ABSTRACT

The role of stearoyl-CoA desaturase in the regulation of cardiac metabolism

INTRODUCTION

Cardiovascular disease (CVD) is responsible for 3.9 million deaths in Europe [1] and approximately 800,000 deaths in the United States [2] annually. It remains the leading cause of death and long-term disability on both continents. Changes in lifestyle and diet (i.e., an increase in the consumption of processed food products) as a consequence of progressive industrialization coincide with a higher risk of developing CVD [3]. Although still debated, one of the dietary recommendations that seeks to lower the incidence of coronary heart disease is the replacement of saturated fatty acids (SFAs) with monounsaturated fatty acids (MUFAs) or polyunsaturated fatty acids (PUFAs) [4]. The fatty acid (FA) profile of an individual is influenced by dietary fat intake and the action of endogenous enzymes that participate in FA metabolism, especially those that are involved in the desaturating reaction (e.g., stearoyl-CoA desaturase; SCD) [5,6]. The desaturase index reflects the substrate-to-product ratio. It has been used as an indirect measure of the action of SCD and is a useful tool for assessing the risk of cardiovascular mortality [7]. A correlation was found between an elevated plasma palmitoleate (16:1)-to-palmitate (16:0) ratio (i.e., an indirect indicator of SCD activity) and a higher occurrence of heart failure [8]. Another study found an association between the plasma desaturation index and heart rate, linking the action of this enzyme with arrhythmia and cardiovascular mortality [9]. A recent study of the Chinese population that included 2447 participants linked high 16:0 and 16:1n-7 levels with hypertension [10]. High SCD activity is associated with metabolic syndrome, a set of metabolic abnormalities that correlate with a higher risk of CVD, in both adults [6] and children [11]. Data that suggest that the FA ratio is an indicator of SCD activity have not been validated against any specific measure of enzyme activity. Therefore, any link between these two should be considered with caution [12]. Nonetheless, the FA ratio and SCD activity are postulated to be valuable for the assessment of CVD risk, suggesting an important role for SCD in the regulation of heart metabolism. Research that seeks to decipher the actions of SCD may have clinical importance. The present review discusses recent findings on SCD in terms of cardiac metabolic reprogramming.

STEAROYL-COA DESATURASE STRUCTURE AND FUNCTION

Stearoyl-CoA desaturase is a transmembrane protein that is localized in the endoplasmic reticulum. It catalyzes the biosynthesis of MUFAs via the desaturation of SFAs, which are synthesized de novo or supplied in the diet. Stearyl-CoA desaturase catalyzes the reaction of introducing a single double bond during cis-formation between the 9th and 10th carbon atoms of long-chain fatty acyl-CoA (Fig. 1). Thus, SCD is also referred to as Δ9-desaturase. Palmitoyl-CoA (16:0) and stearoyl-CoA (18:0) are the most often utilized reaction substrates, which are converted to palmitoleoyl-CoA (16:1) and oleoyl-CoA (18:1), respectively [13]. Monounsaturated fatty acids are important substrates for the synthesis of
triglycerides (TGs), cholesterol esters, phospholipids, and wax esters [14]. Intracellular levels of MUFAs influence membrane fluidity, signal transduction, cell growth, and cell differentiation [15]. Excessively high levels of MUFAs can induce tumorigenesis [16]. Stearoyl-CoA desaturase has three conserved histidine motifs that are responsible for binding Fe$^{2+}$, which is required for electron flow [17,18]. Desaturation is an aerobic process that requires oxygen as a final electron acceptor and NAD(P)H as a donor of electrons. The electrons flow through cytochrome b5 reductase (FADH$_2$), to cytochrome b5, to SCD, and finally to O$_2$, which is reduced to H$_2$O [18] (Fig. 1).

Studies of mouse strains that have a mutation of the SCD1 gene provided evidence that SCD1 is an important control point in lipid metabolism and body weight regulation [19]. Mice with targeted disruption of the SCD1 gene exhibited higher energy expenditure, lower body adiposity, and higher insulin sensitivity and were resistant to diet-induced obesity [18]. SCD1 was found to be specifically repressed during leptin-mediated weight loss. Leptin-deficient ob/ob mice that lacked SCD1 exhibited markedly lower adiposity, despite higher food intake [20]. Additionally, SCD1 deficiency completely reversed the hypometabolic phenotype and hepatic steatosis in ob/ob mice [20] and attenuated fasting-induced liver steatosis in peroxisome proliferator-activated receptor-a (PPARα)-deficient mice [21]. The lack of SCD1 expression also improved the action of insulin in skeletal muscles and prevented diet-induced hepatic insulin resistance in mice [22]. Much evidence indicates that the direct anti-steatotic and anti-diabetic effects of SCD1 deficiency result from lower tissue lipid content that is caused by higher FA oxidation and lower lipid synthesis [23].

**ISOFORMS OF STEAROYL-COA DESATURASE**

Four isoforms of murine SCD have been characterized, the genes for which are localized on chromosome 19 and span six exons. SCD1 (transcript 4844 bp → 355 aa) and SCD2 (transcript 5453 bp → 358 aa) are expressed in almost all tissues, especially in white adipose tissue, brown adipose tissue, the eyelids, the skin, the Harderian gland, the preputial gland, and the liver, whereas SCD2 is not expressed in the liver in adult mice [24-27]. The nucleotide sequences of murine SCD1 and SCD2 have 85% and 82% homology, respectively, with human SCD1 (i.e., one of two isoforms [SCD1 and SCD5] that have been characterized in humans [28]). SCD3 (transcript 3470 bp → 359 aa) is expressed in the skin, Harderian gland, and preputial gland [17,29,30]. SCD4 (transcript 3072 bp → 353 aa) is expressed exclusively in the murine heart [29,30]. The SCD4 amino acid sequence has over 77% sequence homology with the other SCD isoforms, and SCD4 shares 100% identity with the other SCD isoforms with its three histidine motifs [13,26]. The reason for the expression of several SCD isoforms is still not well understood, but different physiological roles for these isoforms have been suggested. Three SCD isoforms are expressed in the heart, where only SCD4 is repressed by leptin, whereas SCD1 and SCD2 are not [13,26]. The regulation of SCD expression also appears to be tissue-specific. For example, PUFAs repress the expression of SCD1 and SCD2 but not SCD4 in the heart, and all of these isoforms are induced by the liver X receptor α agonist [26]. More research is needed to confirm tissue-specific regulation of the actions of SCD.

**SCD1 REGULATES CARDIAC SUBSTRATE UTILIZATION**

The work that the heart performs requires a large amount of adenosine triphosphate, which derives from oxidative phosphorylation in mitochondria and glycolysis and guanosine triphosphate formation in the citric acid cycle. Different energy substrates can be consumed by the heart, including FAs, glucose, lactate, ketones, pyruvate, and amino acids. A shift of substrate preference is a key factor for maintaining cardiac function, which is tightly controlled by the activity of several enzymes and transporters. The specific types of substrates that are utilized and their metabolic by products affect cardiac efficiency [31]. Although FAs are a plentiful source of energy, they are also very inefficient because more oxygen is utilized for their oxidation relative to glucose oxidation. This suggests the possibility of applying pharmacological agents to shift substrate preference toward glucose as an energy substrate to preserve or improve the mechanics of the heart [32,33].

SCD1 influences myocardial substrate utilization. Higher glucose oxidation and lower fat oxidation were observed in the heart in SCD1$^{-/-}$ mice [34] (Fig. 2). These effects were related to the upregulation of insulin signaling and an increase in glucose uptake that was caused by an increase in tyrosine phosphorylation of the insulin receptor and the activation of downstream molecules [34]. SCD1 deficiency also altered the availability of FAs, reflected by a decrease in FA uptake in the cytosol and transport across the mitochondrial membrane (Fig. 2). The uptake of FAs is facilitated by transport proteins, including FA translocase (CD36) and FA transport protein (FATP), the levels of which were lower in the heart in SCD1$^{-/-}$ mice compared with controls. Similarly, SCD1 inhibition decreased FATP1 protein levels in HL-1 cardiac muscle cells [35]. SCD1 deficiency also resulted in
a disturbance of mitochondrial FA transport, reflected by lower levels of carnitine palmitoyltransferase (CPT1) at both the mRNA and protein levels [34]. Importantly, free FAs must be conjugated to carnitine by CPT1 before entering mitochondria [36]. Energy metabolism in the heart has been shown to be regulated by the activity of adenosine monophosphate-activated protein kinase (AMPK). AMPK phosphorylation results in increases in FA oxidation, glucose uptake, and glycolysis [37]. An increase in AMPK phosphorylation was found in the liver and skeletal muscle in SCD1-deficient mice, whereas no such activation was observed in the heart. This indicates that AMPK does not play a role in metabolic changes that are observed in the heart in SCD1-deficient mice [34]. Moreover, in HL-1 cardiomyocytes, SCD inhibition did not alter the phosphorylation of either AMPK or its downstream target acetyl-CoA carboxylase (ACC) [35].

Metabolic pathways in the heart are rapidly regulated by the activity of enzymes and transporters and can be regulated on a long-term basis. Such long-term regulation occurs through modulation of the rate of transcription by specific transcription factors, such as PPARs. Upon their activation, PPARs can promote FA oxidation together with ketone body synthesis in the heart [38]. An increase in cardiac lipid oxidation and alterations of glucose metabolism and insulin resistance were found in transgenic mice with the heart-specific overexpression of PPARα. Notably, these mice developed a cardiac phenotype that resembled cardiomyopathy that is seen in obesity and diabetes, reflected by specific ventricular hypertrophy and lipid accumulation [39]. This lipotoxic form of cardiomyopathy that was caused by PPARα overexpression was ablated by CD36 knockout [40]. The expression of PPARα significantly decreased in the myocardium in SCD1−/− mice. This decrease was accompanied by a significant drop in the mRNA levels of CPT1 and acetyl-CoA oxidase, whose genes are targets for PPARα [34]. A similar effect was found in HL-1 cells that were treated with a SCD inhibitor [35]. These results suggest a link between SCD1 and PPARα. Palmitoleic acid, which is a product of the SCD1-mediated desaturation of palmitic acid, led to PPARα activation in peripheral tissues through an increase in PPARα binding to its DNA consensus sequence (PPRE) [41]. Furthermore, PPARα activity in the heart can be regulated by oleate, which is de novo-produced by SCD [42]. Thus, the observed decrease in intracellular PUFA content in SCD1-deficient mice [34] might cause the downregulation of PPARα, in which PUFAs were shown to influence SCD1 expression [43]. A study of SCD1−/− and PPARα−/− double-knockout mice suggested that SCD1 can act independently of PPARα [35].

**SCD1 REGULATES LIPID METABOLISM IN THE HEART**

Both circulating non-esterified FAs and those that are bound to lipoproteins are the main source of lipids in the heart, because de novo FA synthesis in the myocardium is very limited. Fatty acid uptake and oxidation are tightly regulated, any imbalance of which results in the accumulation of long-chain FAs and an increase in intracellular TGs...
and toxic derivatives of unoxidized palmitoyl-CoA, such as ceramide [44]. Lower levels of free FAs (FFAs) and ceramide were reported in the heart in SCD1-/- mice compared with wildtype mice [34]. Similarly, the inhibition of SCD1 in HL-1 cells resulted in a decrease in FFA, TG, and ceramide levels. These changes were accompanied by lower levels of lipogenic proteins, such as ACC and fatty acid synthase (FAS). Decreases in the expression of these two enzymes were also found in the heart in SCD1-/- mice [35].

Acetyl-CoA carboxylase- and FAS-encoding genes are under the control of sterol regulatory element binding proteins (SREBPs). Inactive SREBP precursors undergo proteolytic cleavage when intercellular lipid levels drop. Following cleavage, the amino terminus enters the nucleus and enhances the expression of genes that participate in lipid synthesis, including ACC and FAS [45]. A significant drop in the expression of SREBP1c was observed in SCD1-/- mice, with a consequent decrease in the expression of lipogenic genes. Likewise, in an in vitro model that employed HL-1 cells, SCD1 inhibition decreased SREBP1, FAS, and ACC protein levels [35].

In addition to influencing lipogenesis, SCD1 can also influence lipolysis (i.e., TG hydrolysis to DAG). SCD1 inhibition in cardiomyocytes decreased the levels of adipose triacylglyceride lipase (ATGL) protein, which initiates the intracellular catabolism of TAG. Concurrently, a significant drop in G0/G1 switch 2 (G0S2) protein levels was observed in HL-1 cells after SCD1 inhibition [35]. G0S2 acts as an inhibitor of ATGL, and G0S2 deficiency evokes the de-repression of cardiac lipolysis [46]. The effect of SCD1 inhibition on G0S2 expression was confirmed in an in vivo study in SCD1-/- mice, in which a decrease in G0S2 transcript levels was found [35]. SCD1 also appears to play an important role in regulating subsequent steps of lipolysis, including DAG hydrolysis. This process is mediated by hormone-sensitive lipase (HSL) [46]. SCD1 inhibition in cardiomyocytes resulted in an increase in the enzymatic activity of HSL, thus confirming the importance of SCD protein in regulating lipid metabolism in the heart [35].

ROLE OF SCD1 IN MAINTAINING HEART FUNCTION AND STRUCTURE

The effect of alterations of substrate utilization that are caused by SCD1 deficiency on excitation-contraction coupling machinery in the heart has been investigated. Metabolic alterations in the heart in SCD1-/- mice did not induce any changes in cardiac function [34]. However, further studies of the structure of the heart that performed transthoracic echocardiography and Doppler flow analysis indicated some structural changes in the heart in SCD1-/- mice compared with wildtype mice. SCD1 ablation increased the weight of the left ventricle (LV) by 15% and enlarged the diameter of the LV. Nevertheless, the thicknesses of the LV posterior and anterior walls were not significantly affected by SCD1 deficiency. Moreover, no differences in heart rate, systolic/diastolic function, the myocardial performance index, or blood flow velocity across the mitral valve in early diastole were observed between SCD1-/- and wildtype mice. Importantly, excitation-contraction coupling is energy-dependent, and any uncontrolled shift in substrate utilization can disturb heart function.

Slight changes in ectopic intracellular myocardial and pericardial lipid deposition are associated with alterations of cardiac performance. Functional alterations of the myocardium were described in obese leptin-resistant Zucker diabetic rats [47], db/db mice, and leptin-deficient ob/ob mice [48]. Hypertrophic remodeling in the LV was shown to be accompanied by increases in TG and ceramide levels and cardiomyocyte apoptosis [34, 48]. SCD1 was shown to be a crucial enzyme that regulates cardiomyocyte metabolism in the pathogenesis of lipid-induced heart disease. Significant increases in SCD1 expression and activity were observed in the myocardium in ob/ob and db/db mice and obese diabetic rats [49]. Moreover, increases in SCD1 expression were shown to favor lipid droplet accumulation in isolated neonatal rat cardiac myocytes that were incubated with palmitate [50]. Studies of acyl-CoA synthase transgenic mice, which are prone to the development of severe lipotoxic cardiomyopathy [51], reported increases in SCD1 and SCD4 expression. Significant decreases in LV diameter and LV mass were found in ob/ob/SCD1-/- double mutant mice compared with ob/ob mice, with no alterations of LV wall thickness [19]. However, the LV mass/body weight ratio was significantly increased in double knockout mice. Measurements of the myocardial performance index showed significant differences between ob/ob mice and ob/ob/SCD1-/- mice. Moreover, a Doppler flow analysis revealed a 57% decrease in the E/Ea ratio in ob/ob mice compared with wildtype mice, indicating diastolic dysfunction in ob/ob mice [19]. The E/Ea ratio in the heart in ob/ob/SCD1-/- decreased compared with wildtype mice, and SCD1 deficiency increased the E/Ea ratio by 27% in the heart in these mice compared with ob/ob mice. Altogether, SCD1 ablation was shown to improve cardiac function in obese leptin-deficient ob/ob mice.

Higher levels of ceramide cause the apoptosis of cardiac myocytes, which can result in LV chamber expansion, contractile dysfunction, and impairments in diastolic filling, thus contributing to cardiomyopathy that is observed in the setting of obesity and diabetes [47]. The accumulation of intramyocardial ceramide and TGs was also observed in obese leptin-deficient ob/ob mice [19]. Interestingly, SCD1 gene knockout resulted in a decrease in TG and ceramide content in the heart in ob/ob/SCD1-/- mice compared with control ob/ob mice [19]. The decrease in ceramide levels in the SCD1-deficient heart appeared to be attributable to a decrease in de novo synthesis, reflected by decreases in serine palmitoyltransferase activity and gene expression and a reduction of the incorporation of [14C]palmitate into ceramide [19].

The ceramide pathway is one of the most important lipoapoptotic routes in cardiomyocytes [52]. A decrease in ceramide content that was caused by SCD1 deficiency resulted in a lower rate of apoptosis in the heart in ob/ob mice. Two key markers of ceramide-induced apoptosis—nitric oxide (NO) production (measured by inducible nitric oxide synthase [iNOS] activity) and caspase-3 activity—were significantly reduced in the heart in ob/ob/SCD1-/- double knockout mice [19]. Decreases in the activity of iNOS and caspase-3 might result from the inhibition of de novo ceramide synthesis that is caused by SCD1 deficiency. Bielawska et al. [53] proposed that ceramide upregulates iNOS expression and increases NO production, which causes
an increase in apoptosis. The downregulation of caspase-3 was linked with the action of ceramide [54,55]. Ravid et al. [55] showed that ceramide-mediated apoptosis was blocked by the general caspase inhibitor Boc-D-fluoromethylketone. Ruvolo et al. [56] found that exogenous ceramide can downregulate antiapoptotic factor Bcl-2 expression and phosphorylation, thereby activating caspase-3 and apoptosis. The antiapoptotic effect of Bcl-2 may occur via the modulation of ceramide production and prevention of ceramide-mediated caspase activation [56,57]. An increase in Bcl-2 mRNA levels was observed in the heart in ob/ob;SCD1-/- double mutant mice compared with ob/ob controls. Thus, an increase in Bcl-2 gene expression could be another factor that contributes to the downregulation of caspase-3 activity and a lower rate of apoptosis in the heart in ob/ob;SCD1-/- mice.

An increase in the oxidation of palmitate through CPT1 is involved in the production of apoptosis in cardiomyocytes. Palmitate-induced cell death was enhanced by carnitine, a cofactor that is required for palmitate transport into mitochondria via CPT1 [58]. CPT1 mRNA levels decreased in the heart in ob/ob;SCD1-/- mice compared with ob/ob mice [19]. Therefore, lower FA mitochondrial oxidation could account for the decrease in apoptosis in the heart in SCD1-deficient ob/ob mice. A reduction of cardiomyocyte apoptosis was shown to improve cardiac function [48], and the inhibition of lipid-induced apoptosis that is caused by SCD1 deficiency might be directly responsible for the improvement in heart function in ob/ob mice.

A reduction of SCD1 activity was also shown to exert beneficial effects on heart function in animal models of diet-induced obesity. Rats that were fed a high-carbohydrate, high-fat diet developed eccentric hypertrophy, which is characteristic of higher preload, defined by an increase in the internal diameter on the LV in diastole without changes in the relative wall thickness [59]. Consequently, these rats exhibited impairments in systolic function. Additionally, diastolic, systolic, and stroke volumes and cardiac output were all elevated in high-carbohydrate, high-fat diet fed rats, with no changes in heart rate [59]. These detrimental changes in cardiac function were accompanied by an increase in SCD activity and reversed by α-linoleic acid, docosahexaenoic acid, and eicosapentenoic acid, all of which suppress SCD1 expression [60]. These findings emphasize the importance of SCD for the regulation of heart function.

**LIPIDS IN THE DEVELOPMENT OF PHYSIOLOGICAL AND PATHOLOGICAL LEFT VENTRICULAR HYPERTROPHY**

Cardiac hypertrophy is the abnormal enlargement or thickening of the heart muscle, associated with extensive physiological or pathological remodeling of the heart structure. Pathological remodeling is typically characterized by cardiac myocyte loss, fibrosis, and cardiac dysfunction [61,62]. In contrast, physiological remodeling is characterized by cardiac enlargement, associated with cell survival and normal heart function [63]. Lipidomic profiling revealed that significant changes in lipid content and composition and the regulation of lipid metabolism pathways between physiological and pathological cardiac hypertrophy [63].

Our group found increases in the expression of SCD1- and SCD2-encoding genes and other lipogenic protein-encoding genes, together with the activation of SREBP-1 and Akt signaling pathways, under conditions of physiological hypertrophy that was induced by endurance training, in contrast to the heart that presented pathological hypertrophy that was induced by abdominal aortic banding [61]. These data indicate that although the myocardium has a low capacity for de novo lipogenesis, lipogenic genes play significant roles in the control of cardiac metabolism and remodeling. The effects of physiological and pathological hypertrophy on cardiac lipid metabolism are more complex. Although the expression of lipogenic genes and the levels of FA transport proteins (e.g., CD36 and FATP1) were unaltered or reduced, pathological hypertrophy led to cardiac TG and DAG accumulation compared with the sham group. A possible explanation for this phenomenon is a decrease in lipolysis, reflected by an increase in G0S2 protein content (i.e., a small basic protein that functions as an endogenous inhibitor of adipose triglyceride lipase, a key enzyme in intracellular lipolysis), an increase in the phosphorylation of hormone-sensitive lipase at Ser565, and decreases in the protein levels of DAG lipase α (DAGLα) and DAGLβ, which attenuate TG and DAG content [61]. Taken together, all of these data show that activation of lipogenesis and reduction of lipolysis may play a role in the mechanism underlying the differences between physiological and pathological cardiac hypertrophy.

**FUTURE REMARKS**

The available data from SCD1-deficient mice highlight the importance of SCD in cardiac metabolic reprogramming. The shift of substrate utilization toward glucose that is evoked by the loss of SCD appears to be favorable in terms of cardiomyocyte protection against apoptosis and improvements in heart function in obese mouse models. Future research should focus on elucidating the mechanisms by which SCD plays a role in the heart with regard to both physiology and pathology. Myocardial substrate utilization affects the development and progression of heart failure. One intriguing possibility is to employ SCD inhibitors to regulate myocardial metabolism and improve cardiac function. Such a possibility, however, remains to be investigated. The role of SCD4, which is a cardiac-specific isoform of SCD, also requires further investigation.

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Rola desaturazy stearoilo-CoA w regulacji metabolizmu serca

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Słowa kluczowe: kwas tłuszczowy, utylizacja glukozy, niewydolność serca, przerost mięśnia sercowego, ceramidy

STRESZCZENIE

Metabolizm energetyczny serca jest ścisłe regulowany, a zaburzenia w zużyciu substratów energetycznych w kardiomiocytach prowadzą do zaburzeń funkcji skurczowej oraz powodują przebudowę lewej komory serca. W przypadku zaburzeń metabolicznych w sercu przewodzących do rozwoju chorób sercowych, znane są nadużywania, które mogą prowadzić do powstania ceramidów. Ceramidy to grupa lipidów, które są produkowane w wyniku enzymatycznego rozkładowania endogenezowanych kwasów tłuszczowych, a ich wzrost może być związany z zaburzeniami metabolizmu serca.

Desaturaza stearoilo-CoA (SCD) jest enzymem, który syntetyzuje mono- i dwukwasowe kwas tłuszczowe, a jego wzrost może być związany z zaburzeniami metabolizmu serca. Wymienione w teksie badania sugerują, że obniżenie wyraźnie ekspresji SCD poprawia funkcję skurczową i rozkurczową lewej komory serca w przypadku mięśniowaty dysfunkcji.”

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