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Title: Global intensification of N fertilisation increases allergenic proteins and may spread coeliac pathology

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Abstract: Background
Fertilisation of cereal crops with nitrogen (N) has increased in the last five decades. In particular, the fertilisation of wheat crops increased by nearly one order of magnitude from 1961 to 2010, from 9·84 to 93·8 kg N ha-1 y-1. We hypothesized that this intensification of N fertilisation would be associated with the increased pathology of coeliac disease in human populations. An increase in the per capita intake of gliadin proteins, the group of gluten proteins principally responsible for the development of coeliac disease, would be the responsible factor.
Methods and Findings
We conducted a global meta-analysis of available reports that supported our hypothesis: wheat plants growing in soils receiving higher doses of N fertilizer have higher total gluten, total gliadin, □/□-gliadin, □-gliadin and □-gliadin contents and higher gliadin transcription in their grain. We thereafter calculated the per capita annual average intake of gliadins from wheat and derived foods and found that it increased from 1961 to 2010 from approximately 2·4 to 3.8 (+ 1·4 ± 0·18 kg, mean ± SE), i.e. increased by 58 ± 7·5%. Finally, we found that this increase was positively associated with the increase in the rates of coeliac disease in all the available studies with temporal series of coeliac disease.
Interpretation
The impacts and damage of over-fertilisation have been observed at an environmental scale (e.g. eutrophication and acid rain), but a potential direct effect of over-fertilisation is thus also possible on human health (coeliac disease).

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Global intensification of N fertilisation increases allergenic proteins and may spread coeliac pathology

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Key words: Global intensification of N fertilisation; wheat; allergenic proteins; gluten proteins; coeliac pathology

Summary

Background

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Introduction

The demand for and application of nitrogen (N) fertilizer in cropland at a global scale are continuously increasing. The global use of N fertilizers has increased substantially from 11·3 Tg N y^{-1} (0·9 g N m^{-2} y^{-1}) in 1961 to 107·6 Tg N y^{-1} (7·4 g N m^{-2} y^{-1}) in 2013.[1] The data provided in the last International Nitrogen Initiative Conference indicated that the global consumption of N fertilizers increased 33% from 2000 to 2013.[2] FAO (Food and Agriculture Organization of the United Nations) data indicated that the recent (2014-2018) intensification of N fertilisation at global and regional scales has affected most of the world, but with regional differences, with increases, from highest to lowest, of 29·1, 24·5, 17·6, 9·0, 5·4, 4·8, 4·1, 2·5 and 1·3% in eastern Asia, southern Asia, Latin America and the Caribbean, eastern Europe & central Asia, sub-Saharan Africa, North America, western Asia, northern Africa and Oceania, respectively, and a decrease of 1·5% in western Europe.[3]

Wheat (*Triticum* sp.) is currently the most widely planted crop and continues to be the most important food grain for humans.[4] Furthermore, despite a decrease of direct flour food products intake has occurred in some countries such as the United States of America, there is still a net increase in per capita annual wheat flour intake due to an additional flour intake from the extra flour used as food additive that has increased the net gluten anual intake per person from 4·1 kg in 1970 to 5·4 kg in 2000.[5] Wheat crops currently cover an area of 217 × 10^6 ha globally.[6] Global wheat yield in recent decades (1961-2015) has continuously increased despite representing a similar area of land (Figure 1).[7–9] The annual amounts of N fertilizer applied to wheat crops have increased globally in the same period from approximately 10 to 100 kg N ha^{-1} y^{-1}. This increase in N fertilisation is associated with an increase in the production of wheat grains and flour per hectare. The fertilisation (kg ha^{-1}), to yield (t ha^{-1}) ratio, however, increased from 0·9 to 3·1 kg N t grains^{-1} during 1961-2010, i.e. the yield-to-fertilisation ratio is now only 3·5 fold what it was 50 years ago.

The protein composition of wheat grains varies depending on genotype and environmental conditions, but wheat proteins are generally deficient in some fundamental amino acids, such as lysine and threonine.[10] Structural proteins of wheat grains are mostly albumins, globulins and amphiphilic proteins[10], whereas storage proteins are gliadins (monomeric proteins) and glutenins (polymeric proteins).[11] N fertilisation generally influences the quantity and quality type of storage proteins (gliadins and gluteins) but has little effect on structural proteins.

Ingestion of wheat gluten can trigger several intolerances and allergic diseases, among which coeliac disease (CD) is the most widespread in humans.[12] The mean prevalence of CD in the general population in Europe and the United States of America (USA) is approximately 1%, [13,14] with some regional differences, e.g. the prevalence of CD is as high as 2-3% in Finland and Sweden but is only 0·2% in Germany. The overall prevalence of CD is now clearly increasing everywhere. The prevalence of CD in the USA was only 0.2% in 1975 but increased 5-fold during the next 25 years.[15] The causes remain elusive but are likely linked to the environmental components of CD (e.g. changes in the quantity and quality of ingested gluten, patterns of infant feeding, the spectrum of intestinal infections and colonization by gut microbiota).

Storage proteins of the gliadin group of gluten are mainly responsible for the CD after autoimmune responses,[12] in genetically susceptible individuals.[16] All three gliadin families, α/β, γ and ω, have been associated with allergic reactions to gluten and with the development of CD in humans.[17–25] The autoimmune response is due to the deamination of glutamine residues in gliadins by human transglutaminase 2 (tTG2) produced in the gut mucosa.[16,26–28] These deaminated peptides can bind to histocompatibility leukocyte antigen (HLA) class II in some humans, which stimulates lymphocyte T cells and triggers an inflammatory response in the gut.[12,14] Most studies have reported that gliadins are most responsible for CD,[17–25] but some studies have reported that the high-molecular-weight glutenins, another group of gluten storage proteins, can also induce these autoimmune responses in some people.[29–31] Glutenins, however, are easily degraded by digestive enzymes, providing mostly di- and tripeptides, whereas gliadins are more resistant to enzymatic degradation, producing mostly oligopeptides that are the main cause of inflammatory responses.[32] HLA-DQ2 and HLA-DQ8 are the HLA genes that have been identified to predispose the development of coeliac disorders and code for HLA class II in T lymphocytes.[31-33] The α-gliadins cause CD[31] both *in vitro*,[34,35] and *in vivo*,[36] without stimulating T cells. Some studies have consistently observed that the allergic response can be due to the sensitivity of HLA to gliadins or to other mechanisms, such as α-gliadin activation of the innate immune system mediated by interleukin 15.[37]

Martre et al. (2016),[38] modelled N-gliadin contents in wheat grains as a function of N partitioning among plant-protein groups and of wheat-plant development and validated the model using 18 experimental studies observing a significant relationship between N-fertilisation rates and the amount of N allocated to gliadins after sowing. More detailed information is now available from experimental data in field conditions including studies with a great variety of wheat genotypes growing in distinct areas of world. This thus allows a more detailed analysis of final gluten and gliadin contents in the grains and flour of wheat as a function of N-fertilisation levels.

We conducted a meta-analysis to determine whether N-fertilisation intensity could be associated with an increase in total gluten, total gliadin and α/β-, γ- and especially ω-gliadin contents and levels of transcription in wheat grains based on all compiled data from the experimental studies that have investigated these relationships (Table 1).

Methods

Data collection

To determine the relationships by meta-analysis between N fertilization increase and gliadins concentration increase in wheat grain we searched PubMed, ISI Web of Science and Google Scholar using the terms: coeliac, coeliac, disease, nitrogen, fertilisation, gliadins, gluten, glutenin grain, wheat, flour, spanning 1960-2019. To obtain the data of the prevalence (percentage of coeliac cases in the total population) or incidence (new cases per 1000 inhabitants and year) of coeliac disease from studies with large and representative sets of population data, adjusted for age and sex, from 1961 to 2010 we have searched PubMed, ISI Web of Science and Google Scholar using the terms: coeliac, coeliac, disease, gluten, health, time, incidence and prevalence, spanning 1960-2019. We also used the FAO data: FAOSTAT http://www.fao.org/faostat/en/#data. (2019) and the other sources cited in each figure caption.

Statistical analyses.

We examined the effects of the intensification of N fertilisation by a meta-analysis of studies reporting the differences of total contents of gluten, total gliadins, α/β-gliadins, γ-gliadins, ω-gliadins and gliadin transcripts (operational taxonomic units) in wheat grains and/or flour under different levels of N fertilisation by calculating the response ratios from each study, as described by Hedges *et al*..[49] The natural-log response ratio (ln*RR*) was calculated as ln $(X_i/X_n) = \ln X_i - \ln X_n$, where X_i and X_n are the values of each observation in treated and control plants, respectively. The sampling variance for each ln*RR* was calculated as $\ln[(1/n_i) \times (S_i/X_i)^2 + (1/n_n) \times (S_n/X_n)^2]$ using the R package metafor 1·9–2,[50] where n_i and n_n, S_i and S_n and X_i and X_n are the sample sizes, standard deviations and mean response values of the treatments and controls, respectively. The natural-log response ratios were determined by specifying studies as random factors using the *rma* model in metafor. The differences of contents of total gluten, gliadins, α/β-gliadins, γ-gliadins, ω-gliadins and gliadin transcripts in wheat grains and/or flour under different levels of N-fertilisation were considered significant if the 95% confidence interval of ln*RR* did not overlap zero. All statistical analyses were performed in RStudio 3.1.2.[51] We analyzed only the variables with >30 observations available at the global scale. We then examined the sensitivities of contents of total gluten, total gliadins, α/β-gliadins, γ-gliadins, ω-gliadins and gliadin transcripts in wheat grains and/or flour under different levels of N-fertilisation using REML estimation in the rma.unl model for metafor.

Results and discussion

Our meta-analysis found that the increase in N-fertilisation rates was associated with increased content of total gluten, total gliadins, α/β-gliadins, γ-gliadins, ω-gliadins and gliadin transcripts in wheat grains (Figure 2). Our analyses also identified a significant relationship (R^2=0.30, P<0·001) between the increase in total gliadin content in wheat grains and the increase in N fertilisation (Figure 3). These results are consistent with several studies observing sensory mechanisms and metabolic signalling pathways that link nutrient availability with the expression of gliadin genes,[39] and also with common farmer knowledge on protein content being strongly affected by N availability, which leads them to adjust the level of nitrogen fertilization to obtain the required protein content for bread making (Shewry et al. 2016)

The per capita annual increase in gliadin intake from wheat and derived foods during 1961-2010 was estimated to be approximately 1·4 kg y^{-1} (+ 58% ± 7.5%) (mean ± SE; Figure 4). This estimation took into account the annual intake of wheat and derivatives at the global scale,[8] the increase in N fertilisation in wheat crops and the relationships between N fertilisation and gliadin increase (Fig. 3 and Table 1). The increase in N-fertilization from approximate 10 to 100 kg N ha^{-1} corresponded to an increase in gliadin contents in grains/flour from 44 to 59 mg g^{-1}, respectively) (Fig 3 and Table 1).

Part of the increase in CD prevalence in populations in recent decades has frequently been attributed to improved diagnosis,[40] with some studies suggesting that the increase in diagnoses was due to increased awareness.[41] Some studies of populations over time, however, have reported an actual increase in CD development in recent decades beyond the improvement of diagnostic efficiency.[42] The increase in diagnosed cases of new coeliac patients may thus be due to more efficient diagnosis, higher awareness and changes in environmental variables associated with this increase in the percentage of a population affected by CD. Our study provides, though, good evidence of a strong potential increase in the average human intake of gliadins by associating the changes in global per capita intakes of wheat and derivatives with the empirical effects of a global 10-fold increase in intensification of N fertilisation during 1961-2010. The contents of digested peptides derived from gliadin in the gut have been demonstrated to be a determinant for the appearance of autoimmunological responses and CD manifestation,[43,44] and the amount of gluten/gliadin necessary to trigger CD in susceptible people can vary but has a threshold.[45]

A comparison of the changes in global per capita intake of gliadins from 1961 to 2010, global per capita intake of gliadins per kg of N fertilizer applied and amount of N fertilizer applied per tonne of harvested wheat grains with data for CD prevalence (percentage of coeliac cases in the total population) or incidence (new cases per 10 000 inhabitants per year) provided by studies of large and representative sets of population data, adjusted for age and sex, identified general trends of increases in prevalence/incidence during this period coinciding with the increase in the application of N fertilizer per tonne of wheat grains produced and with the per capita increase in gliadin intake (Figure S1). The higher per capita ingestion of gluten/gliadin globally in recent decades could thus account for, at least partially, the spread of coeliac pathology in the global human population. New research is though warranted to test this possibility and to figure out why instead the prevalence of CD is comparable between countries in which the intake of gluten is much higher, e.g. Italy with its high consumption of pasta, than in other EU countries where the intake is much lower (European Society for Paediatric Gastroenterology Hepatology and Nutrition). Long-term evolutionary adaptation may play a role there.

There are, moreover, several other possible factors predisposing to the development of CD such as the many substances emitted by humans into the environment. Glyphosate, an increasingly used herbicide, has for example also been demonstrated to predispose the development of CD. T cells, though, can induce an allergic response by other mechanisms such as by their synthesis of antibodies, which can also degrade transglutaminase in the gut mucosa.[46] In fact, CD is a very complex pathology, whose development involves not only environmental factors (gluten) but also genetic factors. Recently, a gen Inc13 has been identified that encodes for a noncoding RNA that blocks and represses the expression of certain inflammatory genes under normal conditions. The Inc13 expression can be inhibited by stimulation and increased expression of inflammatory genes favoring CD.[47] Moreover, the inflammatory over expression of T cells could be also favored under the infestation of certain reovirus that would suppress peripheral regulatory T cell.[48] In any case, we now know that autoimmune responses are generally triggered by gliadin peptides,[28] and these gliadin peptides increase their contents in grains in response to the intensification of N fertilisation.

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Authors' contributions

JP and JS conceived the idea and designed the research, JS gathered the data, JS and JP analysed the data. AG-G, IJ, PC, MO, KK, and OU assisted in the analyses. All authors contributed substantially to the writing and discussion of the paper.

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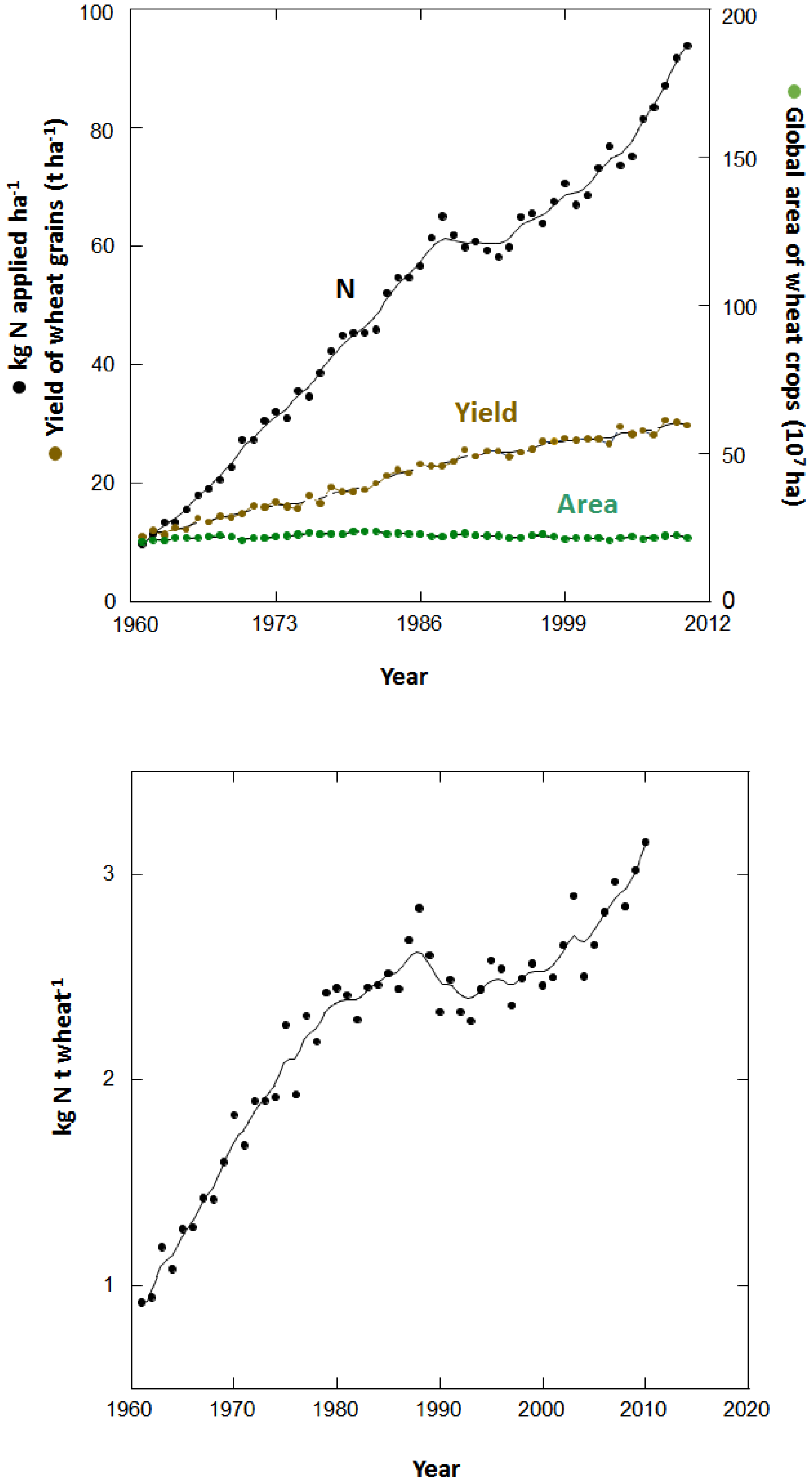
Figure captions

Figure 1. Global N-fertilisation rates (kg N ha^{-1} y^{-1}) in wheat crops. Wheat grain yield (t ha^{-1} y^{-1}) and global annual area of wheat crops (10^7 ha) during 1961-2016 (1961-2010 for N-fertilisation rates) (A). Efficiency of N fertilisation (kg^{-1} N ha^{-1} per tonne of wheat grains) (B). Sources: FAO (2015b, 2019) and Ladha et al. (2016).

Figure 2. Response ratios of contents of total gluten, total gliadins, α/β-gliadins, γ-gliadins and ω-gliadins and gliadin transcripts in wheat grains after an increase in N fertilisation. See Table 1 and References S1 for the sources. The number into parenthesis indicates the number of studies. *** $p < 0.0001$.

Figure 3. Increases in total gliadin contents in wheat grains as a function of the increases in N-fertilisation rates (kg N ha^{-1} y^{-1}). See Appendix 1 in the Supplementary material for the sources.

Figure 4. Per capita gliadin intake (g y^{-1}) and per capita wheat and derivates intake (kg y^{-1}). See Appendix 1 in the Supplementary material for the sources.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Year

Figure 1

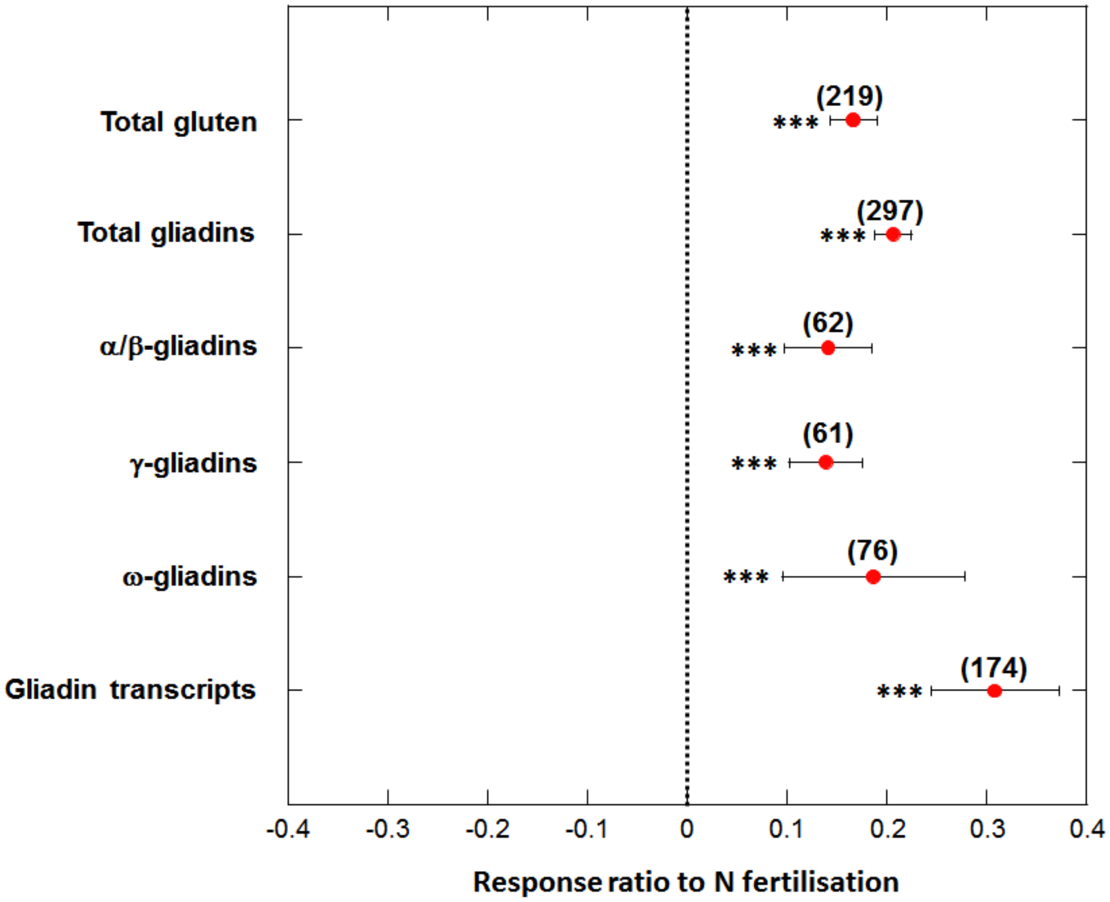

Total gluten
Total gliadins
α/β-gliadins
γ-gliadins
ω-gliadins
Gliadin transcripts
(219)
***
(297)
***
(62)
***
(61)
***
(76)
***
(174)
***
-0.4
-0.3
-0.2
-0.1
0
0.1
0.2
0.3
0.4
Response ratio to N fertilisation

Figure 2

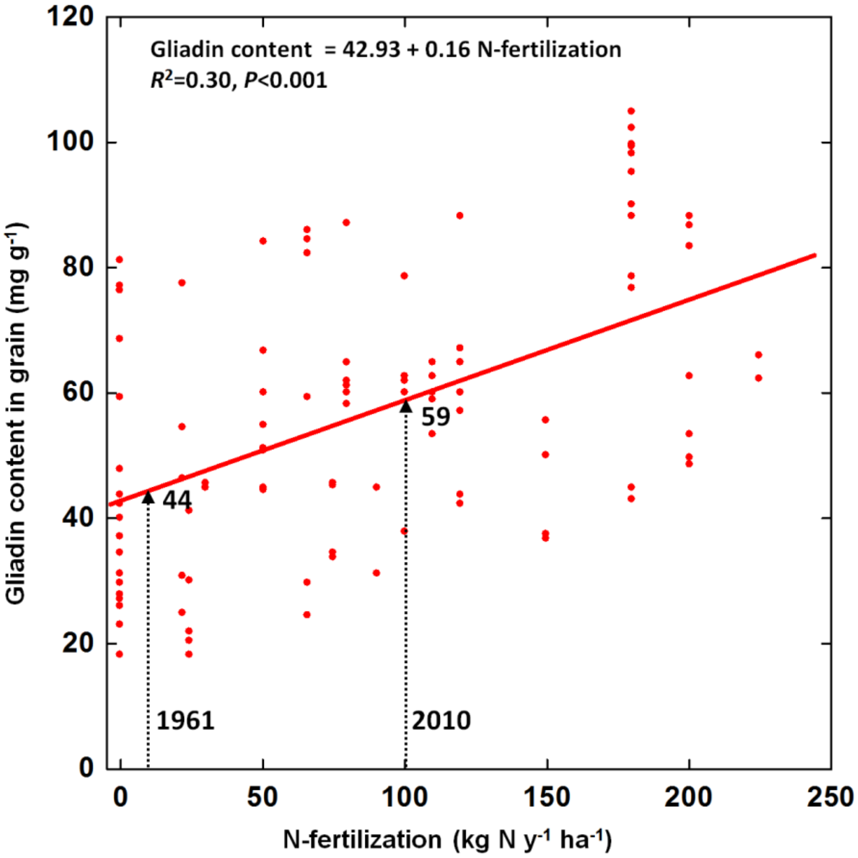
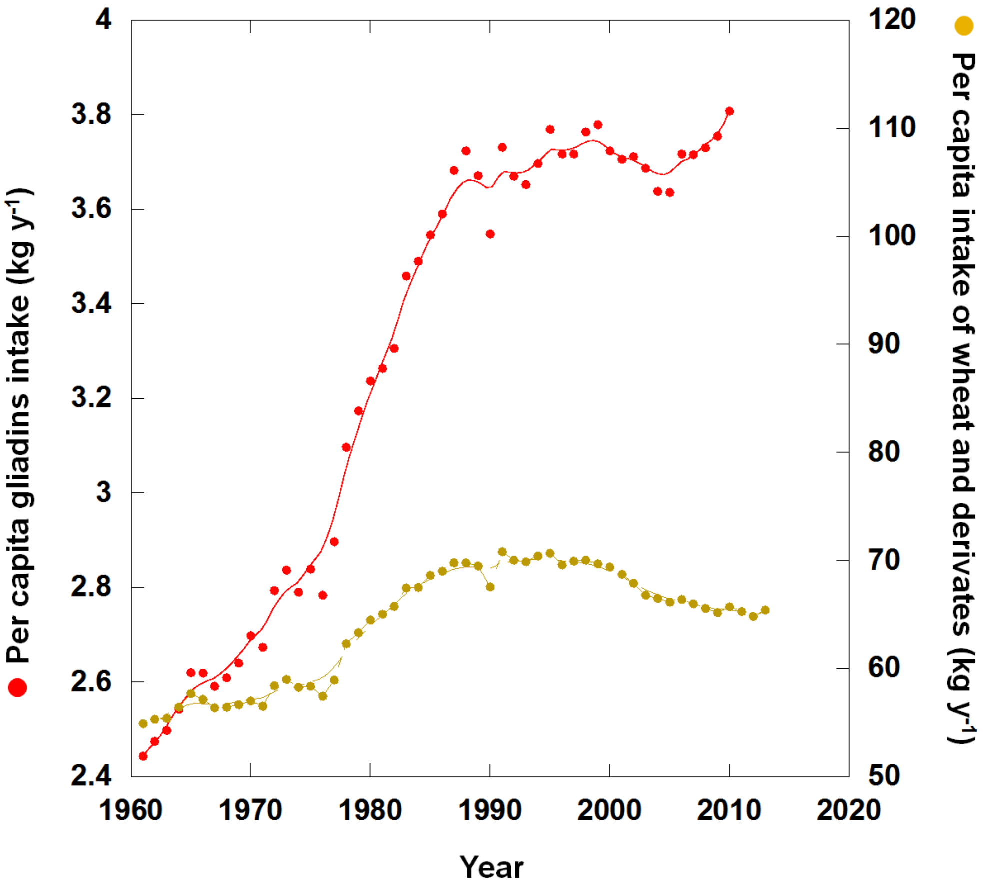

Gliadin content = 42.93 + 0.16 N-fertilization
R^2=0.30, P<0.001
Gliadin content in grain (mg g^{-1})
N-fertilization (kg N y^{-1} ha^{-1})
0
20
40
60
80
100
120
0
50
100
150
200
250
44
59
1961
2010

Figure 3



4
3.8
3.6
3.4
3.2
3
2.8
2.6
2.4
● Per capita gliadins intake (kg y^{-1})
120
110
100
90
80
70
60
50
● Per capita intake of wheat and derivates (kg y^{-1})
1960
1970
1980
1990
2000
2010
2020
Year

Figure 4

Table 1. Response of contents of total gluten, total gliadins, α/β-gliadins, γ-gliadins and ω-gliadins and gliadin transcripts in wheat grains after an increase in N fertilisation.

Bibliographic Source			**Experimental traits**				**Result**
Authors	**Source**	**Year**	**Site**	**Type of experiment**	**N fertilization rates (equivalent at Kg N ha^{-1} y^{-1} if not specified)**	**Genotypes**	**Changes in concentrations in grain and/or flour concentrations in response to increasing N-fertilization**
Abedí et al.	Aus J Crop Sci, 5, 330-336	2011	Experimental Farm Shiraz University (Iran)	Field	0, 120, 240, 360	Shiraz variety of winter wheat	Increases in gluten concentrations in grain
Andruszczak et al.	J Animal Plant Sci, 28, 1476-1484	2018	Bezek experimental station (Poland)	Field	0, 50, 80	*Triticum aesticum* ssp. *spelta*	Increases in total protein and gluten concentrations in grain
Bouacha et al.	J Cereal Sci, 59, 9-14	2014	Two field areas of Tunis	Field	0, 67	Chili, Biskri, Mahmoudi, INRAT69, Karim, Razzak, Omrabiaa and Khiar varieties	Increases in gluten concentrations in grain
Castro et al.	J Cereal Sci, 83, 49-57	2018	Experimental Station J. Hirschhorn (Argentina)	Field	0, 70, 140		Increases in gluten concentrations in grain
Cho et al.	J. Integr Agric, 17, 982-993	2018	Upland crop experimental farm of National Institute of crop Science (Korea)	Field	25, 50, 75	Five Korean wheat varieties	Increases in gluten concentrations in flour. Increases in α + β-gliadin and decreases in ω and γ-gliadin concentrations
Chope et al.	J Agric Food CHem, 14, 4399-4407.	2014	Replicates in five field sites in U.K.	Field	100, 200, 350	Five breadmaking wheat varieties (Cordial, Hereward, Malacca, Marksman and Xi19)	Increases in total gliadin concentrations in grain
Daniel and Triboi	J Cereal Sci, 32, 45-56	2000	Canada	Field	0, 100	Neepawa variety	Increases in total gliadin concentrations in grain
Dubetz et al.,	Can J Plant Sci, 59, 299-305	1979	Canada	Field	0, 50, 100, 150, 200, 250, 300, 350, 400	Neepawa variety	Increases in total protein and total gliadin concentrations in grain

Dubois et al.	BMC Plant Biol, 18, 262	2018	Eight different site sources	Field	0, 105, 165, 225	*Triticum aesticum* ssp. *spelta*	Increases of total epipodes expression of a-gliadin in grain
Ducsay and Lozek	Plant Soil Environ, 50, 309-314	2004	Plant Breeding Station of Sladkovicovo-Novy (Slovakia)	Field	120, 140	Winter wheat	Increases in total protein and gluten concentrations in grain
Fleitas et al.	J Cereal Sci, 80, 119-127	2018	Experimental Station J. Hirschhorn (Argentina)	Field	0, 70, 140		Increases in total gluten concentrations in flour
Fuertes-Mendizabal et al	Eur J Agron	2010	Alava (Spain)	Field	0, 100, 140, 180	Soissons variety	Increases in total gliadin concentrations in grain
Fuertes-Mendizabal et al.	J Sci Food Agric, 93, 2161-2171	2013	Spain	Pot experiment	37, 48 mg ammonium or nitrate per pot	Cezanne variety	Increases in total protein and gliadins concentrations in flour
Galieni et al.	Italian J Agron, 11,662	2016	Experimental field station Teramo University (Italy)	Field	50, 100, 150, 250	*Triticum turgidum* L. subsp. *durum*	Increases in total protein, gluten and gliadins concentrations in flour
García-Molina and Barro	Front Plant Sci, 8, 257	2017	Spain	Greenhouse	0, 22.2, 66.7, 200	Bobwhite variety	Increases in total, α, ω and γ-gliadin and total protein concentrations in flour
Guardia et al.	Agric Ecosys Environ, 265, 421-431	2018	National Center of Irrigation Technology station (Spain)	Field	0, 120	Winter wheat	Increases in total gliadins concentrations in flour
Johansson et al.	J Plant Nutrit Soil Sci, 167, 345-350	2004	Field (Sweden)	Field	0, 70, 140	Sport, Dacke, Dragon and Thasos varieties	Increases in total proteins and gliadins concentrations in flour
Kindred et al.	J Cereal Sci, 48, 46-57	2008	UK	Field	0, 40, 80, 120, 160, 200, 240	Option and Riband varieties	Increases in total proteins and gliadins concentrations in grain
Klikocka et al.	Plant Soil Environ, 5, 230-236	2016	Malice (Poland)	Field	0, 40, 80, 120	Tybalt variety	Increases in gluten concentrations in grain
Knapowski et al.	Plo J Environ St, 18, 227-233	2009	Agricultural experimental Staion of University of Technology and Life Sciences of Minikowo (Poland)	Field	80, 120	Spring wheat	Increases in gluten concentrations in grain
Litke et al.	Agron Res, 16, 500-509	2018	Peterlauki research and Study Farm (Latvia)	Field	0, 60, 90, 120, 150, 180, 210, 240	Skagen variety	Increases in gluten concentrations in grain
Ma et al.	J Sci Food Agric, 89, 1213-1220	2008	Henan Agricultural University Experimental Satation (China)	Field	0, 90, 180, 270, 360, 450	Yumai and Lanko Aizao varieties	Increases in total gliadins concentrations in flour
Makowska et al.	Acta Sci Pol Techn Alimentaria, 7, 29-39	2008	Swadzim Experimental Station (Poland)	Field	0, 50, 100, 150	Durabon, Durabonus, Duraprimus and Rusticano varieties	Increases in gluten concentrations in flour

Martin et al.	New Zeal J Crop Hort Sci, 20, 273-282	1992	Lincoln Research Farm (New Zealand)	Field	0, 50, 100	Batten, Kotare, Oroua, Rongotea, Ruapuna and Tui varieties	Increases in total gliadins concentrations in flour
Morari et al.	Prec Agric, 19, 257-277	2018	Mira (Italy)	Field	70, 120, 130, 160, 180, 200, 240	Biensur variety	Increases in gluten concentrations in flour
Pechanek et al.	Cereal Chem, 74, 800-805	1997	Two different sites (Austria)	Field	0, 180	Three varieties: Capo, Renan and Lindos	Increases in total, α, ω and γ gliadin concentrations in flour
Peltonen and Virtanen	Ceral Chem, 71, 1-5	1994	Experimental Farm of Helsinki University (Finland)	Field	0, 110	Scandinavian, Kadett, Ruso and Reno wheat varieties	Increases in total proteins concentrations but not changes in gliadin concentrations in flour
Pepó et al.	Cereal Res Comm, 33, 825-832	2005	Hungary	Field	30-300	Winter wheat	Increases in gluten concentrations in flour
Pinilla-Quesada and Herrera-Floody	Idesia, 26, 77-81	2008	Chile	Field	0, 220, 250		Increases in gluten concentrations in flour
Plessis et al.	J Exp Bot, 64, 3627-3644	2013	France	Field	40, 60	Seedling from INRA	Increases in total gliadins concentrations in flour
Ralcewicz et al.	J Central Eur Agric, 10, 223-232	2009	Minokowo (Poland)	Field	0, 60, 90, 120	Zebra variety	Increases in gluten concentrations in flour
Rizzello et al.	Appl Environ Microbiol, 81, 3192-3204	2015	Field experimental Station of Mediterranean Agronomic Institute of Bari (Italy)	Field	30, 40, 50, 70	*Triticum turgidum* subsp. *durum*	Increases in total gluten concentrations in grain and flour
Rodrighero et al.	Acta Aci, 37, 175-181	2015	Brazil	Field	0, 50, 100, 150	Quartzo variety	Increases in gluten concentrations in grain
Tea et al.	Cereal Chem, 81, 759-766	2004	Experimental farm of INRA, Grignon, France	Field	40, 60, 120	Soissons variety	Increases in total gliadin concentrations in grain
Varga et al.	Acta Agro Hungarica, 55, 37-48	2007	Research field sation of Faculty of Agriculture (Croatia)	Field	0-194	Marija and Soissons varieties	Increases in gluten concentrations in grain
Wan et al.	J Exp Bot, 64, 161-168	2013	Rothamsted Research station (UK)	Field	100, 200, 350	Cordiale, Hereward, Istabraq, Malacca, Marksman and Xi 19 varieties	Increases in γ-gliadin gene expression
Wan et al.	An Bot, 113, 607-615	2014	Rothamsted Research station (UK)	Field	100, 200, 350	Cordiale, Hereward, Istabraq, Malacca, Marksman	Increases in ω-gliadin gene expression

						and Xi 19 varieties	
Wieser and Seilmeier	J Sci Food Agric, 76, 49-55	1998	Germany	Field	0, 40, 120, 180, 200	Dozent, Monopol, Rektor, Apollo, Ares, Astron, Basalt, Bussard, Herzog, Ignaz, Kanzler, Monopol, Obelisk, Sperber varieties	Increases of α/β -gliadin, ω- and γ-gliadins, total gliadins and gluten concentrations in flour
Wieser et al.	J Agric Food Chem, 56, 6531-6535	2008	Johann Heinrich von Thunen-Institute, Federal Research Institute for Rural Areas, Forestry and Fisheries, in Braunschweig, Germany	Field	84, 168	Batis variety	Increases of α/β -gliadin, ω- and γ-gliadins, total gliadins and gluten concentrations in flour
Wojtkowiak et al.	African J Agric Res, 8, 3778-3783	2013	Research Station of Warmia and Mazury University (Poland)	Field	80, 120	*Spring triticale* cv. Andrus	Increases of α/β -gliadin, no clear effects on ω- and γ-gliadins in grain
Wojtkowiak et al.	J Elementology, 19, 229-242	2014	Research Station of Warmia and Mazury University (Poland)	Field	80, 120	*Spring triticale* cv. Andrus	Increases of total gliadins concentrations in in grain
Wozniak and Makarski	J Elementology, 18, 297-305	2013	Uhrusk Experimental Station belonging to the University of Life Sciences in Lublin (Poland)	Field	90, 150	Opatka variety	Increases in gluten concentrations in grain
Yang et al.	Agronomy, 8, 257	2018	Fields research stations of Idaho and Monatana state Universities (USA)	Field	168, 224, 280	Spring wheat	Increases in gluten concentrations in flour
Xue et al.	J Plant Nutrit and Soil Sci	2015	Futterkamp and Sonke-Nissen-Koog Northern Germany	Field	220, 260	Tobak and Asano varieties	Increases in total gliadin and gluten concentrations in flour
Zecevic et al.	Genetika, 42, 465-474	2010	Grains Research Centre Kragujevac (Serbia)	Field	60, 90, 120		Increases in gluten concentrations in grain
Zhen et al.	J Cereal Sci, 69, 85-94	2016	China Agricultural University Research Center field station, Hebei province, China	Field	180, 240	Zhongmai variety	Increases in gluten concentrations in grain
Zheng et al.	Sci Rep, 8, 11928	2018	Chongzhou and Renshou experimental stations of	Field	0, 75, 150, 225	Shumai 969, Shumai 482, Chuannong 16	Increases of total, α/β -gliadin and ω-gliadins and gluten

			Sichuan Agricultural University, China			and Mianmai 51 varieties	concentrations, no clear effects on ω-gliadins in flour
Zörb et al.	Plant Soil, 327, 225-234	2010	Germany	Pot experiment	0.25, 1.0 and 2.5 g N/pot	Privileg variety	Increases of total gliadin concentrations