Ascorbic acid supplementation improves postprandial glycaemic control and blood pressure in people with type 2 diabetes: Findings of a randomized cross-over trial

Running title: *Ascorbic acid and glycaemic control*

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Abstract

Aims: The primary aim of the study was to investigate whether ascorbic acid (AA) supplementation improves postprandial glucose responses under free living conditions in people with type 2 diabetes. A secondary aim was to investigate the effect of AA supplementation on blood pressure.

Materials and methods: Thirty-one people with type 2 diabetes (26 males/5 females; aged 61.8±6.8 y; duration of diabetes 5.6±4.6 y; HbA1c 7.6±0.7% [mean±SD]) were enrolled in a randomized cross-over study involving 4 months of supplementation with oral AA (2x500 mg/day) and placebo. Participants wore continuous glucose monitors for 48 h and consumed standardized meals pre- and post-supplementation. Measurements included postprandial glucose incremental areas under the curve (iAUC), duration of day in hyper- and hypo-glycaemia, average 24 h and daily postprandial glucose concentrations, HbA_{1c}, insulin, blood pressure and oxidative stress (F_2-isoprostanes).

Results: Following AA supplementation, significant decreases were observed for daily postprandial glucose iAUC (-36%), duration of day in hyperglycaemia (-2.8 h/day) and postprandial hyperglycaemia (-1.7 h/day), average 24 h glucose (-0.8 mmol/L) and daily postprandial glucose (-1.1 mmol/L) concentrations, systolic (-7 mmHg) and diastolic (-5 mmHg) blood pressures, and a specific fraction of free plasma F_2-isoprostanes (-47 pg/ml), when compared to placebo.

Conclusions: People with type 2 diabetes experienced improved postprandial and 24 h glycaemia and decreased blood pressure after 4 months of AA supplementation when compared to placebo. These findings offer evidence for the proposed use of AA as an adjunct therapy to improve glycaemic and blood pressure control in people with type 2 diabetes (#ACTRN12616000276459).

Introduction

Ascorbic acid (AA) is a major water-soluble antioxidant that decreases cellular and tissue oxidative stress [1-3]. Oxidative stress has been proposed as an important underlying causative agent in the pathogenesis of insulin resistance [4] and hyperglycaemia-induced diabetes complications [5]. Furthermore, some studies have shown that AA can improve whole body insulin action in people with type 2 diabetes (T2D) [6,7]. We recently found that four months of AA supplementation increases insulin-mediated peripheral glucose disposal and decreases skeletal muscle oxidative stress during hyperinsulinaemia in people with T2D [8]. Improvements in insulin sensitivity with AA may subsequently promote improvements in glycaemic control. Indeed, findings of some studies [7,9-12] but not others [13] support the efficacy of AA supplementation in improving glycaemic outcomes including lowering of HbA_{1c} and fasting glucose concentrations in people with T2D.

Reliance on general measures such as HbA_{1c} to characterize glycaemic control may fail to account for daily glycaemic excursions that can lead to acute hypoglycaemic events or transient postprandial hyperglycaemia [14]. Use of continuous glucose monitors (CGMs) can overcome such limitations by allowing assessment of daily glucose excursions into hyperglycaemia and hypoglycaemia in free-living conditions. Assessment of postprandial glycaemia is of particular clinical importance, given that evidence from epidemiological and interventional studies implicate postprandial hyperglycaemia as an independent risk factor for cardiovascular disease and cardiovascular events in people with T2D [15-17]. Assessment of hypoglycaemia is also clinically important, as severe hypoglycaemia may result in symptoms such as stupor, unconsciousness or even death, and is considered a potential risk with therapies that improve mean glycaemic control [18]. Currently there is a paucity of data investigating effects of AA supplementation on postprandial glycaemia and daily excursions into hyper- and hypo-glycaemia during free-living conditions in people with T2D.

Control of cardio-metabolic disease risk factors including high blood pressure is important in the management of T2D [19]. Systematic reviews of randomized controlled trials have reported potential beneficial effects of AA supplementation on blood pressure in people with T2D [20,21]. However, the number of studies is limited, and of these studies, further limitations include a lack of specificity for AA only and short supplementation durations [20,22,23]. Thus, there is a need to further explore the efficacy of AA supplementation in people with T2D.

The main aims of the study were to investigate effects of AA supplementation on postprandial glucose responses, and the duration of the day spent in periods of hyper- and hypo-glycaemia using ambulant CGMs. We hypothesized that AA supplementation would attenuate the daily postprandial rise in blood glucose concentrations, thereby lowering the duration of the day spent in hyperglycaemia in people with T2D. An additional aim of our study was to investigate the effects of AA supplementation on blood pressure of participants.

Materials and Methods

Study design and participants

A double-blind, placebo-controlled cross-over study was undertaken in people with T2D. Recruitment occurred via local newspaper advertisements during 2016–2017. Of the individuals screened by phone or email, *n*=43 underwent clinical screening to confirm study eligibility. From these individuals, *n*=31 were enrolled (see **Figure S1** for participation flow and **Table 1** for participant baseline characteristics). Participants ingested a capsule containing AA (500 mg L-ascorbic acid, microcrystalline cellulose, vegetable cellulose and vegetable magnesium stearate; Solgar, Inc., Leonia, USA) or placebo (560 mg gelatine, 8 mg calcium carbonate, vegetable magnesium stearate and vegetable cellulose; Solgar, Inc.) twice daily for four months (123±8 [mean±SD] days). AA and placebo capsules appeared identical and were provided to participants in identical opaque sealed bottles. A minimum one-month washout (46±16 days) separated treatments [7,8]. Concealment and randomization of treatment order (by coin toss) was undertaken by a third party with no direct involvement in the study.

Participation criteria included: having diagnosed T2D that is managed with diet or oral anti-hyperglycaemic medications; age 35–75 y; stable HbA_{1C}≥6.5% and <10.0%; total cholesterol≤6.5 mmol/L; HDL cholesterol≥0.9 mmol/L; triglycerides≤4.0 mmol/L; BMI<35 kg/m^2; systolic blood pressure (SBP)<160 mmHg; diastolic blood pressure (DBP)<90 mmHg; no smoking; no heart murmur, bleeding disorder or haemochromatosis; no comorbid cardiovascular, renal, or liver diseases; not taking vitamin supplements; and not pregnant or planning a pregnancy.

Ethics statement

This study was conducted in accordance with the principles of the Declaration of Helsinki as revised in 2008. All procedures involving human subjects were approved by the Deakin

University Human Research Ethics Committee. Written informed consent was obtained from all subjects prior to participation.

Study protocol

Participants visited our Burwood clinical laboratory over two consecutive days at pre- and post-supplementation time points. On day one, body composition was measured using dual-energy X-ray absorptiometry (Lunar Prodigy, GE Medical Systems, Madison, USA). Participants were fitted with a glucose sensor (Enlite™, Medtronic Minimed, Northridge, USA) and recorder (iPro™2, Medtronic Minimed). Participants received training in capillary blood sampling (Accu-chek Performa; Roche Diagnostics GmbH, Mannheim, Germany). CGM values were calibrated to capillary blood glucose concentrations, which were measured before main meals and before sleep at night. Forty-eight hours of continuous glucose data was extracted from recorders using online software (Medtronic CareLink) commencing at 12:00 am on day two, and the average of each 24 h period was used in subsequent analyses. Blood pressure was taken using an automated sphygmomanometer (HEM-907, Omron Healthcare, Hoofddorp, The Netherlands) that was calibrated to less than 5 mmHg annually using multi-subject auscultation. The average of three consecutive measurements with a 2-min interval between measurements was determined. Participants were given an accelerometer (GTX3+, Actigraph™ LLC, Pensacola, USA) to wear around their waist throughout the 48-h period of CGM recording. Data were extracted from the accelerometer using Actilife software (v6.13.3, Actigraph™ LLC) and average 24 h physical activity energy expenditure was calculated [24]. A minimum of 8 h of daily recording was deemed valid for representing usual activity. On day two, participants underwent fasting blood sampling for measurement of HbA_{1c}, glucose, lipids, plasma insulin, plasma AA, plasma F_2-isoprostanes and renal/liver function. Participants were instructed not to take trial supplements prior to blood sampling.

Standardized diet

A standardized diet (**Table S1**) was provided to participants to consume over the 48-h period of CGM recording. Energy content of the diet was based on individual estimated requirements using the Schofield equation [25], with an activity factor of 1.4 applied. Macronutrient profile of the daily diet, main meals (*n*=3) and snacks (*n*=2), approximated 55% carbohydrate, 25% fat and 20% protein [26,27]. The nutrient composition of the diet was established using Foodworks software (v.9.0 Xyris, Australia). The same diet was consumed by participants during each 48-h testing occasion.

Outcome measures, sample size determination and compliance

The primary outcome of the study was postprandial glucose incremental area under the curve (iAUC) above basal glucose, assessed as a cumulative total (10.5 h) of the 3.5 h postprandial periods after each main meal [26]. A sample size of *n*=22 was determined to provide 95% power (α=0.05, two-tailed t-test) to detect a 50% reduction in postprandial glucose iAUC after 4 months of AA supplementation [26]. Secondary outcomes were duration of the day (h/day) spent in hyperglycaemia (>10.0 mmol/L glucose) and hypoglycaemia (<4.0 mmol/L glucose), average 24 h glucose concentration, mean 10.5 h postprandial glucose concentration, HbA_{1c}, insulin, blood pressure, total 10.5 h postprandial glucose AUC (iAUC above basal glucose *plus* basal glucose AUC) and duration of the day spent in postprandial hyperglycaemia. All measures were assessed pre- and post-supplementation.

Analytical methods

Total plasma AA concentration was measured using an Ultimate 3000 Thermofisher HPLC system with UV detection as described previously [28]. Plasma insulin was measured using a human insulin ELISA (Alpco, Salem, NH, USA) according to the manufacturer's instructions.

Free F_2-isoprostanes were measured in plasma samples using negative chemical ionization gas-chromatography-mass spectrometry (GC-MS) with an Agilent 7983 GC and Agilent 5975 MS according to prior methods [29], with some modifications. F_2-isoprostanes (8,12-iso-iPF2α-VI, 5-iPF2α-VI, 8-iso PGF2α) were measured and quantified using added deuterated internal standards (0.5 ng each of 8,12-iso-iPF2α-VI-d11, 5-iPF2α-VI-d11 and 8-iso PGF2α-d4). F_2-isoprostanes were observed as two main chromatographic peaks, as shown previously [29,30]. Peak 1 contained 5-iPF2α-VI and 8-iso-PGF2α, while peak 2 contained 8,12-iso-iPF2α-VI. Peak 1 concentration was quantified using 569 m/z corrected for the combined responses of 8-iso-PGF2α-d4 (573 m/z) and 5-iPF2α-VI-d11 (580 m/z); while peak 2 concentration was quantified using 569 m/z corrected for 8,12-iso-iPF2α-VI-d11 (580 m/z). The two peak concentrations were added together for total free F_2-isoprostanes concentration.

Compliance and adverse effects

Compliance was determined from capsule counts using the equation: *number of capsules consumed/number of capsules expected to be consumed for full compliance ×100%*. Participants completed a 4-day diet record and a 7-day physical activity recall prior to commencement of each treatment [8] to monitor consistency of diet and physical activity. Participants were contacted monthly to follow-up on regular capsule ingestion, to inform of any treatment changes, and to note any adverse effects to health that may have potentially arisen from the supplementation.

Statistics

AA and placebo trials were compared using ANCOVA (SPSS v.23; IBM, Armonk, USA) with the within-subject difference in post-supplementation responses as the dependent variable and the corresponding difference in pre-supplementation responses as a covariate [31]. Adjusted mean differences between treatments and effect sizes (Cohen's *d*) were determined

from ANCOVA data. Two-way repeated measures ANOVA with Fisher's LSD tests were used to assess within-treatment pre-post changes (Prism v.6.0; Graphpad Software Inc., La Jolla, USA). Paired t-tests or Wilcoxon rank tests were used to compare survey measures of energy intake, vitamin C intake and physical activity energy expenditure [32] between treatments.

Both complete-case (CC) and per-protocol (PP) analyses were performed. Less than 5% of data were missing across randomized participants, therefore we opted for CC instead of intention-to-treat main analyses, as little bias is expected [33]. PP analyses included data of all participants who had complete data, remained consistent in their treatments, and who were at least 80% capsule compliant. To model the impact of missing data, sensitivity analyses of key outcomes were undertaken using "last-observation-carried-forward" (LOCF) imputations and multiple imputations (MI, n=5 iterations) using linear regression.

Data found to violate normality using a D'Agnostino & Pearson test were log-transformed prior to parametric testing and are presented within the results as geometric mean (SD factor). Potential order or carry-over effects were investigated using two-way ANOVAs with treatment order as the between-subject factor. Significance was set at P<0.05 for all analyses.

Results

Participants

Of *n*=31 participants who were initially enrolled, *n*=27 completed the study, and their data were used in the main analyses. Two participants withdrew from the study prior to treatment order randomization, while another participant withdrew after completing the first treatment arm only. Family obligations and/or work commitments were cited as causes of withdrawal. Another participant was withdrawn during the first treatment due to the development of a serious illness that was unrelated to study participation. Capsule compliance was high and similar between AA (geometric mean [SD factor]: 92.5% [1.1]) and placebo (94.3% [1.1]) treatments (*P*=0.94). Exclusions from PP analyses included two participants with relatively poor (<80%) capsule compliance and five participants that altered their anti-hyperglycaemic treatments during the study. No changes in compliance with any other treatments were reported during the study. No significant differences were observed between treatments for self-reported measures of energy intake (AA mean±SD: 8732±2467 kJ/day vs placebo: 8943±1957 kJ/day; *P*=0.69), vitamin C intake (AA geometric mean [SD factor]: 70.6 mg/day [2.3] vs placebo: 61.7 mg/day [3.3]; *P*=0.46) or physical activity energy expenditure (AA mean±SD: 624±441 kcal/day vs placebo: 825±711 kcal/day; *P*=0.13). The only self-reported adverse effect was a feeling of depression in one participant during placebo supplementation.

Main analyses (n=27)

Postprandial glucose

The daily 10.5 h postprandial glucose iAUC decreased significantly (*P*<0.01) during AA supplementation relative to a significantly increased (*P*<0.01) response during placebo (*P*<0.01 between treatments; **Figure 1**). Mean daily 10.5 h postprandial glucose concentration also significantly decreased (*P*=0.04) during AA relative to an increased

(P<0.01) response during placebo (P<0.01 between treatments; Figure 1). The adjusted mean changes in 10.5 h postprandial glucose iAUC and mean postprandial glucose with AA compared to placebo were -410 mmol.min.L^{-1} (Cohen's d=1.32) and -1.1 mmol/L (Cohen's d=1.27), respectively. Total 10.5 h postprandial glucose AUC (iAUC above basal glucose *plus* basal glucose AUC) decreased during AA supplementation relative to a significantly increased (P<0.01) response during placebo (**Table 2**). Raw postprandial glucose data is provided in **Figure S1**.

Duration of day spent in hyper- and hypo-glycaemia

Duration of the day spent in hyperglycaemia significantly decreased (P=0.03) during AA supplementation relative to an increased (P<0.01) response during placebo (P<0.01 between treatments; adjusted mean change vs placebo: -2.8 h/day; Cohen's d=1.15; Figure 1). Duration of time within the 10.5 h postprandial period spent in hyperglycaemia significantly decreased during AA (P=0.04) relative to a significantly increased (P<0.01) response during placebo (Table 2). There was no significant effect of AA on duration of the day spent in hypoglycaemia (Table 2).

Average 24 h glucose and general glycaemic measures

Average 24 h glucose decreased during AA supplementation relative to a significantly increased (P<0.01) response during placebo (P<0.05 between treatments; adjusted mean change vs placebo: -0.8 mmol/L; Cohen's d=0.83; Figure 1). There were no significant differences between or within treatments for HbA_{1c}, fasting glucose or insulin (Table 2).

Blood pressure

Both SBP (P<0.05) and DBP decreased during AA supplementation compared to placebo (P<0.01 between treatments; **Figure 2**). Adjusted mean differences in SBP and DBP with AA compared to placebo were -7 mmHg (Cohen's d=1.50) and -5 mmHg (Cohen's d=1.39),

respectively. Prevalence of hypertension (i.e. SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg) decreased during AA supplementation (pre: 12/27 vs. post: 7/27) and increased during placebo supplementation (pre: 9/27 vs. post: 14/27).

Body composition, lipids, energy expenditure and renal and liver function

There were no significant differences between or within treatments for measures of body composition, serum lipids and accelerometer-measured physical activity energy expenditure (Table 2). AA supplementation had no effect on serum renal or liver function measures, including creatinine, urea, uric acid, bilirubin, albumin (data not shown), estimated glomerular filtration rate, alkaline phosphatase, gamma glutamyltransferase, aspartate aminotransferase and alanine aminotransferase (Table 2)

Plasma AA concentration

AA concentration significantly increased (P<0.01) during AA supplementation compared to placebo (P<0.01 between treatments; adjusted mean change vs placebo: +50.0 µmol/L; Cohen's d=4.23; **Figure 3**).

F_2-Isoprostanes

Total free plasma F_2-isoprostanes concentration (peak 1+peak 2) decreased significantly (P=0.03) during AA supplementation, but was not significantly different when compared to placebo (P=0.11 between treatments; Figure 3). Peak 1 concentration (containing both 5-iPF2α-VI and 8-iso PGF2α) did not change significantly (P=0.17 between treatments; Figure 3). Peak 2 (containing 8,12-iso-iPF2α-VI) significantly decreased (P<0.01) with AA compared to placebo (P=0.01 between treatments; Figure 3). Adjusted mean changes in F_2-isoprostanes concentrations with AA compared to placebo were -37.9 (Cohen's d=0.65), +11.3 (Cohen's d=0.57) and -46.7 pg/ml (Cohen's d=1.05) for total (peak 1+2), peak 1 and peak 2, respectively.

PP analyses (n=20)

Overwhelmingly, findings of the PP analyses were quantitatively and statistically similar to those of the main analyses (see **Tables S2 and S3**). An exception was for fat free mass, which was found to decrease with placebo relative to AA supplementation ($P<0.05$ for comparison between treatments). However, the relatively small magnitude of difference between AA and placebo for fat-free mass (+0.6 kg) is unlikely to be of clinical significance.

Sensitivity analyses

Sensitivity analyses evaluating missing data using LOCF and MI confirmed the statistical significance of key outcome measures reported in the main analyses (see **Table S4**). Two-way ANOVAs using pre-supplementation values and within-supplementation changes with treatment order as the between-subject factor revealed no effect of treatment order on any key outcome measure. This suggests an absence of any carry-over effects of treatments.

Discussion

After four months of supplementation with AA, participants with T2D had a 36% lower daily postprandial glucose iAUC and a -1.1 mmol/L lower mean postprandial glucose concentration when compared to placebo. Improvements in postprandial glycaemia with AA supplementation likely contributed to the significant decreases also observed in the duration of the day spent in hyperglycaemia (-2.8 h/day), time spent in postprandial hyperglycaemia (-1.7 h/day) and average 24 h glucose concentration (-0.8 mmol/L) compared to placebo. These findings are of potential clinical importance given that postprandial hyperglycaemia has been considered as an independent risk factor for cardiovascular disease and cardiovascular events in people with T2D [15-17]. The improvement in total daily time spent in hyperglycaemia is also of potential clinical importance given that the risk of complications in T2D is strongly associated with previous hyperglycaemia [34]. Calculated effect sizes of these glycaemic improvements with AA supplementation indicate that they were all large improvements (Cohen's d>0.80). Magnitude of changes and statistical findings were consistent across CC and PP analyses for these key measures and the vast majority of measures. Moreover, sensitivity modelling of data consistent with intention-to-treat approaches using imputations for missing data confirmed a robustness of the key findings.

Systolic and diastolic blood pressures were significantly decreased by 7 mmHg and 5 mmHg, respectively, after AA supplementation compared to placebo. Calculated effect sizes of these blood pressure improvements with AA supplementation indicate that they were all large improvements (Cohen's d>0.80). Moreover, the calculated prevalence of hypertension was 50% lower following AA supplementation (7/27) when compared with placebo (14/27). Biologically plausible mechanisms of AA in improving blood pressure relate to its potential to enhance nitric oxide (NO) synthesis and bioavailability through its antioxidant actions. AA is thought to scavenge the oxidant superoxide [1] and therefore may decrease NO reactions

with superoxide to limit formation of the potential vasculature-damaging reactive species peroxynitrite [35]. AA has also been shown to preserve concentrations of the endothelial nitric oxide synthase (eNOS) cofactor, tetrahydrobiopterin, thus in turn maintaining NO production via eNOS [36]. Similar to our findings, previous systematic reviews found AA supplementation to lower SBP and DBP by approximately 4-5 mmHg and 3-4 mmHg, respectively, in people with T2D [20,21]. Consistent with prior studies [22,23], many participants in our study group (~40%) were regularly taking anti-hypertensive medications. Thus, our findings lend support to current evidence that AA supplementation can improve blood pressure in people with T2D, even when managed with a primary anti-hypertensive agent(s).

While we cannot confirm the mechanism(s) of postprandial glucose improvement based on our data, we speculate based on prior findings that the improved response occurred via an increase in insulin-mediated peripheral glucose disposal [8]. In that study, AA supplementation also increased skeletal muscle AA concentrations and decreased muscle oxidative stress during hyperinsulinaemia, which is suggestive of an ameliorative antioxidant effect on muscle insulin sensitivity [8]. Paolisso et al. [7] reported decreased plasma free radicals (O_2) along with improved insulin-mediated whole-body glucose disposal in elderly people with T2D using a similar AA dosage regimen to our study. We observed a significant decrease in the concentration of plasma free F_2-isoprostanes during AA supplementation, which was largely a result of decreased concentration of the plasma F_2-isoprostanes fraction co-eluting with 8,12-iso-iPF2α-VI. There has been limited specific investigation of 8,12-iso-iPF2α-VI in people with T2D, although its concentration appears to be sensitive to oxidative stress [37,38] and it is an F_2-isoprostanes isomer that is relatively prevalent in human plasma [39]. Thus, 8,12-iso-iPF2α-VI might be a quantitatively important marker of oxidative stress in plasma. Although AA might decrease oxidative stress via antioxidant actions, the relationship between oxidative stress and postprandial hyperglycaemia [40] also implicates potential indirect

lowering of oxidative stress following improvements in postprandial glycaemia. Our findings, in combination with the above study findings, offer plausible evidence that AA supplementation may decrease oxidative stress and potentially improve glycaemic control in people with T2D.

Interestingly, we did not find a significant improvement in HbA_{1c} despite an improvement in postprandial glucose responses. Previous studies that reported significant improvements in HbA_{1c} after AA supplementation either included more participants [7,9-11], enrolled participants with a higher baseline HbA_{1c} [7,9-11] and/or undertook AA supplementation for longer [11] than we did. Nonetheless, other findings we report are suggestive that HbA_{1c} may be improved given a more prolonged supplementation period. The significantly decreased average 24 h glucose and total postprandial glucose AUC following AA supplementation compared with placebo are predictive of an improvement in HbA_{1c}, given that these measures have been found to correlate strongly with HbA_{1c} variability [41,42]. On average, the wash-out period between treatments was 6.5 weeks, suggesting that there may not have been a complete wash-out of the prior treatment effects with respect to HbA1c. However of note, we did not observe any order or carry-over effect for HbA1c (or any other measures) in the study.

Another potential limitation of our study was that our cohort was predominantly male. We thus lacked adequate females to undertake a reliable gender subgroup analysis. Despite this, it was apparent that all female participants involved in the study followed the same general pattern of improvements across key outcomes measures as did the cohort on whole.

Compliance in the present study was high, and reported adverse effects low, suggesting that AA may be safely and consistently used in addition to a patient's primary diabetes treatment. Given potential side effects with common anti-diabetic medications such as hypoglycaemia and weight gain, the idea that a relatively benign and inexpensive vitamin supplement might

play a role in managing diabetes has particular appeal. Thus, findings of the current study implicate AA supplementation as a potentially useful adjunct therapy in people with T2D for management of both glycaemia and blood pressure.

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Figure legends

Figure 1. Effect of AA supplementation on key glycaemic outcomes in main analyses (*n*=27). Figure 1A shows 10.5 h postprandial glucose iAUC; Figure 1B shows the duration of day spent in hyperglycaemia; Figure 1C shows the average 24 h glucose concentration; and Figure 1D shows the mean daily postprandial glucose concentration. *Denotes significantly different (P<0.05) between points. Statistical analysis for duration of the day spent in hyperglycaemia was made using log-transformed normalised data. Bars represent the mean (or geometric mean for duration of day in hyperglycaemia). Open circles represent individual pre-supplementation data, while closed circles represent post-supplementation data.

Figure 2. Effect of AA supplementation on blood pressure in main analyses (*n*=27). Figure 2A shows systolic blood pressure and Figure 2B shows diastolic blood pressure. *Denotes significantly different (P<0.05) between points. Bars represent the mean. Open circles represent individual pre-supplementation data, while closed circles represent post-supplementation data.

Figure 3. Effect of AA supplementation on plasma concentrations of ascorbic acid and F_2-isoprostanes in main analyses (*n*=27). Figure 3A shows plasma ascorbic acid concentration; Figure 3B shows total free F_2-isoprostane concentration (peak 1 + peak 2); Figure 3C shows F_2-isoprostane peak 1 concentration (containing 5-iPF2α-VI and 8-iso PGF2α); And Figure 4D shows F_2-isoprostane peak 2 concentration (containing 8,12-iso-iPF2α-VI); *Denotes significantly different (P<0.05) between points. Statistical analysis for peak 1 was made using log-transformed normalised data. Bars represent the mean (or geometric mean for peak 1). Open circles represent individual pre-supplementation data, while closed circles represent post supplementation data.

Table 1. Baseline study participant characteristics

Characteristic/Measure	Mean ± SD
n (M,F)	31 (26,5)
Age (y)	61.8 ± 6.8
Height (m)	1.74 ± 0.09
Weight (kg)	88.7 ± 12.1
BMI (kg/m^2)	29.1 ± 3.1
SBP (mmHg)	139 ± 10
DBP (mmHg)	85 ± 9
Duration of diabetes (y)	5.6 ± 4.6
Diabetes management (*n*) - Metformin - Janumet (sitagliptin + metformin) - Sulfonylurea - SGLT2 inhibitor - Lifestyle only	 19 5 6 2 5
Anti-hypertensive (*n*) - ACE Inhibitor - AT Receptor Antagonist - Beta Blocker - Calcium Channel Blocker - Diuretic	 6 5 1 1 1
Other medications (*n*) - Anti-hypercholesterolaemic - Cardiovascular-related - GERD/gout/anti-depressive/anti-inflammatory/other	 18 5 10
HbA_{1c} (%) [mmol/mol]	7.6 ± 0.7 [59.6 ± 5.3]
Fasting glucose (mmol/L)	9.1 ± 1.8
Total cholesterol (mmol/L)	4.7 ± 1.3
LDL-C (mmol/L)	2.5 ± 1.1
HDL-C (mmol/L)	1.3 ± 0.3
Triglycerides (mmol/L)	2.1 ± 1.0
Plasma AA (µmol/L)	41.2 ± 18.4
eGFR ($mL.min^{-1}.1.73m^{-2}$)	79.1 ± 12.3
Urea (mmol/L)	6.3 ± 1.9
ALP (U/L)	75.7 ± 19.0
GGT (U/L)	48.1 ± 39.9
AST (U/L)	24.1 ± 9.2
ALT (U/L)	34.5 ± 16.3

(eGFR: estimated glomerular filtration rate; ALP: Alkaline Phosphatase; GGT: Gamma Glutamyltransferase; AST: Aspartate Aminotrasnferase; ALT: Alanine Aminotransferas

Table 2. Effect of supplementation on body composition, glycaemia, general biochemistry and physical activity (main analysis, *n*=27)

Measure	Placebo		AA		*P* value[†]	Adjusted mean difference (AA – placebo)[‡]	Cohen's *d*[§]
	Pre	*Post*	*Pre*	*Post*			
Weight (kg)	86.5 ± 12.3	86.9 ± 12.1	86.8 ± 12.0	87.1 ± 12.2	0.62	+ 0.2	0.20
BMI (kg/m^2)	29.2 ± 3.3	29.3 ± 3.2	29.2 ± 3.0	29.3 ± 3.0	0.75	+ 0.0	0.13
% Body fat	34.3 ± 5.5	34.6 ± 5.5	35.0 ± 5.2	34.8 ± 5.4	0.76	+ 0.1	0.13
Fat-free mass (kg)	57.8 ± 8.5	57.4 ± 8.3	57.5 ± 8.6	57.5 ± 8.4	0.68	+ 0.1	0.17
Hours per day in hypoglycaemia (<4 mmol/L glucose)	0.0 (1.1)	0.0 (1.1)	0.0 (1.0)	0.0 (1.1)	0.25	+ 0.1	0.47
10.5 h postprandial glucose (total) AUC (mmol.min.L^{-1})	5690 ± 1167[*]	6538 ± 1558	6442 ± 1422	6169 ± 1267[*]	0.02	- 640	1.02
Hours per day in postprandial hyperglycaemia (>10 mmol/L glucose)	2.0 (2.1)[*]	3.7 (2.3)	3.5 (2.3)	2.5 (2.5)[*,**]	0.01	- 1.7	1.10
HbA_{1c} (%) [mmol/mol]	7.4 ± 0.7 [57.4 ± 3.7]	7.6 ± 0.9 [59.6 ± 5.1]	7.7 ± 0.9 [60.7 ± 5.1]	7.6 ± 0.9 [59.6 ± 5.1]	0.36	- 0.2 %	0.37
Fasting glucose (mmol/L)	8.5 ± 1.9	9.0 ± 2.1	9.0 ± 1.8	8.7 ± 1.9	0.15	- 0.5	0.59
Fasting insulin (μIU/mL)	9.4 (1.5)	9.4 (1.8)	9.4 (1.5)	9.2 (1.6)	0.73	- 0.3	0.14
Total cholesterol (mmol/L)	4.6 ± 1.0	4.7 ± 1.1	4.7 ± 1.0	4.9 ± 1.3	0.50	+ 0.1	0.28
LDL-cholesterol (mmol/L)	2.4 ± 0.8	2.5 ± 0.9	2.5 ± 0.8	2.6 ± 1.0	0.78	+ 0.0	0.11
HDL-cholesterol (mmol/L)	1.2 ± 0.3	1.2 ± 0.3	1.2 ± 0.3	1.2 ± 0.3	0.40	- 0.0	0.35
Triglycerides (mmol/L)	1.8 (1.6)	1.8 (1.6)	1.9 (1.5)	2.1 (1.7)	0.06	+ 0.3	0.79
eGFR (mL.min^{-1}.1.73m^{-2})	75.9 (1.2)	73.9 (1.2)	76.3 (1.2)	75.3 (1.2)	0.39	+ 1.0	0.00
ALP (U/L)	70.0 ± 16.7	72.1 ± 20.5	72.9 ± 18.9	74.1 ± 23.2	0.37	+ 1.9	0.37
GGT (U/L)	38.6 (1.9)	38.7 (1.8)	34.1 (1.8)	35.7 (1.8)	0.11	+ 4.1	0.67
AST (U/L)	22.9 (1.4)	22.7 (1.4)	24.2 (1.4)	23.8 (1.5)	0.35	+ 1.1	0.39
ALT (U/L)	31.5 ± 15.9	34.1 ± 14.8	34.9 ± 16.3	36.7 ± 18.2	0.40	+ 1.8	0.35
24 h Accelerometer physical activity energy expenditure (kcal)	396 ± 206	432 ± 277	415 ± 247	419 ± 241	0.68	- 20.3	0.13

(Data are mean ± SD; [†]*P* value for ANCOVA test between treatments; [‡]mean difference between treatments after adjusting for pre-supplementation differences using ANCOVA test; [§]effect size of ANCOVA test; [¶]data was analysed using log-transformed normalised data and is expressed as geometric mean (SD factor); [*]denotes significantly different to Placebo Post; [**]denotes significantly different to AA Pre, $P<0.05$)

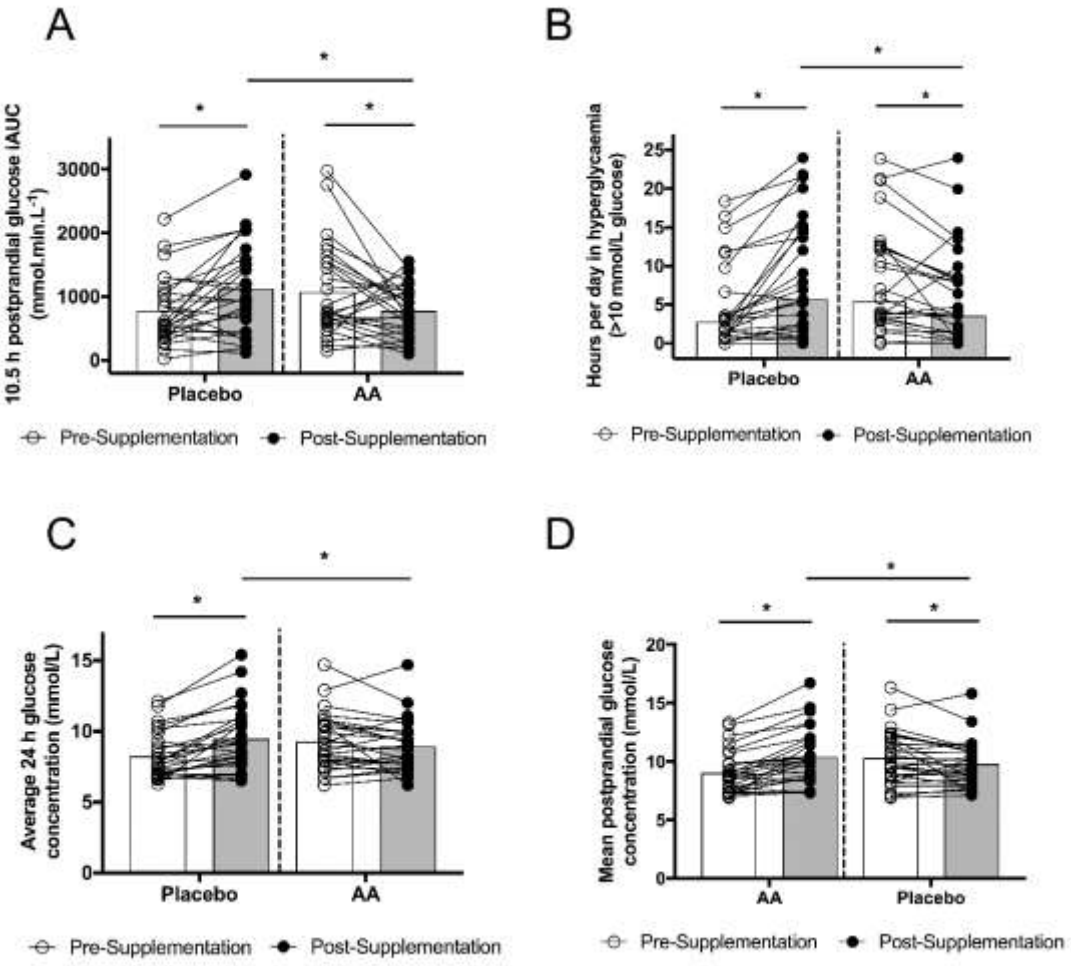

Figure.1

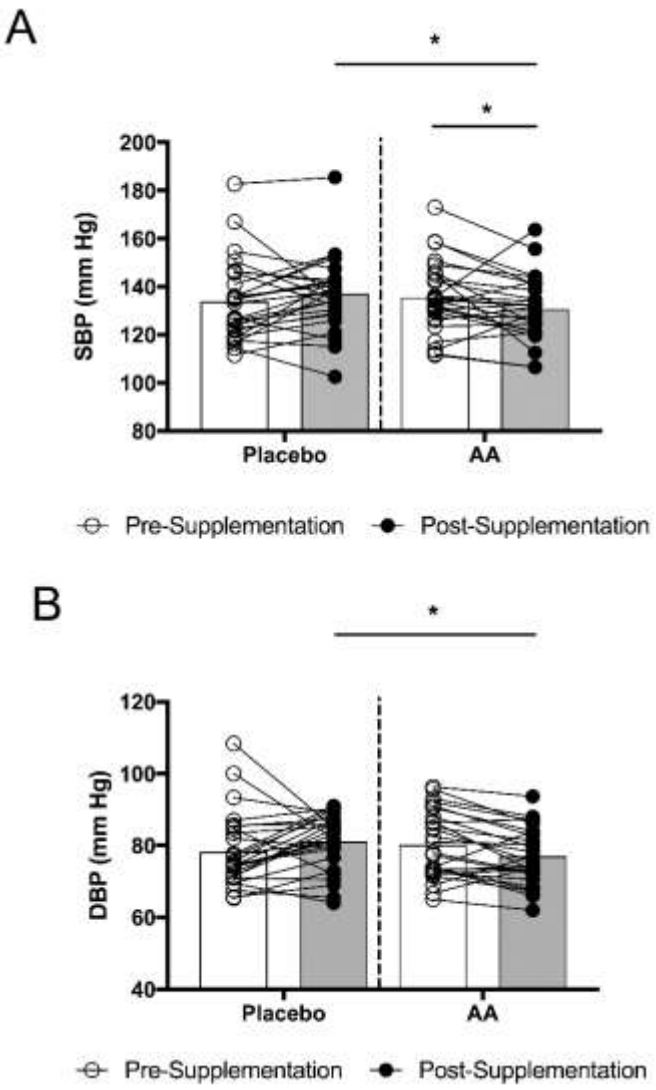

Figure 2.

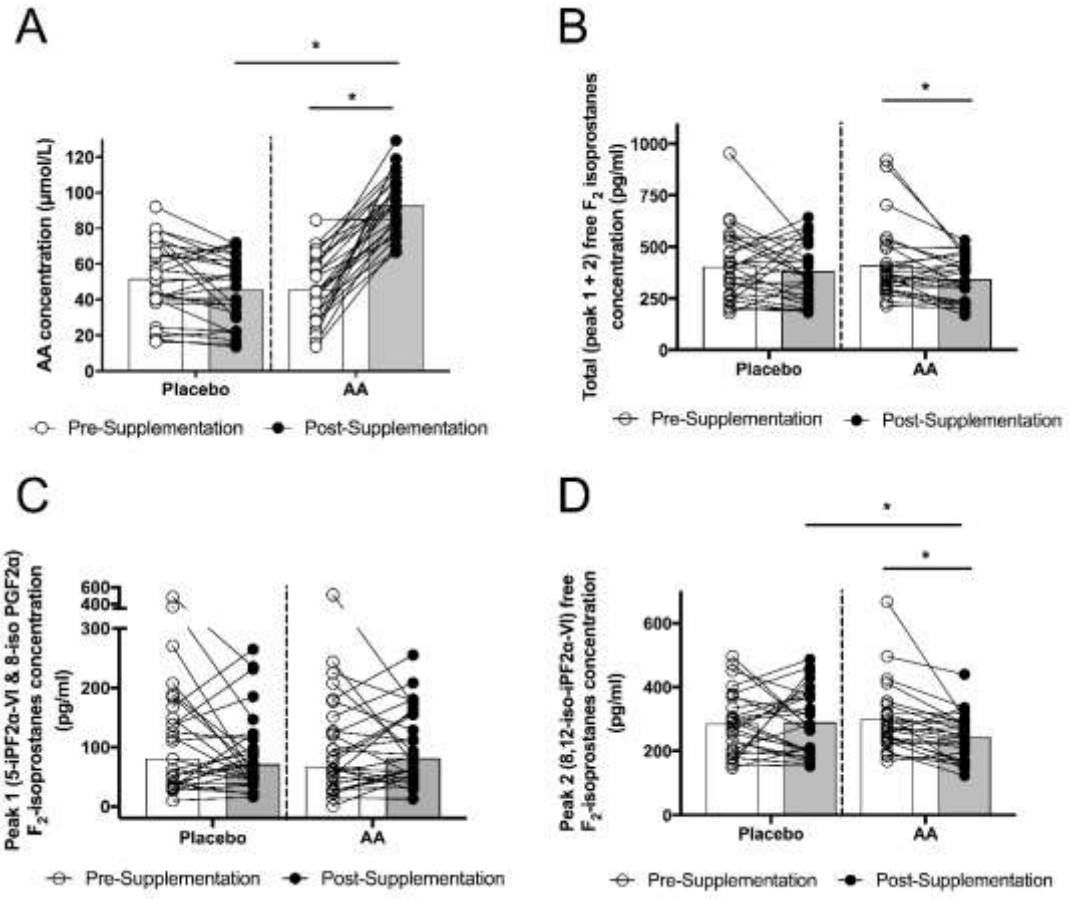

Figure 3.