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Randomized control trials

Effects of rye and whole wheat versus refined cereal foods on metabolic risk factors: A randomised controlled two-centre intervention study

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SUMMARY

Background & aims: Intervention studies investigating the effects of wholegrain intake on glucose and insulin metabolism have provided conflicting results. Aim of this study was the evaluation of glucose and insulin metabolism in response to long-term consumption of rye and whole wheat compared with a diet containing the same amount of refined cereal foods, in individuals with metabolic syndrome from two European locations (Kuopio-Finland/Naples-Italy).

Methods: 146 individuals of both genders, age range 40–65 years with metabolic syndrome, were recruited to this study with parallel groups. After a 2–4 week run-in period, participants were assigned to a diet based on wholegrain (wholegrain group) or on refined cereal products (control group), each one for a duration of 12 weeks. Peripheral insulin sensitivity, assessed by FSIGT, lipids and inflammatory markers were measured before and at the end of intervention.

Results: 61 participants in the control group and 62 in the wholegrain group completed the dietary intervention. Compliance to the two diets was good. At the end of the intervention, insulin sensitivity indices and secretion (S_I, QUICKI, DI, dAIRG) and lipids and inflammatory markers did not change significantly in the wholegrain and control groups as compared with baseline and no differences between the two groups were observed.

Conclusions: Wholegrain cereal foods consumption compared with refined cereals for 12 weeks did not affect peripheral insulin sensitivity.

The study was registered with ClinicalTrials.gov identifier NCT00945854.

1. Introduction

A large body of evidence from observational studies has shown that habitual consumption of cereal fibre and wholegrain foods is consistently associated with reduced risk of type 2 diabetes, metabolic syndrome and CVD.[1] The protective effects of wholegrain cereals against type 2 diabetes and CVD have been attributed to a synergistic action of their components such as dietary fibre, vitamins and other molecules with antioxidant properties, phytoestrogens and micronutrients on several biological functions.

However, the biological mechanisms responsible for the health effects of wholegrain are still unclear. Findings from epidemiological studies suggest that the benefits of wholegrain intake on human

Abbreviations: ARs, alkylresorcinol homologues; BIA, bioelectrical impedance analysis; CVD, cardiovascular disease; dAIRG, dynamic glucose-stimulated insulin response; DI, disposition index; FSIGT, frequently sampled intravenous glucose tolerance test; GI, glycaemic index; hs-CRP, high sensitivity C-reactive protein; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; IL-1ra, interleukin 1 receptor antagonist; IL-6, interleukin 6; OGTT, oral glucose tolerance test; QUICKI, index of fasting insulin sensitivity; S_I, insulin sensitivity index; TNF-α, tumour necrosis factor-α.

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health are related to improved body weight, insulin sensitivity, lipid metabolism, inflammation and antioxidant activity. Since insulin resistance is a key factor in the pathogenesis of type 2 diabetes and CVD, the reduced risk for type 2 diabetes and CVD observed in wholegrain consumers could be mediated by an improvement in insulin resistance. As a matter of fact, increased consumption of wholegrain was associated with higher insulin sensitivity, lower fasting insulin concentration, and lower 2-h glucose concentration after an OGTT in many observational studies.[2–5]

However, so far the results of intervention studies investigating the effects of wholegrain intake on glucose and insulin metabolism have provided conflicting results. Among the clinical trials with positive findings on insulin metabolism, a study in hyperinsulinemic overweight individuals showed that a 6-week period with a wholegrain rich diet, composed of 80% wheat, reduced fasting plasma insulin levels and improved insulin resistance as compared with a refined cereal diet[6]; two other studies reported that high-fibre rye bread consumption compared with refined wheat bread significantly increased the first phase of insulin secretion, suggesting an improved beta cell function,[7] reduced fasting insulin levels and 24-h urinary C-peptide excretion.[8] Other intervention studies observed benefits of wholegrain consumption on plasma cholesterol concentrations,[9,10] systolic blood pressure levels,[11] abdominal fat and hs-CRP,[8,12] but no effect on glucose or insulin metabolism. Finally, some trials did not observe any effect of wholegrain consumption on either insulin sensitivity or metabolic abnormalities, or on inflammatory and oxidative status.[13–15] These contradictory results could be related to either different wholegrain cereals used and/or differences in the pathophysiology of glucose metabolism in the participants in these studies, as well as to differences in approaches used to measure glucose and insulin metabolism.[16]

The primary aim of our study was to evaluate differences in glucose and insulin metabolism, as assessed by FSIGT (frequently sampled intravenous glucose tolerance test) in response to long-term consumption of rye and wholegrain cereal products as compared with a diet containing the same amount of refined cereal foods, in individuals with the metabolic syndrome. The participants were recruited in two European locations (Kuopio, Finland and Naples, Italy) with different assortment of whole-grain products, food culture, genetic and life-style backgrounds. The secondary aim was to investigate the effects of this type of diet on lipid metabolism and inflammatory markers.

2. Subjects and methods

2.1. Population

One hundred and forty six individuals (85 from Kuopio and 61 from Naples) of both genders, age range 40–65 years with the metabolic syndrome, were recruited to participate in the dietary intervention. At screening, health status and medical history of the participants were examined by interview, clinical examination and routine laboratory tests (glucose, lipids, haemoglobin and liver, kidney and thyroid functions). In addition, a 75 g OGTT was carried out to evaluate glucose tolerance and exclude those with undiagnosed diabetes. The diagnosis of metabolic syndrome was based on the National Cholesterol Education Program Criteria.[17] Individuals were excluded if they were diagnosed with diabetes and/or renal failure (serum creatinine $>$ 1.5 mg/dl), liver abnormalities (ALT/AST ratio two times above normal values), anaemia (Hb $<$ 12 g/dl), any other chronic disease or if they used any drug able to influence glucose and lipid metabolism and inflammation (corticosteroid hormones other than inhaled corticosteroids, hypolipidemic or/and anti-inflammatory drugs); however, in the Kuopio study centre the use of cholesterol lowering medications (statins) was allowed.

All participants gave their written informed consent to the study which was approved by the Ethics Committee at the Kuopio University hospital and at the “Federico II” University of Naples.

2.2. Study design

The study was based on a randomized, controlled, parallel group design and consisted of a 2–4 week run-in period, during which the participants were stabilised on their own diet, and a 12-wk test period (Fig. 1). At the end of the run-in period, the participants were randomly assigned to one of two groups: one group consumed a diet based on wholegrain cereal products, most of them with a low postprandial glucose and/or insulin response (wholegrain group), and the other group consumed a diet based on refined cereal products (control group). The randomization was carried out separately at each centre with stratification for sex, age (5 years) and BMI (25–30, 30–35 kg/m^2) by use of random allocation software. Allocation was carried out by personnel not involved in the study; therefore the investigators and the dieticians were aware of the group allocation of the participant only after the randomization process had been performed. During the study, participants were advised not to change their body weight and lifestyle habits such as exercise and alcohol consumption and not to change their medications unless necessary.

At baseline and at 4, 8 and 12 weeks during the intervention, participants underwent clinical investigations including measurements of body weight, waist circumference and blood pressure; fasting blood samples were taken after a 12-h overnight fast for biochemical measurements. After the run-in and at the end of the intervention, participants underwent FSIGT[18]; in addition, BIA

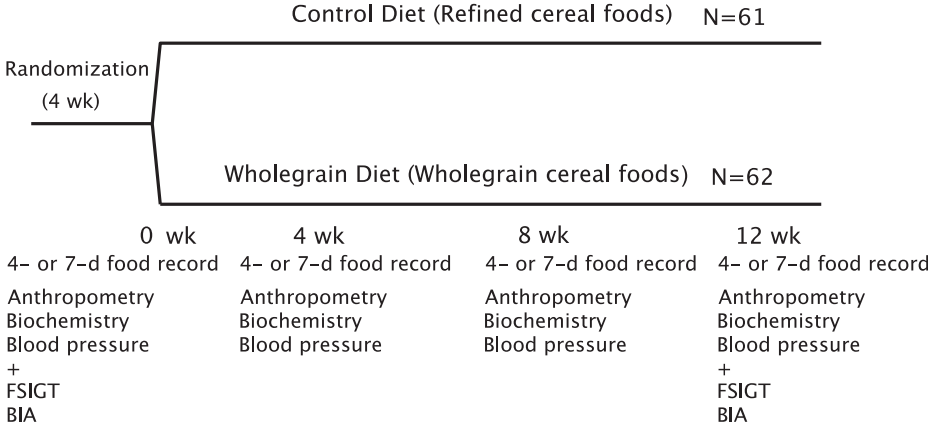

Fig. 1. Study design.

(bioelectrical impedance analysis) was performed to evaluate body composition.

2.3. Experimental diets

Participants were encouraged not to change their habitual meat, dairy products, eggs, fish, fruit, vegetable and fat intake during the study; the only difference between the wholegrain and the control diet was the inclusion of a fixed amount of wholegrain or refined cereal products as the main carbohydrate source. The wholegrain diet in Naples was based on wholegrain products including whole wheat bread (plus some endosperm rye bread), whole wheat pasta, barley kernels, wholegrain oat biscuits and breakfast cereals (all bran sticks and flakes) while the control diet contained commercial products based on refined cereals such as wheat bread, rice, pizza, cornmeal porridge, and breakfast cereals (rice krispies). The wholegrain bread consumed by the Neapolitan participants was 90% sourdough whole wheat bread and 10% endosperm rye bread. In Naples the average GI was 46% for the wholegrain diet and 72% for the control diet.

In Kuopio, the wholegrain and control diets were aimed to include 20–25% of the total daily energy intake as study breads. In the wholegrain diet, the type of bread consumed by the Kuopio participants was 50% commercial wholegrain rye bread, 40% endosperm rye bread, and 10% sourdough whole wheat bread. In addition, participants in Kuopio were advised to replace their habitual potato consumption with 210 g dry weight of whole wheat pasta per week, and were given whole oat biscuits for snacks. In the control group, the participants consumed commercial refined wheat breads, and only 1–2 small portions of rye products were allowed daily.

The wholegrain products used were defined as containing a minimum of 51% wholegrain per dry substance, including the starchy endosperm, germ, and bran, mainly in milled form.[19] Commercial wholegrain rye breads were made with 100% wholegrain flour, and the endosperm rye bread with 100% endosperm rye flour. Both the wholegrain rye bread and the endosperm rye bread were shown to produce a low postprandial insulin response.[20] The sourdough whole wheat bread was shown to have beneficial postprandial glucose and insulin responses.[21] Furthermore, both the Finnish and Italian sourdough whole wheat breads contained high amount of bioactive compounds present in bran like ferulic acid, betaine, choline and alkylresocinols (data not shown).

In order to improve the adherence to the two experimental diets, the test products in both diets were provided free of charge to the participants in amounts sufficient to cover their household consumption for the whole duration of the study. For both diets cereal products represented about 60–80% of the daily carbohydrate intake; the remaining 20–40% of carbohydrates was provided by fruits and vegetables according to participants' usual dietary habits. The intake of sugar and sugar-sweetened drinks was minimal (<30 g/day).

The diets were controlled for energy intake to maintain body weight of participants stable during the whole period of the intervention.

2.4. Dietary assessment

Compliance with the experimental diets was assessed using a 4-d food records in Kuopio and 7-d food records in Naples during the run-in period, i.e. before starting the intervention, and at the 4th, 8th and 12th week of the intervention, to evaluate the energy intake and the nutrient composition of the diets followed by each participant (Fig. 1) (Table 1). Four-day food records included one weekend day. All 4-d food records were analysed with the MICRONUTRICA program version 2.0 (Finnish Social Insurance Institute, Turku, Finland), which utilizes a database of Finnish foods. All 7-d food records obtained in the Italian cohort were analysed by a computerized program using the food database of the Italian National Institute for Foods and Nutrition. If any of the foods recorded did not match a product already included in the database, the nutrient values declared by the producer were added to the database. For food whose nutrients were not declared by the producer, the database value for the most similar product was used. In addition to food records, the intake of test products was followed by daily questionnaires filled in by the subjects in Kuopio and by counting the unused test products in Naples.

Table 1
Energy intake and diet composition at baseline and at the end of the intervention.

	Control group ($n = 61$)		Wholegrain group ($n = 62$)	
	Baseline	12-Week	Baseline	12-Week
Energy (kcal/d)	1719 ± 63[a]	1965 ± 57[b]	1702 ± 62	1900 ± 57[b]
CHO (%)	48 ± 0.7	49 ± 0.6	46 ± 0.6	48 ± 0.6
Protein (%)	18 ± 0.4	17.8 ± 0.3	18 ± 0.4	18.7 ± 0.3[c]
Total fat (%)	31.8 ± 0.6	30.8 ± 0.7	33.5 ± 0.6	31.0 ± 0.7
SAFA (%)	11.0 ± 0.3	10.9 ± 0.3	11.4 ± 0.3	10.2 ± 0.3
MUFA (%)	12.1 ± 0.3	11.9 ± 0.3	12.9 ± 0.3	11.1 ± 0.3
PUFA (%)	4.9 ± 0.2	3.9 ± 0.2	5.3 ± 0.2	4.8 ± 0.2[c]
Cholesterol (mg/day)	228 ± 12	225 ± 9	238 ± 13	207 ± 10
Total fibre (g/day)	21.6 ± 0.8	19.8 ± 0.7	22.7 ± 0.8	32.6 ± 0.7[c]
Cereal fibre (g/day)	11.4 ± 0.6	10.4 ± 0.3	11.9 ± 0.8	24.3 ± 0.9[c]

[a] Mean ± SEM (all such values).
[b] $p < 0.02$ Paired sample t test (12-week versus baseline).
[c] Treatment effects (Wholegrain versus Control) were evaluated by GLM where study centre and baseline values were included as covariates, $p < 0.05$.

The energy and nutrient composition of wholegrain and refined wheat products employed in this trial were measured directly by the manufacturer. All participants received written and oral instructions given by the dietician or nutritionist concerning the diets to follow during the intervention and were supplied with recipes indicating how the products could be used in the best way to ensure good adherence to the diets. Weekly or biweekly the participants visited the clinic to collect new food products. During the visits for the clinical and body weight measurements at 4-week intervals, the participants returned the food records and questionnaires and were encouraged to continue on the study diet.

Plasma total AR concentration, a biomarker of wholegrain wheat and rye intake,[22] was measured at baseline and at the end of the intervention in both groups in order to evaluate the compliance to the experimental diets.

2.5. Blood pressure, anthropometric and body composition measurements

Blood pressure was measured in the supine position in a standardized way after 5–10 min rest with an automatic sphygmomanometer. Body weight was measured on the same calibrated beam balance scale throughout the study. Waist circumference was measured halfway between the lowest rib and the iliac crest. Body composition was measured by bioelectrical impedance (BIA 101S with BODYGRAM software; Akern Srl Bioresearch, Florence, Italy).

2.6. Clinical test and laboratories analyses

Blood samples were drawn after a 12-h overnight fast, from an antecubital vein for the measurements of plasma glucose, insulin, lipid, and inflammatory markers. Peripheral insulin sensitivity was assessed by FSIGT. A glucose dose of 300 mg/kg body weight was

given intravenously followed by a bolus of 0.03 U/kg of insulin injected after 20 min. Blood samples were frequently collected for 3 h for the measurement of plasma glucose and serum insulin concentrations, utilized to calculate the insulin sensitivity index S_I (10^4 $min^{-1}/(\mu U/ml)$).[23,24] Insulin sensitivity at fasting was evaluated by the unitless QUICKI index. First-phase insulin response was evaluated as the average insulin concentration from 2 to 10 min and expressed as dAIRG (μU/ml). The disposition index, DI, that measures the ability of the beta cell to increase its secretion to compensate for insulin resistance, was calculated as $S_I \times$ dAIRG.[25]

In Naples, plasma insulin concentrations were measured by an enzyme-linked immunosorbent assay (ELISA) for the specific determination of biologically active insulin (DAKO Insulin, DAKO Diagnostics, Ely, UK). Plasma glucose, cholesterol, and triglycerides were assayed by enzymatic colourimetric methods (ABX Diagnostics, Montpellier, France; Roche Molecular Biochemicals, Mannheim, Germany; Wako Chemicals GmbH, Neuss, Germany, respectively) on a Cobas Mira autoanalyzer (ABX Diagnostics, Montpellier, France). HDL cholesterol was isolated from plasma by a precipitation method with a sodium phosphotungstate and magnesium chloride solution and measured by the same enzymatic colourimetric method utilized for the analysis of total cholesterol. The LDL cholesterol concentration was calculated according to the formula of Friedwald.

In Kuopio, serum insulin was analysed with a chemiluminescent immunoassay (Advia Centaur, Siemens Medical Solution Diagnostics, Tarrytown, NY, USA), plasma glucose was analysed by the glucose hexokinase method (Konelab System Reagents; Thermo Fisher Scientific, Vantaa, Finland), and serum total, LDL-, and HDL-cholesterol, and triglycerides were analysed using commercial kits (Thermo Electron, Vantaa, Finland).

The inflammatory markers (hs-CRP,TNF-α, IL-6, IL-1ra) were determined in Germany at University of Ulm, in the laboratory of Department of Internal Medicine II-Cardiology, as described by de Mello et al.[26] Measurements were done on plasma EDTA samples. Plasma samples were analysed for AR homologues C17:0–C25:0 according to a gas chromatography–mass spectrometry-single ion monitoring method, using molecular ions for quantification.[27] Samples were divided randomly in 17 batches and analysed in singlets. In each batch, five replicates of a control sample were included randomly in the sequence. The within- and between-day batch variation, determined as the coefficient of variation, was <10%.

Laboratory analyses were performed blind in respect to the assigned treatment.

2.7. Statistical analysis

The main outcome variable in the statistical analysis was insulin sensitivity. The effects of dietary intervention on insulin sensitivity were analysed on 111 of 123 participants completing the study. On the basis of previous studies, a sample size of 120 individuals was calculated to detect a 20% difference in S_I between the two groups with 0.05 significance level and 80% power (type II error = 0.1), assuming a 15% drop-out rate.

Results for continuous variables were presented as mean ± standard error of means (mean ± SEM), unless otherwise stated. Variables with skewed distributions by Shapiro–Wilks test were normalized with logarithmic or a square root transformation and were reported as median (interquartile range).

Energy intake and nutrient composition at baseline and during the intervention were calculated from the food records; the intakes during the intervention were expressed as mean of three food records filled in at 4, 8, and 12 weeks.

A general linear model (GLM) for repeated measures was used to evaluate differences between the groups (calculated as change of the parameter between 12-week and baseline and indicated as Δ) during the intervention including the centre (Naples/Kuopio) and baseline variables as covariates. GLM for univariate analysis was used to assess the difference in the relative change (calculated as the change from baseline) of insulin sensitivity and insulin secretion indices between the groups with the centre and baseline variables as covariates. The effects of dietary intervention on insulin sensitivity between the groups were also analysed in a subgroup of participants having an S_I index below the median value [$<2.8 \times 10^4$ $min^{-1}/(\mu U/ml)$] at baseline. A paired-samples t test was used to examine the changes compared with baseline in variables within each group. For all analyses, the level of statistical significance was set at $p = 0.05$ (two tails). Data were analysed with SPSS for Windows 11.5 (SPSS Inc., Chicago, IL).

3. Results

3.1. Baseline characteristics of the participants

One hundred and twenty three participants (69 in Kuopio and 54 in Naples) completed the dietary intervention: 61 individuals (29M/32F) in the control diet group and 62 individuals (29M/33F) in the wholegrain diet group, while 14 individuals (18.7%) allocated in the control group and 9 (12.6%) in the wholegrain group dropped out because of limited time resources due to work or family-related problems. Of the participants completing the study, 55% had IFG and 27% had IGT at baseline. Furthermore, 92% presented high waist circumference, 52% low HDL cholesterol levels, 34% high fasting plasma triglyceride levels, 65% high systolic and 57% high diastolic blood pressure levels.

Clinical characteristics of participants are reported in Table 2. At the baseline, the wholegrain and control groups were similar with respect of age, body weight, BMI, waist circumference, body composition, blood pressure levels and fasting plasma concentrations of glucose, insulin, lipid, and ARs. The two groups were not different at baseline for insulin sensitivity (S_I and QUICKI) and for beta cell function (dAIRG) (Table 3). There were no differences in fasting plasma concentrations of the inflammation markers hs-CRP, IL-6, IL-1ra, and TNF-α at baseline (Table 4).

3.2. Dietary compliance

Compliance for the wholegrain and the control diets was good. During the intervention, both groups reported to consume the portions of the breads and wholegrain and/or refined cereal based products as advised. In the wholegrain group, the mean daily intake of the test breads in Kuopio was 185 g (of which commercial wholegrain rye bread (mean ± SD) 87 ± 26 g, endosperm rye bread 73 ± 21 g, and sourdough whole wheat bread 26 ± 4 g) and in Naples was 153 ± 44 g (of which sourdough whole wheat bread 139 ± 35 g, endosperm rye bread 14 ± 4 g). In the control group, the mean daily intake of refined wheat breads was 197 ± 46 g and 136 ± 47 g in Kuopio and Naples, respectively. In addition, in Naples the daily consumption of whole wheat pasta was on average 85.6 ± 22.8 g in the wholegrain group and that of refined wheat pasta plus rice plus pizza was on average 78 ± 19.0 g in the control group.

At baseline, the energy intake and nutrient composition of the diets were similar between the wholegrain and the control groups (Table 1). Compared with baseline, both the wholegrain and control groups increased their energy intake (mean value of dietary records at 4, 8 and 12 wk) during the intervention period ($p = 0.001$). However, the proportional nutrient composition did not change in the control group whereas slightly higher protein and PUFA intakes ($p < 0.05$) were observed in the wholegrain group (likely due to the higher protein and PUFA content in wholegrains than in refined grains). As expected, the wholegrain group significantly increased

Table 2
Anthropometric and fasting plasma metabolic parameters at baseline and at the end of the intervention.

	Control group ($n = 61$)			Wholegrain group ($n = 62$)			p for Δ
	Baseline	12-Week	Δ	Baseline	12-Week	Δ	
Weight (kg)	87.8 ± 15.8[a]	87.8 ± 16.1	0.03[b]	88.6 ± 15.8	87.9 ± 18.3	−0.7	0.248[c]
BMI (kg/m^2)	31.3 ± 4.4	31.3 ± 4.5	0.00	31.6 ± 4.6	31.0 ± 5.8	−0.6	0.481
WC (cm)	105.8 ± 10.7	105.8 ± 10.8	0.04	106.7 ± 13.0	106.4 ± 12.8	−0.3	0.621
Lean mass (kg)	56.9 ± 12.3	56.6 ± 12.2	−0.3	56.3 ± 11.3	55.7 ± 11.3	−0.2	0.511
Fat mass (kg)	30.9 ± 9.8	31.2 ± 10.3	0.3	32.3 ± 9.3	32.2 ± 9.4	−0.06	0.375
SBP (mmHg)	135 ± 14	130 ± 17	−5	133 ± 15	128 ± 15	−5	0.849
DBP (mmHg)	86 ± 8	82 ± 9	−4	84 ± 9	81 ± 9	−3	0.526
Glucose (mmol/l)	6.06 ± 0.56	6.00 ± 0.61	−0.06	5.89 ± 0.56	5.94 ± 0.56	0.05	0.310
Insulin (pmol/l)	93.1 ± 45.1	93.1 ± 47.2	0.001	93.8 ± 52.8	103.5 ± 55.6	9.72	0.074
Triglycerides (mmol/l)	1.52 ± 0.72	1.56 ± 0.65	0.03	1.58 ± 1.20	1.65 ± 0.86	0.07	0.745
Cholesterol (mmol/l)	5.28 ± 0.93	5.31 ± 0.90	0.03	5.15 ± 1.09	5.25 ± 1.14	0.10	0.417
HDL-C (mmol/l)	1.14 ± 0.31	1.16 ± 0.31	0.02	1.16 ± 0.36	1.16 ± 0.34	0.001	0.425
LDL-C (mmol/l)	3.41 ± 0.80	3.41 ± 0.78	0.001	3.26 ± 0.98	3.31 ± 0.98	0.05	0.506
Total ARs (nmol/l)	68 ± 55	40 ± 32[d]	−28	75 ± 69	122 ± 96[d]	+47	0.0001

[a] Mean ± SD (all such values).
[b] Δ = change of parameter between 12-week and baseline (all such values).
[c] Treatment effects (Wholegrain versus Control) were evaluated by GLM with the centre and baseline variables included as covariates.
[d] $p < 0.02$ Paired sample t test (12-week versus baseline).

the intake of total and cereal fibre ($p < 0.05$); the differences as compared with the control group being 12.8 and 13.8 g/day, respectively; conversely, in the control group total and cereal fibre did not change after the intervention (Table 1).

Fasting plasma ARs concentrations increased significantly in the wholegrain diet group as compared with baseline and decreased in the control diet group with a significant difference between the two groups at the end of the intervention (+47 versus −28 nmol/l; $p < 0.0001$,GLM analysis) (Table 2). In particular, fasting plasma ARs concentrations increased in the wholegrain group as compared with baseline both in Kuopio (106.4 ± 89.3 versus 93.2 ± 69.8 nmol/l) and in Naples (140.2 ± 102.0 versus 52.0 ± 62.3 nmol/l) and decreased in the control group both in Kuopio (36.4 ± 26.8 versus 72.1 ± 52.1 nmol/l) and in Naples (43.7 ± 38.0 versus 63.4 ± 58.4 nmol/l).

The nutrient intake of the participants in the Kuopio ($n = 69$) and Naples ($n = 54$) study centres was compared at baseline to evaluate any differences of the background diets in the two intervention sites. As compared with Kuopio, in Naples the intake of carbohydrates (50 ± 0.7 versus 44 ± 0.6 E%) and monounsaturated fatty acids (14.6 ± 0.4 versus 10.7 ± 0.3 E%) was higher ($p < 0.05$) while the intake of proteins (17.1 ± 0.4 versus 19.1 ± 0.4 E%), saturated fatty acids (9.8 ± 0.3 versus 12.3 ± 0.3 E%), polyunsaturated fatty acids (4.2 ± 0.2 versus 5.8 ± 0.2 E%), and cereal fibre (8.9 ± 0.5 versus 13.8 ± 0.7 g/day) was lower ($p < 0.05$). These differences in the background diet were maintained during the intervention (data not shown). Due to the different food cultures, the baseline diet in Kuopio was higher in cereal fibre than that in Naples because of the habitual intake of high fibre wholegrain rye bread in Kuopio. During the intervention, in Kuopio, about 40% of the habitual intake of wholegrain rye bread was replaced in the wholegrain diet with endosperm rye bread containing less fibre (7% as compared with 10–14%, respectively). On the contrary, in Naples, during the intervention the participants in the wholegrain diet group replaced the habitual refined grain consumption with wholegrain products.

3.3. Effects of dietary intervention on anthropometric and metabolic parameters

The mean body weight, BMI, waist circumference, fat mass and lean fat mass and systolic and diastolic blood pressure levels did not change during the intervention period in either group (Table 2). Before and at the end of the intervention, BMI was 31.6 ± 4.6 versus 31.0 ± 5.8 kg/m^2 in the wholegrain diet group and 31.3 ± 4.4 versus 31.3 ± 4.5 kg/m^2 in the control diet group; the waist circumference was 106.7 ± 13.0 versus 106.4 ± 12.8 cm in the wholegrain and 105.8 ± 10.7 versus 105.8 ± 10.8 cm in the control diet.

No effects of the wholegrain and control diet on fasting plasma concentrations of glucose, insulin and lipids were observed at the end of the intervention period (Table 2), as well as at 4 and 8 weeks (data not shown); however, fasting plasma insulin concentrations tended to be higher in the wholegrain group.

3.4. Effects of dietary intervention on insulin sensitivity and insulin secretion

At the end of the intervention, S_I, QUICKI, dAIRG and DI did not change significantly in the test and control groups as compared with baseline; no significant differences between the two groups were observed at the end of the intervention period (Table 3) (data

Table 3
Markers of insulin sensitivity and insulin secretion at baseline and at the end of the intervention.

	Control group ($n = 54$)[a]			Wholegrain group ($n = 57$)			p for Δ
	Baseline	12 Week	Δ (%)	Baseline	12 Week	Δ (%)	
S_I	3.32 ± 0.26[b]	3.18 ± 0.22	−4.2[c] (−18; +5)[d]	2.97 ± 0.20	3.05 ± 0.19	+2.7 (−7.8; +12)	0.331[e]
QUICKI	0.37 ± 0.006	0.36 ± 0.006	−1.39 (−2.6; −0.2)	0.36 ± 0.007	0.36 ± 0.007	−1.31 (−2.6; +0.003)	0.937
DI	146 ± 17	160 ± 21	+9.6 (−14; +24)	140 ± 17	144 ± 17	+2.8 (−16; +15)	0.559
dAIRg (2–10 min)	54.2 ± 5.2	55.7 ± 5.4	+2.8 (−8.2; +10.4)	61.4 ± 8.4	60.0 ± 7.8	−2.3 (−15.9; +5.1)	0.415

[a] For 7 participants in the control group and 5 participants in the wholegrain group it was not possible to perform the FSIGT either at baseline or at follow-up for technical reasons.
[b] Mean ± SEM (all such values).
[c] Δ = change of parameter between 12-week and baseline (all such values).
[d] 95% C.I. for Δ percentage (all such values).
[e] Treatment effects (Wholegrain versus Control) were evaluated by GLM with the centre and baseline variables included as covariates.

Table 4
Concentrationsof plasma inflammatory markers at baseline and at the end of the intervention.

	Control group ($n = 61$)		Wholegrain group ($n = 62$)		p for group effect
	Median (interquartile range)		Median (interquartile range)		
	Baseline	12-Week	Baseline	12-Week	
hs-CRP[a] (mg/dl)	1.95 (0.96; 2.56)	1.74 (1.04; 2.95)	1.95 (0.74; 4.12)	1.36 (0.62; 3.34)	0.16[b]
IL-6 (pg/ml)	1.41 (0.84; 2.21)	1.43 (1.07; 2.11)	1.42 (1.01; 2.32)	1.54 (1.12; 2.23)	0.52
IL-1ra (pg/ml)	251 (193; 330)	239 (190; 379)	300 (214; 518)	298 (175; 386)	0.13
TNF-α (pg/ml)	0.62 (0.43; 1.05)	0.63 (0.41; 0.90)	0.73 (0.50; 0.96)	0.68 (0.50; 0.94)	0.84

[a] hs-CRP data on 49 participants in the control and 52 participants in the wholegrain group, untreated with cholesterol lowering medications.
[b] Treatment effects (Wholegrain versus Control) on the change as Δ (12 week − baseline) were evaluated by GLM with the centre and baseline variables included as covariates.

were available in 54 and 57 subjects in the control and wholegrain groups, respectively). An additional analysis was performed on a subgroup of 54 participants (24 in the control group and 30 in the wholegrain group) who were more insulin resistant with S_I below the median value [$<2.8 \times 10^4$ $min^{-1}/(\mu U/ml)$] at baseline. Compared with baseline, in this subgroup S_I and QUICKI did not change after the wholegrain diet (S_I: 1.8 ± 0.1 versus 2.2 ± 0.2; QUICKI: 0.34 ± 0.004 versus 0.34 ± 0.004) and the control diet (S_I: 1.7 ± 0.1 versus 1.9 ± 0.2; QUICKI: 0.34 ± 0.004 versus 0.34 ± 0.004); no differences between the two groups were observed with respect to the changes from baseline between the two intervention groups.

3.5. Effects of dietary intervention on plasma inflammatory markers

Plasma concentrations of hs-CRP, IL-6, IL-1ra, and TNF-α did not change during the intervention, and did not differ between the test and the control group at the end of the intervention (Table 4).

Since there were some individuals at the Kuopio study centre using cholesterol lowering medication ($n = 10$ in the test and 10 in the control group), we also performed the statistical analysis after excluding these individuals. In addition, individuals with high baseline value of hs-CRP (>10 mg/l) in Kuopio ($n = 1$) and in Naples ($n = 1$), both in the control group, were excluded as outliers. There was a trend for a decreased hs-CRP concentration at the end of the wholegrain diet [1.36 mg/dl (0.62; 3.34)] [median (interquartile range)] as compared with baseline [1.95 mg/dl (0.74; 4.12)] ($p = 0.08$).

4. Discussion

Twelve weeks consumption of rye and wholegrain wheat based diets compared with corresponding refined diets did not improve glucose and insulin metabolism nor lipid and inflammatory markers in this randomised, controlled, two-centre intervention study with Finnish and Italian individuals at risk of type 2 diabetes. These results are in line with those of previous interventions showing no effects of wholegrain consumption on the above mentioned markers[14,15] or on insulin sensitivity.[10–12] However, the results of the present study are at variance with the findings of improved insulin sensitivity or first phase insulin response observed in association with an increased consumption of whole wheat[6] or wholegrain rye products in some studies.[7,8,28] The conflicting results on glucose and insulin metabolism in wholegrain intervention studies may be due to differences in the methodologies used for analysing glucose and insulin metabolism or to differences in to the amount and type of wholegrain products of experimental diets (Appendix Table).

In this context, it is worth to underline that our study, using both an adequate sample size and a validated methodology for insulin sensitivity measurement, was unable to show any effect of wholegrain on glucose and insulin metabolism. In fact, peripheral insulin sensitivity was evaluated by FSIGT on a sample of 111 individuals, suitable to detect a clinically relevant effect (20%) of wholegrain on insulin sensitivity with a low risk (0.1) for type II error. Any smaller effect may lack clinical relevance. However, since the test here employed to evaluate insulin sensitivity takes into account predominantly insulin effects at the level of muscle and adipose tissue, it cannot be completely ruled out the possibility of beneficial effects of wholegrain consumption on glucose and insulin metabolism at the splanchnic site and in the postprandial period.

No effects on the first phase insulin secretion by either diet were observed in this study using the data from FSIGT, contrary to the study by Laaksonen et al.,[28] in which insulin secretion calculated from OGTT improved after the rye-pasta diet as compared with the oat–wheat–potato diet. However, the role of gastrointestinal hormones during an OGTT should also be taken into account when assessing the insulin response.

It is also to be considered that in our intervention the daily intake of cereal fibre from the wholegrain diet was lower (24.3 ± 0.9 g) than that consumed in the studies of Juntunen (29.7 ± 6.3 g)[7] and Landberg (58 ± 7.0 g)[8] which, respectively, observed beneficial effects of wholegrain rye products on the acute insulin response and on the fasting insulin and C-peptide concentrations. However, the daily cereal fibre intake in our intervention is in line with the amount associated to a lower risk of type 2 diabetes in epidemiological studies.[1,29] In addition, our daily cereal fibre intake, compared with that very high reported in the Landberg study, is better tolerated by consumers and more sustainable in the long term.

Moreover, in our intervention, the wholegrain diet contained a variety of whole wheat and rye products accompanied by smaller amounts of oat and barley. Statistical analysis performed separately for the two centres, Kuopio and Naples, showed no difference in S_I and insulin secretion between the two intervention groups in the participants of either centre, although in Kuopio the wholegrain group consumed 90% of daily bread as wholegrain and endosperm rye bread and in Naples as whole wheat bread. It is also noteworthy that the wholegrain diet had a lower GI than the control diet in Naples but not in Kuopio. Therefore, we could rule out that in our experiment the absence of any beneficial effect of wholegrain on measured glucose and insulin metabolism could depend on the type of grain (rye or wheat) and environment or culture.

In relation to the relationship between wholegrain consumption and markers of subclinical inflammation, it is worth stressing a reduction of plasma hs-CRP has been observed only when wholegrain and bran rye products were consumed and that the intake of endosperm rye bread is negatively correlated with concentrations of hs-CRP in plasma, as observed respectively by Landberg et al.[8] and de Mello et al.[26]

An additional strength in our intervention was that plasma AR was used as a biomarker for the intake of whole wheat and rye.[22] The food records showed a significant increase in the cereal fibre intake in the wholegrain group and this was confirmed by an increase in plasma ARs concentrations, while conversely, ARs plasma levels were decreased in the control group. These data

indicate a good adherence to the prescribed dietary treatments. The evaluation of the AR homologues in plasma confirmed a higher consumption of rye in Kuopio as compared with a higher consumption of wheat in Naples (data not shown).

Furthermore, during the intervention, participants, according to the study design, maintained stable their body weight, body fat composition and waist circumference that may represent confounding factors on insulin sensitivity and secretion.[2,12,30] Our study has many strengths but also some limitations. In fact, it does not allow to rule out the possibility of a more relevant effect of wholegrain on glucose and insulin metabolism in individuals with more pronounced metabolic derangements. In addition, a more focused evaluation of insulin sensitivity at the liver site would probably be more appropriate to investigate the metabolic impact of wholegrain since the effects of wholegrain are probably mediated by mechanisms acting in the splanchnic region, predominantly in the postprandial period. Further limitation of the study is the treatment duration which could be too short to induce relevant modifications in metabolic parameters which interfere with plasma glucose and insulin regulation.

In conclusion, our randomized, well controlled two-centre intervention clearly shows that rye or wheat based wholegrain diets did not affect peripheral insulin sensitivity and other parameters of glucose metabolism in individuals with the metabolic syndrome. However, it remains to be elucidated with intervention studies of appropriate duration and sample size whether wholegrain consumption is able to reduce the risk of type 2 diabetes or cardiovascular diseases as suggested by observational studies.

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Authors contributions

GR and HM, principal investigators, designed the study and wrote the manuscript together with RG and JL; AAR and RG screened the subjects and supervised the study; US recruited the subjects in Kuopio, planned the diets, coordinated the laboratory analyses, was responsible for the analyses of dietary intake; GP carried out the assessment of insulin sensitivity and secretion and contributed to the data interpretation; RL was responsible for the alkylresorcinol measurements; GC, JL and MK performed the statistical analyses and contributed to the data interpretation; AAR, KP and MU contributed to design the study and revised the manuscript. All the authors reviewed the manuscript critically and approved the final version.

Conflict of interest

All authors had no potential conflict of interest to declare in relation with the content of this article. None of the sponsors had any role in defining the study design or its implementation or in data analysis and interpretation.

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Appendix

Appendix Table
Effects of wholegrain foods on insulin sensitivity in intervention studies.

Reference	Characteristics of participants	Study design	Wholegrain products (intake/d)	Control products (intake/d)	Methods for the evaluation of insulin sensitivity	Insulin sensitivity
Pereira et al., 2002	6 F, 5 M hyperinsulinemic subjects (25–56 y) BMI 30 ± 1 kg/m^2	Randomized crossover 2 × 6 wk separated by a 6–9 wk washout	Breakfast cereal, bread, pasta, muffin, cookies, snacks (80% of consumed grains were wheat, remainder oats, rice, corn, barley, rye). Grains were ground to flour. (6–10 servings)	Refined wheat, rice, and corn (no bran, germ, little fibre)	Euglycemic hyperinsulinemic clamp	↑
Juntunen et al., 2003	20 healthy females (3 with IGT) (59 ± 6 yrs) BMI 28 ± 3 kg/m^2	Randomized crossover 2 × 8 wk separated by a 8 wk washout	High fibre rye bread (includes added rye bran) Minimum of 4–5 portions (24.1–28.1 g), (≥96–140 g)	White wheat bread Amount (≥83 –125 g)	FSIGT	↔
McIntosh et al., 2003	28 M overweight subjects (40–65 y) BMI 30 ± 1 kg/m^2	Randomized crossover 3 × 4 wk No washout	Rye WG diet: 135 g wholemeal bread, 22 g crisp bread, 50 g breakfast cereal Wheat WG diet: 135 g wholemeal bread, 42 g crisp bread, 50 g breakfast cereal (Amount of WG ingredients in a diet = 88 g)	Low fibre foods: white bread (135 g), refined wheat crisp bread (42 g), rice cereal (50 g)	Fasting insulin and glucose concentrations	↔

(*continued on next page*)

Appendix Table (*continued*)

Reference	Characteristics of participants	Study design	Wholegrain products (intake/d)	Control products (intake/d)	Methods for the evaluation of insulin sensitivity	Insulin sensitivity
Andersson et al., 2007	22 F and 8 M subjects with one or more abnormalities of MetS (59 ± 5 yrs) BMI 28 ± 2 kg/m^2	Randomized crossover 2 × 6 wk separated by a 6 −8 wk washout period	3 portions of bread (45 g), 2 portions of crisp bread (12 g), 1 portion of muesli (35 g), 1 portion of pasta (70 g) Planned intake of WG ingredients = 112 g Of which ≥90% was consumed by the subjects Grains (wheat, rye, oat) contained ≥50% WG per dry substance, mainly in milled form	Refined wheat, rye, and corn	Euglycemic hyperinsulinemic clamp	↔
Katcher et al., 2008	23 F and 24 M subjects with MetS: WG group (45 ± 8 y), BMI 36 ± 4 kg/m^2 RG group (47 ± 10 y) BMI 36 ± 5 kg/m^2	Randomized parallel, 12 wk Hypocaloric diet	Bread and rolls, ready-to-eat cereal, brown rice, oatmeal, pasta, salty snacks and snack bars (WG was listed as the first ingredient on the food label) About 5 servings 1 serving was 1 slice of bread, 28 g of ready-to-eat cereal, or 120 ml of cooked cereal/rice/pasta	Refined grains (<0.2 servings of WG foods/d)	OGTT Fasting and 2 h-glucose and insulin concentrations (ISI[a])	↔
Tighe et al., 2010	102 F, 104 M healthy subjects/MetS (52 ± 1 y) BMI 28 ± 0.5 kg/m^2	Randomized parallel, 16 wk (3 treatment groups)	*Group 1*: whole wheat foods, 3 servings (70 −80 g wholemeal bread and 30−40 g WG cereals) *Group 2*: whole wheat foods, 1 serving, and oats, 2 servings	Refined grain foods	Fasting insulin and glucose concentrations (HOMA-IR,[b] Modified QUICKI[c])	↔
Landberg et al., 2010	17 M with prostate cancer (73.5 ± 4.6 y) BMI 27.5 ± 4.6 kg/m^2	Randomized crossover 2 × 6 wk separated a by 2 wk washout	Wholegrain rye and rye bran products: 247 ± 34 g bread, 89 ± 17 g crisp bread, 50 ± 9 g breakfast cereals, 35 ± 10 g porridge (uncooked) (Fibre intake from the test products: 58 ± 7 g)	Refined wheat grain products with added cellulose: 245 ± 39 g bread, 96 ± 19 g crisp bread, 39 ± 15 g breakfast cereals, 29 ± 5 g porridge (uncooked) Fibre intake from the test products: 57 ± 9 g	Fasting insulin concentrations	↑
Giacco et al., 2010	3 F, 12 M healthy subjects (55 ± 8 y) BMI 27 ± 3.0 kg/m^2	Randomized crossover 2 × 3 wk No washout	Wholemeal wheat bread, pasta, rusks and crackers (dietary cereal fibre content = 23 g)	Refined wheat bread, pasta, rusks and crackers (Dietary cereal fibre content = 10 g)	Fasting insulin and glucose concentrations (HOMA-IR[b])	↔
Brownlee et al., 2010	216 healthy subjects no WG consumer (age 46 ± 10 yrs) BMI 30 ± 4.25 kg/m^2	Randomized parallel, 16 wk Treatment groups: *Group 1*: WG 60 g/d for 16 weeks; *Group 2*: WG 60 g/d for 8 weeks followed by 120 g/d until the end of intervention. *Group 3*: RG	Subjects freely selected from the provided foods: Whole wheat bread, shredded wheat, cheerios, porridge oats, brown basmati rice, whole wheat pasta, weetabix, oat bar, WG crisps In all products content of WG was >50% except rice and pasta (Intake of WG ingredients was 74 ± 28.5 g in the group 1 and 115 ± 39.6 g in the group 2)	Refined grain foods (no dietary change)	Fasting insulin and glucose concentrations (modified QUICKI[c])	↔

WG = wholegrain; RG = refined grains; FSIGT = Frequently sampled intravenous glucose tolerance test; ↑ improved; ↔ no effect; wk = week. Food intake expressed as mean ± SD. MetS = Metabolic Syndrome; "or absolute amount".

[a] Insulin sensitivity index.

[b] Homeostatic model assessment.

[c] Quantitative insulin sensitivity check index.

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