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List of Abbreviations

APS	Ammoniumperoxydisulphate
AMP	Adenosine monophosphate
ATP	Adenosine triphosphate
BSA	Bovine Serum Albumin
$CaCl2*2H_20$	Calcium chloride dehydrate
ChREBP	Carbohydrate responsive element-binding protein
CD14	Cluster of differentiation
CO_2	Carbon dioxide
CoA	Coenzyme A
DNA	Deoxyribonucleic acid
DNL	De novo lipogenesis
DTT	Dithiotreitol
EDTA	Etylenediaminetetraacetic acid
EGF	Epidermal growth factor
ER	Endoplasmic reticulum
FFA	Free fatty acids
GLUT-2	Glucose transporter 2
GTP	Guanosine triphosphate
HEPES	(4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)
ICAM	Intercellular adhesion molecule
IGEPAL	Octylphenoxy poly(ethyleneoxy)ethanol
IECs	Intestinal epithelial cells
IL	Interleukin
iNOS	Inducible nitric oxide synthase
IR	Insulin resistance

IRS-1	Insulin receptor substrate 1
ISPF	Isonitrosopropiophenone
JAM	Junctional adhesion molecules
KCl	Potassium chloride
KH_2PO4	Potassium dihydrogen phosphate
KRH-buffer	Krebs-Ringer-HEPES-glucose-glutamine buffer
LBP	LPS binding proteins
LPS	Lipopolysaccharides
MCP-1	Monocyte chemoattractant protein-1
MgSO4	Magnesium sulfate anhydrous
MOPS	3-(N-Morpholino) propan sulfonic acid)
MLCK	Myosin light chain kinase
NaCl	Sodium chloride
NAD+/ NADH	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide-adenine dinucleotide phosphate
NAFLD	Nonalcoholic fatty liver disease
NFDMP	Nonfat dried milk powder
NF-kB	Nuclear factor-kappa B
NO	Nitric oxide
PAF	Platelet- activating factor
PBS	Buffered saline solution
PC	Phosphatidylcholine
PKC	Protein kinase C
PLs	Phospholipids
PFK	Phosphofructokinase
PVDF	Polyvinyidene fluoride membrane

ROS	Reactive oxygen species
RIPA-buffer	Radioimmunoprecipitation assay buffer
SDS	Sodium dodecylsulfate-polyacrylamide
SDS-PAGE	Sodium dodecyl sulfate–polyacrylamide gel electrophoresis
SEM	Standard error of mean
SGLT	Sodium-glucose co-transporter
SREBP1c	Sterol regulatory element binding transcription factor 1c
TBST	Tris-buffered saline with Tween 20
TEMED	Tetramethylethylendiamine
TGF-B	Transforming Growth Factor B
TJs	Tight junctions
TLR	Toll-like receptor
TNF-α	Tumor necrosis factor-alpha
VLDL	Very low density lipoprotein
WHO	World health organization
ZO	Zonula occludens

1. Introduction

1.1 Intestinal barrier dysfunction in the development of metabolic diseases

Intestinal barrier

The gut microbiome has been draw intention and interest since decades [1]. Lifestyle, environment and drugs are factors that influence the composition of microbiota [1]. Pathogens, genetic predisposition, stress but also antibiotics can damage the barrier of the intestine and can lead to infections and diseases [1]. The gastrointestinal tract builds a semipermeable barrier consisting of mucus and cells and plays an important role in the selective entrance of ingestible compound into the system [2]. The outer and inner mucus are protecting the epithelial cells from direct contact with bacteria [2]. The lamina propria, an immunological barrier, is a sub-epithelial connective tissue between the microbiome and the immune cells like T-cells, B-cells, macrophages, and dendritic cells which are responsible for the homeostasis [3]. The trans-cellular pathway, the carrier mediated pathway and the para-cellular pathway are the most common transport system in the intestinal epithelial cells [2]. The epithelial barrier integrity is maintained through intestinal mucus and tight junctions [6]. Tight junctions (TJs) consist in transmembrane proteins including occludin, claudins, junctional adhesion molecules (JAM) and many others (see Figure 1) [4]. TJs hold intestinal epithelial cells together and maintain the intestinal integrity [4]. They form a semipermeable barrier regulating the passage of water, ions and macromolecules through the para-cellular spaces [4]. Through different cellular process with intracellular proteins, TJs are maintaining the permeability of intestinal barrier [5]. TJ proteins interact with actin cytoskeleton and maintain TJs structure [6]. This specific interaction regulates the barrier integrity [6]. Small guanosine triphosphate (GTP)-binding proteins but also tyrosine kinases such as protein kinase C (PKC) are located in TJs and have been shown to maintain the integrity of TJs complexes but also paracellular barrier function [7]. Occludin, the main TJs protein, provides structure and integrity and plays also an important role in the regulation of intestinal permeability [7]. When alterations in the phosphorylation of occludin occur, elevation in reactive oxygen species (ROS) levels and inflammation has been reported leading

to separation of tight junctions [8]. The disruption of TJ proteins leads to intestinal barrier dysfunction and an increased intestinal permeability [8].

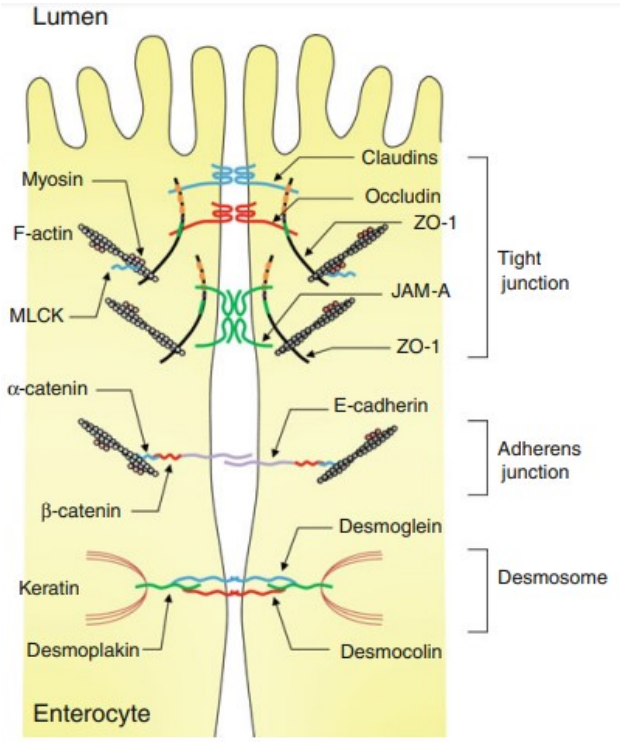

Figure 1: Molecular composition of intestinal epithelial cells [6]. Intestinal epithelial cells (IECs) are forming a layer and act as a barrier against exogenous and endogenous pathogens. Different proteins complexes such as TJs, adherens junctions and desmosomes are present in IECs. TJs compound are occludin, claudins, ZO-1 and JAM-A. These proteins seal IECs together. Occludin, JAM-A and claudins interact with ZO-1 and are fixed to F-actin filament. MLCK is linked to F-actin filament and plays a role in the intestinal permeability via contraction of myosin. Adheres junctions are formed from alpha-, beta–catenin and E-cadherin. Desmosomes are formed from desmoglein, keratin and desmoplakin. Adherens junctions and desmosomes are supporting TJs proteins by binding cells strongly together. Abbreviations: ZO-1: zonula occludens; JAM-A: junctional adhesion molecule-A; MLCK: myosin light chain kinase.

Intestinal permeability

Intestinal permeability is described as the passage of metabolites between the lumen and tissues [9]. Permeability can be increased through different mechanisms leading to imbalance [9]. Transcellular permeability consist in the transport of amino acids, sugars, electrolytes and short chain fatty acids through the epithelial cells [10]; while para-cellular permeability is the transport of substances between epithelium spaces according to their size and charge [10]. Intestinal permeability is regulated by extern factors, cytokines and immune cells [11]. Dysfunction in the intestinal barrier leads to inflammations, food allergies or diseases [11]. Alterations in the Intestinal permeability are associated with increased paracellular transport of lipopolysaccharides (LPS) and luminal bacteria in the systemic circulation [12]. LPS binding proteins (LBP) interact with LPS and bind to toll-like receptor 4 (TLR-4) which is previously associated with cluster of differentiation 14 (CD14) [13]. This interaction stimulates TLR-4 and MyD88-dependent signaling pathways leading to activation of pro-inflammatory responses [13]. This is followed by the stimulation of nuclear factor-kappa B (NF-kB) leading to secretion of tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL), IL-6 but also inducible nitric oxide synthase (iNOS) resulting in a systemic inflammation [14]. In fact, impairment of intestinal barrier dysfunction affects different organs and is the cause of many metabolic diseases such as ulcerative colitis [15]. Increased insulin resistance and lipogenesis have been observed in the liver leading to fatty liver disease [12]. The development of diabetes is caused by an elevation of insulin resistance and inflammation in the adipose tissue [12]. Increased activation of macrophages stimulates formation of cell foam resulting in arteriosclerosis [12]. Many diseases are the consequences of induced-LPS through intestinal barrier dysfunction (see Figure 2) [12]. Indeed, the translocation of LPS plays an important role in the development of various diseases such as inflammatory bowel disease [12].

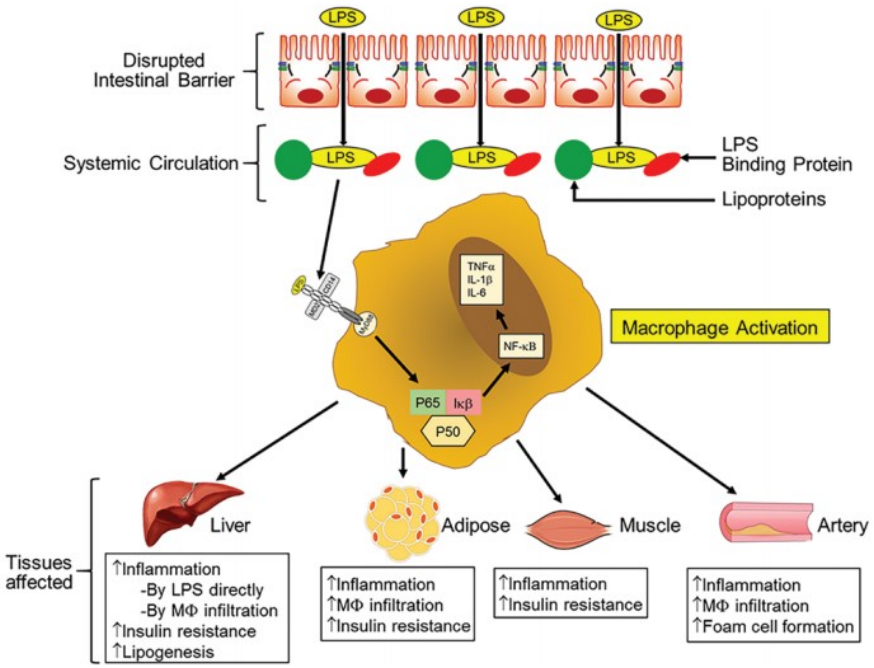

Figure 2: Repercussions of intestinal barrier dysfunction [12]. Increased LBP binds to LPS. This complex binds TLR-4 and activates macrophages. The stimulation of signal pathway of TLR-4 activates NF-kB and produces pro-inflammatory cytokines such as TNF-alpha, IL-10 and -6. The inflammation reaches different organs. Increased insulin resistance and lipogenesis affects the liver and leads to fatty liver disease. Increased insulin resistance and inflammation affects adipose tissue and are the main causes of the development of diabetes. Muscles are also affected by elevation in insulin resistance. The formation of foam cell in the arteries may trigger the development of atherosclerosis. Abbreviations: LPS: lipopolysaccharides; TNF-alpha: tumor necrosis factor alpha; NF-kB: Nuclear factor kappa B; IL: interleukins.

1.2 Fructose in the development of metabolic diseases

Fructose is a monosaccharide present in vegetables and fruits but also used as a sweetener by humans [16]. This specific sugar is a component of sucrose and also of inulin, which is a polymer of fructose [17]. Sucrose, composed in equal amount of glucose and fructose, is the most common sugar produced by plants [18]. Along with high-fructose corn syrup, they represent the most frequent sugars used as additives in the food industry, mainly in beverages but also manufactured food [19]. In the United States the average fructose intake is between 50 and 100 g and has been increasing between 1977 and 2007 but has been stabilizing this couple of years [17&20]. In contrary, the worldwide fructose intake has been increasing by 16% over 20 years [20]. The world health organization (WHO) recommends limiting the total monosaccharide intake to less than 10% per day [17]. Fructose is discussed as one of the risk factor of many metabolic diseases like obesity, nonalcoholic fatty liver disease (NAFLD) and diabetes type 2 [21]. Indeed, a study showed that high-fructose intake around 200g per day caused a 25% increase in metabolic diseases [22]. Excess of fructose consumption can result in fructose malabsorption in the small intestine [23]. In fact, development of metabolic diseases can also be induced with a fructose concentration around 20% [22]. Furthermore, the absorption of fructose is faster when administered with glucose [22]. Glucose and fructose pathways differ that's why the consequences on the health are not the same [24]. Glucose is insulin-dependent while fructose is insulin-independent [24]. Moreover, elevation of fructose intake has been reported to increase translocation of bacterial endotoxin, in contrast to glucose [25]. Fructose intake has been shown to alter intestinal barrier function and to elevate pro-inflammatory cytokines resulting in inflammation of the gut [25]. Indeed, a high-fructose diet alters the intestinal permeability and further promotes the development of metabolic diseases such as NAFLD and vascular endothelial dysfunction [25].

Fructose metabolism

Both, glucose and fructose are monosaccharides and have a similar chemical formula ($C_6H_{12}O_6$) and caloric value (4 kcal/g) [17]. Glucose is taken up via the sodium-glucose co-transporter 1 (SGLT1) on the apical membrane from the lumen into the enterocytes; and further via glucose transporter 2 (GLUT-2) on the basolateral membrane into the portal blood [26]. Fructose is absorbed in the small intestine and passes the intestinal lumen to the enterocytes through apical transport via GLUT-5 [27]. At the basolateral side of enterocytes, GLUT-2 transports fructose from the cytosol into the blood vessels [23]. In the small intestine, 90% of fructose is metabolized to glucose and lactate and is transported via portal blood [23]. Fructose is then transported across the portal vein into the liver [27]. In contrary, glucose is transported into the portal vein without being metabolized [23]. In the hepatocytes, fructokinase phosphorylates fructose to fructose-1-phosphate [28]. Fructokinase is not undergoing a feedback inhibition resulting into depletion of ATP and activation of adenosine monophosphate (AMP) [29]. In contrast, glucose is phosphorylated to glucose-6-phosphate via glucokinase and is then isomerized to fructose-6-phosphate [30]. Indeed, glucokinase is regulated by insulin and is repressed by glucagon [31]. Phosphofructokinase (PFK) catalyzes fructose-6-phosphate to frucose-1,6-bisphosphate [30]. PFK is allosterically regulated by citrate levels and ATP (see Figure 3) [30]. In contrary to glucose pathway, fructose catabolism is not regulated by feedback inhibition [32]. In fact, fructose-1-phosphate is hydrolyzed by triosephosphate isomerase to dihydroxyacetonephosphate and glyceraldehyde bypassing the regulation step of glucose production [32]. Fructose and glucose pathways meet at the dihydroxyacetonephosphate and glyceraldehyde-3-phosphate level [30]. Those substrates are needed for the gluconeogenesis, glycogenesis and lipogenesis [30]. Excess of fructose consumption leads to increased fructose concentrations in the portal vein resulting in activation of several pathways and development of many metabolic diseases such as NAFLD [33].

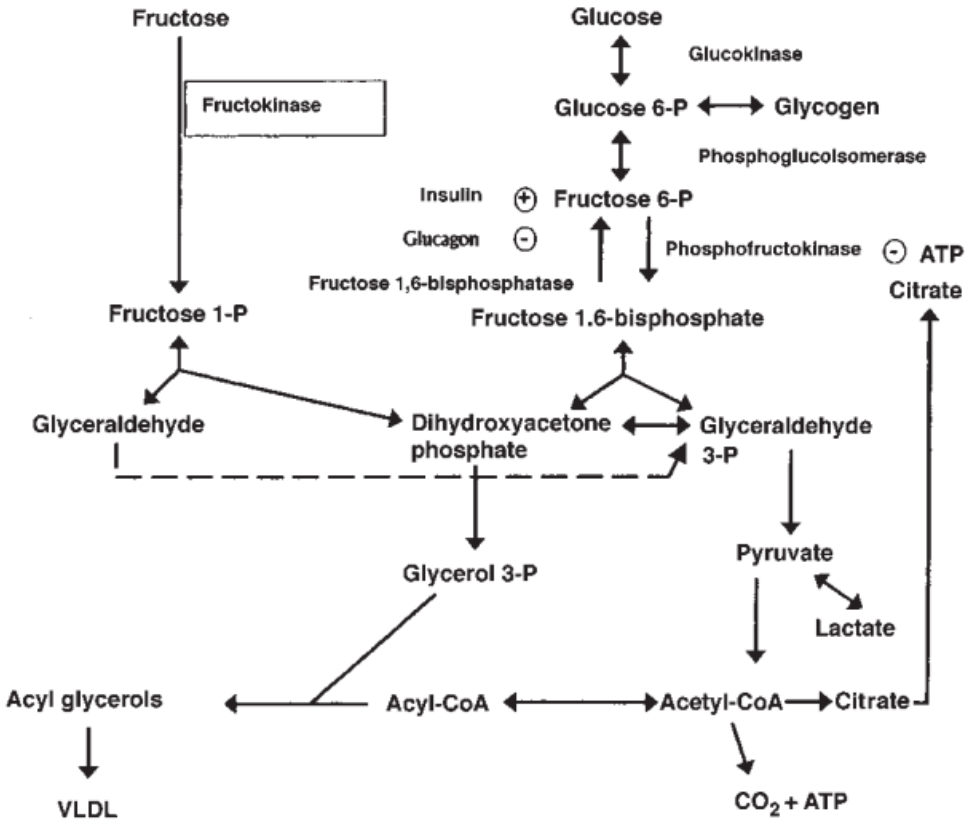

Figure 3: Metabolic pathways of glucose and fructose [30]. Fructokinase phosphorylates fructose to fructose-1-phosphate. After this, fructose-1-phosphate is hydrolyzed to dihydroxyacetonephosphate and glyceraldehyde. On the other hand, glucose is phosphorylated to glucose-6-phosphate via glucokinase and is isomerized to fructose-6-phosphate. Phosphofructokinase (PFK) catalyzes fructose-6-phosphate to frucose-1,6-bisphosphate. Substrates of fructose and glucose pathways are needed for the gluconeogenesis, glycogenesis and lipogenesis. ATP and citrate levels inhibit PFK. Insulin and glucagon regulates glucokinase. Abbreviations: CO_2: carbon dioxide; ATP: adenosine triphosphate; CoA: coenzyme A; VLDL: very low density lipoprotein.

Metabolic diseases

After metabolizing fructose to dihydroxyacetone phosphate and glyceraldehyde 3-phosphate, a small concentration of fructose is found in the blood circulation unlike glucose [34]. That is explained by the regulation of glucose via insulin in contrast to fructose which is not regulated by this hormone [34]. Glyceraldehyde 3-phosphate and other triose phosphate derivate are used for the production of pyruvate, acetyl-CoA and de novo lipogenesis (DNL) [34]. NAFLD is characterized by triglycerides accumulation in the liver [35]. Indeed, evidence suggests that elevation in DNL may contribute in the development of NAFLD [36]. Results of a study showed that chronic fructose intake induces DNL enzymes [36]. In fact, higher fructose concentrations are provided to the liver previously assimilated via portal vein [37]. In contrary to fructose, glucose does not produce metabolites leading to stimulation of DNL [37]. In fact, when fructose reaches the liver, it activates carbohydrate responsive element-binding protein (ChREBP) and sterol regulatory element binding transcription factor 1c (SREBP1c) [38]. Those transcription factors induce the upregulation of enzymes involved in fructolysis, glucose production and DNL [38]. High concentration of fructose in enterocytes increases GLUT-5 expression [34]. Results of a study suggest that ChREBP is a regulator of GLUT-5 expression and plays an important role in fructose-induced metabolic diseases such as diabetes type 2 [39]. Furthermore, chronic fructose intake is associated with increased insulin resistance, hepatic steatosis and hypertriglyceridemia [24]. A human study showed that a high-fructose diet induced visceral and intrahepatic fat [24]. Furthermore, due to stimulation of DNL, an increase in VLDL-triacylglycerol and inhibition in lipid oxidation resulting in hepatic insulin resistance has been documented as consequences of fructose consumption [24]. Lipid accumulation is generating toxic metabolites such as fatty acyl CoA, diacylglycerol and high triglycerides (TGs) concentration [40]. An elevation in insulin receptor substrate 1 (IRS-1) phosphorylation has been observed resulting in reduction of insulin signaling [41]. In fact, the inhibition of free fatty acids (FFA) esterification promotes the circulation of FFA into the liver and causes insulin resistance [42]. Fast phosphorylation of fructose to fructose-1-phosphate results in depletion of ATP followed by a degradation of AMP [37]. This increases purine pathway and elevates the synthesis of uric acid [37]. High uric acid levels in blood can lead to hyperuricemia and is responsible for renal dysfunction (see Figure 4) [37].

Moreover, uric acid activates ROS production through transforming growth factor B (TGF-B) and nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase [43]. The excess of ROS production by mitochondria leads to endoplasmic reticulum (ER) stress and inflammation in the liver [43]. In addition, ER stress is also caused by accumulation of lipids on ER membrane [43].

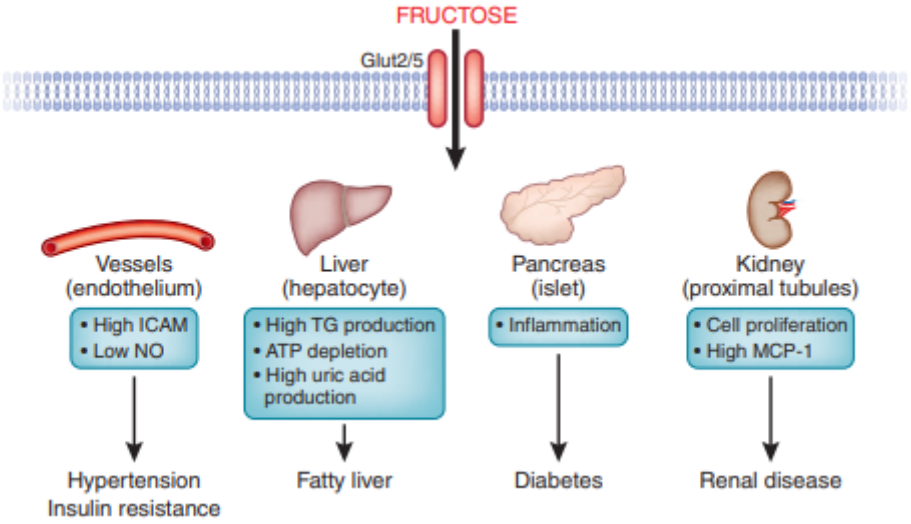

Figure 4: Effects of fructose in the development of metabolic diseases [22]. Fructose intake increases cell proliferation and MCP-1 concentration in the kidney leading to renal disease. High TG production, ATP depletion and elevation in uric acid production have been reported as effects of fructose intake in the liver resulting in fatty liver. Fructose induces inflammation in the pancreas leading to diabetes. Fructose elevates ICAM and inhibits NO leading to hypertension and insulin resistance. Abbreviations: Glut: glucose transporter; ATP: adenosine triphosphate; ICAM: intercellular adhesion molecule; NO: nitric oxide; TGs: triglycerides; MCP-1: monocyte chemoattractant protein-1.

1.3 Phosphatidylcholine: chemical structure, biological function and possible health effects

Cell membranes are composed by the majority of phospholipids (PLs) and are formed from hydrophilic and hydrophobic groups providing the structure with an amphiphilic character [44] .The glycerol phospholipid phosphatidylcholine (PC) is the most abundant phospholipid in eukaryotic cells with a total of 55% lipids [45]. PC is composed of hydrophobic two fatty acid, glycerol, phosphoric acid and a hydrophilic choline group (see Figure 5) [44]. The biological function of these PLs is due to the amphiphilic character [46]. PC along with other PLs forms a bi-layer capable of building cells and organelles membranes [47]. In fact, PC provides structure but also flexibility in the cell membrane [46]. These phospholipids-bilayers act as a selective permeable barrier and regulate absorption of ions and nutrients [47]. PC is found in the external leaf of cellular membrane and plays an important role in the maintain of membrane integrity [47]. In addition, PC contributes in intracellular signaling processes including deoxyribonucleic acid (DNA) replication, transport and secretion [48]. In fact, the fatty acid part is important for the membrane fluidity and furthermore for the creation of lipid rafts [48]. These transport saturated fatty acid and are implicated in cellular proliferation and apoptosis [48]. Another role of PC is the supply of unsaturated fatty acid and acts as a precursor in the synthesis of eicosanoids [48]. In fact, eicosanoids more specifically prostaglandins E1 and E2 and epidermal growth factor (EGF) elevates PC concentration in the luminal side which prevent from bacterial translocation [49]. The hydrophobicity of PC protects the intestinal lumen against bacteria and harmful substances [49]. Also, PC is related in the hepatic secretion of very low density lipoprotein and further in regulating the transport of cholesterol and lipids [47]. In addition, the positively charged choline group, an essential nutrient is a precursor of the neurotransmitter acetylcholine [50]. This amine plays an important role in the modulation of gene expression and cell signaling molecules such as platelet-activating factor (PAF) [50]. Besides being a component of cell membrane, PC is also known as a natural surfactant and is used as additives in the food industry [51]. Moreover, PC is used in pharmaceutical but also industrial fields [51].

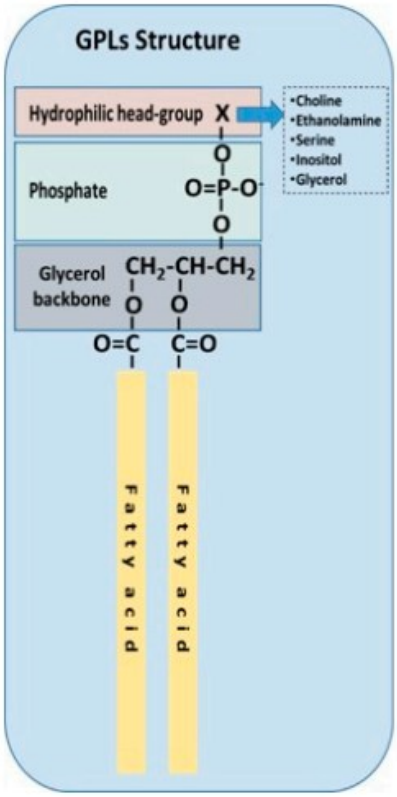

Figure 5: The structure of a phospholipid [46]. Glycerol phospholipids have the same basic components: two fatty acids, a glycerophosphoric acid and a hydrophilic group. PC is positively charged with hydrophilic choline group*.*

Health effects

The application of exogenous PC and lyso-PC was investigated in vitro to assess the possible effects in intestinal epithelial cells [52]. A reduction in F-actin assembly was observed, which is normally involved in the pro-inflammatory response, underlying the possible anti-inflammatory properties of PC [52]. In fact, PC inhibited TNF-aplha and the activation of NF-kB signaling leading to the blockade of MAPK pathway [53]. Indeed, those pathways have already been showed to alter the intestinal permeability resulting in the development of chronic intestinal inflammations such as ulcerative colitis (UC) [53]. In fact, patients diseased with UC have a 70% decrease in PC concentration in the colonic mucus [54]. Furthermore, the supplementation of retarded PC showed an improvement of the disease by increasing PC concentration and restoring the structure and density of the mucus [54]. Moreover, the regeneration

of the hydrophobic layer was observed which is important for the mucosal defense mechanism against diseases [54]. Addition of PC changes the lipid associations in the membrane and impacts membrane-dependent signaling disturbing the initiation of pro-inflammatory molecules [52]. LPS increases phospholipase A2 (PLA2) and damages PC resulting in the impairment of intestinal barrier integrity [55]. On rats, the oral administration of PC seems to prohibit LPS-induced intestinal permeability [55]. In addition, a decrease in plasma endotoxin concentrations was documented in rats treated with PC and further implicating the protective effects on intestinal epithelial cells [56]. Moreover, it has been suggested that PC can repair intestinal barrier dysfunction [56]

Another interesting effect of PC was observed in liver diseases [56]. Under PC treatment, rats with induced liver injury showed amelioration in liver function as well as a decrease in portal pressure [56]. Furthermore, inflammatory cell-infiltration and submucosal oedema were mostly inhibited after PC administration [56]. It has also been established that PC has a hepatoprotective effect and reduces liver fibrosis [56]. In vivo, a PC rich diet demonstrated a positive effect by decreasing fatty acid synthesis and increasing beta-oxidation resulting in decreased TGs and cholesterol levels [57]. In addition to this, PC may block the accumulation of fatty acid in the liver leading to attenuation of NAFLD [57]. Indeed, choline deficiency leads to NAFLD and furthermore to cirrhosis [57]. Besides those effects on the intestinal membrane and liver, PC has also shown benefits in the improvement of cognitive functions [47]. In fact, the anti-oxidative effects of polyunsaturated fatty acids of PC have been used in Parkinson and Alzheimer diseases [47]. On the other hand, a decrease in NSAIDs-induced gastric inflammation has been observed under PC supply [48]. In addition, this PL demonstrated a reduction in rheumatoid arthritis but also hepatic cancer cells [48].

1.4 Objectives

Results of several studies suggest that dietary fructose intake may add to the development of metabolic diseases including non-alcoholic fatty liver disease [24&25]. Studies further suggest that fructose not only through it insulin-independent metabolism but also through more direct effects on intestinal barrier function and subsequently an increased elevated permeation of basolateral endotoxin may add to the subclinical inflammation afflicted with the development of metabolic diseases [24&25]. Results of studies craving model of intestinal barrier dysfunction suggest that phosphatidylcholine may have beneficial effects on intestinal barrier function [55&56]. However, if phosphatidylcholine also has beneficial effects on intestinal barrier function in settings of metabolic diseases and herein especially fructose-induced metabolic alterations has not been studied in great depth yet. Starting from this background the main aim of the present study was to assess if phosphatidylcholine affects fructose-induced alterations of intestinal barrier function.

The following questions were addressed:

1. Does phosphatidylcholine affect fructose-induced changes in intestinal permeability in an everted sac ex-vivo model?
2. Does phosphatidylcholine alter the protein levels of occludin, arginase-2 and ROCK2 phosphorylation in an everted sac ex-vivo model?
3. Does phosphatidylcholine influence the arginase activity in an everted sac ex-vivo model?

2. Materials

2.1 Preparation of everted gut sac experiments

Equipment

Scale	Sartorius AG Göttingen, Germany
Water bath	GFL, Gesellschaft für Labortechnik GmbH, Burgwedel, Germany
Speedvac SPD1030	Thermo Fisher Scientific, Waltham, USA
Oxymix	Air liquide, Schwechat, Austria

Chemical Reagents

Sodium chloride	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Potassium chloride	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Magnesium sulfate anhydrous	Alfa Aesar, Thermo Fischer GmbH&Co, Kandel, Germany
Calcium chloride dehydrate	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
HEPES	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Potassium dihydrogen phosphate	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Bovine Serum Albumin (BSA)	Sigma-Aldrich Chemie GmbH, Steinheim, Germany
Fructose	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Xylose	Merck Chemicals GmbH, Darmstadt, Germany
Sodium deoxycholate	Sigma-Aldrich Chemie GmbH, Steinheim, Germany

Phosphatidylcholine	Sigma-Aldrich Chemie GmbH, Steinheim, Germany
Buffered saline solution (DPBS)	PAN- Biotech GmbH, Aidenbach, Germany
Dichloromethane	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Methanol	Carl Roth GmbH & Co. KG, Karlsruhe, Germany

Buffers and solutions

Table 1: Solution for preparation of KRH-Buffer

	Substances	**Mass**
Solution 1	1.5M NaCl	67.2 g/L
	50mM KCl	3.73 g/L
	12mM MgSO4	1.44 g/L
	20Mm CaCl2*2H_20	2.94 g/L
Solution 2	250 mM HEPES	59.5 g/L
Solution 3	10 mM KH_2PO4	1.36 g/L

Table 2: Preparation of KRH-Buffer for the incubation of everted sac build from small intestinal tissue

KRH buffer	**To 1000 mL**
Solution 1	100 mL
Solution 2	100 mL
Solution 3	100 mL
dd H_20	500 mL
pH	7.4
BSA	2 g
Water	200 mL

Table 3: Preparation of phosphatidylcholine stock solution (10mL)

Substances	Mass
0.6 mM Phosphatidylcholine	4.55 mg
0.15 mM Sodium deoxycholate	0.62 mg
Methanol	5 mL
Dichloromethane	5 mL

2.2 Measurement of arginase activity

Equipment

Tissue-Lyser II	Qiagen, Hilden, Germany
PCE-MSR 100 Magnetic Stirrer	PCE, GmbH, Germany
Centrifuge Z216 MK	HERMLE Labortechnik GmbH, Wehingen, Germany
Mixing block	BIOER, Hangzhou, China
Thermo-shaker	BIOSAN, Riga, Latvia
96-Well-Plate	VWR International GmbH, Darmstadt, Germany
SpectraMax M3 Multi-Mode Microplate Reader	Molecular Devices, Biberach an der Riss, Germany

Chemical Reagents

Tris-hydrochloride	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Triton-X-100	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Manganese (II) chloride tetrahydrate	Alfa Aesar, Thermo-Fischer, GmbH&Co, Kande, Germany

Protease inhibitor complex	Sigma-Aldrich Chemie GmbH, Steinheim, Germany
L-Arginine	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Urea	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Alpha-isonitrosopropiophenone	Sigma-Aldrich Chemie GmbH, Steinheim, Germany
Sulfuric acid (1N)	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Phosphoric acid	Sigma-Aldrich Chemie GmbH, Steinheim, Germany
Absolute ethanol	Merck Chemicals GmbH, Damstadt, Germany

Buffers and solutions

Table 4: Preparation of the homogenization buffer (6mL)

Substances	Volume (µL)
0.1% Triton-X-100	30
Protease inhibitor cocktail	30
50mM Tris-HCl (pH 7.5)	150
10mM $Mncl_2$	300
dd H_20	5490

Table 5: Preparation of the urea standard

	Urea	Volume	Concentration
A	80 mg	10 mL water	800 µg/100µL
B	100 A	900 µL water	0.8 µg/µL
C	500 B	500 µL water	0.4 µg/µL
D	500 C	500 µL water	0.2 µg/µL
E	500 D	500 µL water	0.1 µg/µL
F	500 E	500 µL water	0.05 µg/µL
G	500 F	500 µL water	0.025 µg/µL

2.3 Assessment of intestinal permeability using xylose assay

Equipment

Thermo-shaker	BIOSAN, Riga, Latvia
Centrifuge Z216 MK	HERMLE Labortechnik GmbH, Wehingen, Germany
96-Well-Plate	VWR International GmbH, Darmstadt, Germany
SpectraMax M3 Multi-Mode Microplate Reader	Molecular Devices, Biberach an der Riss, Germany

Chemical Reagents

Xylose – assay kit: Megazyme, Bray, Co. Wicklow, Ireland

Bottle 1: Buffer
Bottle 2: NAD+ plus ATP
Bottle 3: Hexokinase suspension
Bottle 4: XDH/XMR solution
Bottle 5: D-xylose standard solution

Buffers and solutions

Table 6: Preparation of the standard

	Solutions	Volume	Concentration (mg/mL)
A	130 µL of standard solution	1235 µL water	0.5
B	683ul A	683 µL water	0.25
C	683ul B	683 µL water	0.125
D	683ul C	683 µL water	0.0625
E	683ul D	683 µL water	0.03125
F	683ul E	683 µL water	0.015625
G	683ul F	683 µL water	0.007813
H	683ul water	683 µL water	0

Table 7: Preparation of the Mastermix

	Volume
Solution 1	2 000 µL
Solution 2	2 000 µL
Solution 3	100 µL

2.4 Detection of protein levels using Western Blot

Equipment

PVDF-Membranes	BioRad Laboratories, Munich, Germany
Extra Thick Western blotting	Thermo Fisher Scientific, Waltham, USA
Impuls Welding machine	ALLPAX GmbH & Co. KG, Papenburg, Germany
Cover foils	ALLPAX GmbH & Co. KG, Papenburg, Germany

Mini-PROTEAN® Glass Plates	BioRad Laboratories, Munich, Germany
Mini-PROTEAN® Short Plates	BioRad Laboratories, Munich, Germany
Mini-PROTEAN® Spacer Plates	BioRad Laboratories, Munich, Germany
Mini-PROTEAN® Comb 10 well	BioRad Laboratories, Munich, Germany
Mini-PROTEAN® Tetra System Cell	BioRad Laboratories, Munich, Germany
Mini-PROTEAN® Gel Releasers	BioRad Laboratories, Munich, Germany
Orbi-Blotter	Benchmark, Frankfurt, Germany
PowerPac™ HC	BioRad Laboratories, Munich, Germany
Trans-Blot Turbo Transfer System	BioRad Laboratories, Munich, Germany
Mixing Block	BIOER, Hangzhou, China
Multifunctionsrotor PS-M3D	Grant Instruments Ltd, Cambridge, England
Centrifuge Z216 MK	HERMLE Labortechnik GmbH, Wehingen, Germany
ChemiDoc™ Imaging Systems	BioRad Laboratories, Munich, Germany

Chemical Reagents

TRIS	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Acrylamide	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Ammoniumperoxydisulphate	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Tetramethylethylendiamine	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Nonfat dried milk powder	PanReac, Barcelona, Spain
Sodiumdodecylsulfate	Carl Roth GmbH & Co. KG, Karlsruhe, Germany

Dithiothreitol	Sigma-Aldrich Chemie GmbH, Steinheim, Germany
Glycine	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Mercaptoethanol	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Bromophenol Blue	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Glycerol	VWR International GmbH, Darmstadt, Germany
Ethylenediaminetetraacetic acid	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
3-(N-Morpholino) propan sulfonic acid) (MOPS)	Sigma-Aldrich Chemie GmbH, Steinheim, Germany
Tween ® 20	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Octylphenoxy poly(ethyleneoxy)ethanol (IGEPAL)	Sigma-Aldrich Chemie GmbH, Steinheim, Germany
Methanol	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Bovine Serum Albumin (BSA)	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Precision Plus Protein Standard	BioRad Laboratories, Munich, Germany
Potassium chloride	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Sodium chloride	Carl Roth GmbH & Co. KG, Karlsruhe, Germany
Protease inhibitor cocktail	Sigma-Aldrich Chemie GmbH, Steinheim, Germany
Phosphatase inhibitor cocktail 2	Sigma-Aldrich Chemie GmbH, Steinheim, Germany
Phosphatase inhibitor cocktail 3	Sigma-Aldrich Chemie GmbH, Steinheim, Germany

SuperSignal™ West Dura Extended Duration Substrate	Thermo Fisher Scientific, Waltham, USA

Primary antibodies

ROCK 2 (phospho Ser1366) antibody	GeneTex, California, USA
ROCK 2 antidbody	GeneTex, California, USA
Occludin monoclonal antibody	Invitrogen, Thermo Fisher Scientific, Waltham, USA
Beta-Actin antibody	Cell Signaling Technology, Leiden, Netherland
Arginase-2 antibody	Cell Signaling Technology, Leiden, Netherland

Secondary antibodies

Anti-rabbit IgG	Cell Signaling Technology, Leiden, Netherland
Anti-mouse IgG	Cell Signaling Technology, Leiden, Netherland

Buffers and solutions

Table 8: Composition of 10% separating gel for 2 gels with 1 mm Spacer

Substances	Volume (µL)
Aqua bidest	4170
1.5 M Tris (pH 8.8)	2600
30% Acryl/Bis	3330
10% SDS	100
10% APS	100
TEMED	6

Table 9: Composition of 5% stacking gel for 2 gels with 1mm Spacer

Substances	Volume (µL)
Aqua bidest	1512.5
0.5M Tris	625
30% Acryl/Bis	312.5
10% SDS	25
10% APS	25
TEMED	3

Table 10: Preparation of the extraction buffer supplemented with proteinase inhibitors

Substances	*Volume*
Protease Inhibitor cocktail	20 µL
Phosphatase Inhibitor cocktail 2	20 µL
Phosphatase Inhibitor cocktail 3	20 µL
RIPA-Buffer	1 mL

Table 11: Composition of RIPA-Buffer

Substances	*Concentration*
MOPS (3-(N-Morpholino) propan sulfonic acid	20 mM
NaCl	150 mM
EDTA	1 mM
IGEPAL	1 % (v/v)
SDS	0,1 % (w/v)

2.5 Determination of protein concentration according to Bradford

Equipment

96-Well-Plate	VWR International GmbH, Darmstadt, Germany
SpectraMax M3 Multi-Mode Microplate Reader	Molecular Devices, Biberach an der Riss, Germany

Chemical Reagents

Bovine Serum Albumin (BSA)	Sigma-Aldrich Chemie GmbH, Steinheim, Germany
Protein Assay Dye Reagent Concentrate	BioRad Laboratories, Munich, Germany

Buffers and solutions

Table 12: Preparation of the standard

	Protein	Extraction buffer (µL)	Concentration (mg BSA/mL)
A	2 µL BSA Stock solution	198	1.0
B	100 µL A	100	0.5
C	100 µL B	100	0.25
D	100 µL C	100	0.125
E	100 µL D	100	0.0625
F	---	100	0

2.6 Consumables

Piston stroke pipette	Eppendorf AG, Hamburg, Germany
Serological pipette	Sarstedt AG & Co, Nürnbrecht, Germany
Multichannel pipette	Brand GmbH & Co KG, Wertheim, Germany
Pipette tip (10, 200 & 1000 µL)	Sarstedt AG & Co, Nürnbrecht, Germany
Reaction container (0.5, 1.5 & 2 mL)	Sarstedt AG & Co, Nürnbrecht, Germany
Falcon Tubes (15 & 50mL)	Sarstedt AG & Co, Nürnbrecht, Germany
Vortex Reax 2000	Heidolph-Instruments, Schwabach, Germany
Aluminum foil	Carl Roth GmbH & Co KG, Karlsruhe, Germany
Weighing dishes	Carl Roth GmbH & Co KG, Karlsruhe, Germany
Stop watch	Carl Roth GmbH & Co KG, Karlsruhe, Germany
Magnetic Stirrer RSM-10HS	Phoenix Instrument GmbH, Garbsen, Germany

Rotalibo-Magnetic sticks	Carl Roth GmbH & Co KG, Karlsruhe, Germany
Orion 420A pH Meter	Orion Research, Inc., Jacksonville, USA
Rotilabo®-Tubes racks	Carl Roth GmbH & Co KG, Karlsruhe, Germany
Gloves	STARLAB GmbH, Hamburg, Germany
Labpens	VWR International GmbH, Darmstadt, Germany
Forceps	Laboratory equipment
Buffer tank	Laboratory equipment
Nitrogen container	Karlsruher Glastechnisches Werk-Schieder,KGW-Isotherm GmbH, Karlsruhe, Germany
Razor blade	Hugo Herkenrath GmbH & Co.KG, Solingen, Germany

2.7 Software

GraphPad Prism 8.0	GraphPad Software Inc., La Jolla, USA
Image Lab 6.0 Software	BioRad Laboratories, Munich, Germany
SoftMax Pro7 Software	Molecular Devices GmbH, Biberach an der Riss, Germany

3. Methods

3.1 Everted gut sac experiment: an ex vivo approach

3.1.1 Preparation of everted sac

Female C57BL/6J mice were bred in the own animal facility at the University of Vienna. 3-5 months old female naïve C57BL/6J mice were used to obtain small intestinal tissue for the building of everted sacs. After cervical dislocation, the proximal intestinal tissue was collected, rinsed with PBS and transferred to a dish with ice-cold KRH-Buffer. The intestine was then gently placed on a gavage needle using forceps and fixed with a simple knot on one end. The intestinal tissue was then everted above the knot as showed in the Figure 6 [58]. Once the intestine was everted, the first knot was removed and the intestine was cut into equal pieces of approx. 3.5 cm. Each piece of tissue was assigned to an experimental condition. To form the everted sac, one end was closed with a double knot to avoid leakiness. A needle was inserted into the other end of the intestine and 100µL KRH-Buffer was injected before closing the opposite end with a double knot. Before the incubation, length between the knots and the weight of each sac tissue were measured. The everted gut sacs were randomly put into falcon tubes containing different test solutions and the tubes were placed in a water bath at 37ºC for 55 min. During the incubation time, solutions were continuously oxygenated (95%O_2/5%CO_2) [59]. After 55 min, the everted sacs tissue were transferred into a solution of 0.1% xylose dissolved in KRH-Buffer and were incubated for 5 Min at 37ºC to assess permeability. After 5 min incubation, liquid from inside of the sacs was collected in a vial and stored at -20°C for further analysis. The weight of the intestinal tissue without liquid inside was then determined. The tissue was cut in 3 pieces, snap frozen in liquid nitrogen and stored at -80 °C for measuring arginase activity and protein expression of B-Actin, occludin, arginase-2, ROCK 2 and phospho ROCK 2.

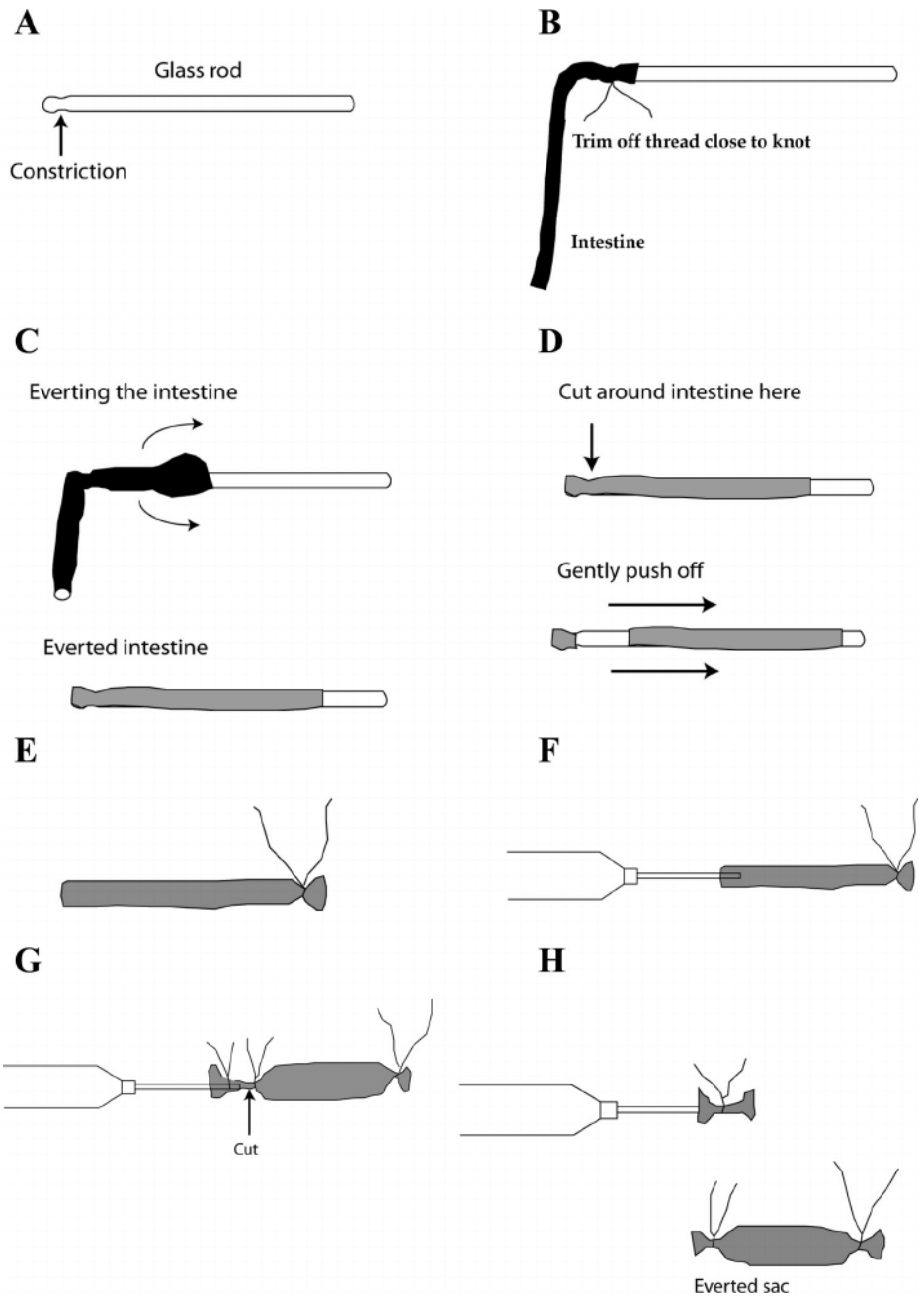

Figure 6: Everted sac technique [58].

3.1.2 Experimental conditions

To analyze the effect of phosphatidylcholine on fructose-induced intestinal barrier dysfunction, the following conditions were used as listed in Table 13. For the preparation of the different solutions and buffers see Table 1-3.

Table 13: Experimental conditions for the incubation of everted sac build from small intestinal tissue

Conditions	Abbreviation
1. Control	C
2. Fructose 5mM	F
3. Control+ Sodium deoxycholate 0.15mM	C/SD
4. Fructose+ Sodium deoxycholate 0.15mM	F/SD
5. Control+ Sodium deoxycholate 0.15mM + Phosphatidylcholine 0.6mM	C/PC
6. Fructose+ Sodium deoxycholate 0.15mM + Phosphatidylcholine 0.6mM	F/PC

3.2 Measurement of arginase activity in duodenal tissue

A protocol to measure the arginase activity in duodenum was established in our lab based on the method reported by Corraliza et al [60]. In short, approximatively 15 mg frozen intestinal tissue was homogenized with 100µL of the homogenization buffer (composition see Table 4) and lysed for 30 sec with a frequency of 30/sec in the Tissue-lyser. Homogenates were centrifuged at 4°C for 10 min at maximum speed. The supernatant was transferred into a new vial and protein concentration was determined according to the method of Bradford. To assess arginase activity, serial dilutions of the samples were made. The dilution of 1/250 was incubated in a heating block at 55°C for 5 min to activate the enzyme. 50µL of L-arginine (0.5M pH 9.7) and 50µL of the activated samples were mixed together. A sample blank was necessary and was made with 50µL sample + 50µL water. The lysate was incubated at 37°C for 1 h and the reaction was stopped by adding 800µL of an acid mixture H_2S0_4:H_3PO_4:H_20 (1:3:7). Meanwhile, a calibration curve was made with an increasing urea concentration between 2.5 and 80µg (see Table 5). 100µL urea in appropriate concentration and 800µL acid mixture were mixed together. After addition of 50µL of 9% alpha-isonitrosopropiophenone (ISPF) dissolved in 100% ethanol, the samples were heated for 45min at 100°C. 200µL of each samples,

sample blanks and standards were pipetted in a 96 well plate. Samples and urea standards were pipetted as triplicates and sample blanks as duplicates. The plate was incubated for 10 min in the Thermo-shaker at 60°C. This step was important to remove the fogginess and the crystals which can interfere with the results. Absorbance was measured with a spectrophotometer at 540nm.

3.3 Measurement of xylose permeation as measure of intestinal permeability

Intestinal permeability of everted gut sacs was analyzed using xylose. In this assay, D-xylose is catalyzed by xylose mutarotase to B-D-xylose [61]. B-D-xylose in the presence of NAD^+ is oxidized by B-xylose dehydrogenase to D-xylonic acid + NADH + H^+ [61]. NADH is then measured at 340nm [61]. The xylose assay was performed using a commercial available kit and xylose concentration was measured as indicated by manufacture. 65 µL from the sample was added to 617.5 µL H_20. 210µL of the samples and standards (standards are made as described in the Table 6) were pipetted in 96 well plate in triplicates. 82 µL of mastermix (see Table 7) was added to each well and incubated in the Thermo-shaker for 4 min at room temperature. After that, the plate was measured at 340 nm in a spectrophotometer and the absorbance A1 was determined. 5µL of the solution 4 was added quickly in each well. The plate was again incubated in the Thermo-shaker for 6 min at room temperature. A second measurement of absorbance at 340 nm was performed to determine absorbance A2. For the calculation A2 was subtracted from A1. The difference between the absorbance was further used to calculate the concentration of samples using a standard curve.

3.4 Measurement of protein levels in intestinal tissue using Western blot

Western blot Method

Western blot is a technique used in molecular biology to detect specific proteins in samples [62]. Proteins are separated based on their molecular weight and are then transferred to a membrane [62]. After the incubation with the primary and secondary antibodies, an antigen-antibody complex is formed and the signal of the target protein can be then detected [62].

Protein lysates

For the extraction of proteins, 100 µL of lysis buffer containing RIPA-buffer (composition see Table 11), protease and phosphatase inhibitors mix (see Table 10) was added to the frozen tissue of each sample. Samples were then homogenized in the Tissue-lyser for 30 sec and centrifuged at 4° for 15 min at maximum speed.

SDS-PAGE

To separate the proteins according to their molecular weight, samples undergo a sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). Therefore, 10% acrylamide separating and 5% stacking gel (composition see Table 8 and 9) were made.

After the preparation of the homogenates, protein concentration of samples was determinate using a commercial available Bradford assay. Samples dilutions were made with extraction buffer (Table 10) to obtain between 1 – 3 µg/µL of protein to target specific needed ones.

After addition of SDS-Loading buffer and dithiothreitol (DTT), samples were heated in a mixing blot at 95°C for 5 min and centrifuged at 4°C at high speed (3.000 x g). 10µL of each samples and 2µL of Ladder solution were then loaded onto the gels. The electrophoresis ran 1.5h at 110 V.

Blotting

After SDS-PAGE, proteins were transferred onto polyvinylidene fluoride membranes (PVDF). For this, filter paper was impregnated with transfer buffer for 10 min. PVDF-membranes were activated in methanol for 10 sec. As shown in Figure 7, a blotting sandwich with filter paper, gel and membrane was prepared. Sandwich blotting was placed in Turbo Blotter and standard SD program was turned on (25V, 1A, 30 min). Proteins were then transferred from gel to membrane. The membranes were left to dry out.

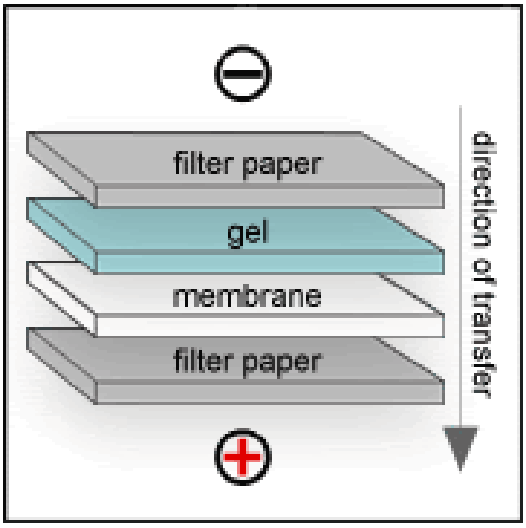

Figure 7: Blotting sandwich [63].

The next day, membranes were activated for 20 sec in methanol, rinsed for 5 sec in water and dyed with Red Ponceau for 3 min. This step allows the visualization of the protein and the confirmation of the protein transfer. The membranes were washed several times in water (each time for 20 sec) and were blocked 1h at room temperature with the appropriate blocking solutions: 2.5 % BSA in 1x TBST for the detection of ß-Actin, phosphorylated and unphosphorylated ROCK2 and 5% nonfat dried milk powder (NFDMP) in 1x TBST for the detection of occludin and arginase-2.

Incubation with primary and secondary antibody

Primary antibodies were previously diluted in the appropriate dilution buffer (see Table 14). Membranes were put in a plastic foil with the corresponding primary antibody and were incubated overnight at 4° C on a rotation machine.

Table 14: Dilution of primary antibodies

Primary antibodies	Dilution factor	Dilution buffer
ß-Actin	1:1000	2.5% BSA in 1x TBST
ROCK 2 (phosphor.)	1:1000	2.5% BSA in 1x TBST
ROCK 2 (unphos.)	1:1000	2.5% BSA in 1x TBST
Occludin	1:500	5% NFDMP in 1xTBST
Arginase-2	1:1000	5% NFDMP in 1x TBST

After the incubation, membranes were washed in 1xTBST for a total time of 30min (3x 10min). Membranes were incubated with secondary antibodies, anti-mouse-HRP or anti-rabbit-HRP, for 1.5h at room temperature. Secondary antibodies were diluted in the appropriate dilution buffer (see Table 15). After incubation with secondary antibodies, membranes were washed 3x10 min with 1xTBST before detection.

Table 15: Dilution of secondary antibodies

Secondary antibodies	Dilution factor	Dilution buffer
Anti-rabbit (ß-Actin)	1:5000	2.5% BSA in 1x TBST
Anti-rabbit (ROCK 2 (phosphor.)	1:5000	2.5% BSA in 1x TBST
Anti-rabbit (ROCK 2 (unphos.)	1:5000	2.5% BSA in 1x TBST
Anti-mouse (Occludin)	1:5000	5% NFDMP in 1xTBST
Anti-mouse (Arginase-2)	1:5000	5% NFDMP in 1x TBST

Detection

The membranes were incubated in peroxidase buffer and luminol solution 1:1 and placed into the dark for 5 min. Membranes were drained of excess developing solution and densitometric analysis were performed using ChemiDoc XRS System. For detecting the bands, the software Image Lab was used and the intensities of bands were measured and calculated for further results.

3.5 Protein determination using the method of Bradford

Bradford is a simple method to determine the total protein concentration in a sample [64]. The complex formed from the protein in the solution and the Bradford reagent causes a shift in the absorption spectrum [64]. The Coomassie dye, in reaction with the protein changes the color of the solution to blue [64]. The absorption spectrum shifted from 465nm to 595nm and it can be measured with a spectrophotometer [64]. Samples and extraction buffer were diluted 1:40 in distilled water. Standard curve was made with different concentrations of BSA and was used to calculate protein

concentration in mg/mL of each sample (see Table 12). 5µL from each standard dilution and each sample were pipetted in triplicates in a 96 well plate. 200µL color reagent (diluted 1:5) was added to each well. Absorbance was then measured for 5 min at 590 nm in a spectrophotometer.

3.6 Statistics

All data are presented as means ± standard error of mean (SEM). Statistical evaluation was performed using Graphpad Software (Prism, version 8.0). Grubbs test was used to identify outliers. Paired t-test was used to determine statistically significant differences between two parameters. One-way ANOVA test was applied to determine statistical differences between different groups. Differences were considered to be significant with a p value of < 0.05.

4. Results

4.1 Effect of fructose on permeability, tight junction protein and arginase activity as well as concentration and on ROCK in small intestinal tissue of everted sacs

To determine the effect of fructose on the intestinal permeability, everted sacs of small intestinal tissue obtained from Janvier mice were incubated with 5mM of fructose for 55 min followed by an additional 5 min incubation with fructose in the presence of xylose. Results are summarized in Figure 8. Permeation of xylose was significantly lower in tissue challenge with fructose when compared to intestinal tissue only incubated with KRH-buffer.

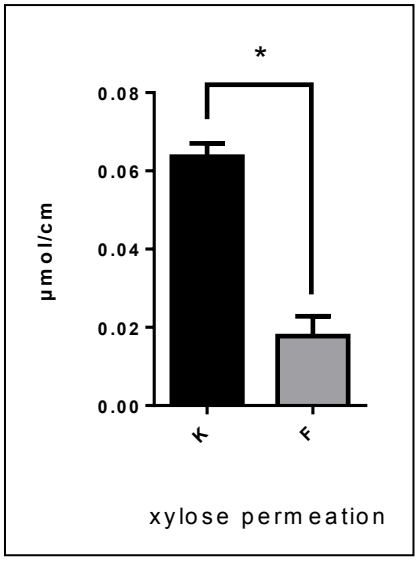

Figure 8: Effect of fructose on the xylose permeation in everted sacs of small intestinal tissue. Control group n= 5; fructose group n= 5. Data are presented as means ± SEM. Abbreviations: K= control; F= 5 mM fructose; * $p<0.05$.

To determine the protein concentration of the tight junction protein occludin, Western blot was performed. As shown in Figure 9, protein levels of occludin were only found lower in everted sac challenged with fructose when compared to controls.

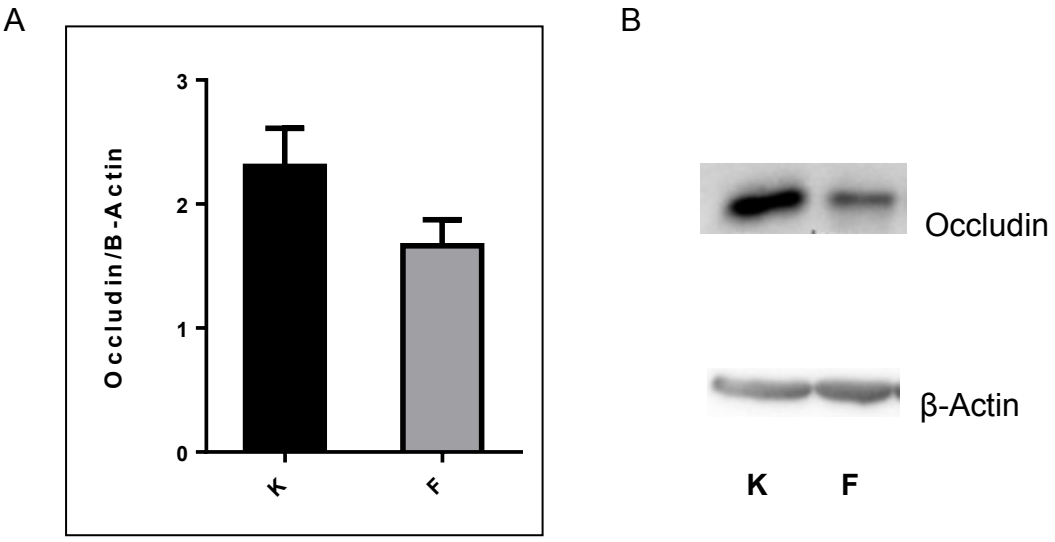

Figure 9: Effect of 5 mM fructose on occludin protein concentration in everted sacs of small intestinal tissue. (A) Relative protein concentration of occludin normalized to β-Actin. (B) Representative picture of a Western blot of occludin and β-Actin as well as densitometric analysis of blots. Control n=6, fructose n=6. Data are presented as means ± SEM. Abbreviations: K= control, F= 5 mM fructose.

While arginase-2 protein levels, being the arginase isoform pre-dominantly found in small intestinal tissue of mice, was not altered, activity of arginase was significantly lower in sac incubated with fructose when compared to sac only incubated with KRH-buffer (see Figure 10).

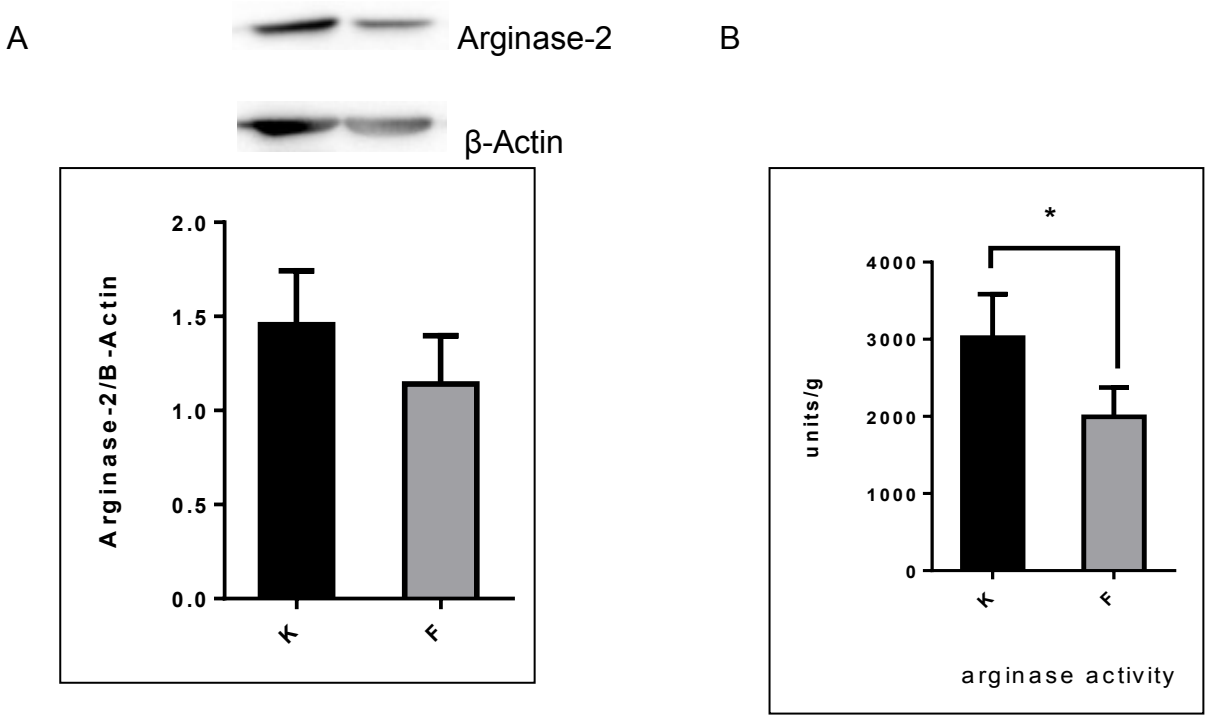

Figure 10: Effect of 5 mM fructose on (A) arginase-2 protein levels and (B) arginase activity in small intestinal tissue of everted sacs. (A) Relative protein concentration of arginase-2 normalized to β-Actin and representative picture of a Western blot of arginase-2 and β-Actin as well as densitometric analysis of blots. Control n=8, fructose n=8. Data are presented as means ± SEM. Abbreviations: K= control; F= 5 mM fructose; * $p<0.05$

As it has been suggested by the results of others that the Rho/Rock signaling pathway is involved in regulation of arginase but also tight junction, phosphorylation of ROCK2 was assessed by Western blot. No differences were found between groups (see Figure 11).

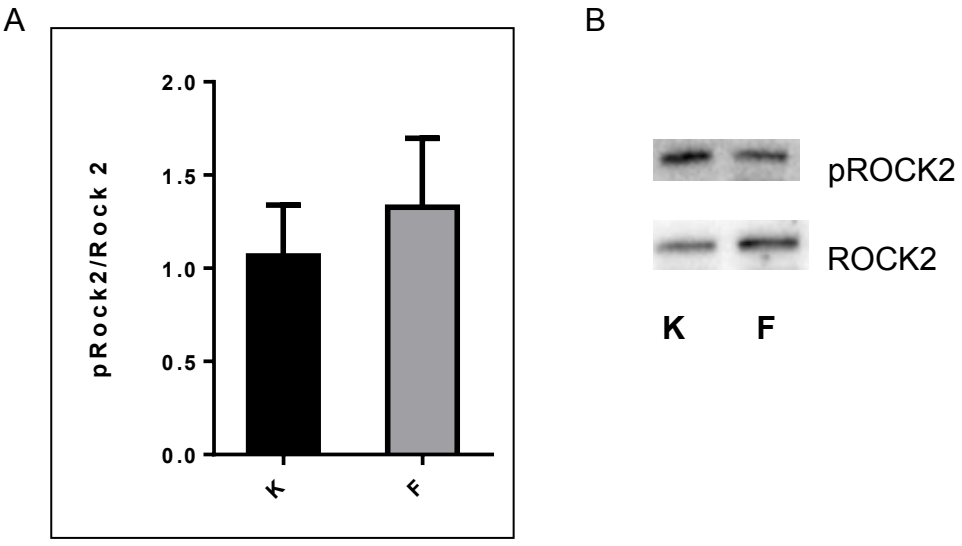

Figure 11: Effect of 5 mM fructose on ROCK2 protein concentration in everted sacs of small intestinal tissue. (A) Relative protein concentration of pROCK2 normalized to total ROCK2. (B) Representative picture of a Western blot of pROCK2 and total ROCK2 as well as densitometric analysis of blots Control n=4, fructose n=3. Data are presented as means ± SEM. Abbreviations: K= control, F= 5 mM fructose; p= phosphorylated.

4.2 Effect of phosphatidylcholine on marker of intestinal permeability, arginase activity and ROCK in everted sacs of small intestinal tissue

To determine if phosphatidylcholine affects fructose-induced alterations of intestinal permeability but also arginase activity, everted sacs of small intestinal tissue were incubated in the presence of 0.6 um phosphatidylcholine. Controls sacs were incubated with vehicle only. Contrasting the findings shown in Figure 4.1, xylose permeation was markedly high in everted sac challenged with fructose in the presence of vehicle. However, due to the lower and high interindividual variability difference didn't reach the level of significance (p=0.1783). The addition of PC attenuates the effects of fructose on xylose permeation (see Figure 12).

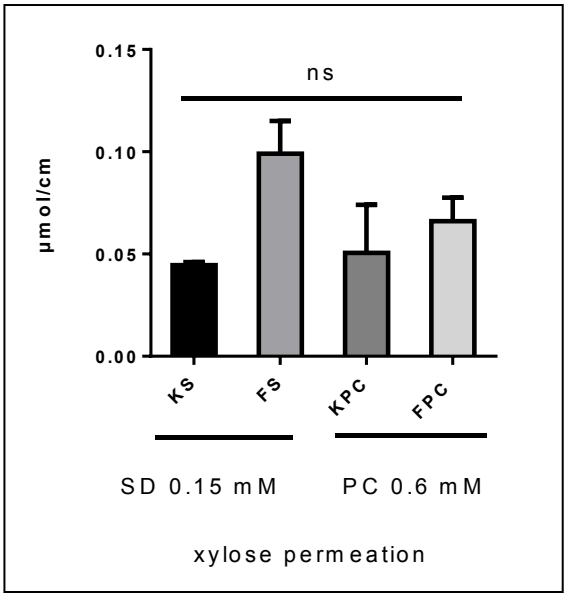

Figure 12: Effect of fructose and PC on xylose permeation in everted sacs of small intestinal tissue. For KS, FS and KPC n =2, FPC n= 3. Data are presented as means ± SEM. Abbreviations: KS= control + sodium deoxycholate; FS= 5 mM fructose + sodium deoxycholate; KPC= control + sodium dexoychlate & phosphatidylcholine (1:4); FPC= 5 mM fructose + sodium deoxycholate & phosphatidylcholine (1:4); ns= not significant; SD= sodium deoxycholate; PC= phosphatidylcholine.

Fructose- and sodium deoxycholate-treated intestinal tissue showed no significant difference compared to intestinal tissue incubated in KRH-buffer and the vehicle (see Figure 13). The addition of phosphatidylcholine did not show any significant differences among the groups (p= 0.9410).

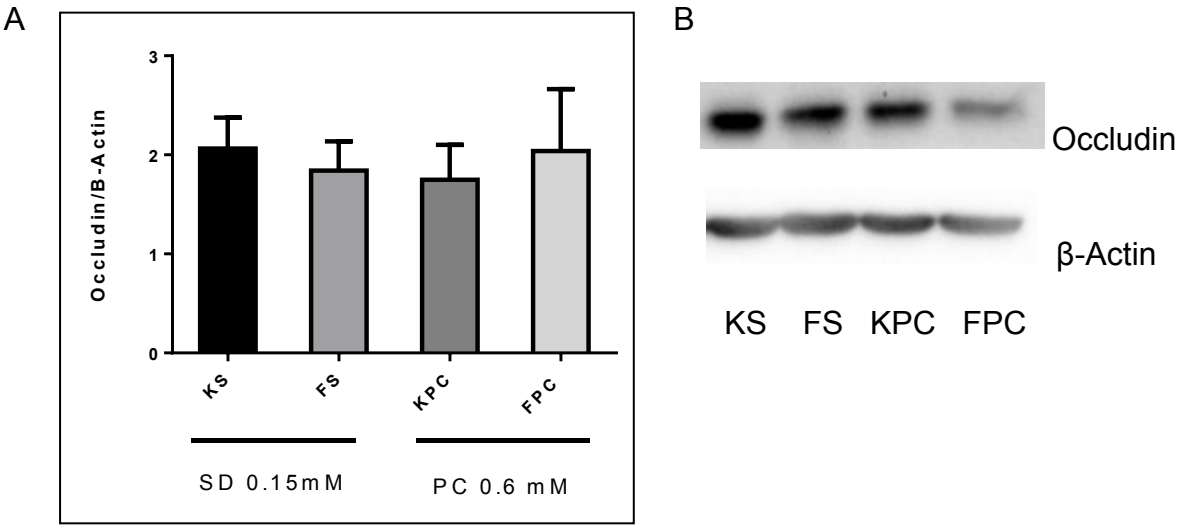

Figure 13: Effect of PC and fructose on occludin tight junction protein concentration in everted sacs of small intestinal tissue. (A) Relative protein concentration of occludin normalized to β-Actin. (B) Representative picture of a Western blot of occludin and β-Actin as well as densitometric analysis of blots. KS, KPC and FPC n=4, FS n=3. Data are presented as means ± SEM. Abbreviations: KS= control + sodium deoxycholate; FS= 5 mM fructose + sodium deoxycholate; KPC= control + sodium dexoycholate & phosphatidylcholine (1:4); FPC= 5 mM fructose + sodium deoxycholate & phosphatidylcholine (1:4); SD= sodium deoxycholate; PC= phosphatidylcholine.

To observe the effect of sodium deoxycholate, the substance was added to control and 5 mM fructose group. Results showed a significant decrease arginase activity in fructose- and sodium deoxycholate-treated tissue (FS) while the activity was increased in intestinal tissue treated with KRF-buffer and sodium deoxycholate (KS). Addition of phosphatidylcholine (KPC) did not show a significant decrease in the arginase activity when compared to FPC (see Figure 14) (p= 0.0060). KS and KPC showed a significant difference among the means.

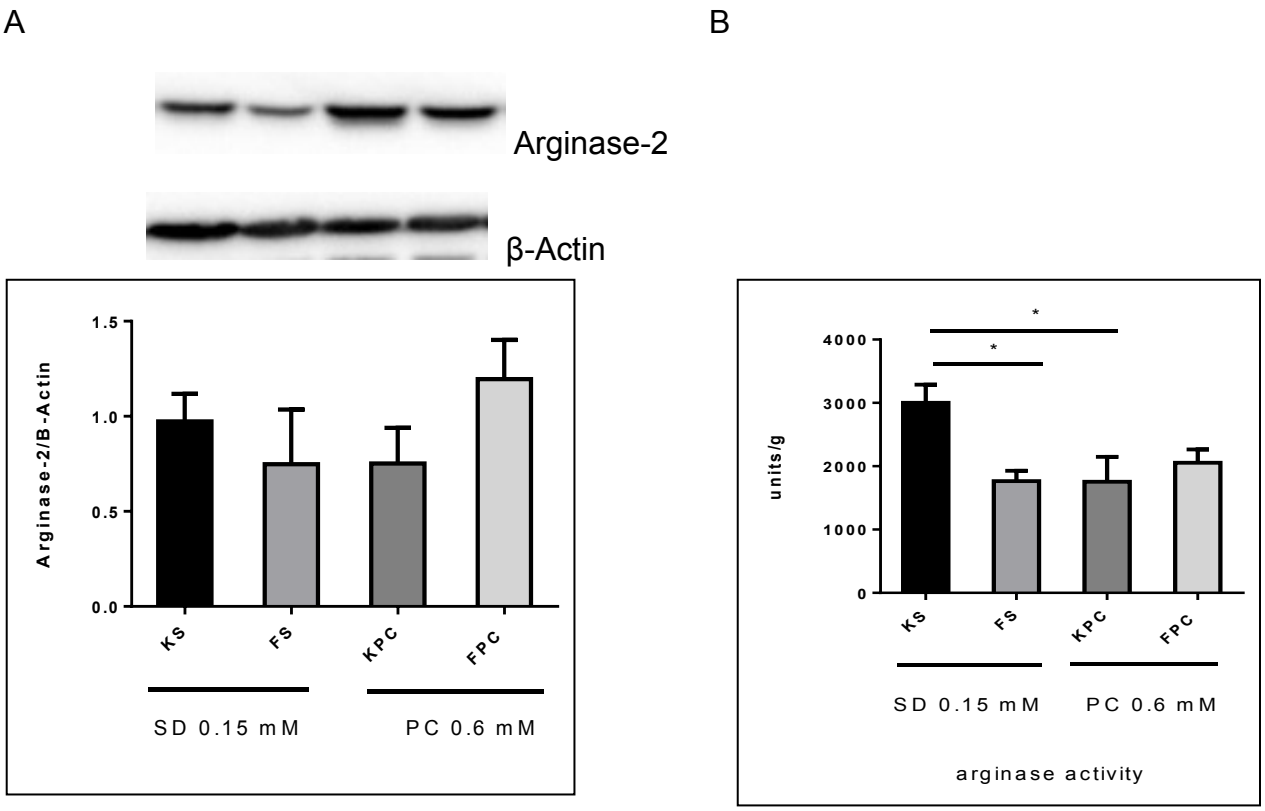

Figure 14: Effect of PC and fructose on (A) intestinal arginase-2 protein levels and (B) arginase activity in everted sacs of small intestinal tissue. (A) Relative protein concentration of arginase-2 normalized to β-Actin and representative picture of a Western blot of arginase-2 and β-Actin as well as densitometric analysis of blots. For KS, FS, KPC and FPC n=4; (B): KS n=9, FS n=7, KPC n=6, FPC n=8. Data are presented as means ± SEM. Abbreviations: KS= control + sodium deoxycholate; FS= 5 mM fructose + sodium deoxycholate; KPC= control + sodium dexoycholate & phosphatidylcholine (1:4); FPC= 5 mM fructose + sodium deoxycholate & phosphatidylcholine (1:4); SD= sodium deoxycholate; PC= phosphatidylcholine; * $p<0.05$

To determine the effect of phosphatidylcholine on intestinal tissue of everted sacs, ROCK2 protein expression was measured. Due to the small number of samples and variation, there was no significant differences between groups (see Figure 15). In addition, those groups had to be excluded from the statistical analysis.

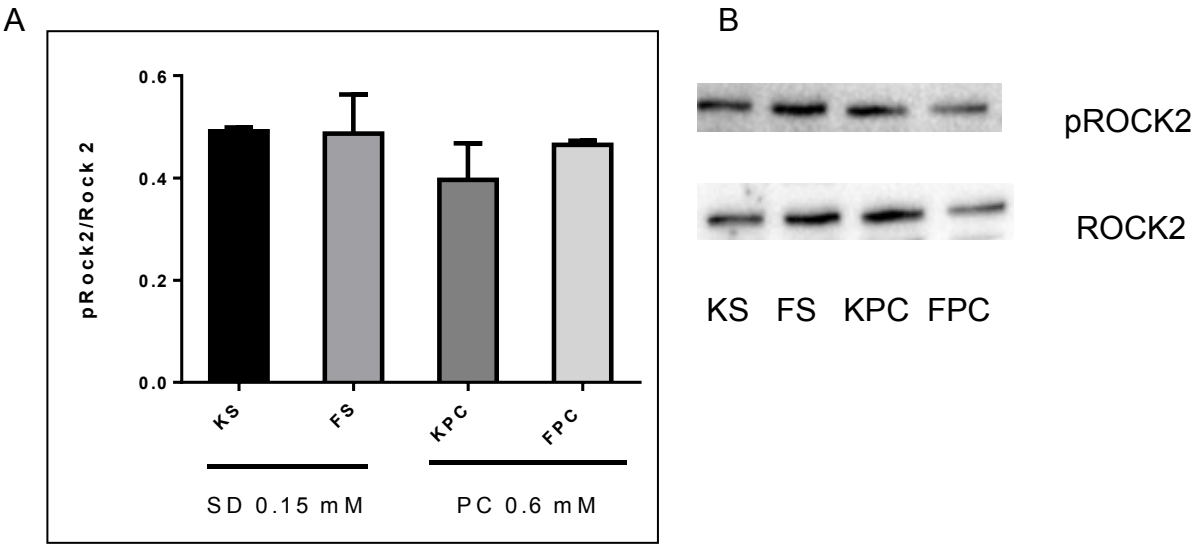

Figure 15: Effect of PC and fructose on ROCK2 protein concentration in everted sacs of small intestinal tissue. (A) Relative protein concentration of pROCK2 normalized to total ROCK2 (B) Representative picture of a Western blot of pROCK2 and total ROCK2 as well as densitometric analysis of blots .For KS, FS, KPC and FPC n=2. Data are presented as means ± SEM. Abbreviations: KS= control + sodium deoxycholate; FS= 5 mM fructose + sodium deoxycholate; KPC= control + sodium dexoycholate & phosphatidylcholine (1:4); FPC= 5 mM fructose + sodium deoxycholate & phosphatidylcholine (1:4); p= phosphorylate; SD= sodium deoxycholate; PC= phosphatidylcholine.

5. Discussion

Fructose has been implicated in the development of many metabolic diseases such as NAFLD but also obesity and diabetes type 2 [65]. Chronic fructose intake has been associated with the loss of TJs protein and alterations of intestinal permeability resulting in intestinal inflammation [24]. Indeed, several studies suggest that fructose intake increases bacterial endotoxin translocation leading to impairments of intestinal barrier function [25]. In fact, LPS stimulates TLR4 signaling leading to the activation of various pro-inflammatory responses causing intestinal barrier dysfunction [25]. On the other hand, several studies showed that phosphatidylcholine, a phospholipid present in the intestinal membrane, has beneficial effects on intestinal barrier function and could be a potential therapeutic tool in the prevention of metabolic diseases such as ulcerative colitis and inflammatory bowel disease [54&55]. The aim of this study was to assess if phosphatidylcholine can alter fructose-induced intestinal barrier alterations.

5.1 Fructose-induced alterations of intestinal permeability are associated with changes of arginase activity but not marked changes in ROCK2 phosphorylation

Fructose has been involved in the development of various metabolic diseases such as NAFLD [24]. Studies suggest that chronic fructose intake can alter the intestinal barrier function and the intestinal permeability by reducing tight junction's proteins [24&25]. In the present study, 5mM fructose was tested in intestinal tissue of everted sacs to assess the effects of this monosaccharide on intestinal barrier function.

Fructose decreases the intestinal permeability and the protein concentration of the tight junction's protein occludin in everted sacs of small intestinal tissue.

In the present study, xylose permeation was significantly lower in everted sacs of intestinal tissue challenged with 5 mM fructose for 60 min with protein levels of the tight junction protein occludin and activity of arginase decrease when compared to control sac. Studies in mice and rats of the own groups and other groups have shown before that chronic intake of fructose can alter intestinal barrier function. Fructose intake has already been shown to reduce tight junction proteins expression in the

small intestine and to increase intestinal permeability [65]. For instance, results of a study showed that occludin and ZO-1 protein concentrations were lower in the small intestine of mice fed for 8 weeks with fructose [66]. Results of a human study suggest that fructose intake does in fact increase the risk of developing a metabolic disease like NAFLD [67]. The increased intestinal permeability is associated with an elevated endotoxin translocation and inflammation but also changes in intestinal microbiota [67]. Rats with non-alcoholic steatohepatitis (NASH) were fed with fructose in the drinking water for 10 weeks; results showed an elevation in endotoxin levels in the portal blood and an increased LPS-TLR4 signaling in the liver which are responsible for the initiation of the systemic inflammation [65]. In line with these findings, results of a human study investigated a fructose-rich diet for 3 days [68]. Increased markers of intestinal permeability such as TLR4 mRNA expression, endotoxin and LBP concentrations were observed after fructose consumption [68]. Liver diseases like NAFLD and NASH are mainly caused by fructose intake through leaky gut [65]. Our results showed a decrease in intestinal permeability in contrary to findings of others. The differences between our findings and those of others might have resulted from the ex vivo model but also the method used to measure the intestinal permeability. On the other hand, our results showed that the loss of occludin tight junction's protein is associated with fructose-treatment in intestinal tissue of everted sacs. However, further experiments are needed to confirm these results. Taken together, despite the appeal discrepancy of xylose permeation and tight junction levels, results still suggest that intestinal permeability was altered even of a rather short challenge with fructose in small intestinal tissue.

Unpublished results of the own group suggest that changes in NO-synthesis are among the crucial factors in the development of fructose-induced intestinal barrier dysfunction. Furthermore, it has also been shown that a supplementation of arginine and citrulline can abolish fructose-induced intestinal barrier dysfunction.

Fructose showed a significant decrease in the arginase activity but arginase-2 protein expression was not altered in everted sacs of small intestinal tissue.

Arginine pathway plays an important role in the intestinal barrier function. When alterations in this pathway appear, damages of intestinal barrier integrity can occur. In brief, L-arginine produces urea and ornithine via the enzyme arginase [69]. Two

isoenzymes have been discovered, arginase-1 is mostly found in liver and arginase-2 in gastrointestinal tract and kidney [69]. On the other hand, L-arginine also produces NO and citrulline via nitric oxide synthase (NOS) [70]. Inducible-, endothelial- and neuronal-NO are knowns as isoforms of NO and plays different roles [70]. NO plays an important role as neurotransmitter in the gut and is able to regulate he gastrointestinal smooth muscle [70]. Both arginase and NOS are competing for L-arginine; inhibition of arginase leads to elevation in NO production [69]. Moreover, it has been suggested that iNOS catalyzes NO production during inflammation and is responsible for the dysfunction of mucosal integrity [70]. Elevation in NO production leads to cytotoxicity in intestinal epithelial cells resulting in the progression of intestinal inflammation such as necrotizing enterocolitis [72]. Results of a previous study on pulmonary arterial endothelial cell culture showed that the inhibition of arginase resulted in higher NO production levels via increased arginase-2 expression [71]. Cell culture of Intestinal epithelial cells of rats were incubated with LPS and NO production was induced [72]. Finding of this study showed that when arginase inhibition was induced with an arginase inhibitor, Nω-Hydroxynor-L-arginine (NOHA), an increased NO production and arginase-2 protein expression was observed in intestinal epithelial cells of rats when compared to control [72]. Results of this study also suggest that an elevated NO production can affect the intestinal barrier integrity leading to increased intestinal permeability [72]. As discussed above, fructose intake leads to dysfunction of intestinal barrier and a loss of tight junction's proteins. In line with these findings, a study performed in rats fed with fructose in drinking water showed that iNOS concentrations were significantly elevated in the small intestine [73]. Moreover, our work was performed only in intestinal tissue of naive young and healthy female mice. Results on intestinal tissue of naïve male mice but also old and diseased ones may be differing. However more studies are needed to delineate the molecular mechanism of fructose regarding iNOS and arginase activity. Results of the present study and those of others suggest that fructose inhibits arginase activity and alter the intestinal barrier integrity. Our results showed a significant decrease in arginase activity but in contrary arginase-2 expression was not altered.

Results of several studies suggest that ROCK may alter arginase activity and has also been proposed to be involved in the regulation of TJs [77&78]. A study suggests that Rho kinase activates iNOS production and can further alter the arginine pathway

[79]. Results of an animal study suggest that the activation of RhoA/ROCK pathway is responsible for the development of intestinal diseases by inducing intestinal inflammation [77].

Phosphorylation of ROCK2 was not altered in fructose challenge small intestinal tissue of everted sacs.

A study performed on isolated small intestinal cells from patients with Crohn's disease showed the relationship between arginase activity and RhoA/ ROCK kinase [78]. An elevated arginase activity, a reduction in NO generation along with an increased concentration of arginase-2 was observed in the submucosal tissue when compared to control [78]. Furthermore, the inhibition of RohA/ROCK signaling pathway resulted in alteration of arginase activity [78]. Indeed, arginase-2 protein concentrations in human intestinal microvascular endothelial cells were also decreased after the blocking of RohA/ ROCK signaling [78]. The stimulation of ROCK pathway increases arginase activity as well as iNOS and has been implicated in epithelial barrier dysfunction further leading to intestinal diseases such as Crohn's disease and inflammatory bowel disease [78]. In addition, ROCK is playing a role in the organization of actin filaments and also the regulation of TJs proteins [74]. It has been shown that ROCK has downstream effects on Rho GTP binding proteins [74]. ROCK2 is divided via caspase-2 or granzyme B [75]. In a study performed in a mouse model with induced-colitis by intra-colonic administration of TNBS solution, the inhibition of ROCK showed an enhancement of the intestinal permeability [75]. Furthermore, the addition of the ROCK inhibitor Y-27632 reduced the loss of occludin and ZO-1 proteins suggesting that the inhibition of ROCK may increase TJs proteins [75]. During intestinal inflammation, myosin light chain (MLC), myosin phosphatase-targeting subunit-1 (MYPT-1) and NF-κB pathway are all activated via ROCK pathways [75]. In fact, several studies suggest that ROCK is involved in intestinal barrier dysfunction leading to inflammation [76&77]. In the present study the challenge of each tissue sacs with fructose were not associated with any changes of the phosphorylation of ROCK. Also, it could be possible that changes in ROCK2 phosphorylation were not detectable due to variation but also time interval. How fructose and ROCK2 interacts is not documented yet. Our results showed an elevated ROCK2 protein expression in fructose-treated intestinal tissue of everted

sacs but this was not significant, due to low sample size. Based on the results showed in the literature, both fructose and ROCK increases the intestinal permeability and are a risk factor for the development of metabolic diseases such as NAFLD. We can further suggest that fructose elevates ROCK2 protein expression.

Intestinal tissue of everted sacs challenged with 5mM fructose showed alterations in intestinal permeability as well as changes in arginase activity. Moreover, changes in arginase-2 expression were observed as well as phosphorylation of ROCK2 in intestinal tissue of everted sacs treated with fructose but didn't reach the level of significance.

5.2 Phosphatidylcholine protects small intestinal tissue against fructose-induced impairment of intestinal barrier function and of arginase activity

Phosphatidylcholine, a phospholipid present in the intestinal membrane, plays an important role in the protection against extern pathogens [80]. Supplementations but also rich diets with this particular phospholipid have been shown to protect intestinal barrier and to have anti-inflammatory properties [54]. Indeed, several studies suggest that phosphatidylcholine has also beneficial effects by preventing the development of various metabolic diseases such as ulcerative colitis but also liver fibrosis [54&55]. The main aim of this study was to asses if phosphatidylcholine can alter the fructose-induced intestinal barrier alterations in intestinal tissue of everted sacs.

Phosphatidylcholine enhances the intestinal permeability and elevates occludin protein levels in fructose-treated intestinal tissue of everted sacs.

The maintenance of hydrophobic layer of PC in the intestinal membrane is important to prevent the development of diseases such as ulcerative colitis [81]. In an animal study, LPS was parenterally administrated to induce gastrointestinal inflammation and deterioration of PC in the intestinal membrane [81]. After this, oral supplementation of PC has been investigated on rats [81]. The increasing intestinal permeability was enhanced and a protective effect on the gastrointestinal tract has been shown in the ileum of rats after 1h of PC treatment [81]. Another study tested the effects of PC in a model of intestinal epithelial cells previously treated with TNF-alpha to induce inflammation [82]. Findings of this study suggest that, the addition of

PC did in fact lead to the inhibition of TNF-α-induced NF-kB signaling cascade in a human model of intestinal epithelial cells, which are responsible for the loss of intestinal barrier integrity [82]. A study investigated the oral administration of PC on rats with liver injury [82]. Results showed a decrease in plasma endotoxin levels in rats treated with PC when compared to control [83]. Moreover, this study also suggests that PC has a protective effect regarding the intestinal barrier integrity by reducing pro-inflammatory responses in the gut [83]. Due to low sample size further experiments are needed to confirm these results. Somewhat in line with the findings of others in the present study, the addition of PC to the incubation buffer enriched with fructose prevented the increase permeation of xylose compared to the respective controls found in everted sacs only challenged with fructose. In fact, the accumulation and transport of PC on the apical side was blocked when TJ protein were disrupted suggesting the positive effects of PC by preventing the loss of TJ [84].

As results shown in 4.1 and unpublished data of the own group suggests that the increase in intestinal permeability observed in everted sacs is associated with a decrease in arginase activity.

Phosphatidylcholine enhances the arginase activity and increases arginase-2 protein expression in fructose-treated intestinal tissue of everted sacs but this was not significant.

The stimulation of TNF-alpha caused by LPS activates mitogen-activated protein kinases (MAPKs) [85]. These are pro-inflammatory factors and play a role in the development of many diseases such as ulcerative colitis [85]. In a study performed on caco-2 cells, applied exogenous PC basolateral or apical inhibited TNF-a-induced nuclear factor-kB (NF-kB) signaling [85]. Another study investigated the effect of a phosphatidylcholine-enriched diet performed in mice-induced arthritis for 8 weeks [86]. Results showed a decrease in iNOS production and inhibition of NO levels after PC treatment [86]. Increased arginase activity is correlated with increased polyamines synthesis following with a decrease in NO production [87]. In a study, the relationship between lyso-PC and polyamines synthesis on smooth muscle cells was tested [88]. The expression of ornithine decarboxylase and cationic amino acid transfer are stimulated through lyso-PC and elevates the production of polyamines [88]. Lyso-PC promotes arginase activity and inhibits NO production [88]. Based on

these findings that PC but also Lyso-PC inhibits NO production and induction of iNOS, we can suppose that PC also enhance arginase activity and have a positive impact on the intestinal barrier [88]. Our results showed that PC increases the arginase activity and that it might leads to the inhibition of NO production which are in line with findings of others. On the other hand, our results showed that PC increased arginase-2 protein expression but was not significant. In fact, a study performed in macrophages suggests that the iNOS production is associated with elevated arginase-2 protein expression [89]. We can suppose that PC inhibits iNOS levels further impacting arginase-2 protein expression by decreasing it. The differences between our findings and those of others might have resulted from low sample size but also from different model used and can't be compared with each other. Indeed, our results could be differing from others due to the use of intestinal tissue from everted sacs as an ex vivo model. PC and lyso-PC mechanism are not fully discovered yet. We suppose that the impact of these phospholipids on arginase activity and NO production are positive; they both showed that iNOS and arginase compete for L-arginine, the common substrate. However further investigations of the mechanism and the possible transporters are needed to understand how PC impact different pathways of L-arginine.

Several studies suggest that ROCK2 is involved in the arginine pathway and can further impact the arginase activity but also the intestinal permeability by reducing TJ proteins [77&78].

Phosphatidylcholine enhances phosphorylation of ROCK2 in fructose-treated intestinal tissue of everted sacs but this was not significant.

A study investigated the possible underling mechanism of PC on polarized caco-2 cells [84]. Results showed that PC can only be transported after being hydrolyzed to lyso-PC from the intestinal mucus into lumen. PC passes the basolateral side via paracellular transport along tights junction's proteins [84]. After this, the choline group of PC, positively charged binds to mucins, negatively charged [84]. A spontaneous phospholipid layer is formed protecting the intestinal mucus from bacteria [84]. On the other hand, choline can also be metabolized to phosphatidylcholine via choline kinase and is then transported into tissue cells [90]. In fact, choline stimulates the activation of AMP-activated protein kinase (AMPK) pathway and further regulates

several enzymes such as SREBP-1c and PPAR-α [91]. These enzymes have been involved in the progression of metabolic diseases such as NAFLD [91]. Indeed, it has been suggested that choline can reduce lipogenesis and elevate oxidation of fatty acid [91]. Stimulation of AMPK plays an important role in the intestinal epithelial cells by maintaining the tight junction's assembly and further preventing the development of intestinal barrier dysfunction [92].

One of the limitations of our study is the low sample size; further studies are needed to determine extensively the role of PC on ROCK2 phosphorylation in intestinal tissue of everted sacs. The mechanism underlined it is still unclear and the repercussion on the intestinal permeability is unknown. Furthermore, our experience was done after sacrificing mice; an oral supplication of PC for a specific period of time can be performed as an alternative. In our work, only PC from egg yolk was used; other type such as soy-PC could show the same or different effects. Egg-PC consists of the majority of unsaturated fatty acid mainly oleic acid but also saturated fatty acid such as palmitic acid [48]. Moreover, we only used one concentration of phosphatidylcholine (0.6 mM); higher concentrations may lead to different results or show the same effects. Based on our results in this study but also results of others listed above, we can suppose how fructose and PC affects the intestinal integrity. However, the mechanism of PC on ROCK has not been documented yet. Furthermore, the positive impact of PC on the intestinal barrier can also influence the phosphorylation of ROCK2 protein levels by decreasing it in intestinal tissue of everted sacs. The reduction of ROCK2 protein levels is associated with a decrease in iNOS production and further arginase-2 protein expression. The possible underlying mechanism may be related to the blockade or inhibition of NF-kB signaling which produces pro-inflammatory molecules further preventing the loss of TJ proteins.

Overall, the addition of phosphatidylcholine enhanced the fructose-induced intestinal permeability by preventing the reduction of TJ protein. Moreover, PC impacts the arginine pathway by improving arginase activity in intestinal tissue of everted sacs challenged with fructose. Due to the low sample size, changes in arginase-2 concentrations and phosphorylation of ROCK2 in fructose-induced intestinal tissue of everted sacs didn't reach the level of significance. Further studies are needed to confirm our results but also to investigate a possible other pathway of PC. However,

a study suggested that an apical transport of PC also exist and can be one of the mechanisms of action of this phospholipid [82].

6. Conclusion

Taken together, our data suggest that fructose affects the intestinal permeability in intestinal tissue of everted sacs. Moreover, fructose-treated intestinal tissue of everted sacs was associated with changes in arginase activity. Furthermore, fructose did not alter arginase-2 levels neither ROCK2 phosphorylation. However, further studies are required to confirm our results but also to determine the underlying mechanism of fructose regarding arginase activity and how it affects the RhoA/ROCK signaling in small intestinal tissue. Our data further suggest that the addition of phosphatidylcholine protects against the effects of fructose-induced impairments of intestinal barrier function. In fact, treatment of PC showed elevation in occludin TJs protein but also arginase activity in small intestinal tissue. Indeed, we suppose that intestinal permeability correlates with arginase activity. Furthermore, due to low sample size, the addition of PC didn't show any changes regarding arginase-2 levels or ROCK2 phosphorylation. PC may be improving fructose-induced intestinal barrier dysfunction by preventing the loss of TJ proteins and could be used for further therapeutic purposes for gastrointestinal diseases but also cancers. Further studies are needed to investigate if similar and long term effects with higher concentrations of phosphatidylcholine could also be found in small intestinal tissue. However, molecular mechanisms are not fully discovered yet and remain to be determined.

7. Summary

A diet rich in fructose has been implicated in the development of many metabolic diseases such as diabetes type 2, obesity and non-alcoholic fatty liver disease (NAFLD). Several studies have been shown that chronic fructose intake alters the intestinal barrier function by reducing tight junctions resulting in impairment of intestinal permeability. Phosphatidylcholine, a phospholipid present in the intestinal membrane, has already been shown to have anti-inflammatory properties and has beneficial effects on health by preventing the development of many metabolic diseases. Starting from this background, the main aim of this study was to assess if phosphatidylcholine affects fructose-induced alterations of intestinal barrier function in an ex vivo model of everted sacs of small intestinal tissue. Therefore, 0.6mM phosphatidylcholine was added to 5 mM fructose and was tested in intestinal tissue of everted sacs from female C57BL/6J mice. After incubation with fructose and phosphatidylcholine, xylose permeation as well as arginase activity were measured. In addition, occludin TJ protein, arginase-2 levels and phosphorylation of ROCK2 were analyzed using Western blot method. 5 mM fructose showed a decrease in intestinal permeability as well as changes in arginase activity. Indeed, alterations in intestinal permeability are associated with changes in arginase activity and RhoA/ROCK signaling. However, fructose did not alter arginase-2 levels or ROCK2 phosphorylation in small intestinal tissue of everted sacs. In contrary, the addition of phosphatidylcholine prevented against the effects of fructose-induced intestinal permeability as well as changes in arginase activity in small intestinal tissue of everted sacs. Our results further showed that PC enhanced both arginase-2 levels and phosphorylation of ROCK2 but due to law sample size, the level of significance was not reached. In pilot experiments ROCK2 protein levels were assessed and suggest being critical in the regulation of arginase and intestinal permeability. As data varied considerably, no differences were found between treatment groups. Taken together the results of the present study suggest that, phosphatidylcholine has in fact an impact on fructose-induced alterations of intestinal barrier function. Moreover, phosphatidylcholine protects intestinal permeability by preventing the loss in occludin tight junction's protein. However, further studies are required to confirm the findings

but also to determine the underlying mechanism of phosphatidylcholine regarding arginase and ROCK2 pathway.

Zusammenfassung

Eine fructosereiche Ernährung wird mit der Entstehung vieler Stoffwechselkrankheiten wie Diabetes Typ 2, Fettleibigkeit und nichtalkoholischer Fettlebererkrankung (NAFLD) in Verbindung gebracht. Mehrere Studien haben gezeigt, dass eine chronische Fruktoseaufnahme die Funktion der Darmbarriere verändert, indem sie die tight junctions reduziert, was zu einer Beeinträchtigung der intestinalen Permeabilität führt. Phosphatidylcholin, ein Phospholipid, das in der Darmmembran vorhanden ist, hat bereits gezeigt, dass es entzündungshemmende Eigenschaften hat und sich positiv auf die Gesundheit auswirkt, indem es die Entwicklung vieler Stoffwechselkrankheiten verhindert. Ausgehend von diesem Hintergrund war das Hauptziel dieser Studie zu beurteilen, ob Phosphatidylcholin die Fructose-induzierten Veränderungen der intestinalen Barrierefunktion in einem Ex-vivo-Modell von intestinalen Everted sacs beeinflusst. Dazu wurde 0,6 mM Phosphatidylcholin zu 5 mM Fruktose gegeben und in Darmgewebe von weiblichen C57BL/6J-Mäusen getestet. Nach Inkubation mit Fructose und Phosphatidylcholin wurden die Xylose-Permeation sowie die Arginase-Aktivität gemessen. Zusätzlich wurden das Occludin-TJ-Protein, der Arginase-2-Spiegel und die Phosphorylierung von ROCK2 mittels Western-Blot-Methode analysiert. 5 mM Fruktose zeigte eine Abnahme der intestinalen Permeabilität sowie Veränderungen der Arginase-Aktivität. In der Tat sind Veränderungen der intestinalen Permeabilität mit Veränderungen der Arginase-Aktivität und der RhoA/ROCK-Signalisierung verbunden. Fruktose veränderte jedoch weder die Arginase-2-Konzentration noch die ROCK2-Phosphorylierung im Dünndarmgewebe der Everted sacs. Im Gegensatz dazu verhinderte die Zugabe von Phosphatidylcholin die Effekte der Fruktose-induzierten intestinalen Permeabilität sowie die Veränderungen der Arginase-Aktivität im Dünndarmgewebe der Everted sacs. Unsere Ergebnisse zeigten weiterhin, dass PC sowohl die Arginase-2-Spiegel als auch die Phosphorylierung von ROCK2 erhöhte, aber aufgrund der gesetzlichen Probengröße wurde das Signifikanzniveau nicht erreicht. In Pilotexperimenten wurden die ROCK2-Proteinkonzentrationen bestimmt, die darauf hindeuten, dass sie bei der Regulierung der Arginase und der intestinalen Permeabilität entscheidend sind. Da die Daten stark variierten, wurden keine

Unterschiede zwischen den Behandlungsgruppen gefunden. Zusammengenommen deuten die Ergebnisse der vorliegenden Studie darauf hin, dass Phosphatidylcholin tatsächlich einen Einfluss auf Fruktose-induzierte Veränderungen der intestinalen Barrierefunktion hat. Darüber hinaus schützt Phosphatidylcholin die intestinale Permeabilität, indem es den Verlust des Occludin-Proteins der tight junction verhindert. Es sind jedoch weitere Studien erforderlich, um die Ergebnisse zu bestätigen, aber auch um den zugrundeliegenden Mechanismus von Phosphatidylcholin in Bezug auf Arginase und den ROCK2-Signalweg zu bestimmen.

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Declaration of Authorship

Hereby, i confirm that the present thesis was written by myself and without any other sources that the literature cited in this present work.

Vienna, 8 February 2021