Acute effect of red meat and dairy on glucose and insulin: a randomized crossover study\textsuperscript{1}

Kirsty M Turner, Jennifer B Keogh, and Peter M Clifton* 

School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, Australia

ABSTRACT

Background: In contrast with some epidemiologic evidence, our previous research showed that a 4-wk diet that was high in low-fat dairy reduced insulin sensitivity compared with the effect of a diet that was high in red meat.

Objective: We investigated whether a dairy meal would produce a greater insulin response than a carbohydrate-matched red meat meal would, which might account for the change in insulin sensitivity.

Design: One meal contained lean red meat, bread, and orange juice, and the other meal contained skim milk, low-fat yogurt, cheese, and bread. Meals were isoenergetic, equal in macronutrient profile, and consumed 1 wk apart. Glucose, insulin, and triglycerides were measured before and at 30, 60, 90, 120, 150, and 180 min after meal consumption. Differences between meals were tested with the use of a repeated-measures ANOVA and paired sample \(t\) tests.

Results: Nineteen men and 24 women [mean \(\pm\) SD age: 50.8 \(\pm\) 16.0 y; body mass index (in kg/m\(^2\)): 30.0 \(\pm\) 3.5] completed the study. Twenty-two participants had normal glucose tolerance, and 21 participants had impaired fasting glucose or impaired glucose tolerance. The red meat meal resulted in a higher glucose response at 30 min after consumption \((P < 0.001)\); however, the glucose total AUC was not different between meals \((P = \text{NS})\). The mean \(\pm\) SEM incremental AUC (\(\text{gAUC}\)) for glucose was significantly higher after the dairy meal than after the red meat meal \((2.23 \pm 0.49\) compared with \(0.88 \pm 0.57 \text{ mmol/L} \cdot \text{h}\), respectively; \(P = 0.004\)). The insulin total AUC and iAUC were not different between meals (iAUC: 159.65 \(\pm\) 20.0 mU/L \cdot 3 h for red meat compared with 167.49 \(\pm\) 24.1 mU/L \cdot 3 h for dairy; \(P = \text{NS}\)).

Conclusions: Lean red meat and low-fat dairy produced a similar glycemic response. The higher glucose response 30 min after consumption of the red meat meal was likely attributable to differences in the glycemic load between orange juice and milk and yogurt. An insulinotropic effect of dairy was not observed. This trial was registered at www.anzctr.org.au as ACTRN12615000164594. \textit{Am J Clin Nutr} 2016;103:71–6.

Keywords: dairy, dietary proteins, insulin sensitivity, red meat, glucose metabolism

INTRODUCTION

The escalating prevalence of overweight and obesity has resulted in a global increase in type 2 diabetes mellitus (T2DM)\textsuperscript{2} (1), which is a disease that increases risks of nephropathy, neuropathy, retinopathy, and cardiovascular disease (2). The burden that T2DM places on the health care system could be reduced with lifestyle modifications because weight loss, exercise, and an improvement in diet quality have been shown to reduce risk of T2DM (3, 4). However, it is not clear if individual dietary components affect insulin resistance and diabetes risk.

Dairy is recommended as a quality source of essential nutrients and as a way to improve bone health (5). A high dairy intake (particularly of low-fat dairy) has been associated with lower risk of T2DM in many prospective studies (6), and dairy has also been shown to reduce risk of developing insulin resistance in young overweight individuals (7). However, there has been some dispute about which products are protective; a recent meta-analysis showed that only yogurt was associated with protection (8), whereas an analysis of the Malmo Diet and Cancer cohort showed that consumers of high-fat cream, fermented milk, and cheese had reduced risk of developing T2DM (9). Intervention studies have had mixed results with regard to the effect of dairy consumption and glycemic control. Higher dairy consumption has been associated with a lower homeostasis model assessment score in some interventions, but other studies have shown no effect (10). A recent meta-analysis of 20 dairy interventions in adults showed no effect of a mean increase of 3.6 servings of dairy/d on cardiometabolic risk factors except for an increase in body weight (11). As in prospective studies, the source and type of dairy varies, and a conclusive role for dairy in reducing diabetes risk has not been established.

For individuals with high red meat consumption, prospective studies have shown increased risk of T2DM (12), although some studies have shown this association only with processed meat and not with unprocessed red meat (13, 14), thereby indicating that other factors may be involved. Processed meats are moderately higher in energy and fat with a lower percentage of protein than in unprocessed meat and can contain 4 times the amount of sodium

\textsuperscript{1}Supported by a University of South Australia Postgraduate Award (to KMT), a South Australian Cardiovascular Research Development Program Fellowship (to JBK), and a National Health and Medical Research Council Principal Research Fellowship (to PMC).

\textsuperscript{2}Abbreviations used: BCAA, branched-chain amino acid; GI, glycemic index; iAUC, incremental AUC; T2DM, type 2 diabetes mellitus.

Received September 14, 2015. Accepted for publication November 4, 2015. First published online December 16, 2015; doi: 10.3945/ajcn.115.123505.
and twice the nitrate, nitrite, and nitrosamine amounts (15). Few clinical trials have evaluated the effect of red meat consumption on glucose metabolism. Fasting glucose and insulin concentrations did not change in a comparison of two 5-wk weight-loss diets, one of which was high in lean red meat and one of which high in soy protein (16), whereas oily fish intake was shown to improve insulin sensitivity compared with the effect of red meat intake in an 8-wk crossover intervention (17). In contrast to epidemiologic evidence, our previous research showed that a 4-wk diet that was high in primarily low-fat dairy reduced insulin sensitivity compared with the effect of a diet high in lean red meat or no dairy (18).

Meal studies that have measured the acute glycemic response to the ingestion of glucose with or without the addition of various protein sources have indicated an insulinotropic effect of whey protein, and a review in this area indicated that there was a dose-dependent effect (19) with amounts >20 g whey/serving leading to substantially higher insulin AUCs and lower glucose AUCs. However, because the whey portion of the protein in cow milk amounts to ~20% (20), and the amount in cheese is negligible, 20 g whey/serving far exceeds usual intake. Whole foods appear to have a stronger health benefit than dietary supplements do, perhaps because of the complex interactions between foods in the digestive system (21, 22), and the overall dietary pattern certainly plays a role (23, 24). Our aim was to investigate whether a meal that was high in whole low-fat dairy products would produce a higher insulin response and, thus, possibly a lower glucose response than would an isonenergetic and carbohydrate-matched meal that contained lean red meat.

METHODS

Participants

Participants were recruited with the use of a public advertisement and were screened for eligibility. Inclusion criteria included overweight and obese men and women >20 of age with normal glucose tolerance or with impaired glucose tolerance or impaired fasting glucose as established by a 75-g oral-glucose-tolerance test. Exclusion criteria included diagnosed diabetes, a history of metabolic illness such as kidney or liver disease. Participants were excluded if they had a known allergy or intolerance to any of the foods provided. Participants were required to visit the University Clinic on 3 occasions as follows: a baseline visit to establish glucose tolerance followed by 2 meal visits. The University of South Australia Human Research Ethics committee approved the study, and all participants provided written informed consent before participating. The trial was registered with the Australian New Zealand Clinical Trials Registry (www.anzctr.org.au; ACTRN12615000164594). AUD $20/visit was offered to the participants at the completion of the study.

Dietary intervention

Two isoenergetic test meals, which were equal in protein, carbohydrate, and fat contents with one meal containing red meat and the other meal containing low-fat dairy products, were consumed by each participant. Table 1 illustrates the macronutrient composition of each meal. All of the food provided was purchased from local commercial markets. The beef fillet was oven roasted, sliced, and frozen into individual portions and reheated before serving. The yogurt was natural with no added sweetener, the milk was nonfat, and the cheese was a reduced-fat cheddar style cheese. Participants were served the meals in the morning after an overnight fast. The meal order was randomized and given ~1 wk apart and at the same time of the morning. At the first meal visit, participants provided a 24-h dietary recall of their previous day’s food intake, and they were asked to replicate this intake on the day before their second meal visit.

Clinical measurements

Participant height was measured with the use of a wall-mounted stadiometer (Seca) at the baseline visit. Body weight was measured with the use of electronic digital scales (Tanita Corp.) with the subject wearing light clothing and without shoes. BMI (in kg/m²) was calculated as weight divided by height. An oral-glucose-tolerance test was performed at the baseline visit to ascertain glucose tolerance. A fasting blood sample was taken before each meal and every 30 min after the meal for a total of 7 time points. Blood for serum

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Nutrient composition of the test meals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amount, g</td>
</tr>
<tr>
<td>Macronutrient, %</td>
<td>25</td>
</tr>
<tr>
<td>Red meat</td>
<td></td>
</tr>
<tr>
<td>Beef fillet, cooked</td>
<td>90</td>
</tr>
<tr>
<td>Bread, wholemeal</td>
<td>59</td>
</tr>
<tr>
<td>Butter, regular salt</td>
<td>13</td>
</tr>
<tr>
<td>Orange juice</td>
<td>250</td>
</tr>
<tr>
<td>Total</td>
<td>—</td>
</tr>
<tr>
<td>Dairy</td>
<td></td>
</tr>
<tr>
<td>Bread, wholemeal</td>
<td>62</td>
</tr>
<tr>
<td>Milk, nonfat</td>
<td>260</td>
</tr>
<tr>
<td>Yogurt, reduced fat</td>
<td>75</td>
</tr>
<tr>
<td>Cheese, reduced fat</td>
<td>36</td>
</tr>
<tr>
<td>Margarine, canola</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>—</td>
</tr>
</tbody>
</table>
was collected in tubes with no additives and allowed to clot at room temperature for 30 min. Blood for plasma was collected in tubes containing sodium fluoride EDTA and stored on ice until processed. Blood samples were separated by centrifugation at 4000 × g at 4°C for 10 min (Universal 32R; Hettich Zentrifugen). Serum and plasma aliquots were stored at −80°C until analysis. Plasma glucose and triglycerides were measured with the use of an automated spectrophotometric analyzer (Konelab 20XTi; Thermo Electron), and serum insulin was measured with the use of commercial ELISA kits (kit 0030N; Alpha Diagnostic).

Analysis

The statistical analysis was performed with SPSS V22 software (IBM). A Kolmogorov-Smirnov test, quantile-quantile plots, and histograms were used to test for the normality of distribution. The insulin incremental AUC (iAUC) was not normally distributed and was log transformed. Differences between meals were tested with the use of a repeated-measures ANOVA and paired samples t tests. The iAUC was calculated with the use of the trapezoidal equation. The primary endpoint was insulin, and on the basis of meal-tolerance tests that we performed previously (25), we had 80% power at \( P < 0.05 \) to see a difference in peak insulin of 11 mU/L and an iAUC of 27 mU/L · 3 h with an SEM for glucose of 0.5 mmol/L and SEM for insulin of 10 mU/L. The secondary endpoint was glucose, and the study had 90% power at \( P = 0.02 \) to see a change in average glucose of 0.3 mmol/L (or a peak change of 0.6 mmol/L) with 42 people completing the assessment. Data are expressed as means ± SEMs except for baseline characteristics, which are expressed as means ± SDs. Significance was set at \( P < 0.05 \).

RESULTS

Nineteen men and 24 women (age: 50.8 ± 16.0 y; BMI: 30.0 ± 3.5) completed the study. Twenty-two participants had normal glucose tolerance, and 21 subjects had impaired fasting glucose or impaired glucose tolerance. Figure 1 outlines the recruitment and withdrawal of participants. The red meat meal resulted in a higher peak glucose response at 30 min postconsumption (7.5 ± 0.2 compared with 6.9 ± 0.2 mmol/L; \( P < 0.001 \)); however, the glucose total AUC was not different between meals (\( P = \text{NS} \)). The iAUC for glucose was significantly higher after the dairy meal than after the red meat meal (2.23 ± 0.49 compared with 0.88 ± 0.57 mmol/L · 3 h, respectively; \( P = 0.004 \)). There was no effect of sex, BMI, or group (normal glucose tolerance compared with impaired glucose tolerance or impaired fasting glucose) when added as factors. The insulin total AUC and iAUC were not different between meals (iAUC 159.6 ± 20.0 mU/L · 3 h for red meat compared with 167.5 ± 24.1 mU/L · 3 h for dairy; \( P = \text{NS} \)). Postprandial triglycerides were not different between meals (Figure 2).

DISCUSSION

Lean red meat and dairy produced a similar metabolic response in this study. The insulin response and glucose total AUC were not different between meals, although the glucose iAUC was higher after consumption of the dairy meal. The amount of carbohydrate was equal between meals; however, the source of carbohydrate may have influenced the response (26). The glycemic index (GI) is a method of ranking carbohydrate-containing foods according to their effect on glucose concentrations (27). Both orange juice and milk are considered low-GI foods (28), but it is possible that the higher GI of orange juice compared with the corresponding amount of dairy (GI: 46 compared with 32, respectively) resulted in the higher peak glucose response at 30 min. The overall glycemic load amounted to 28.1 for the red meat meal and 25.8 for the dairy meal.

Glycemic and insulinemic indexes for most foods are highly correlated, but protein-rich foods can elicit insulin responses that are greater than expected on the basis of their carbohydrate contents (29). Milk and dairy products have a low GI (28) but have been shown to have a high insulin response that is very similar to that of bread (30). For instance, in this study, a 1000-kJ serving of yogurt had an insulin index of 115, whereas beef had an insulin index of 51. Meal studies have indicated that the consumption of various sources of protein with glucose can increase insulin secretion compared with the consumption of glucose alone (31). Protein from cow milk is made up of ~20% whey and ~80% casein (20), and it is the whey portion that is thought to be responsible for the insulinotropic effect (32). Holmer-Jensen et al. (33) showed that consumption of a high-fat test meal that contained 45 g whey hydrolysate raised the 30-min insulin response compared with the effect of consumption of 3 other meals with different sources of milk-derived proteins as follows: α-lactalbumin, whey isolate, and casein-glycomacropeptide. Although these 4 meals were isocaloric and equal in macronutrient profiles, they were much higher in total energy and fat contents than in the current study (~4980 compared with ~2090 kJ, respectively, and 66% energy from fat compared with 36%, respectively). No difference in postprandial triglycerides, glucose, or insulin iAUC was shown between the 4 meals. Another study that examined the postprandial inflammatory response in men after the consumption of 3 meals (i.e., a high-fat control meal, a high-fat dairy meal, and a high-fat control plus milk meal) similarly showed no difference in the glucose or insulin iAUC between meals (34), and the effect on inflammatory biomarkers was inconsistent. Compared with nondairy proteins, whey has shown a glucose-lowering effect. The iAUC for glucose was lower after a meal containing 45 g whey protein than after meals containing the same amount of cod, casein, or gluten protein both in participants with T2DM (35) and in obese participants without T2DM (36).
The elevated plasma concentration of the branched-chain amino acids (BCAAs) leucine, isoleucine, and valine after consumption of a whey meal has been proposed as a mechanism for the insulinotropic effect of whey (32). Certainly leucine and isoleucine, when administered with glucose, have been shown to stimulate insulin secretion and lower blood glucose (37, 38). Holt et al. (29) showed that beef had a larger insulin response per gram than did many carbohydrate-containing foods; however, serum insulin did not change in healthy participants when 50 g glucose was combined with various amounts of beef protein except at the highest dose of 50 g beef protein (39). A computed BCAA analysis of the 2 meals showed no significant difference, and because beef has a similar BCAA profile to that of dairy protein (40), it would be reasonable to expect that beef would have a similar insulin response to dairy as shown in this study.

The current dietary guidelines for dairy in Australia recommend 2–4 servings/d (41), but although 85% of Australians consume dairy products, few of them meet the dietary guidelines (42). The amount of dairy consumed in this study amounted to a little over 2 servings and contained ~3 g whey protein. This amount may not have been enough to elicit the high insulin responses seen in previous studies that examined whey in isolation. Certainly, a review of whey-protein intervention studies showed that amounts >20 g/serving led to increased insulin concentrations and a lowering of blood glucose (19), which were far higher than would be consumed in whole dairy.

Calcium in the dairy meal amounted to 814 mg compared with 88 mg in the red meat meal. Although total fat in the 2 meals was equal, a reduced fat absorption has been shown with higher calcium intakes because calcium combines with fatty acids in the intestine to form calcium soaps that increase fecal fat excretion (43). This process was hypothesized as the reason for lower chylomicron triglycerides in healthy overweight men after intakes of meals that contained both 350 and 793 mg dairy calcium compared with after intake of a low–dairy calcium meal (68 mg), or one supplemented with calcium carbonate (44). However, the iAUCs for total triglycerides, glucose and insulin were not significantly different between meals. Similarly, glycemic control and lipids were not affected by higher dairy and calcium intakes in a comparison of 2 high-protein weight-loss diets (25). Triglycerides were not different between meals in the current study, which may have been due to the overall lower fat load of these meals. The duration of this study was also not long enough for a return to baseline amounts, and thus, it is possible that any late effect of calcium would have been missed. The 3-h measurement period for triglycerides in this study was not a limitation although the length of time was calculated for our primary outcome and not for triglycerides. Other limitations were that a standard test meal was not provided to participants before their meal visits, and a 24-h recall was used to standardize the previous day’s consumption instead of the use of a weighed food diary. These limitation may have resulted in recall bias or underreporting.

In conclusion, glycemic and insulinemic responses were similar for red meat and low-fat dairy when consumed as part of 2 average-sized mixed meals of equal macronutrient and energy contents by overweight and obese individuals with both normal and impaired glucose tolerance. Evaluations of components of a diet and also components of individual foods such as whey, casein, or individual amino acids are important to establish possible mechanisms. However, we eat whole foods as a part of mixed meals rather than as elements in isolation, and thus, it is preferable to evaluate the effect of whole foods on glucose metabolism. The higher glucose response 30 min after consumption of the red meat meal may have been attributable to a small difference in the glycemic load between the orange juice and the milk and yogurt or the type of sugar. An insulinotropic effect of dairy was not observed and could not account for the reduction in insulin sensitivity that we observed in our chronic feeding study (18).
RED MEAT AND DAIRY EFFECTS ON GLUCOSE AND INSULIN

We thank Eva Pedersen and Katja Morsky for assistance with study participants. The authors’ responsibilities were as follows—KMT: conducted the research and had primary responsibility for the final content of the manuscript; KMT and PMC: analyzed the data; JBK and PMC: designed the research; and all authors: wrote the manuscript and read and approved the final manuscript. None of the authors reported a conflict of interest related to the study.

REFERENCES


