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Effect of supplementation with n-trans caffeoyltyramine and n-trans feruloyltyramine on glycemia in prediabetic individuals: Randomized, double-blind, placebo-controlled trial

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ABSTRACT

Background: The global prevalence of elevated blood glucose levels is at an all-time high. For many people, reversal to normoglycemia is possible with diet and lifestyle modification. Dietary bioactives, in the form of functional food formulations or supplements, have attracted interest for their potential to improve glycemic control. *In silico* prediction, validated by preclinical research, has identified novel bioactives, N-trans-Caffeoyltyramine (NCT) and N-trans-Feruloyltyramine (NFT), as potent HNF4α agonists with gluco-regulatory effects. While promising, clinical studies are needed to validate these findings in humans.

Objective: To evaluate the effects of a proprietary dietary supplement containing 120 mg of NCT/NFT on fasting blood glucose and other biomarkers of glycemia in an otherwise healthy population with prediabetes.

Methods: A randomized, double-blind, placebo-controlled parallel arm trial was conducted in Asian Indian adults (Age: 18-50 years; BMI: 25-30 kg/m^2) with prediabetes (fasting blood glucose ≥100 mg/dL and <126 mg/dL) and abdominal adiposity (waist circumference ≥80 cm for females and ≥90 cm for males). A total of 126 participants were randomized to receive either 120 mg/d NCT/NFT (n=63) or placebo (n=63) for 4 weeks. Outcomes were measured at day 0 and day 28. The primary endpoint was fasting blood glucose. Secondary endpoints included fasting insulin, postprandial glucose response, and time spent within and outside of the ideal glucose range (<140 mg/dL) as assessed by continuous glucose monitoring (CGM). Additional outcomes related to CGM will be presented in a subsequent publication. Glycated hemoglobin (HbA1c), serum lipids, and anthropometrics were also measured.

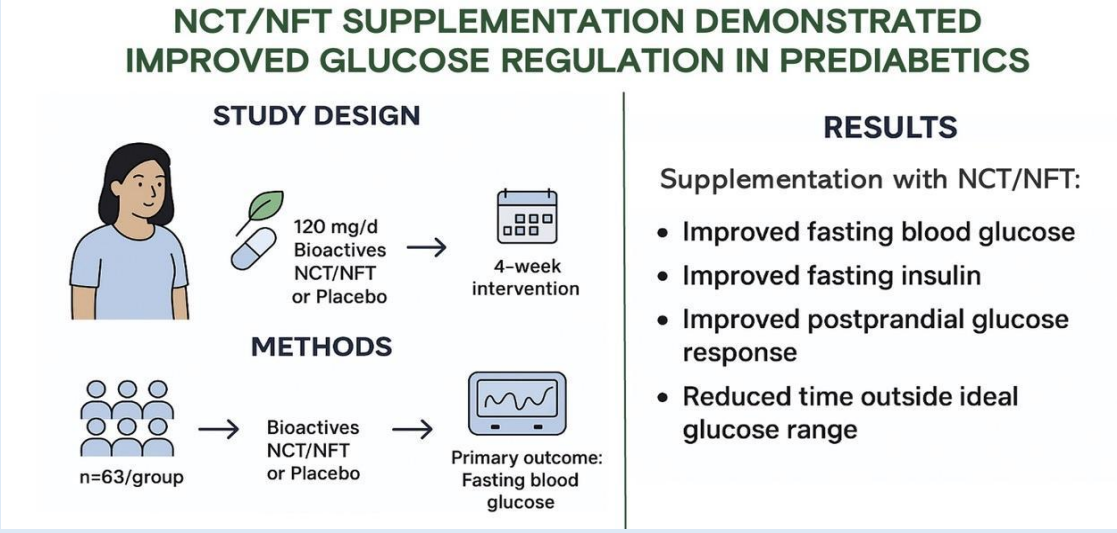
Results: Supplementation with a proprietary sustained-release formula of 120 mg/d NCT/NFT for 4 weeks resulted in significant improvements in the primary outcome measure, fasting blood glucose (NCT/NFT: -4.1 ± 7.1 mg/dL vs. Placebo: -0.7 ± 7.0 mg/dL; p=0.0026). Fasting insulin, postprandial glucose response, and time spent outside of the ideal glucose range were also significantly improved with treatment (p=0.0147, p=0.0036, and p=0.0138, respectively). No changes were observed in other markers of glycemia, lipid profile, or anthropometric measures.

Conclusions: Naturally occurring bioactive compounds, NCT and NFT, act as potent HNF4 α agonists for the part that they play in blood glucose regulation and should be considered as part of a comprehensive approach to support glycemic and overall metabolic health.

Novelty of the Study: This study is among the first randomized, double-blind, placebo-controlled clinical trials to evaluate the effects of N-trans caffeoyltyramine (NCT) and N-trans feruloyltyramine (NFT) supplementation on blood glucose regulation in prediabetic adults. While prior research has focused on outcomes that provide a snapshot in time (e.g. fasting glucose or insulin), this study uniquely integrates both cross-sectional (fasting levels) and continuous endpoints (continuous glucose monitoring), providing a comprehensive assessment of bloglucose regulation through novel bioactive compounds. The findings introduce a new direction for bioactive supplementation targeting individuals with looking to maintain healthy blood glucose regulation.

This trial was registered at clinicaltrials.gov as NCT06417840.

Keywords: dietary supplements, N-trans caffeoyltyramine, N-trans feruloyltyramine, prediabetes, Type 2 Diabetes, glucose regulation, HNF4α, NCT, NFT



Graphical Abstract: NCT/NFT supplementation demonstrated improved glucose regulation in prediabetics.

INTRODUCTION

Glucose homeostasis is central to normal physiological function and is the foundation of long-term metabolic health [1-2]. Insulin, the primary regulator of blood glucose levels, modulates hepatic gluco-regulatory pathways and facilitates glucose uptake into the muscle and adipose cells [1]. In the setting of chronically elevated blood glucose, increased insulin resistance and pancreatic beta cell death have also been implicated as critical mediators [3-4].

Prediabetes, an intermediary stage in the development of insulin resistance-related hyperglycemia, presents a unique opportunity for intervention and in some cases, even reversal to normoglycemia [5]. Prediabetes is defined by blood glucose levels that are higher than normal but not yet high enough to be considered Type 2 Diabetes Mellitus (T2D) [5]. Prediabetes affects the global population at unprecedented levels – 9.1% (464 million) with impaired glucose tolerance and 5.8% (298 million) with impaired fasting glucose [6]. In the U.S., 98 million people have prediabetes, translating to more than 1 in 3 American adults, underscoring the significant public health burden posed by abnormal glucose regulation [7].

Over time, chronic exposure to insulin leads to reduced insulin sensitivity, causing circulating glucose levels to remain high and leaving the pancreas unable to compensate, ultimately resulting in T2D [3-4]. T2D is highly prevalent - affecting almost 600 million (1 in every 9) people worldwide [8-9]and is associated with a myriad of severe complications, including cardiovascular and renal disease and retinopathy [7].

Early intervention can effectively manage hyperglycemia and substantially impede advancement to a full-blown disease state [5]. Pharmacological approaches have demonstrated effectiveness in improving pancreatic β-cell function and increasing insulin sensitivity but may present limitations with regard to adherence, adverse effects, and cost [10]. Lifestyle modification is primarily considered the cornerstone of diabetes prevention [11]. Normoglycemia can be achieved through nutritional interventions, including dietary supplements [5].

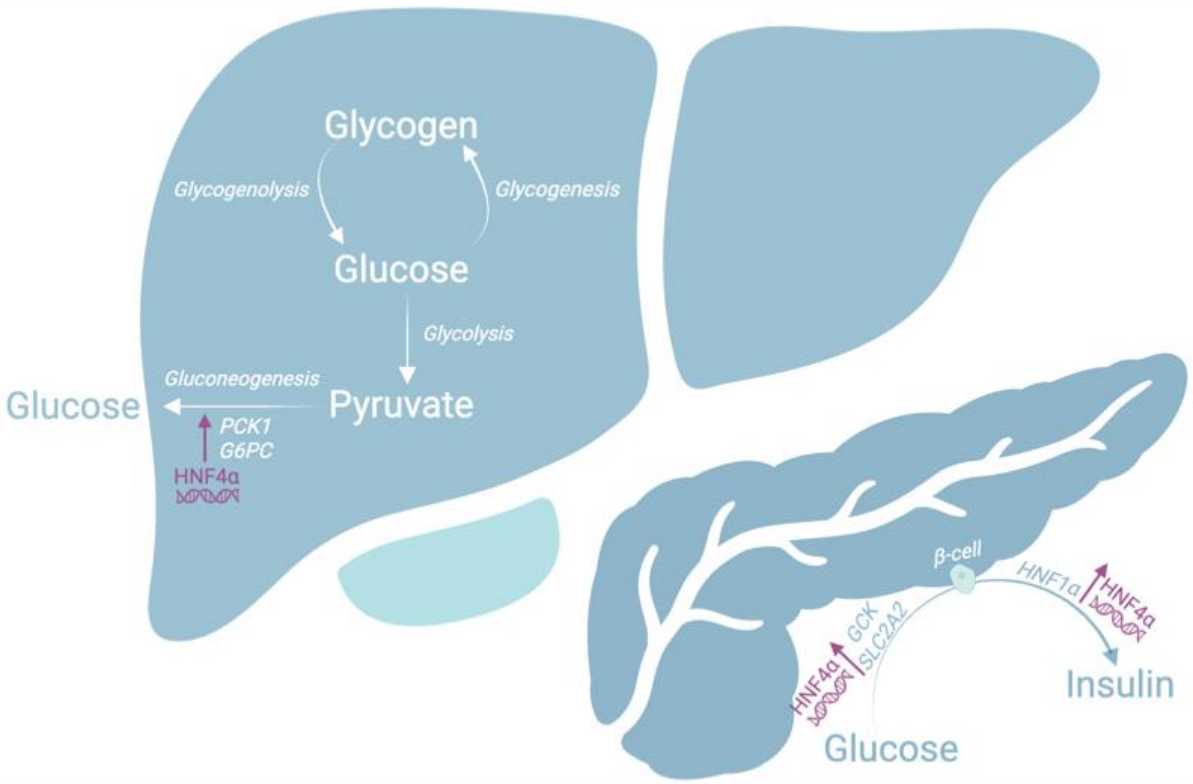


Figure 1. The Roles of HNF4α in Hepatic Glucose Regulation and Pancreatic Insulin Secretion. HNF4α activates genes involved in gluconeogenesis (PCK1, G6PC), glucose sensing (SLC2A2, GCK), and insulin secretion (HNF1α); PCK1: phosphoenolpyruvate carboxykinase 1; G6PC: glucose-6-phosphatase catalytic subunit; SLC2A2: solute carrier family 2 member 2; GCK: glucokinase; HNF1α: hepatocyte nuclear factor 1 alpha

In recent years, the role of dietary bioactives has garnered particular attention for improving glycemic control [12]. Bioactive compounds play a critical role in regulating glucose metabolism by modulating pancreatic insulin secretion, cellular glucose uptake, and hepatic glucose production [13] and can be promising candidates for functional food formulations and supplements. Novel bioactives, N-trans-Caffeoyltyramine (NCT) and N-trans-Feruloyltyramine (NFT), show promise for their effectiveness in regulating blood glucose. Emerging research has highlighted the role of NCT and NFT as hepatocyte nuclear factor 4 alpha (HNF4α) agonists, a key transcription factor often referred to as the 'master metabolic regulator' due to its critical role in both glucose and lipid metabolism [14-15]. Specific roles of HNF4α include glucose sensing and insulin secretion in the pancreas [16] and regulation of gluconeogenesis (figure 1), fatty acid oxidation, and lipid transport in the liver [15,17].

Preclinical studies confirm that NCT and NFT are potent HNF4α agonists with gluco-regulatory effects [18-19]. While promising, clinical studies are needed to validate these findings in humans. To our knowledge, this study is the first to evaluate the efficacy of bioactives NCT and NFT on upregulating HNF4α and improving blood glucose regulation in individuals with hyperglycemia. The effects of HNF4α agonists, NCT and NFT, on glycemic control in a prediabetic population are of particular interest, given the high prevalence of metabolic dysregulation globally and the potential to reverse prediabetes before the clinical manifestation of T2D.

Therefore, the present study was conducted to evaluate the effects of a proprietary dietary supplement containing 120 mg of NCT/NFT on fasting blood glucose and other glycemic biomarkers in an otherwise healthy

population with prediabetes. Exploring the effects of NCT/NFT supplementation on glycemia could offer insights into its potential to reverse prediabetes and its utility as an adjunctive treatment in T2D.

MATERIALS AND METHODS

Study design: This study was conducted at the accredited National Diabetes, Obesity and Cholesterol Foundation (NDOC) in New Delhi, India, and used a randomized, double-blind, placebo-controlled parallel-arm design. Free-living Asian Indian adults (Age: 18-50 years; BMI: 25-30 kg/m^2) with prediabetes (fasting blood glucose ≥100 mg/dL and <126 mg/dL) and abdominal adiposity (waist circumference ≥80 cm for females and ≥90 cm for males) were recruited to participate. Exclusion criteria were a current diagnosis of diabetes (Type 1 or Type 2), recent (within the past 3 months) weight change of ≥5% and/or use of weight-altering interventions (medication, surgery, etc.), history of a chronic gastrointestinal disorder, acute infection, pregnancy or lactation, and excessive alcohol consumption. Eligible participants were randomized to either the control or the intervention arm using computerized random number tables. Block randomization with variable block sizes was used to generate allocation sequences for assigning participants to receive either 120 mg/d sustained release NCT/NFT (treatment) or 280 mg/d microcrystalline cellulose (placebo) for an intervention duration of 4 weeks.

Outcomes were measured at baseline and end-of-study, on days 0 and 28. The primary endpoint was fasting blood glucose as assessed by venipuncture. Secondary endpoints included fasting insulin, continuous and postprandial glycemia as assessed by continuous glucose monitoring (CGM), HbA1c, anthropometrics, and serum lipids. The study design is summarized in Figure 2.

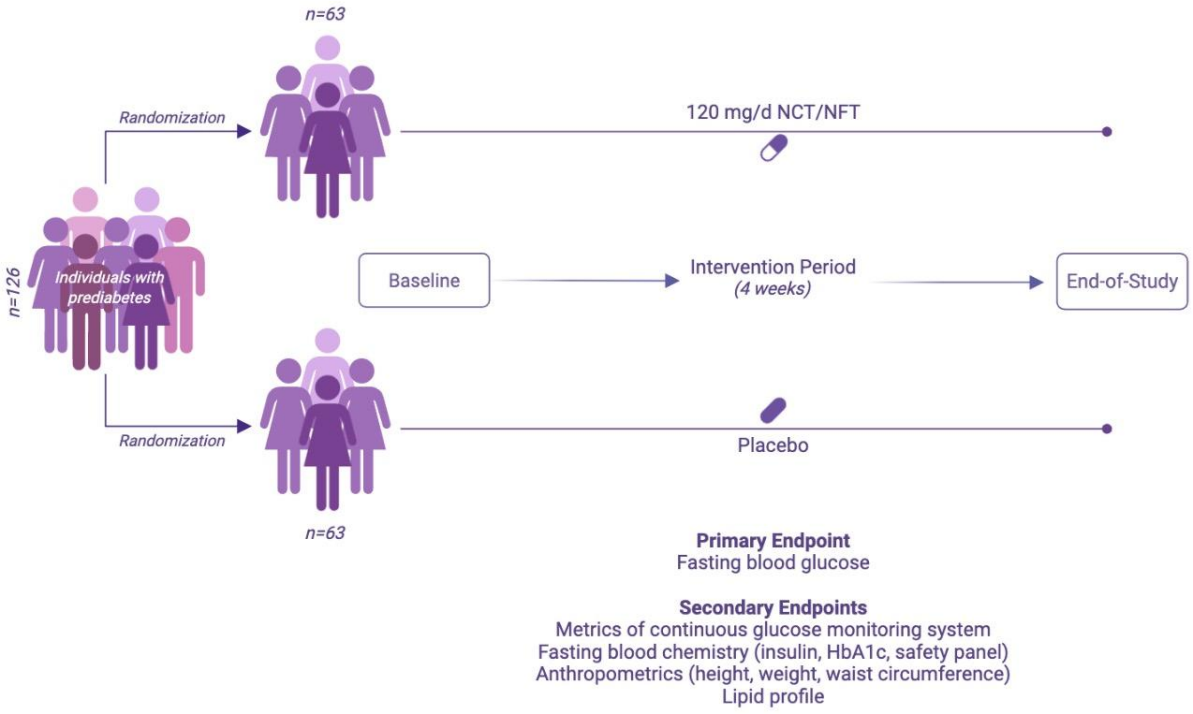


Figure 2. Study Design

The full trial protocol can be found in Supplementary File #1. Written informed consent was obtained from all participants, who were comprehensively briefed on study procedures. Following

recruitment, potential participants underwent a one-week run-in period during which their compliance with the protocol was monitored. Upon completion of the run-in period, participants underwent repeat screening. Those who met the eligibility criteria were randomized to either the intervention or control group.

Participants were advised to follow their habitual dietary pattern and to refrain from making any significant changes to their usual food intake. A 24-hour dietary recall was collected to monitor for significant deviations in intake throughout the intervention. Compliance with the intervention was rigorously monitored. Members of the research team communicated with participants regularly via bi-weekly telephone calls, weekly in-person meetings, and twice-weekly text messages. This open communication allowed for the continuous opportunity to ask questions and discuss issues. Text messaging was used to remind participants of intake instructions. Consumption of the study product was recorded in a compliance log.

Study products were encapsulated and were identical in size, texture, and color. Neither treatment was flavored. All study participants and members of the research team were blinded to intervention allocations. Treatments were distributed in opaque, sealed containers. Personnel involved in the study did not have access to the allocation sequence. Unblinding occurred only after the database lock.

This study was executed in accordance with the ethical principles outlined in the Declaration of Helsinki. It was consistent with the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) Good Clinical Practice (GCP) guidelines (2019), as well as the ethical guidelines of the Indian Council of Medical Research. The study protocol received approval (Registration Number: ECR/1233/Inst/dl/2019) from the Institutional Ethics Committee of the participating center (Fortis C-DOC Hospital for Diabetes, Metabolic Diseases and Endocrinology, New Delhi, India, https://www.fortishealthcare.com/location/fortis-cdoc-chirag-enclave-new-delhi). The trial was duly registered at ClinicalTrials.gov (NCT06417840; 05/16/2024).

Outcome Measures: Due to the high volume of data collected during this study, results will be published in two phases. The current manuscript will cover the following outcome measures, including select CGM endpoints (postprandial response and time in range). All others will be presented in a subsequent publication.

Primary Endpoint: Fasting blood glucose levels were measured. Venous blood samples were drawn from the antecubital vein following an overnight (10-12 hour) fast. Collected samples were centrifuged, and plasma/serum was aliquoted and stored at −80°C until further analysis. Glucose concentrations were determined by an enzymatic colorimetric test (Cobas Integra Analyzer, Roche Diagnostics GmbH, Mannheim, Germany).

Secondary Endpoints

i. Fasting Serum Insulin

   Insulin levels were measured using a chemiluminescence immunoassay (Roche Diagnostics GmbH, Mannheim, Germany).

ii. Continuous Glucose Monitoring (CGM)

   Blood glucose levels were continuously monitored using the latest version of the Guardian 4 CGM system (Medtronic MiniMed, Northridge, CA, U.S.A.), which operates on the principle of electrochemical detection of glucose in subcutaneous interstitial fluid. The sensor was inserted into the subcutaneous tissue of the lateral abdominal wall/upper arm and worn for three consecutive days at baseline (Day 1 to Day 3) and again from Day 28 to Day 31 at

the end of the intervention. Unlike earlier CGM models, the Guardian 4 is factory-calibrated and does not require fingerstick calibration, requiring only a 2-hour warm-up after insertion. It captures and stores 288 glucose readings every 24 hours during participants' routine daily activities. Participants were provided with one standardized test meal during the monitoring period, while all other meals reflected their usual dietary patterns. Data were automatically uploaded daily via the Guardian App to the web-based CareLink software (Medtronic MiniMed), which generated comprehensive glucose summaries. CGM data were analyzed to assess postprandial glucose responses following the standardized meal and to evaluate the duration spent within and outside the normoglycemic range (<140 mg/dL).

iii. Fasting Blood Chemistry

Additional fasting blood parameters were measured including a comprehensive safety panel to monitor participant health and safety during the intervention and glycated hemoglobin (HbA1c), which was calculated using immunoturbidimetry (Biorad Labs, CA, U.S.A.).

iv. Lipid Profile

Serum lipid concentrations were evaluated, including high-density lipoprotein (HDL), low-density lipoprotein (LDL), very-low-density lipoprotein (VLDL), total cholesterol (TC), and triglycerides (TG). Total cholesterol and HDL were measured using the cholesterol oxidase esterase peroxidase (COEP) and direct measure polyethylene glycol (PEG) methods, respectively (Integra Analyzer, Roche Diagnostics GmbH, Mannheim, Germany). Triglycerides were quantified by the glycerol phosphate oxidase-p-aminophenazone (GPO-PAP) method (Cobas Integra Analyzer, Roche Diagnostics GmbH, Mannheim, Germany). LDL and VLDL values were calculated.

v. Anthropometric Measurements

Anthropometric data were collected to monitor changes in body composition. Measurements included waist circumference, body weight, height, and calculation of body mass index (BMI). Waist circumference was measured mid-way between the iliac crest and the lowest margin of the ribs while the individual was standing straight. Height was measured using a stadiometer to the nearest 0.1 cm with the subject's head held in the Frankfort plane. Weight was measured to the nearest 0.1 kg using the InBody 770 Body Composition Analyzer. Subjects were instructed to remove heavy clothing and to stand still with body weight evenly distributed between both feet. BMI was calculated by dividing weight in kilograms by the square of height in meters.

vi. Dietary Data

Dietary intake information was obtained using the 24-hour dietary recall method and analyzed with "DietCal" software (version 15.1.1 Profound Tech Solution; http://dietcal.in/), utilizing nutrient values from the Nutritive Value of Indian Foods database.

Statistical Analysis: Sample size was calculated based on the primary endpoint, fasting blood glucose (FBG). A

reduction in mean FBG from a prediabetic level (110 ± 20 mg/dL) to a normoglycemic level (<100 mg/dL), representing a difference of 10 mg/dL, corresponds to an effect size (δ) of 0.50. Based on this effect size and the aim of achieving 80% statistical power (α=0.05, two-sided), the required sample size was estimated to be 126 participants (n=63 per study arm).

For the primary endpoint (FBG), the effect of the intervention on change from baseline was tested using an analysis of covariance (ANCOVA), with sex, age, and baseline FBG as covariates and intervention as the fixed effect. Secondary endpoints were analyzed in a manner similar to the primary endpoints. Adjustments for multiplicity were not performed; therefore, caution should be used when interpreting these results as anything other than supportive of the primary efficacy findings due to increased potential for Type I error.

Statistical analyses were conducted utilizing SAS Version 9.4 (www.SAS.com). Missing data were assumed to be missing at random and were not imputed. Data was tested for normality and transformed as needed. A p-value of <0.05 was considered statistically significant. The full statistical analysis plan can be found in Supplementary File #2.

RESULTS

This study was completed between June 2024 and February 2025. A total of 360 individuals were screened for eligibility, of whom 126 met inclusion/exclusion criteria and were subsequently enrolled in the study. Enrolled participants were randomized to either the NCT/NFT treatment (n=63) or placebo group (n=63) (Figure 3). All 126 participants completed the study and were included in the analysis. Compliance with the protocol was over 90%.

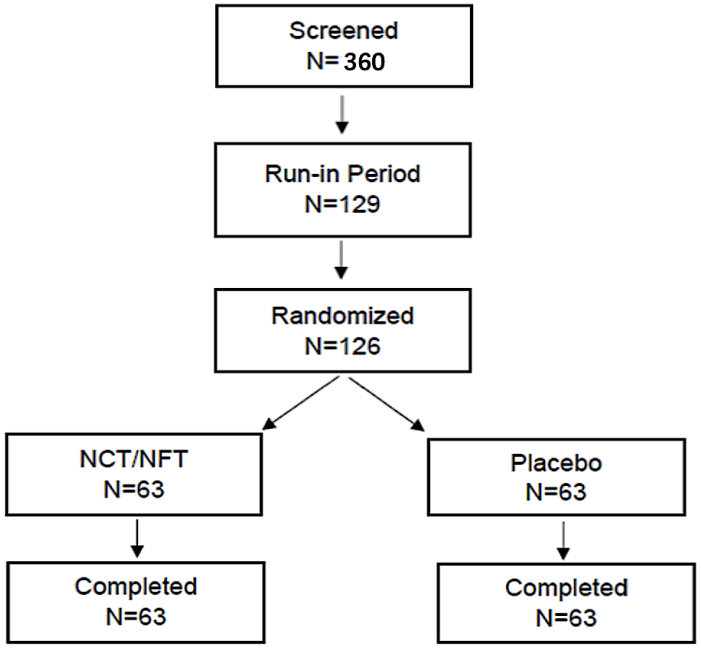


Figure 3. Consort Diagram.

Baseline Characteristics: The baseline characteristics of study participants are provided in Table 1**.** Treatment and placebo groups were comparable in terms of age, BMI, and waist circumference at baseline. Groups also had similar energy and macronutrient intake and maintained their habitual diet throughout the trial.

Table 1. Baseline Characteristics.

	NCT/NFT (n=63)	Placebo (n=63)
Age (years)	43.1 ± 9.4	44.4 ± 9.9
Gender (M:F)	41:22	30:33
BMI (kg/m^2)	27.2 ± 1.6	27.4 ± 1.5
Waist Circumference (cm)	97.7 ± 6.3	97.3 ± 6.5
Fasting Blood Glucose (mg/dL)	108.4 ± 8.3	108.1 ± 6.4
Fasting Insulin (mU/mL)	19.6 ± 11.6	18.2 ± 14.8
HbA1c (%)	5.9 ± 0.4	5.8 ± 0.3
Total Cholesterol (mg/dL)	173.5 ± 39.4	176.9 ± 41.2
HDL (mg/dL)	39.9 ± 9.9	40.4 ± 10.0
LDL (mg/dL)	107.3 ± 32.1	109.5 ± 32.5
VLDL (mg/dL)	30.7 ± 14.9	30.6 ± 15.1
Triglycerides (mg/dL)	149.0 ± 67.3	145.0 ± 55.5

Values are means ± standard deviation. HbA1c: hemoglobin A1c; HDL: high-density lipoprotein; LDL: low-density lipoprotein; VLDL: very-low-density lipoprotein

Fasting Blood Glucose: At baseline, mean FBG levels were comparable between the NCT/NFT and placebo groups (108.4 ± 8.3 mg/dL vs 108.1 ± 6.4 mg/dL, respectively). Following the intervention period, the NCT/NFT group exhibited a mean change from baseline of -4.1 ± 7.1 mg/dL, while the placebo group demonstrated a lesser change of -0.7 ± 7.0 mg/dL (Table 2). While it is appreciated that there is a high degree of variability in this result (as evidenced by the large standard deviation), the retained statistical significance suggests that the effect size is also sufficiently large. The difference in change in fasting blood glucose between the treatment and placebo groups was statistically significant (p=0.0026; Figure 4), indicating a meaningful improvement in fasting glycemia attributable to the treatment.

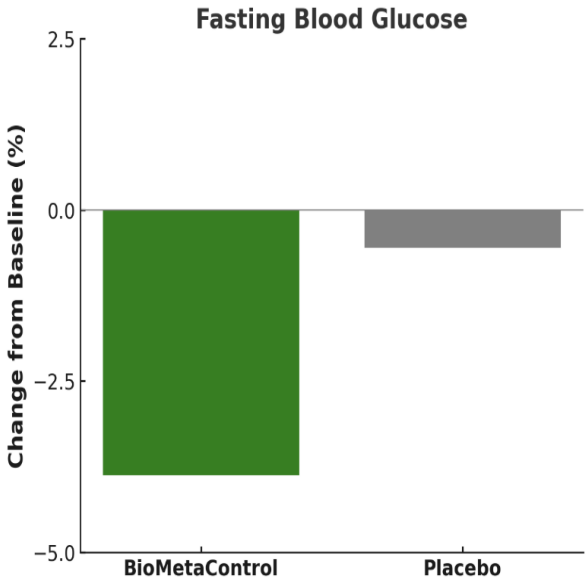


Figure 4. Statistically significant reduction in Fasting Blood Glucose in the NCT/NFT group compared to the placebo group; Asterisk (*) denotes statistical significance p<0.05 (p=0.0026).

Table 2: Fasting Blood Glucose and Insulin at Baseline and End-of-Study.

	Glucose (mg/dL)		Insulin (mU/mL)	
	Day 0	Day 28	Day 0	Day 28
NCT/NFT	108.4 ± 8.3	104.2 ± 7.8	19.6 ± 11.6	19.2 ± 9.8
Placebo	108.1 ± 6.4	107.5 ± 8.9	18.2 ± 14.8	20.9 ± 15.5

Values are presented as means ± standard deviation.

Fasting Insulin: The difference in change from baseline in fasting insulin was also statistically significant between the treatment and placebo groups (p=0.0147; Figure 5). Compared to baseline, fasting insulin in the placebo group increased by 2.7 ± 7.8 mU/mL, whereas values in the NCT/NFT group were reduced (-0.4 ± 8.5 mU/mL; Table 2).

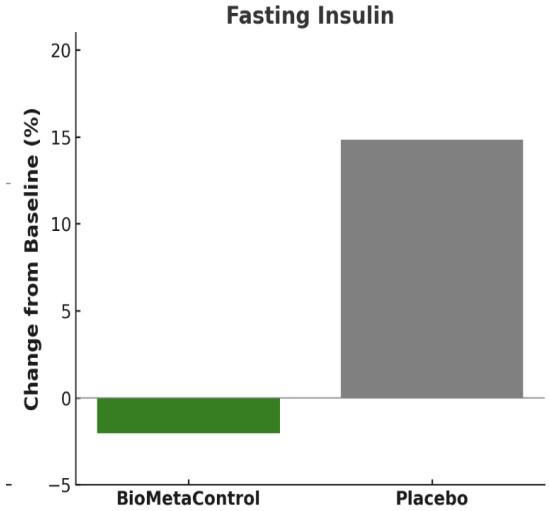


Figure 5. Statistically significant reduction in Fasting Insulin in the NCT/NFT treatment group compared to the placebo group; Asterisk (*) denotes statistical significance p<0.05.

Postprandial Glucose Monitoring: Average 24-hr continuous glucose measures in treatment and placebo groups before and after the intervention are showcased in Figure 6. Baseline blood glucose was higher in the NCT/NFT group, and analyses were adjusted for this. A summary of CGM outcome results is provided in Table 2.

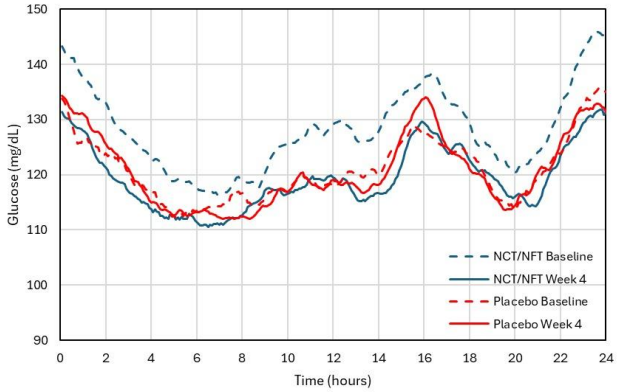


Figure 6: Average 24-Hr Continuous Glucose Measures

This figure shows an average of measured 72-hour blood glucose values illustrated as a 24-hour profile. The X-axis indicates time over a 24-hour continuous glucose monitoring period, and the Y-axis shows blood glucose levels ranging from 90 to 150 mg/dL (5.0 to 7.8 mmol/L). The dotted blue line represents the treatment group at baseline, while the solid blue line shows values after 4 weeks of intervention. The dotted red line represents the placebo group at baseline (n = 63), and the solid red line corresponds to the placebo group after 4 weeks (n = 63).

i. Postprandial AUC

Postprandial AUC was evaluated following the consumption of a standardized meal. After the 4-week intervention, the NCT/NFT-treated group demonstrated a significant decrease in postprandial blood glucose response, while the placebo group was unchanged. This difference was statistically significant (p=0.0036).

ii. Postprandial Hyperglycemia AUC

iii. Similar results (blunted postprandial response in the NCT/NFT treatment group) were observed for postprandial AUC, which focuses on the portion of post-meal AUC where blood glucose is above the normal range (p=0.0001).

Time Spent Within and Outside of Ideal Glucose Range

I. Time Spent Within Ideal Glucose Range

The ideal range for blood glucose levels was defined as <140 mg/dL. As a group with prediabetes, time spent in this range was 75-80%, which was not significantly altered with the NCT/NFT intervention (Absolute: p=0.9598; AUC: p=0.6992).

II. Time Spent Outside of Ideal Glucose Range

In contrast, participants who were treated with NCT/NFT exhibited a mean decrease of 417 minutes spent out of range, representing approximately 10% less time, whereas participants who received a placebo experienced no change (Absolute: p=0.0127; AUC: p=0.0138).

Table 4. Summary of Differences in CGM Outcomes between NCT/NFT and Placebo Groups. Values represent change from baseline and are presented as means ± standard deviations. Asterisk (*) denotes statistical significance (p<0.05). AUC: area under the curve.

Outcome	NCT/NFT	Placebo	p-value
Postprandial (mg/dL) AUC	-1684.1 ± 2479.8	40.6 ± 2279.4	0.0036*
Postprandial Hyperglycemia (mg/dL) AUC	-13283.6 ± 19549.1	6253.6 ± 24073.9	0.0001*
Time in Range (minutes), Absolute	238.7 ± 641.3	88.8 ± 986.7	0.9598
Time in Range (minutes), AUC	19381.5 ± 69911.8	7873.4 ± 106911.8	0.6992
Time out of Range (minutes), Absolute	-417.1 ± 572.0	31.0 ± 777.0	0.0127*
Time out of Range (minutes), AUC	-73027.5 ± 98561.1	4900.9 ± 139809.8	0.0138*

Glycated Hemoglobin (HbA1c): No changes were observed for HbA1c following the intervention, either within or between groups.

Lipid Profile: In this normolipidemic subject population, no further improvement in lipid profile (HDL, LDL, VLDL, TC, or TG) was observed in either group.

Anthropometric Measures: There were no significant changes in weight, BMI, or waist circumference in either group.

Safety and Adverse Events: Clinical chemistry tests were conducted in the NCT/NFT treatment group before and after the intervention. No safety-related events were observed, and all values remained within the normal

range. Further, no adverse events were reported in either study arm related to the study treatment.

DISCUSSION

Nutritional intervention remains the first-line approach for both the treatment and prevention of elevated blood glucose levels [5,11]. Specifically, recent research has uncovered a role for dietary bioactives in improving glycemic control [12-13]. As HNF4α agonists, NCT and NFT, show promise. HNF4α has critical gluco-regulatory functions, including pancreatic insulin release and hepatic glucose production [15-17]. Preclinical studies demonstrate a similar positive response with administration of NCT and NFT [18-19]. The present study sought to confirm the effects of a proprietary dietary supplement containing NCT/NFT on fasting blood glucose and other biomarkers of glycemic control in an otherwise healthy population with prediabetes.

Supplementation with a proprietary sustained-release formulation containing 120 mg/d NCT/NFT for 4 weeks resulted in significant improvements in the primary outcome measure, fasting blood glucose, as well as several secondary endpoints. Fasting insulin was also significantly reduced.

Fasting blood glucose is an essential indicator of overall physiological function [20]. When blood glucose is regulated normally, circulating levels are maintained below a threshold (<100 mg/dL), providing adequate energy to vital organ systems while also ensuring sufficient reserves for future needs [21]. It also preserves the integrity of the vasculature, including both macro- and micro-vessels [22]. High concentrations of glucose in the circulation damage the blood vessels and, over time, impair the delivery of oxygen and nutrients to their physiological targets [22]. Organ system dysfunction, e.g. renal failure, is a common complication secondary to uncontrolled hyperglycemia, as are retinopathy and neuropathy [22]. In this way, glucose homeostasis is central to both metabolic and systemic function.

Glucose metabolism is intricately connected to other metabolic pathways [20]. Significantly, glucose concentration directly influences the secretion of insulin, which modulates metabolic processes to ensure sufficient glucose availability to the cells [20]. By promoting storage and suppressing the catabolism of lipids and proteins, insulin regulates the flux of glucose and its substrates - amino acids and free fatty acids - between the liver, skeletal muscle, and adipose tissue to meet cellular energy needs [20]. Although insulin has key regulatory functions, chronic hyperinsulinemia is associated with weight gain, insulin resistance, and increased risk of obesity and T2D [23]. Therefore, the balance between glucose and insulin is vital for energy homeostasis and broader metabolic function.

Supportive of the primary outcome, postprandial blood glucose response was also significantly improved. Exaggerated spikes in blood glucose levels often precede an over-compensatory insulinemic response, resulting in a subsequent, pronounced drop in circulating glucose levels. Low blood glucose in response to overcompensatory insulin action, or reactive hypoglycemia, disrupts appetite and energy balance, resulting in lethargy, food cravings, impaired focus, and mood disturbances [24]. Study participants reported sustained improvements in energy balance following the intervention.

Time spent within the ideal glucose range was not significantly affected by the intervention, likely because, in the absence of clinically relevant T2D, the study population already spent 75-80% of time with blood glucose levels below 140 mg/dL. In contrast, time spent outside of the ideal blood glucose range was significantly reduced, indicating improved glycemic control. Sustained euglycemia supports energy balance, preserves insulin sensitivity, and reduces metabolic stress, thereby promoting metabolic health [25].

Glycated hemoglobin was unchanged. This is not surprising considering hemoglobin takes 2-3 months to turn over, meaning the 4-week intervention period was likely not sufficient time for a measurable change in glycemic control to occur [26]. The lack of change in lipid profile may be explained by participants being normolipidemic at baseline. The short duration of the intervention period and baseline normality of select endpoints of interest may be considered limitations of this study. Future studies should employ a longer intervention period and target individuals with baseline abnormalities to fully elucidate physiological differences and demonstrate beneficial effects.

The intervention was deemed safe and well-tolerated. Clinical chemistry parameters remained within established reference ranges across both groups. No safety-related events were observed, and no adverse events attributable to the study treatment were reported in either arm.

Taken together, these findings demonstrate marked improvements in glucose metabolism, including regulation of circulating insulin levels and attenuated postprandial glucose excursions. These findings are particularly relevant to a population with prediabetes, who are at high risk of developing T2D. Ameliorating blood glucose regulation in this group can effectively prevent or delay progression to a full-blown disease state [5]. With metabolic dysfunction at unprecedented levels, a significant portion of the population could benefit from metabolic support [7]. As the catalyst in a cascade of physiological functions, including energy balance and fat metabolism, the benefits of supporting healthy blood glucose levels span well beyond basic glycemic control [20].

Existing strategies for metabolic regulation primarily rely on pharmaceuticals, and while the efficacy of such interventions is undisputed, they may be limited in terms of accessibility, affordability, and suitability for long-term use [10]. One such medication is the glucagon-like-peptide receptor agonist GLP-1 RA, which acts on receptors in the pancreas to stimulate glucose-dependent insulin secretion and suppress glucagon secretion, effectively lowering circulating blood glucose [27-28]. Human physiology is complex, oftentimes with multiple pathways working in synergy. In addition to incretin hormones like GLP-1, augmenting HNF4α activity is another way to improve glycemic control. Given that they target different pathways, HNF4α agonists such as NCT and NFT are ideally suited to complement GLP-1 agonists. Indeed, preclinical data show that HNF4α regulates glucose-dependent insulinotropic polypeptide (GIP) expression in intestinal K-cells [29], suggesting that HNF4α agonists like NCT/NFT could enhance endogenous incretin production, amplifying the effects of GLP-1 RAs. Given that NCT/NFT are found in and consumed as part of functional food items like black pepper, onion, garlic, etc., and because their purified form has been assessed for safety and toxicity [30-31], they present as ideal dietary supplement candidates for glycemic regulation.

In a population with impaired glucose regulation, a multifaceted approach harnessing diverse biological pathways in addition to diet and lifestyle modification may offer more effective and sustainable improvements in metabolic outcomes. In this regard, a dietary supplement containing NCT/NFT is well-poised to support other therapeutic strategies as part of a multifaceted framework for blood glucose regulation. When used as an adjunct, NCT/NFT could help reinforce glycemic control, particularly in individuals who struggle to achieve normoglycemia with diet and exercise alone. Overall, these findings support further research into the integration of NCT/NFT alongside established lifestyle measures, which remain the cornerstone of diabetes prevention; however, longer-term and larger studies are needed to confirm sustained benefit.

In conclusion, supplementation with a proprietary sustained release formulation containing 120 mg/d NCT/NFT for 4 weeks significantly improved fasting blood glucose as well as other measures of glycemic control, including fasting insulin, postprandial glucose response, and time spent outside of the ideal blood glucose range. No adverse events were reported, and treatment was well-tolerated. As potent HNF4α agonists, NCT/NFT demonstrate strong potential for a safe option [32-33] for healthy blood glucose regulation and overall metabolic support. The described proprietary dietary supplement containing NCT/NFT is well-positioned to mediate these effects in conjunction with other therapeutic strategies. Taken together, these results highlight the role of NCT and NFT in optimizing glucose metabolism and support their inclusion as a dietary supplement in comprehensive approaches aimed at promoting healthy blood glucose regulation.

Scientific Innovation: The trial utilized a rigorous design to evaluate a proprietary supplement containing NCT and NFT, targeting elevated fasting blood glucose, fasting insulin, postprandial blood glucose, and other blood glucose-related parameters. The significant improvements in cross-sectional and continuous endpoints represent clinically meaningful outcomes rarely observed in short-term dietary interventions, offering a multidimensional view of the bioactives' potential mechanisms. These insights help establish NCT/NFT as promising candidates for functional food formulation and for clinical nutrition strategies to support healthy blood glucose regulation. One such clinical nutrition strategy is for adults weaning off GLP-1 drugs to help maintain healthy blood glucose levels.

Practical Implications: Supplementation with NCT/NFT for just four weeks demonstrated marked improvement in blood glucose regulation endpoints in subjects with prediabetes. A combination of cross-sectional and continuous endpoint assessment provided a comprehensive picture of the potential of these bioactives in helping to improve blood glucose regulation. The proprietary formulation shows potential as a dietary supplement for managing healthy blood glucose and offers a non-pharmacological approach for individuals seeking to lower elevated blood glucose levels or adults seeking to maintain healthy blood glucose levels once they discontinue the use of a glucose-regulating drug. These findings support its further development for commercial and clinical use in metabolic support.

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