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β-Adrenergic control of stearoyl-CoA desaturase 1 repression in relation to sympathoadrenal regulation of thermogenesis

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Mice lacking β-adrenoceptors, which mediate the thermogenic effects of norepinephrine and epinephrine, show diminished thermogenesis and high susceptibility to obesity, whereas mice lacking stearoyl-CoA desaturase 1 (SCD1), which catalyzes the synthesis of monounsaturated fatty acids, show enhanced thermogenesis and high resistance to obesity. In testing whether β-adrenergic control of thermogenesis might be mediated via repression of the SCD1 gene, we found that in mice lacking β-adrenoceptors, the gene expression of SCD1 is elevated in liver, skeletal muscle and white adipose tissue. In none of these tissues/organs, however, could a link be found between increased sympathetic nervous system activity and diminished SCD1 gene expression when thermogenesis is increased in response to diet or cold, nor is the SCD1 transcript repressed by the administration of epinephrine. Taken together, these studies suggest that the elevated SCD1 transcript in tissues of mice lacking β-adrenoceptors is not a direct effect of blunted β-adrenergic signalling, and that β-adrenergic control of SCD1 repression is unlikely to be a primary effector mechanism in sympathoadrenal regulation of thermogenesis. Whether approaches that target both SCD1 and molecular effectors of thermogenesis under β-adrenergic control might be more effective than targeting SCD1 alone are potential avenues for future research in obesity management.

Keywords: thermogenesis; type 2 diabetes; sympathetic nervous system; catecholamines

The sympathoadrenal system, through the release of norepinephrine from sympathetic nerves innervating peripheral tissues and through circulatory epinephrine released by adrenal medulla, plays an important role in the regulation of mammalian heat production.[1,2] Whereas in large mammals, the sites and molecular mechanisms underlying adrenergic control of heat production are poorly understood, studies in small rodents have implicated an elevation in sympathetic nervous system (SNS) activity in brown adipose tissue (BAT) – via β-adrenoceptor activation of its uncoupling protein (UCP1) – as a common mechanism for the stimulation of thermogenesis in response to both dietary and cold stimuli.[3,4] This contention is supported by the demonstrations that both SNS activity and UCP1 are elevated in BAT from rats and mice exhibiting adaptive increases in thermogenesis in response to cold or to overfeeding, and conversely they are both diminished in BAT from animals showing adaptive suppression of thermogenesis in response to fasting or severe food restriction. A major role for the SNS–BAT–UCP1 axis in thermoregulatory thermogenesis is further supported by the demonstration that mice lacking β-adrenoceptors (i.e. β-less mice) and mice deficient in UCP1 show impaired thermogenesis and poor tolerance to cold exposure.[5–7] However, the findings that only the β-less mice, but not the UCP1-deficient mice, are highly susceptible to develop obesity have also underscored the existence, even in small rodents, of mechanisms other than the SNS–BAT–UCP1 axis in β-adrenergic control of thermogenesis.

In the search for insights into these UCP1-independent thermogenic mechanisms under β-adrenergic control, the repression of stearoyl-CoA desaturase 1 (SCD1), a microsomal enzyme that catalyzes the synthesis of monounsaturated fatty acids, is of particular significance. Apart from being components of lipids – that include phospholipids, triglycerides, cholesterol esters, wax esters and alkyldiacylglycerols – these monounsaturated fatty acids also serve as

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mediators of signal transduction[8] that could interfere with the stimulation of thermogenesis in peripheral tissues. Indeed, mice lacking SCD1 show an elevated energy expenditure that confers resistance to obesity[9] and that is associated (in liver and skeletal muscle) with an increase in the activities of phosphatidylinositol 3-kinase and AMP-activated protein kinase[10–12] – two key signalling pathways that have been implicated in the orchestration of substrate flux towards oxidation[13] and thermogenesis.[14,15] These findings, together with the demonstrations that (i) SCD1 is

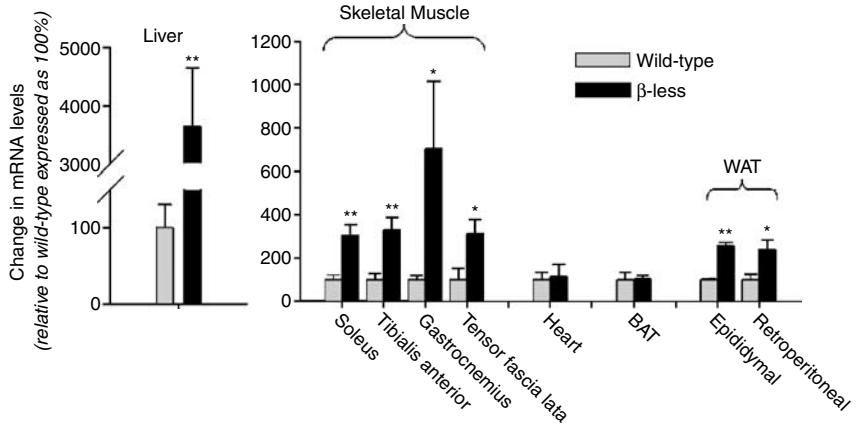

Figure 1 SCD1 mRNA levels in various organs/tissues of β-less mice compared with wild type controls of same age (7 week) and sex (male). All mice originated from the laboratory of Giacobino[6] and fed a standard chow diet. The gene expression analysis was performed by the real-time PCR using iQ SYBR Green Supermix (BioRad Laboratories, Reinach, Switzerland), the data normalized to the ribosomal protein 36B4, and presented relative to wild-type values expressed as 100%. The primers utilized for the mouse were as follows: 36B4 – forward 5′- TTG TGG GAG CAG ACA ATG TG -3′ and reverse 5′- AGT CCT CCT TGG TGA ACA CG -3′; for the mouse SCD1 – forward 5′- TGG GAA AGT GAG GCG AGC AAC TG -3′ and reverse 5′- AGG GAG GTG CAG TGA TGG TGG TG -3′. All data are mean $\pm$ s.e. (as vertical bars) ($n=6$), and were analyzed by the Wilcoxon rank sum test for statistically significant differences indicated as follows: *$P<0.05$ and **$P<0.01$. BAT: BAT (from interscapular area); WAT: white adipose tissue.

Table 1 SCD1 mRNA levels in organ/tissues of rats fed *ad libitum* compared with rats fasted for 3 days, or after 1 week of exposure to cold (4°C) compared with controls at room temperature (22°C)

	Diet			*Cold*			
	SNS	*SCD1*			*SNS*	*SCD1*	
		Fed	*Fasted*			*4°C*	*22°C*
Liver	↑	115±16	0.74**±0.13	Liver	NS	4.52±1.24	106**±20
Skeletal muscle				*Skeletal muscle*			
Soleus	NS	2.80±0.4	1.88±0.78	Soleus	↑	27.70±10.1	5.73*±1.93
Tibialis anterior	?	6.2±0.95	4.02±1.40	Tibialis anterior	↑	9.94±2.3	3.14*±0.74
Gastrocnemius	NS	1.14±0.18	2.18±0.49	Gastrocnemius	↑	5.19±1.76	1.45*±0.32
Tensor fascia lata	?	0.78±0.36	0.87±0.52	Tensor fascia lata	↑	2.57±0.92	1.84±0.6
Heart	↑	2.96±0.61	3.13±0.82	Heart	↑	3.72±1.09	4.36±2.74
BAT	↑	1139±200	774±126	BAT	↑	429±59	3615**±921
WAT				*WAT*			
Epididymal	NS	3730±46	366**±12.6	Epididymal	↑	10370±1749	6463±1155
Retroperitoneal	NS	6260±116	595**±21.8	Retroperitoneal	↑	11640±2574	7101±1770

Abbreviations: BAT, brown adipose tissue; NS, clearly not significantly different; SCD1, stearoyl-CoA desaturase 1; SNS, sympathetic nervous system; WAT, white adipose tissue. Seven-week-old male Sprague–Dawley rats (Elevage-Janvier, France) were caged singly and fed *ad libitum* on chow diet with access to tap water, except while fasting they were given a hypotonic saline solution (50 mM) to drink. The gene expression analysis was performed by real-time PCR using iQ SYBR Green Supermix (BioRad), with the following primers: cyclophilin forward 5′-TCA GGG CTC TTG AAG TCC C-3′ and reverse 5′-CAG AAA ATC ACA GCA GCC AAC-3′; for rat SCD1 forward 5′-TGG GAA AGT GAA GCG AGC AAC CG-3′ and reverse 5′-AGA GGG GCA CCT TCT TCA TCT TCT C-3′. All data are mean $\pm$ s.e. ($n=6$), and were analyzed by Wilcoxon rank sum test for statistically significant differences indicated as follows: *$P<0.05$, **$P<0.01$. The arrows indicate changes in sympathetic nervous system (SNS) activity in specific organs/tissues in response to diet or cold, as reported in literature.[1,18–20] The thick arrows indicate significant increases, whereas thin arrows indicate tendency to increase but failed to reach statistical significance; NS = clearly not significantly different. The symbol '?' indicates 'data not available'.

markedly repressed by leptin,[9] a hormone that stimulates fatty acid oxidation and thermogenesis in peripheral tissues in part through central activation of sympathetic outflow,[13,16] and that (ii) impaired thermogenesis in leptin-deficient (ob/ob) mice can be associated with both an elevation in hepatic SCD1[9] and diminished urinary epinephrine,[17] underscore a potential link between β-adrenergic control of SCD1 repression and thermogenesis.

To test the hypothesis that the repression of SCD1 is under β-adrenergic control, we first investigated whether the gene expression of SCD1 is increased in the absence of β-adrenoceptors by comparing its mRNA levels in various organs and tissues of β-less mice and their wild-type controls. As shown in Figure 1, SCD1 mRNA levels are markedly higher in liver ($+36$ fold), and to a lesser extent in skeletal muscle ($+$threefold to sevenfold) and in white adipose tissue (WAT) (from twofold to threefold) from β-less mice than from wild-type controls. By contrast, no significant between-group differences in SCD1 mRNA levels are observed in heart or interscapular BAT, two organs/tissues in which SNS activity in mice is known to vary in parallel to changes in thermogenesis in response to cold or diet.[1,4] To what extent the association between β-adrenoceptor and SCD1 repression shown here in murine liver, skeletal muscle and WAT might also be dissociated or associated with the functional state of the SNS in response to diet or cold is unknown, as changes in SNS activity in these tissues have not been reported in mice. In the rat, however, the application of kinetic techniques to the assessment of norepinephrine turnover rate in sympathetically innervated organs/tissues has demonstrated considerable heterogeneity in sympathetic outflow in response to diet or cold,[1,18–20] as depicted in Table 1. In particular, SNS activity is higher in liver but not in skeletal muscle nor in WAT in fed than in fasted state, and in response to exposure to cold (at 4°C), SNS activity tends to be higher in skeletal muscle and in WAT but not in liver. Using a similar study design to investigate SCD1 gene expression in these various organs/tissues, we found no repression of SCD1 gene expression in liver during feeding nor in skeletal muscle and in WAT after exposure to cold (Table 1). Furthermore, the lack of difference in the levels of SCD1 mRNA in heart and BAT in response to feeding, as well as in heart in response to cold – despite the well-known increases in SNS activity in these tissues in the rat – is consistent with our data in Figure 1, suggesting no adrenergic control of SCD1 in these tissues. In BAT, the association between elevated SNS activity and repressed SCD1 mRNA levels in the cold contrasts with the lack of a similar inverse association in response to diet. Whereas an α-adrenergic control of SCD1 in BAT in response to cold, but not to diet, could be invoked, the fact that SCD1 gene expression in BAT from β-less mice are not different from controls argues against a role for an SNS–βadrenoceptors-axis in the repression of SCD1. Overall, therefore, the data in Table 1 suggest that sympathetic neural stimulation of thermogenesis in response to diet or cold is unlikely to be mediated through repression of SCD1. A role for repressed SCD1 in the thermogenic actions of circulating epinephrine is also unlikely because, as shown in Figure 2, the administration of epinephrine, at doses known to stimulate maximally resting metabolic rate in rodents, failed to repress the levels of the SCD1 transcript in liver, skeletal muscle or in WAT, despite evidence of efficacy for epinephrine's action in these tissues judging from the increases in gene expression of 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase (PFK-2/FBPase-2), a marker of increased glycolytic rate.[21,22]

How then might these data above that are inconsistent with a role for β-adrenergic repression of SCD1 in relation to sympathoadrenal regulation of thermogenesis be reconciled with the upregulation of this gene in liver, skeletal muscle and WAT from β-less mice. One possibility is that the upregulation of the SCD1 transcript in these mutants is part of the enhanced machinery for synthesizing triglycerides consequential to their diminished thermogenesis. Alternatively (or additionally), an elevated SCD1 might be consequential to metabolic disturbances associated with the development of their excess adiposity, namely hyperinsulinemia, hyperglycemia and/or increased adipokines like tumor necrosis factor-α, some or all of which have been reported to increase SCD1 gene expression in liver, skeletal muscle and WAT.[8] In this context, an upregulation of SCD1 gene expression has also been reported in liver,[9] WAT[23] and

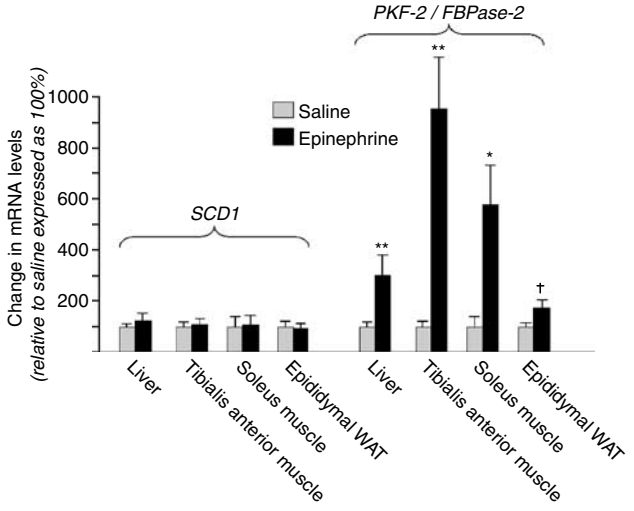

Figure 2 SCD1 and PFK-2/FBPase-2 mRNA levels in various organs/tissues from 7 week old male Sprague–Dawley rats treated with epinephrine compared to controls injected with saline. Epinephrine tartrate (300 μg/kg body weight) or saline were injected subcutaneously on four successive occasions with 2 h intervals on the same day, and the animals were killed 2 h after the last injection (i.e. 8 h after the first injection). The gene expression analysis was performed by real-time PCR, the data normalized to cyclophilin, and presented relative to saline control values expressed as 100%. The primers utilized for cyclophilin and for rat SCD1 are as indicated under Table 1, and that for PFK-2/FBPase-2 as follows: forward 5′- TGG CAG GTC GCC GAA TAC AGC -3′ and reverse 5′- TTG GTC AGC TTC GGC CCA CAG -3′. All data are mean $\pm$ s.e. (as vertical bars) ($n=6$), and were analyzed by the Wilcoxon rank sum test for statistically significant differences indicated as follows: *$P<0.05$, **$P<0.01$; †close to statistical significance ($P=0.08$).

skeletal muscle[24] from other animal models of obesity, and most recently in skeletal muscle from obese humans.[25]

Whatever the explanation, however, our studies investigating SCD1 gene regulation under physiological settings of adaptive changes in thermogenesis in response to diet or to cold argue against a primary role for SCD1 in the effector mechanisms by which the sympathoadrenal system regulates adaptive thermogenesis. Whether, from a therapeutic standpoint, approaches that target both SCD1 and molecular effectors of thermogenesis under β-adrenergic control might be more effective than targeting SCD1 alone are potential avenues for future research in obesity management.

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