

Article

Method of Food Preparation Influences Blood Glucose Response to a High-Carbohydrate Meal

Caroline Hodges¹, Fay Archer¹, Mardiyah Chowdhury¹, Bethany L Evans¹, Disha J Ghelani¹, Maria Mortoglou¹, Fergus M. Guppy^{1,2}



¹ School of Pharmacy and Biomolecular Sciences, University of Brighton, UK

² Centre for Stress and Age-related Disease, University of Brighton, UK

* Correspondence: F.Guppy@brighton.ac.uk

Abstract: The aim of this study was to establish the blood glucose response to different cooking methods of pasta. Participants consumed three identical meals in a random order that were freshly cooked (hot), cooled and reheated. Blood glucose concentrations were assessed before, and every 15 minutes after ingestion of each meal for 120 minutes. There was a significant interaction between temperature and time ($F_{(8,46-372.34)} = 2.75, p = 0.005$), with the reheated (90 minutes) condition returning to baseline faster than both cold (120 minutes) and hot conditions. Blood glucose AUC was significantly lower in the reheated ($703 \pm 56 \text{ mmol}\cdot\text{L}^{-1}\cdot\text{min}^{-1}$) compared with the hot condition ($735 \pm 77 \text{ mmol}\cdot\text{L}^{-1}\cdot\text{min}^{-1}, t_{(92)} = -3.36, p_{\text{bonferroni}} = 0.003$), with no significant difference with the cold condition ($722 \pm 62 \text{ mmol}\cdot\text{L}^{-1}\cdot\text{min}^{-1}$). To our knowledge, the current study is the first to show that reheating pasta causes changes in post-prandial glucose response, with a quicker return to fasting levels in both the reheated and cooled conditions compared with the hot condition. The mechanisms behind the changes in post-prandial blood glucose seen in this study are most likely related to changes in starch structure and how these changes influence glycaemic response.

Keywords: Pasta; Glycemic Index; Resistant Starch

1. Introduction

Dietary carbohydrates are a fundamental constituent of a balanced diet, contributing between 40-70% of energy intake [1], with people relying heavily on staple foods, such as pasta and rice for energy [2,3]. The digestion rates of carbohydrates are determined strongly by the proportions of sugars, starch, and fibre present within the carbohydrate. Refined and starchy carbohydrates are readily hydrolysed into their glucose components by pancreatic amylase and brush border enzymes in the small intestine, whereas dietary fibres (including resistant starch, RS) cannot be hydrolysed in the small intestine [4]. Starch that has undergone retrogradation – a process by which the glucose molecules in starch re-associate with each other in an irregular fashion post-gelatinisation [5] – is known to have a high RS content, and therefore will not be digested as effectively in the small intestine and will undergo fermentation by gut bacteria in the large intestine [6]. Therefore, foods with a high RS content produce a lower glycaemic response and are classed as having a lower Glycaemic Index (GI).

Low GI diets have numerous nutritional benefits and may be effective in management of metabolic syndromes such as obesity and type-2 diabetes mellitus (T2DM), as they produce a lower postprandial blood glucose response [7,8]. There is a body of evidence supporting the link between RS digestion and postprandial hyperglycaemia and insulinaemia, because RS-rich foods are harder to digest and therefore have a lower GI.

33 The preparation of food can also influence the GI properties of a meal, with retrogradation of
34 starch by cooling and reheating, increasing the amount of RS present [9]. However, there is a lack of
35 published evidence in humans that supports the effect of cooking on glycaemic response to a high
36 carbohydrate meal.

37 The aim of this study was to establish the blood glucose response to a pasta meal that was cooked
38 and eaten hot; cooked and eaten after cooling or cooked, cooled and then reheated. We hypothesised
39 that the cooked and eaten hot meal would produce the highest glycaemic response when compared to
40 the other methods of preparation because of its disorganised, amorphous structure and swollen starch
41 granules produced by gelatinization, with the cooked, cooled then reheated meal causing the lowest
42 glycaemic response due to its repeated retrogradation from being cooked, cooled and then reheated.

43 2. Materials and Methods

44 2.1. Participants

45 Forty-five healthy young volunteers (age = 20-24 years) took part in this study. Participants were
46 informed of the experimental protocol both verbally and in writing before giving informed consent.
47 The study protocol was approved by the School of Pharmacy and Biomolecular Sciences Research
48 Ethics Panel (approval number: PABS-REP-2017-05).

49 2.2. Experimental Conditions

50 Participants undertook each experimental condition in a random order decided by a random
51 number generator, with each experimental visit separated by a minimum of 48 hours. Participants
52 were instructed to refrain from performing any strenuous physical activity for 2 days prior to each
53 experimental visit and attended the laboratory after an overnight fast.

54 2.3. Pasta Preparation

55 Three different preparations of white fusilli pasta (Asda Stores Ltd, Leeds, UK) with a simple
56 Tomato and Basil Stir-in pasta sauce (Dolmio®, Mars Inc., Slough, UK) were tested in this study:
57 hot, cold and reheated. Each participant was given 100g (dry weight) of pasta, which was cooked in
58 water for 20 minutes, at a ratio of 566 ml of water to 100g of pasta, with 100g of pasta sauce. The hot
59 pasta meal was freshly cooked, the cold pasta meal was cooked and chilled for 24 hours overnight in
60 a refrigerator at 4°C in a sealed plastic container, while the reheated pasta meal followed the same
61 treatment as the cold condition but was then reheated on the day of the experiment for 3 minutes in a
62 750 W microwave (Proline SM18) on the high setting, with stirring every minute. Each subject was
63 provided with 250 ml of water with their meal, which they were asked to ingest within 15 mins [10].

64 2.4. Blood Glucose Responses

65 Capillary blood samples were collected by the participant by single use lancet from the fingertip
66 before, and every 15 minutes for 120 minutes after ingestion of the meal. Whole blood glucose
67 concentrations were measured using an automatic analyser (Accu-Chek Performa Blood Glucose
68 Meter, Roche Diagnostics).

69 2.5. Calculations and Data Analysis

70 Area under the glucose curve (AUC) was calculated using the conventional trapezoid rule. Blood
71 glucose response was analysed using a 2-way repeated measures ANOVA, and area under the curve
72 was analysed using a 1-way repeated measures ANOVA (Jamovi v 0.9.5.12). Pairwise comparisons
73 were conducted using a Bonferonni post-hoc correction ($p_{\text{bonferonni}}$). Data shown is mean \pm standard
74 deviation unless otherwise stated, with significance accepted if $p < 0.05$.

75 3. Results

76 3.1. Preparation Method

77 There was a significant effect of the preparation method of the pasta ($F_{(2-88)} = 4.40, p = 0.015$), with
 78 blood glucose concentration significantly lower in the reheated condition (5.78 ± 0.91 mmol/L) than
 79 in the hot condition (6.03 ± 1.02 mmol/L, $t_{(88)} = 2.94, p_{\text{bonferroni}} = 0.013$). There were no differences
 80 observed between cold pasta (5.94 ± 0.95 mmol/L) and either hot ($t_{(88)} = 1.10, p_{\text{bonferroni}} = 0.820$), or
 81 reheated ($t_{(88)} = 1.83, p_{\text{bonferroni}} = 0.210$) pasta.

82 3.2. Time

83 Pasta ingestion caused significant increases in blood glucose regardless of the preparation method
 84 ($F_{(3.06-134.81)} = 59.97, p < 0.001$), with significantly increased blood glucose at each time point compared
 85 with previous time up to 30 minutes (Table 1).

Table 1. Post-prandial glucose response

Time (mins)	0	15	30	45	60	75	90	105	120
Hot	5.07 [0.70]	5.91 ^B [0.76]	7.10 ^{B,C} [0.95]	6.68 ^B [1.00]	6.26 ^B [1.23]	5.90 ^B [0.77]	5.97 ^B [0.78]	5.82 ^B [0.78]	5.58 ^B [0.67]
Cold	5.16 [0.57]	5.68 ^A [0.94]	6.63 ^{B,C} [1.08]	6.58 ^B [1.17]	6.09 ^B [0.91]	6.04 ^B [0.91]	5.72 ^A [0.66]	5.72 ^A [0.58]	5.63 [0.65]
Reheated	5.05 [0.44]	5.82 ^B [0.71]	6.94 ^{B,C} [0.92]	6.47 ^B [0.97]	5.85 ^B [0.93]	5.57 ^A [0.74]	5.56 [0.62]	5.46 [0.63]	5.33 [0.59]

Data shown is mean [standard deviation]. All n = 45. A/B significantly different from baseline ($p < 0.05$ / $p < 0.001$). C significantly different from previous time-point ($p < 0.05$)

86 3.3. Preparation Method x Time Interaction

87 There was a significant interaction between preparation method and time ($F_{(8.46-372.34)} = 2.75, p =$
 88 0.005). The reheated condition saw a faster return to baseline compared to both cold and hot conditions,
 89 with the reheated condition seeing a return to baseline values within 90 minutes, compared with 120
 90 minutes in the cold condition, and the hot condition not returning to baseline by the end of the 2-hour
 91 period (Table 1). However, there were no differences at any time point between any condition (Figure
 92 1).

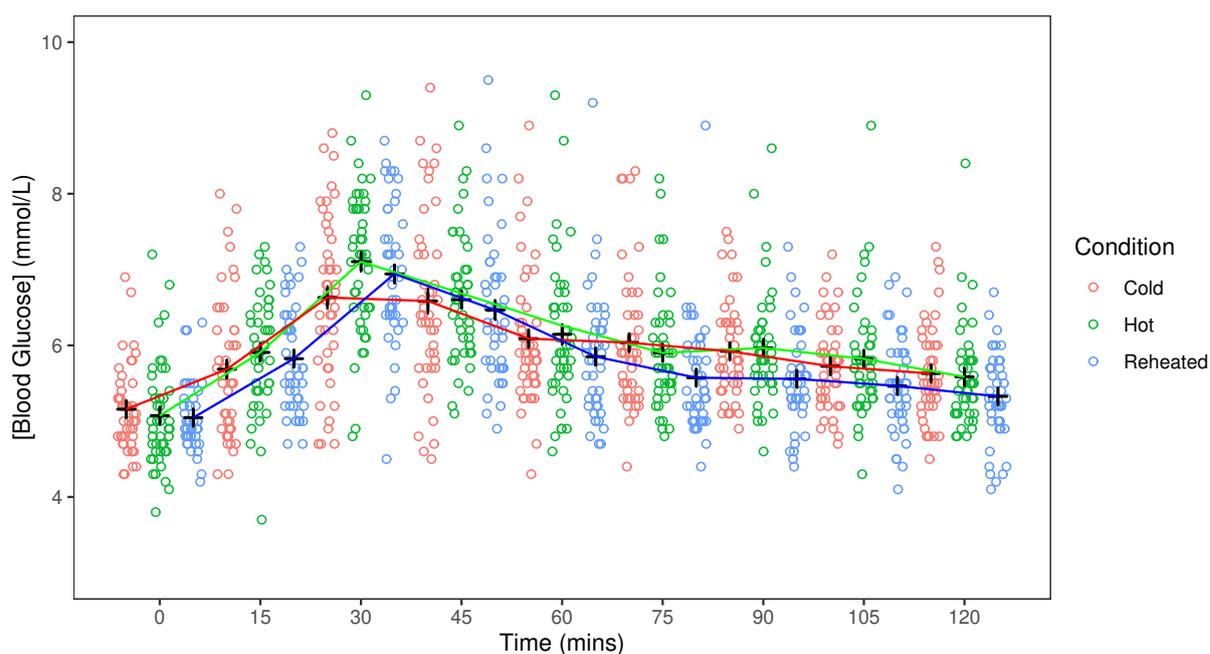


Figure 1. Post-prandial glucose response to either fresh (hot), cold, or reheated pasta. Data shown is mean \pm standard error, alongside individual responses.

93 3.4. Area Under the Curve

94 There was a significant effect of preparation method on AUC ($F_{(2-92)} = 6.19, p = 0.003$). Reheated
 95 pasta had a significantly lower area under the curve ($703 \pm 56 \text{ mmol}\cdot\text{L}^{-1}\cdot\text{min}^{-1}$) than the hot condition
 96 ($735 \pm 77 \text{ mmol}\cdot\text{L}^{-1}\cdot\text{min}^{-1}, t_{(92)} = -3.36, p_{\text{bonferroni}} = 0.003$), with no significant difference with the
 97 cold condition observed ($722 \pm 62 \text{ mmol}\cdot\text{L}^{-1}\cdot\text{min}^{-1}, t_{(92)} = -2.07, p_{\text{bonferroni}} = 0.123$). There was also no
 98 significant difference between the cold and hot condition ($t_{(92)} = -1.29, p_{\text{bonferroni}} = 0.601$, Figure 2).

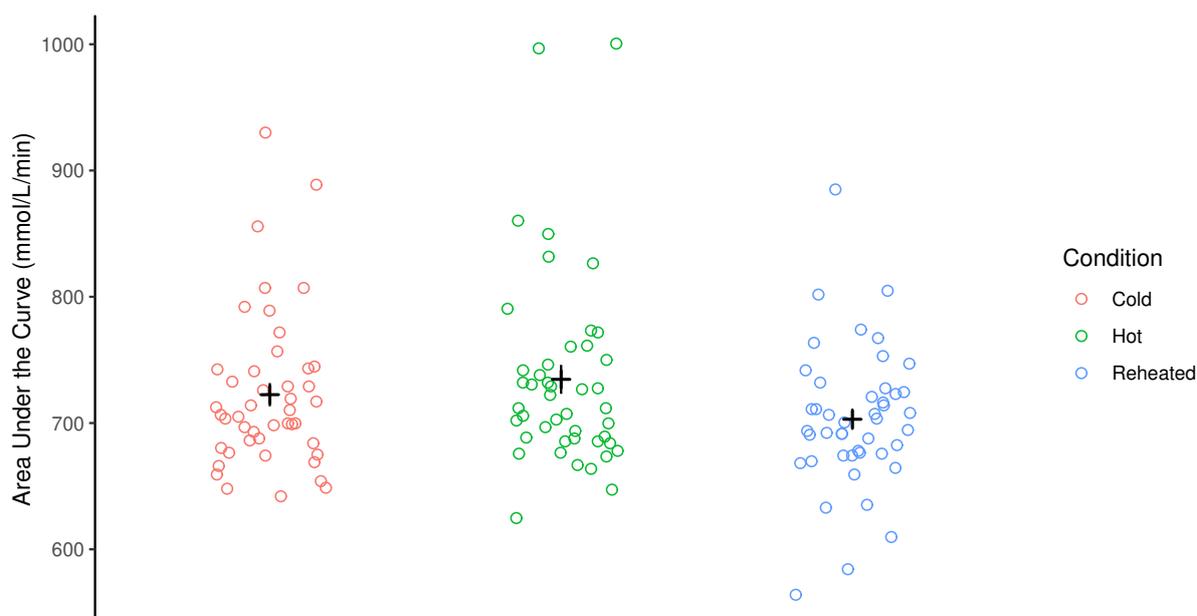


Figure 2. Area under the curve for post-prandial glucose response to three different carbohydrate meal preparations. Data shown is mean \pm standard error alongside individual responses.

99 4. Discussion

100 This study aimed to examine the effect of cooking methodology of pasta on post prandial blood
101 glucose, and found that both cooled, and reheated pasta, were associated with a faster return to
102 baseline blood glucose, compared to the hot condition, while reheated pasta also showed significantly
103 reduced blood glucose AUC, compared to freshly cooked pasta.

104 To our knowledge, the current study is the first to show that reheating pasta causes changes in
105 post-prandial glucose response, with a quicker return to fasting levels in both the reheated and cooled
106 conditions, compared with the hot condition. The mechanisms behind the changes in post-prandial
107 blood glucose seen in this study are most likely related to modifications of starch structure and these
108 subsequently influence glycaemic response. Studies in potatoes, noodles, rice and lentils indicate that
109 cooking and cooling changes the amount of RS present [6,11–16] changing the digestibility of these
110 foods [17,18]. This alteration in chemical structure, in conjunction with changes in amylopectin and
111 amylose crystallisation, may contribute to the indigestibility of starch [19,20]. These retrograded RS
112 molecules form tight structures stabilised by hydrogen bonds [5]. This modified structure means that
113 digestive enzymes (*e.g.* α -amylase) less effectively digest starch [21] resulting in food with a lower GI
114 [22].

115 Another important finding was that there was a significant effect of preparation method on AUC.
116 Sonia *et. al.* [23] found lower blood glucose levels and AUC after consumption of reheated rice,
117 compared with control rice, and suggested this was most likely attributable to higher RS, which would
118 decrease the available carbohydrate content. A similar result was obtained by Lu *et. al.* [24], when
119 comparing freshly cooked white rice with reheated cold-stored parboiled rice.

120 The decreased AUC resulting from reheating is significant because flattening the glucose response
121 by reducing peak rise, reduces post-prandial glucose fluctuations, which has several benefits, such as
122 the reduction of inflammation and oxidative stress, [25]. Schisano *et. al.* [26], reported that exposure of
123 cultured endothelial cells to oscillating glucose concentrations was more deleterious than constant high
124 glucose exposure and induced a metabolic memory after glucose normalisation, as well as causing
125 greater apoptosis. In non-diabetic lean patients, reduced postprandial NF κ B activation in white blood
126 cells resulted from meals which elicited a flatter glycaemic response. [27]. In patients with T2DM
127 glycaemic variability is implicated in coronary artery disease [28]. For example oxidative stress is
128 activated by glycaemic fluctuations [29] and incremental glucose peaks have been shown to correlate
129 with carotid intima-media thickness, which is a surrogate marker for atherosclerosis [30].

130 In conclusion, although it is evident that cooking methodology of pasta influences post-prandial
131 glucose response, with a faster return to baseline in both cooled and reheated pasta, as well as reduced
132 AUC following reheating, further work is needed to understand the mechanisms driving these changes
133 and to ascertain if alterations in chemical structure is the primary factor influencing post-prandial
134 glucose response.

135 **Author Contributions:** Conceptualization, CH and FMG; methodology, CH & FMG; formal analysis, FMG;
136 investigation, FA, MC, BLE, DJG, MM; resources, CH; writing—original draft preparation, CH, FA, MC, BLE, DJG,
137 MM; writing—review and editing, CH, FMG; visualization, FMG; supervision, CH, FMG; project administration,
138 CH, FA, MC, BLE, DJG, MM

139 **Funding:** This research received no external funding

140 **Conflicts of Interest:** The authors declare no conflict of interest.

141 Abbreviations

142 The following abbreviations are used in this manuscript:

143 ANOVA Analysis of Variance
GI Glycaemic Index
144 AUC Area under the Curve
T2DM Type-2 Diabetes Mellitus

145 **References**

- 146 1. Cust A., Skilton M., van Bakel M., *et al.*. Total dietary carbohydrate, sugar, starch and fibre intakes in the
147 European Prospective Investigation into Cancer and Nutrition. *Eur J Clin Nutr.*, **2009**, *63*, S4, S37-60.
- 148 2. Heinemann R.J.B., Fagundes P.L., Pinto E.A., Penteado M.V.C., Lanfer-Marquez U.M., Comparative study of
149 nutrient composition of commercial brown, parboiled and milled rice from Brazil. *J. Food Comp. Anal.*, **2005**,
150 *18*, 287-96.
- 151 3. Huang, M., Li, J., Há, M. A., Riccardi, G., Liu, S., A systematic review on the relations between pasta
152 consumption and cardio-metabolic risk factors. *Nutr. Metab. Cardiovasc. Dis.*, **2017** *27*, 11, 939-48.
- 153 4. Lattimer J., Haub M., Effects of Dietary Fiber and Its Components on Metabolic Health. *Nutrients*, **2010**, *2*, 12,
154 1266-89.
- 155 5. Haralampu S., Resistant starch—a review of the physical properties and biological impact of RS3. *Carbohydr.*
156 *Polym.*, **2000**, *41*, 3, 285-92.
- 157 6. Sajilata M.G., Singhal R.S., Kulkarni P.R., Resistant Starch- A Review. *Comp Rev Food Sci Food Safety*, **2006**, *5*, 1,
158 1-17.
- 159 7. Lin C., Chang D., Wu D., Peng H., Chuang L., Assessment of Blood Glucose Regulation and Safety of
160 Resistant Starch Formula-Based Diet in Healthy Normal and Subjects With Type 2 Diabetes. *Medicine*, **2015**,
161 *94*, 33, e1332.
- 162 8. Raigond P., Ezekiel R., and Raigond B., Resistant starch in food: a review. *J. Sci. Food Agric.*, **2014**, *95*, 10,
163 1968-78.
- 164 9. Robertson M.D., Could resistant starch be the redeemer of dietary carbohydrate? *Pract. Diab.*, **2014**, *31*, 375.
- 165 10. Brouns F., Björck I., Frayn K., Gibbs A.L., Lang V., Slama G., Wolever T.M.S., Glycaemic index methodology.
166 *Nutr. Res. Rev.*, **2005**, *18*, 1, 1-17.
- 167 11. Akerberg A.K., Liljeberg H.G., Granfeldt Y.E., Drews A.W., Björck I.M., An *in vitro* method, based on chewing,
168 to predict resistant starch content in foods allows parallel determination of potentially available starch and
169 dietary fiber. *J. Nutr.*, **1998**, *128*, 651-60.
- 170 12. Wang N., Hatcher D.W., Tyler R.T., Toews T., Gawalko E.J., Influence of cooking and dehulling on nutritional
171 composition of several varieties of lentils (*lens culinaris*). *LWT – Food Science and Technology*, **2009**, *42*, 4, 842-8.
- 172 13. Garcia-Alonso A., Jimenez-Escrig A., Martin-Carron N., Bravo L., Saura-Calixto, F., Assessment of some
173 parameters involved in the gelatinization and retrogradation of starch. *Food Chem.*, **1999**, *66*, 2, 181-7.
- 174 14. Monro J., Mishra S., Blandford E., Anderson J., Genet, R., Potato genotype differences in nutritionally distinct
175 starch fractions after cooking, and cooking plus storing cool. *J. Food Compost. Anal.*, **2009**, *22*, 6, 539-45.
- 176 15. Raatz S.K., Idso L., Johnson L.K., Jackso M.I., Combs G.F., Resistant starch analysis of commonly consumed
177 potatoes: Content varies by cooking method and service temperature but not by variety. *Food Chem.*, **2016**,
178 *208*, 297-300.
- 179 16. Wepner B., Berghofer E., Miesenberger E., Tiefenbacher, K., Citrate starch: application as resistant starch in
180 different food systems, *Starch/Stärke*, **51**, 10, 354-61.
- 181 17. Garcia-Alonso A., Goni I., Effect of processing on potato starch: In vitro availability and glycaemic index.
182 *Nahrung*, **2000**, *44*, 1, 19-22.
- 183 18. Leeman A.M., Barstrom L.M., Björck I.M.E. *In vitro* availability of starch in heat-treated potatoes as related to
184 genotype, weight and storage time. *J. Sci. Food Agri.* **2005**, *85*, 5, 751-6.
- 185 19. Nayak B., De J., Berrios J., Tang J., Impact of food processing on the glycemic index (GI) of potato products.
186 *Food Res. Int.*, **2014**, *56*, 35-46.
- 187 20. Sharma A., Yadav B.S., Yadav R.B., Resistant starch: physiological roles and food applications. *Food Rev. Int.*,
188 **2008**, *24*, 193–234.
- 189 21. Englyst H., Wiggins H.S., Cummings J.H., Determination of the non-starch polysaccharides in plant foods
190 by gas-liquid chromatography of constituent sugars as alditol acetates. *Analyst*, **1982**, *107*, 307-18.
- 191 22. Granito M., Perez S., Valero Y., Quality of cooking acceptability and glycemic index of enriched pasta with
192 legumes. *Rev. Chil. Nutr.*, **2014**, *41*, 4, 425-32.
- 193 23. Sonia, S., Witjaksono, F., Ridwan, R. Effect of cooling of cooked white rice on resistant starch content and
194 glycemic response. *Asia Pac. J. Clin. Nutr.*, **2015**, *24*, 620-5.

- 195 24. Lu L., Monro J., Lu J., Rush E., Effect of Cold Storage, Reheating, and Particle Sizes on In Vitro Glucose
196 Release and Starch Digestibility among Five Rice Products in Auckland, New Zealand. *J. Rice Res.* **2016**, *4*,
197 171.
- 198 25. Wolever T.M.S., Jenkins A.L., Prudence K., Johnson J., Duss R., Chu Y., Steinert R.E., Effect of adding oat
199 bran to instant oatmeal on glycaemic response in humans-a study to establish the minimum effective dose of
200 oat β -glucan. *Food Funct.*, **2018**, *9*, 1692-700.
- 201 26. Schisano B., Tripathi G., McGee K., McTernan P.G., Ceriello A., Glucose oscillations, more than constant high
202 glucose, induce p53 activation and a metabolic memory in human endothelial cells, *Diabetologia*, **2011**, *54*,
203 1219-26.
- 204 27. Dickinson S., Hancock D.P., Petocz P., Ceriello A., Brand-Miller J., High-glycemic index carbohydrate
205 increases nuclear factor-kappaB activation in mononuclear cells of young, lean healthy subjects. *Am. J. Clin.*
206 *Nutr.*, **2008**, *87*, 1188-93.
- 207 28. Benalia M., Zeller M., Mouhat B., Guenancia C., Glycaemic variability is associated with severity of coronary
208 artery disease in patients with poorly controlled type 2 diabetes and acute myocardial infarction. *Diabet. &*
209 *Metab.*, **2019**, *45*, 5, 446-52.
- 210 29. Monnier L., Colette C., Mas E., Michel F., Cristol J.P., Boegner C., Owens D.R., Regulation of oxidative stress
211 by glycaemic control: evidence for an independent inhibitory effect of insulin therapy, *Diabetologia*, **2010**, *53*,
212 562-71.
- 213 30. Esposito K., Ciotola M., Carlen D., Schisano B., Sardelli L., Di Tommaso D., Misso L., Saccomanno F., Ceriello
214 A., Guigliano D., Post-meal glucose peaks at home associate with carotid intima-media thickness in type 2
215 diabetes. *J. Clin. Endocrinol.*, **2008**, *93*, 1345-50.