The effect of buffering a high acid load meal with sodium bicarbonate on postprandial glucose metabolism in humans

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The effect of buffering a high acid load meal with sodium bicarbonate on postprandial glucose metabolism in humans

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Background: High dietary acid load relates to increased risk of type 2 diabetes in epidemiological studies. We aimed to investigate whether buffering a high acid load meal with an alkalising treatment changes post meal glucose metabolism. **Methods**: Non-diabetic participants (n=32) were randomized to receive either $NaHCO_3$ 1680mg or placebo, followed by a high acid load meal in a double-blind placebo-controlled crossover (1-4 weeks apart) study. Thirty (20 males) participants completed the study. Venous blood pH, serum bicarbonate, blood glucose, serum insulin, C-peptide, non-esterified fatty acid (NEFA), and plasma glucagon-like peptide-1 (GLP-1) concentrations were measured at baseline (fasting) and at 15-30min intervals for 3h post meal. **Results**: The treatment was well tolerated. Venous blood pH declined in the first 15min post meal with the placebo ($p=0.001$), but not with $NaHCO_3$ ($p=0.86$) and remained lower with the placebo for 3h ($p_{interaction}=0.04$). The iAUC of pH was significantly higher following the $NaHCO_3$ treatment versus the placebo ($p=0.02$). However, postprandial glucose, insulin, C-peptide, NEFA and GLP-1 were not different between treatments ($p_{interaction}\geq 0.07$). **Conclusions**: An alkalising medication administered pre-meal has no acute effect on glycaemia and insulin response in healthy individuals. Long-term interventions in at-risk populations are necessary to investigate the effect of sustained alkalisation on glucose metabolism.

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Publications and presentations arising from this thesis

1. Williams RS*, Kozan P*, Samocha-Bonet D. The role of dietary acid load and mild metabolic acidosis in insulin resistance in humans. *Biochimie*. 2016;124:171-7.

2. Kozan P*, Blythe JC*, Greenfield JR, Samocha-Bonet D. The effect of buffering high acid load meal with sodium bicarbonate on postprandial glucose metabolism in human – A randomized placebo-controlled study. *Nutrients*. 2017; 9(8):861.

3. Kozan P, Blythe JC, Greenfield JR, Samocha-Bonet D. The effect of buffering high acid load meal with sodium bicarbonate on postprandial glucose metabolism in human – A randomized placebo-controlled study. Abstract, Australian Diabetes Society Annual Scientific Meeting 2017, Perth

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Abbreviations

AUC, Area under the curve

iAUC, incremental area under curve

AIx, augmentation index

ALT, alanine transaminase

AST, aspartate transaminase

BMI, body mass index

CI, confidence interval

CONSORT, Consolidated Standards of Reporting Trials

EDTA, ethylenediaminetetraacetic acid

DPP-IV, dipeptidyl peptidase-4

g, grams

GLUT4, glucose transporter type 4

HbA1c, glycated haemoglobin

HOMA-IR, Homeostatic Model Assessment of Insulin Resistance

H.pylori, Helicobacter pylori

IQR, interquartile range

LDL, low-density lipoprotein

NEAP, net endogenous acid production

PRAL, potential renal net acid load

PWV, pulse wave velocity

RM-ANOVA, repeated measures analysis of variance

RNAE, renal net acid excretion

SD, standard deviation

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1.INTRODUCTION

1.1. THE DIABETES AND OBESITY EPIDEMIC

The type 2 diabetes epidemic has been recognised as a global health crisis by the World Health Organisation. An estimated 422 million adults (8.5% of the global adult population) have diabetes, and its prevalence has doubled since 1980 (1), mirroring the rising prevalence of obesity worldwide. In Australia, the age standardised prevalence of type 2 diabetes increased from 1.5 to 4.4% between 1989-90 and 2014-2015, with an estimated one million Australians having a diagnosis of type 2 diabetes in 2014-2015 (2, 3). The annual cost of treating diabetes and its complications is estimated to be greater than $10 billion in Australia alone (4).

The pathogenesis of diabetes is marked by relative insulin deficiency, where insulin secretion is inadequate to maintain normal blood glucose levels, and insulin resistance at muscle and liver, leading to higher blood glucose levels despite higher insulin concentrations (5). The prevalence of insulin resistance, considered a risk factor or precursor to diabetes, has also been rising (6). Obesity is a leading risk factor (7), and intensive lifestyle intervention is recommended to prevent the progression of prediabetes in those who are overweight or obese, as these subjects carry up to a 10% risk of progression to diabetes per year (5).

The rapid increase in the prevalence of type 2 diabetes, in combination with earlier disease onset, has led to increased public health concern and a greater

focus on the employment of lifestyle strategies that may prevent or delay the onset of type 2 diabetes.

1.2. ACID-BASE HOMEOSTASIS

1.2.1. REGULATION OF ACID-BASE BALANCE

The pH of a solution is the negative logarithm of its hydrogen ion activity. The pH of the human body is tightly regulated between 7.35 and 7.45, and a pH <7.35 is consistent with gain of hydrogen ions (H^+) or loss of bicarbonate ions (HCO_3^-), and is termed acidosis. A pH >7.45 is consistent with loss of hydrogen ions or gain of bicarbonate ions, and is termed alkalosis (8). The body acid/base balance is tightly regulated by various buffer systems to maintain pH in the normal range, and a deviation from the normal range leads to compensation by the respiratory and renal system, ultimately restoring homeostasis.

Metabolic acidosis is the result of ingestion, infusion, or production of a fixed acid. This may involve a reduction in the H^+ elimination through the kidneys, shift of H^+ to the extracellular space, or HCO_3^- loss. Within minutes, the respiratory system can rapidly compensate for metabolic acidosis by increasing ventilation (and respiratory rate) to increase carbon dioxide (CO_2) elimination. The renal system can also compensate for non-renal metabolic acidosis by increasing acid excretion and bicarbonate resorption (8).

1.3. 1.3 DIETARY ACID LOAD

1.3.1. DIETARY ACID LOAD OF COMMON DIETARY PATTERNS

Although obesity is a leading risk factor in the development of type 2 diabetes, diet itself may independently be associated with type 2 diabetes risk (9, 10). Dietary patterns define the quantities, proportions, variety or combinations of different foods in the diet (11), and have a stronger correlation with disease risk, when compared with individual dietary components (9). Consumption of the 'Western diet' has been increasing globally in recent decades (9). The Western diet is typically energy dense, and high in animal proteins such as those derived from red meat and processed meat, as well as refined carbohydrates including high-energy drinks, dessert and snack foods (12). However, Western diet is a misleading term to describe this pattern of eating, which is becoming increasingly common in developing non-Western nations (13).

Dietary protein from animal sources is rich in the sulphur-containing amino acids cysteine and methionine, which when metabolised lead to production of the non-volatile acids hydrochloric acid and hydrogen sulphate (12-14). This process, coupled with deficiency in the buffering potassium and magnesium salts due to inadequate fruit and vegetable intake, renders the Western diet acidogenic in nature. It has been suggested that the high acid load of the Western diet contributes to the development of 'mild metabolic acidosis', a state of relatively low blood pH or serum bicarbonate concentration within the normal range (15).

In contrast to the Western diet, The Prudent diet is rich in fruit, vegetables,

legumes and whole grains (16), and is therefore rich in fibre, magnesium, potassium, folate, and vitamin B6. It is relatively low in fat, particularly saturated fat (12). It has been linked to a reduced incidence of type 2 diabetes compared with the Western dietary lifestyle (17). The Mediterranean diet is based on similar principles to the Prudent diet, but with a higher intake of plant based fats, and moderate consumption of red wine, providing a higher monounsaturated fat and antioxidant content (18). The Mediterranean diet has been shown to prevent type 2 diabetes (18, 19) and improve the risk of cardiovascular disease and mortality (20).

Although there appears to be no universal strategy to prevent the development of type 2 diabetes, maintenance of a normal body weight, in addition to following a Prudent or Mediterranean style diet may moderate this risk (18).

1.3.2. QUANTIFYING DIETARY ACID LOAD

The effect of the acidogenic Western diet on the body's acid/base balance has been gaining increasing interest in recent years. The acidogenic potential of foods can be quantified using two indices calculated from dietary intake, potential renal net acid load (PRAL; (21, 22)) and net endogenous acid production (NEAP; (21)). PRAL is based on the nutrient ionic balance and intestinal absorption rates of protein, phosphorous, potassium, magnesium and calcium as well as the production of sulphate from metabolised protein (21, 22). PRAL may be calculated by the following equation (21):

$$PRAL\left(\frac{mEq}{day}\right) = 0.49 \times Protein\left(\frac{g}{day}\right) + 0.037 \times Phosphorous\left(\frac{mg}{day}\right) - 0.021 \times Potassium\left(\frac{mg}{day}\right) - 0.026 \times Magnesium\left(\frac{mg}{day}\right) - 0.013 \times Calcium\left(\frac{mg}{day}\right)$$

A high PRAL score is reflective of a food with a high acid load, whilst a low (may be negative) score is indicative of a low acid (or alkaline) forming food (15). Foods containing animal proteins have the highest PRAL scores, and fruit and vegetables have the lowest scores. **Table 1** lists the PRAL score of common foods.

NEAP is an alternative score of dietary acidity considering dietary intake of protein and potassium as the main determinants of endogenous acid production and is calculated as follows (15)

$$NEAP\left(\frac{mEq}{day}\right) = 54.5 \times Protein\left(\frac{g}{day}\right) \div Potassium\left(\frac{mEq}{day}\right) - 10.2$$

The Western diet, which typically has a high PRAL score, leads to the net addition of acid to the body (quantified as NEAP), and results in an equivalent consumption of HCO_3^-, which must be replaced through the renal system as the excess acid is excreted. The Western diet has a NEAP score of 34 to 76 mEq/day (23), much higher than the -0.08 to 34 mEq/day reported for the other dietary pattern extreme in terms of acid load, the vegan diet (24)

Table 1: PRAL scores of common foods

Food	PRAL (mEq/100g)
Cheese and dairy	
Parmesan cheese	34.2
Cheddar	26.2
Cottage cheese	8.7
Yoghurt	1.5
Meat/meat products	
Corned beef	13.2
Salami	11.6
Lean beef	7.8
Chicken	7.8
Fish, trout	10.8
Bread/grain products	
Bread, white wheat	3.7
Bread, wholemeal wheat	1.8
Rice, white	4.6
Spaghetti, white	6.5
Fats/oils	
Butter	0.6
Olive oil	0.0
Sugar/sweets	
Milk chocolate	2.4
Honey	-0.3
Sugar	-0.1
beverages	
Beer, draft	-0.2
Whole milk	0.7
Coca-Cola	0.4
Coffee	-1.4
Tea	-1.3
Red wine	-2.4
Legumes/nuts	
Lentils	3.5
Peanut	8.3
Hazelnut	-2.8
Fruit/vegetables	
Apples	-2.2
Banana	5.5
Lemon juice	-2.5
Raisins	-21
Broccoli	-1.2
Carrots	-4.9
Spinach	-14.0

Adapted from Remer *et al.* (22)

The acid excreted through the kidneys can be quantified by the renal net acid excretion (RNAE). In a state of acid/base balance, NEAP is equal to RNAE, which is represented by the following equation (14).

$$RNAE = (U_{NH4}^{+} \times V) + (U_{TA} \times V) - (U_{HCO3}^{-} \times V)$$

U: urine concentration; V: urine flow rate; TA: titratable acid

1.3.3. DIETARY ACID LOAD AND MILD METABOLIC ACIDOSIS

The consumption of dietary protein, which is rich in the sulphur containing amino acids cysteine and methionine, leads to the net production of non-volatile acids such as HCl (hydrochloric acid) and H_2SO_4 (sulphuric acid). These are rapidly buffered, to generate CO_2, which is excreted from the lungs, and Na salts are excreted from the kidneys in the form of NH_4Cl (ammonium chloride) and $(NH_4)_2SO_4$ (ammonium sulphate), resulting in HCO_3^- generation to replace the HCO_3^- consumed while buffering the acid (14). These buffering reactions are described below:

$$HCl + NaHCO_3 \rightarrow NaCl + CO_2 + H_2O$$

$$H_2SO_4 + 2NaHCO_3 \rightarrow Na_2SO4 + 2CO_2 + 2H_2O \qquad (14)$$

Conversely, fruit and vegetables are rich in potassium and magnesium salts, citrate and malate, as well as the amino acid glutamate, and when metabolised hydrogen ions are consumed, therefore increasing pH (25). With aging, there is a gradual loss of renal acid-base regulatory function (26) that may affect the buffering capacity of high acid load diets in older populations. If the excess

consumption of acidogenic food (for example animal protein) is not compensated for by functional homeostatic mechanisms (such as excretion of excess acid from the respiratory and renal system), or offset by the consumption of potassium and magnesium rich fruit and vegetables, which consume excess hydrogen ions, this results in a downward shift in the plasma pH to the lower end of the normal range (14). This has previously been labelled ‘mild metabolic acidosis’ (21, 27, 28), ‘diet induced acidosis’ (29-31), ‘low grade acidosis’ (30, 32), or ‘subacute acidosis’ (30).

1.3.4. The “Alkaline Diet”

The influence of the high acid load Western diet on health and disease as a result of altering the body’s acid/base balance, has gained increasing interest in recent years. The “Alkaline diet” has gained popularity, and is alleged to assist in the treatment of a variety of medical conditions including cancer, osteoporosis, back pain, muscle wasting, and cardiovascular health (26, 33).

Findings from studies exploring the association between dietary acid load and bone and calcium metabolism are mixed (34-39), and influenced by numerous confounders including the use of dietary protein as a surrogate of dietary acid load. Overall, there is no robust evidence from meta-analysis that a reduced dietary acid load or dietary protein intake improved bone health outcomes (40). Many of the other alleged health claims attributed to the alkaline diet, including improved efficacy of chemotherapy medications (26), have also been challenged (41).

1.4. THE EFFECT OF METABOLIC ACIDOSIS ON GLUCOSE HOMEOSTASIS

1.4.1. MECHANISMS UNDERLYING THE RELATIONSHIP BETWEEN BODY ACIDITY AND GLUCOSE HOMEOSTASIS

The mechanisms underlying the relationship between metabolic acidosis and glucose homeostasis remain unclear. *In vitro* evidence supports the hypothesis that acidic pH reduces insulin binding to its receptor. Waelbroek (42) showed that in human lymphocytes, optimal insulin receptor binding occurs at a pH range that is above the physiological range, at 7.6-8.0, and is represented by a bell-shaped curve, where insulin receptor binding decreases at pH above and below this optimal pH range. However, a limitation of this study was that tissue glucose uptake was not measured.

Furthermore, Hayata *et al*. (43) demonstrated that in cultured rat myocytes, the phosphorylation level (and therefore activation) of the insulin receptor was diminished at pH ≤ 7.2, and that the phosphorylation of Akt (a key mediator in the insulin signalling pathway) is reduced at a pH of 6.8, which is far below the physiological range. In addition, they showed that insulin receptor binding and tissue glucose uptake were also impaired at an acidic pH of 7.0 and 6.8 respectively, with no effect reported on the total expression of the insulin receptor.

Whittaker and colleagues' (44) findings were slightly different. They found that treating diabetic ketoacidotic rats with bicarbonate resulted in an increase in the concentration of adipocyte insulin receptors, leading to increased insulin binding. Reciprocally, in the same paper, they reported that inducing metabolic

acidosis with ammonium chloride in non-diabetic rats, led to a reduction in insulin binding, consistent with a reduction in adipocyte insulin receptor concentration (per cell). In addition, they found insulin's binding affinity to the insulin receptor was lower in the diabetic rats only, but the change in receptor concentration appeared to be due to changes in acid-base balance.

It is important to note that in animal and cell models, the pH was often altered outside of the physiological range to induce an adverse effect on glucose metabolism, and therefore its relevance to the *in vivo* situation, in particular in healthy individuals, needs further evaluation. In addition, the authors above (with the exception of Hayata et al) explore changes in insulin binding affinity, and not directly measuring the effect on glucose uptake. Therefore, it remains unclear whether this translates to a change in tissue glucose uptake, and requires further study.

Exploring these mechanisms further in humans, in an interventional study including 16 healthy volunteers, DeFronzo and Beckles (27) used the hyperglycaemic clamp followed by a hyperinsulinaemic-euglycaemic clamp to test the effect of acidifying treatment on insulin secretion and insulin sensitivity. They showed that shifting pH into the low normal range from 7.41 ± 0.01 to 7.37 ± 0.01 by ammonium chloride administration resulted in a decline in insulin sensitivity, as measured by a decrease in glucose infusion rate (GIR, a measure of tissue insulin sensitivity) during the hyperinsulinaemic clamp from 7.42 to 6.42mg/kg/min. The authors concluded that a reduction in blood pH led to a reduction in tissue sensitivity to insulin. Insulin secretion increased to overcome

insulin resistance, indicating beta-cell sensitivity to glucose was enhanced in healthy young individuals. Similarly, correction of metabolic acidosis with sodium bicarbonate in chronic renal failure participants (45) resulted in an increase in GIR during a hyperinsulinaemic-euglycaemic clamp, indicating that correction of acidosis led to an increase in tissue sensitivity to insulin.

Finally, it has been suggested in animal models that a high acid load diet may increase glucocorticoid production, marked by a rise in plasma cortisol, which in turn may contribute to insulin resistance and proteolysis, as well as impacting bone metabolism (45). It is known that in patients with chronic renal failure, metabolic acidosis leads to protein degradation via glucocorticoid stimulation (45). Furthermore, Maurer and colleagues (46) found that alkaline treatment (sodium bicarbonate and potassium bicarbonate) for 7 days caused a significant reduction in mean plasma cortisol from 264 ± 45 to 232 ± 43 mmol/L (p=0.032), as well as a reduction in 24 hour urinary free cortisol.

1.4.2. PLASMA LACTATE AND GLUCOSE HOMEOSTASIS

Lactate is both a body acid and major gluconeogenic precursor in man (47), and plasma lactate has been found to be increased in obesity and diabetes (48). A systemic infusion of lactate in rats during euglycaemic-hyperinsulinaemic clamp studies performed by Vettor *et al.* (49) led to a significant decrease in glucose uptake and utilisation in heart and skeletal muscle, suggesting that lactate is involved in the regulation of peripheral glucose metabolism. The same group (48) also found that GLUT-4 mRNA and protein were also decreased post lactate infusion, suggesting that hyperlactataemia affects the expression of the genes involved in muscle

glucose metabolism. In addition, infusion of lactate was shown by Consoli and colleagues (47) to promote hepatic gluconeogenesis in both diabetic and non-diabetic human subjects. The direct cause and effect relationship between a high acid load diet and body lactate production however, is yet to be established (50).

Many studies have explored the association between plasma lactate and type 2 diabetes risk, and there is substantial observational data correlating higher resting lactate with an increased risk of type 2 diabetes in sedentary individuals. Juraschek and colleagues (51) found in the prospective Atherosclerosis Risk in Communities (ARIC) study of 8045 healthy participants with a 12 year follow up period, that after adjustment of diabetic risk factors, baseline plasma lactate correlated with incident diabetes, with a significant graded relationship across quartiles of plasma lactate. These findings are despite 99% of participants having a resting plasma lactate within the normal range, i.e. ≤2 mmol/L. The authors hypothesised that higher resting lactate within the normal range was a marker of extrahepatic glucose utilisation and lower oxidative capacity, which may herald the development of diabetes. In a cross-sectional sub-study of 1905 participants within the ARIC study (52), the prevalence of diabetes rose across lactate quartiles (with a prevalence of 11%, 14%, 20%, and 30%, across the 1st to 4th quartile respectively, $p_{trend} < 0.0001$), and lactate was also associated with higher fasting blood glucose levels in non-diabetic individuals. Consistent with the findings in the ARIC cohort, a large prospective study of healthy Swedish men (with an average age of 54) followed for 13.5 years, found that participants

who developed diabetes or impaired glucose tolerance during the follow up period had a higher resting lactate at baseline (53).

The association between lactate and insulin resistance may be independent of body weight or adiposity, as suggested by a stronger association between plasma lactate and insulin resistance (by frequently sampled intravenous glucose tolerance test) than body mass index (BMI), in a cohort of healthy lean and obese adults (54). Similarly, using the gold-standard measurement of whole body insulin resistance, the hyperinsulinaemic-euglycaemic clamp in a cohort of 104 men and women, our group (50) reported that fasting plasma lactate was elevated in obese insulin-resistant subjects compared to (BMI and fat matched) obese insulin-sensitive and lean insulin-sensitive subjects, suggesting that elevated lactate may be a marker of insulin resistance irrespective of obesity. Furthermore, in the ARIC study (51), adjustment for BMI and waist circumference did not attenuate the graded rise in type 2 diabetes incidence over increasing plasma lactate quartiles. In the same cohort, a strong positive association between plasma lactate, fasting blood glucose, and prevalence of type 2 diabetes was independent of BMI (52).

Figure 1 summarises the mechanisms proposed to link high dietary acid load with altered glucose metabolism and risk of type 2 diabetes (15).

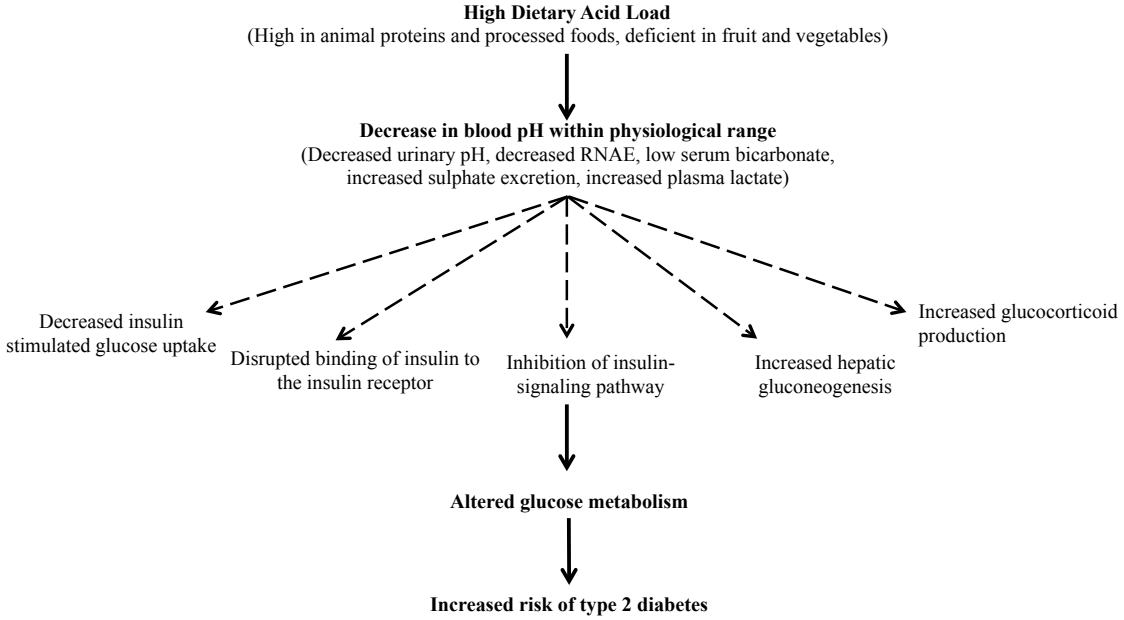

Figure 1: Mechanisms proposed to mediate the effect of dietary acid load on insulin resistance and type 2 diabetes risk

Adapted from Williams et al (15).

1.4.3. SERUM BICARBONATE, pH AND GLUCOSE HOMEOSTASIS

Epidemiological studies have suggested a link between body acidity and glucose homeostasis. In the US National Health and Nutrition Examination Survey cohort (55), a cross-sectional study of 1496 adults, found that serum bicarbonate was inversely associated with fasting insulin, and that participants with serum bicarbonate measured in the highest quartile had fasting insulin concentrations (a marker of insulin resistance) that were ~ 140 pmol/L (20 mU/L) lower than those in the lowest bicarbonate quartile. Furthermore, incremental rise in serum bicarbonate has predicted a lower risk of type 2 diabetes in the Nurses' Health study (56). In that cohort, women with serum bicarbonate above the median had an odds ratio of 0.76 (95% CI 0.6-0.96) for developing diabetes compared to women with serum bicarbonate below the median. The authors concluded that after adjustment for BMI, renal function

and hypertension, plasma bicarbonate levels were predictive of type 2 diabetes. However, cause and effect cannot be established in these studies, and whilst these studies report a relationship between the body acidity marker serum bicarbonate and diabetes risk, whether the increased diabetes risk is a result of increased body acidity, or whether the lower serum bicarbonate is a marker of poor health in general, remains unclear.

Several interventional studies supported a causative role of body pH in glucose homeostasis in healthy individuals or patients with chronic renal failure. As discussed above, in a small cohort of healthy young adults, DeFronzo and Beckles (27) reported that a decrease in arterial blood pH to the lower end of the normal range (from an average of 7.41 to 7.37) by ammonium chloride administration for 3 days, resulted in an increase in insulin resistance, as measured by a reduction in glucose infusion rate during hyperinsulinaemic clamps from 7.42 to 6.42 mg/kg/min. Conversely, correction of overt metabolic acidosis in a small cohort of chronic renal failure patients by administering sodium bicarbonate (1.2g three times a day) for 4 weeks, with arterial blood pH increasing from an average of 7.29 to 7.36, resulted in an increase in glucose infusion rate from 6.44 to 7.38 mg/kg/min (45). Similar to this, administration of sodium bicarbonate (252 mg/kg/day, or 17.6 g/day for a 70 kg person) for two weeks in end stage renal failure patients on haemodialysis led to an improvement in insulin sensitivity measured by hyperinsulinaemic-euglycaemic clamp studies, and an increased insulin secretion in hyperglycaemic clamp studies, changes which were not achieved with sodium chloride (57). Overall, these studies suggest a role for body alkalinisation in improving insulin

sensitivity in chronic renal failure patients. Moreover, Kobayashi *et al.* (58) reported that in chronic renal failure patients, serum bicarbonate was a moderate predictor of insulin resistance as measured by hyperinsulinaemic-euglycaemic clamp ($r = 0.6$; $p < 0.0005$).

A recent larger study by Belassi and colleagues (59) supported these findings. 145 adults with chronic renal failure and type 2 diabetes not treated with insulin were randomised to either open label oral sodium bicarbonate (dose adjusted to achieve a serum HCO_3- of 24-28mmol/L, average dose 58.8 ± 8.4 mg/kg,), or no treatment for one year. The treatment group was shown to have significantly lower plasma insulin concentrations and HOMA-IR, with a concomitant reduction in diabetic therapy dose.

In summary, the majority of the alkalising interventional studies were performed in patients with chronic renal failure, where alkalising treatment is used to correct metabolic acidosis. The effect of bicarbonate therapy in healthy individuals with normal renal function, with a blood pH in the normal range has not been explored. In order to determine whether a shift in pH within the normal range can affect glucose homeostasis, and whether alkalising treatment could be utilised as a therapeutic option to delay or prevent the development of insulin resistance and type 2 diabetes, requires further study.

1.5. DIETARY ACID LOAD, INSULIN RESISTANCE AND TYPE 2 DIABETES

Prospective studies have examined the contribution of dietary acid load to type 2 diabetes risk. In the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort of 66,485 women (21), those with the highest dietary

PRAL quartile (the highest dietary acid load, quantified by diet diaries) had a higher risk of incident diabetes during 14 years of follow up compared to those reporting dietary intake in the bottom quartile (the least acidic diet; HR 1.56 [95% CI 1.29-1.90]). Interestingly, the association was stronger in women in the normal weight range (BMI < 25 kg/m^2, HR 1.96 [95% CI 1.43-2.96]; versus BMI >25 kg/m^2, HR 1.28 [95% CI 1.00-1.64]). Indirectly, these findings were supported by findings in the Nurses' Health study (60), consisting of 84360 women followed for 6 years, whereby women reporting diets rich in calcium, potassium and magnesium (major contributors to the PRAL score) had a lower risk of type 2 diabetes. Furthermore, in a cross-sectional study of 1732 Japanese workers, PRAL and NEAP (calculated from diet diaries) were positively associated with HOMA-IR in normal weight individuals (BMI < 23 kg/m^2) (61). A recent pooled analysis of the Nurses' Health Study, the Nurses' Health Study II, and the Health Professionals' Follow- up Study (62), lends further support to the association between dietary acid load and type 2 diabetes. In this meta-analysis, based on a total cohort of 187,486 subjects with 15,305 new cases of diabetes diagnosed during 4,025,131 person-years of follow-up, after adjustment for diabetic risk factors, the authors have reported that dietary NEAP and PRAL were positively associated with type 2 diabetes incidence, with a HR (95% CI) for highest versus lowest quintile for NEAP: 1.29 (95% CI 1.22-1.37, p<0.0001), and PRAL: 1.29 (95% CI 1.22-1.36, p<0.0001).

However, such significant relationships between dietary acid load indices and glucose regulation or diabetes were not reported in all studies. Xu *et al*. (63) found in a cohort of 911 older Swedish men, that PRAL and NEAP (estimated

from diet diaries) were not significantly associated with insulin resistance, as measured by hyperinsulinaemic-euglycaemic clamp, or with incidence of type 2 diabetes. Similarly, using hyperinsulinaemic-euglycaemic clamp studies in a cohort of chronic renal failure patients and healthy controls, Ikizler *et al.* (64) found that while lower dietary acid load was associated with higher serum bicarbonate concentrations, no relationship was found between dietary acid load and insulin sensitivity. These two studies, using the gold standard clamp technique to measure insulin resistance, questioned the relationships between dietary acid load and insulin resistance.

The inconsistent findings between epidemiological studies with diabetes endpoints and studies measuring insulin resistance directly, most probably stem from the different endpoints/methodologies and relate to the different populations studies and the different sample size.

No study to-date assessed body acid/base status when studying the relationships between dietary acid load and glucose homeostasis. Furthermore, all of the cited studies mentioned above are observational in nature and conclusions regarding causality cannot be drawn. Finally, the studies cited examined the association between long-term dietary acid load and insulin resistance as a common outcome measure. However, to have a chronic effect on glucose metabolism, the alkalizing effect of a low PRAL diet may, or may not, be mediated by acute (that is, immediately post-meal) mechanisms.

1.5.1. NUTRITIONAL CONFOUNDERS IN STUDIES EXAMINING THE RELATIONSHIP BETWEEN DIETARY ACID LOAD AND GLUCOSE HOMEOSTASIS

Despite the evidence linking dietary acid load to type 2 diabetes risk, findings from nutritional studies are likely to be confounded by other nutrients in the diet.

Firstly, individuals following a vegetarian diet have a reduced risk of type 2 diabetes (65). In a cohort of 41,387 North Americans (66), following a vegan (OR 0.38; 95% CI 0.24-0.62), lacto ovo vegetarian (OR 0.62; 0.50-0.76) or pesco vegetarian (OR 0.47, 0.31-0.76) diet resulted in a lower risk of diabetes compared to non-vegetarians, after adjusting for diabetic risk factors, including BMI. Similarly, Valachovicova *et al.* (67) reported that a vegetarian diet was associated with a lower HOMA-IR (a marker of greater insulin sensitivity in non-diabetic populations). The high fibre content of the vegetarian diet is a likely player in the favourable effects reported, as high fibre diets have also been linked to reduced incidence of diabetes (15, 68), potentially through decreasing postprandial insulin demand (17) by lowering the glycaemic response to dietary carbohydrate (69). This may be modulated by slowing intestinal glucose absorption (69), increasing bile acid excretion and the pool size of bile acids, increasing glucagon-like peptide-1 (GLP-1) secretion, and delayed gastric emptying (70). In addition, bacterial fermentation of fibre may increase short-chain fatty acid production, known to reduce hepatic glucose output (71). Finally, high dietary fibre likely plays a role in maintaining favourable gut microbiota composition, which has been linked to improved insulin resistance (15, 72).

The macronutrient proportions of the diet (independent of energy intake) may also influence insulin resistance (15). Dietary animal protein, in particular processed meat and red meat are associated with increased diabetes risk (67, 68, 73, 74). However, this has not been reported with non-animal protein. In particular, Valachovicova's vegetarian cohort was more insulin-sensitive compared to the non-vegetarian cohort, despite their diets containing similar proportions of daily energy intake from fat, carbohydrate, and protein (67). This suggests that the risk of diabetes is likely to be influenced by other dietary factors beyond the macronutrient proportion (such as the fibre content of the meal, and source of protein, as discussed above).

Furthermore, given the documented association between animal protein intake and diabetes risk, iron metabolism may also confound the relationship between diet and glucose metabolism. Fernandez *et al*. (75) reported that subjects with impaired glucose tolerance and reduced insulin sensitivity had a higher concentration of the soluble transferrin receptor (a marker of erythropoiesis) and serum ferritin (indicative of iron stores), suggesting a link between iron and glucose metabolism (15). Conversely, in a population of individuals with liver disease (76), iron depletion with phlebotomy resulted in a reduction in HOMA-IR, whilst a further reduction in iron with desferrioxamine treatment increased insulin binding. Furthermore, in the same study, iron supplementation was found to reduce insulin binding, again implicating this link. The authors proposed that iron modulates the transcription, expression and affinity of the insulin receptor in hepatocytes.

It is important to note that nutritional studies are likely to involve a number of other factors associated with lifestyle choices, including, physical activity, sleep pattern, smoking and alcohol use, all have been found to associate with a healthy dietary patterns (77).

In designing a study to examine the effect of Western diet on glucose homeostasis, a dietary intervention seems most appropriate. However, pharmacological rather than a dietary intervention may be a reasonable alternative to control for the confounding effect of dietary variables.

1.6. THE ASSOCIATION BETWEEN BODY ACIDITY AND CARDIOVASCULAR DISEASE

Insulin resistance and type 2 diabetes are known risk factors for cardiovascular disease. The relationship between body acid / base balance and cardio-metabolic disease risk factors, other than glucose regulatory impairments, has also been explored. In a cross sectional study of 1136 young Japanese students, Murakami *et al.* (78) found that a higher dietary PRAL correlated with elevated diastolic and systolic blood pressure (p_{trend} =0.03 and 0.04, respectively), as well as with LDL and total cholesterol (p_{trend} =0.04 and 0.02, respectively). Similarly, over 14 years of follow up in the Nurses' Health Study cohort, Zhang and colleagues (23) found that dietary NEAP was associated with incident hypertension (RR 1.14 95% [95% CI 1.08-1.41] for highest versus lowest NEAP decile).

Conversely, Engberink *et al.* (28) did not find an association between dietary PRAL or NEAP and incident hypertension over 6 years of follow up in an older

Dutch cohort (n=2241) from the Rotterdam study. The authors suggested that their contradictory findings may be related to the older cohort studied and to the alkaline nature of their diet, making the acid load of this cohort's diet too low to exert an effect on blood pressure (average PRAL -1.5 mEq/d compared to 10 mEq/d in Murakami's cohort).

In conclusion, it is important to consider the potential effect of dietary acid load and body acidity on cardiovascular risk factors in addition to those pertaining to glucose homeostasis.

1.7. STUDY OBJECTIVES AND STUDY HYPOTHESIS

In the present study, I performed a randomised double-blind placebo-controlled crossover study to determine the effect of pre-prandial oral sodium bicarbonate on postprandial glucose metabolism in non-diabetic participants with normal renal function.

I assessed body acidity directly by measuring blood pH and serum bicarbonate, as well as glucose, insulin, C-peptide, non-esterified fatty acid (NEFA), and glucagon-like peptide-1 (GLP-1) concentrations at baseline (fasting) and at 15-30 minute intervals for 3 hours post meal.

I hypothesised that buffering a high acid load meal will attenuate postprandial blood pH decrease and glycaemic response (**Figure 2).** I anticipated that alkalising treatment can be used to offset the acid load of a meal, and thus have

a favourable effect on glucose metabolism in non-diabetic participants with normal renal function.

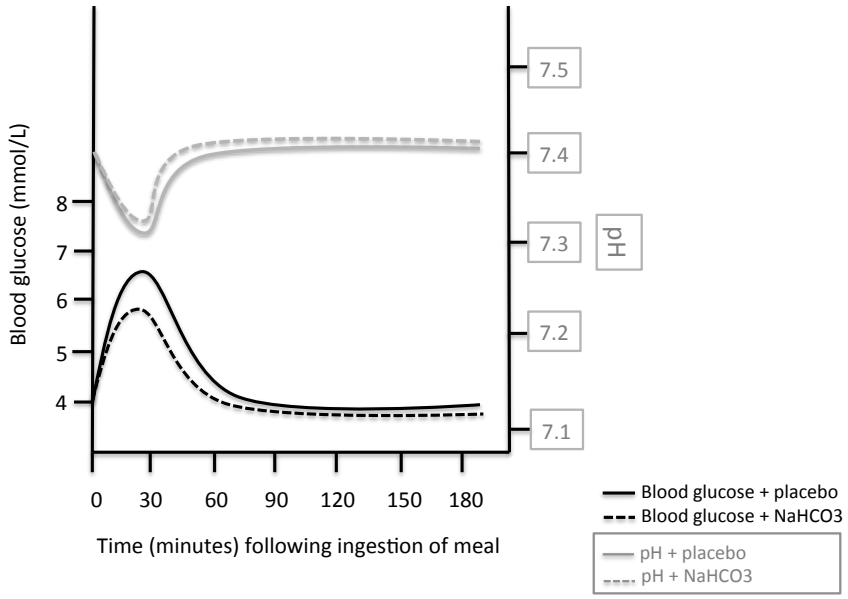

Figure 2: Hypothesised effect of oral sodium bicarbonate treatment (administered at time= 0) on pH and glucose excursion post high acid load meal (Western style breakfast)

Adapted from Jackson Blythe, with permission

The specific aims were:

1. To examine body acidity markers, including venous pH, serum bicarbonate and plasma lactate following a high acid load meal
2. To test whether buffering the meal with sodium bicarbonate (1680 mg) versus placebo attenuates the expected drop in venous blood pH postprandially.

3. To determine whether changing postprandial pH will change glucose metabolism, specifically circulating glucose, insulin, C-peptide, GLP-1 and NEFA excursions.
4. To determine whether sodium bicarbonate administered with a high acid load meal has an effect on cardio-metabolic markers, including blood pressure and arterial stiffness.
5. To determine whether sodium bicarbonate administered with a high acid load meal has an effect on hunger and satiety scores.

2.METHODS

2.1. ETHICS AND TRIAL REGISTRATION

The study was conducted according to the principles outlined in the declaration of Helsinki (79). The study protocol was approved by the St Vincent's Hospital Human Ethics Research Committee (SVH reference 14/157) and all participants provided written informed consent prior to study commencement. The study was registered at *clinicaltrials.gov* (NCT 02501343).

2.2. PARTICIPANTS

2.2.1. PARTICIPANT RECRUITMENT

The study was performed at the Clinical Research Facility at the Garvan Institute of Medical Research (Darlinghurst). Participant recruitment took place from June 2015 until May 2016. Potential participants were recruited through advertisements placed on noticeboards at the Garvan Precinct, and via social media and email distribution lists. In addition, advertisements were placed in free magazines distributed in train stations (Sydney), and at local Returned and Services League (RSL) clubs. Some participants contacted the study team after seeing the study listed on the *clinicaltrials.gov* website.

2.2.2. PARTICIPANT INCLUSION AND EXCLUSION CRITERIA

I aimed to recruit healthy adult men and women. Inclusion and exclusion criteria are listed below.

Inclusion criteria

- Age range: 22-65
- Laboratory parameters: Fasting plasma glucose <7 mmol/L, HbA1c <6.5% (48 mmol/mol)
- Willingness to provide written informed consent and willingness to participate and comply with the study procedures

Exclusion criteria

- Individuals with a personal history of diabetes, or with fasting plasma glucose ≥7 mmol/L and/or HbA1c ≥6.5% (48 mmol/mol)
- Individuals treated with antihypertensive medications, or with a blood pressure ≥140/90 mmHg measured at the Clinical Research Facility
- Individuals with a history of renal disease, serum creatinine > 90 µmol/L and/or eGFR < 60 mL/min/1.73m^2
- Individuals with a history of liver disease, or alanine transaminase (ALT) and/or aspartate transaminase (AST) more than twice the upper limit of the normal range (i.e. ALT or AST >60 U/L)
- Individuals with a history of cardiovascular disease, respiratory disease or inflammatory disease
- Individuals treated with medications known to affect insulin sensitivity (including oral hypoglycaemic agents, anti-psychotic agents, and glucocorticoids)
- Individuals treated with proton pump inhibitors or H2 histamine receptor antagonists

- Individuals treated with calcium supplementation
- Individuals with an unstable body weight in the past 3 months (+/- 2 kg or more)
- Individuals with a history of a psychological illness or condition that may interfere with the participant's ability to understand the requirements of the study
- Pregnant or lactating women
- Individuals who smoke
- Individuals who consume more than 40 g (male) or 20 g (female) of alcohol daily
- Individuals who live out of area and are unable to easily travel to the Clinical Research Facility at the Garvan Institute of Medical Research

2.2.3. PARTICIPANT SCREENING

Interested participants contacted the research team (either PK or the study nurse) via telephone or email. Each participant underwent a telephone screening, and a questionnaire was completed to ascertain whether they were likely to be eligible to participate in the study. If participants were willing to participate, met the inclusion criteria and were able to travel to the Garvan Institute of Medical Research, they were invited to an in-person screening visit performed by a physician (PK). Potential recruits were emailed the participant information sheet and consent form to review prior to their visit.

The screening visit included:

- A detailed discussion regarding the study aims and procedures, and a written consent was obtained prior to proceeding.
- A thorough medical history and examination, including:
    - Medical status and history, current medications (including over the counter medications) and allergies
    - Family history of type 2 diabetes
    - Ethnic origin
    - Alcohol, tobacco and recreational drug use
    - Diet, exercise or weight changes in the preceding 12 month period
    - Weight, height (calculated BMI), waist and hip circumference, pulse rate/rhythm
    - Cardiovascular, respiratory, abdominal and basic neurological examination
- Venesection and blood samples were drawn, to test serum electrolytes concentrations, renal function, liver function, HbA1c, and (non-fasting) blood glucose concentration

Eligible participants were contacted once their pathology results were reviewed (typically within 2-4 days from the screening visit) to arrange their meal studies.

2.3. STUDY DESIGN

2.3.1. OVERVIEW

This was a randomised placebo-controlled double-blind single centre study with a crossover design. Participants were randomised to either receive sodium bicarbonate ($NaHCO_3$, 2*840 mg Sodibic capsules, Aspen Australia, St Leonards, NSW, Australia) with their first meal, or two identically looking placebo capsules (microcrystalline cellulose, Stenlake Compounding Chemist, Bondi Junction, NSW, Australia). The capsules were administered 15 minutes prior to ingestion of the meal, and participants were studied for 3 hours following the meal. Participants returned for the second study, which was procedurally identical, but utilised the alternate treatment, 1 to 4 weeks after the first study.

2.3.2. RANDOMISATION AND BLINDING

Eligible willing participants underwent randomisation prior to the commencement of their first meal study. Each participant was randomised to receive either capsule set (active treatment or placebo) with their first meal.

Randomisation was performed by a Research Nurse unrelated to the study. Study participants and investigators were blinded to the treatment allocation. Randomisation was performed using an online available randomiser (http://www.randomizer.org) by a nurse unrelated to the study.

2.3.3. NUTRITIONAL BREAKDOWN OF THE STUDY MEAL

The study meal was collected from a local McDonald's restaurant (McDonalds Australia Limited®, Sydney, Australia) on the morning of the study, and consisted of two Sausage and Egg McMuffins® and a 250 mL carton of apple fruit drink (Golden Circle®, Brisbane, Australia), purchased ahead of time. The ingredients and nutritional contents of the meal were obtained from the McDonalds and Golden Circle websites (80, 81) and the nutritional breakdown and PRAL score of the meal were calculated using FoodWorks (version 7, Xyris, Australia, **Table 2)**.

Each Sausage and egg McMuffin® consisted of one sausage patty, one egg, a cheese slice, canola oil, and an English muffin. The meals were weighed prior to the study and the average weights of the meal for the placebo and treatment studies were 304 ±10 g and 305 ± 8 g respectively (p = 0.65).

Each 250ml carton of Golden circle® brand apple fruit drink, consisted of water, 25% reconstituted apple juice, sugar, food acids (malic acid, potassium citrate), flavour, and vitamin C.

Table 2: Nutritional breakdown of the study meal [1]

Constituent	Average quantity per meal
Energy, kj (kcal)	3567 (853)
Protein, g	42
Fat, g	39
Saturated fat, g	18
Total carbohydrates, g	79
Sugars, g	31
Sodium, g	1.3
PRAL (mEq)	18.3

[1] Based on information provided by McDonalds® and Golden Circle®
[a]PRAL (potential renal acid load) is calculated from dietary intake as follows,
PRAL (mEq) = 0.49 *X Protein (g)* + 0.037 *X Phosphorus (mg)* – 0.021 *X Potassium (mg)* – 0.026 *X Magnesium (mg)* – 0.013 *X Calcium (mg)*

2.3.4. STUDY MEDICATION AND DOSE CONSIDERATIONS

The main indication for sodium bicarbonate use is to correct metabolic acidosis in the context of chronic kidney disease (82). It also has a utility as an antacid (83), and urinary alkaliser in the context of uric acid renal calculi (84). In healthy individuals, physiological bicarbonate levels are tightly regulated by the kidneys and lungs and most bicarbonate pharmacokinetic parameters are dependent on the physical state of the patient at that point in time. As such, numerical values for some of the pharmacokinetic parameters such as half-life and volume of distribution cannot be determined (83). Oral bicarbonate is well absorbed with a rapid onset of action. Absorption is influenced by stomach acid, and following absorption, in the absence of bicarbonate deficiency in the circulation, excess bicarbonate is rapidly excreted in the urine in conjunction with sodium ions. By employing a crossover study design to collect paired dataset, and by ensuring the participants were following a similar routine in the 48 hours prior to the study, I aimed to minimise the effect of variations in exogenous bicarbonate handling in the body on my results (83).

The recommended dosing of sodium bicarbonate varies with the indication. **Table 3** lists the indications and recommended doses, as found in several international pharmacotherapy resources.

Adverse effects are dose dependent, and include gastrointestinal upset, muscle weakness or cramp, metabolic alkalosis, hypokalaemia (83), or the milk alkali syndrome with concurrent calcium use (85).

Table 3: Recommended dosing of sodium bicarbonate and medical indications

Recommended dose	Reference	Indication
1-5 g daily	EMC UK (83) [a] MIMS USA (84) [b]	Dyspepsia
325 mg to 2 g, 1-2 times a day, maximum 8 g daily	UpToDate (86)	Dyspepsia
Maximum 10 g daily	MIMS USA (84)	Urine alkaliser
1-2 g every 4 hours, maximum 16 g daily	UpToDate (86)	Urine alkaliser
4.8 g daily, uptitrate as required	MIMS USA (84)	Metabolic acidosis in chronic kidney disease
650 mg 2-3 times daily, maximum dose 5.85 g daily	UptoDate (86)	Metabolic acidosis in chronic kidney disease
840 mg daily, uptitrate as required	MIMS Australia (82)	Metabolic acidosis in chronic kidney disease

[a] eMC UK; electronoic Medicine Compendium UK
[b] MIMS USA; Monthly Index of Medical Specialties USA

Limited studies have examined the effect of oral $NaHCO_3$ on serum bicarbonate and blood pH. A relatively large dose of 0.7 ± 0.2 mmol/kg (0.58 g/kg or 4 g for a 70 kg individual) was required in a cohort of chronic renal failure patients (59) to normalise serum bicarbonate (from 21.2 ± 1.9 mmol/L to 26.0 ± 2.0 mmol/L).

Reaich et al (45) administered 1.2 g three times a day (3.6 g daily) to correct acidosis from pH 7.29 to 7.36 in chronic renal failure patients.

The sport literature lists many studies examining the effect of sodium bicarbonate on athletic performance. Miller and colleagues (87) administered 0.3 g/kg of $NaHCO_3$ (approximately 21 g daily in a 70 kg individual) and reported a significant elevation in capillary blood pH of 0.03 pH units ($p<0.05$). Cameron *et al.* (88) found, in a placebo-controlled crossover study, that administering 0.6 g/kg of sodium bicarbonate to elite rugby players resulted in a rise in capillary blood pH, with a difference in mean pH 60 minutes after $NaHCO_3$ ingestion compared to placebo of 0.08 (95% CI 0.07-0.11, $p<0.001$). In addition, they reported a significant increase in mean serum bicarbonate of 6.29 mmol/L (95% CI 3.47-9.11, $p<0.001$), and an attenuation in the pH and bicarbonate decline following high intensity exercise. However, there was no difference in performance markers, and an increase in gastrointestinal side effects was noted following this mega dose of sodium bicarbonate ingestion. These studies indicate that body acidity can be manipulated following sodium bicarbonate administration, but side effects may occur at large doses.

The chosen sodium bicarbonate dose was guided by the study by Reaich *et al.* (45), administering 1.2 g sodium bicarbonate with each meal, 3 times daily. I also consulted the information sources listed in Table 3 and local nephrologists. The dose selected is a common starting dose in chronic renal failure patients at our affiliated St Vincent's Hospital, and it was thought this would be a tolerable

dose for the study participants, and unlikely to cause significant gastrointestinal side effects.

2.4. STUDY PROCEDURES

Participants arrived to the Clinical Research Facility in the morning after an overnight fast (minimum 10 hours). They were requested to avoid exercise and alcohol for 48 hours prior to the study day, and to follow a similar routine and consume a similar evening meal at home the evening before each study. Participants had baseline height, weight (in light clothing), hip and waist circumference, blood pressure, heart rate and arterial stiffness measured. Baseline hunger and satiety scores were taken. Pregnancy was excluded in premenopausal female participants by a urinary pregnancy test.

An 18 gauge intravenous cannula was inserted in the right cubital fossa, and used for blood collection throughout the study. Normal saline (0.9% NaCl) was infused at 20 ml/hour to keep the intravenous line patent. Participants rested in bed for 30 minutes prior to measurements/blood collection, and remained reclined in bed for the duration of the study. They were allowed sips of water (maximum 2 cups for the duration of the study).

Table 4 outlines the meal study data collection timing. Briefly, baseline (fasting) samples were collected at t = -30 minutes. The sodium bicarbonate or placebo capsules were administered after collecting the baseline samples/measurements. Consumption of the meal commenced 15 minutes after administration of the treatment at t = -15, and participants allowed 20

minutes to complete the meal, with t = 0 marking the completion of the meal. Outcome measures, as detailed below, were collected at 15-30 minute intervals following the meal completion, with the last time point at 180 minutes.

As no previous data were available in the literature regarding the effect of the treatment on body acidity markers in healthy human, I performed an interim analysis of the blood pH data after the initial 30 studies (15 participants). Paired T test showed that a drop in blood pH was detected from 0 minutes to 30 minutes following meal ingestion that was greater in the placebo group, but this did not reach statistical significance (mean difference in pH between sodium bicarbonate and placebo groups -0.14 pH units, 95% CI -0.03 to 0.04, P=0.12).

I realised that the change in blood pH is rapid, with blood pH reaching its nadir at the 30 minute timepoint. Therefore, I elected to add collection of blood at additional time points -15 and 15 minutes, from all the following participants (participant 16 onwards, n=15 for these additional time points).

Table 4: data collection points for meal study

Outcome measure	Medium where measured	Time point (minutes)										
		-30	-15	0	15	30	45	60	90	120	150	180
Non-esterified fatty acids	Serum	X				X	X	X	X	X		X
Lactate	Plasma	X			X	X		X	X	X		X
Glucagon-like peptide 1	Plasma	X			X	X		X				X
Blood pressure, arterial stiffness		X				X		X	X	X	X	X
Hunger/Satiety score		X		X		X		X	X	X	X	X
pH, bicarbonate	Blood, serum	X	X		X	X		X		X		X
Glucose, insulin, C-peptide	Serum	XX	X		X	X	X	X	X	X		X

X indicates the timing and frequency of sample collection for each outcome measure. The study capsules were administered immediately following the baseline measurements at t=-30 min. Consumption of the meal commenced immediately following the measurements at t=-15, and the meal was completed at t=0. Time points -15 min and 15 min were only collected in participants 16-30.

2.5. OUTCOME MEASURES

2.5.1. LABORATORY MEASURES

2.5.1.1.Venous blood pH

Two previous studies measured arterialised blood pH (27, 45), however, I elected to assess venous blood pH, due to the complexity and risks associated with frequent arterial blood sampling. The peripheral venous pH is approximately 0.02 to 0.04 pH units lower than the arterial pH of 7.35-7.45 (89-91). Venous and arterial pH correlate reasonably well (89, 91, 92), but this was particularly tested in acidotic patients, with most of the data in this area collected in diabetic ketoacidosis. Gokel *et al.* (89) found that arterial and venous pH correlated moderately well in healthy individuals with a pH in the normal physiological range ($r^2 = 0.595$), and very well in acidotic patients presenting with uraemia or diabetic ketoacidosis ($r^2 = 0.979$).

Venous blood gases were analysed, as indicated in Table 4, by St Vincent's Pathology (Sydpath, Sydney, Australia). The samples were collected in blood gas syringes, transported on ice, and processed swiftly within 15 minutes of collection using the Radiometer ABL 700 Blood Gas Analyzer (Diamond Diagnostics, Holliston, MA).

2.5.1.2.Serum bicarbonate

Serum bicarbonate and other serum electrolytes (including sodium, potassium, chloride, urea and creatinine) were analysed as indicated in Table 4, by St Vincent's Pathology (Sydpath, Sydney) using the Roche Diagnostics Modular System (Indianapolis, IN).

2.5.1.3.Plasma lactate

Plasma samples were collected as indicated in Table 4, in 4ml ethylenediaminetetraacetic acid (EDTA) tubes, centrifuged immediately (3500 x g for 7 minutes at 4 °C), plasma was aliquoted, snap frozen on dry ice, and stored at -80°C until analysis. Plasma lactate was measured using the YSI (2300 Stat Plus; Yellow Springs Instruments, Yellow Springs, OH).

2.5.1.4.Blood glucose

Whole blood glucose was measured throughout the study (Table 4). Blood was collected into 4mL fluoride oxalate tubes and measured immediately using the YSI (2300 Stat Plus; Yellow Springs Instruments, Yellow Springs, OH).

2.5.1.5.Serum insulin, C-peptide and non-esterified fatty acids (NEFA)

Blood was collected in 8.5 ml serum separation (gel) tubes, as indicated in Table 4, centrifuged immediately (3500 x g for 7 minutes at 4 °C), serum was then collected and aliquoted, snap frozen on dry ice, and stored at -80°C until analysis. Serum insulin and C-peptide were measured by radioimmunoassay (EMD Millipore, St Charles, MO, intra-assay CVs were 4 and 7% respectively). Serum NEFA concentrations were measured by a colorimetric kit (Wako Diagnostics, Richmond, VA, intra-assay CVs 5%). Serum insulin, C-peptide and NEFA concentrations were measured at the Garvan Institute of Medical Research.

2.5.1.6.Plasma glucagon-like peptide-1 (GLP-1)

GLP-1 is the predominant mediator of prandial insulin synthesis and secretion. It is an incretin hormone, explaining the greater stimulatory effect that oral glucose has on insulin secretion compared with intravenous glucose (93). GLP-1 is rapidly released from the intestinal L-cells in response to nutrients (93). It also inhibits inappropriate postprandial glucagon secretion (94), slows gastrointestinal motility (95), stimulates satiety (94), promotes insulin biosynthesis (93), and enhances glucose disposal (96). Fasting concentrations of GLP-1 are low, and postprandial secretion is biphasic (97). The initial early phase (15-30 minutes) is mediated by neural and endocrine factors, and the sustained second phase (30-60 minutes) is stimulated by the direct intraluminal contact between ingested nutrients and the intestinal mucosa (97). GLP-1 is rapidly degraded by dipeptidyl peptidase-4 (DPP4) following secretion, resulting in a short half-life of 1-1.5 minutes in the circulation (94).

GLP-1 released from intestinal L-cells is N-terminally truncated, resulting in GLP-1 (7-37) and GLP-1 (7-36) amide. The sum of these components represents the total GLP-1 concentration secreted from the intestinal L-cells (97). Blood for total GLP-1 analysis (Table 4) was collected into chilled 4ml EDTA coated tubes with DDP-IV inhibitor and trasylol (aprotinin), to prevent DPP-IV and protease activity, respectively, as previously described (97). 2 ml of blood was added to each tube with a syringe and placed on ice immediately following collection, centrifuged as above, aliquoted, snap frozen on dry ice, and stored at -80°C until analysis.

Total GLP-1 was measured using radioimmunoassay (EMD Millpore, St Charles, MO, intra-assay CV 10%) after extraction of plasma with 95% ethanol according to the manufacturer's instructions at the Garvan Institute of Medical Research.

2.5.2. HAEMODYNAMIC MEASURES

2.5.2.1.Blood pressure and heart rate

Blood pressure was measured, as indicated in Table 4, on the left arm by manual sphygmomanometer (WelchAllyn, Arden North Carolina) that was periodically calibrated. Heart rate was measured by the Sphygmocor transducer at the time of applanation tonometry.

2.5.2.2.Applanation tonometry

Central wave form analysis is a method of quantifying arterial stiffness. The augmentation index (AIx), is a well described index of arterial stiffness, and is

associated with coronary artery disease (98) as well as cardiovascular risk and mortality (99, 100).

As the ventricle contracts, it creates a forward pressure, as well as a reflected wave returning from the periphery to the heart. If the arteries are elastic the pulse wave velocity (PWV) is increased, the reflected wave arrives back at the aortic root during the diastole. In stiffened vessels, the reflected wave arrives back sooner, during systole, and increases, or "augments" the forward systolic pulse pressure, from which the augmentation index is calculated. **Figure 3** shows a graphic representation of the derivation of the AIx.

The AIx is automatically calculated by the instrument by dividing the difference between the second systolic peak and first systolic peak (or inflection point), also known as augmentation pressure, by the difference between the second systolic peak and the diastolic pressure (the pulse pressure) x 100%, as in the formula below.

$$\text{AIx} = \frac{Augmentation\ pressire}{pulse\ pressure} \text{ x } 100\%$$

The disadvantage of this technique is that the AIx calculated can be influenced by heart rate (which affects ventricular contraction) as well as age and height (101). However, my crossover study design ensures the latter two variables are controlled for.

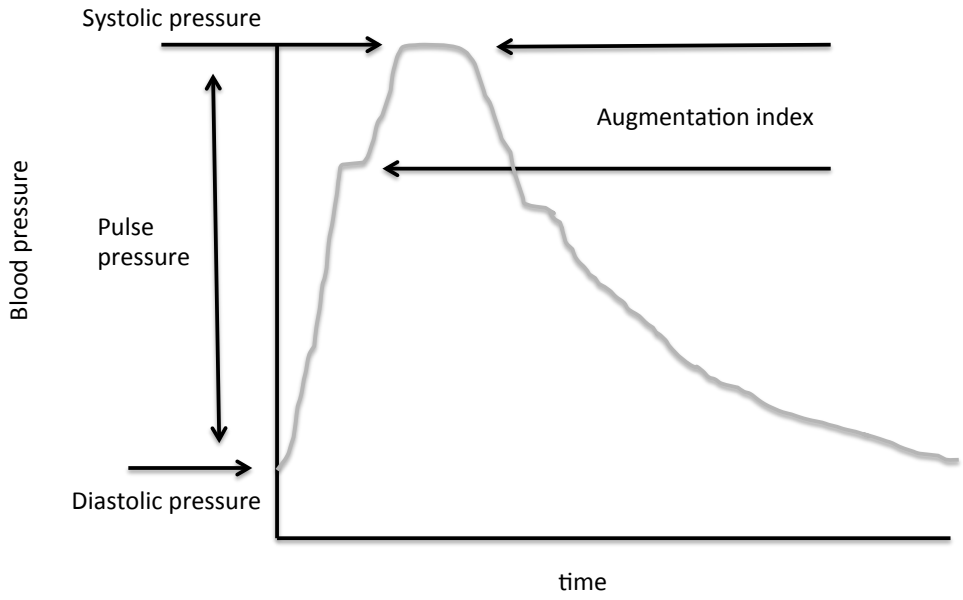

Figure 3: Graphic representation of the derivation of the Augmentation index
Adapted from Antonini-Canterin et al (102).

Applanation tonometry of the radial artery was performed using a highly sensitive transducer (Sphygmocor, AtCor Medical Inc., Australia, Table 4). A measurement was repeated if the operator accuracy index was <80%.

2.5.3. HUNGER AND SATIETY SCALE

Hunger and Satiety scales are a subjective quantification of appetite, and are influenced by both physiological and psychological factors (103). Raben et al (103) found that despite similar appetite profile curves with two identical meals in 9 subjects, subjective appetite scores were not easily reproduced. However, Flint et al (104) showed that visual appetite scores could be reproduced despite variations in repeatability coefficients. Incongruity amongst studies in this area may be influenced by the statistical analysis performed (104), but generally, and similar to other subjective measures, have limitations.

The participants were asked to rank on a scale of 1 to 10 their hunger (how hungry they were feeling) with 0 representing not hungry at all and 10 representing most hungry). They were also asked to rank their satiety (how full they felt) with 0 representing not full at all and 10 representing most full (**Figure 4**). These were performed at the times indicated in Table 4.

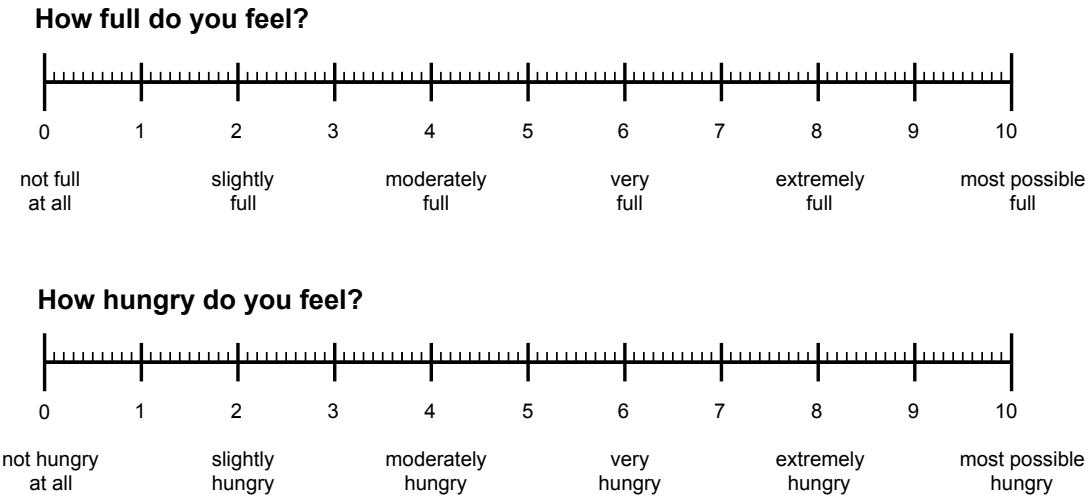

Figure 4: Hunger and satiety scale

2.6. STATISTICAL ANALYSIS

A-priori power analysis calculation was performed to detect a change in postprandial glucose excursions, measured by area under the curve of the glucose concentrations postprandially, based on a similar cohort studied by our group at the Clinical Research Facility at the Garvan Institute previously. Sample size calculation was based on detecting a 15% decrease in blood glucose incremental area under the curve (iAUC) with sodium bicarbonate *vs.* placebo, with a power $1-\beta \geq 0.8$ and α of 0.05 (2 tailed). Thirty participants were

required to complete the study, as this sample size was deemed sufficient to detect a difference in the AUC of venous blood pH with the treatment.

All normally distributed data is expressed as mean ± SD, and non-normally distributed data expressed as median and interquartile range (IQR). Non-normally distributed data were logarithmically transformed prior to statistical analysis.

Incremental AUC (iAUC) for pH, bicarbonate, lactate, glucose, insulin, C-peptide, GLP-1, NEFA, arterial stiffness, heart rate, and blood pressure was calculated using the trapezoidal method, and compared using a paired t-test.

Two-way repeated measure ANOVA tests were conducted to assess differences in the response to the meal with sodium bicarbonate *vs* placebo, where p_{time} indicates the main effect of time (i.e. the meal on its own), $p_{treatment}$ indicates the main effect of the treatment throughout the meal, and $p_{interaction}$ indicates the effect of the interaction between time and treatment. *P* values < 0.05 were considered significant.

Blood samples, blood pressure, arterial stiffness and hunger and satiety scores were collected at t= -30 min (baseline), then immediately after ingestion of the meal (0 min), then postprandially at 30, 45, 60, 90, 120, 150 and 180 min (Table 4). A sub-cohort (n = 15) had additional blood sampled 15 min after administration of the treatment (t = -15) and 15 min post meal ingestion (t = 15, Table 4). These time points were added after an interim examination of the

blood pH data revealed that the predefined collection times (-30 and +30 min) may miss a rapid effect on venous blood pH. Data collected at -15 and 15 min were removed in the repeated measure ANOVA analyses, so that the tests were performed on the whole cohort (n = 30).

A one-sample t-test was performed with t = 15 min data to assess the change in venous blood pH from baseline to 15 mins after meal ingestion (n = 15).

Statistical analysis was carried out using SPSS version 23 (SPSS Inc, Chicago, IL). GraphPrism version 6.07 (GraphPad Software, Inc, La Jolla, CA) was used to construct figures.

3.RESULTS

3.1. STUDY PARTICIPANTS

Figure 5 summarises the flow of participants in the study. 146 potential participants contacted the research team regarding the study. Of those, 108 were excluded following telephone screening, for either not meeting the inclusion criteria (n=22), declining to participate after receiving further information (n=48), or living outside of Sydney (n=8). Of those not meeting the inclusion criteria (n=22), they were excluded due to a history of hypertension (n=6), type 2 diabetes (n=3), unstable body weight (n=4), chronic disease (n=4), smoking (n=3), or concerns over their ability to provide informed consent (n=2). A further 30 individuals showed initial interest, but did not reply to further correspondence. The remaining 38 potential participants presented to the Clinical Research Facility for a screening visit.

Four potential participants were excluded due to not meeting the inclusion criteria. Specifically, one person was diagnosed with a haemolytic disorder at screening, another subsequently commenced a proton pump inhibitor treatment for *H.pylori* infection prior to the first meal study, and the remaining two were diagnosed with hypertension and type 2 diabetes at screening. Two further participants changed their mind and withdrew prior to the first study day.

Thirty two eligible participants (20 men and 12 women) met the inclusion criteria, provided informed written consent, underwent randomisation, and

completed the first meal study. Two female participants withdrew consent and dropped out following the first meal study. One due to difficulties with venesection, and the other found the meal was unpalatable to consume. A total of 30 participants completed the two study meals and were included in the data analyses.

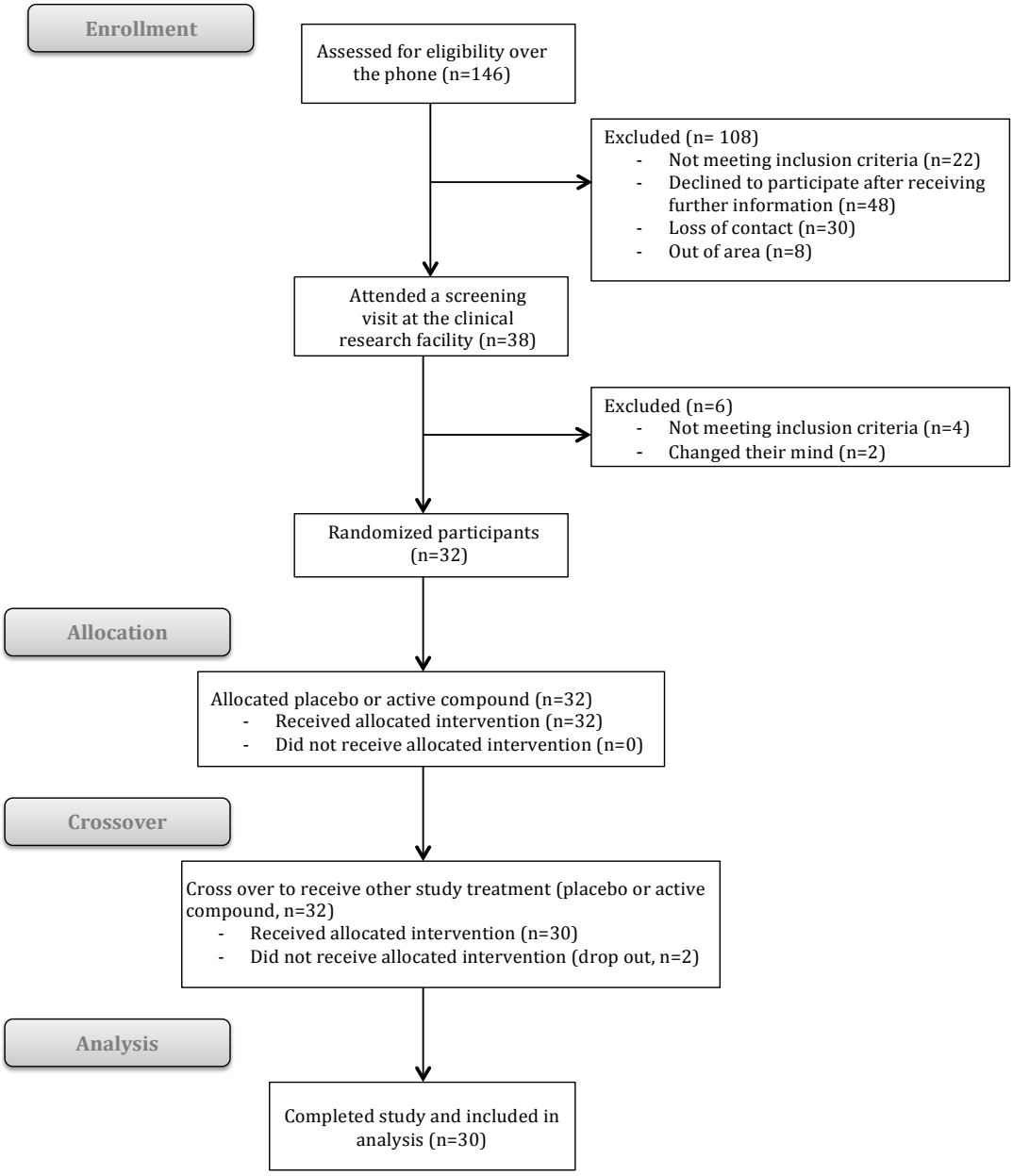

Figure 5: CONSORT diagram demonstrating study recruitment and participant progress

Adapted from Schulz et al (105).

Participant baseline characteristics are reported in **Table 5.** Of the 30 participants who completed the study, the majority were male (n=20, 66.7%), and Caucasian (n=24, 80%). A third of participants had a family history of type

2 diabetes (n=10, 33.3%). Twelve participants (40%) were sedentary and the remaining engaged in 1-6 hours/week of moderate physical activity. The mean age was 31.5 (IQR, 24.0-44.0) years, with a median BMI in the upper normal range at 24.0 (IQR, 22.1-25.9) kg/m^2. They were normotensive (mean systolic blood pressure: 120 ± 12 mmHg; mean diastolic blood pressure: 77 ± 7 mmHg), with normal fasting blood glucose concentration with a mean of 4.6 ± 0.4 mmol/L. HOMA-IR was slightly higher than expected at 2.3 (IQR, 1.7-3.6). Fasting venous blood pH was in the mid physiological range (7.39 ± 0.02), as expected and renal function was normal.

Table 6: Characteristics of study cohort

n (Men)	30 (20)
Age (years)	31.5 (24.0-44.3)
Waist circumference (cm)	83.0 (77.0-91.0)
Weight (kg)	74.4 ± 14.9
BMI (kg/m^2)	24.0 (22.1-25.9)
Family history of type 2 diabetes, (n)	10
*Sedentary lifestyle (n)	12
Systolic blood pressure (mmHg)	120 ± 12
Diastolic blood pressure (mmHg)	77 ± 7
**Fasting blood glucose (mmol/L)	4.6 ± 0.4
**Fasting serum insulin (mU/L)	10.8 (8.0-17.3)
***HOMA-IR	2.3 (1.7-3.6)
Serum creatinine (µmol/L)	81.1 ± 10.1
**Fasting venous pH	7.39 ± 0.02

Data expressed as mean ± SD, or median with interquartile range (IQR)
*Sedentary lifestyle: no regular strenuous activity
**Average of 2 measurements
*** HOMA-IR, homeostatic model assessment of insulin resistance. [glucose]*[insulin]/22.5

3.2. THE EFFECT OF SODIUM BICARBONATE ADMINISTERED PRIOR TO A HIGH ACID LOAD MEAL ON MARKERS OF BODY ACIDITY

3.2.1. VENOUS BLOOD PH

A one-sample T test at t=-15 (collected in participants 16-30, n=15) showed a decline in pH from baseline to -15 minutes in both groups, which was an unexpected finding. This decline was statistically significant in the placebo group (mean 0.012, 95%CI 0.003, 0.021, *p*=0.01), but not in the sodium bicarbonate group (mean 0.315, 95% CI -0.006, 0.018, *p*=0.03).

A significant postprandial effect on venous blood pH was noted with the meal ($p_{time} < 0.001$, **Figure 6A**), with an early decline in blood pH with the placebo, but not with the sodium bicarbonate. Specifically, a one-sample t-test at t = 15 min (n = 15) revealed that while venous blood pH under the placebo condition had decreased significantly from baseline (mean: 0.018, 95% CI: 0.009, 0.027; p = 0.001), this was not the case with the $NaHCO_3$ treatment (mean: 0.001, 95% CI: -0.015, 0.018; p = 0.86). This finding is supported by a significant time-treatment interaction ($p_{interaction} = 0.04$, Figure 6A), and a difference in the overall pH iAUC between the placebo and bicarbonate treatment (**Figure 6B**).

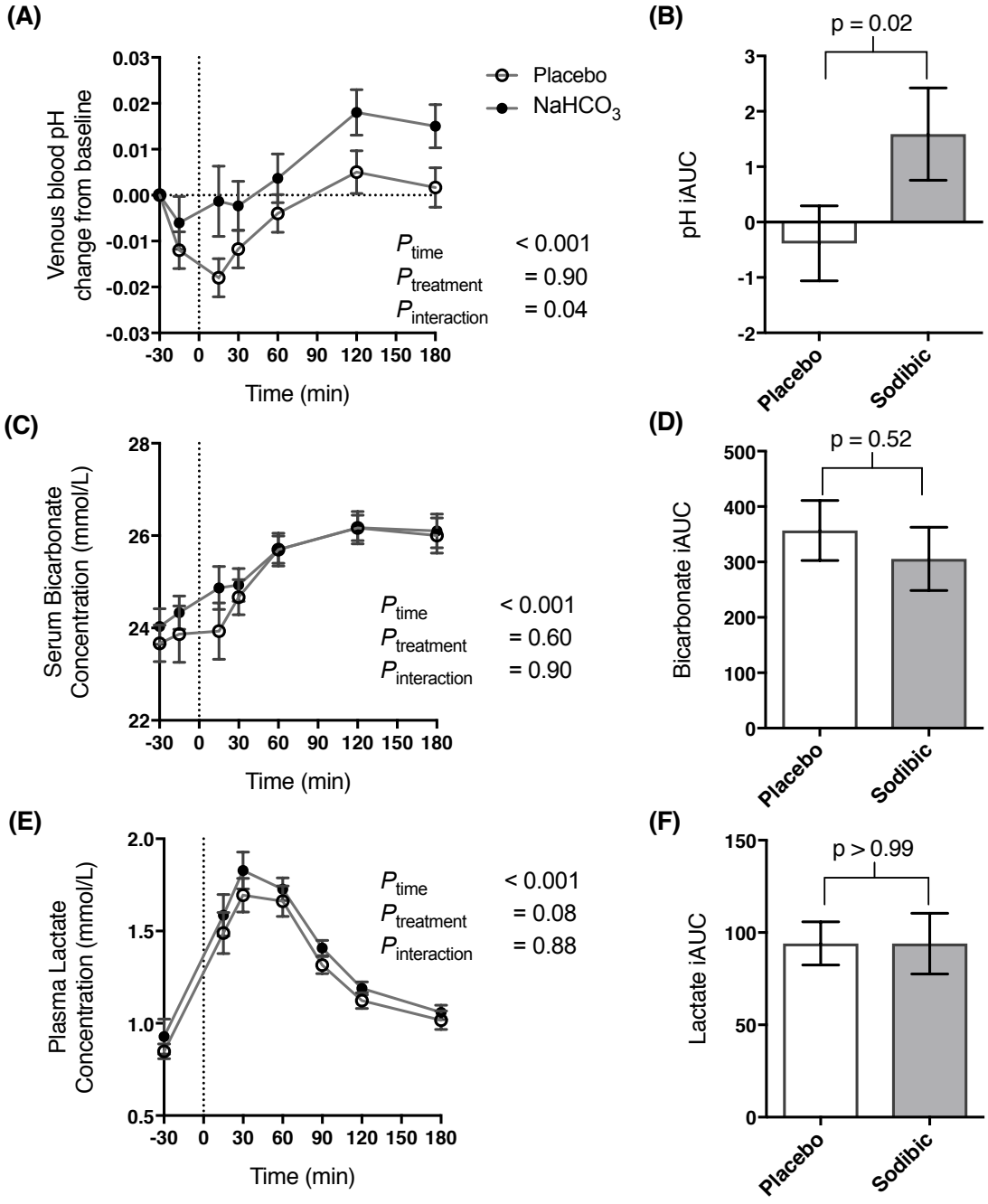

Figure 6: The effect of oral NaHCO3 (1680 mg) administered prior to a high acid load meal on venous blood pH (A), pH iAUC (B), serum bicarbonate (C) bicarbonate iAUC (D), plasma lactate (E) and lactate iAUC (F), comparing NaHCO3 (solid circle) and placebo (hollow circle). Treatment was administered at t = -30 min and the meal was completed at t = 0 (indicated by a dotted line). The effect of the treatment versus placebo on the postprandial outcome measure was tested by two-way repeated measure ANOVA and the difference in iAUC was tested by paired t-test, with P values indicated on the graphs. All data presented as mean ± SEM.

3.2.2. SERUM BICARBONATE

Serum bicarbonate increased in the first 60 minutes following the meal, and then plateaued between 60 and 180 minutes (**Figure 6C**). There was no significant difference in serum bicarbonate iAUC between the placebo and $NaHCO_3$ treatments (p_{iAUC} =0.52, **Figure 6D**).

The two way RM-ANOVA revealed a significant increase in serum bicarbonate concentration over time (p_{time}<0.001), without significant treatment effect or time-treatment interaction ($p_{treatment}$=0.60 and $p_{interaction}$=0.90).

3.2.3. PLASMA LACTATE

Plasma lactate concentrations followed a bell shape curve, increasing following the meal, and peaking at 30 minutes post prandially, then declining (**Figure 6E**). There was no significant difference in plasma lactate iAUC between the placebo and $NaHCO_3$ treatments (p_{iAUC} >0.99, **Figure 6F**).

The two way RM-ANOVA revealed that a significant change in plasma lactate over time (p_{time}<0.001), but the effects of the treatment and the time-treatment interaction were not significant ($p_{treatment}$=0.08 and $p_{interaction}$=0.88).

3.3. THE EFFECT OF SODIUM BICARBONATE GIVEN PRIOR TO A HIGH ACID LOAD MEAL ON MARKERS OF GLUCOSE HOMEOSTASIS

3.3.1. BLOOD GLUCOSE

As expected, blood glucose increased following the meal, peaking at 15-30 minutes and returning to baseline by 90 minutes post prandially (**Figure 7A**). There was no significant difference in blood glucose iAUC between the placebo and $NaHCO_3$ treatments (p_{iAUC}= 0.21, **Figure 7B**).

Consistently, RM-ANOVA showed a significant main effect of time (p_{time} <0.001), but not treatment ($p_{treatment}$ = 0.87) or time-treatment interaction ($p_{interaction}$ = 0.77, Figure 7A).

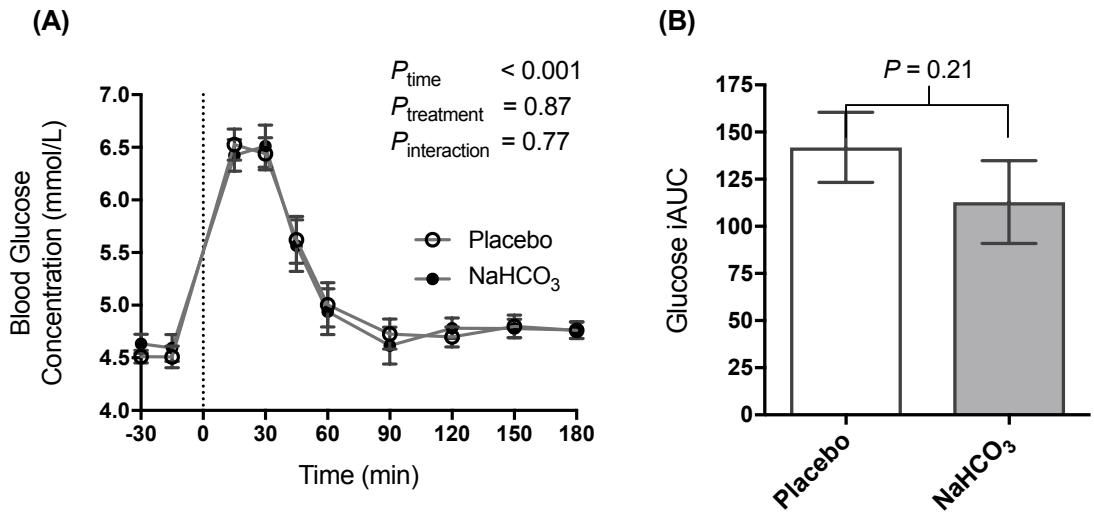

Figure 7: The effect of oral NaHCO3 (1680 mg) administered prior to a high acid load meal on blood glucose (A), glucose iAUC (B), comparing NaHCO3 (solid circle) and placebo (hollow circle). Treatment was administered at t = -30 min and the meal was completed at t = 0 (indicated by a dotted line). The effect of the treatment versus placebo on the postprandial outcome measure was tested by two-way repeated measure ANOVA and the difference in iAUC was tested by paired t-test, with P values indicated on the graphs. All data presented as mean ± SEM.

3.3.2. SERUM INSULIN

Serum insulin concentrations peaked at 30 minutes following the meal and then declined (**Figure 8A**). There was no significant difference in serum insulin iAUC between the placebo and $NaHCO_3$ treatment (p_{iAUC}=0.84, **Figure 8B**).

Similarly, RM-ANOVA analysis showed a significant main effect of time (p_{time} <0.001), but not treatment ($p_{treatment}$ = 0.85) or the time-treatment interaction ($p_{interaction}$ = 0.40, Figure 8A).

3.3.3. SERUM C-PEPTIDE

Serum C-peptide peaked at 30-60 minutes postprandially, with a subsequent decline (**Figure 8C**). There was no significant difference in serum C-peptide iAUC between the placebo and $NaHCO_3$ treatment (p_{iAUC}=0.39, **Figure 8D**).

Similarly, RM-ANOVA showed a significant main effect of time (p_{time} <0.001), but not treatment ($p_{treatment}$ = 0.29) or time-treatment interaction ($p_{interaction}$ = 0.29, Figure 8C).

3.3.4. PLASMA GLUCAGON-LIKE PEPTIDE-1 (GLP-1)

Plasma GLP-1 rose following the meal, peaking at 15 minutes, then declined and reached a nadir at 60 minutes before rising again at 180 minutes (**Figure 8E**). Overall, plasma GLP1 iAUC was not significantly different between the placebo and $NaHCO_3$ treatment (p_{iAUC}= 0.43, **Figure 8F**).

Furthermore, two-way ANOVA analysis revealed a significant main effect of time (p_{time} =0.02) and while the effect of treatment was not significant ($p_{treatment}$ =0.43), the time-treatment interaction was trending significance ($p_{interaction}$ =0.07).

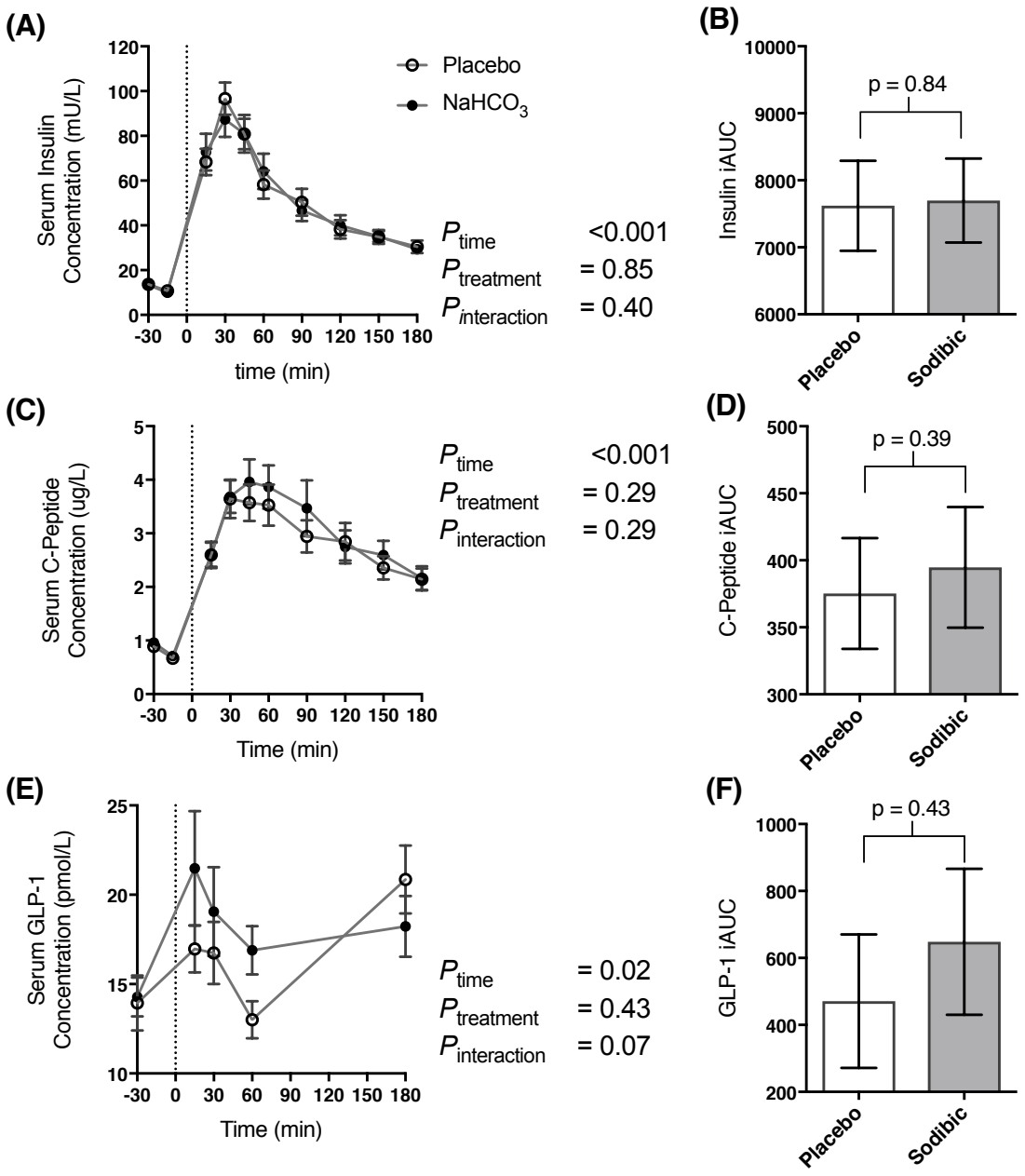

Figure 8: The effect of oral NaHCO3 (1680 mg) administered prior to a high acid load meal on serum insulin (A), insulin iAUC (B), serum C-peptide (C), C-peptide iAUC (D), plasma GLP-1 (E), GLP-1 iAUC (F), comparing NaHCO3 (solid circle) and placebo (hollow circle). Treatment was administered at t = -30 min and the meal was completed at t = 0 (indicated by a dotted line). The effect of the treatment versus placebo on the postprandial outcome measure was tested by two-way repeated measure ANOVA and the difference in iAUC was tested by paired t-test, with P values indicated on the graphs. All data presented as mean ± SEM. Abbreviations: GLP-1, glucagon-like peptide 1.

3.3.5. INSULIN:C-PEPTIDE, GLUCOSE:INSULIN, AND GLUCOSE:C-PEPTIDE RATIOS

Glucose:insulin, glucose:C-peptide, and insulin:C-peptide ratios, surrogate markers for insulin sensitivity, beta cell function, and hepatic insulin clearance, respectively (93, 106, 107), were unaffected by the alkalising treatment (data not shown). There was no significant difference in the iAUC between the placebo and $NaHCO_3$ treatment (insulin:C-peptide, p_{iAUC}=0.7; glucose:insulin, p_{iAUC}=0.18; and glucose:C-peptide, p_{iAUC}=0.71).

Similarly, the two-way ANOVA analysis, showed a significant main effect of time (p<0.001 for all three indices), however, the effects of treatment and the time-treatment interaction were not significant ((insulin:C-peptide, $p_{interaction}$=0.53; glucose:insulin, $p_{interaction}$=0.11; and glucose:C-peptide, $p_{interaction}$=0.71).

3.3.6. SERUM NON-ESTERIFIED FATTY ACIDS (NEFA)

Serum NEFA concentrations were suppressed postprandially (Figure 9A), with no significant difference in the iAUC between the placebo and $NaHCO_3$ treatments (p_{iAUC}= 0.87, **Figure 9B**). RM-ANOVA revealed a significant effect of time (p_{time}<0.001), but not treatment ($p_{treatment}$=0.97), or time-treatment interaction ($p_{interaction}$=0.69).

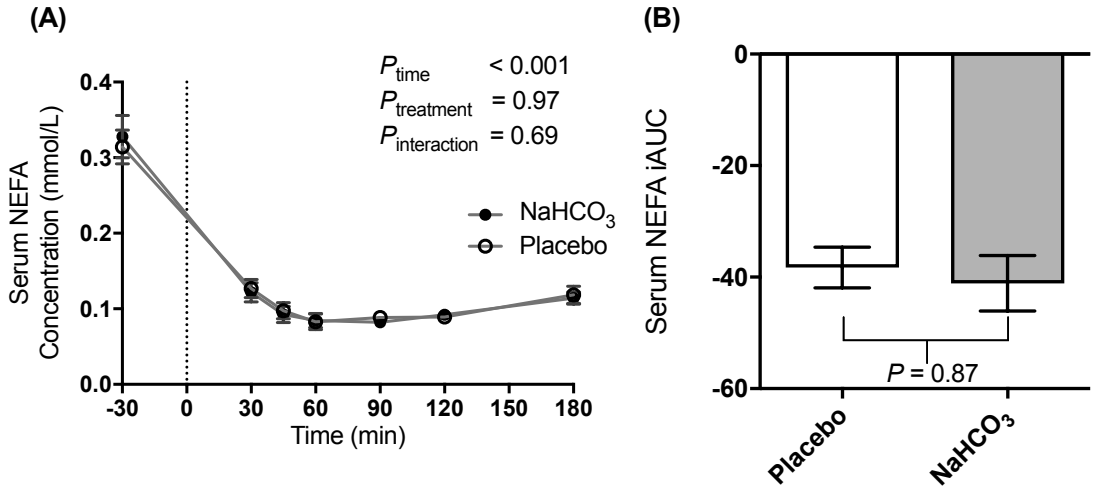

Figure 9: The effect of oral NaHCO3 (1680 mg) administered prior to a high acid load meal on serum NEFA (A) NEFA iAUC (B), comparing NaHCO3 (solid circle) and placebo (hollow circle). Treatment was administered at t = -30 min and the meal was completed at t = 0 (indicated by a dotted line). The effect of the treatment versus placebo on the postprandial outcome measure was tested by two-way repeated measure ANOVA and the difference in iAUC was tested by paired t-test, with P values indicated on the graphs. All data presented as mean ± SEM. Abbreviations: NEFA, non-esterified fatty acid.

3.4. THE EFFECT OF SODIUM BICARBONATE GIVEN PRIOR TO A HIGH ACID LOAD MEAL ON BLOOD PRESSURE AND ARTERIAL STIFFNESS

3.4.1. BLOOD PRESSURE

Systolic and diastolic blood pressure decreased significantly following the meal (**Figure 10**). RM-ANOVA showed a significant effect of time for both **(**systolic BP p_{time} =0.02; diastolic BP p_{time} <0.001).

The effect of the treatment on systolic and diastolic blood pressure was not significant ($p_{treatment}$ =0.20 and $p_{treatment}$ =0.28 for systolic and diastolic blood

pressure, respectively). Similarly, the time-treatment interaction was not significant ($p_{interaction}$=0.68 and $p_{interaction}$=0.28 for systolic and diastolic blood pressure, respectively).

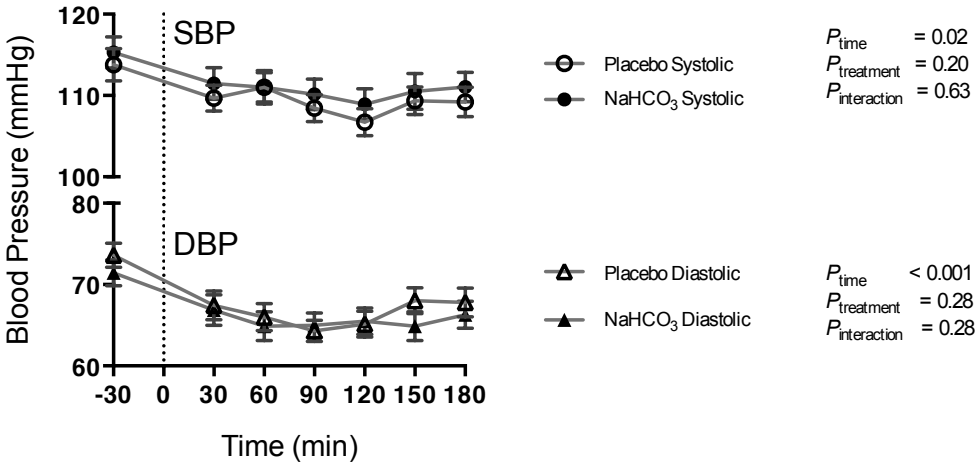

Figure 10: The effect of oral NaHCO3 (1680 mg) administered prior to a high acid load meal on systolic and diastolic blood pressure, comparing NaHCO3 (solid symbol) and placebo (hollow symbol). Treatment was administered at t = -30 min and the meal was completed at t = 0 (indicated by a dotted line). The effect of the treatment versus placebo on the postprandial outcome measures was tested by two-way repeated measure ANOVA and the difference in iAUC was tested by paired t-test, with P values indicated on the graphs. All data presented as mean ± SEM. Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure.

3.4.2. ARTERIAL STIFFNESS

Arterial stiffness, measured by the AIx, decreased following the meal (**Figure 11A**). Two-way repeated measure ANOVA test revealed the significant main effects of time (p_{time} <0.001) and treatment ($p_{treatment}$ <0.001) on the AIx, with a trend to a significant difference between the active treatment and placebo in the time-treatment interaction ($p_{interaction}$ =0.07).

However, no difference in the overall AIx iAUC between treatments was observed (p_{iAUC}=0.98, **Figure 11B**).

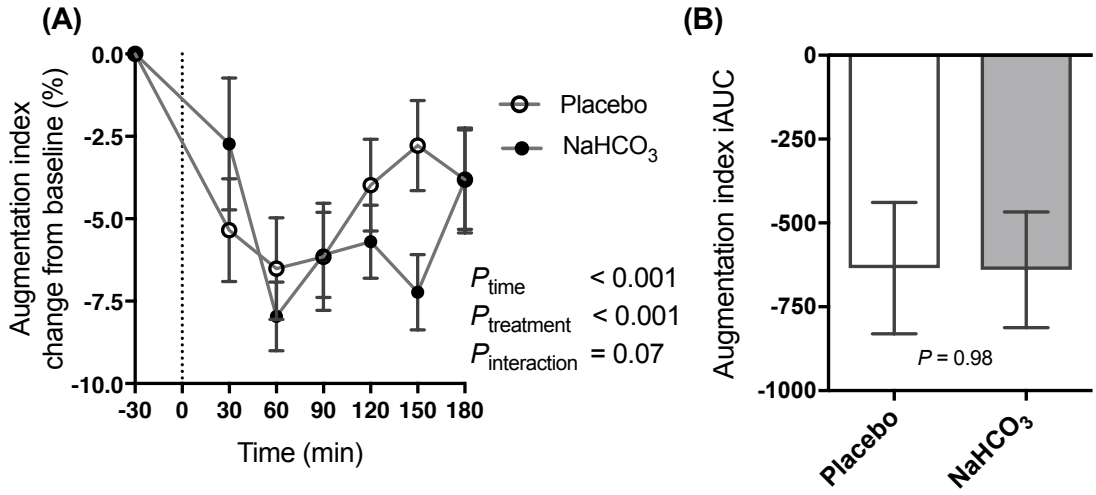

Figure 11: The effect of oral NaHCO3 (1680 mg) administered prior to a high acid load meal on the augmentation index (A) and the augmentation index iAUC (B), comparing NaHCO3 (solid circle) and placebo (hollow circle). Treatment was administered at t = -30 min and the meal was completed at t = 0 (indicated by a dotted line). The effect of the treatment versus placebo on the postprandial outcome measure was tested by two-way repeated measure ANOVA and the difference in iAUC was tested by paired t-test, with P values indicated on the graphs. All data presented as mean ± SEM.

3.5. THE EFFECT OF SODIUM BICARBONATE GIVEN PRIOR TO A HIGH ACID LOAD MEAL ON HUNGER AND SATIETY SCORES

Satiety rapidly increased following the meal, and gradually declined from 0 minutes (**Figure 12A**). The RM-ANOVA test revealed a significant main effect of time (p_{time} <0.001) and treatment ($p_{treatment}$ =0.04) on satiety. However, the time-

treatment interaction was not significant ($p_{interaction}$ =0.52). The Satiety iAUC showed a tendency towards a significant increase in satiety with the $NaHCO_3$ treatment (p_{iAUC}=0.07, **Figure 12B**).

Conversely, hunger rapidly decreased following the meal and gradually increased over the following 3 hour period (Figure 12A). The RM-ANOVA test revealed a significant main effect of time on hunger (p_{time} <0.001). However, the effect of the treatment ($p_{treatment}$ =0.14) and the time-treatment interaction ($p_{interaction}$ =0.43) were not significant. Hunger iAUC was not significantly affected by the treatment (p_{iAUC}=0.55, **Figure 12C**).

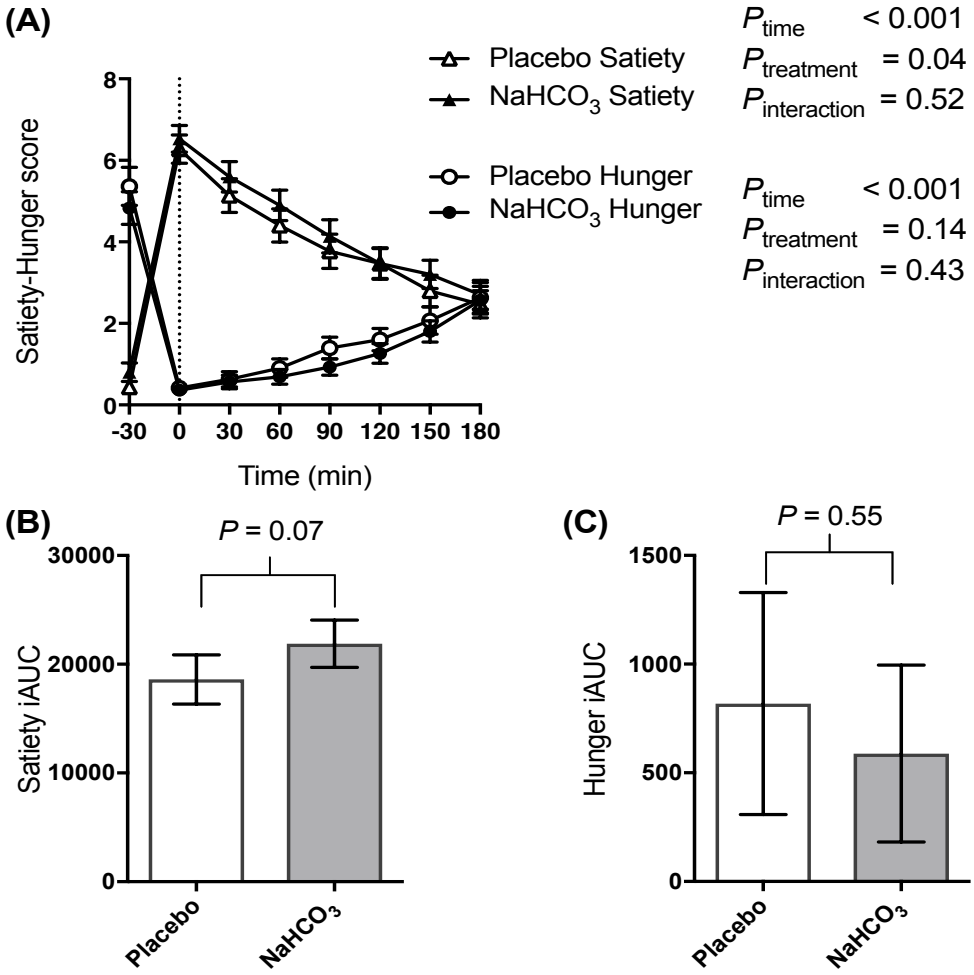

Figure 12: The effect of oral NaHCO3 (1680 mg) administered prior to a high acid load meal on satiety and hunger scores (A), satiety iAUC (B), and hunger iAUC (C), comparing NaHCO3 (solid circle) and placebo (hollow circle). Treatment was administered at t = -30 min and the meal was completed at t = 0 (indicated by a dotted line). The effect of the treatment versus placebo on the postprandial outcome measure was tested by two-way repeated measure ANOVA and the difference in iAUC was tested by paired t-test, with P values indicated on the graphs. All data presented as mean ± SEM.

4.DISCUSSION

4.1. SUMMARY OF THE FINDINGS

My primary finding is that sodium bicarbonate treatment attenuates the observed postprandial decline in pH with placebo, in a cohort of non-diabetic, normotensive individuals with normal renal function. However, despite the attenuation in pH decline seen with the treatment, there was no significant effect on glucose homeostasis following the meal.

Whilst it has previously been hypothesised that a high dietary acid load can decrease blood pH (108), this is the first study I am aware of, that clinically demonstrates a decline in venous blood pH following a high acid load meal, in the context of a meal tolerance test. However, whether this is the effect of the meal *per se*, or its acid load remains to be established, as a low-PRAL meal comparator was not tested in the present study. In any case, the postprandial pH decline has been attenuated by sodium bicarbonate administration.

Consistent with the lack of significant difference in glucose excursion between the two treatments, I found that postprandial insulin and C-peptide excursion were not significantly different between the sodium bicarbonate and placebo treatments. Therefore, I conclude, that sodium bicarbonate treatment prior to a high acid load meal, did not lead to a significant change in postprandial insulin secretion.

There was a trend towards increased plasma GLP-1 levels postprandially with sodium bicarbonate administration compared to placebo. However, as this enhanced GLP-1 response was not associated with a corresponding increase in insulin response, the clinical significance of this finding remains unclear.

Additionally, although hunger was unchanged, self-reported satiety was increased with sodium bicarbonate administration, which may be a result of the gastrointestinal side effects from the medication. I also considered whether this could be due to delayed gastric emptying related to higher GLP-1 concentrations, however, the difference in GLP-1 excursion did not reach statistical significance.

There was no significant effect of the alkalising treatment on the secondary outcome measures (including lactate, NEFA, blood pressure and arterial stiffness).

4.2. Discussion of the findings

4.2.1. Effect of sodium bicarbonate treatment on glucose metabolism

4.2.1.1.The effect of sodium bicarbonate administration on blood glucose, insulin, and C-peptide concentrations

There are several possible reasons why I did not detect a change in glycaemia with sodium bicarbonate treatment. Firstly, it is possible that the change in pH caused by the sodium bicarbonate treatment was too marginal to cause a

significant change in glucose metabolism. This could be related to the low dose of sodium bicarbonate used, or alternatively to the highly effective physiological buffering systems in healthy individuals.

I elected to administer 1680 mg of oral sodium bicarbonate prior to the meal, similarly to the dose administered in chronic renal failure patients (109). In chronic renal failure patients, this dose increased serum bicarbonate, without significant adverse effects. Larger doses of oral sodium bicarbonate have been associated with more impressive blood pH increases. In an elite athlete cohort, Cameron et al (88) administered 0.3 kg/kg of sodium bicarbonate, a dose that in a 70 kg man is more than 10-fold the dose administered here. Whilst that study documented a significant difference in blood pH and serum bicarbonate, large doses, typically used in cohorts of athletes, have been associated with substantial gastrointestinal side effects (87, 88), rendering any potential clinical application impractical. In the present study, I found an increase in self-reported satiety with sodium bicarbonate treatment, which may have been due to mild (unreported) gastrointestinal effects. Whilst the difference in venous pH detected between the two treatments in my study was small, it is similar to that reported by Buclin et al (38) when comparing a 4 day low-PRAL (alkaline) versus high-PRAL (acidifying) dietary intervention.

It has recently been shown that interstitial fluid pH is lower in patients with type 2 diabetes compared to non-diabetic controls, and suggested to play a causative role in insulin resistance (110). Whilst I detected a small change in venous blood pH in my study, it is possible that sodium bicarbonate

administration caused a more substantial effect on interstitial pH, but this has not been tested here. A change in the pH in the interstitium may not be as efficiently buffered as blood pH (110), and may be more likely to influence insulin receptor density and affinity (42, 44). However, at least acutely, this did not result in a change in glucose metabolism. Whilst it is possible to measure pH changes in the interstitial fluid of human skeletal muscle (111), this was not performed in the present study.

Metabolic acidosis in chronic renal failure patients is known to contribute to insulin resistance (57, 58), and previous studies have detected an increase in insulin sensitivity after correction of metabolic acidosis in a chronic renal failure population (45, 59). Therefore, correction of acidosis in individuals with a pH below the physiological range appears to have an effect on glucose homeostasis. However, alkalising treatment in populations with overt acidaemia is likely to have a different physiological effect compared with an adjustment of the pH within the normal range in a non-acidotic population with normal renal function, such as my study population.

Despite a significant difference in plasma pH between the sodium bicarbonate and placebo treatments, a change in serum bicarbonate was not detected. The very small change in serum bicarbonate concentration seen immediately after the administration of the study capsules was not statistically significant, and was quickly normalised. The lack of detectable change in serum bicarbonate concentration may be related to the sensitivity of the bicarbonate assay.

Alternatively, rapid buffering of the exogenous bicarbonate may have occurred before the 15 minute venesection.

Previous studies with longer term outcomes (days to weeks of alkaline treatment) have used the gold standard hyperinsulinaemic-euglycaemic clamp protocol to explore the relationship between sodium bicarbonate administration and insulin sensitivity. However, I elected to utilise a high acid load meal tolerance test, as it is a more relevant to the scientific question and due to my interest in the acute glycaemic effect of the alkaline treatment postprandially. I did not expect in the acute study settings to change insulin resistance.

4.2.1.2.The effect of sodium bicarbonate administration on glucagon-like peptide-1 (GLP-1) secretion

There was a trend towards higher plasma GLP-1 levels following sodium bicarbonate administration, but this did not reach statistical significance. The difference in plasma GLP-1 concentration measured between the bicarbonate and placebo treatments was similar to the difference in GLP-1 measured by our group in another study (97) with pre-prandial ingestion of glutamine, an amino acid known to enhance GLP-1 secretion with concomitant increases in insulin secretion (97). However, in the current study, the GLP-1 rise did not result in an increase in insulin, and therefore may mean that the observed increase in GLP-1 concentrations is clinically irrelevant.

Furthermore, it is possible that a pH change in the lumen of the ileum with sodium bicarbonate treatment resulted in modulation of ion transporter activity,

thus stimulating GLP-1 secretion. Nevertheless, small changes in the extracellular pH are unlikely to affect the potassium and calcium channels involved in GLP-1 secretion (112).

In summary, the long term implications of postprandial circulating GLP-1 enhancement with bicarbonate and the mechanism(s) involved in this response require further investigation.

4.2.1.3.The effect of sodium bicarbonate administration on non-esterified fatty acids (NEFA)

NEFA are linked to adverse metabolic outcomes, including insulin resistance and the propensity for diabetes, particularly in the context of obesity (113), through their effect on the early steps of the insulin signalling pathway (114). NEFA are derived from adipose tissue, and their release is inhibited by insulin. However, the suppressive effect of insulin on fatty acid mobilisation decreases with insulin resistance. Counter-intuitively, adiposity doesn't correlate directly with plasma NEFA levels, as NEFA release is downregulated as adipose tissue stores expand (115).

NEFA have a short plasma half-life of 2-4 minutes, and concentrations are typically higher after an overnight fast, and fall following a meal, due to the insulin response, which is dependent on the carbohydrate content of the meal and adipose insulin resistance (115). Serum NEFA concentrations were suppressed after the meal and suppression level was not different between the

placebo and sodium bicarbonate treatments, consistent with the lack of change in serum insulin concentrations.

4.2.2. THE EFFECT OF SODIUM BICARBONATE TREATMENT ON CARDIOVASCULAR OUTCOMES

4.2.2.1.The effect of sodium bicarbonate treatment on arterial stiffness

The AI decreases postprandially proportional to the carbohydrate content of the meal, and dependent on the insulin response (116). Insulin-resistant subjects can have an attenuated postprandial AIx response which has been suggested to confer an increased cardiovascular risk (117).

Whilst there was no effect of sodium bicarbonate treatment on systolic or diastolic blood pressure, I found that there was a trend towards a greater decline in arterial stiffness following sodium bicarbonate administration compared to placebo. The reason behind this difference remains unclear in the absence of a significant difference in serum insulin levels between the two treatments.

One possible explanation is that the administered sodium bicarbonate may affect arterial stiffness directly. A study in haemodialysis patients found that increasing dialysate bicarbonate concentrations were associated with a reduction in arterial stiffness measured using a pulse wave analyser (118). However, the mechanism for this phenomenon requires further investigation.

4.2.3. STRENGTHS AND LIMITATIONS OF THE STUDY

The strengths of the present study are the robust double blind randomised crossover placebo-controlled design and direct measurements of blood pH and glucose metabolism in a high acid load meal context. However, the present study has some limitations. Firstly, this study did not target insulin-resistant individuals and therefore the findings can only be applicable to a relatively healthy population. While several studies have reported improvement in insulin sensitivity after correcting metabolic acidosis in chronic renal failure patients (45, 59), reparation of an abnormally low pH may be physiologically different to increasing pH within the physiological range in healthy individuals with respect to its impact on glucose metabolism. Additionally, this study only examined the acute effect of a relatively low dose bicarbonate supplementation on glucose metabolism. The effect on insulin resistance cannot be studied in the acute settings and therefore, a chronic intervention is necessary to establish whether an alkalising treatment will correct glucose metabolism in individuals with prediabetes. This could potentially be investigated through administration of sodium bicarbonate with meals over a period of weeks to months, with a hyperinsulinaemic-euglycaemic clamp assessing changes in insulin sensitivity due to the treatment. Furthermore, a low-acid load meal comparator was not tested, and finally, while increased venous blood pH was detected with the bicarbonate treatment, there was no detectable change in serum bicarbonate. While this was unexpected, it may be related to the sensitivity of the serum bicarbonate assay.

4.3. CONCLUSION

The alkaline diet is proposed to counter the excessive acid load of the Western diet, with many suggested health benefits. There is some evidence, mainly from epidemiological studies, to suggest that adherence to an alkaline or low PRAL diet may be protective against insulin resistance and type 2 diabetes. Furthermore, metabolic acidosis in chronic kidney disease patients is thought to contribute to insulin resistance, and correction of metabolic acidosis has been shown to improve insulin resistance in chronic renal failure patients.

In the present study, my working hypothesis was that buffering a high acid load meal with an alkaline treatment will attenuate postprandial blood pH decrease and glycaemic response after the meal. I found that despite the attenuation of pH decline achieved with the sodium bicarbonate treatment, no effect on circulating glucose or insulin secretion was documented in this non diabetic cohort who had normal renal function. Based on these findings, I conclude that any beneficial glycaemic effects reported in previous studies from adhering to a low acid load diet long term are unlikely to be explained by an acute effect on postprandial glycaemia.

Due to the acute nature of my study involving a meal tolerance test, I am not able to comment on the effect of alkalising treatment on insulin sensitivity, and a chronic intervention study administering an alkaline treatment (or a low PRAL diet) over weeks or months in insulin-resistant individuals will be necessary to establish the relationship between body acid / base balance and insulin

resistance. Furthermore, considering my findings against findings in chronic renal failure patients, suggests that correction of full-blown acidosis may have a different effect on glucose metabolism compared with an upward shift in pH which is already within the physiological normal range in healthy individuals.

Although the literature exploring the relationship between acid / base balance and glucose homeostasis remains conflicting, my study suggests that a single pre-prandial bicarbonate supplementation does not have a clinically meaningful effect on postprandial glycaemia, in healthy cohorts.

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