Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

**Faculty of Natural Resources
and Agricultural Sciences
Department of Food Science**

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Abstract

Child obesity and overweight increase dramatically in Sweden and globally. Studies show besides genetics, that lifestyle such as parental feeding behaviors have a significant impact on the weight of the children. Whole grain (WG) intake has been associated with reduced risk of developing type 2 diabetes, cardiovascular diseases (CVD) and their preconditions or risk factors, such as weight gain, blood pressure and inflammation. The inverse association with weight is emphasized in several observational studies, whereas a few intervention studies show no or marginal weight loss. Hitherto, no intervention studies on the role of WG intake for body weight management in children have been conducted. Alkylresorcinols (AR) are lipophilic phenolic compounds found mainly in the bran of rye and wheat among commonly consumed foods, which makes them to suitable biomarkers for WG intake. The ratio between the two homologues C17:0 and C21:0 shows the origin of the WG (~1.0 rye, ~0.1 common wheat and ~0.01 durum wheat). The early Stockholm Obesity Prevention Program (STOPP) is an ongoing intervention study on families with two overweight parents or one obese parent and their children. The intervention started when the children were one year old and will continue until the age of six, with continuous examinations and blood sampling after one, three and six years. Coaches have been used in order to introduce the families to a healthier lifestyle, steadier sleeping patterns and a healthier diet. The aim of the present study was to examine AR concentrations in plasma, as a biomarker of WG wheat and rye intake and was associated with changes in body mass index (BMI) of the subjects in the Early STOPP-study. Plasma samples has previously been analyzed from the first year in the intervention, when the infants were one year old. In this study, plasma samples were analyzed from the families when the children were three years old. The results showed that the AR in the plasma were not associated with BMI of the children. Moderate positive correlations for total AR in plasma were found between the 3-year old children and the mothers and fathers at the 1-year follow-up, as well as the fathers at the 3-year follow-up, which could imply that the AR reflects the true WG intake for 3-year old children. However, the self-declared WG intake did not correlate with the measured AR concentrations in the plasma, most likely due to the impact of other non-dietary determinants of plasma AR concentrations in small children. This implies that AR are not suitable as biomarkers of WG intake in children.

Keywords: Alkylresorcinols, Obesity, Overweight, Whole grain rye, Whole grain wheat, Whole grains, C17:0/C21:0

Sammanfattning

Barnfetma och -övervikt ökar dramatiskt i Sverige och i världen. Studier visar att utöver gener, så har livsstilsfaktorer, såsom föräldrarnas matningsrutiner, en signifikant effekt på barnens vikt. Fullkornsintag har blivit associerat med reducerad risk för att utveckla diabetes typ 2 och hjärt- och kärlsjukdomar genom att förhindra riskfaktorer som viktuppgång, högt blodtryck och inflammationer. Att fullkorn förhindrar viktuppgång är påvisat i flera observationsstudier, dock visar ett fåtal interventionsstudier ingen eller en marginell viktnedgång. Än så länge har inga interventionsstudier genomförts för att observera fullkornets roll i viktkontroll hos barn. Alkylresorcinoler (AR) är fettlösliga fenoler, främst förekommande i råg- och vetekli bland vanliga livsmedel, vilket gör dem till passande biomarkörer för fullkornsintag. Ration mellan de två homologerna C17:0 och C21:0 avslöjar fullkornskällan (~1,0 råg, ~0,1 vete och ~0,01 durumvete). Early Stockholm Obesity Prevention Program (STOPP) är en pågående interventionsstudie på familjer med två överviktiga föräldrar eller en fet förälder och deras barn. Interventionsstudien började när barnen var ett år gamla och kommer pågå till att de är sex år gamla, med kontinuerliga utvärderingar och blodprovstagningar efter ett, tre och sex år. Coacher har introducerat familjerna till en hälsosammare livsstil, mer regelbundna sömnrutiner och en hälsosammare diet. I denna studie uppmättes koncentrationer av AR i plasman hos Early STOPP-deltagarna, för att undersöka relationen mellan intaget av fullkornsråg och – vete och förändringar av body mass index (BMI). Plasmaprover har tidigare blivit analyserade från första året i interventionen, när barnen var ett år gamla. I denna studie analyserades plasmaprover från familjerna när barnen var tre år gamla. Resultatet visade att AR i plasman inte var associerat med barnens BMI. Måttliga positiva korrelationer för total AR i plasman kunde observeras mellan barnen år tre och föräldrarna i 1-årsuppföljningen, samt med papporna i 3-årsuppföljningen, vilket kan tyda på att AR reflekterar fullkornsintaget för 3-åriga barn. Dock korrelerade inte det självdeklarerade fullkornsintaget med de uppmätta AR-koncentrationerna i plasman, förmodligen på grund av icke-kostrelaterade faktorers inverkan på AR-koncentrationerna hos små barn. Detta tyder på att AR inte är lämpliga som biomarkörer för fullkornsintag hos barn.

Nyckelord: Alkylresorcinoler, Fetma, Övervikt, Fullkornsråg, Fullkornsvete, Fullkorn, C17:0/C21:0

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Abbreviations

AR	Alkylresorcinol
CVD	Cardiovascular Disease
Early STOPP	Early Stockholm Obesity Prevention Program
FFQ	Food Frequency Questionnaires
GC/MS	Gas Chromatography Mass Spectrometry
GLM	Generalized Linear Model
RCT	Randomized Controlled Trial
SPE	Solid Phase Extraction
TFAA	Trifluoroacetic anhydride
WG	Whole Grain

1 Background

1.1 Overweight and obesity

Overweight and obesity are considered to be epidemic diseases that occur not only in the industrialized parts of the world, but also in the developing countries. Worldwide, obesity has more than doubled the past 35 years (WHO, 2015a) and there is no indication of a retrenchment (OECD, 2014). The prevalence of overweight and obesity is highest among Americans (61 % overweight and 27 % obesity) and lowest among South East Asians (22 % overweight and 5 % obesity) (WHO, 2015b). "Globesity" (global obesity) is one of the most severe health problems mankind is dealing with, considering all the health related complications that may possibly come with the disease (Ibid). Type 2 diabetes, cardiovascular diseases and some types of cancer are all obesity related diseases (ibid). The number of obese adults increased tremendously between the years 1995 and 2000, with an increase of more than 30 % (WHO, 2015a). Meanwhile OECD (2014) reported that one in five children in the OECD countries is overweight. WHO declared that 42 million infants and young children were overweight or obese in 2013 and that by 2025, the number will reach to 70 million with the current trend (WHO, 2015c). In order to break the current trend with the increasing childhood obesity it is crucial to take preventive measures at a local, regional and global level (WHO, 2015d). Such measures should include physical activity, limitations regarding the intake of sugars and fats and increasing the intake of fruits, vegetables, legumes and whole grains (WG) (ibid).

A simple and established method of measuring overweight and obesity is by using the body mass index (BMI) which is the weight (kg) divided by the square of the height (m) of a person (Svensson, 2014). A BMI over 25 is considered to be overweight while a BMI over 30 is associated with obesity. The same index can be used for children. However, in order to be able to compare children across age and gender groups, a modified index is used, BMI SDS (Body Mass Index Standard Deviation Score) (Svensson, 2014). BMI SDS is a measure of relative weight and is calculated by using weight, height, age and gender and is based on a reference population.

1.2 Parental and child obesity

Nearly 50 % of the Swedish population is currently overweight or obese and the numbers are increasing (Folkhälsomyndigheten, 2014a; SCB, 2012). These numbers have increased with more than 13 percent units during the past 20 years. No Swedish national data is available for overweight and obesity among children, however data from healthcare services show alarming numbers among children born 2001-2008, even though the trend do not seem to be increasing (Table 1).

Child obesity is to a high extent heritable, but also dependent of environmental factors like parental feeding behaviors (Svensson, 2014; Lo *et al*., 2015). This is causing the children of obese parents to be vulnerable of becoming obese already at an early stage of life (Moraeus *et al*., 2012). The child feeding practices are suggested to be an important factor in the fate of the future weight of the children (Lo et al., 2015). The diet of the parents is most often given also to their children and thus unhealthy eating of the parents will pass on to their children. Many of the children that are overweight or obese tend to remain so through adolescence and adulthood (Svensson, 2014; Sun Guo *et al*., 2007). Prevention at an early stage is therefore highly effective and crucial (Moraeus *et al*., 2012). Since obesity is associated with a number of chronic diseases such as type 2 diabetes, cardiovascular disease (CVD) and cancer, it also increases the mortality rate and thus obese children have a lower life expectancy than the average normal weighed child (Folkhälsomyndigheten, 2014b).

Table *1. Prevalence of overweight and obesity among children born 2001-2008. Annual report from the county council of Stockholm 2013 (Barnhälsovården, 2013.)*

Year of birth	Overweight %	Obesity %
2001	10.6	2.2
2002	9.3	2.1
2003	9.3	1.9
2004	9.3	1.8
2005	9.5	1.9
2006	9.4	1.9
2007	9.1	1.8
2008	9.4	1.8

1.3 Whole grain

WG are intact, ground or flaked kernels of cereals, including the bran, the germ and the starchy endosperm, or where these components are present in the same proportions that naturally occur in the grains (FDA, 2015). Refined grain is based on the starch endosperm only. In the refining process, the outer components and the germ are excluded. The bran is rich in dietary fiber and associated bioactive compounds which are considered to be beneficial for human health (Landberg, 2009a). The germ is rich in essential fatty acids, minerals and vitamins. WG foods are thus nutritious whereas the refined products contain little beyond starch and protein (ibid).

1.4 Evidence for beneficial health effects of whole grains

The Swedish National Food Agency recommends WG instead of refined grains whenever cereals are to be consumed, due to the variety of micronutrients that are available in the bran (NNR, 2012). WG are also a source of dietary fibers, which are considered to be beneficial for health in several ways (Landberg, 2009a). WG intake has been associated with reduced risk of developing type 2 diabetes, CVD and their preconditions or risk factors, such as weight gain, high blood pressure and inflammation as well as cancer (SLV, 2013). The association with weight is emphasized in several observational studies (Harland & Garton 2007; Ma *et al*., 2012; Steffen *et al*., 2003). However, several intervention studies show no or marginal weight loss related to the consumption of WG (Pol *et al*. 2015). Short-time interventions are believed to be the reason behind the conflicting results between observational and interventional studies, thus more extensive Randomized control trials are necessary to carry out (ibid). Hitherto there are no intervention studies on the role of WG intake of children and the impact on weight.

1.5 Methods for whole grain intake assessment

Food diaries and food records are commonly used methods to prospectively assess the food intake of an individual person or in a household (Landberg, 2009a). However, these methods have some limitations due to several reasons. For example, they result in a high participant-burden which require highly motivated participants in order to obtain accurate results. Moreover, over- and under reporting is common with these methods as well as memory lapses (Berdanier, Dwyer & Heber, 2014; van Dam & Hu, 2015). These and other limitations with self-reporting methods leads to systematic and random measurement errors in the intake estimation which may in worst case lead to incorrect conclusions in studies conducted. A specific problem associated with self-reported intake of WG is the difficulty that the consumers may have to recognize WG products among other products. Moreover, a uniform definition for WG products is lacking as well as food composition data on WG products (Söderholm *et al*., 2015; van Dam & Hu, 2015). Due to these problems, WG intake may be challenging to estimate accurately and thus a biomarker may be used as a complement to the dietary assessment method for intake ranking and or as a tool to validate other methods.

1.6 Alkylresorcinols in whole grains

Alkylresorcinols (AR) (Figure 1) are lipophilic phenolic compounds found mainly in wheat (300-784 µg/g) and rye (568-1231µg/g) among commonly consumed foods. They can also be found in lower amounts in barley (8-210 µg/g) and in very low amounts (<5 µg/g) in maize and in garden peas (Landberg, 2009a). They are present almost exclusively in the bran and not found in any high amounts in the refined flour or in the germ, which makes them to suitable biomarkers for WG products (ibid). The AR found in cereal grains mainly consist of saturated 5-n-alkyl-derivates with odd alkyl chain length, in the range of 17-25 carbon atoms, although unsaturated derivates can be found as well (ibid). Rye is the cereal with the highest occurrence of unsaturated AR.

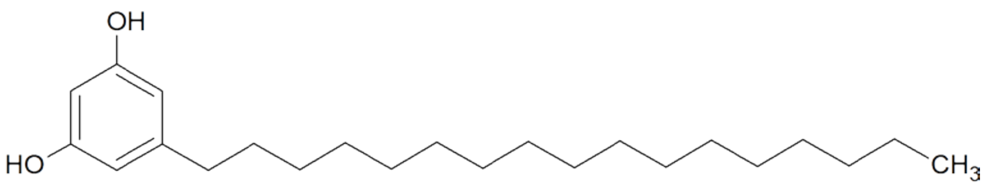

Figure 1. Alkylresorcinol C17:0. (Wikipedia)

1.7 Alkylresorcinols as biomarkers of whole grain wheat and rye in plasma

AR are absorbed by humans and have a similar metabolism as tocopherols (Marklund et al., 2012). Studies have been conducted to validate AR in plasma as biomarkers for WG wheat and rye intake among free-living subjects as well as under controlled conditions. These studies have shown that AR reflects WG intake adequately in populations with a regular and stable WG intake (Andersson *et al*., 2015). By determining the ratio between two of the AR homologues in plasma, C17:0/C21:0, the source of WG can be traced (Landberg, 2009a). The AR C17:0/C21:0 ratio is typically ~1.0 in rye, ~0.1 in common wheat and ~0.01 in durum wheat (Landberg, 2009a; Chen *et al*., 2004). The ratio in plasma is to some extent determined by other non-dietary factors than the source of WG intake. A ratio > 0.15 indicates rye intake to some extent (Ross, 2011). The ratios have been shown to reflect trends of WG consumed in plasma from different European countries (Kyrö *et al*., 2014).

AR seem to fulfill many of the criteria for being suitable as biomarkers. They remain stable under cooking and preparation of foods, are absorbed and metabolized similarly in different individuals and are simple to quantify (Söderholm, 2015; Landberg *et al*., 2006). In addition, studies have shown a moderate reproducibility over a 2-3 month period (Andersson *et al*., 2015). However, there are some limitations connected to the use of AR as biomarkers. First, the half-time ($t_{1/2}$) of intact plasma AR are reported to be 5 h, which is a relatively short time considering that blood samples usually are collected after a night of fasting (Söderholm *et al*., 2015). Along with a short half-life, day to day variation in the WG intake, will cause fluctuations which leads to lower precision in estimating the long-term average AR concentration in plasma, unless intake is frequent and stable (Andersson *et al*., 2011). Currently, studies are ongoing to evaluate AR-metabolites in urine and adipose tissue as alternative biomarkers of WG intake (Marklund *et al*., 2012; Wierzbicka *et al*., 2015).

1.8 The Early STOPP

The Early Stockholm Obesity Prevention Program (STOPP) is an ongoing randomized controlled (RCT) intervention study that aims to prevent children of obese or overweight parents to become obese or overweight (Svensson, 2014). Families included in the study have been recruited from child healthcare centers in Stockholm and both child and parents are included in the study. To be included either both parents should be at least overweight or one parent should be obese.

The study consisted of three treatment groups, an intervention group, a control group and a reference group. The control group had similar health background as the intervention group but with no interventions. The reference group included normal-weighed family members (BMI <25).The intervention started when the infants were one year of age and will continue until the children are at the age of six. During the course of the study, families have been regularly examined and followed up to obtain data on weight, length/height, BMI and other variables of health as well as blood samples after 1, 3 and 6 years. The diet of the families has been followed up through annual food diaries. The total number of participating families included were 242. Of these, 182 were high risk families and 60 low risk families (ibid). For an overview of the experiment see Table 2.

The intervention has been carried out with the use of coaches to improve the life-style, dietary habits, physical activities and sleeping patterns of the family in order for the child to avoid risk factors that could cause overweight or obesity. The dietary recommendations have complied with the general recommendations according to SLV (2015). However, the condition of each specific family have been considered in the coaching process. Thus each family have received a unique dietary recommendation and not all families have been advised to increase the WG intake (Svensson, 2015 - personal communication).

Table 1. *Overivew of the Early STOPP.*

Study group	N (families)	Parental weight	Risk group	Intervention
Intervention	91	Overweight or obese	High	Yes
Control	91	Overweight or obese	High	No
Reference	60	Normal	Low	No

1.9 The aim of this study

The aim of this study was to analyze the concentrations of AR, as biomarkers for WG wheat and rye intake, in blood plasma from families that have participated in the Early STOPP-project in order to investigate if WG intake of wheat and rye (indicated by AR) differed between groups and if it was associated with BMI of family members. Moreover, the intake of different WG (assessed by the C17:0/C21:0 ratio) and BMI was also investigated. The hypothesis was that a difference in WG wheat and rye intake between groups will be reflected by differences in plasma AR concentrations and that AR concentrations will be inversely associated with BMI of both children and their parents. If such a correlation exist, a further hypothesis was that a high proportion of WG rye over wheat, measured by the AR C17:0/C21:0 ratio would be beneficially associated with BMI of parents and children.

2 Materials and Methods

2.1 Subjects and samples

Plasma samples taken at 3 year follow-ups from families participating in the Early STOPP study (227 samples from 115 families) were analyzed for the study. The plasma samples and raw data on various variables, as the data from the 1-year follow ups, the BMI of the individuals and food diaries, were obtained from the Early STOPP investigator team at Karolinska Institutet. All individuals and families were encoded.

2.2 Analysis of alkylresorcinols in plasma

Plasma samples were analyzed according to Wierzbicka *et al*. (2015) with some minor changes. Briefly, samples (0.2 mL) were extracted with ether (3*3 mL) and put in a dry-ice ethanol batch. The combined extracts were evaporated in a stream of nitrogen and dissolved in 1 mL methanol. Samples were purified using Oasis ®MAX SPE cartridges. The cartridges were conditioned with 0.1M NaOH in MeOH (3:7), upon which MeOH was added to the samples in order to elute neutral lipids. Finally, formic acid (2%) was added, to elute the AR. Samples were evaporated with a nitrogen stream and then silylated with Trifluoroacetic anhydride (TFAA). The samples were finally analyzed with a gas chromatograph mass spectrophotometer. In total, 7 batches were analyzed and quality controls (n=4) were included in each batch to estimate within and between batch variation. All subjects from each family were analyzed in the same batch, to ensure highest precision.

2.3 Statistical analysis

Plasma AR concentrations were log-transformed in order to improve normality. Differences in plasma AR concentration between groups were assessed by one way ANOVA after 1 year and 3 years separately. Generalized Linear Model (GLM) with sex, family group, BMI of mother and father and total plasma AR concentration as predictors of BMI at 1 or 3 years as response variables in order to find potential factors affecting BMI in children at 1 and 3 years of age. Spearman's rank correlation coefficients were calculated for correlations between variables. Minitab version 16 and SAS version 9.3 were used for statistical analyses. $P<0.05$ was considered statistically significant.

3 Results and Discussion

3.1 Precision for determination of alkylresorcinols

The within- and between-batch variations (Table 3) were calculated based on four quality control (QC) samples in each batch, except from batch 3, where only three QC-samples could be included due to waste of one sample.

The within- and between-batch variation reflects the precision associated with a certain method. Random errors are the reason behind precision. A coefficient of variation (CV) below 15 % is acceptable precision when assessing AR, considering the low concentration in plasma. The estimated within- and between-batch variation was <15% for all homologues except for C23:0 and C25:0. The high variations for these homologues could be due to instrumental discrimination, considering that these specific homologues have the longest carbon chains and thus are the least polar of the homologues with highest boiling point. Due to the long lipophilic characteristic of C23:0 and C25:0, losses might have occurred in the SPE-purification, when the lipids were eluted from the samples. It is likely that these specific homologues performed differently from the other homologues also in the quantification in the GC-MS, which has a better sensitivity for low molecular weight compounds. Both within- and between batch CV: s were considered adequate (<20 %).

Table 3. Within batch *coefficient of variation (%) for different homologues and for total AR in QC-samples (quality control) for batch 1-8 and between batches coefficient of variation (BB).*

	C17	C19	C21	C23	C25	Total AR
1	11	3.1	7.9	4.4	14	6.2
2	8.6	5.8	5.8	6.0	24	6.7
3	15	8.5	10.6	19.0	29	13
4	2.1	4.1	2.3	0.98	2.6	2.3
5	11	10	9.2	5.2	12	6.2
6	1.7	1.9	2.8	0.38	5.8	1.3
7	10	6.3	5.2	5.9	5.5	5.8
8	2.3	3.5	1.6	1.6	2.8	1.5
BB	11	6.6	5.9	13	19	8.3

3.2 The impact of treatment on alkylresorcinols

No statistical significant differences in plasma AR concentrations were found between the treatment groups (Table 4 and 5). These results are unexpected, since the intervention group was expected to have the highest WG intake due to the conducted intervention. However, as mentioned, it is possible that not all families in the intervention group actually were recommended to increase the WG intake. Another possible scenario would be that the subjects did not follow the advice.

The mean AR concentrations appears to have been higher among the 1-year old infants compared to when they were 3 years. Since infants mostly consume formula, which seldom consist of wheat and rye, it is unexpected that the AR concentrations were present at such high concentrations. A potential explanation is that infants metabolize AR differently than at the age of 3 years. This should be investigated further. Enzyme activity may differ for children at 1 and 3 years of age. The AR C17:0/C21:0 ratio was higher at the measurement at the age of 3 years, which indicates that they consumed more rye than when they were 1 year old infants.

Table 4. *AR concentration in the plasma of the children at the 1 year follow-up. Mean ± SD (Median).*

	Reference group	Control	Intervention	P-value*
Total AR	275 ± 251 (221)	227 ± 181 (164)	174 ± 220 (86)	0.087
C17:0/C21:0	0.11 ± 0.08 (0.08)	0.11 ± 0.07 (0.10)	0.13 ±0.10 (0.12)	0.887

*P-value < 0.05 is significant.

Table 5. *AR concentration in the plasma of the children at the 3 year follow-up. Mean ± SD (Median).*

	Reference group	Control	Intervention	P-value*
Total AR	86 ±73 (65)	122 ± 137 (65)	90 ± 64 (83)	0.834
C17:0/C21:0	0.20 ± 0.13 (0.16)	0.20 ± 0.09 (0.18)	0.22 ± 0.12 (0.21)	0.725

*P-value < 0.05 is significant.

3.3 Factors influencing the BMI of children

Total AR in plasma, treatment group, sex or the BMI of the parents were not significantly influencing the BMI of the child at the 1 or 3- year follow-up (Table 6 and 7). The results do not comply with the observational studies on adults, where an inverse association between the total AR in plasma and the BMI of the subjects have been found (Harland & Garton 2007; Ma *et al*., 2012; Steffen *et al*., 2003). A suggestion is that the total AR may not reflect the true WG intake since AR as biomarkers for WG intake have not been validated in children. This is supported by unexpectedly high AR concentrations among children at the age of 1 and lower concentrations at the age of 3, despite expectedly higher WG wheat and rye consumption at the age of 3. The type of treatment does not appear to have a significant impact on the BMI. This as well indicates that AR may not be suitable as biomarkers for WG in children.

The BMI of the parents have no significant impact on the BMI of the children at the age of 1 or 3, which is in conflict with studies that have determined the BMI of the parents to correlate with the BMI of the children (Bralic *et al*., 2005). A possible explanation for this could be that an eventual weight gain among children in the high-risk group of being overweight will come later in life.

Table *6. Factors influencing the BMI of the child at the 1 year follow-up.*

Factor	P-value*
Total AR	0.596
Treatment	0.465
Sex	0.857
BMI of father	0.832
BMI of mother	0.762

*P-value < 0.05 is significant.

Table *7. Factors influencing the BMI of the child at the 3 year follow-up.*

Factor	P-value*
Total AR	0.891
Treatment	0.295
Sex	0.194
BMI of father	0.996
BMI of mother	0.459

*P-value < 0.05 is significant.

3.4 Correlations of alkylresorcinols between parents and children

The Spearman´s correlation was determined for total AR as well as the AR C17:0/C21:0 ratio at the 1- and 3-year follow-ups (Table 8 and 9). A moderate positive correlation for total AR in the plasma of the mothers between year 1 and 3 was observed (Figure 2), as well as for the fathers (Figure 3). The correlation for AR C17:0/C21:0 ratio in the plasma of the mothers between year 1 and 3 was moderate (Figure 4). The correlations observed shows that the intake among parents are rather stable over time and that the relative WG rye to wheat is also stable during the follow-ups.

There is also a moderate positive correlation between the total AR in the plasma of the 3-year old children and the fathers year 1 and 3, respectively the mothers year 1 (Table 8). However, there is no significant correlation between the total AR in the 3-year follow-up between the children and the mothers. One explanation could be that the diet of the mothers differs between year 1 and 3. The first years following after a childbirth could be a challenging time for the mother, since pregnancy and breast-feeding may have an effect on the health and weight of the mother. Thus it is not inconceivable that this in turn affects the diets of the mothers, especially when the period of breast-feeding is at its end. This is however not supported by the positive correlation between the total AR of the mothers between year 1 and 3, which implies that the WG intake of the mothers stayed constant during the follow-ups (Figure 2). The fact that the AR of the 3-year old children seem to correlate with the parents (disregarding from mothers year 3) could imply that AR actually reflects the true WG intake. No similar correlation could be found in the plasma of the children at the 1-year follow-up and the parents. The results could imply that the 3-year old children consumed a similar diet as the parents, assuming that the total AR reflects the consumed WG, whereas the 1-year old infants consumed a different diet. However it is highly unlikely that the infants consumed the high amounts that the results suggests (Table 4), thus a more likely explanation is that AR do not work as a biomarker for WG in infants.

Table 8. Spearman´s correlation Total AR year 1 and year 3 for children (C), fathers (F) and mothers (M). (P-value *).

	C 1	C 3	F 1	F 3	M1	M 3
C 1		-0.14 (0.453)	0.07 (0.536)	-0.15 (0.409)	0.04 (0.720)	0.09 (0.577)
C 3			0.51 (0.007)	0.36 (0.044)	0.42 (0.016)	0.19 (0.237)

*P-value < 0.05 is significant.

Table 9. Spearman´s correlation C17:0/C21:0 year 1 and year 3 for children (C), fathers (F) and mothers (M). (P-value*).

	C 1	C 3	F 1	F 3	M1	M 3
C 1		0.03 (0.867)	0.05 (0.644)	0.05 (0.804)	0.23 (0.019)	0.10 (0.558)
C 3			0.14 (0.506)	0.40 (0.059)	0.27 (0.140)	-0.03 (0.888)

*P-value < 0.05 is significant.

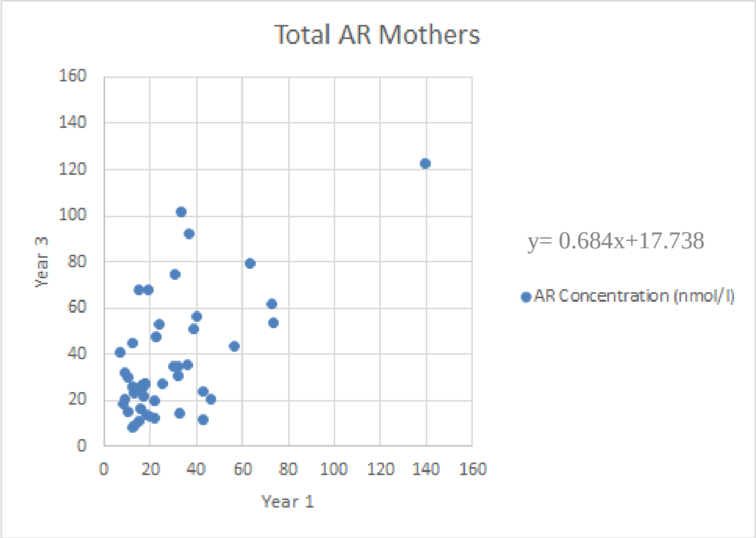

Figure 2. Total AR concentration (nmol/l) in plasma from mothers at the 1- and 3- year follow-ups. Spearman's r = 0.43 (P = 0.003) and was based on 45 persons due to missing data.

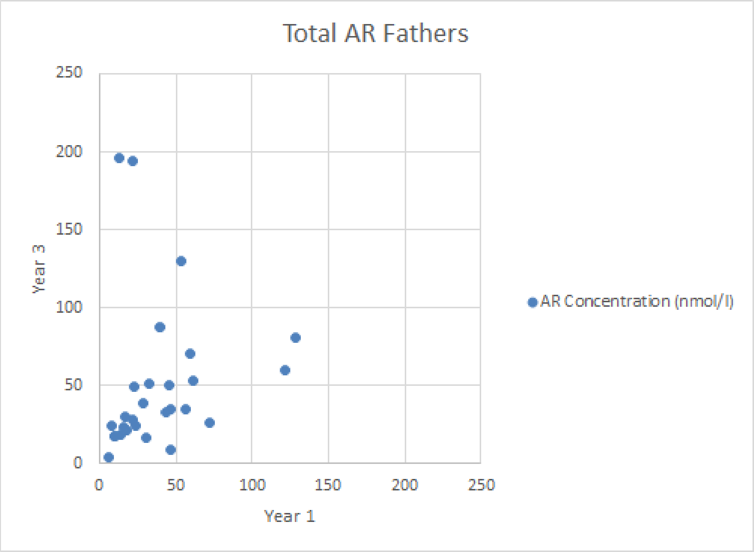

Figure 3. Total AR concentration (nmol/l) in plasma from fathers at the 1- and 3- year follow-ups. Spearman's r = 0.43 (P = 0.021) and was based on 29 persons due to missing data. One abnormal value (531) was excluded from the graph.

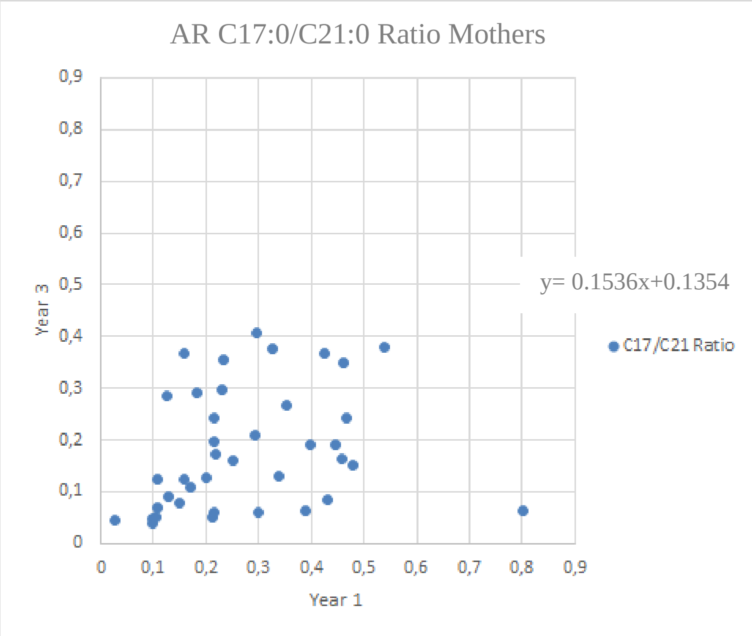

Figure 4. AR C17:0/C21:0 ratio in plasma from mothers at the 1- and the 3-year follow-ups. Spearman's r = 0.43 (P = 0.006) and was based on 39 persons due to missing data.

3.5 The validity of plasma alkylresorcinol concentrations as a biomarker of whole grain wheat and rye intake in children

No statistical significant correlation between the self-reported WG intake (total or the sum of wheat, rye and barley) and total AR was observed for the children at the 3-year follow-up (Table 10). Moreover, no significant correlation between the declared WG intake (general or wheat, rye and barley) and the assessed AR C17:0/C: 21:0 ratio was observed. This is in contrast to many studies conducted in adults under controlled and free-living conditions (Andersson et al., 2015; Ross, 2011).

As stated, self-declared food diaries can be deceiving since under and over reports are commonly occurring sources of errors. Specific difficulties in the food declaration of children could also further aggravate the method. Parents of small children can be expected to have less time and thus do not have time to prioritize the food diaries. Moreover, parents may feel ashamed if they do not feed their children healthy food, which in turn could cause them to misreport and claim that they consumed more WG than they actually did.

Plasma AR concentration in children may be affected by other factors than diet to a larger extent than for adults. This may be a reason for the lack of a correlation between reported intake and plasma AR. This is supported by the fact that children at one year old has 5-10 times higher AR concentrations than their parents.

Since there are no previous studies on AR as biomarkers for WG in children, the only available values to use in comparison are from adults. The studies on adults show a good validity concerning AR as biomarkers for WG. The results however only applies to adults, thus infants or small children may have a different metabolism and absorption of AR. This needs further investigations.

Table *10. Spearman´s correlation between self-declared WG intake and AR concentrations in the plasma of the children at the 3 year follow-up and P-value* in parenthesis. Correlation was based on data from 51 children.*

	Total AR	C17:0/C21:0
WG intake	0.14 (0.346)	0.17 (0.223)
WG intake (Wheat, rye & barley)	0.23 (0.112)	0.24 (0.090)

*P-value < 0.05 is significant.

4 Conclusion

No significant correlations between the AR concentrations in the plasma of the children and their BMI at 1 or 3 year of age was observed. Moderate positive correlations for total AR in plasma were found between the 3-year old children and the mothers and fathers at the 1-year follow-up, as well as the fathers at the 3-year follow-up, which could imply that the AR reflects the true WG intake in 3-year old children. However, no correlation between the assessed AR and the self-declared WG intake was found in the 3-year follow-up. This implies that AR are not suitable as biomarkers for WG intake in children, possibly due to other factors than intake that may be important for the plasma AR concentration among children. Such factors could be related to the metabolism of AR.

References

Andersson, A., Marklund, M., Diana, M., Landberg, R. (2011). Plasma Alkylresorcinol Concerntrations Correlate with Whole Grain Wheat and Rye Intake and Show Moderate Reproducibility over a 2- to 3-Month Period in Free-Living Swedish Adults. *The Journal of Nutrition*, vol. 141, pp. 1712-1718.

Barnhälsovården (2013). *Barnhälsovård. Årsrapport 2013*. http://www.vardgivarguiden.se/Omraden/BVC/Om-barnhalsovarden/Arsrapporter/Rapport-BHV-2013/

Berdanier, C.D., Dwyer, J., Heber, D. (2014). HANDBOOK of NUTRITION and FOOD. 3 Edition. New York: Taylor & Francis Group.

Bralic, I., Vrdoljak, J., Kovacic, V. (2005) Associations Between Parental and Child Overweight and Obesity Collegium. *Collegium Antropologicum*, vol. 29, pp.481-486.

Chen, Y., Ross, A.B., Aman, P. & Kamal-Eldin, A. (2004). Alkylresorcinols as Markers of Whole Grain Wheat and Rye in Cereal Products. *Journal of Agricultural and Food Chemistry,* vol. 52, pp.8242-8246.

Folkhälsomyndigheten (2014a). *Fler har fetma och övervikt*. http://www.folkhalsomyndigheten.se/nyheter-och-press/nyhetsarkiv/2014/februari/fler-har-fetma-och-overvikt/ [2015-06-10]

Folkhälsomyndigheten (2014b). *Folkhälsan i Sverige. Årsrapport 2014.* https://www.folkhalsomyndigheten.se/pagefiles/17825/Folkhalsan-i-Sverige-arsrapport-2014.pdf [2015-07-20]

Harland, J.I., Garton, L.E. (2007). Whole-grain Intake as a Marker of Healthy Body Weight and Adiposity. *Public Health Nutrition,* vol. 11, pp.554-563.

Kyrö, C., Olsen, A., Bueno-de-Mesquita, HB., Skeie, G., Loft, S., Åman, P., Leenders, M., Dik, VK., Siersema, PD., Pischon, T., Christensen, J., Overvad, K., Boutron-Ruault, MC., Fagherazzi G., Cottet, V., Kühn, T., Chang-Claude, J., Boeing, H., Trichopoulou, A., Naska, A., Oikonomidou, D., Masala, G., Pala, V., Tumino, R., Vineis, P., Mattiello, A., Peeters, PH., Bakken, T., Weiderpass, E., Asli, LA., Sánchez, S., Jakszyn, P., Sánchez, MJ., Amiano, P., Huerta, JM., Barricarte, A., Ljuslinder, I., Palmqvist, R., Khaw, KT., Wareham, N., Key, TJ., Travis, RC., Slimani, N., Freisling, H., Ferrari, P., Gunter, MJ., Murphy, N., Riboli, E., Tjønneland, A., Landberg, R. (2014). Plasma Alkylresorcinol Concentrations, Biomarkers of Whole-Grain Wheat and Rye Intake, in the European Prospective Investigation into Cancer and Nutrition (EPIC) Cohort. *British Journal of Nutrition*, vol. 111, pp. 1881-1890.

Landberg, L. (2009a). *Alkylresorcinols as Biomarkers of Whole Grain Wheat and Rye Intake*. Diss. Uppsala: Swedish University of Agricultural Sciences.

Landberg, R., Kamal-Eldin, A., Andersson, S-O., Johansson, J-E., Zhang, J-X., Hallmans,G., Åman, P. (2009b). Reproducibility of Plasma Alkylresorcinols during a 6-Week Rye Intervention Study in Men with Prostate Cancer. *The Journal of Nutrition*, vol. 139, pp. 975-980.

Landberg, R., Linko, A-M., Kamal-Eldin, A., Vessby, B., Adlercreutz, H., Åman, P. (2006). Human Plasma Kinetics and Relative Bioavailability of Alkylresorcinols after Intake of Rye Bran. *The Journal of Nutrition*, vol. 136, pp. 2760-2765.

Lo, K., Cheung, C., Lee, A., Tam, W.W.S., Keung, V. (2015). Associations between Parental Feeding Styles and Childhood Eating Habits: A Survey of Hong Kong Pre-School Children. *PLoS One*, vol. 10, pp.1-11.

Ma, J., Ross, A.B., Shea, M.K., Bruce, S.J., Jacques, P.F., Saltzman, E., Lichtenstein, A.H., Booth, S.L., McKeown, N.M. (2012). Plasma Alkylresorcinols, Biomarkers of Whole-Grain Intake, Are Related to Lower BMI in Older Adults. *The Journal of Nutrition*, vol. 142, pp. 1859-1864.

Marklund, M. (2012). *Alkylresorcinol Metabolites. Candidate Biomarkers for Whole Grain Wheat and Rye Intake*. Diss. Uppsala: Swedish University of Agricultural Sciences.

Marklund, M., Landberg, R., Andersson, R., Åman, P., Kamal-Eldin, A. (2012). Alkylresorcinol Metabolism in Swedish Adults Is Affected by Factors Other Than Intake of Whole-Grain Wheat and Rue. *Journal of Nutrition*, vol. 142, pp. 1479-1486.

Moraeus, L., Lissner, L., Yngve, A., Poortvliet, E., Al-Ansari, U., Sjöberg, A. (2012). Multi-Level Influences on Childhood Obesity in Sweden: Societal Factors, Parental Determinants and Child´s Lifestyle. *International Journal of Obesity*, vol. 36, pp. 969-976.

Nordic Council of Ministers (2012). *Nordic Nutrition Recommendations (NNR)*. http://norden.diva-portal.org/smash/get/diva2:704251/FULLTEXT01.pdf [2015-07-15]

Organisation for Economic Cooperation and Development (OECD). (2014). OBESITY Update. http://www.oecd.org/health/Obesity-Update-2014.pdf

Pol, K., Christensen, R., Bartels, E.M., Raben, A., Tetens, I., Kristensen, M. (2013). Whole Grain and Body Weight Changes in Apparently Healthy Adults: a Systematic Review and Meta-Analysis of Randomized Controlled Studies. *The American Journal of Clinical Nutrition*, vol. 98, pp. 872-884.

Ross, A., B. (2011). Present Status and Perspectives on the Use of Alkylresorcinols as Biomarkers of Wholegrain Wheat and Rye Intake. *Journal of Nutrition and Metabolism*, vol. 2012.

Statistiska centralbyrån (SCB). (2012). *Vi växer på bredden*. http://www.scb.se/sv_/Hitta-statistik/Artiklar/Vi-vaxer-pa-bredden/ [2015-06-20]

Steffen, L.M., Jacobs, Jr., D.R., Murtaugh, M.A., Moran, A., Steinberger, J., Hong, C-P., Sinaiko, A.R. (2003). Whole Grain Intake Is Associated with Lower Body Mass and Greater Insulin Sensitivity among Adolescents. *American Journal of Epidemiology*, vol. 158, pp. 243-250.

Sun Guo, S., Wu, W., Chumlea, W.C., Roche, A.F. (2002). Predicting Overweight and Obesity in Adulthood from Body Mass Index Values in Childhood and Adolescence. *The American Journal of Clinical Nutrition*, vol. 76, pp. 653-658.

Svenska livsmedelsverket (SVL). (2013). *Fullkorn. Faktaserie från livsmedelsverket*. http://www.livsmedelsverket.se/globalassets/matvanor-halsa-miljo/kostrad-matvanor/stod-till-varden/fakta-fullkorn.pdf [2015-07-15]

Svensson, V. (2014). *Family-related Obesity Risk Factors and Dietary Behaviours in High-risk Populations: Associations with Child Weight Development*. Diss. Stockholm: Karolinska Institutet.

Söderholm, P.P., Koskela, A.H., Lundin, J.E., Tikkanen, M.J., Adlercreutz, H.C. (2009). Plasma Pharmacokinetics of Alkylresorcinol Metabolites: New Candidate Biomarkers for Whole-Grain Rye and Wheat Intake. *The American Journal of Clinical Nutrition*, vol. 90, pp. 1167-1171.

U.S. Food and Drug Administration. (2015). *The Scoop on Whole Grains*. http://www.fda.gov/ForConsumers/ConsumerUpdates/ucm151902.htm [2015-08-01]

van Dam, R.B., Hu, F.B. (2008). Are Alkylresorcinols Accurate Biomarkers for Whole Grain Intake? *The American Journal of Clinical Nutrition*, vol. 87, pp.797-798.

Wierzbicka, R., Wu, H., Franek, M., Kamal-Eldin, A., Landberg, R. (2015). Determination of Alkylresorcinols and their Metabolites in Biological Samples by Gas Chromatography-Mass Spectrometry. *Journal of Chromatography B*, vol. 1000, pp. 120-129.

World Health Organization (WHO). (2015a). *Obesity and overweight*. http://www.who.int/mediacentre/factsheets/fs311/en/ [2015-07-25]

World Health Organization (WHO). (2015b). *Overweight and obesity*. http://www.who.int/gho/ncd/risk_factors/overweight_text/en/ [2015-08-08]

World Health Organization (WHO). (2015c). *Commission on Ending Childhood Obesity*. http://www.who.int/end-childhood-obesity/en/ [2015-08-08]

World Health Organization (WHO). (2015d). *Facts and figures on childhood obesity*. http://www.who.int/end-childhood-obesity/facts/en/ [2015-08-08]