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UNCOVERING THE COMPLEX METABOLIC ROLE OF SILENCING MEDIATOR OF RETINOID AND THYROID HORMONE RECEPTORS (SMRT)

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Dedication

I dedicate this work to my supporters beyond the academy, without whom, I surely would have been torn asunder:

To my family

Anne—for instilling within me the fire and determination to accomplish anything
Baba—for tempering my passions with prudence and consideration
Deniko—for being my balancing pole on the tightrope

Tete, Eniste, Selin—bana ailenin gercek anlamini gosterdiginiz icin

Aunt Toni—for always speaking with me openly and compassionately, a kindred spirit

Hala, Murat Dayi, Tina, Konner, Kate, the Cousins—for your continued unconditional love and support

To my friends

Yuni, Emma, Whitney, David, Jake, Cyn—for your loyalty, companionship, and understanding, and for enriching my life in ways nobody else could

And to Chris

For breaking me free of my chrysalis, and catalyzing my transformation. What I have learned from you, an education could never teach me. Thank you for always being in my corner, and for helping me see the big picture. Words cannot capture my deepest gratitude

Thank you all for helping me walk the path of success

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Chapter I: Advancing Our Understanding of Type II Diabetes Mellitus Metabolism via the Study of Nuclear Corepressors

1.1: Type II Diabetes Mellitus

1.1.1: Domestic and Global Healthcare Burden of T2DM

Over the last 60 years, the domestic prevalence of type II diabetes mellitus (T2DM) has increased by nearly an order of magnitude, with less than 1% of Americans diagnosed with diabetes in 1958[1] compared to 9.4% in 2015[2]. However, the U.S. population has essentially doubled during this period[3], and thus, analyzing the expansion of diabetes using population percentages underestimates the alarming acceleration of diagnoses; when considering absolute statistics, the number of Americans living with T2DM has actually increased by nearly 2000% during this period, from 1.58 million to 30.2 million individuals[1,4]. Currently, T2DM is the 7th leading cause of death in the U.S.[5], surpassing AIDS and breast cancer combined[6]. This translates to a financial burden of $327 billion in 2017, accounting for direct healthcare costs (pharmacological treatment plans) and loss in workforce productivity[7]; in fact, this value is conservative as it excludes the contribution of other substantial factors that cannot be directly measured, including millions of undiagnosed individuals and the associated decrease in workforce productivity and quality of life, the cost of prevention programs not considered under standard medical costs, over-the-counter medications used to treat complications of T2DM, impact on family members of the patient, and more[7]. Despite its ranking for leading causes of

death in the U.S., the price tag of T2DM makes it the most expensive medical condition in America[8].

The explosion of T2DM has surpassed epidemic proportions, affecting global populations more than ever before. The pandemic status of T2DM is reflected in a 2016 report by the World Health Organization where, between the years 1980 and 2014, the number of individuals living with T2DM increased from 108 million to 422 million[9]; at the time this summary was released, the direct global annual cost of T2DM was $827 billion[9,10], though with the increasing rate of diagnoses, this figure has likely surpassed $1 trillion today. Particularly concerning is the vulnerability of specific global communities to T2DM, the susceptibility of which is presumably attributed to genetics; while all ethnic groups have observed this frightening expansion, African, and especially Eastern Mediterranean populations, have seen unprecedented increases in prevalence of 4% and 7.8%, respectively, over the last 35 years[9]. The problem of T2DM has become such that the world public health agenda is focusing specifically on reducing the impact of T2DM via policies that promote healthy living habits (diet, exercise, curbing use of tobacco/alcohol, etc.), with the ambitious aim of halting the rise in obesity and diabetes by the year 2025[9].

1.1.2: T2DM Pathophysiology

While T2DM can, in most cases, be prevented with proper diet and exercise practices, nearly all diagnosed patients (with the rare exception of remissions resulting from bariatric surgery) maintain lifelong T2DM status[11]. The reason for this is because over time, sustained

diet-induced hyperglycemia elicits compensatory increases in insulin production, which in turn causes insulin resistance. This can eventually become a runaway feedback loop, where pancreatic beta cells, in an attempt to satisfy increasing systemic insulin demand, are stressed to a tipping point and dysfunction en masse[12,13]. This leaves the individual in a complex, insulin resistant state while also unable to endogenously produce appreciable amounts of insulin; if left unmanaged, hyperglycemia, which has detrimental long-term impacts on all tissues[14], becomes a serious health concern: retinopathy, amputations, kidney failure, and eventually, premature death are all potential complications of T2DM[15]. Additionally, excess body weight, an independent risk factor for T2DM, is observed in 90% of T2DM patients[16]. This common comorbidity is germane when discussing the pathology of diabetes, as the inflammation associated with obesity serves to advance T2DM progression by further disrupting insulin signaling and worsening insulin resistance[17,18]; the impact of inflammation will be specifically addressed this later in this chapter. Clinically, it is important to note that once the deteriorating metabolic profile progresses to an official diagnosis of T2DM, hyperglycemia no longer depends solely on lifestyle choices, and becomes the default physiological state as a function of a lack of insulin action. Unfortunately, because of the two arms that contribute to T2DM pathology (lack of insulin production and insulin resistance), exogenous insulin alone as a treatment option is not optimal, especially when excess insulin can act as an exacerbating factor in disease state progression[19]. Therefore, policies focusing on prevention/early intervention are extremely important when addressing the national and global crisis of T2DM.

1.1.3: Adipose Tissue Physiology

Metabolism is a complex process that occurs at all levels of physiology. As such, homeostatic regulation is achieved through the coordinated activity of a variety of metabolically critical tissues. For example, 70-90% of dietary sugars are stored as glycogen in skeletal muscle[20], regulated primarily by the translocation of GLUT4 upon insulin signaling[21]; in liver, expression of AMPK suppresses glucose and lipid synthesis, while increasing fatty acid beta oxidation[22]; the central nervous system, too, plays a vital role in maintaining energy balance through the brain's connection to signaling in the pancreas, impact on feeding behavior, and more[23,24]. Perhaps most influential, as far as the synchronization of local and systemic metabolic signaling is concerned, is adipose tissue, which is now appreciated for its potent autocrine, paracrine, and endocrine functions[25,26,27].

Adipose tissue comes in many different forms, which, depending on its metabolic purpose and location, can exert drastically different effects on the system. For instance, brown adipose tissue (BAT) behaves in a fundamentally different way than white adipose tissue (WAT), both of which are distinct from metabolically intermediary beige or brite adipose tissue. Unlike WAT, notorious for its direct role in obesity and excess lipid accumulation[28], the primary function of BAT is heat production through inefficient energy expenditure[29]; upon exposure to cold, non-shivering thermogenesis is accomplished via expression of UCP1, which uncouples the mitochondrial respiratory chain, allowing for the dissipation of chemical energy as heat[30]. While the metabolic dysfunction commonly associated with obesity lends WAT a poor image,

in addition to its lipid-storing properties, WAT actually expresses a diverse profile of adipokines that play a critical role in the maintenance of homeostasis, influencing processes ranging from energy utilization, vascularity, and even inflammation[31]. Consider the balance between adipocyte leptin, adiponectin, and MCP-1 signaling, none of which depend directly on the other but are still informed by each's cumulative downstream effects. While each factor assumes a critical function in the maintenance of metabolic homeostasis, they exert their influence through disparate pathways, and at varying proximities, underscoring the amalgamative and regulatory role of adipocytes. Leptin, circulating levels of which correlate tightly with adiposity, acts distally in the brain as a signal for satiety, serving to suppress non-hedonistic appetite and promote lipolytic pathways[32]. On the other hand, adiponectin, displaying both endocrine and autocrine function, sensitizes metabolic tissues like muscle, liver, and adipose tissue to insulin while also promoting adipogenesis, exerting a primarily anabolic, rather than catabolic, effect on the system[33,34]. The adipocyte must then coordinate its expression of these signals, which fluctuate depending on the metabolic state, with secretion of chemoattractants like MCP-1 that act in a paracrine fashion. MCP-1 is responsible for potentiating macrophage infiltration in WAT[35]; in the context of obesity, the pro-inflammatory effect of macrophage infiltration can oppose insulin sensitization via interference with insulin signaling at the level of the insulin receptor[36], while synergistically promoting lipolysis[37]. This, combined with phagocytic clearance of WAT free fatty acids (FFAs) from recruited macrophages, ameliorates the metabolic consequences of adipocyte hypertrophy. When chronic, however, inflammation loses its

protective quality, subtracting from the regulatory capacities of adiponectin and leptin. In addition to its own hormone production, WAT adipocytes present with a gamut of receptors, ranging from those for catecholamines, vitamin D, estrogen, angiotensin II, and more[26], demonstrating the multifunctionality of adipose tissue, and the complex cross-talk it is involved in.

WAT may be further categorized by its distribution; subcutaneous, which lies just beneath the skin, or visceral, which is situated within the visceral cavity, surrounding internal organs. Beginning with the anatomical position, it becomes quickly evident that these two depot types influence metabolism differently; unlike its subcutaneous counterpart, which has a broader, systemic network of vasculature, blood from visceral WAT drains directly into the liver via the portal vein, providing the liver with direct access to FFAs and inflammatory cytokines[38]. Further, the morphology of adipocytes within these depots vary; adipocytes present in visceral depots are typically more saturated in lipids than those in subcutaneous ones[38,39]; this increase in size promotes insulin resistance, hyperlipolysis, and a weakened avidity for triglyceride storage compared to the smaller adipocytes generally associated with subcutaneous WAT[40]. Because of the limited metabolic flexibility of adipocytes present in visceral WAT compared to subcutaneous WAT, it is believed that visceral adiposity is generally unhealthier and more susceptible to perturbations that give rise to the dysregulation characteristic of metabolic syndrome. Not surprisingly, visceral adipose tissue from obese humans often display higher levels of inflammation than that found subcutaneously[41,42,43]; this is often attributed to increased presence of macrophages, recruited to aid in the management of lipid saturation[44]. However,

both WAT depot types participate in the recruitment of macrophages, the inflammatory signaling from which has been shown to be a hallmark in the development of insulin resistance in models of obesity and T2DM[45].

1.1.4: Obesity, T2DM, and Inflammation

In its broadest sense, inflammation is a protective, generalized response to tissue malfunction, evolved to mitigate continued dysregulation by isolating the disruptive agent or process and restoring homeostasis[46]. Depending on the trigger that initiates inflammation, which can range from microbial invasion to physical injury to metabolic dyssynchrony[46,47,48], the cascade of signaling events and downstream consequences that typify it can vary drastically; inflammation may be acute or low grade, short-lived or chronic, mediated exogenously (allergens, virulence factors, etc.) or endogenously (products of apoptosis, crystallized inducers, etc.)[48]. For example, an acute inflammation response caused by bacterial infection results in the production of a specialized set of mediators (vasoactive amines, among many others) by resident macrophages that allow for increased blood flow to the area, which in turn enables localization of neutrophils to release their toxic contents (reactive oxygen species, cathepsin G, etc.) in order to overcome the invader[48,49]; the non-specific nature of the neutrophil attack results in collateral damage to the host, and so a primary end focus of this type of inflammation is that of tissue repair and the immunosuppressive response[49]. The events defining the subtler inflammation seen under metabolically stressful conditions, however, are entirely different. For example, malfunctioning adipocytes in obese individuals secrete CC-

chemokine ligand 2, a macrophage recruitment factor, when presently resident-macrophages fail to meet homeostatic demand[49]; while the increase in macrophage presence aids in the host's ability to adapt to over-nutrition by enhancing lipid clearance and adipocyte turnover, if left unresolved, production of factors like TNF-α or IL-6 may actually sustain or even worsen the metabolic state of the microenvironment. However, unlike with the inflammatory response observed upon the detection of exogenous pathogens, cellular destruction is not the priority; therefore, rather than healing, a critical end-goal for this type of inflammation is to expediently restore homeostasis before new set-points are established and disease states propagated.

It is important to appreciate the diversity in inflammation—the causes, physiological effects, and pathological outcomes can all vary, yet still serve to ameliorate tissue perturbations. Much of this variation is mediated by the status of resident macrophages, which drastically impact the course of inflammation based on their number and/or activation state. Though discussed later in more detail, the model of M1/M2 macrophage activation has been used to successfully describe pathogen-related inflammation, whereas the more recently characterized MMe macrophage activation state better explains the low-grade inflammation seen with obesity. While the mechanisms underpinning acute, infection-induced inflammation are far better understood than those defining the pervasive inflammation closely associated with diseases like T2DM[50], common to all types of inflammation is an increase in cytokine/chemokine signaling which typically results in an enrichment of immune cell presence and/or activity.

The association between metabolic disease and inflammation has long been established. Far downstream the consumption of a meal, dietary glucose is ultimately destined to either participate in a glycation reaction, or pass through the mitochondrial electron transfer chain[51]. Both of these essential biological processes, however, generate reactive oxygen species (ROS); while our bodies are equipped with anti-oxidative mechanisms to overcome basal levels of ROS, chronic hyperglycemia caused by over-nutrition can lead to a detrimental accumulation of ROS[51,52]. The highly oxidative capacity of these factors triggers the activation of pro-inflammatory pathways evolved to contain the damage these molecules cause[52,53]. As mentioned earlier, the inflammatory signaling characteristic of the adipose tissue microenvironment in those with obesity and/or T2DM can quickly transform from protective to deleterious. These pro-inflammatory signals may originate from adipocytes themselves or from resident immune cells in the adipose tissue in a context-dependent fashion. For instance, hyperinsulinemia under conditions of ROS-generating glucotoxicity is an independent factor for increased adipocyte TNF-α production[54]. TNF-α serves to interfere with insulin action at the level of the insulin receptor such that sugar influx does not exceed the lipid storage capacity of the adipocyte[54,55]. When the accrual of stress signaling in hypertrophic adipocytes is not successfully mitigated by these protective mechanisms, however, the activation of apoptotic pathways may be initiated[56]. As this process advances, the release of certain lipid species and, specifically, expression of chemoattractants result in the recruitment of macrophages for the phagocytotic clearance of lipids[57]; these cells, however, typically exacerbate pro-

inflammatory signaling in the microenvironment via a distinct set of cytokines, which has an unintended, harmful metabolic side effect on adipose tissue as a whole.

Often, the onset of T2DM is preceded by increased concentrations of pro-inflammatory biomarkers expressed by macrophages. In fact, dying adipocytes can even be histologically identified by the crown-like structures formed by lipid-consuming macrophages that the dot the perimeter of these cells[58]. However, not all macrophages are necessarily pro-inflammatory; depending on the conditions, these immune cells polarize to display either pro-inflammatory (M1) or anti-inflammatory (M2) function[59]. Classically, this M1-M2 archetype has been used to describe the initiation and resolution of inflammation during infection. However, the inflammation associated with obesity and T2DM is sterile, and so evaluation of the role of macrophages in metabolic disease using this binary paradigm may not be the best way to consider the role of macrophages in obesity and T2DM. Indeed, recent studies focusing on how macrophages may contribute towards metabolic aberrations have uncovered a distinct, mixed macrophage phenotype: the metabolically activated (MMe) macrophage[60]. While the pro-inflammatory cytokine expression profile of MMe macrophages mimics that of M1 macrophages, their identification using cell surface markers for M1 macrophages is unsuccessful[61]. Also, the activation of this macrophage phenotype is distinct; unlike with the potentiation of M1 polarization by detection of LPS[62,63], MMe macrophages are activated by free fatty acids, specifically the species palmitate[61,64]. MMe macrophages play different roles in the regulation of metabolic homeostasis, dependent on the stage of obesity progression; in

early-onset where adipocytes insulin resistance begins, release of damaging pro-inflammatory cytokines acts to further insulin resistance[60,61]. However, in late-onset, where adipocytes undergo apoptosis in appreciable amounts, MMe macrophages can begin to display an M2 phenotype wherein excessive palmitate buildup activates PPARγ[60,61], a key adipogenic and insulin sensitizing factor (discussed later). Either way, the unique role of MMe macrophages in exacerbating inflammation is undeniable, making it perhaps the most germane macrophage subtype to consider in the context of metabolic disease. Vis-à-vis the management of primary outcomes associated with T2DM pathology, specifically insulin resistance, the net effect of chronic inflammation can be detrimental—with the continuum of patient care already compromised by a lack of sound pharmacological options aiming to restore insulin sensitivity, the additional obstacles presented by chronic inflammation are not insignificant.

1.1.5: Current Treatment Options

While lifestyle interventions (better diet and exercise practices) are necessary for disease state improvement, due to the interdependent dysfunctions that drive T2DM pathology, most patients are unsuccessful in overcoming their condition with exercise/diet changes alone, and eventually require pharmacotherapy[65,66]. Because chronic treatment with exogenous insulin in T2DM patients prior to late stage diabetes may potentially exacerbate the underlying metabolic problem by worsening insulin resistance[67], pharmacotherapies have evolved to lower blood sugar levels by a number of other mechanisms. For example, metformin primarily functions by decreasing hepatic glucose production in a glucose-dependent fashion[68], the sulfonylureas

act on remaining beta cells to stimulate endogenous insulin secretion[69], while α-glucosidase inhibitors exert their effect by binding polysaccharide-cleaving enzymes in the gut, resulting in decreased absorption of dietary sugars[70]. The GLP-1 agonists, a class of drugs that include recent pharmaceutical success Byetta (exenatide), function as anti-hyperglycemic agents through multiple mechanisms (postprandial suppression of glucagon release, slowed gastric emptying, improved insulin granule release, etc.) and have shown promise for the management of T2DM[71]. Meanwhile, SGLT2 inhibitors, some of which have been lauded for their cardioprotective effects in addition to managing blood sugar levels, exert their influence by binding renal proteins involved in reabsorption of glucose, promoting excretion of dietary sugars through the urine[72]. Though some of these drugs peripherally improve insulin sensitivity, the only class of drugs that act specifically to ameliorate insulin resistance are the thiazolidinediones (TZDs)[66,73]. TZDs function as potent agonists of PPARγ[74], a powerful modulator of metabolic activity[75]. Specifically, increased activity of PPARγ leads to improved insulin sensitivity through both upregulated expression of adiponectin, a known insulin-sensitizing factor secreted by adipocytes[34], and increased adipogenic activity, yielding smaller, more insulin sensitive adipocytes[34,76]. Increases in the transcriptional activity of PPARγ may also block expression of proteins that promote inflammation[77,78].

1.1.6: Gaps in Treatment Options

The primary objective of current pharmacological options in the management of T2DM is to ameliorate hyperglycemia. However, while these drugs address elevated blood glucose

via a number of clever modalities, hyperglycemia is not a cause of advanced T2DM, but rather, a symptom. While it is important to maintain healthy blood sugar levels to avoid complications of T2DM, it is equally, if not more, important for the patient's long-term prognosis to address the two underlying forces that drive the advancement of T2DM: progressive, severe dysregulation of both insulin production and insulin sensitivity. Endogenous insulin production and secretion is orchestrated in a complex, glucose-dependent fashion by the beta cells of the pancreatic islet of Langerhans, the cessation of which occurs once compensatory increases in mass induce enough stress to trigger apoptotic pathways[79]. While in type I diabetes (T1D) beta cell death occurs via a different mechanism—an attack mounted by the immune system, wiping out virtually any possibility of self-regulated insulin production[80]—those with T1D nonetheless face similar problems regarding insulin deficits as those with T2DM. Because impaired insulin production is a hallmark of both T1D and T2DM, current research is exploring unique strategies for beta cell replacement, including use of stem cells, transplantation, and more, though these strategies remain to be more fully developed[81]. Fortunately, because insulin sensitivity remains intact, type I diabetics can effectively palliate glycaemia with exogenous insulin.

This is not the case with T2DM. Severe insulin resistance negates much of the therapeutic effects of exogenous insulin, necessitating interventions aiming to re-sensitize metabolic tissues. This is especially important for the disconcerting prediabetic state, where Butler at al. show a 40% deficit in beta cell mass (suggesting appreciable levels of apoptosis)

in obese individuals with impaired fasting glucose[82]; in fact, a number of studies have provided evidence demonstrating that beta cell mass is already decreased by approximately 50–80% at the time of T2DM diagnosis[83,84,85]. While drugs like the sulfonylureas may boost the efficiency of remaining beta cells, beta cell death effectively cements disease progression in individuals with T2DM unless the effect insulin resistance is also lessened. The TZDs are the only class of drugs used in the management of T2DM that explicitly address this pathological mechanism. However, because TZDs act as full agonists of PPARγ, life-threatening side effects in the form of hepatoxicity, fluid retention, osteopenia, and congestive heart failure have been observed[86], thus limiting its clinical application. This gap in reliable, insulin sensitizing drugs necessitates a better understanding of pathways that regulate insulin sensitivity and other metabolic endpoints such that new, clinically sound R_X-interventions addressing insulin resistance may be developed in tandem with those that seek to restore beta cell function.

1.2: Nuclear Corepressors

1.2.1: Biological Function and Mechanism

Homeostasis is defined as the tendency toward a relatively stable equilibrium between interdependent elements, especially as maintained by physiological processes. As such, maintenance of metabolic homeostasis is a molecularly complicated process that depends on the coordinated interpretation of signaling from a variety of sources. Most proteins involved in biological pathways play relatively one-dimensional, specialized roles as intermediaries in

signaling cascades; however, the more complex job of sensing and responding to multiple signals from these pathways converges on nuclear coregulators. These large, scaffold proteins associate with a range of transcription factors that play key roles in metabolism; once complexed, the coregulator binds regulatory regions of transcription factor target genes in a sequence-specific fashion, and is then able to fine-tune transcriptional activity either up or down by subsequent recruitment of chromatin remodeling cofactors[87]. The presence or absence of ligand for these transcription factors determines whether the nuclear coregulatory partner is a coactivator or a corepressor. Generally speaking, agonist-bound transcription factors will pair with coactivators to potentiate transcription, whereas corepressors complex with antagonist-bound or ligand-absent transcription factors to silence gene expression[87,88]. As one might suspect, upon association to DNA, nuclear coactivators like peroxisome proliferator-activated receptor gamma, coactivator 1 alpha (PGC1α) or steroid receptor coactivator 1 will modify chromatin via intrinsic histone acetyltransferase activity[89]. Nuclear corepressors, on the other hand, recruit histone deacetylases (HDACs), along with a host of other cofactors, to tighten DNA around histones, decreasing the accessibility of gene promoter regions to transcriptional machinery[90].

1.2.2: Silencing Mediator of Retinoid and Thyroid Hormone Receptors

Silencing Mediator of Retinoid and Thyroid Hormone Receptors (SMRT), or NCoR2, is a nuclear corepressor that has been identified, along with its homolog NCoR1, as a critical modulator of metabolic processes. Discovered first in the 1990s in an attempt to explain nuclear

receptor repression, it was found that both NCoR1 and SMRT bind unliganded thyroid hormone receptor and retinoic acid receptor, influencing their activity epigenetically[91,92]. Since then, a number of targets for SMRT have emerged, representing a variety of biological pathways in addition to metabolism, ranging from development to circadian rhythm[87]. One prominent family of metabolic factors regulated by SMRT includes the PPAR transcription factors, notably PPARγ. PPARγ is as a master effector of metabolism through axes of insulin sensitivity, adipogenesis, and more[93]; groups studying SMRT through a variety of genetic mouse models frequently cite PPARγ derepression as the mechanism driving their metabolic phenotype, usually characterized by increased adiposity and improved insulin sensitivity[87].

SMRT, which is an extremely large, ~270 kDa protein, contains a number of distinct domains that, together, enable it to have specific, repressive activity. Beginning with the repression domains (RDs), these three regions grant SMRT its repressive capabilities by enabling interactions with members of the core repression complex—class I HDACs, chiefly HDAC3[87,94]. Additionally, the RDs aid in the interaction of SMRT with a number of other proteins to enhance repression, like class IIA or III HDACs[87,95]. The two SANT-like domains of SMRT are also involved in its down-regulatory function by synergistically promoting HDAC activity. One of these domains resides in the broader deacetylase activation domain, and is responsible for the recruitment and activation of HDAC3[87,96]. The other is found in the histone interaction domain, which preferentially binds deacteylated histone tails to reduce stochastic acetylation and further repression[87,97]. While the RDs and SANT-like domains enable SMRT

to have repressive capabilities, the receptor interaction domains (RIDs) are responsible for the specificity of SMRT. The three RIDs contain leucine-rich CoRNR boxes, regions that interact with nuclear receptors via their ligand-binding domains[87,98]. Via alternative splicing, a number of SMRT isoforms with modified versions of the aforementioned domains may be generated: each has varying affinities for targets and responsiveness to post-translational modifications[87,99,100], enabling SMRT to broadly regulate signaling per environmental cues.

1.2.3: Canonical Role of SMRT in T2DM and the Current State of Research

Because of the broad range of interacting partners of SMRT, its physiological role is multifarious. Its function in a number of biological processes is already well understood; for example, in development, the essential role of SMRT in cell fate determination/lineage progression (specifically for the formation of the central nervous system and heart) is evidenced by the unviability of mouse models where SMRT is homozygously ablated[101,102]. The metabolic involvement of SMRT is also well documented; however, while its ability to influence outputs important for T2DM, notably insulin sensitivity, energy expenditure, and weight gain, has been demonstrated by other groups studying SMRT, the phenotypes reported do not necessarily align with each other[87]. This lack in consensus comes despite the fact that the mechanism driving the metabolic effects of SMRT loss (which, depending on the genetic model, may even move in entirely opposite directions) is, in each case, attributed to PPARγ derepression[87]. While the interaction between SMRT and PPARγ is unrefuted, the disparate metabolic profiles observed in the various models employed previously suggest there is more to the story of how

SMRT exerts its influence on metabolism beyond its traditional association to PPARγ. Further, notwithstanding the present study, all models interrogating the metabolic role of SMRT have utilized systemic knockout models—this has made it difficult to locate the origin of phenotypic discrepancies, necessitating tissue-specific models to better interrogate the comprehensive function of SMRT as it relates to obesity and T2DM. This overview of the current state of the field is expanded on in depth in the Discussion of Chapter II.

1.3: Project Overview

1.3.1: Significance

In the year 2015, 9.4% of the American population suffered from diabetes[2]. With prevalence only accelerating, these 30.3 million Americans represent a fraction of the current 84 million prediabetics[2], 70% of whom are predicted to ultimately progress to diabetes[103]. These figures are also reflected in the cost of healthcare; the American Diabetes Association recently released data demonstrating a 26% increase in the financial burden of T2DM treatment strategies over a five-year period, representing a $327 billion toll on the U.S. in 2017[7]. While T2DM may be prevented with proper diet and exercise practices, nearly all diagnosed patients (with the rare exception of remissions resulting from bariatric surgery) maintain lifelong T2DM status[11].

Though lifestyle changes are a prerequisite for disease state improvement, most patients eventually require pharmacological intervention[65,66]. Unfortunately, however,

pharmacotherapies to address T2DM have evolved in such a way that have left clinicians exclusively with palliative options. To address the root of the problem and improve prognoses in a long-term fashion, the issue of insulin resistance must be expressly addressed, in tandem with insulin production. While certain currently available drugs peripherally improve insulin sensitivity, there exist no safe R_X options that specifically ameliorate insulin resistance; with a lack of curative interventions, reversal of T2DM progression remains an unrealistic goal. Until a refocusing of industrial efforts on developing ways to address T2DM beyond its symptoms, valuable resources that could otherwise be dedicated to research initiatives directed at better understanding other leading causes of death in the U.S. will remain unavailable.

1.3.2: Rationale

The gap in reliable insulin sensitizing drugs necessitates a better understanding of basic metabolic pathways that regulate insulin sensitivity and other endpoints such that new interventions may be developed. Previous groups had begun this process by leveraging a variety of generalized knockout models interrogating nuclear coregulators NCoR1 and SMRT, corepressors that modulate the activity of metabolically critical transcription factors. Because of both the broad regulatory functions of these metabolic corepressors and the non-specific nature of the models utilized, however, the findings reported by these groups have been contradictory[87]. This muddles the picture of whether these factors may be reliably targeted for the treatment of T2DM; the lack of consensus on the comprehensive roles of NCoR1 and

SMRT inspired the generation of tissue-specific models to better parse apart these factors' contributions.

From 2001-2011, NCoR1 had been extensively studied in the context of various metabolic tissues including liver, muscle, and adipocytes[87,104,105,106,107,108,109]. In the last 20 years, however, no such tissue-specific model had existed for SMRT, which motivated our group to generate the first adipocyte-specific SMRT knockout (adSMRT$^{-/-}$) mouse model. Our rationale was based on what was then known regarding the functions of SMRT; while an analysis of the conclusions reached through various generalized models reveals that SMRT's comprehensive metabolic role is not unanimously agreed upon, these groups did consistently report increased adiposity and improved adipocyte insulin sensitivity as a result of PPARγ derepression[87]. The concept of SMRT overseeing the activity of PPARγ is important because PPARγ is a powerful effector of adipogenesis and adiponectin expression[110]; in fact, the thiazolidinedione (TZD) class of drugs, used as a second line treatment in the management of T2DM, targets PPARγ—however, while TZDs represent the only pharmacological option to specifically address insulin resistance, pharmacodynamics have limited the clinical application of TZDs due to unsafe side effects[111]. Therefore, attaining a more holistic understanding of SMRT by characterizing its function through specific metabolic tissues will pave the way for the development of safer, insulin sensitizing drugs.

1.3.3: Central Hypothesis

SMRT is one of a few factors that endogenously regulate pharmacological targets of insulin sensitivity. While SMRT has been established as metabolically significant, its broad yet poorly defined involvement in the repression of many other important modulators of homeostasis underscores the need to better understand how SMRT is able to integrate and synchronize signaling from disparate metabolic sources. The overall objective of the study is to pinpoint exactly this, by focusing on how SMRT exerts its influence through adipocytes. When juxtaposing data from the Cohen Lab's previous, generalized SMRT knockout mouse with findings from our adSMRT$^{-/-}$ mice, it becomes evident that the role of SMRT in regulating metabolic homeostasis is not straightforward.

The comprehensive spectrum of transcription factors, representing multiple biological pathways, that are regulated by SMRT along with incongruences in metabolic outcomes at the cellular and systemic level between our two models suggest that SMRT exerts a multifaceted, tissue-specific influence on the phenotype. Thus, our central hypothesis is that SMRT influences metabolic processes through the adipocyte as they relate to T2DM in a complex, tissue-specific fashion. We believe that SMRT amalgamates signaling from a number of pathways (e.g. inflammation, lipid metabolism, etc.), and that it is able to moderate the confluence of various metabolic events through the adipocyte, a major effector of metabolic homeostasis.

1.3.4: Specific Aims

Our central hypothesis is addressed by the following specific aims, designed to clarify uncertainty surrounding the role of SMRT as a result of the phenotypic incongruences reported by groups leveraging generalized knockout models. This will be achieved not only by comparing and contrasting differences between the metabolic profiles of adSMRT$^{-/-}$ mice and models previously reported, but also by describing the driving mechanism, specifically by examining the influence of PPARγ and inflammatory activity.

1) Characterize the phenotypic profile of adSMRT$^{-/-}$ mice at both the cellular and systemic level.

This aim may be partitioned into the following subaims:

A) Interrogate the metabolic phenotype on a cellular level by assessing adipocyte i) insulin signaling, ii) lipogenesis, and iii) morphology.

B) Interrogate the metabolic phenotype on a systemic level by assessing mouse i) weight, ii) body composition, iii) insulin/glucose tolerance, iv) eating behavior, v) fuel usage preferences, and vi) serum hormones.

2) Define the mechanism driving the metabolic phenotype.

This aim may be partitioned into the following subaims:

A) Interrogate transcriptional changes in key metabolic genes by assessing i) specific PPARγ downstream targets and ii) all other unconsidered pathways (via RNA sequencing).

B) Interrogate the level and contribution of adipose tissue inflammation by assessing i) macrophage infiltration, ii) changes in the adipocyte secretome, and iii) alterations in the gut microbiome.

Chapter II: Adipocyte-Specific Loss of SMRT Impairs Metabolism, Independent of PPARγ Activity

2.1: Introduction

While the adipocyte was once considered a passive vehicle for energy storage, this one-dimensional understanding has since expanded in light of research demonstrating the active role of adipose tissue in metabolism[25,26,112]. As an endocrine organ, adipocytes collectively express and secrete a vast profile of adipokines that influence a diverse array of metabolic processes[113]; as such, adipocyte dysregulation is a major contributor to the pathogenesis of metabolic disease[114,115,116]. In the setting of over-nutrition, for example, adipokine production shifts towards factors that induce insulin resistance[117,118] – while this may be protective from the perspective of the hypertrophic adipocyte by attenuating additional lipid storage, the net effect of insulin resistance is deleterious on a macrophysiological scale[119]. In addition to exacerbating the general development of metabolic syndrome, adipocyte insulin resistance itself can have a compounding metabolic consequence by propagating dysregulated lipolysis and release of fatty acids that interfere with signaling in other metabolic tissues[120,121].

Regarding the adipocyte's ability to maintain homeostasis, it is evident that nuclear cofactors play a critical role by regulating the transcription of metabolically essential genes. For example, the coactivator PGC1α is a master regulator for mitochondrial remodeling[122]; when exposed to cold, stimulation of the sympathetic nervous system activates *Pgc1α* through the

PKA/CREB pathway, which in turn strongly induces expression of *Ucp1* in brown adipose tissue, thus increasing thermogenesis[123]. In white adipose tissue, the Silencing Mediator of Retinoid and Thyroid Hormone Receptors (SMRT, or NCoR2) has been identified as an important factor in the maintenance of metabolic signaling[88]. SMRT is a nuclear corepressor, responsible for decreasing the transcriptional activity of its target transcription factors (TFs); in the absence of ligand, TFs complex with SMRT, leading to recruitment of factors with histone deacetylase activity (particularly, HDAC3) at the TF promoter site and, ultimately, transcriptional repression[124,125].

Nearly all genetic models seeking to better understand SMRT's molecular and macrophysiological roles *in vivo* have reported metabolic phenotypes with significant increases in weight or adiposity, coupled with alterations in insulin sensitivity and energy expenditure[87]. Because of its strong association to PPARγ, a powerful effector of adipogenesis, insulin sensitivity, and glucose metabolism[126,127], phenotypes resulting from perturbations of *Smrt* are often ascribed to PPARγ derepression. However, while there is clear consensus that SMRT strongly influences metabolism, results from these various knock-out and knock-in models, discussed at length posteriorly, have led to conflicting conclusions[87]. In addition, a ChiP-Seq study by Raghav et al. in 3T3-L1 cells suggested that SMRT is not in fact recruited to PPARγ target genes during adipogenesis, and instead regulates 3T3-L1 adipogenesis via effects on KAISO and C/EBPb[128]. Thus, the comprehensive role of SMRT in the adipocyte *in vivo* remains poorly understood, and even its targets remain unclear.

To more rigorously define the role of SMRT in the adipocyte, we generated an adipocyte-specific SMRT knockout (adSMRT$^{-/-}$) mouse by crossing homozygous floxed *Smrt* mice with adiponectin-Cre mice on a C57/BL6 background. We were able to show that SMRT deficiency in the adipocyte does not actually lead to obesity, even when mice are challenged with a high-fat diet. Even more surprising was learning that PPARγ activity remained entirely unaffected by loss of SMRT, a strong departure from the mechanism traditionally attributed to metabolic dysfunction as a consequence of SMRT ablation. Despite this, we identified a striking, metabolically deleterious phenotype defined by glucose intolerance, adipocyte insulin resistance, and more; this was combined with aberrant adipose tissue inflammation, which will be discussed in Chapter III. These findings suggest that the obesity phenotypes of prior generalized SMRT models were due at least in part to the effects of SMRT loss in tissues beyond the adipocyte.

What is so profound about the conclusions of the studies detailed within, however, is that the metabolic results discussed in Chapter II are accompanied, and at least in part driven, by changes in immune signaling. Demonstrated through the results of RNA sequencing (RNAseq), flow cytometry, and conditioned media experiments, we found that adipocyte-specific loss of SMRT promotes a pro-inflammatory adipose tissue microenvironment due to alterations in the adipocyte secretome, which strongly informs the status of those metabolic phenotypes discussed below. While all inflammatory and non-metabolic findings will be explored in Chapter III, it is important to note that this is the first time SMRT has been reported

as an integrator of metabolic and inflammatory signaling via the adipocyte in the maintenance of homeostasis, and that the data expounded on in Chapter II go hand in hand with those discussed in Chapter III.

2.2: Results

2.2.1: Generation of the Adipocyte-Specific SMRT Knockout (adSMRT$^{-/-}$) Mouse

To define the role of SMRT in the adipocyte, we generated an adipocyte-specific SMRT knockout mouse (adSMRT$^{-/-}$) by crossing mice homozygously floxed for *Smrt* (the generation of which is described previously[129]) with mice hemizygously expressing Cre driven by the adiponectin promoter on a C57/BL6 background (Fig. 1A). Therefore, half of the resultant offspring lost *Smrt* expression specifically in adipocytes, as evidenced by a lower molecular weight band (fig. 1B). Fidelity of the genotype was validated by qRT-PCR analysis of isolated adipocytes and their associated stromal vascular components from primary, mature fat tissue. Here, we observe a significant decrease in *Smrt* expression of ~80% in the adipocytes of knockout mice compared to wildtype; the associated stromal vascular fraction (SVF) showed no change in *Smrt* expression (Fig. 1C), confirming specificity of the model.

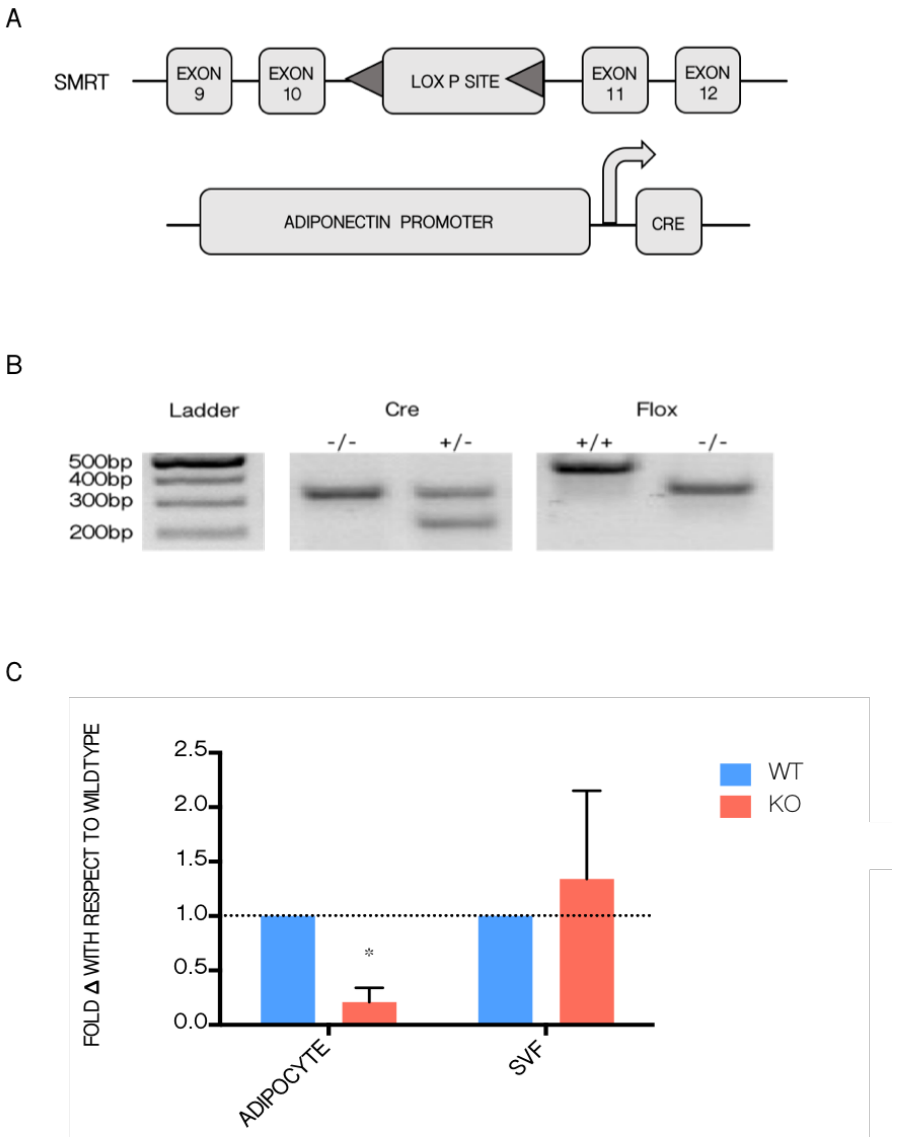


Figure 1: Genetics of adSMRT$^{-/-}$ Mouse. A: *Smrt* receives a single loxP site on both alleles, inserted 187 bp downstream of exon 11; mice hemizygously expressing adiponectin-driven *Cre* excise this site such that a frameshift mutation is introduced, rendering SMRT protein non-functional exclusively in adipocytes. **B**: mice expressing *Cre* display two bands at ~320bp and ~250bp while wildtype mice display a darker band (two overlapping bands) at ~320bp; all mice used in this study are floxed on both alleles, displaying a band at ~450bp; Cre$^{+/-}$, Flox$^{+/+}$ mice are crossed with Cre$^{-/-}$, Flox$^{+/+}$ mice to generate adSMRT$^{-/-}$ mice. **C**: qRT-PCR model validation shows that adSMRT$^{-/-}$ mice have ~80% reduction in *Smrt* expression exclusively in adipocytes while the associated stromal vascular fraction remains unaffected. N=5 per genotype. Data are means ±SEM; *P < 0.01.

2.2.2: Adipocyte-Specific Loss of SMRT Does Not Cause Obesity

In prior in vivo SMRT models, whether created by knockout or RID knockin mutations, mice demonstrated variable degrees of increased adiposity[87]. However, on both standard chow and 45% high-fat diets (HFD), no differences in weight were observed between adSMRT$^{-/-}$ and wildtype mice (Fig 2A,B). In addition, DEXA scanning showed no changes in body fat percentage or bone mineral density (Fig. 2C-E), though metabolic cage studies indicated increased total caloric intake, specifically during the light cycle, as well as increased respiratory exchange ratio (RER) in adSMRT$^{-/-}$ mice (Fig. 2F,G); in fact, when analyzing the diurnal RER patterns between genotypes, adSMRT$^{-/-}$ mice demonstrate metabolic inflexibility by an inability to efficiently switch to fat utilization as an energy source (indicated by depressed amplitudes) during the resting light cycle (Fig. 2H). Data from the metabolic cages also showed a significant decrease in ambulatory (horizontal) and rearing (vertical) activity (Fig. 2I,J) in adSMRT$^{-/-}$ mice, though these complex behaviors are too multi-faceted to reliably attribute to SMRT loss in adipocytes, and is beyond the focus of the manuscript. Additionally, we assessed for adipocyte hypertrophy via histological analyses, and found no significant differences in adipocyte size between adSMRT$^{-/-}$ and wildtype mice for both subcutaneous and epididemal fat tissue (Fig. 2K-N).

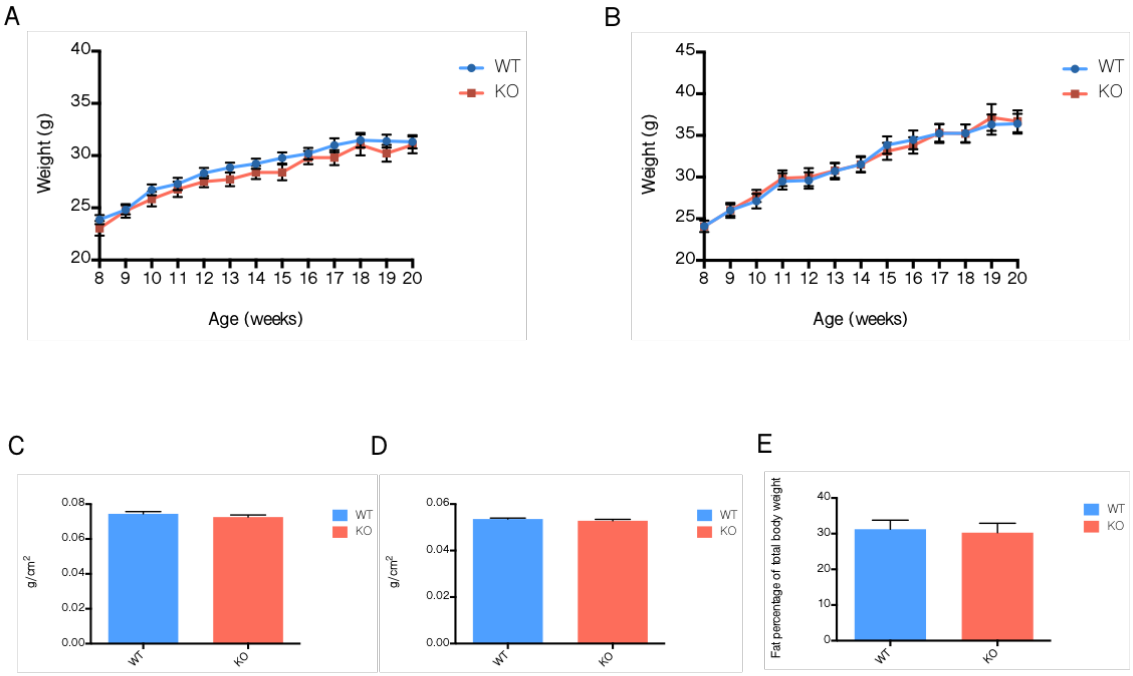


Figure 2: adSMRT$^{-/-}$ Mice Exhibit Altered Energy Consumption and Utilization without Obesity or Adipocyte Hypertrophy. A, B: total body weight measured weekly between ages 8–20 weeks for mice on a chow diet (A) and mice fed a 45% high-fat diet (HFD) (B). N=15–25 per genotype. Data are means ±SEM. **C-E:** determinations of femur bone mineral density (C), total bone mineral density (D), and body fat percentage (E) by DEXA scanning for 20-week old mice fed a 45% HFD. N=10–15 per genotype. Data are means ±SEM.

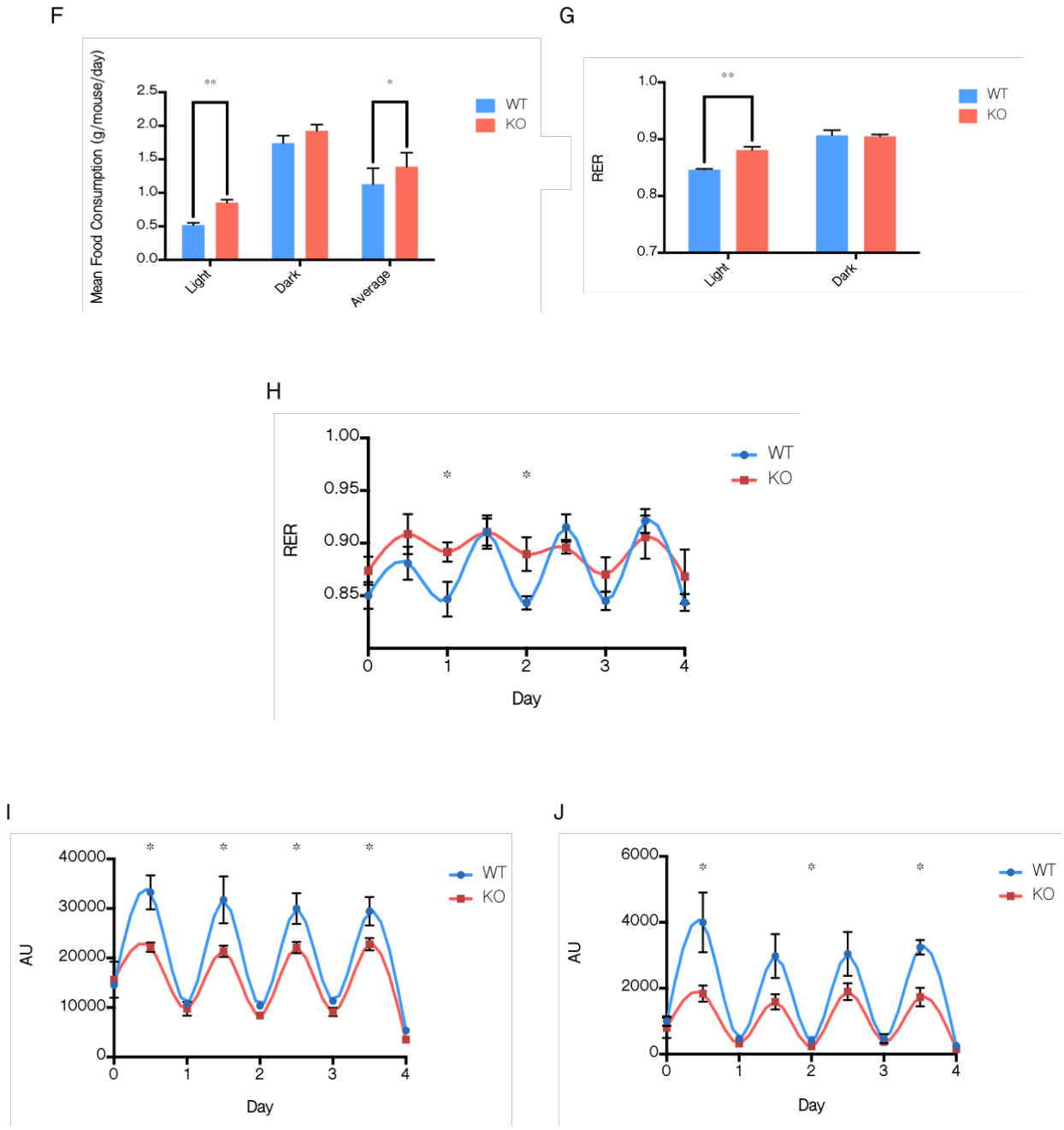


Figure 2 (continued): F-J: determinations of average food consumption (F), RER (G, H), ambulatory activity (I), and rearing activity (J) for 20-week old mice fed a 45% HFD by metabolic cage analysis. N=4 per genotype. Data are means ±SEM; *P < 0.05, **P < 0.001.

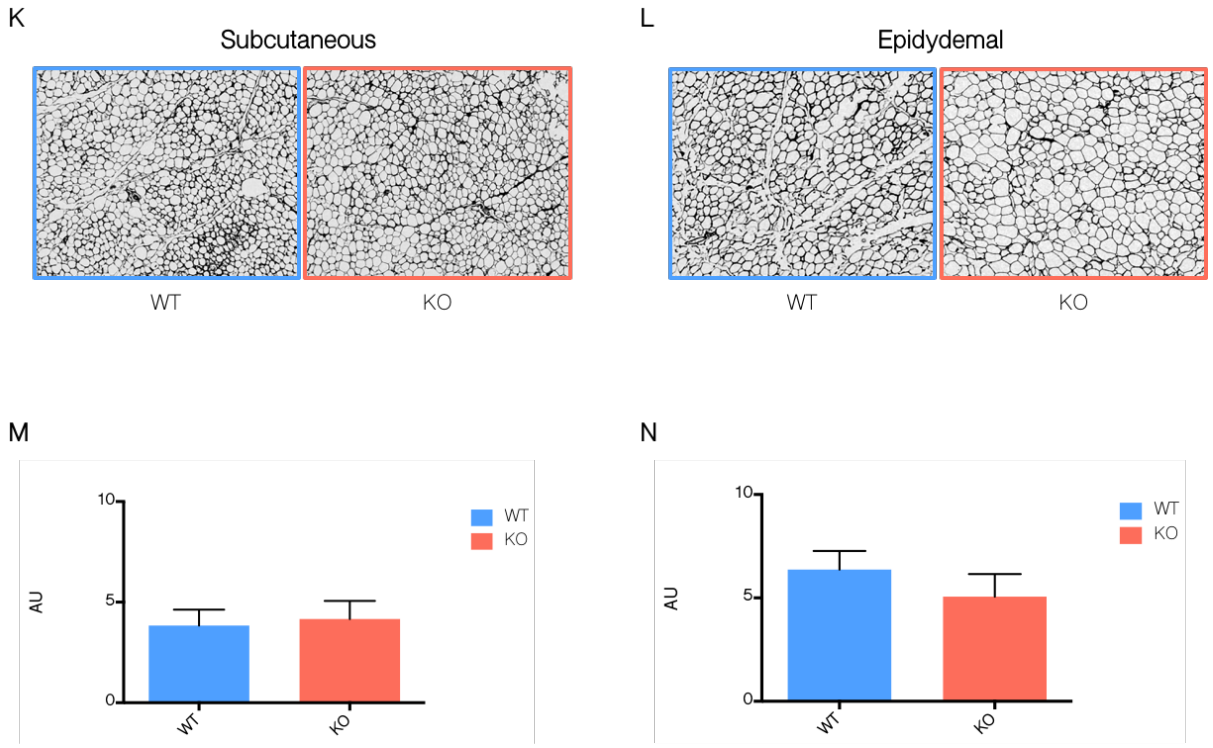


Figure 2 (conitnued): K-N: sample histological sections of H&E-stained subcutaneous (K) and epidydemal (L) adipose tissue for mice fed a 45% HFD, and the respective quantifications for adipocyte size via ImageJ quantification of negative space (M, N).

2.2.3: adSMRT$^{-/-}$ Mice Exhibit Glucose Intolerance and Adipocyte Insulin Resistance

Though adSMRT$^{-/-}$ mice did not develop obesity when fed either standard chow or a 45% high fat challenge diet, SMRT deficiency still negatively impacted adipocyte function and metabolic homeostasis. adSMRT$^{-/-}$ mice fed a 45% HFD displayed significant glucose intolerance by ~20% compared to wildtype, though glucose tolerance remained unaffected between genotypes for mice fed a chow diet (Fig. 3A-D). Thus, further experimentation was conducted exclusively on mice fed a 45% HFD. In fact, adipocytes isolated from adSMRT$^{-/-}$ mice fed a 45% HFD (but not those from mice fed a standard chow diet) exhibited impaired

insulin sensitivity as measured by the ratio of AKT:pAKT signaling following treatment with insulin (Fig. 3E,F), a result inconsistent with our previous, generalized SMRT$^{+/-}$ mice. Finally, insulin tolerance tests yielded a potentially confounding result, demonstrating improved systemic insulin sensitivity (Fig. 3G,H); while adipocyte insulin resistance and systemic glucose intolerance are usually accompanied by impairments in systemic insulin tolerance, there exist plausible reasons as to why this may be occurring (e.g. deficiencies at the level of the pancreas) that have simply not yet been explored.

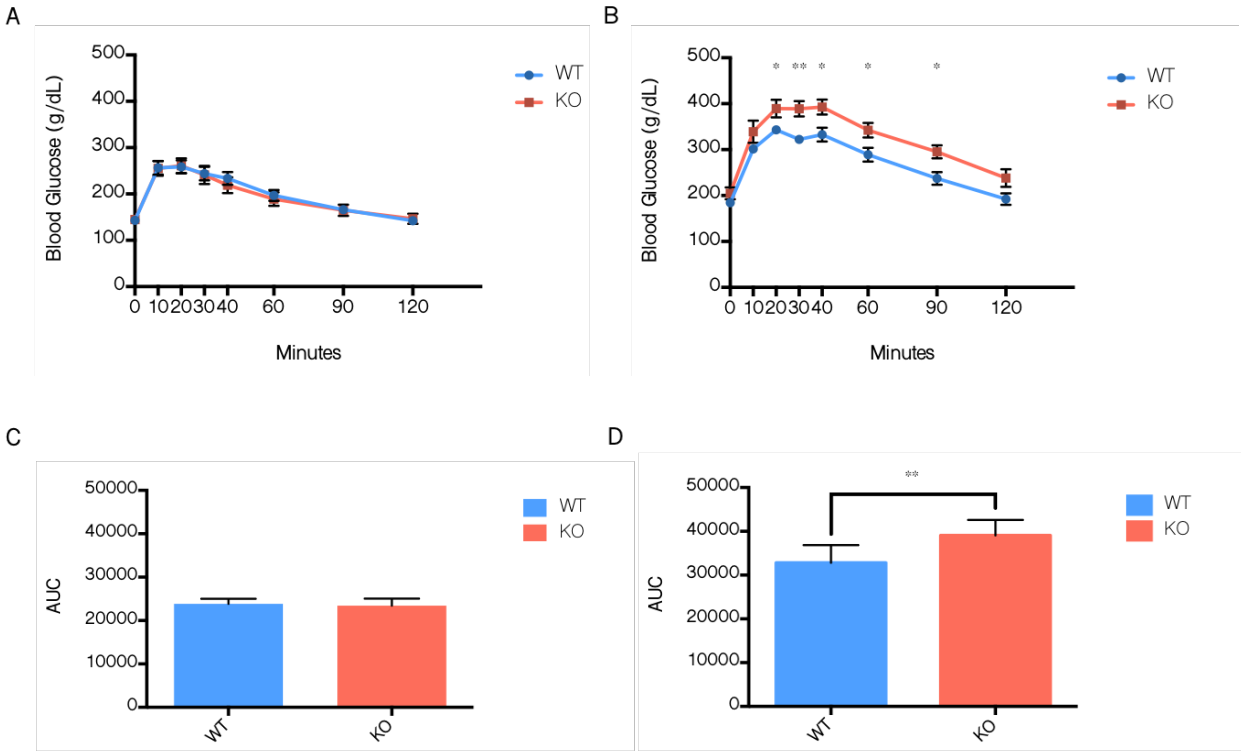


Figure 3: adSMRT$^{-/-}$ Mice are Glucose Intolerant with Insulin Resistant Adipocytes. A-D: intraperitoneal glucose tolerance tests (IP-GTTs) for 20-week old mice fed a chow (A) and 45% high-fat diet (HFD) (B), and their respective area under the curve quantifications (C, D). N=12-13 per genotype for chow GTT data, N=7-11 for HFD GTT data. Data are means ±SEM; *P < 0.05, **P < 0.005.

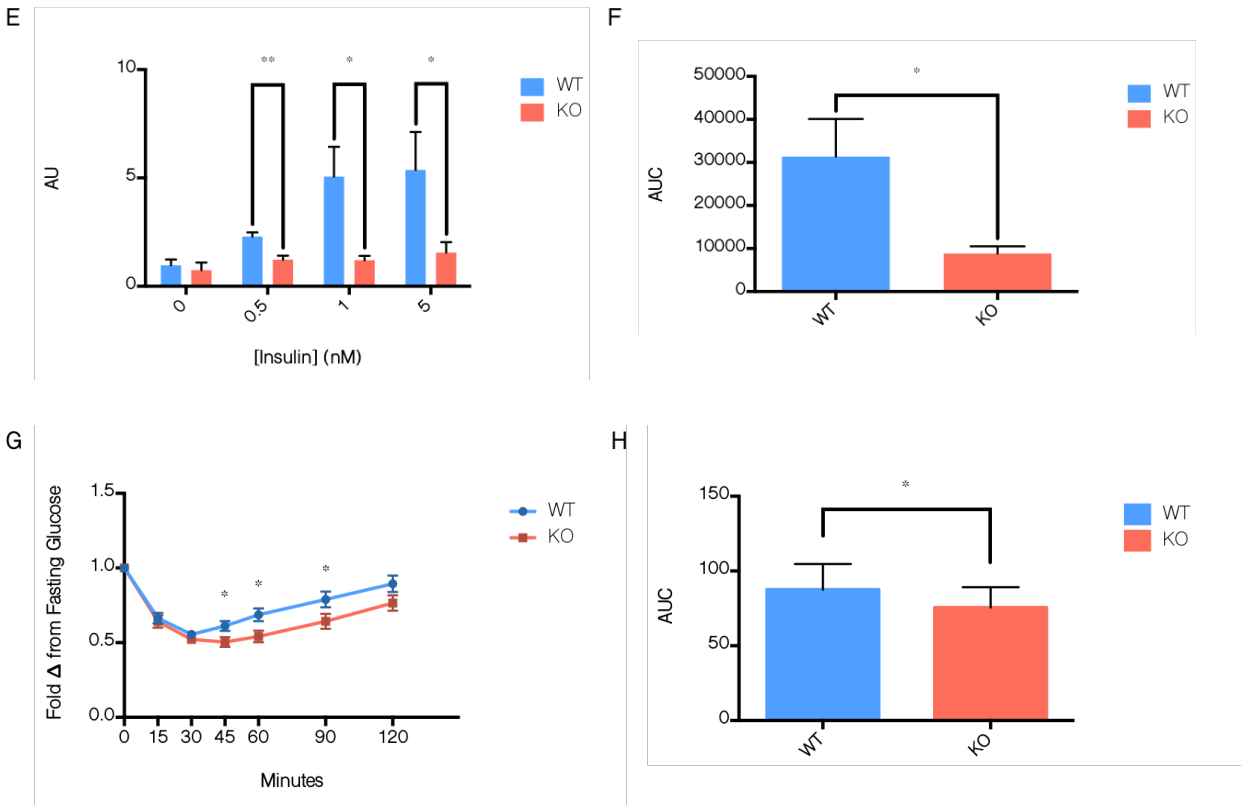


Figure 3 (continued): E, F: adipocyte insulin sensitivity from mice fed a 45% HFD, determined by Western blotting for AKT:pAKT signaling and plotted as a quantification of band intensity (E), and the respective AUC measurement (F). N=7 per genotype. Data are means ±SEM; *P < 0.05, **P < 0.005. **G,H:** intraperitoneal insulin tolerance tests (IP-ITTs) for 20-week old mice fed a 45% high-fat diet (HFD) (G), and the respective area under the curve quantification (H). Data are means ±SEM; *P < 0.05.

2.2.4: PPARγ Derepression Fails to Explain Phenotype

In previously employed mouse models interrogating SMRT, the mechanism driving any phenotypes of weight gain, fat mass expansion, and improved adipocyte insulin sensitivity was thought to be a result of PPARγ derepression due to loss of SMRT[87]. However, our phenotype (specifically, no change in weight nor adipocyte size/number, coupled with a decrease in adipocyte insulin sensitivity) suggested that this might not be the case. We therefore assessed

the expression of PPARγ target genes Fabp4, Gyk, Pck1, Ucp1, and Cd36[130] in adSMRT$^{-/-}$ isolated adipocytes by qRT-PCR, and observed no generalized increase in these downstream factors (Fig. 4A-E); this suggests that altered PPARγ transcriptional activity in adipocytes is not the mechanism for the metabolic effects seen in adSMRT$^{-/-}$ mice. Additionally, when assessing lipogenesis following treatment with radiolabeled glucose and insulin at varying doses, we observed no changes in the ability of adipocytes to incorporate glucose into triglycerides (Fig. 4F), which indirectly supports the idea that PPARγ derepression is not driving our metabolically deleterious phenotype.

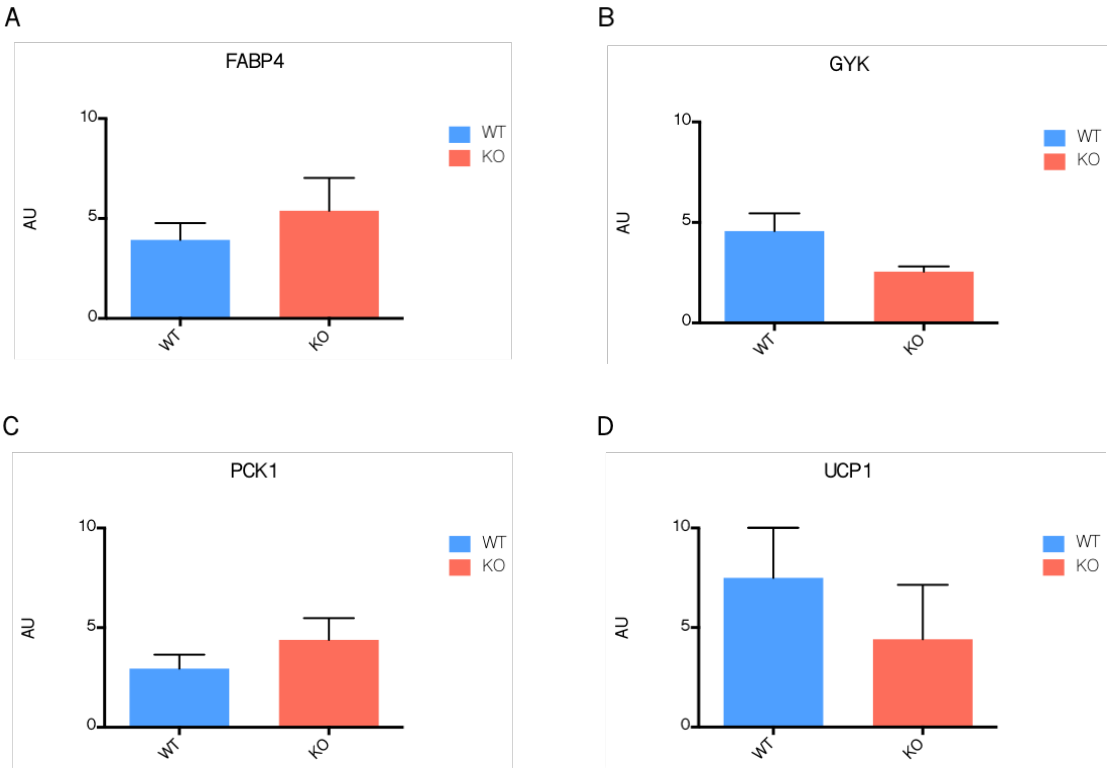


Figure 4: PPARγ Derepression Fails to Explain the Phenotype. A-E: qRT-PCR expression data from isolated adipocytes for PPARγ downstream targets *Fabp4, Gyk, Pck1, Ucp1, and Cd36.*

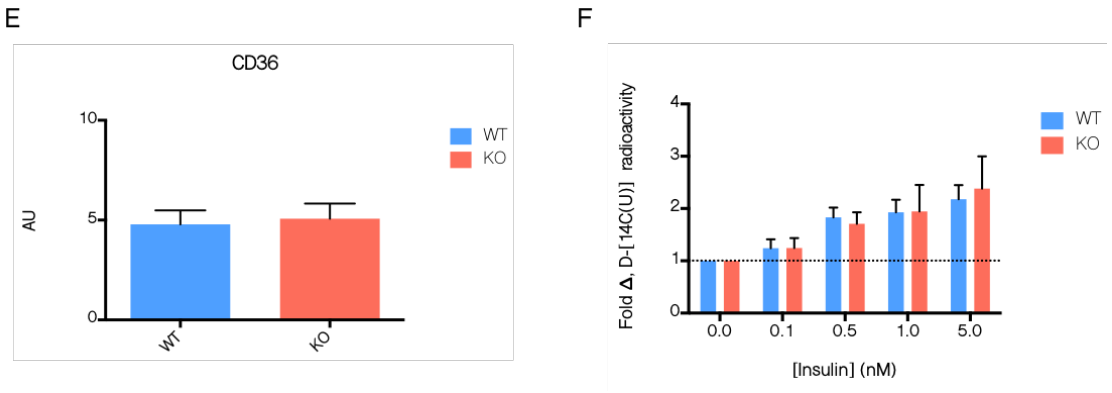


Figure 4 (continued): N=7 per genotype. Data are means ±SEM. **F:** lipogenesis assay determining ability of adipocytes to incorporate radiolabeled glucose into triglycerides upon stimulation with insulin; plotted as fold change in radioactivity from baseline. N=8 per genotype. Data are means ±SEM.

2.3: Discussion

SMRT is a corepressor for nuclear receptors and other transcription factors, and is structurally related to its homolog NCoR1[131]. However, SMRT and NCoR1 have distinct functions, as homozygous knockout of either is embryonic lethal, demonstrated by Jepsen et al[132]. Because of this lethality, two approaches have been used to develop models to study the metabolic functions of SMRT and NCoR1 *in vivo*: a generalized heterozygous knockout, or various mutations of the RIDs[87]. In the case of the latter, for NCoR1, Astapova et al. found that NCoRΔID mice demonstrated increased thyroid hormone-dependent energy expenditure, a result of increased sensitivity[105]. However, Nofsinger et al. showed that when *Smrt* is analogously mutated, this somewhat beneficial outcome is replaced by reduced respiration and energy expenditure[133]; $SMRT^{mRID}$ mice also showed an increase in fat

mass/adipogenesis, via PPARγ derepression. A similar model also interrogating SMRT ($SMRT^{mRID1}$), utilized by Reilly et al. and Fang et al., yielded a mouse with decreased mitochondrial function and worsened insulin resistance[134,135], whereas Sutanto et al., utilizing a heterozygous knockout ($SMRT^{+/-}$) model, reported a mixed metabolic outcome: fat mass expansion/enhanced caloric intake combined with improved adipocyte insulin sensitivity, again likely due to PPARγ derepression[129]. Because these findings, together, are somewhat contradictory, later studies have focused on tissue-specific knockout models; Yamamoto et al. studied NCoR1 through a muscle-specific knockout model[109], whereas Li et al. did the same, but in adipocytes[136]. However, no such study existed for SMRT – to this end, we generated an adipocyte-specific SMRT knockout ($adSMRT^{-/-}$) mouse to rigorously define the role of SMRT in the adipocyte, and how it affects metabolic homeostasis.

We were initially surprised to find that certain classical parameters, such as weight and adiposity, remained unaltered in $adSMRT^{-/-}$ mice, suggesting that altered SMRT function does not influence those characteristics through the adipocyte; this is in stark contrast to findings described by other groups, where these measures were strongly impacted due to systemic loss of SMRT as a result of PPARγ derepression. However, when considering metabolic functions that have a truer association to T2DM pathology, for example insulin sensitivity or glucose tolerance, it becomes evident that SMRT plays a critical role in the maintenance of homeostasis through the adipocyte, as significant dysregulation of both was observed. This comes in addition to aberrations in other metabolic readouts, like respiratory exchange ratio

(RER) or feeding behavior. We were also surprised that the dramatic metabolic phenotype observed in adSMRT$^{-/-}$ mice develops independently of PPARγ transcriptional activity as evidenced by qRT-PCR experiments demonstrating that the expression of PPARγ downstream targets were unaltered. This is particularly interesting considering the results of prior models, which support an association between SMRT and obesity/PPARγ activity[87,137]; the results of this study, therefore, indicate that the obesity observed in other models likely result from SMRT deficiency beyond the adipocyte, possibly via alterations in the central nervous system.

Interestingly, when comparing the adSMRT$^{-/-}$ mouse to the NCoR1$^{-/-}$ adipocyte-specific knockout model utilized by Li et al.[136], the divergence in metabolic phenotypes is stark. While increased food intake was reflected in both models, all other metabolic parameters move in opposite directions. For example, adipocyte-specific NCoR1$^{-/-}$ mice demonstrated increases in both total weight, and subcutaneous/visceral adipose depot mass, whereas these readouts are unaffected in adSMRT$^{-/-}$ mice. Further, Li et al. showed improved glucose tolerance and adipocyte insulin sensitivity in their knockouts, while adSMRT$^{-/-}$ mice were worsened for both parameters. Combined with increased PPARγ transcriptional activity, Li et al. concluded that loss of NCoR1 in the adipocyte was metabolically protective, via increased expression of PPARγ target genes[136]. In contrasting these models, the idea that NCoR1 and SMRT play distinct roles in the regulation of metabolic processes through the adipocyte is strongly supported. However, in the absence of changes in PPARγ transcriptional activity, we were left with the question of what mechanism may be driving this phenotype; to address this,

we took an unbiased approach by examining the gene expression profile of adipose tissue via RNAseq, gaining new insights regarding SMRT's role as an integrator of metabolic and inflammatory signaling as discussed in Chapter III.

2.4: Methods

2.4.1: Model Generation

Floxed *Smrt* mice have been reported previously[138]. To generate knockout adSMRT$^{-/-}$ mice experimental cohorts, floxed *Smrt* mice (SMRT$^{loxP/loxP}$) were crossed with mice hemizygously expressing *Cre* recombinase under the control of the adiponectin promoter (Adipoq-Cre$^{+/-}$) [B6.FVB-Tg(Adipoq-cre)1Evdr/J; Jackson Laboratory]. Using this line, *Cre* is expressed exclusively in white and brown adipose tissue, without activation in resident macrophages. Resulting cohorts were either wildtype (Adipoq-Cre$^{-/-}$ SMRT$^{loxP/loxP}$) or knockout (Adipoq-Cre$^{-/+}$ SMRT$^{loxP/loxP}$). All experimental mice were developed on a C57/B6J strain background, and littermate controls from the cohorts developed were used in experiments.

2.4.2: Animal Husbandry

Unless otherwise stated, mice used in this study were male, generated on a C57/BL6 background in-house, and fed a standard chow diet until 8 weeks of age, at which point a 45% HFD (Teklad Custom Research Diet, TD.06415; Envigo, Madison, WI), matched to our standard chow diet (Teklad Global 18% Protein Diet 2018; Envigo, Madison, WI), was provided

ad libidum for 12 weeks. All mice were sacrificed at 20 weeks of age via isofluorane overdose; euthanasia was confirmed via cervical dislocation. Weights were taken weekly, and genotype was determined via PCR using tail clippings obtained at weaning (3 weeks of age). Tissues collected for experimentation were either snap frozen in liquid nitrogen and stored at -80°C or immediately used. All mice were housed under standard conditions, and no single-housed mice were used for experimentation. Animals were treated in accordance with protocols approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Chicago.

2.4.3: Intraperitoneal Glucose/Insulin Tolerance Tests (IP-GTT/ITT)

Following 12 weeks of 45% HFD feeding, mice were fasted for 4 hours, at which point baseline blood glucose readings were obtained from all mice via tail vein blood sampling following application of local anesthetic (2% viscous lidocaine, Water-Jel, Carlstadt, NJ). For GTTs, dextrose was injected intraperitoneally at a concentration of 1 g/kg body weight. Blood glucose levels were measured at 10, 20, 30, 40, 60, 90, and 120 minutes following injection. For ITTs, insulin (Humalog) was injected intraperitoneally at a concentration of 0.5 U/kg body weight. Blood glucose levels were measured at 15, 30, 45, 60, 90, and 120 minutes following injection. All blood glucose measurements were taken using a Freestyle Lite Glucometer (Abbott Laboratories, Abbott Park, IL). Percentage difference in blood glucose clearance was determined by measuring and comparing areas under the curve (AUC) between wildtype and knockout.

2.4.4: Adipocyte Insulin Sensitivity

Primary whole fat tissue from mice fed a 45% HFD was thoroughly minced in KRBH supplemented with 4% endotoxin-free BSA, 0.5 mM glucose, and 1 mM PIA. Adipocytes were then isolated by the addition of T1 Collagenase (Worthington Biochemical Corporation, NJ), and subsequent incubation at 37°C for 30 minutes with agitation (125 rpm). The resultant mixture was passed through a cell filter to remove debris and hand-spun to achieve separation. Adipocytes were placed into clean tubes and washed in BSA-free KRBH to then be treated with increasing doses of 0, 0.5, 1, and 5 nM insulin for 15 minutes. The reaction was halted and samples were prepared for SDS-PAGE via cell lysis by addition of RIPA buffer and subsequent sonication at an amplitude of 40 Hz with an ultrasonic processor (Sonics & Materials, Inc., Newtown, CT). Samples were nutated at 4°C for 30 minutes and then centrifuged at 4°C at 13,200 rpm for 30 minutes to isolate the infranatant. Protein concentrations were determined by BCA protein assay (23227, Thermo Scientific, Waltham, MA) to standardize protein concentration loaded into gel. After addition of dye, samples were then reduced using 2-mercaptoethanol, boiled for 10 minutes, and analyzed via SDS-PAGE. Insulin sensitivity was determined by pAKT:AKT. Primary antibodies utilized included anti-AKT (2920S, Cell Signaling), and anti-phosphorylated AKT targeting serine 473 (4060S, Cell Signaling).

2.4.5: Dual-energy x-ray absorptiometry (DEXA)

Body composition was measured by DEXA (Lunar PIXImus densitometer system, GE Healthcare) using PIXImus 2 software. The system was calibrated according to manufacturer's

instructions prior to the start of the experiment. Mice were anesthetized prior to imaging using a mixture of Ketamine and Xylazine (80–120 mg/kg body weight of Ketamine and 5–10 mg/kg body weight of Xylazine injected intraperitoneally).

2.4.6: Metabolic Cages

Indirect calorimetric measurements were carried out using the LabMaster System (TSE Systems, Chesterfield, MO) on individually housed mice, maintained under standard housing conditions. Mice were provided ad libitum access to food and water. Following a two-day acclimation period, O_2 consumption, CO_2 production, energy expenditure, ambulatory activity, and food/water consumption were monitored over 30-minute periods for five consecutive light-dark cycles over. The respiratory exchange ratio (RER) was calculated as the ratio of O_2 consumption to CO_2 production.

2.4.7: Histology

Adipose tissue samples were fixed for 24–48 hours in 4% paraformaldehyde at 4°C. Cassettes containing samples were then washed with and stored in 70% EtOH short-term. Samples were sent to the University of Chicago Human Tissue Resource Center core for sectioning (5 μm), paraffin embedding, and hematoxylin and eosin (H&E) staining of the sections were performed using standard techniques. Images of sections were taken at 10X magnification and adipocyte cell size was analyzed using a custom macro script on Fiji (ImageJ); size was quantified by measuring negative space within cell borders.

2.4.8: Gut Microbiome

Fecal samples were collected from mice at 8, 14, and 20 weeks of age. The harvesting method employed ensured that stool pellets were fresh, and did not come into contact with any materials other than the sterile tubes used for collection. All samples were stored at -80°C until DNA extraction. DNA was extracted from fecal samples using standard, published protocols (Wang et al., 2009). Samples were then sequenced by MiSeq at the Next Generation Sequencing Core in the Biosciences Division at Argonne National Laboratory. DNA sequences were analyzed by Quantitative Insights into Microbial Ecology (QIIME). Samples with less than 3,000 sequences were excluded from the analyses. Operational taxonomic units (OTUs) were picked at 97% sequence identity using the GreenGenes Database (2013). Analysis of similarities (ANOSIM) was performed using QIIME to examine the impacts of genotype, diet, and age on fecal microbiota. The number of permutations was 10,000 or the maximum number of permutations allowed by the data. Permutation test with 10,000 permutations was performed using R to compare the UniFrac distances of animals between cohorts.

2.4.9: Quantitative Real Time Polymerase Chain Reaction (qRT-PCR)

Adipocytes and associated stromal vascular fraction (SVF) were isolated from perigonadal fat depots. Adipocyte and SVF isolation followed the same procedure described above for adipocyte isolation in the preparation of Western blot samples, up until hand-spinning. At this point, samples were spun at 300xg at 4°C for 8 minutes to separate adipocytes while also pelleting the SVF. RNA was then prepared from each fraction separately using a

commercially available kit (Quick-RNA MiniPrep Kit, ZymoResearch, CA). Following analysis for purity and concentration, RNA was then reverse transcribed to cDNA using qScript cDNA mix. Utilizing ThermoFisher's TaqMan Gene Expression Assays (FAM probes) and the TaqMan Fast Advanced materials (4444964) and protocol for cycling (CFX Connect Real-Time PCR Detection System), all biological replicates were run in triplicate for 11 RNAseq-identified significantly differentially expressed targets. Each plate was repeated once for a total of two technical replicates per biological replicate. 18S Eukaryotic rRNA Endogenous Control was used as a reference gene (4333760F).

2.4.10: Statistical Analyses

Unless otherwise noted, data are presented as means ± SEM with statistical significance determined using a two-tailed, Student's t-test to compare wildtype and adSMRT-/- groups. A threshold of $p < 0.05$ was used to identify statistically significant data. Graph generation and statistical methods were carried out in GraphPad Prism, version 6.0, and Microsoft Excel.

Chapter III: Inflammation Plays a Causal Role in the Development of adSMRT$^{-/-}$ Phenotypes

3.1: Introduction

It has been well-established that obesity and T2DM are characterized by adipose tissue inflammation[139], and it has been shown that metabolically activated (MMe) macrophages, recruited by adipocytes to aid in the clearance of excess lipid, can worsen and sustain the pro-inflammatory microenvironment[60]. In the short term, this impairs proper function of the adipocyte, significantly contributing to altered energy storage and utilization[60]. While the mechanisms by which the adipocyte regulates the infiltration of MMe macrophages is not fully understood, the findings discussed here begin to shed light as to how nuclear cofactors, specifically SMRT, may coordinate this chemotaxis via the adipocyte, which in turn influences metabolic regulation both locally and systemically.

Previous studies that have focused on better understanding the metabolic role of SMRT have concluded nearly unanimously that their findings were driven, mechanistically, by PPARγ derepression. Upon discovering that this canonical pathway remained unaffected in our tissue-specific model, and thus did not explain our metabolic phenotype, we took an unbiased, broad approach at ascertaining the mechanism responsible for the adSMRT$^{-/-}$ deleterious metabolic profile. By taking a snapshot of the gene expression profile via RNAseq experiments, we were surprised to find that pathways involved in immunity/inflammation were strongly upregulated in knockout mice compared to wildtype. The reason this was unexpected was because while

inflammation and obesity/T2DM are closely associated with respect to pathology, this was the first time, to our knowledge, SMRT's direct involvement in immune function in the context of metabolism had been described.

Because inflammatory signaling to the adipocyte, be it autocrine or paracrine, is a primary cause for adipocyte insulin resistance[141] (and thus, impaired glucose uptake), SMRT's involvement in the synchronization of metabolic and inflammatory signaling is particularly germane when explaining the phenotype of the adSMRT$^{-/-}$ mouse. The adverse metabolic outcomes discussed in Chapter II combined with the aberrant adipose tissue inflammation discussed below suggests that alterations in the adipocyte secretome of adSMRT$^{-/-}$ mice inform the adipose tissue microenvironment to be pro-inflammatory via heightened influence of MMe macrophages. These findings offer a novel function for SMRT, overseeing the cross-talk between adipocytes and inflammation-modulating macrophages for the regulation of physiological parameters relevant to T2DM, distinct from the development of obesity.

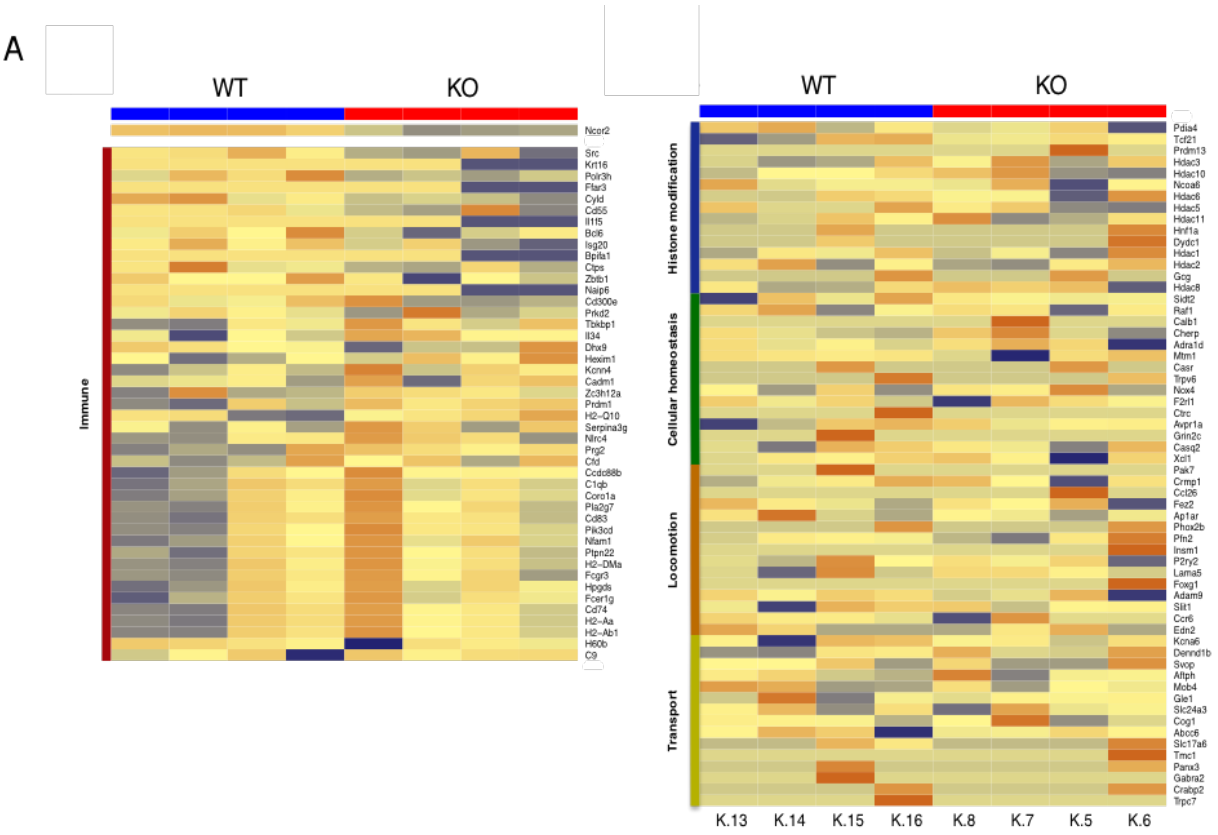
3.2: Results

3.2.1: RNA Sequencing Indicates Dysregulation of Immune Pathways

Because our data did not support altered PPARγ transcriptional activity in adSMRT$^{-/-}$ adipocytes, we next wanted to assess large-scale gene expression changes induced by loss of this corerpressor to identify possible mechanisms that would explain the knockout phenotype. We therefore performed RNAseq analysis of primary adipose tissue derived from

wildtype and adSMRT$^{-/-}$ mice. We found that 67 genes were differentially expressed by at least 50% between our genotypes at a false discovery rate of < 0.05. Of these genes, nearly all play roles in immune pathways/are involved in pro-inflammatory processes (Fig. 5A,B). From these 67 genes, 10 were selected for qRT-PCR validation on isolated adipocytes and the associated SVF (Fig. 5C) to both confirm RNAseq findings as well as identify the source of inflammatory signaling (adipocyte, resident immune cell, or both); we found that of these pro-inflammatory genes, most were significantly upregulated in adipocytes; any genes that were not identified as significantly differentially expressed still trended strongly in this direction, in both adipocyte and SVF samples (Fig 5D-M). Together, these surprising data led us to the conclusion that SMRT loss in the adipocyte causes inflammatory dysregulation in adipose tissue, which in turn informs the microenvironment and resident immune cells to drive local insulin resistance and glucose intolerance.

A



B

Diet	|FD|	FDR			nominal p-value		
		≤0.01	≤0.05	≤0.1	≤0.01	≤0.05	≤0.1
HFD	≥0	27	77	126	358	809	1307
	≥1.5	26	67	104	254	369	427
	≥2	8	25	37	84	109	114

C

Gene	Fold Δ	Nominal p-value	FDR-corrected p-value
KCNN4	2.820595921	1.06E-04	2.71E-02
H2-AA	1.705269784	5.66E-08	9.33E-05
FCER1G	2.020902893	8.01E-05	2.43E-02
NFAM1	2.0363704	3.17E-04	4.80E-02
CD83	1.926523788	3.73E-07	3.08E-04
SLAMF9	1.869950396	1.17E-07	1.50E-04
HPGDS	3.067365319	3.03E-04	4.79E-02
PRKCB	2.220677667	1.60E-07	1.68E-04
CLEC7A	2.278366754	1.40E-06	8.95E-04
PLA2G7	2.0139111	5.82E-06	3.36E-03
NCOR2	0.554016174	1.76E-11	1.02E-07

Figure 5: RNA Sequencing Indicates Dysregulation of Immune Pathways in adSMRT$^{-/-}$ Mice. A-C: RNA sequencing data for whole fat samples from mice fed a 45% HFD, plotted as a heatmap of select genes representing molecular functions spanning immunity, histone modification, homeostasis, movement, and membrane transport; red, blue, and yellow colors represent upregulation, downregulation, or no change, respectively, relative to the average expression level for that gene across all eight biological replicates (each column represents a biological replicate) (A); summarizing table identifying differentially expressed genes between wildtype and adSMRT$^{-/-}$ samples at various fold change (FD) and false-discovery rate (FDR) cutoffs (67 differentially expressed genes identified at a FDR of < 0.05 and FD of > 1.5) (B); FDs and FDR-corrected p-values of genes selected for qRT-PCR validation (C); N=4 per genotype.

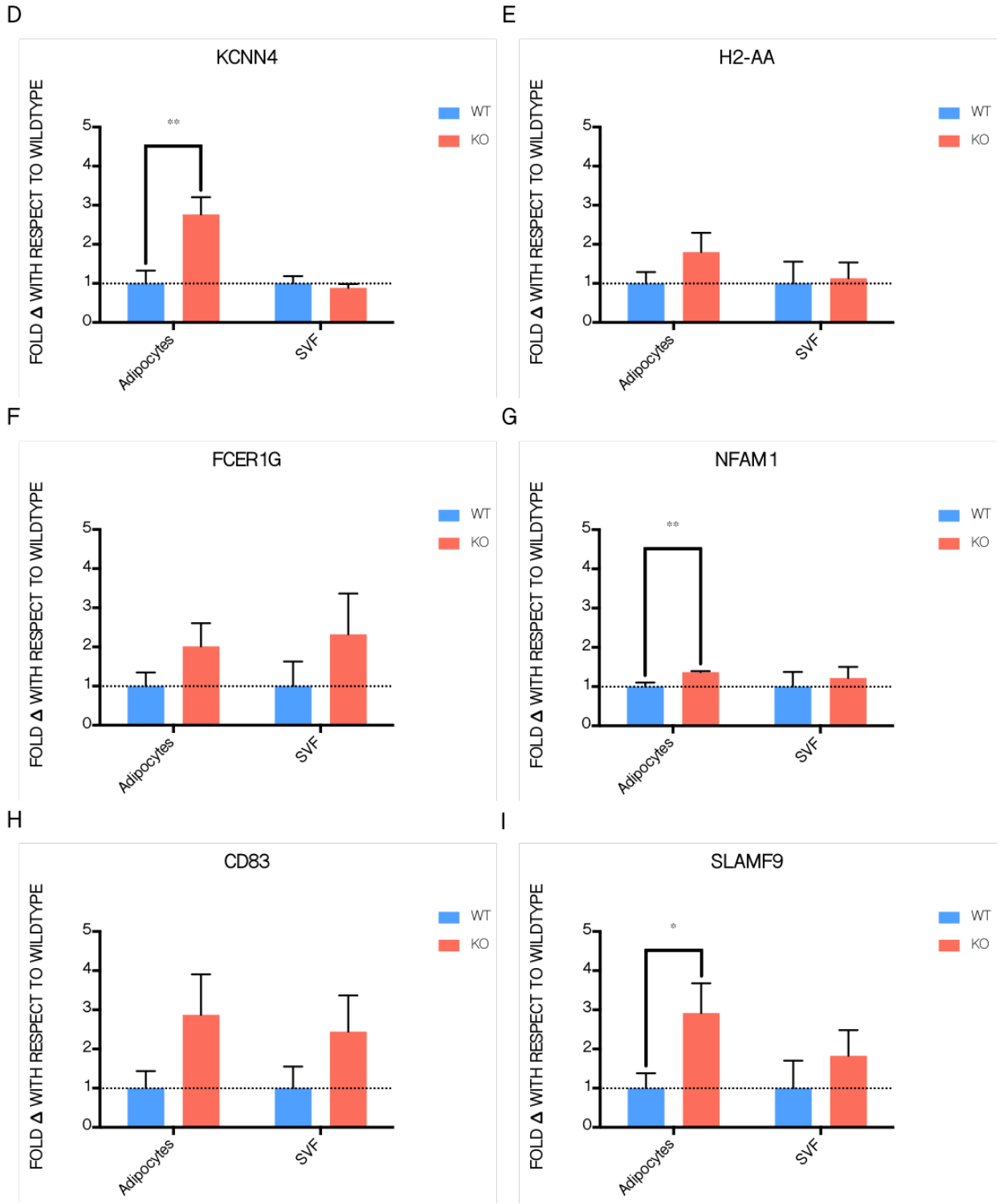


Figure 5 (continued) D-M: qRT-PCR validation of RNAseq via genes identified in table C, expressed as fold change with respect to wildtype for both isolated adipocytes and the associate stromal vascular fraction. N=3 per genotype per sample type (adipocyte vs SVF). Data are means ±SEM; *P < 0.1, **P < 0.05.

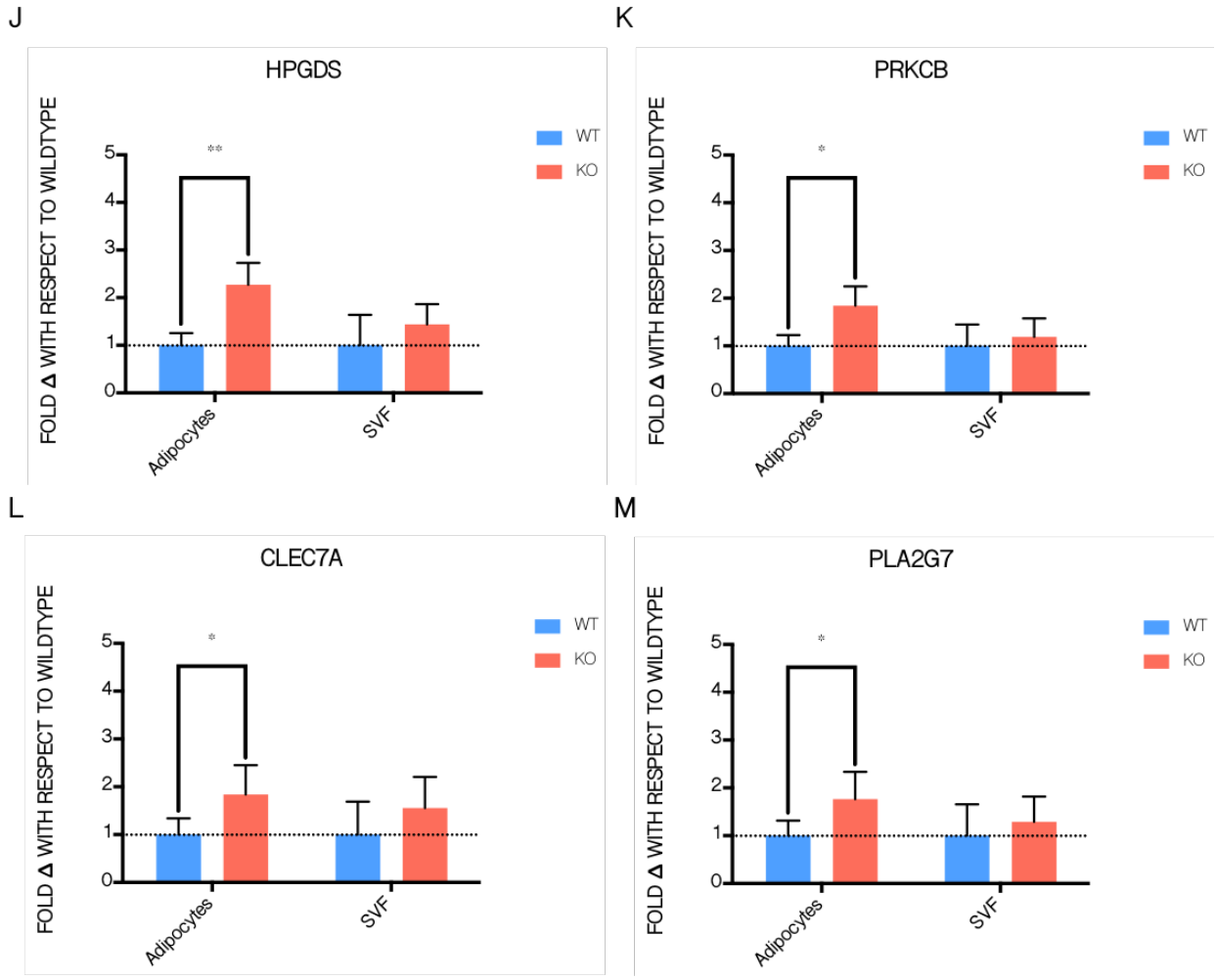


Figure 5 (continued) D-M: qRT-PCR validation of RNAseq via genes identified in table C, expressed as fold change with respect to wildtype for both isolated adipocytes and the associate stromal vascular fraction. N=3 per genotype per sample type (adipocyte vs SVF). Data are means ±SEM; *P < 0.1, **P < 0.05.

3.2.2: Increased Presence of Metabolically Activated Macrophages in adSMRT$^{-/-}$ Adipose Tissue

To better understand the status of the microenvironment, we conducted flow cytometry on the adipose tissue SVF in our mice. We selected two general markers for macrophage

identification (CD11b, F4/80) from which we gated for M1 (CD38, CD274) and M2 (CD36, CD206) macrophages. While CD11b and F4/80 markers were analyzed independently, cells would only be considered macrophages and interrogated for M1/M2 phenotypes if cells expressed both markers. In our adSMRT$^{-/-}$ samples, we found a significant increase in the proportion of F4/80 positive cells, while an increase cells expressing both CD11b and F4/80 trended towards significance. Meanwhile, wildtype expressed significantly more non-macrophage immune cells, indicated by the proportion of CD11b, F4/80 negative cells. From the CD11b+F4/80+ gate, a significant decrease in CD206 (M2) positive cells was observed (Fig. 6A-D).

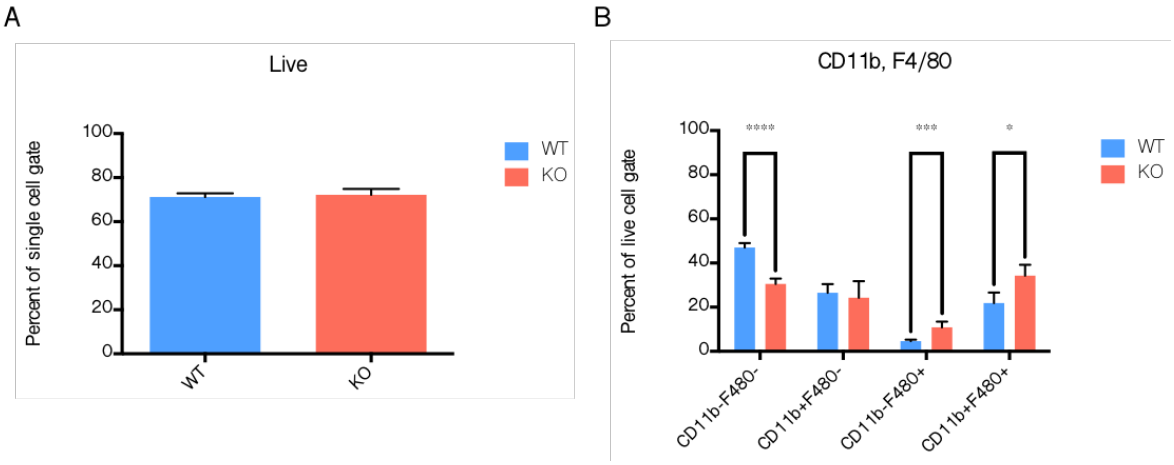


Figure 6: Flow Cytometry Suggests Decreased Anti-Inflammatory Activity in Adipose Tissue of adSMRT$^{-/-}$ Mice. A-D: flow cytometric data for isolated SVF, with all data presented as a percentage of cells from the previous gate (indicated on axes); only cells positive for both macrophage markers CD11b and F4/80 were considered when analyzing for M1/M2 phenotypes, and any samples that contained <65% live cells were excluded from analyses. N=5-8 per genotype. Data are means ±SEM; *P < 0.1, **P < 0.05, ***P < 0.005, ****P < 0.0005.

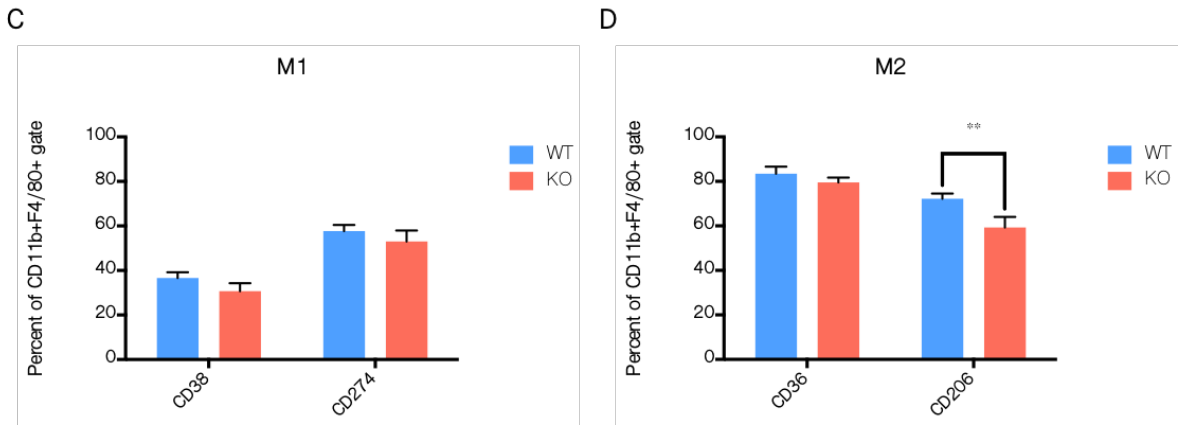


Figure 6 (continued): A-D: flow cytometric data for isolated SVF, with all data presented as a percentage of cells from the previous gate (indicated on axes); only cells positive for both macrophage markers CD11b and F4/80 were considered when analyzing for M1/M2 phenotypes, and any samples that contained <65% live cells were excluded from analyses. N=5-8 per genotype. Data are means ±SEM; *P < 0.1, **P < 0.05, ***P < 0.005, ****P < 0.0005.

From these data, we hypothesized that dysregulated adipocyte signaling was altering the dynamics of adipose tissue macrophage infiltration, so we conducted conditioned media experiments. Naïve macrophages were cultured in media conditioned from adipose tissue derived from wildtype and adSMRT$^{-/-}$ mice, and qRT-PCR was utilized to assess gene expression patterns of cultured macrophages using markers for M1 (Il-1, Tnfα), M2 (Arg1, Fizz1), and the mixed, metabolically activated MMe (Abca1, Plin2) macrophage – a macrophage phenotype which has been shown to proliferate in the presence of certain adipocyte secreted factors, e.g. palmitate[140].

For the M1 phenotype, adSMRT$^{-/-}$ demonstrated a striking, significant increase in the expression of marker Il-1, while the increase in Tnfα trended toward significance (Fig. 7A).

Further in support of an enflamed adipose tissue microenvironment, M2 marker Arg1 was significantly differentially underexpressed in adSMRT$^{-/-}$ samples (Fig. 7B). Finally, markers for MMe phenotypes Abca1 and Plin2 were both significantly and strongly upregulated in knockout samples (Fig. 7C). This strongly indicated that the microenvironment of our adSMRT$^{-/-}$ adipose tissue, informed by altered signaling/secretions of the adipocyte, encourage resident macrophages to take on a pro-inflammatory, M1/MMe character, both of which explain the local pro-inflammatory phenotype that is driving the overall deleterious metabolic outcome.

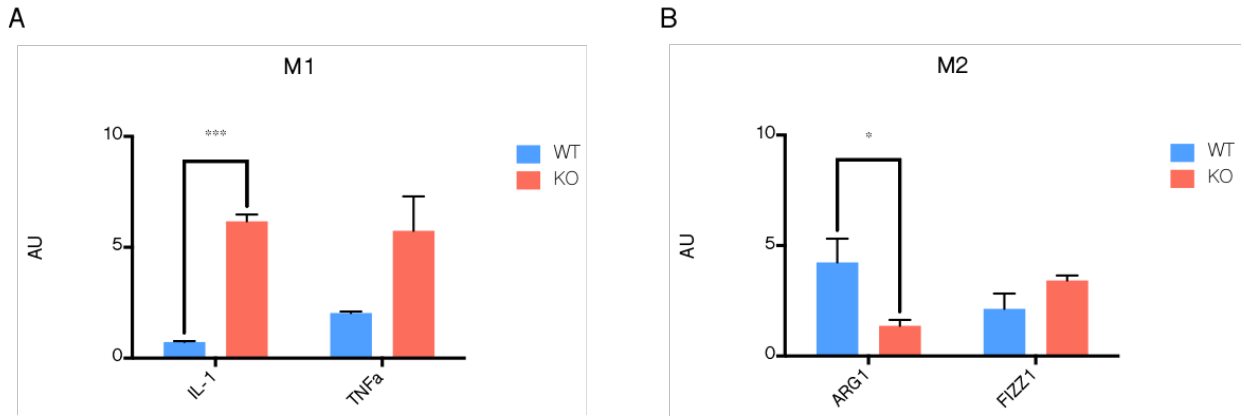


Figure 7: Adipose Tissue Microenvironment is Enriched for Metabolically Activated Macrophages in adSMRT$^{-/-}$ Mice. A-C: following incubation in media conditioned from wiltype and adSMRT$^{-/-}$ whole fat, naïve macrophages were analyzed via qRT-PCR for expression of M1 (A), M2 (B), and MMe (C) markers to determine whether alterations in the KO adipose tissue secretome influence macrophage differentiation. N=3 per genotype. Data are means ±SEM; *P < 0.05, **P < 0.005, ***P < 0.0005.

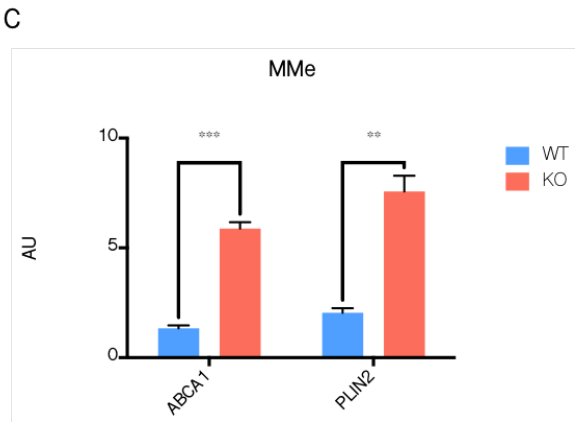


Figure 7 (continued): A-C: following incubation in media conditioned from wiltype and adSMRT$^{-/-}$ whole fat, naïve macrophages were analyzed via qRT-PCR for expression of M1 (A), M2 (B), and MMe (C) markers to determine whether alterations in the KO adipose tissue secretome influence macrophage differentiation. N=3 per genotype. Data are means ±SEM; *P < 0.05, **P < 0.005, ***P < 0.0005.

3.2.3: Other inflammatory trends

Given the results of the conditioned media studies, it is undeniable that SMRT is critical not only for proper metabolic function, but also for appropriate immune signaling via the adipocyte; loss of SMRT alters the adipocyte's secretome in such a way that metabolic and inflammatory signaling are disrupted in tandem. Because of the complex network of factors that regulate inflammatory processes, however, we wanted to be measure the potential contributions of well-established effectors of immunity, such as perturbations in gut microbiome communities, that may also be contributing to the metabolic aberrations discussed in Chapter II. To this end, we tested for differences in gut flora species and found that while mice fed a 45% HFD saw a strong shift in the composition of their gut flora from bacteroidetes to firmicutes

compared to their chow counterparts (the anticipated outcome following high-fat diet feeding), there were no genotype-dependent changes in the microbiome (Fig. 8A-D).

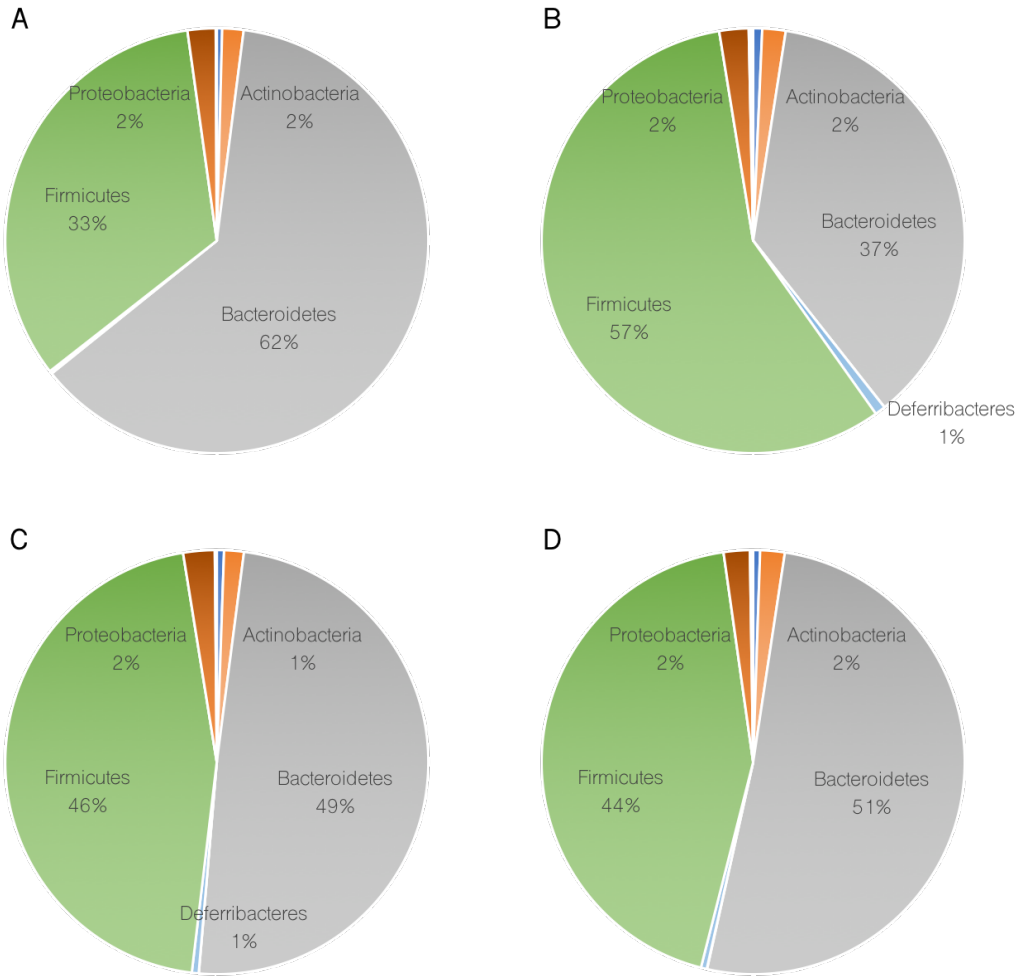


Figure 8: Alterations in Gut Flora are Dependent on Diet but not Genotype. A-D: following 12 weeks of either chow or 45% HFD feeding, phylum diversity of gut microbiota was compared along variables of either diet (A, B) or genotype (C, D). Charts A, C represent the initial composition of the microbiome (before 12 weeks of diet), whereas charts B, D represent the gut flora populations after 12 weeks of diet. Species that represent <1% of the total composition are not reported. N=12 per genotype or diet comparison.

Additionally, to begin identifying potential specific molecules that may be causal agents for the overall phenotype, we became interested in determining whether factors commonly associated with metabolic and inflammatory disease were up- or down-regulated in the serum of wildtype and adSMRT$^{-/-}$ mice fed a 45% HFD. While local inflammation reasonably explains much of the metabolic phenotype, alterations in serum proteins may have unexpected effects by acting distally on tissues peripheral to adipose depots. Because of technical limitations, 15 serum samples per genotype were pooled, yielding one representative sample per genotype; for this reason, these studies were not powered well enough to report a conclusive finding. However, we did observe trends that supported the adipose tissue phenotype, for example down regulation of FGF-21, a stimulator of glucose uptake in adipocytes, or upregulation of Lipocalin-2, BLC, or C5a, all of which promote pro-inflammatory recruitment of immune cells (Fig. 9A).

A

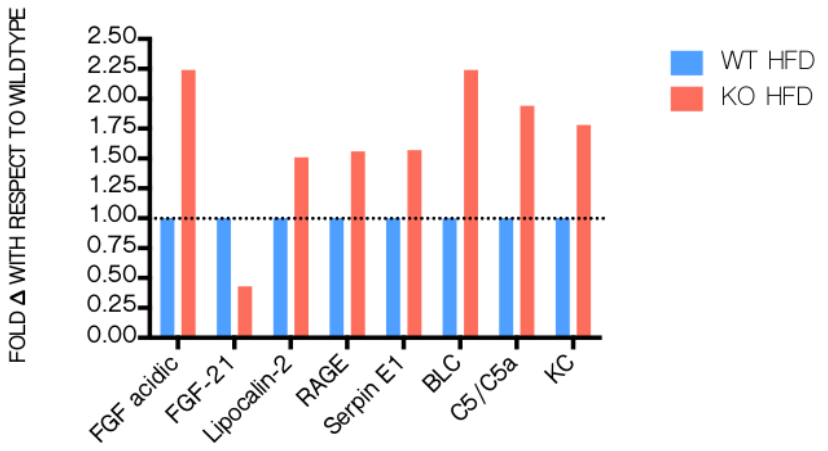


Figure 9: Trends in Serum Factors Support Increased Inflammatory Signaling in adSMRT$^{-/-}$ Mice. **A:** relative expression of select adipokines and chemokines (those showing >1.5-fold change) in blood serum, determined using commercially available antibody array kits from R&D Systems (ARY013/ARY006). Technically, N=1, since one pooled sample representing each genotype was used; however, both serum samples were generated by pooling 15 different biological replicates per genotype.

3.3: Discussion

Because the canonical paradigm of SMRT impacting metabolism through PPARγ/obesity did not hold for the adSMRT$^{-/-}$ mouse, we took an unbiased approach to define gene transcription changes in adipose tissue by performing RNAseq analysis. The data from this study revealed that genes involved in immunity (specifically, pro-inflammatory processes) were strongly upregulated in the adipose tissue of adSMRT$^{-/-}$ mice. Since we used whole adipose tissue, containing both adipocytes and resident immune cells, to generate our RNAseq data, we next confirmed our RNAseq results by interrogating the expression levels for a subset of RNAseq-identified genes in both adipocytes and the SVF fraction, separately.

While nearly all genes trended in the anticipated pro-inflammatory direction for both sample types, we found that the majority of genes that were significantly differentially expressed originated from adipocytes.

We next performed flow cytometry studies and showed alterations in M1:M2 macrophage ratios, though this was mainly driven by decreases in the expression of M2 macrophage marker CD206. We therefore focused on determining whether metabolically activated macrophages (MMe) could be responsible for driving the phenotype, as the influence of MMe macrophages have recently been shown to lead to detrimental metabolic phenotypes[61], much like those effects discussed in Chapter II. Ultimately, taking into consideration the "mixed" MMe macrophage, rather than focusing exclusively on the binary M1:M2 paradigm which developed as a way to explain the initiation and resolution of inflammation during classic infection, likely reveals a more accurate reflection of changes in the adipose tissue microenvironment. Through conditioned media experiments, we found that the secretome of our adSMRT$^{-/-}$ mice adipose tissue significantly stimulated MMe macrophage differentiation; qRT-PCR experiments confirmed that naïve macrophages, after being exposed to media conditioned by adSMRT$^{-/-}$ adipose tissue, strongly express MMe markers *Abca1* and *Plin2*, as well as M1 marker *Il-1*, in contrast to exposure from wildtype fat tissue media. Coates et al. have established that MMe macrophages play important roles in the pro-inflammatory signaling involved in adipocyte insulin resistance and clearance of FFAs from adipocytes[60]; thus, increased MMe macrophage infiltration, caused by changes in adipocyte

signaling via decreased *Smrt* expression likely explains the overall phenotype in our adSMRT$^{-/-}$ mice.

While we did not observe outwardly obvious physiological alterations between adSMRT$^{-/-}$ and wildtype mice (e.g. obesity), when considering the combination of metabolic and immune dysregulations, it is clear that SMRT functions as an integrator of both types of signaling via the adipocyte in order to achieve homeostasis. The net effect of SMRT loss appears to mimic the beginnings of diabetes, underscored by insulin resistance and inflammation. In fact, loss of SMRT impairs proper immune signaling in the adipocyte so much so, that it alters its secretome enough to change the adipose tissue microenvironment, evidenced by a significant increase in metabolically activated (MMe) resident macrophages. Because of the close relationship between metabolic dysregulation and pro-inflammatory signaling, the finding that SMRT directly influences immunological processes in tandem with metabolic ones offers a novel perspective on the role of nuclear corepressors in development of diseases like T2DM.

As mentioned in Chapter II, the results of from our adSMRT$^{-/-}$ mice are a complete departure from those described in the NCoR1$^{-/-}$ model utilized by Li et al. The amplification of inflammation in the adipose tissue of adSMRT$^{-/-}$ mice, in conjunction with increased MMe macrophage influence and no change in PPARγ target genes, suggests that the mechanism by which SMRT influences metabolism via the adipocyte is entirely different from its analog, NCoR1. In contrasting these models, the idea that NCoR1 and SMRT play distinct roles in the regulation of metabolic processes through the adipocyte is strongly supported. Specifically,

SMRT is able to impact metabolism through inflammatory processes by integrating metabolic and immune signaling in a way that, to our knowledge, has not been reported with other nuclear corepressors (e.g. NCoR1, RIP-140, SUN-CoR, Alien, Hairless)[87,142].

Future studies will aim to define the molecular events leading to altered adipocyte secretory function, and what these secreted factors are that lead to MMe macrophage adipose infiltration. One possibility is that SMRT deficiency results in altered lipolytic function/FFA secretion from adipocytes as a result of dysregulated immune signaling – since FFAs, particularly the lipid species palmitate, have been shown to activate MMe macrophages[60,61,143,144]. Determination of such a factor, though, is beyond the current scope of the manuscript. However, these data do suggest that SMRT is a key sensor in adipocytes that simultaneously regulate adipose tissue insulin sensitivity and macrophage infiltration.

Taken together, our data suggest that SMRT loss in the adipocyte induces dysregulation of genes involved in inflammatory processes, which in turn alters signaling to the microenvironment in such a way that stimulates resident macrophages in the adipose tissue of our mice to take on a MMe/M1 phenotype. This then promotes the development of a host of deleterious metabolic outcomes, including local insulin resistance and glucose intolerance, ultimately offering a previously unconsidered role for SMRT in the maintenance of metabolic homeostasis. In sum, our data indicate that SMRT functions as an integrator of metabolic and inflammatory signals to maintain physiological homeostasis.

3.4: Methods

3.4.1: Flow Cytometry

Fluorochrome labeled cells were analyzed according to the following workflow. Following analysis for cell size and granularity (FSC-A vs SSC-A), a gate for analysis of single cells was applied. Of this population of cells, a gate for live cells was selected (dertermined by calcein blue). From this subset, macrophages were identified by gating for cells that were both CD11b (557396, Abcam) and F4/80 (123117, Abcam) positive. For cells both CD11b and F4/80 positive (gate applied), M1 macrophages were identified via proinflammatory markers CD38 (562770, BD Biosciences) and CD274 (124313, BD Biosciences), while M2 macrophages were identified via antiinflammatory markers CD36 (562702, BD Biosciences) and CD206 (565250, BD Biosciences). Analyses were conducted using a Canto-II or LSRII flow cytometer (BD Biosciences) and data were analyzed using FlowJo software v.9.4.11.

3.4.2: Conditioned Media

Adipose tissue from wildtype and knockout mice were cultured separately in RPMI serum free media overnight to obtain conditioned media. Hematopoietic stem-cells were then obtained from the long bones of a young, wildtype male mouse, and plated with L-cell conditioned media for six days, followed treatment with adipose tissue-conditioned media at concentrations of 1:10, 1:100, 1:1000 for 24 hours. Macrophages were then collected and processed for RNA isolation and subsequent conversion to cDNA for qRT-PCR analysis to determine macrophage differentiation phenotype. Markers used for identifying M1-type cells

include Il-1b and Tnfα (Mm00434228_m1, Mm00443258_m1); markers used for identifying M2-type cells include Arg1 and Fizz1 (Mm00475988_m1); markers used for identifying metabolically activated MMe-type cells include Abca1 and Plin2 (Mm00442646_m1, Mm00475794_m1). All probes were obtained via Life Technologies, and qRT-PCR was conducted as previously described.

3.4.3: Gut Microbiome

Fecal samples were collected from mice at 8, 14, and 20 weeks of age. The harvesting method employed ensured that stool pellets were fresh, and did not come into contact with any materials other than the sterile tubes used for collection. All samples were stored at -80°C until DNA extraction. DNA was extracted from fecal samples using standard, published protocols (Wang et al., 2009). Samples were then sequenced by MiSeq at the Next Generation Sequencing Core in the Biosciences Division at Argonne National Laboratory. DNA sequences were analyzed by Quantitative Insights into Microbial Ecology (QIIME). Samples with less than 3,000 sequences were excluded from the analyses. Operational taxonomic units (OTUs) were picked at 97% sequence identity using the GreenGenes Database (2013). Analysis of similarities (ANOSIM) was performed using QIIME to examine the impacts of genotype, diet, and age on fecal microbiota. The number of permutations was 10,000 or the maximum number of permutations allowed by the data. Permutation test with 10,000 permutations was performed using R to compare the UniFrac distances of animals between cohorts.

Chapter IV: Considerations for Future Investigations of SMRT, T2DM

4.1: Uncovering the Complex Metabolic Role of Silencing Mediator of Retinoid and Thyroid Hormone Receptors: Future Directions

The findings detailed within highlight a previously unconsidered role for SMRT in the regulation of metabolism through its modulation of immunological pathways. While a link between SMRT and inflammation has been suggested in the past, this is the first time a direct connection has been demonstrated. The results of this investigation are particularly interesting, as the overall metabolic phenotype of the adSMRT$^{-/-}$ mouse does not align with those of generalized knockout models of SMRT, suggesting that the metabolic profile described by other groups interrogating SMRT was influenced in large part by loss of SMRT in metabolic tissues beyond the adipocyte. This is underscored by the fact that the mechanism driving the deleterious adSMRT$^{-/-}$ phenotype is likely due to increased presence of pro-inflammatory MMe macrophages in the adipose tissue of our mice, a strong departure from the traditional explanation of increased PPARγ activity cited by other groups. This signifies that the regulatory functions of SMRT through the adipocyte are unique, and influence systemic and local metabolic endpoints in a way that is not necessarily recapitulated in tissues like muscle, liver, or pancreas.

In the most concise terms, our findings can be summarized as follows: loss of SMRT in the adipocyte causes changes in the gene expression profile of adipose tissue, specifically

upregulation of pro-inflammatory pathways. This change in signaling originates primarily from adipocytes, which in turn alters the adipocyte secretome, subsequently stimulating metabolic activation of resident macrophages. An increase in MMe macrophage influence likely exacerbates inflammation in the adipose tissue microenvironment such that proper adipocyte insulin signaling is disrupted, causing adipocyte insulin resistance. This ultimately leads to worsened systemic glucose tolerance, along with a host of other metabolic dysregulations (e.g., changes in feeding behavior, apparent RER inflexibility, etc.), illustrating the distinct regulatory role SMRT plays through the adipocyte. While this story sufficiently explains our novel conclusions, there remain a number of questions that, once answered, will yield a more comprehensive picture of how SMRT contributes to the maintenance of metabolic homeostasis through the adipocyte.

First and foremost is the question of what factor, specifically, is driving the proliferation of the MMe phenotype in our model. Conditioned media experiments have demonstrated that secreted factors from adSMRT$^{-/-}$ adipose tissue trigger metabolic activation in naïve macrophages in a way that wildtype adipose tissue does not. While it is clear that adipocyte-specific loss of SMRT alters the gene expression profile amply enough to elicit a significant change in adipocyte secreted factors, we have not yet identified what these changes in the secretome are that enable the potentiation of the MMe macrophage phenotype. It has recently been shown that the lipid species palmitate is sufficient for the metabolic activation of macrophages; therefore, one way to address this gap in our mechanistic understanding would

be through lipidomics assays on the cultured media itself, to identify discrepancies in the abundance of specific lipid species between adSMRT$^{-/-}$ and wildtype. Should these studies reveal no changes in palmitate or other lipids in our conditioned media, a broader metabolomics analysis may be carried out, wherein key metabolic factors, beyond lipids, would be considered. These studies would provide, at the very least, a starting point in understanding how SMRT loss in the adipocyte ultimately leads to a secretome that favors MMe macrophage activation, and may even potentially identify new factors that promote said activation.

In a similar vein, studies investigating the mechanism further upstream would be apropos; as important for our mechanistic understanding as identifying the secreted factor(s) responsible for the metabolic activation of adipose tissue macrophages in our adSMRT$^{-/-}$ mice may be, so is characterizing the initial changes in gene expression that enable the secretome to be modified in the way it is. While our RNAseq experiments were useful in leading us to interrogate resident adipose tissue macrophage phenotypes by illustrating the involvement of SMRT in regulating pro-inflammatory pathways, RNAseq provides essentially no granularity with respect to understanding the cascade of events that led to this altered gene expression profile. Identifying the foremost changes in gene expression caused by SMRT loss will clarify what events take place that lead to an altered secretome. One way to address this would be through ChIP-seq studies. As mentioned in Chapter II, studies in 3T3-L1 cells that refute the paradigm of SMRT interaction with PPARγ during adipogenesis exemplify the lack of consensus regarding targets of SMRT *in vivo*, warranting a better understanding of the primary

axes through which SMRT exerts its regulatory function, specifically as it relates to regulation of immune pathways. A comprehensive ChIP-seq experiment, identifying regions of DNA (and thus target genes) SMRT associates to under various environmental conditions (e.g. chow- versus HFD-fed) will provide a stronger mechanistic explanation for how SMRT regulates inflammatory processes that feedback on metabolic endpoints.

Refocusing on the role of immune cells in the development of our adSMRT$^{-/-}$ metabolic profile—while we are confident that the adSMRT$^{-/-}$ adipose tissue secretome strongly informs macrophages to take on an MMe phenotype *ex vivo*, the applicability of our findings would be significantly bolstered by showing increased MMe macrophage presence *in vivo* as well. Because our conclusion that an altered, pro-inflammatory adipose tissue microenvironment (as a result of dysregulated adipocyte gene expression and secreted factors) is what drives the increase in MMe macrophages, it should follow that adSMRT$^{-/-}$ adipose tissue is enriched for MMe macrophages *in vivo*. This can be accomplished by flow cytometry on isolated adipose tissue macrophages; while we have already carried out these studies using markers for M1/M2 polarization, a relatively easy follow-up experiment would be to repeat the flow cytometry experiment using primary adipose tissue, including markers for the MMe phenotype.

Further, because our hypothesis that the development of adipocyte insulin resistance in adSMRT$^{-/-}$ mice is a result of inflammatory signaling from MMe macrophages, it would be valuable to conduct a study that confirms the source from which signaling disruptive to insulin action originates. The alternative to MMe macrophages being the primary contributor to insulin

resistance would be adipocytes themselves (signaling in an autocrine fashion), or, most likely, a combination of both. The most straightforward way to determine this would be to evaluate mRNA and protein expression levels for cytokines produced by both adipocytes and immune cells and that are classically associated with insulin resistance, like TNFα, IL-1β, and IL-6; specifically, this would be accomplished by qRT-PCR and Western blotting on isolated adipocytes and the associated stromal vascular fraction (containing resident macrophages).

While most outstanding questions related to this study are mechanistic, a few remain with respect to characterization of the phenotype. One issue with the study of SMRT is that it interacts with many different transcription factors, and does so more as a fine-tuner of target gene expression than a binary on-off switch. These two qualities inherent to SMRT make for a subtler phenotype, the nuance of which may very well be masked by the noise of idiosyncratic differences unique to each mouse, e.g. predisposition to stress, position in established social hierarchies, etc., or simply by the robustness of typical biological processes. This is especially true when SMRT is knocked out in a tissue specific fashion. Indeed, we were not able to identify any notable metabolic disparities between adSMRT$^{-/-}$ and wildtype mice fed a chow diet; only when challenged with a 45% HFD did the phenotype described begin to emerge. This begs the question of how the metabolic profile of adSMRT$^{-/-}$ mice would be different if challenged even further by a 60% HFD, or if the timeline for HFD feeding lasted 16 or 20 weeks, instead of 12 weeks. A 2017 study from the Becker lab has already demonstrated the temporal nature of macrophage phenotypes as a function of disease state progression in diet

induced obesity—consequently, it would be valuable to assess how the adSMRT$^{-/-}$ phenotype would be changed or exacerbated if the metabolic challenge was heightened. As an addendum to the concept of temporality influencing our phenotype, it is worth noting that adiponectin, the promoter of which drives Cre expression and thus the genotype in adSMRT$^{-/-}$ mice, is not expressed until late adipogenesis. This means that the effect of SMRT loss is obscured until adipocytes are mature enough to express adiponectin, which could have nullifying effects regarding the magnitude of our phenotype. Future studies could overcome this by generating conditional knockouts using newer gene-editing technologies, such as CRIPSR.

Throughout this investigation, the results of most experiments have supported the overarching paradigm of adipocyte-specific ablation of SMRT having a metabolically deleterious effect vis-à-vis inflammation. However, what has not yet been discussed is the confounding result of the insulin tolerance tests reviewed in Chapter II. The reason the outcome of the ITT, which showed improved systemic insulin sensitivity in adSMRT$^{-/-}$ mice, is confusing is because worsened glucose tolerance is typically a downstream effect of insulin resistance. While we did observe local adipocyte insulin resistance, systemic insulin sensitization should outweigh this effect with respect to glucose tolerance, since most sugar is deposited at skeletal muscle. One possible reason for the improvement of systemic insulin sensitivity in the face of glucose intolerance could be because of a defect at the level of insulin production/granule release in the pancreas, wherein chronically reduced circulating insulin concentrations cause insulin sensitization in peripheral tissues (except in adipose tissue, where inflammatory signaling

persists). If SMRT oversees cross-talk between adipocytes and the pancreas, then it is plausible that adipocyte-specific loss of SMRT could impair beta cell function. While studies aiming to establish SMRT-mediated links between the adipocyte and pancreas are beyond the scope of this project, one may begin answering this question by conducting immunohistochemical analyses on pancreatic cross sections, staining for indicators of beta cell health. Additionally, blood may be collected during GTTs for downstream analysis of insulin levels via ELISA; this was attempted initially, but due to technical limitations, was underpowered and should be repeated.

While there are many other interesting directions that could be taken with this investigation, one final consideration as it relates to this project specifically is that of lipolysis. If the MMe macrophage phenotype observed arises due to increased FFA release, the mechanism for this is most likely increased lipolytic activity; this would not only support the spike in metabolic activation of resident macrophages, but would also corroborate adipocyte insulin resistance, which is a primary driver of lipolysis. This could be studied by carrying out a glycerol assay (the cleavage of the glycerol backbone from the triglyceride acting as a proxy for lipolysis), wherein glycerol levels in adipocytes are determined using a commercially available kit under conditions of no treatment, treatment with isoproterenol, treatment with insulin, and treatment with both.

4.2: Other Considerations for Investigations of SMRT

There are a number of future directions for the study of SMRT not specific to this project that would forward our understanding in the field of nuclear corepressors. When knocking out SMRT in adipocytes, we find that the adSMRT$^{-/-}$ mouse not only fails to phenocopy generalized knockout models, but even directly opposes them for a number of metabolic parameters. This indicates that the regulatory function of SMRT in the adipocyte is distinct from that in other metabolic tissues; however, the question of how SMRT mediates the generally protective phenotype observed in global knockouts remains. Since the adSMRT$^{-/-}$ model is the first time SMRT has been studied in a tissue-specific fashion, there is still a need to further characterize the metabolic function of SMRT in other tissues like muscle, liver, and pancreas. Therefore, one interesting avenue to pursue would be to generate other tissue-specific knockouts of SMRT, aiming to more fully parse its specific regulatory roles. This could be extended beyond metabolic tissues to macrophages, which also express SMRT. Considering the direct involvement of SMRT in the regulation of immune pathways in the adipocyte, it would be particularly illuminating to determine how SMRT maintains homeostasis through immune cells. By reiterating the experiments carried out in the study of adSMRT$^{-/-}$ mice in other tissue-specific models and contrasting the results with those described earlier, we may attain a more holistic understanding of the specific yet integrative roles of SMRT in various tissues that allow for coherent systemic regulation of homeostasis.

As mentioned in Chapter I, SMRT undergoes alternative splicing, giving rise to a number of isoforms that are optimized for specific needs of the organism (tissue type, developmental stage, and environmental cues all play a role in the generation of specific SMRT isoforms). However, beyond confirming that these isoforms exist, and that they have varying substrate affinities, we know very little about the downstream functional impact of these alternatively spliced forms of SMRT. Because SMRT has never been studied in an isoform-specific fashion, determining the regulatory differences between each will shed much light onto how SMRT maintains metabolic homeostasis. In fact, interrogating SMRT isoforms would strongly compliment the conclusions reached from future tissue-specific knockout models, as phenotypic differences between each model may be, at least in part, explained by abundance of specific SMRT isoforms for a given tissue. This could be addressed by use of small interfering RNA (siRNA); siRNAs trigger endonucleolytic cleavage of mRNA in a strict sequence-specific fashion to post-transcriptionally silence gene expression, and may be used to target the isoforms of SMRT by exploiting minor nucleotide differences[145].

One technical consideration for the future study of SMRT is that of access to consistent and specific antibodies. While a number of highly-reviewed SMRT antibodies are commercially available for certain species (e.g., human), none exist for mouse, which is the primary modality for the study of NCoR1/SMRT. While this obstacle may seem trivial, it presents unique challenges in establishing validity of the genetic model, as gold-standard techniques for the determination of protein expression, like Western blots, cannot be leveraged. This forces

researchers to resort to second line options of model confirmation, like qRT-PCR, which may not estimate the level of protein expression with full accuracy. Additionally, other experiments that rely on the SMRT protein itself, such as co-immunoprecipitations, must also be foregone as these depend on the availability of a high-quality, SMRT antibody. Therefore, it would be valuable to the field if a dedicated effort was made with respect to the generation of effective antibodies in animals most frequently used for these kinds of studies.

4.3: Optimizing Efforts in Addressing the T2DM Pandemic

The value of academic research cannot be understated as it relates to the advancement of our fundamental understanding of modern diseases. Without basic science to define the molecular networks through which signaling aberrancies can propagate, we would not have the foundation that enables the development of treatments for complex diseases like T2DM. It is this same kind of investigation that allowed Donald Steiner to discover proinsulin in the 1960s, which not only propelled the field of endocrinology forward through our new understanding of hormone production and protein precursors in general, but also had a profound applied impact by enabling pharmaceutical companies to manufacture more effective forms of insulin for diabetics. However, as mentioned at the beginning of this manuscript, the state of T2DM care (at least from a pharmaceutical perspective) has plateaued at "management", when it should be evolving towards "cure". While this does not imply that funding should be decreased in the area of T2DM research, it would be valuable to consider whether the allocation of new funding towards prevention and education would be more

efficient in reducing the prevalence and financial burden of T2DM. Which has a greater beneficial impact: a dollar toward research or a dollar towards prevention?

This should be a priority question for policy makers. The requirement for basic metabolic research will always remain: monogenic diabetes and the noxious environments of those who are consistently forced to make poor lifestyle choices are not elements that can be reasonably addressed in the near future. There will be large populations belonging to either category for some time, and these individuals will continue to rely on the advancements made in modern medicine, enabled by basic science research. However, for those who are in socioeconomic positions to access resources for healthier lifestyles but choose not to, perhaps funding for programs that aim to rework pervasive factors that play into our decision making (culture, mental health, etc.) will be more effective. After all, a very common comorbidity of obesity/T2DM is depression[146], which robs patients of their motivation to comply with treatment plans. As far as the development of T2DM is concerned, an ounce of prevention is worth a pound of cure; therefore, in addition to dedicating funding towards basic science research that will forward our molecular understanding of T2DM (much like the study of SMRT detailed within), a more concrete effort should be made in prioritizing education of youth and their families on the impact of unhealthy living.

References

1. Centers for Disease Control and Prevention. (2017) Long-term Trends in Diabetes. https://www.cdc.gov/diabetes/statistics/slides/long_term_trends.pdf.

2. Centers for Disease Control and Prevention. (2017) Diabetes Report Card 2017. https://www.cdc.gov/diabetes/pdfs/library/diabetesreportcard2017-508.pdf.

3. United States Census Bureau. (2019) US Population by Year. https://www.census.gov/data/tables.html.

4. Centers for Disease Control and Prevention. (2017) National Diabetes Statistics Report, 2017. https://www.cdc.gov/diabetes/pdfs/data/statistics/national-diabetes-statistics-report.pdf.

5. Centers for Disease Control and Prevention. (2016) National Vital Statistics Reports: Deaths: Leading Causes for 2016. https://www.cdc.gov/nchs/data/nvsr/nvsr67/nvsr67_06.pdf.

6. American Diabetes Association. (2017) Fast Facts - Data and Statistics About Diabetes. https://professional.diabetes.org/content/fast-facts-data-and-statistics-about-diabetes.

7. American Diabetes Association. (2018) Economic Costs of Diabetes in the U.S. in 2017. Diabetes Care, 41(5), 917–928. doi:10.2337/dci18-0007.

8. American Diabetes Association. (2018) American Diabetes Association Releases "Economic Costs of Diabetes in the U.S." Report at Annual Call to Congress Event Urging Legislators to Make Diabetes a National Priority [Press release]. http://www.diabetes.org/newsroom/press-releases/2018/economic-cost-study-call-to-congress-2018.html.

9. World Health Organization. (2016) Global Report on Diabetes. https://apps.who.int/iris/bitstream/handle/10665/204871/9789241565257_eng.pdf?sequence=1.

10. NCD Risk Factor Collaboration (NCD-RisC). (2016) Worldwide trends in diabetes since 1980: a pooled analysis of 751 population-based studies with 4.4 million participants. Lancet, 387(10027), 1513–1530. doi:10.1016/S0140-6736(16)00618-8.

11. Cho, Y. M. (2014) A gut feeling to cure diabetes: potential mechanisms of diabetes remission after bariatric surgery. Diabetes & Metabolism Journal, 38(6), 406–415. doi:10.4093/dmj.2014.38.6.406.

12. Plaisance, V., Waeber, G., Regazzi, R., Abderrahmani, A. (2014) Role of microRNAs in islet beta-cell compensation and failure during diabetes. Journal of Diabetes Research, 2014, 618652. doi:10.1155/2014/618652.

13. Fonseca, V. A. (2009) Defining and characterizing the progression of type 2 diabetes. Diabetes Care, 32 Suppl 2, S151–S156. doi:10.2337/dc09-S301.

14. McDonnell, M. E., Umpierrez, G. E. (2012) Insulin therapy for the management of hyperglycemia in hospitalized patients. Endocrinology and Metabolism Clinics of North America, 41(1), 175–201. doi:10.1016/j.ecl.2012.01.001.

15. American Diabetes Association. (2019) Complications. http://www.diabetes.org/living-with-diabetes/complications/.

16. Barnes, A. S. (2011) The epidemic of obesity and diabetes: trends and treatments. Texas Heart Institute Journal, 38(2), 142–144.

17. de Luca, C., Olefsky, J. M. (2008) Inflammation and insulin resistance. FEBS Letters, 582(1), 97–105. doi:10.1016/j.febslet.2007.11.057.

18. Shoelson, S. E., Lee, J., Goldfine, A. B. (2006) Inflammation and insulin resistance. The Journal of Clinical Investigation, 116(7), 1793–1801. doi:10.1172/JCI29069.

19. Lebovitz, H. E. (2011) Insulin: potential negative consequences of early routine use in patients with type 2 diabetes. Diabetes Care, 34 Suppl 2, S225–S230. doi:10.2337/dc11-s225.

20. Jensen, J., Rustad, P. I., Kolnes, A. J., Lai, Y. C. (2011) The role of skeletal muscle glycogen breakdown for regulation of insulin sensitivity by exercise. Frontiers in Physiology, 2, 112. doi:10.3389/fphys.2011.00112.

21. Brady, M. J., Saltiel, A. R. (1999) Closing in on the cause of insulin resistance and type 2 diabetes. The Journal of Clinical Investigation, 104(6), 675–676. doi:10.1172/JCI8216.

22. Long, Y. C., Zierath, J. R. (2006) AMP-activated protein kinase signaling in metabolic regulation. The Journal of Clinical Knvestigation, 116(7), 1776–1783. doi:10.1172/JCI29044.

23. Roh, E., Kim, M. S. (2016) Brain Regulation of Energy Metabolism. Endocrinology and Metabolism (Seoul, Korea), 31(4), 519–524. doi:10.3803/EnM.2016.31.4.519.

24. Levin, B. E., Routh, V. H. (1996) Role of the brain in energy balance and obesity. American Journal of Physiology, 271, R491–500. doi:10.1152/ajpregu.1996.271.3.R491.

25. Coelho, M., Oliveira, T., Fernandes, R. (2013) Biochemistry of adipose tissue: an endocrine organ. Archives of Medical Science, 9(2), 191–200. doi:10.5114/aoms.2013.33181.

26. Kershaw, E. E., Flier, J. S. (2004) Adipose Tissue as an Endocrine Organ. The Journal of Clinical Endocrinology & Metabolism, 89(6), 2548–2556. doi:10.1210/jc.2004-0395.

27. Ahima, R. S., Flier, J. S. (2000) Adipose Tissue as an Endocrine Organ. Trends in Endocrinology and Metabolism, 11(8), 327-332. doi:10.1016/S1043-2760(00)00301-5.

28. Vazquez-Vela, M. E. F., Torres, N., Tovar, A. R. (2008) White Adipose Tissue as Endocrine Organ and Its Role in Obesity. Archives of Medical Research, 39(8), 715-728. doi:10.1016/j.arcmed.2008.09.005.

29. Ravussin, E., Galgani, J. E. (2011) The implication of brown adipose tissue for humans. Annual Review of Nutrition, 31, 33–47. doi:10.1146/annurev-nutr-072610-145209.

30. Matthias, A., Ohlson, K. B., Fredriksson, J. M., Jacobsson, A., Nedergaard, J., Cannon, B. (2000) Thermogenic responses in brown fat cells are fully UCP1-dependent; UCP2 or UCP3 do not substitute for UCP1 in adrenergically or fatty acid-induced thermogenesis. Journal of Biological Chemistry, 275(33), 25073–25081. doi:10.1074/jbc.M000547200.

31. Gnacinska, M., Malgorzewicz, S., Stojek, M., Lysiak-Szydlowska, W., Sworczak, K. (2009) Role of adipokines in complications related to obesity: a review. Advances in Medical Sciences, 54(2), 150–157. doi:10.2478/v10039-009-0035-2.

32. Kelesidis, T., Kelesidis, I., Chou, S., Mantzoros, C. S. (2010) Narrative review: the role of leptin in human physiology: emerging clinical applications. Annals of Internal Medicine, 152(2), 93–100. doi:10.7326/0003-4819-152-2-201001190-00008.

33. Achari, A. E., Jain, S. K. (2017) Adiponectin, a Therapeutic Target for Obesity, Diabetes, and Endothelial Dysfunction. International Journal of Molecular Sciences, 18(6), 1321. doi:10.3390/ijms18061321.

34. Fu, Y., Luo, N., Klein, R. L., Garvey, W. T. (2005) Adiponectin promotes adipocyte differentiation, insulin sensitivity, and lipid accumulation. Journal of Lipid Research, 46(7), 1369–1379. doi:10.1194/jlr.M400373-JLR200.

35. Kanda, H., Tateya, S., Tamori, Y., Kotani, K., Hiasa, K., Kitazawa, R., ... Kasuga, M. (2006) MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. The Journal of Clinical Investigation, 116(6), 1494–1505. doi:10.1172/JCI26498.

36. Lauterbach, M. A., Wunderlich, F. T. (2017) Macrophage function in obesity-induced inflammation and insulin resistance. European Journal of Physiology, 469(3-4), 385–396. doi:10.1007/s00424-017-1955-5.

37. Thomas, D., Apovian, C. (2017) Macrophage functions in lean and obese adipose tissue. Metabolism: Clinical and Experimental, 72, 120–143. doi:10.1016/j.metabol.2017.04.005.

38. Ibrahim, M. M. (2010) Subcutaneous and visceral adipose tissue: structural and functional differences. Obesity Reviews, 11(1), 11-18. doi:10.1111/j.1467-789X.2009.00623.x.

39. Wajchenberg, B. L. (2000) Subcutaneous and Visceral Adipose Tissue: Their Relation to the Metabolic Syndrome. Endocrine Reviews, 21(6), 697–738. doi:10.1210/edrv.21.6.0415.

40. Parlee, S. D., Lentz, S. I., Mori, H., MacDougald, O. A. (2014) Quantifying size and number of adipocytes in adipose tissue. Methods in Enzymology, 537, 93–122. doi:10.1016/B978-0-12-411619-1.00006-9.

41. Fontana, L., Eagon, J. C., Trujillo, M. E., Scherer, P. E., Klein, S. (2007) Visceral Fat Adipokine Secretion Is Associated With Systemic Inflammation in Obese Humans. Diabetes, 56(4), 1010-1013. doi: 10.2337/db06-1656.

42. Verboven, K., Wouters, K., Gaens, K., Hansen, D., Bijnen, M., Wetzels, S., ... Jocken, J. W. (2018) Abdominal subcutaneous and visceral adipocyte size, lipolysis and inflammation relate to insulin resistance in male obese humans. Nature: Scientific Reports, 8(1), 4677. doi:10.1038/s41598-018-22962-x.

43. Alvehus, M., Burén, J., Sjöström, M., Goedecke, J., Olsson, T. (2010) The human visceral fat depot has a unique inflammatory profile. Obesity, 18, 879-883. doi: 10.1038/oby.2010.22.

44. Kosteli, A., Sugaru, E., Haemmerle, G., Martin, J. F., Lei, J., Zechner, R., Ferrante, A. W. (2010) Weight loss and lipolysis promote a dynamic immune response in murine adipose tissue. The Journal of Clinical Investigation, 120(10), 3466–3479. doi:10.1172/JCI42845.

45. Castoldi, A., Naffah de Souza, C., Câmara, N. O., Moraes-Vieira, P. M. (2016) The Macrophage Switch in Obesity Development. Frontiers in Immunology, 6, 637. doi:10.3389/fimmu.2015.00637.

46. Ashley, N. T., Weil, Z. M., Nelson, R. J. (2012) Inflammation: Mechanisms, Costs, and Natural Variation. Annual Review of Ecology, Evolution, and Systematics, 43, 385-406. doi: 10.1146/annurev-ecolsys-040212-092530.

47. Chen, L., Deng, H., Cui, H., Fang, J., Zuo, Z., Deng, J., ... Zhao, L. (2017) Inflammatory responses and inflammation-associated diseases in organs. Oncotarget, 9(6), 7204–7218. doi:10.18632/oncotarget.23208.

48. Medzhitov, R. (2008) Origin and physiological roles of inflammation. Nature, 454(7203), 428-435. doi: 10.1038/nature07201.

49. Freire, M. O., Van Dyke, T. E. (2013) Natural resolution of inflammation. Periodontology, 2000, 63(1), 149–164. doi:10.1111/prd.12034.

50. Shen, H., Kreisel, D., Goldstein, D. R. (2013) Processes of sterile inflammation. Journal of Immunology, 191(6), 2857–2863. doi:10.4049/jimmunol.1301539.

51. Kawahito, S., Kitahata, H., Oshita, S. (2009) Problems associated with glucose toxicity: role of hyperglycemia-induced oxidative stress. World Journal of Gastroenterology, 15(33), 4137–4142. doi:10.3748/wjg.15.4137.

52. Forrester, S. J., Kikuchi, D. S., Hernandes, M. S., Xu, Q., Griendling, K. K. (2018) Reactive Oxygen Species in Metabolic and Inflammatory Signaling. Circulation Research, 122(6), 877–902. doi:10.1161/CIRCRESAHA.117.311401.

53. Mittal, M., Siddiqui, M. R., Tran, K., Reddy, S. P., Malik, A. B. (2014) Reactive oxygen species in inflammation and tissue injury. Antioxidants & Redox signaling, 20(7), 1126–1167. doi:10.1089/ars.2012.5149.

54. Cawthorn, W. P., Sethi, J. K. (2008) TNF-alpha and adipocyte biology. FEBS Letters, 582(1), 117–131. doi:10.1016/j.febslet.2007.11.051.

55. Hotamisligil, G. S. (1999) The role of TNFα and TNF receptors in obesity and insulin resistance. Journal of Internal Medicine, 245, 621-625. doi:10.1046/j.1365-2796.1999.00490.x.

56. Khan, S., Khan, S. (2014) ER stress in adipocytes and insulin resistance: Mechanisms and significance (Review). Molecular Medicine Reports, 10, 2234-2240. https://doi.org/10.3892/mmr.2014.2532.

57. Cinti, S., Mitchell, G., Barbatelli, G., Murano, I., Ceresi, E., Faloia, E., Wang, S., Fortier, M., Greenberg, A. S., Obin, M. S. (2005) Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans. Journal of Lipid Research, 46(11), 2347–2355. doi:10.1194/jlr.M500294-JLR200.

58. Murano, I., Barbatelli, G., Parisani, V., Latini, C., Muzzonigro, G., Castellucci, M., Cinti, S. (2008) Dead adipocytes, detected as crown-like structures, are prevalent in visceral fat depots of genetically obese mice. Journal of Lipid Research, 49, 1562–1568. doi: 10.1194/jlr.M800019-JLR200.

59. Boutens, L., Stienstra, R. (2016) Adipose tissue macrophages: going off track during obesity. Diabetologia, 59(5), 879–894. doi:10.1007/s00125-016-3904-9.

60. Coats, B. R., Schoenfelt, K. Q., Barbosa-Lorenzi, V. C., Peris, E., Cui, C., Hoffman, A., ... Becker, L. (2017) Metabolically Activated Adipose Tissue Macrophages Perform

Detrimental and Beneficial Functions during Diet-Induced Obesity. Cell Reports, 20(13), 3149–3161. doi:10.1016/j.celrep.2017.08.096.

61. Kratz, M., Coats, B. R., Hisert, K. B., Hagman, D., Mutskov, V., Peris, E., ... Becker, L. (2014). Metabolic dysfunction drives a mechanistically distinct proinflammatory phenotype in adipose tissue macrophages. Cell Metabolism, 20(4), 614–625. doi:10.1016/j.cmet.2014.08.010.

62. Meng, F., Lowell, C. A. (1997) Lipopolysaccharide (LPS)-induced macrophage activation and signal transduction in the absence of Src-family kinases Hck, Fgr, and Lyn. The Journal of Experimental Medicine, 185(9), 1661–1670. doi:10.1084/jem.185.9.1661.

63. Wang, N., Liang, H., & Zen, K. (2014). Molecular mechanisms that influence the macrophage m1-m2 polarization balance. Frontiers in Immunology, 5, 614. doi:10.3389/fimmu.2014.00614.

64. Riera-Borrull, M., Cuevas, V. D., Alonso, B., Vega, M. A., Joven, J., Izquierdo, E., Corbi, A. L. (2017) Palmitate Conditions Macrophages for Enhanced Responses toward Inflammatory Stimuli via JNK Activation. Journal of Immunology,199(11), 3858–3869. doi: 10.4049/jimmunol.1700845.

65. American Diabetes Association. (2013) Standards of medical care in diabetes—2013. Diabetes Care, 36 Suppl 1, S11–S66. doi:10.2337/dc13-S011.

66. Fowler, M. J. (2007) Diabetes treatment, Part 2: oral agents for glycaemic management. Clinical Diabetes, 25(4), 131–134. 10.2337/diaclin.25.4.131.

67. Lebovitz, H. E. (2011) Insulin: potential negative consequences of early routine use in patients with type 2 diabetes. Diabetes Care, 34 Suppl 2, S225–S230. doi:10.2337/dc11-s225.

68. Viollet, B., Guigas, B., Sanz Garcia, N., Leclerc, J., Foretz, M., Andreelli, F. (2012) Cellular and molecular mechanisms of metformin: an overview. Clinical Science (London, England), 122(6), 253–270. doi:10.1042/CS20110386.

69. Aquilante, C. L. (2010) Sulfonylurea pharmacogenomics in Type 2 diabetes: the influence of drug target and diabetes risk polymorphisms. Expert Review of Cardiovascular Therapy, 8(3), 359–372. doi:10.1586/erc.09.154.

70. van de Laar, F. A. (2008) Alpha-glucosidase inhibitors in the early treatment of type 2 diabetes. Vascular Health and Risk Management, 4(6), 1189–1195. doi:10.2147/VHRM.S3119.

71. Khan, M., Ouyang, J., Perkins, K., Nair, S., Joseph, F. (2015) Determining predictors of early response to exenatide in patients with type 2 diabetes mellitus. Journal of Diabetes Research, 162718. doi:10.1155/2015/162718.

72. Hsia, D. S., Grove, O., Cefalu, W. T. (2017) An update on sodium-glucose co-transporter-2 inhibitors for the treatment of diabetes mellitus. Current Opinion in Endocrinology, Diabetes, and Obesity, 24(1), 73–79. doi:10.1097/MED.0000000000000311.

73. Soccio, R. E., Chen, E. R., Lazar, M. A. (2014) Thiazolidinediones and the promise of insulin sensitization in type 2 diabetes. Cell Metabolism, 20(4), 573–591. doi:10.1016/j.cmet.2014.08.005.

74. Della-Morte, D., Palmirotta, R., Rehni, A. K., Pastore, D., Capuani, B., Pacifici, F., … Lauro, D. (2014) Pharmacogenomics and pharmacogenetics of thiazolidinediones: role in diabetes and cardiovascular risk factors. Pharmacogenomics, 15(16), 2063–2082. doi:10.2217/pgs.14.162.

75. Usuda, D., Kanda, T. (2014) Peroxisome proliferator-activated receptors for hypertension. World Journal of Cardiology, 6(8), 744–754. doi:10.4330/wjc.v6.i8.744.

76. Mansour, M. (2014) The roles of peroxisome proliferator-activated receptors in the metabolic syndrome. Progress in Molecular Biology and Translational Science, 121, 217-266. doi:10.1016/B978-0-12-800101-1.00007-7.

77. Martin, H. (2010) Role of PPAR-gamma in inflammation. Prospects for therapeutic intervention by food components. Mutation Research, 690, 57-63. doi: 10.1016/j.mrfmmm.2009.09.009.

78. Zhang, Y., Zhan, Chen, J. Q., Gao, Y., Chen, L., Kong, Y., Zhong, X. J., Liu, M. Q., Chu, J. J., Yan, G. Q., Li, T., He, M., Huang, Q. R. (2015) Pharmacological activation of PPARgamma ameliorates vascular endothelial insulin resistance via a non-canonical PPARgamma-dependent nuclear factor-kappa B trans-repression pathway. European Journal of Pharmacology, 754, 41-51. doi:10.1016/j.ejphar.2015.02.004.

79. Fu, Z., Gilbert, E. R., Liu, D. (2013) Regulation of insulin synthesis and secretion and pancreatic Beta-cell dysfunction in diabetes. Current Diabetes Reviews, 9(1), 25–53.

80. Simmons, K. M., Michels, A. W. (2015) Type 1 diabetes: A predictable disease. World Journal of Diabetes, 6(3), 380–390. doi:10.4239/wjd.v6.i3.380.

81. Kieffer, T. J., Woltjen, K., Osafune, K., Yabe, D., Inagaki, N. (2017) Beta-cell replacement strategies for diabetes. Journal of Diabetes Investigation, 9(3), 457–463. doi:10.1111/jdi.12758.

82. Butler, A. E., Janson, J., Bonner-Weir, S., Ritzel, R., Rizza, R. A., Butler, P. C. (2003) β-Cell Deficit and Increased β-Cell Apoptosis in Humans With Type 2 Diabetes. Diabetes, 52(1), 102-110. doi:10.2337/diabetes.52.1.102.

83. Chen, C., Cohrs, C. M., Stertmann, J., Bozsak, R., Speier, S. (2017) Human beta cell mass and function in diabetes: Recent advances in knowledge and technologies to understand disease pathogenesis. Molecular Metabolism, 6(9), 943–957. doi:10.1016/j.molmet.2017.06.019.

84. Leahy, J. L., Hirsch, I. B., Peterson, K. A., Schneider, D. (2010) Targeting β-cell function early in the course of therapy for type 2 diabetes mellitus. Journal of Clinical Endocrinology and Metabolism, 95, 4206-4216.

85. Leahy, J. L. (2005) Pathogenesis of type 2 diabetes mellitus. Archives of Medical Research, 36(3), 197-209. doi: 10.1016/j.arcmed.2005.01.003.

86. Nesto, R. W., Bell, D., Bonow, R. O., Fonseca, V., Grundy, S. M., Horton, E. S., Le Winter, M., Porte, D., Semenkovich, C. F., Smith, S., Young, L. H., Kahn, R. (2004) Thiazolidinedione use, fluid retention, and congestive heart failure: a consensus statement from the American Heart Association and American Diabetes Association. Diabetes Care. 27, 256–63. doi: 10.2337/diacare.27.1.256.

87. Mottis, A., Mouchiroud, L., Auwerx, J. (2013) Emerging roles of the corepressors NCoR1 and SMRT in homeostasis. Genes & Development, 27(8), 819–835. doi:10.1101/gad.214023.113.

88. Feige, J. N., Auwerx, J. (2007) Transcriptional coregulators in the control of energy homeostasis. Trends in Cell Biology, 17(6), 292-301. doi: 10.1016/j.tcb.2007.04.001.

89. Dasgupta, S., Lonard, D. M., O'Malley, B. W. (2014) Nuclear receptor coactivators: master regulators of human health and disease. Annual Review of Medicine, 65, 279–292. doi:10.1146/annurev-med-051812-145316.

90. Lazar M. A. (2003) Nuclear receptor corepressors. Nuclear Receptor Signaling, 1, e001. doi:10.1621/nrs.01001.

91. Chen, J. D., Evans, R. M. (1995) A transcriptional co-repressor that interacts with nuclear hormone receptors. Nature, 377, 454–457. doi:10.1038/377454a0.

92. Hörlein, A. J., Näär, A. M., Heinzel, T., Torchia, J., Gloss, B., Kurokawa, R., Ryan, A., Kamei, Y., Söderström, M., Glass, C. K. (1995) Ligand-independent repression by the thyroid hormone receptor mediated by a nuclear receptor co-repressor. Nature, 377, 397–404. doi: 10.1038/377397a0.

93. Ahmadian, M., Suh, J. M., Hah, N., Liddle, C., Atkins, A. R., Downes, M., Evans, R. M. (2013) PPARγ signaling and metabolism: the good, the bad and the future. Nature Medicine, 19(5), 557–566. doi:10.1038/nm.3159.

94. Li, H., Leo, C., Schroen, D. J., Chen, J. D. (1997) Characterization of Receptor Interaction and Transcriptional Repression by the Corepressor SMRT. Molecular Endocrinology, 11(13), 2025–2037. https://doi.org/10.1210/mend.11.13.0028.

95. Aranda, A., Pascual, A. (2001) Nuclear Hormone Receptors and Gene Expression. Physiological Reviews, 81(3), 1269-1304. doi:10.1152/physrev.2001.81.3.1269.

96. Guenther, M. G., Barak, O., Lazar, M. A. (2001) The SMRT and N-CoR corepressors are activating cofactors for histone deacetylase 3. Molecular and Cellular Biology, 21(18), 6091–6101. doi:10.1128/mcb.21.18.6091-6101.2001.

97. Yu, J., Li, Y., Ishizuka, T., Guenther, M. G., Lazar, M. A. (2003) A SANT motif in the SMRT corepressor interprets the histone code and promotes histone deacetylation. The EMBO Journal, 22(13), 3403–3410. doi:10.1093/emboj/cdg326.

98. Cohen, R. N., Brzostek, S., Kim, B., Chorev, M., Wondisford, F. E., Hollenberg, A. N. (2001) The Specificity of Interactions between Nuclear Hormone Receptors and Corepressors Is Mediated by Distinct Amino Acid Sequences within the Interacting Domains. Molecular Endocrinology, 15(7), 1049–1061. doi:10.1210/mend.15.7.0669.

99. Goodson, M. L., Jonas, B. A., Privalsky, M. L. (2005) Alternative mRNA splicing of SMRT creates functional diversity by generating corepressor isoforms with different affinities for different nuclear receptors. The Journal of Biological Chemistry, 280(9), 7493–7503. doi:10.1074/jbc.M411514200.

100. Faist, F., Short, S., Kneale, G. G., Sharpe, C. R. (2009) Alternative splicing determines the interaction of SMRT isoforms with nuclear receptor-DNA complexes. Bioscience Reports, 29(3), 143-149. doi: 10.1042/BSR20080093.

101. Jepsen, K., Gleiberman, A. S., Shi, C., Simon, D. I., Rosenfeld, M. G. (2008) Cooperative regulation in development by SMRT and FOXP1. Genes & Development, 22(6), 740–745. doi:10.1101/gad.1637108.

102. Jepsen, K., Solum, D., Zhou, T., McEvilly, R. J., Kim, H. J., Glass, C. K., Hermanson, O., Rosenfeld, M. G. (2007) SMRT-mediated repression of an H3K27 demethylase in progression from neural stem cell to neuron. Nature, 450, 415-419. doi:10.1038/nature06270.

103. Tabák, A. G., Herder, C., Rathmann, W., Brunner, E. J., Kivimäki, M. (2012) Prediabetes: a high-risk state for diabetes development. Lancet (London, England), 379(9833), 2279–2290. doi:10.1016/S0140-6736(12)60283-9.

104. Feng, X., Jiang, Y., Meltzer, P., Yen, P. M. (2001) Transgenic targeting of a dominant negative corepressor to liver blocks basal repression by thyroid hormone receptor and increases cell proliferation. Journal of Biological Chemistry, 276(18), 15066-72. doi:10.1074/jbc.m011027200.

105. Astapova, I., Lee, L. J., Morales, C., Tauber, S., Bilban, M., Hollenberg, A. N. (2008) The nuclear corepressor, NCoR, regulates thyroid hormone action in vivo. Proceedings of the National Academy of Sciences of the United States of America, 105(49), 19544–19549. doi:10.1073/pnas.0804604105.

106. Astapova, I., Vella, K. R., Ramadoss, P., Holtz, K. A., Rodwin, B. A., Liao, X. H., ... Hollenberg, A. N. (2011) The nuclear receptor corepressor (NCoR) controls thyroid hormone sensitivity and the set point of the hypothalamic-pituitary-thyroid axis. Molecular Endocrinology, 25(2), 212–224. doi:10.1210/me.2010-0462.

107. Fozzatti, L., Lu, C., Kim, D. W., Park, J. W., Astapova, I., Gavrilova, O., … Cheng, S. Y. (2011) Resistance to thyroid hormone is modulated in vivo by the nuclear receptor corepressor (NCOR1). Proceedings of the National Academy of Sciences of the United States of America, 108(42), 17462–17467. doi:10.1073/pnas.1107474108.

108. Alenghat, T., Meyers, K., Mullican, S. E., Leitner, K., Adeniji-Adele, A., Avila, J., … Lazar, M. A. (2008) Nuclear receptor corepressor and histone deacetylase 3 govern circadian metabolic physiology. Nature, 456(7224), 997–1000. doi:10.1038/nature07541.

109. Yamamoto, H., Williams, E. G., Mouchiroud, L., Cantó, C., Fan, W., Downes, M., … Auwerx, J. (2011) NCoR1 is a conserved physiological modulator of muscle mass and oxidative function. Cell, 147(4), 827–839. doi:10.1016/j.cell.2011.10.017.

110. Heikkinen, S., Auwerx, J., Argmann, C. A. (2007) PPARgamma in human and mouse physiology. Biochimica et Biophysica Acta, 1771(8), 999–1013. doi:10.1016/j.bbalip.2007.03.006.

111. Rizos, C. V., Elisaf, M. S., Mikhailidis, D. P., Liberopoulos, E. N. (2009) How safe is the use of thiazolidinediones in clinical practice? Expert Opinion on Drug Safety, 8(1), 15-32. doi: 10.1517/14740330802597821.

112. Choe, S. S., Huh, J. Y., Hwang, I. J., Kim, J. I., Kim, J. B. (2016) Adipose Tissue Remodeling: Its Role in Energy Metabolism and Metabolic Disorders. Frontiers in Endocrinology, 7, 30. doi:10.3389/fendo.2016.00030.

113. Khan, A. A., Hansson, J., Weber, P., Foehr, S., Krijgsveld, J., Herzig, S., Scheideler, M. (2018) Comparative Secretome Analyses of Primary Murine White and Brown Adipocytes Reveal Novel Adipokines. Molecular & Cellular Proteomics, 17(12), 2358-2370. doi:10.1074/mcp.RA118.000704.

114. Rezaee, F., Dashty, M. (2013) Role of Adipose Tissue in Metabolic System Disorders: Adipose Tissue is the Initiator of Metabolic Diseases. Journal of Diabetes & Metabolism, 13(8), 2155-6156. doi:10.4172/2155-6156.S13-008.

115. Ordovas, J. M., Corella, D. (2008) Metabolic syndrome pathophysiology: the role of adipose tissue. Kidney International. Supplement 111, S10–S14. doi:10.1038/ki.2008.517.

116. Bremer, A. A., Devaraj, S., Afify, A., Jialal, I. (2011) Adipose Tissue Dysregulation in Patients with Metabolic Syndrome. The Journal of Clinical Endocrinology & Metabolism, 96(11), 1782–1788. doi: 10.1210/jc.2011-1577.

117. Ouchi, N., Parker, J. L., Lugus, J. J., Walsh, K. (2011) Adipokines in inflammation and metabolic disease. Nature Reviews, Immunology, 11(2), 85-97. doi:10.1038/nri2921.

118. Chatterjee, T. K., Stoll, L. L., Denning, G. M., Harrelson, A., Blomkalns, A. L., Idelman, G., Rothenberg, F. G., Neltner, B., Romig-Martin, S. A., Dickson, E. W., Rudich, S., ... Weintraub, N. L. (2009) Proinflammatory phenotype of perivascular adipocytes: influence of high-fat feeding. Circulation Research, 104(4), 541-549. doi:10.1161/CIRCRESAHA.108.182998.

119. Nolan, C. J., Ruderman, N. B., Kahn, S. E., Pedersen, O., Prentki, M. (2015) Insulin resistance as a physiological defense against metabolic stress: implications for the management of subsets of type 2 diabetes. Diabetes, 64(3), 673-686. doi:10.2337/db14-0694.

120. Avramoglu, R. K., Basciano, H., Adeli, K. (2006) Lipid and lipoprotein dysregulation in insulin resistant states. Clinica Chimica Acta, 368(1–2), 1-19. doi:10.1016/j.cca.2005.12.026.

121. Delarue, J., Magnan, C. (2007) Free fatty acids and insulin resistance. Current Opinion in Clinical Nutrition and Metabolic Care, 10(2), 142–148. doi:10.1016/j.cca.2005.12.026.

122. Fernandez-Marcos, P. J., Auwerx, J. (2011) Regulation of PGC-1α, a nodal regulator of mitochondrial biogenesis. The American Journal of Clinical Nutrition, 93(4), 884S-90. doi:10.3945/ajcn.110.001917.

123. Liu, C., Lin, J. D. (2011) PGC-1 coactivators in the control of energy metabolism. Acta biochimica et biophysica Sinica, 43(4), 248–257. doi:10.1093/abbs/gmr007.

124. Mihaylova, M. M., Shaw, R. J. (2013) Metabolic reprogramming by class I and II histone deacetylases. Trends in endocrinology and metabolism: TEM, 24(1), 48–57. doi:10.1016/j.tem.2012.09.003.

125. Horwitz, K. B., Jackson, T. A., Bain, D. L., Richer, J. K., Takimoto, G. S., Tung, L. (1996) Nuclear receptor coactivators and corepressors. Molecular Endocrinology, 10(10), 1167–1177. doi:10.1210/mend.10.10.9121485.

126. Rangwala, S. M., Lazar, M. A. (2004) Peroxisome proliferator-activated receptor gamma in diabetes and metabolism. Trends in Pharmacological Sciences, 25, 331-336. doi:10.1016/j.tips.2004.03.012.

127. Miles, P. D., Barak, Y., He, W., Evans, R. M., Olefsky, J. M. (2000) Improved insulin-sensitivity in mice heterozygous for PPAR-gamma deficiency. The Journal of Clinical Investigation, 105(3), 287–292. doi:10.1172/JCI8538.

128. Raghav, S. K., Waszak, S. M., Krier, I., Gubelmann, C., Isakova, A., Mikkelsen, T. S., Deplancke, B. (2012) Integrative genomics identifies the corepressor SMRT as a gatekeeper of adipogenesis through the transcription factors C/EBPbeta and KAISO. Molecular Cell, 46, 335–350. doi:10.1016/j.molcel.2012.03.017.

129. Sutanto, M. M., Ferguson, K. K., Sakuma, H., Ye, H., Brady, M. J., Cohen, R. N. (2010) The silencing mediator of retinoid and thyroid hormone receptors (SMRT) regulates adipose tissue accumulation and adipocyte insulin sensitivity in vivo. The Journal of Biological Chemistry, 285(24), 18485-95. doi:10.1074/jbc.M110.107680.

130. Kyoto Encyclopedia of Genes and Genomes. (2019) PPAR signaling pathway - Mus musculus (mouse). https://www.kegg.jp/kegg-bin/show_pathway?org_name=mmu&mapno=03320&mapscale=&show_description=hide.

131. Cohen, R. N., Putney, A., Wondisford, F. E., Hollenberg, A. N. (2000) The Nuclear Corepressors Recognize Distinct Nuclear Receptor Complexes. Molecular Endocrinology, 14(6), 900–914. doi:10.1210/mend.14.6.0474.

132. Jepsen, K., Hermanson, O., Onami, T. M., Gleiberman, A. S., Lunyak, V., McEvilly, R. J., Kurokawa, R., Kumar, V., Liu, F., Seto, E., Hedrick, S. M., Mandel, G., Glass, C. K., Rose, D. W., Rosenfeld, M. G. (2000) Combinatorial Roles of the Nuclear Receptor Corepressor in Transcription and Development. Cell, 102(6), 753-763. doi:10.1016/S0092-8674(00)00064-7.

133. Nofsinger, R. R., Li, P., Hong, S. H., Jonker, J. W., Barish, G. D., Ying, H., Cheng, S. Y., Leblanc, M., Xu, W., Pei, L., Kang, Y. J., Nelson, M., Downes, M., Yu, R. T., Olefsky,

J. M., Lee, C. H., Evans, R. M. (2008) SMRT repression of nuclear receptors controls the adipogenic set point and metabolic homeostasis. Proceedings of the National Academy of Sciences of the United States of America, 105(50), 20021–6. doi:10.1073/pnas.0811012105.

134. Reilly, S. M., Bhargava, P., Liu, S., Gangl, M. R., Gorgun, C., Nofsinger, R. R., … Lee, C. H. (2010). Nuclear receptor corepressor SMRT regulates mitochondrial oxidative metabolism and mediates aging-related metabolic deterioration. Cell Metabolism, 12(6), 643–653. doi:10.1016/j.cmet.2010.11.007.

135. Fang, S., Suh, J. M., Atkins, A. R., Hong, S. H., Leblanc, M., Nofsinger, R. R., … Evans, R. M. (2011) Corepressor SMRT promotes oxidative phosphorylation in adipose tissue and protects against diet-induced obesity and insulin resistance. Proceedings of the National Academy of Sciences of the United States of America, 108(8), 3412–3417. doi:10.1073/pnas.1017707108.

136. Li, P., Fan, W., Xu, J., Lu, M., Yamamoto, H., Auwerx, J., … Olefsky, J. M. (2011) Adipocyte NCoR knockout decreases PPARγ phosphorylation and enhances PPARγ activity and insulin sensitivity. Cell, 147(4), 815–826. doi:10.1016/j.cell.2011.09.050.

137. Yu, C., Markan, K., Temple, K. A., Deplewski, D., Brady, M. J., Cohen, R. N. (2005) The nuclear receptor corepressors NCoR and SMRT decrease peroxisome proliferator-activated receptor gamma transcriptional activity and repress 3T3-L1 adipogenesis. Journal of Biological Chemistry, 280(14), 13600–5. doi:10.1074/jbc.M409468200.

138. Shimizu, H., Astapova, I., Ye, F., Bilban, M., Cohen, R. N., Hollenberg, A. N. (2015) NCoR1 and SMRT play unique roles in thyroid hormone action in vivo. Molecular and Cellular Biology, 35(3), 555–565. doi:10.1128/MCB.01208-14.

139. Luft, V. C., Schmidt, M. I., Pankow, J. S., Couper, D., Ballantyne, C. M., Young, J. H., Duncan, B. B. (2013) Chronic inflammation role in the obesity-diabetes association: a case-cohort study. Diabetology & Metabolic Syndrome, 5(1), 31. doi:10.1186/1758-5996-5-31.

140. Tiwari, P., Blank, A., Cui, C., Schoenfelt, K., Zhou, G., Xu, Y., Shah, A., Khan, S., Rosner, M. R., Becker, L. (2018) Metabolically activated macrophages in mammary adipose tissue link obesity to triple-negative breast cancer. bioRxiv, doi: 10.1101/370627.

141. Liang, C. P., Han, S., Senokuchi, T., Tall, A. R. (2007) The macrophage at the crossroads of insulin resistance and atherosclerosis. Circulation Research, 100, 1546–1555. doi:10.1161/CIRCRESAHA.107.152165.

142. Privalsky, M. L. (2004) The Role of Corepressors in Transcriptional Regulation by Nuclear Hormone Receptors. Annual Review of Physiology, 66, 315. doi:10.1146/annurev.physiol.66.032802.155556.

143. Hubler, M. J., Kennedy, A. J. (2016) Role of lipids in the metabolism and activation of immune cells. The Journal of Nutritional Biochemistry, 34, 1–7. doi:10.1016/j.jnutbio.2015.11.002.

144. Bhargava, P., Lee, C. H. (2012) Role and function of macrophages in the metabolic syndrome. Biochemical Journal, 442(2), 253-62. doi:10.1042/BJ20111708.

145. Kisielow, M., Kleiner, S., Nagasawa, M., Faisal, A., Nagamine, Y. (2002) Isoform-specific knockdown and expression of adaptor protein ShcA using small interfering RNA. The Biochemical Journal, 363, 1–5. doi:10.1042/0264-6021:3630001.

146. Katon W. J. (2008) The comorbidity of diabetes mellitus and depression. The American Journal of Medicine, 121(11 Suppl 2), S8–S15. doi:10.1016/j.amjmed.2008.09.008.