Death by lipids: The role of small nucleolar RNAs in metabolic stress

Excess fatty acid accumulation in nonadipose tissues leads to cell dysfunction and cell death that is linked to the pathogenesis of inherited and acquired human diseases. Study of this process, known as lipotoxicity, has provided new insights into the regulation of lipid homeostasis and has revealed new molecular pathways involved in lipid-induced cellular stress. The discovery that disruption of specific small nucleolar RNAs protects against fatty acid–induced cell death and remodels metabolism *in vivo* opens new opportunities for understanding how nutrient signals influence cellular and systemic metabolic homeostasis through RNA biology.

Fatty acids perform many critical cellular functions. These amphipathic molecules are building blocks of membrane lipids that demarcate the boundaries of cells and organize biochemical reactions within intracellular organelles. Fatty acids also serve as precursors for the biosynthesis of signaling lipids, such as ceramides and hydroxylated fatty acids, which regulate physiological processes. Moreover, fatty acids are nutrients that can be oxidized to produce ATP or efficiently stored as triglycerides. They are major nutrients for tissues with high energy demands, such as muscle, and a critical source of potential energy for organisms during periods of fasting. Under physiological conditions, cellular fatty acid supplies derive from *de novo* synthesis, recycling of other lipid classes, and import of exogenous lipids, distinct processes that must be coordinated with cells’ needs for these lipids for diverse functions.

Failure to balance acquisition and use of fatty acids underlies the pathophysiology of human diseases, ranging from rare monogenic disorders of metabolism to common complications of metabolic syndrome and type 2 diabetes mellitus (T2DM). In the 1970s and 1980s, clinicians discovered that disorders presenting as hypoglycemia in the setting of viral illness or fasting could be linked to mutations in genes encoding enzymes in fatty acid oxidation (1). Evidence of tissue dysfunction—failure of the liver to produce critical ketone bodies during starvation or frank damage to skeletal and cardiac muscle—coupled with observations of triglyceride buildup in these tissues suggested that excess lipid was toxic (2). Similarly, associations between triglyceride accumulation and myopathy in the tissues of children and young adults with neutral lipid storage diseases suggested that excess lipid impairs muscle function (3). More recently, as the prevalence of obesity, metabolic syndrome, and T2DM has increased globally, there is increasing recognition that nonalcoholic fatty liver disease and diabetic cardiomyopathy are diseases in which tissue level accumulation of excess lipids, related to hyperlipidemia and/or insulin resistance, is associated with significant impairment of organ function (4, 5).

From the bedside to the bench

These clinical presentations inspired animal studies in the laboratory to address key questions regarding the pathogenesis of these diseases. Knockout mouse models of monogenic disorders of fatty acid oxidation and dietary and genetic models of metabolic syndrome and T2DM phenocopy both tissue level lipid accumulation and organ dysfunction. Fatty acid oxidation disorders are characterized by impaired utilization in the setting of a normal supply of fatty acids, whereas models of metabolic syndrome and T2DM are examples of supply exceeding the capacity to metabolize fatty acids. These models recapitulate the lipid-induced activation of endoplasmic reticulum (ER) and oxidative stress observed in tissues of affected patients (6–9). Other genetically modified mouse models with tissuespecific overexpression of proteins involved in fatty acid transport have provided compelling evidence that tissue lipid overload precipitates similar toxicity, even in the absence of insulin resistance, hyperglycemia, hyperlipidemia, or other systemic metabolic perturbation (10–12).

Clinically, accumulation of lipid droplets in nonadipose tissue is the sine qua non of lipid overload states when supply exceeds demand. Fatty acids are delivered to tissues in the form of circulating lipoprotein particles from which fatty acids are released by lipoprotein lipase–mediated hydrolysis at the capillary endothelium, by endocytosed lipoproteins that are hydrolyzed intracellularly, and through uptake of free fatty acids liberated by adipose tissue lipolysis. In cells and in tissues that do not need the fatty acids for membrane biogenesis, synthesis of signaling molecules, or oxidation, fatty acids are re-esterified to glycerol, and resulting triglycerides are stored in the neutral lipid core of intracellular lipid droplets. Our understanding of the regulation of dynamic lipid trafficking into and out of these organelles has been advanced as the identities and function of proteins associated with the limiting monolayer of the lipid droplet have been illuminated (13).

Several physical features of lipid droplets in nonadipose tissue have the potential to contribute to cellular and organ level dysfunction. Lipid droplet membrane contact sites with the ER, mitochondria, peroxisomes, lysosomes, and Golgi provide potential platforms through which particularly abundant or large lipid droplets may interfere with the functions of other organelles (14). The

© 2020 Schaffer Published under exclusive license by The American Society for Biochemistry and Molecular Biology, Inc.
physical imposition of large lipid droplets also has the potential to alter cytoskeletal structures and perturb the biochemical reactions or trafficking events they regulate (15). Moreover, the ability of lipid droplets to recruit proteins to their surface raises the possibility that sequestration of proteins at these sites could be maladaptive (16). The precise contributions of such mechanisms to the pathogenesis of lipotoxicity remains to be determined.

On the other hand, evidence in cell culture models of fatty acid overload suggests that triglyceride molecules per se are inert with respect to lipotoxicity. Excess saturated long-chain fatty acids (e.g. palmitate) are substantially more toxic than unsaturated long-chain fatty acids (e.g. olate), and toxicity is inversely associated with cells’ capacity to incorporate the excess lipid into triglycerides (17). The ability to synthesize triglyceride from palmitate can be increased by up-regulation of stearoyl-CoA desaturase overexpression, which converts saturated palmitate into monounsaturated palmitoleate, or by exposure to mixtures of both saturated and unsaturated fatty acids (18). Importantly, these strategies that increase incorporation of excess fatty acids into triglyceride stores are associated with enhanced cell tolerance of the lipid load, whereas genetic disruption of triglyceride synthesis pathways renders cells similarly susceptible to saturated and monounsaturated fatty acid–induced cell death. Collectively, these data suggest that the toxicity of excess fatty acids is precipitated either by lipid that is not effectively incorporated into triglyceride stores in lipid droplets or by fatty acids released from these dynamic structures through ongoing cycles of lipolysis.

Studies in mice show that diverse genetic manipulations alter lipid droplet homeostasis and impact lipotoxicity in different ways. In the heart, only some of these data support the hypothesis that triglyceride accumulation is protective. In models in which lipotoxicity results from increased fatty acid import into cardiomyocytes, systolic heart failure is observed both when incorporation of lipid into triglyceride droplets is increased (10) and when excess lipid is diverted to alternate metabolic fates, resulting in little triglyceride accumulation (11). Models with knockout or overexpression of enzymes in triglyceride homeostasis, such as adipose tissue triglyceride lipase and diacylglycerol acyltransferase show seemingly divergent consequences of cardiomyocyte triglyceride accumulation on cardiac function (19–21). Knockout of the lipid droplet protein perilipin 5 limits the ability of the myocardium to accumulate triglyceride droplets during the stress of fasting, leading to myocardial oxidative stress and impaired cardiac systolic function (22). These seemingly disparate observations regarding the effects of triglyceride accumulation on cardiac performance underscore the complexities of metabolic stress responses and the limits of our understanding of lipid homeostasis in tissues.

The many paths to lipotoxicity

The metabolic fates of fatty acids underlie many cellular response pathways that are initiated by lipotoxicity (Fig. 1). Excess free fatty acids are readily incorporated into glycerolipids and phospholipids. Supplementation of the media of cultured cells with saturated long-chain fatty acids, which are particularly effective in inducing cell death, generates complex lipid species with increasingly saturated acyl chains (23). Incorporation of excess phospholipids, many with fully saturated long-chain fatty acyl chains, into membranes can negatively impact organelle shape and size, membrane barrier properties, and the function of integral membrane proteins that are critical for proper organelle function. Increases in the saturation of fatty acyl chains can also impair the ability of precursor lipids to serve as efficient substrates for downstream reactions. For example, because disaturated diacylglycerols are poor substrates for diacylglycerol acyltransferase, these species are not effectively stored as triglycerides (24). Similarly, saturated phosphatidylglycerol, formed in cells supplemented with palmitate, is a poor substrate for synthesis of the critical mitochondrial lipid, cardiolipin (25). Excess saturated long-chain fatty acids also promote de novo synthesis of ceramides, which contribute to lipotoxic cell death in many, although not all, cell types (26, 27).

Lipotoxicity has particularly profound effects on homeostasis and function of the ER and mitochondria. Membrane remodeling may be an important initiator of endoplasmic stress early in lipotoxicity. In both high-fat diet and ob/ob models of obesity and metabolic syndrome, induction of ER stress impairs signaling downstream of the insulin receptor, and treatment with chemical chaperones restores glucose homeostasis (6, 28). In addition, lipid-induced ER stress in pancreatic β-cells inhibits protein synthesis and secretion (29). In the mitochondria of palmitate-supplemented cultured cells, saturated lipid overload initiates mitochondrial permeability transition, increases uncoupling, and is linked to cytochrome c release (25, 30, 31). In animal models of lipotoxicity, tissues are exposed to a more physiological mix of unsaturated and saturated fatty acid species. In these models, even modest levels of lipid overload stimulate post-translational modifications of signaling proteins that regulate mitochondrial dynamics and lead to mitochondrial fission (32).

Lipotoxicity precipitates oxidative stress in cells and in tissues. On one hand, oxidative metabolism of fatty acids can help
to dispose of the nutrient load. This is supported by observations that in cultured cells and in animal models of tissue lipid overload, activation of AMP-activated protein kinase can mitigate lipotoxicity (23, 33). However, in cells with limited endogenous antioxidant capacity, up-regulation of metabolism occurs at the cost of increased mitochondrial production of reactive oxygen species (ROS) through the oxidative phosphorylation electron transport chain. In addition, oxidative stress is also precipitated by extreme ER stress and by lipid-induced activation of protein kinase C that up-regulates superoxide production by NADPH oxidase (34). Much of the in vitro evidence for oxidative stress during lipotoxicity derives from studies that use fluorescent indicators to report on levels of ROS. Although these measures are indirect and lack specificity, oxidative stress leaves a trail of modifications of nucleic acids, proteins, and lipids. In addition, oxidative stress alters the redox balance and induces expression of enzymatic scavengers of ROS. These footprints of oxidative damage are seen in animal models of lipid overload and phenocopy findings in human disease states (9, 35).

Excess fatty acids initiate numerous signaling cascades that contribute to the pathogenesis of lipotoxicity. Increased production of diacylglycerol molecules activates both classical and atypical forms of protein kinase C (36). Fatty acids and their metabolites also initiate signaling through stress kinases, death receptors, apoptosis pathways, and NFκB (37). Oxidative modification of cellular macromolecules not only impairs their physiological functions but also contributes to ER stress and apoptotic signaling (38). There has been some controversy regarding the contributions of fatty acid engagement of pattern recognition receptors on the cell surface (39, 40). Each of these pathways has the potential to contribute to dysfunction or death of cells in organs faced with lipid overload. In addition, fatty acid excess is an important stimulator of chronic inflammatory responses through the function of fatty acids as activators of immune cells (41).

**Rounding up new suspects**

Phenotype-driven forward genetic screens have advanced our understanding of the regulation of cellular responses to lipid overload in mammalian cells. Such approaches rely on random disruption of genetic loci or knockdown, followed by positive selection of cells that survive better under lipotoxic conditions or by negative selection of cells that have enhanced sensitivity to lipotoxicity (24, 35, 42, 43). Genetic disruptions achieved by short hairpin RNA, siRNA, and CRISPR are typically designed to target open reading frames. Screens using these approaches have identified protein-coding genes, including enzymes that catalyze triglyceride synthesis and regulators of glycerolipid synthesis (24, 43). By contrast, retroviral promoter trap mutagenesis approaches rely on gene disruption by proviral integration, which occurs proximal to active RNA polymerase II promoters, regardless of coding potential. Consistent with this, our laboratory’s promoter trap lipotoxicity screen yielded not only protein-coding genes (35, 44, 45), but also a long noncoding RNA (46) and a locus that hosts small nucleolar RNAs (snoRNAs) (47). It is possible that snoRNA-hosting loci were favored in this retroviral promoter trap approach, as bioinformatic analyses suggest that snoRNAs evolved from mobile genetic elements, and loci that contain snoRNAs are hot spots for integration of mobile elements (48). Regardless of the approach and the locus identified, key follow-up studies of newly identified genes in lipotoxicity screens have included complementation of disrupted loci in mutant or knockdown cells, targeted knockdown in WT or naive cells, and elucidation of mechanism.

Our laboratory’s identification of the ribosomal protein L13α locus (Rpl13a) gene in the promotor trap screen for resistance to lipotoxicity was unanticipated. The locus encodes a small globular protein, which functions in the large subunit of the ribosome, as well as four intronic snoRNAs: U32α, U33, U34, and U35α. Both the coding and noncoding RNAs from this locus are processed from a single transcript, and expression of all of these RNA products was diminished by integration of the promoter trap virus (47). Neither the RPL13α protein nor the snoRNAs had previously been implicated in lipid metabolism or metabolic stress responses. However, in complementation studies, only constructs that expressed the complete pre-mRNA, but not the mRNA and not a pre-mRNA with snoRNAs removed from the introns, were capable of restoring WT sensitivity to lipotoxicity in mutant cells. Furthermore, antisense-mediated knockdown targeting the Rpl13a snoRNAs, but not other box C/D snoRNAs, rendered WT cells resistant to lipotoxicity. Additionally, expression of the pre-mRNA and snoRNAs from this locus, but not the mRNA, increased during metabolic stress. These studies established a new role for the Rpl13a snoRNAs in the response to metabolic stress that is independent of effects on fatty acid uptake, β-oxidation, or triglyceride stores.

snoRNAs are a class of small noncoding RNAs that are predominantly localized to the nucleolus. In eukaryotes, these sequences range from ~80 to 200 nucleotides in length and include conserved box C/D or box H/ACA motifs (49). In the human genome, the majority of snoRNAs are encoded within introns of “hosting” genes and are processed out of intron lariats that are generated during splicing, whereas a small number of snoRNAs are independently transcribed. Proteins bound to nascent and to mature snoRNA sequences serve roles in the biogenesis and function of the mature small nucleolar ribonucleoprotein (snoRNP) particles. Box C/D snoRNPs contain fibrillarin, a methyltransferase, and box H/ACA snoRNAs contain dyskerin, a pseudouridine synthetase. Most mature snoRNPs target nascent rRNAs, snRNAs, and tRNAs for 2′-O-methylation or pseudouridylation of specific nucleotides, with specificity determined by a short stretch of sequence complementarity between the snoRNA and the target RNA sequence. A few snoRNAs direct other modifications or cleavage of their targets (50, 51). The biological consequences of these snoRNA-directed chemical modifications are established for some snoRNAs, which have been shown to be critical for rRNA biogenesis, structure, and activity (50, 52). Current estimates place the total number of snoRNAs across the human genome at 2064 (53). Among these, targets have been experimentally determined for many snoRNAs, but there remain snoRNAs for which targets are predicted.
but not proven experimentally and “orphan” snoRNAs whose targets are not yet known.

In addition to the mutant with disruption of the Rpl13a locus, two independent mutant cell lines isolated from our genetic screens are protected against lipotoxicity through mechanisms related to snoRNA biology. First, cells haploinsufficient for the protein SMD3, a core component of the spliceosome, are resistant to lipotoxicity (44). Haploinsufficiency of SMD3 decreases levels of the U4 and U5 small nuclear ribonucleoproteins, key players in pre-mRNA splicing, and lead to decreases in abundance of snoRNA-containing intron lariats that serve as precursors in snoRNA biogenesis. In these cells, levels of all mature intronic snoRNAs are decreased, even though splicing to produce mRNAs remains unperturbed. Second, short hairpin RNA–mediated targeting of nuclear export factor 3 (NXF3) also renders cells resistant to lipotoxic stress (42). The vast majority of snoRNAs are localized to the nucleolus, and lipotoxic stress increases the appearance of snoRNAs in the cytoplasm (54). Knockdown of NXF3 increases the abundance of Rpl13a snoRNAs in the cytoplasm and abrogates the increase in cytosolic snoRNAs with lipotoxic stress, whereas overexpression of NXF3 decreases the abundance of these snoRNAs in the cytoplasm (42). These studies, coupled with the observation that NXF3 binds a broad range of snoRNAs, suggest that NXF3 may function as a transporter to traffic snoRNAs between the nucleus and cytoplasm. Thus, these studies identify steps in the biogenesis and trafficking of snoRNAs as important regulators of noncanonical functions of snoRNAs in lipotoxicity.

Deficiency of Rpl13a snoRNAs not only leads to resistance to lipotoxic stress, but also mitigates against palmitate-induced ER and oxidative stress. Rpl13a–haploinsufficient cells do not up-regulate ER stress signaling pathways in response to lipotoxic stimuli, but maintain normal responses to the ER stress inducers tunicamycin and thapsigargin (47). This suggests that distinct signals engage the ER stress machinery in these settings. On the other hand, Rpl13a–deficient cells are resistant to H2O2-induced as well as palmitate-induced cell death, and they have diminished propagation of reactive oxygen species compared with WT cells following treatment with H2O2. These findings underscore the importance of oxidative stress in the progression to lipotic cell death.

A deeper understanding of the role of Rpl13a snoRNAs in metabolic responses has come from in vivo loss–of–function models that facilitate analysis of metabolic physiology. Early studies indicated that short-term antisense knockdown of Rpl13a snoRNAs protects against acute lipopolysaccharide-induced oxidative stress in the liver (47). For longer-term metabolic analyses, the genomic organization of the Rpl13a snoRNAs posed unique challenges for achieving selective knockdown of the intronic snoRNAs without perturbing expression of the host protein coding sequences. By using homologous recombination to knock in an alternate locus lacking snoRNAs, our laboratory generated a mouse model with constitutive germline loss of the four Rpl13a snoRNAs but retained WT levels of the RPL13a protein (55). Given the lack of alterations of fatty acid metabolism in Rpl13a-deficient cells (47), the finding of enhanced systemic glucose homeostasis in this model was unanticipated (55). These mice have heightened systemic glucose tolerance, enhanced oxidative metabolism of glucose in pancreatic islets, and increased glucose-stimulated insulin secretion in isolated islets. Remodeling of islet metabolism in the setting of loss of the Rpl13a snoRNAs occurs without major changes in the islet transcriptome. These islets are also resistant to oxidative stress, secondary to high glucose, H2O2, or menadione, a feature that likely explains the resistance of these animals to chemically or genetically induced pancreatic β-cell death and diabetes. Studies are under way to evaluate the physiological responses to lipotoxicity in different organs in this model. Conditional knockout models in which the Rpl13a snoRNAs can be manipulated in specific cell types and in which loss of function can be temporally separated from developmental processes will be important to extend these analyses and are under development.

Questions and controversies

Our discovery of a new role for Rpl13a snoRNAs in the response to metabolic stress adds to a growing list of unexpected physiological functions for snoRNAs. snoRNA expression is altered in a number of cancers, leading to the proposal that snoRNAs could serve as biomarkers and regulators of tumor biology (56, 57). Some direct associations between snoRNA expression and oncogenesis may relate to requirements for up-regulation of the protein synthesis machinery in rapidly growing cells, but snoRNAs can also function as tumor suppressors or as regulators of cancer cell signaling (58, 59). Prader–Willi syndrome, a neurodevelopmental syndrome associated with multiple endocrine abnormalities has been mapped to a region of chromosome 15 that contains several snoRNAs and protein-coding genes (60). A mouse model with targeted deletion of one of these snoRNAs, Snord116, in the mediobasal hypothalamus provides strong evidence that loss of this box C/D snoRNA underlies the hyperphagia observed in humans with Prader–Willi (61). Given the large number of snoRNAs across the genome, the Rpl13a snoRNAs may represent a subset of snoRNAs that will ultimately be identified to function in metabolic regulation.

Throughout biology, form follows function. A corollary is that the function of the Rpl13a snoRNAs in lipotoxicity and metabolic regulation likely relates to the ability of these non-coding RNAs to form ribonucleoproteins that target other RNA molecules for site-specific modifications. It is well-established that the four Rpl13a snoRNAs direct 2'-O-methylation on rRNAs (62). Indeed, compared with WT cells, macrophages with knockout of the Rpl13a snoRNAs demonstrate loss of methylation at sites known to be targeted by U32a, U33, U34, and U35a on the 18S and 28S rRNAs (63). Whether disruption of these specific rRNA methylations alters ribosome function in a way that leads to metabolic reprogramming remains to be determined (Fig. 2).

On the other hand, the repertoire of snoRNAs as a class of noncoding RNA regulators extends beyond 2'-O-methylation and pseudouridylation of nascent rRNAs in the nucleolus. Box C/D snoRNAs can direct methylation of tRNAs (51), lead to additional modifications such as acetylation (64), target
mRNAs in ways that regulate alternative splicing (65), and serve as precursors for smaller RNA species that have miRNA-like functions (66). Identification of snoRNP proteins in cells during lipotoxicity could provide important clues to the biochemistry that these noncoding RNA machines direct. Bioinformatic prediction of targets beyond rRNA sequences will be challenging, given the modest length of interaction between snoRNAs and their targets and the observation that some antisense homology mismatch is tolerated (67). Powerful insights may come from experimental approaches that leverage high-throughput methods for genome-wide characterization of specific nucleotide modifications and apply these to snoRNA loss-of-function models, provided that these methods are sufficiently sensitive to detect modifications on a broad range of cellular RNAs (68). The appearance of Rpl13a snoRNA outside the nucleus, particularly during metabolic stress, suggests the possibility of novel snoRNA targets in the cytoplasm (54).

The Rpl13a snoRNAs may have critical roles in regulating cellular responses to the environment that involve mechanisms beyond ribonucleoprotein-directed modifications of target RNAs in the cell in which they are produced. snoRNA function has been linked to major signaling cascades that modulate cellular function. In the setting of lipotoxic stress, snoRNAs bind and activate the dsRNA-dependent protein kinase PKR, a stress response kinase involved in innate immunity and known to regulate translation through phosphorylation of EIF2α (69). In addition, both energy metabolism and translation are regulated by signaling through mTOR, raising the possibility that snoRNA regulation of nutrient stress could involve changes in mTOR signaling (70). Beyond intracellular roles, the Rpl13a snoRNAs are secreted into the circulation and direct 2′-O-methylations in distant tissues, indicating the capacity for these noncoding RNAs to function in cell-to-cell communication (63).

Conclusions

Modern explorations of biology have uncovered links between seemingly disparate molecules and pathways. Some of these discoveries illuminate fundamental mechanisms that are utilized in multiple molecular scenarios to accomplish biological tasks. Other discoveries support the theory that evolutionary pressures select for efficient coordination of related biological functions through shared molecules and mechanisms. Our studies of lipotoxicity have revealed unanticipated connections between metabolic regulation and snoRNAs that could reflect new applications for established functions of this class of noncoding RNAs and/or novel functions that link processes for crosstalk. The intriguing metabolic phenotypes of mice with loss of function of the Rpl13a snoRNAs suggest that altered snoRNA expression or function could contribute to metabolic health and disease in humans.

Funding and additional information—This work was supported in part by National Institutes of Health Grants R01 DK064989, R01 DK108357, and DP1 DK119141 (to J. E. S.). The content is solely the responsibility of the author and does not necessarily represent the official views of the National Institutes of Health.

Conflict of interest—The author’s spouse is employed by and holds equity in Casma Therapeutics, a company focused on developing therapeutics that target the autophagy pathway for disorders that include nonalcoholic fatty liver disease.
Abbreviations—The abbreviations used are: T2DM, type 2 diabetes mellitus; ER, endoplasmic reticulum; ROS, reactive oxygen species; snoRNA, small nucleolar RNA; snorNP, small nucleolar ribonucleoprotein.

References
1. Stanley, C. A., Hale, D. E., Coates, P. M., Hall, C. L., Corkey, B. E., Yang, W., Kelley, R. I., Gonzales, E. L., Williamson, J. R., and Baker, L. (1983) Medium-chain acyl-CoA dehydrogenase deficiency in children with non-ketotic hypoglycemia and low carnitine levels. *Pediatr. Res.* 17, 877–884 CrossRef Medline
2. DiMauro, S., and DiMauro, P. M. (1973) Muscle carnitine palmitoyltransferase deficiency and myoglobinuria. *Science* 182, 929–931 CrossRef Medline
3. Chanarin, I., Patel, A., Slavin, G., Wills, E. J., Andrews, T. M., and Stewart, G. (1975) Neutral-lipid storage disease: a new disorder of lipid metabolism. *Br. Med. J.* 1, 553–555 CrossRef Medline
4. James, O., and Day, C. (1999) Non-alcoholic steatohepatitis: another disease of affluence. *Lancet* 353, 1634–1636 CrossRef Medline
5. Boudina, S., Sena, S., Theobald, H., Sheng, X., Wright, J. J., Hu, X. X., Aziz, S., Johnson, J. I., Bugger, H., Zaha, V. G., and Abel, E. D. ED. (2007) Mitochondrial energetics in the heart in obesity-related diabetes: direct evidence for increased uncoupled respiration and activation of uncoupling proteins. *Diabetes* 56, 2457–2466 CrossRef Medline
6. Ozcan, U., Cao, Q., Yilmaz, E., Lee, A. H., Iwakoshi, N. N., Ozdelen, E., Tuncman, G., Gorgun, C., Glimcher, L. H., and Hotamisligil, G. S. (2004) Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. *Science* 306, 457–461 CrossRef Medline
7. Ljubkovic, M., Gressette, M., Bulat, C., Cavar, M., Bakovic, D., Fabijanic, D., Grkovic, I., Lemaire, C., and Marinovic, J. (2019) Disturbed fatty acid oxidation, endoplasmic reticulum stress, and apoptosis in left ventricle of patients with type 2 diabetes. *Diabetes* 68, 1924–1933 CrossRef Medline
8. Anderson, E. J., Kypson, A. P., Rodriguez, E., Anderson, C. A., Leb, E. D., and Neufeld, P. D. (2009) Substrate-specific derangements in mitochondrial metabolism and redox balance in the atrium of the type 2 diabetic human heart. *J. Am. Coll. Cardiol.* 54, 1891–1898 CrossRef Medline
9. Chiu, H. C., Kovacs, A., Ford, D. A., Hsu, F. F., Garcia, R., Herrero, P., Saffitz, J. E., and Schaffer, J. E. (2001) A novel mouse model of lipotoxic cardiomyopathy. *J. Clin. Invest.* 107, 813–822 CrossRef Medline
10. Yagyu, H., Chen, G., Yokoyama, M., Hirata, K., Augustus, A., Kako, Y., Jeon, Y. G., Nahmgoong, H., Han, K. H., Kim, J., Kim, S., Choe, S. S., and Kim, J. B. (2019) During adipocyte remodeling, lipid droplet configurations regulate insulin sensitivity through F-actin and G-actin Reorganization. *Mol. Cell Biol.* 39, e00210–19 CrossRef Medline
11. Wilfling, F., Wang, H., Haas, J. T., Krahmer, N., Gould, T. J., Uchida, A., Kienesberger, P. C., Nagendran, J., Waller, T. J., Young, M. E., Eizirik, D. L., and Pipeleers, D. G. (2001) Inverse relationship between cytotoxicity of free fatty acids in pancreatic islet cells and cellular triglyceride accumulation. *Diabetes* 50, 1717–1777 CrossRef Medline
12. Meneses, J. F., Haradwaj, K. G., Ileda, S., Yamashita, H., Yagyu, H., Schaffer, J. E., Yu, Y. H., and Goldberg, I. J. (2009) DGAT1 expression increases heart triglyceride content but ameliorates lipotoxicity. *J. Biol. Chem.* 284, 36312–36323 CrossRef Medline
13. Kuramoto, K., Okamura, T., Yamaguchi, T., Nakamura, T. Y., Wakabayashi, S., Morinaga, H., Nomura, M., Yanase, T., Otsu, K., Usuda, N., Matsuura, S., Inoue, K., Fushiki, T., Kojima, Y., Hashimoto, T., Sakai, F. et al. (2012) Perilipin 5, a lipid droplet-binding protein, protects heart from oxidative burden by sequestering fatty acid from excessive oxidation. *J. Biol. Chem.* 287, 23852–23863 CrossRef Medline
14. Borradaile, N. M., Han, X., Harp, J. D., Gale, S. E., Ory, D. S., and Schaffer, J. E. (2006) Disruption of endoplasmic reticulum structure and integrity in lipotoxic cell death. *J. Lipid Res.* 47, 2726–2737 CrossRef Medline
15. Piccolis, M., Bond, L. M., Kampmann, M., Pulimeno, P., Chitrajai, C., Jayson, C. B. K., Vaites, L. P., Boland, S., Lai, Z. W., Gabriel, K. R., Elliott, S. D., Paulo, J. A., Harper, J. W., Weissman, J. S., Walter, T. C., and Farre, R. V. Jr. (2019) Probing the global cellular responses to lipotoxicity caused by saturated fatty acids. *Mol. Cell* 74, 32–44.e8 CrossRef Medline
16. Ostrander, D. B., Sparagana, G. C., Amoscato, A. A., McMillin, J. B., and Dowhan, W. (2001) Decreased cardiolipin synthesis correlates with cytochrome c release in palmitate-induced cardiomyocyte apoptosis. *J. Biol. Chem.* 276, 38061–38067 CrossRef Medline
17. Shimabukuro, M., Zhou, Y. T., Levi, M., and Unger, R. H. (1998) Fatty acid-induced beta-cell apoptosis: a link between obesity and diabetes. *Proc. Natl. Acad. Sci. U.S.A.* 95, 2498–2502 CrossRef Medline
18. Ludden, R. C., Ory, D. S., and Schafer, J. E. (2001) Palmitate-induced apoptosis can occur through a ceramide-independent pathway. *Biochem. J.* 369, 14890–14895 CrossRef Medline
19. Ozcan, U., Yilmaz, E., Ozcan, L., Furuhashi, M., Vaillancourt, E., Smith, D. G., Wang, H., Haas, J. T., Krahmer, N., Gould, T. J., Uchida, A., Kienesberger, P. C., Nagendran, J., Waller, T. J., Young, M. E., Eizirik, D. L., and Pipeleers, D. G. (2001) Inverse relationship between cytotoxicity of free fatty acids in pancreatic islet cells and cellular triglyceride accumulation. *Diabetes* 50, 1717–1777 CrossRef Medline
20. Koshkin, V., Dai, F. F., Robson-Doucette, C. A., Chan, C. B., and Wheeler, M. B. (2008) Limited mitochondrial permeabilization is an early manifestation of palmitate-induced lipotoxicity in pancreatic beta-cells. *J. Biol. Chem.* 283, 7936–7948 CrossRef Medline
21. Joseph, J. W., Koshkin, V., Saleh, M. C., Sivitz, W. I., Zhang, C. Y., Lowell, B. B., Chan, C. B., and Wheeler, M. B. (2004) Free fatty acid-induced beta-cell defects are dependent on uncoupling protein 2 expression. *J. Biol. Chem.* 279, 51049–51056 CrossRef Medline

ASBMB AWARD ARTICLE: snRNAs and lipotoxicity
65. Kishore, S., and Stamm, S. (2006) The snoRNA HBII-52 regulates alternative splicing of the serotonin receptor 2C. *Science* **311**, 230–232 CrossRef Medline

66. Brameier, M., Herwig, A., Reinhardt, R., Walter, L., and Gruber, J. (2011) Human box C/D snoRNAs with miRNA like functions: expanding the range of regulatory RNAs. *Nucleic Acids Res.* **39**, 675–686 CrossRef Medline

67. Kehr, S., Bartschat, S., Stadler, P. F., and Tafer, H. (2011) PLEYX: efficient target prediction for box C/D snoRNAs. *Bioinformatics* **27**, 279–280 CrossRef Medline

68. Dai, Q., Moshitch-Moshkovitz, S., Han, D., Kol, N., Amariglio, N., Rechavi, G., Dominissini, D., and He, C. (2017) Nm-seq maps 2’-O-methylation sites in human mRNA with base precision. *Nat. Methods* **14**, 695–698 CrossRef Medline

69. Youssef, O. A., Safran, S. A., Nakamura, T., Nix, D. A., Hotamisligil, G. S., and Bass, B. L. (2015) Potential role for snoRNAs in PKR activation during metabolic stress. *Proc. Natl. Acad. Sci. U.S.A.* **112**, 5023–5028 CrossRef Medline

70. Morita, M., Gravel, S. P., Chenard, V., Sikstrom, K., Zheng, L., Alain, T., Gandin, V., Avizonis, D., Arguello, M., Zakaria, C., McLaughlan, S., Nouet, Y., Pause, A., Pollak, M., Gottlieb, E., et al. (2013) mTORC1 controls mitochondrial activity and biogenesis through 4E-BP-dependent translational regulation. *Cell Metab.* **18**, 698–711 CrossRef Medline