ABSTRACT – KEYNOTE ADDRESS

Diabetes, Obesity and the Central Role of the Adipocyte in Maintaining Systemic Homeostasis

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Epidemiologically, *obesity* is frequently associated with the *metabolic syndrome* and poses an *increased risk* for the development of type 2 diabetes and *cardiovascular disease*. However, the molecular links that connect the phenomenon of obesity *per se* with insulin resistance are not known. Uncontrolled expansion of adipose tissue, through an increase in both adipocyte size and number, is a hallmark of obesity. Over the past decade, a number of mechanisms have been proposed to explain the phenotypic changes associated with obesity-related metabolic disorders. Among them, *inflammation, lipotoxicity, fibrosis* and the *unfolded protein response (UPR)* have attracted significant attention.

Dysfunctional adipose tissue during states of nutrient excess can promote systemic insulin resistance. Under conditions of obesity, the ability of adipose to buffer excess nutrients is reduced and the secretion of inflammatory cytokines is increased. Importantly, secretion of the anti-lipotoxic hormone adiponectin is lowered. Our work has further demonstrated that an enhanced capacity for lipid storage in adipocytes protects against metabolic disturbances, even in the context of massive nutrient excess, by over-expressing adiponectin constitutively. We recently published an additional model in which we demonstrated that "the world's most obese mouse" can preserve perfect metabolic health, provided the fat expansion occurs in a "healthy" fashion, i.e. as a combination of enhanced adipogenesis, appropriate vascularization and reduced fibrosis and inflammation. This mouse also achieved its protective high-level obesity through massive induction of adiponectin, downstream of altered mitochondrial function. An opposite phenotype can be observed in models of lipodystrophy, whereby the storage capacity of adipose is limited and adiponectin expression is completely ablated.

Elevation of ceramide levels is believed to be central to the pathogenesis of diabetic complications. *De novo* ceramide synthesis begins with the condensation of palmitoyl CoA and serine, catalyzed by serine palmitoyltransferase (SPT), the rate-limiting enzyme in ceramide production. Saturated long-chain free fatty acids stimulate *de novo* ceramide synthesis by fueling the pathway with substrate and inducing expression of at least one biosynthetic enzyme (for instance, SPT). Numerous inflammatory agents increase ceramide accumulation, including LPS, and cytokines (TNFa, IL-1, and PAI-1) (reviewed in (32)). *Ceramidases* catalyze the reversible deacylation of ceramide to generate sphingosine and free fatty acids. Recent data from the Lyons group, in addition to our own data, suggest that adiponectin receptors function to activate a neutral ceramidase activity. The sphingosine and sphinganine produced via ceramidase serve as substrates for sphingosine kinases, which produce the potently bioactive sphingolipid Sphingosine-1-phosphate (S-1-P). Ceramide has emerged as a key regulator of numerous cellular events, including the initiation of apoptosis and the inhibition of insulin action. We have demonstrated an essential role of adiponectin on cardioprotective actions in the context of caspase-8 induced cardiac myocyte cell death. Sphingolipid measurements are therefore an integral part of all the genetic manipulations that we perform, in order to gain a better understanding of the role of adiponectin on this important class of lipid effectors which play a central role in cellular homeostasis.

Combined, it is apparent that adipocytes in whole, through their function as nutrient storage cells and as key endocrine cells, play a role in the pathogenesis of the metabolic syndrome, and moreover, have the capability to exert profound effects on whole body metabolism. Adiponectin is a central player in this process.
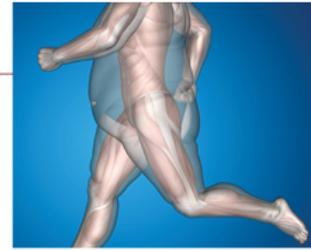
ABSTRACTS – SESSION I

Adipose Tissue MicroRNAs Constitute a Major Fraction of Circulating Exosomal microRNAs and Modulate Hepatic mRNA Expression

C. Ronald Kahn, Thomas Thomou, and Marcelo A. Mori

C. Ronald Kahn, MD
Mark K. Iacocca Professor of Medicine
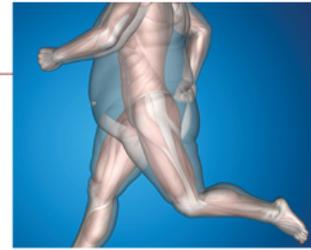Harvard Medical School

MicroRNAs (miRNAs), i.e., small non-coding RNAs, are differentially expressed in different fat depots, regulated in obesity, and undergo a broad down-regulation in adipose tissue with aging in a Dicer-dependent manner. To better understand the role of altered miRNAs in fat we generated mice with a fat-specific knockout of Dicer. ADicerKO mice developed a form of partial lipodystrophy with loss of intra-abdominal and subcutaneous fat, increase and whitening of brown fat, insulin resistance and dyslipidemia. miRNAs are also found in the circulation, primarily in exosomes. To determine if adipose tissue contributes to the circulating miRNA pool and if changes in circulating miRNAs originating from fat tissue might have systemic effects by altering mRNA translation in distant cells and tissues, we isolated circulating exosomal miRNAs from serum of ADicerKO and control mice by differential ultra-centrifugation. Profiling of exosomal miRNAs content by qPCR of all known miRNAs revealed a marked reduction in circulating miRNAs in the serum of ADicerKO mice. A subset of exosomal miRNAs was also reduced in human serum from HIV lipodystrophy patients (low Dicer expression) and from generalized lipodystrophy patients (very little fat tissue) when compared to healthy subjects. In addition, when ADicerKO mice received either a subcutaneous, brown or epididymal fat transplant from control mice, exosomal miRNAs were restored in the circulation of brown and subcutaneous transplanted ADicerKO mice suggesting that adipose tissue is a major source of exosomal circulating miRNAs. To test if these exosomal miRNAs could influence gene expression in secondary target cells, when we introduced miRNAs into adipocyte exosomes and co-cultured those with hepatic cells, we observed miRNA-dependent regulation of key hepatic metabolic regulators such as FGF-21. This could also be demonstrated in vivo using a FGF21 3'UTR reporter. Taken together these findings support the notion that adipose tissue constitutes a major contributor of circulating miRNAs, and that these miRNAs may exhibit specific mRNA-regulatory effects on other cells and organs.

Physiological Signaling by Adipose Tissue in Hunger and Energy Homeostasis

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The rising prevalence of obesity is driven by environmental factors such as changes in diet and levels of physical activity. However, within a given environment, some people develop severe obesity which is strongly influenced by inherited factors. To try to identify the genes and therefore the mechanisms involved in regulating weight, we have studied a cohort of individuals with severe, early onset severe obesity (n=6000) called the Genetics of Obesity Study (GOOS). Candidate gene studies in this cohort have led to the identification of patients with mutations in multiple genes involved in leptin-melanocortin signalling. Whole exome sequencing is proving to be an increasingly important tool in understanding the genetic heterogeneity associated with obesity leading to the discovery of multiple new genes. The discovery of how genetic variation at an individual and at a population level contributes to weight gain can drive further understanding of the molecular and physiological pathways involved in weight regulation and suggest targets for drug discovery and for therapeutic intervention.

Discovery of A Novel Class of Naturally-Occurring Lipids with Anti-Diabetic and Anti-inflammatory Effects

MM Yore[a], I Syed[a], PM Moraes-Vieira[b], T Zhang[b], MA Herman[c], E Homan[c], J Lee, S Chen, OD Peroni, A Hammarstedt, R Patel, TE McGraw, U Smith, A Saghatelian[d] and BB Kahn[d]

(Same letter denotes shared authorship position.)

Barbara B. Kahn, MD
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Increased adipose tissue (AT) lipogenesis is associated with enhanced insulin sensitivity. Mice overexpressing Glut4 in AT (AG4OX) have elevated AT lipogenesis and increased glucose tolerance in spite of obesity and elevated circulating fatty acids. To determine if the lipid profile contributes to improved glucose homeostasis in AG4OX, we performed untargeted lipidomic analysis of AT. This revealed a 16-18-fold increase in a novel class of lipids in AG4OX AT vs wildtype mice. Using a targeted Mass Spec approach, we identified 16 novel lipid family members with multiple isomers based on structural variations. These lipids are branched fatty acid esters of hydroxy fatty acids or FAHFAs. We studied the biologic effects of the isomers of palmitic acid hydroxy stearic acid or PAHSAs. PAHSAs are present at highest levels in brown and white adipose tissue with lower levels in many other tissues. PAHSA levels are acutely regulated by fasting. Most isomers are reduced 50-65% in serum and subcutaneous AT of insulin-resistant vs insulin-sensitive people. Nearly all isomers in humans correlate remarkably strongly with insulin sensitivity determined by euglycemic clamp. PAHSAs are also reduced in subcutaneous white AT in mice fed a High Fat Diet. A single oral dose of PAHSAs lowers ambient glycemia and enhances glucose tolerance in insulin-resistant obese mice while stimulating GLP1 and insulin secretion. PAHSAs also augment insulin stimulated glucose uptake and Glut4 translocation to the plasma membrane in adipocytes. PAHSAs suppress inflammatory processes in immune cells in vitro and decrease proinflammatory cytokines in adipose tissue macrophages in vivo. Biological effects of PAHSAs are mediated through lipid-responsive GPCRs. We have identified several enzymes that hydrolyze FAHFAs. One of these appears to play a major role in hydrolyzing FAHFAs in the pancreas. A gain-of-function mutation in this hydrolytic enzyme is associated with Maturity Onset Diabetes of the Young type 8. In summary, we identified a novel class of lipids that are made in mammalian tissues. These lipids improve glucose-insulin homeostasis and are anti-inflammatory. In conclusion, restoration of the low PAHSA levels in insulin-resistant people may be effective to treat or prevent type 2 diabetes.
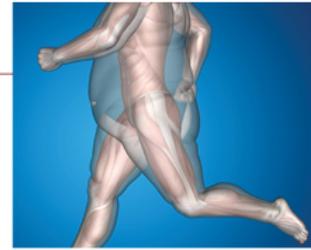
ABSTRACTS – SESSION II

Cavefish evolution as a natural model for metabolic diseases

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Understanding the genetic basis of adaptation has broad implications not only for a basic understanding of evolution, but also for human pathologies given that many human diseases are a consequence of mis-adaptation to modern societies. The emerging cavefish model system *Astyanax mexicanus* has become an important fish species to address adaptation to extreme environments due to its unique ecology and the availability of genetic tools (e.g. QTL mapping) and genomic resources. We have recently established A. mexicanus as a model for comparative physiology to address the question of how these fish have adapted to their nutrient poor environments

Cave environments are typically dark and as a consequence nutrient deprived. We have previously shown that cavefish acquired impressive adaptations such as hyperphagia (increased appetite), starvation resistance and altered feeding behaviors to cope with these harsh conditions. Here, we have focused on the fatty livers and symptoms reminiscent of diabetes these fish develop. Interestingly, we detected only very low insulin levels in cavefish (compared to surface or zebrafish) partially due to lower numbers of beta-insulin producing cells in the pancreas. In addition, cavefish display strong insulin resistance when administered with ectopic insulin. Despite the consequential elevated and highly fluctuating blood glucose levels, cavefish live long and healthy lives, probing the question whether they have acquired mechanisms allowing them to cope with extreme nutritional levels.

Taking advantage of the newly available genome of *Astyanax mexicanus* and tissue specific RNA-Seq data, we identified mutations in the insulin receptor of cavefish most likely responsible for the observed insulin resistant phenotype. Importantly, the same mutations were found in cases of Type-II diabetic patients in human populations. Our findings in independently derived cavefish populations suggest that cavefish are inherently insulin resistant, potentially as an additional strategy to acquire better starvation resistance. We are currently using genome editing to functionally test these and other candidate mutations in zebrafish and cavefish itself to study in detail the molecular mechanisms underlying the adaptation of cavefish to the extreme and nutrient poor environments, thereby providing potential new insights into human health.
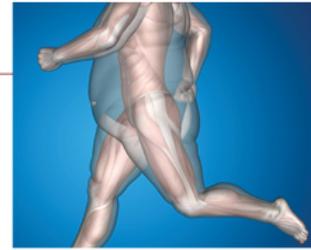
Opposing Proteostasis Pathways Drive Thrifty Metabolism and Survival During Nutrient Deprivation

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Thrifty genetic pathways contribute to elevated fat mass when food is plentiful, permitting extended survival when food is scarce. Although widely theorized, direct evidence for thrifty genetic pathways is generally lacking. We performed a genome-wide RNAi screen in *Caenorhabditis elegans* to identify fat regulatory genes indispensable for the physiologic response to nutrient deprivation. Here we show that two opposing pathways involved in protein homeostasis are principal determinants of nutrient rationing and starvation survival. Specifically, reduced function of genes involved in protein synthesis activates a metabolic defense program characterized by increased fat storage and extended starvation survival, whereas reduced function of protein turnover genes dramatically reduces fat mass and starvation survival. We hypothesize that the ancient pathways that govern cellular protein turnover were co-opted in order to regulate long term, triglyceride energy stores and their rationing during nutrient deprivation. As thrifty metabolic shifts activated in defense against starvation may contribute to obesity during nutritional excess, these findings may have implications for metabolic disease.

Circadian Rhythms in Adipose Tissue

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The earth's daily rotation on its axis accounts for the ~24 hour day:night cycle. This has had a profound impact on physiology in nearly all organisms and has led to the science of circadian biology or chronobiology. Animal studies have identified the suprachiasmatic nucleus (SCN) and light sensing regions of the brain as the dominant regulators of the body's response to the light: dark cycle. More recently, molecular studies have uncovered the central circadian genes and proteins. Many of these have been determined to be members of the PAS (Period/Arnt/Singleminded) family of transcription factors or interactive partners thereof. These genes and proteins display a robust oscillatory expression profile in the SCN. By manipulating the onset and length of the light:dark periods, the expression profile of circadian regulatory genes can be manipulated and, in man, this often leads to the symptoms associated with "jet lag". Moreover, global transcriptional microarray analyses have determined that multiple metabolic genes within the SCN and CNS change their expression level throughout the 24 hour light:dark cycle. This same phenomenon is conserved in both white and brown adipose tissue depots as well as multiple metabolic peripheral organs (bone, liver, kidney, marrow). The oscillation of circadian gene expression is maintained at the single cell level, occurring in fibroblasts and mesenchymal stromal cells of adipose tissue and bone. Over-expression or loss of function of several core circadian regulatory genes can inhibit or promote adipogenesis in pre-adipocytes. *In vitro*, circadian gene oscillation can be induced in pre-adipocytes by exposure to exogenous chemicals including nuclear hormone receptor ligands such as glucocorticoids or heme-related ligands of the circadian transcription factor, Rev-erb α/β. Unlike the SCN, the circadian clock of peripheral tissues can be manipulated *in vivo* by changing meal timing. An independent "food entrainable oscillator" is postulated to regulate this phenomenon. Studies in animal and human subjects have determined that the time of day when meals are ingested and alterations in sleep patterns can impact an individual's food choices and a subsequent weight gain. With the technological developments associated with industrialization, urbanization, and a "24/7" lifestyle, chronobiology may contribute in part to the rising frequency of obesity across the globe.
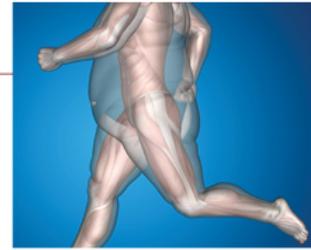
The Mitochondrial Calcium Uniporter: A Key Integrator of Cellular Metabolism

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Mitochondria are capable of transporting and buffering large amounts of calcium via a channel called the uniporter. Calcium transport through the mitochondrial uniporter alters cytosolic calcium waves and consequently impacts calcium-regulated cellular events. In addition, mitochondrion itself is a calcium-responsive organelle. Changes in cytoplasmic calcium concentration are sensed by mitochondrial calcium-binding proteins and modulate mitochondrial reactions. For example, increased ATP demand in the cytoplasm during calcium signaling event is met by activation of the TCA cycle by calcium ions.

The mitochondrial calcium uniporter lies at the center of mitochondrial calcium signaling however its regulation and function in normal physiology and disease remain elusive. The uniporter is composed five subunits: three membrane-spanning proteins (MCU, MCUb and EMRE), and two EF-hand domain containing, membrane-associated proteins (MICU1 and 2). MCUb, MICU1 and MICU2 are regulators of mitochondrial calcium uptake, whereas MCU and EMRE are essential for uniporter function. Moreover, when expressed together, MCU and EMRE form a functional channel, but neither alone is sufficient for calcium conductance.

Electrophysiology of mitochondria from MCU knockout cells and mutational analyses established that MCU is the pore forming subunit of the channel. EMRE, on the other hand, is a metazoan specific protein and in species like *D. discoideum*, MCU can conduct calcium without an EMRE homolog. To better understand the mechanism of EMRE function in calcium conductance in animals, we grafted sequences from DdMCU to human MCU and tried to bypass its EMRE dependence. Surprisingly, substitution of eight amino acids in human MCU with their DdMCU counterparts was enough to bypass EMRE function. On the recently solved MCU structure, these eight amino acids map to an unstructured region near the membrane after the calcium pore. We hypothesize that this region is stabilized by EMRE to form a calcium exit path. Without EMRE, calcium cannot exit the pore, and is in a closed confirmation. Consistent with this hypothesis, MCU and EMRE directly interact in this region, as well as along their transmembrane domains. Detailed understanding of the biochemistry of the uniporter enables mechanistic studies on its regulation in health and disease.
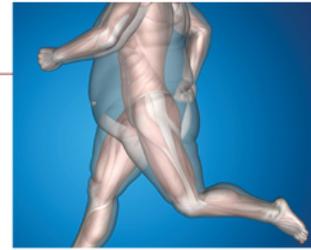
ABSTRACTS – SESSION III

White, Brown and Beige Fat: Basic Biology and Novel Thermogenic Pathways

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We have identified several key regulators in the adipose lineage over the past decades, including PPARg, PGC1a and PRDM16. These studies have led to the conclusion that there at least 3 distinct fat cell types: white, brown and beige. Increases in the amounts or activities of classical brown fat or beige fat in experimental models can have anti-obesity and anti-diabetes effects. We have approached the function of beige fat by purifying mitochondria from both brown and beige fat and asking about difference through the use of isobaric-tagging linked protein mass spectrometry. We show now that the beige fat has a second thermogenic pathway, in addition to the UCP1-mediated thermogenesis. Mitochondria from beige fat run a novel and robust futile cycle of creatine-a creatine phosphate cycle, which dissipates chemical energy via hydrolysis of the high energy phosphate on creatine. We call this pathway the creatine futile cycle (CFC). Beige but not brown fat cell mitochondria are stimulated to respire when supplied with creatine and this creatine acts sub-stoichiometrically, with regard to ADP. Finally, the proteins of this creatine-PO3 cycle are induced when UCP1 KO mice are adapted to the cold. Moreover, chemical interference with this creatine cycle in UCP1 KO mice causes a dramatic drop in body temperature without an alteration in shivering. These data illustrate a novel and robust pathway of energy expenditure centered on creatine metabolism in beige adipose tissues. We have also found post-translational modifications of UCP1 itself that serve as a rheostat regulating proton leak in the mitochondria of thermogenic cells. Finally we will discuss how this new information might lead to new therapeutics for metabolic diseases in humans.

Therapeutic Activators of Brown Fat

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Both environmental and pharmacological activators of brown adipose tissue (BAT) have been effective in rodent models at increasing energy expenditure, insulin action, consumption of ingested glucose and triglycerides, and improvement of the metabolic profile. With last decade's discovery of functional BAT in humans there has been an intense, renewed effort in identifying therapeutic activators of brown fat that will lead to metabolic benefit. Underlying these pursuits are two complicating factors that make the study of human BAT distinctly challenging: its mass and activity cannot be easily measured even with the current standard of PET/CT imaging; and very little is known about the physiological roles of human BAT, so there is little consensus for the appropriate therapeutic endpoint. Progress is being made on both fronts, and in this context, several types of interventions are being evaluated, including mild cold exposure, adrenergic agonists and other small molecules, and novel peptides/proteins. Short-term cold exposure has been shown to improve whole-body insulin sensitivity, and three decades of clinical trials with β3-adrenregic receptor agonists displayed promise in improving glucose tolerance. BAT-derived peptides/proteins may improve hepatic steatosis. The next advances in the field will involve identifying the full range of mechanisms by which human BAT contributes to metabolic health and determining which of the therapeutic activators, alone or combination, offer the best combination of safety, efficacy, and cost.
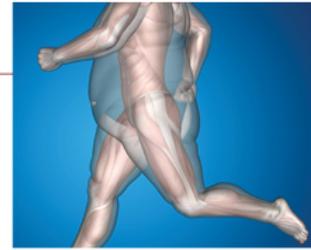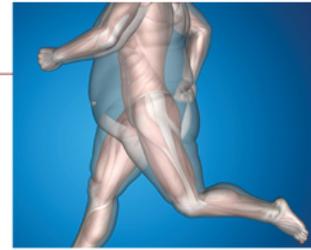
Adipose Tissue and Lipid Imaging

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Harvard Medical School

Quantification of adipose tissue and tissue lipid content has gained substantial attention due to the detrimental metabolic effects of their accumulation. The obesity epidemic and growing prevalence of sarcopenic obesity highlight the importance of being familiar with non-invasive imaging methods capable of measuring whole-body fat, muscle and liver lipid content, marrow fat, and brown adipose tissue. Many imaging techniques provide biomarkers that are linked to cardiometabolic risk, insulin sensitivity and skeletal integrity and represent important outcomes for therapeutic interventions. CT, MRI and MR spectroscopy are key methods for non-invasive determination of fat content and this lecture aims to familiarize attendees with the state-of-the-art in adipose tissue and lipid quantification.

Insights from Lipidomics

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Metabolic diseases present particular difficulty for clinicians because they are often present for years before becoming clinically apparent. We investigated whether metabolite profiles can predict the development of diabetes in the Framingham Heart Study. In a "lipidomics" analysis in the Framingham Heart Study, we found that lipids of relatively lower carbon number and double bond content were associated with an increased risk of diabetes, whereas lipids of higher carbon number and double bond content were associated with decreased risk. To explore potential mechanisms that modulate the distribution of plasma lipids, we also performed lipid profiling in the setting of perturbational experiments in hospital patients, including oral glucose tolerance testing, pharmacologic interventions and acute exercise testing. Lipids associated with increased diabetes risk (particularly triacylglycerols or TAGs) fell in response to insulin action; in turn, these TAGs were elevated in the setting of insulin resistance. These studies identify a novel relationship between lipid acyl-chain content and diabetes risk, demonstrate how lipidomic profiling could also aid in clinical risk assessment beyond standard risk factors, and highlight enzymes including specific lipid elongases and desaturases for further exploration in the context of diabetes. We will provide an update of our biomarker studies of prediabetes in multiple cohorts, highlighting our use of novel mass spectrometry techniques to as well information from genome wide scans of common and rare variants to understand the genetic architecture of the human metabolome.
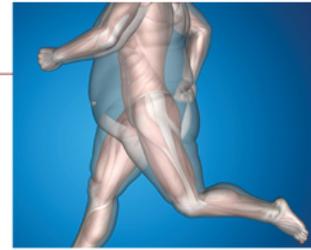
Metaflammation, Immunometabolism and Metabolic Diseases

Gökhan S. Hotamisligil, MD, PhD. Harvard TH Chan School of Public Health, Department of Genetics and Complex Diseases and Sabri Ülker Center for Metabolic Research, Boston, MA

Gökhan S. Hotamisligil, MD, PhD
James Stevens Simmons Professor of Genetics and Metabolism
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Proper coordination of cellular metabolism and its integration with immune response is paramount to function of cells, organs, and organisms. The endoplasmic reticulum is the main site for protein and lipid synthesis, trafficking, and the storage of cellular calcium. ER also plays a significant role in adaptation to metabolic fluctuations and their integration to immune response. This dynamic is disrupted by metabolic stress of chronic metabolic diseases such as obesity and diabetes in animal models and humans. Restoration of the ER adaptive folding responses by genetic or chemical means improve metabolic homeostasis in preclinical models and humans. Therefore, understanding the compositional, structural, and functional regulation of the ER and the mechanisms giving rise to its dysfunction remain limited beyond the canonical unfolded protein response (UPR). We are interested in exploring this aspect of organelle function and understanding both the upstream metabolic signals that influence ER and networks of integration with metaflammation. In recent studies, we discovered interactions between inflammatory signaling pathways and the unfolded protein response during metabolic stress and pathological interactions between the endoplasmic reticulum and mitochondria that disrupt the function of these organelles. Metabolic stress results in a unique pattern of maladaptive responses emanating from the ER, in part due to posttranslational modification of critical UPR sensors, such as IRE1, and activation of novel signaling paths that could compromise the ER function in the inflammatory environment of obesity and lead to abnormal glucose and lipid metabolism. Here, I will present emerging evidence integrating metaflammation to endoplasmic reticulum and mitochondria function, and how these molecular mechanisms may be exploited to understand chronic inflammatory diseases and leveraged to design novel and effective preventive and therapeutic strategies.