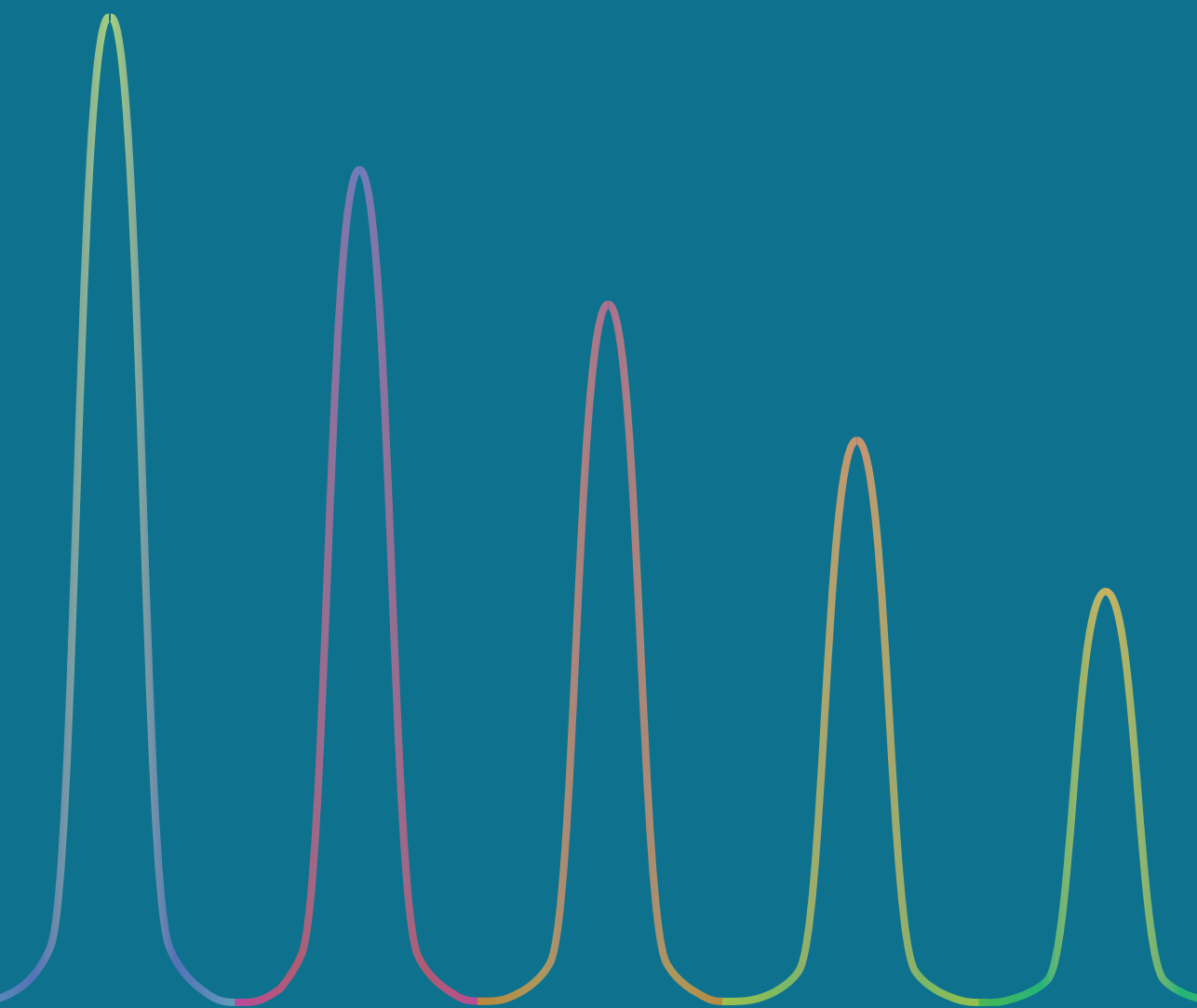
Discovery and characterization of new glucosylated metabolites: pathophysiological consequences

Meijer, H.N.J.



Chapter 6

General discussion and perspectives for future research

General discussion

The topic of this thesis is the discovery and characterization of new glucosylated metabolites and their pathophysiological consequences. The lysosomal enzyme glucocerebrosidase (GBA) is a retaining β-glucosidase that cleaves glucose from the glycosphingolipid glucosylceramide (GlcCer) employing the double displacement mechanism [1]. During regular hydrolysis of GlcCer by GBA, the glucose moiety of the substrate becomes linked to the nucleophile E340 of the enzyme and is next released to water as acceptor [1, 2]. It was recognized with *in vitro* experiments that alternatively, via a so-called transglucosylation reaction, GBA transfers the bound glucose moiety onto cholesterol (Chol) to form glucosyl-β-cholesterol (GlcChol) [3-5]. Likewise, the cytosol-facing retaining β-glucosidase GBA2 is able to perform transglucosylation, again generating from the donor GlcCer the product GlcChol [3]. It has become subsequently clear that normally GBA degrades GlcChol and GBA2 generates the glucosylated sterol [3]. However, in Niemann Pick disease type C (NPC), where cholesterol accumulates in lysosomes due to an inherited defect in its efflux, even GBA generates GlcChol via transglucosylation [3]. Natural occurrence of GlcChol has been demonstrated in several cells and tissues, but its physiological role still remains to be elucidated. Yet, the observed formation of GlcChol via transglucosylation raises the intriguing question whether other metabolites besides cholesterol might comparably act as acceptors in transglucosylation. Key candidates in this respect are compounds similar in structure to Chol.

One of the major aims of this thesis work was to develop a sensitive LC-MS/MS method to quantify newly discovered glycosylated metabolites, like glucosylated 7-dehydrocholesterol (Glc7DHC), glucosylated vitamin D_3 ($GlcD_3$) and glucosylated desmosterol (GlcDesm) in human body fluids and tissue. This goal was reached by further development of the LC-MS/MS method for quantitative measurement of GlcChol [3] (**Chapter 2** of this thesis). With the desired method in place we could demonstrate the *in vitro* formation and degradation of GlcDesm (**Chapter 3** of this thesis) and that of Glc7DHC and $GlcD_3$ (**Chapter 4** of this thesis). Furthermore, we demonstrated the presence of Glc7DHC and GlcDesm in biological samples (**Chapter 2**, **Chapter 3** and **Chapter 4** of this thesis). In **Chapter 5** of this thesis the transglycosylation potential of the broad-specific β-glucosidase (GBA3) is discussed. Transglucosylation by GBA3 has not yet been detected and its ability to perform transxylosylation warrants further investigation. Xylosylated cholesterol occurs [6], however xylosylation of 7-dehydrocholesterol is unclear (see later in this discussion).

The research described in this thesis excites directions for further investigations that are discussed below.

Perspectives for future research

New metabolites to be explored

The first direction of new research to be discussed is the search for additional metabolites, related in structure to cholesterol, that are transglucosylated as well. Besides being structurally related to 7DHC and Desm, cholesterol is a precursor for other metabolites such as oxysterols, bile acids, and steroid hormones [7]. As the chemical structure of these downstream metabolites resembles their precursors, they are conceivably also subject to transglucosylation. The three different classes of metabolites are separately discussed below.

A. Oxysterols.

Oxysterols are associated to several diseases, like atherosclerosis, AD, multiple sclerosis, Huntington's disease, Niemann Pick type C disease, Parkinson's disease, amyotrophic lateral sclerosis (ALS) [8], Smith-Lemli-Opitz Syndrome (SLOS, a disease with dysfunctional DHCR7) [8-12], autism spectrum disorders and spastic paraplegia type 5 [8]. Formation of oxysterols from Chol, 7DHC or Desm occurs via members of the cytochrome P450 family, in particular 27-hydroxylase (CYP27A1), 24-hydroxylase (CYP46A1), 7α-hydroxylase (CYP7A1), Cytochrome P450Scc (CYP11A1) or via autoxidation processes (oxidation and epoxidation) [8, 13, 14]. Oxysterols formed by auto-oxidation, like 7-hydroxyperoxycholesterol, 7-ketocholesterol and 7β-hydroxycholesterol, appear during oxidative stress and have been found in association with atherosclerosis, Parkinson's disease, Niemann Pick disease type C and SLOS [8, 13, 14]. Furthermore, they occur in our diet [13]. The enzymatically formed oxysterols activate LXRs [14], but are also important in bile acid and steroid production [8].

In brain and adrenal glands CYP46A1 is responsible for the conversion of cholesterol into the oxysterol 24S-hydroxycholesterol [15-17]. 24S-hydroxycholesterol is important in cholesterol homeostasis in the brain. It crosses the blood-brain barrier and thus allows exit of cholesterol building block from the brain. Via the blood circulation 24S-hydroxycholesterol moves towards the liver for further conversion into bile acids [17, 18]. The enzyme CYP46A1 is also able to convert Desm to two oxysterol products, 24S,25-epoxycholesterol and 27-OH-desmosterol. It can convert 7-DHC into the oxysterols 24-hydroxy-7-dehydrocholesterol (24-(OH)7DHC) and 25-hydroxy-7-dehydrocholesterol (25-(OH)7DHC [8, 19].

B. Steroid hormones

Steroid hormones regulate numerous processes in the central nervous system and in peripheral organs. They act through binding with specific nuclear receptors and mediate gene transcription, inducing behavioral and developmental responses and metabolic actions. Steroid hormones are thought to cross the blood-brain barrier by diffusion [20, 21]. Their structure exists of a tetracyclic (cyclopentaphenanthrene) skeleton, for which Chol is the main precursor [21,

22]. Formation of steroid hormones takes place in the adrenal gland, skin, testis and placenta. The enzyme CYP11A1 modifies cholesterol, to pregnenolone, the precursor of progesterone, cortisol and testosterone [8, 23-26]. On the other hand, desmosterol is also converted by CYP11A1 to the pregnenolone derivative 5-pregnene-3β,20β-diol, which has a similar structure to the cholesterol derivative pregnenolone [27, 28]. In the skin CYP11A1 transforms 7DHC into 7-dehydropregnenolone (7DHP), which is a precursor for several steroidal 5,7-dienes and 4,7-dienes, like 7-dehydroprogesterone [29-33]. The 5,7-diene precursors and secosteroidal products have inhibitory effects on cell proliferation and induce differentiation without calcemic activity [34]. In the skin, D_3 is also an substrate for CYP11A1, resulting in the formation of 20-hydroxycholecalciferol ($20(OH)D_3$) [35, 36], which exhibits antiproliferative activity and inhibits human melanoma growth [36, 37].

C. Bile acids

Bile acids are of great importance for intestinal nutrient absorption, biliary secretion, maintenance of metabolic homeostasis by acting as signaling molecules in lipid, glucose and energy metabolism. They are amphipathic molecules with powerful detergent properties, due to their tetracyclic skeleton with hydroxyl and carboxyl groups on the opposite site of the hydrophobic tail. The complete conversion of cholesterol to bile acids involves 17 distinct enzymes. These enzymes modify the steroid ring and the side chain of cholesterol. The biosynthetic pathway consists of two paths, the neutral bile acid pathway (classic pathway) initiated by the enzyme 7α-hydroxylase (CYP7A1) and the acidic pathway, initiated by 27-hydroxylase (CYP27A1) [15, 38]. CYP7A1 is present in tissues like liver, macrophages, retina and brain nerve cells. Inside the liver the main production of bile acids occurs (200 to 600 mg bile acid per day). CYP7A1 catalyzes the conversion of cholesterol into the oxysterol 7α-hydroxycholesterol [15, 39, 40]. 7α-Hydroxycholesterol is a precursor for the primary bile acids cholic acid and chenodeoxycholic acid. As a negative feedback loop, CYP7A1 activity is inhibited by the oxysterol 7-ketocholesterol, which is produced by CYP7A1 itself via 7DHC oxidation to 7-ketocholesterol or via radical attack by reactive oxidative species (ROS) on cholesterol [41, 42]. Furthermore, CYP7A1 converts 7DHC into 7α,8α-epoxycholesterol [8, 41, 43], a metabolite specifically present in plasma of SLOS patients [43]. CYP27A1, initiating the acidic pathway, is present in liver and lungs, and converts cholesterol to 27-hydroxycholesterol and other oxysterols like cholestenoic acid [15, 38]. Mice with a homozygous knockout, show hepatomegaly, increased cholesterol synthesis and affected fatty acid and bile acid synthesis [38]. In humans the deficiency of CYP27A1 leads to cerebrotendinous xanthomatosis (CTX)[44]. A disease associated with accumulation of cholesterol in the Central Nerves System (CNS) [45], excretion of bile alcohols and low fecal bile acid excretion [38, 46]. Some cases even develop hepatomegaly [47], as observed in CTX mice [38]. Furthermore, CTX is associated with osteoporosis and high chance on bone fractures [48, 49]. This might relate to 7DHC and D_3, both being

a substrate for CYP27A1. In SLOS patients 7DHC levels are elevated, as well as levels of 25(OH)D_3 [51]. SLOS patients do not show association with osteoporosis, indicating that elevation of 7DHC and 25(OH)D_3 protects against osteoporosis [8, 43, 50] In CTX patients, 7DHC levels are also elevated [52], while conversion of D_3 into 25(OH)D_3 by CYP27A1 is impaired[53], resulting in lower levels of 25(OH)D_3 [54]. This might explain why SLOS patient do not develop osteoporosis, while CTX patients do.

Within the three discussed classes of metabolites above transglycosylation of some compounds might occur. After selection of the most likely candidates, comparable research as performed in this thesis (**Chapter 2**, **Chapter 3** and **Chapter 4**) should be considered.

Glucosylated metabolites: the missing link?

The biological function and pathophysiological relevance of glucosylated metabolites in the body is a complete new field of research. In this paragraph it is discussed which kind of impact those metabolites might have, based on literature on the non-glycosylated metabolites.

Gaucher disease (GD) is due to inherited defects in GBA, the lysosomal glucocerebrosidase responsible for breakdown of the glycosphingolipid glucosylceramide (GlcCer) [55-57]. Consequently, GlcCer metabolism is disturbed. Gaucher disease has may manifest with various symptoms ranging from hypersplenism, skeletal complications, hepatomegaly and neurodegeneration [56, 58-61]. Interestingly patients with the same mutation develop different phenotypes, as even twins with the same genetic background show heterogeneous phenotypes [62]. A phenomenon which is also observed in CTX patients [46] and Desmosterolosis, in which the same mutation leads to different phenotypes [63]. Some of the noted symptoms in Gaucher disease patients might point to disturbances in the pathways of desmosterol, 7DHC and cholesterol metabolism. If these pathways are indeed involved than the differences between twins might be related to their food intake, as desmosterol, 7DHC and cholesterol are compounds that are present in our food [14, 64-69]. The discovery of GlcChol is of great interest. We suspect that, as GlcChol levels are increased in plasma of Gaucher patients [3], glucosylated 7DHC and Desm might be present in Gaucher patients as well. GD is not the only disease in which GlcChol was discovered, also in NPC patients elevated levels of GlcChol were detected [3].

The discovery of GlcChol in both of these diseases makes us wonder whether in other disease, like SLOS with high 7DHC levels and Desmosterolosis (defect in DHCR24, resulting in high levels of Desm) [70, 71], also metabolites as Glc7DHC and GlcDesm might be present. Other diseases warranting such investigations are vitamin-D-dependent Rickets, CTX and Lathosterolosis (defect in SC5D, with decreased levels of 7DHC) [72, 73]. All these diseases might have glucosylated metabolites playing a role in the pathology of the disease. An overview of the pathways of Desm, 7DHC and Chol is presented in Figure 1.

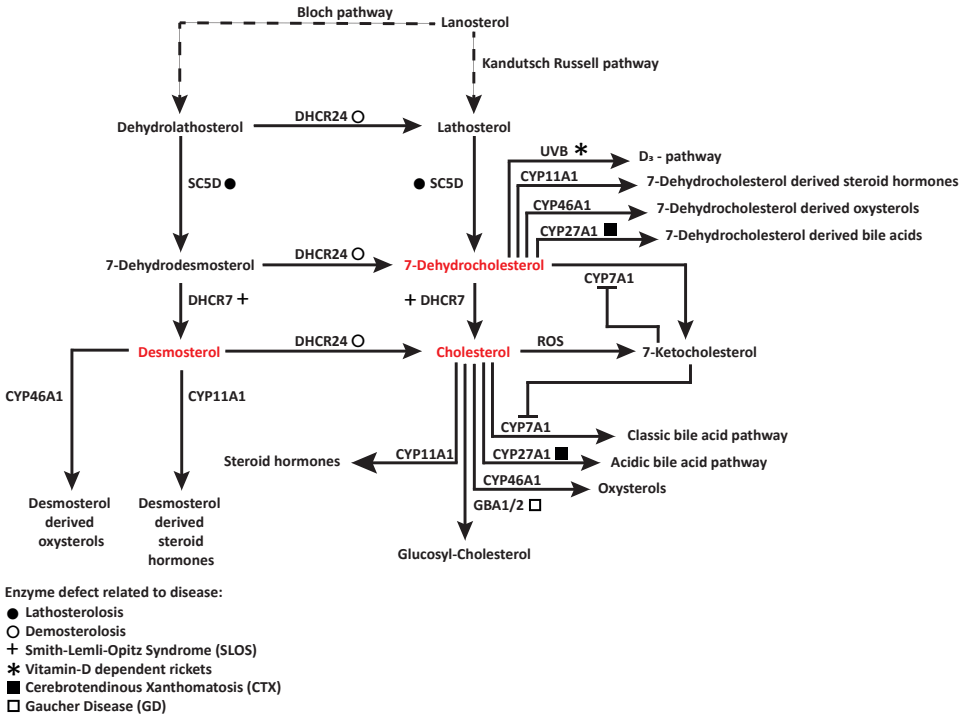


Figure 1. Pathways of desmosterol, 7-dehydrocholesterol and cholesterol related to disease.
Displaying the pathways of desmosterol, 7-dehydrocholesterol and cholesterol. Involved enzymes are Sterol C5-desaturase (SC5D), 7-Dehydrocholesterol Reductase (DHCR7), 24-Dehydrocholesterol Reductase (DHCR24), 7α-hydroxylase (CYP7A1), Cytochrome P450Scc (CYP11A1), 27-hydroxylase (CYP27A1), 24-hydroxylase (CYP46A1), glucocerebrosidase 1 and 2 (GBA1/2). Defective enzymes are marked and related to the corresponding diseases, lathosterolosis, desmosterolosis, Smith-Lemli-Opitz Syndrome (SLOS). Vitamin-D dependent rickets, Cerebrotendinous Xanthomatosis (CTX) and Gaucher Disease (GD).

Potential acceptor abnormality that might promote causing glucosylated metabolites abnormalities

Table 1a summarizes literature on key metabolites of the 7DHC/D_3 and cholesterol pathways for various relevant diseases including Niemann Pick type C. In NPC elevated levels of both GlcCer and Chol are reported [3]. Earlier research showed that in plasma of NPC patients elevated levels of GlcChol occur as compared to healthy individuals. Table 1b shows a hypothetical prediction of glucosylated metabolite in various relevant diseases.

Table 1a. Metabolites in disease. *Levels of lathosterol (Latho), cholesterol (Chol), 7-dehydrocholesterol (7DHC), desmosterol (Desm), vitamin D_3 (D_3), 25-hydroxyvitamin D_3 (25(OH)D_3), 1α-25-dihydroxyvitamin D_3 (1,25(OH)2D_3) for each of the diseases Lathosterolosis, SLOS, Desmosterolosis, Cerebrotendinous Xanthomatosis (CTX), Vitamin D dependent Rickets and Gaucher Disease Type I, II and III and Niemann Pick Disease Type A, C1 and C2. Elevated indicated by +, decreased indicated by -, normal levels indicated by N. * Levels of D_3 are not measured within Desmosterolosis, but patients show osteosclerosis, which is related to high levels of D_3. Therefore levels are expected to be either elevated or normal. ** As levels of D_3 are unmentioned for Vitamin D- dependent rickets patients, we suspect based on that the enzyme defect is downstream of D_3 formation that levels are either elevated or normal. *** Levels of Desm are only mentioned for Type C1, due to the large overlap between Type C1 and C2 it is suspected that levels of Desm in Type C2 are also elevated as in Type C1.*

Disease		Enzyme Defect	Latho	Chol	7DHC	Desm	D_3	25(OH)D_3	1,25(OH)$_2D_3$
	Lathosterolosis [72-77]	SC5D	+	-/N	-/N	-	-		
	SLOS [72, 78, 79]	DHCR7		-	+	-	+		
	Desmosterolosis [63, 70, 80-86]	DHCR24		-/N	+/N	+	+/N*		
	CTX [44-46, 48, 49, 52, 54, 87-89]	CYP27A1	+	+/N	+		+/N	-	N
Vitamin D - dependent Rickets	*Type 1A [90-92]*	CYP27B1					+/N**	+/N	-
	Type 1B [90-93]	CYP2R1					+/N**	-	N
	Type 2A [90-92, 94-96]	VDR					+/N**	+/N	+
Gaucher Disease	*Type I [3, 97-103]*	GBA		-				-/N	
	Type II [101]	GBA		-					
	Type III [101, 104]	GBA		-					
Niemann Pick Diseases	*Type A [105-107]*	SMPD1		+					
	Type C1 [3, 108-110]	NPC1		+		+			
	Type C2 [3, 111-113]	NPC2		+		+***			

In NPC elevated levels of both GlcCer and Chol are reported. Earlier research showed that in plasma of NPC patients elevated levels of GlcChol occurs as compared to healthy individuals. Table 1b shows a hypothetical prediction of glucosylated metabolites.

Table1b. Hypothetical prediction of Glucosylated compounds in disease. *Shows the predicted levels of the glucosylated compounds GlcChol, Glc7DHC, GlcDesm and $GlcD_3$. Known levels of are indicated in bold. Elevated indicated by +, decreased indicated by -, normal levels indicated by N, undetected is indicated by a U, unpredicted is indicated by a blank spot.*

	Disease	GlcChol	Glc7DHC	GlcDesm	$GlcD_3$
	Lathosterolosis *[72-77]*	-/N	-/N	-	-
	SLOS *[72, 78, 79]*	-	+	-	+
	Desmosterolosis *[63, 70, 80-86]*	-/N	+/N	+	+/N
	CTX *[44-46, 48, 49, 52, 54, 87-89]*	+/N	+		+/N
Vitamin D - dependent Rickets	*Type 1A* *[90-92]*				+/N
	Type 1B *[90-93]*				+/N
	Type 2A *[90-92, 94-96]*				+/N
Gaucher Disease	*Type I* *[3, 97-103]*	**+**	**N**	**+**	**U**
	Type II *[101]*				
	Type III *[101, 104]*				
Niemann Pick Diseases	*Type A* *[105-107]*	+			
	Type C1 *[3, 108-110]*	**+**	+	+	+
	Type C2 *[3, 111-113]*	**+**	+	+	+

Untargeted discovery of glycosylated metabolites

The human body was known to contain glucosylated metabolites, ranging from the simplest glycosphingolipid GlcCer [114] to GlcChol [3, 4]. This thesis demonstrates that also Glc7DHC, $GlcD_3$ and GlcDesm occur. More unknown glucosylated metabolites might be present within the human body. Hydrophobic alcohol acceptors X (X = R-OH) might be suitable for formation of a R-β-glucoside. To detect these a so-called 'Transbody' substrate was collaboratively designed and synthesized with the Bio-organic Synthesis department, Leiden Institute of Chemistry. This 'Transbody' is a modified glucose donor to be used in reverse metabolomics (Figure 2).

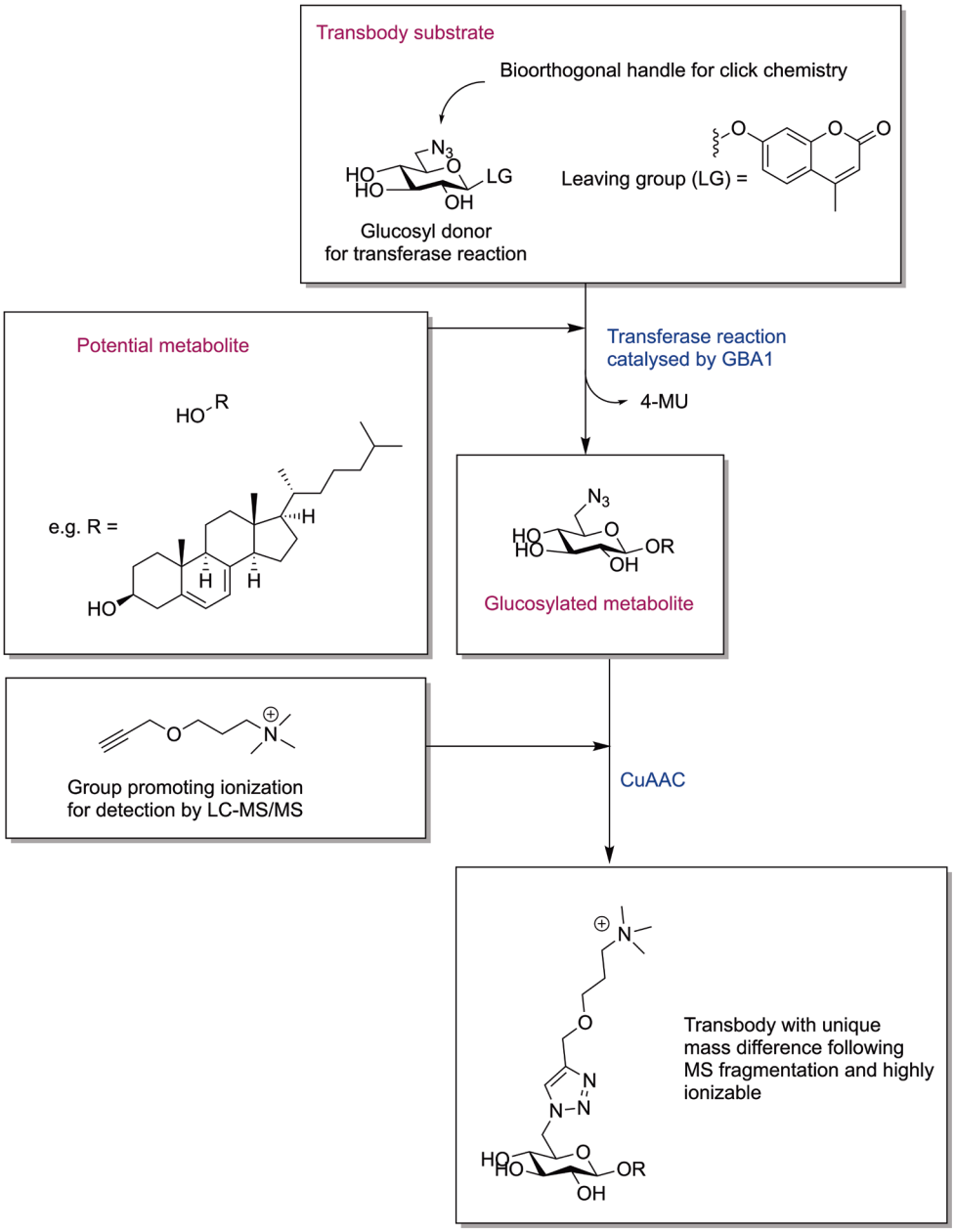


Figure 2. Reverse metabolomics workflow for the discovery of glucose-modified metabolites.

As ‘Transbody’ substrate a C_6-azide-4MUGlc was synthesized. Incubation with recombinant GBA and selected tissue lysates or selected pure alcohols, are expected to result in novel C_6-azide-GlcX. These should be detectable with a specifically developed LC-MS/MS method. For this purpose, the azide should be converted to a group promoting ionization and detection by formation of a unique mass difference following MS fragmentation. Trial experiments were conducted, in order to test the principle. We were able to setup a specific LC-MS/MS method for the detection of C_6-azido-GlcChol. With this method we tested the ability of GBA and GBA2 to transglucosylated the given ‘Transbody’ substrate, C_6-azide-4MUGlc, into C_6-azido-GlcChol. Unfortunately, the ability of GBA to transglucosylate was four times less for the ‘Transbody’ than for the normal 4MUGlc substrate and GBA2 was not able to transglucosylate the ‘Transbody’. An obstacle was the click-chemistry needed to convert the azide group into the ionization and detection promoting group. Further optimization is still required. Alternatively, another modification at C_6 of the glucose-donor substrate is designed preventing the need for click chemistry. Studies on this are presently undertaken at the departments of Medical Biochemistry and Bio-organic Synthesis.

The development of the ‘Transbody’ gives the opportunity to detect known and unknown glucosylated metabolites in biological samples. In **Chapter 2** we present a method for measuring the known glucosylated metabolites GlcChol, Glc7DHC, $GlcD_3$ and GlcDesm within biological samples, such as spleen, plasma, breastmilk and skin. As a limited cohort for each biological sample was available, increasing sample numbers are required for further research. This further research includes the ‘Transbody’ to detect unknown glucosylated metabolites.

Formation and occurrence of Xylosylated metabolites

In line with the conclusion of **Chapter 5** of this thesis, we suggested that xylosylated metabolites might be good substrates for GBA3. The lysosomal β-glucosidase, glucocerebrosidase (GBA) has shown the ability to hydrolyze β-glucosidic substrates and transglucosylates sterols such as cholesterol, 7-dehydrocholesterol and desmosterol to the glucosylated forms GlcChol, Glc7DHC and GlcDesm. Besides hydrolyzing β-glucosidic substrates, GBA is also able to use xylose as sugar donor and perform transxylosylation, at least reaction cholesterol. By transxylosylation xylosyl-cholesterol is formed and this metabolite can act as an acceptor for another xylose to form di-xylosyl-cholesterol as well [6]. As cholesterol can be xylosylated, we conducted some trial experiments on xylosylating 7-dehydrocholesterol. In these experiments GBA has shown potential in transxylosylating 7DHC into Xyl7DHC. These findings prompt further exploration of transxylosylation of 7DHC, the role of Xyl7DHC within the body and the ability of GBA3 to degrade xylosylated compounds.

Conclusion

This thesis describes new LC-MS/MS detection and quantification of discovered glucosylated metabolites Glc7DHC, $GlcD_3$ and GlcDesm. Detection of these metabolites was possible within human samples, such as skin, plasma, breastmilk, but also spleen of healthy and diseased patients. The detection of the glucosylated metabolites allows follow-up research in Gaucher disease patients. It will be of interest to establish whether Glc7DHC and GlcDesm are abnormal in diseases such as SLOS and Desmosterolosis. For this patient material, such as plasma, should be investigated. Even in other diseases such as lathosterolosis, CTX, Vitamin D-dependent rickets and Niemann Pick diseases quantification of GlcChol, Glc7DHC, $GlcD_3$ and GlcDesm should be considered. They might provide clues for novel biomarkers and therapies. The observed natural occurrence of the described glucosylated metabolites prompt a search for additional glucosylated metabolites, within the groups of cholesterol-look-a-like structures, such as oxysterols, steroid hormones and bile acids. New approaches, as the described reversed metabolomics approach for untargeted discovery of glycosylated compounds, might be of great use in this respect. Finally, further investigations on the xylosylation ability of GBA and the ability of GBA3 to degrade xylosylated compounds deserves attention.

References

1. Kallemeijn, W.W., *Activity-based probes for retaining beta-glucosidases: novel tools for research and diagnostics.* 2014, Amsterdam: Amsterdam.
2. Miao, S., et al., *Identification of Glu340 as the active-site nucleophile in human glucocerebrosidase by use of electrospray tandem mass spectrometry.* J Biol Chem, 1994. **269**(15): p. 10975-8.
3. Marques, A.R., et al., *Glucosylated cholesterol in mammalian cells and tissues: formation and degradation by multiple cellular beta-glucosidases.* J Lipid Res, 2016.
4. Akiyama, H., et al., *Cholesterol glucosylation is catalyzed by transglucosylation reaction of beta-glucosidase 1.* Biochem Biophys Res Commun, 2013. **441**(4): p. 838-43.
5. Akiyama, H., et al., *Novel sterol glucosyltransferase in the animal tissue and cultured cells: evidence that glucosylceramide as glucose donor.* Biochim Biophys Acta, 2011. **1811**(5): p. 314-22.
6. Boer, D.E., et al., *Human glucocerebrosidase mediates formation of xylosyl-cholesterol by β-xylosidase and transxylosidase reactions.* J Lipid Res, 2021. **62**: p. 100018.
7. Ikonen, E., *Mechanisms for Cellular Cholesterol Transport: Defects and Human Disease.* Physiological Reviews, 2006. **86**(4): p. 1237-1261.
8. Zmyslowski, A. and A. Szterk, *Oxysterols as a biomarker in diseases.* Clin Chim Acta, 2019. **491**: p. 103-113.
9. Smith, D.W., L. Lemli, and J.M. Opitz, *A NEWLY RECOGNIZED SYNDROME OF MULTIPLE CONGENITAL ANOMALIES.* J Pediatr, 1964. **64**: p. 210-7.
10. Tint, G.S., et al., *Defective cholesterol biosynthesis associated with the Smith-Lemli-Opitz syndrome.* N Engl J Med, 1994. **330**(2): p. 107-13.
11. Cunniff, C., et al., *Clinical and biochemical spectrum of patients with RSH/ Smith-Lemli-Opitz syndrome and abnormal cholesterol metabolism.* Am J Med Genet, 1997. **68**(3): p. 263-9.
12. Rossi, M., et al., *Vitamin D status in patients affected by Smith-Lemli-Opitz syndrome.* J Inherit Metab Dis, 2005. **28**(1): p. 69-80.
13. Brzeska, M., K. Szymczyk, and A. Szterk, *Current Knowledge about Oxysterols: A Review.* J Food Sci, 2016. **81**(10): p. R2299-r2308.
14. Olkkonen, V.M., H. Gylling, and E. Ikonen, *Plant sterols, cholesterol precursors and oxysterols: Minute concentrations-Major physiological effects.* J Steroid Biochem Mol Biol, 2017. **169**: p. 4-9.
15. Chiang, J.Y.L., *Bile Acid Metabolism and Signaling. Comprehensive Physiology,* 2013. **3**(3): p. 1191-1212.
16. Lutjohann, D., et al., *Cholesterol homeostasis in human brain: evidence for an age-dependent flux of 24S-hydroxycholesterol from the brain into the circulation.* Proc Natl Acad Sci U S A, 1996. **93**(18): p. 9799-804.
17. Lund, E.G., et al., *Knockout of the cholesterol 24-hydroxylase gene in mice reveals a brain-specific mechanism of cholesterol turnover.* J Biol Chem, 2003. **278**(25): p. 22980-8.

18. Bjorkhem, I., et al., *Importance of a novel oxidative mechanism for elimination of brain cholesterol. Turnover of cholesterol and 24(S)-hydroxycholesterol in rat brain as measured with 18O2 techniques in vivo and in vitro.* J Biol Chem, 1997. **272**(48): p. 30178-84.
19. Goyal, S., et al., *Oxidation of 7-dehydrocholesterol and desmosterol by human cytochrome P450 46A1.* J Lipid Res, 2014. **55**(9): p. 1933-43.
20. Sakamoto, H., et al., *Rapid signaling of steroid hormones in the vertebrate nervous system.* Front Biosci (Landmark Ed), 2012. **17**: p. 996-1019.
21. Kawata, M., *Roles of steroid hormones and their receptors in structural organization in the nervous system.* Neurosci Res, 1995. **24**(1): p. 1-46.
22. Moss, G.P., *Nomenclature of steroids (Recommendations 1989), in Pure and Applied Chemistry.* 1989. p. 1783.
23. Slominski, A.T., et al., *Characterization of a new pathway that activates lumisterol in vivo to biologically active hydroxylumisterols.* Sci Rep, 2017. **7**(1): p. 11434.
24. Chung, B.C., et al., *Human cholesterol side-chain cleavage enzyme, P450scc: cDNA cloning, assignment of the gene to chromosome 15, and expression in the placenta.* Proc Natl Acad Sci U S A, 1986. **83**(23): p. 8962-6.
25. Staple, E., W.S. Lynn, Jr., and S. Gurin, *An enzymatic cleavage of the cholesterol side chain.* J Biol Chem, 1956. **219**(2): p. 845-51.
26. Shikita, M. and P.F. Hall, *The stoichiometry of the conversion of cholesterol and hydroxycholesterols to pregnenolone (3beta-hydroxypregn-5-en-20-one) catalysed by adrenal cytochrome P-450.* Proc Natl Acad Sci U S A, 1974. **71**(4): p. 1441-5.
27. Goodman, D.S., J. Avigan, and H. Wilson, *The in vitro metabolism of desmosterol with adrenal and liver preparations.* J Clin Invest, 1962. **41**: p. 2135-41.
28. Goodman, D.S., J. Avigan, and H. Wilson, T*he metabolism of desmosterol in human subjects during triparanol administration.* J Clin Invest, 1962. **41**: p. 962-71.
29. Slominski, A., et al., *A novel pathway for sequential transformation of 7-dehydrocholesterol and expression of the P450scc system in mammalian skin.* Eur J Biochem, 2004. **271**(21): p. 4178-88.
30. Slominski, A., G. Ermak, and M. Mihm, *ACTH receptor, CYP11A1, CYP17 and CYP21A2 genes are expressed in skin.* J Clin Endocrinol Metab, 1996. **81**(7): p. 2746-9.
31. Slominski, A.T., et al., *Cytochrome P450scc-dependent metabolism of 7-dehydrocholesterol in placenta and epidermal keratinocytes.* Int J Biochem Cell Biol, 2012. **44**(11): p. 2003-18.
32. Slominski, A.T., et al., *Sequential metabolism of 7-dehydrocholesterol to steroidal 5,7-dienes in adrenal glands and its biological implication in the skin.* PLoS One, 2009. **4**(2): p. e4309.

33. Guryev, O., et al., *A pathway for the metabolism of vitamin D3: unique hydroxylated metabolites formed during catalysis with cytochrome P450scc (CYP11A1).* Proc Natl Acad Sci U S A, 2003. **100**(25): p. 14754-9.
34. Slominski, A.T., et al., *Products of vitamin D3 or 7-dehydrocholesterol metabolism by cytochrome P450scc show anti-leukemia effects, having low or absent calcemic activity.* PLoS One, 2010. **5**(3): p. e9907.
35. Zbytek, B., et al., *20-Hydroxyvitamin D3, a product of vitamin D3 hydroxylation by cytochrome P450scc, stimulates keratinocyte differentiation.* J Invest Dermatol, 2008. **128**(9): p. 2271-80.
36. Slominski, A.T., et al., *Cytochromes p450 and skin cancer: role of local endocrine pathways.* Anticancer Agents Med Chem, 2014. **14**(1): p. 77-96.
37. Skobowiat, C., et al., *Noncalcemic 20-hydroxyvitamin D3 inhibits human melanoma growth in in vitro and in vivo models.* Oncotarget, 2017. **8**(6): p. 9823-9834.
38. Dubrac, S., et al., *Role of CYP27A in cholesterol and bile acid metabolism.* J Lipid Res, 2005. **46**(1): p. 76-85.
39. Mast, N., et al., *Cholesterol binding to cytochrome P450 7A1, a key enzyme in bile acid biosynthesis.* Biochemistry, 2005. **44**(9): p. 3259-71.
40. Russell, D.W., *The enzymes, regulation, and genetics of bile acid synthesis.* Annu Rev Biochem, 2003. **72**: p. 137-74.
41. Shinkyo, R., et al., *Conversion of 7-dehydrocholesterol to 7-ketocholesterol is catalyzed by human cytochrome P450 7A1 and occurs by direct oxidation without an epoxide intermediate.* J Biol Chem, 2011. **286**(38): p. 33021-8.
42. Brown, A.J. and W. Jessup, *Oxysterols: Sources, cellular storage and metabolism, and new insights into their roles in cholesterol homeostasis.* Mol Aspects Med, 2009. **30**(3): p. 111-22.
43. Griffiths, W.J., et al., *Sterols and oxysterols in plasma from Smith-Lemli-Opitz syndrome patients.* J Steroid Biochem Mol Biol, 2017. **169**: p. 77-87.
44. Salen, G. and R.D. Steiner, *Epidemiology, diagnosis, and treatment of cerebrotendinous xanthomatosis (CTX).* J Inherit Metab Dis, 2017. **40**(6): p. 771-781.
45. Menkes, J.H., J.R. Schimschock, and P.D. Swanson, *Cerebrotendinous xanthomatosis. The storage of cholestanol within the nervous system.* Arch Neurol, 1968. **19**(1): p. 47-53.
46. Zadori, D., et al., *Different phenotypes in identical twins with cerebrotendinous xanthomatosis: case series.* Neurol Sci, 2017. **38**(3): p. 481-483.
47. Huidekoper, H.H., et al., *Hepatotoxicity due to chenodeoxycholic acid supplementation in an infant with cerebrotendinous xanthomatosis: implications for treatment.* Eur J Pediatr, 2016. **175**(1): p. 143-6.
48. Martini, G., et al., *Long-term bone density evaluation in cerebrotendinous xanthomatosis: evidence of improvement after chenodeoxycholic acid treatment.* Calcif Tissue Int, 2013. **92**(3): p. 282-6.

49. Federico, A., et al., *Cerebrotendinous xanthomatosis: pathophysiological study on bone metabolism.* J Neurol Sci, 1993. **115**(1): p. 67-70.
50. Wassif, C.A., et al., *27-Hydroxylation of 7- and 8-dehydrocholesterol in Smith-Lemli-Opitz syndrome: a novel metabolic pathway.* Steroids, 2003. **68**(6): p. 497-502.
51. Movassaghi, M., et al., *Vitamin D levels in Smith-Lemli-Opitz syndrome.* American journal of medical genetics. Part A, 2017. **173**(10): p. 2577-2583.
52. de Sain-van der Velden, M.G., et al., *Elevated cholesterol precursors other than cholestanol can also be a hallmark for CTX.* J Inherit Metab Dis, 2008. **31 Suppl 2**: p. S387-93.
53. Shinkyo, R., et al., *Metabolism of vitamin D by human microsomal CYP2R1.* Biochem Biophys Res Commun, 2004. **324**(1): p. 451-7.
54. Gupta, R.P., K. Patrick, and N.H. Bell, *Mutational analysis of CYP27A1: assessment of 27-hydroxylation of cholesterol and 25-hydroxylation of vitamin D.* Metabolism, 2007. **56**(9): p. 1248-55.
55. Gaucher, P.C., *De l'epitheliome primitif de la rate, hypertrophie idiopathique de la rate sans leucemie.* PhD thesis, Faculte de Medicine, Paris, 1882.
56. Goldblatt, J., *Type I Gaucher disease.* J Med Genet, 1988. **25**(6): p. 415-8.
57. Beutler, E., Grawobski, G.A., , *Gaucher disease, in: C.R. Scriver,W.S. Sly, D. Valle (Eds.), The Metabolic and Molecular Bases of Inherited Disease.* 8th ed. 2001, New York: McGraw-Hill.
58. Sidransky, E., *New perspectives in type 2 Gaucher disease.* Adv Pediatr, 1997. **44**: p. 73-107.
59. Eblan, M.J., O. Goker-Alpan, and E. Sidransky, *Perinatal lethal Gaucher disease: a distinct phenotype along the neuronopathic continuum.* Fetal Pediatr Pathol, 2005. **24**(4-5): p. 205-22.
60. Sidransky, E., *Gaucher disease: complexity in a "simple" disorder.* Mol Genet Metab, 2004. **83**(1-2): p. 6-15.
61. Biegstraaten, M., et al., *'Non-neuronopathic' Gaucher disease reconsidered. Prevalence of neurological manifestations in a Dutch cohort of type I Gaucher disease patients and a systematic review of the literature.* J Inherit Metab Dis, 2008. **31**(3): p. 337-49.
62. Lachmann, R.H., et al., *Twin pairs showing discordance of phenotype in adult Gaucher's disease.* QJM, 2004. **97**(4): p. 199-204.
63. Zolotushko, J., et al., *The desmosterolosis phenotype: spasticity, microcephaly and micrognathia with agenesis of corpus callosum and loss of white matter.* Eur J Hum Genet, 2011. **19**(9): p. 942-6.
64. Moreau, R.A., B.D. Whitaker, and K.B. Hicks, *Phytosterols, phytostanols, and their conjugates in foods: structural diversity, quantitative analysis, and health-promoting uses.* Progress in Lipid Research, 2002. **41**(6): p. 457-500.

65. Piironen, V., J. Toivo, and A.M. Lampi, *Natural Sources of Dietary Plant Sterols.* Journal of Food Composition and Analysis, 2000. **13**(4): p. 619-624.
66. Clark, R.M., et al., *Desmosterol in human milk.* Lipids, 1983. **18**(3): p. 264-6.
67. Carlson, S.E., *Infant Formula,* U.o.K.M. Center, Editor. 2005: Canada.
68. Weingartner, O., et al., *Plant sterol ester diet supplementation increases serum plant sterols and markers of cholesterol synthesis, but has no effect on total cholesterol levels.* J Steroid Biochem Mol Biol, 2017. **169**: p. 219-225.
69. Mouritsen, O.G., et al., *Effects of seaweed sterols fucosterol and desmosterol on lipid membranes.* Chem Phys Lipids, 2017. **205**: p. 1-10.
70. FitzPatrick, D.R., et al., *Clinical phenotype of desmosterolosis.* Am J Med Genet, 1998. **75**(2): p. 145-52.
71. Waterham, H.R., et al., *Mutations in the 3beta-hydroxysterol Delta24-reductase gene cause desmosterolosis, an autosomal recessive disorder of cholesterol biosynthesis.* Am J Hum Genet, 2001. **69**(4): p. 685-94.
72. Brunetti-Pierri, N., et al., *Lathosterolosis, a novel multiple-malformation/mental retardation syndrome due to deficiency of 3beta-hydroxysteroid-delta5-desaturase.* Am J Hum Genet, 2002. **71**(4): p. 952-8.
73. Krakowiak, P.A., et al., *Lathosterolosis: an inborn error of human and murine cholesterol synthesis due to lathosterol 5-desaturase deficiency.* Human Molecular Genetics, 2003. **12**(13): p. 1631-1641.
74. Ho, A.C., et al., *Lathosterolosis: a disorder of cholesterol biosynthesis resembling smith-lemli-opitz syndrome.* JIMD Rep, 2013. **12**: p. 129-34.
75. Rossi, M., et al., *Clinical phenotype of lathosterolosis.* Am J Med Genet A, 2007. **143a**(20): p. 2371-81.
76. Anderson, R., et al., L*athosterolosis: A Relatively Mild Case with Cataracts and Learning Difficulties.* JIMD Rep, 2019. **44**: p. 79-84.
77. Kuriyama, M., et al., *High levels of plant sterols and cholesterol precursors in cerebrotendinous xanthomatosis.* J Lipid Res, 1991. **32**(2): p. 223-9.
78. Porter, F.D. and G.E. Herman, *Malformation syndromes caused by disorders of cholesterol synthesis.* J Lipid Res, 2011. **52**(1): p. 6-34.
79. Yu, H. and S.B. Patel, *Recent insights into the Smith-Lemli-Opitz syndrome.* Clin Genet, 2005. **68**(5): p. 383-91.
80. Allen, L.B., et al., *Desmosterolosis and desmosterol homeostasis in the developing mouse brain.* J Inherit Metab Dis, 2019. **42**(5): p. 934-943.
81. Zerenturk, E.J., et al., *Desmosterol and DHCR24: unexpected new directions for a terminal step in cholesterol synthesis.* Prog Lipid Res, 2013. **52**(4): p. 666-80.
82. Dewind, L.T., *Hypervitaminosis D with osteosclerosis.* Arch Dis Child, 1961. **36**: p. 373-80.

83. Schaaf, C.P., et al., *Desmosterolosis-phenotypic and molecular characterization of a third case and review of the literature.* American journal of medical genetics. Part A, 2011. **155A**(7): p. 1597-1604.
84. Dias, C., et al., *Desmosterolosis: an illustration of diagnostic ambiguity of cholesterol synthesis disorders.* Orphanet J Rare Dis, 2014. **9**: p. 94.
85. Rohanizadegan, M. and S. Sacharow, *Desmosterolosis presenting with multiple congenital anomalies.* Eur J Med Genet, 2017.
86. Andersson, H.C., L. Kratz, and R. Kelley, *Desmosterolosis presenting with multiple congenital anomalies and profound developmental delay.* Am J Med Genet, 2002. **113**(4): p. 315-9.
87. Mast, N., et al., *Cytochrome P450 27A1 Deficiency and Regional Differences in Brain Sterol Metabolism Cause Preferential Cholestanol Accumulation in the Cerebellum.* J Biol Chem, 2017. **292**(12): p. 4913-4924.
88. Berginer, V.M., et al., *Osteoporosis and increased bone fractures in cerebrotendinous xanthomatosis.* Metabolism, 1993. **42**(1): p. 69-74.
89. Gelzo, M., et al., *Age-related changes of cholestanol and lathosterol plasma concentrations: an explorative study.* Lipids Health Dis, 2019. **18**(1): p. 235.
90. Kim, C.J., *Vitamin D dependent rickets type I.* Korean journal of pediatrics, 2011. **54**(2): p. 51-54.
91. Acar, S., K. Demir, and Y. Shi, *Genetic Causes of Rickets.* Journal of clinical research in pediatric endocrinology, 2017. **9**(Suppl 2): p. 88-105.
92. Elder, C.J. and N.J. Bishop, *Rickets.* The Lancet, 2014. **383**(9929): p. 1665-1676.
93. Casella, S.J., et al., *A possible genetic defect in 25-hydroxylation as a cause of rickets.* J Pediatr, 1994. **124**(6): p. 929-32.
94. Brooks, M.H., et al., *Vitamin-D-dependent rickets type II. Resistance of target organs to 1,25-dihydroxyvitamin D.* N Engl J Med, 1978. **298**(18): p. 996-9.
95. Marx, S.J., et al., *A familial syndrome of decrease in sensitivity to 1,25-dihydroxyvitamin D.* J Clin Endocrinol Metab, 1978. **47**(6): p. 1303-10.
96. Rosen, J.F., et al., *Rickets with alopecia: an inborn error of vitamin D metabolism.* J Pediatr, 1979. **94**(5): p. 729-35.
97. Hughes, D., et al., *Gaucher Disease in Bone: From Pathophysiology to Practice.* J Bone Miner Res, 2019.
98. Vergara, C., et al., *AB0804 Bone Manifestations in Patients with Gaucher Disease.* Annals of the Rheumatic Diseases, 2014. **73**(Suppl 2): p. 1069.
99. Mikosch, P., et al., *Patients with Gaucher disease living in England show a high prevalence of vitamin D insufficiency with correlation to osteodensitometry.* Mol Genet Metab, 2009. **96**(3): p. 113-20.
100. Mikosch, P. and D. Hughes, *An overview on bone manifestations in Gaucher disease.* Wien Med Wochenschr, 2010. **160**(23-24): p. 609-24.

101. Baris, H.N., I.J. Cohen, and P.K. Mistry, *Gaucher disease: the metabolic defect, pathophysiology, phenotypes and natural history.* Pediatr Endocrinol Rev, 2014. **12 Suppl 1**: p. 72-81.
102. Ginsberg, H., et al., *Reduced plasma concentrations of total, low density lipoprotein and high density lipoprotein cholesterol in patients with Gaucher type I disease.* Clin Genet, 1984. **26**(2): p. 109-16.
103. Pocovi, M., et al., *Beta-glucocerebrosidase gene locus as a link for Gaucher's disease and familial hypo-alpha-lipoproteinaemia.* Lancet, 1998. **351**(9120): p. 1919-23.
104. Schwartz, I.V.D., et al., *Characteristics of 26 patients with type 3 Gaucher disease: A descriptive analysis from the Gaucher Outcome Survey.* Molecular genetics and metabolism reports, 2017. **14**: p. 73-79.
105. Storch, J. and Z. Xu, *Niemann-Pick C2 (NPC2) and intracellular cholesterol trafficking.* Biochim Biophys Acta, 2009. **1791**(7): p. 671-8.
106. Liou, H.L., et al., *NPC2, the protein deficient in Niemann-Pick C2 disease, consists of multiple glycoforms that bind a variety of sterols.* J Biol Chem, 2006. **281**(48): p. 36710-23.
107. Conte, C., et al., *Niemann-Pick Type A Disease: Behavior of Neutral Sphingomyelinase and Vitamin D Receptor.* International journal of molecular sciences, 2019. **20**(9): p. 2365.
108. Vanier, M.T., *Complex lipid trafficking in Niemann-Pick disease type C.* J Inherit Metab Dis, 2015. **38**(1): p. 187-99.
109. Xie, C., et al., *Cholesterol is sequestered in the brains of mice with Niemann-Pick type C disease but turnover is increased.* J Neuropathol Exp Neurol, 2000. **59**(12): p. 1106-17.
110. Yerushalmi, B., et al., *Niemann-pick disease type C in neonatal cholestasis at a North American Center.* J Pediatr Gastroenterol Nutr, 2002. **35**(1): p. 44-50.
111. Vanier, M.T., *Lipid changes in Niemann-Pick disease type C brain: personal experience and review of the literature.* Neurochem Res, 1999. **24**(4): p. 481-9.
112. Vanier, M.T., *Niemann-Pick disease type C.* Orphanet J Rare Dis, 2010. **5**: p. 16.
113. Hashimoto, N., et al., *Cholesterol-dependent increases in glucosylceramide synthase activity in Niemann-Pick disease type C model cells: Abnormal trafficking of endogenously formed ceramide metabolites by inhibition of the enzyme.* Neuropharmacology, 2016. **110**(Pt A): p. 458-469.
114. Sandhoff, K. and T. Kolter, *Biosynthesis and degradation of mammalian glycosphingolipids.* Philos Trans R Soc Lond B Biol Sci, 2003. **358**(1433): p. 847-61.