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1 **Inhibition of the glycaemic response by onion: a comparison between**
2 **lactose-tolerant and lactose-intolerant adults**

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16 to declare.

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18 This pilot study compared inhibition of the glycaemic response to glucose by a
19 dietary source of quercetin glucosides (onion) in lactose-tolerant adults (n =
20 12) and lactose-intolerant adults (n = 12). We hypothesised that lactose-
21 intolerant people (who do not express lactase) will retain intact quercetin
22 glucosides that can inhibit glucose uptake via the glucose transporter SGLT1
23 whereas lactose-tolerant people (who do express lactase) will hydrolyse
24 quercetin glucosides to free quercetin which does not inhibit glucose uptake.
25 In a glucose tolerance test, reduction of peak glucose levels by an onion meal
26 was higher in lactose-intolerant people than lactose-tolerant people (44.2%
27 versus 19.3%, $p = 0.04$). Incremental area under the blood glucose curve was
28 reduced more in lactose-intolerant people, but was not statistically
29 significantly (54.5% versus 42.1%, $p = 0.42$). A diet containing quercetin
30 glucosides may be of greater benefit for glycaemic control in lactose-intolerant
31 people than in lactose-tolerant people.

32

33 **INTRODUCTION**

34 A recent consensus report concluded that there is convincing evidence that a
35 low glycaemic index (GI) diet reduces the risk of type 2 diabetes and coronary
36 heart disease, probably reduces the risk of obesity and possibly reduces the
37 risk of some cancers.¹ Some dietary polyphenols can modify the apparent GI
38 of foods by reducing glucose absorption.² These include quercetin glucosides,
39 which have been shown to inhibit the active transport of glucose from the
40 luminal side of the brush border into the small intestine via the sodium-
41 dependent glucose transporter SGLT1. By contrast, free quercetin does not
42 inhibit SGLT1.^{3,4}

43 Onions are a major dietary source of quercetin glucosides, and the glucosides
44 in an onion meal can be completely hydrolyzed in the human small intestine to
45 quercetin, as demonstrated in ileostomy patients.⁵ The β -glycosidase lactase
46 phlorizin hydrolase (LPH) is responsible for hydrolysis on the luminal side of
47 enterocytes.⁶ LPH is expressed in lactose-tolerant people but not in lactose-
48 intolerant people. Hence, we hypothesised that quercetin glucosides may be
49 less effective at blocking glucose uptake in lactose-tolerant people (because
50 they can hydrolyse quercetin glucosides to the aglycone with LPH) compared
51 to lactose-intolerant people. We tested this hypothesis using an onion meal,
52 since onions are a rich source of quercetin glucosides (mainly quercetin-4'-O-
53 glucoside and quercetin-3,4'-O-diglucoside), but contain very little free
54 quercetin⁷, and onions with a higher flavonoid content have been shown to
55 inhibit glucose uptake to a greater extent than onions containing lower
56 concentrations.⁸

57 **METHODS**

58 *Participants*

59 The characteristics for the lactose-tolerant and lactose-intolerant participants
60 respectively were (SD): Age: 27.6 (4.6) years (8 F; 4 M) and 29.8 (7.4) (7 F, 5
61 M) ($p = 0.4$); BMI (kg/m^2) (SD): 22.0 (3.4) and 24.2 (4.3) ($p = 0.18$). Exclusion
62 criteria: under 18 years; pregnant; diabetic, history of blood disorders. Data on
63 contraceptive pill use was not collected. All participants gave written consent
64 and participant information was coded. The protocol was approved by
65 University of Hertfordshire Ethics Committee (protocol number:
66 LMS/PG/UH/00187).

67 *Onion meal*

68 Fresh red onions, bought locally, were peeled, homogenised in water (23%
69 w/v), boiled for 15 min, cooled to room temperature and filtered. Quercetin
70 and quercetin glucosides were analysed by reverse phase HPLC.⁷

71 *Lactose Tolerance Test*

72 Lactose intolerance was measured by a hydrogen breath test using a Gastro®
73 analyser (Rochester, England). Lactose (25g dissolved in 220 ml water) was
74 given to participants after an overnight fast and breath hydrogen was
75 measured over 2 hours. Hydrogen levels 20 ppm above baseline were
76 classified as lactose intolerance.⁹

77 *Glucose Tolerance Test*

78 Participants fasted overnight for at least 10 h before the study commenced
79 (between 09.00 and 09.30) and were allowed to eat their normal evening meal.
80 Finger prick capillary blood samples were obtained after the overnight fast
81 and at 15, 30, 60, 90 and 120 minutes after drinking a glucose solution (50 g
82 food grade glucose (Holland and Barrett, UK) dissolved in 220 ml water). The
83 same blood collection regime was then repeated on a subsequent day after
84 participants had consumed 220 ml of a filtered onion meal (23% w/v)
85 containing glucose (50 g). Participants were not randomised: all participants
86 were designated as either lactose-tolerant or lactose-intolerant and all
87 received glucose alone and glucose plus filtered onion meal ie participants
88 acted as their own controls. Glucose was measured with an EKF glucose
89 analyser (Cardiff, UK). Incremental area under the time glucose curve (IAUC)
90 was calculated using a linear trapezoidal method in Excel, taking the fasting
91 blood glucose concentration as baseline. The study was conducted at
92 University of Hertfordshire.

93 *Statistical analysis*

94 Percentage changes in peak glucose and blood glucose IAUC values were
95 calculated for glucose control versus glucose plus onion with each participant
96 acting as their own control. The mean changes for peak glucose and blood
97 glucose IAUC were then compared between lactose-tolerant and lactose-
98 intolerant groups in Excel by paired two-tailed t-tests and using two-sample
99 unequal variance. Quality of variance was tested for by an F test. Two-way
100 repeated measures ANOVA was performed to examine interactions between
101 time and treatment (SPSS version 22; IBM Corp., Armonk, New York, USA).

102 Because these interactions were significant, Fisher's least significant
103 difference post-hoc analysis was used.

104

105 **RESULTS**

106 The onion meal contained 2.0 µg/ml quercetin, 39.5 µg/ml quercetin-4'-O-
107 glucoside, 3.5 µg/ml quercetin-3-O-glucoside and 26.5 µg/ml quercetin-3,4'-
108 O-diglucoside. Consuming an onion meal reduced the glycaemic response in
109 both lactose-tolerant and lactose-intolerant people as determined by glucose
110 IAUC and peak blood glucose concentration (Fig. 1 and Table 1). There was a
111 statistically significant greater reduction in glycaemic response, as measured
112 by changes in peak blood glucose using paired t-tests, by the onion meal in
113 lactose-intolerant people compared to lactose-tolerant people (44.2% and
114 19.3% respectively, $p = 0.04$) (Table 1). The onion meal also caused a greater
115 reduction in blood glucose IAUC in lactose-intolerant people compared
116 lactose-tolerant people, but this was not statistically significant (54.5% versus
117 42.1% respectively, $p = 0.42$) (Table 1). In two-way repeated measures
118 ANOVA, interactions between time and glucose \pm onion treatment were highly
119 significant for lactose-tolerant participants ($p = 0.007$) and for lactose
120 intolerant participants ($p = 0.007$). Post-hoc comparisons showed that
121 inhibition of glucose uptake by the filtered onion meal occurred from 30
122 minutes to 120 minutes in the lactose-tolerant participants whereas in the
123 lactose-intolerant group it only lasted until 60 minutes (Fig. 1).

124

125 **DISCUSSION**

126 In this pilot study we found a significantly greater inhibition of peak glucose
127 concentrations by an onion meal in lactose-intolerant people compared to
128 lactose-tolerant people. This supports our hypothesis that LPH in lactose-
129 tolerant people is hydrolysing quercetin glucosides in the onion meal and that
130 this reduces the ability of the quercetin glucosides to inhibit glucose uptake.
131 Nevertheless, the onion meal inhibited glucose uptake in both groups, and
132 various factors may have contributed to this. Firstly, reduced glucose uptake
133 in lactose-tolerant people may be related to inhibition of GLUT2 by quercetin
134 produced from the hydrolysis of quercetin glucosides by LPH. Quercetin has
135 been shown to inhibit GLUT2, and transport of glucose from the gut to the
136 blood stream requires not only luminal glucose uptake into enterocytes via
137 SGLT1, but also release from the basal membrane of enterocytes into the
138 blood stream via GLUT2.⁴ Secondly, both lactose-tolerant and intolerant
139 people may express other glucosidases able to cleave quercetin glucosides.
140 Thirdly, onions are rich in the soluble fibre inulin, and some types of dietary
141 fibre reduce postprandial glycaemia. However, current results on the glucose-
142 lowering effects of inulin are inconsistent.¹⁰

143 Consuming dietary flavonoid glucosides is an interesting approach to reducing
144 the glycaemic response to a meal, and this aligns with a recent conclusion of
145 the International Carbohydrate Quality Consortium that overall diet, rather
146 than just the GI values of individual foods, is important when evaluating the
147 potential health risks of sugary foods consumed as part of a meal.¹ Our small
148 pilot study cannot rule out that other components in the onion meal are
149 responsible for the reduced glucose uptake in the presence of onion.

150 Nevertheless, it does raise the possibility that a diet containing quercetin
151 glucosides (and possibly other flavonoid glucosides hydrolysed by LPH) may
152 be of greater benefit for glycaemic control in lactose-intolerant people than for
153 lactose-tolerant people. Hence, lactose tolerance could be a confounding
154 factor in studies that compare glycaemic responses to diets between regions
155 of the world where lactose tolerance is low, such as the Mediterranean basin,
156 with regions where lactose tolerance is high, such as Northern Europe.

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194

195

196

197 *Legend to Figure 1*

198 Blood glucose concentrations after consumption of glucose (•) or glucose plus
199 an onion meal (■) in (a) lactose-tolerant adults and (b) lactose-intolerant
200 adults. Data are means \pm SEM (n = 12). Post-hoc Fisher's least significant
201 difference test * p < 0.05; ** p < 0.01; *** p < 0.001.

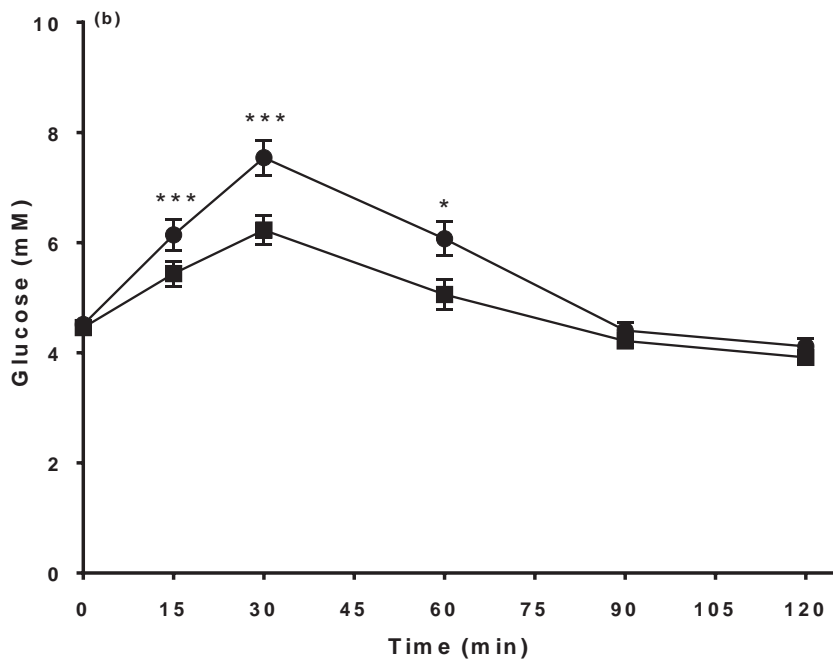
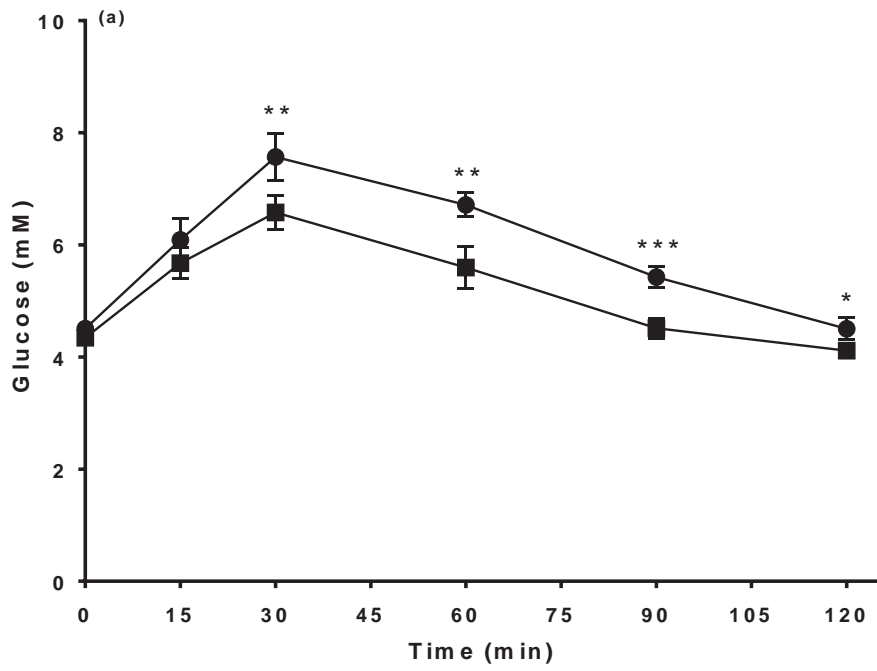


Table 1. Effects of an onion meal on peak rise in blood glucose and incremental area under the blood glucose - time curve (IAUC) in lactose-tolerant and lactose-intolerant adults.

	IAUC (mM x min)			Reduction in IAUC by onion		Δ Blood glucose (mM) ^a			Reduction in Δ glucose by onion	
	Glucose control	Glucose + onion	p	%	p ^b	Glucose control	Glucose + onion	p	%	p ^b
Lactose-tolerant	186.4 (16.74)	109.3 (16.77)	0.0037	42.06 (7.50)		3.06 (0.34)	2.24 (0.26)	0.083	19.28	
Lactose-intolerant	130.2 (14.20)	57.1 (16.01)	0.0034	54.53 (12.72)	0.425	3.03 (0.25)	1.77 (0.27)	0.0038	44.19	0.042

Values are expressed as means (SEM). ^a Peak glucose at 30 min minus fasting glucose. ^b Lactose-tolerant versus lactose-intolerant