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Abstract

Type 2 diabetes (T2D) is a public health problem of great concern. Globally it is increasing and it generates a huge economic burden to society and problems for the individuals. Whole grain has shown to have a protective role against T2D in epidemiological studies and underlying mechanisms are poorly understood, but decreased energy intake, higher insulin sensitivity and improved glucose tolerance have been suggested and partly confirmed in studies. Alkylresorcinols (AR) are phenolic lipids found in different homologs only in the outer parts of wheat and rye among commonly consumed foods. It is used as a biomarker of whole grain for rye and wheat intake. The Finnish Diabetes Prevention Study (DPS) started in 1993 with the purpose to evaluate if an intensive diet- and exercise program could prevent or delay T2D in persons with impaired glucose tolerance (IGT). The aim of this study was to investigate if there was a difference in whole grain cereal intake among non-diabetics and people who developed diabetes under a period of 8 years. A fraction of the subjects in the (DPS) was studied. The aim was also to investigate differences in whole grain intake between participants who got lifestyle intervention and those who did not. Plasma AR was measured and used as a proxy of whole grain rye and wheat intake. Quality control samples were included in each batch to ensure adequate precision. AR were extracted with diethyl ether and further cleaned up with solid phase extraction (SPE). AR were made more volatile through derivatisation with trifluoroacetic anhydride (TFAA) and analyzed with gas chromatography-mass spectrometry (GC-MS). The coefficient of variation (CV) within batches were $\leq$ 9% and between batches $\leq$ 15% except of one homolog, C25. No significant difference was found in total AR concentration between the lifestyle intervention and control groups or between subjects who developed diabetes during follow up and non-diabetic controls. Even if there was no difference in plasma AR between the lifestyle intervention- and control groups there was a significant difference in fiber intake (p=0.001). The Finnish population consumes large amounts of whole grain in the form of rye bread. When the subjects were advised to raise their consumption of dietary fiber during the lifestyle intervention, it is likely they did this with other foods than rye and wheat sources. The main findings of this study were a positive correlation between the AR C17/C21 ratio and time to T2D diagnosis (p=0.027) and the fact that the AR C17/C21 ratio was higher within non-diabetics than diabetics (p=0.025). This indicates that individuals with a high intake of whole grain rye develop T2D later than those with a low intake, or does not develop it at all, i.e. that whole grain rye is protective. This should be confirmed with more sophisticated analysis of data in the present study but that is out of scope for this study.

Keywords: T2D, whole grain, rye, wheat, C17/C21, biomarker, alkylresorcinols, analysis, gas chromatography- mass spectrometry (GC-MS).

Sammanfattning

Typ 2-diabetes är ett stort folkhälsoproblem. Prevalensen ökar globalt och genererar en stor ekonomisk kostnad för samhälle och lidande för individen. Det har i epidemiologiska studier visat sig att fullkorn skyddar mot typ 2-diabetes. Mekanismerna bakom fullkorns positiva inverkan är inte kartlagda men reducerat energiintag, ökad insulinkänslighet och ökad glukostolerans är faktorer som har föreslagits och delvis bekräftats i studier. Alkylresorsinoler (AR) är fenoliska lipider vilka endast finns i de yttersta skikten av råg och vete bland vanliga livsmedel. AR används idag som biomarkör av fullkornsintag av råg och vete. En finsk studie i förebyggande av diabetes, The Finnish Diabetes Prevention Study (DPS), startade 1993 med syfte att utvärdera ett intensivt diet- och träningsprogram för personer med nedsatt glukostolerans. Syftet med den här studien var att undersöka om det fanns en skillnad i fullkornsintag mellan de som inte utvecklat- och de som utvecklat typ 2 diabetes under en åtta års period. Studien gjordes på en del av deltagarna i DPS. Syftet var också att undersöka fullkornsintaget hos de som fick livsstils intervention och de som inte fick det. Fullkornsprodukterna råg och vete mättes genom AR i plasma. Kontrollprover användes för att säkerställa precision. AR extraherades med dietyl eter och renades med fast-fas-extraktion. AR gjordes mer flyktiga med derivatisering av trifluoracetylacetat och analyserades med gas kromatografi-mass spektrometri (GC-MS). CV värdet av kontrollproverna inom batcherna var $\leq$ 9% och mellan batcherna $\leq$ 15% med undantag av homolog C25. Det var ingen signifikant skillnad mellan totala AR och interventionsgrupperna eller mellan totala AR och de som utvecklade diabetes och de som inte gjorde det. Även om det inte fanns en skillnad mellan AR intag och interventionsgrupperna fanns det en signifikant skillnad i fiberintag (p=0.001). Den finska populationen konsumerar mycket fullkorn och den största delen utgörs av rågbröd. När deltagarna fick rådet att öka sitt fiberintag gjordes det troligtvis genom andra källor än via råg- och vete produkter. De viktigaste fynden i denna studie är det positiva förhållandet mellan AR kvoten C17/C21 och tiden till att utveckla typ 2-diabetes (p=0.027) och den högre kvoten av C17/C21 hos icke diabetiker än diabetiker (p=0.025). Detta indikerar att individer med ett högt intag av fullkornsråg senare utvecklar typ 2 diabetes än de med ett lågt intag samt att en högre fullkornskonsumtion av råg kan förebygga en utveckling av diabetes typ 2. Resultaten bör bekräftas med mer avancerade analysmetoder men det ligger utanför detta kandidatarbete.

Nyckelord: Typ 2-diabetes, fullkorn, råg, vete, C17/C21, alkylresorcinoler, biomarkör, analys, gas kromatografi-mass spektrometri (GC-MS).

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Abbreviations

AR	Alkylresorcinols
CV	The coefficient of variation
CVD	Cardiovascular diseases
DPS	The Finnish Diabetes Prevention Study
FFQ	Food frequency questionnaire
GC-MS	Gas chromatography-mass spectrometry
HDL	High density cholesterol
IGT	Impaired glucose tolerance
SD	Standard deviation
SPE	Solid phase extraction
T2D	Type 2 diabetes
TFAA	Trifluoroacetic anhydride

1 Introduction

1.1 Background

1.1.1 Diabetes

Type 2 diabetes (T2D) is a public health problem of great concern worldwide. It is increasing globally due to population growth, urbanization, aging, physical inactivity and obesity (Wild et al., 2004). The increase is most drastic in developing countries where the western diet is adopted in the same time as a change in lifestyle is implied. In year 2010 the global prevalence of T2D was 6.4% and the estimation for year 2030 is 7.7%. In numbers, 285 million people had diabetes year 2010 and 439 million are expected to have it year 2030 (Shaw, Sicree & Zimmet, 2010). This increase will generate a huge economic burden for societies and problem for individuals. More preventive actions are needed to lower the prevalence of T2D (Zhang et al., 2010). The health burden is mostly due to atherosclerotic vascular diseases, neuropathic and microvascular complications that follows with T2D. Examples of clinical complications are reduced eye vision, kidney failure, heart attacks, stroke, amputation and sexual dysfunction (American diabetes association, 2014).

Insulin resistance is a prestage of T2D and is defined as an inability for the liver, adipose tissue and skeletal muscles to respond to insulin and this may develop into T2D when the B cells in the pancreas fail to produce enough insulin. Type 1 diabetes is different from type 2 with no production of insulin at all. When it debut there can still be production but with time it will decline. Type 1 diabetes is an autoimmune disease with destruction of the beta cells (Egan & Dienneen, 2014). T2D stands for 90-95% of all diabetes cases. It often develops asymptomatically with hyperglycemia which can have and injurious effect on many organs before the disease is discovered (American diabetes association, 2014). T2D may develop as a result of the metabolic syndrome. Except of abdominal obesity two out of the following four criteria are needed for a diagnosis of the metabolic syndrome; elevated blood pressure, high serum triglycerides, low levels of high density cholesterol (HDL) and elevated fasting glucose (both for levels corresponding to con-

firmed diabetes and levels indicating on pre-diabetes) (Alberti, Zimmet & Shaw, 2006).

1.1.2 Whole grain

Whole grain are in epidemiological studies associated with beneficial health outcomes such as reduced risk of developing T2D (de Munter et al., 2007, Cho et al., 2013, Parker et al., 2013), cardiovascular diseases (CVD) (Flight and Clifton, 2006) and some cancers (Kyrø et al., 2014, Schatzkin et al., 2008). Whole grain shall consist of an intact, cracked, flaked or ground kernel after the removal of the inedible parts (husks and hulls). The bran, germ and endosperm should be present in the proportions as they naturally are in the intact kernel (Willem van der Kamp et al., 2014). The nutrients in whole grain are mainly concentrated in the bran and germ and consist of resistant starch, oligosaccharides, certain fatty acids, molecules with antioxidant properties, vitamins, minerals and phenolic compounds. In refined grains, the bran and most of the germ have been removed during processing. The endosperm remains and is rich in starch and low in nutrients (Slavin et al., 1999). The mechanism behind the benefits of whole grain consumption and T2D is not fully understood. Studies have shown that whole grain consumption offer protection by mechanisms as decreased energy intake, higher insulin sensitivity and improved glucose tolerance (Ludwig DS et al., 1999). Dietary fibers slower the absorption and digestion of carbohydrates and there will be a lower glucose response and less insulin is required. Once the fibers are processed the vitamins, minerals, fatty acids and phytochemicals are free to be absorbed (Slavin et al., 1999). Scandinavians consume more whole grain than US and UK (Jacobs et al., 2001). It is difficult to compare studies of whole grain intake due to vague and different definitions of whole grain foods. Therefore have objective ways of measuring whole grain intake been requested (Lang and Jebb, 2003).

1.2 Biomarkers

There is a lot of research about food intake and risk of diseases as endpoints. Except of bias from unclear definitions of whole grain there are also common random and systematic errors from gathering information by self-reporting as food frequency questionnaires (FFQ). For example under- or over reporting of food intake, subjects report what is believed to be healthy or asked for or what is thought the investigator wants to hear. Instead of collecting all the information in this subjective manner has there been a need for an objective way of measuring (Lissner et al., 2007). A biomarker is a substance that can be measured in body fluids as blood and urine and it has a biological effect on humans. Also biomarkers can be biased by factors as health status, biochemical availability, age and genetics. The optimal way to avoid bias is to combine both biomarkers and subjective measurements such as food frequency questionnaires, 24 hour recall et cetra (Willett, 2012).

1.3 Alkylresorcinol as a biomarker of whole grain rye and wheat

There has been a lot of research to investigate if the phytochemical alkylresorcinols (AR) can be a potential biomarker of wholegrain (Chen et al., 2004, Landberg et al., 2008b, Ross, 2012, Magnusdottir et al., 2013). AR, 1,3-dihydroxy-5-alkylbenzene, are phenolic lipids. They are found in a wide variety of derivate in a number of bacteria and fungi but most are of plant origin. Interesting for the food area is the significant amount found in some members of the family Gramine (Kozubek and Tyman, 1999). Higher levels have been found in wheat (489-1429 µg/g), rye (720-761 µg/g) and triticale (439-647µg/g). Lower levels have been found in barley (42-51 µg/g) and nothing in millet and maize (Ross et al., 2003b).

ARs are located in high concentrations in the bran fraction (Landberg et al., 2008a) and only in minute quantities in the endosperm (Ross and Kochhar, 2009). With this location they were though of suitable biomarkers of whole grain rye and wheat intake (Landberg et al., 2008a). Several studies have also shown that the concentration of ARs in plasma corresponds to the intake of whole grain rye and wheat intake (Landberg et al., 2008b, Landberg et al., 2009a, Linko-Parvinen et al., 2007). Other factors that make ARs good biomarkers are that they are absorbed by humans, they remain undestroyed during baking or other heat treatment (Ross et al., 2003a) and they can be measured in plasma and their metabolites can be measured in urine (Landberg et al., 2009).

1.4 The chemistry of alkylresorcinols

AR molecules consist of a resorcinol with an alkyl group (Figure 1). They have an amphiphilic character due to the hydrophilic dihydroxylbenzene group (resorcinol) and the lipophilic alkyl group. In rye and wheat, the main homologs have odd numbered alkyl chains with 17-25 hydrocarbons (Kozubek and Tyman, 1999). A study performed by Ross et al (2001) found the proportions of each alkyl group in rye to be C17:0 23%, C19:0 32%, C 21:0 26%, C23:0 11% and C25:0 8%. It differs between types of rye product but broadly the proportions are as mentioned. In wheat it is more or less similar but with a lower amount of C17:0 and slightly higher of C21:0 (Ross et al., 2003b). The alkyl chains are mostly saturated >80%, but unsaturation does also occur, especially in rye (Ross et al., 2003b).

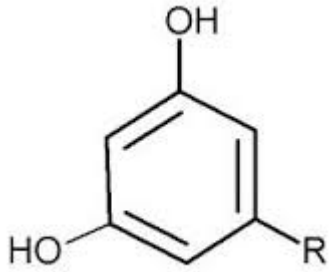

Figure 1. The structure of alkylresorcinols. The most common homologs in cereals have an odd numbered alkyl chain with 17-25 hydrocarbons on position 5 (R).

The ratio of the AR homologs C17:0/C21:0 has been found to distinguish between cereal species (Chen et al. 2004). The ratio in wheat is 0.1 and in rye 1.0. This can be used to distinguish between rye and wheat in food products (Chen et al., 2004). This ratio can also reflect the relative intake of rye and wheat in plasma samples (Linko-Parvinen et al., 2007). A study made by Magnusdottir et al. (2014) showed that a higher ratio was associated with better insulin sensitivity. Although the ratio in rye is 1.0 it is normally between 0.6-0.8 in fasting plasma samples (Linko-Parvinen et al., 2007). There is need of more research to conclude why the ratio is lower but it probably has to do with a faster metabolism of the homolog C17:0 (Ross, 2012).

Whole grain consists of many components and the mechanisms of the benefits with whole grain are still obscure. In the case of ARs they have been thought to have antioxidant properties but the results have been poor compared to alpha-tocopherol *in vitro* (Kamal-Eldin et al., 2001). They do have antifungal and anti-microbial activities (Kozubek and Tyman, 1999). *In vitro* experiments have shown an anticancer effects and an ability to inhibit enzymes. One inhibition that has been observed is a precursor step of forming triacylglycerol. Studies has also shown that a diet high in AR has led to lower levels of cholesterol in the plasma in animal trials (Ross, Kamal-Eldin & Aman, 2004).

1.5 The Finnish Diabetes Prevention Study (DPS)

The DPS study started in 1993 with the aim to evaluate if an intensive diet and exercise program could prevent or delay T2D in people with impaired glucose tolerance (IGT). It was also evaluated what effect the program had on the atherosclerotic vascular diseases and of the rate of cardiovascular events. Test subjects were divided in an lifestyle intervention and a control group. The control group did annually receive basic information about the benefits with improved diet, weight reduction and physical activity. The subjects in the lifestyle intervention group received individual guiding several times each year. The guiding included

advice about diet with the aim of reducing weight and in reducing the intake of saturated fats and increase intake of dietary fiber. Each individual received support with their physical activity. A consistent weight reduction and a higher tolerance for glucose were observed in the lifestyle intervention group during the follow up years. After 4 years, 11% of the subjects in the lifestyle intervention group had diabetes and the number in the control group was 23%. The lower incidence of diabetes in the lifestyle intervention group shows that T2D can be prevented and/or delayed with change of lifestyle (Tuomilehto et al., 2001). There has been many follow up studies on DPS after the end of the lifestyle intervention (4 years after start up). Except of the aspect of T2D and obesity, researchers have been looking on how lifestyle changes can affect depression (Ruusunen et al., 2012). There has also been studies on the possibility that these lifestyle changes can affect the telomere length which is considered a biomarker for aging (Hovatta et al., 2012).

1.6 Aim

The aim of this study was to investigate if there was a difference in whole grain intake among non-diabetics and people who developed diabetes during an 8 years period after the study started (irrespective of dietary intervention). The aim was also to evaluate the difference in whole grain wheat and rye intake between subjects who were in the lifestyle intervention group compared with subjects in the control group. Whole grain wheat and rye was measured by plasma AR.

2 Materials and Methods

2.1 Samples

From the DPS study did we get access to a subsample of 100 samples from subjects who developed T2D and 100 control subjects, irrespective of treatment. In total 186 plasma samples collected between the years 1996-1998 were used. The samples were analyzed with the knowledge who had developed T2D and those who had not. The batches were run with 50/50 from each group. The samples had been stored in -70 °C and were thawed in room temperature before analysis.

2.2 Method

The method used for quantifying ARs in plasma was the one designed by Landberg, Åman & Kamal-Eldin (2009b), with small modifications. ARs were extracted with diethyl ether. The samples were further cleaned up with a solid phase extraction robot (SPE). The final elute in the SPE was made with 2 % formic acid in MeOH instead of 2% acetic acid in MeOH as in the method of Landberg et al (2009b). Another deviation from the method was that the derivatization of the ARs was done with 200 µl trifluoroacetic anhydride (TFAA) instead of silylation. Thereafter the samples were incubated in 40 °C for 30 minutes and transferred to vials with inserts. The vials with TFAA was left to dry in 60 °C and then 20 µl of undecane was added as solvent. Finally, the samples were analyzed by gas chromatography-mass spectrometry (GC-MS) with EI-ionization. Quality control samples (n=4) were included in each batch to verify the within- and between batch precision (CV<15%). An internal standard, AR C20, was used for calibration. For further details about the method see Landberg et al (2009b).

2.3 Statistics

One-way ANOVA, regression and correlation analyses were used for statistical inference. ANOVA was used to evaluate differences in total AR, sex, BMI, total fiber/1000kcal and the AR C17:0/C21:0 ratio between the lifestyle intervention- and control groups. The same method was used to calculate the differences between diabetics and non-diabetics for total AR, total fiber/1000kcal and the AR C17:0/C21:0 ratio. Regression analysis was conducted with total AR as dependent variable and dietary fibre, BMI and sex as independent variables. Correlation analysis was conducted with total AR and the AR C17/C21 ratio, the AR C17/C21 ratio and time to diabetes diagnosis, the AR C17/C21 ratio and total fiber/1000kcal, the AR C17/C21 ratio and carbohydrate (E%) and carbohydrate (E%) and total fiber/1000kcal.

3 Result

The alkylresorcinol concentrations in plasma samples were analyzed in seven batches. Table 1 illustrates the coefficient of variation (CV) within the batches of the quality control samples. The CV was <15% in all batches.

Table 1. *The CV of the homologs within the batches and mean value of CV for each homolog. The mean value was used as an estimator of the within-subject variation.*

	$CV_{within\ batch}$				
Batch	**C17**	**C19**	**C21**	**C23**	**C25**
1	2	3	3	9	5
2	4	3	3	5	7
3	5	5	4	8	12
4	6	4	4	9	9
5	3	6	3	4	11
6	6	3	6	5	9
7	8	6	4	5	9
Mean	5	4	4	6	9

The CV between the batches of each homolog of the quality control samples were ≤15% except of C25. The CV of total value of the batches is low, 6.0 (Table 2).

Table 2. *The mean value of AR for each homolog for each batch of quality control samples (nmol/L). The mean value (nmol/L), standard deviation (SD) and CV (%) of each homolog between the batches and of the total amount of AR between the batches.*

Batch	**C17**	**C19**	**C21**	**C23**	**C25**	**Total AR**
1	9	27	33	10	10	88
2	9	28	38	12	8	95
3	10	30	36	8	6	90
4	10	31	39	8	6	94
5	9	28	37	9	7	92
6	11	32	39	10	6	100
7	12	35	39	10	9	104
Mean	10	31	37	10	7	95
SD	1	3	2	1	2	6
CV	12	9	6	15	22	6

There was no significant difference in total AR concentration between the lifestyle intervention and control groups, or between sex or BMI classes within the dietary intervention groups (Table 3). A multiple regression analysis where total AR was regressed on BMI and sex separately or together was not statistically significant p=0.228 (data not shown). Even if there was no difference in total AR between the lifestyle intervention and control groups, there was a significant difference for total fiber/1000 kcal, p=0.001, Table 3 (Figure 2 for distribution). Based on the recommendations for women to consume ~ 2000kcal and men ~2500kcal/day (Mann & Truswell, 2007) did women in the intervention group have an intake of 27g fiber/day and in the control group 22.2g/day. Men in the intervention group have an intake of 33.8g fiber/day and 27.8g fiber/day in the control group (based on median value of fiber intake/1000kcal, Table 3). The difference of the AR C17:0/C21:0 ratio between the lifestyle intervention and control groups was not significant (Table 3). The lowest value of AR within the subjects was 11.5 and the highest 419.4 nmol/L (data not shown).

Table 3. *The average and median of total AR in the case- and control group. The average and median of total AR in aspect of sex and BMI within the case and control groups. Total fiber and the AR C17/C21 ratio between the lifestyle intervention and control groups (p=0.05).*

		Intervention n=94			**Control n= 92**		
		Average	*Median*	*T test*	*Average*	*Median*	*P value*
Total AR*		78.5	59.4		77.3	55.2	0.875
Sex	Female	80.6	59.4		74.4	53.7	
	Male	74.3	62.1	0.622	82.6	59.4	0.595
BMI	(23.5-30.0)	77.8	65.7		89.2	62.2	
	(30.1-47)	79.1	58.5	0.922	64.9	45.5	0.078
Total fiber/ (1000kcal)		14.0	13.5		12.2	11.1	0.001
C17/C21		0.4	0.4		0.38	0.35	0.471

* Analyses done on log transformed values of AR to aquire normal distribution (Figure 3).

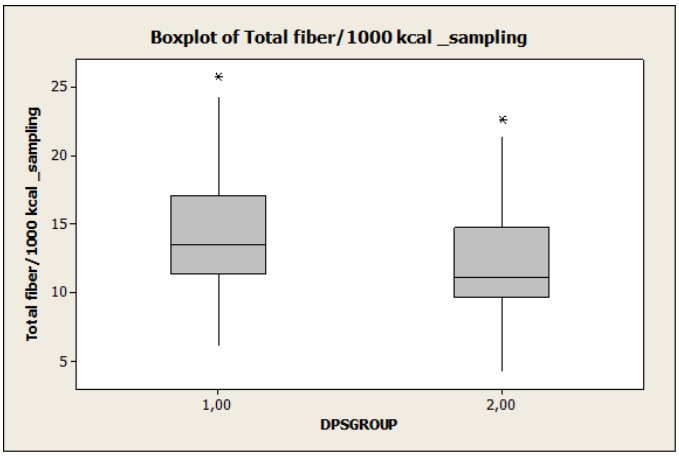

Figure 2. A boxplot of the total fiber intake/1000kcal in the intervention and control groups. (1= intervention, 2= control)

No significant difference in total AR (log transformed to acquire normal distribution, Figure 3) or fiber intake/1000kcal between diabetics and non-diabetics was found (Table 4). However, the AR C17:0/C21:0 ratio was significantly higher among non-diabetics than for diabetics, p= 0.025, (Figure 4 for distributions). No correlation between total AR and sex or total AR and BMI at baseline was found, irrespective of other variables as lifestyle intervention or control groups and diabetics/non-diabetics (data not shown).

Table 4. Plasma total AR, the AR C17:0/C21:0 ratio and total fiber/1000kcal intake among diabetics and non-diabetics ($p<0.05$).

	Diabetics n= 90		**Non-diabetics n=96**		
Variable	*Mean*	*Median*	*Mean*	*Median*	*p-value*
Total AR*	82.1	60.3	74.1	55.4	0.514
C17:0/C21:0	0.36	0.32	0.42	0.025	0.025
Total fiber/ 1000kcal	12.8	12.6	13.34	12.2	0.356

* Analyses done on log transformed values of AR to aquire normal distribution (Figure 3).

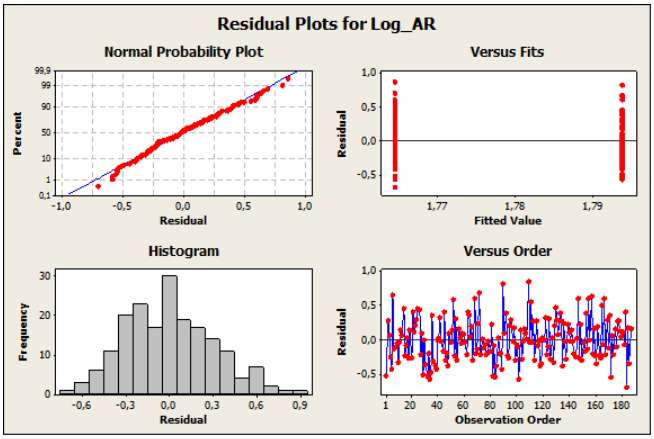

Figure 3. Normal distribution after log transformation of total AR.

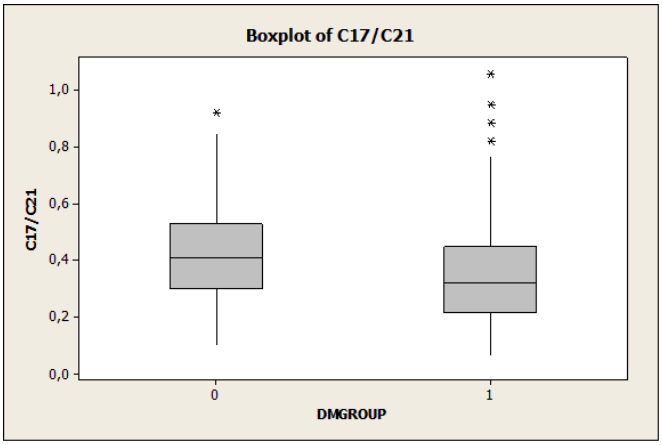

Figure 4. Boxplot of the AR C17:0/C21:0 ratio between non-diabetics and diabetics. (0=non-diabetic, 1=diabetic)

Total plasma AR was not correlated with the AR C17:0/C21:0 ratio. Moreover, a significant positive correlation was found between the AR C17:0/C21:0 ratio and time to development of T2D. Other significant correlations were between the AR C17:0/C21:0 ratio and total fiber/1000kcal, AR C17:0/C21:0 ratio and carbohydrate (E%) and carbohydrate (E%) and total fiber/1000kcal (Table 5).

Table 5. *Correlation between variables (p<0.05).*

Correlations			
Variable	*Variable*	*r*	*P-value*
Total AR	C17/C21	0.0006	0.749
C17/C21	Time to diabetes diagnosis	0.026	0.027
C17/C21	Total fiber/1000kcal	0.053	0.001
C17/C21	Carbohydrate (E%)	0.021	0.046
Carbohydrate (E%)	Total fiber/1000kcal	0.197	<0.0001

4 Discussion

The within- and between batch difference of CV for the quality control samples were <15% except of homolog C25:0. There has been a consistent problem in the lab with this homolog due to its somewhat different behavior in the GC-MS. The reasons for higher CV may be related to a high boiling point and polarity of this homologue. Reasons for variations could also be due to insufficient extraction with diethyl ether or that some of the substance did not come out from the GC-MS, or were delayed giving them another retention time. The reason for the lowest CV for AR C19:0 and C21:0 is probably due to their similarity with the internal standard, C20:0. Even if C25:0 had a high CV it is also the homolog that affects the total amount of AR least. C25:0 represents the lowest part of the total content of AR, C17:0- C25:0 (Ross et al., 2003b). There were also some disparities with C23:0. This is probably due to the possible overlap of retention time with cholesterol in the GC-MS. The amount of cholesterol in the blood is much higher than the homolog C23:0. They are not in the same selected visible window in the GC-MS but it can disturb the measurements of the homolog C23:0. However, overall, the results were acceptable.

In this study, there was no difference in total AR between the intervention and control groups. However, a significant difference in total dietary fiber intake between the lifestyle intervention and control groups was found, with a higher intake in the intervention group. Finland is a country with high intake of dietary fiber and rye is the largest source. Finns do not seem to associate rye with a healthier lifestyle due to its already well-established role in the diet (Prättälä, Elasoja & Mykkänen, 2001). The subjects in the intervention group were advised to raise their consumption of dietary fiber. This was most likely done with other sources than cereals such as vegetables, fruits and beans. The median of the AR C17:0/C21:0 ratio was 0.40 in the intervention group and 0.35 in the control group, indicating that also the rye intake was somewhat increased but the difference was not significant. The AR C17:0/C21:0 ratio was higher compared to other studies which corresponds well with the higher consumption of rye in Finland compared with other Nordic countries. Previous studies have shown that a high rye intake, measured with the AR C17:0/C21:0 ratio is associated with insulin sensi-

tivity (Magnusdottir et al. 2014), but whole grain rye intake may already be high in the present study and no further improvement may be possible.

There was also no significant difference in total AR or total fiber between diabetics and non-diabetics. Very interesting findings of the present study were the positive correlation with the AR C17:0/C21:0 ratio and time to diagnosis of T2D and higher value of the AR C17:0/C21:0 ratio for non-diabetics than diabetics. The positive correlation with the AR C17:0/C21:0 ratio and time to diagnosis of T2D is in accordance with a study made by Magnusdottir et al (2014) and may support the idea that a diet rich in whole grain rye increase the insulin sensitivity and thereby increase the time to diagnosis of T2D. This specific finding of rye consumption could be an important aspect in the diet for preventing T2D.

Earlier studies have found a difference in total AR between men and women (Montonen et al., 2012, Landberg et al., 2013) but no such difference was found in this study.

The latest edition of the Nordic nutrition recommendations (2012) recommends a daily intake of dietary fiber between 25-35 g/day. The fiber intake in this study (Table 3) more or less reflects what is found in earlier studies in Finland. A cohort study of 22 000 finns found the median consumption to be 26 g/day (Pietinen et al., 1996). The Finnish population consumes more dietary fiber than many other countries. The mean consumption of dietary fiber for males in China is 19.4 g/day and17.6 g/day for women (Wang et al., 2011), the average intake in Unites States is between 15.7-17.0 g/day depending on ethnic group (King, Mainous & Lambourne, 2012). A too low intake in children has been noted in Germany (Alexy, Kersting & Sichert-Hellert, 2006)) and Belgium (Bosscher, Van Caillie-Bertrand & Deelstra, 2002) and in England (Muñoz et al., 1997).

The spread of AR values is expected and seen in other studies. Extremely high values may be due to consumption of whole grain rye products just before blood sampling. The median value of AR in the lifestyle intervention and control groups (Table 3) are in the same range as some studies (Landberg et al., 2013, Ross et al., 2012) and others have a higher baseline value (Magnusdottir et al., 2013). It could be expected that the value would be higher than in studies done in other countries due to the high consumption of whole grain products in Finland.

The positive correlations between carbohydrate E% and fiber/1000kcal, AR C17:0/C21:0 ratio and carbohydrate and AR C17:0/C21:0 ratio and fiber/1000kcal are obvious due to the fact they are from the same source.

An improved method for the analysis of the homolog C25:0 is needed and could be a subject for future research. Further studies should also verify the current findings about the AR C17:0/C21:0 ratio and its delayed onset of T2D.

More advanced statistical methods will be used to investigate the data from the current study. But this is out of the scope of the current study.

5 Conclusion

No significant difference in plasma AR and thereby intake of whole grain cereals was found between diabetics and non-diabetics or between the lifestyle intervention and control groups. At the same time there was a higher consumption of fiber in the intervention group. The increase of fiber intake in the intervention group is probably from other sources than whole grain wheat and rye. The AR C17:0/C21:0 ratio was positively correlated with time to diagnosis of T2D and the C17:0/C21:0 ratio was higher within non-diabetics than diabetics. This indicates that a high whole grain rye intake may have a positive impact on the development of T2D.

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