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Whole grain intakes, determined by dietary records and plasma alkylresorcinol concentrations, is low among pregnant women in Singapore

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Running title: Wholegrain intake in Singapore

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ABSTRACT

Objective: To quantify wholegrain intakes in pregnant women in Singapore in order to provide the first detailed analysis of wholegrain intakes in an Asian country and in pregnant women. **Design:** Analysis of 24-h diet recalls in a cross-sectional cohort study and analysis of a biomarker of wholegrain intake (plasma alkylresorcinols) in a subset of subjects. **Setting:** The Growing Up in Singapore Towards healthy Outcomes-mother offspring cohort study based in Singapore. **Subjects:** 998 pregnant mothers with complete 24-h recalls taken during their 26-28th week of gestation. Plasma samples from a randomly select subset of 100 subjects were analysed for plasma alkylresorcinols. **Results:** Median (IQR) whole grain intakes for the cohort and the 30% who reported eating whole grains were 0 (IQR 0, 9) and 23.6 (IQR 14.6, 44.2) g/day respectively. Plasma alkylresorcinol concentrations were very low [median (IQR)=9 (3, 15) nmol/l], suggesting low intakes of wholegrain wheat in this population. Plasma alkylresorcinols were correlated with wholegrain wheat intake (Spearman's $r=0.35$; $p<0.01$). **Conclusions:** Wholegrain intakes among pregnant mothers in Singapore was well below the 2 - 3 (60-95 g) servings of whole grains per day recommended by the Singapore Health Promotion Board. Efforts to increase wholegrain intakes should be supported to encourage people to choose whole grains over refined grains in their diet.

Key words: whole grain, Singapore, pregnant women, alkylresorcinols, dietary intake

INTRODUCTION

Wholegrain intakes have consistently been associated with a reduced risk of many diet-related diseases, including cardiovascular diseases, metabolic syndrome, diabetes, obesity and some cancers.^{1,2,3} Unlike refined grains, whole grains contain all of their anatomical components i.e. bran, germ and endosperm in the proportions found naturally in the grain.⁴ However, the most commonly consumed cereal products are based on refined grains, where the bran and germ are removed, resulting in lower nutrient density (Table 1).

During pregnancy, beyond prevention of related complications such as gestational diabetes,⁵ whole grains may also be important for foetal development due to their high amounts of the methyl donors, folate and betaine.^{6,7} An adequate dietary supply of these compounds may be provide an advantageous environment for the programming of the epigenome by meeting the increased need for methyl groups in rapidly dividing cells.^{8,9}

The consistent association between wholegrain intake and reduced risk of diseases has led to specific whole grain recommendations in a number of countries, including at least 48 g (3

servings)/d in the United States, to at least 75 g/8.5 MJ in Denmark and Sweden.¹⁰ In the Asia-Pacific region, current recommendations are limited to ‘make half your cereal servings whole’ in Australia, ‘eat plenty of breads and cereals, preferably wholegrain’ in New Zealand, and ‘of 5-7 cereal servings, make 2-3 servings whole grain’ in Singapore.¹¹ Where it has been studied, mean wholegrain intake has been found to be well below recommended intake, with recent estimates of intakes of 23 g/d in Irish teenagers,¹² 47 g/d in Scandinavian adults,¹³ and 13 g/d among US children and adults.^{14,15}

At present there is little information on wholegrain intakes in South East Asian countries and no comprehensive studies published in English. The Singapore National Nutrition Survey (SNNS) reported that average wholegrain intake among Singaporean adults is low, though around 50 % of the population eat wholegrain products at least once a month.¹⁶ However there no quantitative information on wholegrain intakes among pregnant women, despite whole grains being recommended as part of a healthy diet during pregnancy.¹⁷

In this study, we aimed to gain insight into wholegrain intakes among pregnant Singaporean women, by performing a retrospective cross sectional study on expectant mothers from the Growing Up in Singapore Towards healthy Outcome (GUSTO) parent-offspring cohort study. As estimating wholegrain intake from dietary records is complicated due to a lack of knowledge about identifying wholegrain foods, as well as variation in the amount of wholegrain ingredients in different foods, we also used plasma alkylresorcinols, a biomarker of wholegrain intake,¹⁸ as a non-subjective control of the questionnaire-based estimates of wholegrain intake in a subset of 100 mothers. To our knowledge, this work is the first detailed assessment of wholegrain intakes in an Asian population and in pregnant women.

MATERIALS AND METHODS

Study design

We used data from the GUSTO study, a longitudinal parent-offspring cohort study in Singapore, which aims to investigate the role of developmental factors in early life to development of metabolic diseases later in life. The study has been described in detail elsewhere.¹⁹ In brief, pregnant women attending antenatal care (<14 weeks’ gestation) in KK Women’s and Children’s Hospital (KKH) and National University Hospital (NUH), which house the major public maternity units in Singapore, were recruited into the GUSTO study from June 2009 to September 2010. The inclusion criteria included age range between 18-50 years, intention to finally deliver in KKH or NUH, intend to reside in Singapore for the next 5 years, and willingness to donate cord, cord blood, and placenta. Only Chinese, Malay, and

Indian women whose parents were from the same ethnic background were included in the study. Women with pre-existing health conditions such as type 1 diabetes mellitus, current depression or mental health-related disorders were excluded. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the Institutional Review Boards of the KKH and NUH. Written informed consent was obtained from all subjects/patients on recruitment.

Maternal characteristics

Data on ethnicity, maternal age and educational level were collected from the participants during recruitment and information about cigarette smoking and alcohol consumption habits during pregnancy was gathered during the 26-28 week pregnancy clinic visits. A 24-h dietary recall was also administered to ascertain participants' diet during pregnancy and for subsequent determination of whole grain intakes. At the same clinic visit, maternal weight and height of participants were measured for calculation of body mass index. Fasting blood samples were drawn for subsequent analyses of plasma alkylresorcinols. The participants also underwent oral glucose tolerance testing (OGTT) and subsequently had their gestational diabetes mellitus (GDM) status diagnosed based on World Health Organization criteria.²⁰

Estimation of dietary whole grain and alkylresorcinol intakes

Total whole grain, wholegrain wheat, non-wheat whole grain (wholegrain rice, oats and corn – other whole grains were not recorded in any of the diet recalls) and alkylresorcinol intakes were determined from the 24-h diet recall obtained from the mothers at 26-28 week of gestation. Food pictures were shown during the interview to obtain more accurate measures of portion sizes. Wholegrain contents in cereal products were converted to grams of whole grain per day based on information supplied on-pack, by manufacturers or estimated from available recipes. The wholegrain definition used was that of the Whole Grains Council,⁴ which is used for nutrition labelling guidelines by the Health Promotion Board of Singapore. Alkylresorcinol intake from foods containing wholegrain wheat was estimated based on data available for specific foods^{21,22,23} or estimates based on the average known flour content in each food or food group. Data were corrected for dry weight, based on water content reported in local food composition tables.²⁴ The 24 h recalls were validated against three day food diaries in 400 subjects. While whole grain intake was not determined for the three day food

diaries, the Spearman's correlation coefficient for dietary fibre was 0.49, and 0.47 for total carbohydrate suggesting agreement between the two methods.

Measurement of plasma alkylresorcinol concentrations

In order to verify estimates of wholegrain intake from the 24-hr diet recalls, fasting plasma alkylresorcinols were analysed in a randomly selected subset of 100 subjects from the total cohort of 988 subjects with eligible diet records (see below). Plasma alkylresorcinols were analysed using a modification of a normal phase LC-MS/MS method,²⁵ after extraction with diethyl ether. The modification entailed the use of a shorter column (100 mm x 2 mm, Polaris NH₂, Agilent, Santa Clara, CA, USA, instead of a 250 mm x 2 mm column) and a shortening of the chromatographic run time from 15 minutes to 8 minutes. Results did not differ for samples analysed using the updated method compared with the new method. Matrix matched calibration was used, and two control samples were run in triplicate with each batch. Calibration curve standards were run in random order throughout each sequence. Intra-batch repeatability for each control plasma sample was <10% and inter-batch repeatability <15%.

Statistical analyses

Maternal characteristics between the two groups (whole grain vs non-whole grain consumers) were compared using independent sample t-tests for continuous variables and chi-square tests for categorical variables. Dietary whole grain and alkylresorcinol intakes were assessed for normality using the Kolmogorov-Smirnov test, Q-Q plots and histograms. Normality testing indicated that the data were left-skewed, so non-parametric tests were used for further analyses. Spearman rank correlation coefficients were calculated for the associations between plasma alkylresorcinol and estimates of total dietary wholegrain and alkylresorcinol intake (n=100). Associations between wholegrain intake and plasma alkylresorcinols were analysed with and without adjustment for total energy intake. All statistical calculations were performed using SPSS v. 16.0 (SPSS Inc., Chicago, IL, USA). Two sided p values less than 0.05 were considered statistically significant.

RESULTS

Characteristics of wholegrain food consumers and non-consumers

Of the 1162 GUSTO participants recruited, 988 participants had valid 24-hour diet recalls, which were used to determine wholegrain intake. Of the 988 subjects, only 293 (29.6%) reported eating any whole grain in the 24-h diet recall (Figure 1A). Of these, mothers of

Indian ethnicity had the greatest frequency of wholegrain consumption (44%). Participants who were younger ($p=0.011$), had lower educational level ($p=0.0002$), smoked regularly before pregnancy ($p=0.001$), had exposure to smoking before and during pregnancy ($p<0.001$ for both), and had no intake of prenatal supplements ($p=0.06$) were less likely to consume whole grain foods (Table 2).

Whole grain intakes did not vary by maternal body mass index (BMI) at 26–28 weeks of gestation, gestational diabetes mellitus, alcohol consumption before and during pregnancy or parity (data not shown).

Dietary wholegrain and alkylresorcinol intakes

In the whole cohort, wholegrain intake was highly skewed towards low consumption, with few of those participating in the study eating more than 50 g of whole grain/d (Figure 1A). The median (IQR) whole grain intake of the cohort was 0 (IQR 0, 9) g/day ($n=988$). Among those who reported consuming any whole grain in their diet ($n=293$; 29.6%), the median whole grain intake was 23.6 (IQR 14.6, 44.2) g/day (Figure 1B). In this group, 63% ($n=185$) consumed wholegrain wheat, which corresponded to a median intake of 18.1 (IQR 9.4, 35.9) g/d and equivalent to a median of 7.22 (IQR 3.68, 11.68) mg of alkylresorcinols/d. Non-wheat whole grain was consumed by 49.1% ($n=144$) of the whole grain consumers (median 31.4 g/day; IQR 15.88, 44.2). As alkylresorcinols are only found in high amounts in wheat and rye, the non-wheat sources of whole grain identified from the diet records (rice, corn and oats) do not add to alkylresorcinol intake.

Association of plasma alkylresorcinols with wholegrain and alkylresorcinols intakes ($n=100$)

Of the 100 subjects analysed for plasma alkylresorcinols, only 6 had plasma concentrations above 60 nmol/L (Figure 1C), which corresponds to a suggested threshold for regular intake of at least some wholegrain wheat.¹⁸ The median total plasma alkylresorcinol concentration was 9.26 (IQR 2.6, 15.35) nmol/L. The relative percentages of the individual alkylresorcinol homologues was C17:0 (3 %), C19:0 (22 %), C21:0 (50 %), C23:0 (17 %) and C25:0 (8 %), indicating that wheat was the source of the alkylresorcinols.²⁶

Plasma alkylresorcinols correlated with total whole grain, whole grain wheat and alkylresorcinol intake, but not with the intake of non-wheat whole grains. Correlations were significant for all alkylresorcinol homologues measured and strongest for C21:0 and C23:0 (Table 3).

Among subjects who reported consumption of wholegrain wheat products (n=24), plasma alkylresorcinol concentrations were higher (median 21.34 nmol/L; IQR 6.73, 59.48) than those not reporting any consumption of wholegrain wheat (median 7.37 nmol/L; IQR 2.54, 13.69; $p=0.03$). There was no difference in plasma alkylresorcinol concentration between those reporting eating non-wheat whole grains and those who did not eat any whole grains (data not shown). Adjustment for energy intake did not affect the relationships between wholegrain intake and plasma alkylresorcinols.

Key food sources of whole grains in the diets

Of the wholegrain foods eaten, wholemeal bread was the most commonly eaten, followed by oat products, 'ethnic breads' (e.g. Indian flatbread) and whole grain breakfast cereals (Table 4). Corn-based products were also a relatively commonly consumed source of whole grains. Brown rice was only reported as being eaten by 4.3% of the study participants.

DISCUSSION

The median wholegrain intakes among pregnant women in Singapore was low (0 g/d) and below government guidelines for wholegrain intake. Among participants who did report eating whole grains, intakes were also well below recommendations (23.6 g/d). Plasma alkylresorcinols in the subpopulation were distributed similarly to the wholegrain intakes, confirming the findings from the 24-h diet recall data that wholegrain intakes were indeed low. The low whole grain intakes in this cohort are consistent with low intakes among the adult Singapore population, as reported in the recent Singapore National Nutrition Survey.¹⁶ Internationally, this is low compared with European countries, where median wholegrain intakes among Swedish women of fertile age were 30 g/d,²⁷ 48 g/d in Denmark,²⁸ and around 12 g for men and women between the ages of 19 and 44 in the United Kingdom.²⁹ Data on whole grain consumption during pregnancy is lacking, though extrapolating from Brazilian data¹⁷ would suggest that whole grain intake during pregnancy is also low (mean < 5 g/d).

In our cohort of pregnant women, consuming whole grains was associated with a number of healthy behaviours and indicators of higher-socioeconomic status, including not smoking, having a high educational level, and a tendency to consume dietary supplements prior to pregnancy. Associations of greater wholegrain intake with older age and higher educational attainment, but not smoking, have also been observed among UK adults.²⁹ Participants of Indian ethnicity had a greater likelihood of eating whole grains, compared with those of Chinese or Malay ethnicity, which we speculate is due to the greater use of whole grains in

Indian cuisine. There was no association between eating whole grains and incidence of gestational diabetes or body mass index, though overall intakes were so low among wholegrain consumers that an effect on these parameters would be unlikely.

In addition to diet recalls to assess wholegrain intake, the study results were confirmed using plasma alkylresorcinols, a non-subjective measurement of wholegrain intake to confirm the results of the 24-h diet recalls. The advantages of using a biomarker in an observational study is that it does not rely on the population having any background knowledge of what whole grain foods are, nor any bias towards over reporting if participants over-emphasise healthier foods in their diet. However, a limitation of using plasma alkylresorcinols as a biomarker of wholegrain intake is that they do not capture brown rice intake, as brown rice does not contain any alkylresorcinols²¹. This is unlikely to have a large impact on the overall interpretation of our current results as brown rice was not recorded as being commonly eaten by this cohort (Table 4). Moreover, based on the NNS, the mean intake of brown rice intake was also low, estimated at 16 g/d compared with 101 g/d for wholemeal bread and wholegrain breakfast cereals¹⁶. This would suggest that wholegrain wheat intakes are far greater than brown rice intakes and that plasma alkylresorcinols should be able to capture a large proportion of wholegrain intakes in the Singaporean population.

The median fasting plasma alkylresorcinol concentration in this population was 9 nmol/L, which is low compared with the few population based studies that have been published to date. Median fasting plasma alkylresorcinol concentrations in elderly US citizens was 20 nmol/L,³⁰ while the geometric mean of fasting plasma alkylresorcinol concentrations measured in a subsample of women in a cohort from Northern Sweden was 38 nmol/L.³¹ Based on the mean plasma response to alkylresorcinol intakes across 9 studies,¹⁸ a plasma alkylresorcinol concentration of 9 nmol/L would be expected from the intake of 1.3 mg of alkylresorcinols/d, the amount found in approximately 80 g of cooked egg noodles.²³ Plasma alkylresorcinol concentrations from controlled whole grain-free diets (but still containing refined wheat) are generally below 60 nmol/L,¹⁸, confirming that the plasma alkylresorcinol concentrations in this study reflect very low intakes of whole grains, and possibly wheat products in general.

Although the plasma alkylresorcinol concentrations measured were low, they were still significantly correlated with reported wholegrain, wholegrain wheat and alkylresorcinol intakes. These correlations were in the same range as those previously reported for food frequency questionnaire-based estimates of whole grain intake,¹⁸ and were higher for homologues C21:0 and C23:0. As the association of plasma alkylresorcinols with wholegrain intake still holds in this population, we propose that being pregnant does not alter the uptake

and metabolism of alkylresorcinols in a way that would confound their use as a biomarker of wholegrain intake during pregnancy. Correlations between dietary recall of wholegrain intakes and plasma alkylresorcinols in the range of 0.25-0.35 appear moderate, and may be seen to raise questions about the relationship between these two. However these correlations are a combination of measurement error for the dietary recall method for whole grains, combined with the many factors that lead to inter-individual differences, including absorption and metabolism of alkylresorcinols, and time since last meal. The deviation (error) from actual intake for both the recall and biomarker are unrelated, so that the use of both methods helps to provide a better picture of actual whole grain intake. In this case, while the overall distribution is similar (Figure 1), the low measured alkylresorcinol concentrations suggested that intakes were lower than that reported from the recall measures.

This study represents the first comprehensive analysis of wholegrain intakes in an Asian population. While some recent reviews have suggested that whole grain may be important for improving health in some Asian populations,^{32,33,34} there is little data available on wholegrain intakes. Daily whole grain intakes in China were estimated to have dropped from 104 g to 24 g from 1982 to 2002 (against a background of 510-402 g/d total grain consumption),³⁵ suggesting that while there may be a tradition of whole grain intake in some Asian populations, economic development or greater availability leads to reduced wholegrain intake. The finding that wholegrain intakes among pregnant Singaporeans are low is a starting point for efforts to encourage greater availability of wholegrain foods and increase consumption of whole grains.

The low rates of wholegrain consumption among pregnant women in this study are of concern given the substantial evidence based observational data that suggests that whole grains are an important part of a healthy diet.¹ In particular, some whole grains are key sources of the methyl-donors folate and betaine, which play a role in preventing neural tube defects,^{36,37} as well as a diverse range of vitamins and minerals, and dietary fibre. While there may be efforts to increase whole grain consumption at a government level,³⁸ these data suggest that among pregnant women, few are seeking out whole grain versions of cereal products, or that such products may not be widely available. A public-private partnership to promote eating whole grains first in Denmark resulted in an increase of wholegrain intake from 32 g/d to 55 g/d²⁸ demonstrating that whole grain 'campaigns' can lead to substantial increases in intake.

In this observational study, we have found both subjective and non-subjective evidence that whole grain intakes among pregnant Singaporeans are very low. This may have implications

for long-term disease risk and dietary intake of nutrients important for foetal development. Overall research on wholegrain intakes and health benefits among South East Asian populations is lacking, and more research is needed to define the potential public health benefits of higher intakes.

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CONFLICT OF INTERESTS

Peter Gluckman, Keith Godfrey and Yap Seng Chong have received reimbursement for speaking at conferences sponsored by companies selling nutritional products. They are part of an academic consortium that has received research funding from Abbott Nutrition, Nestec and Danone. Irma Silva-Zolezzi and Katherine Macé are Nestec SA employees, and Alastair Ross is a former employee of Nestec SA, the research arm of the Nestlé food and beverage company. The other authors have no financial or personal conflict of interests.

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Table 1. Comparison of the macronutrient, B-vitamins, and alkylresorcinol content[†] of whole grain (WG) wheat with refined (RG) wheat and white rice with brown rice.

	Energy (kJ/100 g)	Carbohydrate (g/100 g)	Protein (g/100 g)	Fat (g/100 g)	Dietary fibre (g/100 g)	Folate (μ g/100 g)	Betaine (mg/100 g)	Choline (mg/100 g)	Thiamine (mg/100 g)	Riboflavin (mg/100 g)	Niacin (mg/100 g)	Vitamin B ₆ (mg/100 g)	Alkylresorcinols (mg/100 g)
Whole grain wheat	1610	70.6	11.6	2.9	13.1	50	90	20	0.47	0.15	4.65	0.41	50
Refined wheat	1697	83.4	9.8	2.2	4.1	26.5	23	10	0.21	0.03	1.04	0.13	5
Difference WG – RG	-87	-12.8	1.8	0.7	9.0	23.5	67	10	0.26	0.12	3.61	0.28	45
Brown rice	1967	79.9	10.4	3.3	4.7	15.6	3	8	0.44	0.06	6.00	0.67	0
White rice	1694	88.4	9.0	0.8	1.1	10.3	3	10	0.05	0.06	0.57	0.64	0
Difference WG – RG	2	8.5	1.5	2.5	3.5	5.2	0	-2	0.40	0	5.43	0.02	0

[†] Data from the Swedish National Food Composition Database³⁹ for all nutrients except for betaine and choline⁶, and alkylresorcinols²³

Table 2. Maternal characteristics according to intake of whole grain in the diet (N=988).

	No whole grain intake		Consumed whole grain		<i>p</i> -value [†]
	N	%	N	%	
Ethnicity					0.0001
Chinese	374	69.3	166	30.7	
Malay	217	83.1	44	16.9	
Indian	104	55.6	83	44.4	
Age					0.011
less than equal 21	35	89.7	4	10.3	
more than 21	660	69.5	289	30.5	
Education [‡]					0.0002
Up to secondary school	231	78.6	63	21.4	
Post-secondary school	273	77.1	81	22.9	
University	186	56.2	145	43.8	
Smoked regularly before pregnancy					0.0005
No	587	68.3	272	31.7	
Yes	108	83.7	21	16.3	
Smoke exposure before pregnancy					0.0003
No	350	61.9	215	38.1	
Yes	345	81.6	78	18.4	
Smoke exposure during pregnancy					0.0003
No	398	64.2	222	35.8	
Yes	297	80.7	71	19.3	
Any prenatal supplements during pregnancy [§]					0.0592
No	181	65.8	94	34.2	
Yes	442	72.3	169	27.7	

[†]*P*-values were obtained using chi-square tests.

[‡]N= 979

[§]N= 886

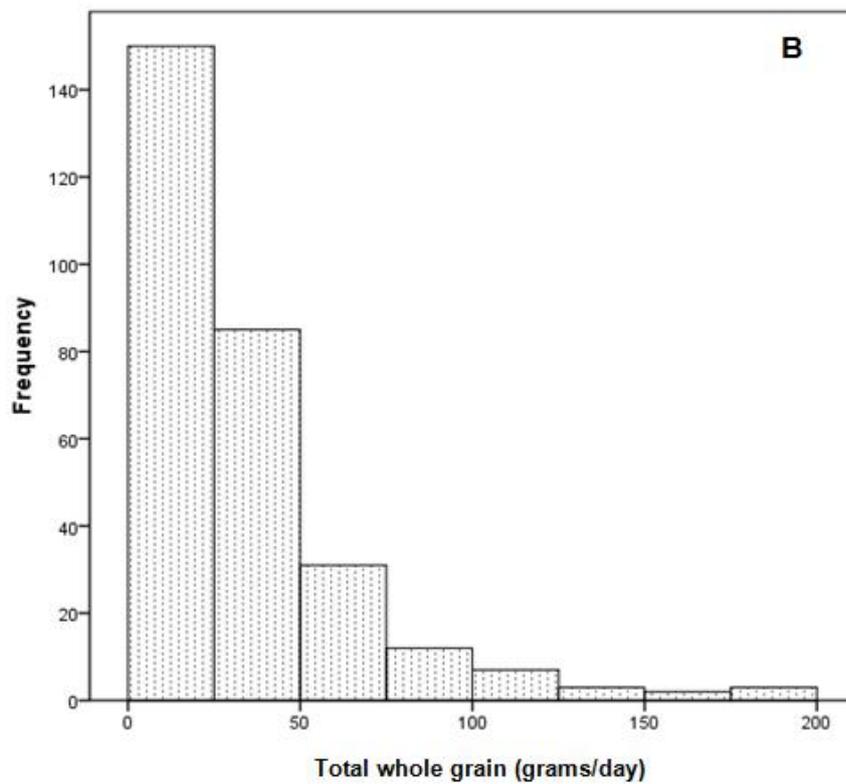
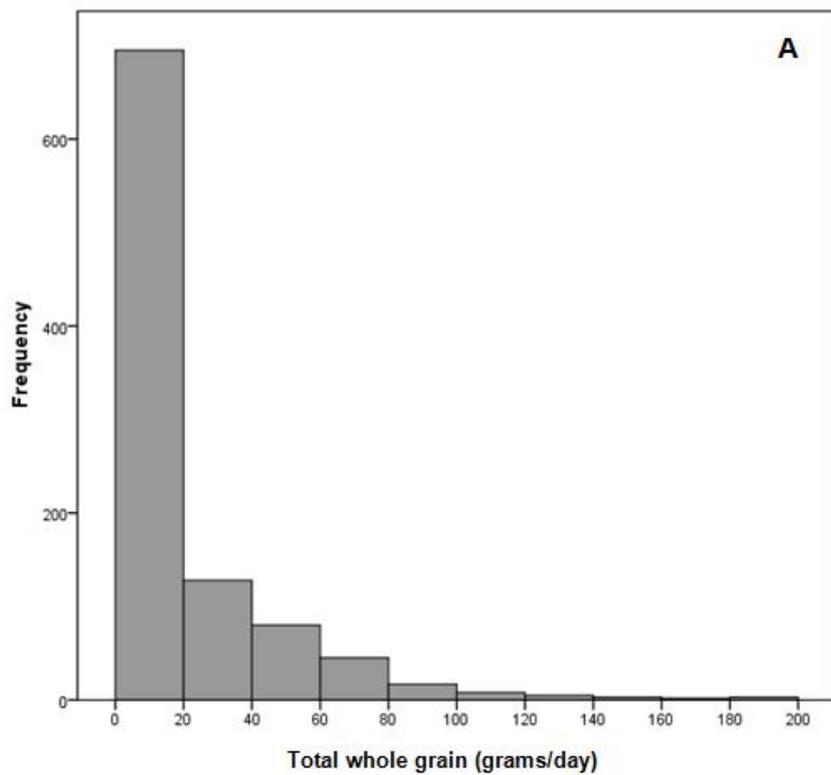
Table 3. Spearman's correlations between individual and total plasma alkylresorcinol homologues and estimates of whole grain and alkylresorcinol intakes (n=100). C17:0, C19:0, C21:0, C23:0 and C25:0 are the main alkylresorcinol homologues present in plasma.

	C17:0	C19:0	C21:0	C23:0	C25:0	Total plasma alkylresorcinols
Total wholegrain intake (g/day)	0.25*	0.13	0.31**	0.29**	0.13	0.26**
Wholegrain wheat (g/dry)	0.24*	0.22*	0.42**	0.36**	0.27**	0.35**
Non-wheat wholegrain (g/day)	0.16	0.02	0.05	0.12	-0.05	0.07
Total alkylresorcinol intake (mg/day)	0.26*	0.24*	0.41**	0.37**	0.29**	0.35**

* Spearman's correlation $p < 0.05$; **Spearman's correlation $p < 0.01$

Table 4. Food sources of wholegrain wheat and other wholegrains in the cohort and their frequency of intake (% of people consuming the food).

	Freq (%) n=988
Foods made of whole grain wheat	
Wholemeal bread	10.0
Ethnic breads (e.g., chapati, paratha, puri)	5.1
Breakfast cereal with wholegrain wheat flour	4.4
Cereal drink (with whole wheat flakes and rice)	2.0
Wholegrain wheat biscuits/cookies	2.6
Uppuma (thick porridge made of wholegrain wheat)	1.5
Foods made of other whole grain	
Oats and oats products (biscuits, cookies, muesli bar)	5.6
Corn and corn products (sweetcorn cream style, corn chips)	4.3
Brown rice	4.3
Wholegrain corn breakfast cereal	2.5
Barley	0.2



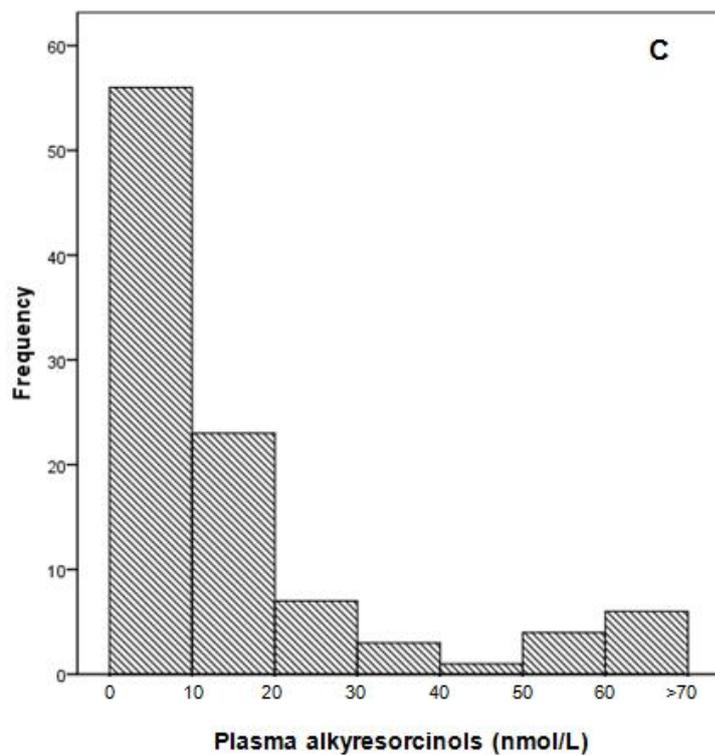


Figure 1. Distribution of measures of wholegrain intakes in pregnant women in the GUSTO cohort. Wholegrain intake in the entire cohort (n=988) was estimated by 24 h recall (A); distribution of wholegrain intakes among subjects reporting consumption of whole grains (n= 293) (B) and distribution of plasma alkylresorcinols among a subset of 100 women from the same cohort (C).