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Ancient wheat species and human health: Biochemical and clinical implications

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Abstract

Wheat is the major staple food in many diets. Based on the increase in worldwide mortality attributable to diet-related chronic diseases, there is an increasing interest in identifying wheat species with greater health potential, more specifically for improved anti-oxidant and anti-inflammatory properties. In particular, ancient varieties (defined as those species that have remained unchanged over the last hundred years) are gaining interest since several studies suggested that they present a healthier nutritional profile than modern wheats. This manuscript reviews the nutritional value and health benefits of ancient wheats varieties, providing a summary of all *in vitro*, *ex vivo*, animal and human studies that have thus far been published. Differences in chemical composition, and biochemical and clinical implications of emmer, einkorn, spelt, khorasan and various regional Italian varieties are discussed. Although many studies based on *in vitro* analyses of grain components provide support to the premise of a healthier nutritional and functional potential of ancient wheat, other *in vitro* studies performed are not in support of an improved potential of ancient varieties. In the light of existing evidence derived from *in vivo* experiments, the ancient wheat varieties have shown convincing beneficial effects on various parameters linked to cardio-metabolic diseases such as lipid and glycaemic profiles, as well as the inflammatory and oxidative status. However, given the limited number of human trials, it is not possible to definitively conclude that ancient wheat varieties are superior to all modern counterparts in reducing chronic disease risk.

Keywords: Ancient wheat; Old wheat; Health; Review; Nutrition; Grain

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Contents

1. Introduction

Consumed by billions of people, wheat (*Triticum spp.*) is the major staple food in many diets, providing a large proportion of the daily energy intake. It is a cereal grain derived originally from the Levant

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region, but is currently cultivated worldwide. In 2016, the global production of wheat exceeded 749 million tonnes, making it the second most-cropped cereal after maize. About 95% of the wheat produced is *Triticum aestivum*, a hexaploid species usually called "common", "bread" or "soft" wheat [1]. The remainder is primarily comprised of *Triticum durum* or "durum" wheat, a tetraploid species predominantly used for making pasta. Within the context of a balanced diet, wheat represents a healthy source of multiple nutrients, dietary fiber and bioactive compounds, especially if consumed as a whole-grain. Regular whole-grain consumption has been extensively associated with reduced levels of the most relevant risk factors for cardio-metabolic diseases such as total and LDL-cholesterol, triglycerides, blood glucose, blood pressure and body mass index [2]. Recently, a meta-analysis confirmed the association between the consumption of whole grains and a substantial and significant decreased risk for cardiovascular disease, cancer, and all cause and cause specific mortality [3].

The mechanisms by which wheat confers protective effects on human health are attributed to the physical properties and structure of grains (granular size of semolina, amount and type of fiber, quantity and quality of phytochemicals, amylose and amylopectin content) [4]. Given the increased worldwide mortality attributable to nutrient- or diet-related chronic diseases, over the last years, there is currently a great interest in improving wheat to ameliorate health potential [5]. In particular, ancient wheat species have gained increasing attention since several studies have suggested that they could present a healthier and a better nutritional profile than modern wheats, by providing more vitamins, minerals and nutraceutical compounds [6–8]. In addition, given that ancient varieties are cultivated with environmentally sustainable organic agriculture, and given the current concerns for environmental sustainability, these varieties may represent an alternative potential [9].

The aim of this review is to present the available information on ancient wheat species against the backdrop of recent findings, by reporting all the results derived from *in vitro* cell models, *ex vivo* and animal studies, as well as *in vivo* human intervention trials.

2. Ancient wheat species

Although there is no precise definition, it is generally accepted that ancient wheat has remained unchanged over the last hundred years. In contrast, modern species have been extensively modified and subject to cross-breeding in what is commonly referred to as the "Green Revolution". This term was developed to refer to a set of research and technological transfer initiatives that occurred between the 1930s and the late 1960s. The Green Revolution was initiated by Strampelli, who was among the first, in Europe and in the World, to systematically apply Mendel's laws to traits such as rust resistance, early flowering and maturity and short straw. As a consequence, Italian wheat production doubled, an achievement that during the fascist regime was referred to as the "Wheat Battle" (1925–1940) [10]. After the Second Word War, some of Strampelli's wheat varieties were used as parents in breeding programmes in many countries in a phase of the Green Revolution, defined as Norman Borlaug's Green Revolution. This phase was instrumental in the development of the high-yielding varieties [10]. Thereafter, during the 1960s, research was concentrated on improving the storage protein quality, thereby increasing the technological properties. Agronomists bred cultivars of maize, wheat, and rice that were generally referred to as "high-yielding varieties" based on a higher capacity for nitrogen-absorption than other varieties. High levels of nitrogen in the soils causes the lodging of wheat before harvest. Therefore, semi-dwarfing genes were bred to improve to reduce both lodging and the maturation cycle. The principle results of this revolution were the development of modern varieties characterized by higher yield, a reduced susceptibility to diseases and insects, an increased tolerance to environmental stresses, a homogeneous maturation (to optimize harvest) and a higher gluten content (to improve bread and pasta quality). Whilst these intensive breeding programs helped to increase production and technological quality, a concomitant decrease in genetic variability as well as a gradual impoverishment of the nutritional and nutraceutical properties of the wheat occurred, mainly determined by the complete replacement of ancient local breeds with modern varieties.

The most common ancient wheat species commercially available are einkorn (*Triticum monococcum*), emmer (*Triticum dicoccum*), khorasan (*Triticum turgidum ssp. turanicum*) and spelt (*Triticum spelta*). In addition, there are several heritage cultivars of both *Triticum aestivum* and *Triticum durum* that remained unchanged over the years, namely *Russello, Senatore Cappelli, Timilia or Tumminia and Urria (Triticum durum),* as well as *Autonomia B, Frassineto, Gentil Rosso, Inallettabile, Maiorca, Sieve, Solina, and Verna* (*Triticum aestivum).*

Einkorn wheat (*Triticum monococcum*) was one of the first crops domesticated approximately 12,000 years ago in the Near East, alongside emmer wheat (*Triticum dicoccum*) [11]. Typically, einkorn was cultivated on marginal agricultural land, being able to survive in harsh environments and poor soils where other species of wheat could not survive. Spelt wheat (*Triticum spelta*) represents a hexaploid series of the *Triticum* genome constitution, which is characterized by a great adaptation to a wider range of environments. Khorasan wheat (*Triticum turgidum ssp. turanicum)* is an ancient free-threshing ("naked") grain type with an appearance similar to that of common wheat. In recent years, given that the ancient species are generally cultivated in organic or traditional low-input farming, the trend towards low-impact and sustainable agriculture, combined with increased attention to the nutritional aspects of food, has led to the rediscovery of regional forgotten crops, especially in Italy. Notwithstanding their generally lower yields, a significant recovery in the cultivation of these ancient and local wheat cultivars is presently underway.

2.1. Phytochemical composition of ancient vs modern wheat species

There is extensive literature coverage reporting that the phytochemical composition of wheat is strongly influenced by genotype, environment and genotype-environmental interactions. It is noteworthy that this provides a challenge when comparing varieties, as genetic potential can only be evaluated accurately by performing cultivar comparisons within the same environment. In this section, a brief overview of the information will be provided, given that the nutritional composition of spelt [6], einkorn [7], and khorasan [8] have been subjects of previous reviews. Recently, a comprehensive review was presented by Shewry and Hey, in which data from the HEALTHGRAIN project were combined with data from other studies in order to determine whether spelt, emmer and einkorn differed from modern wheat in the contents and composition of bioactive components [12]. The HEALTHGRAIN project is the largest study comparing ancient and modern wheat in the same laboratories using the same methodology of evaluation [12]. Within this project, dietary fiber and major groups of phytochemicals in 5 lines of einkorn, emmer and spelt, and 161 modern wheat cultivars were investigated. Differences in concentration of fiber and phytochemicals reported in the HEALTHGRAIN project are shown in Table 1.

Available data show that ancient wheat cultivars are generally lower in some components such as dietary fiber, and higher or characteristic in other components like polyphenols. Nevertheless, studies on the bioactive components of wheat (including minerals, trace elements, vitamins, carotenoids, polyphenols and alkylresorcinols), predominantly contained in the outer layers and in the germ of the wheat kernel, reported wide variability in content, which was dependent on genetic factors, growing season environments and locations [13]. Starch composition, as well as protein content and composition, are key determinants of texture, as well as nutritional and technological quality traits in wheat. Compared with soft wheat, einkorn showed a

Table 1
Contents of fiber and phytochemicals in wheat cultivars in comparison to modern wheat (data obtained from the HEALTHGRAIN study [12])

Component (DM)	Fiber %	Total phenolic acids μg/g	Folate μg/g	Phytosterols μg/g	Alkylresorcinols μg/g	Total tocols μg/g	Ferulic acid μg/g	α-tocopherol μg/g
Einkorn	11.0 (9.3–12.8)	615 (449–816)	0.58 (0.43–0.68)	1054 (976–1187)	595 (545–654)	57.0 (42.7–70.2)	298 (207–442)	9.1 (7.0–12.1)
Emmer	9.8 (7.2–12.0)	779 (508–1161)	0.69 (0.52–0.94)	857 (796–937)	581 (531–714)	36.4 (29.0–57.5)	476 (323–711)	7.7 (6.4–8.6)
Spelt	12.0 (10.7–13.9)	579 (382–726)	0.58 (0.50–0.65)	928 (893–963)	605 (490–741)	46.2 (40.2–50.6)	365 (223–502)	11.0 (9.9–12.5)
Modern	15.1 (11.5–18.3)	657 (326–1171)	0.56 (0.32–0.77)	844 (241–677)	432 (421–677)	49.8 (27.6–79.7)	396 (181–742)	13.5 (9.1–19.9)

Data are expressed as mean (range).
DM = dry matter.

lower content of both total and resistant starch (mean value: 655 *vs* 685 g/kg dry matter (DM) and 25.6 *vs* 30–88 g/kg DM respectively) [7]. However, the amount of amylose molecules, that are digested more slowly, was higher than the amount of amylopectin molecules, thereby lowering both glucose and insulin levels in the blood after meals [14] and maintaining satiety for longer periods [15]. By evaluating the average protein content, einkorn protein values were 59% higher than those of modern wheat [16], but the bread-manufacturing quality of storage proteins were poor, making it better suited to the preparation of cookies or pasta [17]. The comparative analysis of lipids and fatty acid composition in einkorn and soft wheat germ revealed a higher content of lipids (+50%) in einkorn, with a greater proportion of monounsaturated fatty acids (+53%), and lower polyunsaturated (−8%) and saturated fatty acids (−21%) [16].

With respect to phytochemicals, einkorn showed the highest concentration of phytosterols and tocols (1054 and 57 μg/g DM respectively), but this difference was mostly marked in the HEALTHGRAIN dataset [12]. In addition, einkorn, khorasan wheat and emmer wheat cultivars showed the highest content of total carotenoids (2.26, 6.65 and 8.23 μg/g DM respectively) and lutein (7.28, 4.9 and 2.7 μg/g DM), the major carotenoid with respect to all the other species [18,19]. Of interest, several lines of einkorn showed lutein values from three to eight-fold higher than soft wheat and two-fold greater than those for durum wheat. Some authors suggested that the higher carotenoid content in einkorn-made products could be a result of lower processing losses, linked to lower lipoxygenase activity [7].

The smaller size of ancient wheat species increases the ratio of bran to flour, thereby determining a concentration effect of trace elements present in higher proportion in the bran fraction. A comparison of ancient and modern wheat species highlighted that emmer, einkorn and spelt mainly differed from other species for higher concentrations of magnesium, phosphorus, selenium and zinc [20]. In the HEALTHGRAIN project 150 lines of bread wheat representing diverse origin and 25 lines of durum, spelt, einkorn and emmer wheat species were analyzed for variation in micronutrient concentrations in grain. Spelt, einkorn and emmer wheats appeared to contain higher selenium concentration in grain than bread and durum wheats [21]. Several authors have also described higher concentrations of iron, zinc, manganese and cupper in einkorn, detecting a large genotypic variation [22]. In contrast to minerals, the phytic acid content tends to be 40% lower in spelt than in modern wheat [6].

Although much information on emmer, einkorn and spelt have been reviewed previously, less information is available comparing the functional components between heritage and durum cultivars and their modern counterparts. Interestingly, the most relevant scavenging effect was found for the variety *Verna*, in comparison to 4 additional varieties and 1 modern variety respectively [23]. Results showed that antioxidant activity is mostly influenced by flavonoid (both bound and free) content and by the ratio flavonoids/polyphenols. However, Laus and colleagues measured anti-oxidant activity in a large number of Italian ancient and modern varieties, and reported minimal differences claiming that modern varieties had not lost potential over the last 100 years of breeding [24]. Although the overall amount of total phytochemical compounds in both durum and soft heritage cultivars generally appears be largely comparable to that of modern species, the qualitative phytochemical profile was shown to differ [25,26]. Through the use of liquid chromatography coupled with time-of-flight mass spectroscopy analysis, both heritage, durum and soft wheat varieties, were shown to be comprising a higher number of phenolic isoforms and a greater total of unique isoforms than the commercial varieties analyzed. The 6 soft heritage genotypes analyzed (*Gentil Bianco, Gentil Rosso, Frassineto, Marzuolo d'Aqui, Marzuolo Val Pusteria,* and *Verna*) were characterized by an elevated number of isoforms for apigenin-6-C-arabinoside-8-Chexoside, vicenin-2, glycosylated pinosylvin, dihydroferulic acid and procyanidin B-3 [26]. Of interest, *Gentil Rosso* was characterized by the unique compound double glycosilated pinosylvin, whereas *Verna* was characterized by isovitexin-2″-O-rhamnoside and orientin/isoorientin [26]. Of the 2 heritage durum varieties analyzed, *Senatore Cappelli* was showing to have 3 unique compounds (vanillin, pinosylvin, sinapic acid) [25]. Included in the study, the ancient KAMUT® khorasan wheat was analyzed and showed to contain 2 unique compounds namely coumarin and ferulic acid isomer. These collective results indicate that ancient/heritage wheats may represent a valuable source of biodiversity, especially as regards phenolic compounds [25,26].

3. Ancient wheat species: *in vitro*, *ex vivo* and animal studies

3.1. In vitro studies

Cell model systems permit a more rapid and extensive screening to evaluate antioxidant and anti-inflammatory properties. Few *in vitro* studies have reported positive anti-oxidant and anti-inflammatory effects for ancient Italian wheat varieties and for the khorasan wheat variety. From the evaluation of cell viability in primary cultures of neonatal rat cardiomyocytes incubated with wheat extracts, the cultivar, *Verna* (irrespective of dose) was the most effective antioxidant, whilst the modern variety *Palesio* was the least [23]. In addition, supplementation of human HepatomaG2 (HepG2) cells with the bio-accessible fraction of digested cookies made from various whole-grain flour preparation showed a greater reduction in reactive oxygen species-mediated fatty acid peroxidation by the KAMUT® khorasan wheat in comparison to Italian-grown khorasan wheat and *Claudio* [27].

The effects on gut bacteria was also evaluated. The soluble dietary fiber fraction, which provides fermentative substrates for colon bacteria, is mainly constituted by resistant starch and non-starch polysaccharides, with principal effects on glucose and lipid absorption, gut bacterial composition and anti-cancer activity [28]. The soluble dietary fiber fraction from various ancient and modern durum wheats, as potential prebiotic substrates for the selective proliferation of *B. Pseudocatenulatum* B7003 and *L. Plantarum* L12 was evaluated. Among the cultivars tested, the ancient KAMUT® khorasan grain and the modern variety Solex were both shown to have the most promising potential to promote the growth of both tested strains *in vitro* [28].

Table 2
Animal model trials to evaluate the impact of wheat (ancient and modern cultivars) to improve risk factors related to nutrient-related chronic diseases

Reference	Animal model Information	Disease	Study design	Ancient grain + processing conditions	Control grain + processing conditions	Main results (ancient *vs.* control)
Gianotti et al., 2011 [29]	Wistor rats n: 64 m/f: 64/0 Age: 30 days	Clinically healthy	Parallel trial of 7 wk with 4 interventions. After 7 wk, each group divided into 2 subgroups. One subgroup administered DOX for 36 h	*Triticum turgidum* WG KAMUT® brand khorasan Bread preparation 2 groups 1: Sourdough (SKB) 2: Baker's Yeast (KB) Bread cut in cubes administered to animals *ad libertum*	Positive control: WG *Triticum durum* bread prepared with baker's yeast. Negative control: standard rat chow. Administered to animals *ad libertum*	Reduction: ROM. ROM also lower in SKB than KB in blood plasma. DOX administration caused significant increase in ROM in controls with no change for SKB and KB. DOX administration negatively affected TAC in controls, with no effect for SKB and KB.
Benedetti et al., 2012 [30]	Wistor rats n: 64 m/f: 64/0 Age: 30 days	Clinically healthy	Parallel trial of 7 wk with 3 interventions. After 7 wk, each group divided in 2 subgroups. One subgroup administered DOX for 36 h	*Triticum turgidum* WG KAMUT® brand khorasan Bread preparation 2 groups 1: Sourdough (SKB) 2: Baker's Yeast (KB) Bread cut in cubes administered to animals *ad libertum*	WG *Triticum durum* bread prepared with baker's yeast. Bread cut in cubes administered to animals *ad libertum*	No histological signs of inflammation in the livers for SKB and KB. After DOX administration increased liver levels of glutathione, peroxidase and thioredoxin reductase activity, GSH, MDA and AOPP in controls, with no change for SKB and KB.
Carnevali et al., 2014 [31]	Wistor rats n: 24 m/f: 24/0 Age: 30 days	Clinically healthy	Parallel trial of 7 wk with 2 interventions. After 7 wk, each group divided in 2 subgroups. One subgroup administered DOX for 36 h	*Triticum turgidum* WG KAMUT® brand Khorasan pasta (KP) administered to animals *ad libertum*	WG *Triticum durum* pasta (CP) administered to animals *ad libertum*	Confirmation of results reported by Benedetti et al., 2012. Gut metabolome increases in succinate for KP and increases in ethanol, propionate and putrescine for CP. Negative histological effects on duodenum morphology of control (flattened mucosa, shortening of the villi, high lymphocyte infiltration) for CP with no effects for KP
Thorup et al., 2015 [32]	Zucker rats n: 40 m/f: 40/0 Age: 7 wk	Zucker diabetic fatty rats (inbred model resemblinghuman T2DM)	Parallel design trial of 9 wk with 5 interventions	1:*Triticum dicoccum* (Emmer) 2: *Triticum monococcum* (Einkorn) 3: *Triticum spelta* WG of the above (1,2,3) were included in rat chow at a concentration of 50%. Administered to animals *ad libertum*	Positive control: Refined *Triticum aestivum* Negative control rye 50% WG mixed with 50% rat chow. Administered to animals *ad libertum*	Reduction: total plasma cholesterol, LDL-cholesterol, development and progression of diabetes due to down-regulation of various hepatic genes (emmer, einkorm and rye). Improvement: glycemic index (spelt and rye).
Gorelick et al., 2017 [33]	Rats n: 50 m/f: 0/50 Age: 6 wk	Non-Obese Diabetic (NOD) rats	Parallel design trial of 72 days with 5 interventions	1:*T. aestivum,* 2:*T. turgidum ssp. dicoccoides* 3. *T. turgidum ssp. dicoccum.* WG of the above (1,2,3) were included in standard rat diet at a concentration of 20%. Administered to animals *ad libertum*	1:Low diabetogenic (non-wheat diet) 2: *T. aestivum* WG of *T. aestivum,* was included in standard diet at a concentration of 20% Administered to animals *ad libertum*	No onset of diabetes for 3 ancient wheats. Diabetes onset in both controls. Reduction: cholesterol, glucose, IFN-gamma in all ancient landraces. Increases: insulin and Il-10 in all ancient landraces.

AOPP = advanced oxidation protein product; DOX = doxorubicin (inducer of oxidative stress); GSH = glutathione; IL- interleukin; INF-gamma = Interferon-gamma; m/f = male/female; MDA = malondialdehyde; ROM = reactive oxygen molecules; TAC = total anti-oxidant capacity; T2DM = Type 2 Diabetes Mellitus; WG = whole grain.

3.2. Studies on animal models

To the best of our knowledge, only 5 studies aimed at comparing the effects of ancient wheat cultivars *versus* modern wheat cultivars have been reported, all using rat models. The 5 studies reported are presented in Table 2. The first 3 studies reported improvements in antioxidant and inflammation parameters in the blood plasma [29] and hepatic tissues [30,31] in clinically healthy rats fed with *Triticum turgidum* KAMUT® brand khorasan in comparison to modern wheat varieties. In all 3 studies, rats were submitted to exogenous oxidative stress induced from an intraperitoneal injection of DOX (doxorubicin). The histologic evaluation of the hepatic tissue of rats showed complete protection from the onset of the DOX-induced inflammation when provided a diet of khorasan wheat bread [30]. The histological evaluation of the duodenum and spleen of rats fed with modern durum pasta for 7 weeks provided an inflammatory profile that resembling wheat sensitivity, whilst rats fed with khorasan wheat pasta showed normal histological characteristics. In addition, khorasan wheat pasta-fed rats showed a lower oxidative status under basal conditions and an improved response to exogenous oxidative stress.

Moreover, modifications in the fecal metabolite profiling, provided distinctive differences between the two experimental diets, suggesting the development of a different microbiota in the two groups [31].

The remaining 2 studies reported the use of rat models suited to studying diabetes, and included Zucker diabetic fatty rats [32] and non-obese diabetic rats [33] to compare effects induced by either ancient wheat cultivars or their modern counterparts. In the study of Thorup et al. (2015), the effects of spelt, emmer and einkorn diets on the glycaemic control, plasma lipid profile, hepatic genes and acute glycaemic responses in Zucker diabetic fatty rats were examined. After a 9-week dietary intervention period, the development and progression of type 2 diabetes mellitus was less pronounced for the group fed with ancient wheat varieties compared with modern wheat [32]. As suggested by the authors, this might be attributable to a down-regulation of the PPAR-α, GLUT2, SREBP-1c and SREBP-2, key regulatory genes involved in glucose and fat metabolism. In the most recent study of Gorelick et al. (2017), it was shown that rats receiving wheat from local landraces or ancestral species displayed a lower incidence of Type 1 diabetes mellitus (T1DM) and related complications compared to animals fed a modern wheat variety. This study is the first to suggest that ancient wheat sources may lack T1DM linked epitopes, thus reducing the incidence of T1DM [33].

3.3. Studies on immune toxicity

Several studies have explored the immune toxicity profile for celiac disease of ancient wheat cultivars with respect to modern varieties. *In vitro* and *ex vivo* studies have provided conflicting results thus far.

Data derived from a limited number of studies indicate that, on average, ancient wheat, though not all the varieties, express lower levels of immunoreactive T-cells [34]. Research using protein extracts from both ancient and modern wheat varieties demonstrated a large variation in immune responses depending on genotype, as measured by epitope-specific T-cell responses. The cytotoxicity of spelt was found to be similar to *Triticum aestivum* [35], whilst emmer generally appeared to be less immunoreactive, but more immunoreactive than einkorn [34]. Despite lower reactivity, einkorn and emmer nonetheless produced reactions in 25% to 38% of tested patients' T cells [36]. Such variability underscores the fact that wheat varieties are safe for individuals with celiac disease.

Moreover, when comparing spelt and *Triticum aestivum*, there is a similar inhibition of cell growth, activation of apoptosis, release of nitric oxide, release of tissue transglutaminase, and alteration of transepithelial electrical resistance on Caco-2/Tc7 and K562 (S) cell agglutination [37]. Regarding emmer, T-cell activity and the release of interferon-gamma from 4 children with celiac disease differed widely after exposure to 9 landraces of emmer and *Triticum aestivum* [38], confirming that individuals with celiac disease react differently to the gluten profiles of ancient wheats. Gianfrani and colleagues compared the immune toxicity of 2 lines of einkorn wheat with modern wheat varieties by using celiac patient–derived gliadin reactive T-cell lines and organ cultures of jejunal biopsies [39]. Their findings showed that gliadins from both einkorn lines can stimulate celiac mucosal polyclonal T-cell lines with a magnitude of responses comparable to common wheat gliadins, as indicated by interferon-gamma production and cell proliferation. On the other hand, a subsequent paper investigating how *in vitro* gastro-intestinal digestion affects the immune toxic properties of gliadin from einkorn (compared to modern wheat), demonstrated that gliadin proteins of einkorn are sufficiently different from those of modern wheat, thereby determining a lower immune toxicity following *in vitro* simulation of human digestion [40].

A recent study evaluating the *in vitro* chemokine response of peripheral blood mononucleated cells from non-celiac gluten sensitivity patients to both modern and ancient wheat genotypes concluded that modern grains can over-activate the production of CXCL10, a chemokine produced predominantly by neutrophils, macrophages and resident cells with an active role in triggering tissue inflammation [41].

Although there is insufficient evidence to suggest that ancient wheat varieties prevent gluten-related disorders, several studies have shown that a diet based on less-immunoreactive wheat products, with fewer amounts and types of reactive prolamins and fructans, may help in the improvement of gastrointestinal and/or systemic symptoms of some auto-immune or chronic diseases (eg, irritable bowel syndrome, *etc.*) [34]. These less-immunoreactive varieties, like einkorn, may be good targets for slowing the development of disease in populations genetically predisposed to celiac disease and other wheat sensitivities [42].

4. Ancient wheat species and human health: Human studies and clinical implications

Despite the identification of various groups of bioactive components in wholegrain cereals with favorable *in vitro* anti-oxidant capacity, evidence for a comparable function *in vivo* is lacking [9]. Although research on cell model systems support the premise that bioactive anti-oxidant compounds act *via* different, complex and synergistic mechanisms *in vivo*, these effects have not been convincingly validated by human intervention trials.

To the best of our knowledge, only 13 studies which were specifically designed to compare the effects of ancient and modern wheat cultivars on humans have been published [43–45]. As presented in Table 3, six were conducted on clinically healthy individuals, 2 on diabetic patients, 2 on celiac patients, 1 on irritable bowel syndrome participants, 1 acute coronary syndrome patients and 1 Baker's asthma or wheat allergy sufferers. Ten studies were conducted in Italy [45,47–55], 7 of which investigated the potential functional efficacy of ancient wheat on circulatory parameters, addressing risk factors of oxidative stress/pro-inflammatory markers together with traditional risk measurements. Although concrete functional benefits are difficult to ascertain from random individual human trials, since they are subject to differences and/or limitations in experimental design, participant number and participant characteristics in the case of parallel arm studies, results unanimously suggest that the consumption of products made with ancient wheat varieties ameliorate not only pro-inflammatory/anti-oxidant parameters (where investigated) but also glycaemic and lipid status. The same effects were not evident after the consumption of products made from commercially available modern varieties. Effects and possible mechanisms of ancient wheat components on glycaemic, lipid and mineral profiles, as well as on inflammatory and oxidative states are shown in Fig. 1.

Interesting information on the functional potential of the ancient soft wheat variety, *Verna* [45,55], and khorasan wheat under the brand name KAMUT® [48,51,52,54] is available from multiple studies using the same experimental design. In all instances, randomized cross-over trials were implemented, providing added comparative strength in that each participant consumed both ancient and modern wheat products during the respective dietary interventions. The *Verna* variety repeatedly imposed a significant beneficial effect on total cholesterol, LDL-cholesterol, as well as for blood glucose [45,55]. The collective studies of Sofi et al. (2010) and Sereni et al. (2016), report the same effects on human subjects, irrespective of whether the grain had been cultivated in organic or conventional systems, implicating a potential genetic functionality of *Verna* variety that transcended cultivation method. For the first time, a wheat variety, in this case *Verna*, was reported to determine an increase in circulating endothelial progenitor cells, whereas a significant worsening effect was evident after consumption of the modern variety *Blasco* [55]. The same study showed that two additional ancient varieties did not have the

Table 3
Human intervention trials to evaluate the impact of wheat (ancient and modern cultivars) to improve risk factors related to nutrient-related chronic diseases

Reference	Subject Information	Disease	Study design	Ancient grain + processing conditions	Control grain + processing conditions	Main results (ancient *vs.* control)
Yenagi et al., 2001 [43]	n: 22 m/f: 8/14 Age: 42–71 yrs. BMI: 22.4±2.4 (m) 23.2±3.1 (f)	Type 2 Diabetes Mellitus	Total duration 6-wk. parallel trial	*Triticum dicoccum* (Emmer) WG flour unleavened bread (200-300 g/day)	*Triticum aestivum* WG flour unleavened bread (200-300 g/day)	Reduction: Total cholesterol, triglycerides, LDL-cholesterol
Bakhoj et al., 2003 [44]	n: 11 m/f: 11/0 Age: 25±2 yrs. BMI: 23±4	Clinically healthy	one single meal test	3 types of WG *Triticum monococcum* (Einkorn) wheat bread: A: honey-salt leavened (127 g) B: crushed WG (129 g) C: commercial leavening (127 g)	*Triticum aestivum* Commercial Danish bread (118 g)	Reduction: GIP only when the einkorn bread was processed through honey–salt leavened and as a crushed WG bread
Sofi et al., 2010 [45]	n: 20 m/f: 11/9 Age: 21–61 yrs. BMI: 26.1±2.5 (m) 24.8±4.9 (f)	Clinically healthy	10-wk. RCT with 10-wk. wash-out period Total duration: 7.5 months	*Triticum aestivum* Verna semi-WG heritage variety. Sourdough artisan bread processing (150 g/day)	*Triticum aestivum* mixed modern semi-WG varieties. Commercial bread processing (150 g/day)	Reduction: Total cholesterol, LDL-cholesterol, IL-8, whole blood viscosity, erythrocyte filtration
Armentia et al., 2012 [46]	n: 66 m/f: 45/21 Age: 28.6±12.9 yrs. BMI: Absent	Baker's asthma or wheat allergy	Oral and bronchial challenges, prick test	*Triticum spelta* grain extract (5 mg protein/ml for oral, bronchial and prick tests)	*Triticum aestivum* (cultivar *Astral*) grain extract. (5 mg protein/ml for oral, bronchial and prick tests)	Reduction: Wheal area and percentage of positive challenge tests
Ghiselli et al., 2013 [47]	n: 20 m/f: 11/9 Age: 21–61 yrs. BMI: 26.1±2.5 (m) 24.8±4.9 (f)	Clinically healthy	10-wk. RCT with a 10-wk. wash-out period. Total duration: 7.5 months	*Triticum durum* semi-WG Senatore Cappelli heritage variety. Artisan pasta processing (low T slowing drying) (70 g/day)	Mixed modern semi-WG *Triticum durum* wheat varieties. Commercial pasta (70 g/day)	Reduction: Total cholesterol, whole blood viscosity, RBC deformability
Sofi et al., 2013 [48]	n: 22 m/f: 8/14 Age: 50.5±12 yrs. BMI: 23.3±3.6	Clinically healthy with high cardiovascular risk profile	8-wk. RCT with a 8-wk. wash-out period. Total duration:6 months	*Triticum turgidum* KAMUT® brand khorasan semi-WG wheat Artisan pasta processing (low T slow drying) (70 g/day). Artisan sourdough preparation for bread (150 g/day). Biscuits and crackers- artisan bakery	Mixed modern semi-WG *Triticum durum* and *Triticum aestivum* wheat varieties Artisan pasta processing (low T slow drying) (70 g/day). Artisan sourdough preparation for bread (150 g/day). Biscuits and crackers- artisan bakery	Reduction: Total cholesterol, LDL-cholesterol, glucose, TBARs, TNFα, IL-6, IL-12, VEGF Increase: K^{+}, Mg^{2+}
Zanini et al., 2013 [49]	n: 12 m/f: 4/8 Age: 44.5±10 yrs. BMI: Absent	Celiac disease on GFD	Cross-over trial with a single dose of gluten on day 0, 14, 28	*Triticum monococcum* (Einkorn) wheat 2.5 g of wheat flour in gluten-free pudding	*Amygluten* (pure gluten) and *rice* 2.5 g of gluten in gluten-free pudding	No significant conclusions
Taneyo Saa et al., 2014 [50]	n: 30 m/f: 4/26 Age: 37±7 yrs. BMI: Absent	Clinically healthy	parallel trial with 3-month total duration	*Triticum turgidum* KAMUT® khorasan WG Processing conditions: Absent 118 g/day pasta 88 g/day baked products	*Triticum durum* WG wheat Processing conditions: Absent 118 g/day pasta 88 g/day baked products	Increase: SCFA, phenol compounds, and an increase in health-promoting mutualists of the gut microbiota
Sofi et al., 2014 [51]	n: 20 m/f: 7/13 Age: 18–59 yrs. BMI: 22.3±4.1	Irritable Bowel Syndrome (moderate)	6-wk. RCT with a 6-wk. wash-out period. Total duration:4.5 months	*Triticum turgidum* KAMUT® brand khorasan semi-WG wheat. Artisan processing conditions for pasta and bread as in Sofi et al., 2013	Mixed modern semi-WG *Triticum durum* and *Triticum aestivum* wheat varieties. Artisan processing conditions for pasta and bread as in Sofi et al., 2013	Reduction: IL-6, INF-gamma, IL-ra, Il-4, Il-17, MCP-1, VEGF Improvement: gastrointestinal symptoms (abdominal distention, bloating, tiredness, pain and quality of life)
Whittaker et al., 2015 [52]	n: 22 m/f: 13/9 Age: 47–75 yrs. BMI: 26.9±4.4	Acute coronary syndrome	8-wk. RCT with a 8-wk. wash-out period. Total duration:6 months	*Triticum turgidum* KAMUT® brand khorasan semi-WG wheat. Artisan processing conditions for pasta and bread as in Sofi et al., 2013	Mixed modern semi-WG *Triticum durum* and *Triticum aestivum* wheat varieties. Artisan processing conditions for pasta and bread as in Sofi et al., 2013	Reduction: Total cholesterol, LDL-cholesterol, glucose, insulin, TNFα Increase: Mg^{2+}
Zanini et al., 2015 [53]	n: 7 m/f: 1/6 Age: 37±7.3 yrs. BMI: 22.8±3.1	Celiac disease in remission for 1 yr. on GFD	Total duration 60-day intervention trial	*Triticum monococcum* (Einkorn) wheat (100 g/day) in the of water biscuits	None	Increase: villous atrophy and recurrence of dermatitis herpetiformis
Whittaker et al., 2016 [54]	n: 21 m/f: 14/9 Age: 64±10.9 yrs. BMI: 27.9±4.7	Type 2 Diabetes Mellitus	8-wk. RCT with a 8-wk. wash-out period. Total duration:6 months	*Triticum turgidum* KAMUT® brand khorasan semi-WG wheat. Artisan processing conditions for pasta and bread as in	Mixed modern semi-WG *Triticum durum* and *Triticum aestivum* wheat varieties. Artisan processing conditions	Reduction: Total cholesterol, LDL-cholesterol, glucose, insulin,

Table 3 (*continued*)

Reference	Subject Information	Disease	Study design	Ancient grain + processing conditions	Control grain + processing conditions	Main results (ancient *vs.* control)
				Sofi et al., 2013	for pasta and bread as in Sofi et al., 2013	monocyte and granulocyte ROS production, VEGF, IL-1ra
Sereni et al. 2016 [55]	n: 45 m/f: 32/13 Age: 25–75 yrs. BMI: 25.5±4.4	Clinically healthy	8-wk. RCT with 3 consecutive interventions 1: Verna in organic or conventional agriculture 2: Blasco 3: Gentil Rosso or Autonomia B	*Triticum aestivum* 1) Verna heritage variety 2) Gentil Rosso and Autonomia B heritage varieties. Processing conditions: not presented (bread: 80 g/day)	*Triticum aestivum* Blasco wheat variety Processing conditions: not presented	Reduction: Total cholesterol, LDL-cholesterol, glucose Increase: Circulating endothelial progenitor cells only after Verna consumption

BMI = body mass index (kg/m^{-2}); GFD = gluten free diet; GIP = glucose-dependent insulinotropic polypeptide; IL = interleukin; INF-gamma = Interferon-gamma; LDL = Low-Density Lipoprotein; m/f = male/female; MCP-1 = Monocyte Chemotactic Protein-1n = number; RBC = red blood cell; RCT = randomized cross-over trial; ROS = Reactive Oxygen Species; SCFA = short chain fatty acids; TBARs = thiobarbituric acid reactive substances; TNFα = Tumor Necrosis Factor alpha; VEGF = Vascular Endothelial Growth Factor; WG = whole grain.

same functional strength as *Verna*, and of the two, *Gentil Rosso* performed better than *Autonomia B* [55].

Four separate human intervention trials [48,51,52,54] provide additional support for the functional potential of KAMUT® khorasan wheat. Collectively, the number of participants superseded the limitation of each individual study, and cross-comparisons between studies were feasible given that the experimental duration of each individual study was equivalent. Of relevance, the populations analyzed covered a wide spectrum, including healthy individuals at risk for cardiovascular disease, otherwise healthy participants with irritable bowel syndrome, and two chronic disease populations on drug prevention therapy, namely acute coronary syndrome and type 2 diabetes mellitus, respectively. During each dietary intervention, participants "replaced" all other cereals with either the KAMUT® khorasan wheat or modern products, which in turn, were respectively prepared from semi-wholegrain semolina or flour (organically grown and processed), using identical artisan-based transformation procedures. Given that lifestyle habits and medicinal therapy were maintained constant throughout each trial, the beneficial changes observed were attributable to the replacement diet with minimal interference by extraneous factors. The functional effects, attributable only to KAMUT® khorasan wheat, impacted on selected glycemic, lipid, oxidant and inflammatory parameters, also evident in the chronic disease populations notwithstanding medicinal therapy.

Regarding the risk markers pertaining to low-grade inflammation, for which there is very little information, only through a collective cross-examination of a wide spectrum of populations, it was possible to assess effects of KAMUT® khorasan wheat on specific underlying risk parameters. Tumor Necrosis Factor-α (TNF-α) is a potent protagonist in the induction of pro-inflammatory gene expression [56]. Of great relevance, there appears to be a threshold baseline level of TNF-α in human subjects above which khorasan wheat products induce a significant decrease in TNFα, [48,52] and below which there is no significant effect [51,54]. The same trend is evident for Interleukin (IL)-6. Both TNF-α and IL-6 are well-known risk factors in the development of cardiovascular disease and type 2 diabetes mellitus, and "healthy individuals at significant risk" may benefit from the consumption of an ancient wheat with enhanced functional potential within an already established diet plan such as the Mediterranean diet. The pleiotropic effects of medicinal therapy, were likely responsible for the notably lower baseline levels of TNF-α and IL-6 in the type 2 diabetes mellitus patients, and in IL-6 levels in acute coronary syndrome patients [52,54], and no additional impact was provided by the consumption of khorasan wheat products. In

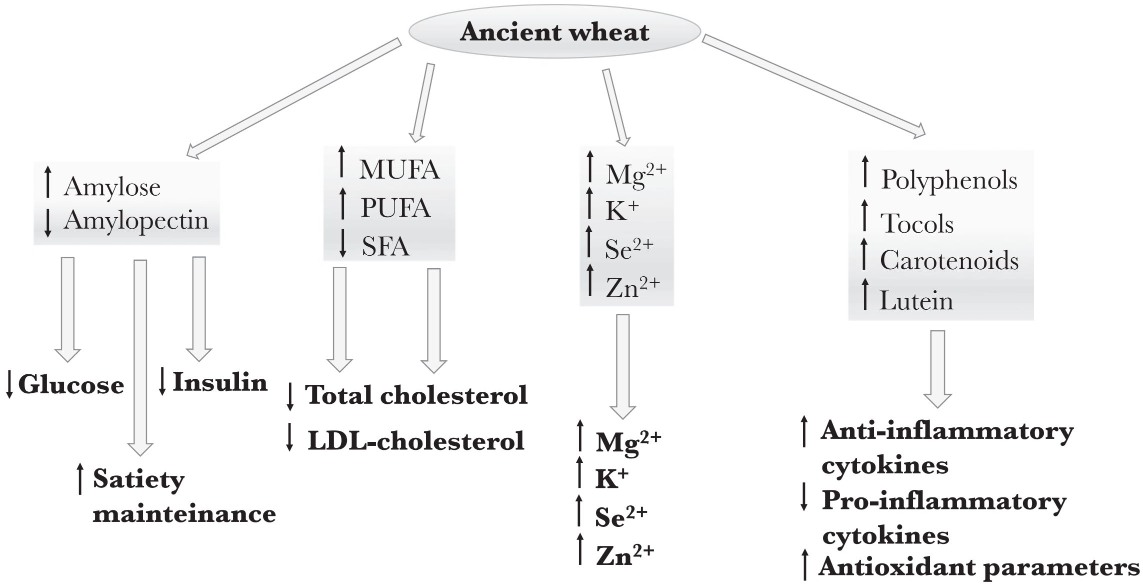

Fig. 1. Effects and possible mechanisms of ancient wheats components on glycaemic, lipid and mineral profiles, and on inflammatory and oxidative states.

contrast, even though β-blockers are suggested to attenuate TNF-α, baseline levels in the acute coronary syndrome patients were comparative to the asymptomatic population, resulting in additive effect ascribed to the consumption of khorasan wheat. Increased baseline IL-6 levels also considered a biomarker for irritable bowel syndrome were reported, and a significant decrease in IL-6 and concomitant improvement in symptomology was noted after the consumption of khorasan wheat [48]. Interestingly, khorasan wheat impacted on decreasing pro-inflammatory Vascular Endothelial Growth Factor (VEGF) regardless of the baseline level, which was higher in the two chronic disease populations. Strategies aimed at reducing VEGF levels are considered to have therapeutic significance as increased (overexpressed) VEGF is a proven causative agent in increased permeability of endothelial cells (leakage), and in regulating subsequent inflammatory responses, resulting in the progression of vascular complications [57].

In a human intervention with 30 healthy participants, a KAMUT® khorasan-based diet (over a period of 3 months) was characterized by the release of short chain fatty acids and phenol compounds, as well as by a slight increase in health-promoting mutualists of the gut microbiota, in comparison to that of a modern durum wheat diet [50]. Gut bacteria are involved in releasing bound phenolic compounds from dietary fiber, thereby facilitating host absorption.

5. Conclusions and future trends

The increasing interest on ancient wheat cultivars is based on their characteristic nature, particularly appropriate for low-input and organic managements, and on the “perceived” higher nutritional value of their flour, with respect to modern wheats. Findings derived from human studies suggest that the consumption of ancient wheat products ameliorate pro-inflammatory/anti-oxidant parameters, as well as glycaemic and lipid status. However, the mechanisms responsible for these beneficial effects are not completely understood. In addition, given that the overall number of human intervention trials conducted to date are numerically insufficient, it is not possible to definitively conclude that ancient wheat varieties are superior to all commercial, modern wheat counterparts in reducing chronic disease risk. Although recent studies provide a positive evaluation of the dietary merit of ancient varieties, their contents of bioactive components differ little from modern wheat species [12]. Nevertheless, taking all studies performed into consideration, it is evident that higher quantitative measurements do not appear to be strictly associated with improved functional performance, and cannot be considered reliable indicators of qualitative potential. Moreover, the attempt to select wheat varieties with greater health potential simply based on assessments of bioactive compounds content and *in vitro* anti-oxidant activity, is a challenge. This is especially relevant to secondary metabolites, such as phenolic compounds, which are reported to be both variable and not highly heritable [58]. Additionally, the final concentrations of ingested phytochemicals are result of bioavailability after breakdown and absorption at the level of the intestine [59]. Therefore, the functional efficacy is evident from synergistic effects between combinations of various breakdown components, not necessarily the respective un-metabolized precursors, and often at the lowest serum concentrations [60]. These synergistic effects between combinations of various components, which may or may not be in the same structure as that present in semolina or flour, generates a great challenge in attempting to select varieties to improve health simply based on standard quantitative phytochemical laboratory analyses.

In conclusion, research performed on ancient wheat varieties is scarce, but available information raises interesting considerations that necessitate deliberation, especially when drawing conclusions on health benefits. Given that there are no linear correlations between quantitative bioactive compound measurements and functional potential, the best option in investigating the functional potential of these varieties would be to include the use of cell model systems to screen for potential candidates, with follow-up trials on human subjects.

Competing interests

The Authors declare that they have no competing interests and the research is not being supported by any commercial.

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