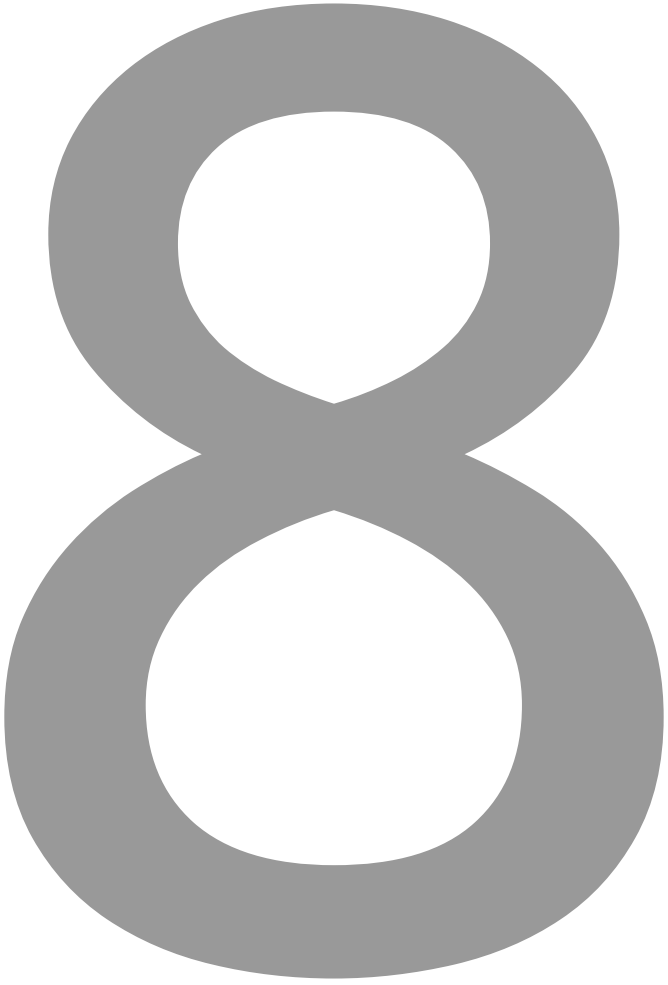
Tuberculosis & type 2 diabetes

Vrieling, F.

CHAPTER



Summarizing Discussion

Frank Vrieling [1]

[1] Department of Infectious Diseases, Leiden University Medical Center, Leiden, The Netherlands

Introduction

Diabetes mellitus type 2 (DM) is a major risk factor for developing active tuberculosis (TB) disease, yet the causal mechanisms driving this association remain largely elusive. As the incidence of DM is rising, especially in TB endemic countries, it is important to identify the relevant immunological and metabolic processes that underlie TB-DM comorbidity, because such insights will facilitate optimal treatment, diagnosis and prevention. In this thesis, we have started to unravel key factors underlying the association between TB and DM using two approaches. Firstly, we identified and analyzed human macrophage subsets and studied the interactions between these human cells and a major pathogen, *Mycobacterium tuberculosis* (*Mtb*), and the specific metabolic changes involved using well-controlled *in vitro* systems. Next, we employed metabolomics to determine the impact of concurrent TB-DM on circulating metabolites in patient cohorts *ex vivo*. Here we discuss and synthesize these results (summarized in **Figure 1**) and discuss their implications for TB-DM biology, treatment, diagnosis and prevention.

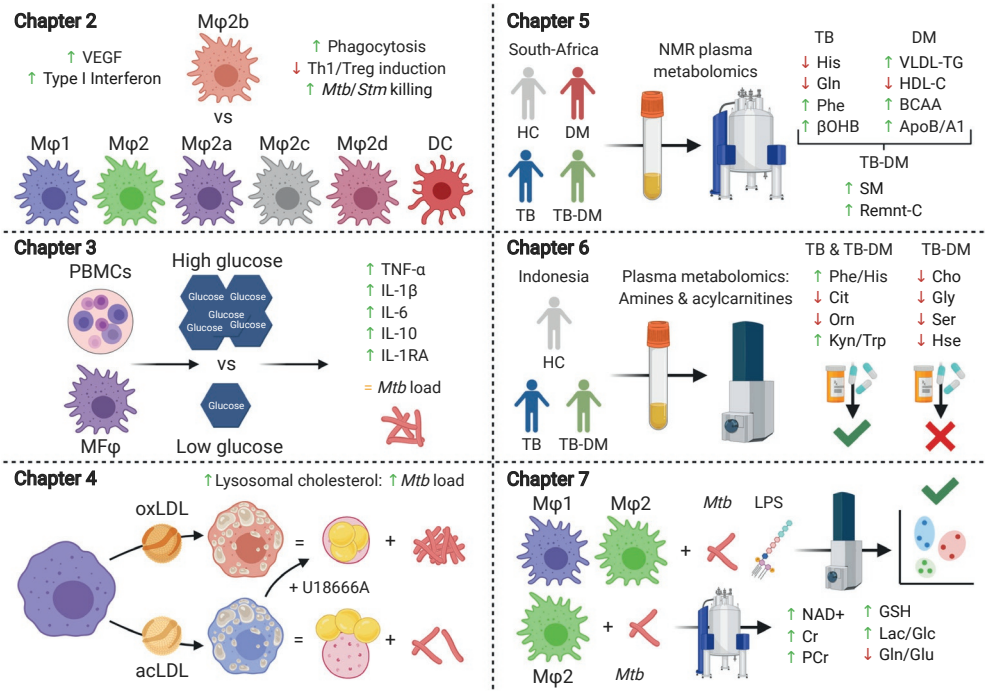


Figure 1: Schematic overview of thesis results by chapter.

Human macrophages and macrophage subsets as a model for TB(-DM)

Macrophages are key players during the *Mtb* infection cycle as they are the preferential habitat of *Mtb*. For this reason, many TB researchers use primary macrophages as a model to study *Mtb* infection dynamics *in vitro*. An important hallmark of macrophages is their high functional plasticity, which also governs their capacity to eradicate intracellular pathogens such as *Mtb*. While originally classified as either pro-inflammatory Mφ1 or anti-inflammatory Mφ2, there has recently been increasing appreciation of higher diversity within the macrophage activation spectrum (1, 2). As imbalances in tissue macrophage polarization have been demonstrated to exacerbate various diseases including TB (3), targeting macrophage differentiation holds merit as a potential therapeutic approach. The importance of macrophage function in the infectious process of *Mtb* is illustrated in **Chapter 2**, in which we characterized various Mφ2 subsets. We identified Mφ2b macrophages (induced by combined exposure to lipopolysaccharide (LPS) and IgG + ovalbumin immune complexes) as a polarized subset with potent antimicrobial activity against both *Mtb* and *Salmonella* Typhimurium, and unexpectedly, with a relatively poor capacity to induce Th1 and Treg responses compared to Mφ2a/c/d. The observed reduced bacterial load was not the result of diminished phagocytosis. Proteomics and transcriptomics analyses both showed that Mφ2b macrophages were characterized by a marked type I interferon signature, and they secreted relatively high levels of vascular endothelial growth factor (VEGF), an important mediator of granulomatous inflammation and angiogenesis in both mice (4) and zebrafish (5), in response to stimulation with a mixture of LPS, interferon-γ (IFN-γ) and *Mtb* lysate. Moreover, repolarization of Mφ2 subsets towards Mφ2b successfully induced an anti-mycobacterial phenotype, indicating that Mφ2b polarization could potentially be targeted as host-directed treatment of infectious diseases.

As macrophage polarization is strongly intertwined with cellular metabolism, this also provides an interesting model for studying TB-DM interactions. First, we examined whether hyperglycemia, the major hallmark of DM, could directly modulate functional macrophage cytokine responses to, or macrophage infection by *Mtb* (**Chapter 3**). Fasting blood glucose levels normally range between 4.4-6.6 mM in non-diabetics, but can reach levels as high as 25 mM during diabetic hyperglycemia. Many DM-associated complications can be attributed to hyperglycemia, including diabetic ketoacidosis and micro- and macrovascular disease (6). We found that *in vitro* differentiation of human macrophages under high glucose conditions (25 mM D-glucose) increased Mφ2 cytokine production (TNF-α, IL-6, IL-10, IL-1RA) in response to stimulation with LPS or *Mtb* lysate. Similar results have been obtained by other groups using both primary macrophages (7) and THP-1 cells (8). However, high glucose levels did not affect macrophage cytokine production or mycobacterial outgrowth after live *Mtb* infection. Furthermore, while several studies previously reported impaired macrophage phagocytosis as a result of hyperglycemia (9, 10), we did not observe significant differences in uptake of fluorescent

beads or mycobacteria when comparing macrophages cultured in hyperglycemic to euglycemic conditions.

Taken together, these results do not support a direct role for hyperglycemia during DM-associated TB. However, as is intrinsic to all *in vitro* research, our model of DM-associated hyperglycemia is reductionist in nature, calling for careful interpretation of its results. Firstly, only the direct effect of high glucose levels on macrophages was assessed, while other cell types and cell-cell interactions involved in TB immunity were not taken into account. Hyperglycemia has previously been shown to hamper neutrophil mobilization (11) and antibacterial activity (12), reduce immunoglobulin production by B cells (13) and induce T cell hyperresponsiveness (14). Secondly, it can be questioned whether a relatively short term incubation under hyperglycemic conditions accurately mimics the metabolic complexities of chronic DM *in vivo*, which also elicit changes in epigenetics (15). It would be of interest to study whether the observed phenotypes from **Chapter 3** can be recapitulated *ex vivo* using cells and/or serum from DM patients. For example, metabolic activation of macrophages by combined exposure to high glucose levels, insulin and palmitate resulted in a distinct pro-inflammatory phenotype which resembled adipose tissue macrophages isolated from obese humans and mice (16, 17). Finally, only the direct effects of hyperglycemia on macrophage function and *Mtb* infection were assessed. Potential indirect effects of high circulating glucose levels include the formation of advanced glycation end-products (AGEs), which can modulate macrophage inflammatory pathways (18, 19) and have been implicated in TB-associated hyperglycemia and disease severity (20). In conclusion, while our results clearly showed that hyperglycemia did not directly promote macrophage *Mtb* infection *in vitro*, other effects of hyperglycemia during TB-DM pathogenesis *in vivo* cannot be excluded.

Lipids in TB-DM: parallels between TB and atherosclerosis

As we did not find evidence for direct modulation of macrophage *Mtb* infection by elevated glucose levels, we next investigated other relevant metabolic characteristics of DM. Both T1DM and T2DM are often accompanied by dyslipidemia, characterized by aberrant circulating lipid levels. Diabetic dyslipidemia is accompanied by elevated triglycerides, increased low-density lipoprotein (LDL) levels (in case of T1DM), decreased high-density lipoprotein (HDL) levels and small dense LDL particles (21). Together, these changes in blood lipid profile put DM patients at increased risk of developing cardiovascular disorders (CVD) such as atherosclerosis, a disease which exhibits some striking pathophysiological similarities with TB. Likewise, TB is also associated with elevated risk of CVD and peripheral arterial disease, although the underlying mechanisms remain unclear (22, 23). A key event during both TB and atherosclerosis is the formation of lipid-loaded macrophages so-called foam cells, which form an integral part of both TB granulomas and atherosclerotic plaques (24, 25). *Mtb* has been demonstrated to reprogram macrophage metabolism to accumulate intracellular lipid droplets (26-28), as *Mtb* requires host-derived lipids and

cholesterol as a source of carbon (29-32). Moreover, hypercholesterolemia has been shown to negatively impact the immune response to TB (33). Therefore, we hypothesized that DM-associated changes in lipid metabolism could be involved during TB-DM.

Atherosclerotic plaque formation is initiated by extravasation of blood monocytes through the endothelium in response to local inflammatory signals, an event which is thought to be driven by oxidized low-density lipoprotein (oxLDL) particles deposited in the sub-endothelial space (34). OxLDL is a pathologically modified lipoprotein which is formed as result of oxidative stress, and circulating oxLDL levels are elevated in DM patients (35, 36). In the artery wall, oxLDL is taken up by macrophages through scavenger receptor-mediated phagocytosis, culminating in foam cell formation (37). Interestingly, oxLDL was found to accumulate in granulomas of *Mtb*-infected guinea pigs (38), and thus represented a potential connection between TB and DM. We found that treatment with increasing concentrations of oxLDL induced a dose-dependent lipid accumulation in human macrophages, which strongly supported intracellular *Mtb* survival after infection (**Chapter 4**). Mechanistically, oxLDL induced lysosomal cholesterol accumulation, which was accompanied by reduced colocalization of *Mtb* with functional lysosomes. Treatment with acetylated LDL (acLDL), a modified lipoprotein which is taken up by similar pathways but does not accumulate in lysosomes (39), did not result in increased *Mtb* outgrowth unless combined with a lysosomal cholesterol transport inhibitor, highlighting that the subcellular localization of cholesterol was pivotal for the effect of oxLDL. These results were in line with earlier observations that cholesterol accumulation, for instance in the lysosomal storage disorder Niemann-Pick Disease Type C (NPC), can inhibit phagosome maturation and autophagy in macrophages, both involved in controlling intracellular infections (40-43). A study by Fineran *et al.* showed that mycobacterial infection itself triggered a NPC-like phenotype in macrophages which supported mycobacterial intracellular persistence (44), indicating that modulation of this pathway could be part of the pathogen's defense machinery. In agreement with these results, cholesterol depletion restored phagosome maturation in *Mycobacterium avium* infected macrophages (45).

While these results demonstrated that DM-associated lipids can promote *Mtb* infection *in vitro*, it was unknown whether lipid metabolism would be similarly altered in patients with concurrent TB-DM. In contrast to DM, TB is often accompanied by malnutrition and wasting syndrome (46). In **Chapter 5**, we analyzed plasma ^{1}H-nuclear magnetic resonance (NMR) spectroscopy biomarker profiles of TB, DM and TB-DM TB patients from South-Africa to examine the respective correlations of these markers with patients' lipid metabolism. As expected, DM patients presented with dyslipidemia, characterized by hypertriglyceridemia and decreased HDL-cholesterol, while TB patients showed clear signs of wasting disease in the form of low blood levels of amino acids. Interestingly, TB-DM presented with hallmarks of both wasting and dyslipidemia, seemingly reflecting a metabolic 'tug-of-war' between TB and DM and resulting in relatively high interindividual variation. Another explanation for this heterogeneity could be variations in DM duration,

as the TB-DM group consisted of both newly diagnosed and chronic diabetics. Besides high levels of triglycerides and low levels of HDL-C, TB-DM patients showed disease interaction-specific increases in remnant-like lipoprotein particles and sphingomyelin levels, both of which are associated with increased atherogenesis (47-49). Finally, we were able to confirm that circulating oxLDL levels are elevated in DM patients from this cohort (**Chapter 4**). This is an important observation as DM patients are the specific population at increased risk for developing active TB, indicating a possible relationship between increased circulating lipid, in particular oxLDL levels and TB. Although this increase was not observed in the TB-DM patient group, oxLDL levels showed a strong positive correlation with plasma triglyceride levels in these patients, implying that oxLDL levels were associated with severity of dyslipidemia.

Together, **Chapter 4** and **Chapter 5** support a role for atherogenic changes as risk factors during TB-DM development, both *in vitro* and *in vivo*. A major contributor to atherosclerosis is oxidative stress (50). Both TB and DM are associated with increased generation of free radicals and a reduction in anti-oxidative capacity (50-53), which can lead to formation of modified proteins and lipids such as oxLDL and AGEs. Moreover, DM and hyperglycemia increase expression of oxLDL scavenger receptors such as CD36 in macrophages (54-56), which has also been shown to promote *Mtb* growth by mediating uptake of surfactant lipids (57). Combined with the observed changes in circulating lipids, these changes could drive monocytes and macrophages towards foam cell formation during TB-DM, potentially contributing to granuloma caseation, a pathognomonic feature of TB (58). In support of this, histological analysis of TB patient lung biopsies showed that DM and dyslipidemia correlated with enlarged areas of caseous necrosis (59). Previous studies already established that *Mtb* can utilize host lipids for intracellular growth (57, 60). In **Chapter 4**, we demonstrated that lipid accumulation can also directly interfere with macrophage mycobacterial growth inhibition, depending on their intracellular localization. Accelerated foam cell biogenesis during TB-DM could therefore support both primary *Mtb* infection by interfering with mycobacterial growth inhibition and promoting (future) reactivation by providing a nutritionally rich niche for replication. These results support further investigation into the beneficial effects of lipid lowering drugs for treatment of TB-DM. Various studies have demonstrated the efficacy of statins for adjunctive therapy for TB (61-64), although some of these did not observe a protective effect of statin-usage against TB in patients with DM (65, 66). Another paper showed a reduced incidence of latent TB infection in DM patients receiving the cholesterol-lowering drug ezetimibe (67). Recently, antagonists of PCSK9 (68), a protein which regulates LDL-receptor expression, have shown promise for treatment of atherosclerosis, and it would therefore be of interest to investigate whether PCSK9 inhibitors could also benefit patients with TB-DM.

Despite the apparent similarities between atherosclerosis and TB, multiple questions remain to be answered. A recent study reported that triglycerides and not cholesteryl esters were the dominant storage lipids in granulomas of *Mtb*-infected

marmosets and rabbits, a process which was regulated by TNF-α signaling through activation of the caspase cascade and mammalian target of rapamycin complex 1 (mTORC1) (69). These results suggest that TB-associated foam cell biogenesis may be reliant on activation of specific intracellular signaling pathways and not on uptake of cholesterol-rich lipoproteins, as is the case during atherosclerosis. In addition, another recent paper found protective role for lipid droplet formation during *Mtb* control by supporting eicosanoid production in murine macrophages (70). However, neither paper assessed the impact of DM on lipid metabolism and foam cell formation during TB. It would be of interest to study granuloma lipid composition in an *in vivo* DM model and/or TB-DM patients. Simultaneously, the presence of oxidized lipid species in granuloma macrophages could be determined to substantiate potential involvement of oxLDL. Furthermore, while our results provide evidence for defective phagolysosomal killing in oxLDL-treated macrophages, the participating molecular players remain to be identified. Lysosomal lipid storage disorders can interfere with phagolysosomal function through dysregulated Ca^{2+}-signaling (43, 71) and autophagy (41, 42), however, pharmacological modulation of these pathways could not rescue the oxLDL-induced phenotype (**Chapter 4**). Finally, due to the cross-sectional design of the study described in **Chapter 5**, the observed atherogenic changes cannot be causally linked to TB-DM pathophysiology as of yet. Some studies have reported a DM-independent protective effect of obesity on active TB development (72-74), further illustrating the complexity of the TB-DM conundrum. Longitudinal studies which assess and manipulate TB-DM patient lipid profiles in large prospective cohorts will be necessary to conclusively validate a possible link between atherogenic changes and TB-DM development.

Metabolomics of TB-DM: what have we learned?

The first usage of a primitive form of metabolomics can be traced back thousands of years to ancient physicians from China, Babylon and Egypt, who evaluated characteristics of urine to diagnose and predict onset of disease, including detection of diabetes by its sweet smell and taste (75). Since its first modern application in 1971 by Pauling *et al.* (76), metabolomics has developed into a powerful tool to study pathophysiological processes of human disease. Over the past decade researchers have successfully employed metabolomics to identify biomarkers for various human diseases, including Alzheimer's (77), cancer (78), and diabetes (79). In concert with the rise of immunometabolism as a field of research, metabolomics has also attracted growing attention as a method to study the impact of infectious diseases on host metabolism (80), for instance leading to the development of diagnostic signatures for sepsis (81). A recent study by Weiner *et al.* highlighted the potential of metabolomics for TB research (82), as they reported a prognostic metabolite signature for active TB with 69% sensitivity at 75% specificity within 5 months prior to TB diagnosis. Importantly, some of the observed metabolic changes

were already present 12 months before active disease was diagnosed, and can therefore potentially be used for timely identification of TB progressors (83).

We used targeted metabolomics to analyze the overall effects of TB-DM on plasma metabolic profiles in two different patient cohorts, respectively from South-Africa (**Chapter 5**) and Indonesia (**Chapter 6**), while employing different measurement platforms. In both studies, TB and TB-DM patients had signs of wasting as represented by strongly decreased levels of many amino acids. Specifically, low levels of circulating histidine showed very high predictive power for TB and TB-DM in both studies, which is in agreement with previous plasma metabolic profiling of TB patients (84). Weiner *et al.* showed that histidine levels started to deviate from 9 months prior to manifestation of clinical TB (82), substantiating its promise for early detection of TB. In contrast to histidine, plasma phenylalanine concentrations were increased in both TB patient cohorts. The ratio of phenylalanine over histidine (Phe / His) was a more potent signature for TB classification, as expected. In line with this, both amino acids were part of the 10 metabolite prognostic signature described by Weiner *et al.* (82). Other early changes in amino acid levels reported in this paper included low concentrations of citrulline and tryptophan with increasing kynurenine levels over time proximal to disease onset, all of which were in agreement with our own results from **Chapter 6**. Previous studies demonstrated similarly low levels of tryptophan and/or elevated concentrations of kynurenine in serum (84-86) or pleural fluid (87) from TB patients. This increased ratio of kynurenine over tryptophan (Kyn / Trp) is indicative of a higher activity of the immunoregulatory enzyme indoleamine 2,3-dioxygenase (IDO) which catalyzes the rate-limiting step of tryptophan catabolism and has been implicated in attenuated control of *Mtb* infection (73). Citrulline is an urea cycle intermediate which can be used to synthesize arginine, a process which has been demonstrated to contribute to the anti-mycobacterial response in macrophages (88, 89) and T-cells (90). Importantly, antibiotic treatment resulted in normalization to levels observed in healthy controls for the majority of the TB-associated amines investigated. In conclusion, we find that both TB and TB-DM are associated with major changes in amino acid levels; these results are validated by other metabolomics studies using different technical platforms as well cohorts from different geographical regions, thus enhancing the plausibility of our results.

Importantly, the results from **Chapter 5** and **Chapter 6** are suggestive of alterations in liver function during TB-DM. Our measurements reported in **Chapter 5** showed that TB-DM resulted in disease interaction-specific increases in remnant-like lipoprotein particles, sphingomyelins and LDL-triglyceride content, suggesting that concurrent TB-DM affects the function of hepatic enzymes involved in lipoprotein and lipid biosynthesis. Successive lipolysis by lipoprotein lipase (LPL) and hepatic triglyceride (HTGL) mediates conversion of triglyceride-rich chylomicrons and very low-density lipoprotein particles to intermediate density lipoprotein and LDL particles (91). The observed changes in lipoprotein size and content could therefore reflect a disruption of the relative activities of LPL and HTGL.

Sphingomyelin synthesis is mediated by serine palmitoyltransferase (SPT). Hepatic SPT activity was found to be elevated by inflammatory signaling, leading to alterations in lipoprotein composition (92). In line with these results, in **Chapter 6** we reported lower levels of glycine, serine, threonine and homoserine in TB-DM compared to TB patients. Low levels of these amino acids are associated with non-alcoholic fatty liver disease (NAFLD) development, potentially through their role in glutathione (GSH) synthesis (93, 94), although we did not observe significant alterations in GSH concentrations during TB-DM. Additionally, plasma concentrations of free choline were extremely low in TB-DM patients, and choline deficiency has been similarly connected to NAFLD (95, 96). Finally, we find that anti-TB therapy resulted in increased levels of metabolic markers of drug-induced liver injury, and it was previously demonstrated that streptozotocin-induced diabetes exacerbated liver injury and steatosis resulting from anti-tubercular treatment in rats (97). While NAFLD development as a common comorbidity of DM is widely recognized, much less is known about the occurrence of non-alcoholic hepatic steatosis during TB. Autopsy studies spanning multiple decades have demonstrated that TB is often associated with fatty liver infiltration (98-100), however, it is unclear whether this is directly related to disease or to comorbidities such as alcohol abuse or malnutrition. In conclusion, observed changes in plasma metabolic profiles from **Chapter 5** and **Chapter 6** could be related to increased liver dysfunction and/or damage as a result of concurrent TB-DM, calling for increased liver function monitoring in patients, especially during antibiotic treatment.

A major question which remains to be answered is whether the metabolic changes reported in **Chapter 5** and **Chapter 6** are truly specific for TB or TB-DM, or simply a reflection of ongoing oxidative stress and/or inflammation. For example, low levels of histidine, one of the strongest individual biomarkers for TB, have also been reported in patients with rheumatoid arthritis (101), sepsis (102) and obesity (103). It would therefore be of great interest to directly compare the metabolic effects of TB to other respiratory diseases in patients with or without concurrent DM, such as sarcoidosis or lung cancer, as this could both establish their association with disease and provide additional information on the involved pathological mechanisms. Additionally, future metabolomics studies on TB-DM will need to advance from cross-sectional to prospective patient cohorts akin to Weiner *et al.* (82) to substantiate a possible causal role of specific metabolites for TB-DM development, preferentially including a treatment arm to investigate the effect of modulating the circulating levels of a metabolite of choice such as statins. Finally, to effectively utilize potential diagnostic or predictive metabolic biomarker signatures for TB or TB-DM in clinical or field settings, current methodologies for quantification of these metabolites will have to be translated to user-friendly tests. An example of this could be the development of paper-based metabolic assays using metabolite-specific bioluminescent sensor proteins, a technique which has been successfully demonstrated for analysis of phenylalanine concentrations in finger-prick samples of phenylketonuria patients (104).

Finally, we performed cellular metabolomics using *Mtb*-infected macrophages

to increase our insights into the fundamental effects of *Mtb* infection on macrophage metabolism (**Chapter 7**). ^{1}H-NMR revealed that macrophage *Mtb* infection induced elevated levels in nicotinamide-adenine-dinucleotide (NAD^+), creatine and creatine phosphate, glutamine catabolism and glycolysis. These results were corroborated by analysis of in-house and previously published (105) RNA-sequencing datasets of *Mtb*-infected macrophages revealed alterations in gene expression of key enzymes regulating these metabolic pathways. The role of NAD^+ metabolism during macrophage *Mtb* infection is of particular interest, as multiple recent papers have demonstrated the importance of maintaining adequate NAD^+ pools for macrophage activation (106-108). NAD^+-boosting therapy has shown potential for treatment of various conditions (108), and these results prompt further investigation into the importance of NAD^+ metabolism during macrophage *Mtb* infection. Another recent paper revealed that SLC6A8-mediated creatine uptake supported anti-inflammatory interleukin-4 (IL-4) polarization in macrophages, while simultaneously suppressing pro-inflammatory IFN-γ-mediated macrophage activation (109). As the results from **Chapter 2** illustrate the importance of polarization state for macrophage anti-mycobacterial functions, it would be of interest to study whether increased intracellular creatine levels differentially influence the capacity of macrophage subsets to control *Mtb* infection.

Concluding comments

In this thesis we present evidence derived from *in vitro* experiments and from *ex vivo* observational data which collectively suggest a pathogenic role of atherogenic lipid species during TB development. Mechanistically, increased intracellular levels of lipids and cholesterol could support *Mtb* survival by interfering with macrophage antimicrobial functions, while also providing increased access to nutrients and carbons sources during chronic disease. Although the results from our observational data by definition can only highlight correlations between dyslipidemia and TB-DM and not demonstrate causality, several interventional studies have demonstrated that lipid-lowering drugs such as statins have potential as adjunctive TB therapy (61-64). Besides their effect on blood cholesterol levels, statins have also been shown to modulate bacterial intracellular growth and virulence *in vitro*, making them an interesting candidate for drug repurposing (63, 110, 111). Future prospective studies should elucidate whether pharmacological normalization of blood lipid levels can reduce the risk of DM patients to develop active TB, or lead to improved TB and TB-DM treatment outcomes when given in conjunction with standard antibiotic regimens. To further dissect and determine the contribution of individual metabolic changes on DM-associated *Mtb* infection, future studies will be needed, including animal models of TB-DM, prospective interventional human cohort studies, as well as novel holistic and mechanistic models such as organs-on-chips, to determine mechanisms of action. These studies are important given the rapidly increasing TB-DM co-epidemic, which will have great impact on future global health if not controlled in time.

References

1. Amit I, Winter DR, & Jung S (2015) The role of the local environment and epigenetics in shaping macrophage identity and their effect on tissue homeostasis. *Nat.Immunol.* 17(1):18-25.
2. Lavin Y, Mortha A, Rahman A, & Merad M (2015) Regulation of macrophage development and function in peripheral tissues. *Nat.Rev.Immunol.* 15(12):731-744.
3. Sica A & Mantovani A (2012) Macrophage plasticity and polarization: in vivo veritas. *J.Clin.Invest* 122(3):787-795.
4. Harding JS*, et al.* (2019) VEGF-A from Granuloma Macrophages Regulates Granulomatous Inflammation by a Non-angiogenic Pathway during Mycobacterial Infection. *Cell reports* 27(7):2119-2131.e2116.
5. Walton EM*, et al.* (2018) Cyclopropane Modification of Trehalose Dimycolate Drives Granuloma Angiogenesis and Mycobacterial Growth through Vegf Signaling. *Cell host & microbe* 24(4):514-525.e516.
6. Marcovecchio ML, Lucantoni M, & Chiarelli F (2011) Role of chronic and acute hyperglycemia in the development of diabetes complications. *Diabetes Technol Ther* 13(3):389-394.
7. Grosick R, Alvarado-Vazquez PA, Messersmith AR, & Romero-Sandoval EA (2018) High glucose induces a priming effect in macrophages and exacerbates the production of pro-inflammatory cytokines after a challenge. *J Pain Res* 11:1769-1778.
8. Moganti K*, et al.* (2017) Hyperglycemia induces mixed M1/M2 cytokine profile in primary human monocyte-derived macrophages. *Immunobiology* 222(10):952-959.
9. Restrepo BI, Twahirwa M, Rahbar MH, & Schlesinger LS (2014) Phagocytosis via complement or Fc-gamma receptors is compromised in monocytes from type 2 diabetes patients with chronic hyperglycemia. *PLoS.One.* 9(3):e92977.
10. Montoya-Rosales A, Castro-Garcia P, Torres-Juarez F, Enciso-Moreno JA, & Rivas-Santiago B (2016) Glucose levels affect LL-37 expression in monocyte-derived macrophages altering the Mycobacterium tuberculosis intracellular growth control. *Microbial pathogenesis* 97:148-153.
11. Fainsod-Levi T*, et al.* (2017) Hyperglycemia Impairs Neutrophil Mobilization Leading to Enhanced Metastatic Seeding. *Cell reports* 21(9):2384-2392.
12. Javid A*, et al.* (2016) Hyperglycemia Impairs Neutrophil-Mediated Bacterial Clearance in Mice Infected with the Lyme Disease Pathogen. *PloS one* 11(6):e0158019.
13. Sakowicz-Burkiewicz M*, et al.* (2013) High glucose concentration impairs ATP outflow and immunoglobulin production by human peripheral B lymphocytes: involvement of P2X7 receptor. *Immunobiology* 218(4):591-601.
14. Martinez N*, et al.* (2014) Chromatin decondensation and T cell hyperresponsiveness in diabetes-associated hyperglycemia. *Journal of immunology* 193(9):4457-4468.
15. Ahmed M, de Winther MPJ, & Van den Bossche J (2017) Epigenetic mechanisms of macrophage activation in type 2 diabetes. *Immunobiology* 222(10):937-943.
16. Kratz M*, et al.* (2014) Metabolic dysfunction drives a mechanistically distinct proinflammatory phenotype in adipose tissue macrophages. *Cell Metab* 20(4):614-625.

17. Coats BR, *et al.* (2017) Metabolically Activated Adipose Tissue Macrophages Perform Detrimental and Beneficial Functions during Diet-Induced Obesity. *Cell reports* 20(13):3149-3161.
18. Son S, *et al.* (2017) Advanced glycation end products impair NLRP3 inflammasome-mediated innate immune responses in macrophages. *The Journal of biological chemistry* 292(50):20437-20448.
19. Pertynska-Marczewska M, *et al.* (2004) Advanced glycation end products upregulate angiogenic and pro-inflammatory cytokine production in human monocyte/macrophages. *Cytokine* 28(1):35-47.
20. Podell BK, *et al.* (2012) Non-diabetic hyperglycemia exacerbates disease severity in Mycobacterium tuberculosis infected guinea pigs. *PloS one* 7(10):e46824.
21. Dake AW & Sora ND (2016) Diabetic Dyslipidemia Review: An Update on Current Concepts and Management Guidelines of Diabetic Dyslipidemia. *Am J Med Sci* 351(4):361-365.
22. Huaman MA, Henson D, Ticona E, Sterling TR, & Garvy BA (2015) Tuberculosis and Cardiovascular Disease: Linking the Epidemics. *Trop Dis Travel Med Vaccines* 1.
23. Wang SH, *et al.* (2017) Tuberculosis increases the risk of peripheral arterial disease: A nationwide population-based study. *Respirology* 22(8):1670-1676.
24. Russell DG, Cardona PJ, Kim MJ, Allain S, & Altare F (2009) Foamy macrophages and the progression of the human tuberculosis granuloma. *Nature immunology* 10(9):943-948.
25. Moore KJ & Freeman MW (2006) Scavenger receptors in atherosclerosis: beyond lipid uptake. *Arteriosclerosis, thrombosis, and vascular biology* 26(8):1702-1711.
26. Ouimet M, *et al.* (2016) Mycobacterium tuberculosis induces the miR-33 locus to reprogram autophagy and host lipid metabolism. *Nature immunology* 17(6):677-686.
27. Mahajan S, *et al.* (2012) Mycobacterium tuberculosis modulates macrophage lipid-sensing nuclear receptors PPARgamma and TR4 for survival. *J.Immunol.* 188(11):5593-5603.
28. Singh V, *et al.* (2012) Mycobacterium tuberculosis-driven targeted recalibration of macrophage lipid homeostasis promotes the foamy phenotype. *Cell host & microbe* 12(5):669-681.
29. Santucci P, *et al.* (2016) Experimental Models of Foamy Macrophages and Approaches for Dissecting the Mechanisms of Lipid Accumulation and Consumption during Dormancy and Reactivation of Tuberculosis. *Frontiers in cellular and infection microbiology* 6:122.
30. Maurya RK, Bharti S, & Krishnan MY (2018) Triacylglycerols: Fuelling the Hibernating Mycobacterium tuberculosis. *Frontiers in cellular and infection microbiology* 8:450.
31. Pandey AK & Sassetti CM (2008) Mycobacterial persistence requires the utilization of host cholesterol. *Proceedings of the National Academy of Sciences of the United States of America* 105(11):4376-4380.
32. Peyron P, *et al.* (2008) Foamy macrophages from tuberculous patients' granulomas constitute a nutrient-rich reservoir for M. tuberculosis persistence. *PLoS pathogens* 4(11):e1000204.
33. Martens GW, *et al.* (2008) Hypercholesterolemia impairs immunity to tuberculosis. *Infection and immunity* 76(8):3464-3472.

34. Miller YI*, et al.* (2011) Oxidation-specific epitopes are danger-associated molecular patterns recognized by pattern recognition receptors of innate immunity. *Circulation research* 108(2):235-248.
35. Toshima S*, et al.* (2000) Circulating oxidized low density lipoprotein levels. A biochemical risk marker for coronary heart disease. *Arteriosclerosis, thrombosis, and vascular biology* 20(10):2243-2247.
36. Njajou OT*, et al.* (2009) Association between oxidized LDL, obesity and type 2 diabetes in a population-based cohort, the Health, Aging and Body Composition Study. *Diabetes/ metabolism research and reviews* 25(8):733-739.
37. Park YM (2014) CD36, a scavenger receptor implicated in atherosclerosis. *Exp Mol Med* 46:e99.
38. Palanisamy GS*, et al.* (2012) Uptake and accumulation of oxidized low-density lipoprotein during Mycobacterium tuberculosis infection in guinea pigs. *PLoS.One.* 7(3):e34148.
39. Yancey PG & Jerome WG (1998) Lysosomal sequestration of free and esterified cholesterol from oxidized low density lipoprotein in macrophages of different species. *Journal of lipid research* 39(7):1349-1361.
40. Huynh KK, Gershenzon E, & Grinstein S (2008) Cholesterol accumulation by macrophages impairs phagosome maturation. *The Journal of biological chemistry* 283(51):35745-35755.
41. Sarkar S*, et al.* (2013) Impaired autophagy in the lipid-storage disorder Niemann-Pick type C1 disease. *Cell reports* 5(5):1302-1315.
42. Schwerd T*, et al.* (2016) Impaired antibacterial autophagy links granulomatous intestinal inflammation in Niemann-Pick disease type C1 and XIAP deficiency with NOD2 variants in Crohn's disease. *Gut*.
43. Shen D*, et al.* (2012) Lipid storage disorders block lysosomal trafficking by inhibiting a TRP channel and lysosomal calcium release. *Nature communications* 3:731.
44. Fineran P*, et al.* (2016) Pathogenic mycobacteria achieve cellular persistence by inhibiting the Niemann-Pick Type C disease cellular pathway. *Wellcome open research* 1:18.
45. de Chastellier C & Thilo L (2006) Cholesterol depletion in Mycobacterium avium-infected macrophages overcomes the block in phagosome maturation and leads to the reversible sequestration of viable mycobacteria in phagolysosome-derived autophagic vacuoles. *Cellular microbiology* 8(2):242-256.
46. Schwenk A & Macallan DC (2000) Tuberculosis, malnutrition and wasting. *Curr Opin Clin Nutr Metab Care* 3(4):285-291.
47. Dong J*, et al.* (2006) Adenovirus-mediated overexpression of sphingomyelin synthases 1 and 2 increases the atherogenic potential in mice. *Journal of lipid research* 47(6):1307-1314.
48. Ruuth M*, et al.* (2018) Susceptibility of low-density lipoprotein particles to aggregate depends on particle lipidome, is modifiable, and associates with future cardiovascular deaths. *Eur Heart J* 39(27):2562-2573.
49. Varbo A, Benn M, & Nordestgaard BG (2014) Remnant cholesterol as a cause of ischemic heart disease: evidence, definition, measurement, atherogenicity, high risk patients, and present and future treatment. *Pharmacol Ther* 141(3):358-367.

50. Giacco F & Brownlee M (2010) Oxidative stress and diabetic complications. *Circulation research* 107(9):1058-1070.
51. Ceriello A*, et al.* (2002) Evidence for an independent and cumulative effect of postprandial hypertriglyceridemia and hyperglycemia on endothelial dysfunction and oxidative stress generation: effects of short- and long-term simvastatin treatment. *Circulation* 106(10):1211-1218.
52. Palanisamy GS*, et al.* (2011) Evidence for oxidative stress and defective antioxidant response in guinea pigs with tuberculosis. *PloS one* 6(10):e26254.
53. Venketaraman V*, et al.* (2008) Glutathione levels and immune responses in tuberculosis patients. *Microbial pathogenesis* 44(3):255-261.
54. Lu H*, et al.* (2013) High glucose induces upregulation of scavenger receptors and promotes maturation of dendritic cells. *Cardiovascular diabetology* 12:80.
55. Fukuhara-Takaki K, Sakai M, Sakamoto Y, Takeya M, & Horiuchi S (2005) Expression of class A scavenger receptor is enhanced by high glucose in vitro and under diabetic conditions in vivo: one mechanism for an increased rate of atherosclerosis in diabetes. *The Journal of biological chemistry* 280(5):3355-3364.
56. Chen M*, et al.* (2001) Diabetes enhances lectin-like oxidized LDL receptor-1 (LOX-1) expression in the vascular endothelium: possible role of LOX-1 ligand and AGE. *Biochem Biophys Res Commun* 287(4):962-968.
57. Dodd CE, Pyle CJ, Glowinski R, Rajaram MV, & Schlesinger LS (2016) CD36-Mediated Uptake of Surfactant Lipids by Human Macrophages Promotes Intracellular Growth of Mycobacterium tuberculosis. *Journal of immunology* 197(12):4727-4735.
58. Kim MJ*, et al.* (2010) Caseation of human tuberculosis granulomas correlates with elevated host lipid metabolism. *EMBO molecular medicine* 2(7):258-274.
59. Dong Z*, et al.* (2017) Hemostasis and lipoprotein indices signify exacerbated lung injury in tuberculosis with diabetes comorbidity. *Chest*.
60. Genoula M*, et al.* (2018) Formation of Foamy Macrophages by Tuberculous Pleural Effusions Is Triggered by the Interleukin-10/Signal Transducer and Activator of Transcription 3 Axis through ACAT Upregulation. *Frontiers in immunology* 9:459.
61. Su VY*, et al.* (2017) Statin Use Is Associated With a Lower Risk of TB. *Chest* 152(3):598-606.
62. Lai CC*, et al.* (2016) Statin treatment is associated with a decreased risk of active tuberculosis: an analysis of a nationally representative cohort. *Thorax* 71(7):646-651.
63. Parihar SP*, et al.* (2014) Statin therapy reduces the mycobacterium tuberculosis burden in human macrophages and in mice by enhancing autophagy and phagosome maturation. *J Infect Dis* 209(5):754-763.
64. Dutta NK*, et al.* (2016) Statin adjunctive therapy shortens the duration of TB treatment in mice. *J Antimicrob Chemother* 71(6):1570-1577.
65. Kim MC*, et al.* (2019) Association between Tuberculosis, Statin Use, and Diabetes: A Propensity Score-Matched Analysis. *Am J Trop Med Hyg* 101(2):350-356.

66. Kang YA*, et al.* (2014) The effects of statin use on the development of tuberculosis among patients with diabetes mellitus. *Int J Tuberc Lung Dis* 18(6):717-724.
67. Tsai IF*, et al.* (2017) Potential effect of ezetimibe against Mycobacterium tuberculosis infection in type II diabetes. *Respirology* 22(3):559-566.
68. Shapiro MD & Fazio S (2017) PCSK9 and Atherosclerosis - Lipids and Beyond. *Journal of atherosclerosis and thrombosis* 24(5):462-472.
69. Guerrini V*, et al.* (2018) Storage lipid studies in tuberculosis reveal that foam cell biogenesis is disease-specific. *PLoS pathogens* 14(8):e1007223.
70. Knight M, Braverman J, Asfaha K, Gronert K, & Stanley S (2018) Lipid droplet formation in Mycobacterium tuberculosis infected macrophages requires IFN-gamma/HIF-1alpha signaling and supports host defense. *PLoS pathogens* 14(1):e1006874.
71. Lloyd-Evans E*, et al.* (2008) Niemann-Pick disease type C1 is a sphingosine storage disease that causes deregulation of lysosomal calcium. *Nature medicine* 14(11):1247-1255.
72. Yen YF*, et al.* (2017) Obesity/overweight reduces the risk of active tuberculosis: a nationwide population-based cohort study in Taiwan. *Int J Obes (Lond)* 41(6):971-975.
73. Lin HH*, et al.* (2017) Association of obesity, diabetes, and risk of tuberculosis: two population-based cohorts. *Clin Infect Dis*.
74. Lonnroth K, Williams BG, Cegielski P, & Dye C (2010) A consistent log-linear relationship between tuberculosis incidence and body mass index. *Int J Epidemiol* 39(1):149-155.
75. Echeverry G, Hortin GL, & Rai AJ (2010) Introduction to urinalysis: historical perspectives and clinical application. *Methods Mol Biol* 641:1-12.
76. Pauling L, Robinson AB, Teranishi R, & Cary P (1971) Quantitative analysis of urine vapor and breath by gas-liquid partition chromatography. *Proceedings of the National Academy of Sciences of the United States of America* 68(10):2374-2376.
77. Varma VR*, et al.* (2018) Brain and blood metabolite signatures of pathology and progression in Alzheimer disease: A targeted metabolomics study. *PLoS Med* 15(1):e1002482.
78. Armitage EG & Ciborowski M (2017) Applications of Metabolomics in Cancer Studies. *Adv Exp Med Biol* 965:209-234.
79. Pallares-Mendez R, Aguilar-Salinas CA, Cruz-Bautista I, & Del Bosque-Plata L (2016) Metabolomics in diabetes, a review. *Ann Med* 48(1-2):89-102.
80. Zurfluh S*, et al.* (2018) The role of metabolomic markers for patients with infectious diseases: implications for risk stratification and therapeutic modulation. *Expert Rev Anti Infect Ther* 16(2):133-142.
81. To KK*, et al.* (2015) Lipid mediators of inflammation as novel plasma biomarkers to identify patients with bacteremia. *J Infect* 70(5):433-444.
82. Weiner J*, et al.* (2018) Metabolite changes in blood predict the onset of tuberculosis. *Nature communications* 9(1):5208.
83. Duffy FJ*, et al.* (2019) Immunometabolic Signatures Predict Risk of Progression to Active Tuberculosis and Disease Outcome. *Frontiers in immunology* 10:527.

84. Weiner J, 3rd*, et al.* (2012) Biomarkers of inflammation, immunosuppression and stress with active disease are revealed by metabolomic profiling of tuberculosis patients. *PloS one* 7(7):e40221.
85. Suzuki Y*, et al.* (2012) Serum indoleamine 2,3-dioxygenase activity predicts prognosis of pulmonary tuberculosis. *Clin Vaccine Immunol* 19(3):436-442.
86. Feng S*, et al.* (2015) Analysis of serum metabolic profile by ultra-performance liquid chromatography-mass spectrometry for biomarkers discovery: application in a pilot study to discriminate patients with tuberculosis. *Chin Med J (Engl)* 128(2):159-168.
87. Suzuki Y*, et al.* (2013) Indoleamine 2,3-dioxygenase in the pathogenesis of tuberculous pleurisy. *Int J Tuberc Lung Dis* 17(11):1501-1506.
88. Lange SM*, et al.* (2019) L-Arginine Synthesis from L-Citrulline in Myeloid Cells Drives Host Defense against Mycobacteria In Vivo. *Journal of immunology*.
89. Rapovy SM*, et al.* (2015) Differential Requirements for L-Citrulline and L-Arginine during Antimycobacterial Macrophage Activity. *Journal of immunology* 195(7):3293-3300.
90. Lange SM*, et al.* (2017) l-Citrulline Metabolism in Mice Augments CD4(+) T Cell Proliferation and Cytokine Production In Vitro, and Accumulation in the Mycobacteria-Infected Lung. *Frontiers in immunology* 8:1561.
91. Goldberg IJ*, et al.* (1982) Lipoprotein metabolism during acute inhibition of hepatic triglyceride lipase in the cynomolgus monkey. *The Journal of clinical investigation* 70(6):1184-1192.
92. Memon RA*, et al.* (1998) Endotoxin and cytokines increase hepatic sphingolipid biosynthesis and produce lipoproteins enriched in ceramides and sphingomyelin. *Arteriosclerosis, thrombosis, and vascular biology* 18(8):1257-1265.
93. Mardinoglu A*, et al.* (2017) Personal model-assisted identification of NAD(+) and glutathione metabolism as intervention target in NAFLD. *Mol Syst Biol* 13(3):916.
94. Gaggini M*, et al.* (2018) Altered amino acid concentrations in NAFLD: Impact of obesity and insulin resistance. *Hepatology* 67(1):145-158.
95. Sharpton SR, Ajmera V, & Loomba R (2019) Emerging Role of the Gut Microbiome in Nonalcoholic Fatty Liver Disease: From Composition to Function. *Clin Gastroenterol Hepatol* 17(2):296-306.
96. Leermakers ET*, et al.* (2015) Effects of choline on health across the life course: a systematic review. *Nutr Rev* 73(8):500-522.
97. Srivastava RK, Sharma S, Verma S, Arora B, & Lal H (2008) Influence of diabetes on liver injury induced by antitubercular drugs and on silymarin hepatoprotection in rats. *Methods Find Exp Clin Pharmacol* 30(10):731-737.
98. JONES JM & PECK WM (1944) INCIDENCE OF FATTY LIVER IN TUBERCULOSIS WITH SPECIAL REFERENCE TO TUBERCULOUS ENTERITIS. *JAMA Internal Medicine* 74(5):371-374.
99. Amarapurkar A & Agrawal V (2006) Liver involvement in tuberculosis--an autopsy study. *Trop Gastroenterol* 27(2):69-74.
100. Amarapurkar A & Ghansar T (2007) Fatty liver: experience from western India. *Ann Hepatol* 6(1):37-40.

101. Gerber DA (1975) Low free serum histidine concentration in rheumatoid arthritis. A measure of disease activity. *The Journal of clinical investigation* 55(6):1164-1173.
102. Su L*, et al.* (2015) Dynamic changes in amino acid concentration profiles in patients with sepsis. *PloS one* 10(4):e0121933.
103. Niu YC*, et al.* (2012) Histidine and arginine are associated with inflammation and oxidative stress in obese women. *Br J Nutr* 108(1):57-61.
104. Yu Q*, et al.* (2018) Semisynthetic sensor proteins enable metabolic assays at the point of care. *Science* 361(6407):1122-1126.
105. Blischak JD, Tailleux L, Mitrano A, Barreiro LB, & Gilad Y (2015) Mycobacterial infection induces a specific human innate immune response. *Sci Rep* 5:16882.
106. Cameron AM*, et al.* (2019) Inflammatory macrophage dependence on NAD(+) salvage is a consequence of reactive oxygen species-mediated DNA damage. *Nature immunology* 20(4):420-432.
107. Minhas PS*, et al.* (2019) Macrophage de novo NAD(+) synthesis specifies immune function in aging and inflammation. *Nature immunology* 20(1):50-63.
108. Rajman L, Chwalek K, & Sinclair DA (2018) Therapeutic Potential of NAD-Boosting Molecules: The In Vivo Evidence. *Cell Metab* 27(3):529-547.
109. Ji L*, et al.* (2019) Slc6a8-Mediated Creatine Uptake and Accumulation Reprogram Macrophage Polarization via Regulating Cytokine Responses. *Immunity*.
110. Hennessy E, Adams C, Reen FJ, & O'Gara F (2016) Is There Potential for Repurposing Statins as Novel Antimicrobials? *Antimicrob Agents Chemother* 60(9):5111-5121.
111. Dutta NK*, et al.* (2019) Adjunctive host-directed therapy with statins improves tuberculosis-related outcomes in mice. *J Infect Dis*.