Dietary acid load, metabolic acidosis and insulin resistance - lessons from cross-sectional and overfeeding studies in humans

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Abbreviations used: GIR, Glucose infusion rate; NEAP, Net endogenous acid potential; Ob_{res}, Obese insulin-resistant; Ob_{sen}, Obese insulin-sensitive; PRAL, Potential renal acid load; RNAE, Renal net acid excretion; SFAT, Subcutaneous fat; TEI, Total energy intake

ABSTRACT

Background: Western diets rich in animal protein and poor in fruit and vegetables increase body acid load, which is predictive of type 2 diabetes risk independent of BMI. The relationships between dietary acid load, mild metabolic acidosis and insulin resistance remain unclear.

Objective: To assess the association between dietary acid load, body acid/base markers and peripheral insulin resistance at baseline and following a short-term overfeeding intervention.

Design: Insulin sensitivity (hyperinsulinemic-euglycemic clamp) was assessed in healthy individuals (n=104, 49 men). Plasma lactate, a marker of metabolic acidosis was measured and diet diaries analyzed for acid load scores (potential renal acid load, PRAL and net endogenous acid production, NEAP). The cohort was grouped into lean and overweight/obese and the latter further classified as insulin-sensitive (Ob_{sen}) and insulin-resistant (Ob_{res}) based on hyperinsulinemic-euglycemic clamp glucose infusion rate (GIR, top tertile vs. bottom 2 tertiles). A subset of forty non-obese individuals was overfed (+1250 kcal/day, 45% fat) for 28 days and studies repeated.

Results: Ob_{sen} and Ob_{res} were matched for adiposity (BMI and fat mass, both P=1). Fasting plasma lactate was higher in Ob_{res} (0.9±0.0 mmol/L) compared with both lean (0.7±0.1 mmol/L, P=0.04) and Ob_{sen} (0.7±0.0 mmol/L, P=0.02). No difference in plasma lactate was noted between lean and Ob_{sen} (P=1). Overfeeding was characterized by an increase in dietary acid load scores PRAL (P=0.003) and NEAP (P=0.05), a reduction in GIR necessary to maintain euglycemia (P=0.03) and an increase in fasting plasma lactate (P=0.02). The change in lactate was inversely associated with the change in GIR (r=-0.36, P=0.03).

Conclusions: Mild metabolic acidosis, measured by plasma lactate, aligns with insulin resistance independent of obesity and is induced by short-term increases in energy and dietary

acid load in healthy humans. Further studies are required to determine whether buffering mild metabolic acidosis improves insulin resistance and reduces diabetes risk.

INTRODUCTION

Insulin resistance is closely associated with obesity and precedes the development of type 2 diabetes. In lean healthy adults, the induction of mild metabolic acidosis by ammonium-chloride administration over three days decreased insulin sensitivity (1). Conversely, correction of metabolic acidosis in chronic renal failure patients, following four weeks bicarbonate treatment, increased insulin sensitivity (2).

A diet with a persistently high acid load can cause blood pH to decrease towards the lower end of the normal physiological range (3). This disequilibrium in acid/base balance, if not compensated for by homeostatic mechanisms or dietary modification can lead to the development of chronic mild metabolic acidosis (4, 5). The Western diet is characteristically high in animal proteins that when metabolized generate sulfate, a major contributor to dietary acid load (6). This is typically accompanied by inadequate consumption of fruit and vegetables, rich in mineral cations and bicarbonate precursors that have an alkalizing effect when digested (6, 7).

The acidogenic potential of foods can be calculated using potential renal net acid load (PRAL) (8) and net endogenous acid production (NEAP) scores (4). PRAL takes into account the nutrient ionic balance and intestinal absorption rates of protein, phosphorous, potassium, magnesium and calcium as well as the metabolism of protein in the production of sulfate (8, 9). A positive PRAL score indicates an acid forming potential, while a negative score indicates an alkaline forming potential (10). NEAP is based on the dietary intake of protein and potassium as the main determinants of endogenous acid production (4). For example, the NEAP score of a Western diet was ~48 mEq/d (10) and a strict to moderately strict vegan diet

was ~15 mEq/d (11). A high score is therefore indicative of the consumption of animal proteins in quantities not sufficiently compensated for by intake of fruit and vegetables.

In healthy individuals, PRAL and NEAP have been shown to provide a reliable estimation of the diet-dependent component of daily renal net acid excretion (RNAE) (4, 8, 9, 12). A high RNAE as well as other markers of mild metabolic acidosis including decreased urinary pH (12, 13), a high anion gap (the difference between measured anions and cations in serum) (14-16) and increased serum lactate (a small component of the anion gap) have consistently been associated with insulin resistance (17, 18) and type 2 diabetes risk (18, 19).

Thus, dietary acid load may be an important factor in the development of insulin resistance and type 2 diabetes (6, 16). The aim of the present study was to assess the association between dietary acid load, body acid/base markers and peripheral insulin sensitivity before and after a short-term overfeeding intervention. We hypothesized that a higher dietary acid load would be associated with mild metabolic acidosis, as indicated by elevated plasma lactate, and insulin resistance, as measured by hyperinsulinemic-euglycemic clamp.

SUBJECTS AND METHODS

The study was conducted in the Clinical Research Facility at the Garvan Institute of Medical Research, Sydney. Study protocols were approved by St Vincent's Hospital Human Research Ethics Committee, Sydney and participants provided written informed consent.

Participants

Cross-sectional insulin-sensitive and insulin-resistant cohort

One hundred and four non-diabetic individuals with an average age of 45±1 years were recruited between 2007 and 2013, as part of two separate studies. The cohort was stratified based on BMI into lean (n=20, 9 males, BMI 22.6±0.3 kg/m^2) and overweight/obese (n=84, 40 males, BMI 34.5±0.6 kg/m^2) groups. Overweight/obese individuals were further classified based on hyperinsulinemic-euglycemic clamp glucose infusion rate (GIR) with separate cut offs for males and females into insulin-sensitive (Ob_{sen}, top tertile; n=27, 13 males) and insulin-resistant (Ob_{res}, bottom 2 tertiles; n=57, 27 males). Exclusion criteria included treatment with medications that affect glucose metabolism, excessive alcohol intake, smoking, unstable body weight in the preceding three months, known renal, cardiac or liver disease and a personal history of diabetes.

Short-term overfeeding intervention

A subset of 40 non-obese individuals (20 males, aged 37±2 years, BMI 25.6±0.6 kg/m^2) underwent a short-term overfeeding intervention, as previously described (20-22). Participants were studied at three time points: baseline, day 3 and day 28 of overfeeding. The study was registered at ClinicalTrials.gov (NCT00562393).

Diets

Baseline diet was not supervised and individuals were self-selecting their foods. Prior to the baseline study individuals recruited to the overfeeding intervention received a 3 day standardized diet, calculated based on estimated energy requirements with a target nutritional composition of 30% fat, 15% protein and 55% carbohydrate. Participants were then overfed for 28 days with a target of 1250 kcal/day above baseline energy requirements, based on 45% fat, 15% protein and 40% carbohydrate, as previously described (21). Briefly, during overfeeding participants were asked to supplement their daily diet with three energy dense snacks (~240 kcal; e.g. mixed nuts, cheesecake, potato crisps) and a liquid oil-based supplement added to a dairy dessert (Benecalorie, ~525 kcal), which were all provided (21). Participants were asked to fill in food diaries before the commencement of the study and during overfeeding. Diet diaries were based on daily weighed food records and analyzed using FoodWorks 7 (Xyris, Australia).

Estimation of acid load

Dietary acid load was estimated using PRAL (10) and NEAP (4) scores:

1. PRAL (mEq/d) = (0.49 * protein [g/d]) + (0.037 * phosphorous [mg/d]) – (0.021 * potassium [mg/d]) – (0.026 * magnesium [mg/d]) – (0.013 * calcium [mg/d])
2. NEAP (mEq/d) = (54.5 * protein [g/d] / potassium [mEq/d]) – 10.2

Hyperinsulinemic-euglycemic clamp studies

Peripheral body insulin sensitivity was assessed using a 2-hour hyperinsulinemic-euglycemic clamp with insulin infusion rate of either 60 or 80 mU/m^2/min. Variable GIR was applied to achieve a blood glucose target of 5.0 mmol/L. The same procedure was repeated post 28 days of overfeeding (20, 21). Insulin sensitivity was expressed as GIR necessary to maintain euglycemia normalized to average serum insulin in the last 30 minutes of the clamp divided

by fat-free mass (FFM). Indirect calorimetry (Parvomedics, UT, USA) was performed at baseline following 30 minutes of supine rest and during clamp steady state.

Body composition and abdominal fat distribution

Dual-energy X-ray absorptiometry (DXA) was performed to evaluate body fat and FFM (Lunar DPX-Lunar Radiation, Madison, WI, USA). Abdominal visceral (VFAT) and subcutaneous (SFAT) adipose tissue distribution and liver fat were evaluated by computed tomography (CT, Gemini GXL; Phillips, the Netherlands) at baseline and 28 days of overfeeding, as previously described (20, 21).

Measurement of metabolites in blood

Blood glucose and plasma lactate were measured by YSI 2300 (YSI Life Sciences, Yellow Springs, Ohio, USA) and serum insulin by radioimmunoassay (Linco Research, St Charles). HOMA-IR was calculated as fasting plasma insulin (mU/L) * fasting blood glucose (mmol/L) / 22.5.

Statistical analysis

Data are presented as mean ± SEM, unless stated otherwise. Insulin and lactate data were logarithmically transformed. Baseline differences were assessed by one-way ANOVA and Tukey posthoc analyses. Repeated measure ANOVA was used to assess the effect of overfeeding on metabolic outcomes, the change in dietary intake and dietary acid load scores. To assess the relationships between variables, Pearson and Spearman correlation coefficients were calculated for normally- and abnormally- distributed variables, respectively. P-value of less than 0.05 was considered significant. Statistical analyses were carried out using SPSS 22 Statistical pack (Chicago, IL, USA).

RESULTS

Baseline anthropometric and metabolic characteristics of the cohort

Baseline characteristics of the cohort (n=104) are reported in **Table 1**. The lean group was younger than the obese groups. Ob_{res} and Ob_{sen} individuals were matched for age, weight, BMI, total body fat and central abdominal fat, as evaluated by DXA. Fasting blood glucose was lower in lean compared to both Ob_{res} and Ob_{sen}. Fasting serum insulin was elevated in Ob_{res} compared with both Ob_{sen} and lean. Fasting insulin concentration in Ob_{sen} was intermediate between that measured in Ob_{res} and lean individuals. By design, insulin resistance, measured by GIR necessary to maintain euglycemia during the hyperinsulinemic-euglycemic clamp was lower and almost half in Ob_{res} individuals compared to both Ob_{sen} and lean individuals (lean 0.6±0.0 *vs.* Ob_{sen} 0.6±0.0 *vs.* Ob_{res} 0.3±0.0 $\mu mol \cdot min^{-1} \cdot FFM^{-1} \cdot [Insulin]^{-1}$, respectively). HOMA-IR, a surrogate of insulin resistance was greater in Ob_{res} individuals compared to both Ob_{sen} and lean individuals (**Figure 1A**). Whilst plasma lactate concentration, a marker of metabolic acidosis, was within the normal clinical range of less than 2 mmol/L, plasma lactate was higher in Ob_{res} individuals compared to both the lean and Ob_{sen} groups. No difference was observed between the Ob_{sen} and lean groups (P=1, **Figure 1B**).

Dietary intake and acid load scores at baseline

Energy intake and macro- and micro- nutrient intake were not different between groups at baseline (**Table 2**). Accordingly, no differences were observed in scores of dietary acid load PRAL and NEAP.

Insulin sensitivity in association with dietary acid load and lactate at baseline

Fasting plasma lactate was positively associated with HOMA-IR (**Figure 1C**) and inversely associated with insulin sensitivity by GIR (**Figure 1D**). Dietary acid load scores PRAL and NEAP were not associated with insulin sensitivity (either HOMA-IR or GIR; all $P \geq 0.1$) or plasma lactate (both $P \geq 0.7$).

The effect of overfeeding on anthropometric and metabolic variables

The effects of overfeeding on anthropometric and metabolic variables were reported previously (20). Body weight increased by 0.6±0.1 and 2.7±0.3 kg at day 3 and 28, respectively ($P<0.001$) and increases were similarly observed in total body fat, VFAT, SFAT and liver fat (all $P<0.001$). Fasting blood glucose (4.5±0.1, 4.7±0.1 and 4.6±0.1 mmol/L at baseline, day 3 and day 28 of overfeeding, respectively, $P<0.001$) and serum insulin (65.5±3.3, 80.3±4.5 and 77.2±3.6 pmol/L at baseline, day 3 and day 28 of overfeeding, respectively, $P<0.001$) increased with overfeeding and GIR decreased from 54.8±2.8 to 50.3±2.5 µmol/kg FFM/min with overfeeding ($P=0.03$). Plasma lactate concentration increased with overfeeding ($P=0.02$, **Figure 2A**).

Dietary intake and acid load scores during overfeeding

Average energy intake increased by 1,120±105 kcal/d during the overfeeding period, with total fat intake increasing from 78±6 to 157±7 g/d (35±1 to 45±1% of total energy intake [TEI]), protein from 92±7 to 121±6 g/d (19±1 to 16±1% TEI) and carbohydrate from 207±11 to 283±18 g/d (45±1 to 38±1% TEI, **Table 3**). Dietary acid load estimated by PRAL and NEAP increased from baseline to overfeeding (**Figure 2B and C**, respectively). While some negative PRAL scores were observed at baseline, all PRAL scores were above zero during overfeeding (Figure 2B).

Insulin sensitivity in association with dietary acid load and plasma lactate in response to overfeeding

An inverse association was observed between the change in protein intake and the change in GIR from baseline to overfeeding (r=-0.38, P=0.03). At day 28 of overfeeding NEAP was positively associated with fasting insulin (r=0.54, P=0.002) and HOMA-IR (r=0.48, P=0.006). Comparatively, at the same time point an inverse relationship was observed between dietary potassium intake and fasting insulin (r=-0.41, P=0.02). Whilst dietary intake of potassium, magnesium and calcium increased with overfeeding (all $P \leq 0.001$, Table 3), there was an inverse association between the change in these mineral cations and the change in plasma lactate with overfeeding (**Figure 3A and B**). Moreover, the change in insulin sensitivity with overfeeding was inversely associated with the change in plasma lactate (**Figure 3C**).

DISCUSSION

Long-term consumption of a diet with high acid load can cause blood pH to decrease towards the lower end of the normal physiological range. This diet-induced disruption of the body's acid/base balance (8) has been shown to be associated with increased risk of type 2 diabetes (10). Here, we show that fasting plasma lactate, a marker of metabolic acidosis, was higher in obese insulin-resistant individuals compared to equally obese insulin-sensitive individuals. The obese insulin-sensitive individuals had a similar lactate concentration to that measured in lean individuals, suggesting that elevations in lactate are associated with insulin resistance and are independent of obesity *per se*. We also establish that short term overfeeding and increases in dietary acid load were associated with the development of mild metabolic acidosis, which may have contributed to the increase in peripheral insulin resistance.

A widespread consensus exists that dietary intake can significantly influence the body's acid/base balance (4, 8, 12). Long term consumption of a diet with a high ratio of acid to base precursors has been shown to induce mild metabolic acidosis measured by an increase in RNAE (4). The present experimental overfeeding intervention, achieved by supplementation of the diet with energy dense snacks, was characterized by an increase in dietary acid load. In a prospective study of 66,500 women of varying BMI, high PRAL and NEAP scores were associated with increased risk of type 2 diabetes (10). Interestingly, this association was even stronger in normal weight women than those who were overweight/obese (10), suggesting that the acid load of the diet is detrimental even in the absence of obesity. The relationship between dietary acid load and diabetes incidence was not supported in another cohort of 911 elderly overweight men (23). However, in this relatively small cohort only 115 new events of diabetes were reported, which limits the power of this interpretation.

While PRAL and NEAP are markers derived from subjective diet diary reporting, circulating lactate, a small component of the anion gap, is a marker of body acid/base balance (6). Elevations in plasma lactate within the normal range are indicative of mild metabolic acidosis and predictive of type 2 diabetes incidence in the Atherosclerosis Risk in Communities (ARIC) study (19). In the present study, fasting plasma lactate was higher in obese insulin-resistant individuals compared to obese insulin-sensitive individuals matched for BMI, total body fat and central abdominal fat, suggesting that lactate is higher in insulin-resistant individuals regardless of adiposity. Consistent with these findings, a previous study reported that lactate concentration was more strongly associated with insulin resistance (by frequently sampled intravenous glucose tolerance test) than BMI in a cohort of healthy lean and obese adults (17). Similarly, in the ARIC study, adjustment for BMI and waist circumference did not attenuate the graded rise in type 2 diabetes incidence over increasing plasma lactate quartiles (19).

Whilst increased plasma lactate was associated with decreased insulin sensitivity, the mechanisms underlying this association are not clear. In animal models, lowering pH in cultured rat myoblasts disrupted insulin binding to its receptor (24). Moreover, systemic infusion of lactate decreased insulin-stimulated glucose uptake in rat muscle during hyperinsulinemic clamp (25, 26). This was accompanied by a decrease in Akt phosphorylation (26), a key downstream regulator of the insulin signaling cascade. Further studies are necessary to confirm these mechanisms in humans.

Plant-based foods such as fruit and vegetables are a key source of potassium and magnesium, major contributors to the dietary alkali load (16). In patients with chronic renal disease, supplementation of the diet with fruit and vegetables has been reported to have a comparable

effect to oral sodium bicarbonate in correcting metabolic acidosis over a one year trial (27). This is supported in the present study, where elevations in plasma lactate during overfeeding were inversely associated with dietary intake of potassium and magnesium. This suggests that dietary intake of these cations may promote a more favorable body acid/base balance. Moreover, it has previously been demonstrated that a high fiber vegetarian diet rich in fruit and vegetables was associated with the maintenance of insulin sensitivity over time in non-obese individuals (28). At day 28 of overfeeding in the present study, higher intake of dietary potassium was associated with lower fasting insulin, a marker of insulin sensitivity. However, the increased intake of potassium and magnesium with overfeeding was not due to greater intake of fresh fruit and vegetables, as evidenced by the unchanged intake of dietary fiber. Rather, the observed increase in these dietary cations was due to the consumption of potassium-rich snacks such as potato crisps and orange juice as well as mixed nuts, which are a rich source of magnesium. The overall increase in acid load scores and plasma lactate during the short-term overfeeding intervention therefore suggests that the intake of these alkalizing nutrients was not sufficient to compensate for the consumption of acidifying nutrients.

The main strengths of this study are twofold. The stratification of the overweight/obese individuals to insulin-resistant and insulin-sensitive groups in the cross-sectional analysis enabled the dissection of the contribution of insulin resistance from that of obesity *per se*. The overfeeding study was designed to trace the events leading to a mild decrease in insulin sensitivity and enabled the evaluation of a controlled short-term obesogenic environment on metabolic acidosis. However, acid/base balance was not measured directly by blood/urinary pH, RNAE or serum bicarbonate. The subjective nature of the diet diaries is additionally a known limitation of nutritional based clinical studies. Furthermore, testing the net effect of

switching from high- to low- acid load diet should be performed in an isocaloric environment to eliminate the confounding effect of the increase in energy intake, and more importantly the increases in abdominal visceral and liver adiposity.

CONCLUSIONS

Mild metabolic acidosis, as indicated by circulating lactate, (i) aligns with insulin resistance independent of obesity and (ii) is induced by short-term increases in energy and dietary acid load in healthy humans. Further interventional and longitudinal studies are required to determine whether the buffering of mild metabolic acidosis improves insulin resistance and reduces diabetes risk.

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Authors' contributions to manuscript

DS-B, JRG and LKH: designed and conducted research; DLC: conducted research; RSW: analyzed data, performed statistical analysis and wrote the paper; RSW and DS-B: had primary responsibility for the final content; and all authors: read and approved the final manuscript. None of the authors had a conflict of interest.

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Table 1: Baseline anthropometric and metabolic characteristics in lean, insulin-sensitive and insulin-resistant overweight/obese groups

	Lean	**Overweight/Obese**	
	(n=20, 9M)	***Insulin-Sensitive*** ***(Ob_{sen})*** ***(n=27, 13M)***	***Insulin-Resistant*** ***(Ob_{res})*** ***(n=57, 27M)***
Age (years)	34 ± 3	50 ± 2[a]	46 ± 2[b]
Weight (kg)	67 ± 1	100 ± 3[a]	100 ± 3[b]
BMI (kg/m^2)	22.6 ± 0.3	34.7 ± 1.1[a]	34.4 ± 0.7[b]
Total body fat (kg)	19 ± 1	44 ± 3[a]	44 ± 2[b]
Total body fat (%)	28 ± 2	44 ± 2[a]	45 ± 1[b]
Central abdominal fat (kg)	1.3 ± 0.1	3.0 ± 0.1[a]	3.3 ± 0.1[b]
Fasting blood glucose (mmol/L)	4.4 ± 0.1	4.8 ± 0.1[a]	4.8 ± 0.1[b]
Fasting serum insulin (pmol/L)	57 ± 4	80 ± 6[a]	140 ± 11[b,c]

Data are expressed as mean ± SEM.

Statistical significance by one-way ANOVA, with Tukey post-hoc

[a]$P < 0.05$ Ob_{sen} *versus* lean

[b]$P < 0.05$ Ob_{res} *versus* lean

[c]$P < 0.05$ Ob_{res} *versus* Ob_{sen}

Table 2: Baseline daily dietary intake and acid load scores in lean, insulin-sensitive and insulin-resistant overweight/obese groups

Intake per day	**Lean**	**Overweight/Obese**	
		Insulin-Sensitive ***(Ob_{sen})***	***Insulin-Resistant*** ***(Ob_{res})***
Energy (kcal)	2030 ± 140	1885 ± 105	2040 ± 115
Protein (g)	99 ± 8	103 ± 8	99 ± 6
Total Fat (g)	82 ± 8	74 ± 6	83 ± 6
Saturated Fat (g)	30 ± 3	27 ± 2	34 ± 2
Monounsaturated Fat (g)	30 ± 3	28 ± 2	32 ± 3
Polyunsaturated Fat (g)	14 ± 2	14 ± 2	12 ± 1
Carbohydrate (g)	216 ± 14	190 ± 11	215 ± 14
Dietary fiber (g)	24 ± 2	22 ± 1	21 ± 1
Sodium (mg)	2891 ± 288	2601 ± 199	2483 ± 165
Potassium (mg)	2951 ± 157	2980 ± 163	2996 ± 136
Magnesium (mg)	319 ± 25	360 ± 28	346 ± 16
Calcium (mg)	768 ± 100	789 ± 64	886 ± 54
Phosphorous (mg)	1595 ± 125	1590 ± 113	1598 ± 80
PRAL (mEq) (range)	27.5 ± 4.6 (4.5–62.1)	27.2 ± 6.1 (-21.1–115.1)	24.3 ± 2.8 (-22.9–74.0)
NEAP (mEq) (range)	60.5 ± 3.9 (39.0–95.7)	64.9 ± 5.0 (27.2–119.4)	59.7 ± 2.2 (27.9–106.5)

Data are expressed as mean ± SEM and based on 2 or 3 day diet diaries completed prior to the commencement of the study. Diet diaries were completed by 16 lean (7 males), 26 (12 males)

overweight/obese insulin-sensitive and 53 (25 males) overweight/obese insulin-resistant individuals.

Abbreviations: NEAP, net endogenous acid production; PRAL, potential renal acid load.

Table 3: Daily dietary intake in non-obese individuals at baseline and overfeeding

Intake per day	**Baseline**	**Overfeeding**	**P-Value**
Energy (kcal)	1930 ± 110	3050 ± 142	<0.001
Total fat (g)	78 ± 6	157 ± 7	<0.001
Total fat % of energy	35 ± 1	45 ± 1	<0.001
Saturated fat (g)	30 ± 3	54 ± 3	<0.001
Saturated fat % of energy	42 ± 1	36 ± 1	<0.001
Monounsaturated fat (g)	29 ± 2	76 ± 3	<0.001
Monounsaturated fat % of energy	41 ± 1	51 ± 1	<0.001
Polyunsaturated fat (g)	12 ± 1	18 ± 1	<0.001
Polyunsaturated fat % of energy	18 ± 1	12 ± 1	<0.001
Protein (g)	92 ± 7	121 ± 6	<0.001
Protein % of energy	19 ± 1	16 ± 1	<0.001
Carbohydrate (g)	207 ± 11	283 ± 18	<0.001
Carbohydrate % of energy	45 ± 1	38 ± 1	<0.001
Dietary fiber (g)	22 ± 1	25 ± 1	0.11
Sodium (mg)	2521 ± 192	3182 ± 203	0.007
Potassium (mg)	2829 ± 143	3445 ± 157	<0.001
Magnesium (mg)	302 ± 17	403 ± 20	<0.001
Calcium (mg)	752 ± 66	1174 ± 68	<0.001
Phosphorous (mg)	1498 ± 93	1965 ± 99	<0.001

Data are expressed as mean ± SEM and based on 3 day diet diaries (n=31).

Difference from baseline by paired t-test.

Figure Legends

Figure 1: Difference in insulin sensitivity and plasma lactate between lean and overweight/obese insulin-sensitive (Ob_{sen}) and insulin-resistant (Ob_{res}) individuals and the association between lactate and measures of insulin sensitivity at baseline.

Baseline differences in HOMA-IR (A) and plasma lactate concentration (B) between groups, and associations between lactate and insulin sensitivity by HOMA-IR (C) and GIR (D). Differences by one-way ANOVA with Tukey post-hoc are noted, $^{**}P \leq 0.01$, $^{*}P < 0.05$. Scatter dot plot with mean ± SEM (HOMA-IR) or median with interquartile range (lactate) are given. Also depicted are the line of fit and the 95% confidence curves that were obtained from linear regression (C and D). GIR, glucose infusion rate.

Figure 2: Plasma lactate and dietary acid load scores at baseline and overfeeding in non-obese healthy individuals.

The effect of overfeeding on plasma lactate (A) and the change in the dietary acid load scores PRAL (B) and NEAP (C) with overfeeding. Repeated measures ANOVA was performed and differences from baseline are noted $^{*}P \leq 0.05$, $^{**}P \leq 0.01$. Scatter dot plot with either interquartile range (lactate) or mean ± SEM (dietary acid load scores) are given. Dietary-based data were available in 31 individuals that returned diet diaries at both baseline and overfeeding periods. BL, baseline; OF, overfeeding; NEAP, net endogenous acid production; PRAL, potential renal acid load.

Figure 3: Associations between plasma lactate, intake of dietary alkalinizing mineral cations and insulin sensitivity.

The association between change in plasma lactate and dietary intake of potassium (A) and magnesium (B) and between change in plasma lactate and GIR (C) with overfeeding. Dietary

data were available in 31 individuals that returned diet diaries at both baseline and overfeeding. Depicted are the line of fit and the 95% confidence curves that were obtained from linear regression. GIR, glucose infusion rate