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EDITORIAL

Assembling fats and sugar for cardiac protection

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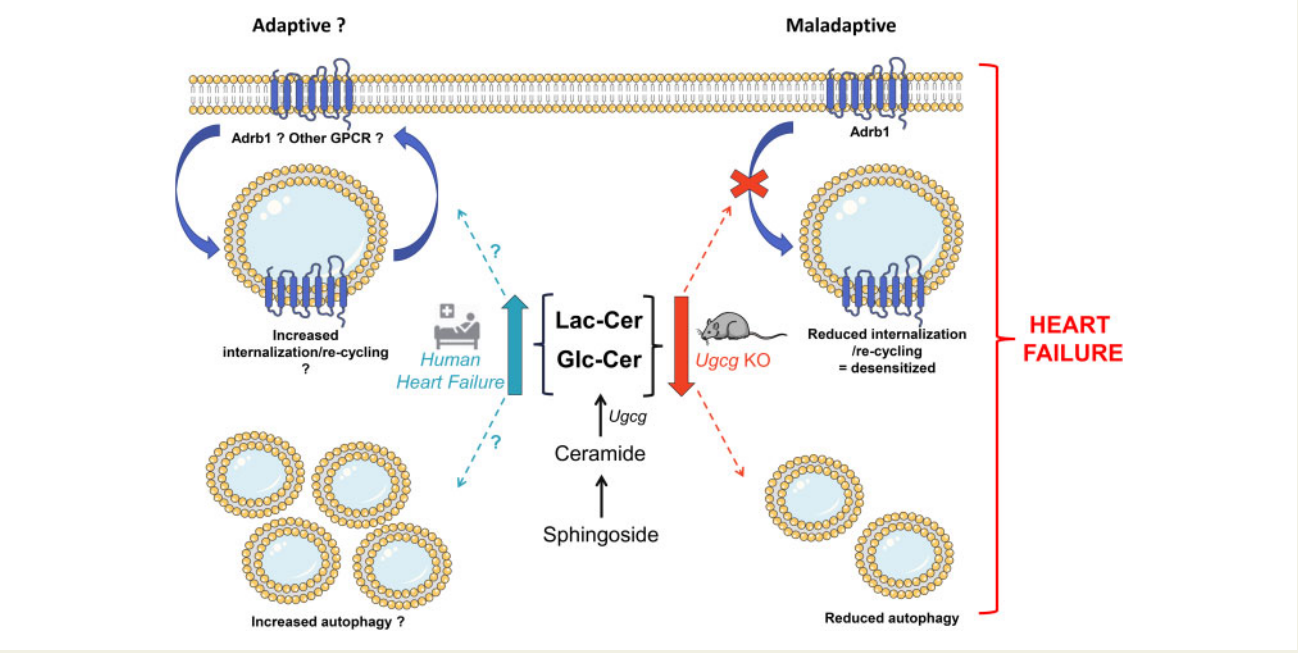
This editorial refers to 'Glucosylceramide synthase deficiency in the heart compromises beta1-adrenergic receptor trafficking', by L. Andersson *et al.*, doi:10.1093/eurheartj/ehab412.



Graphical Abstract Cardiac myocytes synthesize glucosyl- (and gactosyl-) ceramides from ceramide through glucosyl-ceramide synthase (encoded by *Ugcg*). As demonstrated from the phenotype of *Ugcg*–/– mice (right-hand side), these sphingosides are necessary for the internalization and recycling of membrane G-protein-coupled receptors, such as the beta1-adrenergic receptor (Adrb1), to maintain receptor coupling. *Ugcg* deficiency leads to Adrb1 desensitization and loss of cardiac contractile reserve. *Ugcg*–/– mice also exhibit reduced autophagy (illustrated with a reduced number of autophagosomes). These (and other defects of the endosomal pathway) lead to progressive heart failure and premature death. Cardiac muscle extracts from heart failure patients (left-hand side), however, are enriched in Glc-Cer and Lac-Cer, raising the possibility that these changes represent an adaptation mechanism of the cardiac muscle to stress. Whether these increased sphingosides actually optimize GPCR (including Adrb1) recycling/coupling and/or the endolysosomial/autophagy pathway for improved contractility/cell survival remains hypothetical. Adrb1, adrenergic receptor, beta-1 subtype; GPCR, G-protein-coupled receptor; Lac-Cer, lactosyl-ceramide; Glc-Cer, glucosyl-ceramide; Ugcg: UDP-glucose ceramide glucosyltransferase.

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Sphingosides are complex lipids indispensable for cell membrane integrity and signalling. They are synthesized from sphingosine by the addition of an aliphatic lipid chain; on the polar side, they can either remain unsubstituted (yielding ceramide) or can be substituted with several sugars (e.g. glucosyl- and galactosyl-ceramides) allowing their interaction with surface proteins while inserted in lipid membranes through their hydrophobic tail. These complex lipids are known to concentrate in lipid 'rafts' and caveolae which are 'hubs' for mechanosensing, receptor-mediated signalling, and transcytosis through the endosomal pathway,[1] among others. While lipotoxic ceramides have been involved in several cardiovascular diseases, e.g. atherosclerosis and myocardial infarction,[2] the role of their biochemical transformation to more complex sphingosides in the normal functioning of the cardiac muscle has remained elusive. Aside from rare monogenic diseases causing specific thesaurismoses (e.g. Fabry disease), the fact that single nucleotide polymorphisms (SNPs) for key enzymes involved in their synthesis have been associated with cardiac phenotypes in genome-wide association studies (GWAS)[3] suggests a role for some of these lipid intermediates.

In a previous study, an increase in sphingosides was identified in cardiac extracts from patients with ischaemic disease.[4] In the paper published in this issue of the *European Heart Journal*,[5] the same authors describe a more systematic lipidomic analysis in human cardiac extracts; among numerous changes in energy substrates, they again found increased glucosyl-ceramides (Glc-Cer) and lactosyl-ceramides (Lac-Cer) in hearts from patients with heart failure with reduced ejection fraction (HFrEF) from ischaemic cardiomyopathy [compared with ischaemic 'controls' with normal ejection fraction (EF), or with patients with structural heart disease but normal EF]. To test the causality of such biochemical modifications in the cardiac phenotype, they generated a mouse line with cardiac-specific genetic deletion of the key enzyme responsible for the synthesis of glucosyl-ceramide (encoded by *Ugcg*); while no phenotype was observed under basal conditions until 9–10 weeks of age, upon ageing to 6 months, the mice exhibited exacerbated adverse left ventricular (LV) remodelling (excentric hypertrophy with LV dilation and fibrosis) and increased mortality due to LV failure. At 9–10 weeks, the *Ugcg*–/– mice already had increased cardiac mRNA levels of markers of cardiac dysfunction and fibrotic remodelling, and impaired contractile response to dobutamine. To further discriminate the role of the initial metabolic disturbance (vs. long-term consequences of the genetic defect), they studied the effect of acute disruption [with a small interfereing RNA (siRNA) approach] of the Ugcg-dependent glucosyl-(and lactosyl-)ceramide formation in a myocyte cell line (HL-1); whole-genome RNAseq revealed down-regulation of transcripts that regulate cellular protein transport and lysosomal processes; consistently, depletion of Glc-(and Lac-)Cer was associated with disruption of the endolysosomal pathway and decreased autophagic flux. As the retrograde (membrane towards intracellular) endosomal pathway is well known to participate in the internalization and recycling of membrane G-protein-coupled receptors (GPCRs), they next focused on the function and coupling of the beta1-adrenergic receptor (Adrb1), a key regulator of cardiac contractility and remodelling. Upon stimulation with the pan-beta-adrenergic activator, isoproterenol, *Ugcg*–/– myocytes responded with less cAMP generation than myocytes from littermate controls. Using heterologous expression systems, they found defects in Adrb1 internalization, associated with its persistent desensitization upon stimulation with isoproterenol. Altogether, the data point to a role for Glc-Cer and (downstream) Lac-Cer formation for the adaptation of the cardiac muscle to stress, including through the preservation of beta1-adrenergic signalling (*Graphical Abstract*).

The data contain interesting information that could extend the interpretation beyond the authors' main conclusion. Clearly, the observations point to an adaptive role of Glc-Cer helping the cardiac muscle to maintain its integrity and function in the face of stress. While the *Ugcg* deletion experiments would support that, the demonstration could be completed with a gain-of-function experiment, showing that up-regulation of Glc-Cer synthesis confers a benefit in terms of remodelling and mortality from HF. From a translational perspective, it would also be important to verify if similar conclusions apply to LV remodelling of non-ischaemic origin; the fact that sphingoside levels were low in 'control' patients with normal EF but with structural heart disease (hypertrophy from valvular disease) suggests that this adaptive mechanism may only be triggered late in the disease evolution, i.e. when HF with low EF is established, and potentially irreversible; would any intervention on sphingosides levels still be useful then?

Linking the protective role of sphingosides to the maintenance of beta1-adrenergic signalling and, particularly, to cAMP production in cardiac myocytes, may seem paradoxical, especially in view of the failure of many inotropic interventions that increase cAMP in HF trials.[6] A number of experimental studies have emphasized the importance of cAMP compartmentation and targeted signalling (i.e. increases confined to the submembrane space)[7] to avoid deleterious effects of more diffuse cAMP increases throughout the cell (leading to calcium overload and its consequences on oxidant stress, transcriptional responses, and apoptosis). This aspect is not examined in the models used here. Also, while the authors focus on Adrb1, the effect of sphingosides on receptor distribution in subcellular compartments is very likely to operate equally on many other cardiac GPCRs not studied here, some of which may well protect against adrenergic or ischaemic stress. Beta2-adrenoreceptors would deserve consideration,[8] as well as beta3-adrenoreceptors, which were shown to protect against cardiac hypertrophy and fibrosis in pre-clinical models.[9–11] Recent work also proposed a protective role for alpha1-adrenoceptors in the stressed heart.[12] On the other 'limb' of the autonomic system, cardiac muscarinic cholinergic type 2 receptors (m2-AchRs) are well known to internalize and become sequestered in caveolae,[13] where sphingolipids are known to be particularly concentrated; would potentiation of muscarinic cholinergic receptor sequestration further tilt the balance between ortho- and parasympathetic drive, known to be already altered in the course of HF? Clinically, this is typically assessed through measurements of heart rate variability. While the authors report longitudinal measurements of (unchanged) heart rate in their mouse model, a more complete spectral analysis of heart rate variability by implanted telemetry would allow direct testing of a potential impact of sphingoside levels.

Finally, the impact of sphingosides on the endolysosomal pathway, and the ensuing autophagic flux, is equally likely to account, in part, for the observed phenotype. Many studies have highlighted the protective role of several forms of autophagy in the face of cardiac stress,[14,15] albeit at an appropriate intensity. There, we are faced with uncertainty regarding the stoichiometry between Glc-Cer (or Lac-

Cer) concentrations and autophagic flux. Would the levels observed in cardiac muscle from ischaemic patients drive autophagy at levels that still protect the heart? Also could any pharmacological intervention on the Ugcg pathway be harnessed in a sufficiently precise manner to avoid deleterious effects of exaggerated autophagy? These questions will need to be answered before the intriguing observations in the study of Anderson *et al.*[5] can be translated therapeutically.

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