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GLYCAEMIC PROFILE AND INSULIN RESPONSE AFTER CONSUMING TRITICALE FLAKES

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*Daily intake of cereal fibre reduces incidence and progression of metabolic diseases. Very little is known on how triticale (*Triticosecale*) influences human health and its role in regulating carbohydrate metabolism. The aim of the study was to investigate glycaemic and insulin response in blood after consuming whole grain triticale cereal flakes. A group of twelve healthy, young people, aged from 18 to 30 years participated in the test. The participants in fasted state were given equivalent carbohydrate amounts of triticale cereal and reference food (glucose solution). Post-prandial blood glucose and plasma insulin concentrations were measured according to Brouns* et al. *(2005). Whole grain triticale cereal flakes elicited lower metabolic responses compared to glucose solution. Intake of the triticale cereal flakes induced significantly lower incremental insulin area (iAUC 0–120 min) 1672.9 ± 619.85 than glucose solution 2646.65 ± 1260.56 and showed lower insulinemic indices (II) 68 ± 19.0 (*$p < 0.05$*). A low insulin incremental peak was associated with less severe late post-prandial hypoglycaemia. Our study showed that triticale cereal product caused low acute insulinemic response and improved glycaemic profiles, similarly to the rye products studied before. The results also suggested that the triticale cereal flakes could have beneficial appetite regulating properties. Thus, triticale flakes would be a wonderful option for functional breakfast cereal mixtures that might influence course of metabolic syndrome prevention*

Key words: *Triticale, glycaemic response, insulinemic response, healthy subjects.*

INTRODUCTION

In industrialised nations, the majority of carbohydrate-containing foods used in the human diet are quickly digested, absorbed and give a rise to “spikes” of high blood glucose and insulin. In addition, highly processed diets may lead to a dramatic rise in the prevalence of metabolic syndrome, type 2 diabetes and other non-communicable diseases. In 2014, the World Health Organisation (WHO) estimated that 422 million adults were living with diabetes and this number had increased approximately four times since 1980. This reflects risk factors, such as overweightness and obesity increase. Modification of the risk factors through dietary intervention offers a great potential to decrease the incidence of metabolic syndrome and type 2 diabetes (Anonymous, 2016).

There is evidence that a Mediterranean-style diet (MedDiet) that is rich in fruits, vegetables, legumes, cereals, olive oil, and nuts provides a protective effect against the most prevalent diseases such as cancer, cardiovascular diseases, as well as metabolic syndrome and type 2 diabetes. The protective role of the MedDiet against these health outcomes has also been shown consistently in the long-term PREDIMED (2003–2011) randomised trial (Babio *et al*., 2014; Buil-Cosiales *et al*., 2016). A reason explaining why the MedDiet influences our health positively has been attributed to its low-glycaemic properties and dietary fibre (DF) content that is associated with bioactive ingredients (Babio *et al*., 2014; Buil-Cosiales *et al*., 2016; Salas-Salvadó *et al*., 2016)

Recent studies highlight that specific cereal fibre may have a positive impact on human health (Evans *et al*., 2015; Liu *et al*., 2015; Aune *et al*., 2016; Hajishafiee *et al*., 2016).

The most important cereal grains in the daily diet globally are wheat, rice and corn. In Europe, the most important grains are oats, barley and rye (Gennari, 2015). In Northern and Eastern Europe, rye traditionally plays an important role due to its breeding traditions and highly beneficial impact on health (Mykkänen, 2012). Several mechanisms as mediators of favourable outcomes in metabolic processes have been discussed, e.g. whole rye grain is a good source of various phytochemicals, phenolic acids, alkylresorcinols and lignans (Smith and Tucker, 2011; Belobrajdic and Bird, 2013; Meynier *et al.*, 2015). However, modern consumers are more familiar to wheat than rye and prefer wheat flavour instead of rye flavour, regardless of wheat intake in a refined form.

Increasing incidence of various chronic diseases has created the need to look for alternative foods e.g. cereal based functional foods. It is well documented that consumption of rye results in positive health benefits (Mykkänen, 2012). In recent time, researchers have focused on a new cereal species — triticale (*Triticosecale*). Triticale is a hybrid of wheat (*Triticum* sp.) and rye (*Secale* sp.) and is mainly used as animal food. As a hybrid plant, it combines many good qualities obtained from both species. Several studies in Canada and Poland had carried out to determine if fractionation of triticale grain can be utilised to procure value-added components (McGoverin *et al.*, 2011; Fraś *et al.*, 2016). These components include: specific proteins, starches, β-glucan, pentosans, fibre (soluble and insoluble) and tocols. Moreover, the levels of dietary fibre and lignans in triticale may be high enough for use in high fibre food products, which are beneficial for human health (Hosseinian *et al.*, 2009; Rakha *et al.*, 2011; Nakurte *et al.*, 2012; Agil *et al.*, 2014; Agil *et al.*, 2016).

Up to now, use of triticale in the human diet has been very little studied.

Triticale is not a suitable grain for bread baking, because of lack of gluten. However, chemical composition of grain suggests that triticale may be a wonderful option for production of breakfast cereals (Wrigley *et al.*, 2010).

Usually, after ingestion, food starch is rapidly digested and absorbed as glucose, potentiating a hyperglycaemic response and stimulating insulin secretion. Glucose absorption in tissue may result in hypoglycaemia. A repeated hyper- and hypoglycaemic cycle appears as a result of insulin resistance and type 2 diabetes, thereby contributing to obesity. Thus, an improved insulin economy might contribute to the health benefits. We know that rye products induce low acute insulinemic responses and improved glycaemic profiles. The hypothesis of our study was that triticale has insulinemic response similar to that of rye. The aim of the study was to investigate the effect of consumption of triticale flakes on carbohydrate metabolism, and evaluate frequency of glycaemic and insulin response after consumption.

MATERIALS AND METHODS

Experimental group. Twelve healthy and non-smoking volunteers (9 women and 3 men) aged 22.4 ± 3.7 years with normal body mass index: 22.3 ± 2.9 kg/m^2 took part in the experiment. All of them had normal fasting blood glucose concentrations. Participants were not undergoing any use of medicaments or other substances like food supplements or functional food that could have influenced blood glycaemia. The study period was from January until March 2016. All participants gave their consent to take part in the experiment with possibility of withdrawing from the study at any time if they desired. The Ethics Committee of Rīga Stradiņš University approved the study. Two volunteers terminated their participation in the study due to personal reasons.

Experimental design. The testing protocol demanded that the participants arrived at the laboratory at 8:00 a.m. after 10–12 hours of night fasting. The participants were encouraged to standardise their meal patterns during the experimental period. They were asked to avoid foods that are rich in DF (e.g. legumes) the day before the test-day. They were also asked to avoid alcohol and excessive physical exercise in the evening before the experiment. The first basal blood sample (at t = 0) was taken in fasting status. The concentration of glucose was determined in the capillary blood, and the insulin level in peripheral venous blood. After the consumption of the meal, blood analyses were taken six times at 15, 30, 45, 60, 90, and 120 minutes. The blood samples were analysed for glucose and insulin concentration. The test was repeated for each participant four times: a standard meal was used on the first three times, and on 4th visit only triticale cereal meal was given. The participants were instructed to finish the meal within 10–15 minutes.

Test meals. The standard meal contained of 50 g glucose that was dissolved in 250 ml water. Triticale cereal meal was prepared in the laboratory of the Latvia University of Agriculture, Faculty of Food Technology. Triticale grain used in preparation of the meal was cv. 'Tulus' cultivated at the Norwegian Institute of Bioeconomy Research (Norway). The grains were cleaned, washed and soaked in water at a ratio of 1:2 (grains to water) for 24 ± 1 h at 22 ± 2 °C. After soaking, water was drained and cereal grains were flaked using a manual flaker (Eschenfelder, Germany). The thickness of obtained flakes was 1.2 ± 0.3 mm. Thereafter, flaked cereals were dried using a microwave-vacuum "Musson-1" (OOO Ingredient, Russia). Drying time and temperature for 5 kg flaked triticale cereals were 56 ± 2 min at 40 ± 5 °C, respectively. Chemical analysis of triticale flakes was conducted to determine concentrations of total starch, total sugars, resistant starch (RS), and DF (-soluble and insoluble-). The analyses were conducted in the laboratory of Eurofins Polska. Available carbohydrate concentration was calculated by subtracting RS from total starch concentration. The triticale meal contained 50 g available carbohydrates; thus one portion triticale flakes was 103 g. In addition, 250 ml water was added.

Laboratory methods. Capillary blood was taken with a *Sarstedt* glucose microvete containing 200 μl sodium fluoride. Glucose determination was done by hexokinase method with an analyser Architect c8000 (ABBOTT). The intra assay coefficient of variation (CV) was < 3%. Blood samples (4 ml) were collected by venipuncture using a Greiner bio-one vacuette containing anticoagulant-spray-dried lithium heparin. The collected samples were ultra-centrifuged immediately. Centrifuged serum samples were assayed within 1–2 hours. Serum insulin was measured using a chemiluminescent immunometric assay with an analyser Immunilite 2000 Systems (The Quality System of Siemens Healthcare Diagnostic Products Ltd.). The CVs were 3.0% and 3.5% within- and between-assay, respectively. The samples collected at each time were analysed within the same run.

Statistical analyses. Replication of blood glucose and insulin levels was n = 10. No formal power calculations were conducted for the metabolism studies, since estimates of the required number of subjects under similar conditions are available in the literature. Ten participants were sufficient to detect a 10% difference in a glucose and insulin ratings, in a paired design with a power of 80% and a level of significance at $p < 0.05$ (Brouns *et al.*, 2005). The average of the three standard meal evaluations were used for statistical analysis. Results are expressed as mean ± SD. Cumulative changes in postprandial plasma glucose and insulin responses for each meal were quantified as the incremental area under the 120 min. response curve (iAUC), which was calculated by using the trapezoidal rule with fasting concentrations as the baseline and truncated at zero. The insulinemic index (II) was calculated from the 120 min. incremental post-prandial area for serum insulin by using glucose solution (II = 100) as a reference. Any negative areas tended to be small and these were ignored. The significance of differences ($p < 0.05$) between the products at different time points was evaluated by the Wilcoxon test for paired observations using SPSS 22.0 software (SPSS Inc. Chicago).

RESULTS

Blood glucose responses. The meal containing triticale cereal flakes caused a significantly lower peak of glucose concentration than the corresponding glucose solution ($p < 0.05$). Moreover, this meal also induced significantly lower incremental area (iAUC 0–120 min) than the standard meal ($p < 0.05$) (Fig. 1, Table 1). The glycaemic index of triticale was obtained in our previous study (Havensone *at el.*, 2017). The postprandial peak of glucose values for all meals included in the test occurred at 30 min, with the lowest value at 120 min. The early incremental blood glucose area (iAUC 0–30) indicated clear difference in effect between triticale cereals and glucose solution. Some differences in glycaemic profiles were also observed between the meals in the late phase (iAUC 90–120). The glucose solution showed a sharp postprandial decline and tended to drop below fasting concentration. In contrast, intake of the triticale cereal flakes showed a slower postprandial decrease. Significant differences in blood glucose were recorded at specific time of points (time × treatment $p = 0.005–0.009$), however no significant differences were observed at 30 and 45 minutes $p = 0.33$ and 0.15, respectively).

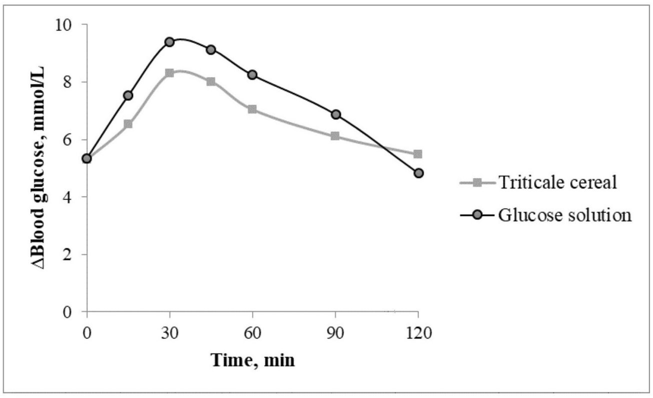

Fig. 1. Mean blood glucose (mmol/L) concentration after a standard test meal and triticale cereal flakes at times from 0 to 120 min; (n = 10).

Table 1

RESPONSE OF BLOOD GLUCOSE CONCENTRATION AFTER INTAKE OF MEALS

Meals	Glucose incremental peak mmol·min/L	Glucose iAUC (0-30 min) mmol·min/L	Glucose iAUC (0-120 min) mmol·min/L	GI %
Glucose solution	4.03 ± 0.77	39.69 ± 9.89	142.00 ± 41.58	100 ± 0.00
Triticale flakes	2.97 ± 0.87	24.74 ± 7.91	89.57 ± 22.63	66 ± 18.00

Values are means ± SD, n = 10, significantly different, $p < 0.05$.

Serum insulin responses. The mean insulin concentration in blood showed a similar pattern to that of blood glucose, resulting in the insulinemic index (II) 68 ± 19.0 (Fig. 2, Table 2). However, after intake of glucose solution the peak of serum insulin occurred in 60 minute, for triticale cereal flakes in 45 minute. A low insulin incremental peak was associated with less severe late post-prandial hypoglycaemia. Intake of the triticale cereal flakes induced significantly lower incremental insulin area (iAUC 0–120 min), compared to that of glucose solution ($p < 0.05$). Significant differences in serum insulin were recorded at times15, 30, 90, 120 min (time × treatment $p = 0.005–0.009$), but there were no significant differences between insulin responses after consumption of triticale cereal flakes and glucose solution at 45 and 60 minutes ($p = 0.074$ and 0.141, respectively). The insulin response at 30 min (iAUC 0–30) was significantly lower after consumption of triticale flakes than after intake of glucose solution ($p = 0.006$).

DISCUSSION

The present study showed that triticale cereal flakes acted similarly to rye products, as they induced low acute insulinemic responses and showing improved glycaemic profiles (Nilsson *et al.*, 2008; Rosén *et al.*, 2009; Rosén *et al.* 2011). For triticale cereal flakes the II was 68 ± 19, which is similar to the II for rye products (II range from 61 to 73) (Rosén *et al.*, 2011). However, comparison of the II of these

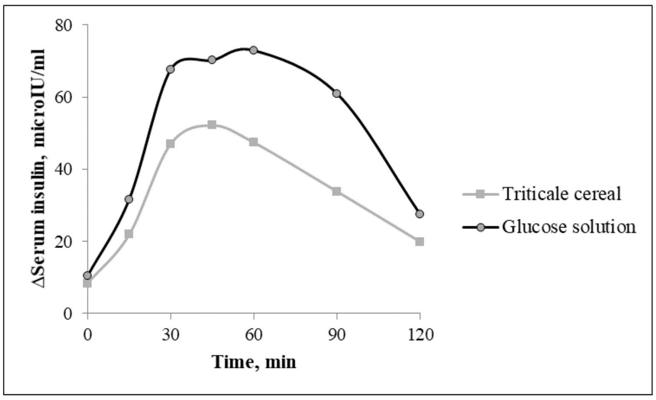

Fig. 2. Mean serum insulin concentration (microIU/ml) after intake of a standard test meal and triticale cereal flakes at times 0 to 120 min (n =10).

Table 2

CONCENTRATION OF SERUM INSULIN AFTER INTAKE OF THE TEST MEALS

Meals	Insulin incremental peak (mmol·min/L)	Insulin iAUC (0–30 min) (mmol·min/L)	Insulin iAUC (0-120 min) (mmol·min/L)	II %
Glucose solution	62.65 ± 40.85	445.56 ± 208.20	2646.65 ± 1260.56	100 ± 0.00
Triticale flakes	43.77 ± 15.97*	296.46 ± 115.40**	1672.79 ± 619.85*	68 ± 19.00

Values are means ± SD, n = 10. *differences are statistically significant, $p < 0.05$, ** $p = 0.006$

two grains might be biased because of different forms (flakes, bread and porridge) of products used in the studies. The low post-prandial insulin response of triticale was examined by the glycaemic response at different times. Our study showed that triticale cereal flakes tended to result in a lower glucose peak and prolonged postprandial glucose response. A lower insulin surge seems to be related to a more prolonged postprandial glucose response above the fasting level. The prolonged postprandial normoglycaemia was in turn positively correlated with the feeling of subjective satiety (Rosén *et al*., 2011; Poppitt, 2013). This correlation suggests that the triticale cereal could possess beneficial appetite regulating properties. Based on recent and our studies we might further hypothesise that a whole grain triticale cereal breakfast slows down acute insulin response, it might reduce depression of blood glucose below baseline levels (reactive hypoglycaemia) in the late postprandial phase and possibly has lower energy intake at subsequent meals compared to commercial grain breakfast with high II (Nilsson *et al*., 2008; Isaksson *et al*., 2011; Peters *et al*., 2011). There is some evidence that glucose kinetics affects this process little and that particularly the secretion of insulin in response to food intake, induces satiety (Flint *et al*., 2007; Blaak *et al*., 2012). Insulin can influence appetite by stimulating appetite centres in the brain, interacting with satiety gut peptides and modulating substrate oxidation in the liver (Flint *et al*., 2007).

Postprandial hyperinsulinemia has been identified as a risk marker for the development of metabolic syndrome and 2 type diabetes (Giacco *et al*., 2014).

The effect on appetite and metabolic responses from whole grain rye and probably also whole grain triticale is most likely mediated by the large content of dietary fibre, but may also be due to bioactive compounds present in the kernel and other structures of grain (Isaksson *et al*., 2011; Rosén *et al*., 2011). Whatever the mechanisms involved, a lower postprandial plasma insulin response promotes a beneficial effect on human health outcomes (Blaak *et al*., 2012). Therefore, in recent years, nutritional research has focused on the identification of carbohydrate-rich foods that have a lower insulinemic index, such as rye and barley. Our study also confirmed that whole-grain triticale cereal flakes have relatively low insulin responses, despite their high carbohydrate concentration.

CONCLUSION

Triticale cereal flakes possess a beneficial insulin profile which could be in line with appetite regulating properties. Our results suggest that triticale could be used in breakfast cereals helping in prevention of metabolic syndrome and positively influencing course of metabolic syndrome.

Further studies are needed with metabolic syndrome patients in order to identify other potentially protective bioactive substances in triticale.

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TRITIKĀLES PĀRSLU IETEKME UZ PĒCMALTĪTES GLIKĒMIJU UN INSULĪNA SEKRĒCIJU

Pasaulē un Latvijā pieaug komplekso vielmaiņas traucējumu — metaboliskā sindroma, otrā tipa cukura diabēta — izplatība. Izprotot metabolisko traucējumu attīstību un īstenojot efektīvu profilaksi, var novērst to turpmāku izplatību. Uztura un ēšanas paradumu maiņa ir ietekmējams riska faktors. Tieši pilngraudu produktu lietošana un graudaugu šķiedrvielu daudzums uzturā vistiešākā veidā samazina mirstību. Pēdējo desmit gadu laikā tradicionālo graudaugu klāstu — kviešus, rudzus, auzas un miežus — papildina inovatīvi pilngraudi — tritikāle un kailgraudi. Tritikāle (X *Triticosecale* Whittmack) iegūta, krustojot kviešus (*Triticum*) un rudzus (*Secale*). Šo graudu ietekme uz glikēmiju un insulinēmiju ir pētīta maz. Darba mērķis ir izanalizēt pilngraudu tritikāles pārslu ietekmi uz pēcmaltītes glikēmiju un insulinēmiju un noteikt tritikāles pārslu insulīna indeksu (II). Pētījumam atlasīja 18 līdz 30 gadus vecus veselus jauniešus. Dalībnieki laboratorijā ieradās no rīta un tukšā dūšā saņēma standarta glikozes šķīduma vai tritikāles pārslu maltītes. Uzreiz pēc standarta maltītes (glikozes šķīduma) vai tritikāles pārslu parauga (kas saturēja 50,0 gramu asimilējamo ogļhidrātu) pirmā kumosa sākās laika atskaite, un attiecīgi sešas reizes (resp., 15., 30., 45., 60., 90. un 120. minūtē) tika paņemti kapilāro asiņu paraugi, lai noteiktu glikozes koncentrāciju, un venozās asinis, lai noteiktu serumā insulīnu. No sērijveida pētījumā iegūtajiem analīžu datiem, izmantojot inkrementālās AUC (*area under curve*) vērtības, aprēķināja tritikāles pārslu II. Tritikāles pārslas uzrādīja statistiski ticami zemāku insulīna sekrēciju (iAUC 0–120 min), 1672,9 ± 619,85, salīdzinot ar glikozes šķīdumu 2646,65 ± 1260,56 ($p < 0.05$). Pēc tritikāles pārslu maltītes zemākas maksimālās insulīna vērtības izraisīja mazāk izteiktu glikēmijas līmeņa samazinājumu vēlīnajā fāzē — 120. minūtē. Aprēķinātais tritikāles pārslu II: 68 ± 19%. Tritikāles pilngraudu pārslas ir ogļhidrātiem bagāts produkts ar zemāku II. Iegūtie dati liecina, ka tritikāles pārslu ietekme uz insulinēmiju ir līdzīga rudzu un miežu pilngraudu produktiem. Tie izraisa mazāku pēcmaltītes reaktīvo hipoglikēmiju. Tas, iespējams, var modelēt labāku sāta sajūtu un samazināt turpmākajās maltītēs apēsto ēdiena apjomu. Savukārt mazāks kaloriju daudzums labvēlīgi ietekmē ķermeņa svaru, kas ir viens no riska faktoriem kompleksajiem vielmaiņas traucējumiem. Lai pilnībā novērtētu tritikāles pārslu ietekmi uz ogļhidrātu vielmaiņu, nepieciešams izanalizēt kādi struktūrelementi tritikāles graudā labvēlīgi ietekmē pēcmaltītes insulīna sekrēciju.