commentary

The two Cs: ceramide and cardiomyopathy[1]

Godfrey S. Getz, *Associate Editor*[2]

Department of Pathology, University of Chicago, Chicago, IL 60637

The paper published in this issue of the *Journal* (1) highlights the great importance of lipid metabolism, fatty acid metabolism, and, particularly, ceramide homeostasis in cardiac function. While fatty acid is a preferred fuel for the energy of cardiac contractility, an imbalance between fatty acid and glucose metabolism may result in the generation of lipotoxic metabolites that disturb the function in the heart, in severe cases leading to cardiomyopathy, especially dilated cardiomyopathy. Rodent models of this pathology and altered metabolic homeostasis include obesity, often associated with diabetes and increases in the expression of fatty acid transport protein; of fatty acyl CoA synthase; and of either PPARα or PPARγ nuclear hormone receptors regulating fatty acid metabolism. The precise mechanism of cardiac dysfunction is not fully established.

The paper by Park et al. (1) represents a further probing of the model established in Goldberg's laboratory about 5 years ago (2). In an effort to determine the possible role of an extravascular pool of lipoprotein lipase (LPL), they developed a mouse in which LPL was expressed in and tethered to cardiomyocyte plasma membrane by a glycosylphosphatidyl inositol anchoring sequence (LPLgpi). This transgene was driven by a myosin heavy chain promoter. These mice exhibited increased lipid uptake and oxidation, ceramide accumulation, and a dilated cardiomyopathy, with decreased functional cardiomyocyte shortening. The mechanism of this cardiac dysfunction was not clear.

IS THE ACCUMULATION OF THE CARDIAC CERAMIDE RESPONSIBLE FOR THE CARDIOMOPATHY?

They now provide strong evidence that the increased accumulation of intracardiac ceramide is a major factor in the lipotoxicity by interrupting its biosynthesis by pharmacologic or genetic interventions. Ceramide is a sphingolipid consisting of sphingosine coupled by an N-acyl bond to a long chain fatty acid, predominantly a saturated fatty acid. The rate limiting enzyme of ceramide biosynthesis is serine palmitoyl transferase (SPT), which condenses palmitoyl CoA with serine, producing 3 keto sphinganine (3).

SPT is made up of two subunits (LCB1 and LCB2), both of which are required for SPT function. The enzyme has a high selectivity for palmitoyl CoA. It is the activity of this enzyme that has been attenuated with the specific inhibitor, myriocin. A heterozygous knockout of LCB1 achieved a down-regulation of the gene and its function. Much of the biochemical phenotype of the LPLgpi was reversed by either approach, along with the return of the ceramide concentration to wild-type levels. Myriocin treatment reversed the increases in heart weight, cardiac levels of sphingomyelin, ceramide, glycogen, pyruvate dehydrogenase kinase 4 (PDK4), fatty acid oxidation, and left ventricular diameter, and decreases in mRNA levels for CD36, acyl CoA synthase, fatty acid transport protein, glucose oxidation, as well as cardiac efficiency and functional shortening of the ventricle. With genetic reduction of ceramide synthesis, there were similar changes: reduced cardiac ceramide and reduced PDK4.

Thus the overexpression of LPL on the surface of cardiomyocytes seems to redirect energy metabolism more toward fatty acid metabolism and away from glucose oxidation, and these changes are reversed by reduction of ceramide levels. Additionally, the heart failure markers, ANF and BNP, which are both elevated in the enlarged hearts, are reduced markedly by either pharmacologic or genetic treatment of the rate of ceramide biosynthesis.

As impressive as are these responses, not all changes are fully reversed. The LPLgpi transgenic mice exhibit a notable reduction in viability. While the use of long-term myriocin treatment improves viability, it does not fully restore this to that of wild-type mice. This suggests that the ceramide accumulation may not fully account for the pathology of these LPLgpi mice. There are a number of gene product changes that are not reversed by the inhibition of ceramide biosynthesis. These include the elevation of diacylglycerol; the reduction in the glucose transporter 4 expression; and the reduction in some proteins of fatty acid metabolism, PPARα, acyl CoA oxidase, and carnitine palmitoyl transferase. Whether these unreversed changes contribute to the sustained impairment of viability of these mice remains to be determined. It was of interest that

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[2] To whom correspondence should be addressed.
e-mail: g-getz@uchicago.edu

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myriocin treatment of wild-type mice did not alter cardiac ceramide, probably attributable to the up-regulation of LCB1 and LCB2 in these treated mice. This may well be the case, but it is noteworthy that plasma sphingomyelin is reduced in both transgenic mice and in wild-type mice after myriocin treatment. Plasma sphingomyelin is probably synthesized in the liver, where hepatic ceramide serves as a substrate. This suggests that the up-regulation of LCB1 and LCB2, while seen in the heart, does not occur in the liver. This needs to be checked, and if the differences in tissue regulation are confirmed, the mechanisms should be accounted for.

Ceramide is derived from two fatty acid sources: the first is in the biosynthesis of 3 keto-sphinganine, and the second in the synthesis of ceramide by the N acylation in the sphinganine producing dihydroceramide, which is then oxidized to the bioactive ceramide (3). In the first case catalyzed by SPT, there is a high specificity for palmitoyl CoA. It is worth reminding ourselves that LPL has a predeliction for the hydrolysis of the *sn*-1 and *sn*-1′ fatty acid of the lipoprotein bound triglyceride (4), often occupied by palmitic acid. On the other hand, a variety of acyl CoAs can be employed for the N acylation of the sphinganine. Indeed there is a variety of isoforms of dihydroceramide synthases, which exhibit differing tissue distributions (5) and which generate subsets of ceramides containing various fatty acids and perhaps different functions. The changes in ceramide and plasma sphingomyelin responses to myriocin treatment may reflect the outcome of such tissue regulatory specificities. The fatty acid composition of cardiac and hepatic ceramide subspecies merits further attention.

The precise mechanisms by which ceramide accumulation leads to the metabolic changes and cardiomyopathy are yet to be elucidated. In many situations ceramide is proapoptotic (6), but in this model of cardiomyopathy, no increase in apoptosis was observed. Park et al. (1) noted an increase in pAKT and its downstream target G3K-3β, which upon phosphorylation promotes glycogen synthesis. These changes are also reversed by myriocin treatment. Ceramide has been implicated as a mediator of insulin resistance, in part by its action on intracellular trafficking of AKT (7). As with any good study, many new questions are generated.

TO WHAT EXTENT MAY CERAMIDE BE A MEDIATOR OF DILATED CARDIOMYOPATHY IN OTHER MURINE MODELS?

With the overexpression of PPAR α (8), PPAR γ (9), acyl CoA synthase (10), and fatty acid transport protein (11), an increase in ceramide levels has been observed in association with cardiac pathology. In each of these models, an increase in fatty acid oxidation is shown with a reduction of glucose oxidation. In contrast to the LPLgpi, the increment of ceramide was accompanied by cardiac apoptosis in the PPARγ and acyl CoA synthase models. But there does not appear to be a direct correlation between the extent of ceramide accumulation and the presence or absence of apoptosis, with ranges of ceramide increases from 30% to 330%. It is likely that other metabolic changes modify this relationship. The strategy of Park et al. (1) for the interruption of ceramide biosynthesis has not been applied to these models. This would be worthwhile to further understand the mechanisms at play and perhaps to develop additional therapeutic approaches to cardiac dysfunction. Park et al. (1) have demonstrated that long-term myriocin therapy is well tolerated.

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