Exploring the mechanisms underlying the phenotype of MCAD deficiency with Systems Medicine

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Appendices

Summary

Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency (OMIM 201450), a deficiency of an isoenzyme of the mitochondrial fatty acid oxidation (mFAO) pathway, is one of the most prevalent inborn errors of metabolism (IEM). MCAD deficiency is also the most common IEM of the mFAO pathway. The MCADD prevalence at birth was 1/8300 in the Netherlands between 2007 and 2015 (**Chapter 1**). mFAO plays an important role in energy homeostasis during fasting and conditions of high-energy demand. Under these conditions, the liver uses the mFAO pathway to generate ketone bodies and the ATP that is required for *de novo* glucose production. Thereby the mFAO is of critical importance for fuel supply to the peripheral tissues. Untreated MCAD-deficient (MCADD) children run a severe risk of life-threatening low blood glucose and low ketone body levels (hypoketotic hypoglycemia). Nevertheless, MCADD individuals have been reported to tolerate 18-24 hours of fasting. Specifically, a combination of prolonged fasting together with an additional trigger, such as airway or gastrointestinal infections or cold exposure, can put untreated MCADD individuals at risk. Even within the subpopulation of individuals with a homozygous loss-of-function c.985A>G mutation in the MCAD-encoding *ACADM* gene, symptoms can range from fatal hypoketotic hypoglycemia early in life to a complete lack of symptoms throughout life. Genetic and environmental modifiers could play a role in the heterogeneity in vulnerability to or robustness against metabolic stress. How this vulnerability or robustness arises is as yet unclear.

In my thesis I have therefore taken a systems medicine approach to get a better insight in the complex etiology of MCADD. My overarching question has been what elicits hypoketotic hypoglycemia in some MCAD patients and what protects others. I investigated which modifying factors within and outside the pathway may push metabolism from an apparently healthy state into towards hypoketotic hypoglycemia and which factors may keep the metabolic network safely away from this dangerous state. In order to answer this question, I evaluated MCAD deficiency in a computational model (*i.e. in silico*), in mice *in vivo* and in human cells *in vitro*.

I first investigate the behavior of a published computational model of isolated mFAO in rat liver (**Chapter 2**), since I intended to use this model as a basis to construct a human model version later. I unraveled the origin of the sharp decline of the mFAO flux that occurred in the model high substrate concentrations. This flux decline was clearly related to the promiscuity of the mFAO enzymes, *i.e.* the fact that they accept multiple homologous substrates, which compete for binding to the active site of the enzyme. I identified a vicious cycle, that started at medium-chain ketoacyl-CoA thiolase (MCKAT) and propagated throughout the system, leading to low mFAO flux. Herein, promiscuity of MCKAT was both necessary and sufficient to elicit the flux decline. MCKAT had insufficient capacity to cope with high substrate influx and consequently, it's ketoacyl-CoA substrates started to accumulate. This accumulation was amplified by the preceding enzyme, leading to accumulation of upstream CoA esters, including acyl-CoA esters, and sequestration of free CoA (CoASH). Since CoASH is an essential cofactor of MCKAT and acyl-CoA esters are MCKAT products, its activity was further inhibited. In addition, CoASH is also a substrate of other enzymes in the mFAO pathway, and thereby its sequestration efficiently communicated the 'traffic jam' at MCKAT to the entire pathway, ultimately resulting in low mFAO flux. Finally, I found that the vicious cycle was aggravated by a high NADH concentration. The latter causes product inhibition of the mFAO and thereby enhances the accumulation of CoA esters and sequestration of CoASH.

In **Chapter 3**, I investigated which metabolic pathways contribute to the robustness of fasted MCAD-KO mice, since in these mice low blood glucose or ketone body concentrations had never been observed. The octanoyl-CoA oxidation capacity of liver mitochondria from MCAD-KO mice was lower than that of WT mice. This lower capacity was not compensated for by higher protein levels of the short- and long-chain isoenzymes SCAD and LCAD in the MCAD-KO mice. We did however observe subtle adaptations in the expression of genes encoding enzymes that take CoASH, $NAD(P)^+$ or NAD(P)H as a substrate or product, as well as enzymes involved in detoxification. These adaptations may contribute to the robustness of the MCAD-KO mice and warrant further investigation. These results corroborate the important role of CoASH and NADH for mFAO function shown in **Chapter 2**.

In **Chapter 4**, I investigated the hepatic metabolism of cold-exposed, fasted MCAD-KO mice in response to severe energetic stress. Under these conditions the MCAD-KO mice showed for the first time, lower plasma glucose levels than the WT. In addition, the MCAD-KO mice had lower hepatic amino acid levels and higher liver weights, liver total triglycerides, liver medium-chain triglycerides and higher plasma medium-chain acyl-carnitine levels. In the most severely affected MCAD-KO mice, we also observed hepatic inflammation, which is in line with the trend towards liver inflammation observed in fasted MCAD-KO mice in **Chapter 3**. The results suggest an important role of liver mFAO in glucose homeostasis during cold stress in combination with fasting. MCAD-KO mice appeared to use amino acids an alternative fuel to compensate for the deficient mFAO in the generation of ATP, glucose, and ketone bodies, while excess medium-chain fatty-acid intermediates were exported and rerouted directly or indirectly by chain elongation into triglycerides.

In **Chapter 5**, I investigated how pathways surrounding the mFAO, notably those that affect CoASH, modulate the metabolic phenotype when MCAD or other acyl-CoA dehydrogenases are deficient. This should give insights in modifying factors that may play a role in the different disease presentation of individual patients. I first adapted the computational model that was used in Chapter 2 by incorporating the available human kinetic parameters. Moreover, I added a number of pathways involved in acyl-CoA metabolism. These modifications stabilized the mFAO and prevented the flux decline observed in Chapter 2. Simulated fluxes and metabolite profiles reflected the relative disease severities of SCAD, MCAD, and VLCAD deficiency in humans. Repression of the futile cycle of acyl-CoA hydrolysis and reactivation, by modulating the substrate specificity of CPT1, impaired the mFAO flux specifically in the MCAD deficient model. The results give a first indication how genetic modifiers related to the surrounding pathways could affect mFAO function in MCADD patients.

In **Chapter 6**, I investigated whether there are metabolic adaptations in cultured skin fibroblasts of MCADD individuals that distinguish symptomatic from asymptomatic MCADD individuals. The fibroblasts of most of the asymptomatic MCADD individuals resembled those of their symptomatic counterparts. Some of the asymptomatic children were however diagnosed at a young age and one might wonder if they were asymptomatic because of the treatment or because of their genetic make-up. One MCADD individual, however, was diagnosed at the age of 30 and was genuinely asymptomatic throughout life, without any treatment. The fibroblasts of this person secreted acyl-carnitines at a higher rate and had higher concentration of SCAD. Other differences between the proteome of this person and all others, were not reproducible. I suggest that the differences may be larger when the cells depend more on the mFAO, e. g. when the glucose concentration is lowered further.

This thesis has added to the understanding of MCAD deficiency by using a combination of Systems Medicine tools. The results obtained by computational modeling and experiments with mice and patient-derived cells, suggest an important the role of CoASH, its conjugated thioesters and NAD(P)(H) in mFAO function and MCAD deficiency. The human version of the computational model (**Chapter 5**) will be an important tool to integrate experimental data from human tissue and obtain a better understanding of MCAD deficiency. The groundwork and discussions laid out in this thesis provide opportunities for follow-up studies to unravel the etiology of heterogeneous phenotypes in MCAD deficiency. I hope that in the future, the obtained result will enable more personalized diagnoses, prognoses and treatment strategies for MCAD deficiency.

Samenvatting

Midden-keten acyl-CoA dehydrogenase (MCAD) deficiëntie (OMIM 201450) is een enzymdefect in de mitochondriële vetzuuroxidatieroute. MCAD-deficiëntie is een van de meest voorkomende aangeboren stofwisselingsziektes. Het is ook het meest voorkomende aangeboren defect in de vetzuuroxidatie. In Nederland was de prevalentie bij geboorte 1/8300 tussen 2007 en 2015 (**Hoofdstuk 1**). De mitochondriële vetzuuroxidatie speelt een belangrijke rol in onze energiehuishouding, met name tijdens vasten of als we veel energie verbruiken. Onder deze omstandigheden zorgt vetzuuroxidatie in de lever voor de productie van ketonlichamen en voor de ATP die nodig is om nieuwe glucose te maken. Hiermee is dit proces van essentieel belang voor de brandstofvoorziening van de organen in het lichaam. Als MCAD-deficiënte (MCADD) kinderen niet worden behandeld, lopen ze een ernstig risico op levensbedreigende lage bloedsuikerwaarden en lage concentraties van ketonlichamen. Dit wordt hypoketotische hypoglycemie genoemd. Toch is beschreven dat MCADD-patiënten 18 tot 24 uur kunnen vasten. Het is de combinatie van langdurig vasten met een extra risicofactor, zoals een luchtweg- of darminfectie of blootstelling aan kou, die gevaarlijk is voor jonge MCADD-patiënten. Er is echter een grote klinische heterogeniteit in deze patiëntengroep. Zelfs binnen de subpopulatie van patiënten met dezelfde c985A>G mutatie, die het enzym volledig inactiveert, kunnen de symptomen variëren van een fatale hypoketotische hypoglycemie tot een levenslang ontbreken van enige ziekteverschijnselen die met MCADD in verband kunnen worden gebracht. Genetische en omgevingsfactoren kunnen een rol spelen in de heterogeniteit van deze patiënten groep. Wat de gevoeligheid van individuele patiënten precies bepaalt, is echter niet duidelijk. In mijn proefschrift heb ik daarom de aanpak van de 'systeemgeneeskunde' gevolgd om een beter inzicht te krijgen in het ontstaan van symptomen bij MCADD. Mijn overkoepelende vraag is wat ervoor zorgt dat bij sommige patiënten een hypoketotische hypoglycemie optreedt, terwijl anderen hiertegen beschermd lijken. Ik heb onderzocht welke modulaties binnen en buiten de vetzuuroxidatie, de stofwisseling vanuit een schijnbaar gezonde toestand naar een hypoketotische hypoglycemie kunnen duwen, maar ook welke factoren juist bescherming bieden tegen deze gevaarlijke toestand. Om deze vragen te kunnen beantwoorden heb ik MCAD-deficiëntie onderzocht door middel van computersimulaties en experimenten met muizen en menselijke cellen.

Ik ben begonnen het gedrag van een reeds bestaand computermodel van de geïsoleerde vetzuuroxidatie in rattenlever te onderzoeken (**Hoofdstuk 2**). Dit model zou ik later in **Hoofdstuk 5** nodig hebben als basis voor een model van de menselijke vetzuuroxidatie. Computersimulaties brachten een scherpe daling van de snelheid van vetzuuroxidatie bij hoge substraatconcentratie aan het licht. Ik heb het mechanisme dat hiervoor verantwoordelijk is, ontrafeld. De daling van de snelheid had duidelijk te maken met de promiscuïteit van de betrokken enzymen. Hiermee bedoel ik dat de enzymen verschillende, op elkaar lijkende substraten accepteren, welke op hun beurt concurreren voor de binding aan het katalytische centrum van het enzym. Ik kwam een vicieuze cirkel op het spoor die zijn oorsprong had in het enzym middenketen ketoacyl-thiolase (MCKAT), zich voortplantte door het gehele systeem en uiteindelijk leidde tot het instorten van de vetzuuroxidatiesnelheid. De promiscuïteit van MCKAT was een noodzakelijke en voldoende voorwaarde voor dit verschijnsel. MCKAT had te weinig katalytische capaciteit om een hoge instroom van vetzuren te kunnen verwerken. Daardoor begonnen keto-acyl-CoA moleculen, de substraten van MCKAT, op te hopen. Deze metabolietstapeling werd versterkt door

productremming van het voorgaande enzym, zodat ook acyl-CoA metabolieten stroomopwaarts begonnen op te hopen. Omdat dit allemaal thioesters van coenzym A zijn, leidde dit tot een tekort aan beschikbaar coenzym A. Nu zijn de acyl-CoA metabolieten tevens producten van MCKAT en is vrij coenzym A een substraat van dit enzym. Het tekort aan coenzym A en de overmaat van acyl-CoA had daardoor een sterk remmende werking op MCKAT. Coenzym A is bovendien een essentiële cofactor voor veel andere enzymen in de vetzuuroxidatie. Het tekort aan coenzym A communiceerde de ontstane opstopping bij MCKAT daardoor snel naar de rest van het systeem. Dit resulteerde uiteindelijk in een sterk verlaagde vetzuuroxidatiesnelheid. Ten slotte kwam ik erachter dat de vicieuze cirkel versterkt werd door een hoge NADH concentratie. NADH zorgt voor productremming van de vetzuuroxidatie en verergert de ophoping van esters van coenzym A en de uitputting van vrij beschikbaar coenzym A.

In **Hoofdstuk 3** heb ik onderzocht welke stofwisselingsprocessen kunnen bijdragen aan de robuustheid van muizen waarin het gen dat codeert voor MCAD, was uitgeschakeld door genetische modificatie. In deze muizen waren nog niet eerder lage bloedsuikerwaardes of concentraties van ketonlichamen aangetoond tijdens vasten. De levers van muizen zonder MCAD hadden een lagere katalytische capaciteit voor de oxidatie van octanoyl-CoA dan de niet-gemodificeerde muizen. Deze lagere capaciteit werd niet gecompenseerd door verhoogde eiwitniveaus van de isoenzymen korte-keten- en lange-keten-acyl-CoA dehydrogenases, respectievelijk SCAD en LCAD. Wel nam ik subtiele aanpassingen waar in de expressie van genen die coderen voor enzymen die coenzym A, $NAD(P)^+$ of NAD(P)H als substraat of product hebben, alsmede in enzymen die betrokken zijn bij ontgiftingsreacties. Deze aanpassingen kunnen mogelijkerwijs bijdragen aan de robuustheid van de muizen zonder MCAD en zijn de moeite waard om verder te onderzoeken. De resultaten versterken ook mijn eerdere bevindingen in **Hoofdstuk 2** dat coenzym A en NADH belangrijk zijn voor het functioneren van de vetzuuroxidatie.

In **Hoofdstuk 4** heb ik de leverstofwisseling onderzocht wanneer muizen zonder MCAD werden blootgesteld aan een combinatie van koude en vasten, wat een grote energetische uitdaging teweegbrengt. Onder deze omstandigheden vond ik voor het eerst lagere bloedglucosewaarden in muizen zonder MCAD dan in de niet-gemodificeerde muizen. Bovendien hadden de muizen zonder MCAD lagere aminozuurconcentraties in de lever. Ze hadden ook een zwaardere lever en een hogere concentratie van triglycerides in de lever (zowel de totale concentratie als die van de midden-keten triglycerides), en hogere concentraties van midden-keten acyl-carnitines in het bloed. In de muis die het sterkst was aangedaan door de afwezigheid van MCAD, nam ik ook een ontsteking van de lever waar. Dit past bij mijn waarneming in **Hoofdstuk 3** dat de muizen zonder MCAD al tijdens gewoon vasten enige neiging tot ontsteking van de lever hebben. De resultaten suggereren dat vetzuuroxidatie in de lever een belangrijke rol speelt in de homeostase van glucose tijdens vasten in combinatie met kou. Muizen zonder MCAD leken aminozuren te gebruiken als alternatieve brandstof om te compenseren voor de defecte vetzuuroxidatie, zodat ze toch ATP en glucose konden maken. De overmaat van midden-keten vetzuren werd door de lever geëxporteerd in de vorm van acyl-carnitines of ingebouwd in triglycerides, al dan niet na verlenging van de vetzuurketens.

In **Hoofdstuk 5** heb ik de invloed onderzocht van de stofwisselingsroutes die in contact staan met de vetzuuroxidatie, met name degenen waarin coenzym A betrokken is. Ik heb het

effect van deze processen op het MCADD fenotype bestudeerd door middel van computersimulaties van de leverstofwisseling. Dit kan inzicht geven in welke factoren een rol spelen in het ziektebeeld van individuele patiënten. Eerst heb ik het computermodel uit Hoofdstuk 2 aangepast door er de beschikbare kinetische parameters voor menselijke enzymen in te voegen. Verder heb ik een aantal processen toegevoegd die betrokken zijn bij de omzettingen van acyl-CoA. Deze aanpassingen maakten de vetzuuroxidatie stabieler en voorkwamen de het instorten van de vetzuuroxidatieflux die ik in **Hoofdstuk 2** had bestudeerd. De gesimuleerde snelheden en metabolietprofielen weerspiegelden de ernst van SCAD-, MCAD- en VLCAD- deficiëntie in mensen. Toen ik de niet-productieve cyclus van acyl-CoA hydrolyse en reactivering onderdrukte door de substraatspecificiteit van CPT1 te veranderen, bleek dit de vetzuuroxidatie met name in het MCADD model te remmen. De resultaten geven een eerste indruk hoe genetische verschillen met betrekking tot de processen rondom de vetzuuroxidatie uiteindelijk de vetzuuroxidatie zelf kunnen beïnvloeden in MCADD-patiënten.

In **Hoofdstuk 6** heb ik tenslotte gezocht naar aanpassingen in de stofwisseling waarmee we onderscheid kunnen maken tussen MCADD-patiënten met en zonder symptomen. Ik heb dit onderzocht in gekweekte fibroblasten van de huid die waren afgenomen van individuele MCADD-patiënten. De fibroblasten van symptomatische en asymptomatische patiënten leken sterk op elkaar. Sommige asymptomatische patiënten hadden hun diagnose echter op jonge leeftijd gekregen en je kunt je afvragen of ze asymptomatisch waren vanwege hun behandeling of vanwege hun genetische achtergrond. Eén MCADD-patiënt was echter pas gediagnosticeerd op 30-jarige leeftijd en was werkelijk levenslang asymptomatisch gebleven, zonder enige behandeling. De fibroblasten van deze persoon vertoonden een hogere uitscheiding van acyl-carnitines een hogere concentratie van SCAD. Andere verschillen tussen het proteoom van de fibroblasten van deze persoon en dat van alle anderen waren niet herhaalbaar. Ik stel voor dat de verschillen mogelijk groter zullen zijn als de cellen meer afhankelijk worden gemaakt van vetzuuroxidatie, bijvoorbeeld door de glucoseconcentratie verder omlaag te brengen.

Met mijn proefschrift hoop ik te hebben bijgedragen aan het begrip van MCADD door gebruik te maken van methodes uit de systeemgeneeskunde. De resultaten die ik heb verkregen door middel van computersimulaties en experimenten met muizen en cellen van patiënten, suggereren dat coenzym A, esters van coenzym A en NAD(P)H een belangrijke rol spelen in het functioneren van de vetzuuroxidatie wanneer MCAD afwezig of inactief is. De menselijke versie van het computermodel (**Hoofdstuk 5**) kan een belangrijk hulpmiddel zijn om experimentele gegevens uit menselijke weefsels te integreren en om een beter begrip te krijgen van MC3ADD. De basis die ik in dit proefschrift heb gelegd, biedt mogelijkheden voor vervolgstudies om het heterogene ziektebeeld van MCADD verder te ontrafelen. Ik hoop dat de resultaten in de toekomst persoonlijkere diagnoses, prognoses en behandelingen voor MCADD-patiënten mogelijk zullen maken.

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Biography

Anne-Claire Martines was born on October 10th 1981 in Willemstad, Curaçao, and grew up in Jandoret, with her parents and sister. In 1999, she graduated Maria Immaculata Lyceum (VWO) in Willemstad. Due to her keen interest in technical, biological and medical aspects of chemistry, she decided to follow a path to educate herself and gain experience in these fields and hopefully in this way make the world a better place and human health as optimal as possible. Therefore, after her secondary education, she started her Chemical Technology study at the University of Twente. She followed the Process Technology track with the subdiscipline Process and Plant design. During her studies she took time to amongst others start a student-focused computer company (Procyon Computers, Arnhem) to particularly help new students flying in from Curaçao and other Dutch Antilles islands with a computer. This is because, as at that time, due to some technological limitations compared to now, is was not easy for the new students to integrate into the culture, fight homesickness and focus on their studies. Receiving a computer with internet access upon arrival was therefore very helpful. After this, to gain work experience she undertook a 4-month internship at Teijin Aramid, Arnhem, where she did process plant optimization and design for cyclohexylamine production, under the supervision of Eric Bekx, prof. Louis van der Ham. During her studies, she also followed a self-devised minor in Biochemistry at the Radboud University in Nijmegen. Subsequently, finished her study (MSc) with a one-year internship at DSM Anti-Infectives, Delft, under the supervision of Maarten van de Graaf, Els Schulten, prof. Vincent Nierstrasz, prof. Louis van der Ham and prof. Henk van den Berg. There she designed a biomass-separation process for the sustainable large-scale production of succinic acid using microorganisms. To continue on her journey, she followed a second Master study in Medical Biochemistry at the Radboud University in Nijmegen. There, she followed her 1-year master thesis internship in Medical Biochemistry under the supervision of Richard Rodenburg, Peter Willems and pediatrician prof. Jan Smeitink in the Pediatrics department and the Radboud Institute for Molecular Life Sciences of the Radboudumc. During this internship, she studied the effect of the antioxidant SkQ1 on cell-biological consequences of Complex I-deficient patient cell lines. She also followed a 6-month minor research internship in Radboudumc's Experimental Clinical Chemistry research group, under the supervision of Guus Kortman and group-leader prof. Dorine Swinkels, and gained more knowledge in Clinical Chemistry. During and after her internship, she studied the effect of iron-rich and iron-deficient enteric bacteria on mRNA expression and protein levels of the iron-binding protein NGAL, in intestinal epithelial cells *in vitro*. In the clinical chemistry field, NGAL was mostly known for its association with kidney injury. Together with her literature thesis discussing the (potential) function of NGAL and potential ways the use of NGAL in diagnostics and therapy, a novel research line in this area was started in Dorine's group. Shortly after, she went on to write two grant proposals with Dorine, of which one was granted, and publish a review in kidney iron metabolism in Nat. Rev. Nephr., under Dorine. Her discovery during her studies that the systems biology and systems medicine approach have the potential to significantly improve diagnostics and prognostics in patients with an inborn deficiency and improve our understanding of human health in general, sparked her interest in these fields and the PhD research she presented in this thesis. Her PhD was carried out under the supervision of prof. Barbara Bakker and prof. Dirk-Jan Reijngoud, in collaboration several other PI's and researchers and with clinicians of the Pediatrics department. Herein, as the thesis title indicates, she explored the mechanisms underlying the phenotype of MCAD deficiency by using a systems medicine approach. During her PhD she presented at several national and international conferences and obtained a travel grant for a large systems biology conference in Melbourne, Australia, and a best-oral-presentation price at the Coenzyme A workshop in Marseille, France. After her PhD, she will take a well-deserved short sabbatical and will further pursue her interests in improving human health by applying the systems medicine approach and working closely with researchers, clinician's and healthcare providers.

Portfolio

Description	Year	Workload (ECTS)
Teaching Molecular Cell Physiology course 2017	2017	0.5
Life Sciences with Industry workshop	2017	1.4
Supervisor of intern Emmalie Jager (6-month internship)	2017	4.0
PhD Day 2017 organization committee - Advisor in board of committee	2016	8.0
ISGSB2016 conference, Jena, Germany	2016	0.5
Mathematica - Basic principles II	2016	0.3
Entrepreneurship and Valorization for medical sciences	2016	5.0
European Student Council Symposium 2016	2016	0.5
Prize for best oral presentation at Coenzyme A workshop in France	2016	0.5
Coenzyme A workshop, Marseille, France	2016	1.0
In silico life: Constraint-based modelling at genome scale	2016	0.9
PhD Day 2016 organization committee - Sponsorship team member	2016	4.0
BIOSB2016 conference	2016	0.5
Teaching Molecular Cell Physiology course 2016	2016	1.0
Teaching for PhD students	2016	2.0
Supervisor of intern Marcel Vieira Lara (6-month internship)	2016	4.0
Python course for Life Scientists	2015	0.9
Ethics in Research and Scientific Integrity (FMW2151)	2015	2.0
PhD Day 2015 (attended as participant)	2015	1.0
Introduction into R	2015	1.4
BioSB course: RNA-seq data analysis	2015	0.9
Personal effectiveness	2015	0.5
BCN Statistics Course 2015	2015	2.0
BIOSB2015 conference	2015	0.5
BioSB course: Kick start R	2015	0.3
Teaching Molecular Cell Physiology course 2015	2015	0.5
BioSB course: Pattern Recognition	2015	3.0
SB@NL 2014 conference	2014	0.5
Microbiological safety	2014	1.0
ICSB 2014 conference, Melbourne, Australia	2014	0.5
ISGSB2014 conference, Durham, UK	2014	0.5
Teaching Molecular Cell Physiology course 2014	2014	0.5
Advanced Lecture Course on Systems Biology (Sysbio 2014), Innsbruck, Austria	2014	2.0
SB@NL 2013 conference	2013	0.5
Presentation Skills	2013	0.5
Two-day course on Mass Spectrometry	2013	0.6
Project Management and GSMS Introduction	2013	2.0
Tools and approaches of systems biology	2013	3.0
International Conference on Systems Biology 2013 (ICSB 2013), Copenhagen, Denmark	2013	0.5
Mathematica - Basic principles I	2013	0.3

List of publications

Martines AMF, Wetzels JFM, Swinkels DW. Ijzer en Nierschade. Capita Selecta Nefrologie, Nummer 2, 2012

Martines AMF, Masereeuw R, Tjalsma H, Hoenderop JG, Wetzels JFM, Swinkels DW. Iron metabolism in the pathogenesis of iron-induced kidney injury. Nat Rev Nephrol. 2013 Jul;9(7):385–98

van Eunen K, Volker-Touw CML, Gerding A, Bleeker A, Wolters JC, van Rijt WJ, Martines ACMF, Niezen-Koning KE, Heiner RM, Permentier H, Groen AK, Reijngoud DJ, Derks TGJ, Bakker BM. Living on the edge: Substrate competition explains loss of robustness in mitochondrial fatty-acid oxidation disorders. BMC Biol. 2016;14(1):1–15

Martines A-CMF, van Eunen K, Reijngoud D-J, Bakker BM. The promiscuous enzyme medium-chain 3-keto-acyl-CoA thiolase triggers a vicious cycle in fatty-acid beta-oxidation. PLOS Comput Biol. 2017;13(4):1–22.