 99% powered to detect such a decrease in MRI-derived fat fraction at $\alpha = 0.01$. Extrapolating from the epidemiological association of the rs738409-GG genotype with fatty liver in insulin-resistant patients,[2] the benefit in this group may be twice as high as in the general population (24%); if so, we would likewise be 99% powered to detect a reduction *significantly greater than* 12% at $\alpha = 0.01$.

One-sample Wilcoxon tests will likewise be used for post-program *vs.* baseline insulin resistance and body weight as secondary outcomes. We do not anticipate significant fibrotic differences after 4 months on this intervention, but will report pre-post liver fibrosis values. Baseline and post-program semi-structured interviews, as in Aim 1, will be used to assess satisfaction, adherence and dropout.

1. Reeve, B.B., et al., *Psychometric evaluation and calibration of health-related quality of life item banks: plans for the Patient-Reported Outcomes Measurement Information System (PROMIS).* Medical care, 2007. **45**(5): p. S22-S31.
2. Craig, C.L., et al., *International physical activity questionnaire: 12-country reliability and validity.* Medicine & Science in Sports & Exercise, 2003. **35**(8): p. 1381-1395.
3. Kiernan, M., et al., *The Stanford Leisure-Time Activity Categorical Item (L-Cat): a single categorical item sensitive to physical activity changes in overweight/obese women.* International journal of obesity (2005), 2013. **37**(12): p. 1597.
4. Dalton, M., et al., *Preliminary validation and principal components analysis of the Control of Eating Questionnaire (CoEQ) for the experience of food craving.* European journal of clinical nutrition, 2015. **69**(12): p. 1313.
5. Tsenkova, V., J.M. Boylan, and C. Ryff, *Stress eating and health. Findings from MIDUS, a national study of US adults.* Appetite, 2013. **69**: p. 151-155.
6. Burgess, E., et al., *Profiling motives behind hedonic eating. Preliminary validation of the Palatable Eating Motives Scale.* Appetite, 2014. **72**: p. 66-72.
7. Norman, G.J., et al., *Reliability and validity of brief psychosocial measures related to dietary behaviors.* International Journal of Behavioral Nutrition and Physical Activity, 2010. **7**(1): p. 56.
8. Stunkard, A.J. and S. Messick, *The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger.* J Psychosom Res, 1985. **29**(1): p. 71-83.
9. Saslow, L.R., et al., *A randomized pilot trial of a moderate carbohydrate diet compared to a very low carbohydrate diet in overweight or obese individuals with type 2 diabetes mellitus or prediabetes.* PloS one, 2014. **9**(4): p. e91027.
10. Phinney, S.D. and J.S. Volek, *The Art and Science of Low Carbohydrate Living: An Expert Guide to Making the Life-saving Benefits of Carbohydrate Restriction Sustainable and Enjoyable*. 2011: Beyond Obesity.
11. Bernstein, R.K., *The diabetes diet : Dr. Bernstein's low-carbohydrate solution*. 1st ed. 2005, New York: Little, Brown and Co. xi, 291 p.
12. Volek, J.S., et al., *Carbohydrate restriction has a more favorable impact on the metabolic syndrome than a low fat diet.* Lipids, 2009. **44**(4): p. 297-309.
13. Ruskin, D.N. and S.A. Masino, *The nervous system and metabolic dysregulation: emerging evidence converges on ketogenic diet therapy.* Frontiers in neuroscience, 2012. **6**.
14. Bistrian, B.R., et al., *Nitrogen metabolism and insulin requirements in obese diabetic adults on a protein-sparing modified fast.* Diabetes, 1976. **25**(6): p. 494-504.
15. Dashti, H.M., et al., *Beneficial effects of ketogenic diet in obese diabetic subjects.* Molecular and cellular biochemistry, 2007. **302**(1-2): p. 249-256.
16. Mavropoulos, J.C., et al., *The effects of a low-carbohydrate, ketogenic diet on the polycystic ovary syndrome: a pilot study.* Nutr Metab (Lond), 2005. **2**: p. 35.
17. Kivelä, K., et al., *The effects of health coaching on adult patients with chronic diseases: A systematic review.* Patient education and counseling, 2014. **97**(2): p. 147-157.
18. Folkman, S., *Positive psychological states and coping with severe stress.* Social Science and Medicine, 1997. **45**: p. 1207-1221.
19. Folkman, S., *The case for positive emotions in the stress process.* Anxiety, Stress & Coping, 2008. **21**: p. 3-14.
20. Lazarus, R.S., A.D. Kanner, and S. Folkman, *Emotions: A cognitive-phenomenological analysis*, in *Theories of emotion*, R. Plutchik and H. Kellerman, Editors. 1980, Academic Press: New York. p. 189-217.
21. Williams, D.M., *Exercise, affect, and adherence: an integrated model and a case for self-paced exercise.* Journal of sport & exercise psychology, 2008. **30**(5): p. 471.

22. Coyne, T., et al., *Dietary satisfaction correlated with adherence in the Modification of Diet in Renal Disease Study.* Journal of the American Dietetic Association, 1995. **95**(11): p. 1301-1306.
23. Ahlgren, S., et al., *Development of a preliminary diabetes dietary satisfaction and outcomes measure for patients with type 2 diabetes.* Quality of Life Research, 2004. **13**(4): p. 819-832.
24. Wing, R.R. and S. Phelan, *Long-term weight loss maintenance.* Am J Clin Nutr, 2005. **82**(1 Suppl): p. 222S-225S.
25. Larsen, J.K., et al., *Binge eating and exercise behavior after surgery for severe obesity: a structural equation model.* Int J Eat Disord, 2006. **39**(5): p. 369-75.
26. van Strien, T., *Ice-cream consumption, tendency toward overeating, and personality.* Int J Eat Disord, 2000. **28**(4): p. 460-4.
27. Van Strien, T., et al., *The Stice model of overeating: tests in clinical and non-clinical samples.* Appetite, 2005. **45**(3): p. 205-13.
28. van Strien, T. and M.A. Ouwens, *Counterregulation in female obese emotional eaters: Schachter, Goldman, and Gordon's (1968) test of psychosomatic theory revisited.* Eat Behav, 2003. **3**(4): p. 329-40.
29. Daubenmier, J., et al., *Mindfulness Intervention for Stress Eating to Reduce Cortisol and Abdominal Fat among Overweight and Obese Women: An Exploratory Randomized Controlled Study.* Journal of obesity, 2011. **2011**.
30. Kristeller, J.L. and R.Q. Wolever, *Mindfulness-based eating awareness training for treating binge eating disorder: the conceptual foundation.* Eating disorders, 2010. **19**(1): p. 49-61.
31. Craig, J., *Meal replacement shakes and nutrition bars: Do they help individuals with diabetes lose weight?* Diabetes Spectrum, 2013. **26**(3): p. 179-182.
32. Heymsfield, S., et al., *Weight management using a meal replacement strategy: meta and pooling analysis from six studies.* International journal of obesity, 2003. **27**(5): p. 537-549.
33. Fry, J.P. and R.A. Neff, *Periodic prompts and reminders in health promotion and health behavior interventions: systematic review.* Journal of medical Internet research, 2009. **11**(2).
34. Baumeister, R.F. and K.D. Vohs, *Self-Regulation, Ego Depletion, and Motivation.* Social and Personality Psychology Compass, 2007. **1**(1): p. 115-128.
35. Chow, C.K., et al., *Effect of Lifestyle-Focused Text Messaging on Risk Factor Modification in Patients With Coronary Heart Disease: A Randomized Clinical Trial.* JAMA, 2015. **314**(12): p. 1255-1263.
36. Redfern, J., et al., *Development of a set of mobile phone text messages designed for prevention of recurrent cardiovascular events.* European journal of preventive cardiology, 2014. **21**(4): p. 492-499.