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ORIGINAL ARTICLE

The role of acetic acid on glucose uptake and blood flow rates in the skeletal muscle in humans with impaired glucose tolerance

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BACKGROUND/OBJECTIVES: Previous studies support the glucose-lowering effect of vinegar. However, the effect of vinegar on muscle glucose metabolism and endothelial function has not been studied in humans. This open, randomized, crossover, placebo-controlled study aims to investigate the effects of vinegar on muscle glucose metabolism, endothelial function and circulating lipid levels in subjects with impaired glucose tolerance (IGT) using the arteriovenous difference technique.

SUBJECTS/METHODS: Eight subjects with IGT (4 males, age 46 ± 10 years, body mass index 30 ± 5) were randomised to consume 0.50 mmol vinegar (6% acetic acid) or placebo before a mixed meal. Plasma samples were taken for 300 min from the radial artery and the forearm vein for measurements of glucose, insulin, triglycerides, non-esterified fatty acids (NEFAs) and glycerol. Muscle blood flow was measured with strain gauge plethysmography. Glucose flux was calculated as the arteriovenous difference of glucose multiplied by the blood flow rates.

RESULTS: Vinegar compared with placebo: (1) decreased arterial plasma insulin ($P_{overall} < 0.001$; $P_{75\,min} = 0.014$, $\beta = -42$), (2) increased forearm blood flow ($P_{overall} < 0.001$; $P_{240\,min} = 0.011$, $\beta = 1.53$; $P_{300\,min} = 0.023$, $\beta = 1.37$), (3) increased muscle glucose uptake ($P_{overall} < 0.001$; $P_{60\,min} = 0.029$, $\beta = 2.78$) and (4) decreased arterial plasma triglycerides ($P_{overall} = 0.005$; $P_{240\,min} < 0.001$, $\beta = -344$; $P_{300\,min} < 0.001$, $\beta = -373$), without changing NEFA and glycerol.

CONCLUSIONS: In individuals with IGT, vinegar ingestion before a mixed meal results in an enhancement of muscle blood flow, an improvement of glucose uptake by the forearm muscle and a reduction of postprandial hyperinsulinaemia and hypertriglyceridaemia. From this point of view, vinegar may be considered beneficial for improving insulin resistance and metabolic abnormalities in the atherogenic prediabetic state.

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INTRODUCTION

Type 2 diabetes is associated with a marked increase in cardiovascular disease.[1] Increased risk factors for coronary heart disease before the onset of type 2 diabetes have been also shown in several populations.[2,3] The potential to prevent type 2 diabetes in high-risk individuals by lifestyle intervention has been established in several clinical trials.[4,5] In this point of view, there is increasing interest in identifying diet patterns that could favourably affect insulin resistance and metabolic abnormalities in the prediabetic state.

Vinegar has been used extensively since the era of Hippocrates as an antifungal and antibacterial agent.[6] However, over the last decades there has been an increasing interest on the metabolic effects of vinegar. Recent studies provide evidence that vinegar/acetic acid can evoke beneficial effects on glucose metabolism, in healthy subjects[7–11] and in patients with insulin resistance or diabetes mellitus.[12–17]

The main constituent of vinegar is acetic acid, an organic acid that gives vinegar its characteristic smell and sour taste. Additional components in vinegar are other organic acids (formic, lactic, malic, citric, succinic and tartaric), amino acids and peptides, mineral salts, vitamins and polyphenolic compounds.[18] The mechanisms by which vinegar improves glucose metabolism are still obscure. Previous studies in healthy subjects[19] and patients with type 1 diabetes[20] have shown that vinegar delays gastric emptying. Moreover, acetic acid has been shown to inhibit disaccharidase activity in the small intestine, resulting in blocking the complete digestion of starch molecules.[21] Vinegar at bedtime has been shown to reduce fasting morning glycaemia;[14] results consistent with an effect of acetic acid on the glycolysis/gluconeogenesis cycle in the liver. Glucose regulation by insulin depends on the suppression of endogenous glucose production and stimulation of peripheral glucose disposal.[22] Skeletal muscle is considered as an important tissue for glucose disposal in response to insulin, especially in the postprandial state; during this period, the effect of insulin on blood flow is an important component of its stimulation of glucose uptake.[23–26] In rats, acetic acid feeding enhanced glycogen repletion in liver and skeletal muscle apparently by reducing xyulose-5-phosphate accumulation in the liver, as well as phosphofructokinase-1 activity in skeletal muscle.[27–29] These metabolic changes are consistent with reduced glycolysis and promotion of glycogen synthesis. Moreover, the chronic intake of vinegar has been reported to decrease triglyceride levels and reduce total cholesterol and low-density

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lipoprotein cholesterol in several animal[30–34] and few human[35,36] studies. In addition, vinegar has shown to protect from lipid accumulation in liver and skeletal muscle.[37] As the accumulation of excess lipid in the peripheral tissues disturbs insulin signalling, the effect of acetate on the reduction of lipid contends in the skeletal muscle and liver may lead to an improvement of glucose tolerance and insulin resistance.

However, the acute effects of vinegar on endothelial function and glucose metabolism in muscle have not been studied in humans. The aim of this study was to investigate the effect of vinegar on (1) plasma glucose, insulin and lipid levels and (2) blood flow rates and glucose uptake by the forearm muscles in patients with impaired glucose tolerance (IGT), using the arteriovenous difference technique across the forearm muscle, after the consumption of a mixed meal in order to create a metabolic environment, permitting the interaction of insulin and substrates to be investigated under conditions as close to physiological as possible.[38]

SUBJECTS AND METHODS

Study design

This was an open, randomized, crossover, placebo-controlled study, where repeated measurements were recorded on two different days separated by 1 week (±2 days); in each day the participants were given vinegar or placebo before a mixed meal. The whole study's duration was 52 weeks.

Subjects

Eight subjects with IGT (4 males, age 46 ± 10 years, body mass index 30 ± 5) were included in the study. All subjects were subjected to an oral 75-g glucose tolerance test (owing to the positive family history of diabetes) within the last month prior to the study, and had 2-h glucose values between 7.8 (140 mg/dl) and 11.0 mmol/l (199 mg/dl) and fasting glucose values below 7 mmol/l (126 mg/dl).[39] None of the subjects had any systemic disease. All subjects were not taking any medication therapy before or during the study. All were recreationally active, but none were elite-trained. Their diet and exercise programme was stable during the last 2 months. The subjects were instructed not to consume vinegar or acetic acid-containing products for 2 weeks prior to the study. The study was approved by the hospital ethics committee, and subjects gave written informed consent.

Experimental protocol

All subjects were admitted to 'Attikon' University Hospital (Haidari, Greece) at 0700 hours after an overnight fast and had the radial artery in one hand (A) and a deep forearm vein (V) in the contralateral arm catheterized.[23,26] Half an hour after catheterisation, the subjects were randomly assigned to consume vinegar (30 ml wine vinegar (0.50 mmol) containing 6% acetic acid and 20 ml water) or placebo (50 ml water). After 5 min, the subjects consumed a meal composed of bread, cheese, turkey ham, orange juice, butter and a cereal bar (557 kcal, 75 g carbohydrates, 26 g protein and 17 g fat).

Blood samples were collected from both sides preprandially and at 15–60-min intervals for 300 min post the meal for measurements of glucose (Yellow Springs Instruments, Yellow Springs, OH, USA) and from the radial artery for measurements of insulin (RIA, Linco Research, St Charles, MO, USA), glycerol, triglycerides and non-esterified fatty acids (NEFAs) (Roche Diagnostics, Mannheim, Germany). A full blood count was performed preprandially for measurement of haematocrit. Blood flow was measured immediately before collection of each blood sample in the forearm using mercury strain gauge plethysmography (Hokanson, Bellevue, WA, USA) in the same arm as the catheterized deep forearm vein.[23]

Calculations

The values obtained from the two preprandial samples were averaged to give a '0 time value'. Because blood flow was used in the calculation of fluxes, the plasma levels (P) of glucose were converted to whole blood (B) using fractional haematocrit (Ht): B = P (1–0.3 Ht).[23,26,40,41]

Incremental areas under the curve (iAUC) were calculated by the trapezoid rule from the start of the meal to 300 min after subtracting baseline values from postprandial values ($iAUC_{0-300}$). Glucose uptake by muscle was calculated as the arteriovenous difference of glucose multiplied by the blood flow rates.

Statistical analysis

Results are presented as mean ± s.d. Normality tests were applied to each dependent variable. Comparisons between groups were performed with a linear mixed model (with time-treatment interaction and baseline measurements as fixed effects and subject-specific random effects) to incorporate within-subjects varying in time correlations. The estimates, significant values and time of occurrence were calculated for each of the dependent variables that were found significant. Supplementary tests included one-way analysis of variance for each iAUC of the dependent variables. Note that $P_{overall}$ denotes the overall (between groups) and P_{min} the statistically significant (within time) *P*-values from the time–treatment interaction model (SPSS Inc., Chicago, IL, USA).

RESULTS

Vinegar consumption was well tolerated. Neither nausea nor vomiting was reported.

Glucose metabolism

Plasma insulin levels raised postprandialy in the patients who had consumed placebo, whereas after the consumption of vinegar postprandial insulin spikes were decreased ($P_{overall} < 0.001$ and $P_{75\,min} = 0.014$, $\beta = -42$) (Figure 1a and Table 1). As a result, vinegar compared with placebo reduced incremental insulin levels by 33%.

As shown in Figure 1, arterial plasma glucose levels were similar between the two groups. Venous plasma glucose levels ($iAUC_{0-300\,min}$ 176 ± 171 vs 222 ± 270 mM min, in vinegar and

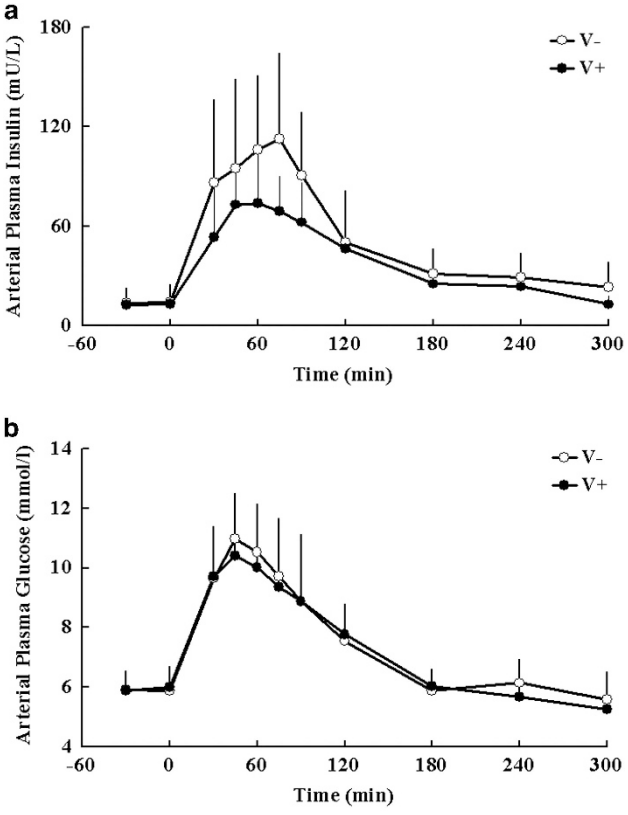

Figure 1. Arterial plasma insulin ($P_{overall} < 0.001$, $P_{75\,min} = 0.014$ with $\beta = -42$) (**a**), and glucose ($P_{overall}$ = not significant) (**b**) levels in subjects consuming vinegar (V+) or placebo (V−). Results are presented as mean ± s.d. $P_{overall}$ values represent overall comparison (linear mixed model) between the two groups across time. At $t = 0$ min, a mixed meal was given.

Table 1. Time of significance, estimates and s.e. of time–treatment coefficients (β), level of significance and 95% confidence intervals for the dependent variables

Variable	*Time–treatment interaction with occurrence of time of significance*	*Estimate*	*s.e.*	*Significance*	*95% Confidence interval, lower bound*	*95% Confidence interval, upper bound*
Arterial plasma insulin	(Group: vinegar) × (time: 75 min)	−41.978	16.891	0.014	−75.35	−8.605
Muscle blood flow	(Group: vinegar) × (time: 240 min)	1.534	0.595	0.011	0.358	2.71
Muscle blood flow	(Group: vinegar) × (time: 300 min)	1.372	0.595	0.023	0.196	2.548
Arterial plasma triglycerides	(Group: vinegar) × (time: 240 min)	−344.05	106.367	0.001	−554.201	−133.904
Arterial plasma triglycerides	(Group: vinegar) × (time: 300 min)	−372.8	106.367	0.001	−582.951	−162.654
Muscle glucose uptake	(Group: vinegar) × (time: 60 min)	2.785	1.263	0.029	0.29	5.28

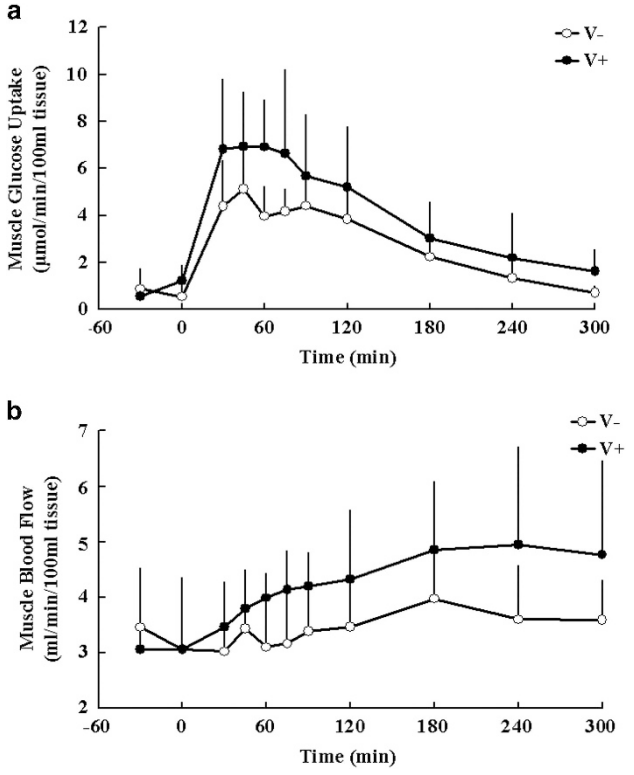

Figure 2. Forearm muscle glucose uptake ($P_{overall} < 0.001$, $P_{60\,min} = 0.029$ with $\beta = 2.78$) (**a**), and muscle blood flow ($P_{overall} < 0.001$, $P_{240\,min} = 0.011$ with $\beta = 1.53$ and $P_{300\,min} = 0.023$ with $\beta = 1.37$) (**b**), in subjects consuming vinegar (V+) or placebo (V−). Results are presented as mean ± s.d. $P_{overall}$ values represent overall comparison (linear mixed model) between the two groups across time. At $t = 0$ min, a mixed meal was given.

placebo groups, respectively) and arteriovenous differences ($iAUC_{0–300\,min}$ 310 ± 293 vs 209 ± 105 mM min, in vinegar and placebo groups, respectively) were also similar between the two groups.

However, muscle glucose uptake was increased after the meal consumption in the vinegar group compared with the placebo group by 35% ($P_{overall} < 0.001$ and $P_{60\,min} = 0.029$, $\beta = 2.78$). Postprandial glucose uptake was dominated by that occurring 0–120 min after the meal, during the time when arterial insulin levels rose rapidly (Figure 2a and Table 1).

Forearm blood flow

Forearm blood flow rates were similar in the fasting state. In the patients who had consumed placebo, blood flow rates were unaffected by increased postprandial insulin levels and remained blunted throughout the experiment. In contrast, vinegar ingestion resulted in an increase of blood flow rates compared with baseline

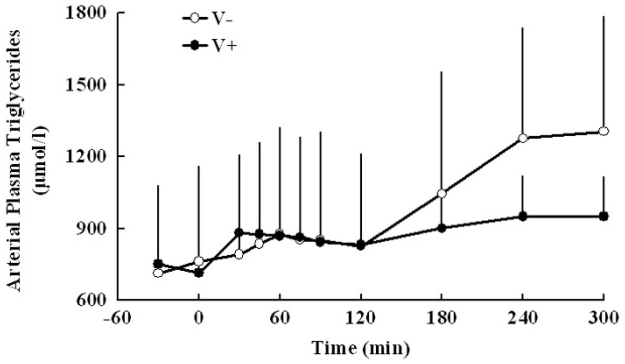

Figure 3. Arterial plasma triglycerides ($P_{overall} = 0.005$, $P_{240\,min}$ and $P_{300\,min} < 0.001$ with $\beta = -344$ and -373, respectively) in subjects consuming vinegar (V+) or placebo (V−). Results are presented as mean ± s.d. $P_{overall}$ values represent overall comparison (linear mixed model) between the two groups across time. At $t = 0$ min, a mixed meal was given.

(peak–baseline blood flow, $P = 0.011$). Consequently, vinegar compared with placebo resulted in a threefold increase of the incremental blood flow rates ($P_{overall} < 0.001$; $P_{240\,min} = 0.011$, $\beta = 1.53$; $P_{300\,min} = 0.023$, $\beta = 1.37$) (Figure 2b and Table 1).

Lipid metabolism

Fasting plasma triglyceride levels were not different between the two groups. Postprandial plasma triglycerides increased steadily in the placebo group and by 240 min reached maximal value. In the vinegar group, postprandial hypertriglycaemia was less evident, resulting in decreased total plasma triglyceride levels by 48% ($P_{overall} = 0.005$; $P_{240\,min} < 0.001$, $\beta = -344$; $P_{300\,min} < 0.001$, $\beta = -373$) (Figure 3 and Table 1).

Fasting plasma NEFA and glycerol levels were not different between the two groups. Postprandial plasma NEFA values showed a similar pattern in both groups: their levels decreased within 60–90 min, remained suppressed until 240 min and then gradually returned to baseline ($iAUC_{0–300\,min}$ −32 ± 31 vs −40 ± 25 nmol/l min, in vinegar and placebo groups, respectively). In accordance with NEFA, postprandial glycerol levels were suppressed to the same extent in both groups ($iAUC_{0–300\,min}$ −0.4 ± 2 vs −0.5 ± 1 nmol/l min, in vinegar and placebo groups, respectively).

DISCUSSION

In this study, we have investigated the effect of vinegar on circulating plasma glucose and insulin levels, as well as blood flow rates and glucose uptake by the forearm muscles, in patients with IGT. To our knowledge, this is the first report examining the effect of vinegar on glucose metabolism in the skeletal muscle in humans.

In our study, vinegar consumption reduced postprandial plasma insulin levels. This finding is in accordance with previous reports showing that vinegar supplementation reduces postprandial hyperinsulinaemia in healthy subjects,[7,9,10,12,19] as well as in subjects with insulin resistance and type 2 diabetes.[12,16] However, in our study, plasma glucose levels were not altered by vinegar ingestion. This finding is not in agreement with previous experiments showing that vinegar co-ingestion decreased postprandial hyperglycaemia in insulin-resistant subjects.[12] These discrepancies could be explained, at least in part, by differences in the kind of the test meal following acetic acid ingestion, as our test meal had lower glycaemic index (52) and contained less carbohydrates (75 g) and more dietary fibres (3.3 g) compared with the test meal used in the previous study (estimated as glycaemic index 64, 87 g carbohydrates and 2.7 g dietary fibres).[42] This assumption is based on recent findings showing that vinegar reduced postprandial glycaemia in patients with type 2 diabetes when added to a high-, but not to a low-glycaemic index meal.[16] Interestingly, our data showed that even when vinegar is added to a low-glycaemic index meal, it could have a favourable effect on insulin-stimulated glucose uptake by the skeletal muscles, suggesting an improvement in insulin sensitivity.

Skeletal muscle is considered as the most important tissue for glucose disposal in response to insulin, especially in the postprandial period.[22] In our study, vinegar ingestion enhanced glucose disposal by the forearm muscles, suggesting an improvement in insulin action. Previous experiments have showed that insulin-mediated increases in blood flow and insulin's effects on tissue glucose uptake and metabolism are tightly coupled processes and therefore are important determinants of tissue sensitivity to insulin.[23–26] Impairment of the vasodilatory response in insulin-sensitive tissues may partly account for insulin resistance in insulin resistance states.[23–25] Interestingly, a recent study showed that the increase in blood flow rates after a mixed meal seen in control subjects is impaired in all stages of type 2 diabetes, including subjects with IGT, suggesting that this defect could be an early marker of insulin resistance that precedes the development of type 2 diabetes.[24] In good correlation with these findings, our study showed that blood flow rates remained blunted throughout the 5-h test period in the group consuming placebo, despite postprandial hyperinsulinaemia. In contrast, in the group consuming vinegar, blood flow rates were increased after the meal although postprandial insulin levels were decreased compared with their respective values in the group consuming placebo. The effects of insulin on blood flow are mediated by an increase in endothelium-derived nitric oxide, which is produced by the endothelial nitric oxide synthase.[43] Nonetheless, a recent trial in healthy humans suggests that vinegar intake for 3 days can enhance fasting flow-mediated vasodilation via upregulation of endothelial nitric oxide synthase activity.[43] This effect could account for, at least in part, a dose-dependent, biphasic induction of endothelial nitric oxide synthase phosphorylation, most likely via cAMP-dependent protein kinase and the AMPK pathway.[43] These observations propose that the increased blood flow by vinegar may serve as an important step that could lead to an improvement in vascular reactivity and endothelial function, and finally to an improvement in insulin action in skeletal muscle.

Our results are in agreement with previous animal experiments supporting an effect of acetic acid on glucose metabolism in skeletal muscle.[27,28] In rats, acetic acid has been shown to enhance glycogen repletion, attributed to the accumulation of glucose 6-phosphate due to the suppression of glycolysis.[27] The same effect has been reported in horses after exercise; in this case, acetate supplementation resulted in an enhanced rate of muscle glycogen resynthesis during the first 4 h following the exercise period compared with the control treatment.[44] Moreover, earlier animal studies provide evidence that acetate treatment might increase the gene expression of myoglobin and glucose transporter (*Glut4*) genes in the skeletal muscle via AMPK activation.[37] Although the intracellular pathways of glucose metabolism were not investigated in our study, these animal studies suggest that other factors, besides increased blood flow, could also mediate the effects of vinegar on muscle glucose metabolism.

It must be noted that it seems somehow contradictory why on one hand, vinegar induced a higher muscle blood flow and glucose disposal rate with less insulinaemia, whereas on the other hand, no significant effect on plasma glucose was observed. An obvious interpretation would be that there is additional glucose entering the circulation, potentially from endogenous glucose production. However, this is quite unlikely as the previous studies have shown that vinegar ingestion at bedtime reduces fasting morning glycaemia, results consistent with a positive effect of acetic acid on the glycolysis/gluconeogenesis cycle in the liver.[14] An alternative possibility is that the small sample size was not enough to indicate a difference in the mild postprandial hyperglycaemia in subjects with IGT (a-posterior calculated statistical power ~ 0.8). In any case, although in the present study vinegar did not reduce plasma glucose levels *per se*, its positive effects on reducing hyperinsulinaemia and improving insulin action in the skeletal muscle are encouraging, but should be confirmed by larger trials.

Previous data regarding the lipid-lowering effect of vinegar are derived from animal models or from a few human trials; in most animal[30–32,34,45–48] and all human[35,36,49,50] studies, vinegar was chronically administered. To our knowledge, this is the first study investigating the acute effects of vinegar on lipid metabolism in humans. In our study, vinegar ingestion decreased postprandial triglyceride levels, suggesting a higher triglyceride turnover. These findings correlate well with previous animal experiments showing that chronic administration of acetic acid reduces serum and hepatic triglyceride levels.[30,32,47] Besides these findings in metabolically healthy animals, additional chronically administered acetate treatments in obese[31] and/or type 2 diabetic[34] rats have been shown to result in a reduction of plasma triglyceride levels. In contrast, our results are not in agreement with a previous study in rabbits examining the acute effects of vinegar intake on lipid profile. In this study, addition of 10 ml vinegar to a hypercholesterolaemic diet resulted in a decrease in total cholesterol, low-density lipoprotein cholesterol, oxidized low-density lipoprotein and apolipoprotein B levels; however, levels of triglycerides, high-density lipoprotein cholesterol and apolipoprotein A were not affected by vinegar intake.[33]

As far as humans are concerned, our results are in agreement with a double-blind, placebo-controlled trial in obese subjects during a 12-week period of either 15 or 30 ml vinegar intake showed that both vinegar doses resulted in a decrease of serum triglyceride levels, as early as week 4.[38] They are also in good correlation with the results of another study in patients with hyperlipidaemia; in this trial, the consumption of 30 ml of apple vinegar twice a day for 8 weeks was effective in reducing the serum levels of total cholesterol, triglyceride and low-density lipoprotein cholesterol, along with a nonsignificant tendency of increasing high-density lipoprotein cholesterol.[35] Our findings are in contrast with a prospective randomized, double-blind, placebo-controlled clinical study conducted in 114 non-diabetic subjects consuming 30 ml of apple vinegar for 8 weeks; in this trial, there was no evidence on vinegar impacting triglycerides. The results of this study should, however, be considered with caution as this study had several limitations; the most important one is the mixed group (one-third of the participants were on statin and/or fish oil treatment).[50]

The underlying mechanisms explaining the effects of vinegar on lipid metabolism are still obscure. Previous animal studies suggest that the hypolipidaemic effects of acetic acid on triglyceride levels could be attributed to the inhibition of the metabolic pathways of

lipogenesis in the liver, through the activation of AMPK, an inhibitor of fatty acid and sterol synthesis.[30,34] It has been shown that AMPK activation decreases sterol regulatory element-binding protein-1; the suppression of sterol regulatory element-binding protein-1 activity results in the reduction of both the mRNA level and the activity of ATP-citrate lyase, which has an important role in supplying acetyl-CoA to pathways of cholesterogenesis and fatty acid synthesis.[30,34] Moreover, the addition of vinegar in animals chronically fed with a high-cholesterol diet increases the expression of the acyl-CoA oxidase gene, suggesting that acetate might increase fatty acid oxidation, attenuating the cholesterol-mediated increase in the hepatic triglyceride concentration and finally suppressing the elevation of plasma triglycerides.[30,34] However, this mechanism could not explain the results of the present study, as vinegar ingestion had no acute effect on plasma levels of NEFAs and glycerol. As a result, although chronic administration of vinegar could have an impact on fatty acid metabolism,[34,46] our study showed that the acute administration of vinegar in insulin-resistant subjects with IGT did not affect lipolysis. A possible explanation of these findings could be that the acute intake of vinegar increases insulin sensitivity of the adipose tissue (in accordance with its insulin-sensitizing effect in skeletal muscle), increasing thus the lipoprotein lipase activity, an enzyme, which is responsible for the postprandial clearance of triglycerides, and its activity is impaired in insulin-resistant subjects,[23–25] with no effect on hormone-sensitive lipase, which is regulating lipolysis. However, a type II error cannot be excluded (a-posterior calculated statistical power ~ 0.8), and further studies with increased number of participants are warranted to shed light on the mechanisms mediating the acute effects of vinegar on adipose tissue.

Although the arteriovenous difference technique has allowed insights into the glucose fluxes across the forearm muscles, some limitations should be borne in mind when considering the results. First, the present study has not been registered as a clinical trial and as a result it may be considered as an exploratory study. Second, the number of participants was relatively small, so the findings reported do not provide strong evidence of the effects of vinegar, but are indicative of an effect. This was mainly due to the invasive nature of the technique and the need for repeating the experiment after 1 week. However, owing to the crossover design of the study, our data were sufficient for reaching statistical significance in most metabolic parameters. On the other hand, the likelihood of type II statistical errors in markers, which were not found to be significant, cannot be excluded.

In summary, our study showed that in subjects with IGT, vinegar ingestion before a mixed meal results in an enhancement of muscle blood flow rates, an improvement of glucose uptake by the forearm muscle and a reduction of postprandial hyperinsulinaemia and hypertriglyceridaemia. In this point of view, vinegar can perhaps be considered beneficial for improving insulin resistance and metabolic abnormalities in the prediabetic state.[51] Although vinegar is a safe product, widely available and affordable, further long-term clinical trials with an increased number of participants are warranted before definitive health claims can be made.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

1 Haffner SM, Lehto S, Ronnemaa T, Pyorala K, Laakso M. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. *N Engl J Med* 1998; **339**: 229–234.
2 Tominaga M, Eguchi H, Manaka H, Igarashi K, Kato T, Sekikawa A. Impaired glucose tolerance is a risk factor for cardiovascular disease, but not impaired fasting glucose. The Funagata Diabetes Study. *Diabetes Care* 1999; **22**: 920–924.
3 Mykkanen L, Kuusisto J, Pyorala K, Laakso M. Cardiovascular disease risk factors as predictors of type 2 (non-insulin-dependent) diabetes mellitus in elderly subjects. *Diabetologia* 1993; **36**: 553–559.
4 Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA *et al.* Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med* 2002; **346**: 393–403.
5 Tuomilehto J, Lindstrom J, Eriksson JG, Valle TT, Hamalainen H, Ilanne-Parikka P *et al.* Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *N Engl J Med* 2001; **344**: 1343–1350.
6 Johnston CS, Gaas CA. Vinegar: medicinal uses and antiglycemic effect. *MedGenMed* 2006; **8**: 61.
7 Johnston CS, Buller AJ. Vinegar and peanut products as complementary foods to reduce postprandial glycemia. *J Am Diet Assoc* 2005; **105**: 1939–1942.
8 Johnston CS, Steplewska I, Long CA, Harris LN, Ryals RH. Examination of the antiglycemic properties of vinegar in healthy adults. *Ann Nutr Metab* 2010; **56**: 74–79.
9 Leeman M, Ostman E, Bjorck I. Vinegar dressing and cold storage of potatoes lowers postprandial glycaemic and insulinaemic responses in healthy subjects. *Eur J Clin Nutr* 2005; **59**: 1266–1271.
10 Ostman E, Granfeldt Y, Persson L, Bjorck I. Vinegar supplementation lowers glucose and insulin responses and increases satiety after a bread meal in healthy subjects. *Eur J Clin Nutr* 2005; **59**: 983–988.
11 Sugiyama M, Tang AC, Wakaki Y, Koyama W. Glycemic index of single and mixed meal foods among common Japanese foods with white rice as a reference food. *Eur J Clin Nutr* 2003; **57**: 743–752.
12 Johnston CS, Kim CM, Buller AJ. Vinegar improves insulin sensitivity to a high-carbohydrate meal in subjects with insulin resistance or type 2 diabetes. *Diabetes Care* 2004; **27**: 281–282.
13 Mitrou P, Raptis AE, Lambadiari V, Boutati E, Petsiou E, Spanoudi F *et al.* Vinegar decreases postprandial hyperglycemia in patients with type 1 diabetes. *Diabetes Care* 2010; **33**: e27.
14 White AM, Johnston CS. Vinegar ingestion at bedtime moderates waking glucose concentrations in adults with well-controlled type 2 diabetes. *Diabetes Care.* 2007; **30**: 2814–2815.
15 Johnston CS, White AM, Kent SM. Preliminary evidence that regular vinegar ingestion favorably influences hemoglobin A1c values in individuals with type 2 diabetes mellitus. *Diabetes Res Clin Pract* 2009; **84**: e15–e17.
16 Liatis S, Grammatikou S, Poulia KA, Perrea D, Makrilakis K, Diakoumopoulou E *et al.* Vinegar reduces postprandial hyperglycaemia in patients with type II diabetes when added to a high, but not to a low, glycaemic index meal. *Eur J Clin Nutr* 2010; **64**: 727–732.
17 Petsiou EI, Mitrou PI, Raptis SA, Dimitriadis GD. Effect and mechanisms of action of vinegar on glucose metabolism, lipid profile, and body weight. *Nutr Rev* 2014; **72**: 651–661.
18 Cocchi M, Durante C, Grandi M, Lambertini P, Manzini D, Marchetti A. Simultaneous determination of sugars and organic acids in aged vinegars and chemometric data analysis. *Talanta* 2006; **69**: 1166–1175.
19 Liljeberg H, Bjorck I. Delayed gastric emptying rate may explain improved glycaemia in healthy subjects to a starchy meal with added vinegar. *Eur J Clin Nutr* 1998; **52**: 368–371.
20 Hlebowicz J, Darwiche G, Bjorgell O, Almer LO. Effect of apple cider vinegar on delayed gastric emptying in patients with type 1 diabetes mellitus: a pilot study. *BMC Gastroenterol* 2007; **7**: 46.
21 Ogawa N, Satsu H, Watanabe H, Fukaya M, Tsukamoto Y, Miyamoto Y *et al.* Acetic acid suppresses the increase in disaccharidase activity that occurs during culture of caco-2 cells. *J Nutr* 2000; **130**: 507–513.
22 Dimitriadis G, Mitrou P, Lambadiari V, Maratou E, Raptis SA. Insulin effects in muscle and adipose tissue. *Diabetes Res Clin Pract* 2011; **93**: S52–S59.
23 Dimitriadis G, Mitrou P, Lambadiari V, Boutati E, Maratou E, Panagiotakos DB *et al.* Insulin action in adipose tissue and muscle in hypothyroidism. *J Clin Endocrinol Metab* 2006; **91**: 4930–4937.
24 Lambadiari V, Mitrou P, Maratou E, Raptis A, Raptis SA, Dimitriadis G. Increases in muscle blood flow after a mixed meal are impaired at all stages of type 2 diabetes. *Clin Endocrinol (Oxf)* 2012; **76**: 825–830.
25 Mitrou P, Boutati E, Lambadiari V, Maratou E, Papakonstantinou A, Komesidou V *et al.* Rates of glucose uptake in adipose tissue and muscle in vivo after a mixed meal in women with morbid obesity. *J Clin Endocrinol Metab* 2009; **94**: 2958–2961.

26 Dimitriadis G, Mitrou P, Lambadiari V, Boutati E, Maratou E, Koukkou E *et al.* Glucose and lipid fluxes in the adipose tissue after meal ingestion in hyperthyroidism. *J Clin Endocrinol Metab* 2006; **91**: 1112–1118.
27 Fushimi T, Sato Y. Effect of acetic acid feeding on the circadian changes in glycogen and metabolites of glucose and lipid in liver and skeletal muscle of rats. *Br J Nutr* 2005; **94**: 714–719.
28 Fushimi T, Tayama K, Fukaya M, Kitakoshi K, Nakai N, Tsukamoto Y *et al.* The efficacy of acetic acid for glycogen repletion in rat skeletal muscle after exercise. *Int J Sports Med* 2002; **23**: 218–222.
29 Sakakibara S, Yamauchi T, Oshima Y, Tsukamoto Y, Kadowaki T. Acetic acid activates hepatic AMPK and reduces hyperglycemia in diabetic KK-A(y) mice. *Biochem Biophys Res Commun* 2006; **344**: 597–604.
30 Fushimi T, Suruga K, Oshima Y, Fukiharu M, Tsukamoto Y, Goda T. Dietary acetic acid reduces serum cholesterol and triacylglycerols in rats fed a cholesterol-rich diet. *Br J Nutr* 2006; **95**: 916–924.
31 Lozano J, Juárez-Flores B, Pinos-Rodríguez J, Aguirre-Rivera J, Álvarez-Fuentes G. Supplementary effects of vinegar on body weight and blood metabolites in healthy rats fed conventional diets and obese rats fed high-caloric diets. *J Med Plants Res* 2012; **6**: 4135–4141.
32 Moon Y, Choi D, Oh S, Song Y, Cha Y. Effects of persimmon-vinegar on lipid and carnitine profiles in mice. *Food Sci Biotechnol* 2010; **19**: 343–348.
33 Setorki M, Asgary S, Akram E, Rohani AH, Khazzaei M. Acute effects of vinegar intake on some biochemical risk factors of atherosclerosis in hypercholesterolemic rabbits. *Lipids Health Dis* 2010; **9**: 10.
34 Yamashita H, Fujisawa K, Ito E, Idei S, Kawaguchi N, Kimoto M *et al.* Improvement of obesity and glucose tolerance by acetate in type 2 diabetic Otsuka Long-Evans Tokushima Fatty (OLETF) rats. *Biosci Biotechnol Biochem* 2007; **71**: 1236–1243.
35 Beheshti Z, Chan Y, Nia H, Hajihosseini F, Nazari R, Shaabani M *et al.* Influence of apple cider vinegar on blood lipids. *Life Sci J* 2012; **9**: 2431–2440.
36 Kondo T, Kishi M, Fushimi T, Ugajin S, Kaga T. Vinegar intake reduces body weight, body fat mass, and serum triglyceride levels in obese Japanese subjects. *Biosci Biotechnol Biochem* 2009; **73**: 1837–1843.
37 Yamashita H, Maruta H, Jozuka M, Kimura R, Iwabuchi H, Yamato M *et al.* Effects of acetate on lipid metabolism in muscles and adipose tissues of type 2 diabetic Otsuka Long-Evans Tokushima Fatty (OLETF) rats. *Biosci Biotechnol Biochem* 2009; **73**: 570–576.
38 Coppack SW, Frayn KN, Humphreys SM, Whyte PL, Hockaday TD. Arteriovenous differences across human adipose and forearm tissues after overnight fast. *Metabolism* 1990; **39**: 384–390.
39 American Diabetes Association. Standards of medical care in diabetes--2013. *Diabetes Care* 2013; **36**: S11–S66.
40 Coppack SW, Fisher RM, Gibbons GF, Humphreys SM, McDonough MJ, Potts JL *et al.* Postprandial substrate deposition in human forearm and adipose tissues in vivo. *Clin Sci (Lond)* 1990; **79**: 339–348.
41 Frayn KN, Shadid S, Hamlani R, Humphreys SM, Clark ML, Fielding BA *et al.* Regulation of fatty acid movement in human adipose tissue in the postabsorptive-to-postprandial transition. *Am J Physiol* 1994; **266**: E308–E317.
42 Jenkins DJ, Wolever TM, Taylor RH, Barker H, Fielden H, Baldwin JM *et al.* Glycemic index of foods: a physiological basis for carbohydrate exchange. *Am J Clin Nutr* 1981; **34**: 362–366.
43 Sakakibara S, Murakami R, Takahashi M, Fushimi T, Murohara T, Kishi M *et al.* Vinegar intake enhances flow-mediated vasodilatation via upregulation of endothelial nitric oxide synthase activity. *Biosci Biotechnol Biochem* 2010; **74**: 1055–1061.
44 Waller AP, Geor RJ, Spriet LL, Heigenhauser GJ, Lindinger MI. Oral acetate supplementation after prolonged moderate intensity exercise enhances early muscle glycogen resynthesis in horses. *Exp Physiol* 2009; **94**: 888–898.
45 Budak NH, Kumbul Doguc D, Savas CM, Seydim AC, Kok Tas T, Ciris MI *et al.* Effects of apple cider vinegars produced with different techniques on blood lipids in high-cholesterol-fed rats. *J Agric Food Chem* 2011; **59**: 6638–6644.
46 Kondo T, Kishi M, Fushimi T, Kaga T. Acetic acid upregulates the expression of genes for fatty acid oxidation enzymes in liver to suppress body fat accumulation. *J Agric Food Chem* 2009; **57**: 5982–5986.
47 Moon YJ, Cha YS. Effects of persimmon-vinegar on lipid metabolism and alcohol clearance in chronic alcohol-fed rats. *J Med Food* 2008; **11**: 38–45.
48 Shishehbor F, Mansoori A, Sarkaki AR, Jalali MT, Latifi SM. Apple cider vinegar attenuates lipid profile in normal and diabetic rats. *Pak J Biol Sci* 2008; **11**: 2634–2638.
49 Kahraman N, Mesci B, Oguz A, Tamer G, Kahraman C, Sagun G *et al.* The effect of vinegar on postprandial glycemia. Does the amount matter?. *Acta Endo (Buc)* 2011; **7**: 577–558.
50 Panetta C, Jonk Y, Shapiro A. Prospective randomized clinical trial evaluating the impact of vinegar on lipids in non-diabetics. *World J Cardiovasc Dis* 2013; **3**: 191–196.
51 Haffner SM, Mykkanen L, Festa A, Burke JP, Stern MP. Insulin-resistant prediabetic subjects have more atherogenic risk factors than insulin-sensitive prediabetic subjects: implications for preventing coronary heart disease during the prediabetic state. *Circulation* 2000; **101**: 975–980.