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Metabolic, Anthropometric and Blood Pressure Effects of Adding Two Kiwifruit or Bottled Water into the Diets of People with Pre-Diabetes: A Randomised, Parallel Group, Intervention Study

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Abstract

People with pre-diabetes may be reluctant to add fruit to their diets due to concerns around sugars. Our objective was to measure outcomes associated with potential adverse effects of ingesting fruit sugars while assessing metabolic benefits from eating nutrient-rich kiwifruit. Thirty-four people with pre-diabetes were randomized to receive two kiwifruit or 250 mL bottled water per day for 12-weeks; two people withdrew. The primary outcomes were between-group differences for glycated haemoglobin (HbA1c) and plasma vitamin C concentration. Secondary outcomes included anthropometry, blood pressure, lipids, uric acid,

glycaemic-, inflammatory- and oxidative-markers. Mean (95% confidence interval) vitamin C intake and plasma concentration increased by 170 (141, 200) mg/d (P<0.001) and 11 (5, 17) µM (P = 0.001), respectively, in the kiwifruit compared with the control group. There was no between-group difference for HbA1c or for anthropometric, blood pressure or metabolic markers except for Trolox equivalent antioxidant capacity (TEAC) assay, for which there was a mean decrease of 27 (3, 51) mM (P = 0.027) in the plasma of the kiwifruit compared with the control group. The inclusion of two kiwifruit per day into the diets of people with pre-diabetes raised vitamin C intake and status without causing metabolic disturbance due to the sugars contained in the fruit. The reason behind a reduction in TEAC in the kiwifruit group despite an increase in plasma vitamin C is unclear. For any beneficial effect on HbA1c to become evident, it may require a larger sample and a longer intervention.

Keywords
Pre-diabetes; fruit; kiwifruit; sugars; glycated haemoglobin

1. Introduction

Fruits are a valuable component of the human diet because of the wide range of vitamins, minerals, dietary fibre and other bioactives they supply, for which reason regular fruit consumption is advised by health authorities around the world [1]. Amongst fruit, kiwifruit are relatively rich in nutrients with an exceptionally high complement of vitamin C [2]. However, kiwifruit, like most fruit, are also rich in fruit sugars, in particular glucose and fructose. Dietary glucose may increase exposure to hyperglycaemia and risk of long-term medical complications typical of diabetes that arise from the widespread systemic effects of high blood glucose concentrations [3]. Although less glycaemic than glucose, fructose has been implicated in metabolic dysregulation through its unregulated entry into lipid metabolism [4], including a triglyceride raising effect [5]. These effects have been found particularly when fructose has been used in doses outside the range of what would be consumed in a well- balanced diet [6]. Additionally, hepatic fructose phosphorylation may deplete cellular phosphate leading to AMP degradation, which generates uric acid, a purine breakdown product [7]. Elevated uric acid as a result of fructose consumption has been implicated in gout and as an independent risk factor for hypertension [8]. The epidemiological link between fructose, hyperuricaemia, gout and hypertension is strongest for sweetened beverage intake [9, 10], although a role for whole fruit has not been ruled out [10]. Therefore, it is important to ensure that the benefits of consuming kiwifruit are not counteracted by putative negative effects of ingesting fruit sugars.

In an acute setting in people with normal glucose tolerance, we have found that two kiwifruit ingested as a preload 30 min before a main meal significantly reduced the amplitude of the glycaemic response to the main meal compared with a carbohydrate-matched meal consumed in one sitting [11]. For effectiveness, any acute effects of lowered postprandial glycaemia need to translate into long-term benefits, potentially expressed in people with pre-diabetes as a change in glycated haemoglobin (HbA1c). From a public health perspective, it's also necessary that the intervention is acceptable for people to adopt as a lifelong dietary habit [12].

This study was designed therefore as a 12-week randomised controlled trial in which people with pre-diabetes would incorporate either two kiwifruit (intervention) or water (control) into their diet each day while monitoring a range of physical and metabolic parameters. Our hypothesis was that the addition of kiwifruit into the diet would increase circulating vitamin C whilst producing no adverse metabolic or physical effects in the kiwifruit intervention group. The primary outcomes would be HbA1c and plasma vitamin C concentration with secondary outcomes comprising anthropometry, blood pressure, wellbeing, biomarkers of glycaemia, insulin resistance, dyslipidaemia, oxidative stress, and immune function.

2. Materials and Methods

2.1 Study Design

The experiment was a single blinded, randomised, parallel group study in which participants either ate two kiwifruit (intervention group) or drank 250 mL bottled water (control group) per day over a 12-week period. Kiwifruit were provided as whole fruit weighing approximately 100 g each after peeling the skin. There was a three week lead in period in which the participants were asked to not consume kiwifruit (Figure 1). Measurements of outcome were made at the start and at the end of the intervention period.

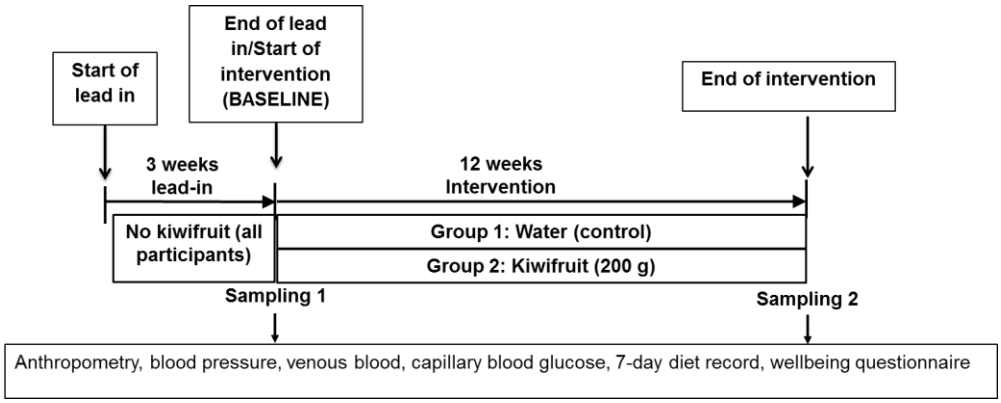

Figure 1 Plan of study to determine 12-week effects of consuming two kiwifruit daily.

2.1.1 Ethics Statement

The trial was approved by the New Zealand Human and Disabilities Ethics Committee of the New Zealand Ministry of Health (HDEC, no. 18/CEN/2) and from a Māori perspective on behalf of the Marae Te Mauri o Rangitāne o Manawatū (Council of Elders) and Tanenuiarangi Manawatū Incorporated (Iwi Authority). Trial registration was with the Australia New Zealand Clinical Trials Registry (ANZCTR no. ACTRN12618000329268).

2.2 Recruitment and Screening

Potential pre-diabetic participants were contacted by online notification within Plant & Food Research, by flyers posted at Plant & Food Research, Massey University, Central Public Health Organisations (CPHO), Diabetes Trust and local newspaper. The study was briefly described in all forms of recruitment advertising. Volunteers who self-identified as being pre-diabetic were invited

to attend the Plant & Food Clinical Trials Unit to be screened by the trial investigators for eligibility. The inclusion criteria were normal weight, overweight or obese males and females aged 18 to 70 y with HbA1c in the range 40 – 55 mmol/L, a range that encompasses the values of 41 – 49 mmol/mol used to define pre-diabetes [13]. Exclusion criteria were intolerance of kiwifruit, a planned change in diet or medication within the trial period, and diagnosis of any illness or gastrointestinal disorder within the 3 weeks prior to the trial. A screening test for HbA1c was carried out on a fingerprick blood sample using a HemoCue HbA1c analyser (Angelholm, Sweden). The nature of the study and the involvement and responsibilities of participants was described to the volunteers who were also given an information sheet containing study details. All participants signed an informed consent form. The participants received a $20 supermarket voucher for attending pre-screening and a $100 supermarket voucher for each testing session.

2.3 Assignment, Treatments and Intervention

From 77 respondents, 14 men and 20 women were identified as eligible to take part in the study and randomly assigned to the intervention (n = 18) and control (n = 16) arms of the trial (Figure 2, CONSORT flow diagram). Randomisation was undertaken by a biostatistician not involved in the practical aspect of the trial. Assignment concealment was achieved with the use of sealed envelopes containing the treatment arm for each participant.

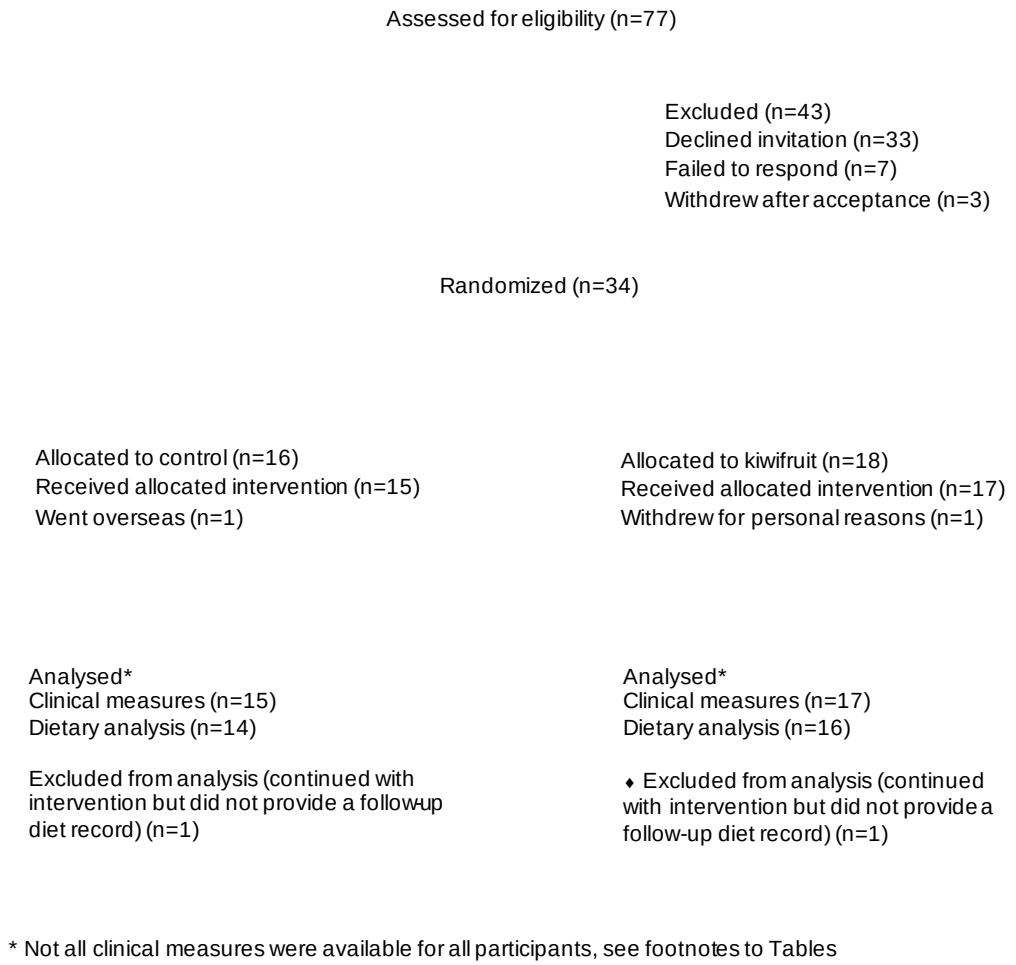

Figure 2 CONSORT flow diagram.

Kiwifruit (Actinidia chinensis var. chinensis 'Zesy002') marketed as Zespri® SunGold Kiwifruit of export quality grade were supplied by Zespri® over the course of the trial (Zespri International Limited, Mount Maunganui, New Zealand). Fourteen kiwifruit were given to participants each week of the intervention period to ensure a constant supply of fruit in good condition. The water (control) was supplied as 250 mL bottled sparkling water (Lightly Sparkling Spring Water, Woolworths, Auckland, New Zealand).

Participants in both groups were asked to continue with their customary diet for fifteen weeks (3 weeks lead-in, 12 weeks intervention period) and not to consume vitamin supplements. During the lead-in period all participants were asked not to consume kiwifruit and for those assigned to the control group, not to eat kiwifruit during the intervention period. For the 12-week intervention, participants in the kiwifruit group were asked to eat two kiwifruit and in the control group to drink 250 mL bottled water each day 30 minutes before breakfast. To check compliance, each participant filled in a daily form on which the time of ingesting the intervention and breakfast were indicated. To assess well-being, participants were asked to record how well they were tolerating the intervention including change in bowel habits.

2.4 Food Diaries, Clinic Visits and Blood Sampling

Two 7-day food diaries were kept, the first covering the last seven days of the lead-in period, and the second at the end of the 12-week intervention (Figure 1). Participants were trained in dietary recording and provided with written instructions and electronic scales. Food diaries were taken as a means of assessing changes in nutrient intakes between groups that could affect the primary outcomes (HbA1c and plasma vitamin C) independent of the intervention foods.

Participants attended the Plant & Food Research clinic at the end of the lead-in period (baseline) and after 12 weeks of the intervention after a 10-12 hour overnight fast (Figure 1). Blood pressure, height, weight, waist and hip circumference were taken during each visit. Blood pressure was measured using an OMRON model HEM-7322 automatic monitor (OMRON Healthcare Group, Kyoto, Japan). Height was measured to the nearest 0.1 cm and weight to the nearest gram using a Seca model 213 stadiometer and Seca scales model 762, respectively (Seca GmbH, Hamburg, Germany). Weight was measured with shoes off, light clothing and pockets emptied. A cloth tape measure was used to measure waist and hip circumference to the nearest 0.1 cm. Waist measurement was conducted on bare skin or over a thin item of clothing.

A capillary blood sample was taken by finger prick to measure fasting blood glucose concentration. Venous blood was drawn from each participant's arm by a trained phlebotomist into four Becton Dickinson vacutainer tubes comprising one 4 ml and one 10 ml lavender potassium EDTA tube; one 5 ml gold serum separator tube (SST II Advance); and one 6 ml green heparin tube (Becton Dickinson & Co., Franklin Lakes, NJ, USA).

2.5 Blood Processing and Analyses

Fasting blood glucose concentration was determined using a HemoCue™ Glucose 201 DMRT Analyzer (Angelholm, Sweden). The 10 ml lavender and the green tubes were placed on ice immediately after sampling. Plasma was separated after the whole blood had been centrifuged at 1000 g for 15 min. Plasma aliquots were stored in 1 ml Eppendorf tubes at -80 ^{o}C until analysis. For vitamin C determination, 0.2 mL plasma was mixed with 0.2 mL 10% metaphosphoric acid/2 mM

EDTA, stood on ice for 5 min, and centrifuged (16,000 g, 10 min, 4°C), and the supernatant stored at -80°C until analysis. The 4 ml lavender and the gold tubes were delivered to an Internationally Accredited Medical Laboratory (MedLab Central, Palmerston North, New Zealand). HbA1c was measured in whole blood using a D-100™ HPLC system (Bio-Rad Laboratories, CA, USA). The following analyses were undertaken on a Cobas 702 auto-analyser (Roche Diagnostics, Basel, Switzerland). Serum lipids kits CHOL2, HDLC4, and TRIGL were used for the measurement of total-, high-density lipoprotein cholesterol and triglycerides, respectively. Low-density lipoprotein cholesterol was calculated using the Friedewald equation. Plasma was tested for glucose, uric acid, and C-Reactive Protein (CRP) using GLUC3, UA2 and CRP4 kits, respectively.

Plasma insulin was analysed on a Roche Cobas e411 instrument after polyethylene glycol (PEG) precipitation of immunoglobulins (Canterbury Health Laboratories, Christchurch, New Zealand). Plasma lactate dehydrogenase (LDH) was determined using a proprietary kit (Abcam®, Cambridge, United Kingdom, ref. ab102526). Plasma was diluted 1/50 in assay buffer supplied in the kit and the assay was run according to manufacturer's instructions. LDH activity in samples was calculated from the change in optical density at 450 nm once activity of the samples reached the linear range (between 10 and 40 mins of incubation at 37 degrees). NADH generated from LDH activity was calculated from the standard curve and subsequently activity of LDH (mU/ml) was calculated by the equation ((nmol NADH/(change in optical density x volume of sample added to the reaction in ml x sample dilution factor). One unit of LDH is the amount of enzyme that catalyses the conversion of lactate to pyruvate to generate 1 µmol of NADH per minute at pH 8.8 at 37 degrees.

Plasma total antioxidant capacity was measured as Trolox equivalents using a proprietary kit (Abcam®, Cambridge, United Kingdom, ref. ab65329). The Trolox equivalent antioxidant capacity (TEAC) assay measures the antioxidant capacity of a given substance, as compared to the standard, Trolox, and is often used to measure the antioxidant capacity of foods, beverages and nutritional supplements. Plasma samples were masked 1:1 in protein mask supplied in the kit to measure small molecule antioxidant capacity. Plasma samples were then diluted 1/1500 in double distilled water and the assay was carried out according to manufacturer's instructions. Optical density was read at 570 nm after incubation at room temperature on an orbital shaker for 90 minutes. TEAC (nM) in samples was calculated using the equation (Trolox calculated from standard curve (nmol) / sample volume (µL) x sample dilution factor).

Plasma superoxide dismutase (SOD) activity was assayed in undiluted plasma samples using a proprietary kit (Abcam®, Cambridge, United Kingdom, ref. ab65354). The assay was run according to manufacturer's instructions. SOD activity (inhibition rate %) of samples was calculated using the OD at 450 nm following a 20 minute incubation at 37 degrees and adjusted by the OD of three blanks (a water control blank, a sample with no enzyme working solution blank and a water with no enzyme solution blank) using the equation ((blank1-blank3)-(sample-blank2))/(blank1-blank3) x 100.

Plasma interleukin 6 (IL-6) and tumour necrosis factor alpha (TNF-α) assays were carried out with undiluted plasma samples using the Human IL-6 and TNF-α High sensitivity ELISA kits (eBioscience refs. BMS213HS and BMS223HS) according to manufacturer's instructions (Affymetrix, Santa Clara, CA, USA). Optical density was measured at 450 nm and concentrations of IL-6 and TNF-α were calculated from standard curves.

2.6 Power Calculation and Statistical Analysis

A sample size of 20 per group with a conservative within-person correlation of 0.6 (in-house data) would be required to detect a difference between groups in HbA1c of 5.5 mmol/mol given a SD of 7.7 with 80% power and alpha set at the 5% level of significance [14]. The sample could be reduced to a minimum of 16 per treatment given a more homogenous group having a within-person correlation of 0.7.

All statistical analysis was undertaken in Stata 17.0 (StataCorp, Texas). To estimate the effect of kiwifruit compared to water, outcome variables were included as dependent variables in a linear regression model, with treatment group as an independent variable and adjusted for baseline. Mean differences and 95% confidence intervals (CI) were reported for all outcomes as well as p-values. $P<0.05$ was considered statistically significant. As the effect size is represented by a mean difference in change, the change for the outcome variable was described using mean and standard deviation (SD), as well as baseline values. Residuals of all models were plotted and visually assessed for homoskedasticity and normality. No adjustment for multiple comparisons was made.

3. Results

The flow of participants through the study is given in Figure 2. Mean (SD) percentage compliance with ingestion of the study foods was 94.4% (11.2) and 93.3% (7.4) for the control and intervention groups, respectively. Two participants in the kiwifruit group reported transient diarrhoea throughout the intervention period, one associated with a stomach bug that lasted 6 days, the other with diarrhoea alone over 3 days.

The age of participants ranged from 39 to 73 y, and body mass index (BMI) ranged from 19.8 to 47.7 kg/m^2. Characteristics of the participants are given in Table 1.

Table 1 Demographic characteristics by randomized group (n = 32).

Characteristic		Total	Control group	Intervention group
Sex	Male	14	9	5
	Female	18	6	12
Age, y – mean (SD)		56.1 (9.5)	57.0 (10.9)	55.3 (8.3)
BMI, kg/m^2 – mean (SD)		30.6 (6.4)	30.6 (5.7)	30.5 (7.2)

BMI = Body mass index (n = 42); SD = standard deviation.

Of the participants, 18 were Caucasian, 1 was Asian, and the remainder were of mixed race, predominantly Polynesian. The mean (SD) fingerprick HbA1c at screening was 44.3 (3.4) mmol/mol with a range of 40 to 54 mmol/mol.

3.1 Diets

The mean (SD) macronutrient composition of the diets at baseline, calculated as percent of energy of protein, fat and carbohydrate, were 19% (3.5), 38% (5.9) and 40% (5.8) for the control

group and 19% (3.6), 38% (5.0) and 40% (8.6) for the intervention group. These macronutrients contributed around 97% of the diet's energy with the remainder provided by fibre and alcohol. The mean (SD) changes in proportional energy from protein, fat and carbohydrate during the intervention were +0.5% (2.3), -1.0% (7.7) and -0.1% (7.3) for the control group and -0.4% (3.2), -3.5% (4.7) and +3.7% (6.6) for the kiwifruit group, indicating a small reduction in the proportion of energy from fat with a corresponding increase in the proportion of carbohydrate in the diets of the kiwifruit intervention group.

An analysis of fruit serves and selected nutrients of the diet records is given in Table 2. Fruit serves are given as these would be expected to increase in the intervention group due to the inclusion of two kiwifruit into the diet. Dietary energy intake has been calculated as the inclusion of kiwifruit energy by the intervention group compared with the non-energy containing water given to the control group could have generated a between-group difference in energy intakes. Fibre and vitamin C intakes were calculated as these nutrients are contained in kiwifruit.

Table 2 Mean (SD) dietary intake per day before and during the 12-week intervention period.

Dietary factor	Kiwifruit intervention		Water intervention		Mean (95% CI) difference in change between groups	p-value
	Lead-in period Mean (SD)	End stage Mean change (SD)	Lead-in period Mean (SD)	End stage Mean change (SD)		
Energy (kJ)	8770 (2928)	-903 (2225)	8787 (2349)	-1141 (1417)	232 (-1011, 1475)	0.705
Fibre (g)	22.6 (8.1)	0.1 (6.0)	26.6 (7.4)	-3.1 (6.0)	1.6 (-2.5, 5.8)	0.422
Vitamin C (mg)	96 (74)	150 (76)	87 (54)	-13 (52)	170 (141, 200)	<0.001
Fruit serve	1.3 (1.3)	0.4 (1.0)	1.3 (0.9)	-0.4 (0.5)	0.8 (0.4, 1.2)	<0.001

Kiwifruit group (n = 16) water group (n = 14)

The mean energy intake of both groups declined during the intervention period while the number of fruit serves and vitamin C intake increased in the kiwifruit group compared with the water group.

Anthropometric and blood pressure data are given in Table 3.

Table 3 Mean (SD) anthropometric and blood pressure measurements at baseline and at 12-weeks.

	Kiwifruit intervention		Water intervention			
	Mean (SD) at baseline	Mean change (SD) at 12-wk	Mean (SD) at baseline	Mean change (SD) at 12-wk	Mean (95% CI) difference in change between groups	p-value
Weight (kg)	85.3 (22.9)	1.5 (2.1)	92.9 (22.6)	0.0 (2.3)	1.4 (-0.2, 3.1)	0.089

Waist circumference (cm)	95.6 (16.5)	2.9 (5.0)	101.9 (13.0)	0.7 (2.9)	2.3 (-0.8, 5.4)	0.138
Hip circumference (cm)	110.5 (11.2)	0.7 (3.5)	112.1 (10.0)	-1.2 (2.4)	2.1 (-0.1, 4.2)	0.064
Systolic blood pressure (mmHg)	145.4 (25.2)	-3.5 (12.3)	138.1 (20.1)	1.0 (20.2)	-2.7 (-14.4, 8.9)	0.633
Diastolic blood pressure (mmHg)	90.9 (12.4)	1.5 (7.4)	88.7 (12.8)	0.3 (9.6)	1.4 (-4.7, 7.6)	0.637

Weight, blood pressure, and waist and hip circumference did not change significantly between the two groups. Nor were there perceived differences in change in general well-being over the course of the study.

All metabolic outcomes are given in Table 4.

Table 4 Mean (SD) metabolic markers at baseline and at 12-weeks.

Metabolic markers	Kiwifruit intervention		Water intervention		Mean (95% CI) difference in change between groups	p-value
	Mean (SD) at baseline	Mean change (SD) at 12-weeks	Mean (SD) at baseline	Mean change (SD) at 12-weeks		
HbA1c (mmol/mol)	42 (3)	0 (3)	41 (3)	-1 (1)	1 (-1, 2)	0.450
Vitamin C (µM)	33 (15)	8 (12)	30 (12)	-2 (5)	11 (5, 17)	0.001
Blood glucose (mmol/L)	5.9 (0.8)	0.0 (0.5)	6.3 (1.0)	0.1 (0.5)	-0.2 (-0.6, 0.2)	0.406
Insulin (pmol/L)	86 (61)	12 (44)	93 (36)	7 (25)	1 (-21, 23)	0.936
Uric acid (mmol/L)	0.35 (0.08)	-0.01 (0.04)	0.37 (0.10)	0.00 (0.03)	-0.02 (-0.05, 0.01)	0.267
Total cholesterol (mmol/L)	5.3 (0.9)	-0.1 (0.6)	5.4 (1.1)	0.2 (0.6)	-0.3 (-0.8, 0.2)	0.213
Triglyceride (mmol/L)	1.7 (1.0)	-0.1 (0.6)	1.7 (0.9)	0.3 (0.7)	-0.4 (-0.9, 0.1)	0.137
HDL cholesterol (mmol/L)	1.3 (0.3)	-0.1 (0.2)	1.4 (0.5)	0.0 (0.1)	0.0 (-0.1, 0.1)	0.584
LDL cholesterol (mmol/L)	3.2 (0.8)	-0.1 (0.5)	3.2 (0.9)	0.0 (0.5)	-0.2 (-0.6, 0.2)	0.430
Ratio total:HDL cholesterol	4.1 (1.3)	0.0 (0.7)	4.3 (1.5)	0.2 (0.6)	-0.2 (-0.7, 0.2)	0.344
C-reactive protein (mmol/L)	3.4 (3.9)	0.2 (3.0)	2.8 (2.9)	-0.2 (0.8)	0.5 (-1.2, 2.3)	0.527
IL6 (pg/ml)	4.2 (3.9)	-0.3 (2.0)	3.0 (2.1)	-0.4 (2.7)	0.3 (-1.5, 2.1)	0.698
TNF-alpha (pg/ml)	0.3 (0.2)	-0.2 (0.2)	0.4 (0.3)	-0.4 (0.5)	0.0 (-0.2, 0.1)	0.713
Trolox equivalents (mM)	233 (34)	-26 (43)	245 (29)	-7 (31)	-27 (-51, -3)	0.027
SOD activity (% inhibition)	73 (18)	20 (16)	69 (10)	15 (13)	8 (-1, 16)	0.072
LDH activity (mU/ml)	152 (49)	3 (87)	207 (96)	-49 (80)	2 (-37, 41)	0.916

Serum was used for lipids analyses; plasma was used for all other metabolic markers except whole blood for HbA1c

Kiwifruit group n = 17 at baseline except SOD activity (n = 16): Water group n = 15 at baseline except HbA1c and blood glucose (n = 14)

Kiwifruit group n = 17 at 12-weeks except insulin (n =16): Water group n = 14 at 12-weeks except immune markers (n = 15), HbA1c and blood glucose (n = 13)

There was no mean difference in change in HbA1c between groups while there was an increase in mean plasma vitamin C concentration in the kiwifruit compared with the control group. For all other metabolic outcomes there was no mean difference in change following the intervention period with the exception of Trolox equivalents in the TEAC assay, for which there was a decrease in the kiwifruit compared with the control group.

4. Discussion

For the primary outcomes, plasma vitamin C concentration increased in the people who incorporated two kiwifruit into their diets daily while there was no between-group difference in change in HbA1c. As a result of the intervention, there were no between-group differences for blood pressure or for the anthropometric measures. For the metabolic outcomes, there was a reduction in the Trolox equivalents for the kiwifruit group compared with the control group. There were no other group differences in lipids or for the biomarkers of glycaemia, immune function and oxidative capacity. There was an increase in fruit serves by the kiwifruit group relative to the control group, accompanied by an increase in vitamin C intake by the kiwifruit group. The macronutrient composition of the diet during the study was stable for the control group while for the kiwifruit group there was a slight increase in the proportion of energy from carbohydrate with a corresponding reduction in the proportion of fat energy. This slight shift in macronutrient energy is consistent with the introduction of kiwifruit, being a low-fat/high carbohydrate food, into the diet.

Kiwifruit have a vitamin C content of around 140 mg per fruit, relatively high compared with other commonly consumed fruits in New Zealand such as apples (6 mg) and oranges (78 mg) per fruit [15]. In placebo-controlled trials, vitamin C supplementation at a dose of 1000 mg/d has improved glycaemic control in people with type 2 diabetes [16-18]. Although there was an increase in plasma vitamin C concentration in our kiwifruit supplemented group, there was no effect on markers of glycaemic control including HbA1c, fasting glucose and insulin concentrations. This is consistent with high dose (1000 mg/d) vitamin C supplementation producing a beneficial effect whereas a dose of 500 mg/d over 6-weeks was ineffectual on markers of glycaemic control [18]. However, these results do not exclude the possibility that chronic vitamin C intakes of less than 1000 mg/d could provide glycaemic benefit to people with pre- or type 2 diabetes [19], possibly via vitamin C rich fruits in the diet [20].

Eating 2-3 kiwifruit per day, containing 280-420 mg vitamin C over 28-weeks has been found to reduce platelet aggregation and circulating triglycerides [21]. A triglyceride-lowering effect via an increased vitamin C intake is plausible given that vitamin C supplements have been found to lower triglyceride concentrations [22]. However, a lack of effect on circulating triglycerides in our study is consistent with a meta-analysis of randomised controlled kiwifruit intervention trials [23].

From a meta-analysis of randomised controlled trials, vitamin C supplementation at a median dose of 500 mg/d has been found to lower blood pressure [24]. Typically, in these trials, vitamin C is given at doses higher than those obtainable by dietary means. Perhaps for this reason, fruit and vegetable interventions, including kiwifruit interventions, have generally been ineffective at reducing blood pressure [23, 25]. The lack of an effect in the present study is consistent with the literature. Previously however, we found a favourable effect on systolic blood pressure in Asian people assigned to eat two kiwifruit per day for 7-weeks compared with a control group (unpublished data).

Fruit intake has been inversely associated with plasma C-reactive protein (CRP) concentration, a marker of inflammation [26]. In intervention trials, various fruits and fruit juices have been found to have beneficial effects on circulating high sensitivity CRP (hs-CRP) and tumour necrosis factor alpha (TNF-α), a pro-inflammatory cytokine [27]. In a kiwifruit/control crossover intervention, improvements in hs-CRP and interleukin-6 (IL-6), (but not TNF-α) were found after 4-weeks of incorporating two green kiwifruit into the diet of men with slightly elevated hs-CRP at baseline [28]. In contrast, there was no between-group difference in change in hs-CRP for people randomly assigned to eat either four gold kiwifruit or two freeze-dried bananas per day for 4-weeks [29]. The different outcomes could be due to the variety of kiwifruit or to the inflammatory status of the participants. In our study, there was no effect on CRP, TNF-α, or IL-6 comparing the kiwifruit and control groups.

In observational work, fruit intake has been associated with plasma total antioxidant capacity [30]. Increasing the intake of fruit and vegetables from 5 to 10 servings per day for 15 days has been found to raise the oxygen radical absorbance capacity (ORAC) in healthy people [31]. Acutely, the ingestion of 300 grams of kiwifruit raises plasma total antioxidant capacity [32]. In a 4-week crossover study in which participants ate 1 or 2 gold kiwifruit each day, there was no change over time in the ferric reducing activity of plasma (FRAP) but there was increased resistance to the hydrogen peroxide oxidation of lymphocytes [33]. In our study we assessed plasma Trolox equivalent antioxidant capacity (TEAC), and the activities of superoxide dismutase (SOD) and lactate dehydrogenase (LDH). The only between-group difference in change, despite an increase in plasma vitamin C concentration, was a decrease in TEAC in the kiwifruit group compared with the control group. How well TEAC reflects antioxidant status in people with diabetes is unclear, as people with pre-diabetes were found to have lower TEAC compared to people with type 2 diabetes [34]. Additionally, comparisons of antioxidant measures between people with and without diabetes are inconsistent [35] and a definitive measurement of oxidative stress is incompletely resolved [36-38]. Hence, the clinical significance of a mean decrease in TEAC in vitamin C enriched plasma between the kiwifruit and the control group is unclear. Overall, fruit interventions in the form of fruit juices have been found to raise vitamin C concentration [39] and the recommendation given to people with diabetes in New Zealand is to eat at least 3-4 servings of fruit each day [40, 41].

Contrary to this recommendation is the advice given in some publications and websites purporting to list fruits that people should avoid based on the total sugar or fructose content of the fruits [42-44]. Such contrasting information between social media sources and that given by health authorities could lead to confusion, particularly given the influence that websites and social networking sites have on people's behaviour [45]. Of concern, there is evidence that the public's trust in information is no different between website sources and government health agencies [46]. Nevertheless, there is a unifying message from Diabetes New Zealand and the aforementioned listed websites around limiting sugar intake whilst taking account of the food source of sugar [41, 43, 44]. There is evidence that sugars, including fructose, are only detrimental to glycaemic control and other metabolic outcomes in the context of excess dietary energy intake [47, 48]. Whole fruit is helpful in this regard as it has a low energy density, for example, kiwifruit contains 224 kJ/100g whereas biscuits/cookies are up to ten times as energy dense [15]. The low energy-density and the high nutrient-density of fruit may explain inverse associations between fruit intake, incident diabetes and diabetic complications found in large observational studies [49, 50]. To test the effect of a fruit intervention in people with type 2 diabetes, 63 participants were randomized to either eat

more or fewer than two pieces of fruit per day over a 12-week period, generating a mean difference of 175 g/d in fruit intake between groups with the difference in fruit intake having no effect on HbA1c, body weight or waist circumference [51]. Christensen and colleagues surmised that changing fruit intake in a diet results in substitution rather than addition to intake [51], a plausible explanation given the satiety of whole fruit [52, 53]. Our data are consistent with this work in that adding 200 g of kiwifruit each day for 12-weeks into the diets of people with pre-diabetes had no effect on HbA1c, body weight or waist circumference. This is perhaps unsurprising given that there was no difference in energy, fibre or in sugars intake between groups as a result of the intervention.

At the extreme end of fruit intake, people have been placed on predominantly fruit diets supplemented with avocado and nuts for up to 24 weeks [54]. The carbohydrate in the diet (65% of energy) was essentially sugar (sucrose, fructose and glucose) with just a small amount of starch coming from bananas. The participants maintained good health throughout the study period despite an estimated sugar intake of 350-400 g/d, on average losing weight, reducing systolic blood pressure and improving the lipid profile [54, 55]. Such metabolic improvements are contrary to the disturbances found when people consume excess energy, some of which is provided by sugar [56].

One sugar in particular that has been associated with metabolic disturbance is fructose via its hepatic metabolism resulting in the production of uric acid [7]. Even the amount of fructose contained in one apple has been found sufficient to transiently raise postprandial uric acid concentration [57]. However, a diet high in fruit and vegetables is associated with relatively high urinary alkalinity [58] creating a physiological environment which predisposes to the elimination of uric acid from the body [59]. This is consistent with the observation that fruit intake has been inversely associated with serum uric acid [60] and a reduction in serum uric acid concentration over three months when hyperuricaemic participants raised their fruit intake from 86 to 200 g/d and soybean products from 71 to 100 g/d [61]. Cross-sectional and interventional data reinforce the contention that excess energy is a major underlying factor in the aetiology of metabolic disease and that the healthfulness of a diet needs to be assessed with regard to the whole diet rather than focussing on single foods or nutrients [48].

5. Conclusions

In summary, daily consumption of two kiwifruit by prediabetic individuals was found to increase plasma vitamin C concentration without increasing metabolic risk due to sugars intake. A limitation of the work was the small sample. While adequately powered to detect a difference in HbA1c of 5.5 mmol/mol, it appears that the potential for change in HbA1c by eating two kiwifruit per day is less than this and that a larger sample and possibly a longer intervention would be required to provide definitive evidence. A strength of the study was the large number of factors considered covering outcomes that could have been adversely affected by sugars while measuring potential benefits of increased fruit intake.

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Author Contributions

S.Mishra., J.M., A.L. and B.V. designed the intervention. J.M. obtained the funding. The intervention was undertaken by H.D., S.M., and co-ordinated by S.Mishra, K.B-H. conducted antioxidant analyses, D.H. and J.H. analysed the data. B.V. and J.M. wrote the draft manuscript. All authors edited the manuscript.

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Competing Interests

The authors have declared that no competing interests exist.

References

1. Herforth A, Arimond M, Álvarez-Sánchez C, Coates J, Christianson K, Muehlhoff E. A global review of food-based dietary guidelines. Adv Nutr. 2019; 10: 590-605.
2. Richardson DP, Ansell J, Drummond LN. The nutritional and health attributes of kiwifruit: A review. Eur J Nutr. 2018; 57: 2659-2676.
3. Garg A, Grundy SM, Koffler M. Effect of high carbohydrate intake on hyperglycemia, islet function, and plasma lipoproteins in NIDDM. Diabetes Care. 1992; 15: 1572-1580.
4. Tappy L, Lê KA, Tran C, Paquot N. Fructose and metabolic diseases: New findings, new questions. Nutrition. 2010; 26: 1044-1049.
5. Wang DD, Sievenpiper JL, de Souza RJ, Cozma AI, Chiavaroli L, Ha V, et al. Effect of fructose on postprandial triglycerides: A systematic review and meta-analysis of controlled feeding trials. Atherosclerosis. 2014; 232: 125-133.
6. Livesey G. Fructose ingestion: Dose-dependent responses in health research. J Nutr. 2009; 139: 1246S-1252S.
7. Tappy L, Lê KA. Metabolic effects of fructose and the worldwide increase in obesity. Physiol Rev. 2010; 90: 23-46.
8. Jossa F, Farinaro E, Panico S, Krogh V, Celentano E, Galasso R, et al. Serum uric acid and hypertension: The Olivetti heart study. J Hum Hypertens. 1994; 8: 677-681.
9. Stewart DJ, Langlois V, Noone D. Hyperuricemia and hypertension: Links and risks. Integr Blood Press Control. 2019; 12: 43-62.
10. Choi HK, Curhan G. Soft drinks, fructose consumption, and the risk of gout in men: Prospective cohort study. BMJ. 2008; 336: 309-312.
11. Lubransky A, Monro J, Mishra S, Yu H, Haszard JJ, Venn BJ. Postprandial glycaemic, hormonal and satiety responses to rice and kiwifruit preloads in Chinese adults: A randomised controlled crossover trial. Nutrients. 2018; 10: 1110.

12. Greaves CJ, Sheppard KE, Abraham C, Hardeman W, Roden M, Evans PH, et al. Systematic review of reviews of intervention components associated with increased effectiveness in dietary and physical activity interventions. BMC Public Health. 2011; 11: 119.
13. Health Navigator New Zealand. HbA1c test-diagnosing diabetes and pre-diabetes [Internet]. Auckland: Health Navigator New Zealand; 2021 [cited date 2021 August 18th]. Available from: https://www.healthnavigator.org.nz/health-a-z/h/hba1c-testing/.
14. Incani M, Sentinelli F, Perra L, Pani MG, Porcu M, Lenzi A, et al. Glycated hemoglobin for the diagnosis of diabetes and prediabetes: Diagnostic impact on obese and lean subjects, and phenotypic characterization. J Diabetes Investig. 2015; 6: 44-50.
15. The New Zealand Institute for Plant and Food Research Limited and the Ministry of Health. [cited date 2021 August 29th]. Available from: https://www.foodcomposition.co.nz/.
16. Dakhale GN, Chaudhari HV, Shrivastava M. Supplementation of vitamin C reduces blood glucose and improves glycosylated hemoglobin in type 2 diabetes mellitus: A randomized, double-blind study. Adv Pharmacol Sci. 2011; 2011: 195271.
17. Mason SA, Rasmussen B, van Loon LJ, Salmon J, Wadley GD. Ascorbic acid supplementation improves postprandial glycaemic control and blood pressure in individuals with type 2 diabetes: Findings of a randomized cross-over trial. Diabetes Obes Metab. 2019; 21: 674-682.
18. Afkhami-Ardekani M, Shojaoddiny-Ardekani A. Effect of vitamin C on blood glucose, serum lipids & serum insulin in type 2 diabetes patients. Indian J Med Res. 2007; 126: 471-474.
19. Das UN. Vitamin C for type 2 diabetes mellitus and hypertension. Arch Med Res. 2019; 50: 11-14.
20. Will JC, Ford ES, Bowman BA. Serum vitamin C concentrations and diabetes: Findings from the third national health and nutrition examination survey, 1988-1994. Am J Clin Nutr. 1999; 70: 49-52.
21. Duttaroy AK, Jørgensen A. Effects of kiwi fruit consumption on platelet aggregation and plasma lipids in healthy human volunteers. Platelets. 2004; 15: 287-292.
22. McRae MP. Vitamin C supplementation lowers serum low-density lipoprotein cholesterol and triglycerides: A meta-analysis of 13 randomized controlled trials. J Chiropr Med. 2008; 7: 48-58.
23. Suksomboon N, Poolsup N, Lin W. Effect of kiwifruit on metabolic health in patients with cardiovascular risk factors: A systematic review and meta-analysis. Diabetes Metab Syndr Obes Targets Ther. 2019; 12: 171-180.
24. Juraschek SP, Guallar E, Appel LJ, Miller III ER. Effects of vitamin C supplementation on blood pressure: A meta-analysis of randomized controlled trials. Am J Clin Nutr. 2012; 95: 1079-1088.
25. Elsahoryi N, Patterson CC, McKinley MC, Neville CE, Baldrick FR, Mulligan C, et al. The effect of increased fruit and vegetable consumption on systolic and diastolic blood pressure in six randomized controlled fruit and vegetable intervention trials: A pooled analysis. Proc Nutr Soc. 2017; 76: E56.
26. Esmaillzadeh A, Kimiagar M, Mehrabi Y, Azadbakht L, Hu FB, Willett WC. Fruit and vegetable intakes, C-reactive protein, and the metabolic syndrome. Am J Clin Nutr. 2006; 84: 1489-1497.
27. Hosseini B, Berthon BS, Saedisomeolia A, Starkey MR, Collison A, Wark PA, et al. Effects of fruit and vegetable consumption on inflammatory biomarkers and immune cell populations: A systematic literature review and meta-analysis. Am J Clin Nutr. 2018; 108: 136-155.

28. Gammon CS, Kruger R, Conlon CA, von Hurst PR, Jones B, Stonehouse W. Inflammatory status modulates plasma lipid and inflammatory marker responses to kiwifruit consumption in hypercholesterolaemic men. Nutr Metab Cardiovasc Dis. 2014; 24: 91-99.
29. Hunter DC, Skinner MA, Wolber FM, Booth CL, Loh JM, Wohlers M, et al. Consumption of gold kiwifruit reduces severity and duration of selected upper respiratory tract infection symptoms and increases plasma vitamin C concentration in healthy older adults. Br J Nutr. 2012; 108: 1235-1245.
30. Wang Y, Yang M, Lee SG, Davis CG, Koo SI, Chun OK. Dietary total antioxidant capacity is associated with diet and plasma antioxidant status in healthy young adults. J Acad Nutr Diet. 2012; 112: 1626-1635.
31. Cao G, Booth SL, Sadowski JA, Prior RL. Increases in human plasma antioxidant capacity after consumption of controlled diets high in fruit and vegetables. Am J Clin Nutr. 1998; 68: 1081-1087.
32. Harasym J, Oledzki R. Effect of fruit and vegetable antioxidants on total antioxidant capacity of blood plasma. Nutrition. 2014; 30: 511-517.
33. Brevik A, Gaivao I, Medin T, Jorgenesen A, Piasek A, Elilasson J, et al. Supplementation of a western diet with golden kiwifruits (Actinidia chinensis var.'Hort 16A':) effects on biomarkers of oxidation damage and antioxidant protection. Nutr J. 2011; 10: 54.
34. Gunawardena HP, Silva R, Sivakanesan R, Ranasinghe P, Katulanda P. Poor glycaemic control is associated with increased lipid peroxidation and glutathione peroxidase activity in type 2 diabetes patients. Oxid Med Cell Longev. 2019; 2019: 9471697.
35. Maritim AC, Sanders A, Watkins Iii JB. Diabetes, oxidative stress, and antioxidants: A review. J Biochem Mol Toxicol. 2003; 17: 24-38.
36. Huang D, Ou B, Prior RL. The chemistry behind antioxidant capacity assays. J Agric Food Chem. 2005; 53: 1841-1856.
37. Pellegrini N, Vitaglione P, Granato D, Fogliano V. Twenty-five years of total antioxidant capacity measurement of foods and biological fluids: Merits and limitations. J Sci Food Agric. 2020; 100: 5064-5078.
38. Peng WK, Chen L, Boehm BO, Han J, Loh TP. Molecular phenotyping of oxidative stress in diabetes mellitus with point-of-care NMR system. NPJ Aging Mech Dis. 2020; 6: 11.
39. Tonin FS, Steimbach LM, Wiens A, Perlin CM, Pontarolo R. Impact of natural juice consumption on plasma antioxidant status: A systematic review and meta-analysis. Molecules. 2015; 20: 22146-22156.
40. Diabetes New Zealand. Pre-diabetes [Internet]. Wellington: Diabetes New Zealand; [cited date 2021 August 29th]. Available from: https://www.diabetes.org.nz/pre-diabetes.
41. Diabetes New Zealand. Diabetes and healthy food choices [Internet]. Wellington: Diabetes New Zealand; 2019 [cited date 2021 August 29th]. Available from: https://static1.squarespace.com/static/5a1b161b6957daf4c4f3b326/t/5d2529d9f503ce00012bbc0f/1562716644700/Diabetes+and+Healthy+Food+Choices.pdf.
42. Zou F, Zhao X, Wang F. A review on the fruit components affecting uric acid level and their underlying mechanisms. J Food Biochem. 2021; 45: e13911.
43. Madormo C. 9 fruits to avoid if you have diabetes [Internet]. Harlan: Taste of home; 2021 [cited date 2021 August 29th]. Available from: https://www.tasteofhome.com/collection/fruits-for-diabetics-to-avoid/.

44. The Urban Diabetes Network. Twenty of the worst fruits for diabetics [Internet]. The Urban Diabetes Network; 2019 [cited date 2021 August 29th]. Available from: https://www.theurbandiabetesnetwork.com/20-of-the-worst-fruits-for-diabetics/.
45. Wang Z, Walther JB, Pingree S, Hawkins RP. Health information, credibility, homophily, and influence via the Internet: Web sites versus discussion groups. Health Commun. 2008; 23: 358-368.
46. Ye Y. Correlates of consumer trust in online health information: Findings from the health information national trends survey. J Health Commun. 2010; 16: 34-49.
47. Choo VL, Viguiliouk E, Mejia SB, Cozma AI, Khan TA, Ha V, et al. Food sources of fructose-containing sugars and glycaemic control: Systematic review and meta-analysis of controlled intervention studies. BMJ. 2018; 363: k4644.
48. Khan TA, Sievenpiper JL. Controversies about sugars: Results from systematic reviews and meta-analyses on obesity, cardiometabolic disease and diabetes. Eur J Nutr. 2016; 55: 25-43.
49. Du H, Li L, Bennett D, Guo Y, Turnbull I, Yang L, et al. Fresh fruit consumption in relation to incident diabetes and diabetic vascular complications: A 7-y prospective study of 0.5 million Chinese adults. PLoS Med. 2017; 14: e1002279.
50. Li S, Miao S, Huang Y, Liu Z, Tian H, Yin X, et al. Fruit intake decreases risk of incident type 2 diabetes: An updated meta-analysis. Endocrine. 2015; 48: 454-460.
51. Christensen AS, Viggers L, Hasselstrom K, Gregersen S. Effect of fruit restriction on glycemic control in patients with type 2 diabetes--a randomized trial. Nutr J. 2013; 12: 29.
52. Holt SH, Miller JC, Petocz P, Farmakalidis E. A satiety index of common foods. Eur J Clin Nutr. 1995; 49: 675-690.
53. Hakim BNA, Yahya HM, Shahar S, Manaf ZA. Influence of fruit and vegetable intake on satiety and energy intake: A review. Sains Malays. 2018; 47: 2381-2390.
54. Meyer BJ, Van Der Merwe M, Du Plessis DG, De Bruin EJ, Meyer AC. Some physiological effects of a mainly fruit diet in man. S Afr Med J. 1971; 45: 191-195.
55. Meyer BJ, de Bruin EJ, Du Plessis DG, van der Merwe M, Meyer AC. Some biochemical effects of a mainly fruit diet in man. S Afr Med J. 1971; 45: 253-261.
56. Pang G, Xie J, Chen Q, Hu Z. Energy intake, metabolic homeostasis, and human health. Food Sci Hum Wellness. 2014; 3: 89-103.
57. White SJ, Carran EL, Reynolds AN, Haszard JJ, Venn BJ. The effects of apples and apple juice on acute plasma uric acid concentration: A randomized controlled trial. Am J Clin Nutr. 2018; 107: 165-172.
58. Welch AA, Mulligan A, Bingham SA, Khaw KT. Urine pH is an indicator of dietary acid-base load, fruit and vegetables and meat intakes: Results from the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk population study. Br J Nutr. 2008; 99: 1335-1343.
59. Kanbara A, Seyama I. Effect of urine pH on uric acid excretion by manipulating food materials. Nucleosides Nucleotides Nucleic Acids. 2011; 30: 1066-1071.
60. Zykova SN, Storhaug HM, Toft I, Chadban SJ, Jenssen TG, White SL. Cross-sectional analysis of nutrition and serum uric acid in two Caucasian cohorts: The AusDiab study and the Tromso study. Nutr J. 2015; 14: 49.
61. Zhang M, Gao Y, Wang X, Liu W, Zhang Y, Huang G. Comparison of the effect of high fruit and soybean products diet and standard diet interventions on serum uric acid in asymptomatic

hyperuricemia adults: An open randomized controlled trial. Int J Food Sci Nutr. 2016; 67: 335-343.