



Sveriges lantbruksuniversitet  
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# The effects of rye products on postprandial metabolites

Effekterna av rågprodukter på postprandiala metaboliter

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## Abstract

During the last few years, a strong increase in prevalence of metabolic diseases like diabetes mellitus type 2, obesity and cardiovascular diseases could be observed. Certainly, lifestyle and nutrition contribute to their development to a high percentage.

Rye is known to be a healthy cereal since it contains numerous nutrients, dietary fibers and phytochemicals. Its features result in interesting effects on metabolism which are often examined by approaches in the field of metabolomics.

Recent studies found out that rye has desirable influences on metabolites like amino acids (homocysteine, leucine, isoleucine, tryptophan), ketone bodies, short chain fatty acids and metabolites involved in the tricarboxylic acid cycle. Many of those metabolites can be linked to insulin production and sensitivity, improved intestinal flora, or to an induced catabolic state in the body. In fact, rye consumption does provoke lower insulin responses, longer satiety and weight loss. But many biochemical mechanisms still are unclear and need to be examined.

*Keywords:* rye, metabolites, metabolomics, insulin, diabetes, metabolic diseases



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## Abbreviations

AMPK	AMP activated protein kinase
ATP	Adenosin triphosphate
AUC	Area under the curve
BCAA	Branched-chain amino acids
GIP	Gastric inhibitory polypeptide
GLP-1	Glucagon-like peptide-1
MS	Mass spectrometry
mTORC1	Mammalian target of rapamycin complex 1
NMR	Nuclear magnetic resonance spectroscopy
NADH	Nicotinamid adenine dinucleotide
PC	Phosphatidylcholine
SCFA	Short chain fatty acids

# 1 Introduction

During the last years, a strong increase in prevalence of metabolic diseases like diabetes mellitus type 2, obesity and cardiovascular diseases could be observed (Mozumdar, Liguori 2011). They can be summarized as the metabolic syndrome. In 2014, 387 Mio people in the world suffered on diabetes and estimations predict a further increase in the next years, leading to a prevalence of about 592 Mio patients in 2035. Those diseases not only impair quality of life and lead to earlier deaths, but they also cause higher costs for health care (IDF 2014).

The development of diseases related to metabolic syndrome can be genetically caused, however lifestyle and nutrition contribute to an even higher percentage. Therefore it is becoming more and more important to understand the underlying mechanisms and evaluate strategies for prevention (IDF 2014).

Certainly, some foodstuffs are considered healthier than others. For example whole grain cereal products have a beneficial effect on metabolism (Sahyoun et al. 2006) and especially (whole grain) rye seems to be outstanding due to its insulin response. It induces an insulin response with a significantly lower area under the curve (AUC) than wheat, while the glucose AUC is the same (Juntunen et al. 2002). This means that less insulin is needed and produced to regulate glucose levels. So there must be other mechanisms besides glucose level in the blood that influence insulin response.

A promising approach to examine biochemical pathways is metabolomics. It is a tool for identifying and distinguishing relevant metabolites and has led to important findings regarding pathways (Hollywood et al. 2006). Thus many studies aiming to investigate metabolic responses are located in the field of metabolomics.

Therefore the aim of this work was to summarize selected studies that engage in the effects of consumption of different rye products on human metabolites.

## 2 Rye

### 2.1 The plant

Rye belongs to the tribe Triticeae and is closely related to wheat and barley. It is a cereal and its grains consist of three main milling parts: the germ which is the embryo and rich in protein, the endosperm that nourishes the germ with its high starch content and the bran that protects germ and endosperm with firm layers of different cells. The rye endosperm contains fibers in higher amounts than other cereals, which is a special feature (Delcour 2010).

### 2.2 Production of rye grains

The first traces of rye plants in Turkey can be dated back to 6600 BC. Since 4440 BC, single grains of rye were found among wheat grains in central Europe. The number of findings increases noticeably since the pre-Roman Iron Age especially around the Black Sea, however rye was still rather considered a weed. Clues for intentional cultivation in Europe are found starting from the Roman Ages and a strong increase can be seen in the Middle Ages. A reason for the success of rye cultivation is the change in harvesting techniques, which compensate the former advantages of wheat and its easy harvesting. Especially on sandy soils and in unfavorable climate conditions, rye thus became a main crop plant (Behre 1992).

Today rye is still a popular crop, although a steady decrease in worldwide production since the 1960s can be observed. The former amount of 35 Mio tons halved during the last 55 years and came to 16 Mio tons in 2013 (FAO 2015). Wheat production in contrast tripled from 222 Mio tons to 716 Mio tons. The



major part, 89.6%, of total rye production in 2013 took place in Europe, primarily in Germany and Poland (FAO 2015).

Of the rye grain for internal use in the EU 41.1% are used for animal feed, 35.6% for human consumption, 17.6% are processed in the industry and 5.8% are used as seeds. This leads to a human consumption of rye and rye products of about 6 kg per year and European head, which is only a minor amount compared to the overall intake of cereals of 130 kg (European Commission 2013).

### 2.3 Nutrients and other compounds

Rye products are rich in nutrients like proteins, carbohydrates, minerals or vitamins. But depending on additives and processing, moderate to strong differences can appear for example in energy, fiber or magnesium content, as it can be seen in Table 1.

Besides nutrients there are several dietary fibers in rye and rye products. They can be divided into soluble and non-soluble fibers. Soluble fibers attract water molecules and form gels, which leads to bulking and slower digestion. Insoluble fibers also bulk but increase the speed of intestinal passage. Both kinds of fibers can be fermented by gut microbiota (The National Academies 2005). The most present fibers in rye are arabinoxylans,  $\beta$ -glucans, fructans and cellulose. Together they may contribute to up to until 20% of the compounds in commercial crisp bread (Rakha et al. 2010).

Also numerous bioactive phytochemicals can be found in rye. For example alkylresorcinols are phenolic lipids which are typical for whole grains of wheat as well as rye. They are often used to assess whole grain rye intake (Ross et al. 2004). Benzoxazinoids are other bioactive compounds (Hanhineva et al. 2011). Lignans are found in the highest concentrations in rye, compared to other cereals. As phytoestrogens they might be a promising bioactive compound in rye having estrogenic properties (Smeds et al. 2007). Phenolic acids have beneficiary effects on health mostly due to their antioxidant activity, which might for example counteract cardiovascular diseases (Crozier et al. 2009; Pihlava et al. 2015).

However, processing (heat, enzymes...) of rye grains can lead to degradation of fibers and loss of the bioactivity of secondary plant compounds (Boskov Hansen et al. 2002). Therefore the types of rye food should be differentiated and their processing should be considered.

Table 1: *Nutrients per 100g (USDAa; USDAb; USDAc)*

	<i>Rye grain</i>	<i>Rye bread (soft)</i>	<i>Rye flour dark</i>
<b>Water g</b>	10.60	37.30	10.75
<b>Energy kcal</b>	338	259	325
<b>Protein g</b>	10.34	8.50	15.91
<b>Total lipid g</b>	1.63	3.30	2.22
<b>Carbohydrate, by difference g</b>	75.86	48.30	68.63
<b>Fiber, total g</b>	15.1	5.8	23.8
<b>Sugars, total g</b>	0.98	3.85	2.31
<b>Calcium, mg</b>	24	73	37
<b>Iron, mg</b>	2.63	2.83	4.97
<b>Magnesium, mg</b>	110	40	160
<b>Phosphorus, mg</b>	332	125	499
<b>Potassium, mg</b>	510	166	717
<b>Sodium, mg</b>	2	603	2
<b>Zinc, mg</b>	2.65	1.14	5.04
<b>Vitamin C mg</b>	0.0	0.4	0.0
<b>Thiamin mg</b>	0.316	0.434	0.316
<b>Riboflavin mg</b>	0.251	0.335	0.251
<b>Niacin mg</b>	4.270	3.805	4.270
<b>Vitamin B-6 mg</b>	0.294	0.075	0.443
<b>Folate, DFE µg</b>	38	151	33
<b>Vitamin B-12 µg</b>	0.00	0.00	0.00
<b>Vitamin A, RAE µg</b>	1	0	1
<b>Vitamin A, IU</b>	11	7	11
<b>Vitamin E mg</b>	0.85	0.33	2.73

### 3 Methods - Metabolomics

Metabolites are small molecules that function as intermediates or products of metabolism. They are sensitive to intrinsic factors like age, health status and diurnal cycle as well as extrinsic factors like nutrients, physical activity and drugs (Goodacre 2007). But there is also a close relationship to the gut microbiome which produces unique compounds that can be found in human serum or urine samples (Goodacre 2007).

Metabolomics aims to identify and quantify every single metabolite, known as the metabolome, and thus is used as a tool to draw conclusions about biochemical processes in a system under defined states and at several time points. Located at the end of the "-omics" chain (genomics, transcriptomics, proteomics), it is considered a promising approach compared to genomics or proteomics, because it mostly reflects phenotypical responses (Hollywood et al. 2006). Additionally, metabolites can be highly amplified by enzymes and become easily measurable and they amount to a moderate number of 2645 different metabolites in humans (Goodacre 2007).

In contrast, human proteins add up to about 1 Mio different ones and originate from 31,897 genes (Goodacre 2007). The system of proteins and genes is very complex and affected by unclear interactions, which makes it difficult to determine which genotype leads to phenotypical appearances in the body. Thus examining extrinsic factors like nutritional interventions on genes or proteins and trying to observe effects turns out to be complicated, especially if a basic and broad understanding of underlying mechanisms is desired (Hollywood et al. 2006).

A common approach in metabolomic studies is to analyse blood, urine or tissue samples with nuclear magnetic resonance spectroscopy (NMR) or mass spectrometry (MS), optionally coupled with a chromatography to obtain even more precise outcomes. Resulting in big data volumes, this should be followed by a robust statistical analysis (multivariate or univariate) in order to draw accurate conclusions and meaningful interpretations (Lenz, Wilson 2007).

## 4 Studies

The following studies were used to examine effects of rye on plasma metabolites in humans. They all compare wheat products to rye products and can be separated in 3 groups according to their design. In the first type (1.A/1.B) different single test meals were provided to the subjects and postprandial blood samples were analyzed. Thus, the digestion and absorption of food compounds and their short term interaction with the body could be characterized. In the second type (2.A/2.B/2.C), a long term intervention period of 6-8 weeks was performed, where the subjects consumed defined amounts of rye and wheat products daily. Before and afterwards, samples of the fasting blood were withdrawn. In this way, long term effects of nutrition on metabolism can be demonstrated. The third type of studies (3.A) is a combination of the first two ones. It consists of a long term intervention followed by a standardized test meal, examining how nutrition can influence the body's reaction to a challenge. In the following sections the studies will be referred to their introduced numbers.

### Type 1 studies

#### **1.A Postprandial differences in the plasma metabolome of healthy Finnish subjects after intake of a sourdough fermented endosperm rye bread versus white wheat bread (Bondia-Pons et al. 2011)**

In this study, 16 healthy subjects randomly received a single test meal which consisted of either refined endosperm sourdough rye bread or refined white wheat bread. After 1-2 weeks they respectively received the other test bread. They were advised to fast 10-12 h over night before the test. Plasma samples were taken at fasting state and at 9 time points after the test meal (15 – 240 min). Glucose was analyzed with glucose dehydrogenase enzymatic photometric assay, and insulin with a chemiluminescent immunoassay. 255 metabolites were identified and quantified with a gas chromatography coupled with a gas chromatography and

time of flight mass spectrometry (GC x GC-TOF/MS). The metabolites with interesting results were amino acids and organic acids (2-ketobutyrate, picolinic acid, pyruvate, 2-ketoglutarate, citrate, malonate).

### **1.B Metabolomics Reveals Differences in Postprandial Responses to Breads and Fasting Metabolic Characteristics Associated with Postprandial Insulin Demand in Postmenopausal Women (Moazzami et al. 2014)**

Twenty healthy participants received three test meals, which consisted of respectively one kind of bread, in 1-2 week intervals. The breads were refined rye bread, whole-meal rye bread and refined wheat bread. Before the intake of the test meal, a fasting blood sample was withdrawn and afterwards, postprandial blood samples were taken at 5 time points. A radioimmunoassay was used to measure insulin, NMR was used to analyze 48 metabolites and LC-MS was used to analyze 189 metabolites. The most significant findings were the branched chain amino acids leucine and isoleucine.

Type 2 studies

### **2.A Metabolomic Analysis of Plasma Metabolites That May Mediate Effects of Rye Bread on Satiety and Weight Maintenance on Postmenopausal Women (Lankinen et al. 2011)**

In this study, 39 women took part in an intervention trial. It had a cross-over design with two 8 week periods. The subjects consumed 4-5 slices of bread every day, which was either high fiber rye bread or white wheat bread. Before and after the intervention, fasting blood samples were taken. Then, cholesterol was measured with an enzymatic colorimetric method, lipids were analyzed with Ultra Performance Liquid Chromatography and other metabolites were analyzed with GC x GC-TOF/MS. The metabolites of interest were ribitol, indoleacetic acid and ribonic acid.

### **2.B Nuclear Magnetic Resonance-Based Metabolomics Enable Detection of the effects of a Whole Grain Rye and Rye Bran Diet on the Metabolic Profile of Plasma in Prostate Cancer Patients (Moazzami et al. 2011)**

This study was performed with 17 men. Each person completed two 6 week trials with a diet rich in rye (whole grain or bran) and with a diet with refined wheat products enriched with cellulose each. At the end of a period, fasting blood samples were withdrawn. Several metabolites (thereof betaine, dimethylglycine, ketone bodies) were analyzed with NMR and homocysteine with HPLC.

**2.C Metabolomics reveals the metabolic shifts following an intervention with rye bread in postmenopausal women - a randomized control trial (Moazzami et al. 2012)**

In this study, 33 women were recruited. They took part in two cross-over 8 week intervention periods with refined wheat bread or high fiber whole grain rye bread each. In those periods, the test breads contributed to 20% of energy intake every day. After the run-in period and the test periods, fasting plasma samples were analyzed. NMR was used to examine metabolites (thereof amino acids, betaine, dimethylglycine) and an enzymatic colorimetric method to quantify cholesterol.

Type 3 studies

**3.A Postprandial glucose metabolism and SCFA after consuming wholegrain rye bread and wheat bread enriched with bioprocessed rye bran in individuals with mild gastrointestinal symptoms (Lappi et al. 2014)**

In this study, 21 subjects took part. It was designed as an intervention with a four-week run-in period with white wheat bread followed by two crossover four-week test periods with whole grain rye bread or white wheat bread with bioprocessed rye bran. The intake of bread was 6-10 slices per day. At the end of each period, a test meal with white wheat bread was consumed. Before the test meal and at four time points after it, plasma samples were taken. Glucose was measured with a glucose hexokinase enzymatic photometric method and insulin with a chemiluminescent immunoassay. Short chain fatty acids (SCFA) were analyzed with gas chromatography.

## 5 Effects on metabolites

In the following, a short overview of metabolites will be given that were influenced differently by rye consumption than by wheat. Afterwards, they will be further examined and specific findings from the studies in section 4 will be presented.

Firstly, rye had an impact on amino acid concentrations in the plasma. One of them is homocysteine, a biomarker and a risk factor of cardiovascular diseases (Joubert, Manore 2006). An enzyme using betaine can transform it to methionine and dimethylglycine (Frolkis et al. 2010). Further, leucine and isoleucine are branched chain amino acids that function as predictive biomarkers for type 2 diabetes (Lynch, Adams 2014). The amino acid tryptophan can be degraded and transformed in different ways (Frolkis et al. 2010). In the kynurenine pathway, it might generate pro-inflammatory metabolites (Bosco et al. 2003). So an alternative transformation for example to serotonin is favored.

Secondly, several organic acids involved in energy metabolism showed interesting results. Some of them were intermediates in the tricarboxylic acid cycle. It generates energy out of oxaloacetate and acetyl-CoA, a common product of catabolic processes, together forming citrate (Frolkis et al. 2010). Enzymatic oxidation of citrate provides products like NADH that can be used in mitochondria to form ATP, a central energy storing molecule. Alterations in the intermediates' concentrations indicate a change in the energy metabolism (Bondia-Pons et al. 2011).

Acetoacetate, acetone and 3-hydroxybutyrate are known as the ketone bodies. They are synthesized in the liver when the body is in a fasting state and short in glucose, so fatty acids are broken down to acetyl-CoA which then is used to produce ketone bodies. The ketone bodies are released into the blood from where tissues, especially brain and heart, can absorb them and use them for energy production by tricarboxylic acid cycle (Fukao et al. 2014). Higher concentrations of ketone bodies indicate an enhanced breakdown of fatty acids.

Furthermore, short chain fatty acids can be linked to the energy state in a body. In a study with mice, butyrate supplements prevented obesity during a high-fat intervention. They also improved insulin sensitivity as well as energy expenditure due to for example higher thermogenesis (Gao et al. 2009). Previous studies showed that acetate in fact can increase AMP/ATP ratio in cells (Sakakibara et al. 2006).

## 5.1 Amino Acids

### 5.1.1 Homocysteine/Betaine

Homocysteine is a biomarker and a risk factor of cardiovascular diseases (Joubert, Manore 2006). Its levels in blood are dependent on nutrition and exercise (Joubert, Manore 2006). It can be transformed to methionine by betaine-homocysteine-S-methyltransferase 1, which transfers a methyl group from betaine to homocysteine and thus forming methionine and dimethylglycine (Frolkis et al. 2010).

Whole grain cereals contain high amounts of betaine, enabling the enzymatic transformation of homocysteine and therefore lowering its plasma concentrations. Findings in a recent study (2.B) underline this hypothesis. Fasting levels of betaine were higher after a diet high in rye (from whole grain cereals as well as bran products) and simultaneously dimethylglycine was higher whereas homocysteine was lower. The control group received a diet with white wheat bread. A similar study (2.C) also found significantly higher betaine and dimethylglycine levels after the subjects have eaten a high-fiber rye bread. Another study (1.A) independently found a rise in methionine levels after rye consumption while it fell after wheat bread intake, which might also underline the observations about homocysteine metabolism. Additionally, this study observed in the rye groups a higher increase of 2-ketobutyrate, which is a degradation product of amino acids, especially homocysteine. Another approach on homocysteine metabolism is to examine the key enzyme betaine-homocysteine-s-methyltransferase1, which interestingly is down-regulated by insulin (Ratnam et al. 2006). So a lower insulin level caused by rye might enhance the enzyme's activity and thus shifts the equilibrium to the side of the products dimethylglycine and methionine, which might have amplified the effect of betaine (Moazzami et al. 2012).



### 5.1.2 Leucine and Isoleucine

The branched chain amino acids (BCAA) leucine and isoleucine have a lower fasting concentration after 8wk treatment with rye than with wheat, as observed in study 2.C. Already a single test meal (1.B) shows differences in leucine and isoleucine responses. Only after wheat consumption, an increase of those amino acids can be observed. This indicates that there is a difference in absorption of proteins between rye and wheat as well as a long term effect of rye consumption on leucine and isoleucine concentration. BCAA are predictive biomarkers for type 2 diabetes (Wang et al. 2011; Wurtz et al. 2013). So rye might be protective in the development of diabetes, since previous studies found out that constantly high levels of BCAA activate the protein complex mTORC1 in skeletal muscles (Lynch, Adams 2014). This protein complex then induces phosphorylation of other proteins that firstly promote insulin resistance and secondly promote degradation of muscle protein, resulting in higher plasma levels of amino acids (Lynch, Adams 2014).

### 5.1.3 Tryptophan

After a single test meal (1.A), picolinic acid, which is a breakdown product from tryptophan in the kynurenine pathway, was significantly decreased in the rye group. It induces a pro-inflammatory pathway in macrophages thus there seems to be a favorable change in the way tryptophan is processed (Bosco et al. 2003). Possibly, after rye intake, serotonin might be synthesized preferably instead of kynurenine (Frolkis et al. 2010). Increased concentrations of serotonin regulate neuropeptide Y production and therefore increase satiety. This fits very well to findings in other studies where rye consumption resulted in better satiety than foods with similar energy content. So besides insulin also tryptophan could be responsible for these findings (Rosén et al. 2011). In another study (2.A), plasma metabolites after 8 week consumption of high-fiber rye bread and white wheat bread were measured during fasting state. Significant changes in ribitol, indoleacetic acid and ribonic acid were observed, they were found higher after rye bread intake. Those molecules function as precursors and breakdown products in tryptophan metabolism, however tryptophan levels did not differ.

### 5.1.4 Glutamic acid, Tyrosine, Lysine

Study 1.A showed effects of rye also on other amino acids. There was a decrease of tyrosine and glutamic acid after 30min in both the rye and the wheat group, but after rye consumption, the decrease was less pronounced. Lysine was higher in both groups and a stronger increase could be observed after rye consumption. There are no hypotheses to explain these results yet.

## 5.2 Glucose and insulin

Rye bread intake does not induce a significantly different glucose response compared to wheat bread, however a lower insulin AUC response is provoked (Juntunen et al. 2002).

In study 1.B, a lower insulin response was observed after the two rye breads than after the refined wheat bread. The authors found a positive correlation to BCAA responses, which might contribute to the effects on insulin.

Study 3.A showed, that long-term consumption of rye products leads to a significantly lower insulin response (after 120 min) although the same test meal was consumed. A suggested explanation is that the first-phase insulin secretion was slightly improved and therefore less insulin is needed later on to control glucose levels.

This phenomenon was investigated more closely by the following studies. A study examining postprandial differences of glucose and insulin levels between whole-meal wheat bread and pure glucose found that neither the peak concentration nor the AUC of plasma glucose differed. However insulin peak and AUC was significantly lower in the whole-meal wheat group. So the differences in insulin levels cannot be explained by glucose influx rate, but rather by other specific compounds in bread (Priebe et al. 2008).

To determine those other specific compounds, a further study compared food forms and fiber contents of cereal foods to insulin and incretin (Gastric inhibitory polypeptide (GIP) and Glucagon-like peptide-1 (GLP-1)) responses. Although  $\beta$ -glucan rye bread had highest total dietary fiber, it did not generate the most favorable insulin response. The main factors for decreased insulin response, however, could not be determined since both addition of whole kernels and addition of soluble fiber ( $\beta$ -glucan) had an impact. What could be shown is that rather the kind of fiber was responsible for the effect than the amount of it. Also the form of cereals the food and the food structure played a role, as whole kernels seemed to have an impact as well as a firmly pressed pasta which allows only 56% of *in vitro* starch hydrolysis. The study could also confirm a positive correlation between incretin and insulin concentration in blood which indicates an up-regulation of insulin response by high GLP-1 and GIP levels (Juntunen et al. 2002).

Similar findings were made by another study that compared postprandial glucose, insulin, incretins and c-peptide after a test meal with white wheat bread vs. rye bread with different fiber contents (Juntunen et al. 2003). The AUCs of insulin after rye breads were smaller and the GIP response was decreased. But there were no differences in AUC of insulin within the rye breads, whereas the AUC of GIP in traditional rye bread was significantly different from endosperm

rye bread. Thus different contents of rye fibers did not result in different insulin responses. Some attempts to explain were the differences in soluble fiber and structures of continuous matrix and starch granules. The starch granules after baking were bigger in rye and coated by amylose, causing a slower hydrolysis (Juntunen et al. 2003).

Phytochemicals from rye probably do not have an impact on glucose or insulin (Lappi et al. 2013).

### 5.3 Organic acids and ketone bodies

The tricarboxylic acid cycle generates energy out of oxaloacetate and acetyl-CoA, common products of catabolic processes, together forming citrate. Enzymatic oxidation of citrate provides products like NADH that can be used in mitochondria to form ATP, a central energy storing molecule (Frolkis et al. 2010).

Pyruvate, the precursor of acetyl-CoA, decreased after a single test meal in study 1.A more in the refined rye bread group than in the wheat group, suggesting a stronger inhibition of aerobic glycolysis after rye intake. But, since pyruvate is involved in many biochemical processes, this is very hypothetical. Alpha-ketoglutarate increased more in the wheat group. While citrate increased after rye consumption, it decreased after wheat. It was the opposite in succinate. The latter metabolites remain rather unexplained. Also malonate, which is a competitive inhibitor of succinate dehydrogenase II in respiratory chain and in tricarboxylic acid cycle, was elevated in the rye group.

Some more organic acids are more present in plasma after a single rye test meal (Bondia-Pons et al. 2011). One study (1.A) found higher levels of phenylacetate, which can be either a degradation product of phenylalanine or a microbial product.

#### 5.3.1 Ketone bodies

Acetoacetate, acetone and 3-hydroxybutyrate are known as the ketone bodies. They are synthesized in the liver when the body is in a fasting state and short in glucose. Fatty acids are broken down to acetyl-CoA which then is used to produce ketone bodies (Fukao et al. 2014).

Two of the ketone bodies, 3-hydroxybutyric acid and acetone, were found to be higher during fasting state after a diet rich in rye (2.B) indicating a more catabolic state. The authors trace this catabolic state back to simultaneously found lower insulin levels, as it is an anabolic hormone. Also the average weight reduction of 2% in the subjects affirms the hypothesis.

## 5.4 Lipids

The study 2.A could not find any significant differences in lipid metabolites between rye and wheat bread.

### 5.4.1 Short chain fatty acids (SCFA)

A recent study (3.A) showed higher plasma concentrations of butyrate after a standardized meal when a period of high rye intake preceded. Especially whole grain rye bread had also an enhancing effect on propionate. The authors proposed microbial fermentation of the dietary fibers as a reason for increased levels of SCFA. In a study with mice, butyrate supplements prevented obesity during a high-fat intervention (Gao et al. 2009). They also improved insulin sensitivity as well as energy expenditure due to for example higher thermogenesis (Gao et al. 2009). Another effect of SCFA on metabolism involves the hepatic enzyme AMPK. It is activated by a high AMP/ATP ratio which occurs when a cell had a higher energy consumption than capture (Hu et al. 2010). Previous studies showed that acetate in fact can increase AMP/ATP ratio in cells (Sakakibara et al. 2006) and butyrate could activate AMPK in intestinal epithelial cells. The enzyme inhibits fatty acid and cholesterol biosynthesis and glucose production while it increases fatty acid oxidation. This leads to a more catabolic state which is favored particularly regarding the development of metabolic diseases. Apart from that, activation of AMPK in epithelial cells also led to improved epithelial barrier function (Peng et al. 2009).

### 5.4.2 Cholesterol

Rye products seem to increase total cholesterol in blood, yet not in a clinically relevant extent (0.2 mmol/l) (2.A). Investigations by another research group (2.C) confirmed higher total serum cholesterol and also LDL cholesterol was found to be significantly higher after rye intake compared to white wheat. Betaine in plasma, which can be increased after rye intake, might contribute to LDL cholesterol production (Olthof et al. 2005).

## 6 Conclusions

The effects of rye and wheat on certain metabolites are very different. Many of the metabolites can be linked to the development of metabolic diseases and seem to be influenced more beneficially after rye consumption.

A more catabolic state is induced by rye, which is indicated by higher fasting levels of ketone bodies in the blood after long term consumption of rye products (Moazzami et al. 2011). Long term consumption also promotes a catabolic state by enabling higher SCFA levels after a test meal (Lappi et al. 2014), which activate AMPK and therefore inhibit fatty acid and cholesterol biosynthesis and glucose production (Sakakibara et al. 2006). Already a single test meal of rye can influence energy metabolism, as it is shown on the intermediates of tricarboxylic acid cycle (Bondia-Pons et al. 2011). Yet the findings on this pathway are rather inconsistent, making it difficult to interpret the results.

Rye also has an impact on tryptophan-related metabolites after a single test meal as well as after regular consumption (Bondia-Pons et al. 2011; Lankinen et al. 2011). The concentration of the amino acid itself is not different in rye or wheat, but its breakdown products and predecessors indicate that there might be a change in kinetics. It is possible that increased satiety observed after rye consumption can be traced back to tryptophan and serotonin metabolism.

Finally, rye also leads to differences in insulin production and sensitivity. This effect is on the one hand reflected and mediated by amino acids. Leucine and isoleucine, which occur in lower postprandial concentrations as well as in fasting state after long term rye consumption, play a key role here (Moazzami et al. 2012; Moazzami et al. 2014). They are biomarkers for the future development of diabetes type 2 and probably also responsible for insulin resistance (Wang et al. 2011; Lynch, Adams 2014). Moreover, betaine and homocysteine interact with insulin levels. High concentrations of insulin can inhibit an enzyme that converts possibly harmful homocysteine to methionine (Ratnam et al. 2006), but betaine, which is present in rye, could again promote this conversion and compensate the

insulin effect (Moazzami et al. 2012). On the other hand, also SCFA are involved in insulin regulation as they can improve insulin sensitivity (Gao et al. 2009). They are more present in plasma after long time rye consumption (Lappi et al. 2014) indicating that there is a better possibility to regulate levels.

Still the question remains what is the reason for different postprandial plasma levels of these metabolites. A difference in rye grains is that there are more fibers in the endosperm, but in general, the amounts of carbohydrates or proteins in wheat or rye grains are very similar.

A study examining the influence of different cereals on insulin and incretins demonstrated that not the total content of fibers but the kind of fibers and also the food structure had a major impact (Juntunen et al. 2003). Analogous to this, it seems to be concerning single metabolites. So the kind of product (bread, porridge, pasta) and its structure, processing and of course the part of grain plays an important role determining digestion, absorption and microbial fermentation in the human body.

Despite all those positive effects, long-term rye consumption also increased cholesterol levels in the plasma in two of the presented studies (Lankinen et al. 2011; Moazzami et al. 2012). So an excessive intake should probably be avoided.

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