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Evaluation of a Synthetic Spermidine–Spermine Supplement with Arginine and BB12: Safety Outcomes and Beclin-1 Modulation in a Randomized Placebo-Controlled Pilot Trial in Older Adults

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Evaluation of a Synthetic Spermidine–Spermine Supplement with Arginine and BB12: Safety Outcomes and Beclin-1 Modulation in a Randomized Placebo-Controlled Pilot Trial in Older Adults.

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Abstract

Background: The aging population is expanding with the proportion of adults over 60 years projected to double by 2050. Healthspan extension, the maintenance of functional resilience across older adulthood is critical. Cellular aging drives a diminished healthspan. Polyamines are crucial for cellular health, yet declining intracellular polyamine concentrations correlate with increasing age. Nutraceutical interventions that modulate polyamine pathways are of growing interest; however, many lack evaluation of safety and exploration of biological mechanisms.

Objective: We conducted a placebo-controlled randomized pilot study to assess the safety and mechanistic effects of Cellitas®, a proprietary oral supplement combining synthetic spermidine and spermine, arginine and the probiotic *Bifidobacterium animalis subsp. Lactis* (Trial ID NCT06445569, 31/5/2024-retrospective registration). Forty-two pre-diabetic adults aged over 50 years were assigned to a placebo or Cellitas® arm and followed for 24 weeks. Safety endpoints included adverse events and changes to biochemical, haematological, glycaemic and inflammatory cytokine profiles. Exploratory endpoints included levels of Beclin-1 in serum. Beclin-1 initiates autophagy, coordinating autophagosome formation and has important functions in endocytic trafficking, apoptosis regulation, membrane dynamics and cellular stress response

Results and Conclusion: Cellitas® was safe with no serious adverse events or adverse shifts in clinical biomarkers. Serum Beclin-1 increased over 24 weeks, with no change observed with the placebo, consistent with a possible biological effect on Beclin-1 pathways. This is the first preliminary evidence on the safety of a synthetic polyamine–arginine–probiotic formulation, demonstrating a favourable safety profile. Further trials are important to fully elucidate its effects on autophagic signalling.

Introduction

Global population aging is accelerating, with adults aged greater than 60 years projected to exceed 2.1 billion by 2050 [WHO, 2017]. Although life expectancy continues to rise, healthspan, the period of life lived in good metabolic, immune, and functional health, has not kept pace. Age-related declines in physiological resilience contribute to multimorbidity and impaired recovery from medical conditions [Niccoli and Partridge (2012)]. Among the multiple biochemical correlates of aging, a decrease in intracellular polyamines has been described in pre-clinical models and during human aging [Zhang et al. (2019), Alsaleh et al. (2020), Elworthy et al. (1989), Das et al. (1982), Ferioli et al. 1975]. Polyamines have essential cellular functions, including regulation of cell growth, proliferation, differentiation, apoptosis and autophagy [Pegg et al. 1982]. Their depletion will halt cell growth and proliferation. Key polyamines in mammalian cells are spermidine, spermine and putrescine. Polyamines can be sourced from dietary intake, endogenous biosynthesis, and production by the gut microbiota [Nakamura et al. (2019); Nishimura et al (2006); Eisenberg et al. (2009)]. Several studies have reported that administration of spermidine extends the lifespan of yeast, flies and worms, and effects human immune cells in an autophagy-dependent manner [Hofer et al. (2024), Alsaleh et al. (2020), Gupta et al. (2013)]. Spermidine is thought to promote autophagy through inhibition of acetyltransferase EP300, modulation of transcriptional regulators, and facilitation of autophagosome formation [Pietrocola et al. 2015]. Spermidine can also promote mitophagy, a specialized form of autophagy, in pre-clinical models [Zhang et al. 2023, Fairley et al. 2023, Liang et al. 2021, Eisenberg et al. 2016, Puleston et al. 2019]. Spermine is a less-studied polyamine yet has been identified as anti-inflammatory factor and an inhibitor of monocyte activation, suppressing the synthesis of TNFα and other proinflammatory cytokines in human immune cells. Spermine also reduces inflammasome assembly in pre-clinical models [Levy et al. 2015 and Zhang et al. 1999].

Dietary supplementation of spermidine has been associated with health-promoting effects (Hofer et al. 2022; Schroeder et al. 2021; Schwarz et al 2022]. Interestingly, increased levels of whole-blood spermidine are linked to longevity in healthy nonagenarians and centenarians [Pucciarelli et al. 2012]. Furthermore, epidemiological studies and preclinical research correlate elevated dietary polyamine uptake with reduced overall mortality and diminished cardiovascular-related mortality [Kiechel et al. 2018; Eisenberg et al. 2016; Wang et al 2020]. Despite growing interest in polyamine supplements, few formulations have undergone evaluation of safety or mechanistic effects in humans. Polyamine supplements often use plant- or yeast-derived

spermidine. Synthetic spermidine and spermine may offer advantages in chemical definition and reproducibility, but their combined safety profile in humans is uncharacterised. This study explores a synthetic polyamine (spermidine and spermine) formulation, combined with arginine and the probiotic *Bifidobacterium animalis* subsp. *lactis* (BB-12). Arginine is a precursor for the synthesis of polyamines and essential for normal T-cell proliferation and function and for nitric oxide generation. [Carriche et al. 2021; Lorin et al. 2014; Paulo et al. 2007]. Low levels of dietary L-Arginine have been associated with high levels of C-reactive protein (CRP), a biomarker of inflammation [Nazarian et al. 2019]. The probiotic *Bifidobacterium animalis* subsp. *lactis* BB-12, is reported to modulate gut microbiome and increase gut polyamine levels in humans, mucosal immunity and metabolic function, stimulating the induction of regulatory T cells and the production of the anti-inflammatory cytokine Interleukin-10 [Kibe et al. 2014]. Arginine and the probiotic BB-12 components of the supplement are of interest as age-related chronic inflammation or 'inflammaging' and dysbiosis are hallmarks of aging (López-Otín et al. 2022) yet few clinical studies have examined their effects on serum biomarkers.

The combined effects of arginine and BB-12 with synthetic polyamines (spermidine and spermine) had not been evaluated in a clinical study. To address this knowledge gap, we conducted a first-in-humans randomized, placebo-controlled pilot study evaluating the safety of the novel supplement Cellitas® comprising synthetic spermidine, synthetic spermine, arginine, and BB-12, administered for up to 24 weeks in adults aged over 50 years with pre-diabetes. Adverse events, comprehensive biochemical and haematological profiles, glycaemic control, lipid and inflammatory cytokine profiles were assessed. Additionally, we explored its effects on Beclin-1, a master regulator of autophagy initiation involved in autophagosome nucleation, apoptosis regulation, endocytic trafficking, and cellular stress responses. Our findings inform future trials targeting cellular health in an aging population.

Materials and Methods

Study Design and Ethics This randomized, double-blind, placebo-controlled pilot study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. The protocol was prospectively approved by an independent Institutional Review Board (Sterling IRB; reference K03-21-01-T0014) and registered with ClinicalTrials.gov (NCT06445569) retrospectively. All participants provided written informed consent prior to enrolment. A CONSORT flow diagram can be seen in Fig1 and FigS1. Forty-two males and females aged over 50 years with a Body Mass Index (BMI) of 25.0–45.0 kg/m² were recruited. Inclusion and exclusion criteria were predetermined prior to screening (Supplementary Methods S1 and Figure S2).

Randomization and Blinding Participants were randomized (1:1) to receive either the active intervention or placebo. Both participants and the clinical/laboratory research teams remained blinded to treatment allocation throughout the initial 12-week phase, prior to the open-label crossover phase (FigS3 and Supplementary Methods S4). The study employed a unidirectional crossover design for the placebo arm. Participants in the active arm received Cellitas® continuously for 24 weeks. Participants in the placebo-crossover arm received a matched placebo for the first 12 weeks, then crossed over to the active product for the remaining 12 weeks (Weeks 12–24) in an open-label fashion. The daily active dose consisted of three capsules containing Arginine, Spermidine and Spermine) and one stick packet of *Bifidobacterium animalis* subsp. *Lactis* (FigS5). Clinical visits occurred at Screening, Baseline (Day 0), Week 12, and Week 24. Participants fasted for 12 hours prior to all blood draws.

Outcome Measures The primary objective was to evaluate safety of the Cellitas® supplement (hereafter referred to as Cellitas) in pre-diabetic older adults. Endpoints included changes in fasting blood glucose (FBG), fasting insulin (FI), glycated haemoglobin (HbA1c), and dynamic glucose handling via an Oral Glucose Tolerance Test (OGTT). inflammatory markers (TNF-α, CRP, IL-6), lipid profiles, and safety assessments, the number and severity of Adverse Events (AEs)- FigS6. The secondary objective was exploratory, examining effects on serum levels of the autophagy biomarker Beclin-1.

Laboratory Assessments Clinical laboratory assessments were conducted by SGS Central Laboratory Services (ISO 15189 accredited). Routine biochemistry, lipids, and high-sensitivity C-reactive protein (hsCRP) were quantified on a Roche Cobas 8000 series analyzer. Hematological parameters were analyzed on a Sysmex XN-Series analyzer. Cytokines (IL-6, TNF-α) were quantified using Meso Scale Discovery (MSD) multiplex assays. Serum Beclin-1 was quantified using a commercial sandwich ELISA (Novus Biologicals, USA). Further details are described in Supplementary Methods S6.

S*tatistical Analysis* Consistent with CONSORT guidelines for pilot trials, no formal power calculation was performed; the sample size (n=42) was selected to estimate effect sizes for future definitive studies. Data normality was assessed using the Shapiro-Wilk test. Within-group changes were analyzed using paired Student's t-tests (parametric) or Wilcoxon matched-pairs signed-rank tests (non-parametric). Analyses were performed using GraphPad Prism version 11.0, with p-values <0.05 conservatively interpreted as indicative of potential significant trend.

Results

Baseline Characteristics of the Study Population

52 volunteers aged over 50 years were screened and 42 recruited to the study, with 21 participants allocated to the Cellitas group and 21 participants to the placebo group. Two participants were lost to follow-up after screening (Fig1 and S1). Participants average age was 57.6 years and not significantly different between the Cellitas arm and the placebo arm. Biological sex, ethnicity and baseline BMI (body mass index) were of comparable magnitude in both groups. There was no significant within-in group change in BMI from day 0 to week 24 in the Cellitas arm (p=0.46) and no within-group change of note from day 0 to week 12 in the placebo arm (p=0.25). Overall, these data suggest that the study cohorts exhibited homogeneity in terms of baseline demographics; no clinically relevant disparities in age, weight, ethnicity or BMI were observed between the randomized groups (Table 1).

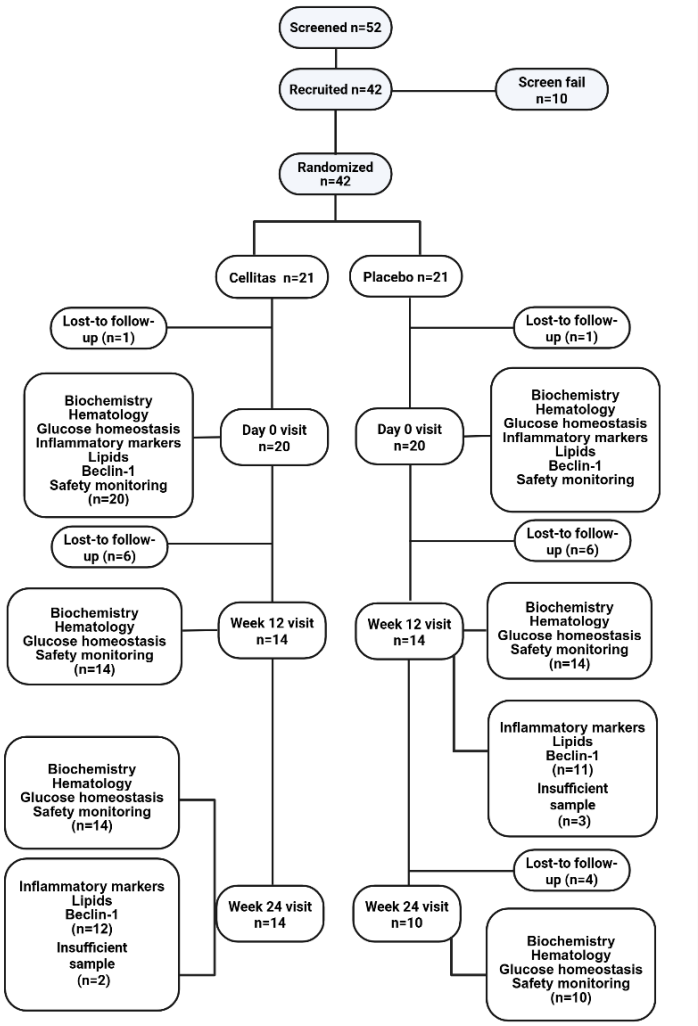


Fig1. CONSORT flow diagram. The flowchart details participant progression through the 24-week randomized, double-blind, placebo-controlled pilot trial. This includes the phases of enrollment, allocation, follow-up, and final analysis. Abbreviations: CONSORT, Consolidated Standards of Reporting Trials; n, number of participants. A larger print detailed version can be seen in Figure S1. Recruitment to the study started 2021-12-20 and pilot study was completed on 2023-06-08.

Parameter	Cellitas	Placebo
Participants (n=)	20	20
Sex (number)		
Male	5	6
Female	15	1
Age (years)		
Mean ± SD	56.3 ± 4.74	57.7 ± 5.2
Range	50–67	51–67
BMI (kg/m²)	33.38 ± 5.47	33.88 ± 5.06
Ethnicity:		
European ancestry	7	8
African ancestry	11	11
Not reported	2	1

Table 1. Baseline demographic and anthropometric characteristics of the study participants. Data are presented as number of participants (n) for categorical variables or Mean Standard ±Deviation (SD) for continuous variables. Abbreviations: BMI, Body Mass Index

Stability of Glycemic and Insulinemic Indices Post-Intervention in Cellitas group.

Due to participant attrition limiting the statistical power of the originally planned crossover comparison, analyses were restricted to longitudinal within-group changes. This approach allowed for the evaluation of temporal effects of the Cellitas intervention over the 24-week period. Glycated Hemoglobin (HbA1c), Fasting Blood Glucose (FBG), Fasting Insulin (FI) and Oral Glucose Tolerance Test (OGTT) were measured. HbA1c values represent long-term glycemic control with longitudinal values for HbA1c presented (Fig2a). Levels demonstrated stability throughout the intervention, recorded at 5.99 ± 0.24% (mean ± SD) at baseline, 5.89 ± 0.22% at Week 12, and 5.86 ± 0.24% at Week 24. Detailed analysis of the changes between time points, including mean differences, standard deviations (SD), and 95% confidence intervals (CI), is provided in Table S2. No significant mean differences were observed for any interval (Day 0-Week 12, p=0.34; Week 12-24, p=0.56; Day 0-Week 24, p=0.18). FBG concentrations remained stable throughout the 24 weeks (Fig2b) with mean measurements of 94.57 ± 10.02 mg/dL, 96.86 ± 5.67 mg/dL, and 93.21 ± 7.19 mg/dL at Day 0, Week 12, and Week 24, respectively. There were no significant mean differences or CI shifts between any time points (Table S2). Raw serum FI levels at each time point are visualized in Fig1c. FI levels exhibited a non-significant upward trend from baseline (8.88 ± 5.02 µIU/mL) to Week 12 (11.54 ± 5.96 µIU/mL) and Week 24 (12.71 ± 5.96 µIU/mL). The analysis of mean differences and 95% CI's confirmed that these changes were not statistically significant across any time comparison (p=0.06 for Day 0-12; p=0.54 for Week 12-24; p=0.10 for Day 0-24) (Table S2). The OGTT measured the dynamic response to glucose at 30, 60, 90, and 120 minutes post-ingestion (Fig1 e-h). Changes in mean differences were observed only at the 30- and 60-minute intervals (Table S2). At 30 minutes, a significant decrease in the mean difference was observed between week 12 and Week 24 (p=0.04). At 60 minutes, the mean difference significantly increased from Day 0 to Week 12

(p=0.002) but subsequently decreased from Week 12 to Week 24 (p=0.03). No significant differences were observed at 90 or 120 minutes.

Glycemic Stability in the Placebo Group.

Longitudinal changes to HbA1c, FBG, FI and OGTT were measured within the placebo group across three key intervals: the placebo phase (Day 0 to Week 12), the subsequent crossover phase where participants received Cellitas (Week 12 to Week 24), and the full study duration (Day 0 to Week 24). HbA1c remained stable throughout the study, with raw longitudinal values presented in Fig3a. Mean HbA1c levels showed no significant fluctuation, recorded at 6.01 ± 0.19% at baseline, 5.91 ± 0.27% at Week 12, and 5.92 ± 0.34% at Week 24. A detailed analysis of these changes, including mean differences, standard deviations SD, and 95% CI, is provided in Table S3. No statistically significant differences were observed across any time interval (Day 0-Week 12, p=0.11; Week 12-24, p=0.83; Day 0-Week 24, p=0.12) Fig3a. FBG levels remained in the clinically stable range throughout the 24-week period (Fig2b). During the placebo phase, mean levels shifted non-significantly from 95.48 ± 7.81 mg/dL at Day 0 to 99.14 ± 11.47 mg/dL at Week 12. Following the crossover to the Cellitas intervention, levels at Week 24 were recorded at 95.90 ± 8.71 mg/dL. As shown in Table S3, no significant within-group fluctuations in mean difference were detected between any time points, indicating that the glycemic status of the participants remained unchanged by the protocol. Raw FI concentrations across the study duration are visualized in Fig3c. Levels were recorded at 15.67 ± 9.92 µIU/mL at baseline, decreasing non-significantly to 13.89 ± 11.21 µIU/mL after 12 weeks of placebo, and subsequently measuring 16.72 ± 8.52 µIU/mL at Week 24. Analysis of mean differences (Table S3) confirmed no significant changes during the placebo phase (Day 0-Week 12, p=0.12), the crossover phase (Week 12-Week 24, p=0.10), or across the full study duration (Day 0-Week 24, p=0.91). OGTT dynamic glucose response at 30, 60, 90, and 120 minutes post-ingestion during the initial placebo phase (Day 0-Week 12), glucose excursions remained stable across all time points (Fig3d-g). Following the crossover to the Cellitas intervention (Weeks 12-24), a reduction in glucose levels was observed at the 120-minute post-load interval. As detailed in Table S3, the mean difference for this period was -20.3 mg/dL (SE 7.61; 95% CI: -37.51 to -3.09; p=0.03).

No significant changes were observed at the 30, 60, or 90-minute intervals during this phase. Overall, in both participant groups, Cellitas was well-tolerated, with no incidence of glycemic adverse events or adverse reactions recorded in either the Cellitas or placebo arms.

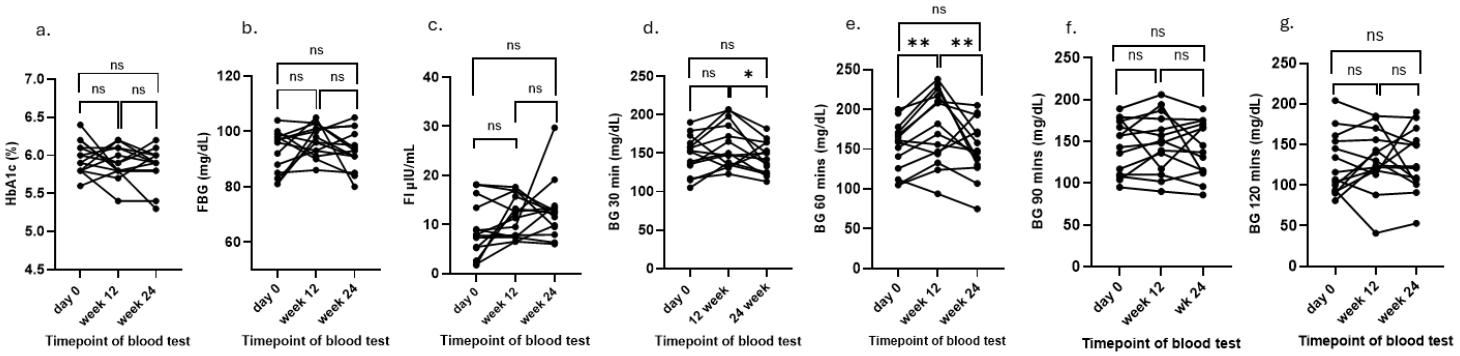


Fig2. Longitudinal Changes in Glycemic Parameters Across Study Timepoints in the Cellitas Group. The graphs display raw data values for (a) HbA1c, (b) FBG, (c) FI and (d-e) OGTT (at 30, 60, 90, and 120 min). Due to the normal distribution of the data, a paired t-test was employed to compare time intervals (Day 0, Week 12, Week 24). Statistical significance was defined as p<0.05*. Abbreviations: FBG, Fasting Blood Glucose; FI, Fasting Insulin; HbA1c, Glycated Hemoglobin; OGTT, Oral Glucose Tolerance Test; ns-not statistically significant

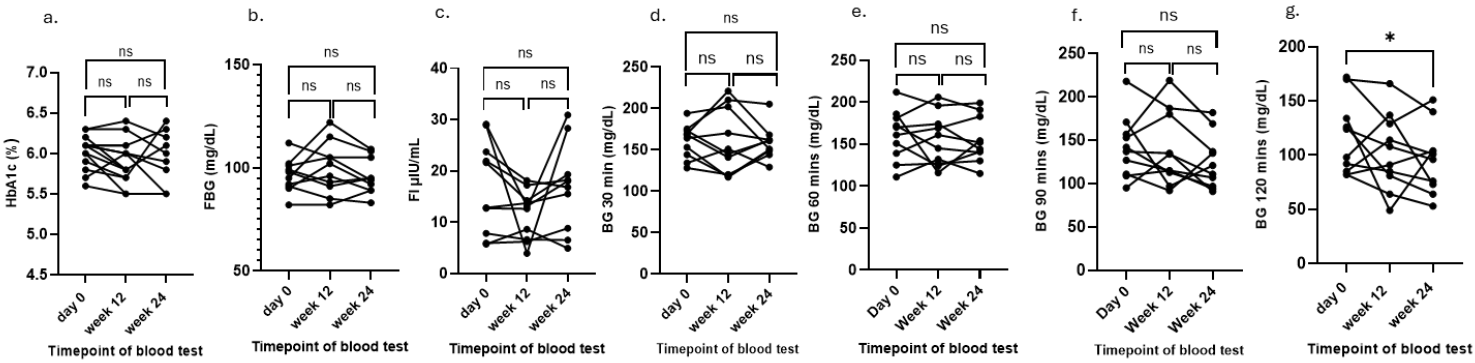


Fig3. Longitudinal Changes in Glycemic Parameters Across Study Phases in the Placebo Group. The graphs display raw data values for (a) HbA1c, (b) FBG, (c) FI and (d-g) OGTT (at 30, 60, 90, and 120 min. Due to the non-normal distribution of the data, a Wilcoxon matched-pairs signed-rank test was employed to compare time intervals (Day 0, Week 12, Week 24). Statistical significance was defined as p<0.05*. Abbreviations: FBG, Fasting Blood Glucose; FI, Fasting Insulin; HbA1c, Glycated Hemoglobin; OGTT, Oral Glucose Tolerance Test. ns-not statistically significant

Evaluation of Systemic Safety through Haematological, Biochemical and Clinical Monitoring.

To evaluate the safety of the Cellitas nutraceutical, we analyzed within-group changes in biochemistry profiles (urea, creatinine, electrolytes, liver function tests, and uric acid). In the Cellitas arm, no significant within-group deviations were observed between baseline, Week 12, and Week 24 (mean difference, SEM, 95% CI values in Table 2). This stability was mirrored in the placebo group, where parameters remained unchanged both during the initial placebo phase (Day 0 to Week 12) and following the crossover to active treatment (Week 12 to Week 24) –(Table 3). Specific analysis of hematological parameters (Full Blood Count-FBC and differential white cell) indicated that all values remained within normal physiological limits in the Cellitas group with no significant mean difference between all timepoints of the study (Table S7). In the placebo measurements of the same biochemical (Table S8) and hematological parameters also remained stable across all timepoints, 0-12 (placebo phase) 12-24 (Cellitas phase) and 0-24 (all phases).

Table 2. Changes in biochemical and electrolyte parameters with Cellitas.

Parameter	Time period	Mean Difference	SE	95% CI	p-value[a]
Albumin	0–12	-0.01	0.05	(-0.12, 0.10)	0.793
	12–24	0.05	0.08	(-0.14, 0.24)	0.575
	0–24	0.05	0.07	(-0.12, 0.22)	0.544
ALP	0–12	-1.00	2.88	(-7.22, 5.22)	0.734
	12–24	2.70	1.67	(-1.08, 6.48)	0.141
	0–24	2.90	3.45	(-4.92, 10.72)	0.424
ALT	0–12	-5.28	4.45	(-14.91, 4.34)	0.257
	12–24	1.00	1.43	(-2.23, 4.23)	0.502
	0–24	0.10	1.40	(-3.07, 3.27)	0.945
AST	0–12	-5.78	4.65	(-15.84, 4.27)	0.236
	12–24	0.60	1.86	(-3.61, 4.81)	0.755
	0–24	-0.90	2.47	(-6.50, 4.70)	0.725
BUN	0–12	0.14	0.88	(-1.77, 2.06)	0.875
	12–24	-1.40	1.14	(-3.99, 1.19)	0.253
	0–24	-1.50	1.02	(-3.81, 0.81)	0.177
Calcium	0–12	-0.01	0.08	(-0.19, 0.17)	0.935
	12–24	0.10	0.07	(-0.06, 0.26)	0.195
	0–24	0.09	0.10	(-0.15, 0.33)	0.426
Chloride	0–12	0.14	0.67	(-1.30, 1.59)	0.834
	12–24	-1.30	0.51	(-2.47, -0.12)	0.063
	0–24	-0.80	0.87	(-2.67, 1.07)	0.359
Creatinine	0–12	-0.01	0.01	(-0.04, 0.02)	0.521
	12–24	-0.05	0.03	(-0.12, 0.02)	0.177
	0–24	-0.08	0.04	(-0.17, 0.01)	0.082
Potassium	0–12	-0.23	0.24	(-0.75, 0.30)	0.368
	12–24	0.00	0.11	(-0.25, 0.25)	0.999
	0–24	-0.30	0.35	(-1.10, 0.50)	0.418
Sodium	0–12	1.14	0.61	(-0.17, 2.46)	0.084
	12–24	-1.30	0.59	(-2.65, 0.05)	0.057
	0–24	0.30	0.97	(-1.91, 2.51)	0.766
Uric acid	0–12	-0.13	0.21	(-0.59, 0.32)	0.534
	12–24	-0.15	0.29	(-0.82, 0.52)	0.627
	0–24	-0.24	0.22	(-0.74, 0.26)	0.310

Table 3. Changes in biochemical and electrolyte parameters with placebo.

Parameter	Time period	Mean Difference	SE	95% CI	p-value[a]
Albumin	0–12	0.00	0.06	(-0.12, 0.12)	0.999
	12–24	-0.04	0.07	(-0.19, 0.11)	0.574
	0–24	-0.06	0.07	(-0.21, 0.09)	0.405
ALP	0–12	-1.92	3.38	(-9.24, 5.39)	0.579
	12–24	-1.80	2.91	(-8.39, 4.79)	0.552
	0–24	-7.30	5.01	(-18.64, 4.04)	0.180
ALT	0–12	-0.50	1.24	(-3.19, 2.19)	0.695
	12–24	0.80	2.32	(-4.46, 6.06)	0.739
	0–24	0.40	1.92	(-3.94, 4.74)	0.840
AST	0–12	0.78	1.84	(-3.20, 4.78)	0.678
	12–24	0.20	1.61	(-3.44, 3.84)	0.904
	0–24	-0.20	1.20	(-2.91, 2.51)	0.871
BUN	0–12	0.85	1.43	(-2.23, 3.95)	0.560
	12–24	0.70	0.72	(-0.91, 2.31)	0.354
	0–24	0.00	1.04	(-2.36, 2.36)	0.999
Calcium	0–12	-0.02	0.11	(-0.26, 0.22)	0.854
	12–24	-0.03	0.06	(-0.15, 0.09)	0.604
	0–24	-0.10	0.08	(-0.27, 0.07)	0.237
Chloride	0–12	-0.57	0.78	(-2.24, 1.10)	0.474
	12–24	0.66	0.69	(-0.91, 2.25)	0.360
	0–24	1.22	0.91	(-0.87, 3.31)	0.216
Creatinine	0–12	-0.03	0.02	(-0.07, 0.00)	0.053
	12–24	0.00	0.04	(-0.09, 0.10)	0.927
	0–24	-0.02	0.03	(-0.10, 0.05)	0.516
Potassium	0–12	-0.03	0.09	(-0.23, 0.16)	0.709
	12–24	0.02	0.08	(-0.16, 0.20)	0.808
	0–24	0.00	0.06	(-0.13, 0.13)	0.999
Sodium	0–12	0.14	0.59	(-1.13, 1.42)	0.813
	12–24	-0.80	0.44	(-1.80, 0.20)	0.104
	0–24	0.100	0.66	(-1.38, 1.58)	0.882
Uric acid	0–12	-0.25	0.26	(-0.80, 0.29)	0.332
	12–24	0.02	0.25	(-0.55, 0.59)	0.938
	0–24	-0.35	0.24	(-0.89, 0.19)	0.177

Data in Table 2 and Table 3 are presented as mean difference and standard error (SE). 95% CI, Confidence Interval. Abbreviations: **ALP**, alkaline phosphatase; **ALT** ,alanine aminotransferase; **AST**, aspartate aminotransferase; **BUN**, blood urea nitrogen. **Units:** Albumin (g/dL); ALP, ALT, and AST (U/L); BUN, Calcium, Creatinine, and Uric Acid (mg/dL); Chloride, Potassium, and Sodium (mmol/L). *a*- For each measure a paired T-Test was employed to assess the differences between the time periods in pairs, comparing day 0 vs. week 12; week 12 vs. Week 24 and day 0 vs Week 24. Significance was set at $p<0.05$.

Safety and Tolerability Monitoring. Continuous monitoring of adverse events (AEs) and adverse reactions (ARs) confirmed the systemic safety and tolerability of the intervention. No local or systemic AR's were reported in either the Cellitas or placebo groups. Furthermore, AE's attributed to the study supplement were observed. In the Cellitas

	AE	SAE	AR	SAR	Details
Cellitas (day 0 to week 24	1	0	0	0	AE - Mild flatulence (*n*=1)
Placebo (day 0 to week 12)	2	2	0	0	AE- Mild indigestion (*n*=1) AE- Mild rash (*n*=1) SAE – rectal bleeding (*n*=1) SAE –hypertension (*n*=1)
Placebo (week 12 to week 24)	0	0	0	0	Not applicable

Table 4. Summary of adverse events and reactions reported during the study. Incidence and classification of adverse events. The table displays the number of Adverse Events (AE), Serious Adverse Events (SAE), Adverse Reactions (AR), and Serious Adverse Reactions (SAR) recorded for the Cellitas and Placebo groups, along with specific clinical details of each occurrence.

group, a single mild adverse event was reported, which was classified as unrelated to the study protocol. In the placebo group, four events were recorded during the initial placebo phase: two mild AEs and two serious adverse events (SAEs). notably, no adverse events occurred during the subsequent crossover phase where placebo participants received the active Cellitas intervention (Table 4). Collectively, these data indicate that daily supplementation with Cellitas presented no safety concerns within the parameters of this study.

Effect of Cellitas Intervention on Systemic Beclin-1 Concentrations.

To assess longitudinal changes in systemic Beclin-1, we evaluated within-group differences over the 24-week Cellitas intervention period. This approach was selected to account for the high inter-individual variability observed in peripheral blood markers at baseline. In the Cellitas group, baseline (Day 0) Beclin-1 concentrations were 0.62 ± 0.27 ng/mL (95% CI: 0.45–0.79; range: 0.18–1.20 ng/mL). Following 24 weeks of supplementation, mean levels increased significantly to 0.82 ± 0.43 ng/mL (95% CI: 0.55–1.10; range: 0.39–1.76 ng/mL). This represented a significant within-group elevation in circulating Beclin-1 compared to baseline (p=0.04) (Fig4a). In the placebo group, baseline (Day 0) Beclin-1 levels were 0.51 ± 0.20ng/mL (95% CI: 0.38–0.64 ng/mL; range: 0.24–0.89 ng/mL). Unlike the Cellitas group, placebo participants demonstrated no significant within-group change in circulating Beclin-1 concentrations at Week 12 (p=0.49) (Fig4b). Collectively, these findings suggest that 24 weeks of Cellitas supplementation significantly increases systemic Beclin-1 concentrations compared to baseline. The stability of Beclin-1 levels in the placebo group further supports the specificity of this effect to the active intervention.

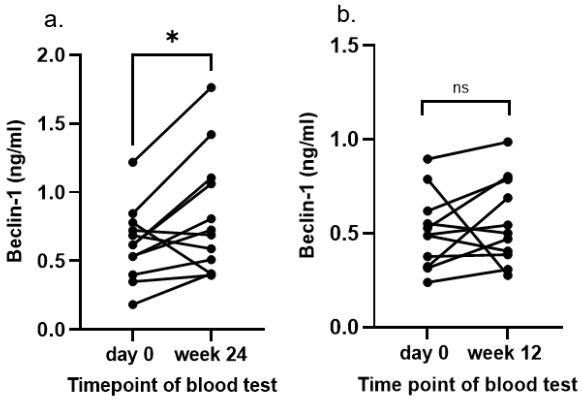


Fig4. Beclin-1 levels in Cellitas (a) and Placebo (b) groups. Plots show raw venous blood values comparing baseline (Day 0) to study endpoints (Week 24 for Cellitas; Week 12 for Placebo). A paired t-test was used to assess the within-group differences between the time periods in pairs. Significance was set at $p<0.05$*. ns – not statistically significant.

Impact of Cellitas on Lipid Metabolism and Cardiovascular risk biomarkers

To assess cardiovascular safety, lipid profiles including Low-Density Lipoprotein (LDL), High-Density Lipoprotein (HDL), and Triglycerides (TG) were monitored. In the Cellitas group, HDL concentrations ranged from 42 to 80 mg/dL at baseline (mean 54.8 ± 11.35 mg/dL) and 35 to 84 mg/dL at Week 24 (mean 56.67 ± 14.7 mg/dL)-(Fig5a). No significant mean difference was found between time points (Mean Diff 2.58; SEM 2.73; 95% CI -3.42 to 8.58; p=0.36). LDL levels remained stable over the 24-week intervention (Fig5b). Baseline values ranged from 57 to 142 mg/dL (mean 100.1 ± 24.06 mg/dL). At Week 24, levels were of similar magnitude, ranging from 57 to 125 mg/dL (mean 96.5 ± 24.58 mg/dL). Data followed a normal distribution, and no significant mean difference was observed after 24 weeks (Mean Diff -3.58; SEM 8.69; 95% CI -22.71 to 15.55; p=0.69). Triglyceride levels showed no significant fluctuations in the Cellitas arm (Figure 5c), values ranged from 54 to 246 mg/dL at Day 0 (mean 116.0 ± 65.2 mg/dL) and 61 to 302 mg/dL at Week 24 (mean 99.75 ± 65.04 mg/dL). Despite non-normal distribution, the mean difference analysis showed no significant change (Mean Diff -16.25; SEM 16.56; 95% CI -52.71 to 20.21; p=0.61).

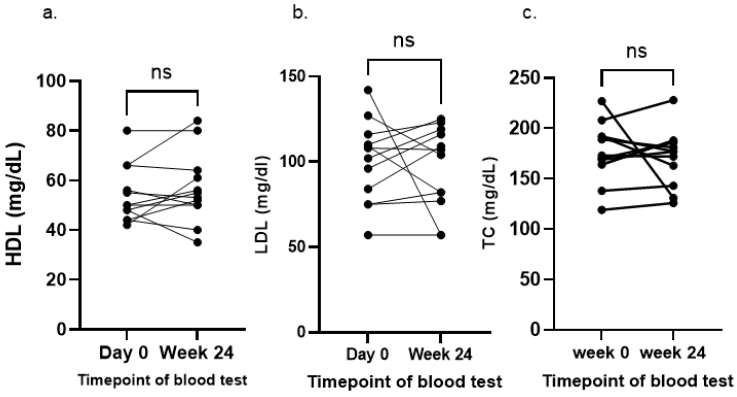


Fig5. Longitudinal changes in venous blood lipid parameters in the Cellitas group. Data are presented as raw values at Day 0 and Week 24 of (a)HDL-high density lipoprotein; (b)LDL-low density lipoprotein; (c) TG-triglycerides in the Cellitas group. A paired t-test was used to assess within-group differences between the time periods in pairs for LDL and HDL. TG was assessed with a Wilcoxin matched-pairs signed rank test due to non-normal distribution. Significance was set at $p<0.05$. ns-not statistically significant.

Similarly, in the placebo group HDL values ranged from 35 to 72 mg/dL at baseline (mean 52.36 ± 11.98 mg/dL) and 29 to 70 mg/dL at Week 12 (mean 49.36 ± 11.77 mg/dL)- (Fig6a). Statistical analysis confirmed no significant change during the placebo phase (Mean Diff -3.00; SEM 2.24; 95% CI -7.99 to 1.99; p=0.21). LDL cholesterol showed no significant deviations. Values at Day 0 ranged from 74 to 172 mg/dL (mean 109.2 ± 30.46 mg/dL) and at Week 12 ranged from 63 to 129 mg/dL (mean 98.36 ± 24.2 mg/dL). No significant mean difference was detected over the placebo phase (Mean Diff -10.82; SEM 6.05; 95% CI -24.3 to 2.66; p=0.10) (Fig6b). In the placebo group baseline TG measurements ranged from 80 to 432 mg/dL (mean 142.7 ± 99.1 mg/dL) and Week 12 measurements ranged from 69 to 328 mg/dL (mean 137.5 ± 75.83 mg/dL). No significant mean difference was observed (Mean Diff -5.18; SEM 14.95; 95% CI -38.5 to 28.13; p=0.99) Fig6c. Collectively, these data confirm that the

intervention was well-tolerated with respect to lipid metabolism, as the study duration yielded no clinically significant fluctuations in pro-atherogenic lipoprotein fractions.

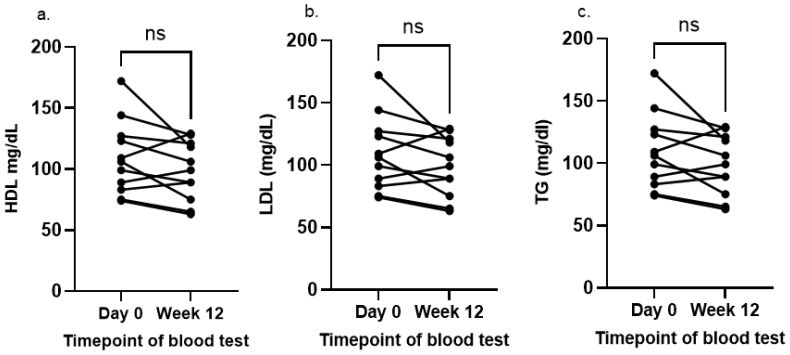


Fig6. Longitudinal changes in venous blood lipid parameters in the placebo group Data are presented as raw values at Day 0 and Week 12 of (a) HDL-high density lipoprotein; (b) LDL-low density lipoprotein; (c) TG-triglycerides in the placebo group (placebo phase). A paired t-test was used to assess within-group differences between the time periods in pairs, comparing day 0 vs. Week 12. for LDL and HDL. TG was assessed with a Wilcoxin matched-pairs signed rank test due to non-normal distribution Significance was set at $p<0.05$. ns–not statistically significant.

Assessment of Systemic Inflammatory Markers and Cytokine Profiles.

Systemic inflammatory status was evaluated by quantifying C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α). TNF-α concentrations followed a normal distribution in both groups. In the Cellitas group, levels exhibited stability. Baseline values ranged from 0.7 to 1.8 pg/mL (mean 1.14 ± 0.41 pg/mL), and Week 24 values ranged from 0.7 to 3.0 pg/mL (mean 1.34 ± 0.76 pg/mL), with no significant within-group change detected (p=0.24) (Fig7a)**.** In the placebo arm, values ranged from 0.5 to 1.7 pg/mL at baseline (mean 1.03 ± 0.37 pg/mL) and 0.3 to 2.0 pg/mL at Week 12 (mean 1.13 ± 0.45 pg/mL). There was no significant mean difference between Day 0 and Week 12 (Mean Difference 0.1; SEM 0.12; 95% CI -0.16 to 0.36; p=0.42) (Fig8a). CRP data displayed distinct distributional characteristics between groups. In the Cellitas group data followed a non-normal distribution and were analyzed using the Wilcoxon matched-pairs signed-rank test. Values at Day 0 ranged from 0.38 to 16.07 mg/L (mean 6.39 ± 4.76 mg/L). At Week 24, values ranged from 1.05 to 65.0 mg/L (mean 10.47 ± 17.47 mg/L). Despite the wide range at Week 24, there was no statistically significant within-group change (Mean Difference 4.08; SEM 5.18; 95% CI -7.33 to 15.49; p=0.57) (Fig7b). CRP values in the placebo group (Fig8b) were normally distributed. Values ranged from 0.46 to 6.3 mg/L at baseline (mean 2.17 ± 1.89 mg/L) and 0.47 to 5.79 mg/L at Week 12 (mean 2.19 ± 1.86 mg/L). No significant mean difference was observed (Mean Difference 0.03; SEM 0.22; 95% CI -0.45 to 0.50; p=0.91). Data for IL-6 followed a non-normal distribution in both cohorts; therefore, within-group differences were assessed using the Wilcoxon matched-pairs signed-rank test. In the Cellitas intervention arm, IL-6 levels showed no significant within-group changes between baseline and Week 24. Baseline values ranged from 2.5 to 19.8 pg/mL (mean 5.71 ± 6.5 pg/mL), and Week 24 values ranged from 2.4 to 24.5 pg/mL (mean 7.1 ± 7.8 pg/mL). Statistical analysis confirmed no significant mean difference over the study period (Mean Difference 0.09; SEM 0.05; 95% CI -0.027 to 0.195; p=0.06) (Fig7c). Similarly, in the placebo group, no significant change was observed (p=0.06). Values ranged from 2.5 to 7.3 pg/mL at Day 0 (mean 3.29 ± 1.4 pg/mL) and from 2.4 to 6.3 pg/mL at Week 12 (mean 2.93 ± 1.04 pg/mL) (Fig8c). Collectively, these results indicate that long-term Cellitas supplementation did not result in significant elevations in pro-inflammatory cytokines or acute-phase reactants.

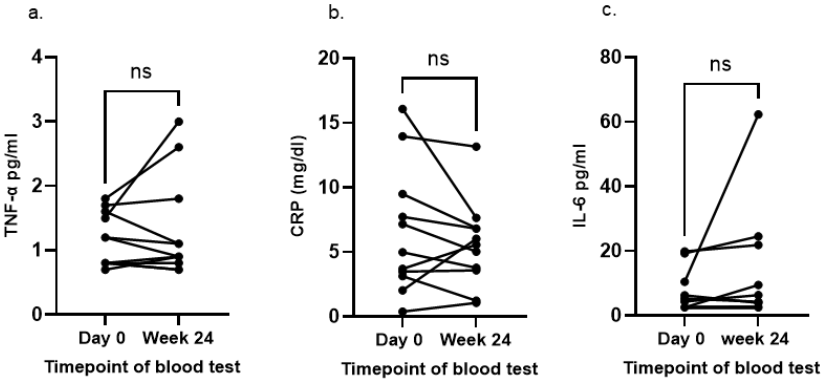


Fig7. Within-in group longitudinal changes in venous blood inflammatory biomarkers with Cellitas. Changes in inflammatory markers (a) TNF-α, (b) CRP and (c) IL-6 in blood over time. Graphs display raw venous blood values for the Cellitas group (Day 0 vs. Week 24). Statistical significance between paired time points for TNFα was determined by a paired *t*-test. IL-6 and CRP data had a non-normal distribution and was assessed with a Wilcoxon matched-pairs signed rank test. Significance was set at $p < 0.05$. Abbreviation: CRP- C-reactive , ns– not statistically significant.

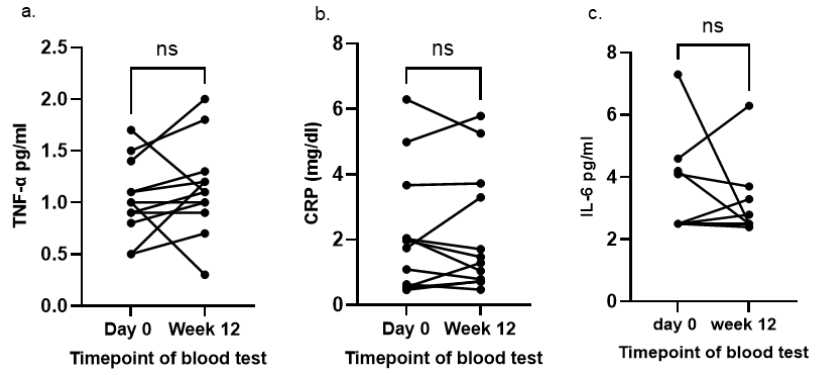


Fig8. Changes in inflammatory markers (a) TNF-α, (b) CRP and (c) IL-6 in blood over time. Graphs display raw venous blood values for the placebo group (Day 0 vs. Week 12). Statistical significance between paired time points for TNFα and CRP was determined by a paired *t*-test. IL-6 data was assessed with a Wilcoxon matched-pairs signed rank test as data was no-normal distribution. Significance was set at $p < 0.05$. Abbreviation:CRP- C-reactive protein, ns- not significant.

Discussion

Amongst the many nutraceutical supplements available to healthcare providers and consumers, few have gone through safety testing and efficacy analysis. The data presented here represent the first-in-human randomized, placebo-controlled pilot study on the safety of an oral supplement containing synthetic polyamines, spermidine (4.HCl) and spermine (4.HCl), in combination with the probiotic *Bifidobacterium animalis* subsp. *lactis* BB-12® and arginine. Our findings contribute to a growing body of evidence suggesting that exogenous spermidine is well-tolerated. Previous investigations have established a baseline for safety using various delivery matrices. The SmartAge trial (Schwarz et al 2022.) utilized a wheatgerm-derived product (1.2 mg spermidine) in a cohort with subjective cognitive decline, reporting no adverse safety signals. Similarly, Bruno et al. (2025) and Senekowitsch et al. (2023) demonstrated that extracts from rice germ (3.3 mg/day) and *Chlorella*/soybean (7.5 mg/day) were consumed without reported complications, supporting the safety of plant-based spermidine complexes. Moving toward higher concentrations, recent data indicates that 40 mg/day of *Saccharomyces cerevisiae*-derived spermidine-3.HCl was well-tolerated in healthy older men for up to 28 days [Keohane et al. 2024]. Our findings extend this current understanding to synthetic spermidine and spermine. The absence of safety concerns or adverse events related to the Cellitas supplement in this trial supports the current evidence that polyamine supplementation provides a significant margin of safety. This is maintained when in combination with arginine and BB-12.

These results complement existing safety data by providing early mechanistic insights. We observed a 32% within-group increase in Beclin-1 serum levels following six months of supplementation with this synthetic spermidine, spermine, *B. animalis* subsp. *lactis* (BB-12), and arginine formulation. Beclin-1 is a protein and serves as a critical scaffold protein for the PI3KC3 complex (class III phosphatidylinositol 3-kinase complex), acting as a central hub that integrates various cellular cues to govern autophagy and cellular trafficking [Oberstein et al. 2007]. Autophagy is a conserved degradation process in various species. It involves the formation of autophagosomes, enclosed by a double membrane, containing macromolecules and cellular components for recycling. Autophagy is key in preserving cell homeostasis [Eisenberg-Lerner 2009; He et al. 2009; Huett et al. 2010]. As a primary initiator of autophagosomal formation, Beclin-1 interacts with VPS34 (a type III phosphatidylinositol 3-kinase) to generate phosphatidylinositol-3-phosphate (PI3P). This biochemical signaling recruits essential autophagy-related (ATG) proteins and LC3 to the phagophore, facilitating the maturation and eventual lysosomal fusion of the autophagosome [Zhong et al. 2009; Matsunaga et al. 2009; Zeng et al. 2006] Beyond its canonical role in macroautophagy, Beclin-1 is a versatile regulator of multiple cell death pathways, including mitophagy, apoptosis, and ferroptosis [Zeng et al. 2024; Furuta et al. 2005; Zhou et al. 2020; Song et al. 2018]. Its interaction with the Bcl-2 protein family allows it to modulate the delicate threshold between cell survival and programmed death. By regulating these divergent pathways, Beclin-1 is involved in the efficient clearance of toxic proteins and damaged organelles and it is essential for autophagy and maintaining systemic cellular balance. The observed increase in Beclin-1 in our study cohort of older adults is pertinent. High levels of circulating Beclin-1 have been proposed as a marker of better clinicopathological status in several disease states and low levels of Beclin-1 are associated with increased disease severity and poorer outcomes in conditions such as type 2 diabetes mellitus and linked to decreased cellular health and the progression of age-related pathologies. [Naguib et al. 2021; Zheng et al 2018; Zhou et al 2013; Qui et al. 2014]. Furthermore, targeting the restoration of Beclin-1 levels through nutritional intervention represents a potential strategy for risk stratification and therapy, warranting further investigation. As Beclin-1 is a central biological pathway that can promote autophagy, the ability of Cellitas to increase levels of serum Beclin-1 is promising. However, the precise mechanisms underlying these systemic increases remain to be fully elucidated. Future research should focus on identifying mechanisms and specific patient subpopulations in larger trials that may derive the greatest benefit from Beclin-1 modulation and any off-target effects in complex disease states.

The BB-12 and arginine components of this supplement are also of interest, as Matsumoto et al (2019) reported that a combination of oral BB-12 and arginine increased polyamine production in the gut in a randomized placebo-controlled trial with older people.. BB-12 with arginine in older adults also upregulates blood spermidine levels and may reduce the risk of atherosclerosis [Matsumoto, 2020; Matsumoto et al. 2019]. The underlying mechanisms of these observations remain unclear. Some evidence suggests that autophagy may be an effector mechanism through which probiotics provide their protective effects through changes to autophagy-related genes [Inaba et al (2016) and Bakic et al. (2020)] and Beclin-1 [Kim et al (2010); Wu et al. (2017); Bonfili et al. (2017); and Andrade-Oliveira et al. (2015)]. Orlando et al. (2025) recently reported that a probiotic treatment restored autophagy in pre-clinical models, as evidenced by the normalization of LC3-II and Beclin-1 levels. These studies suggest that probiotics can affect Beclin-1 and autophagy-related genes in preclinical models, but mechanistic exploration will be important in clinical trials with older people where dysbiosis is common [Haran et al. 2021].

Cellitas supplementation appeared to influence dynamic glucose handling to some extent, although kinetic profiles varied between cohorts. Prolonged administration in the continuous arm reduced early-phase excursions (30 and 60 minutes), whereas the crossover group exhibited late-phase reductions (120 minutes) during the active period. However, these findings must be interpreted with caution given the exploratory pilot design and limited sample size. Larger, definitive trials are required to confirm efficacy and elucidate the mechanisms underlying these temporal differences. The intervention was well-tolerated with no glycemic adverse events.

There are limitations to this study that are typical of pilot designs, including a small sample size, a lack of randomization with stratification by sex, participant ethnicity being largely people with African ancestry and an intervention window of 24 weeks, all of which are typical characteristics of pilot studies (Shader, 2015). The cross-over phase utilized an open-label design, providing a pragmatic assessment of the intervention's effects in a real-world context, though this does preclude the use of double-blinding. While these preliminary findings are intended to inform future research rather than provide definitive extrapolations to the broader population, they offer a meaningful basis for further investigation. As this supplement contained spermidine, spermine, arginine

and BB-12, conclusions on the effects of each individual ingredient could be drawn with further research examining each component separately. Arginine as a metabolic substrate to support cellular homeostasis and *B. animalis* subsp. *lactis* may further influence systemic markers axis and this requires further mechanistic validation in larger scale trials. Although serum Beclin-1 levels were elevated, our current data cannot confirm whether this translated to altered downstream autophagic flux and cellular metabolism. In conclusion, this randomized controlled trial demonstrates that a six-month nutritional intervention, Cellitas, comprising spermidine, spermine, arginine, and *Bifidobacterium animalis* subsp. *lactis* BB-12 was well-tolerated and exhibited a favorable safety profile in pre-diabetic adults aged 50 years and older. Notably, the intervention was associated with a 32% increase in circulating levels of the autophagy-related protein Beclin-1 within this cohort. Further mechanistic investigations are required to characterize the effects on autophagic signaling, specifically by evaluating the intracellular downstream targets of Beclin-1.

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Conflicts of Interest: LJ is a Board member and SN is a co-founder of Kalin Health LLC.

Data Availability Statement The datasets generated and analyzed during the current study are not publicly available due to ethical restrictions and patient confidentiality requirements established by the IRB. However, de-identified individual participant data, alongside the study protocol, are available from the corresponding author upon reasonable request. Access will be granted to researchers who provide a methodologically sound proposal and following the execution of a formal data sharing agreement.

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Supplementary Materials and Methods

Fig. S1. CONSORT flow diagram.

The flowchart details participant progression through the 24-week randomized, double-blind, placebo-controlled pilot trial. This includes the phases of enrollment, allocation, follow-up, and final analysis. A total of 42 participants were recruited and randomized in a 1:1 ratio to receive either the active intervention (Cellitas) or a visually matched placebo for the initial 12-week double-blind period. Following this, the placebo group crossed over to the active arm, and all remaining participants consumed Cellitas in an open-label fashion from Week 12 to Week 24. Specific reasons for exclusion prior to randomization, as well as study withdrawals or loss to follow-up during both the blinded and open-label phases, are indicated at each respective stage. Abbreviations: CONSORT, Consolidated Standards of Reporting Trials; n, number of participants.

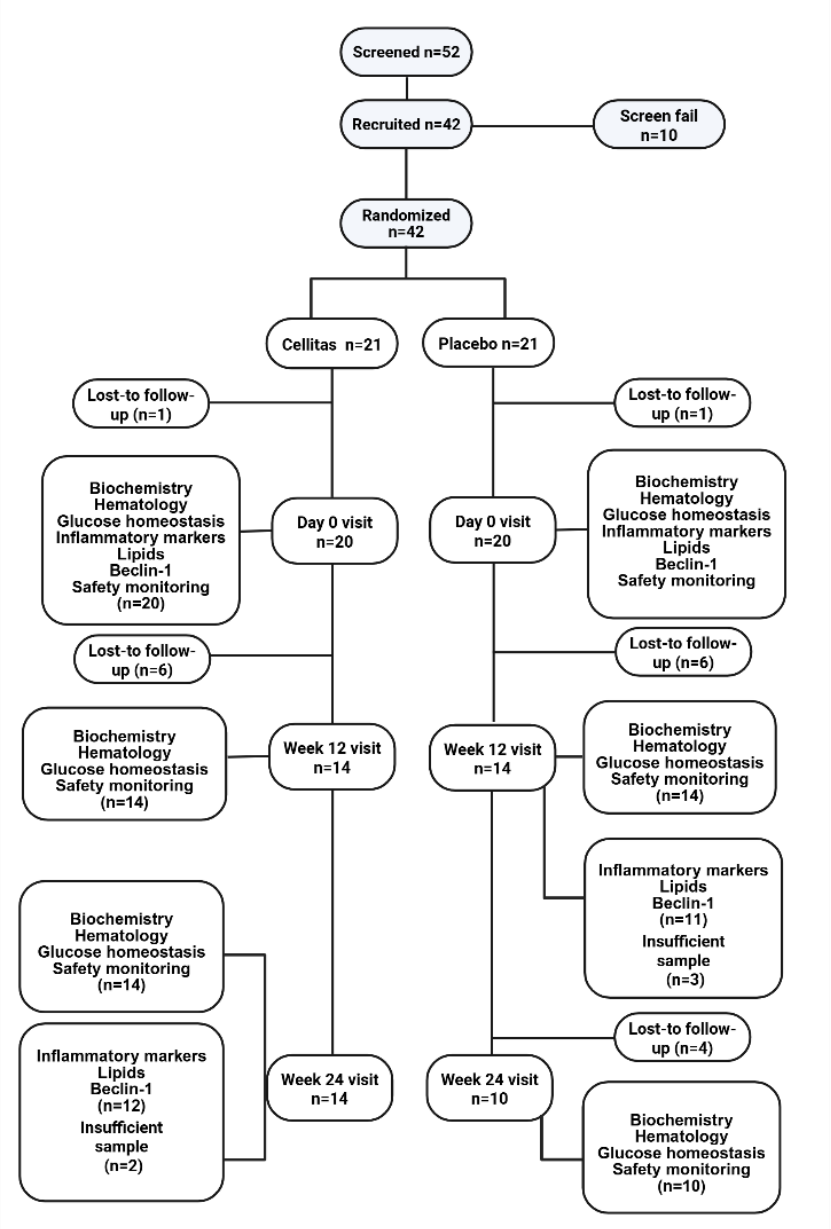


Figure S2.

Study Design and Ethics This was a randomized, double-blind, placebo-controlled pilot study (non-CTIMP) conducted at Lifedoc Health and Lifedoc Research, Memphis in the United States. The primary objective was to evaluate the safety, tolerability, and metabolic efficacy of the nutraceutical Cellitas® (referred to as Cellitas) in older adults with pre-diabetes. The study protocol was approved by an independent Institutional Review Board (Sterling IRB; reference K03-21-01-T0014) prior to the commencement of recruitment. The trial was conducted in accordance with the ethical principles of the Declaration of Helsinki and Good Clinical Practice (GCP) guidelines. The study was registered retrospectively with ClinicalTrials.gov
(Identifier: NCT06445569). Participants were identified by LifeDoc Health during routine appointments and in accordance with ethical approvals.

Inclusion criteria
1. Male and female participants who are at least 50 years of age (inclusive) at the time of signing the informed consent form.
2. In good general health and good oral health (no active or uncontrolled diseases, infections, or conditions).
3. Have a BMI within the range of 25.0 to 45.0 kg/m2 (inclusive).
4. Impaired glucose metabolism evidenced by either fasting glucose of 100 to 125 mg/dl at screening or an HbA1c between 5.7 and 6.4%.
5. Female participants of childbearing potential (i.e., participants who are not surgically sterilized or not post-menopausal (defined as amenorrhea for greater than 1 year), or transgendered males with retained ovaries and uterus) must agree to use a medically approved method of birth control for at least one month prior (or three months prior in the case of oral or long-acting injective contraceptives) to the first dose of study product and throughout the study or abstain from heterosexual intercourse throughout the duration of the study and have a negative urine pregnancy result at baseline. For males of reproductive potential: use of condoms, abstinence from heterosexual intercourse, or other methods to ensure that their partners (if able) do not become pregnant during the course of the study.
6. Willing and able to agree to the requirements and restrictions of this study, be willing to give voluntary consent, be able to understand and read the questionnaires, and carry out all study-related procedures.

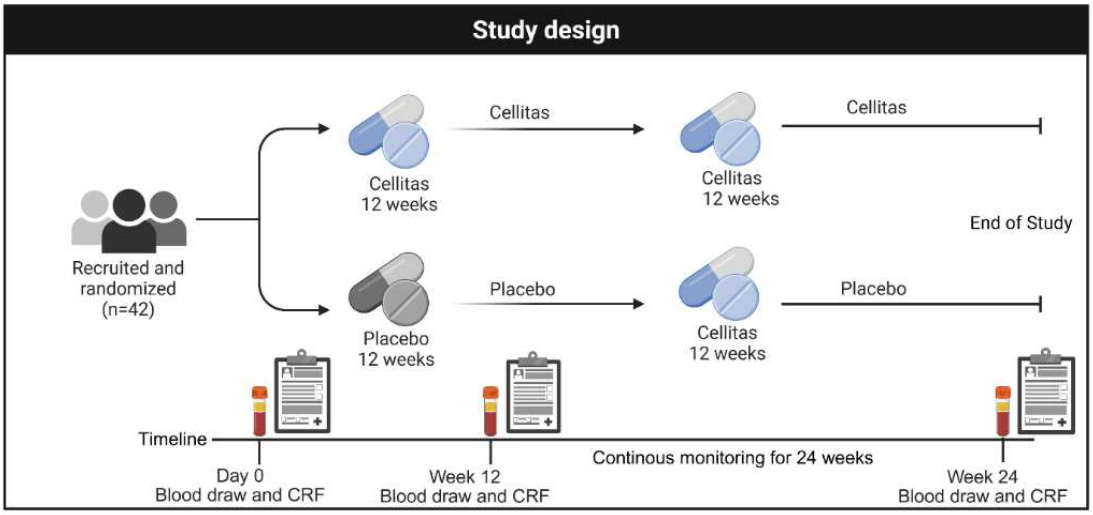
Exclusion criteria
1.Participant has a medical history of uncontrolled hypertension (i.e., ≥160 mmHg systolic and/or ≥100 mmHg diastolic) Participant has a medical history of heart disease and/or cardiovascular disease, kidney disease (dialysis or renal failure), hepatic impairment or disease, or Type 1 or Type 2 diabetes.
3. Participant has a medical history of unstable thyroid disease, previously diagnosed major affective disorder, psychiatric disorder that required hospitalization in the year prior to screening, immune disorder (i.e., HIV/AIDS), or a history of cancer (except localized skin cancer without metastases or in situ cervical cancer) within 5 years prior to screening visit.
4. Female participants who are lactating, pregnant, or planning to become pregnant during the study.
5. Have a known intolerance, sensitivity, or allergy to any of the study products or their ingredients, or any of the excipients used in the formulation.
6. Have a known intolerance, sensitivity, or allergy to milk.
7. Any condition or abnormality that, in the opinion of the Investigator, would compromise the safety of the participant or the quality of the study data.
9. Currently taking dietary supplements other than vitamins
10. Taking any prescription medication at the time of randomization that is known to impact blood sugar and or blood sugar metabolism, as per Principal Investigator's (PI) discretion.
11. Use of certain medications/supplements/foods/drinks in defined timeframes (see footnote)
12. History of alcohol or substance abuse in the 12 months prior to screening.
13. Receipt or use of an investigational product in another research study within 28 days prior to baseline/Visit 2.

Footnote: Prohibited prescribed and over-the-counter medications
Prohibited within 2 weeks or 7 half-lives (whichever is longer) prior to baseline visit and for the duration of the study, unless stated otherwise:
- Antibiotics: Used within 4 weeks prior to baseline will lead to exclusion; can be prescribed by the PI during the study as required by standard of care.
- Potassium sparing diuretics
- Nitroglycerin and other nitrates
- Antidiabetic/Blood sugar lowering medications
- Any blood thinning medications (i.e., anticoagulant/antiplatelet drugs)
- Antidepressants- Use is permitted if dosage is maintained throughout the study
- Anxiolytics
- Antipsychotics
- Other anticholinergics/antispasmodics
- Calcium-channel blockers - Use is permitted if dosage is maintained throughout the study
- Opioids

Dietary supplements/food/drink
Prohibited within 2 weeks or 7 half-lives (whichever is longer) prior to baseline and for the duration of the study
Foods/drinks rich in probiotics or prebiotics (e.g., yogurt, sauerkraut, kombucha)
Probiotics, prebiotics, or synbiotics
Any herbs and supplements with hypoglycemic potential
Any herbs or supplements that induce weight loss
Any blood sugar lowering herbs or supplements
Any blood thinning herbs or supplements.

Figure S3.

Schematic of the unidirectional crossover study design and participant flow. Following screening and recruitment, eligible participants were randomized to receive either the active investigational product (Cellitas) or a while participants in the continuous active arm maintained their Cellitas regimen. Consequently, all enrolled participants consumed the active product for the final 12 weeks of the 24-week trial Abbreviations: CRF–case report form.



S4. *Randomization and Blinding*

Prior to the study, a randomization list (coded/blinded) was generated by the unblinded team. Upon arrival to the clinic at the screening visit, participants reviewed the informed consent form (ICF) and provided written informed consent. All participants who signed the ICF received a unique participants ID. Eligible participants who completed the screening process were then randomized to receive either Cellitas or placebo in a 1:1 non-stratified ratio and assigned a unique randomization code. Participants were randomized into two groups (n=21 per group) a computer-generated randomization sequence. To maintain the double-blind design, randomization and product dispensing were managed by a dedicated unblinded team member who was not involved in data collection or clinical assessment. Both the participants and the blinded investigative team remained unaware of their treatment allocation throughout the first phase of the trial. The second phase of the study was open-label; after 12 weeks of randomized, blinded product administration, the remaining 12 weeks was unblinded, with all participants to be administered Cellitas. Participants were instructed on the use of the products, according to label instructions. Laboratory based researchers were blinded for the duration of the study. Unblinding only occurred in the case of an urgent reportable SAE/ medical situation requiring knowledge of the study product assignment.

Fig. S5. *Composition of Cellitas and placebo supplements*

Active ingredients – dose per day 3 capsules	**Placebo ingredients - dose per day 3 capsules ∞**
1 g Arginine	Hypromellose
8 mg Spermidine	Hypromellose acetate succinate
3 mg Spermine	Gellen gum
1 powdered stick packet containing: 17 billion CFU of BB-12 (*Bifidobacterium animalis subsp. lactis*).	1 powdered stick packet containing: Microcrystalline cellulose

Ingredients were dissolved in cup of room temperature water daily and consumed with or without food and recommended to be consumed around the same time each day. Placebo physically and visually matched oral Cellitas dose.

Fig. S6. Adverse event reporting

The term " Adverse Event" AE) refers to any untoward medical occurrence related or not to the supplement (Cellitas)
The term "Serious Adverse Event" (SAE) refers to any untoward medical occurrence related or not to the supplement that may lead to death, persistent or significant disability, requires hospitalization or prolongation of a hospitalization period, provokes invalidity, significant or permanent incapacity, or results in congenital anomaly or malformation.
AE classification was defined as: • Mild – Events require minimal or no treatment and do not interfere with the participant's daily activities. • Moderate – Events result in a low level of inconvenience or concern with the study intervention(s). Moderate events may cause some interference with functioning. • Severe – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating. Of note, the term "severe" does not necessarily equate to "serious".
All AEs had their relationship to study intervention assessed by the clinician who examines and evaluates the participant. The degree of certainty about causality will be graded using the categories below: • Related – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study intervention administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study intervention (de-challenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory re-challenge procedure if necessary.
• Suspected – There is some evidence to suggest a causal relationship, and the influence of other factors is unlikely. However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the study intervention, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (de-challenge). Re-challenge information is not required to fulfil this definition. • Not Related – The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician. All AEs and SAEs were collected from the start of administration of the study product (Day 0) until the last study visit. All SAEs will be followed until resolution, stabilization, the event is otherwise explained, or the participant is lost to follow-up.

Supplementary Methods S7.

Data Collection and Procedures

After a screening visit, clinical appointments occurred at baseline (Day 0), Week 12, and Week 24. At each visit**,** 50 mls venous blood samples were collected following an overnight fast for the analysis of glycemic indices (HbA1c, fasting glucose, insulin) and safety biomarkers (hematology and biochemistry tests). An Oral Glucose Tolerance Test (OGTT) was performed at Day 0, Week 1 and Week 24. Safety was monitored continuously through the recording of adverse events (AEs) and adverse reactions (ARs). Beclin-1, Inflammatory biomarkers (CRP, IL-6 and TNFα) and lipid profiles were measured at two timepoints – day 0 and week 24 for the Cellitas group and day 0 and week 12 for the placebo group.

Compliance data was assessed and documented at each visit. Compliance was assessed by direct questioning and counting returned capsules and sticks during the site visits and documented in the source documents and Case Report Form (CRF). During the study (from screening until completion of the final/End of study visit participants abstained from taking prescription drugs, nonprescription drugs and any other dietary supplements.

Safety Assessments

Vital Signs. The vital signs (systolic and diastolic blood pressure and heart rate) was captured as per the site's standard operating procedures and captured in the source documents and CRF. The same recording method and the same type of equipment was used for each participant throughout the study.

Clinical Safety Laboratory Assessments

The participants underwent a blood draw for complete blood count and basic metabolic panel assessment.

Blood draws will be performed in compliance with the standard laboratory procedures. Laboratory parameters that are out of acceptable ranges and clinically significant as determined by the Investigator were followed up and assessed at the Investigator's discretion. Clinically significant values were reported as AEs and participants were re-evaluated for continuing eligibility

List of safety laboratory assessments: Hematology Panel - Hemoglobin, Hematocrit, red blood cell (RBC) count, red cell distribution width (RDW), RBC Indices mean cell volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), White Blood Cell count and differential* (abs), and Platelet count.

Clinical Chemistry Panel -Alanine aminotransaminase (ALT), Aspartate aminotransaminase (AST), Alkaline phosphatase, Albumin, Blood Urea Nitrogen (BUN), Creatinine, Sodium, Potassium, Chloride, Calcium, Uric acid, Metabolic biomarkers (for glucose control) -HbA1c, Fasting glucose, Fasting insulin, Oral glucose tolerance tests (OGTT).

Protocol accessibility

The trial protocol may be obtained upon written request to the corresponding author.

Table S2. *Longitudinal Changes in Glycemic Parameters Across Study Phases in the Cellitas Group*

Parameter	Time period	Mean Difference	SE	95% CI	p-value[a]
FBG (mg/dL)	0–12	4.07	2.38	(-1.08, 9.22)	0.139
	12–24	-3.64	2.10	(-8.18, 0.89)	0.123
	0–24	0.43	2.86	(-5.76, 6.62)	0.916
FI (µlU/ml)	0–12	2.72	1.32	(-0.14, 5.58)	0.104
	12–24	1.17	1.83	(-2.80, 5.14)	0.903
	0–24	3.89	2.18	(-0.82, 8.61)	0.119
HbA1c (%)	0–12	-0.06	0.06	(-0.20, 0.07)	0.422
	12–24	-0.03	0.06	(-0.16, 0.09)	0.720
	0–24	-0.10	0.07	(-0.25, 0.05)	0.220
OGTT 30 (mg/dL)	0–12	11.92	6.03	(-1.11, 24.97)	0.064
	12–24	-15.28	6.65	(-29.66, -0.90)	0.035*
	0–24	-3.35	5.91	(-16.13, 9.42)	0.754
OGTT 60 (mg/dL)	0–12	24.28	6.25	(10.76, 37.80)	0.002*
	12–24	-26.85	11.20	(-51.22, -2.48)	0.030*
	0–24	-2.57	8.66	(-21.29, 16.15)	0.777
OGTT 90 (mg/dL)	0–12	4.57	6.33	(-9.11, 18.25)	0.196
	12–24	-7.00	8.26	(-24.85, 10.85)	0.363
	0–24	-2.42	5.35	(-13.99, 9.13)	0.675
OGTT 120 (mg/dL)	0–12	5.28	7.95	(-11.90, 22.47)	0.509
	12–24	-2.07	9.83	(-23.32, 19.18)	0.683
	0–24	3.21	10.86	(-20.25, 26.68)	0.826

Data are presented as mean difference, standard error (SE) and 95% Confidence Interval (CI). *a*- for each measure a paired t-test was used to assess the within-group differences between the time periods in pairs, comparing day 0 vs. week 12; week 12 vs. Week 24 and day 0 vs Week 24. Significance was set at $p<0.05$ * Abbreviations: **FBG**, Fasting Blood Glucose; **FI**, Fasting Insulin; **HbA1c**, Glycated Hemoglobin; **OGTT**, Oral Glucose Tolerance Test at 30, 60, 90 and 120 minutes.

Table S3. *Longitudinal Changes in Glycemic Parameters Across Study Phases in the Placebo Group*

Parameter	Time period	Mean Difference	SE	95% CI	p-value[a]
FBG (mg/dL)	0–12	4.357	2.833	(-1.764, 10.479)	0.420
	12–24	-3.700	2.171	(-8.611, 1.211)	0.137
	0–24	-0.300	2.276	(-5.448, 4.848)	0.758
FI (µIU/ml)	0–12	-3.714	2.217	(-8.503, 1.075)	0.135
	12–24	5.300	2.840	(-1.124, 11.724)	0.084
	0–24	-0.290	2.446	(-5.824, 5.244)	0.557
HbA1c (%)	0–12	-0.092	0.054	(-0.209, 0.025)	0.165
	12–24	0.010	0.046	(-0.094, 0.114)	0.892
	0–24	-0.130	0.076	(-0.302, 0.042)	0.270
OGTT 30 (mg/dL)	0–12	3.714	7.122	(-11.671, 19.100)	0.638
	12–24	-1.400	10.160	(-24.376, 21.576)	1.000
	0–24	-2.100	5.984	(-15.637, 11.437)	0.646
OGTT 60 (mg/dL)	0–12	8.000	10.670	(-15.041, 31.041)	0.451
	12–24	-0.100	6.207	(-14.140, 13.940)	0.878
	0–24	-5.900	6.181	(-19.883, 8.083)	0.414
OGTT 90 (mg/dL)	0–12	7.231	12.970	(-21.032, 35.493)	0.839
	12–24	-11.500	9.609	(-33.238, 10.238)	0.241
	0–24	-17.600	8.626	(-37.113, 1.913)	0.092
OGTT 120 (mg/dL)	0–12	1.429	11.240	(-22.848, 25.705)	0.903
	12–24	-6.200	9.927	(-28.657, 16.257)	0.507
	0–24	-20.300	7.609	(-37.512, -3.088)	0.028*

Data are presented as mean difference, standard error (SE) and 95% Confidence Interval (CI).*a*- paired T-Test was employed to assess the differences between the time periods in pairs, comparing day 0 vs. week 12; week 12 vs. Week 24 and day 0 vs Week 24. Significance was set at $p<0.05$* Abbreviations: **FBG**, Fasting Blood Glucose; **FI**, Fasting Insulin; **HbA1c**, Glycated Hemoglobin; **OGTT**, Oral Glucose Tolerance Test at 30, 60, 90 and 120 minutes.

Table S7. *Longitudinal changes in hematological parameters across all timepoints in the Cellitas group*

Parameter	Time Period	Mean Difference	SE	95% CI	p-value∞
MCH (pg)	0-12	0.04	0.14	-0.26 to 0.35	0.768
	12-24	0.66	0.78	-1.11 to 2.43	0.420
	0-24	-0.91	0.80	-2.72 to 0.90	0.286
Lymphocytes (×10^3/µL)	0-12	-0.19	0.16	-0.53 to 0.15	0.239
	12-24	-0.31	0.17	-0.69 to 0.07	0.097
	0-24	-0.01	0.13	-0.31 to 0.32	0.99
Hemoglobin (g/dL)	0-12	1.03	0.86	-0.87 to 2.94	0.261
	12-24	-0.08	0.28	-0.72 to 0.56	0.792
	0-24	-0.01	1.02	-2.36 to 2.34	0.991
Hematocrit (%)	0-12	0.39	2.66	-5.64 to 6.42	0.892
	12-24	-0.23	2.54	-5.98 to 5.52	0.931
	0-24	0.16	0.78	-1.62 to 1.94	0.843
Eosinophils (×10^3/µL)	0-12	0.02	0.02	-0.04 to 0.07	0.552
	12-24	-0.01	0.01	-0.04 to 0.07	0.341
	0-24	0.02	0.02	-0.05 to 0.02	0.554
Platelets (×10^3/µL)	0-12	-14.20	6.67	-29.28 to 0.88	0.061
	12-24	14.00	11.38	-11.75 to 39.75	0.251
	0-24	-0.200	11.25	-25.64 to 25.24	0.992
Neutrophils (×10^3/µL	0–12	0.04	0.43	-0.96 to 1.05	0.921
	12–24	-0.06	0.46	-1.12 to 1.01	0.912
	0–24	-0.01	0.30	-0.69 to 0.67	0.974
WBC (×10^3/µL)	0-12	0.10	0.28	-0.61 to 0.81	0.731
	12-24	0.38	0.32	-0.44 to 1.20	0.284
	0-24	0.48	0.47	-0.72 to 1.68	0.312
MCV(fL)	0-12	0.56	0.67	-0.99 to 2.10	0.431
	12-24	0.44	0.41	-0.51 to 1.40	0.312
	0-24	0.40	0.49	-0.73 to 1.53	0.441
Basophils (×10^3/µL	0-12	-0.01	0.01	-0.05 to 0.02	0.341
	12-24	-0.01	0.01	0.05 to 0.02	0.351
	0-24	-0.01	0.02	-0.03 to 0.03	0.991

Data are presented as mean difference, standard error (SE) and 95% Confidence Interval (CI). ∞ - paired T-Test was employed to assess the differences between the time periods in pairs, comparing day 0 vs. week 12; week 12 vs. Week 24 and day 0 vs Week 24. Significance was set at $p<0.05$* **MCHC**, mean corpuscular hemoglobin concentration; **MCH**, mean corpuscular hemoglobin; **WBC**, white blood cell count, **MCV**- mean corpuscular volume.

Table S8. *Longitudinal changes in hematological parameters across all timepoints in the placebo group*

Parameter	Time period	Mean Difference	SE	95% CI	p-value[a]
MCHC (g/dL)	0–12	-0.25	0.33	(-0.96, 0.46)	0.463
	12–24	-0.21	0.33	(-0.97, 0.55)	0.543
	0–24	-0.30	0.27	(-0.92, 0.32)	0.302
MCH (pg)	0–12	-0.05	0.24	(-0.58, 0.47)	0.818
	12–24	-0.03	0.14	(-0.37, 0.30)	0.827
	0–24	0.04	0.21	(-0.46, 0.54)	0.844
Lymphocytes ($\times 10^3$/µL)	0–12	0.02	0.12	(-0.23, 0.28)	0.862
	12–24	-0.04	0.08	(-0.23, 0.15)	0.657
	0–24	-0.09	0.16	(-0.47, 0.29)	0.606
Hemoglobin (g/dL)	0–12	-0.92	0.72	(-2.50, 0.64)	0.225
	12–24	1.26	1.02	(-1.06, 3.58)	0.251
	0–24	-0.03	0.20	(-0.48, 0.42)	0.884
Hematocrit (%)	0–12	-0.30	0.32	(-1.01, 0.41)	0.378
	12–24	0.88	0.55	(-0.37, 2.13)	0.148
	0–24	0.31	0.71	(-1.31, 1.93)	0.676
Eosinophils ($\times 10^3$/µL)	0–12	0.00	0.01	(-0.02, 0.02)	1.000
	12–24	-0.01	0.01	(-0.05, 0.03)	0.591
	0–24	-0.01	0.01	(-0.05, 0.03)	0.591
Platelets ($\times 10^3$/µL)	0–12	-7.27	6.25	(-21.21, 6.66)	0.270
	12–24	9.42	12.14	(-20.27, 39.13)	0.467
	0–24	-0.85	5.25	(-13.72, 12.01)	0.631
Neutrophils ($\times 10^3$/µL)	0–12	0.06	0.25	(--0.46,0.59)	0.800
	12–24	-0.05	0.46	(--1.12, 1.01)	0.910
	0–24	0.01	0.29	(-0.69, 0.67)	0.970
WBC ($\times 10^3$/µL)	0–12	0.02	0.11	(-0.38, 0.43)	0.90
	12–24	0.21	0.24	(-0.36, 0.78)	0.410
	0–24	0.02	0.26	(-0.60,0.65)	0.270
MCV(fL)	0–12	0.30	0.67	(0.05,0,80)	0.66
	12–24	0.37	0.18	(-1.78, 1.28)	0.08
	0–24	-0.25	0.64	(-1.78, 1.28)	0.71

Data are presented as mean difference, standard error (SE) and **CI**, confidence interval; **MCHC**, mean corpuscular hemoglobin concentration; **MCH**, mean corpuscular hemoglobin; **WBC**, white blood cell count, **MCV**- mean corpuscular volume. *a*- For each measure a paired T-Test was employed to assess the differences between the time periods in pairs, comparing day 0 vs. week 12; week 12 vs. Week 24 and day 0 vs Week 24. Significance was set at $p<0.05$.