Effects of peppermint oil (Mentha piperita L.) on cardiometabolic and other health related outcomes: a parallel placebo randomized controlled trial

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Abstract

Background: There is growing speculation that peppermint may target the mechanisms central to cardiometabolic pathophysiology, though there has yet to be any randomized interventions, examining the efficacy of peppermint supplementation on cardiometabolic outcomes. This trial aimed to examine the effect of peppermint supplementation on cardiometabolic and other health indices following a 20-day supplementation period. Methods: A randomized, placebo-controlled parallel study design was adopted (NCT05071833). Thirty-six healthy adults were

assigned into either peppermint or placebo trial arms, of which they drank 50 μL of either peppermint or peppermint flavoured placebo, diluted in 100 mL of water twice per day for 20 days. Participants were blinded to their trial arm assignment, lead investigators and those analyzing the data were blinded until the data were analyzed and those involved in collecting the data were aware of trial arm allocation. The primary outcome was systolic blood pressure, and secondary measurements included anthropometric, energy expenditure, substrate oxidation, blood lipid, diastolic blood pressure/resting heart rate, psychological wellbeing, and sleep efficacy. All measurements were obtained at baseline and after the 20-day intervention period. Results: There were significantly greater reductions in the primary outcome (-4.53mmHg (95% CI = -8.39 - -0.66) d=-0.81) and in triglycerides (-0.30mmol/L (95% CI = -0.52 - -0.08) d=-0.92) in the peppermint group compared to placebo. Furthermore, both state (-5.43 (95% CI = -11.33 - -0.56) d=-0.73) and trait (-5.18 (95% CI = -10.76 - -0.40) d=-0.74) anxiety indices improved statistically in the peppermint arm compared to placebo. No other statistically significant findings were observed. Conclusion: As both hypertension and high triglyceride levels are important parameters for the aetiology and severity of cardiometabolic disease, this trial indicates that twice daily peppermint supplementation (50μL) may represent an effective means to prophylactically enhance cardiometabolic health. Furthermore, given the negative effects of anxiety on health-related quality of life and psychological wellbeing, peppermint may also be effective in improving both state and trait anxiety.

Keywords: peppermint; cardiovascular disease; blood pressure; metabolic health

1. Introduction

Cardiometabolic disease encompasses a cluster of cardiovascular and metabolic abnormalities, including insulin resistance, hypertension, atherogenic dyslipidemia, low high-density lipoproteins (HDL), high triglycerides, high adiposity, reduced oxidation of lipids, high body

mass index, large waist to hip ratio, atherosclerosis and poor glucose regulation [1, 2]. Globally, the incidence of these aforementioned abnormalities is expanding rapidly [3]. Cardiometabolic disease is recognized as the predominant cause of global mortality, associated with significant global healthcare utilization and expenditure [4].

Pharmaceutical intervention is the predominant treatment approach for cardiometabolic disease, and angiotensin-converting enzyme inhibitors, betablockers, calcium antagonists, diuretics, and lipid-lowering therapies are the most commonly adopted approaches [5, 6]. However, whist these medicines are unequivocally effective for the treatment of cardiometabolic disease, their long-term efficacy has yet to be established [7], and substantial adverse effects, remain commonplace [8]. These side effects, in addition to global overreliance of daily prescription medication [9], suggest that natural cost-effective approaches are necessary for the management of cardiometabolic disease [10].

Dietary practices are considered one of the principal approaches for non-pharmaceutical prevention and management of cardiometabolic disease [11]. However, maintaining effective nutritional patterns have been shown to be difficult to accomplish [12]; making dietary supplementation a potentially appealing treatment and prevention modality [10]. Importantly, medicinal plants have received considerable attention in the treatment of risk factors for the development of cardiometabolic disease [13]. Peppermint (Mentha piperita L.) is a recurrent flowering plant that cultivates predominantly in western Europe and North America. Peppermint itself is a hybrid amalgamation of both spearmint (Mentha Spicata) and water mint (Mentha Aquatica). The peppermint plant contains a diverse chemical profile, including menthol, flavonoids, menthone, and menthyl acetate [14]. Peppermint possesses a broad range of biological activities, including digestive, choleretic, carminative, antiseptic, antibacterial, antiviral, antispasmodic, antioxidant, anti-inflammatory, myorelaxant,

expectorant, analgesic, tonic, and vasodilatory properties [15, 16], and has importantly been shown through toxicology analyses to be safe for ingestion [17].

Importantly, owing specifically to its antioxidant, anti-inflammatory, and vasodilatory properties, there is growing speculation that peppermint ingestion may target the mechanisms central to cardiometabolic pathophysiology, and thus confer significant cardiometabolic benefits [18]. To date, very limited analyses have investigated the influence of peppermint supplementation on cardiometabolic outcomes. Barbalho et al. [19] showed that twice daily supplementation of peppermint (20 g of peppermint leaves in 200 mL water) for 30-days, mediated significant reductions in both low-density lipoproteins (LDL) cholesterol and systolic blood pressure. Meamarbashi & Rajabi, [20] revealed that a once daily peppermint oil ingestion (0.05 mL in 500 mL water) for 10-days produced significant reductions in systolic blood pressure, diastolic blood pressure and resting heart rate. However, neither of the aforementioned investigations featured a control group, meaning that it cannot be conclusively determined that the improvements were decisively attributable to peppermint supplementation, as opposed to other external mechanisms.

1.1 Rationale

At the current time, there has yet to be any randomized intervention studies, comparatively examining the efficacy of supplementation using peppermint oil on cardiometabolic outcomes. Therefore, with preliminary evidence suggesting a positive effect of peppermint ingestion, further placebo-controlled investigations concerning its influence on cardiometabolic outcomes may be of both practical and clinical relevance.

1.2 Aim

The aim of the current study was to investigate the influence of 20-days of twice daily peppermint oil supplementation on cardiometabolic and other health related indices in healthy adults compared to placebo. The primary objective of this randomized trial is to examine the influence of peppermint supplementation on systolic blood pressure relative to placebo. Its secondary objectives are to determine whether peppermint supplementation influences on other risk factors associated with and as a function of cardiometabolic disease.

1.3 Hypotheses

In relation to the primary outcome, it is expected that supplementation with peppermint will mediate significant reductions in systolic blood pressure compared to placebo. Furthermore, for the secondary outcomes, peppermint will produce improvements in cardiometabolic, and other health related parameters compared to placebo.

2. Methods

2.1 Study design

This investigation represents a 20-day parallel, randomized placebo-controlled trial (Figure 1). Participants were tested on two occasions i.e. baseline and 20-days and randomized by a computer program (Random Allocation Software) to either the peppermint or placebo groups. Participants were blinded to their trial arm assignment, lead investigators and those analyzing the data were blinded until the data were analyzed and those involved in collecting the data were aware of trial arm allocation. The 20-day supplementation period was adopted in accordance with Sinclair et al. [21], and the protocol designed according to the updated guidelines for reporting parallel group randomized trials [22]. All experimental testing took place in the morning in a ≥10 h fasted state, with participants having avoided strenuous exercise, alcohol, and nutritional supplements for 24 h and caffeine for 12 h prior to data collection [21]. The study was registered prospectively (NCT05071833) and approved by an institutional ethical review board (HEALTH 0016).

2.1.1 Inclusion criteria:

Inclusion criteria were, the capacity to give informed consent, 18 years of age and above, non-smoker and a BMI < 30.

2.1.2 Exclusion criteria:

Exclusion criteria were, pregnancy, 65 years of age and above, diabetes or any other metabolic/uncontrolled hypertensive conditions, allergy to peppermint, habitual consumption of peppermint products and not regularly taking medication or antioxidant supplements.

2.2 Sample size

Power calculations were performed for the primary outcome variable, i.e., the between-groups change in systolic blood pressure. This showed that a total sample size of 36 was necessary to provide 80% power to detect a minimally important clinical difference (MCID) of 6 mmHg between groups [23], accounting for a loss to follow up rate of 10%.

2.3 Participants

The present study was conducted at the University of Central Lancashire in the United Kingdom. Both males and females of diverse race and ethnicities who lived in Preston and the surrounding areas were recruited. Recruiting materials were placed in the local community, public bulletin boards, as well as via social media. Participants were recruited during November 2021–July 2022 and formal data collection took place from January 2022-August 2022. Participants attended an eligibility, enrolment, and familiarization session prior to the commencement of formal data collection at the University of Central Lancashire. All participants provided informed consent in written form and completed a Par-Q screening form

before taking part, in compliance with principles outlined in the declaration of Helsinki and the Oviedo Convention.

2.4 Dietary intervention

After the conclusion of their baseline data collection session, participants were provided with either peppermint oil (*Mentha piperita L.*) or placebo. Participants in the peppermint group were required to consume 50 µL of peppermint oil (100% essential oil; Piping Rock Health, UK) which was diluted in 100 mL of water twice daily using a dropper: once in the morning and again in the evening [19]. Those in the placebo group consumed 50 µL of peppermint cordial (Schweppes, Schweppes Geneva) which they diluted into 100 mL of water twice daily using a dropper. This approach to placebo preparation has been shown by previous intervention trials to provide an effective blinding strategy [24]. All supplementation/ placebo was kept refrigerated throughout the 20 days.

In accordance with Sinclair et al. [21], participants were encouraged to maintain their habitual diet and exercise routines and asked to refrain from consuming any multivitamin or antioxidant supplements. For their post-intervention data collection session, in order to examine blinding efficacy, all participants were asked whether they felt that they had been allocated to the peppermint or placebo group. In both trial arms, loss to follow up was monitored as were any adverse events. For their post-intervention data collection session, all participants were also asked to return any unused supplementation to determine their % compliance.

2.5 Data collection

2.5.1 Anthropometric measurements

Anthropometric measures of mass (kg) and stature (m) (without shoes) were used to calculate the body mass index (BMI) (kg/m^2). Stature was measured using a stadiometer (Seca, Hamburg, Germany) and mass using weighing scales (Seca 875, Hamburg, Germany). In addition, body composition was examined using a phase-sensitive multifrequency bioelectrical impedance analysis device (Seca mBCA 515, Hamburg, Germany) [25], allowing percentage body fat (%) and fat mass (kg) to be quantified. Finally, waist circumference was measured at the midway point between the inferior margin of the last rib and the iliac crest and hip circumference around the pelvis at the point of maximum protrusion of the buttocks, without compressing the soft tissues [26], allowing the waist-to-hip ratio to be quantified. Anthropometric measures were obtained on three occasions and the mean value extracted for analysis.

2.5.2 Energy expenditure and substrate oxidation

Respiratory gases were collected using a gas analysis system (MetaLyser 3B system, Cortex Biophysic, Leipzig, Germany). The experimental laboratory was maintained using an air-conditioning system at a fixed ambient temperature of 20 °C. To quantify resting energy expenditure and substrate oxidation, participants laid supine for a period of 20 min, and data were extracted and averaged over the final 17 min [27]. Resting fat and carbohydrate oxidation rates (g/min) were quantified using stoichiometric formulae [28] (Equations (1) and (2)), assuming negligible protein utilization. To quantify resting metabolic rate (RMR) (kcal/day) the formula of Weir, [29] was adopted (Equation (3)).

$$\textbf{Carbohydrate (g/min)} = (4.55 \times VCO_2) - (3.21 \times VO_2) \quad (1)$$

$$\textbf{Fat (g/min)} = (1.67 \times VO_2) - (1.67 \times VCO_2) \quad (2)$$

$$\textbf{RMR (kcal/day)} = [(3.941 \times VO_2) + (1.1106 \times VCO_2)] \times 1440 \quad (3)$$

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199 2.5.3 Blood lipid testing

200 Capillary blood samples were also collected via finger-prick using a disposable lancet after
201 cleaning with a 70% ethanol wipe. Capillary triglyceride, total cholesterol, and glucose levels
202 (mmol/L) were immediately obtained using three handheld analyzers (MulticareIn, Multicare
203 Medical, Arezzo, Italy) and capillary hemoglobin levels (g/L) using a single handheld analyzer
204 (HemoCue, Ängelholm, Sweden). From these outcomes, LDL cholesterol (mmol/L) was firstly
205 quantified using the Anandaraja et al. [30] formula with total cholesterol and triglycerides as
206 inputs. In addition, high-density lipoprotein (HDL) cholesterol (mmol/L) was also calculated
207 by re-arranging the Chen et al. [31] equation to make HDL the product of the formulae. Both
208 of these approaches have been shown to have excellent similarity to their associated lipoprotein
209 values examined using immunoassay techniques r = 0.948 − 0.970 32. The ratios between total
210 and HDL cholesterol and between LDL and HDL cholesterol levels were also determined in
211 accordance with Millán et al. [32]. Finally, the triglycerides and glucose index (TyG index)
212 was calculated as the natural logarithm of the product of plasma glucose and triglycerides
213 divided by two [33].

214

215 2.5.4 Blood pressure and resting heart rate

216 Blood pressure (mmHg) and resting heart rate (beats/min) measurements were undertaken in
217 an up-right seated position at the end of the above-described resting energy expenditure test.
218 Both peripheral measures of systolic and diastolic blood pressure and resting heart rate were
219 measured via a non-invasive, automated blood pressure monitor (OMRON M2, Kyoto, Japan),
220 adhering to the recommendations specified by the European Society of Hypertension [34].
221 Three readings were undertaken, each separated by a period of 1 min [35], and the mean of the
222 last 2 readings used for analysis.

223

224 2.5.5 Questionnaires

225 Sleep quality is diminished in patients with cardiometabolic disease [36], therefore general
226 sleep quality was examined using the Pittsburgh sleep quality index (PSQI) [37], daytime
227 sleepiness using the Epworth Sleepiness Scale [44] and symptoms of insomnolence via the
228 Insomnia Severity Index [38]. These questionnaires were utilized co-operatively to provide a
229 collective representation of sleep efficacy. The PSQI measure consists of 19 individual items,
230 creating 7 components (subjective sleep quality, sleep latency, sleep duration, sleep efficiency,
231 sleep disturbance, use of sleep medication and daytime dysfunction) that produce one global
232 score ranging from 0 to 21, with lower scores denoting a healthier sleep quality. The Epworth
233 Sleepiness Scale a list of 8 scenarios in which tendency to become sleepy is rated on a scale of
234 0-3. The total score is the sum of these responses and ranges from 0 to 24, with higher scores
235 indicating increased sleepiness. The Insomnia Severity Index features 7 questions in which
236 sleep difficulty is rated on a scale of 0-4. The total score is the sum of these responses and
237 ranges from 0 to 28, with higher scores indicating greater sleep difficulty.

238

239 Furthermore, as psychological wellbeing is lower in those with cardiometabolic disease [39],
240 general psychological wellbeing was examined using the COOP WONCA questionnaire [40],
241 depressive symptoms using the Beck Depression Inventory [41], and state/trait anxiety with
242 the State Trait Anxiety Inventory (STAI) [42]. Once again, these scales were utilized
243 conjunctively to provide a collective depiction of psychological wellbeing. The COOP
244 WONCA questionnaire is comprised of 6 scales (physical fitness, feelings, daily activities,
245 social activities, change in health and overall health) designed to measure functional health
246 status on a scale ranging from 1 to 5. The final score is the mean of the 6 scales, with a higher
247 score indicating reduced functional health. The Beck Depression Inventory is a 21-item

questionnaire in which depressive symptoms are rated on a scale of 0-3. The total score is the sum of these responses and ranges from 0 to 63, with higher scores indicating greater depression. Finally, the State Trait Anxiety Inventory uses 20 items to assess trait anxiety and 20 to examine state anxiety, rated on a scale of 0-4. The total score for both trait anxiety and state anxiety is the sum of these responses for each component and scores range from 20 to 80, with higher scores denoting greater anxiety.

2.6 Statistical analysis

All continuous experimental variables are presented as mean and standard deviations. Comparisons between the two groups in % compliance were undertaken using linear mixed effects models, with group modelled as a fixed factor and random intercepts by participants. All analyses of the intervention-based data were performed on an intention to treat basis. To determine the effects of the intervention on all of the outcome measures, differences in the changes from baseline to 20-days between the two groups were examined using linear mixed effects models with group modelled as a fixed factor and random intercepts by participants adopted. For linear mixed models the mean difference between groups in change from baseline to 20-days (*b*), and 95% confidence intervals of the difference are presented. Effect sizes were calculated for the changes from baseline to 20-days between the two groups, using Cohen's *d*, in accordance with McGough, & Faraone, [43]. Cohen's *d* values were interpreted as 0.2 = small, 0.5 = medium, and 0.8 = large [44].

Blinding efficacy was examined using a one-way chi-squared (X^2) goodness of fit test. Finally, changes from baseline to 20-days in the experimental parameters were used to create binary variables i.e. improve/ didn't improve for each participant. Pearson chi-square tests of independence were also used to undertake bivariate cross-tabulation comparisons between the two trial groups, specifically to test differences in the number of participants who exhibited

improvements in the experimental outcomes. Probability values for all chi-square analyses in this trial were calculated using Monte-Carlo simulation. All analyses were conducted using SPSS v27 (IBM, SPSS), and statistical significance for all analyses was accepted as the P≤0.05 level.

3. Results

3.1 Baseline characteristics

Characteristics of participants are presented in Table 1.

@@@TABLE 1 NEAR HERE@@@

3.2 Loss to follow up, adverse events & compliance

Total loss to follow up in each group were peppermint (n=0) and placebo (n=1), and number of adverse effects were peppermint (n=0), placebo (n=0) (Figure 1). There were no significant differences (P=0.382) in compliance between peppermint (90.03±6.34%), placebo (88.34±6.34%) groups.

@@@FIGURE 1 NEAR HERE@@@

3.3 Blinding efficacy

Of the 35 participants that completed the trial 53% (n=19) correctly identified their designated trial arm, the chi-squared test was non-significant ($X^2_{(1)} = 0.26$, P=0.612) indicating that an effective blinding strategy was adopted.

3.4 Anthropometric measurements

No statistically significant differences (P>0.05) in anthropometric parameters were found (Table 2).

@@@TABLE 2 NEAR HERE@@@

3.5 Energy expenditure and substrate oxidation

No statistically significant differences (P>0.05) in energy expenditure and substrate oxidation parameters were found (Table 2).

3.6 Blood lipids

Improvements in triglycerides and TyG index were significantly greater in the peppermint arm compared to placebo (Table 2). For triglycerides the chi-squared test was significant ($X^2_{(1)}$ = 6.42, P=0.011) and 73% and 33% of participants exhibited improvements in the peppermint and placebo groups respectively. No other statistically significant differences (P>0.05) in blood lipid values were found.

3.7 Blood pressure and resting heart rate

Improvements in systolic and diastolic blood pressure were significantly greater in the peppermint arm compared to placebo (Table 2). For systolic blood pressure the chi-squared test was significant ($X^2_{(1)}$ = 5.11, P=0.024) and 83% and 44% of participants exhibited improvements in the peppermint and placebo groups respectively. No other statistically significant differences (P>0.05) in blood pressure and resting heart rate values were found.

3.8 Questionnaires

Improvements in STAI trait and STAI state were significantly greater in the peppermint arm compared to placebo (Table 2). No other statistically significant differences (P>0.05) in questionnaire values were found.

4. Discussion

The current study aimed to investigate the influence of 20-days of twice daily peppermint supplementation on cardiometabolic and other health-related indices in healthy adults compared to placebo. To date, this represents the first investigation to explore the effects of peppermint on cardiometabolic and other health-related indices using a parallel placebo-randomized controlled trial. The primary aim of this trial was to determine whether peppermint supplementation improved systolic blood pressure compared to placebo, whereas the

secondary aim(s) were to explore the effects of peppermint on other risk factors for cardiometabolic disease.

In relation to the primary outcome, in agreement with our hypothesis and the findings of both Barbalho et al. [19] and Meamarbashi & Rajabi [20] linear mixed model and chi-square analyses importantly showed that significantly greater improvements in systolic blood pressure were evident in the peppermint group in relation to placebo with a large effect size. It is proposed that this observation was mediated due to the presence of menthol in the peppermint supplementation. As an active agonist of transient receptor potential melastatin 8 (TRPM8) channels present in vascular smooth muscle [45], the vasodilatory effect of menthol mediated as a function of opening of vascular TRPM8 channels. This allows calcium entry into the endothelium [46], which stimulates nitric oxide production [47] and hyperpolarization of vascular smooth muscle cells [48]. Arterial hypertension is the most common preventable risk factor for cardiometabolic disease [49] and represents the greatest single risk factor contributing to the global burden of disease and to global all-cause mortality [50]. Therefore, the observations from this trial appear to have considerable clinical relevance and suggest that peppermint supplementation may be important in the management of hypertension.

In addition to the primary outcome, in further support of our hypotheses the findings also confirmed that both triglycerides and the TyG index were significantly attenuated with large effect sizes in the peppermint group in relation to placebo. As no changes in glucose were evident, it is clear that reductions in total TyG index were mediated as a function of the corresponding attenuation in triglycerides values. Previous analyses have shown that peppermint possesses anti-lipidemic benefits [11], although this is the first investigation to show improvements in triglycerides following peppermint supplementation. The mechanism responsible for this observation has not been explored in human participants, however animal models have shown that the antioxidant properties of peppermint decrease lipid peroxidation

in the plasma and tissues [15]. Furthermore, animal models have shown that peppermint oil raises hepatic glutathione level, enhance liver function and antioxidant activity which has also been proposed as an underpin mechanism in the hypolipidemic effects of peppermint [51]. Taking into account its positive influence on triglycerides; it is important that future investigations seek to explore and utilize the mechanistic pathways of peppermint supplementation in order to further enhance health-related outcomes.

Regardless, elevated triglyceride concentrations contribute to increased risk of cardiometabolic disease [52], and whilst pharmaceutical agents are effective in the management of hypertriglyceridemia, they are associated with substantial side-effects [53] and high levels of global healthcare expenditure. As such, the findings from this investigation lend support to the concept that peppermint supplementation may be important in the preventative management of hypertriglyceridemia.

In addition to the improvements in physiological measures of blood pressure and triglycerides shown in the peppermint trial arm. The current investigation also importantly showed that this condition was able to mediate statistical improvements both state and trait anxiety indices with moderate effect sizes. This observation concurs with those of Abdelhalim et al. [54] who also found using a randomized controlled trial that peppermint produced significant improvements in anxiety. The mechanism responsible for the improvements in psychological wellbeing shown in the peppermint group is not currently known and requires further exploration. However, peppermint has been shown to attenuate the secretion of cortisol from the adrenal gland in animal models [55], which has been shown to be linked to the presence of anxiety in humans [56]. Regardless, the observations from the current trial indicate that peppermint supplementation may be important in improving both state and trait anxiety.

Overall, the current placebo randomized controlled trial was shown to be associated with a good level of blinding efficacy, higher compliance, a low number of adverse events and

a very low dropout rate. These observations, allied to the significant improvements in blood pressure, blood lipid and anxiety indices in the peppermint trial arm indicate that this supplement there appears to represent an effective means to enhance cardiometabolic and psychological wellbeing. However, as with any trial, this investigation is not without limitations. Firstly, whilst this study observed positive effects of peppermint supplementation on cardiometabolic and psychological parameters, it was beyond the scope of the measurements obtained within this trial to elucidate the mechanistic origins for these improvements. It is important therefore that future investigations seek to better understand and potentially utilize these mechanistic pathways of peppermint supplementation to further improve health-related outcomes. Furthermore, although the findings from this investigation indicate that peppermint supplementation may be an effective approach to prophylactically improve cardiometabolic disease risk, as participants in this trial were healthy, it remains unknown as to whether peppermint supplementation would mediate such improvements in patients with existing cardiometabolic disease. Therefore, it is essential that future randomized intervention trials seek to examine the efficacy of peppermint supplementation in pathological populations. Finally, although participants in both arms were instructed to maintain their habitual diet and exercise routines; as many of the experimental variables are influenced by exercise and nutritional status, that physical activity and nutritional intake were not monitored may serve as a limitation to this trial. Therefore, subsequent randomized interventions may seek to quantify the effects of peppermint supplementation whilst at the same time physical activity throughout the intervention period via continuous actigraphy.

5. Conclusion

The current randomized controlled trial aimed to investigate the influence of peppermint supplementation on cardiometabolic, and other health related indices compared to placebo. The current trial supported our primary hypothesis that ingestion of twice daily solution of

peppermint oil (50 μL) is able to mediate improved systolic blood pressure compared to placebo and also secondary predictions concerning triglyceride concentrations and anxiety indices. As both hypertension and high triglyceride levels are important parameters for the aetiology and severity of cardiometabolic disease, this trial indicates that peppermint supplementation may represent an effective means to prophylactically enhance cardiometabolic health. Furthermore, given the negative effects of anxiety on health-related quality of life and psychological wellbeing, peppermint may also be effective in improving both state and trait anxiety. Future randomized intervention trials should now seek to explore the efficacy of peppermint supplementation in pathological populations with established cardiometabolic abnormalities at baseline.

Funding

This trial received no funding.

CRediT authorship contribution statement

Jonathan Sinclair: Conceptualization, Formal analysis, Methodology, Supervision, Writing – review & editing, Heidi Murray: Data curation, Formal analysis, Methodology, Vicki Smith: Data curation, Formal analysis, Methodology, Nevin Tom: Data curation, Formal analysis, Methodology, Tessy Clarence Cruz: Data curation, Formal analysis, Methodology, Paul John Taylor: Formal analysis, Methodology, Supervision, Writing – review & editing, Stephanie Dillon: Formal analysis, Methodology, Supervision, Writing – review & editing, Gareth Shadwell: Formal analysis, Methodology, Supervision, Writing – review & editing, Bobbie Butters: Formal analysis, Methodology, Supervision, Writing – review & editing, Lindsay Bottoms: Conceptualization, Formal analysis, Methodology, Supervision, Writing – review & editing.

Disclosures

All authors of this study confirm there are no conflicts of interest to declare. This was an investigator-initiated study, and the design, management, and analysis of this trial was completely independent of anyone other than the authors.

References

1. Ahmed, C. S., Jiang, H., Chen, J. Y., & Lin, Y. H. (2018). Traffic-related particulate matter and cardiometabolic syndrome: A review. Atmosphere, 9(9), 336.
2. Montes, A., Perez-Bey, A., Corral-Pérez, J., Velázquez-Díaz, D., Opazo-Díaz, E., Fernandez-Santos, J. R., & Ponce-González, J. G. (2021). Maximal fat oxidation capacity is associated with cardiometabolic risk factors in healthy young adults. European Journal of Sport Science, 21(6), 907-917.
3. Harding, S., Silva, M. J., Molaodi, O. R., Enayat, Z. E., Cassidy, A., Karamanos, A., & Cruickshank, J. K. (2016). Longitudinal study of cardiometabolic risk from early adolescence to early adulthood in an ethnically diverse cohort. BMJ Open, 6(12), e013221.
4. Danaei, G., Lu, Y., Singh, G. M., Carnahan, E., Stevens, G. A., Cowan, M. J., & Kobayashi, J. (2014). Cardiovascular disease, chronic kidney disease, and diabetes mortality burden of cardiometabolic risk factors from 1980 to 2010: a comparative risk assessment. Lancet Diabetes & Endocrinology, 2(8): 634–647.
5. Morgan, T. O., Anderson, A. I., & MacInnis, R. J. (2001). ACE inhibitors, beta-blockers, calcium blockers, and diuretics for the control of systolic hypertension. A

6. Binesh, M. M., & Adeli, K. H. (2010). Pharmacological management of metabolic syndrome and its lipid complications. Daru. 2010; 18(3): 146–154.merican Journal of Hypertension, 14(3), 241-247.
7. Canoy, D., Copland, E., Nazarzadeh, M., Ramakrishnan, R., Pinho-Gomes, A. C., Salam, A., ... & Rahimi, K. (2022). Antihypertensive drug effects on long-term blood pressure: an individual-level data meta-analysis of randomised clinical trials. Heart, 108(16), 1281-1289.
8. Nguyen, Q., Dominguez, J., Nguyen, L., & Gullapalli, N. (2010). Hypertension management: an update. American health & drug Benefits, 3(1), 47–56.
9. Kallivayalil, R. A. (2008). Are we over-dependent on pharmacotherapy?. Indian Journal of Psychiatry, 50(1), 7.
10. Smith, S. S., Oei, T. P., Douglas, J. A., Brown, I., Jorgensen, G., & Andrews, J. (2008). Confirmatory factor analysis of the Epworth Sleepiness Scale (ESS) in patients with obstructive sleep apnoea. Sleep Medicine, 9(7), 739-744.
11. Chiva-Blanch, G., & Visioli, F. (2012). Polyphenols and health: Moving beyond antioxidants. Journal of Berry Research, 2(2), 63-71.
12. Varkevisser, R. D. M., Van Stralen, M. M., Kroeze, W., Ket, J. C. F., & Steenhuis, I. H. M. (2019). Determinants of weight loss maintenance: a systematic review. Obesity reviews, 20(2), 171-211.
13. Cicero, A. F., Fogacci, F., & Colletti, A. (2017). Food and plant bioactives for reducing cardiometabolic disease risk: an evidence based approach. Food & function, 8(6), 2076-2088.
14. Meamarbashi, A. (2014). Instant effects of peppermint essential oil on the physiological parameters and exercise performance. Avicenna Journal of Phytomedicine, 4(1), 72-78.

15. Duke, J.A (2002). Bogenschutz-Godwin MJ, duCellier J, Duke PAK. Handbook of medicinal herbs. Boca Raton: CRC Press; 2002. pp. 562–564.
16. McKay, D. L., & Blumberg, J. B. (2006). A review of the bioactivity and potential health benefits of peppermint tea (Mentha piperita L.). Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives, 20(8), 619-633.
17. Nair, B. (2001). Final report on the safety assessment of Mentha Piperita (Peppermint) Oil, Mentha Piperita (Peppermint) Leaf Extract, Mentha Piperita (Peppermint) Leaf, and Mentha Piperita (Peppermint) Leaf Water. International Journal of Toxicology, 20, 61-73.
18. Minihane, A. M., Vinoy, S., Russell, W. R., Baka, A., Roche, H. M., Tuohy, K. M., & Calder, P. C. (2015). Low-grade inflammation, diet composition and health: current research evidence and its translation. British Journal of Nutrition, 114(7), 999-1012.
19. Barbalho, S. M., Machado, F. M. V. F., Oshiiwa, M., Abreu, M., Guiger, E. L., Tomazela, P., & Goulart, R. A. (2011). Investigation of the effects of peppermint (Mentha piperita) on the biochemical and anthropometric profile of university students. Food Science and Technology, 31, 584-588.
20. Meamarbashi, A., & Rajabi, A. (2013). The effects of peppermint on exercise performance. Journal of the International Society of Sports Nutrition, 10(1), 1-6.
21. Sinclair, J., Bottoms, L., Dillon, S., Allan, R., Shadwell, G., & Butters, B. (2022). Effects of Montmorency Tart Cherry and Blueberry Juice on Cardiometabolic and Other Health-Related Outcomes: A Three-Arm Placebo Randomized Controlled Trial. International Journal of Environmental Research and Public Health, 19(9), 5317.
22. Moher, D., Hopewell, S., Schulz, K. F., Montori, V., Gøtzsche, P. C., Devereaux, P. J., & Altman, D. G. (2012). CONSORT 2010 explanation and elaboration: updated

494 guidelines for reporting parallel group randomised trials. International Journal of
495 Surgery, 10(1), 28-55.
496 23. Makai, P., IntHout, J., Deinum, J., Jenniskens, K., & Wilt, G. J. V. D. (2017). A network
497 meta-analysis of clinical management strategies for treatment-resistant hypertension:
498 making optimal use of the evidence. Journal of General Internal Medicine, 32, 921-930.
499 24. Dillon, S. A., Walker, M., & Sinclair, J. K. (2016). The Effect of Peppermint oil on
500 Strength Performance in Resistance Trained Men. Medicine & Science in Sports &
501 Exercise, 48(5S), 245-246.
502 25. Bosy-Westphal, A., Jensen, B., Braun, W., Pourhassan, M., Gallagher, D., & Müller,
503 M. J. (2017). Quantification of whole-body and segmental skeletal muscle mass using
504 phase-sensitive 8-electrode medical bioelectrical impedance devices. European Journal
505 of Clinical Nutrition, 71(9), 1061-1067.
506 26. Czernichow, S., Kengne, A. P., Huxley, R. R., Batty, G. D., De Galan, B., Grobbee, D.,
507 & ADVANCE Collaborative Group. (2011). Comparison of waist-to-hip ratio and other
508 obesity indices as predictors of cardiovascular disease risk in people with type-2
509 diabetes: a prospective cohort study from ADVANCE. European Journal of Preventive
510 Cardiology, 18(2), 312-319.
511 27. Kelly, B., King, J. A., Goerlach, J., & Nimmo, M. A. (2013). The impact of high-
512 intensity intermittent exercise on resting metabolic rate in healthy males. European
513 Journal of Applied Physiology, 113(12), 3039-3047.
514 28. Frayn, K. N. (1983). Calculation of substrate oxidation rates in vivo from gaseous
515 exchange. Journal of Applied Physiology, 55(2), 628-634.
516 29. Weir, J. D. V. (1949). New methods for calculating metabolic rate with special
517 reference to protein metabolism. The Journal of physiology, 109(1-2), 1.

30. Anandaraja, S., Narang, R., Godeswar, R., Laksmy, R., & Talwar, K. K. (2005). Low-density lipoprotein cholesterol estimation by a new formula in Indian population. International Journal of Cardiology, 102(1), 117-120.
31. Chen, Y., Zhang, X., Pan, B., Jin, X., Yao, H., Chen, B., & Chen, H. (2010). A modified formula for calculating low-density lipoprotein cholesterol values. Lipids in Health and Disease, 9(1), 1-5.
32. Millán, J., Pintó, X., Muñoz, A., Zúñiga, M., Rubiés-Prat, J., Pallardo, L. F., & Pedro-Botet, J. (2009). Lipoprotein ratios: physiological significance and clinical usefulness in cardiovascular prevention. Vascular health and risk management, 5, 757–765.
33. Romero, F., Simental-Mendía, L. E., González-Ortiz, M., Martínez-Abundis, E., Ramos-Zavala, M. G., Hernández-González, S. O., & Rodríguez-Morán, M. (2010). The product of triglycerides and glucose, a simple measure of insulin sensitivity. Comparison with the euglycemic-hyperinsulinemic clamp. The Journal of Clinical Endocrinology & Metabolism, 95(7), 3347-3351.
34. O'brien, E., Asmar, R., Beilin, L., Imai, Y., Mallion, J. M., Mancia, G., & European Society of Hypertension Working Group on Blood Pressure Monitoring. (2003). European Society of Hypertension recommendations for conventional, ambulatory and home blood pressure measurement. Journal of Hypertension, 21(5), 821-848.
35. Pickering, T. G., Hall, J. E., Appel, L. J., Falkner, B. E., Graves, J., Hill, M. N., & Roccella, E. J. (2005). Recommendations for blood pressure measurement in humans and experimental animals: part 1: blood pressure measurement in humans: a statement for professionals from the Subcommittee of Professional and Public Education of the American Heart Association Council on High Blood Pressure Research. Hypertension, 45(1), 142-161.

36. Matricciani, L., Paquet, C., Fraysse, F., Grobler, A., Wang, Y., Baur, L., & Olds, T. (2021). Sleep and cardiometabolic risk: a cluster analysis of actigraphy-derived sleep profiles in adults and children. Sleep, 44(7), zsab014.
37. Buysse, D. J., Reynolds III, C. F., Monk, T. H., Berman, S. R., & Kupfer, D. J. (1989). The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry research, 28(2), 193-213.
38. Morin, C. M., Belleville, G., Bélanger, L., & Ivers, H. (2011). The Insomnia Severity Index: psychometric indicators to detect insomnia cases and evaluate treatment response. Sleep, 34(5), 601-608.
39. Gheshlagh, R. G., Parizad, N., & Sayehmiri, K. (2016). The relationship between depression and metabolic syndrome: systematic review and meta-analysis study. Iranian Red Crescent Medical Journal, 18(6), e26523.
40. Bentsen, B. G., Natvig, B., & Winnem, M. (1999). Questions you didn't ask? COOP/WONCA Charts in clinical work and research. Family practice, 16(2), 190-195.
41. Wang, Y. P., & Gorenstein, C. (2013). Psychometric properties of the Beck Depression Inventory-II: a comprehensive review. Brazilian Journal of Psychiatry, 35, 416-431.
42. Spielberger, C.D. Gorsuch, R.L. Lushene, R. Vagg, P.R. Jacobs, G.A. (1983). Manual for the State-Trait Anxiety Inventory; Consulting Psychologists Press: Palo Alto, CA, USA, 1983.
43. McGough, J. J., & Faraone, S. V. (2009). Estimating the size of treatment effects: moving beyond p values. Psychiatry (Edgmont), 6(10), 21-29.
44. Cohen, J. (1988). Statistical Power Analysis for the Behavioral Sciences (2nd ed.). Hillsdale, NJ: Lawrence Erlbaum Associates, Publishers.
45. Johnson, C. D., Melanaphy, D., Purse, A., Stokesberry, S. A., Dickson, P., & Zholos, A. V. (2009). Transient receptor potential melastatin 8 channel involvement in the

regulation of vascular tone. American Journal of Physiology-Heart and Circulatory Physiology, 296(6), 1868-1877.

46. Zholos, A., Johnson, C., Burdyga, T., & Melanaphy, D. (2011). TRPM channels in the vasculature. Transient Receptor Potential Channels, 707-729.
47. Cohen, R. A., & Vanhoutte, P. M. (1995). Endothelium-dependent hyperpolarization: beyond nitric oxide and cyclic GMP. Circulation, 92(11), 3337-3349.
48. Félétou, M., & Vanhoutte, P. M. (2009). EDHF: an update. Clinical Science, 117(4), 139-155.
49. Oparil S, Acelajado MC, Bakris GL, Berlowitz DR, Cífková R, Dominiczak AF, Grassi G, Jordan J, Poulter NR, Rodgers A, Whelton PK. (2018). Hypertension. Nature Reviews Disease Primers, 22 (4), 18014.
50. Forouzanfar, M. H., Liu, P., Roth, G. A., Ng, M., Biryukov, S., Marczak, L., & Murray, C. J. (2017). Global burden of hypertension and systolic blood pressure of at least 110 to 115 mm Hg, 1990-2015. JAMA, 317(2), 165-182.
51. Badal, R. M., Badal, D., Badal, P., Khare, A., Shrivastava, J., & Kumar, V. (2011). Pharmacological action of Mentha piperita on lipid profile in fructose-fed rats. Iranian Journal of Pharmaceutical Research: IJPR, 10(4), 843-848.
52. Yuan, G., Al-Shali, K. Z., & Hegele, R. A. (2007). Hypertriglyceridemia: its etiology, effects and treatment. CMAJ, 176(8), 1113-1120.
53. Marston, N. A., Giugliano, R. P., Im, K., Silverman, M. G., O'Donoghue, M. L., Wiviott, S. D., & Sabatine, M. S. (2019). Association between triglyceride lowering and reduction of cardiovascular risk across multiple lipid-lowering therapeutic classes: a systematic review and meta-regression analysis of randomized controlled trials. Circulation, 140(16), 1308-1317.

54. Abdelhalim, A. R. (2021). The effect of Mentha piperita L. on the mental health issues of university students: A pilot study. Journal of Pharmacy & Pharmacognosy Research, 9(1), 49-57

55. . Albishi, F. M., Albeshi, S. M., Alotaibi, K., Alhussain, N., Kamal, A., Sultan, A. A., & Aljishi, M. J. (2020). The Effect of Menthol on Anxiety and Related Behaviors in Mice. Bahrain Medical Bulletin 42(4):274-276.

56. Polk D. E., Cohen S., Doyle W. J., Skoner D. P., Kirschbaum C (2005). State and trait affect as predictors of salivary cortisol in healthy adults, Psychoneuroendocrinology, 2005, vol. 30 3(pg. 261-272)10.1016/j.psyneuen.2004.08.004

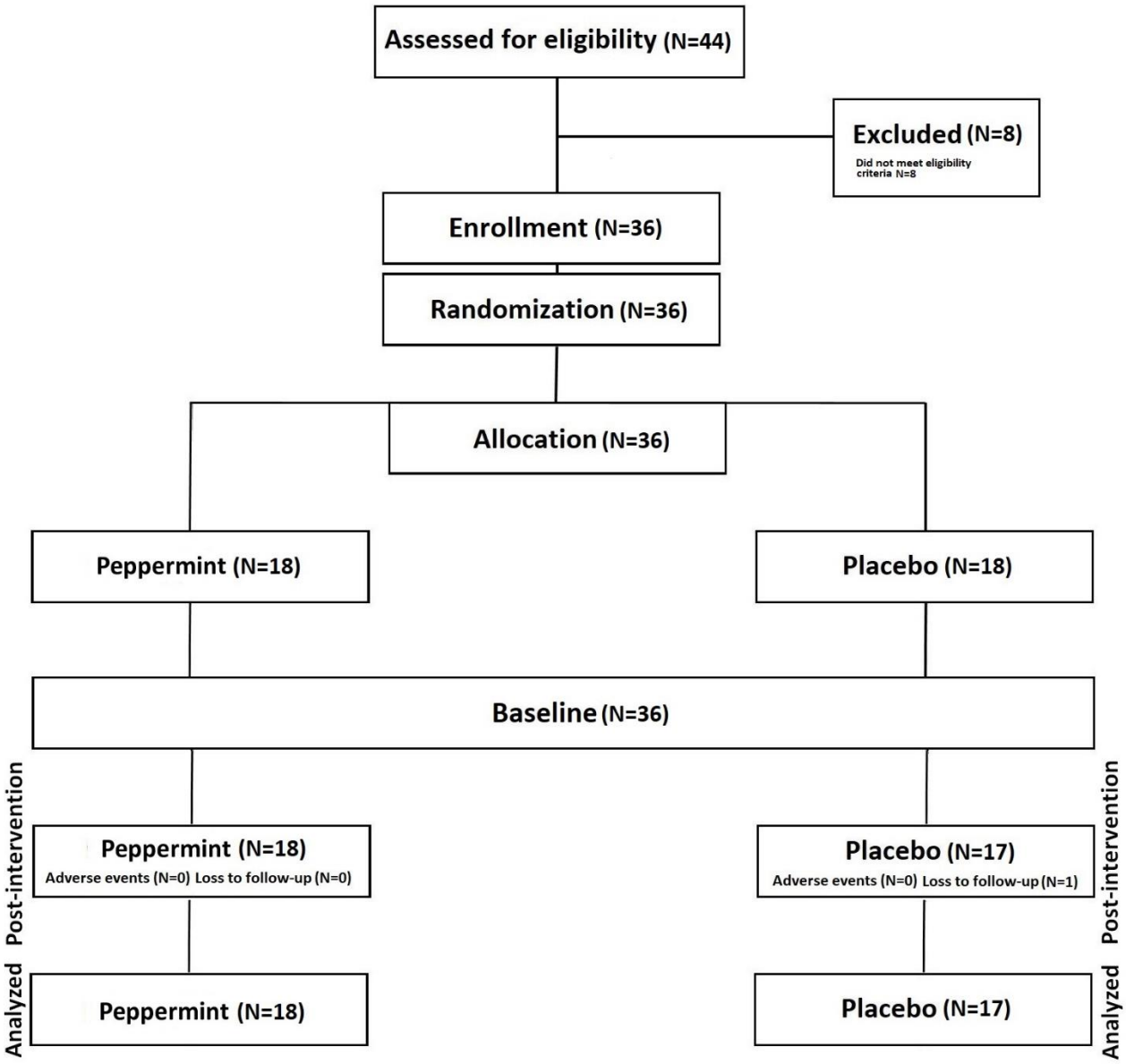

Figure 1: Consort diagram showing of participant flow throughout the study.

Table 1: Baseline characteristics (mean & SD) for both placebo and peppermint groups.

	All		Placebo		Peppermint	
	Mean	*SD*	**Mean**	*SD*	**Mean**	*SD*
Age (years)	28.51	9.80	26.59	5.44	30.33	12.53
Mass (kg)	69.49	9.82	71.65	9.96	67.46	9.50
Stature (m)	1.69	0.09	1.70	0.09	1.67	0.09
BMI (kg/m^2)	24.32	2.35	24.61	1.91	24.04	2.73
Sex (m/f)	23/ 12		11/ 6		12/ 6	
Ethnicity	Caucasian = 22 /Asian = 13		Caucasian = 11/Asian = 6		Caucasian = 11 /Asian = 7	

635 Table 2: Experimental measurements as a function of each trial arm.

	Placebo				**Peppermint**				***b***	**95% CI**	**P-value**	***d***
	Pre		**Post**		**Pre**		**Post**					
	Mean	*SD*	**Mean**	*SD*	**Mean**	*SD*	**Mean**	*SD*				
Mass (kg)	71.65	9.96	71.55	9.63	67.46	9.50	67.22	9.61	-0.14	-0.73-0.45	0.628	-0.17
Fat mass (kg)	17.68	5.65	17.70	5.08	16.64	5.67	16.89	5.64	0.24	-1.24-1.72	0.742	0.11
BMI (kg/m^2)	24.61	1.91	24.58	1.83	24.04	2.73	23.96	2.84	-0.05	-0.25-0.14	0.593	-0.18
Body fat (%)	24.65	7.89	24.75	7.23	24.74	7.51	25.17	7.29	0.33	-1.69-2.35	0.741	0.11
Waist circumference (m)	84.03	8.09	82.12	7.30	81.14	4.48	79.83	5.36	0.60	-2.49-3.70	0.695	0.13
Waist:hip ratio	0.87	0.13	0.83	0.06	0.83	0.05	0.82	0.06	0.03	-0.04-0.10	0.378	0.30
Resting carbohydrate oxidation (g/min)	0.30	0.07	0.28	0.10	0.26	0.08	0.23	0.09	-0.01	-0.07-0.06	0.851	-0.06
Resting fat oxidation (g/min)	0.03	0.02	0.03	0.02	0.02	0.02	0.03	0.02	0.01	0.00-0.02	0.113	0.55
Resting kcal carbohydrates (kcal/min)	1.19	0.27	1.12	0.40	1.03	0.32	0.92	0.34	-0.04	-0.30-0.23	0.784	-0.09
Resting kcal fats (kcal/min)	0.27	0.19	0.28	0.16	0.20	0.15	0.32	0.23	0.10	-0.01-0.21	0.069	0.64
% Carbohydrate rest (%)	82.42	8.81	79.31	10.37	83.37	9.09	73.98	16.63	-6.28	-14.43-1.87	0.127	-0.53
% Fats rest (%)	17.58	8.81	20.69	10.37	16.63	9.09	26.02	16.63	6.28	-1.87-14.43	0.127	0.53
RMR (kcal)	2106.70	545.34	2011.53	588.03	1776.66	518.37	1773.32	446.46	91.83	-258.82-442.48	0.598	0.18
Cholesterol (mmol/L)	3.56	0.19	3.52	0.10	3.79	0.51	3.48	0.38	-0.27	-0.54-0.00	0.051	-0.68
LDL cholesterol (mmol/L)	1.89	0.49	1.83	0.43	2.08	0.50	1.89	0.17	-0.13	-0.41-0.15	0.348	-0.32
HDL cholesterol (mmol/L)	1.31	0.37	1.33	0.34	1.35	0.15	1.29	0.12	-0.08	-0.16-0.001	0.053	-1.10
Total:HDL ratio	2.88	0.57	2.79	0.50	2.90	0.50	2.83	0.29	0.02	-0.27-0.31	0.891	0.05
LDL:HDL ratio	1.59	0.56	1.50	0.49	1.60	0.46	1.55	0.26	0.04	0.22-0.30	0.751	0.11
Glucose (mmol/L)	4.45	1.05	4.58	1.00	4.22	0.56	4.32	0.73	-0.03	-0.50-0.43	0.879	-0.05
Triglycerides (mmol/L)	1.43	1.03	1.49	0.96	1.49	0.43	1.25	0.33	***-0.30***	***-0.52 - -0.08***	***0.010***	***-0.92***
TyG index	8.35	0.38	8.44	0.26	8.47	0.30	8.32	0.29	***-0.26***	***-0.46 - -0.06***	***0.012***	***-0.90***
Haemoglobin (g/L)	148.47	33.34	145.18	23.46	145.94	15.75	153.11	18.15	10.46	-3.96-24.90	0.150	0.50
Systolic blood pressure (mmHg)	118.53	9.53	119.00	10.42	118.44	9.19	114.39	10.70	***-4.53***	***-8.39 - -0.66***	***0.023***	***-0.81***
Diastolic blood pressure (mmHg)	75.29	9.84	76.18	6.21	76.72	7.04	73.78	7.83	***-3.83***	***-7.45 - -0.21***	***0.039***	***-0.73***

Resting heart rate (beats/min)	64.41	9.00	65.12	9.05	68.78	13.04	66.56	10.60	-2.93	-7.14-1.28	0.167	-0.48
Beck depression inventory	4.41	4.09	5.24	4.84	3.89	4.36	3.11	4.13	-1.60	-3.92-0.72	0.170	-0.47
COOP WONCA	1.89	0.56	1.95	0.60	1.76	0.47	1.68	0.50	-0.14	-0.46-0.18	0.380	-0.30
STAI state	34.24	10.27	40.06	11.71	31.28	10.09	30.89	11.69	***-5.43***	***-11.33 - -0.56***	***0.040***	***-0.73***
STAI trait	38.76	10.89	43.00	11.43	34.39	11.10	33.44	11.42	***-5.18***	***-10.76 - -0.40***	***0.038***	***-0.74***
PSQI	5.29	2.44	4.94	2.22	4.72	2.78	3.78	2.44	-0.59	-1.86-0.68	0.351	-0.32
Insomnia severity index	6.24	4.41	5.71	3.65	5.94	5.43	4.72	4.61	-0.69	-2.76-1.37	0.500	-0.23
Epworth sleepiness scale	6.00	4.02	5.59	3.84	6.72	4.11	5.67	3.46	-0.64	-1.19-0.62	0.307	-0.35

Notes: *bold text = significant difference in the changes from baseline to 20-days between the two groups (negative values denote that reductions in the peppermint group exceeded those in placebo), b = mean difference between groups in change from baseline to 20-days, 95% CI = confidence intervals of the mean difference & d = Cohen's d.* ***Abbreviations:*** *RMR = resting metabolic rate, TyG index = Triglyceride-glucose index, STAI = State-Trait Anxiety Inventory questionnaire, BMI = body mass index & PSQI = Pittsburgh Sleep Quality Index questionnaire.*

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