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From molecule to men: Inhibition of intestinal glucose absorption by polyphenols and plant extracts for reducing the glycemic response

Christine Schulze

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„ So eine Arbeit wird eigentlich nie fertig, man muss sie für fertig erklären, wenn man nach Zeit und Umständen das Mögliche getan hat.“

Johann Wolfgang von Goethe

Table of Content

1. Introduction

1.1 Background and general introduction

The worldwide prevalence of overweight and obesity has risen dramatically over the last 20 years and has led to an explosive increase in type 2 diabetes mellitus (T2DM) incidence. In 2011, the estimated number of people with diabetes mellitus accounted for almost 370 million adults worldwide and is predicted to increase to approximately 552 million cases in 2030 [1]. In Germany alone, 7 million people had diabetes in 2012 corresponding to 9% of the German population with increasing numbers of around 300.000 people per year (Deutscher Gesundheitsbericht Diabetes 2012, www.diabetesde.org). Diabetes mellitus is an endocrine and metabolic disorder characterized by high blood glucose levels (hyperglycemia), resulting from defects in insulin secretion, insulin action or both, causing disturbances in carbohydrate, protein and fat metabolism. The two major types of the disease are type 1 diabetes mellitus (T1DM) and T2DM. T1DM is characterized by a complete deficiency of insulin due to an autoimmune destruction of the pancreatic β-cells. Since insulin is the primary anabolic hormone that regulates blood glucose concentrations, type 1 diabetics require a continuous supply of exogenous insulin for survival. T1DM accounts for only 5 to 10% of all diagnosed diabetes cases [352]. In contrast, T2DM accounts for more than 90% of all diabetes cases [2] representing thus the most common form of diabetes. The development of T2DM is influenced by genetic factors, aging and peripheral insulin resistance. But also profound changes in the quality, quantity and source of food consumed, combined with physical inactivity are main factors contributing to the increase in obesity and T2DM in many western countries [3, 4]. Especially the continuous supply of carbohydrates in form of snacks and beverages that provide a large amount of rapidly absorbable sugars causes a permanent stimulation of insulin secretion. This has been considered as a prime factor in the pathogenesis of hyperglycemia and T2DM. In addition, the disposition of carbohydrate energy also contributes to the pathogenesis of hepatic steatosis and fatty infiltration as well as obesity, secondarily promoting the genesis of hyperglycemia and T2DM. Hyperglycemia is an important contributing factor in the development of serious micro-and macrovascular complications in diabetic patients [5]. But also in patients with impaired glucose tolerance (IGT), macrovascular complications are already present suggesting that an intervention before the diagnosis of diabetes can be beneficial for the prevention of both, the development of diabetes as well as the

progression of macrovascular diseases [6]. According to a study published in 2006, 1.490.000 deaths from ischaemic heart disease and 709.000 from stroke were related to blood glucose levels above the optimum, accounting for 21% and 13% of all deaths from these conditions [7]. The mortality rate of diabetes mellitus is comparable with that of deaths from smoking (4.8 million), high cholesterol (3.9 million), and obesity (2.4 million) [7]. Several epidemiological studies suggest that isolated post-challenge hyperglycemia is stronger related to the progression of atherosclerosis and the risk of cardiovascular mortality in patients with T2DM and IGT than is isolated fasting hyperglycemia [8, 9, 10, 11, 12, 13, 14]. Patients with IGT without therapy of postprandial hyperglycemia have a 50% higher risk to develop CVD, while 10-21% were prognosed to have microvascular complications including retino-, nephro- or neuropathy by the time diabetes was diagnosed [15]. A study in elderly Americans showed that the prevalence of cardiovascular diseases was similar in normoglycemic subjects and those with isolated fasting hyperglycemia but higher in subjects with isolated postprandial hyperglycemia [16]. Another study including 474 individuals with T2DM identified post-challenge glucose spikes as the strongest determinant of carotid intimal-medial thickness (IMT) independent of other atherosclerotic risk factors including plasma lipids, blood pressure and waist-to-hip ratio [17]. Controlling postprandial hyperglycemia has thus been suggested as an important measure in the prevention and management of T2DM.

The suppression or the delay of monosaccharide release from starch and various oligo- and dissacharidases by intestinal enzymes including α-glucosidase and α-amylase has been already recognized as a therapeutical approach for the modulation of postprandial hyperglycemia in patients with IGT and T2DM. Acarbose, an alpha-glucosidase inhibitor that delays the release and in turn the absorption of glucose from the small intestine, significantly decreases the risk of cardiovascular events in patients with IGT and T2DM [18, 19]. However, acarbose has limitations as it does not affect glucose absorption after ingestion of large amounts of glucose when delivered as the monosaccharide as for example in fruit juices and beverages [20]. Therefore it has been suggested that the inhibition of transporters responsible for absorption of dietary glucose from the intestinal tract could be another promising option to diminish postprandial hyperglycemia.

The intestinal transport of dietary glucose is mediated by two transporters; the high-affinity sodium-dependent glucose co-transporter 1 (SGLT1) and the low affinity glucose transporter 2 (GLUT2). While uptake of glucose through the apical enterocyte membrane is generated by SGLT1, facilitated glucose transport through the basolateral membrane is exclusively mediated by GLUT2. There is evidence that GLUT2 could be involved in glucose absorption across the apical membrane at high luminal glucose concentrations

[21]. The signaling processes required for GLUT2 translocation however are initiated by transport of glucose via SGLT1 [22, 23, 24, 25] confirming the prime role of SGLT1 in glucose absorption and as a target in the management of hyperglycemia.

The prime therapy in patients with IGT and T2DM are lifestyle changes including increased physical activity, dietary changes and weight reduction [26, 27, 28, 29, 30]. However, when lifestyle interventions alone can no longer maintain good blood glucose control, patients with T2DM will additionally require pharmacotherapy including antihyperglycemic drugs in combination with or without subcutaneous insulin injections. Many of the antidiabetic drugs used have limitations such as undesirable side effects but also contraindications due to the presence of co-morbid diseases in the patients [31, 32]. Thus, their widespread clinical use is limited and the demand for new therapeutic options, especially antihyperglycemic agents with lesser side effects is desirable.

There is growing evidence that secondary plant metabolites, especially polyphenols can modulate postprandial rise in blood glucose levels. Polyphenols are mainly consumed with a vegetarian diet rich in fruits, vegetables, tea and other plant-based food products representing a versatile mixture of a large number and diversity of components. Recent studies have shown antihyperglycemic properties of several polyphenolic plant-based foods that can be linked additionally to α-glucosidase and α-amylase inhibitory activity [33] to an inhibition of intestinal glucose absorption [34]. Studies using two-electrode-voltage clamp technology demonstrated the potential of selected dietary polyphenols from the group of flavonoids to inhibit human SGLT1 [35]. Moreover, phlorizin, a dihydrochalcone which was first isolated from the bark of an apple tree in 1835 [36] has long been known as very effective natural inhibitor of SGLT1 [37]. Based on the phlorizin structure (Figure 1) several pharmaceutical companies have developed SGLT2 inhibitors that suppress glucose reabsorption from the kidney resulting in an improvement of hyperglycemia in patients with T2DM [38]. Up to now, two SGLT2 inhibitors were approved for the treatment of type 2 diabetes; dapagliflozin in the EU and canagliflozin in the USA (see Figure 1).

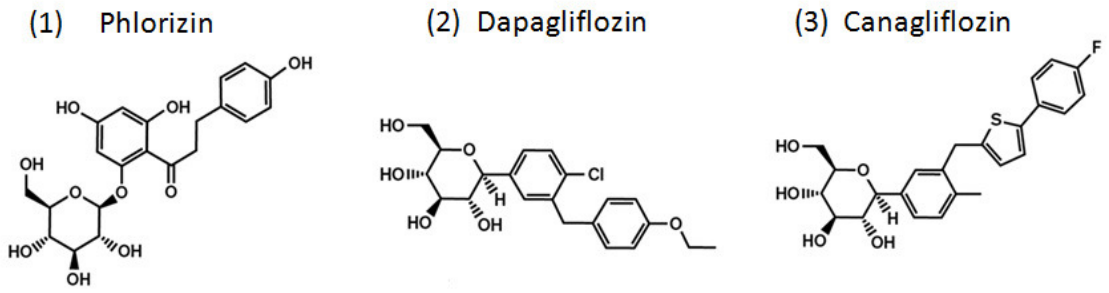

Figure 1: Chemical structures of SGLT inhibitors
(1) The nonselective SGLT inhibitor phlorizin and **(2-3)** human SGLT2-specific inhibitors which are in clinical use.

However, SGLT2 inhibitors have first to be taken up into blood circulation to reach the target side of action. Thus, inhibition of intestinal SGLT1 by orally ingested dietary polyphenols is achieved more easily, as in the intestine highest concentrations compared to any other site in the body can be reached [39].

In search for new food constituents that could reduce intestinal glucose absorption for altering plasma glucose responses and prevent rapid increases in plasma insulin levels, the federal ministry of education and research (BMBF) funded the research project with the title "Der Glucose Transporter SGLT: Schlüsselmolekül zur Prävention von Diabetes". The project was embedded in the funding priority "Biomedizinische Ernährungsforschung" including a total of 5 partners from university and industry with different responsibilities (see Appendix, Table 14).
The focus of the overall project was to investigate and identify plant extracts as well as isolated polyphenolic compounds that can efficiently diminish postprandial rise in blood glucose concentration by inhibiting intestinal monosaccharide transporters with focus on SGLT1.

1.2 Intestinal glucose absorption with focus on SGLT1

Glucose is the major energy source for most energy-dependent processes in the majority of living organisms. Since glucose and other sugars are polar molecules that cannot easily cross lipid bilayers of cells by passive diffusion, they require specific transporter proteins for absorption, distribution and excretion within the mammalian organism. There are two classes of integral membrane proteins responsible for the movement of glucose and other monosaccharides across cell membranes throughout the body. First, the facilitative glucose transporters (GLUTs) coded by the solute carrier (SLC) 2 gene family, and second the sodium-coupled glucose cotransporters (SGLTs) encoded by the SLC5 gene family [40]. While GLUTs are uniporter that passively and selectively let monosaccharides permeate down a concentration gradient, the uptake of glucose and other sugars by SGLTs is mediated in a secondary active manner together with Na^+-ions. To date, 14 members of the GLUT family and 6 members of the SGLT family could be identified in humans [41, 42, 40, 43, 44]. Three transporters play an important role in the intestinal absorption of monosaccharides.

1.2.1 The classical model of glucose absorption

The “classical model” of glucose absorption as found in physiology text books comprises only two transporters; SGLT1 expressed at the apical membrane (AM) and GLUT2 expressed at the basolateral membrane (BLM). Figure 2 shows a schema of monosaccharide transport across the cell membranes of an enterocyte. The uphill transport of glucose and additionally of galactose via SGLT1 is coupled to the co-transport of 2 Na^+ down an electrochemical potential gradient across the AM [45]. This gradient is maintained by the basolateral located Na^+/K^+-ATPase, which pumps the co-transported sodium ions out across the BLM [46]. Besides SGLT1, glucose transporter 5 (GLUT5) is expressed at the AM. GLUT5 proteins exhibit no transport activity for glucose, but mediate transport of fructose into the enterocytes [47, 48, 49]. The exit of glucose, galactose and fructose from the cytosol of enterocytes into the blood circulation is mediated by facilitative diffusion via GLUT2 in the BLM [50, 51].

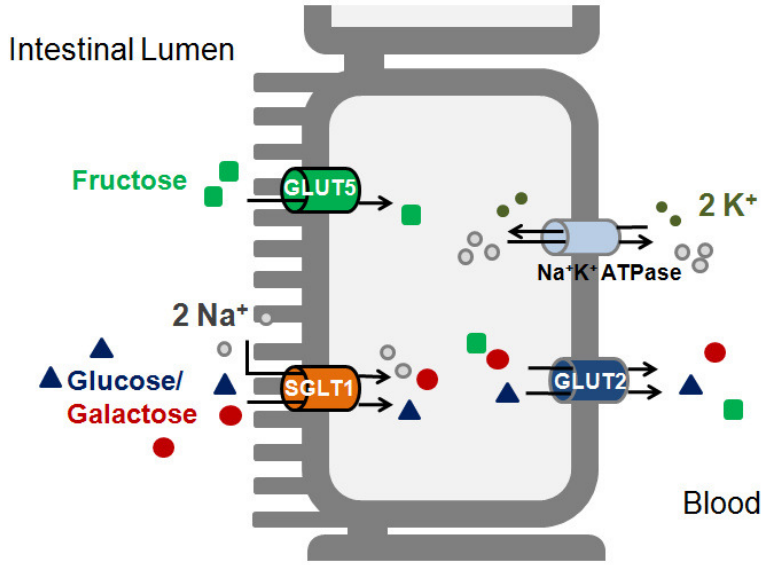

Figure 2: Schema of monosaccharide transport across the intestinal mucosa
SGLT1 and GLUT5 are found in apical membranes while GLUT2 and Na^+/K^+-ATPase are basolateral proteins.

SGLT1 is a high-affinity, low-capacity transporter for D-glucose and D-galactose with highest abundance in the AM of the proximal part of the small intestine (duodenum and proximal jejunum). This is the major side of intestinal glucose absorption [52, 53, 54, 55]. In addition to glucose and galactose other hexoses such as α-methyl-glucopyranoside (α-MDG) and 3-O-methylglucose (3-OMG) could be identified as substrates for SGLT1 [56]. SGLT1 is also expressed in the proximal straight S3-segment of the renal tubule [57] as well as in other tissues such as brain and heart [54, 58]. The apparent Km for D-glucose *in vitro* is 2-6 mM, whereas a value between 6-23 mM has been suggested from *in vivo* studies [59, 60, 61]. Different from SGLT1, GLUT2 is a low-affinity transporter for glucose with a Km of ~17 mM [62] that is expressed in the small intestine but also in liver, kidney and pancreatic β-cells, tissues that require low affinity systems to allow proper influx at physiological glucose concentrations [63, 64]. Dependent on the nutritional state, GLUT2 in the liver facilitates either glucose uptake or efflux [65]. In the kidney and intestinal absorptive cells it mediates glucose absorption or reabsorption [66]. GLUT2 is essential for glucose-induced stimulation of insulin secretion from pancreatic β-cells, thereby acting as a glucose sensor. In addition, low expression levels of GLUT2 were also found in the brain suggesting that GLUT2 in brain could serve a similar sensing function [67, 68].

1.2.2 Proposed alternative routes for glucose absorption

It is generally accepted that SGLT1 exclusively mediates D-glucose movement across the AM at low luminal glucose concentrations, while GLUT2 is the main efflux transporter in the BLM [69]. However, the observation that SGLT1 is in saturation at glucose concentrations of < 10-30 mM [70] that are below the suggested luminal glucose concentration of 50 to 300 mM present immediately after a sugar rich meal [71, 72, 73, 74] led to the assumption that additional mechanisms may be involved. Several theories have been proposed to explain transepithelial glucose transport at high luminal glucose concentrations.

In the mid-1980 it was suggested that passive paracellular flux mediates bulk absorption by a so called solvent drag [75, 76, 77, 78]. The supporters of this theory assumed that the passive component of glucose absorption is caused by an increase in paracellular permeability due to an widening of tight junctions mediated either by an activation of SGLT1 or by the high luminal glucose concentration, allowing large amounts of solvent and other nutrients (solutes) to enter the paracellular space, dragging glucose with them and then diffusing into the portal system [76]. However, animal studies performed with non-membrane permeable substrates such as mannitol or L-glucose in the presence of high luminal sugar concentrations revealed only negligible increases in the blood concentrations of these substrates, indicating that a paracellular transport does not contribute to glucose bulk absorption at high luminal concentrations [79, 80].

More recently Kellet and co-workers suggested GLUT2 as the major pathway of glucose mass absorption across the AM at high luminal glucose concentrations [21]. This concept assumes a rapid translocation of GLUT2 from an internal vesicle pool into the apical membrane in response to an activation of protein kinase $C_{\beta II}$, initiated by SGLT1-mediated transport of glucose across the AM [22, 23, 24, 25]. It was further proposed that GLUT2 insertion into the apical membrane underlies a regulation by insulin [81, 82] by which GLUT2 translocates to the AM in the absence of insulin and re-translocates into cytosolic vesicles in the presence of insulin. Thus, in the insulin resistant state, GLUT2 is thought to be maintained in the apical membrane, thereby creating conditions for an even more pronounced dietary sugar uptake observed under these conditions. However, the theory of GLUT2 as the major transport mechanism for glucose absorption across the AM at high luminal glucose concentrations was challenged by studies in GLUT2-deficient mice that revealed no alterations in the kinetic of intestinal glucose absorption following an oral glucose tolerance test (OGTT) [83] similar to individuals with the Fanconi-Bickel syndrome, carrying inactivating mutations in GLUT2 [84]. In addition, Dyer et al. [85] failed

to detect functional GLUT2 in apical membrane vesicles (AMV) from healthy and/or diabetic patients indicating that GLUT2 is not permanently expressed at the luminal membrane of the intestine of diabetic patients. Stümpel et al. [83] proposed an alternative transcellular route as major pathway for glucose movement across the BLM that does not require functional GLUT2. In this pathway glucose crosses the AM via SGLT1 into the enterocyte followed by immediate phosphorylation to glucose-6-phospate (G6P) and entry into the endoplasmatic reticulum (ER). There, G6P is hydrolyzed into glucose and phosphate and the free glucose is released across the BLM via exocytosis.

Although the exact mechanism of glucose absorption at high luminal concentrations still remains a matter of debate, there is no doubt that SGLT1 plays an essential role in intestinal glucose absorption, since SGLT1-deficient mice ($SGLT1^{-/-}$) develop a severe malabsorption when fed glucose or galactose. This is consistent with the glucose-galactose malabsorption syndrome present in humans with mutations in the SLCA1-gene [86, 87, 88, 69]. However, mice heterozygous for SGLT1 showed no adverse effects or growth retardation on a glucose containing diet [88], suggesting that a partial reduction of intestinal glucose absorption via SGLT1 may be a promising therapeutical option to prevent excessive high postprandial blood glucose concentrations in patients with IGT and T2DM.

1.2.3 Structure of SGLT1

SGLT1 was first cloned from the rabbit intestine by the group of Hediger and Wright in the mid-1980s [89]. To date, 10 SGLT1 proteins have been identified in different mammals. Except of chicken SGLT1, these proteins consist of 605 (pig) to 665 (rat, mouse) amino acids and share more than 80% identity in the amino acid sequence of the human SGLT1 [57, 61, 90, 91]. Different from its essential role in intestinal glucose absorption, SGLT1 plays only a minor role in glucose reabsorption in the kidney [92]. While only ~10% of the filtered glucose is reabsorbed via SGLT1 located in the S3-segment of the renal tubule [56], ~90% of the glucose is reabsorbed via the low-affinity, high-capacity transporter SGLT2 located in the S1-segment of the proximal renal tubule [93, 92, 94]. SGLT2 thus represents the prime transport system for glucose bulk reabsorption in the kidney. Different from SGLT1, cotransport of Na^+ and glucose via SGLT2 occurs with a 1:1 stoichiometry [95]. Dependent on the species, SGLT1 and SGLT2 share about 60 to 80% identity in the primary structure [57]. Figure 3 illustrates the secondary structure and the amino acid sequence of the human SGLT1 as proposed by Wright and collegues [92]. The protein consists of 664 amino acids with 14 transmembrane α-helices (TMH)

connected by 13 surface domains (loops) located either intra-or extracellularly. Both, the C-and N-termini were proposed to face the extracelluar side of the plasma membrane [96]. Studies on structure/function relationships suggest that the N-terminal half of the protein (TMH 4-5) is involved in sodium binding, while the C-terminal half of the protein, in particular TMH 10-13 is involved in sugar binding (recognition) and translocation [97, 98, 99, 87]. In addition, interactions between the N-and C-terminal domains of the protein have been described to be important for cotransport of Na^+ and glucose across the AM [100].

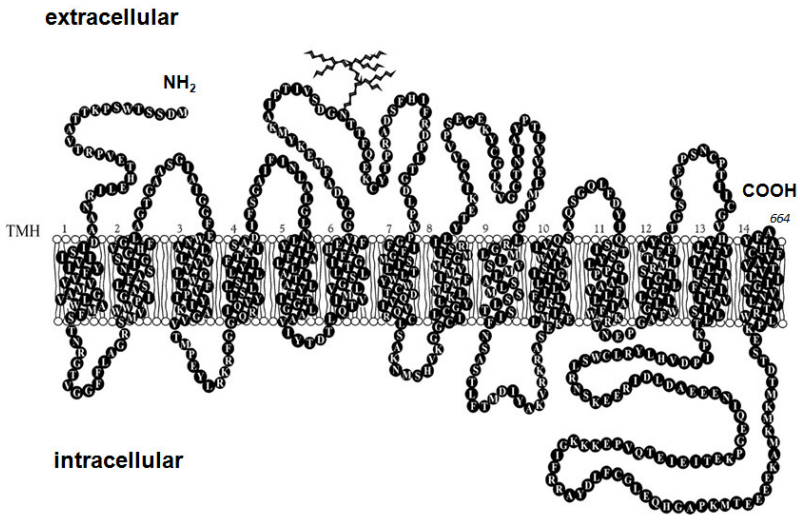

Figure 3: The secondary structure and amino acid sequence of human SGLT as proposed by Wright [92]

1.2.4 Regulation of SGLT1 and its role in postprandial hyperglycemia

Studies investigating the impact of different dietary monosaccharides on SGLT1 expression (metabolizable vs. non-metabolizable or SGLT1-substrates vs. non-SGLT1 substates) in the intestine revealed an up-regulation in the presence of all of the different sugars, indicating that neither transport via SGLT1 nor metabolism is required to induce SGLT1 gene expression [101, 102, 103, 104, 105]. It was also shown that the up-regulation of SGLT1 is mediated by intestinal sweet taste receptors (T1R2 + T1R3) via the G-protein α-gustducin, both found in AMs of enteroendocrine cells. Mice lacking a functional α-gustducin or the sweet receptor subunit T1R3 showed no up-regulation of SGLT1 expression in response to dietary sugars and artificial sweeteners as observed in wild-type mice [106]. Dietary stimuli such as carbohydrate rich diets [107, 108, 109, 110, 103, 111, 112, 69] cause also gastrointestinal hormone secretion and this has been described to change SGLT1 activity in the small intestine as for example glucagon-like

peptide 2 (GLP-2) [113, 114], glucagon 37 [115] as well as insulin [116]. A decrease in the SGLT1 protein abundance that resulted in a diminished rate of intestinal hexose absorption has been described for the gastrointestinal peptide cholecystokinin [117] as well as for leptin that reaches the intestinal lumen after its release by mucosal gastric cells [118, 119]. Both hormones thus can regulate intestinal sugar absorption in the postprandial state and this may also play a key role for changes in SGLT1 expression observed in animal models of obesity and T2DM [120, 121, 122, 123, 124, 125] as well as in patients with T2DM [112]. Increased levels of the gut glucose transporters in obesity and T2DM could promote a more rapid glucose absorption in response to a sugar-rich meal and the resultant higher postprandial blood glucose peak observed under these conditions may be an indicator of that. In isolated brush border membrane vesicles (BBMV), Dyer et al. [112] showed that the rapidity of glucose absorption was approximately threefold faster in tissues of type 2 diabetics as compared to control subjects. In addition to the increases reported in protein and mRNA levels of SGLT1, mRNA levels of brush border enzymes such as sucrase as well as mRNA levels of basolateral GLUT2 were also increased in duodenal samples of type 2 diabetics but not in control subjects. It was recently suggested that a downregulation of SGLT1 expression could in addition to changes in GLP-1 and GIP levels, contribute to the rapid improvement of glycemic control observed after Roux-en Y gastric bypass surgery (RYGB) in patients with morbid obesity or T2DM [126, 125]. In addition, the impact of SGLT1 in the regulation of postprandial hyperglycemia was shown in studies in mice lacking RS1 (regulatory subunit 1) [127]. This protein is a membrane-associated intracellular protein that inhibits release and trafficking of intracellular vesicles containing SGLT1 from the trans-Golgi network to the plasma membrane [128, 129]. Co-expression of RS1 and SGLT1 in *Xenopus* oocytes as well as overexpression of RS1 in the renal epithelial cell line LLC-PK1 decreased SGLT1 mRNA, SGLT1 protein as well as the rate of SGLT1-mediated glucose uptake [130, 131, 132]. Thus, an up-regulation of SGLT1 and intestinal glucose transport capacity would be expected to lead to a rapid uptake of dietary glucose with resulting portal hyperglycemia. Indeed, in mice lacking functional RS1, SGLT1 expression as well as postprandial glucose absorption in the small intestine was drastically increased, leading to a higher peak concentration of glucose in the portal vein compared to wild-type mice [127]. Furthermore, mice without RS1 developed an obesity phenotype. A suppression of intestinal glucose absorption by inhibitors of SGLT1 for example could therefore slow down glucose uptake and blunt postprandial portal glycemic excursions and thus may be helpful to improve the metabolic condition in patients with IGT and T2DM.

1.3 Dietary polyphenols

1.3.1 Classification, structures and dietary sources

Polyphenols are a large group of phytochemicals widely dispersed throughout the plant kingdom. They are produced in secondary plant metabolism where they play an important role in plant morphology (e.g. pigmentation), growth and reproduction as well as in the plant's defense systems against pathogens and predators [133]. Major dietary sources of polyphenols are fruits, vegetables, tea, coffee, red wine, chocolate, cereals as well as herbs and spices [134, 135] where they are found as complex mixtures. The estimated daily intake of polyphenols with a diet rich in fruits and vegetables is approximately 1 g [136]. In subjects with a low intake of fruits and vegetables, beverages such as coffee, tea and wine are the most important source of polyphenols [137]. To date, more than 8.000 polyphenolic compounds have been identified and described in literature, ranging from simple molecules such as phenolic acids to highly polymerized structures, such as tannins [138]. Polyphenols are characterized by at least one aromatic ring with one or more hydroxyl groups attached. The majority of the plant polyphenols usually occurs in glycosylated form linked to different sugar units and acylated sugars at different positions of the polyphenol skeleton [134]. They can be divided into several classes dependent on the number of phenolic rings and to the structural elements binding these rings [139]. The three main subclasses comprise flavonoids, phenolic acids and stilbenes.

The largest group of polyphenols is represented by the flavonoids. The basic flavonoid structure consists of a 15-carbon basic skeleton (flavan) which is arranged by two aromatic rings (ring A and B) connected by a 3-carbon bridge forming a closed heterocyclic ring (ring C) with the aromatic ring A. Like all phenolic compounds, flavonoids are synthesized as metabolites of the shikimate pathway. The aromatic B-ring and the heterocyclic C-ring are considered to originate from the amino acid phenylalanine, whereas the aromatic A-ring is build from three units of malonyl-CoA [134]. According to the modification of the central C-ring such as degree of saturation and oxidation, flavonoids can be further divided into several structural subclasses including flavones, flavonols, dihydroflavonols, flavan-3-ols, flavanones, isoflavones and anthocyanidins. For example, flavonoles (e.g. quercetin, kaempferol, isorhamnetin) and flavones (e.g. apigenin, luteolin) have a similar C-ring structure with a double bond at the 2-3 position and an oxygen atom at the C4 position but different from flavones, flavonoles carry an additional hydroxyl group at position 3 of the C-ring. Figure 4 (framed in green) illustrates

the flavan backbone (scattered frame) and the flavonoid subclasses as well as selected representatives of each goup. Among the flavonoids, flavonols as well as their glycosides are the most widely distributed compounds with quercetin and kaempferol as the main representatives [133]. However, their content in the diet as well as the daily intake is quite low and was estimated to range between 20 and 35 mg per day [140]. The most common flavonol in the diet is quercetin. Primary sources of quercetin and its glycosylated derivatives are apples, broccoli, berries, tea and curly kale [141], but the highest concentration is found in onions. Typical levels of quercetin in onions range from 28.4 to 48.6 mg/100 g, whereas the amount in apples ranges between 2.1 to 7.2 mg/100 g [142]. The predominant forms of quercetin in onions are quercetin-3-4'-O-glucoside (Q3,4'-glc) and quercetin-4'-O-glucoside (Q4'glc), while in apple peel quercetin mainly occurs in O-linkage to glucose, rhamnose, galactose and rutinose at position 3 of the flavan backbone [143, 142]. High amounts of kaempferol and kaempferol derivatives are found for example in curly kale [144]. Flavones are much less common in fruits and vegetables than flavonols. Luteolin and its derivatives have been identified in several edible plants such as carrots, rosemary, chocolate, pomegranate and kale [145], while the most abundant sources of apigenin are leafy herb parsley and dried flowers of chamomile [146]. Anthocyanins are an important group of water-soluble plant pigments which are responsible for the color of flowers and fruits of higher plants [133]. In the human diet they are found in red wine, vegetables (e.g. onions, red cabbage, radishes) and in higher amounts in fruits such as berries [147]. Flavan-3-ols such as catechin and epicatechin are the major flavonoids found in green tea [148].

Although there are limited numbers of flavonoid aglycones, the structural features such as hydroxylation and glycosylation patterns on all 3 rings of the flavon skeleton make them one of the largest and diverse groups of phytochemicals in the plant kingdom [149]. Naturally, the majority of flavonoids, except for flavanols, do not usually appear in plants as aglycones but rather occur in β-glycosylated (O-glycosylated or C-glycosylated) form with glucose or rhamnose as predominant sugars [144]. Glycosylation increases the polarity of the flavonoid molecule, which is necessary for its storage in plant cell vacuoles. Additionally to glucose and rhamnose other sugars such as galactose, arabinose, xylose as well as glucuronic acids have also been found in linkage to flavonoids [150]. More than 80 different sugars including mono-, di-, tri- and tetramonosaccharides have been identified in linkage with flavonoids, preferentially bond to the 3-position [151]. The number of glycosyl moieties can vary from one to five; but the majority of flavonoids occur in linkage to one to three sugar moieties [152].

Another group of polyphenols with structural similarities to the flavonoids are chalcones and dihydrochalcones (Figure 4, framed in red). Formerly, they were classified among the minor flavonoids but they are now regarded as a separate group because their chemical structure cannot by derived from the flavan structure [153]. Biosynthetically they are immediate precursors in the biosynthesis of flavonoids carrying an open-chain three carbon structure that links the A and the B rings [154]. The most prominent dihydrochalcones are phloretin and its glycoside phlorizin (phloretin-2'-glucoside) with high abundance in apples and apple products [37]. The concentration of phlorizin and phloretin is highly dependent on the variety of apples and is formerly found in the apple peel and in lower concentrations in the pulp. For example, the amount of phlorizin and phloretin in the peel of the apple variety *Reineta* ranges between 83 and 418 and 53-100 mg per kg fresh weight, respectively, while the amount of phlorizin in the pulp is approximately between 16 and 20 mg/kg fresh sample [155] The artificial sweetener neohesperidin dihydrochalcone also belongs to the group dihydrochalcones and is used as additive in the food industry. The non-flavonoids group is generally classified according to the number of carbons and comprises compounds such as simple phenolics, phenolic acids, stilbenes, coumarins, lignans and xanthones. The simplest structures consist of a single phenolic ring substituted either by alcoholic, aldehydic or carboxylic acid groups which can be further glycosylated such as in the case of arbutin or salidroside (Figure 4, framed in black). The phenolic acids or phenolcarboxylic acids comprises two major groups; the hydroxybenzoatic acids with a C6-C1 skeleton (Figure 4, framed in blue) and the hydroxycinnamic acids with a C6-C3 carbon skeleton with a double bond in the side chain (Figure 4, framed in yellow) that may have a cis- or trans configuration [156]. Among flavonoids, hydroxycinnamic acids are the most abundant phenolic compounds found in almost every plant [157]. The most common and well known hydroxycinnamic acids are p-coumaric acid, ferulic acid, caffeic acid and sinapic acid which are present in a large variety of fruits and vegetables such as apples, pears, grapes, blueberries, cereal brans, broccoli, lettuce and kale [158]. They can occur as free carboxylic acids, as esters with hydroxylic acids such as flavonoids, quinic acids or carbohydrates or as amides with amino acids [156]. For example in kale (*Brassica oleracea L.)* the majority of flavonoids occur as hydroxycinnamic acid esters of kaempferol and quercetin glycosides [159]. The most abundant hydroxycinnamic acid derivate is chlorogenic acid (5-O-caffeoylquinic acid), an ester of caffeic acid and quinic acid. It occurs in a number of fruits and vegetables such as apples, berries, artichoke and kale and is the major polyphenolic compound found in coffee [160, 159]. It was estimated that the daily consumption of chlorogenic acid (CGA) in coffee drinkers is approximately 0.5-1 g per day, while the

consumption in non-coffee drinkers is less than 100 mg per day [160]. Dependent on the variety, the amount of CGA in fresh apples reaches values between 30 and 60 mg/kg and 208 mg/l in apple juice [161]. It has been estimated that phenolic acids account for approximately on third of the total phenol consumption (dependent on coffee consumption), while flavonoids account for two thirds [2000].

Another group of polyphenolic compounds that are synthesised from cinnamic acid derivatives are the stilbenes which are characterized by two aromatic rings joined by an ethylene bridge (C6-C2-C6) as shown in Figure 4 (framed in purple). They are present in plants as cis- or trans- isomers with trans-configuration as the most common form and occur in monomeric form as well as dimeric, trimeric and polymeric stilbenes, the so-called viniferins [163]. They are not widespread in food plants. The major dietary sources of stilbenes are grapes and red wine from the cultivated species *Vitis vinifera* L. [164, 165]. The stilbene monomer resveratrol (3,4',5-trihydroxystilbene) is the most widely known representative of this group and is found in more than 70 plant species with high abundance in grapes, berries, peanuts and red wine [166]. The predominant form of trans-resveratrol in plant-derived food is trans-piceid, the 3-β-glucoside of resveratrol [167]. For example in red wine trans-piceid reaches concentrations up to 50.8 mg/l [168].

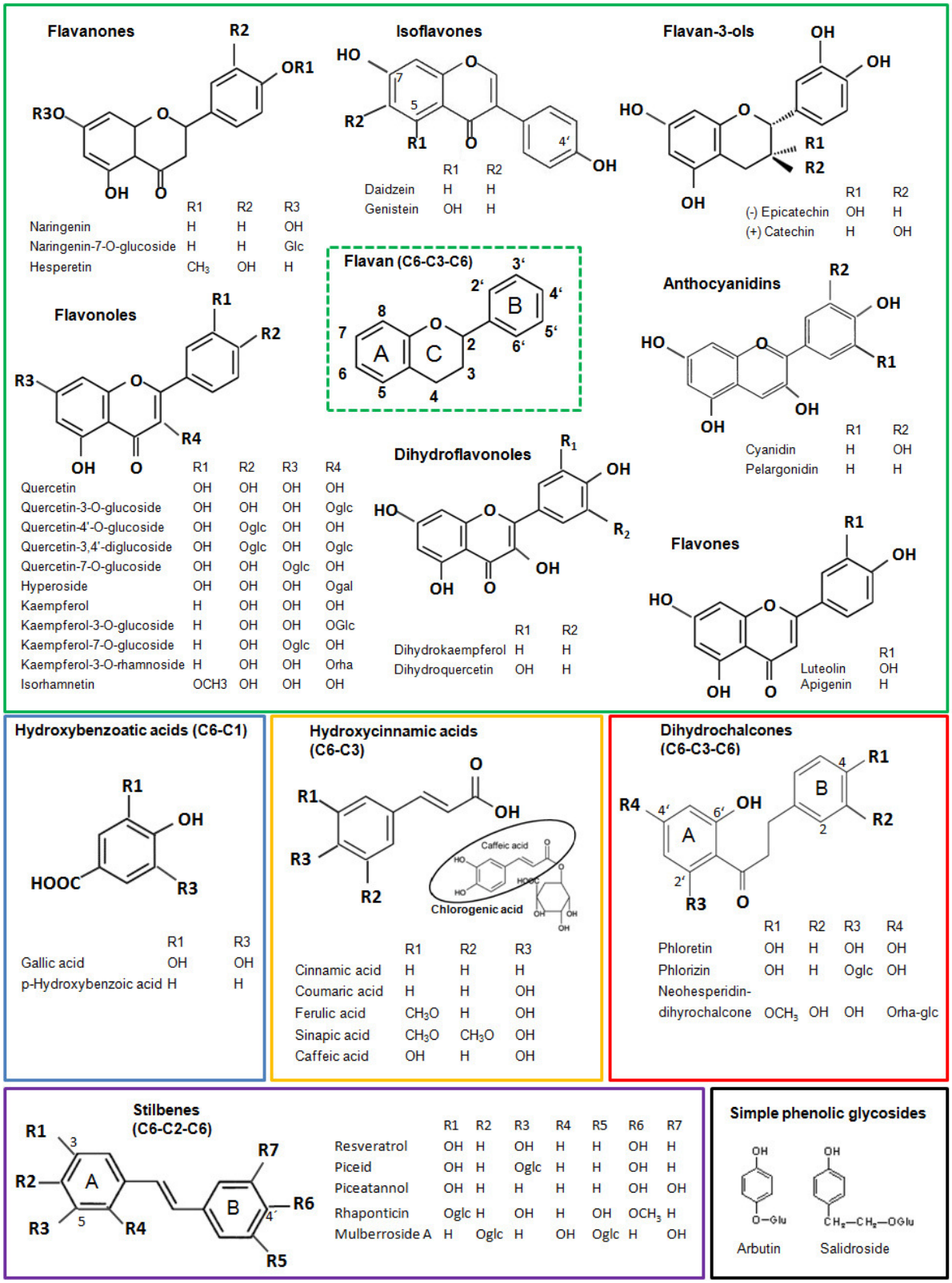

Figure 4: Chemical structures of polyphenols

1.3.2 Bioavailability and metabolism of dietary polyphenols

The bioavailability of dietary polyphenols is essential for biological effects in humans and the knowledge on polyphenol absorption and metabolism is also important to understand their activity. The absorption rate and bioavailability of dietary polyphenols in humans depends on several factors such as dietary source, chemical structure, inter-subject variability (enzyme activity, gut microbiota) as well as the food matrix [169]. For example, bioavailability of quercetin and quercetin-3-O-glucoside in pigs was significantly higher after administration together with a 17% fat-containing diet compared to a 3% fat-containing control diet [170]. In addition, bioaccessibility (the amount of compound in the gut available for absorption) of CGA was reduced by ~20 % when coffee was mixed with milk, probably due to the binding of CGA to milk proteins [137]. Competition or interactions between individual polyphenolic compounds during intestinal digestion has also been shown to influence their absorption [171].

Digestion and absorption of polyphenols occurs mainly in the small Intestine. The majority of polyphenols e.g. hydroxycinnamic acids, flavonoid glycosides as well as procyanidins are resistant against gastric acid hydrolysis and thus reach the small intestine in intact form [172, 173, 160, 174]. However, absorption at the gastric level has been described for several flavonoid aglycones such as quercetin as well as daidzein and genistein but not for their glycosides [175, 176]. In addition, absorption of anthocyanins as well as hydroxycinnamic acids including CGA from the stomach has been demonstrated in several animal studies [177, 178, 179, 180, 181, 139] and may explain the early appearance of certain sufated or glucuronic acid conjugates of CGA and hydroxycinnamic acids in human plasma following coffee consumption [182, 183].

Polyphenols are preferentially absorbed as aglycones since they are relatively lipophylic and can diffuse passively across biological membranes. However, most of the polyphenolic compounds occur as glycosides, esters or polymers that cannot be absorbed in their native form. Thus, they have first to be hydrolyzed by intestinal enzymes or degraded by the colonic microflora before absorption [144]. Deglycosylation by intestinal enzymes is the first step in absorption and metabolism of dietary polyphenolic glycosides, especially of flavonoid glucosides [184, 185, 186, 187]. Lactase-phlorizin hydrolase (LPH) is a surface-bound hydrolase of the small intestinal BBM and is usually responsible for hydrolysis of lactose into galactose and glucose. Additionally, it can hydrolyse ß-O-glycosidic bonds as commonly found in polyphenols. LPH exhibits a broad substrate specifity for hydrolysis of flavonoid-O-β-D-glucosides such as quercetin-glucosides [185], daidzein glucosides [186] as well as of kaempferol and luteolin glucosides [184]. However,

the highest affinity has been shown for the dihydrochalcone phlorizin [188]. Aglycones released from LPH can diffuse passively across the apical membrane into the enterocyte where they are conjugated [189]. It has been shown that LPH activity and abundance in apical membrane vesicles (AMV) isolated from the intestine of diabetic patients were increased 1.4 to 2.2-fold compared to LPH activity and abundance in AMV isolated from the intestine of healthy subjects [85].

An alternative pathway involved in the hydrolysis of O-β-D-glucosides is the cytosolic β-glucosidase (CBG) expressed within the epithelial cells. The enzyme is able to hydrolyze a broad range of glycosides including glucosides, xylosides, galactosides, arabinosides as well as fucosides [190, 191].). However, CBG-catalyzed hydrolysis requires the transport of the polar glucosides into the epithelial cells, possibly via SGLT1 [173, 192, 193, 194]. It was proposed that the glucose moiety enables these polyphenolic glucosides to be transported by SGLT1, since several polyphenols such as quercetin revealed greater bioavailability after ingestion as glucosides than as aglycone or as rhamnoside [195, 196, 197]. Polyphenols linked to rhamnose can only be absorbed in the large intestine following degradation by the gut microbiota [198, 199].

Hydroxycinnamic acids that occur freely in the diet are rapidly absorbed in the small intestine, where they are conjugated and, in particular glucuronidated within the enterocytes [158]. However, hydroxycinnamic and other phenolic acids are often esterified with organic acids, sugars or lipids that cannot be metabolized in the small intestine due to a lack of endogenous esterases [200]. Thus, the majority of phenolic acids will reach the colon and may be metabolized by the gut microflora [201]. For example in rats fed CGA, excretion of CGA in the urine was low, while microbial produced metabolites of CGA such as m-coumaric acid and hippuric acid were detected as the major metabolites in the urine of these rats [202], similar to the findings in human volunteers [203]. In the small intestine only small amounts of polyphenols (5-10%) are absorbed, while the remaining 95-90% passes the colon where they are metabolised by the gut microbiota and eliminated in the feces or further modified within the colonic cells [204]. Modulation of polyphenols by gut bacteria occur by several mechanisms including hydrolysis, ring-cleavage, decarboxylation, reduction and demethylation [205]. Only a small percentage reaches the systemic circulation and tissues, and very little of the absorbed compounds retains the original structure [206]. During or after absorption through the intestinal epithelium, polyphenols and their microbial metabolites undergo β-glucuronidation, sulfation and methylation. For example, glucuronides are one of the predominant forms of flavonoids found in the blood circulation [207]. The conjugation of polyphenols first occurs within the enterocytes [208, 209]. The generated conjugates then reach the liver, where they are further metabolised and either excreted in the urine or stored in tissues e.g. prostate [210,

211, 212]. Polyphenols can also be excreted into the bile and may be recycled, after deconjugation, through enterohepatic circulation [213]. The majority of the circulating polyphenols occur as glucuronidated and/or sulphated conjugates and among a few exceptions such as phloretin, found in conjugated and free form in rat plasma [209]; no free aglycones are usually present in plasma. However, the bioavailability and the metabolism of polyhenolic compounds is very complex and influenced by many other factors including multidrug resistance proteins and glycoprotein P that contribute to overall bioavailability.

1.3.3 Polyphenols and diabetes mellitus

Epidemiological studies indicate that a diet rich in fruits and vegetables is inversely associated with a lower risk to develop major chronic diseases such as CVD, specific cancers, neurodegenerative diseases and T2DM [214, 215, 216, 217, 151, 218, 219, 220]. It has been proposed that the polyphenols within these foods contribute to the health beneficial effects [221]. In addition health promoting properties of dietary polyphenols have been proposed for other diseases including asthma, osteoporosis, skin damage, aging as well as rheumatoid arthritis and inflammatory bowel disease [222, 223, 224, 225, 226, 227].

The putative role of polyphenols in the prevention or even treatment of diabetes and diabetes-related complications has been described in a large number of studies. The proposed anti-diabetic action of polyphenols is based on the reduction of intestinal absorption of dietary carbohydrates, the improvement of insulin release and insulin sensitivity as well as the reduction of inflammation and oxidative stress [205]. Hyperglycemia has been shown to promote reactive oxygen species (ROS) production and oxidation-related damage in nerves, retina, kidney [228, 229, 230, 231] and pancreatic β-cells [232, 233]. Protection of β-cells from hyperglycemia- and ROS-induced damage has been reported for several polyphenolic compounds [234]. Resveratrol led to an improvement of glucose tolerance, attenuation of β-cell loss and reduction of oxidative stress in pancreatic islets *in vitro* [235] as well as *in vivo* [236]. Dietary polyphenols may also influence glucose metabolism by stimulating peripheral glucose uptake in insulin-sensitive and non-insulin sensitive tissues as shown for several polyphenols [237, 238, 239, 240, 241]. But also suppression of hepatic gluconeogenesis has been described as a possible mechanism for the anti-diabetic effect of several polyphenols [242, 243, 237].

One of the best known properties of polyphenols in carbohydrate metabolism is the inhibition of α-glucosidase (maltase) and α-amylase, the key enzymes responsible for

digestion of dietary carbohydrates to glucose. For example, extracts derived from peels and pulps of several apple varieties were able to inhibit α-glucosidase and α-amylase activity *in vitro* [244]. The flavonoles quercetin and kaempferol as well as some of their derivatives were able to suppress intestinal glucose absorption by inhibiting α-glucosidase or sucrase activity *in vitro* [245, 246, 247]. Moreover, quercetin diminished the rise in postprandial plasma glucose when administered together with a starch or maltose solution in normoglycemic and diabetic rats as well as to volunteers with T2DM [247, 248, 249]. But also hydroxycinnamic acids like chlorogenic acid were found to inhibit α-glucosidase activity *in vitro* [245] as well as *in vivo* [250].

The influence of polyphenolic compounds on intestinal glucose absorption via SGLT1 and on postprandial blood glucose and insulin responses has also been investigated in several *in vitro* and *in vivo* studies. Transport activity of SGLT1 was markedly inhibited in the presence of green tea polyphenols such as epicatechin gallate and epigallocatechin gallate [251]. But also other flavonoids such as quercetin monoglucosides, luteolin as well as naringenin significantly inhibited SGLT1-mediated glucose uptake *in vitro* [252, 35]. Rosmarinic acid, the major phenolic constituent of Greek sage herbal tea, significantly decreased rat intestinal SGLT1 levels in diabetic animals 14 days after daily intake with the drinking water [253]. Extracts derived from apple and peach leaf could be identified as potential inhibitors of SGLT1 that improved plasma glucose profiles following a glucose challenge in healthy mice [254, 255]. Recent human studies suggested that beverages such as apple juice or decaffeinated coffee as well as berries may improve short-term glycemic control [205].

2. Aim of the thesis

The aim of the present thesis as part of the overall project was to investigate and identify plant extracts and isolated polyphenolic compounds in their ability to inhibit glucose absorption by intestinal monosaccharide transporters with a focus on the sodium-coupled glucose cotransporter SGLT1. For assessment of the inhibitory effects of candidate compounds 3 different test systems were employed:

1. The first test system used the heterologous expression of the human SGLT1 transporter in *Xenopus* oocytes and the two electrode voltage clamp for analysis of transport currents. Responses of SGLT1 to 1-O-methyl-alpha-D-glucopyranoside (α-MDG), a non-metabolizable analogue of glucose were determined in the absence and the presence of the test compounds under voltage clamp conditions. By use of radiolabelled substrates, test compounds were tested also regarding their ability to inhibit the glucose transporter GLUT2.

2. The second approach took the most effective compounds, identified in the oocyte system into the mouse intestine *in vitro* to test their ability to inhibit glucose absorption using the “everted gut ring” and “everted gut sac” techniques and by employing radiolabelled α-MDG as a substrate for SGLT1.

3. Putative hypoglycemic effects were finally assessed *in vivo*

    a. in mice by use of an oral glucose tolerance test in the absence or the presence of the most promising extracts and constituents thereof.

    b. in healthy young men challenged with an oral dose of 75 g glucose and additionally in obese volunteers by administration of 104 g white bread (equivalent to 50 g carbohydrates) with prior administration of selected plant extracts or mixtures thereof.

3. Methods and Material

3.1 Methods

3.1.1 Synthesis and analysis of the plant extracts

The complete analysis and parts of the production of the curly kale, onion and apple extracts used in the present work were done by Dr. Bettina Schwanck under the supervision of Prof. Wolfgang Blaschek from the Department of Pharmaceutical Biology of the Christian-Albrechts-University of Kiel during her PhD thesis (for detailed information see [188]).

3.1.2 *In vitro* screening using the *Xenopus laevis* oocyte expression system

Xenopus oocytes are a superior expression system used for electrophysiological analysis of ion channels and rheogenic transporters. The advantages of the oocyte expression system are the easy handling and the low expression levels of endogenous channels and/or carriers (transporter). Injected cRNA, coding for the ion channel or transporter of interest is generally translated efficiently and the synthesized proteins are transported to the cell membrane accessible for electrophysiological measurements. But also for non-electrogenic transporters like glucose transporter 2 (GLUT2) *Xenopus* oocytes provide a robust system for functional analysis. By use of radiolabelled substrates it is possible to investigate transport and inhibition of selected substrates and/or inihibitors.

3.1.2.1 Isolation and preparation of Xenopus laevis oocytes

The *Xenopus* oocyte expression system was used to study interactions of plant extracts and their individual constituents (e.g. flavonoids, phenolic acids etc.) with the monosaccharide transporters SGLT1 and GLUT2. *Xenopus* frogs were anesthetized with 0.7 g/l 3-aminobenzoic acid ethyl ester dissolved in 1.5 l of water and animals were kept in anesthetizing solution for 20-30 minutes. During the laparotomy, frogs were bedded on the back and accessorily surrounded by an anesthesia soaken tissue. Oocytes were harvested from parts of ovaries through a small (1 cm) laparotomy incision. The follicular cell layer was removed with collagenase A (1.75 mg/ml) in Ca^{2+} free Barth's solution at room temperature for 90 min with slow agitation. Then, oocytes were washed several

times starting with Ca^{2+} free Barth's solution and then with standard Barth's solution to remove any remaining enzymatic activity. After the manual separation of stage V-VI oocytes by use of pasteur-plast pipets (Ratiolab GmbH, Dreieich, Germany), oocytes were maintained in Barth's solution supplemented with 0.1 g/l gentamycin and 0.56 g/l sodium pyruvate at 17°C. The frog was allowed to recover from operation in a separate tank. After final surgery, frogs were killed by an anaesthetic overdose.

3.1.2.2 cRNA microinjection in oocytes

One day after the isolation, oocytes were micro-injected with 23 nl of solution containing 1 µg/µl cRNA coding for the human SGLT1 or with 13.8 nl of solution containing 1 µg/µl cRNA coding for the human GLUT2 using the Drummond nanoinjector. Therefore a very thin glass capillary (tip diameter ≤ 20 µm) was filled with mineral oil before attachment to the injector. After loading the capillary with the cRNA solution, the injection was carried out in undamaged oocytes, located on a grid surrounded by Barth's solution. The injection capillaries were pulled using a standard electrode puller. The tip of the capillary was broken under a microscope until achieving a diameter of about 10-15 µm. To allow protein expression, injected and non-injected oocytes were stored in Barth's solution supplemented with 0.1 g/l gentamycin and 0.56 g/l sodium pyruvate at 17°C for 3-4 days before the experimental procedure.

3.1.2.3 Electrophysiological recordings using two-electrode voltage-clamp (TEVC)

Operating mode of the TEVC

Since SGLT1 is an electrogenic transporter, all studies on hSGLT1-expressing oocytes could be performed using the TEVC technique. This technology allows the measurement of whole cell currents through ion channels or electrogenic transporter in *Xenopus* oocytes while holding the membrane potential at a constant value. Since the ion current, necessary to keep the potential constant, is directly proportional to the ions transported through the channels and/or transporters, transport or inhibition of a compound can easily be tested. As the name refers to, the measuring device consists of two glass microelectrodes which are both inserted into the oocyte (Figure 5B). One electrode (potential electrode) records the intracellular membrane potential while the second (current electrode) passes a current to maintain the membrane at a constant potential. Both electrodes are filled with 3 M KCl. The membrane potential electrode is connected to a TEC-05 amplifier where the signal is compared to the voltage clamp command given by

a generator. The difference of these signals is applied as a current through the current-delivering electrode across the membrane and to the bath-grounding electrode (Figure 5C). The electrogenic ion fluxes across the membrane were measured as a deflection from the baseline current. The whole setup was carefully grounded and shielded against external currents with a Faraday cage.

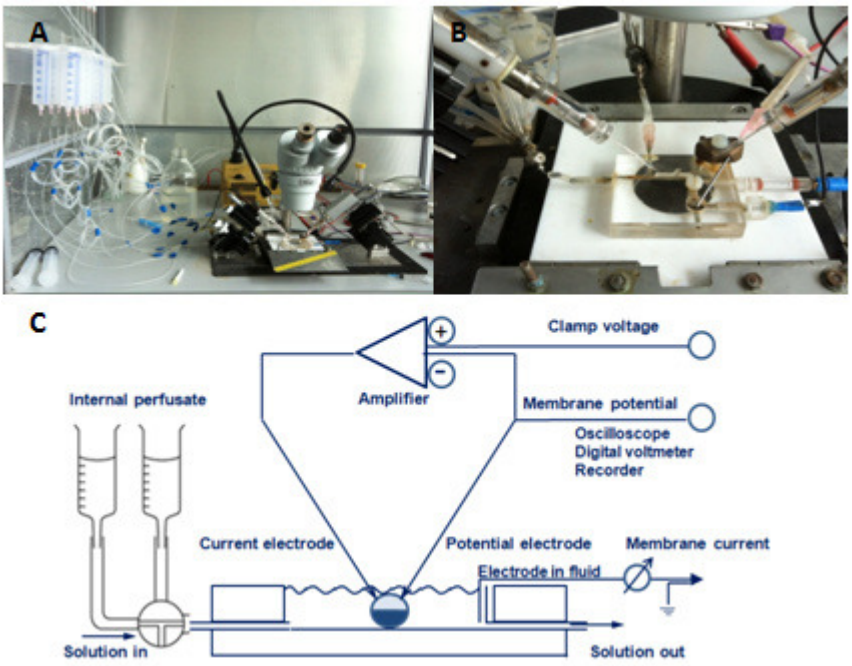

Figure 5: TEVC apparatus and measurement principle

Measurement procedure

To reduce the amount of test solution required for substrate application during TEVC experiments, oocytes were placed in a glass-covered chamber [256] as shown in Figure 6A and 6B. Oocytes were voltage-clamped and subsequently 100 µl of the test solution was applied to a funnel at the chamber inflow. The chamber volume was rapidly (200-400 ms) exchanged by operating a suction pump connected to the chamber outflow (Figure 6C) and transport currents were measured at -60 mV (Figure 6D) using the TEC-05 amplifier and CellWorks software. Current-voltage (*I-V*) relations were measured in 20 mV steps in the potential range of -160 to -80 mV, and the current generated by the α-MDG transport at a given membrane potential was calculated as the difference of the currents measured in the presence and the absence of α-MDG or α-MDG plus the compound to be tested (Figure 6D). Since the test compounds were partly solved in DMSO, ethanol or methanol, solvent-induced currents in the presence of 1 mM α-MDG

were substracted from the currents generated in the presence of the test compounds. The measurement results were evaluated using Visual Basic macros and transferred to Microsoft Excel.

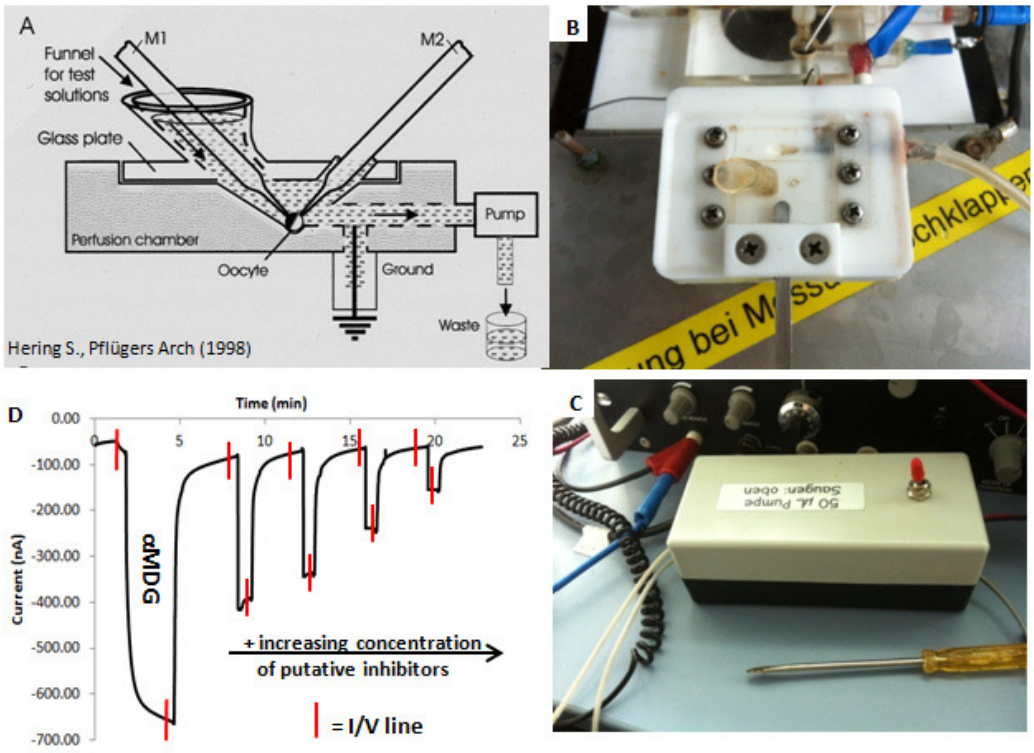

Figure 6: Experimental setting with the mini-chamber used for TEVC measurements of plant material with limited availability
(A, B) Setting of the incubation chamber, (C) α-MDG-induced transport currents in the presence or absence of increasing concentrations of an inhibitor measured over time, (D) manually-controlled suction pump.

3.1.2.4 Uptake of radiolabelled substrates into oocytes expressing GLUT2

Since the glucose transporter 2 (GLUT2) is a non-electrogenic transporter, all studies in hGLUT2-expressing oocytes were performed using ^{3}H-labelled 2-desoxy-D-glucose (2-DG), a GLUT2-specific glucose derivative that is rapidly and completely phosphorylated after uptake via GLUT2. Comparable to α-MDG, 2-DG does not leave the cell after phosphorylation as it is trapped and not further hydrolyzed [257]. Four days after cRNA-injection, 5 to 10 oocytes were incubated in 250 µl of Barth's solution (pH 7.4) containing 1 mM non-labelled 2-DG and 1.8 µM tritium-labelled 2-DG [2 µCi/ml] in the presence or the absence of the compound to be tested. After 30 min incubation on a shaking incubator at RT, incubation medium was removed; oocytes were washed 3 times in icecold Barth's

solution and lysed individually in scintillation vials by addition of 100 µl 10% SDS and agitation for 1 h at RT. After mixing with 3 ml scintillation cocktail (Rotiscint®Eco plus, Roth) the signals were detected in a liquid scintillation counter. Results were expressed as picomol of 2-DG taken up per oocyte over 30 min or as percentage of 2-DG per oocyte per 30 min.

3.1.3 Screening using isolated murine small intestinal segments

Techniques including the everted gut ring and everted gut sac technique are commonly used to study intestinal absorption processes. The majority of studies on glucose absorption in the small intestine (SI) are performed with jejunal tissues as the main site of uptake of carbohydrate digestion products. For better comparison of *in vitro* data (oocyte expression system) with those performed in intestinal segments, the SGLT1-specific glucose analogue α-MDG was also used as a substrate on mouse SGLT1. Since it is exclusively transported by SGLT1, GLUT2-dependent processes are excluded. All procedures regarding tissue preparation [258, 259] and incubations were preformed with freshly prepared Krebs buffer pre-oxygenated for 1 h immediately before usage. For the everted gut sac experiments, sacs were additionally gassed with carbogen during the whole experimental period for up to 1 h. The incubation time of 1 h was chosen because it could be demonstrated in a variety of studies that under the conditions chosen, tissues remain viable up to approximately 120 min [260, 261, 262, 263, 264].

3.1.3.1 Uptake studies performed in everted gut rings

After a 6-h fast, male C57BL/6N mice were anesthesized with isofluran and killed by cervical dislocation. The abdomen was opened and the small intestine was removed and rapidly flushed with icecold Krebs buffer. The intestine was divived into 3 parts and the middle part, the jejunum, was used for the experiments. After gentle evertion with a thin metal rod, the jejunum was cut into segments (rings) of approximately 1 cm in length. Always 3 rings were subsequently placed in one well of a 24-well plate filled with 1 ml carbogen-pregassed Krebs buffer (pH 7.4) supplemented with 1 mM unlabelled α-MDG plus [^{14}C]-labelled α-MDG [0.3 µCi] in the presence or in the absence of increasing concentrations of the potential inhibitor or of the solvent. By vigorous shaking (180 oscillation/min) everted gut rings were incubated for 2 min at 37°C. After removal, rings were washed 3 times in icecold Krebs buffer and blotted carefully on both sides to remove excess moisture and dried overnight in scintillation vials at 50°C. The next day, rings were

weighed, dissolved in 200 µl BIOSOL™ tissue solubilizer and incubated at 50°C for 2 h. To decolorize the samples, 20 µl of hydrogen peroxide was added to the vials and incubated at room temperature for another 1 h. 3 ml of BIOSCINT™ Szintillations Cocktail was added to the samples and counted for radioactivity. The measurements were expressed as picomoles of α-MDG per mg of tissue dry weight.

3.1.3.2 Uptake studies performed in everted gut sacs

The removal and the eversion of the small intestine were carried out as described before. The jejunum was cut into 4 segments of 3.5 to 4 cm in length. Sacs were filled with Krebs buffer (pH 7.4), tightened at both ends with thread and immediately incubated in continuously carbogen-gassed Krebs buffer (pH 7.4) supplemented with 1 mM unlabelled α-MDG and 1 µM [^{14}C]-labelled α-MDG [0.3 µCi] for a maximum of 55 min. To reduce the unstirred water layer adjacent to the epithelial surface, samples were vigorous shaken (180 oscillation/min). Before and after transfer of the sacs into the incubation tubes, 10 µl of the incubation medium was transferred into scintillation vials every 5 minutes and measured for radioactivity. After an incubation time of 15 min, the putative inhibitors were added to the incubation solution and incubation continued for another 15 min. To assess whether the inhibition of SGLT1 was reversible or not, sacs were removed, washed in Krebs buffer (37°C) for 5 min and transferred into incubation tubes containing freshly prepared α-MDG (labelled + unlabelled) solution followed by a incubation for another 15 to 20 min. Sacs were removed, washed three times in icecold Krebs buffer, blotted carefully on both sides to remove excess moisture and cut open to drain serosal fluid into small tubes. Tissue was dried overnight, weighted and digested in BIOSOL™ tissue solubilizer. Radioactivity was measured in the serosal and incubation fluid and additionally in the tissues and calculated for the total concentration of α-MDG (1 mM). Finally the disappearance rate of α-MDG from the mucosal solution (calculated as negative slope value by exponential regression analysis in the absence of the putative inhibitor (before addition and after removal of inhibitor) was compared. A more negative slope value indicates a steeper decline translating into a more rapid disappearance of α-MDG from the incubation solution.

3.1.4 *In vivo* screening for antihyperglycemic effects of plant extracts in mice

3.1.4.1 Experimental animals and feeding trial

Male C57BL/6N mice were used throughout all experiments. Animals were housed conventionally at 22°C and a 12:12 h light/dark cycle, with free access to tap water and food. Until the age of 8 weeks all mice received a chow diet (Ssniff GmbH, Soest, Germany; cat. no. V1534). At the age of 8 weeks animals were divided into 8 groups with similar mean body weights (n=10). Four groups were fed thereafter a high fat diet containing 60 energy percent of fat (Ssniff GmbH, Soest, Germany; cat. no. E15741-34) or a control diet (Ssniff GmbH, Soest, Germany; cat. no. E15000-04) for 12 weeks. The composition of the experimental diets is shown in Table 1. The C57BL/6 mouse was chosen as animal model because it develops typical characteristics of the human metabolic syndrome including obesity and hyperglycemia when fed a high-fat diet [353]. All procedures were done in accordance with the German guidelines for animal care concerning experimental animal protection and approved by the state of Bavaria (Regierung von Oberbayern) ethics committee (Reference number: 55.2-1-54-2532-22-11).

Table 1: Food composition of the diets used in the mice studies

	Carbohydrate	**Protein**	**Fat**
	Chow diet		
Source	Corn starch	Soy bean	Soy bean oil
% kcal	58	33	7
	Control diet		
Source	Corn starch/ Maltodextrin	Casein	Soy bean oil
% kcal	66	23	11
	High fat diet		
Source	Corn starch	Casein	Beef tallow/soy bean oil
% kcal	21	19	60

3.1.4.2 Oral glucose tolerance test

At the end of the 12-week feeding trial, 6-h fasted mice underwent an oral glucose tolerance test (OGTT) with 300 µl of a 20% glucose solution (60 mg glucose) supplemented with or without 12.24 mg apple extract (=15 mM phlorizin), 14 mg onion extract (=15 mM Q4'glc), 15 mM phlorizin or 15 mM Q4'glc. Blood samples were collected

from the tail tip before and 15, 30, 60, 90 and 120 min after the glucose load with subsequent blood glucose measurement using the glucometer Freestyle Lite. After 120 min mice were aneasthetized and killed by cervical dislocation.

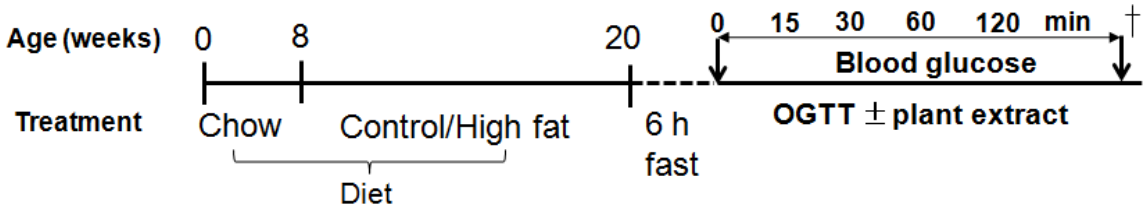

Figure 7: Schedule of the *in vivo* experiments performed in male C57BL/6N mice

3.1.5 *A*ntihyperglycemic effects of plant extracts in humans

All studies were carried out in the human study center of the Else Kröner-Fresenius Center for Nutritional Medicine (Technische Universität München, Freising, Germany). The study protocols were approved by the ethical committee of the Technische Universität München (#2867/10 and #5084/11) and correspond to the Declaration of Helsinki. All subjects provided written informed consent before their inclusion into the study and were free to withdraw from the study at any time with no obligations.

3.1.5.1 Subjects

Before participation in the studies, subjects underwent a medical examination including a complete clinical chemistry check, determination of fasting blood glucose concentration and anthropometric measurements. Participants were excluded if they had an acute or chronic disease, including any metabolic abnormality, any kind of allergy or food intolerance or a fasting blood glucose values ≥ 110 mg*dl^{-1}. Habitual smokers and volunteers that have used any prescribed medication and/or dietary supplement within 2 weeks of entering the study were excluded. To further limit potential confounding factors, individuals were excluded if they reported daily intense sports activities (> 8 hours/wk). All studies were carried out in the Human Study Center between 8 and 12 a.m. after a 12-h fast.

OGTT study

A total of twenty five healthy young men were recruited for the study by advertisement at the campus of the Technische Universität München in Weihenstephan. Always 10 to 15 of them participated in at least one of the four test series.

Table 2: Anthropomethric data of the volunteers participating in the OGTT study
Data are mean ± SD (SEM) of n volunteers

Extract	n	Age (years)	Body height (cm)	Body weight (kg)	BMI (kg/m^2)
Apple	10	23.5 ± 3.1 (1.0)	181.5 ± 6.5 (2.1)	75.0 ± 7.8 (2.5)	22.8 ± 1.6 (0.5)
Onion	15	24.1 ± 2.0 (0.6)	182.4 ± 5.7 (1.8)	77.2 ± 7.3 (2.3)	23.2 ± 1.2 (0.4)
Curly kale	10	24.5 ± 1.9 (0.6)	182.0 ± 6.4 (2.0)	75.9 ± 6.5 (2.0)	23.0 ± 1.4 (0.5)
Grapevine	10	24.0 ± 2.2 (0.7)	184.2 ± 5.8 (1.8)	77.0 ± 8.0 (2.5)	22.8 ± 1.6 (0.5)

White bread (WB) study

A total of seventeen overweight participants (men and women) were recruited for this study. Always ten of them participated in one or more of the seven test series.

Table 3: Anthropomethric data of the volunteers participating in the WB study
Data are mean ± SD (SEM) of n volunteers

Extract	n	♂/♀	Age (years)	Body height (cm)	Body weight (kg)	BMI (kg/m^2)
Apple	10	5/5	55.7 ± 7.5 (2.5)	169.7 ± 9.3 (3.1)	95.4 ± 11.6 (3.9)	32.8 ± 5.0 (1.7)
Onion	10	4/6	57.1 ± 7.5 (2.5)	168.7 ± 8.4 (1.8)	93.5 ± 12.6 (4.2)	32.4 ± 5.1 (1.7)
Curly kale	10	4/6	56.5 ± 8.4 (2.8)	169.3 ± 8.7 (2.9)	91.5 ± 13.1 (4.4)	31.4 ± 5.5 (1.8)
Grapevine	10	4/6	54.3 ± 8.3 (2.8)	169.1 ± 8.9 (3.0)	89.8 ± 13.6 (4.5)	30.9 ± 5.2 (1.7)
Apple+Curly kale	10	6/4	55.3 ± 8.2 (2.7)	170.4 ± 9.3 (3.1)	92.4 ± 12.3 (4.1)	31.5 ± 5.2 (1.7)
Apple+Onion	10	5/5	55.0 ± 7.5 (2.5)	170.6 ± 8.8 (2.9)	93.4 ± 13.2 (4.4)	31.7 ± 5.3 (1.8)
Acarbose	10	4/6	54.6 ± 7.8 (2.6)	169.4 ± 8.6 (2.9)	93.1 ± 13.0 (4.3)	32.0 ± 5.5 (1.8)

3.1.5.2 Study design

Volunteers were requested to refrain from flavonoid-rich foods and beverages, including fruits, vegetables, juices, coffee, tea and chocolate 1 day before the test days. Subjects from the WB study were additionally asked not to consume wholemeal and flatulence causing food products like cereals, cabbage, onions etc. After a 12-hour fast, a permanent venous catheter was placed in the subject's arm vein and after a 15 min adaption period

the first blood sample was drawn. Then subjects from both groups received 150 ml of water. After a period of either 30 min (OGTT study) or 10 min (WB study) further blood samples were taken before (0 min) and 15, 30, 45, 60,120 and 180 min after consuming either 75 g glucose dissolved in 250 ml water (OGTT study) or 104 g white bread (=50 g carbohydrates) together with 250 ml water (WB study). Volunteers were asked to eat the full amount of white bread within 10 minutes or to drink the glucose solution within 2 minutes. A second test day was performed equally but subjects had to ingest the encapsulated extracts either 30 min (OGTT study) or 10 min (WB study) prior to the test meals. During the OGTT study, participants additionally collected 24 h urine, which was fractionated into different collection periods, 0-3 h, 3-8 h and 8-24 h and analyzed afterwards for glucose, creatinine and osmolarity.

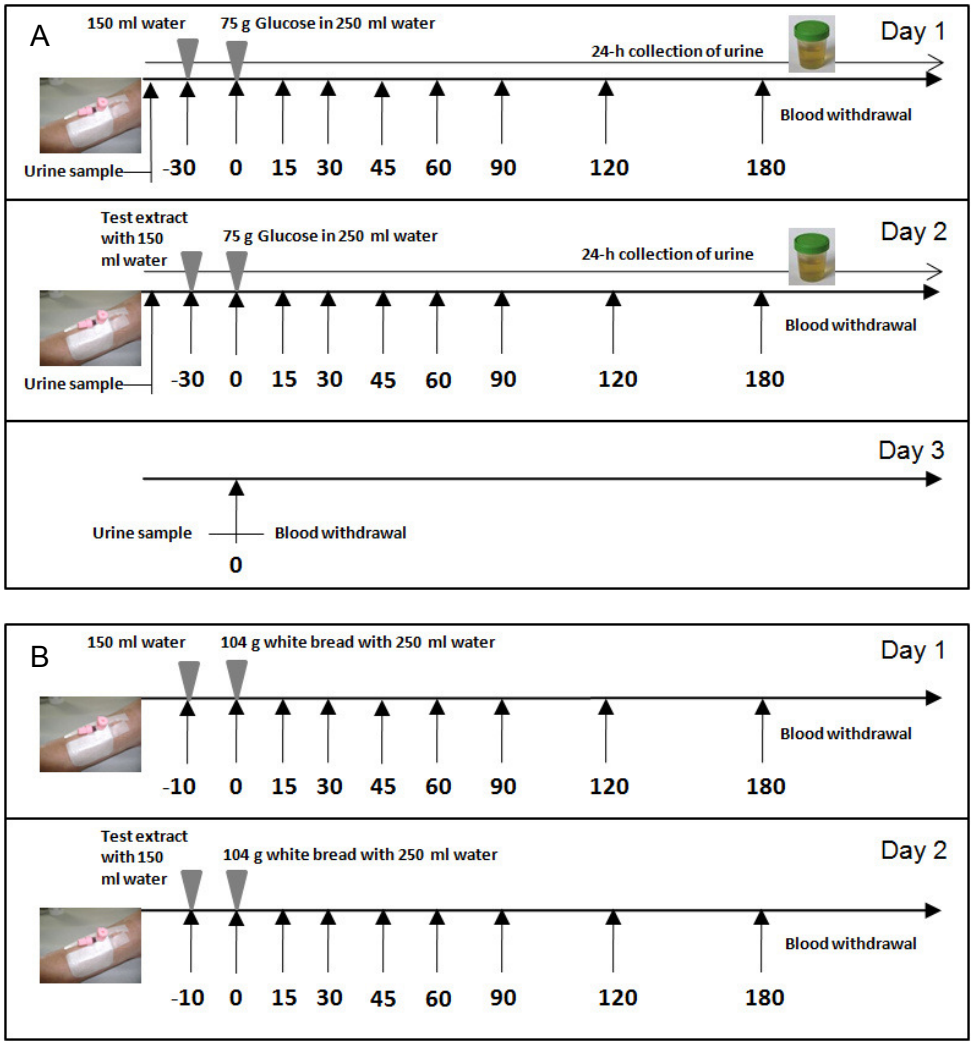

Figure 8: Schedule of the OGTT (A) and WB (B) study

3.1.5.3 Blood and urine sampling

Blood was collected into 4.9 mL EDTA monovettes (SARSTEDT) and was immediately centrifuged for 10 min at 3000 g and 20°C except for glucose concentration determination which was done before centrifugation using Super GL easy+ (OGTT study) or HemoCue 201 RT Analyzer. Plasma samples were aliquoted and stored at -80°C until analysis.
The 24 h urine collection started after ingestion of the plant extracts. The first pass of urine, which was collected before placing the permanent venous catheter, was collected as spontaneous urine in 100 ml urine beaker (JUNGHANS). The 24 h urine collection was done by use of 1 l plastic flasks. For every collection period, subjects had to use a new flask. To avoid bacterial degradation of glucose and polyphenolic compounds, flasks were filled with sodium acide (0.1%) and stored in cool bags during the entire collection period. The urine volume was determined by weighing. Urine samples were aliquoted and stored at -80°C until further analysis. Urine osmolarity was measured immediately after receiving the urine samples from the subjects in non-freezed samples.

3.1.5.4 Clinical parameters in plasma and urine

Whole blood and urine glucose

For the OGTT study, glucose levels from whole blood were determined using the Super GLeasy+ measuring system based on the glucose oxidase method according to the manufacturer's instructions (Super GLeasy+, Möhnesee, Germany). Because of difficulties with the Super GL easy+-handling, the measurement system for the WB study was changed to the HemoCue® Glucose 201 RT Analyzer (HemoCue GmbH, Germany), which measures whole blood glucose based on the glucose dehydrogenase reaction using dual wavelength photometer. Glucose in urine was analyzed with the Glucose Hexokinase FS-Kit (DiaSys International, Holzheim, Germany) according to the manufacturer's instructions.

Plasma Insulin (ELISA)

Determination of plasma insulin was carried out according to the manufacturer's instructions using an insulin enzyme-linked immunosorbent assay (ELISA; Dako Ltd, Cambridgeshire, UK) based on two monoclonal antibodies. The photometric reading was done in a Varioskan platereader.

Plasma and urine creatinine

The amount of creatinine excreted daily by an individual is relatively constant. Thus, urinary creatinine levels were used to normalize the rate of excretion of other analytes in the urine like glucose. Plasma and urine creatinine was analyzed enzymatically with a creatinine kit (DiaSys International, Holzheim, Germany) based on the Jaffé reaction. Urine samples were diluted 1:50 with aqua dest., plasma samples were measured undiluted according to the manufacturer's instruction. All samples were measured in duplicate.

Urine Osmolarity

Osmolarity in urine was determined with a semi-micro osmometer (Knauer, Berlin, Germany) based on the freezing-point depression using 150 µl of the undiluted urine sample. All samples were measured in triplicate.

3.1.6 Transport kinetics

3.1.6.1 Calculation of I_{max}/ V_{max}, K_m and IC_{50} value

The kinetic parameters of transport K_m and I_{max} (TEVC) or V_{max} (intestinal segments) were calculated from least-square fits of at least three data points based on the Michaelis-Menten equation using the following formulas:

$I = I_{max} * [S]*(Km + [S])^{-1}$ or $V = V_{max}*[S]*(Km + [S])^{-1}$

whereas I represents the transmembrane current (TEVC), V the velocity (intestinal segments), [S] the substrate concentration, I_{max} and V_{max} the maximum rate of transport at satiable substrate concentrations and K_m the Michaelis-Menten constant. K_m is the half-saturation constant referring to the substrate concentration at which a half maximum rate of transport is achieved.

The method of least squares is based on the fitting of a curve to a set of measurements as close as possible. The fitting process consists of finding the value of the curve parameters that minimize the sum of squares of the differences between the measurements and the preselected curve. Therefore initially estimated values are inserted into the equation, calculated and compared to the values of the measured data set (generated current in nA). The differences to the measured data points become squared

and summed up. Using specific algorithms, estimation of the equation parameters becomes more and more refined. The minimization of the sum of squares is automatically calculated until no more significant reduction is achievable. For the determination of K_m and I_{max} at least 4 data points were used (varying substrate and inhibitor concentrations). The analysis was performed with Microsoft Office Excel 2007 using specially generated macros.

The determination of the IC_{50} value was also estimated with the method of least squares based on the Michaelis Menten equation using the following formulas:

$$I = I_{max} * (1- [I_n]*(I_nC_{50} + [I_n])^{-1}) \quad \text{or} \quad V = V_{max} * (1- [I_n]*(I_nC_{50} + [I_n])^{-1})$$

whereas I represents the transmembrane current, V the velocity (intestinal segments), [In] the inhibitor concentration, I_{max} and V_{max} the maximum rate of transport at saturable substrate concentrations and I_nC_{50} the inhibitor concentration at which a half maximal inhibition of the rate of transport is achieved.

3.1.7 Statistical Analysis

The graphical and statistical analysis of the results was performed with the programs Prism 4 (GraphPad, San Diego, USA) and the software SAS (Statistical Analysis System). In all experiments, a probability of $p \leq 0.05$ (*) was significant, whereas $p \leq 0.01$ (**) and $p \leq 0.001$ (***) were defined as statistically highly significant.

Oocyte expression system:

For all experiments using *Xenopus leavis* oocytes, data are given as mean ± SEM of n oocytes from N donor frogs. For the TEVC experiments all data are calculated at a membrane potential of -60 mV. Statistically significant differences were determined by one-way ANOVA followed by Dunnett's post test (control as reference) using Graphpad Prism 4.

Everted gut ring experiments:

The everted gut ring experiments were performed with tissues of at least 3 mice. Data are given as mean ± SEM of 3 rings from N mice. Statistically significant differences were determined by one-way ANOVA followed by Dunnett's posthoc test (control as reference) using Graphpad Prism 4.

In vivo mouse studies:

Data are given as mean ± SEM from N mice. Changes in blood glucose concentrations from the baseline concentrations were calculated by subtracting the fasting value from the highest value and were presented as incremental (Δ) concentrations. The incremental area under the curve (iAUC) for blood glucose was calculated from the incremental concentrations by using the trapezoid rule. Statistical analysis was carried out using the statistic program SAS (Version 9.2; SAS Institute Inc, Cary, USA). All blood data were analyzed using two-way ANOVA with repeated measures for one factor (time). iAUCs were analyzed by one-factor analysis without repeated measures. All tests were adjusted for multiple comparisons by means of Tukey-Kramer post-hoc test. In all experiments, a probability of $p \leq 0.05$ (*) was significant, whereas $p \leq 0.01$ (**) and $p \leq 0.001$ (***) were defined as statistically highly significant.

Human studies:

All results were expressed as means ± SEM. Changes in blood glucose and plasma insulin concentrations from the baseline concentrations were calculated by subtracting the fasting value from the highest value and are presented as incremental (Δ) concentrations. The incremental area under the curve (iAUC) for blood glucose and plasma insulin was calculated from the incremental concentrations by using the trapezoid rule. Statistical analysis of the results were carried out using the statistic program SAS. Differences between blood glucose and plasma insulin concentrations were carried out by two-way ANOVA with repeated measures. Differences between the iAUCs were analyzed by one-way ANOVA with repeated measures. All tests were adjusted for multiple comparisons by means of Tukey-Kramer post-hoc test. In all experiments, a probability of $p \leq 0.05$ (*) was considered significant, whereas $p \leq 0.01$ (**) and $p \leq 0.001$ (***) were defined as statistically highly significant.

3.2 Material

3.2.1 Plant material and natural compound library

3.2.1.1 Plant material

3.2.1.1.1 Curly kale

The flavonoid enriched curly kale extract was generated by the project partner BioActive Food GmbH and was analyzed by HPLC-DAD as described in the PhD thesis of Dr. Bettina Schwanck [188]. It could be shown that curly kale contains high amounts of glycosylated and partially acylated derivatives of flavonoids and hydroxycinnamic acids. By gradient elution, a separation of different classes of compounds was achieved. Over 50 polyphenolic compounds were detected and most of them could be identified. Depending on the extraction method the amounts of different glucosylated and acylated flavonoids in the extracts varied. Flavonoids containing up to 5 glucose residues and different patterns of linked polyphenolic acids are characteristic for curly kale. Furthermore, different hydroxycinnamic acid derivatives could be found. The quantification of flavonoids was performed after hydrolysis into the aglycones. The curly kale extract revealed a total content of flavonoids of 14.1 mg/100 mg curly kale dry extract calculated as hyperoside. An intended partial hydrolysis resulted in kaempferol and quercetin as the main aglycones with amounts of 5.2 mg/100 mg and 3.3 mg/100 mg dry mass, respectively. Minor amounts of isorhamnetin (0.4 mg/100 mg dry mass) could also be detected. Moreover some glucosylated compounds were present, e.g. kaempferol-7-O-glucoside, kaempferol-3-O-glucoside and quercetin-7-O-glucoside. Liberated sinapic acid, ferulic acid and caffeic acid were also identified.

3.2.1.1.2 Onion extract

The onion extract used for all experiments was provided by the Wellness & Health Care GmbH (wHc-Service GmbH, Neuenbürg, Germany). Information about extract raw material or extraction method was unfortunately not provided by the company. The onion extract was analyzed for polyphenolic compounds by HPLC-DAD performed and described by Schwanck [188]. The analysis of the flavonoid enriched onion extract by HPLC/ESI-(-)-MS^2 without prior hydrolysis identified quercetin derivatives as the most

abundant compounds, whereas Q4'glc, Q3,4'diglc and the aglycon quercetin itself represented the main components with a total of 20.4% of all detected flavonols. The total amount of flavonoids within the onion extract was 18.7 mg/100 mg onion dry extract, calculated as hyperoside. Q4'glc had the highest amount with 9.5 mg/100 mg dry mass followed by quercetin with 5.4 mg/100 mg dry mass and Q3,4'diglc with 5.2 mg/100 mg dry mass. Other flavonols that could be detected were isorhamnetin, the methylated form of quercetin, and kaempferol but in 30 to 50-times lower concentration than the quercetin derivatives.

3.2.1.1.3 Apple extract

During this project, several apple varieties and preparations (apple pomace, apple core etc.) were analyzed for polyphenolic compounds by HPLC (see [188]).
The apple extract used throughout the present work that was provided by the project partner BioActive Food GmbH. The total amount of polyphenolic compounds was calculated as catechin-equivalent and was found to be 44 mg/100 mg dry extract. The major polyphenoles are presented in Table 4. Phlorizin was detected as the dominant flavonoid with an amount of 16 mg/100 mg dry plant material, followed by quercetin with an amount of 12.4 mg/100 mg dry extract and chlorogenic acid (5.57 mg/100 mg dry extract). Kaempferol, isorhamnetin and phloretin could be also identified but in minor amounts.

Table 4: Amount of major polyphenolic compounds identified in the apple extract

Polyphenol	**(mg/100 mg dry mass)**
Phlorizin	16
Phloretin	0.32
Quercetin	12.43
Kaempferol	0.36
Isorhamnetin	0.297
Chlorogenic acid	5.57

3.2.1.1.4 Grapevine shoot extract Vineatrol®30

Vineatrol®30 was developed and produced jointly by Breko GmbH (Bremen, Germany) and Actichem (Montauban, France). It is a grapevine shoot extract from the species *Vitis Vinifera* cultivated in the Bordeaux region of france (www.breko.de) and produced by water/ethanol extraction.

Vineatrol®30 contains mainly compounds belonging to the family of stilbenes. It is rich in the stilbene monomer trans-resveratrol as well as in resveratrol oligomers, whereas trans-resveratrol and its dimer ε-viniferin had the quantitatively highest amount with 7.7% and 14.6%, respectively. Further stilbene derivatives found in Vineatrol®30 were the monomer trans-piceatannol, the dimer ampelopsin A as well as the tetramers hopeaphenol and r-2-viniferin (Table 5).

Table 5: **Ratio of resveratrol monomers and oligomers present in Vineatrol®30**
Adapted from Nevo-Koch [354] page 3

Resveratrol monomers and oligomers	Amount (%) in Vineatrol®30
Monomers	
trans-Resveratrol	7.7
trans-Piceatannol	0.6
Dimers	
ε-Viniferin	14.6
Ampelopsin A	3.4
Tetramers	
r-Viniferin (Visitin B)	2.5
r-2-Viniferin (Visitin A)	1.6
Hopeaphenol	1.8

3.2.1.2 Amount of plant extracts administered in the human intervention trials

Plant extracts were provided as capsules. **Table 6** shows the amount of plant extracts administered and the total amount of flavonoids (curly kale and onion extract), stilbenes (grapevine extract) or phlorizin (apple extract) within these extracts. The mixture extracts were combined differently. While the curly kale + apple extract mixture contained 600 mg flavonoids and 450 mg phlorizin, the onion+ apple extract mixture contained 300 mg onion flavonoids and 225 mg phlorizin.

Table 6: Amount of plant extracts and polyphenols administered in the human studies

Plant extracts	Scientific name	Amount of plant extracts ingested (g)	Amount of capsules	Amount of flavonoids/stilbenes/phlorizin (mg)
Curly kale	*Brassica oleracea* var. *sabellica L.*	4.4	11	600
Onion	*Allium cepa* L.	3.1	9	600
Grapevine	Vitis vinifera	3.7	12	600
Apple	*Malus domestica* BORKH	2.8	13	450
Apple+Curly kale	-	3.7	13	600+450
Apple+Onion	-	3.3	12	300+225

3.2.1.3 Natural compound library

Table 7: Polyphenol and supplier information

Polyphenols / MW (g/mol)	**Supplier**
Dihydrochalcones	
Phlorizin / 436	PytoLab GmbH & Co.KG, Vestenbergsreuth
Phloretin / 274	PytoLab GmbH & Co.KG, Vestenbergsreuth
Neohesperidin-dihydrochalcon / 612	PytoLab GmbH & Co.KG, Vestenbergsreuth
Aspalathin / 452	PytoLab GmbH & Co.KG, Vestenbergsreuth
Isoliquiritin / 418	PytoLab GmbH & Co.KG, Vestenbergsreuth
Isoliquiritigenin / 256	PytoLab GmbH & Co.KG, Vestenbergsreuth
Cardamomin / 270	PytoLab GmbH & Co.KG, Vestenbergsreuth
Homobutein /286	PytoLab GmbH & Co.KG, Vestenbergsreuth
Isoflavones	
Sophoricosid / 432	PytoLab GmbH & Co.KG, Vestenbergsreuth
Genistein / 270	PytoLab GmbH & Co.KG, Vestenbergsreuth
Genistin / 432	PytoLab GmbH & Co.KG, Vestenbergsreuth
Flavonoles	
Quercetin / 302	PytoLab GmbH & Co.KG, Vestenbergsreuth
Quercetin-4'-O-glucosid / 464	PytoLab GmbH & Co.KG, Vestenbergsreuth
Quercetin-3-O-glucosid / 464	PytoLab GmbH & Co.KG, Vestenbergsreuth
Quercetin-7-O-glucosid / 464	PytoLab GmbH & Co.KG, Vestenbergsreuth
Quercetin-3,4'-O-diglucosid / 626	Extrasynthese SAS, 69726 GENAY, FRANCE
Quercitrin / 448	Extrasynthese SAS, 69726 GENAY, FRANCE
Hyperosid / 448	Carl Roth GmbH, Karlsruhe, Germany
Rutin / 610	PytoLab GmbH & Co.KG, Vestenbergsreuth

Polyphenols / MW (g/mol)	**Supplier**
Kaempferol / 286	PytoLab GmbH & Co.KG, Vestenbergsreuth
Kaempferol-3-O-glucosid / 448	PytoLab GmbH & Co.KG, Vestenbergsreuth
Kaempferol-7-O-glucosid / 448	TransMit GmbH, Marburg, Germany
Kaempferol-3-O-rutinosid / 594	Extrasynthese SAS, 69726 GENAY, FRANCE
Isorhamnetin / 316	Carl Roth GmbH, Karlsruhe, Germany
Flavanones	
Naringenin-7-O-glucosid / 434	Extrasynthese SAS, 69726 GENAY, FRANCE
Naringin / 580	Extrasynthese SAS, 69726 GENAY, FRANCE
Narirutin / 580	Extrasynthese SAS, 69726 GENAY, FRANCE
Naringenin / 272	Extrasynthese SAS, 69726 GENAY, FRANCE
Flavones	
Apigenin / 270	Carl Roth GmbH, Karlsruhe, Germany
Apigenin-7-O-glucosid / 432	PytoLab GmbH & Co.KG, Vestenbergsreuth
Apigenin-8-C-glc / 432	PytoLab GmbH & Co.KG, Vestenbergsreuth
Apigenin-6-C-glc / 432	PytoLab GmbH & Co.KG, Vestenbergsreuth
Saponarin / 594	PytoLab GmbH & Co.KG, Vestenbergsreuth
Luteolin / 286	Carl Roth GmbH, Karlsruhe, Germany
Luteolin-7-O-glucosid / 448	Extrasynthese SAS, 69726 GENAY, FRANCE
Luteolin-3',7-O-glucosid / 610	Extrasynthese SAS, 69726 GENAY, FRANCE
Luteolin-8-C-glc / 448	Extrasynthese SAS, 69726 GENAY, FRANCE
Luteolin-6-C-glc / 448	Extrasynthese SAS, 69726 GENAY, FRANCE
Polyphenolic acids	
Chlorogenic acid / 354	Carl Roth GmbH, Karlsruhe, Germany
Ferulic acid / 194	Carl Roth GmbH, Karlsruhe, Germany
Gallic acid / 170	Carl Roth GmbH, Karlsruhe, Germany
Caffeic acid / 180	Carl Roth GmbH, Karlsruhe, Germany
p-coumaric acid / 164	TransMit GmbH, Marburg, Germany
Sinapic acid / 224	TransMit GmbH, Marburg, Germany
Stilbenes	
Polydatin / 390	PytoLab GmbH & Co.KG, Vestenbergsreuth
2,3,4',5-Tetrahydroxystilbene- 2-glucoside / 406	PytoLab GmbH & Co.KG, Vestenbergsreuth
trans-Resveratrol / 228	Sigma Aldrich, Hamburg, Germany
Ampelopsin A / 470	a kindly gift of Dr. Lisa Marie Bode from the MRI, Karlsruhe, Germany
cis-Resveratrol / 228	
trans-Piceatannol / 244	
a,b-Dihydroresveratrol / 230	
ε-Viniferin / 454	
Mulberrosid A / 568	
Hopeaphenol / 907	
Pinosylvin (trans-Piceid) / 212	PytoLab GmbH & Co.KG, Vestenbergsreuth

Polyphenols / MW (g/mol)	Supplier
Others	
Arbutin / 272	PytoLab GmbH & Co.KG, Vestenbergsreuth
Salicin / 286	PytoLab GmbH & Co.KG, Vestenbergsreuth
Salidroside / 300	PytoLab GmbH & Co.KG, Vestenbergsreuth
Gastrodin / 286	PytoLab GmbH & Co.KG, Vestenbergsreuth
Agnuside / 466	PytoLab GmbH & Co.KG, Vestenbergsreuth

3.2.2 Buffer and solutions

Barth's solution:

Barth's solution was prepared in a larger volume and stored at 4°C. pH was set to 7.4 using HCL or NaCl and was always adjusted before each use.

NaCl	88	mM
KCL	1	mM
$MgSO_4$	0.82	mM
$Ca(NO3)_2$	0.33	mM
$CaCl_2$	0.41	mM
$NaHCO_3$	3	mM
HEPES	6	mM
TRIS	4	mM

Krebs buffer:

Krebs buffer was always made fresh before use and gassed for 1 hour with 95% O_2 and 5% CO_2. pH was set to 7.4 using either MES or HEPES.

NaCl	119	mM
KCL	24.7	mM
$CaCl_2$	2.5	mM
$MgSO_4$	1.2	mM
KH_2PO_4	1.2	mM
$NaHCO_2$	25	mM

3.2.3 Chemicals and reagents

Table 8: List of chemicals and reagents used during this work

Substance	Supplier
[14C]- α-MDG *spec. activity: 300 mCi/mmol*	Hartmann Analytic GmbH, Germany
[3H]- 2-DG *spec. activity: 5.3 Ci/mmol*	Hartmann Analytic GmbH, Germany
2-(N-morpholino)ethansulfonsäure (MES)	Merck, Darmstadt, Germany
2-desoxy-D-glucose (2-DG)	Carl Roth GmbH &Co KG, Karlsruhe, Germany
3-aminobenzoic acid ethyl ester	Sigma Aldrich, Hamburg, Germany
3-aminobenzoic acid ethyl ester	Sigma Aldrich, Hamburg, Germany
BIOSCINTTM Szintillations Cocktail	National Diagnostics, Atlanta, U.S.A
BIOSOLTM tissue solubilizer	National Diagnostics, Atlanta, U.S.A
Calcium Nitrate tetrahydrate	Merck, Darmstadt, Germany
Collagenase A	Roche Diagnostics GmbH, Germany
Creatinine	Sigma Aldrich, Hamburg, Germany
Creatinine Kit	DiaSys Diagnostic Systems GmbH, Holzheim, Germany
d-Glucose monohydrate	Roth GmbH &Co KG, Karlsruhe, Germany
Dimethylsulfoxide (DMSO)	Sigma Aldrich, Hamburg, Germany
EDTA monovettes	Sarstedt AG & Co.,Nümbrecht, Germany
Gentamycin	Roth GmbH &Co KG, Karlsruhe, Germany
Glucose Hexokinase Kit	DiaSys Diagnostic Systems GmbH, Holzheim, Germany
HEPES	Merck, Darmstadt, Germany
Hydrogen peroxide (30%)	Carl Roth GmbH &Co KG, Karlsruhe, Germany
Insulin ELISA Kit	Dako Ltd, Cambridgeshire, UK
Isoflurane (narcotica)	Baxter Deutschl. GmbH, Unterschleißheim, Germany
Magnesium sulphate	Merck, Darmstadt, Germany
MES	Carl Roth GmbH &Co KG, Karlsruhe, Germany
Nitrogen (liquid)	Linde, Pullach, Germany
Phlorizin	Sigma Aldrich, Hamburg, Germany
Potassium chloride (KCl)	Merck, Darmstadt, Germany
Rotiscint® Eco plus	Roth GmbH &Co KG, Karlsruhe, Germany
Sodium azide	Sigma Aldrich, Hamburg, Germany
Sodium Chlorid	Merck, Darmstadt, Germany
sodium dodecyl sulfate (SDS)	Roth GmbH &Co KG, Karlsruhe, Germany
Sodium Hydrogencarbonate	Roth GmbH &Co KG, Karlsruhe, Germany
Sodium Pyruvate	Sigma Aldrich, Hamburg, Germany
Trishydroxymethylaminomethane (TRIS)	Merck, Darmstadt, Germany
α-D-methyl-glucopyranoside (α-MDG)	Sigma Aldrich, Hamburg, Germany
20% Glucose solution	B. Braun Melsungen AG, Melsungen, Germany

3.2.4 Consumables for the human study

Table 9: List of consumables and kits used during this work

Consumables/Kits	Supplier
EDTA monovettes	Sarstedt AG & Co.,Nümbrecht, Deutschland
d-Glucose monohydrate	Roth GmbH &Co KG, Karlsruhe, Deutschland
Insulin ELISA Kit	Dako Ltd, Cambridgeshire, UK
Creatinine Kit	DiaSys Diagnostic Systems GmbH, Holzheim, Deutschland
Glucose Hexokinase FS-Kit	DiaSys Diagnostic Systems GmbH, Holzheim, Deutschland
d-Glucose monohydrate	Roth GmbH &Co KG, Karlsruhe, Germany
Golden butter toast	Lieken Brot-und Backwaren GmbH, Garrel, Germany
Urine beaker	Dr. JUNGHANS medical GmbH, Germany

3.2.5 Technical equipment and software

Table 10: List of instruments and software used during this work

Apparatus/Software	Supplier
CellWorks software	npi electronic, Tamm, Germany
electrode puller	Zeitz Instrumente GmbH, München, Germany
TEC-05 amplifier	Npi electronic, Tamm, Germany
Electronic Data Chart Recorder Model BD40	Kipp and Zonen, Delft, Netherlands
Drummond nanoinjector	World Precision Instruments, Berlin, Germany
Liquid scintillation counter	Perkin Elmer Wallac GmbH, Freiburg,Germany
Super Gleasy+	Hitado Diagnostic Systems GmbH, Möhnesee, Germany
HemoCue® Glucose 201 RT Analyzer	HemoCue GmbH, Germany
Freestyle Lite	Abbott GmbH & Co. KG, Germany
Varioskan	Thermo Elektron Corporation, München, Germany
Osmometer	Knauer, Berlin, Germany
Graphpad Prism 4	GraphPad, San Diego, USA
Statistical Analysis System (SAS)	Version 9.2; SAS Institute Inc, Cary, USA
Freestyle Navigator	Abbott GmbH & Co. KG, Wiesbaden, Germany

4. Results

A total of 40 plant extracts of various fruits and vegetables and more than 70 individual polyphenolic compounds provided by our project partners and others were tested in the course of the BMBF-funded project by use of the TEVC. Individual extracts were found to be partially transported via hSGLT1 or that they contained free sugars (glucose, galactose) that were taken up by hSGLT1. Based on these preliminary findings the extraction processes for some of the extracts were optimized by the project partners and free sugars were removed. From a total of 40 plant extracts initially studied, 4 were taken into more careful analysis in the various test systems with findings presented in the present work.

4.1 Electrophysiological experiments (TEVC) in oocytes expressing hSGLT1

4.1.1 Concentration-dependent transport of α-MDG

To compare our data with those found in literature we first determined the apparent kinetic constant Km for α-MDG transport via SGLT1. By use of increasing concentrations of α-MDG with 4 mM as highest concentration, current-voltage (*I-V*) relations were measured in 20 mV steps in the potential range of -160 to 80 mV (Figure 9A). Since SGLT1 cotransports two sodium ions along with 1 molecule α-MDG, the currents induced by different α-MDG concentrations were highest at the most negative membrane potential of -160 mV. The Km value was calculated from the currents generated by α-MDG transport at a membrane potential of -60 mV and by Michaelis-Menten kinetics defined as 1.02 ± 0.3 mM (Figure 9B). This value is nearly identical with the value (1.06 ± 0.03 mM) reported by Bormans et al. [265].

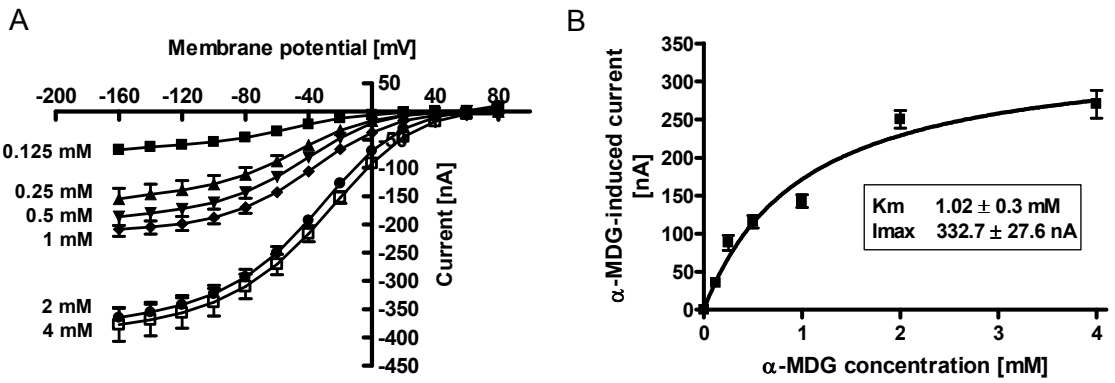

Figure 9: Concentration-dependent transport currents with α-MDG as a substrate and calculation of Km and Imax in oocytes heterologously expressing hSGLT1
Concentration dependent α-MDG-induced transport currents of hSGLT1 either as (A) current-voltage (I/V) relationship in the potential range of 80 to -160 mV or (B) at a membrane potential of -60 mV with calculated Km and Imax values. Data are mean values of 4-7 oocytes ± SEM.

4.1.2 Transport of individual plant extracts via hSGLT1

To exclude possible transport of the plant extracts by human SGLT1 expressed in oocytes, extracts were first tested in the absence of the transporter substrate α-MDG. Figure 10A shows the current-voltage relationship obtained in oocytes expressing hSGLT1 after the addition of 1 mM α-MDG, or of curly kale extract (1 mg/ml), onion extract (1 mg/ml), grapevine extract (0.1 mg/ml) and apple extract (0.1 mg/ml). To simplify the comparability between the data generated by TEVC, they are presented additionally to the I/V-curves (Figure 10A) as bar diagrams at a membrane potential of -60 mV (Figure 10B). Whereas α-MDG evoked a mean transport current of ≈ 160 nA at -60 mV, none of the extracts induced any significant current indicating the complete removal of sugar residues via the extraction process and the lack of transport of any contained polyphenols in the plant extracts.

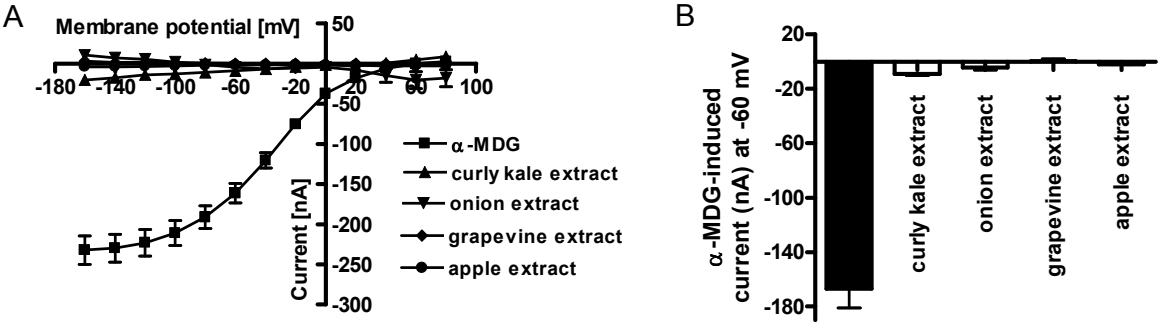

Figure 10: Transport of individual plant extracts via hSGLT1
Comparative analysis of transport currents induced by α-MDG (1 mM), by curly kale extract (1 mg/ml), onion extract (1 mg/ml), grapevine extract (0.1mg/ml) and apple extract (0.1 mg/ml) in *Xenopus* oocytes expressing hSGLT1. (A) Data presented as current-voltage (I/V) characteristic in the potential range of 80 to -160 mV or (B) at a membrane potential of -60 mV. Data are mean values of 4-7 oocytes ± SEM.

4.1.3 Inhibition of hSGLT1 by plant extracts, calculation of IC_{50} and demonstration of reversibility of inhibition

To determine whether there is an interaction between hSGLT1 and selected plant extracts, current-voltage relationships were measured in the potential range of -160 to 80 mV (Figure 11). The current generated by the transport at a given membrane potential was calculated as the difference of the currents measured in the presence and the absence of 1 mM α-MDG plus or minus increasing concentrations of either curly kale extract (A), onion extract (B), grapevine extract (C) or apple extract (D). As shown in Figure 11, all 4 plant extracts were able to inhibit α-MDG-induced currents significantly and in a concentration dependent manner in the potential range of -160 mV to -20 mV.

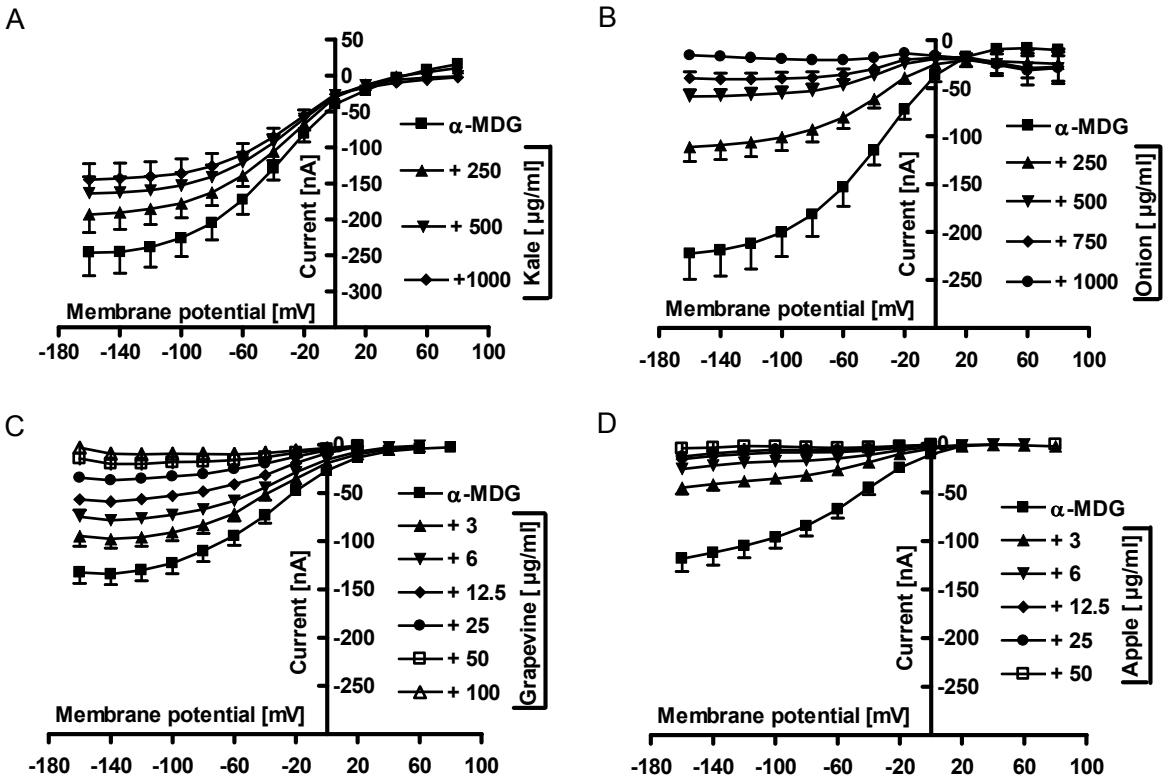

Figure 11: Concentration-dependent inhibition of the α-MDG-induced transport current via hSGLT1 in the presence of plant extracts
Inhibition of the α-MDG (1 mM)-induced current by extracts derived from curly kale (A), onion (B), grapevine (C), and apple (D). Measurements were performed in the presence of increasing concentrations of the plant extracts at membrane potentials ranging from 80 mV to -160 mV. Mean values of 5-8 oocytes ± SEM.

IC_{50} values were derived from Michaelis-Menten kinetics (for details see chapter 3.1.6.1) with apparent IC_{50} values found for the apple and for the grapevine extract of 2.1 ± 0.2 µg/ml and 9.5 ± 1.2 µg/ml, respectively (Figure 12). The onion extract showed slightly weaker inhibition of the current induced by α-MDG with an IC_{50} value of 326.5 ± 57.0 µg/ml. The curly kale extract revealed an IC_{50} value of 1629.9 ± 368.2 µg/ml, indicating only moderate inhibition of hSGLT1.

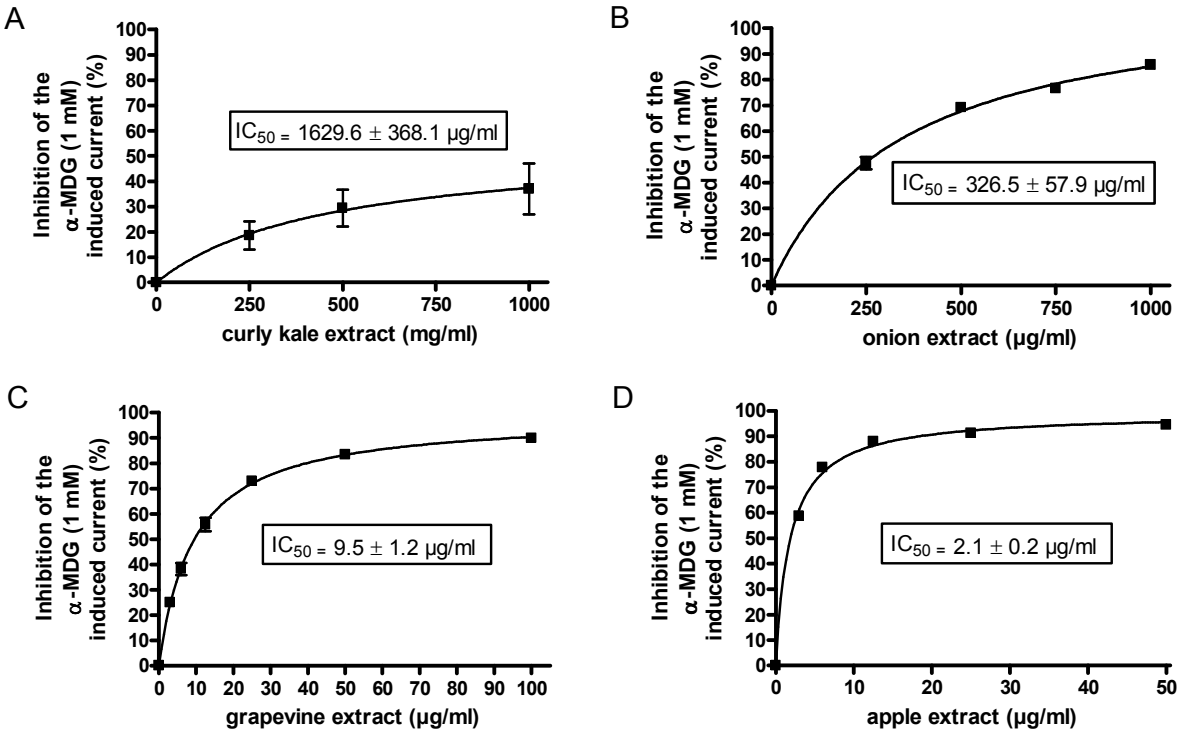

Figure 12: Concentration-dependent inhibition of α-MDG (1 mM)-induced transport currents by the different plant extracts
The determination of the IC50 values was based on the least squares for a Michaelis Menten equation using Graph pad prism 4. Calculations were performed at a membrane potential of -60 mV. Mean values of 5-6 oocytes ± SEM.

Reversibility of inhibition was investigated by flushing oocytes with 1 mM α-MDG at the end of the experiments. Figure 13 shows the α-MDG-induced current at -60 mV after addition of the highest inhibitor concentration followed by a washout with Barth's solution for 2 min. Inhibition of α-MDG-mediated currents by the 4 plant extract was reversible with currents reaching values comparable to those before the addition of the extracts.

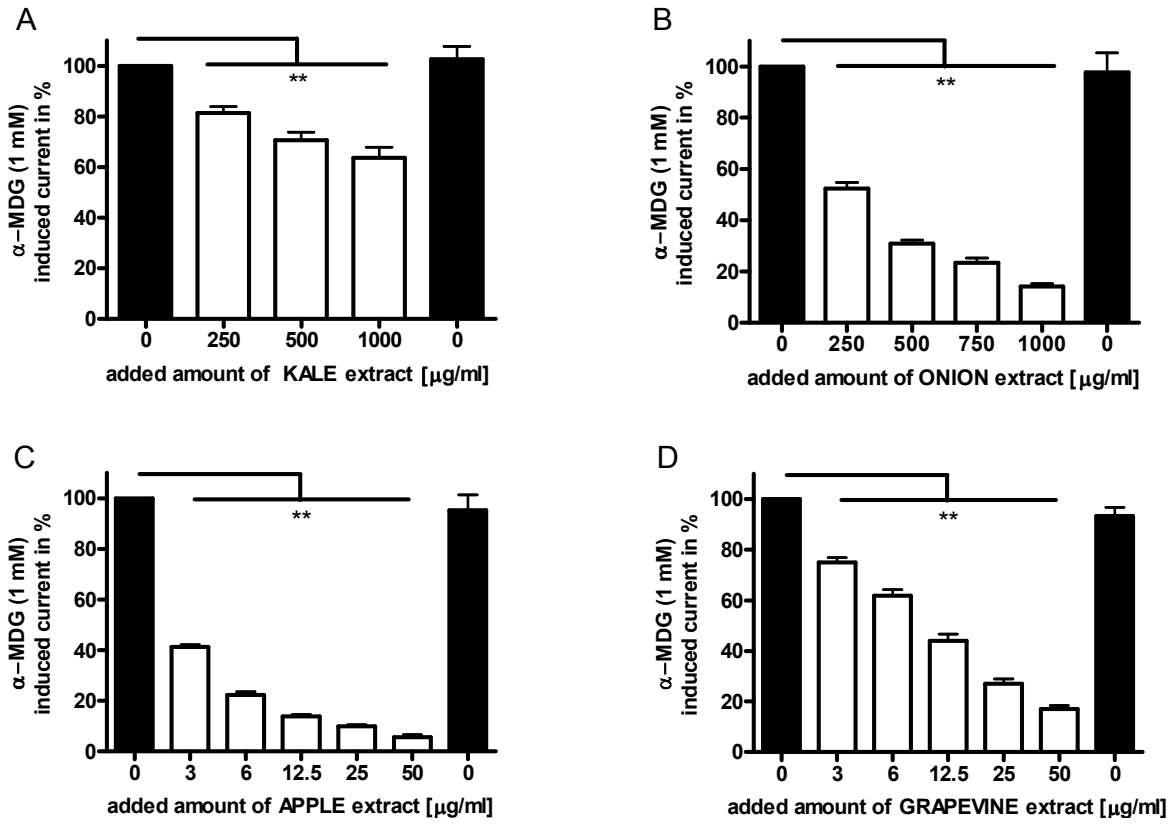

Figure 13: Reversible inhibition of α-MDG-mediated transport currents of hSGLT by plant extracts
Inhibition of the α-MDG (1 mM)-induced current by extracts derived from curly kale (A), onion (B), apple (C), and grapevine (D). Measurements were done using increasing concentrations of the plant extracts at a membrane potential of -60 mV. Mean values of 5-8 oocytes ± SEM. Statistical differences were evaluated by one-way ANOVA with Dunnett's posthoc test. $p < 0.05$ *, $p < 0.01$**

4.1.4 Inhibition of hSGLT1 by polyphenolic compounds identified in curly kale-, onion-, grapevine- and apple extracts

To identify the active compounds responsible for the inhibition of hSGLT1 by the plant extracts, individual polyphenolic compounds were analyzed for their potential to reduce currents induced by α-MDG transport via SGLT1. Polyphenolic compounds were also tested in the absence of α-MDG which could reveal transport of the test compound. The most abundant polyphenols found in curly kale, onion and apple extracts were flavonoids and hydroxycinnamic acids. Dihydrochalcones were additionally found in the apple extract. Figure 14 shows an overview of selected hydroxycinnamic acids, flavonoles, flavanonoles (dihydrokaempferol) and dihydrochalcones with their potency for inhibition of α-MDG (1 mM)-induced transport currents (Figure 14A). Additionally, the same compounds were analyzed for transport via hSGLT1 (Figure 14B). For a better comparison between the compounds (with a few exceptions), they were all applied at

0.1 mM concentration and currents were measured at a membrane potential of -60 mV. For the calculation of IC_{50} values, the compounds were tested at different concentrations (see Appendix, Table 15). Current measurements revealed no significant transport of any of the compounds by hSGLT1 when compared to the currents in the presence of Barth's solution only. Some polyphenols (kaempferol-7-O-glucoside, Q3,4'diglc, phlorizin, trans-p-coumaric acid) partially inhibited the holding current of voltage-clamped oocytes (expressed as negative current) but this occurred also in the absence of 1 mM α-MDG and thus must be an unspecific interaction.

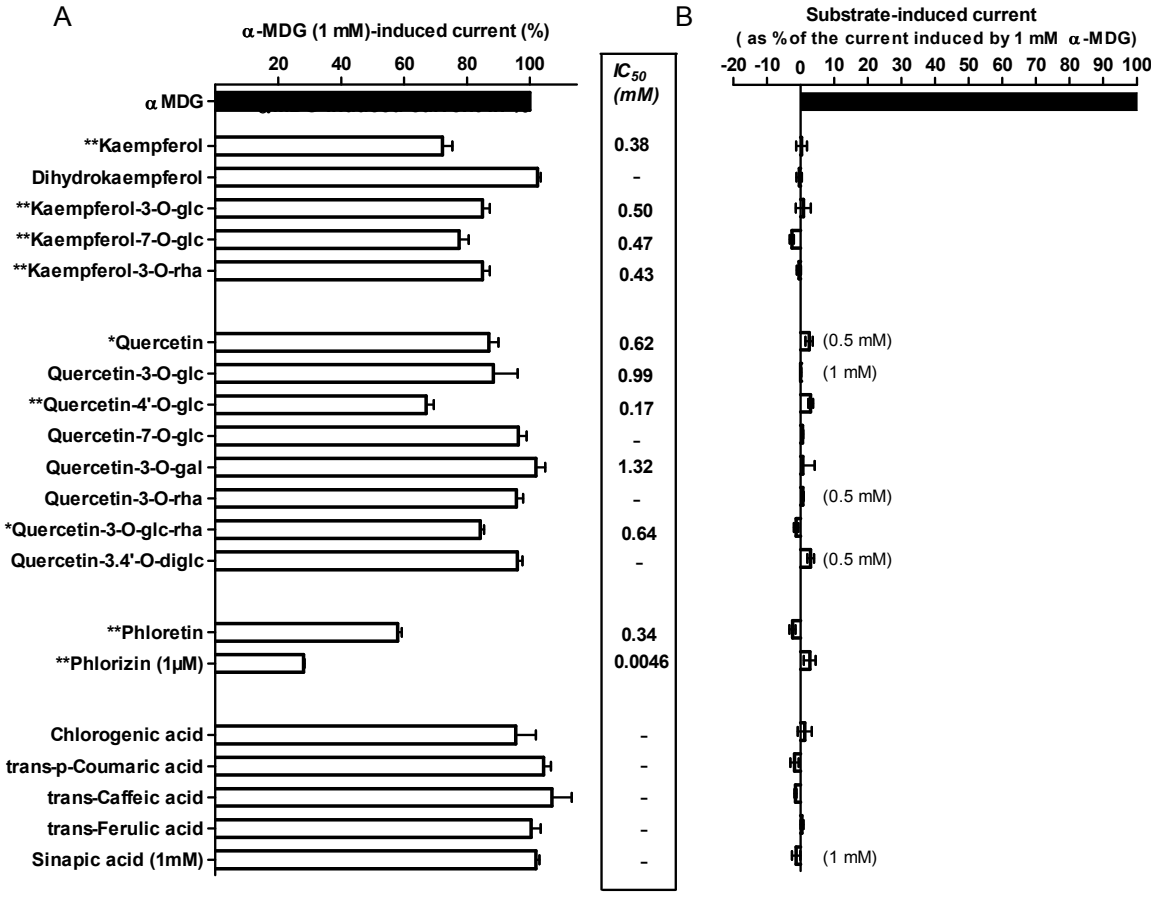

Figure 14: Inhibition of α-MDG-mediated transport currents of hSGLT1 by various flavonoids and hydroxycinnamic acids and transport currents induced by the single polyphenolic compounds

(A) Inhibition of the α-MDG-induced transport currents (1 mM) at -60 mV in the presence of hydroxycinnamic acids, flavonoles, flavanonoles and dihydrochalcones. (B) Currents induced by hydroxycinnamic acids, flavonoles, flavanonoles and dihydrochalcones at -60 mV given as the percentage of the current induced by 1 mM α-MDG. All compounds were added in a concentration of 0.1 mM. The numbers in parenthesis denote the substrate concentration when another concentration than 0.1 mM was used. All values were corrected for the shifts of the zero line due to the presence of the solvent used (DMSO, ethanol or methanol). Negative values define an inhibition of the basal membrane conductance. Mean values of 4-6 oocytes ± SEM. Statistical differences were evaluated by one-way ANOVA with Dunnett's posthoc test. $p < 0.05$ *, $p < 0.01$**

Curly kale extract

Kaempferol and quercetin, the main aglycones found in the curly kale extract (see chapter 3.2.1.1.1) showed only moderate inhibition of hSGLT1 with IC_{50} values of 0.38 ± 0.04 mM and 0.62 ± 0.12, respectively (Figure 14A). Additionally, glycosylated derivatives of kaempferol or quercetin with sugar moieties located at either position 3 (kaempferol-3-O-glucoside) or position 7 (kaempferol-7-O-glucoside, quercetin-7-O-glucoside) were detected in minor amounts. However, none of them further increased inhibition of the α-MDG-induced current (Figure 14A). Kaempferol-3-rhamnoside (K3rha) showed moderate inhibition comparable to the inhibition observed for its aglycone kaempferol. The flavanonole dihydrokaempferol showed no significant inhibition of hSGLT1 at a concentration of 0.1 mM. Simple phenolic acids including sinapic acid, ferulic acid and caffeic acid showed also no significant inhibition of hSGLT1 at concentrations ranging from 0.025 to 1 mM (see Appendix, Table 15).

Onion extract

The major phenolic constituents identified in the onion extract are quercetin and glycosylated quercetin derivatives (see chapter 3.2.1.1.2). The 4'-O-glycosylated quercetin derivative spiraeosid was found in highest concentration in the onion extract with an amount of 0.1 mg/mg extract dry mass, followed by Q3,4'diglc and quercetin. While 4'-O-glycosylated quercetin derivative revealed the strongest inhibition of α-MDG-mediated transport via hSGLT1 with an IC_{50} of 0.17 ± 0.02 mM, quercetin showed only moderate inhibition (Figure 14A). Q3,4'diglc even failed to inhibit hSGLT1 at a concentrations of 0.1 mM but also at a higher concentration of 0.25 mM (see Appendix, Table 15). Further quercetin derivatives with glucose moieties attached to other positions of the aromatic rings such as quercetin-3-O-glucoside (Q3glc) or with other sugar moieties such as quercetin-3-O-galactoside, quercetin-3-O-rhamoside (Q3rha) or quercetin-3-O-glucorhamnoside showed also much lower affinity for inhibition of SGLT1 when compared to Q4'glc.

Apple extract

The predominant polyphenols in the apple extract were phlorizin, followed by quercetin and CGA (see chapter 3.2.1.1.3). Minor amounts of phloretin, kaempferol and isorhamnetin were also detected. CGA showed no significant inhibition of the α-MDG-induced currents and quercetin as well as kaempferol showed only a slight reduction in transport currents induced by α-MDG with IC_{50} values of 0.62 ± 0.05 mM and 0.38 ± 0.03

mM, respectively (Figure 14A). Phlorizin, a well-known inhibitor of SGLT1, showed the strongest inhibition of hSGLT1 with an IC_{50} value of 0.46 ± 0.19 µM thus around 800-fold stronger than its aglycone phloretin with an IC_{50} value of 0.34 ± 0.02 mM.

Grapevine shoot extract

The grapevine shoot extract contains mainly stilbenes including the monomer trans-resveratrol as well as di-and tetramers thereof (see chapter 3.2.1.1.4). Trans-resveratrol, the most prominent representative of this group and dihydro-resveratrol, a metabolite of trans-resveratrol formed in the intestine by microbiota showed only slight inhibition of the current induced by 1 mM α-MDG with an IC_{50} for trans-resveratrol of 0.51 ± 0.09 mM (Figure 15A). Pinosylvin, mulberroside A and 2,3,4',5-tetrahydroxystilben-2-O-glucoside (2,3,4',5-THSG) failed to inhibit α-MDG-induced hSGLT1 currents whereas trans-piceid, the 3-beta-D-glucopyranoside of trans-resveratrol as well as ampelopsin A, a dimer of resveratrol showed strong inhibition of hSGLT1 comparable to Q4'glc identified in the onion extract. Interestingly ε-viniferin and hopeaphenol, the di- and tetramer of resveratrol showed also comparatively strong inhibition of hSGLT with IC_{50} values of 8.0 ± 0.48 and 11.2 ± 0.6 µM, respectively. Current measurements revealed no significant transport of any of the stilbenes by hSGLT1 when compared to the currents in the presence of Barth's solution only (Figure 15B).

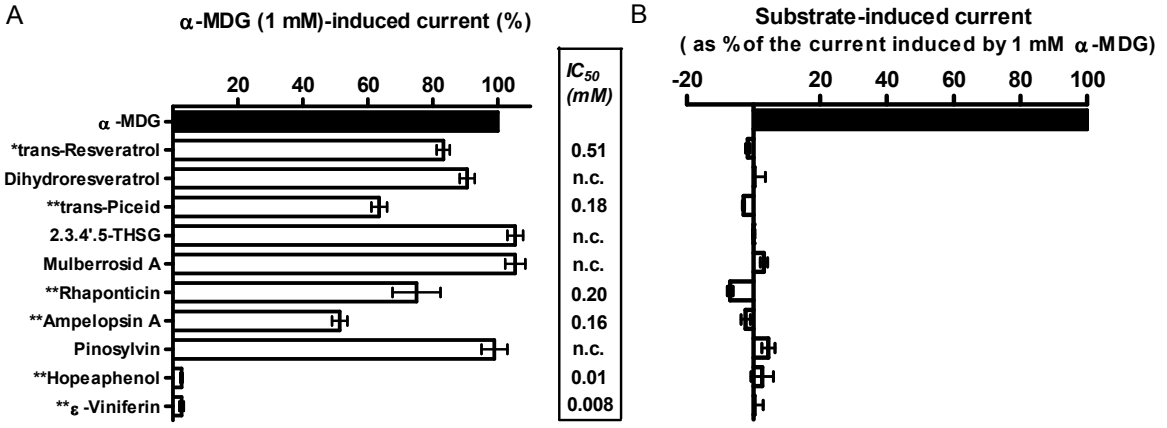

Figure 15: Inhibition of α-MDG-mediated transport currents of hSGLT1 by stilbenes and transport currents induced by the tested stilbenes
(A) Inhibition of the α-MDG-induced transport currents (1 mM) at -60 mV in the presence of stilbenes. (B) Currents induced by stilbenes at -60 mV given as the percentage of the current induced by 1 mM α-MDG. All compounds were added in a concentration of 0.1 mM. All values were corrected for the shifts of the zero line due to the presence of the solvent used (DMSO). Negative values define an inhibition of the basal membrane conductance. Mean values of 4-6 oocytes ± SEM. Statistical differences were evaluated by one-way ANOVA with Dunnett's posthoc test. $p< 0.05$ *, $p< 0.01$**

4.2 Uptake experiments in oocytes overexpressing hGLUT2

4.2.1 Studies on specifity of different glucose analogues

Since the glucose transporter GLUT2 is an uniporter without cotransport of ions, uptake experiments were done by use of radiolabelled substrates. A study performed by Weber et al. [266] demonstrated the presence of endogenous glucose transporter in *Xenopus laevis* oocytes. Since experiments with radiolabelled substrates are more sensitive to uptake by endogenous transporters compared to measurements using TEVC, we first investigated different glucose analogues concerning their uptake in *Xenopus* oocytes injected either with 23 nl water (control) or 23 nl of cRNA coding for human GLUT2.
As demonstrated in Figure 16, glucose was transported in both, H_2O as well as hGLUT2 injected oocytes. In contrast, the SGLT1 specific glucose analogue α-MDG was neither transported by H_2O nor by hGLUT2 injected oocytes. 2-DG, which is a well established substrate of the facilitative glucose transporter, but not of SGLT1, was not transported by the endogenous oocyte glucose transporters and therefore, 2-DG was used as substrate for all studies carried out on GLUT2.

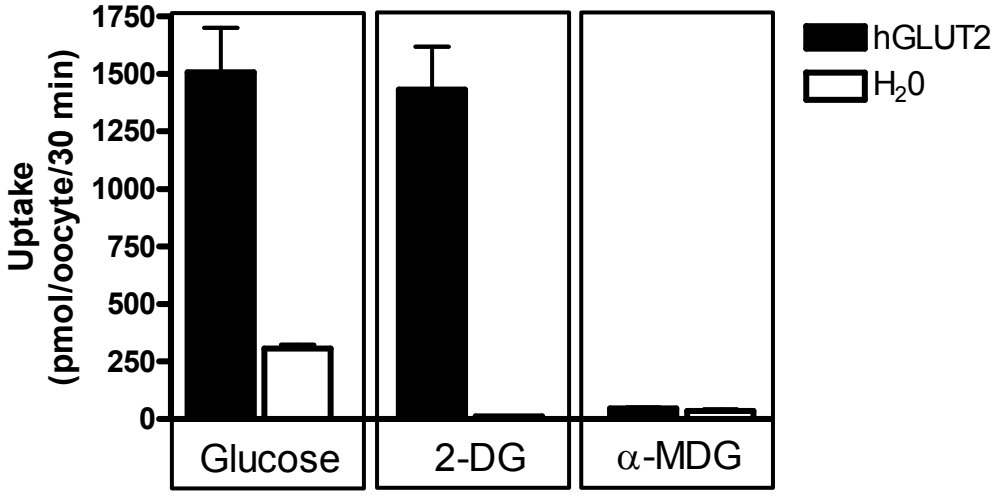

Figure 16: Substrate-specific transport by hGLUT2 heterologously expressed in *Xenopus* oocytes
Uptake of glucose, 2-DG or α-MDG in oocytes injected either with H_2O (white bars) or cRNA coding for hGLUT2 (black bars). Data are mean values ± SEM of 2 biological (donor frog) and 5-10 technical (oocytes) replicates.

4.2.2 Inhibition of hGLUT2 by the plant extracts

To test whether the plant extracts derived from curly kale, onion, grapevine and apple are able to inhibit glucose transport via GLUT2 they were added to the incubation solution (1 mM α-MDG) in a concentration of 0.25 mg/ml. As shown in Figure 17 only the curly kale extract failed to cause a significant reduction of 2-DG uptake into oocytes overexpressing hGLUT2. Whereas the onion and apple extracts reduced 2-DG uptake via hGLUT2 by 78% and 80%, respectively, the grapevine shoot extract abolished 2-DG transport via hGLUT2.

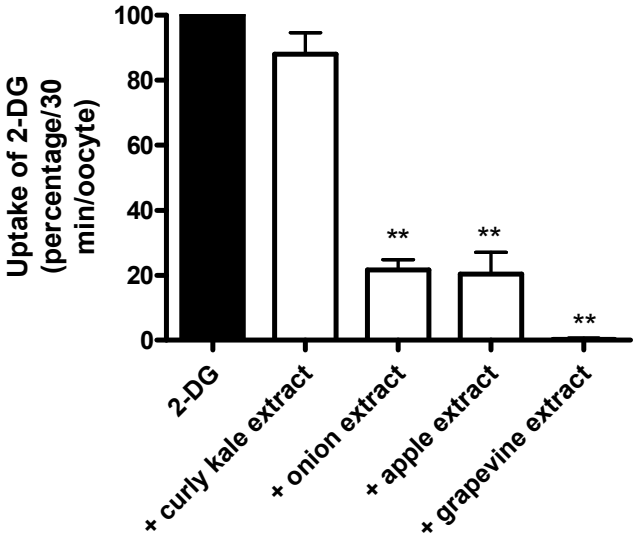

Figure 17: Inhibition of 2-DG transport mediated by hGLUT2 in the presence of plant extracts
Uptake of 2-DG (1 mM) in oocytes overexpressing hGLUT2 in the absence (black bar) or the presence of 0.25 mg/ml of the plant extracts (white bars). Data are mean values ± SEM of 3 biological (donor frog) and 5-8 technical (oocytes) replicates. Statistical differences were evaluated by one-way ANOVA with Dunnett's posthoc test. $p < 0.05$ *, $p < 0.01$**

4.2.3 Inhibition of hGLUT2 by plant extract-derived polyphenols

To identify the active components responsible for the inhibition of hGLUT2, selected polyphenolic compounds were examined for the inhibition of hGLUT2. Figure 18 represents an overview of the compounds analyzed. Polyphenols were all solved in DMSO and added to the incubation solution from a 10 mM stock solution. Therefore the final concentration of DMSO in the incubation solution was always 0.1% which was proven to have no effect on 2-DG uptake into oocytes overexpressing hGLUT2.

From the kaempferol derivatives, kaempferol-3-O-glucoside and kaempferol-7-O-glucoside, of the curly kale extract, strongly decreased 2-DG transport via hGLUT2 by 84% and 69%, respectively. Quercetin and its 3-O-galactoside derivative (hyperoside) were also strong GLUT2 inhibitors with transport reduction by 65% and 85%, respectively. Phloretin, a well known inhibitor of GLUT2, decreased 2-DG transport via GLUT2 by 82%, whereas chlorogenic acid and phlorizin failed to significantly inhibit hGLUT2-mediated uptake of 2-DG.

Within the stilbene group, trans-resveratrol, trans-piceid, mulberroside A and rhaponticin showed no significant inhibition of hGLUT2 at a concentration of 0.1 mM. 2,3,4',5-tetrahydroxystilben-2-O-glucoside and ampelopsin A caused inhibition by 15% and 35%, respectively, whereas the resveratrol dimer ε-viniferin as well as the resveratrol tetramer hopeaphenol almost completely impeded transport of 2-DG via hGLUT2. The inhibition of hGLUT2 by ε-viniferin and hopeaphenol was clearly stronger than that of phloretin, so far considered as the strongest natural inhibitor of GLUT2.

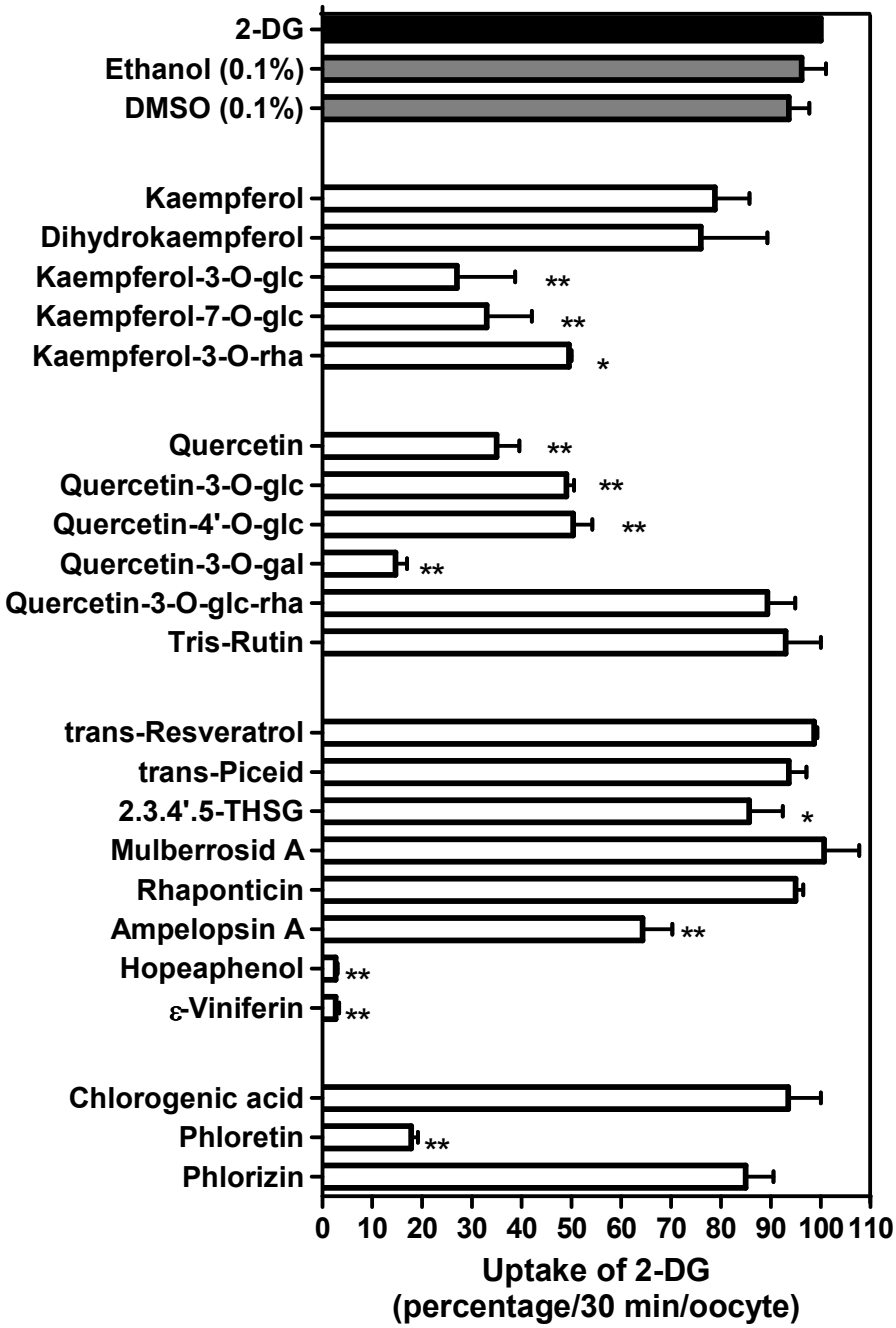

Figure 18: Inhibition of 2-DG transport by hGLUT2 in the presence of selected polyphenolic compounds
Inhibition of the 2-DG transport into oocytes in the presence of polyphenolic compounds or 0.1% DMSO or ethanol. All compounds were added in a concentration of 100 µM. Data are mean values ± SEM of 3 biological (donor frog) and 6-10 technical replicates (oocytes). Statistical differences were evaluated by one-way ANOVA with Dunnett's posthoc test. $p < 0.05$ *, $p < 0.01$**

The dose-dependent inhibition of GLUT2 was determined for ε-viniferin, hopeaphenol and phloretin (Figure 19), ε-Viniferin and hopeaphenol revealed IC_{50} values of 8.8 ± 5.3 µM and 16.2 ± 3.5 µM comparable to the IC_{50} of phloretin (16.1 ± 8.0 µM).

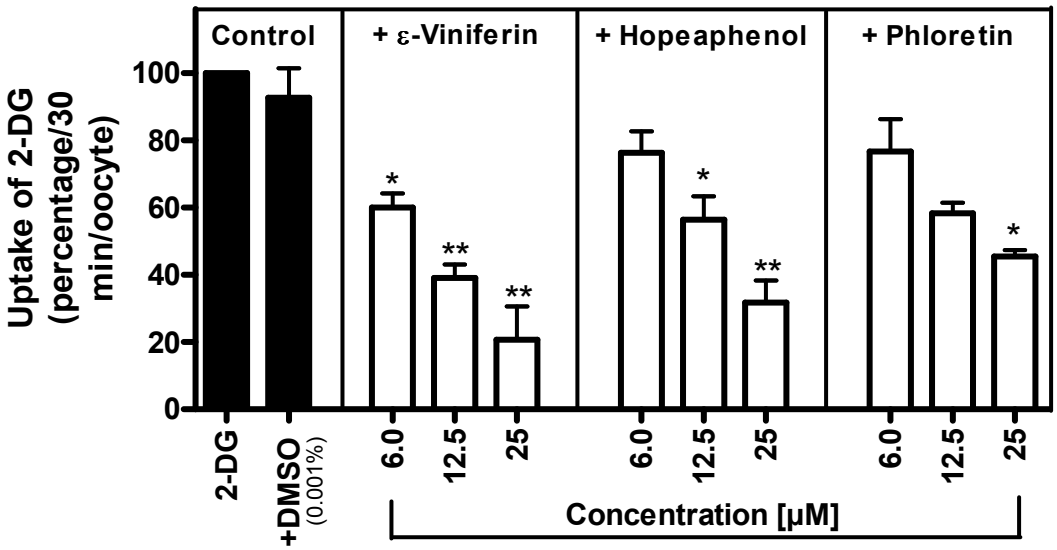

Figure 19: Dose-dependent inhibition of 2-DG transport via GLUT2 in the presence of phloretin, ε-viniferin and hopeaphenol
Dose-dependent inhibition of hGLUT2 expressed in oocytes by ε-viniferin, hopeaphenol and phloretin. Data are mean values ± SEM of 2-3 biological (donor frog) and 6-10 technical replicates (oocytes). Statistical differences were evaluated by one-way ANOVA with Dunnett's posthoc test. $p< 0.05$ *, $p< 0.01$**

4.3 Uptake experiments using isolated intestinal segments from mice

To determine whether the plant extracts and constituents thereof also interact with SGLT1 naturally expressed in the small intestine, uptake experiments were performed in isolated intestinal segments from C57BL/6N mice.

4.3.1 Time and concentration dependent uptake of α-MDG into everted gut rings

Time-dependent α-MDG uptake experiments (Figure 20) revealed linear influx within the first 3 min of incubation (r^2 =0.9896) that reached a plateau with approximately 4.0 nmol/mg at 5 min. Based on this all uptake experiments in mice tissues were performed with 2 min incubation time. Basic transport parameters on α-MDG uptake via SGLT1 were very similar to those reported for either rat or mouse intestinal rings [267, 69].

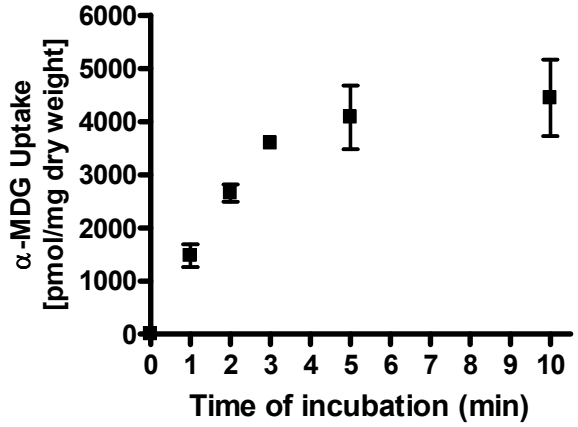

Figure 20: Time-dependent uptake of α-MDG into jejunal murine everted gut rings
Time-dependent uptake of α-MDG into the jejunum of male C57BL/6N mice (1 mM α-MDG in medium). Studies were performed at 37°C in incubation buffer at pH 7.4. Data are expressed as mean ± SEM of 3 technical replicates (rings) and at least 3 biological replicates (mice).

SGLT1 in the small intestine of mammals becomes saturated at glucose concentration between 10 and 30 mM with Km values ranging from 3-6 mM [70]. Jejunal everted gut rings were incubated at increasing concentration of non-labelled α-MDG (0-40 mM) supplemented with 1 µM [14C]-labelled α-MDG. K_m values and V_{max} were calculated by use of Michaelis-Menten kinetics (see chapter 3.1.6.1). Figure 21 shows the uptake of α-MDG in everted gut rings at different α-MDG concentrations. The calculated Km value was 6.5 ± 1.1 mM and the maximal velocity for α-MDG uptake accounted to 17.1 ± 1.8 µmol/mg dry weight/min. The calculated Km value was in good agreement with the Km value determined in rat jejunal segments by the group of Ader et al. [192] with a value of 5.9 mM.

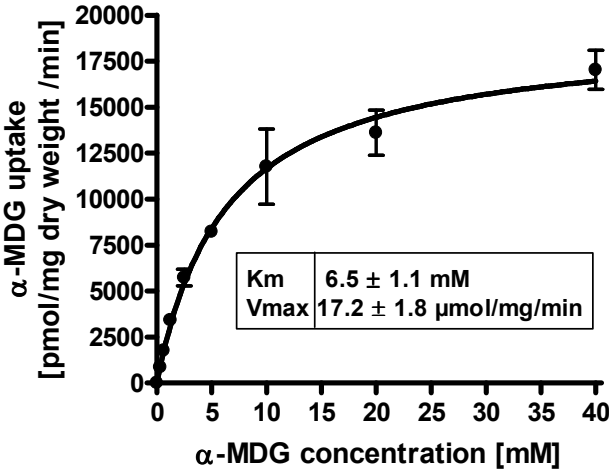

Figure 21: Concentration-dependent uptake of α-MDG into murine jejunal everted gut rings
Concentration-dependent uptake of α-MDG in jejunal gut rings of C57BL/6N mice (0.06-40 mM of total α-MDG in mucosal medium) measured over 2 min. Studies were performed at 37°C, pH 7.4. Data are expressed as mean ± SEM of n=3 technical replicates of n=3 mice.

4.3.2 Inhibition of α-MDG uptake in everted gut rings by plant extracts

To investigate the role of apple, onion, grapevine and curly kale extract on glucose absorption in isolated everted gut rings, increasing concentrations (see Table 11) were added to the α-MDG solution (1 mM).

As shown in Figure 22C, apple extract revealed the strongest and dose-dependent inhibition of SGLT1-mediated α-MDG uptake with a decrease of more than 50% at a concentration of 0.012 mg/ml. The amount of apple extract necessary to inhibit half maximal uptake of 1 mM α-MDG revealed a value of 0.009 ± 0.002 mg/ml extract, which was, compared to the onion extract (IC_{50}=2.6 ± 0.9 mg/ml) almost 300 fold stronger (Table 11, Figure 22B). The grapevine shoot extract showed compared to the strong inhibitory effect observed in the oocyte expression system, less effective inhibition of SGLT1 in mouse jejunum (Table 11). The reason for this may be the poor solubility which could not be improved by a higher amount of DMSO added to the incubation solution.

The curly kale extract only inhibited absorption of α-MDG at high luminal concentrations (Figure 22A) with 15 mg/ml curly kale extract causing only 53% reduction of the α-MDG absorption (Table 11).

Table 11: Inhibition of SGLT1-mediated α-MDG uptake by selected plant extracts performed in mouse everted gut rings

Represented in green are the IC_{50} values, unless calculated. Data are represented as mean ± SEM from 3 technical replicates of n ≥ 3 mice. Conc. = concentration

Extracts	Inhibition of [^{14}C] α-MDG uptake via murine SGLT1 [%]				
Conc. (mg/ml)	1.5	3	6	9	other conc. (mg/ml)
Onion IC_{50}=2.6±0.9	35.3±9.9	54.7±8.1	67.3±3.2	72.3±3.2	80.0±3.6 (12); 84.3±0.9 (15)
Curly kale IC_{50}=13.8±5.1	-	-	-	36.6±10.7	52.9±5.7 (15)
Conc. (mg/ml)	0.0125	0.025	0.05	0.1	other conc. (mg/ml)
Apple IC_{50}=0.009±0.002	57.1±2.0	64.6±3.4	71.2±3.7	82.8±2.5	87.3±1.0 (0.2); 90.7±1.6 (0.4)
Grapevine	-	-	-	30.6±6.4	38.1±8.1 (0.2); 61.1±6.3 (0.4)

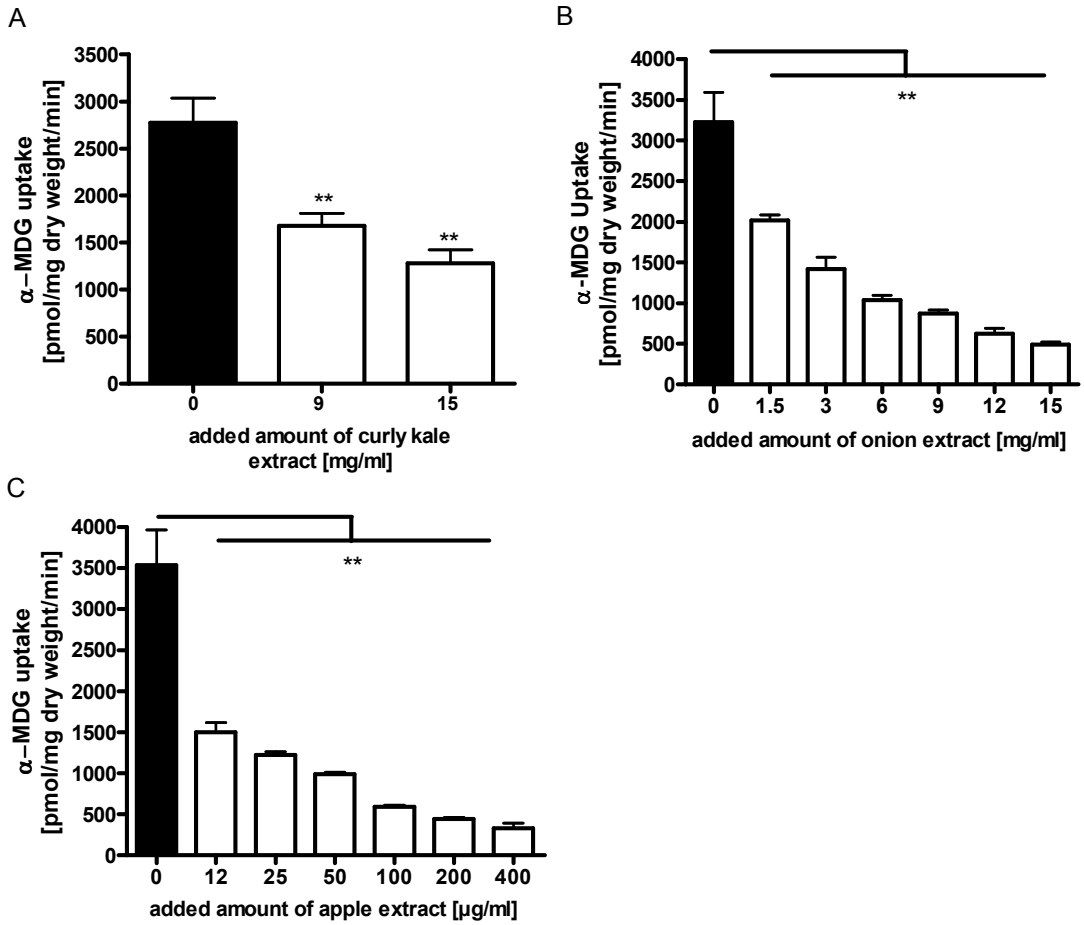

Figure 22: Dose-dependent inhibition of SGLT1-mediated α-MDG uptake into murine everted gut rings by selected plant extracts

Dose-dependent inhibition of SGLT1-mediated α-MDG uptake into murine everted gut rings by extracts derived from curly kale (A), onion (B) or apple (C). Data are represented as mean ± SEM from 3 technical replicates of at least 3 mice. Statistical differences were evaluated by one-way ANOVA with Dunnett's posthoc test. $p < 0.05$ *, $p < 0.01$**

4.3.3 Inhibition of α-MDG uptake in everted gut rings by phlorizin, ε-viniferin and Q4'glc

Q4'glc showed only a moderate inhibition of SGLT1 in mouse intestinal rings with a decrease of α-MDG uptake by 25% at a concentration of 1 mM Q4'glc and of 35% at a concentration of 2 mM (Figure 23C). In contrast, ε-viniferin, revealed a very strong inhibition of SGLT1. In the presence of 0.1 mM ε-viniferin, uptake of α-MDG was reduced by 50% (Figure 23B). Hopeaphenol showed only moderate inhibition of α-MDG-mediated uptake with a significant inhibition of 36% at a concentration of 0.4 mM (Figure 23D). But similar to the poor solubility observed for the grapevine extract, hopeaphenol also showed partly a precipitation at the end of the 2 min incubation period. The calculated IC_{50} value for SGLT1 inhibition by ε-viniferin was 34.8 ± 9.7 µM and this around 8-fold weaker than phlorizin with an IC_{50} value of 4.2 ± 0.02 µM. As shown in Figure 23A phlorizin was able to lower α-MDG uptake by 90% at a concentration of 0.1 mM.

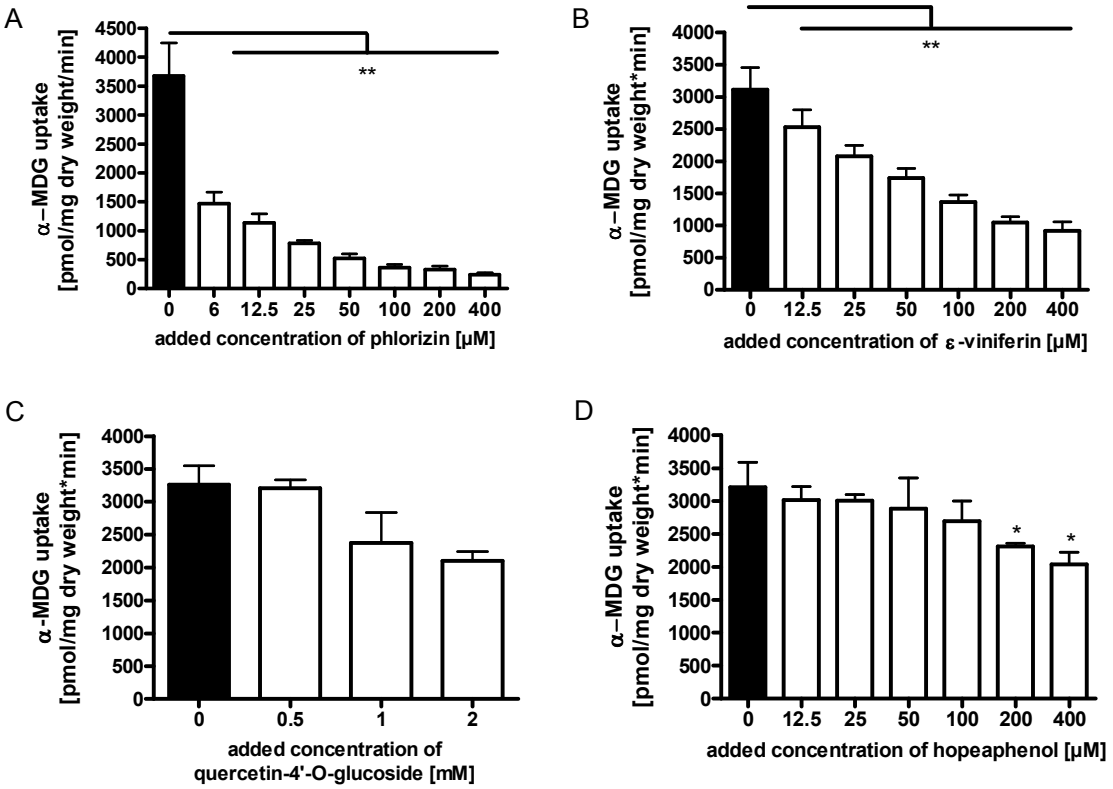

Figure 23: Dose-dependent inhibition of SGLT1-mediated α-MDG uptake into murine everted gut rings by selected polyphenolic compounds
Dose-dependent inhibition of SGLT1-mediated α-MDG uptake into murine everted gut rings by phlorizin (A), ε-viniferin (B), quercetin-4'-O-glucoside (C) or hopeaphenol (D). Data are represented as mean ± SEM from 3 technical replicates of 3 to 5 mice. Statistical differences were evaluated by one-way ANOVA with Dunnett's posthoc test. $p < 0.05$ *, $p < 0.01$**

4.3.4 Reversibility of SGLT1 inhibition in everted intestinal sacs

With the everted gut ring technique it is not possible to determine whether the inhibition of α-MDG uptake by SGLT1 is reversible or not. For this reason we performed experiments using jejunal everted sacs.

4.3.4.1 Proof of concept

We first tested whether the disappearance of α-MDG from the mucosal incubation solution is dependent on a functional SGLT1. Therefore, inhibition of SGLT1 by phlorizin and experiments in SGLT1-deficient mice were conducted. Figure 24A shows the disappearance of 1mM α-MDG from the mucosal solution after transfer of the jejunal everted sacs into the incubation vessels. In tissues from C57BL/6N wild-type (WT) mice, α-MDG in medium decreased rapidly over time. After 50 min of incubation 65% of the mucosal α-MDG had disappeared from the incubation solution, taken up into jejunal tissue (Figure 24C) and delivered to the serosal fluid (Figure 24B). The decrease of α-MDG from the mucosal solution could be stopped completely after addition of 50 µM phlorizin, indicating that transport was mainly mediated by SGLT1. This was confirmed by use of jejunal everted sacs from SGLT1$^{-/-}$ mice, showing no or minimal loss of α-MDG from the incubation solution - even smaller than in the presence of 50 µM phlorizin. Residual amounts of radioactivity in tissue can most likely be attributed to adherant α-MDG.
With these experiments we were able to show the functionality of the test system, which was further used to determine reversibility of SGLT1 inhibition by selected plant compounds.

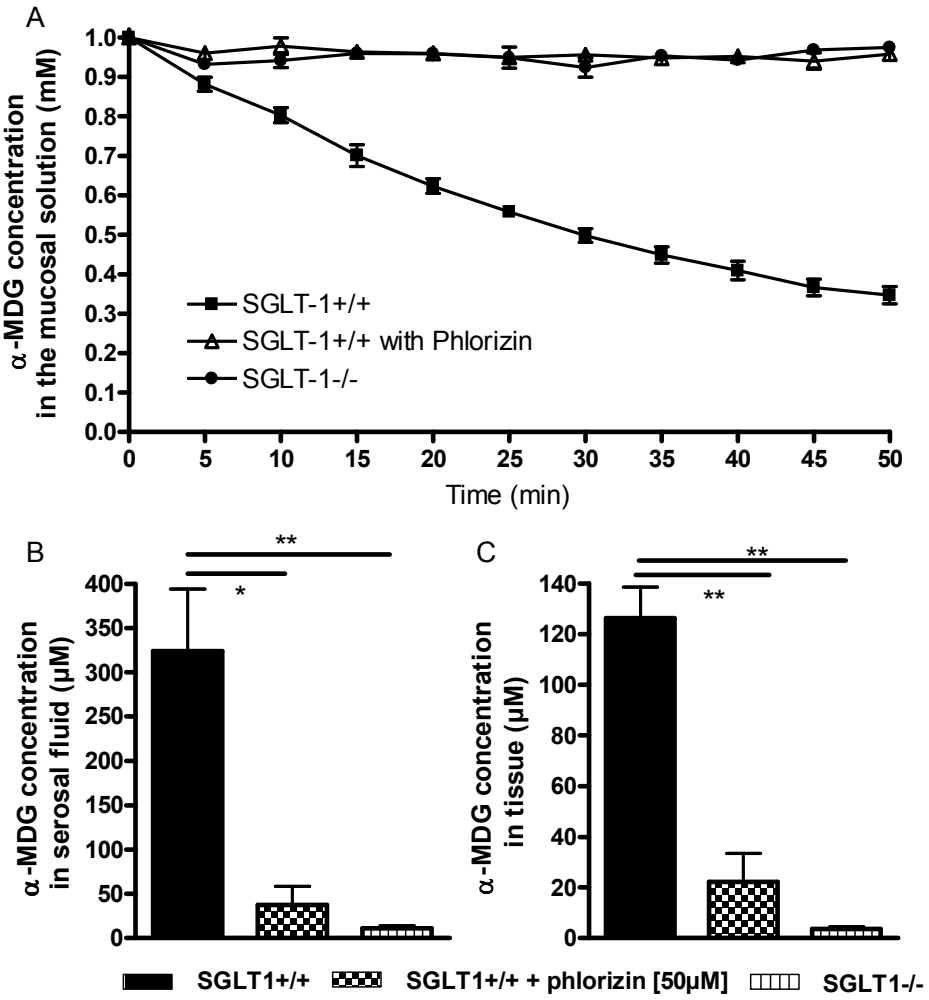

Figure 24: α-MDG disappearance from the medium and appearance in tissues and serosal medium in murine jejunal everted gut sacs
Disappearance of α-MDG from the mucosal incubation solution via SGLT1 (A) and appearance of α-MDG in the serosal fluid (B) and intestinal tissue (C) after 50 min of incubation. Statistical differences were evaluated by one-way ANOVA with Dunnett's posthoc test. $p < 0.05$ *, $p < 0.01$**

4.3.4.2 Reversibility of SGLT1 inhibition by apple extract, phlorizin and ε-viniferin

To assess the reversibility of SGLT1 inhibition caused by the most effective inhibitors everted gut sac experiments were carried out.

Figure 25A shows the disappearance of α-MDG from the mucosal solution that immediately decelerated after addition of 25 µg/ml of apple extract. The subsequent washout of the inhibitor for 5 min revealed the reversible nature of SGLT1 inhibition with an almost identical slope in the α-MDG disappearance ratio (-0.025 before addition of apple extract and -0.022 after removal of apple extract).

After addition of 50 µM phlorizin or 50 µM ε-viniferin to the incubation solution, the loss of α-MDG from the incubation solution also stopped completely (Figure 25B; C). The almost

identical slope values (-0.032 and -0.034) before and after addition of phlorizin to the incubation solution demonstrated the reversible nature of SGLT1 inhibition by phlorizin (Figure 25B). In contrast, incubation with ε-viniferin revealed a reduced slope value (-0.017) after washout compared to the initial slope value (-0.036) suggesting a non-reversible type of inhibition (Figure 25C). Extending the washout-phase also did not cause a recovery of the α-MDG disappearance rate as shown in Figure 25D.

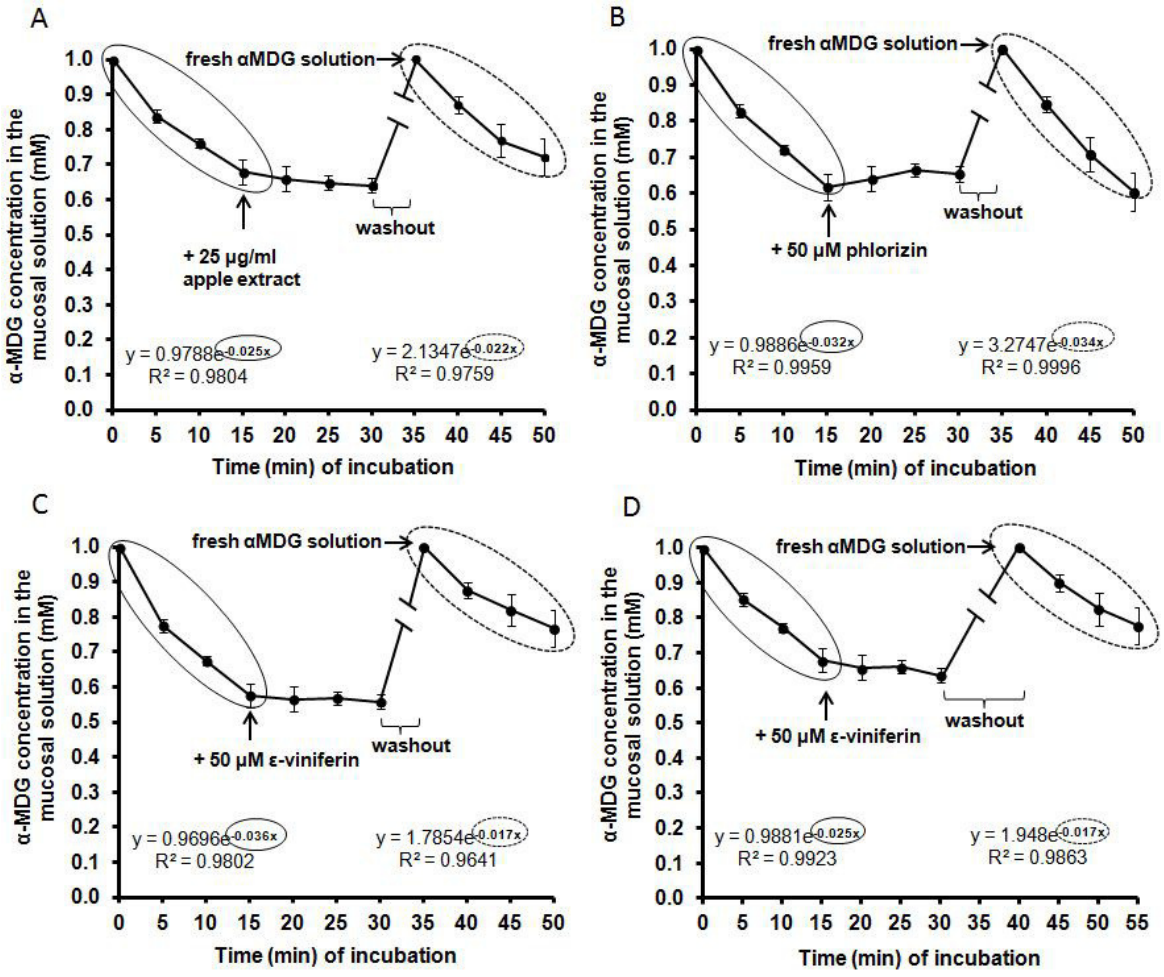

Figure 25: Jejunal disappearance of α-MDG from the mucosal solution in the absence or the presence of selected plant compounds
The decrease of α-MDG is exponential and decelerates or stops immediately after addition of either 25 µg/ml apple extract (A), 50 µM phlorizin (B) or 50 µM ε-viniferin (C, D). The washout of apple extract and phlorizin (A, B) reveals the reversible nature of the inhibition demonstrated by the almost identical slope values (circled values) calculated by exponential regression. The different slope values before addition and after washout of ε-viniferin indicate a non-reversible type of inhibition (C) which could not be recovered even after washout for 10 min (D) in Krebs buffer. Data are presented as mean values ± SEM from 4 technical replicates and n ≥ 1 biological replicate (mouse).

4.4 Antihyperglycemic effects of selected plant extracts *in vivo* in mice

4.4.1 Body weight and 6-h fasting blood glucose in mice fed a control or high fat diet

As shown in Figure 26B, body weight was significantly higher ($p<0.0001$) in mice fed the high fat diet for 12 weeks compared to mice fed a control diet for 12 weeks (40.2 ± 0.6 g and 28.1 ± 0.3 g, respectively). In addition, blood glucose values after a 6 h fast were significantly higher ($p<0.0001$) in high fat fed mice compared to mice fed a control diet (Figure 26A) indicating impaired glucose tolerance.

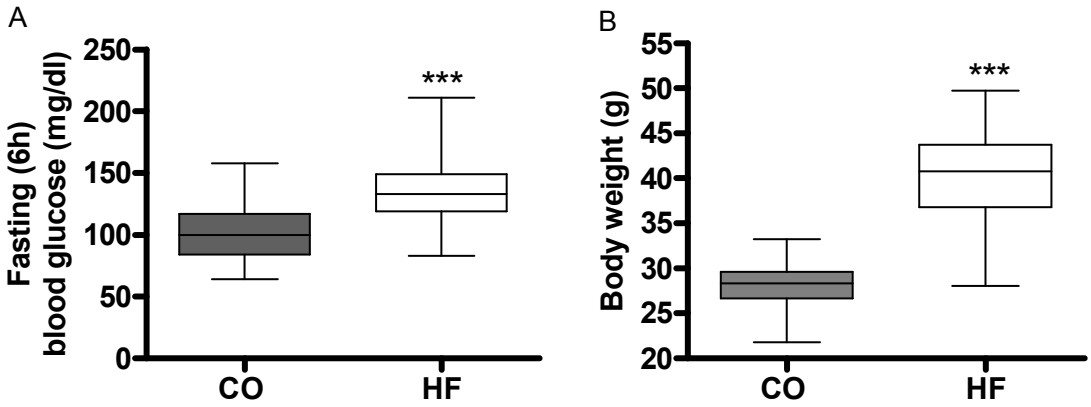

Figure 26: Body weight and fasting blood glucose of C57BL/6N mice fed control or high fat diets
6-h fasting blood glucose values (A) and body weight (B) of male C57BL/6N mice after feeding a control (CO) or high fat diet (HF) for 12 weeks. Data are presented as mean ± SEM of n=60 mice per group. Statistical differences were evaluated by unpaired t-test. $p< 0.05$ *,$p <0.01$**, $p<0.001$***

4.4.2 Effects of apple and onion extracts on postprandial glycemic responses in mice fed a control diet

After the 12-week feeding trial, mice underwent a standard OGTT as performed in humans by receiving the same amount of glucose independent of body weight.
Since the plant extracts as well as phlorizin and Q4'glc are not soluble in water, they were solved in an ethanol/water mixture (apple extract, phlorizin) or DMSO/water mixture (onion extract, Q4'glc) and afterwards added to the glucose solution with a final 3% solvent content. To exclude possible effects on glucose response to the solvent, the same amount of solvent was added to the glucose solution (vehicle group). The amount of phlorizin and Q4'glc (15 mM) provided was defined based on a study of Takii et al. [268] showing a significant inhibition of glucose absorption after an acute administration of 15 mM phlorizin together with a 10% glucose solution to normoglycemic mice.

Figure 27 shows the glycemic response (Δ) in normoglycemic mice after administration of 60 mg glucose (OGTT) or 60 mg glucose together with either 12 mg apple extract or 14 mg onion standardized for their amount of phlorizin (15 mM) or Q4'glc (15 mM). Additionally the same amount of phlorizin (1.96 mg) or Q4'glc (2.08 mg) was tested for lower blood glucose levels.

The administration of apple extract, onion extract and Q4'glc with the glucose solution showed no statistical significant decrease in the rise of blood glucose concentrations at any time point compared to the vehicle groups (Figure 27A, B). In addition, the iAUCs for the blood glucose response after 60 and 120 min also failed to reveal any significant differences between mice receiving the vehicle OGTT and mice receiving the OGTT with the test compounds. However, phlorizin suppressed the increase in postprandial blood glucose levels 15 min after the administration ($p<0.001$), although iAUCs did not show any significant changes (Figure 27C, D).

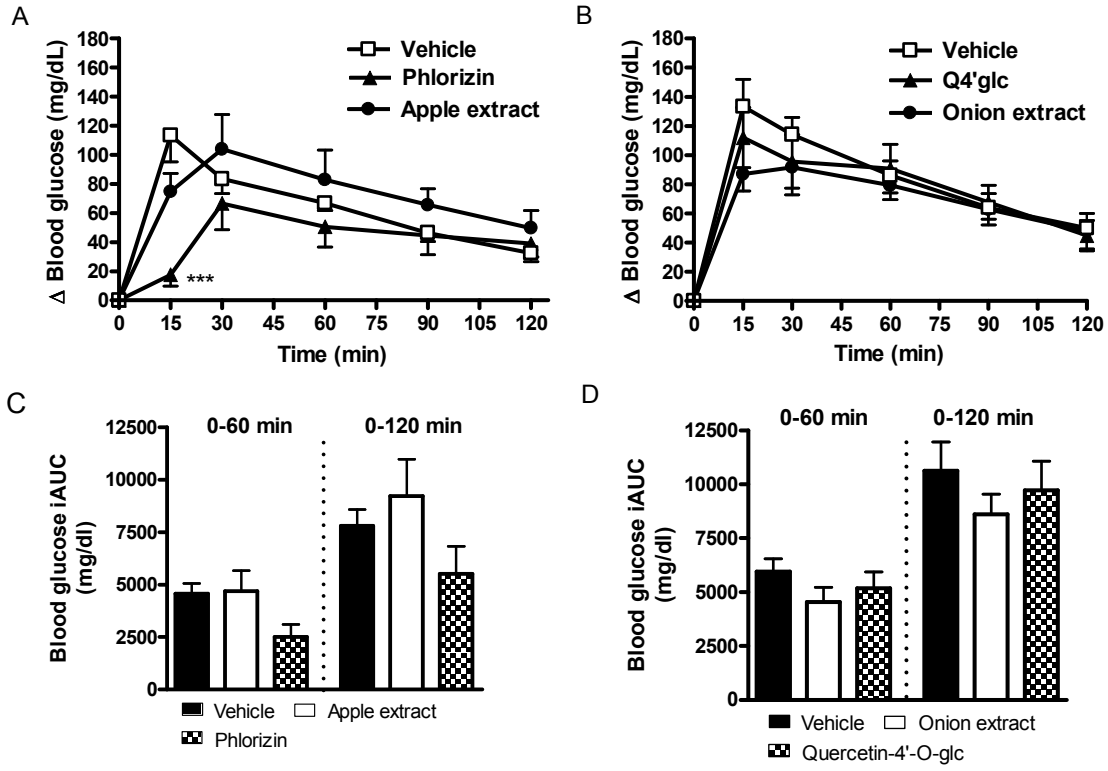

Figure 27: Blood glucose response (OGTT) in C57BL/6N mice fed a control diet
Blood glucose response curves (A,B) and blood glucose iAUCs (C,D) following 20% glucose ingestion (60 mg of glucose per mice) with or without 12.24 mg apple extract and 15 mM phlorizin (A, C) or 14 mg onion extract and 15 mM quercetin-4'-O-glc (B, D) in control diet fed mice. Data are mean ± SEM of n=10 mice. Statistical differences were evaluated by two-way ANOVA with repeated measures for one factor for A and B and one-way ANOVA without repeated measures for C and D. $p< 0.05$*,$p< 0.01$**, $p< 0.001$***

4.4.3 Effects of apple and onion extract on postprandial glycemic responses in mice fed a high fat diet

Figure 28A shows the glycemic response (Δ) in mice fed a high fat diet for 12 weeks followed by an OGTT with 60 mg glucose in the presence or in the absence of 12 mg apple extract or 15 mM phlorizin and Figure 28B with the onion extract and Q4'glc. Except for the latter, blood glucose increases were significantly reduced by the test agents in the animals with impaired insulin sensitivity. Apple extract significantly reduced the increase in blood glucose concentration 15 min ($p<0.001$) after administration of glucose (Figure 28A) while onion extract caused a significant reduction 15 min ($p<0.001$) and 30 min ($p=0.017$) after administration of glucose (Figure 28B). This was reflected in a significant decrease of the iAUC for 0-60 min ($p=0.044$) after apple extract treatment. Phlorizin administration in the same amount as found in the apple extract, together with glucose, led also to a significant decrease in the blood glucose response after 15 min ($p=0.001$) and caused a significant difference in the iAUC at 0-60 min ($p=0.009$). The onion extract led to a decrease in the iAUCs at 0-60 min ($p=0.0026$) and 0-120 min ($p=0.007$), while Q4'glc did not cause a significant change in the iAUCs.

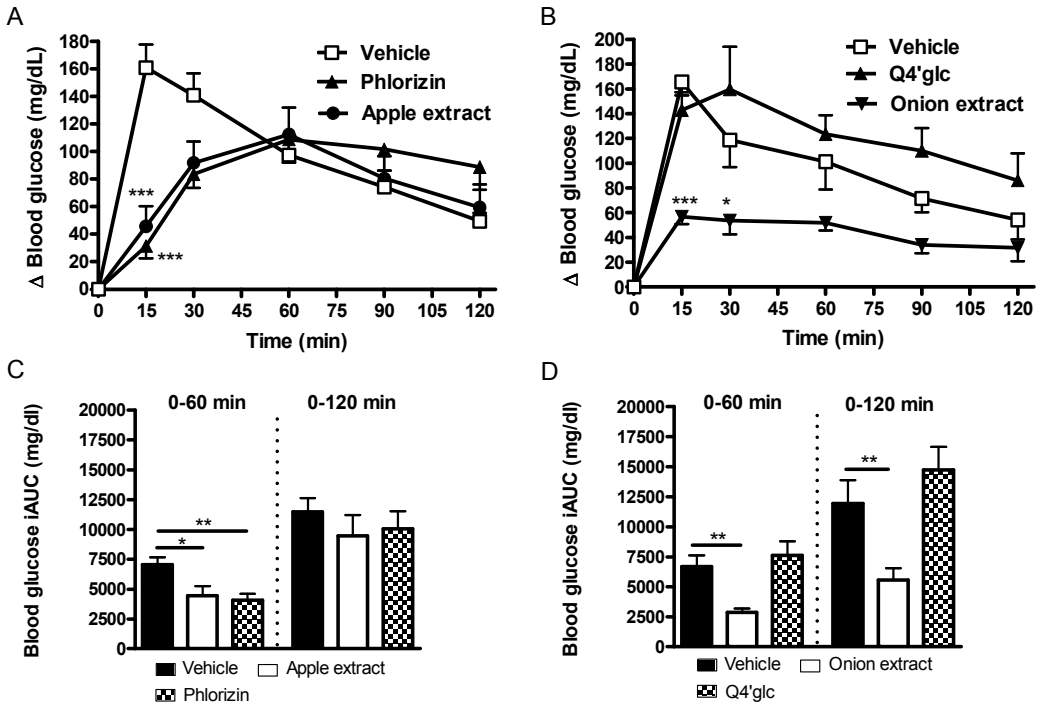

Figure 28: Blood glucose response (OGTT) in C57BL/6N mice fed a high fat diet
Blood glucose response curves (A,B) and blood glucose iAUCs (C,D) following 20% glucose ingestion (60 mg of glucose per mice) with or without 12.24 mg apple extract and 15 mM phlorizin (A, C) or 14 mg onion extract and 15 mM quercetin-4'-O-glc (B, D) in high fat diet fed mice. Data are mean ± SEM of n=10 mice. Statistical differences were evaluated by two-way ANOVA with repeated measures for one factor for A and B and one-way ANOVA without repeated measures for C and D. $p< 0.05$*,$p< 0.01$**, $p< 0.001$***

4.5 Antihyperglycemic effects of plant extracts *in vivo* in humans

4.5.1 Effects of plant extracts on glucose and insulin response in healthy young men (OGTT study)

To assess whether the plant extracts also display an effect in humans, OGTTs in the absence or the presence of the plant extracts were performed. Except for the apple extract, the amount of plant extracts given to the volunteers was standardized for the total amount of flavonoids (curly kale, onion) or the stilbenes (grapevine extract) which accounted for 600 mg. The amount of apple extract was chosen based on a phlorizin content of 450 mg phlorizin (see chapter 3.2.1.1.5)

The ingestion of curly kale, onion, grapevine or apple extract 30 min before the OGTT did not affect basal glucose or insulin levels (see Appendix, Table 16). As shown in Figure 29A-D, the incremental (Δ) postprandial blood glucose response was not statistically significant different at any time. Intake of grapevine extract 30 min prior to the test OGTT tended to result in a significant higher blood glucose response compared to the reference OGTT without extract administration but failed to reach statistical significance (p=0.052).

The changes in postprandial plasma insulin concentration during the OGTTs were also not significant at any time point (Figure 29E-G). However, ingestion of 2.8 g apple extract 30 min before the test OGTT resulted in a significantly lower plasma insulin level 30 min (p=0.036) after the OGTT compared to the reference OGTT (Figure 29H).

Although there was no significant change in the blood glucose and plasma insulin iAUC calculated at a given time point, the ingestion of the apple extract resulted in a significantly lower blood glucose iAUC within the first 45 min after the test OGTT compared to the reference OGTT (Table 12A). Moreover, plasma insulin iAUC was also significantly reduced within the first 90 (Table 12A). In contrast, administration of the grapevine extract, 30 min prior to the OGTT resulted in significant higher blood glucose iAUC over the first 60 min with a significant higher plasma insulin iAUC over the first 45 min compared to the reference OGTT (Table 12B). The ingestion of 4.4 g curly kale extract 30 min prior to the OGTT led to no changes in the incremental blood glucose AUC but slightly, yet significantly increased plasma insulin iAUC over 15 and 30 min compared to the reference OGTT (Table 12C). No significant changes in blood glucose and plasma insulin iAUCs were observed after ingestion of onion extract (Table 12D).

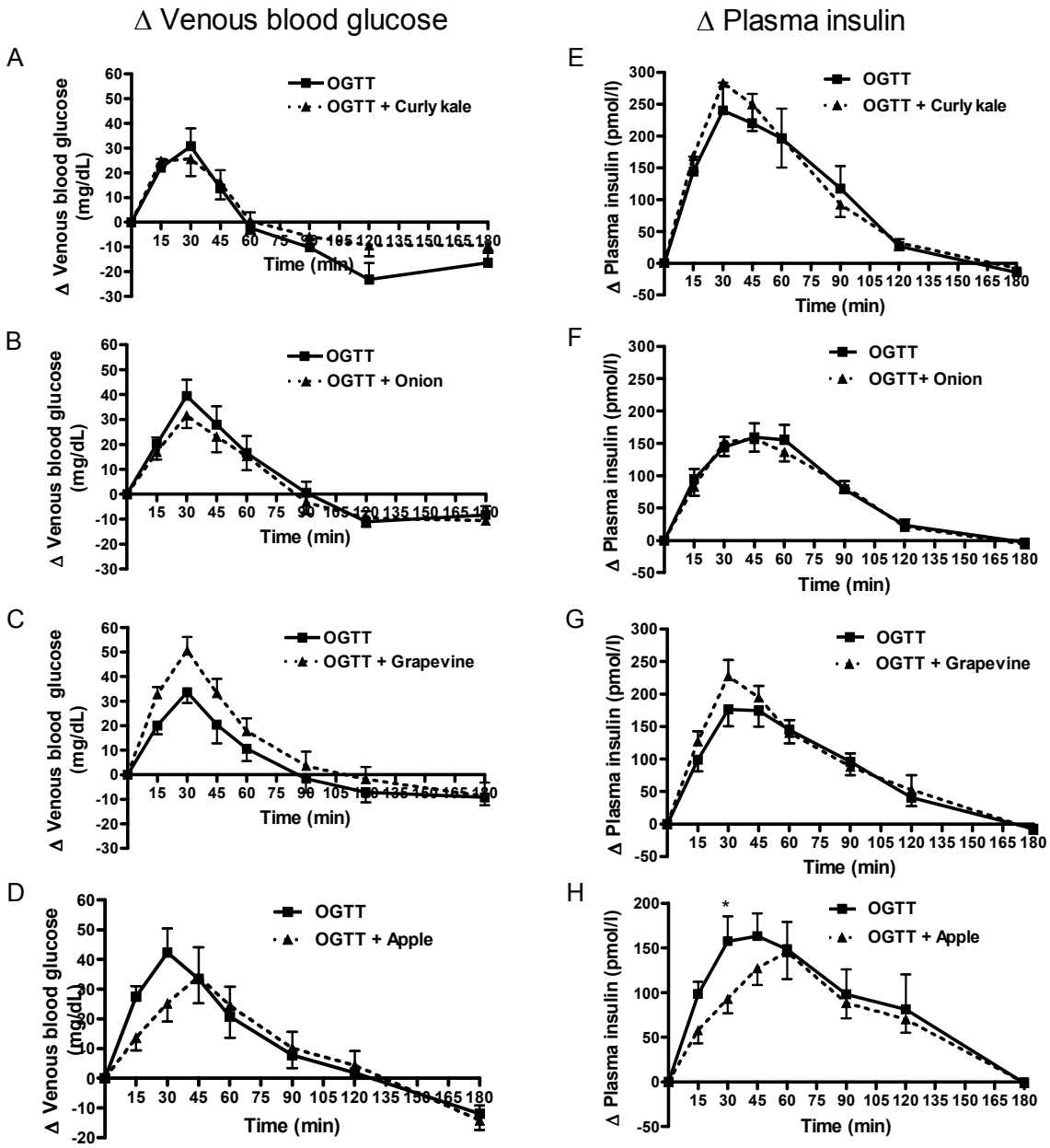

Figure 29: Blood glucose and insulin responses after the OGTT in the absence or the presence of the plant extracts in healthy volunteers

The mean (± SEM) incremental (Δ) blood glucose concentration (A-D) and plasma insulin concentration (E-H) in 10 (C, D, G, H), 11 (A, E) or 15 (B, F) healthy young men after the OGTT or the OGTT with prior ingestion of 4.4 g curly kale extract, 3.1 g onion extract, 1.7 g grapevine extract or 2.8 g apple extract, standardized for their amount of polyphenols. Statistical differences were evaluated with two-way ANOVA with repeated measures and multiple comparison posthoc test (Tukey Kramer test). $p < 0.05$*

Table 12: Postprandial blood glucose and plasma insulin iAUC in healthy subjects
Mean incremental blood glucose and plasma insulin area under the curve (iAUC) in healthy young men after the OGTT without (Reference) or the OGTT with either 2.8 g apple extract (A), 1.7 g grapevine extract (B), 4.4 curly kale extract (C) or 3.1 g onion extract (D). All values are means ± SEM, n=10 (A, B), n=11 (C) or n=15 (D). Significant differences in the iAUCs of either blood glucose or plasma insulin were evaluated by use of one-way ANOVA with repeated measures and multiple comparison posthoc test (Tukey Kramer test). $p < 0.05$ *; $p < 0.01$ **

A	Glucose iAUC (mg/dl)			Insulin iAUC (pmol/l)		
Time (min)	Reference	**Apple extract**	p-value	Reference	**Apple extract**	p-value
0-15	418.7±26.7	314.9±31.7*	0.046	683.7±117.9	452.4±107.5	0.072
0-30	1154.5±94.1	818.6±100.3*	0.031	2548.9±367.7	1601.4±320.2*	0.012
0-45	1934.6±217.5	1476.8±186.5*	0.043	4974.4±674.2	3271.3±507.4**	0.005
0-60	2553.6±360.5	2129.8±308.5	0.131	7332.7±1033.2	5332.6±738.3**	0.005
0-90	3408.3±592.2	3074.3±501.3	0.426	11070.1±1862.1	8874.9±1306.6*	0.036
0-120	3977.9±776.1	3716.9±600.4	0.637	13796.3±2819.0	11294.0±1696.7	0.129
0-180	4526.3±939.3	4272.8 ± 694.7	0.702	16269.4±4069.0	13452.2±2132.9	0.257
B	Glucose iAUC (mg/dl)			Insulin iAUC (pmol/l)		
Time (min)	Reference	**Grapevine extract**	p-value	Reference	**Grapevine extract**	p-value
0-15	288.8±26.6	384.0±22.8**	0.005	868.6±134.9	1078.8±116.1	0.1
0-30	830.3±65.6	1147.5±67.1**	0.002	3059.6±372.8	3862.4±353.6**	0.002
0-45	1374.0±130.0	1914.8±137**	0.007	5814.9±648.6	7153.4±562.4**	0.004
0-60	1744.5±211.7	2436.0±202.3*	0.018	8334.3±3569.7	9794.1±606.2	0.064
0-90	2155.5±347.4	3033.0±308.9	0.052	12196.1±1212.9	13479.2±819.5	0.2
0-120	2301.0±455.9	3336.0±391.2	0.075	14500.2±6299.3	15842.9±1346.8	0.19
0-180	2364.0±564.4	3570.0±555.0	0.098	16018.9±1779.2	17668.2±2016.0	0.21
C	Glucose iAUC (mg/dl)			Insulin iAUC (pmol/l)		
Time (min)	Reference	**Curly kale extract**	p-value	Reference	**Curly kale extract**	p-value
0-15	513.1±26.4	533.9 ±16.2	0.52	1298.2±175.1	1482.7±186.4	0.16
0-30	1257.3±93.0	1260.0±68.4	0.98	4398.2±638.7	5091.9±699.4*	0.036
0-45	1938.5±187.1	1919.5±155.6	0.93	8067.6±1258.3	9311.1±1308.4*	0.041
0-60	2371.8±273.3	2391.1±233.4	0.95	11406.2±1900.7	12875.8±1912.7	0.12
0-90	2881.2±382.7	3008.3±324.2	0.75	16544.4±3074.8	17642.6±2807.2	0.41
0-120	3077.0±466.2	3476.6±348.4	0.39	19140.2±3613.1	19945.3±3068.6	0.57
0-180	3280.2±700.3	4304.9±404.3	0.16	20451.9±3705.7	21605.4±3077.2	0.49
D	Glucose iAUC (mg/dl)			Insulin iAUC (pmol/l)		
Time (min)	Reference	**Onion extract**	p-value	Reference	**Onion extract**	p-value
0-15	318.3±18.8	294.1±23.9	0.25	802.3±114.8	713.0±105.9	0.30
0-30	932.7±68.1	825.2±74.8	0.17	2685.2±279.6	2567.5±357.8	0.68
0-45	1605.0±160.6	1402.1±140.0	0.19	5051.6±463.3	4974.1±630.8	0.63
0-60	2105.3±256.7	1856.9±208.2	0.26	7503.5±687.9	7264.5±830.8	0.77
0-90	2695.1±386.5	2370.4±319.1	0.38	11206.0±1012.5	10758.6±1028.5	0.65
0-120	2870.1 ± 456.8	2510.1 ± 401.8	0.48	12930.2±1142.3	12514.9 ± 1040.6	0.69
0-180	2955.3 ± 529.9	2847.3 ± 677.4	0.89	13900.6±1322.7	13322.8 ± 984.2	0.62

4.5.2 Effects of plant extracts on renal glucose excretion in healthy young men (OGTT study)

It has been demonstrated that ingestion of phlorizin by mammals causes glucosuria due to an inhibition of renal glucose reabsorption [37]. Thus, we were also interested to test whether the plant extracts used in the present work were able to affect glucose reabsorption in the kidney. Volunteers were requested to collect 24-h urine from the time when they ingested the plant extracts.

Figure 30 represents the amount of excreted urine glucose measured over a time period of 24 h. Before the ingestion of the plant extracts (time point 0 min) only physiological concentrations of glucose were detectable in the urine of the volunteers ranging from 0.08 to 0.11 mg glucose/mg creatinine with no significant differences. After ingestion of extracts derived from curly kale, onion or grapevine no increase in the amount of urine glucose could be observed over the whole collection period. But interestingly, intake of apple extract significantly (p=0.0002) increased the amount of urine glucose almost 7-fold within the first 3 h after ingestion of the apple extract reaching initial values after 3-8 h and 8-24 h.

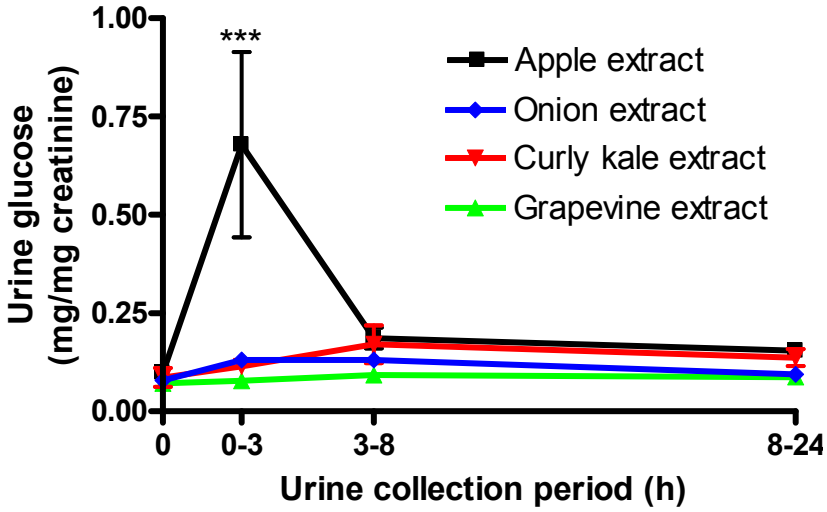

Figure 30: Glucose excretion in urine of healthy volunteers in response to ingestion of the plant extracts
Urine glucose concentration after OGTTs with prior ingestion of extracts derived from apple, onion, curly kale and grapevine. Urinary glucose was measured in different urine fractions (0 h, 0-3 h, 3-8 h and 8-24 h) and normalized to creatinine. Statistical differences were evaluated with two-way ANOVA without repeated measures and multiple comparison posthoc test (Tukey Kramer test). $p < 0.05$*, <0.01**, Data are mean values ± SEM of n=10 (apple, grapevine), n=15 (onion) or n=11 (curly kale) volunteers.

4.5.3 Effects of plant extracts and plant extract mixtures on glucose and insulin responses in obese volunteers (white bread study)

To investigate whether there is a stronger inhibitory effect of the plant extracts on postprandial blood glucose when in addition to inhibition of SGLT1 also glucose release from poly- and oligosaccharides by carbohydrate-hydrolyzing enzymes, obese volunteers received a test meal composed of white bread with or without prior ingestion of plant extracts. Acarbose, a known clinical inhibitor of α-glucosidase was used as a reference.

As expected, ingestion of 50 mg acarbose 10 min before the test meal showed a significant decrease in incremental (Δ) blood glucose concentrations 30 min ($p=0.027$) and 45 min ($p=0.032$) after the ingestion of WB compared to the ingestion of WB alone (Figure 31A). Plasma insulin concentrations were also significantly reduced 30 min ($p<0.001$), 45 min ($p<0.001$), 60 min ($p=0.0011$) and 90 min ($p=0.0011$) after acarbose treatment (Figure 31B). In addition, ingestion of acarbose resulted in a significant lower blood glucose response ($p=0.015$) and plasma insulin response ($p=0.008$) over time. This was also reflected in significant lower blood glucose ($p=0.024$) and plasma insulin ($p=0.0004$) iAUCs over a period of 180 min (Figure 31C, D).

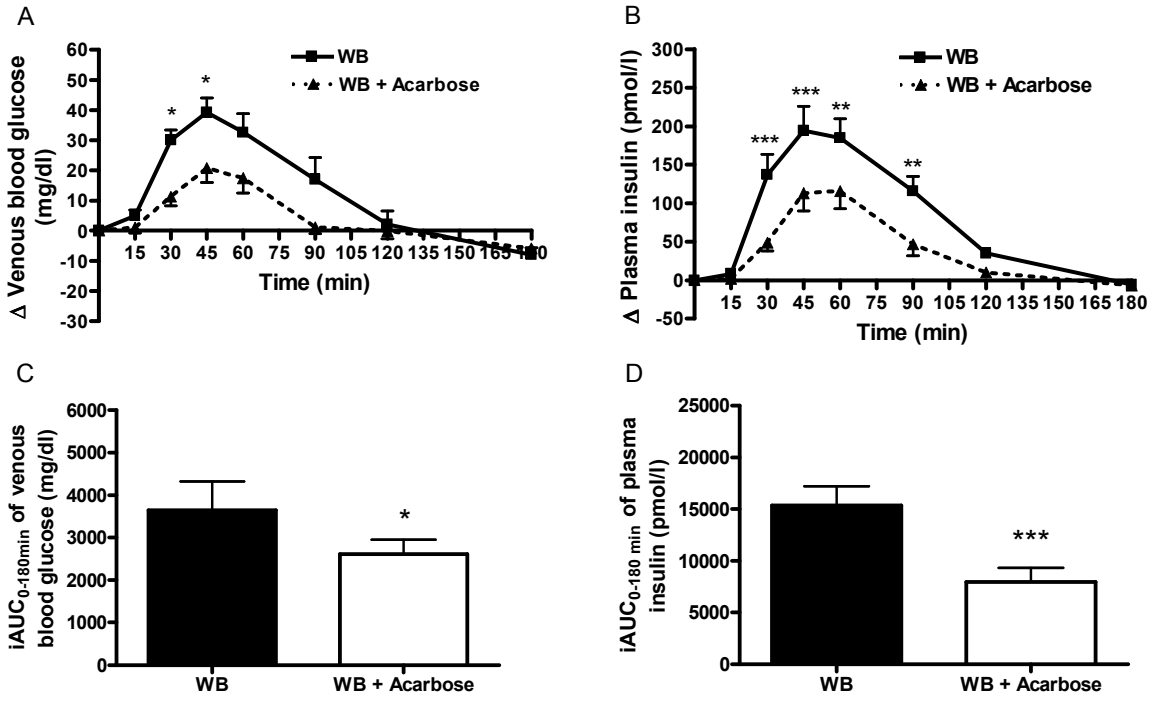

Figure 31: Blood glucose and insulin response after ingestion of WB plus acarbose in obese volunteers

The mean (± SEM) change (Δ) in blood glucose (A) and plasma insulin (B) and the incremental area under the postprandial blood glucose (C) and plasma insulin (D) curves in 10 obese volunteers after ingestion of WB or WB with prior ingestion of 50 mg Acarbose. Statistical differences were evaluated with two-way ANOVA with repeated measures and multiple comparison posthoc test (Tukey Kramer test). $p< 0.05$*; $p< 0.01$**; $p< 0.001$***

The ingestion of curly kale, onion, grapevine and apple extract alone, 10 minutes before the WB meal resulted in no significant changes in blood glucose (Figure 32A-D) or plasma insulin concentrations (Figure 32E-H). Furthermore, no significant differences were observed between the iAUCs for blood glucose or plasma insulin in any of the series (see Appendix, Table 17).

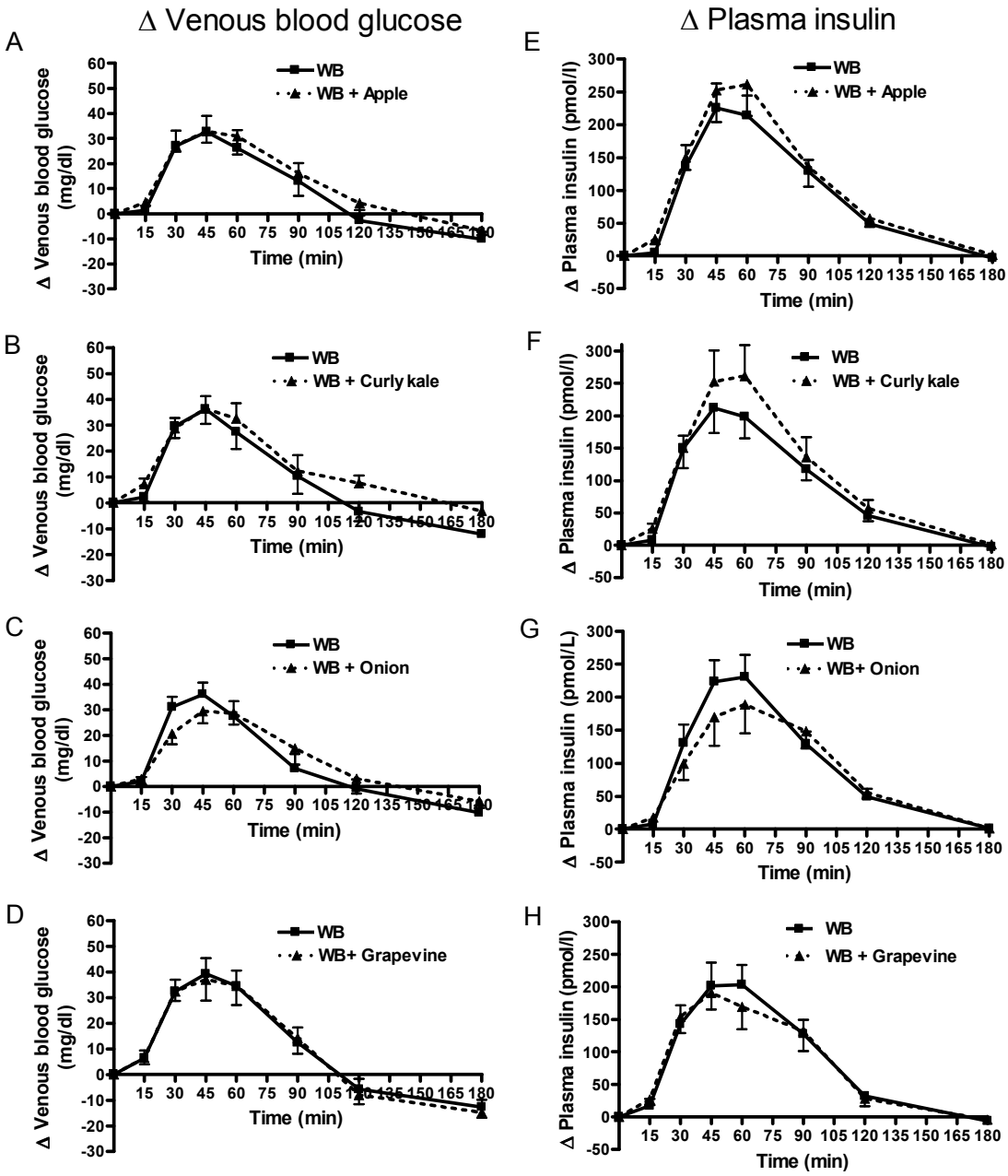

Figure 32: Blood glucose and insulin responses in obese volunteers after ingestion of WB with or without the plant extracts

The mean (± SEM) incremental (Δ) blood glucose (A-D) and plasma insulin concentration (E-H) in 10 obese volunteers after ingestion of WB or WB with prior ingestion of 2.8 g apple extract (A, E), 4.4 g curly kale extract (B, F), 3.1 g onion extract (C, G) or 1.7 g grapevine extract (D, H) standardized for their amount of polyphenols. Statistical differences were evaluated with two-way ANOVA with repeated measures and multiple comparison posthoc test (Tukey Kramer test). $p < 0.05$*; $p < 0.01$**; $p < 0.001$***

Administration of mixtures of the apple extract together with either onion or curly kale extract resulted in a significant decrease in blood glucose levels 30 min (p=0.04), 45 min (p=0.015) or 60 min (p=0.018) after the ingestion of WB alone (Figure 33A, B). In addition, apple plus onion and apple plus curly kale extract combinations caused significant effects over time when compared to the control WB treatment with p-values of 0.028 and 0.022, respectively. However, plasma insulin levels were not altered. (Figure 33C, D).

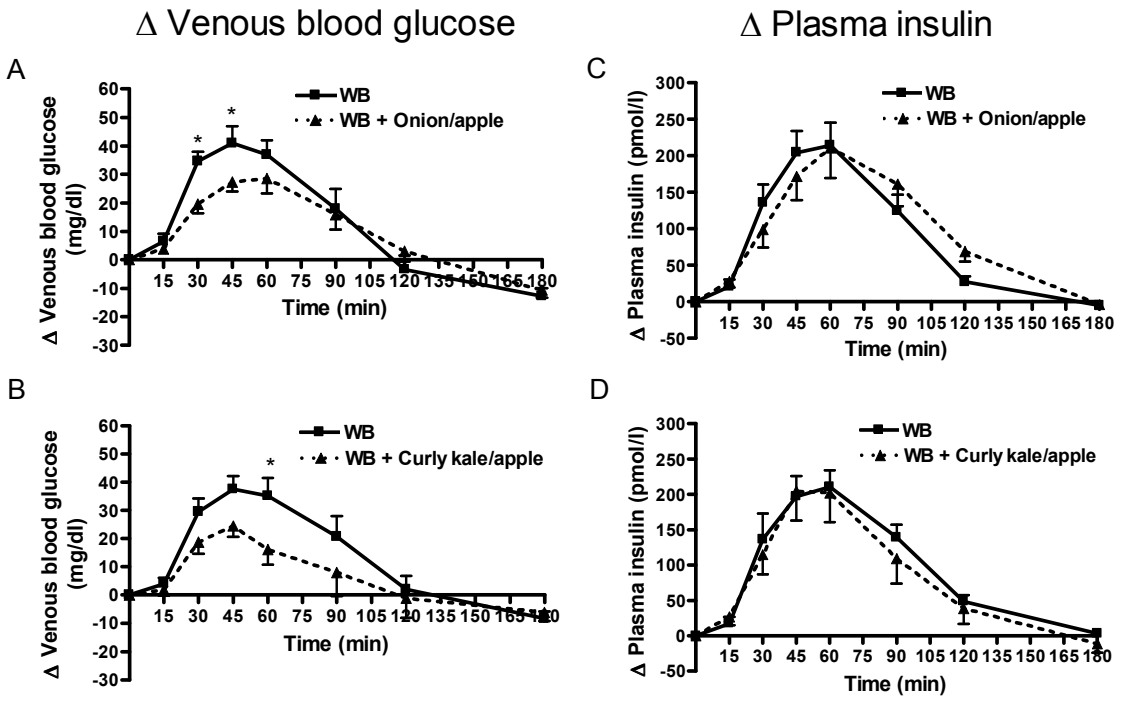

Figure 33: Blood glucose and insulin responses in obese volunteers after ingestion of WB with or without the plant extract mixtures
The mean (± SEM) change (Δ) of blood glucose (A, B) and plasma insulin (C, D) in 10 obese volunteers after ingestion of WB or WB with prior ingestion of combinations of either apple and onion extract (A, C) or apple and curly kale extract (B, D). Statistical differences were evaluated with two-way ANOVA with repeated measures and multiple comparison posthoc test (Tukey Kramer test). $p < 0.05$*; $p < 0.01$**; $p < 0.001$***

Ingestion of the onion plus apple extract mixture caused significantly lower blood glucose iAUC over 30 min, 45 min, 60 min, 90 min and 120 min compared to the blood glucose iAUC after ingestion of WB alone (Table 13B). Similar results were observed for the curly kale plus apple extract mixture (Table 13A) but both plant extract mixtures revealed no significant changes in the plasma insulin iAUCs (Table 13A, B).

Table 13: Postprandial blood glucose and plasma insulin iAUC in obese subjects
Mean postprandial blood glucose and plasma insulin incremental area under the curve (iAUC) in obese subjects after the WB meal without (Reference) or the WB meal with either a mixture of 3.7 g curly kale/apple extract (A) or a mixture of 3.3 g onion/apple extract. All values are means ± SEM, n=10. Significant differences of the iAUCs of either blood glucose or plasma insulin were evaluated by use of one-way ANOVA with repeated measures and multiple comparison posthoc test (Tukey Kramer test). $p < 0.05$ *; $p < 0.01$ **

A	Glucose iAUC (mg/dl)			Insulin iAUC (pmol/l)		
Time (min)	Reference	**Curly kale/ apple extract**	p-value	Reference	**Curly kale/ apple extract**	p-value
0-15	180.0±15.8	164.3±31.7	0.431	300.6±71.3	372.0±90.8	0.533
0-30	584.3±58.6	468.8±56.8	0.07	1624.9±374.3	1605.8±301.3	0.927
0-45	1245.8±126.4	942.8±96.8*	0.028	4296.9±832.5	4172.4±730.2	0.794
0-60	1936.5±193.3	1397.3±132.6*	0.016	7524.0±1098.9	7391.7±1296.2	0.887
0-90	3018.0±336.8	2058.8±246.0*	0.011	13112.1±1459.1	12398.8±2225.8	0.698
0-120	3582.0±458.3	2462.3±396.1*	0.017	16272.3±1636.1	14955.7±2721.3	0.592
0-180	3861.0±586.8	2846.3±581.7*	0.044	18516.3±1668.4	16456.1±3180.7	0.533
B	Glucose iAUC (mg/dl)			Insulin iAUC (pmol/l)		
Time (min)	Reference	**Onion/apple extract**	p-value	Reference	**Onion/apple extract**	p-value
0-15	213.8±20.6	185.3±7.5	0.252	228.1±72.3	284.1±64.2	0.419
0-30	678.0±54.2	517.5±30.1*	0.033	1477.8±300.8	1312.5±295.5	0.558
0-45	1403.3±109.6	1025.3±73.4**	0.008	4106.2± 687.1	3416.8±682.9	0.276
0-60	2163.0±167.6	1600.5±129.3**	0.004	7322.4±1116.3	6359.4±1196.2	0.306
0-90	3328.5±261.6	2580.0±249.3**	0.002	12556.2±1860.4	12089.8±2125.0	0.713
0-120	3915.0±373:8	3175.5±330.1*	0.012	14983.1±2179.1	15691.9±2552.8	0.609
0-180	4209.0±524.7	3571.5±428.7	0.057	15951.5±2189.9	17941.5±2751.5	0.232

5. Discussion

A large number of plants and herbs have been described to have antidiabetic properties. These include mechanisms for improvement of insulin release and improved insulin sensitivity [39]. There is also growing evidence that secondary plant metabolites - especially polyphenols - can modulate the postprandial rise in blood glucose levels due to an interaction with intestinal enzymes and transporters that are responsible for the release and absorption of dietary glucose. Polyphenols are mainly consumed with a diet rich in fruits, vegetables, tea and other plant-based food products and usually represent a versatile mixture of different components. They can reach concentrations up to several hundred micromolar in the lumen of the human gut ([39]; www.phenol-explorer.eu*)* and therefore may be able to reach intestinal glucose transporters and cause their inhibition.

5.1 Influence of polyphenolic plant extracts on SGLT1 and GLUT2 activity *in vitro*

5.1.1 Transport of dietary polyphenols via SGLT1

Only a few studies have investigated the transport of polyphenolic plant compounds by SGLT1 but those demonstrated that arbutin, helicin and gastrodin can be transported by this protein [269, 270, 271]. By use of TEVC we could clearly demonstrate that none of the plant extracts investigated induced a significant transport current. This indicated that the extracts did not contain any sugars and moreover, that none of the contained polyphenolic compounds served as a substrate of the transporter. It is generally considered that aglycones such as flavone, quercetin, daidzein or genistein are absorbed by passive diffusion in the intestinal tract because of their lipophilic characteristics [272, 273, 274]. However, it was postulated that several flavonoid glucosides like Q3glc, Q4'glc and phlorizin are transported by SGLT1 [275, 194, 276]. In addition, trans-piceid, the 3-ß-O-glucoside of trans-resveratrol was taken up across the apical side of Caco-2 cells proposed to be mediated by SGLT1 since uptake of trans-piceid was significantly lower in the presence of glucose and/or phlorizin [187]. The same experiment was repeated with trans-resveratrol but failed to show any significant change in transport rate of glucose, suggesting that resveratrol is absorbed by paracellular diffusion or another mechanism. Experiments in human umbilical vein endothelial cells also suggested an involvement of SGLT1 in the uptake of resveratrol, since the transport rate was reduced in the presence

of phlorizin or glucose as well as after SGLT1 siRNA transfection [277]. In the present work we could clearly demonstrate that flavonoid glucosides such as Q4'glc and Q3glc as well as stilbene-glucosides like trans-piceid are not transported by SGLT1, so are not the parent aglycones which is in accordance with data from Kottra and Daniel [35] performed under the same conditions as in the present work. They failed to detect any transport of numerous, either glycosylated or non-glycosylated flavonoids but demonstrated that various compunds effectively inhibited electrogenic SGLT1-mediated α-MDG uptake. Luminal perfusion of rat small intestine with genistein-7-glucoside (genistin) in the presence of phlorizin resulted in a 2.5-fold increase in genistin absorption compared to genistin perfusion alone, indicating that phlorizin may compete with genistin for hydrolysis by LPH allowing genistin to be taken up by mechanisms not involving SGLT1 [278]. Other studies failed to show any transport of genistin across the apical membrane of Caco-2 cells [279, 274] while Murota et al. [273] additionally observed no transport of quercetin glucosides (Q4'glc, Q3glc and Q3,4'diglc) across the apical membrane of Caco-2 cells. The majority of the studies that proposed transport of polyphenolic glucosides via SGLT1 were performed in isolated murine intestinal segments as well as in cell culture models such as Caco-2 cells that express, in addition to SGLT1 other carriers that could contribute to the bioavailabilty of polyphenolic compounds. Moreover, the postulated uptake of several quercetin glucosides as well as trans-piceid was based on observations that absorption was decreased in the presence of SGLT1 specific inhibitor phlorizin and of glucose. However, phlorizin is also a substrate for LPH and experiments with isolated human LPH showed a rapid deglycosylation of phlorizin [188]. Q3glc and Q4'glc were also identified as substrates for LPH but displayed lower affinities than phlorizin [189, 280, 188, 184]. Sesink et al. [281] and Day et al. [185] observed a reduced deglycosylation of Q3glc and/or Q4'glc in the presence of LPH inhibitor *N*-butyldeoxygalactonojirimycin (NB-DJG) in rat intestinal tissues. This coincided with a reduced appearance of quercetin on the serosal side, suggesting an involvement of LPH in the intestinal absorption of Q3glc and Q4'glc [185]. It was suggested that LPH-mediated deglycosylation of phlorizin inhibits hydrolysis of Q4'glc, thereby reducing the release of free quercetin for passive diffusion. The observation that Q4'glc deglycosylation and transport of free quercetin was equally reduced in the presence of phlorizin and NB-DJG indicates that SGLT1 plays, if any, only a minor role in intestinal uptake of quercetin glucosides *in vivo*. Deglycosylation by LPH was also suggested as initial step for intestinal absorption of daidzein-7-O-glucoside [186]. In addition, Henry-Vitrac et al. [282] observed a deglycosylation of trans-piceid in the presence of purified rat small intestinal fractions containing either LPH or CBG, suggesting that both, LPH and CBG are involved in the release of trans-resveratrol from trans-piceid. Although several other apical transporters (MCT1, PEPT1, SMCT) of

intestinal epithelial cells were discussed to be involved in the absorption of different polyphenolic compounds the precise mechanisms by which they cross the brush border membrane mains to be determined.

In summary, amongst the individual polyphenolic compounds investigated here, only arbutin and gastrodin – already shown before [269, 270, 271] displayed significant transport by human SGLT1 when expressed in *Xenopus* oocytes (see Appendix, Table 15).

5.1.2 Inhibition of SGLT1 and GLUT2 by extracts and polyphenols from apple, onion, curly kale and grapevine

In the present work we demonstrated that extracts and polyphenols from apple, onion and curly kale effectively reduced glucose uptake into *Xenopus leavis* oocytes and into mouse intestinal segments via inhibition of SGLT1. In addition, promising inhibition was also observed in the presence of the grapevine shoot extract Vineatrol®30. Apple extract showed the most effective inhibition of SGLT1 with an IC_{50} value of 2.1 ± 0.2 µg/ml, followed by grapevine shoot extract (IC_{50}=9.5 ± 1.2 µg/ml), onion extract (IC_{50} = 326.5 ± 57.0 µg/ml) and curly kale extract (IC_{50} =1629.9 ± 368.2 µg/ml). Except for the grapevine shoot extract, the results for SGLT1 inhibition in oocytes could be confirmed in isolated murine jejunal segments. However, in all cases a 5 to 10-times weaker affinity (higher EC_{50} values) compared to the values obtained in the oocyte expression system for the same extracts or test compounds were observed. But this was also found for the Km for α-MDG transport. While the Km for SGLT1 in *Xenopus* oocytes revealed a value of 1.02 ± 0.3 mM, the Km in the murine intestinal rings was almost 7-fold higher and accounted to 6.5 ± 1.1 mM. This phenomenon is known and is explained by the presence of an unstirred water layer (UWL) on the mucosal surface [283, 284]. The UWL represents a barrier that impedes access of nutrients [285, 286, 287] and other compounds such as drugs [288] and polyphenols to the carrier side and thus may lead to an underestimation of apparent kinetic parameters of transporter substrates and inhibitors. Additionally, it could be shown that the thickness of UWL was lowered by increasing the agitation rate [289, 290]. For this reason we used a relative high agitation rate of 180 oscillation/min in the experiments. GLUT2-mediated 2-DG uptake into *Xenopus* oocytes injected with cRNA encoding human GLUT2 was also significantly diminished in the presence of apple, onion and grapevine extract but not in the presence of curly kale extract. In mouse intestine however, we failed to measure any uptake of 2-DG indicating that there was no GLUT2 in the apical membrane in this test system, even in the presence of high amounts of glucose

(data not shown). These results were in accordance with a study performed in isolated rat everted gut sleeves that failed to detect any GLUT2 in the apical membrane [291].
The analysis of polyphenolic compounds revealed that flavonoles, dihydrochalcones (apple) as well as hydroxycinnamic acids are the major constituents in apple, onion and curly kale extracts. Among the individual polyphenols tested, phlorizin revealed the strongest inhibition of SGLT1-mediated α-MDG transport in oocytes with an IC_{50} value of 0.46 ± 0.19 µM. In mouse intestine, an IC_{50} value of 4.2 ± 0.02 µM for phlorizin was observed and here this compound also showed highest potency. While phlorizin is a competitive inhibitor of SGLT1 [262, 61, 100, 293] its aglycone phloretin inhibits SGLT1 non-competitively [293] but 800-fold less potent than phlorizin. This demonstrates the critical importance of the glucose moiety at the 2'-position for binding to SGLT1. It has been proposed that phlorizin binding to SGLT1 occurs at both, the sugar-binding site as well as the aglycone-binding site [293, 294]. Phlorizin was also found in an apple extract that effectively inhibited glucose uptake into Caco-2 cells [34]. Next to phlorizin Q3rha (IC_{50}=380 µM) was identified as an effective inhibitor of glucose uptake into Caco-2 cells whereas Q3rha showed only a low inhibition of hSGLT1 in our TEVC experiments with an IC_{50} of 1.28 ± 0.27 mM. This discrepancy might be explained by an additional and even stronger inhibition of GLUT2 by Q3rha shown to be constitutively expressed at the apical membrane of Caco-2 cells [295]. Glucose uptake into Caco-2 cells is mediated mainly by GLUT1 and GLUT2 but only low levels of SGLT1. Therefore this model system may not be applicable to study the fate of polyphenols in intestinal cells.

Among phlorizin as the major compound in the apple extract, other chalcones like phloretin but also aglycones and derivatives of the flavonoles kaempferol and quercetin as well as hydroxycinnamic acids were present in mentionable concentrations. In addition, glycosylated kaempferol and quercetin derivatives but also hydroxycinnamic acids have been identified in the curly kale and/or onion extract. In studies by Manzano and Williamson [34], simple phenolic acids like p-coumaric acid were shown to decrease uptake of glucose into Caco-2 cells. In contrast, trans-p-coumaric acid but also other phenolic acids like CGA showed no significant inhibition of hSGLT1 and hGLUT2 expressed in *Xenopus* oocytes in the present work. These results are in accordance with the work of Johnston et al. [296] showing no change in glucose uptake into Caco-2 cells under sodium-dependent (SGLT1) and sodium-independent (GLUT2) conditions in the presence of phenolic acids including CGA, caffeic acid, p-coumaric acid and ferulic acid. In contrast, several *in vitro* studies could clearly show a decrease in glucose uptake into rat everted gut sacs and rat intestinal membrane vesicles in the presence of CGA [297, 250]. In the study from Welsch et al. [297], CGA at a concentration of 1 mM inhibited

glucose uptake by about 80%, whereas caffeic and ferulic acid in the same amount reduced glucose uptake by only 35% and 38%, respectively. But in accordance with our data, Welsh et al. [297] failed to show a direct interaction with SGLT1 but proposed that the compound may cause a dissipation of the sodium electrochemical gradient essential for the proper function of SGLT1 in tissues. Lacombe and coworkers [298] could further demonstrate that another phenolic acid, tannic acid was able to inhibit Na^+-K^+-ATPase, responsible for maintaining the sodium gradient necessary for the sodium driven glucose transport into the enterocytes. These data together with the data from our TEVC measurements indicate that simple phenolic acids including trans-caffeic, trans-ferulic and sinapic acid as well as chlorogenic and trans-p-coumaric acid do not directly interact with hSGLT1 even at concentrations as high as 1 mM (see Appendix, Table 15) but may modulate intestinal glucose absorption by other mechanisms.

In the present work, quercetin and Q3glc revealed only weak inhibition of hSGLT1 when expressed in *Xenopus* oocytes. Other studies failed to show any significant effect of quercetin and its 3-O-glucoside on glucose uptake via SGLT1 in the oocyte expression system [299, 300]. The comparison of the IC_{50} values of quercetin (IC_{50}=0.62 mM) and Q3glc (IC_{50}=0.98 mM) suggest that the glucose moiety at position 3 reduces binding affinity. Q3,4'diglc bearing a second glucose moiety at position 4' of the B-ring even failed to show any inhibition. A similar reduction of binding affinity by 3-O-glycosylated derivatives was found for kaempferol. But in contrast to quercetin, kaempferol revealed a stronger binding affinity (IC_{50}=0.38 ± 0.07 mM) suggesting that the hydrogen (instead of a hydroxyl group) at position 3' increases binding affinity. This observation is in line with data obtained in isolated rat intestinal segments in which K3rha reduced glucose absorption by 28% whereas Q3rha only revealed a decrease in glucose absorption by 10% [301]. In addition, kaempferol-3-O-(2''-galloyl)-rhamnoside failed to show any inhibition of glucose absorption indicating that a galloyl group additionally attached to the rhamnosyl moiety blocks interaction with SGLT1. In the present work, K3rha also revealed stronger inhibitory strength for hSGLT1 inhibition in the oocyte expression system than Q3rha confirming the results from Rodriguez et al. [301]. The strongest inhibition amongst the quercetin derivatives showed Q4'glc, indicating that a glucose moiety at this position increases binding affinity in accordance with the data from Kottra and Daniel [35]. Since Q4'glc was the dominant representative of the quercetin derivatives within the onion extract it is very likely that the inhibition of the extract was mainly caused by Q4'glc.

In contrast to the results observed for SGLT1, quercetin and several quercetin derivatives showed strong inhibition of GLUT2 in the oocytes expression system (Figure 18). The strongest inhibition of 2-DG-mediated uptake via GLUT2 was caused by

quercetin-3-O-galactoside, followed by quercetin, Q3glc and Q4'glc whereas Q3glc-rha (rutin) showed no inhibition. Similar results were obtained by Kwon et al. [300] using the same expression system. Here, quercetin revealed the strongest inhibition of 2-DG-mediated uptake into oocytes expressing GLUT2, followed by Q3glc and Q4'glc whereas rutin was not an inhibitor of GLUT2. In addition, phloretin but not its glucoside phlorizin revealed strong and significant inhibition of GLUT2 as also observed in our experiments. These results suggest that the inhibition of of hGLUT2 by onion and apple extracts in the present work was mainly caused by quercetin and/or Q4'glc in the onion extract and by quercetin and/or phloretin in the apple extract.

From the plant extract investigated in the present work, curly kale extract showed the weakest inhibition of SGLT1 in the *in vitro* experiments and failed to inhibit GLUT2 expressed in *Xenopus* oocytes.

The analysis of the grapevine extract revealed trans-resveratrol and its dimeric form ε-viniferin as the major components. The screening for hSGLT1 inhibition revealed ε-viniferin as the most effective inhibitor of hSGLT1 with an IC_{50} of 8.0 ± 0.48 µM, whereas trans-resveratrol with an IC_{50} of 510 ± 90 µM showed only modest inhibition. Dihydroresveratrol, a metabolite of trans-resveratrol lacking the double bond between C7 and C8 failed to show any inhibition. Interestingly, an additional glucose moiety at position 3-O of the B-ring (trans-piceid) increased binding affinity.
It was recently demonstrated that trans-resveratrol and its dimer ε-viniferin decreased glucose uptake into porcine small intestine and porcine BBMV via a direct interaction with SGLT1 [302]. In accordance with our results ε-viniferin here also showed a stronger inhibition than its monomer trans-resveratrol. Moreover, ε-viniferin completely blocked glucose uptake into porcine jejunal and ileal vesicles at a concentration of 300 mM; similar to our results obtained in murine everted gut rings in which ε-viniferin at a concentration of 400 µM reduced uptake of α-MDG by almost 70%. The interaction of resveratrol and hopeaphenol with glucose absorption was also investigated by Morikawa et al. [303] but they failed to show any significant effects of hopeaphenol in rat small intestinal tissue even at concentrations of 1 mM. In contrast, we could clearly show that hopeaphenol and ε-viniferin inhibited SGLT1-mediated α-MDG transport into mouse intestinal segments even at lower concentrations but, we also found that this in case of ε-viniferin occured via a non-reversible mechanims. Both, trans-resveratrol and trans-piceatannol were also shown to interact with other transmembrane proteins such as voltage-gated potassium channels Kv1.3 and mitochondrial F0F1-ATPase [304, 305] and both compounds also interacted with model membranes composed of phosphatidylcholine [306]. An interaction of the stilbenes occurred preferentially with the polar headgroups region of the bilayer but

also a permeation of the stilbenes into deeper membrane regions could be observed. Since integral membrane proteins or rather their side chains do interact with lipid bilayers as shown for the C-13 terminal loop of SGLT1 [307], it seems possible that interactions of ε-viniferin and hopeaphenol with phospholipids prevent proper conformational changes of membrane proteins needed for substrate binding, thus leading to an inhibition of transport.

5.2 Effects of plant extracts on glucose absorption *in vivo*

Apple, onion and curly kale extract

OGTT studies

The results from the human studies showed that onion or curly kale extracts standardized for the amount of total flavonoids (600 mg) did not affect blood glucose and plasma insulin levels when given 30 min prior to an OGTT with 75 g of glucose. These results suggest that a flavonoid content of 600 mg is too low to cause significant inhibition of intestinal SGLT1, at least in the presence of high amounts of glucose. In contrast, the ingestion of 2.8 g apple extract containing around 450 mg phlorizin 30 min before the OGTT reduced the iAUC for blood glucose in the first 45 min. This reduction was accompanied with a significant decrease of the iAUC for plasma insulin within the first 90 min. The study by Johnston et al. [308] described similarly that the consumption of cloudy and clear apple juices (400 ml) with equal amounts of glucose (25 g) led to a significant delay of glucose absorption compared to the control, based on an iAUC for plasma glucose that was significantly decreased within the first 30 min. A significant reduction was also seen for plasma insulin concentration within the first 30 min. The authors suggested that the antihyperglycemic effect of the apple juice was mainly caused by phlorizin. We also would propose that the effect seen with the apple extract is mainly caused by the phlorizin contained which represents the dominant component and which revealed the strongest inhibition of SGLT1 in all test systems. However, additional effects of CGA can not be excluded as van Dijk et al. [309] for example demonstrated that ingestion of 1 g CGA 30 min prior to an OGTT with 75 g glucose significantly reduced glucose and insulin responses in overweight men at 15 min. And, similarily, the oral but not intravenous administration of CGA (3.5 mg/kg body weight) in rats 10 min before an oral glucose load caused a reduction in the glycemic peak at 10-15 min [310]. These findings suggest that CGA could modulate glucose absorption by a mechanism independent of SGLT1 as we

failed to demonstrate an effect in the TEVC experiments. It was proposed that CGA could dissipate the Na^+-electrochemical gradient, necessary for a proper SGLT1 activity [297]. However, the apple extract used in the present study contained only 156 mg CGA and the ingestion of 264 mg CGA with decaffeinated coffee failed to show any change in plasma glucose or serum insulin concentrations in the study of Van Dijk et al. [309]. A study in normoglycemic rats orally administered with a aqueous solution of CGA (25 mg/kg body weight) 10 min prior to a glucose load also failed to show any change in the glucose levels [250]. This all suggests that the concentration of CGA in our apple extract was too low to display significant effects and leaves phlorizin as the main effector.

The absence of any effect of the onion extract on postprandial blood glucose concentration when administered 30 min prior an OGTT to healthy volunteers and in normal mice was not expected. Q4'glc the most abundant quercetin derivative within the onion extract was shown to effectively inhibit SGLT1 [35, 192, 252]. In contrast to our results, administration of an ethanolic extract of onion bulb (250 mg/kg) to normoglycemic rats 30 min prior an OGTT (1.5 g/kg) resulted in a significant suppression of blood glucose levels 30 and 60 min after the glucose load [311]. But, different from studies in mice, the onion extract was administered 30 min before the OGTT. Interestingly, Pereira et al. [246] found that the application of quercetin-3-O-glucorhamnoside 30 min prior to an OGTT was able to diminish blood glucose levels, while a simultaneous application had no effect. Application of the onion extract in mice fed a high fat diet resulted in decreased postprandial blood glucose concentrations. Similar results were also observed in a study with type 2 diabetic patients [313]. Here, ingestion of 50 g fresh onion simultaneously with 50 g glucose produced a significant reduction in plasma glucose levels at 60, 90 and 120 min after the glucose load. What made the onion preparations to work in these studies but failed to show an effect in our human study remains to be defined. The high content and effective inhibition of SGLT1 by Q4'glc would have argued for an effect as observed for the extract in our studies in obese mice. However, Q4'glc is rapidly cleaved by LPH into quercetin [188] which is far less active in inhibition of SGLT1. But this also holds true for phlorizin in the apple extract which is rapidly cleaved to phloretin and glucose by LPH with phloretin far less active in inhibition of SGLT1. Amongst other mechanisms able to explain the effects may be the inhibition of the efflux of glucose from epithelial cells via GLUT2 in basolateral membranes which is effectively inhibited by phloretin and which could cause a reduced blood glucose level as well.

A new class of antidiabetic drugs that inhibit SGLT2 in kidney and that partially resemble phlorizin in structure were shown to improve glycaemic control, including reduced fasting

plasma glucose levels, a decrease of glycosylated haemoglobin (HbA1c), and lower postprandial glucose concentrations in patients with T2DM [314, 315]. The effects of the SGLT2 inhibitors are associated with significant renal losses of glucose by effective reduction of glucose re-uptake in S2 and S3 segments. In assessing of whether the plant extracts used in the present work also cause increased renal glucose output, we analyzed urine samples of the human volunteers before and after the OGTT. The ingestion of the apple extract – but not of the two other extracts - indeed resulted in an increased glucose output in urine. This suggested that phlorizin and/or any derivative can enter systemic circulation with a subsequent inhibition of SGLT1 and/or SGLT2 in renal tubules. A preliminary analysis of plasma samples by our project partners from the Christian-Albrechts University of Kiel (Dr. Girreser; Prof. Blaschek, data not shown) were able to detect phloretin and phloretin-glucuronides in the plasma of the volunteers but failed to detect any trace of intact phlorizin in accordance with another human study investigating the bioavailability of apple polyphenols after apple or apple juice consumption [316]. Also in rats fed a single meal supplemented with phlorizin, the investigators were unable to detect traces of intact phlorizin in the plasma of the animals [209]. Only one study in streptozotocin-treated mice was able to detect phlorizin in the plasma of the animals but here phlorizin was fed for 2 weeks [317]. However, the detection of phloretin and its conjugates in plasma suggests substantial absorption and the opportunity that it is active on GLUT-transporters in circulation. Since we were able to show a significant reduction in the plasma insulin curve after treatment with apple extract in healthy young men, it seems possible that circulating phloretin leads inhibition of GLUT2 located at the pancreatic β-cells, resulting in a diminished release of insulin. However, since the decrease in blood glucose and plasma insulin concentrations in the human intervention trial followed a similar pattern with significant differences over the first 45 min (glucose) and 90 min (insulin) after the OGTT, it is more likely that the lower amount of glucose absorbed from the intestinal tract led to a decrease in pancreatic insulin secretion.

In conclusion, phlorizin, given either as pure substance or as part of the apple extract leads to delay in glucose absorption in mice and a reduced increase in blood glucose levels. Similar effects were found in healthy humans for the apple extract while the curly kale and onion extracts failed to show any significant effects.

White bread studies

A variety of food extracts as well as individual polyphenols were reported as able to inhibit α-glucosidase and α-amylase activities *in vitro* as well as *in vivo* (reviewed in [205] and [39]). To proof whether there is a stronger inhibitory effect on the rise in postprandial blood glucose and plasma insulin concentrations by the extracts via an inhibition of starch hydrolysis we additionally investigated extracts and combinations thereof with white bread. We performed this study in elderly obese volunteers with a BMI above 25 kg/m^2 as our studies in mice demonstrated more pronounced effects in obese mice (mice fed a high fat diet for 12 weeks). The volunteers therefore received 104 g white bread (50 g carbohydrates) with or without prior ingestion (10 min before) of capsules containing either curly kale, onion and apple extract or mixtures thereof. The efficacy of plant extracts and their combinations was compared to the effects of acarbose, a well-known inhibitor of intestinal disaccharidases. Whereas impressive effects of acarbose were obtained, none of the extracts significantly affected blood glucose and plasma insulin levels. Although these findings matched crossly those in healthy young volunteers with the OGTT, studies in animals and humans with onion preparations had demonstrated that the active principle of their effects *in vivo* was by inhibition of the release of glucose from oligosaccharides [318, 319]. Especially sucrase and α-glucosidase were significantly inhibited by onion extracts. An oral administration of a single dose of quercetin simultaneously with a starch or maltose solution to normoglycemic and diabetic rats as well as volunteers with T2DM resulted in a significant inhibition of postprandial plasma glucose levels [247, 248, 249] whereas quercetin in combination with glucose did not affect postprandial blood glucose levels [248, 249].

Interestingly, ingestion of onion and curly extracts in combination with the apple extract significantly decreased postprandial blood glucose concentrations without effects on plasma insulin. This suggested synergistic or/and additive effects either on starch and maltooligosaccharide digestion or glucose absorption. *In vitro* studies demonstrated an increased inhibitory potential for α-glucosidase and α-amylase inhibition in the presence of combinations compared to single compounds [320, 33]. As an example, an extract of *Allium sativum* (garlic) combined with *Lagerstroemia speciosa* showed a stronger inhibitory activity against α-glucosidase activity *in vitro* than one alone or the treatment with miglitol, a known α-glucosidase inhibitor [321]. It may well be that the combination of the extracts alters the hydrolysis of phlorizin contained in the apple extracts allowing it to act on SGLT1 or that phlorizin hydrolysis via LPH, because of its higher affinity to LPH, leaves more Q4'glc for inhibition when onion and apple are combined. An additional effect

on basolateral GLUT2 is also conceivable, since it was already described *in vitro* for selected plant extracts and mixtures [322, 34]. Moreover, additive inhibitory effects on glucose absorption in rat intestinal segments were observed when phlorizin and an aqueous extract of *Bauhinia megalandra* – containing mainly kaempferol-3-O-α-rhamnoside - were provided [323]. In the same study, the combined use of kaemperol-3-O-α-rhamnoside and phlorizin reduced glucose absorption into rat intestinal segments by 68%, much more than caused by either one compound alone.

Grapevine extract

A large number of epidemiological studies suggest that the consumption of grape products such as red wine decreases the risk for cardiovascular diseases and diabetes [324, 325, 326, 327, 328]. Especially the polyphenolic fraction within these grape products seems to play an important role in the health promoting effects [329, 330, 331]. Studies in diabetic animals showed an improvement of insulin sensitivity as well as a reduction in oxidative stress after treatment with several grapevine skin and leave extracts [332, 333]. Moreover, the grapevine shoot extract (Vineatrol®30) used in the present work was also able to improve insulin resistance and insulinemia in hamsters fed a high fat diet supplemented with high dose of the extract for 13 weeks [334]. Similarly, Vineatrol®30 supplementation caused a significant reduction in oxidative stress markers which was confirmed by another study performed in rats [335]. However, studies that have investigated effects of grapevine extracts and grapevine polyphenols (especially stilbenes) on blood glucose and plasma insulin levels in response to different carbohydrate loads in humans are very limited. Although our *in vitro* data revealed a strong inhibition by grapevine oligostilbenes for SGLT1 and GLUT2, the *in vivo* data in healthy and obese volunteers did not show any change in postprandial rise in blood glucose or plasma insulin after oral ingestion of a single dose of grapevine extract prior to the administration of different carbohydrate loads. However in rats, application of an ethanolic extract of grapevine leaves (50 mg/kg per body weight) before an OGTT (2 g/kg per body weight) resulted in a significant reduction in rise of blood glucose concentration, suggesting a delayed glucose absorption or a direct stimulation of insulin secretion as mechanism of action [332]. Although these authors did not analyse the grapevine leave extract, Monagas et al. [336] identified flavanols, flavanol oligomers and proanthocyanidins as the major compounds. Proanthocyanidins were also found as the major compounds in a grape seed extract that significantly diminished the rise in blood glucose when ingested at different doses (100 and 300 mg) simultaneously with a high carbohydrate meal

(92 g carbohydrates) in healthy volunteers [337]. The major polyphenolic compounds found in the grapevine shoot extract used in the present work were stilbenes with ε-viniferin as the main compound (0.215 mg/mg dry extract), followed by trans-resveratrol (0.09 mg/mg dry extract) and ampelopsin A (0.037 mg/mg dry extract). For resveratrol when administered at a dose of 10 mg/kg per body weight orally in rats 5 min prior to an OGTT (1 g/kg glucose per body weight) a significant decrease in blood glucose concentration after 90 and 120 min was reported [338]. Other stilbenes (isolated from the bark of *Shorea roxburghii*) were also shown to decrease the rise in blood glucose levels after a sucrose-load in mice [303]. Although trans-resveratrol as well as its glucoside trans-resveratrol 10-C-b-D-glucopyranoside were identified within the extract, both compounds failed to have an effect when orally administered at doses of 100 and 200 mg/kg per body weight 30 min before the sucrose load. Interestingly, in the same study oligostilbenes including hopeaphenol and the resveratrol trimer α-viniferin showed significant effects on plasma glucose levels at doses of 100 or/and 200 mg/kg per body weight within the first 30 min after oral application of sucrose to the mice. These oligostilbenes possessed a moderate inhibition of α-glucosidases. Taken into account the amounts provided to the mice when compared to the 600 mg of stilbenes equivalent to 1.7 g grapevine extract given 10 min prior to a challenge with white bread, the lack of an effect in humans may be not to surprising. While studies on the effects of grapevine extracts on blood glucose and plasma insulin concentrations are limited, the impact of resveratrol on these parameters has been extensively studied. While in several short-term studies in normoglycemic animals no effect of resveratrol on blood glucose concentrations was found [339], other studies reported blood glucose-lowering properties after administration of single doses of resveratrol [340, 338]. Short-term and long-term administration of resveratrol to diabetic animals showed significant blood-glucose lowering effects in the majority of the studies [340, 341, 342, 343, 344, 345] suggesting that resveratrol is a beneficial agent in the treatment of a hyperglycemia in T2DM. Data from the animal studies could be confirmed in long-term studies in volunteers with T2DM [346, 347, 348]. But also in overweight and partly obese people with IGT resveratrol intake over 4 weeks resulted in a significant reduction of peak postmeal blood glucose as well as a reduction of 3-h blood glucose and plasma insulin AUC, while fasting blood glucose levels were not changed [349]. However, resveratrol supplementation to healthy normoglycemic women (for 12 weeks) revealed no beneficial effect on glucose metabolism. This may suggest that a metabolic disturbance is needed to reveal a benefit from resveratrol treatment [350, 351].

6. Summary and conclusions

Modern lifestyles are frequently characterized by a continuous supply of carbohydrates in form of snacks and beverages that cause a permanent stimulation of insulin secretion. This has been considered as a prime factor in the pathogenesis of hyperglycemia and T2DM. Since dietary glucose enters the body via SGLT1 and GLUT2 transporter in the small intestine, inhibition of these proteins could diminish plasma glucose responses and prevent an overshoot in insulin levels. On basis of various *in vitro* and *in vivo* techniques, a number of plant extracts and a large number of isolated polyphenolic compounds (flavonoids, stilbenes, dihydrochalcones, polyphenolic acids, etc.) were tested for their ability to inhibit the intestinal glucose transporters. Amongst the tested extracts, 4 were identified as potent in inhibiting human SGLT1 heterologously expressed in *Xenopus laevis* oocytes. An apple extract showed the strongest inhibition of hSGLT1 followed by extracts derived from grapevine, onion and curly kale. Amongst the polyphenolic compounds tested, none of them revealed a higher potency to inhibit SGLT1 than phlorizin (IC_{50}=0.46 ± 0.19 µM), known for a long time as an effective SGLT1-inhibitor. Nevertheless, some compounds from the stilbene group (ε-viniferin and hopeaphenol) showed an only 10-fold weaker inhibition of hSGLT1 than phlorizin. The results from the oocyte experiments were crossly confirmed in isolated intestinal segments of mice exposed *in vitro* to the extracts and test compounds.

GLUT2 is the main exporter for glucose efflux from intestinal epithelial cells but is also proposed to contribute to glucose absorption across the apical enterocyte membrane by recruitment from intracellular vesicles at high luminal glucose concentration. For this reason, experiments were also performed in *Xenopus* oocytes with heterologous expression of human GLUT2. The apple and the onion extract significantly inhibited GLUT2-mediated 2-deoxy-D-glucose (2-DG) uptake by ~80 %. The grapevine shoot extract inhibited uptake of 2-DG even completely. Individual polyphenols effectively inhibiting hGLUT2 and contained in the extracts were phloretin (apple extract), quercetin-3-galactoside (onion extract) and ε-viniferin or hopeaphenol (grapevine extract).

To proof the effectiveness of the plant extract *in vivo*, oral glucose tolerance test (OGTT) were performed in the absence and/or the presence of selected plant extracts and/or pure substances in mouse models and in human volunteers. In mice, the administration of phlorizin (15 mM) simultaneously with a glucose solution resulted in a significant reduction in rise of blood glucose 15 min postprandially. This was observed in both, mice fed a high

fat diet as well as in mice fed a control diet for 12 weeks. In contrast, administration of apple extract (equivalent to 15 mM phlorizin) together with glucose significantly decreased blood glucose at 15 min postprandial only in mice fed a high fat diet. Administration of Q4'glc (15 mM) or the onion extract (equivalent to 15 mM Q4'glc) together with glucose revealed no effects in normal mice. In high-fat fed mice the onion extract led to a significant lower rise in blood glucose concentration at 15 and 30 min postprandial.

The effectiveness of the preselected plant extracts in human subjects was first tested in healthy volunteers with normal body weight, followed by a second test series including obese subjects. Administration of capsules containing either curly kale, onion or grapevine extract (equivalent to 600 mg flavonoids or stilbenes) 30 min prior to the ingestion of 75 g glucose did not cause any significant reduction in venous blood glucose and plasma insulin concentrations. Only the ingestion of apple extract (equivalent to 450 mg phlorizin) led to a statistically significant decrease in plasma insulin levels 30 min after administration of the glucose solution as well as in the incremental area under the curve for blood glucose and plasma insulin. Glucose output into urine also increased after ingestion of the apple extract suggesting an inhibition of renal glucose reabsoption. In obese subjects 104 g of white bread was administered and the extracts were given 10 min prior to bread intake. None of the plant extracts lowered venous blood glucose or insulin levels. Interestingly, the ingestion of the apple extract together with either curly kale or the onion extract significantly decreased blood glucose levels in response to white bread but failed to decrease plasma insulin levels. As a proof of concept, acarbose, a known α-glucosidase inhibitior was provided 10 min prior to the administration of 104 g white bread and this led to a significant reduction of venous blood glucose and plasma insulin levels over time.

In summary, despite excellent potencies found for SGLT1 and GLUT2 inhibition by extracts *in vitro* and partially *in vivo* in mice, in the human studies far less pronounced effects were observed. This may be best explained by the rather high dose of a natural compound/extract needed in humans and the difficulties to provide those in a proper delivery system. Nevertheless, combinations of extracts proofed to have decent effects on postprandial glucose peaks and that is a starting point for further studies to optimize the extracts for use in food products or in supplements.

7. Zusammenfassung und Schlussfolgerungen

Moderne Lebensstile bedingen sowohl durch Mahlzeitenhäufigkeit aber insbesondere auch durch Snacks und Getränke eine nahezu kontinuierliche Zufuhr an Kohlenhydraten, die nach ihrer Resorption zu einer Stimulation der Insulinsekretion führen. Diese beständige Aktivierung der Insulinfreisetzung wird als maßgeblicher Faktor in der Genese der Hyperglykämie und des T2DM angesehen. Da die Transportproteine für Glucose im Darm (SGLT1, GLUT2) somit am Anfang einer Diät-induzierten Entwicklung des T2DM stehen, erscheint es sinnvoll durch eine Inhibition der Transporter die Kohlenhydratresorption zu verlangsamen oder insgesamt zu vermindern. Im Rahmen der Arbeit wurden daher eine Reihe von Pflanzenextrakten sowie isolierte polyphenolische Verbindungen aus diversen pflanzlichen Lebensmitteln (u.a. Flavonoide, Stilbene, Dihydrochalcone, Polyphenolsäuren etc.) auf ihr Potential geprüft den humanen SGLT1 zu inhibieren. Unter den Extrakten erwiesen sich ein Grünkohl-, ein Zwiebel-, ein Weinreben- und ein Apfelextrakt als potente Inhibitorgemische von humanem SGLT1 nach Expression in Oocyten. Dabei zeigte der Apfelextrakt die stärkste Inhibition, gefolgt vom Weinreben-, Zwiebel und Grünkohlextrakt. Unter den polyphenolischen Reinsubstanzen konnte keine Verbindung identifiziert werden, die den SGLT1 in stärkerem Maße zu hemmen vermag als Phlorizin (IC_{50}=0.46 ± 0.19 µM). Dennoch zeigten einige Verbindungen, vor allem aus der Gruppe der Stilbene (ε-Viniferin und Hopeaphenol), eine nur um den Faktor 10 schwächere Hemmung von hSGLT1 als Phlorizin. Versuche an isolierten Darmsegmenten der Maus bestätigten weitgehend die Ergebnisse aus den Oocytenversuchen und belegten die Fähigkeit der Extrakte zur substantiellen Hemmung der Glucoseresorption.

Neben SGLT1, wurde auch das inhibitorische Potential auf den GLUT2-vermittelten 2-Deoxy-D-Glucose (2-DG) Transport untersucht. Hier zeigte vor allen Apfel- und Zwiebelextrakt eine signifikante Hemmung der 2-DG-Aufnahme um ca. 80%. Mit dem Weinrebenextrakt Vineatrol®30 wurde der Transport von 2-DG sogar vollständig gehemmt. Prominente, in den Extrakten enthaltene Einzelverbindungen wurden als Phloretin (Apfelextrakt), Quercetin-3-galactosid (Zwiebelextrakt) und ε-Viniferin bzw. Hopeaphenol (Weinrebenextrakt) identifiziert.

Um zu untersuchen, inwieweit die Extrakte in der Lage sind die Glucoseresorption *in vivo* im Darm zu hemmen, wurden orale Glucosetoleranztests in Ab- und/oder Anwesenheit ausgewählter Forschungsextrakte und/oder Reinsubstanzen im Mausmodell sowie in Humanstudien durchgeführt. Im Hinblick auf die therapeutische Ausrichtung des Vorhabens und um zu vergleichen, ob es Unterschiede bezüglich der Stärke der Effekte in

gesunden im Vergleich zu adipösen Tieren gibt, wurde einer Gruppe Mäusen jeweils eine Hochfettdiät über 12 Wochen gefüttert. Während die Verabreichung von Phlorizin (15 mM) zusammen mit der Glucoselösung sowohl in Mäusen die eine Hochfettdiät erhielten als auch in Kontrolldiät-gefütterten Tieren zu einer signifikanten Reduzierung des Blutglucoseanstiegs 15 min postprandial führte, konnte dieser Effekt nach Gabe des Apfelextraktes nur in Hochfettdiät-gefütterten Mäusen beobachtet werden. Ebenso resultierte die Verabreichung des Zwiebelextrakts in einer Reduktion des Blutglucoseanstiegs sowohl 15 als auch 30 min postprandial nur in Hochfettdiät-gefütterten Tieren.

In Human-Studien mit normalgewichtigen Probanden führte die Gabe von verkapselten Grünkohlextrakt, Zwiebelextrakt oder Weinrebenextrakten (entsprechend 600 mg Flavonoiden oder Stilbene) 30 min vor der Durchführung eines OGTT zu keiner signifikanten Senkung der Blutglucose- sowie Plasmainsulinkonzentration. Nur die Verabreichung des Apfelextrakts (entsprechend 450 mg Phlorizin) führte zu einer statistisch signifikanten Senkung des Plasmainsulinwertes nach 30 min sowie einer signifikanten Senkung der inkrementalen Fläche unter der Kurve für Plasmainsulin und Blutglucose. Nach Gabe des Apfelextrakts wurde auch ein Anstieg der Glucoseausscheidung im Urin beobachtet was auf eine Hemmung der renalen Rückresorption der Glucose schließen lässt. In weiteren Testserien an übergewichtigen, teilweise adipösen Probanden wurde zusätzlich die Wirkung der Extrakte auf die Freisetzung von Glucose aus Stärke untersucht. Dazu wurden anstatt 75 g Glucose, 104 g Weissbrot verabreicht. Die Gabe von Apfel-, Zwiebel-, Grünkohl- sowie des Weinrebenextraktes 10 min vor der Verabreichung des Weissbrots führte weder zu einer Senkung der Blutglucose noch des Plasmainsulins. Interessanterweise konnte eine signifikante Reduktion der venösen Blutglucose, nicht aber des Plasmainsulins nach Gabe von Mischungen aus entweder Grünkohl- und Apfelextrakt oder Zwiebel- und Apfelextrakt beobachtet werden. Die Gabe von Acarbose als bekannter α-Glucosidase-Inhibitor belegte eindrücklich das Funktionsprinzip der durchegführten Tests mit markanten Wirksamkeiten auf den Blutzuckerverlauf.

Zusammenfassend lässt sich feststellen, dass bemerkenswert starke Hemmungen der intestinalen Glucosetransporter durch ausgewählte Extrakte *in vitro* gefunden werden konnten und diese auch partiell in Mausmodell belegt werden konnten; im Menschen dagegen nur bescheidene Wirkungen zu belegen waren. Dies mag in erster Linie eine Frage der notwendigen Dosis sein, die im Menschen schwerlich über die Extrakte zu erreichen ist. Die beobachteten Effekte bei den Gemischen der Extrakte lassen aber weiterführende Studien als lohnenswert erscheinen.

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List of Figures

List of Tables

List of Abbreviations

2-DG	2-desoxy-D-glucose
3-OMG	3-O-methylglucose
AM	apical membrane
AMV	apical membrane vesicles
ANOVA	analysis of variances
AUC	area under the curve
iAUC	incremental AUC
BLM	basolateral membrane
BMBF	Bundesministerium für Bildung und Forschung
BLMV	basolateral membrane vesicles
BBMV	brush border membrane vesicles
BMI	body mass index
CBG	cytosolic β-glucosidase
CGA	chlorogenic acid
CVD	cardiovascular disease
DE	dry extract
DMSO	dimethylsulphoxide
EGCG	epigallocatechin gallate
ER	endoplasmatic reticulum
EU	European Union
G6P	glucose-6-phospate
GIP	insulinotropic peptide
GLP-1	glucagon-like peptide 1
GLUT	glucose transporter
GlySar	glycyl-sarcosine
HPLC-DAD	High-Performance Liquid Chromatography with Diode-Array Detection
IC_{50}	Inhibitory constant 50
IGT	Impaired glucose tolerance
Imax	Maximum transmembrane current
K3rha	kaempferol-3-O-rhamnoside
KM	Michaelis-Menten constant
LDL	low density lipoprotein
LPH	lactase-phlorizin hydrolase
MCT	monocarboxylate acid transporter
mRNA	messenger RNA
NB-DJG	*N*-butyldeoxygalactonojirimycin
OGTT	oral glucose tolerance test
Q3glc	quercetin-3-O-glucoside
Q3,4'diglc	quercetin-3-4'-O-diglucoside
Q4'glc	quercetin-4'-O-glucoside (spiraeoside)
ROS	reactive oxygen species
RS1	regulatory subunit 1

RT	room temperature
RYGB	Roux-en Y gastric bypass surgery
SGLT	sodium-dependent glucose co-transporter
$SGLT^{-/-}$	SGLT1-deficient
SI	small intestine
SLC	solute carrier
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
TEVC	Two-electrode-voltage clamp
TMH	transmembrane α-helices
TR	sweet taste receptor
USA	United states of america
UWL	unstirred water layer
Vmax	maximum transport velocity
WB	white bread
WT	wild-type
α-MDG	α-methyl-glucopyranoside

Appendix

Table 14: Overview of the institutions involved in the project and their responsibilities

Institution and persons involved	Responsibilities in the project
BioactiveFoodGmbH, Bad Segeberg; **Dr. Henning Vollert**	- Project coordination - Improvement of extraction methods - Providing of plant extracts
Department of Pharmaceutical Biology, Christian-Albrechts University of Kiel; **Dr. Bettina Schwanck, Prof. Dr. Wolfgang Blaschek**	- Project coordination - Development of analytic methods - Identification and quantification of polyphenols - Improvement of extraction methods
IonGate Biosciences GmbH, Frankfurt; **Dr. Inga Bick**	- Assay development and *in vitro* measurements of SGLT1 activity by use of SURFE^{2}R technology
Max-Planck Institut (MPI) for biophysics, Frankfurt; **Dr. Christine Keipert, Prof. Dr. Klaus Fendler**	- Provision of the "*Substanzstoffbibliothek"* and screening of test compounds on SGLT1 activity by use of SURFE^{2}R technology
Molecular nutrition unit, department of food and nutrition, Technical University of Munich; **Christine Schulze, PD Dr. Gabor Kottra, Prof. Dr. Hannelore Daniel**	see Aim of the thesis

Table 15: Overview of the tested polyphenolic compounds and their potential to inhibit hSGLT1 by use of TEVC
Inhibition of SGLT1-mediated α-MDG uptake by polyphenolic compounds performed in *Xenopus* oocytes by use of TEVC. Data are represented as mean ± SEM from 5-8 oocytes.*= poor solubility

Substance	Inhibition of the αMDG induced current via hSGLT1 (TEVC)				
	25 µM	50 µM	100 µM	250 µM	other concentrations used
Chalcones/Dihydrochalcones					
Phlorizin	-				11±3 (0.06 µM); 20±3 (0.12 µM) 37±3 (0.25 µM); 54±3 (0.5 µM)
Phloretin	-	-	-	42 ± 3	23 ± 6 (125 µM); 64 ± 6 (0.5mM)
Neohesperidin-dihydrochalcone	4 ± 8	7 ± 5	12 ± 6	11 ± 6	-
Aspalathin	-	-	-	38 ± 4	54 ± 2 (0.5mM); 72 ± 4 (1mM)
Isoliquiritin	19 ± 10	31 ± 4	49 ± 6	73 ± 5	-
Isoliquiritigenin	-	1 ± 3	2 ± 3	9 ± 3	-
Cardamomin	-2 ± 5	-4 ± 3	-1 ± 4	0 ± 6	-
Homobutein	2 ± 8	1 ± 8	2 ± 8	3 ± 12	-

Substance	Inhibition of the αMDG induced current via hSGLT1 (TEVC)				
	25 µM	50 µM	100 µM	250 µM	other concentrations used
Flavones					
Apigenin	-	-	9 ± 6	-	-
Apigenin-7-O-glc	-	-	24 ± 3	30 ± 3	39.9 ± 5.5 (0.5 mM)
Apigenin-8-C-glc (Vitexin)	-	-	-	-	7.4 ± 3.8 (1 mM)
Apigenin-6-C-glc (Isovitexin)	-	-	-	-	64.7 ± 0.7 (1 mM)
Saponarin (Isovitexin-7-O-glc)	4 ± 5	7 ± 6	12 ± 11	17 ± 12	-
5 min Incubation	20 ± 2	40 ±10	57 ± 7	75 ± 2	-
Baicalein	5 ± 2	7 ± 3	6 ± 6	5 ± 7	-
Baicalein-7-O-glucuronide	-	-	-	-	2.5 ± 4.6 (1 mM)
Luteolin			54 ± 6	*	65 ± 6 (0.16 mM)
Luteolin-7-O-glc	4 ± 4	1 ± 7	6 ± 2	8 ± 8	8.2 ± 2.3 (1 mM)
Luteolin-3',7-O-diglc	2 ± 2	3 ± 2	6 ± 4	7 ± 3	16.1 ± 1.0 (1 mM)
Luteolin-8-C-glc (Orientin)	3 ± 8	4 ± 12	-4 ± 18	5 ± 7	9.6 ± 1.0 (1 mM)
Luteolin-6-C-glc (Homoorientin)	3 ± 7	4 ± 10	7 ± 10	8 ± 7	31.9 ± 1.3 (1 mM)
Flavonoles					
Quercetin	-	-	13 ± 7	-	24.4 ± 6.8 (0.2 mM)
Quercetin-3-O-glc	11 ± 12	7 ± 2	8 ± 11	5 ± 5	50.4 ± 1.2 (1 mM)
Quercetin-4'-O-glc (Spiraeosid)	-	-	49 ± 2	71 ± 1	85.4 ± 1.4 (1 mM)
Quercetin-7-O-glc	5 ± 5	4 ± 13	3 ± 7	10 ± 24	-
Quercetin-3-O-gal	-1 ± 8	-2±10	-2 ± 7	2 ± 5	-
Quercetin-3,4'-O-diglc	5 ± 7	5 ± 6	7 ± 7	2 ± 7	-
Quercetin-3-O-rha	5 ± 2	6 ± 6	8 ± 9	16 ± 7	24 ± 6 (0.5 mM)
Quercetin-3-O-glc-rha	12 ± 4	16 ± 7	20 ± 10	29 ± 5	-
Tris-O-(2-hydrxyethyl)rutin	-1 ± 3	-6 ± 6	-3 ± 6	2 ± 7	-
Kaempferol	-	21 ± 4	28 ± 5	42 ± 10	-
Kaempferol-3-O-glc	-	-	15 ± 5	29 ± 1	54 ± 2 (0.5mM)
Kaempferol-7-O-glc	4 ± 6	14 ± 4	19 ± 1	27 ± 9	-
Isorhamnetin	*	*	*	*	-
Kaempferol-3-O-Rhamnoside	10 ± 9	21 ± 5	26 ± 5	39 ± 4	-

Substance	Inhibition of the αMDG induced current via hSGLT1 (TEVC)				
	25 µM	50 µM	100 µM	250 µM	other concentrations used
Flavanones					
Liquiritigenin	-	-	-	10 ± 5	21 ± 4 (0.5 mM); 37 ± 5 (1 mM)
Liquiritin	-	-	-	4 ± 3	8 ± 3 (0.5mM); 14 ± 4 (1mM)
Naringenin			8 ± 4	12 ± 7	65.5 ± 5.6 (1 mM)
Naringenin-7-O-glc	-	1 ± 0	2 ± 2	-	-
Naringin	6±5	8±4	14±4	34±6	-
Flavanonoles					
Dihydrokaempferol	1 ± 4	1 ± 7	-2 ± 2	6 ± 4	37 ± 5 (1 mM)
Isoflavones					
Daidzein	-	-	-	-	8.4 ± 1.2 (1 mM)
Daidzein-7-O-glc	-	-	-	-	12.7 ± 2.5 (1 mM)
Daidzein-8-C-glc (Puerarin)	22 ± 13	27± 18	15 ± 28	14 ± 19	-
Glycitin	-	-	-5 ± 13	-7 ± 5	-
Genistein	-	-	-	-	21.8 ± 1.9 (1 mM)
Genistein-7-O-glc	-	-	-	-	4.8 ± 3.2 (1 mM)
Sophoricoside	-4 ± 6	10 ± 5	14 ± 6	27 ± 4	-
Anthocyanins					
Cyanidinchloride	*	*	*	*	-
Cyanidin-3-O-glc	13 ± 3	3 ± 6	4 ± 2	1 ± 3	-
Cyanidin-3,5-O-diglc	9 ± 3	0 ± 1	2 ± 2	2 ± 2	-
Pelargonidinchlorid	6 ± 1	18 ± 1	35 ± 1	52 ± 3	-
Pelargonidin-3-O-glc	2 ± 7	7 ± 9	-1 ± 6	-4 ± 7	28.8 ± 0.8 (1 mM)
Pelargonidin-3,5-O-glc	-3 ± 3	1 ± 2	1 ± 2	0 ± 3	-
Stilbenes					
Pinosylvin	-6 ± 6	-1 ± 6	5 ± 6	3 ± 15	-
trans-Resveratrol	5 ± 4	9 ± 3	17 ± 4	*	-
Polydatin	15 ± 7	28 ±10	47 ± 5	67 ± 7	-
2,3,4',5-Tetrahydroxystilben-2-O-glc	-6 ± 4	-8 ± 5	-6 ± 5	-6 ± 8	-
Rhaponticin	10 ± 11	23 ±12	34 ± 14	53 ± 11	-
Mulberroside A	10 ± 15	-1 ± 9	-4 ± 7	1 ± 11	-
Ampelopsin A	22 ± 2	35 ± 6	48 ± 5	59 ± 5	-
ε-Viniferin	74 ± 2	85 ± 1	92 ± 1	-	42 ± 2 (0.06 µM); 59 ± 2 (0.12 µM)
Hopeaphenol	75 ± 3	86 ± 2	91 ± 4	93 ± 6	20 ± 5 (0.03 µM); 33 ± 4 (0.06 µM); 57± 3 (0.12 µM)
Dihydroresveratrol	0 ± 3	10 ± 5	10 ± 2	19 ± 6	-

Substance	Inhibition of the αMDG induced current via hSGLT1 (TEVC)				
	25 µM	50 µM	100 µM	250 µM	other concentrations used
Polyphenolic acids					
Trans-p-coumaric acid	-9 ± 6	5 ± 25	-8 ± 6	-3 ± 10	-
Trans-caffeic acid	0 ± 8	5 ± 15	-7 ± 15	-8 ± 17	-
Trans-ferulic acid	- 11± 3	-12±11	-12 ± 27	-12 ± 23	-
Chlorogenic acid	3 ± 10	8 ± 14	-1 ± 7	4 ± 13	-
Sinapic acid	-	-	-	-	-2 ± 2 (1 mM)
Others					
Salidroside	-	-	-	-	12 ± 4 (1 mM)
Rosin	-	-	-	23 ±8	31 ± 8 (0.5mM); 38 ± 7 (1mM)
Arbutin	-	-	-	-13 ± 0	-22 ± 4 (0.5mM); -37 ± 2 (1mM)
Gastrodin	-	-	-	-5 ± 1	-10 ± 2 (0.5mM); -21 ± 5 (1mM)
Salicin	-	-	-	-	-2 ± 5 (1 mM)
Agnuside	-2 ± 7	5 ± 5	-1 ± 4	4 ± 5	-
Mulberrosid	10 ± 7	-1 ± 5	-4 ± 4	1 ± 5	-

Table 16: Postprandial blood glucose and plasma insulin concentrations in healthy subjects
Mean incremental blood glucose and plasma insulin concentration in healthy young men after an OGTT (Reference) or an OGTT with prior ingestion (30 min) of either 2.8 g apple extract (A), 1.7 g grapevine (B), 4.4 curly kale (C) or 3.1 g onion extract (D). All values are means ± SEM, n=10 (A, B), n=15 (C) or n=11 (D). Significant differences of either blood glucose or plasma insulin were evaluated by use of two-way ANOVA with repeated measures and multiple comparison posthoc test (Tukey Kramer test). $p < 0.05$ *; $p < 0.01$ **, # Time in min

A	Blood Glucose (mg/dl)			Plasma Insulin (pmol/l)		
Time[#]	Reference	**Apple extract**	p-value	Reference	**Apple extract**	p-value
-30	88.2 ± 3.3	82.7 ± 1.1	1.0	32.2 ± 4.1	27.2 ± 1.7	1.0
0	82.2 ± 1.5	82.8 ± 1.4	1.0	25.7 ± 3.3	26.5 ± 2.2	1.0
15	109.6 ± 3.6	96.4 ± 4.6	0.6	124.2 ± 14.6	84.2 ± 14.2	0.7
30	124.5 ± 8.4	108.0 ± 6.0	0.2	183.2 ± 26.8	119.3 ± 15.6*	0.04
45	115.5 ± 11.0	117.0 ± 8.3	1.0	189.0 ± 23.6	153.7 ± 19.2	0.9
60	103.0 ± 10.3	107.3 ± 10.2	1.0	174.3 ± 28.8	171.5 ± 30.4	1.0
90	90.0 ± 8.3	93.0 ± 6.5	1.0	123.7 ± 27.3	115.0 ± 58.1	1.0
120	84.0 ± 7.2	87.2 ± 3.8	1.0	106.9 ± 38.3	96.6 ± 16.2	1.0
180	70.3 ± 2.3	68.6 ± 2.6	1.0	24.4 ± 3.3	25.6 ± 13.7	1.0
B	Blood Glucose (mg/dl)			Plasma Insulin (pmol/l)		
Time[#]	Reference	**Grapevine extract**	p-value	Reference	**Grapevine extract**	p-value
-30	81.3 ± 3.1	80.3 ± 2.6	1.0	32.5 ± 3.1	35.5 ± 3.8	1.0
0	84.5 ± 2.4	80.5 ± 3.3	1.0	31.9 ± 2.7	31.3 ± 2.5	1.0
15	104.6 ± 3.6	113.3 ± 4.7	0.6	131.1 ± 18.6	158.5 ± 17.4	0.97
30	118.2 ± 5.2	131.1 ± 7.7	0.2	208.3 ± 27.1	258.7 ± 26.8	0.28
45	104.9 ± 7.6	113.8 ± 7.5	0.6	206.3 ± 25.2	226.2 ± 18.3	1.0
60	95.1 ± 4.9	98.3 ± 6.3	1.0	176.8 ± 21.8	171.9 ± 18.9	1.0
90	82.9 ± 5.3	84.1 ± 5.7	1.0	127.9 ± 21.1	119.7 ± 20.9	1.0
120	77.4 ± 3.5	78.7 ± 3.6	1.0	73.0 ± 13.9	83.9 ± 23.4	1.0
180	75.3 ± 4.9	71.7 ± 5.3	1.0	24.9 ± 2.8	23.0 ± 2.6	1.0
C	Blood Glucose (mg/dl)			Plasma Insulin (pmol/l)		
Time[#]	Reference	**Curly kale extract**	p-value	Reference	**Curly kale extract**	p-value
-30	84.1 ± 2.4	82.7 ± 4.4	1.0	37.5 ± 6.3	47.7 ± 12.2	1.0
0	84.2 ± 3.8	78.8 ± 2.0	1.0	45.6 ± 7.7	38.8 ± 4.4	1.0
15	106.3 ± 4.9	103.6 ± 2.4	1.0	189.6 ± 25.1	207.4 ± 27.6	1.0
30	115.0 ± 7.2	104.4 ± 7.4	0.8	285.8 ± 46.0	322.3 ± 50.8	0.98
45	97.9 ± 6.0	94.8 ± 7.6	1.0	265.5 ± 48.2	288.7 ± 44.9	1.0
60	82.0 ± 5.3	79.4 ± 5.8	1.0	241.7 ± 47.6	235.1 ± 48.4	1.0
90	74.1 ± 3.7	73.0 ± 3.4	1.0	162.8 ± 37.0	131.2 ± 22.5	1.0
120	61.1 ± 6.0	69.4 ± 4.8	0.97	72.2 ± 9.8	70.8 ± 13.8	1.0
180	67.8 ± 2.7	66.4 ± 3.0	1.0	31.0 ± 4.8	30.5 ± 4.3	1.0
D	Blood Glucose (mg/dl)			Plasma Insulin (pmol/l)		
Time[#]	Reference	**Onion extract**	p-value	Reference	**Onion extract**	p-value
-30	77.7 ± 2.0	76.6 ± 1.5	1.0	33.6 ± 4.1	30.8 ± 2.9	1.0
0	73.0 ± 3.1	77.1 ± 2.0	1.0	27.5 ± 2.3	30.2 ± 2.2	1.0
15	93.2 ± 4.0	94.1 ± 3.4	1.0	122.3 ± 15.7	113.1 ± 14.8	1.0
30	112.5 ± 7.5	108.7 ± 5.3	1.0	171.6 ± 16.3	182.4 ± 22.8	1.0
45	100.9 ± 8.2	100.2 ± 6.8	1.0	186.8 ± 22.9	186.7 ± 19.2	1.0
60	89.5 ± 7.2	92.5 ± 5.7	1.0	183.0 ± 23.9	166.9 ± 15.2	1.0
90	73.6 ± 3.9	73.8 ± 4.1	1.0	106.7 ± 12.1	114.3 ± 11.5	1.0
120	61.9 ± 2.6	67.5 ± 3.3	1.0	51.1 ± 8.3	51.0 ± 5.9	1.0
180	64.7 ± 2.8	66.5 ± 2.1	1.0	24.1 ± 3.7	24.1 ± 2.7	1.0

Table 17: Postprandial blood glucose and plasma insulin iAUC in obese subjects

Mean incremental blood glucose and plasma insulin area under the curve (iAUC) in obese volunteers after a challenge with WB (Reference) or WB with prior ingestion (10 min) of either 2.8 g apple extract (A), 1.7 g grapevine (B), 4.4 curly kale (C) or 3.1 g onion extract (D). All values are means ± SEM of n=10 volunteers. Significant differences in the iAUCs of either blood glucose or plasma insulin were evaluated by use of one-way ANOVA with repeated measures and multiple comparison posthoc test (Tukey Kramer test). $p < 0.05$ *; $p < 0.01$ **

A	Glucose iAUC (mg/dl)			Insulin iAUC (pmol/l)		
Time (min)	Reference	**Apple extract**	p-value	Reference	**Apple extract**	p-value
0-15	202.5±15.0	217.5±9.2	0.93	89.5±24.4	242.0±63.6*	0.01
0-30	640.0±59.0	638.3±26.3	0.81	1202.8±281.3	1611.3±250.2	0.18
0-45	1350.0±129.0	1271.7±56.8	0.57	3969.8±816.1	4684.6±709.0	0.31
0-60	2057.5±206.9	1933.3±119.1	0.55	7320.3±1300.4	8591.9±1415.8	0.2
0-90	3055.8±357.6	3006.7±325.7	0.76	12572.5±1955.8	14656.4±2411.8	0.12
0-120	3535.8±477.3	3680.0±501.9	0.98	15346.5±2229.1	17652.9±2817.1	0.11
0-180	3795.8±587.4	4340.0±626.2	0.66	16919.9±2329.4	19605.5±3051.1	0.09
B	Glucose iAUC (mg/dl)			Insulin iAUC (pmol/l)		
Time (min)	Reference	**Grapevine extract**	p-value	Reference	**Grapevine extract**	p-value
0-15	270.0±22.2	270.8±18.9	0.96	219.9±78.1	293.7±69.8	0.39
0-30	783.0±69.4	783.8±61.9	1.0	1509.7±331.7	1743.5±308.5	0.74
0-45	1542.0±141.1	1525.5±130.9	0.87	4182.6±791.4	4421.6±626.6	0.87
0-60	2316.8±219.8	2284.5±234.4	0.78	7309.9±1258.2	7215.7±878.6	0.78
0-90	3464.3±344.9	3459.0±401.2	0.98	12451.5±1993.0	11923.3±1620.7	0.98
0-120	4007.3±445.4	3994.5±496.8	0.97	15024.4±2300.4	14510.7±2149.1	0.97
0-180	4340.3±568.7	4198.5±532.4	0.81	16163.0±2291.4	15584.0±2425.7	0.81
C	Glucose iAUC (mg/dl)			Insulin iAUC (pmol/l)		
Time (min)	Reference	**Curly kale extract**	p-value	Reference	**Curly kale extract**	p-value
0-15	198.0±14.1	235.5±16.0*	0.02	97.1±28.5	230±60.4*	0.04
0-30	618.0±54.3	687.0±56.4	0.08	1322.4±271.8	1588.2±237.4	0.39
0-45	1293.0±120.6	1358.3±114.5	0.39	4080.1±770.7	4650.0±672.6	0.42
0-60	1951.5±199.3	2057.3±180.9	0.41	7199.5±1261.1	8545.7±1343.2	0.19
0-90	2881.5±350.5	3092.3±319.4	0.49	12018.8±1933.4	14587.0±2288.1	0.07
0-120	3349.5±467.4	3753.8±421.3	0.34	14538.6±2236.0	17560.5±2672.6	0.06
0-180	3610.5±574.7	4611.8±484.2	0.06	15977.2±2425.1	19466.8±2894.5	0.06
D	Glucose iAUC (mg/dl)			Insulin iAUC (pmol/l)		
Time (min)	Reference	**Onion extract**	p-value	Reference	**Onion extract**	p-value
0-15	167.3±13.8	174.8±16.4	0.66	63.2±13.8	144.9±48.1	0.09
0-30	567.8±50.6	504.8±59.7	0.36	1167.2±212.3	1110.0±261.6	0.68
0-45	1224.8±108.9	1033.5±117.3	0.13	3959.8±623.3	3306.1±742.6	0.12
0-60	1854.8±178.5	1622.3±173.7	0.20	7406.1±1029.6	6210.7±1355.9	0.10
0-90	2678.3±299.6	2582.3±270.4	0.73	12698.0±1643.0	11596.8±2079.1	0.33
0-120	3075.8±383.9	3158.3±388.3	0.83	15353.8±1893.0	14818.8±2282.5	0.68
0-180	3354.75±485.1	3689.25±523.0	0.48	16880.2±1991.7	16636.6±2344.0	0.87

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Curriculum Vitae

Name:	Christine Schulze
Geburtsdaten:	06.12.1982, in Halle/Saale
Familienstand:	ledig
Nationalität:	deutsch

Universitäre und schulische Ausbildung

02/2009 bis 11/2013	Promotion an der Technischen Universität München Lehrstuhl für Ernährungsphysiologie
10/2002 bis 07/2008	Studium der Ernährungswissenschaften an der Martin-Luther-Universität Halle-Wittenberg, Abschluss: Diplom-Trophologin
08/1988 bis 06/2002	Abitur am Wilhelm-von-Humboldt-Gymnasium / Halle
02/2000 bis 07/2000	Nazareth-Highschool, Pennsylvania/USA

Berufliche Erfahrungen

05/2007 bis 11/2007	Tätigkeit als wissenschaftliche Hilfskraft am Helmholtz Zentrum für Umweltforschung (UFZ)
10/2006 bis 04/2007	Praktikum bei Nestlé Deutschland AG / Frankfurt am Main Abteilung: Nestlé Health Care Nutrition
01/2005 bis 04/2005	Praktikum am Institut für Ernährung und Prävention/ Berlin

Erklärung

Hiermit versichere ich, dass ich die vorliegende Arbeit

From molecule to men: Inhibition of intestinal glucose absorption by polyphenols and plant extracts for reducing the glycemic response

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Freising, den

Christine Schulze