

# Contents

<b>1 Summary</b>	<b>1</b>
<b>2 Zusammenfassung</b>	<b>3</b>
<b>3 List of abbreviations</b>	<b>7</b>
<b>4 Introduction</b>	<b>11</b>
<b>5 Aims</b>	<b>19</b>
<b>6 Materials and Methods</b>	<b>20</b>
6.1 Chemicals, kits and instruments	20
6.2 Origin and cultures of cell lines	20
6.3 RNA extraction	20
6.4 Plasmid purification from <i>Escherichia coli</i>	21
6.5 Glycerol stocks of bacterial cells	22
6.6 DMSO stocks of mammalian cell lines	23
6.7 Quantification of DNA/RNA	23
6.8 Polymerase chain reaction	23
6.9 Site-directed mutagenesis	24
6.10 Purification of PCR products	25
6.11 Cloning of PCR products into vector systems	26
6.12 Assessment of the transcriptional activities of the promoter regions	26
6.13 RNA interference	27
6.14 <i>In vitro</i> translation of transcription factors	27
6.15 Electrophoretic mobility shift assay	28
6.16 Extraction of nuclear and cytoplasmic proteins	29
6.17 BCA protein assay	30
6.18 Western blot	31

6.19 Chromatin immunoprecipitation (ChIP)	32
6.19.1 Cell fixation	32
6.19.2 Shearing by sonication	33
6.19.3 Immunoprecipitation	33
6.19.4 Wash of the magnetic beads	33
6.19.5 Elution of chromatin, reversion of cross-links and treatment with proteinase K	34
6.20 Reverse transcription of RNA	34
6.21 <i>Ex vivo</i> treatment of rodent tissue specimens with ligands of transcription factors	35
6.22 Statistical analysis	35
6.23 List of chemicals/kits/equipment	36
<b>7 Results</b>	<b>40</b>
7.1 The peptide transporter 1 (PEPT1/Pept1)	40
7.1.1 <i>In silico</i> identification of putative PPAR response elements in human and rodent <i>PEPT1/Pept1</i> promoters	40
7.1.2 Transactivation of the human and rat <i>PEPT1/Pept1</i> promoters by PPAR $\alpha$ and PPAR $\gamma$	42
7.1.3 Binding of <i>in vitro</i> translated PPAR $\gamma$ proteins to human and rat PPRES	44
7.1.4 <i>PEPT1/Pept1</i> promoter constructs featuring wild-type and mutated PPRES transactivation by PPAR $\gamma$ :RXR $\alpha$	45
7.1.5 Relative PEPT1 mRNA quantification with real-time PCR in human cell lines	48
7.1.6 Relative PPAR $\alpha$ , PPAR $\gamma$ and RXR $\alpha$ mRNA quantification with real-time PCR in human cell lines	49
7.1.7 Relative PEPT1 mRNA quantification with real-time PCR in Caco-2 cells upon treatment with ligands for 48 hours in normal growth medium	50

7.1.8 Relative PEPT1 mRNA quantification with real-time PCR in Caco-2 cells treated with ligands for 48 hours in delipidated growth medium_____	51
7.1.9 Relative PEPT1 mRNA quantification with real-time PCR in Caco-2 cells treated for 24 hours in normal growth medium _____	53
7.1.10 Relative PEPT1 mRNA quantification with real-time PCR in Caco-2 cells treated for 24 hours in delipidated growth medium_____	54
7.1.11 Relative RXR $\alpha$ , PPAR $\alpha$ and PPAR $\gamma$ mRNA quantification with real-time PCR in Caco-2 cells treated with siRNA inhibiting the expression of RXR $\alpha$ , PPAR $\alpha$ and PPAR $\gamma$ _____	55
7.1.12 Relative ASBT mRNA quantification with real-time PCR in Caco-2 cells treated with siRNA inhibiting the expression of RXR $\alpha$ , PPAR $\alpha$ and PPAR $\gamma$ _____	57
7.1.13 Relative PEPT1 mRNA quantification with real-time PCR in Caco-2 cells treated with siRNA inhibiting the expression of RXR $\alpha$ , PPAR $\alpha$ and PPAR $\gamma$ _____	58
7.1.14 Relative HNF4 $\alpha$ mRNA quantification with real-time PCR in Caco-2 cells treated with siRNA inhibiting the expression of RXR $\alpha$ , PPAR $\alpha$ , PPAR $\gamma$ and HNF4 $\alpha$ _____	59
7.1.15 Relative RXR $\alpha$ , PPAR $\alpha$ and PPAR $\gamma$ mRNA quantification with real-time PCR in Caco-2 cells treated with siRNA inhibiting the expression of RXR $\alpha$ , PPAR $\alpha$ , PPAR $\gamma$ and HNF4 $\alpha$ _____	60
7.1.16 Relative PEPT1 mRNA quantification with real-time PCR in Caco-2 cells treated with siRNA inhibiting the expression of RXR $\alpha$ , PPAR $\alpha$ , PPAR $\gamma$ and HNF4 $\alpha$ _____	63
7.1.17 Relative PEPT1 mRNA quantification with real-time PCR in Caco-2 transfected with expression plasmid of PPAR $\gamma$ and RXR $\alpha$ and their respective ligands_____	64
7.1.18 Relative ASBT mRNA quantification with real-time PCR in Caco-2 cells co-transfected with expression plasmid of PPAR $\gamma$ and RXR $\alpha$ and their respective ligands_____	65

7.1.19 Relative PEPT1 mRNA quantification with real-time PCR of rat ileal samples treated with PPAR $\alpha$ , PPAR $\gamma$ and RXR $\alpha$ agonists and antagonists	67
7.1.20 Relative ASBT mRNA quantification with real-time PCR of ileal rat samples treated with PPAR $\alpha$ , PPAR $\gamma$ and RXR $\alpha$ agonists and antagonists	68
7.2 The organic anion transporting polypeptides (Oatps)	70
7.2.1 <i>In silico</i> identification of putative response elements in mouse <i>Slco1a1</i> and <i>Slco1a4</i> promoters	70
7.2.2 Transactivation of the mouse <i>Slco1a1</i> and <i>Slco1a4</i> promoters by the human glucocorticoid receptor (GR)	72
7.2.3 Transactivation of the mouse <i>Slco1a1</i> and <i>Slco1a4</i> promoters by the mouse glucocorticoid receptor (mGr)	75
7.2.4 Transactivation of the mouse <i>Slco1a1</i> and <i>Slco1a4</i> promoters by the mouse mPxr:mRxra heterodimer	77
7.2.5 Transactivation of the mouse <i>Slco1a1</i> and <i>Slco1a4</i> promoters by the mouse mPxr:mRxra heterodimer and mGr	78
7.2.6 Transactivation of the mouse <i>Slco1a1</i> and <i>Slco1a4</i> promoters by FXR	79
7.2.7 Transactivation of the mouse <i>Slco1a1</i> and <i>Slco1a4</i> promoters by mShp	81
7.2.8 mOatp1a1 and mOatp1a4 mRNA quantification with real-time PCR of mouse liver specimens treated with mPxr and mGr agonists and antagonists	83

<b>8 Discussion</b>	<b>87</b>
8.1 The peptide transporter 1 (PEPT1/Pept1)	87
8.2 The organic anion transporting polypeptides (Oatps)	94
<b>9 Future directions</b>	<b>97</b>
<b>10 Contributions to original articles</b>	<b>99</b>
<b>11 Literature</b>	<b>100</b>
<b>12 Curriculum vitae</b>	<b>113</b>
<b>13 Appendix</b>	<b>117</b>
<b>14 Acknowledgement</b>	<b>123</b>