Original Research Article

Effects of γ-aminobutyric acid supplementation on glucose control in adults with prediabetes: A double-blind, randomized, placebo-controlled trial

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ABSTRACT

Background: Gamma-aminobutyric acid (GABA) is mainly known as an endogenously produced neurotransmitter. However, GABA intake from dietary sources like tomatoes and fermented foods can be considerable. Studies in rodent models have shown beneficial effects of oral GABA supplementation on glucose homeostasis and cardiovascular health. Still, it is currently unknown whether oral GABA supplementation produces cardiometabolic benefits in humans.

Objectives: This study aimed to investigate whether oral GABA supplementation can improve glucose homeostasis in individuals at risk of developing type 2 diabetes.

Methods: In a randomized, placebo-controlled, double-blind, parallel-arm trial, 52 individuals with prediabetes (classified by impaired glucose tolerance and/or impaired fasting glucose), aged 50 to 70 y with a body mass index ≥25 kg/m2 received either 500 mg GABA 3 times daily or a placebo for 95 days. The primary outcome was the effect of the intervention on glucose response after an OGTT. As exploratory secondary outcomes, markers of glycemic control (glycated hemoglobin, insulin, glucagon, mean amplitude of glycemic excursions, and standard deviation as measured with flash glucose monitoring), cardiovascular health (blood pressure, 24-h blood pressure, circulating triglycerides, cholesterol), and self-reported sleep quality were measured before and after the intervention.

Results: Compared with placebo, GABA supplementation for 95 days did not change the postprandial glucose response (0.21 mmol/L; 95% confidence interval: 0.002, 0.674; P = 0.364). After correction for the false discovery rate, all other outcomes (including fasting plasma GABA concentration) showed no significant effects from GABA intervention at a group level.

Conclusions: GABA supplementation does not change the postprandial glucose response in individuals at risk of developing type 2 diabetes. However, based on findings in secondary outcome measures, further research is warranted in other study populations. Research could focus on the effects of GABA in individuals with advanced diabetes or other cardiometabolic disorders.

This trial was registered at www.clinicaltrials.gov as NCT04303468.

Keywords: GABA, prediabetes, overweight, blood pressure, sleep, metabolic health, glucose, insulin, glucagon, triglycerides

Introduction

GABA is best known as an inhibitory neurotransmitter in the central nervous system. However, GABA is also present in nonneuronal peripheral tissues in concentrations up to micromolar levels [1,2]. In line with this, GABA receptors of both the GABA\textsubscript{A} (ionotropic) and GABA\textsubscript{B} (metabotropic) class have been identified in many non-neuronal cell types, including cells of the immune system, pancreas, liver, adipose tissue, ovaries, adrenal glands, intestinal tract, and kidney [3,4]. Interestingly, GABA is also present at millimolar levels in the human diet and is produced by gut microbiota [5]. Relatively rich sources of GABA are tomatoes (up to 1.9 g/kg), melons (up to 3 g/kg), and fermented food products (up to 10 g/kg in matured cheeses) [6–8]. Functional foods with added GABA have also been developed, and recently a GABA-rich tomato was released in Japan as the first gene-edited food on the market [9].

GABA is also produced in β-cells of the pancreas, and increasing evidence indicates an essential role for GABA in the paracrine and autocrine regulation of insulin and glucagon release [10]. In line with this, pancreatic islets from patients with diabetes are GABA-depleted,
suggesting that disturbed GABA signaling may play a role in the disease [11]. Several studies have explored the effects of exogenous GABA administration in animal models of both type 1 and type 2 diabetes [12–15]. These studies have demonstrated that GABA, administered orally or intravenously for 5 to 16 weeks, can improve insulin sensitivity and glucose tolerance. The proposed underlying mechanisms include a reduction in low-grade inflammation, which often accompanies (pre-)diabetes, and an increase in pancreatic β-cell mass.

Short-term studies into GABA’s potential beneficial metabolic effects in humans have also been published. In a study by Li et al. [16], healthy participants received supplements containing 2 g of GABA as a single dose or 3 times a day for a week. The authors showed that GABA is taken up in the blood when consumed orally and promotes insulin and glucagon production in healthy adults [16]. In a recent study, we showed that GABA is also bioavailable from dietary sources (tomatoes), producing a sharp but relatively short-lasting (<5 h) elevation in plasma GABA concentration after tomato consumption [17]. Therefore, GABA-rich food sources could potentially play a relevant role in preventing type 2 diabetes development.

The prediabetic stage offers an important window of opportunity to prevent further advancement to clinical diabetes [18]. Individuals with prediabetes are characterized by impaired glucose tolerance and/or elevated fasting glucose. Reversal of prediabetes to healthy glucose homeostasis leads to a 56% lower future diabetes risk [19]. Given the evidence from rodent studies, we hypothesize that GABA intake may affect diabetes development [12–15]. However, so far, it is unclear whether acute or long-term intake of GABA can benefit individuals with prediabetes because appropriate intervention studies are lacking to translate these findings to humans.

Therefore, we performed a randomized, placebo-controlled, double-blind, parallel-arm trial investigating the potential beneficial effects of 95 days of oral GABA supplementation on individuals with prediabetes. The intervention was designed to study the postintervention effects of GABA on the postprandial glucose response. It also included secondary outcome measures to explore the effects of GABA on other markers of metabolic health, such as blood pressure, markers of cardiovascular health, circulating GABA concentrations, and sleep quality. At the start of the intervention, the acute effects of GABA on the postprandial glucose response and blood pressure were also assessed.

Methods

The study was approved by the medical ethical committee of Wageningen University and is registered at clinicaltrials.gov under NCT04303468. The study was performed according to the Declaration of Helsinki [20] and took place from September 2020 to September 2021 in the Human Nutrition Research Unit of Wageningen University and Research (the Netherlands).

Study population

Males and females aged 50 to 70, with a BMI above or equal to 25 kg/m², were recruited from an area in and around Wageningen, the Netherlands. An in-house database, flyers, and social media advertisements were all used to reach a sufficient number of responding potential participants. Oral and written information was provided before written informed consent was obtained. Participants were invited to the research facility for a screening session where they underwent an OGTT with 75 g glucose (Beldico, Glucomedics). Blood was drawn with a fingerprick (Freestyle Freedom Lite, Abbott) to assess blood glucose concentration at fasting, 1 h, and 2 h after the glucose drink. Participants with impaired fasting glucose (fasting glucose ≥ 6.1 and ≤ 6.9 mmol/L) and/or impaired glucose tolerance (glucose levels ≥8.6 mmol/L 1 h after an OGTT or/and glucose levels ≥ 7.8 and ≤ 11.1 mmol/L 2 h after an OGTT) were included. Participants were also weighed at screening, and their veins were checked for suitability to draw blood. Participants were excluded from participation according to the following criteria (as assessed by questionnaire): diagnosed with or treated for diabetes; having other conditions that could influence the study results, like liver, pancreatic, cardiovascular, gastrointestinal, or endocrine diseases; use of medication or supplements that could affect the study results, in particular, glucose-regulating drugs or drugs interacting with GABA receptors (eg, benzodiazepines); sensitivity to medical skin adhesives; more than 5 kg weight change in the past 12 weeks; excessive alcohol consumption (>21 glasses/wk on average for males and >14 glasses/wk for females); employment by Wageningen University, Division of Human Nutrition and Health; participation in another trial in the preceding 3 months.

Study design and intervention

The study was designed as a randomized, placebo-controlled, double-blind, parallel-arm trial (Figure 1). Participants received 3 times a day for 95 days (−3 months), either 500 mg GABA capsules (1500 mg/d in total, Swanson Health) or placebo capsules (microcrystalline cellulose, JRS). Participants were asked to take a capsule before each main meal. The study was double-blind; the study team and participants were unaware of the group allocation. The capsules looked identical and were coded by a researcher not involved in the study. Block randomization was also conducted by a researcher not involved in the study. Stratification was based on BMI (below and above 35 kg/m²) and sex.

Baseline and outcome measures were assessed during measurement periods (Figure 1). The intervention was started on day 1. As a primary outcome, the postprandial glucose response was evaluated on day −3 of the measurement period. As exploratory secondary outcomes, we also measured glycemic variability, postprandial insulin and glucagon response, HbA1c, blood pressure, 24-h blood pressure, blood lipids, sleep quality, and GABA and glutamate plasma concentrations. In addition, at the start of the intervention, the acute effects of GABA on the postprandial glucose response and blood pressure were assessed (days 1 and 2). An overview of the activities during the measurement periods is shown in Figure 1. During the intervention period, the participants received new capsules every month. At these monthly visits, the capsules were counted to assess compliance. In addition, participants received a telephone call 1 week after the start of the intervention to increase compliance.

Diet standardization

During the intervention, the participants were asked not to substantially change their habitual food intake and physical activity level. Participants were asked to record a food diary in the 5 days (days −8 to −4, Figure 1) before the baseline measurement on day −3 (Figure 1). At the end of the intervention, participants were asked to repeat (the same meals at the same times) this recorded diet again the 5 days (days 91 to 95, Figure 1) before the final OGTT on day 96 (Figure 1). We introduced this matching of the diet before the OGTT to accurately measure changes in glycemic variability with flash glucose monitoring (FGM) and reduce within-person variability in the OGTT results. The evening before the OGTT, participants also consumed a standardized meal provided by the university (622 kcal, 16.4 g fat, 94 g carbohydrate, 11.4 g fiber, 19 g of hydrate).
FIGURE 1. Flowchart showing an overview of details of interventions and measurements to assess the long-term and acute effects of 3dd 500 mg GABA on different health outcomes. The numbers represent the days of the study, with the treatment starting on day 1 and lasting until day 95. On days $-10$ to $-1$, measurements were done before the intervention, and on days 96 and 97, measurements were done after the intervention and no treatment was given. Measurement period at baseline: day $-10$: flash glucose sensor is placed; day $-8$ to $-4$: food diary is recorded; day $-3$: preintervention OGTT, blood pressure measurement, and sleep quality questionnaire; day $-2$: preintervention 24-h blood pressure. Acute effects: day 1: OGTT while GABA/placebo is already consumed; day 2: 10-h blood pressure monitoring while GABA/placebo is consumed. Endpoint measurements: day 89: flash glucose sensor is placed; days 91 to 95: the diet follows the recorded food diaries; day 96: postintervention OGTT, blood pressure measurement, and sleep quality questionnaire; day 97: post-intervention 24-h blood pressure.

OGTT and biochemical analysis

Postprandial glucose response was assessed by an OGTT in which 75 g glucose was consumed with 200 mL water (Beldico, Gluco-medics). A peripheral venous catheter was inserted into an ante-cubital vein 30 min before the first blood drawing. Blood was drawn at fasting (21 mL) and 30, 60, and 120 min (9 mL) after the glucose drink. Blood tubes (Vacutainers, BD) containing sodium fluoride were used to analyze glucose, and blood tubes containing lithium heparin were used to analyze glucagon, insulin, triglycerides, LDL, and HDL. From these tubes, plasma was prepared by centrifugation at 3000 $g$ for 8 min at $4^\circC$. Blood tubes containing EDTA were used to analyze GABA and glutamate, which were centrifuged at 3000 $g$ for 8 min at $4^\circC$. After separation, plasma samples were stored at $-80^\circC$ until the completion of the study. Glucose, glucagon, and insulin were measured at all-time points of the OGTT. Concentrations of HbA1c (measured in whole EDTA blood analyzed within 3 days from blood drawing), triglycerides, LDL and HDL, GABA, and glutamate were measured in the fasting blood sample. Glucagon (#81520, Crystal Chem) and adiponectin (#DRP300, R&D Systems) were measured with an ELISA kit according to the manufacturer’s protocol. GABA and glutamate concentrations in plasma were determined using a previously validated UPLC-MS/MS method described in full detail elsewhere [21]. In short, GABA and glutamate were extracted from plasma with acetonitrile-containing deuterated GABA and glutamate as internal standards. The extracts were further purified using solid phase extraction, after which they were analyzed on a triple quadrupole mass spectrometer operated in positive electrospray ionization. Concentrations of GABA and glutamate were calculated according to calibration standards that ranged between 3.4 to 2500 ng/mL and 30.9 ng/mL to 22,500 ng/mL, respectively. This method is sufficiently sensitive and robust to detect endogenous circulating GABA [17,21]. All other biochemical analyses (eg, HbA1c, glucose, insulin, lipids, AST/ALT ratio [to rule out hepatotoxicity]) were performed at the Clinical Chemistry and Hematology Laboratory of Geldersche Vallei Hospital using standard clinical laboratory assays.

Flash Glucose Monitoring

Participants were provided a factory-calibrated FGM system (FreeStyle Libre, Abbott) during 2 measurement periods (days $-10$ to $-1$ and 89 to 97). The system measures interstitial glucose concentrations every 15 min. The sensor was attached to the upper arm of the participants and scanning the sensor to allow data transfer was required at least once every 8 h. Only the data collected during the 5 days that participants recorded a food diary were used to analyze glycemic variability. From this data, the standard deviation and the mean amplitude of glycemic excursions (MAGE) were calculated according to previously described methods using the package “CGM shiny” in the R Environment for Statistical Computing version 4.0.2 (R Foundation) [22]. Participants with less than 3 days of available data were excluded from the analysis.

Blood pressure

Blood pressure was measured 2 times (HEM-907 IntelliSense, OMRON) after 5 min of rest in a fasted condition. A third measurement was done if the first 2 measurements varied by more than 10 mmHg; all measurements were averaged. In addition to these separate measurements, 24-h blood pressure (ABPM-50, Contec) was measured by the participants at home the day after the OGTT, starting at 9:00 and ending at 9:00 the next morning. Blood pressure was measured every 30 min during the day (09:00 to 21:00) and every hour during the night (01:00 to 6:00). The cuff was placed on the non-dominant arm for both blood pressure measurements. Participants with less than 70% available data were excluded from the analysis of 24-h blood pressure data only.

Other outcomes

The Pittsburgh Sleep Quality Index (PSQI) was used to assess self-reported sleep quality over the last month. The questionnaire was filled out during the OGTT. The score was calculated as described in the
Acute effects of GABA

The participants started with the intervention 3 days after finishing the baseline measurements (day 1, Figure 1). On the first day of the intervention, the participants underwent another OGTT to assess the potential acute effects of GABA. The first GABA (500 mg) or placebo capsule was consumed with the glucose drink. During the OGTT, the glucose levels were assessed with FGM. On the second day of the intervention, participants wore an ambulatory blood pressure monitor (ABPM-50, Contec) for 10 h while taking their GABA or placebo capsules at prespecified times: 9:00, 13:00, and 18:00. In the figures, these acute effects are also compared to the respective baseline measurements (day − 3 and −2, Figure 1) when no supplement (GABA or placebo) was consumed.

Statistical analysis

The sample size of this study was based on the postprandial glucose response as the primary outcome. A sample size of 46 participants would provide a power of 80% with an α of 0.05 to detect an additive difference of 17%. This estimated effect size and standard deviation were based on intervention effects from the literature, using the glucose concentration 60 min after an OGTT as a proxy for the postprandial glucose response [24]. The sample size was increased by 10% to 52 participants to account for possible dropouts.

All outcomes assessed more than once in a single day (glucose, insulin, glucagon, 24-h blood pressure, and the acute effects of GABA) were statistically evaluated with linear mixed models using IBM SPSS Statistics version 25. The fasting concentration was subtracted from all subsequent values for that day (Δ) to determine the effects on the postprandial response. Treatment and time were added as fixed effects with the preintervention (baseline) values as a covariate. The participant identifier was added as random effect. Time (factor variable) was set as the repeated variable, and the covariance structure was set to AR1. For all linear mixed models, it was tested whether there was a significant interaction between treatment and time and between treatment and baseline outcome values. For none of the outcomes, this was the case. Therefore, the interaction term was not added to the model.

For the other outcomes (HbA1c, fasting glucose, insulin, and glucagon, mean glucose, MAGE, and standard deviation as measured with FGM, HOMA-IR, blood pressure, fasting triglycerides, cholesterol, adiponectin, GABA and glutamate, weight, waist circumference, AST/ALT, and self-reported sleep quality), a 1-factor ANCOVA was performed using R. The linear models (lm) function was used for the statistical analysis, with the formula: “postintervention value” ~ “treatment” + “preintervention value”. The respective baseline value was added as a covariate. The distribution of the residuals was checked for normality by examining Q-Q plots. When the residuals were not normally distributed, the data were logarithmically transformed (insulin, HOMA-IR, triglycerides). Homoscedasticity was assessed by plotting the residuals against the fitted values. Outliers that varied more than 3 standard deviations from the residuals were removed from the analysis. If this was the case (PSQI score), it is mentioned in the results section. The interaction term “treatment”*preintervention value” was checked for significance to determine the homogeneity of the regression slopes. In the case of a significant interaction, this was further investigated using Johnson-Neyman plots [25]. For creating these plots, the R function “johnson_neyman" from the package “interactions” was used [26]. As recommended, the outcomes of the Johnson-Neyman plots were adjusted for multiple testing [27]. Estimates and 95% CIs of the interaction effect are reported.

The outcomes of the statistical assessments were reported with regression parameters, 95% CI, and P value (significant if < 0.05). Using the Benjamini-Hochberg method, P values were also adjusted for the false discovery rate with the R function “p.adjust” [28]. Figures were produced using the R package “ggplot2” [29]. The figure of the study design was created with BioRender.com.

Results

Study population

Fifty-two participants were randomly assigned to receive either the GABA or the placebo-containing capsules. Forty-nine participants completed the intervention (Figure 2). Three participants did not complete the intervention due to perceived adverse effects (tiredness), an inability to honor commitments, and difficult venous access. One participant completed only the secondary outcomes due to difficult venous access at the end of the intervention. Table 1 displays the baseline characteristics of the 51 participants that started the intervention. Notably, the percentage of females included in the study was relatively low (34.6%). Based on capsule count, compliance was high in both groups (GABA: 94% ± 7.4%, placebo: 97% ± 3.8%).

Effects of GABA on glycemic control

Markers of glycemic control were assessed at baseline and the end of the intervention. No significant group differences were found in the postprandial glucose response after an OGTT (0.21 mmol/L; 95% CI: −0.252, 0.674; P = 0.364). Also, the insulin (0.01 μIU/mL; 95% CI: −0.059, 0.086; P = 0.711) and glucagon (0.59 pg/mL; 95% CI: −1.30, 2.48; P = 0.531) response did not show any significant differences (Figure 3). However, there was a significant reduction in fasting glucose concentration after the GABA intervention compared to the placebo group (−0.22 mmol/L; 95% CI: −0.397, −0.045, Table 2). However, this was no longer a significant finding after correction for the false discovery rate. No significant effects were found for HbA1c or markers of glycemic variability as measured with FGM for 5 days. Effects on fasting insulin, glucagon, and HOMA-IR also did not differ significantly between the groups.

Postintervention effects of GABA on exploratory secondary outcomes

As part of the clinical trial, we also explored the effects of GABA on blood pressure, lipid markers, and sleep quality. In addition, the fasting plasma GABA concentrations and that of its precursor, glutamate, were assessed before and after the intervention (~14 h after taking the last capsule).

Blood pressure was measured both at the university by a trained researcher for a single blood pressure measurement and at home with an ABPM for 24 h. No significant treatment group differences were found in blood pressure (Table 3 and Figure 4). In addition, no significant differences were found in fasting plasma triglycerides, HDL, and LDL concentrations (Table 3). The presence of an interaction between the treatment and the baseline value was tested for each outcome. A significant interaction with baseline value was found for postintervention systolic blood pressure and HDL concentration. Therefore, for these outcomes, the baseline value influenced the...
relationship between the treatment and the postintervention value. The regression parameters for the interaction effect of treatment with the respective baseline value were as follows: $-0.37 \text{ mmHg}; 95\% \text{ CI}: -0.718, -0.018; P = 0.040$ for blood pressure, and $0.22 \text{ mmol/L}; 95\% \text{ CI}: 0.012, 0.420; P = 0.038$ for HDL.

Sleep quality was assessed with a PSQI questionnaire. One of the participants in the placebo group had a PSQI score of 10 before the intervention and 1 after the intervention. With an average within-group change in PSQI score of $-0.33 \pm 1.31$, this change of 9 points is considered an outlier. Therefore, this participant’s PSQI score was omitted from the analysis. Excluding the outlier changed the parameters of the model from $\beta = -0.51; 95\% \text{ CI}: -1.320, 0.307; P = 0.21$ with the outlier to $\beta = -0.66; 95\% \text{ CI}: -1.301, -0.012; P = 0.046$ without the outlier. Without the outlier, a significant improvement in self-reported sleep quality after the intervention in the GABA group as compared to placebo was observed (Table 3). However, this was no longer a significant finding after correction for false discovery rate. However, a significant interaction with baseline value was found for the PSQI score with the following regression parameters: $-0.30; 95\% \text{ CI}: -0.554, -0.042; P = 0.023$.

The observed interactions were further investigated and visualized with Johnson-Neyman plots, plotting the estimated effect size and CI against the baseline value (Supplemental Material). Only for the PSQI score, this interaction persisted after correcting for multiple testing. The effect of the GABA treatment on postintervention PSQI score was significant among study participants with a baseline PSQI score of 3.75 or above.

The supplementation of GABA did not significantly elevate the fasting plasma GABA concentration compared to a placebo (Table 3). Also, for glutamate, no differences were observed. Weight, waist circumference, and AST/ALT ratio remained stable during the intervention.

**Acute effects of GABA**

The acute effects of GABA were assessed at the start of the treatment period. Postprandial glucose levels were evaluated with FGM during an OGTT, reflecting interstitial glucose levels rather than blood glucose levels. Compared to a placebo, the glucose response was not different after taking a single GABA capsule ($0.10 \text{ mmol/L}; 95\% \text{ CI}: -0.361, 0.552; P = 0.678$) (Figure 5). Ambulatory blood pressure was measured for 10 h, and GABA or placebo capsules were consumed at
9:00, 13:00, and 18:00 (Figure 6). No significant acute effects of GABA on blood pressure were found (Δ/C0 -5.6 mmHg; 95% CI: -13.1, 2.0; P = 0.146).

Adverse events
No severe adverse events were reported. In the placebo group, 32% of the participants reported mild adverse events, whereas in the GABA group, this was 44% (Table 4). Headache, tingling feeling, light-headedness, and sensitive arms and legs were solely reported in the GABA group.

Discussion
In this double-blind, randomized, placebo-controlled intervention study with adults at risk of developing type 2 diabetes, we assessed whether 95 days of 1500 mg GABA supplementation per day could improve glucose homeostasis, as reflected primarily by the glucose response following an OGTT. Next to the primary outcome, we explored the effects of GABA on a broad spectrum of secondary outcomes for cardiometabolic health and sleep quality. GABA supplementation did not change the postprandial glucose, insulin, and...
Abbreviation: MAGE, mean amplitude of glycemic excursions.

1 Values are presented as mean ± SD. As between-group change, the estimates (β values and 95% CIs are presented derived from a 1-factor ANCOVA with treatment as the main effect and baseline value as a covariate.

2 Log-transformed for normal distribution.

3 Flash glucose monitoring data of maximally 5 consecutive days was used. Participants with fewer than 3 d of glucose data were excluded. Placebo, n = 25 included; GABA, n = 22 included.

4 The adjusted p values were adjusted for false discovery rate using the Benjamini-Hochberg method.

TABLE 3
Postintervention effects of GABA on exploratory secondary outcomes

<table>
<thead>
<tr>
<th></th>
<th>Placebo, n\text{max} = 25</th>
<th>GABA, n\text{max} = 24</th>
<th>Between-group change</th>
<th>P value</th>
<th>Adj. P value</th>
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<td>Baseline</td>
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<td><strong>Blood pressure (mmHg)</strong></td>
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</table>
| Systolic                 | 130 ± 14.49               | -1.15 ± 7.31            | 127 ± 11.83         | 0.03 ± 10.42        | 0.29 (-4.598, 5.181) | 0.905 1.000
| Diastolic                | 78 ± 9.27                 | 1.77 ± 6.06             | 76 ± 9.91           | 1.17 ± 6.55         | -0.71 (-4.345, 2.917) | 0.694 1.000
| 24-h blood pressure (mmHg) |                           |                         |                     |                     |                        |
| Systolic                 | 126 ± 12.97               | -0.07 ± 4.92            | 126 ± 8.28          | -0.79 ± 6.53        | -1.22 (-6.923, 4.486)² | 0.668 1.000
| Diastolic                | 75 ± 7.98                 | -0.09 ± 3.19            | 75 ± 7.09           | 0.07 ± 4.30         | -0.07 (-4.448, 4.301)² | 0.973 1.000
| Blood pressure day (mmHg) |                           |                         |                     |                     |                        |
| Systolic                 | 130 ± 13.71               | -0.27 ± 5.94            | 129 ± 8.79          | -1.35 ± 7.62        | -1.21 (-5.104, 2.684) | 0.534 1.000
| Diastolic                | 78 ± 8.40                 | -0.37 ± 3.27            | 77 ± 7.69           | -0.22 ± 4.80        | 0.09 (-2.368, 2.538) | 0.944 1.000
| Blood pressure night (mmHg) |                           |                         |                     |                     |                        |
| Systolic                 | 116 ± 12.32               | 0.56 ± 5.38             | 115 ± 8.13          | 0.78 ± 6.56         | 0.04 (-3.251, 3.322) | 0.983 1.000
| Diastolic                | 66 ± 7.82                 | 0.93 ± 4.67             | 67 ± 6.31           | 0.88 ± 5.17         | 0.02 (-2.871, 2.916) | 0.987 1.000
| Triglycerides (mmol/L)   | 1.38 ± 0.59               | 0.16 ± 0.38             | 1.58 ± 0.55         | 0.02 ± 0.39         | -0.13 (-0.358, 0.101)³ | 0.266 1.000
| HDL (mmol/L)             | 1.33 ± 0.29               | -0.05 ± 0.09            | 1.31 ± 0.29         | -0.03 ± 0.12        | 0.02 (-0.043, 0.078) | 0.560 1.000
| LDL (mmol/L)             | 3.14 ± 0.79               | 0.04 ± 0.46             | 3.26 ± 1.02         | -0.09 ± 0.39        | -0.10 (-0.327, 0.122) | 0.363 1.000
| Adiponectin (µg/mL)      | 7.28 ± 4.32               | -0.40 ± 1.60            | 8.79 ± 5.04         | 0.04 ± 1.16         | 0.51 (-0.312, 1.330) | 0.218 1.000
| Pittsburgh Sleep Quality Index² | 4.17 ± 2.82 | -0.33 ± 1.31 | 3.45 ± 2.17 | -0.71 ± 1.63 | -0.66 (-1.301, -0.012) | 0.046 0.667
| GABA (ng/mL)             | 20.08 ± 3.45              | -0.22 ± 2.04            | 18.94 ± 3.76        | 0.86 ± 2.15         | 0.68 (-0.329, 1.690) | 0.181 1.000
| Glutamate (ng/mL)        | 3441 ± 1894               | 406 ± 1011              | 4154 ± 2683         | 42 ± 1568           | -251 (-938.4, 345) | 0.465 1.000
| Weight (kg)              | 90.98 ± 10.11             | 0.35 ± 1.43             | 89.13 ± 12.11       | 0.46 ± 1.55         | 0.17 (-0.677, 1.022) | 0.685 1.000
| Waist circumference (mm) | 1048 ± 73.32              | 0.08 ± 25.81            | 1044 ± 82.38        | 4.75 ± 28.46        | 4.18 (-10.896, 19.246) | 0.580 1.000
| AST/ALT (IU/L)           | 0.93 ± 0.2                 | 0.01 ± 0.16             | 0.92 ± 0.24         | -0.01 ± 0.17        | -0.03 (-0.114, 0.061) | 0.542 1.000

1 Values are presented as mean ± SD. As between-group change, the estimates (β values and 95% CI are presented derived from a 1-factor ANCOVA with treatment as the main effect and baseline value as a covariate, unless stated otherwise.

2 Ambulatory 24-h blood pressure measurement on day – 2 (Figure 1); an average per person was calculated. Averages are also split into day (09:00 to 21:00) and night (01:00 to 6:00). Participants with less than 70% of the data available were excluded. Placebo, n = 24 included; GABA, n = 20 included.

3 Log-transformed for normal distribution.

4 As between-group change, the estimates (β values) and 95% CIs are presented derived from linear mixed models with treatment and time as fixed effects, participant identifier as random effect, and preintervention values as a covariate.

5 One participant was excluded from the analysis because their data deviated more than 3 standard deviations from the residuals. GABA, n=24 included; placebo, n=24 included.

6 One participant was excluded as we were unable to measure GABA, possibly due to interference. GABA, n=23 included; placebo, n=24 included.

7 The adjusted P values were adjusted for false discovery rate using the Benjamini-Hochberg method.
FIGURE 4. Ambulatory blood pressure measurements on day -2 and 97 (see Figure 1) of the measurement period, showing (A) systolic blood pressure measurements and (B) diastolic blood pressure measurements over a period of 24 h. Red lines represent the group allocated to the GABA intervention and blue lines represent the group allocated to the placebo. Filled lines represent the measurements taken after the 95-d intervention, whereas dotted lines represent 24-h blood pressure before the intervention. Data are presented as means with SEM error bars. Group differences in 24-h blood pressure were analyzed using linear mixed models, and no significant differences were found.

FIGURE 5. Acute effects on interstitial glucose as measured during a 75-g OGTT with flash glucose monitoring on days -3 and 1 at the start of the intervention. Red lines represent the group allocated to the GABA intervention and blue lines represent the group allocated to the placebo. Filled lines represent the measurements taken when either a GABA or placebo capsule was consumed together with the glucose drink (day 1 at the start of the intervention, see Figure 1), whereas dotted lines represent the preintervention glucose response when no capsules were consumed yet (day -3 before the intervention, see Figure 1). Data are presented as means with SEM error bars. Group differences in the postprandial response were analyzed using linear mixed models, and no significant differences were found.
glucagon response. Although we obtained some indication that GABA supplementation could reduce the fasting blood glucose concentration and improve the sleep quality of the participants, these results did not remain significant after correction for the false discovery rate. Therefore, they can only be interpreted as explorative findings. We observed no effects of GABA supplementation on any of the other secondary outcomes. Additionally, it should be noted that the basal (fasting) plasma levels of GABA did not increase after 95 days of supplementation, suggesting no accumulation at this dosage regimen.

These findings are contrary to our hypothesis, based on previous studies that suggested GABA would benefit individuals at risk of developing diabetes. However, these earlier studies were mostly done with rodent models of diabetes or hypertension. Only a few human studies have been published to which the present intervention trial can be compared. The study by Li et al. [16] looked primarily at the plasma kinetics of GABA in healthy participants after a single dose of 2 g and a repeated dose (3 times daily, 2 g for 7 days). Both the single dose and repeated dose showed an increase in insulin concentrations under both fasting and postmeal conditions. Even with a single GABA dose in the morning, GABA had a significant effect on postmeal insulin later during the day (after lunch and dinner). Glucagon was only raised under fasting conditions, until 4 h after GABA intake. However, no effect on glucose levels was observed in these healthy individuals. The authors did observe a significant decrease in glycated albumin (a short-term measure of glycemic control) after 7 days of repeated dosing. After 95 days, we did not observe any such improvement in HbA1c (a marker of more long-term glycemic control). It is important to note that the dosage employed in that study was not only higher than the dose used in the current study but also exceeded the dosage of GABA supplements and the intake that can be obtained from a GABA-rich diet.

In another study, a single dose of GABA was administered in different doses to patients with type 1 diabetes [30]. Under hypoglycemic conditions, a dose of 600 mg GABA increased glucagon, epinephrine, growth hormone, and cortisol plasma concentrations, but...
there were no effects in a normoglycemic situation. Although we did not evaluate the acute effects of a single dose of GABA on postprandial glucagon and insulin response, we found no alterations in fasting glucagon and insulin plasma levels or the postprandial glucagon and insulin response after 95 days of GABA supplementation.

In line with the results of other studies, we also showed that GABA intake does not acutely change the postprandial glucagon response. This suggests that any acute changes in hormone response after GABA intake do not lead to a change in glucose response in this population, neither acutely nor after 95 d of supplementation. It remains interesting to study the acute effects of GABA on insulin and glucagon response in individuals with (pre-)diabetes in future studies.

Studies performed in rodent models have suggested that GABA is able to restore β-cell function and mass and reduce low-grade inflammation. Untereiner et al. [31] used a diet-induced obesity mouse model for prediabetes and administered GABA via drinking water as a 6 mg/mL solution. Interestingly, they found no effects of GABA (administered in drinking water for 10 wk) in the mice that developed prediabetes-like symptoms. Remarkably, in healthy control mice, they did find an increased β-cell mass, improved glucose tolerance, and increased insulin secretion after GABA administration. Yet, they observed no changes in glucagon secretion or insulin sensitivity. The authors hypothesized that GABA does not impact glucose tolerance in the prediabetic model, potentially because hyperinsulinemia and expansion of β-cell mass already occur during the development of obesity to compensate for insulin resistance. Any potential effect of GABA on increasing β-cell mass and insulin secretion would only be pertinent in a diseased diabetic state, where there has been a loss of β-cell mass and a decrease in insulin secretion.

This theory would be in line with the lack of effect of GABA on glucose tolerance that we observed in the current study. Tian et al. [15] did find an improvement in glucose tolerance and insulin sensitivity in mice after 20 weeks of 2 mg/mL GABA in drinking water together with a high-fat diet. However, they did not assess the effect on β-cell mass, and GABA treatment also reduced weight gain relative to the control, which was not the case in the study by Untereiner et al. [31].

Although the possibility of a chance finding cannot be ruled out, the decrease in fasting plasma glucose by 0.22 mmol/L (95% CI: 0.397, 0.045) following 95 days of GABA intake would be interesting to explore further. The observed decrease was not dependent on the participants’ fasting glucose concentration at the start of the intervention, and this effect size is of the same order of magnitude as found in other studies investigating bioactive food components or lifestyle interventions [32–34]. For example, in a study performed in our institute, Schutte et al. [35] found that a 12-wk, 25% energy restriction trial that successfully induced weight loss led to a reduction in fasting glucose concentration of 0.3 mmol/L in a comparable population [36]. The fasting glucose concentration is normally tightly controlled in a range from 3.9 to 6.9 mmol/L, and even small changes can substantially impact health outcomes. For prediabetes, estimates suggest that a 0.5 mmol/L increase in fasting glucose concentration would be associated with a 2- to 3-fold increased risk of progression to diabetes [36–38]. In this regard, the observed reduction in fasting glucose could be considered of interest for further research. At the same time, effects seen with successful lifestyle interventions are usually broader in terms of their health outcomes. Moreover, the current dosing regimen of 3 times 500 mg per day in combination with this relatively small effect would not be clinically or nutritionally attractive and is likely to result in low compliance.

We also investigated the potential effects of GABA on self-reported sleep quality because GABA supplements are widely advocated for this purpose. Although this secondary outcome measure points toward a potential beneficial effect on sleep quality, it should be interpreted with caution because the finding was no longer significant after correction for the false discovery rate. In addition, an outlier was removed from the data analysis. With an average PSQI score below 5, the included participants can be characterized as good sleepers [23]. The effect of GABA supplementation might be most relevant in poor sleepers, especially since we observed an effect size that is dependent on pre-existing self-reported sleep quality. Studies investigating the effects of GABA on sleep quality in humans are scarce and of short duration [39,40]. Therefore, substantiation for improving sleep quality by GABA remains very limited, and further research focusing on sleep quality in poor sleepers is required. Also, for blood pressure, a possible effect in our data appears to be dependent on pre-existing blood pressure. Although this interaction did not remain statistically significant after a correction for multiple testing, this observation warrants further research in hypertensive individuals. Previous studies also found no effect of GABA under normotensive conditions, which is in line with our results [41,42].

We showed that after 95 days of supplementation, fasting GABA plasma concentrations were not elevated. This is in line with our previous kinetic study [17], in which we observed that plasma levels steeply rise after oral GABA intake but return to baseline values in less than 5 h. Apparently, GABA does not accumulate in plasma; therefore, plasma GABA levels are not a good marker for GABA intake. Another study also showed no accumulation of GABA in plasma after administration of 2 g 3 times a day for 1 week [16]. Both studies indicate that GABA is rapidly cleared from the blood, most likely as a consequence of metabolism or distribution to tissues/organs like the kidney cortex, liver, pineal gland, and pituitary gland [43]. At the same time, positive health effects of other substances, such as green tea catechins, are observed to not depend on prolonged elevated plasma levels [34,44, 45]. Further research should focus on identifying biomarkers of GABA intake, like metabolites or conjugates of GABA, to be able to further investigate any health effects of a GABA-rich diet.

**Strengths and limitations**

The major strengths of this study are its double-blind, randomized, placebo-controlled design and long duration. Next, compliance was high, and the drop-out rate was minimal. However, there are also some limitations of the study that need to be acknowledged. Depending on the comparison, the dosage regimen of 3 daily doses of 500 mg GABA is relatively low compared to the doses given to rodents in previous studies and relatively high compared to the estimated dietary GABA intake [46]. Higher doses of GABA could be administered orally because no severe adverse events of GABA were found up to doses of 18 g [47]. However, it would be impossible to reach a daily intake of this magnitude through dietary intake. This leads us to believe that there is little chance that, with the current dose, we will have missed important effects that GABA could have through dietary intake, at least in this population. In addition, the study was conducted in individuals with a relatively early and still mild stage of prediabetes. This was motivated, on the one hand, by consideration of the status of GABA as a nutritional ingredient and, on the other hand, to circumvent medication use as a possible confounding factor. However, we may well have missed effects that are relevant for more advanced cardiometabolic disorders. Yet, our sample can be considered representative of a large subpopulation in Western Europe aged between 50 and 70 y with modest overweight and beginning prediabetes.
The methods we used also have some limitations to consider when interpreting the results. We standardized the diet before the OGTT to avoid increased variability due to diet and activities in the preceding days [48]. Therefore, standardizing the diet before the OGTT could have reduced the within-person variability. Furthermore, recording a food diary and following a specific diet changes behavior and could affect glucose homeostasis. The participants’ glucose monitor was also not blinded, so they were aware of their glucose levels.

Conclusion

In conclusion, the present study shows no effect of 95 days of oral GABA supplementation on the postprandial glucose response in participants at risk of developing type 2 diabetes. Although we find some indication for a reduction in the fasting plasma glucose concentration, the present results do not provide sufficient evidence to recommend GABA-rich products or GABA supplements to individuals at risk of developing (pre-)diabetes. Further research should clarify the mechanism of action behind the potential health effects of GABA. This is supported by some of the explorative secondary outcomes on sleep quality and hypertension.

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Author contributions

The authors’ responsibilities were as follows—THB, MGJB, MAJ, RFW: designed research; THB: conducted research; THB: performed statistical analysis; THB, MGJB, MAJ, RFW: wrote paper; THB: had primary responsibility for final content; and all authors: read and approved the final manuscript.

Conflict of interest

The authors report no conflicts of interest.

Data availability

Data described in the manuscript, code book, and analytic code will be made available upon request pending.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ajcnut.2023.07.017.

References


