**Antiobese and antidiabetic effects of trientine tetrahydrochloride mediated through the stabilization of spermidine/spermine N1 acetyl transferase-1**

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**Abstract**

Trientine tetrahydrochloride (TETA) is a synthetic polyamine that can be safely administered to patients for the long-term treatment of Wilson disease, a pathology caused by excessive copper storage. Intrigued by its structural resemblance to spermidine, a natural polyamine with anti-aging and pro-autophagic activities, we determined the effects of TETA on whole-body metabolism. In mice, TETA reduced obesity induced by high-fat diet, excessive sucrose or leptin deficiency, as it prevented diabetes and hepatosteatosis. All these effects did not rely on the removal of copper from the organism nor on reduced food intake, but depended on the expression of spermidine/spermine N1-acetyl transferase-1 (SAT1), which was activated by TETA. TETA-induced autophagy depended on SAT1, which is required for the beneficial effects of TETA. Altogether, these results suggest novel health-promoting effects of TETA that might be taken advantage of for the prevention or treatment of obesity.

**Keywords:** spermidine/spermine N1-acetyl transferase-1 (SAT1), Trientine tetrahydrochloride (TETA), spermidine, autophagy, obesity.

**Introduction**

Like spermidine (UICPA name: N-(3-aminopropyl)butane-1,4-diamine), trientine tetrahydrochloride (abbreviated TETA, UICPA name: N,N'-Bis(2-aminoethyl)ethane-1,2-diamine) is a polyamine. Spermidine is a natural compound produced by a biosynthetic pathway present in human cells, but is also produced by the intestinal microbiota and contained in multiple food items (Madeo et al., 2018) while TETA is a fully synthetic product. Spermidine supplementation has the remarkable capacity to extend the lifespan of model organisms (yeast, nematodes, flies and mice) (Eisenberg et al., 2009), (Eisenberg et al., 2016), and a spermidine-rich diet has been linked to reduced mortality in epidemiological studies, in two independent human cohorts from Austria and Northern Italy (Kiechl et al., 2018). The longevity-extending effects of spermidine have been attributed to its capacity to induce autophagy since they are lost in non-mammalian model organisms lacking essential autophagy genes (Eisenberg et al., 2009), as well as in mice deficient for *Atg5* in the myocardium (Eisenberg et al., 2016).Mechanistically, the capacity of spermidine to induce autophagy has been attributed to several effects, the acetyltransferase EP300 inhibition (Pietrocola et al., 2015) and the eIF5A hypusination increase, resulting in the activation of the pro-autophagic transcription factor TFEB (Zhang et al., 2019).

Beyond its longevity-extending effects, spermidine has rather broad health-promoting effects. Thus, it prevents pressure overload-induced heart failure (Eisenberg et al., 2016), improves the efficacy of anticancer immunotherapies (Pietrocola et al., 2016), {LEVESQUEZ 2019 OI}, ameliorates cognition in aging mice (Signor et al., 2017), reduces liver fibrosis (Yue et al., 2017), and prevents high-fat diet (HFD)-induced obesity and diabetes (Fernandez et al., 2017). All these effects depend on autophagy induction (Pietrocola et al., 2016), {LEVESQUEZ 2019 OI}(Signor et al., 2017), (Yue et al., 2017), (Fernandez et al., 2017). TETA is used for the treatment of Wilson disease, in patients that have developed adverse effects against the first-line drug penicillamine (Walshe, 1969), (Klionsky et al., 2016), (Mohr and Weiss, 2019). Wilson disease is caused by the mutation of adenosine triphosphatase copper transporting β gene (*ATP7B*)*,* a copper-extruding P-type ATPase that normally avoids excessive copper accumulation in hepatocytes and other cell types, leading to progressive liver failure, typically with an adult onset (Czlonkowska et al., 2018).TETA is considered as a copper chelator that prevents the intestinal absorption and favors the urinary excretion of excessive copper (Hedera, 2019). TETA can be administered for decades for the long-term treatment of Wilson disease with minor side effects (Mohr and Weiss, 2019), (Litwin et al., 2019).

Given the structural similarities between spermidine and TETA, as well as the fact that both polyamines are metabolized by the same enzyme spermidine/spermine N1-acetyl transferase-1 (SAT1), we addressed the question whether TETA might be used to induce autophagy and/or to improve the course of aging or age-related diseases. Here, we report the unexpected finding that TETA can stimulate the activity of SAT1, thereby setting of a biochemical cascade that protects against obesity and diabetes in mouse models.

**Results**

**Metabolic effects of TETA independent from copper depletion**. Chronic administration of TETA (3000 ppm in the drinking water) to 18-month-old wild-type (WT) C57Bl/6 mice (*late-in-life* feeding), reared under standard conditions, has no major toxic effects, as indicated by the assessment of longevity **(Fig. S1A)**, body weights **(Fig. S1B)**, as well as food and water uptake **(Fig. S1C)**. Of note, at this dose, TETA was unable to deplete heavy metals including copper **(Fig. 1A)**, iron **(Fig. 1B)** and zinc **(Fig. 1C)** in heart, liver and muscle. Moreover, in these organs, TETA was clearly bioavailable, as indicated by its mass spectrometric detection **(Fig. 1D)**, as well as that of its principal metabolites (Lu et al., 2007), N1-monoacetyltriethylenetetramine (MAT) and N1N10-diacetyltriethylenetetramine (DAT) **(Fig. 1E,F)**. Noteworthy, the conversion of TETA into MAT and DAT largely depended on spermidine/spermine N1-acetyl transferase-1 (SAT1) (Cerrada-Gimenez et al., 2011). Commensurate with the structural similarities of the synthetic agent TETA and the natural polyamine spermidine **(Fig. S1D)**, the effects of both compounds on the liver metabolome were largely convergent, though not identical **(Fig. S1E)**.

**TETA increases the activity of spermidine/spermine N1-acetyl transferase-1** (**SAT1**). TETA administration did not affect the spermidine and spermine concentrations in the liver **(Fig. 1G,H)**, yet enhanced that of N1-acetyl spermidine **(Fig. 1I)**, as well as the ratio of N1-acetylated over non-acetylated spermidine **(Fig. 1J)**, as compared to untreated controls. The N1-acetylation of spermidine is mostly catalyzed by SAT1 (Pegg, 2008), and the enzymatic activity of SAT1 was indeed increased by TETA treatment **(Fig. 1K)** although *Sat1* mRNA was not increased **(Fig. 1L, Fig. S1F)**. This may be related to an improvement of SAT1 protein stability because the decline of the enzymatic activity of recombinant SAT1 protein decreased in the presence of liver extracts, an effect that was reduced by addition of TETA **(Fig. S1G,H)**, causing a significant increase in the half-life of SAT1 **(Fig. S1I)**. The ratio of N1-acetyl spermidine over spermidine were significantly reduced in *Sat1-/-* mice and barely elevated by TETA treatment in such mice **(Fig. 1M)**. Non-radioactive 13C isotype-labelled spermidine, injected intraperitoneally, was converted into 13C N1-acetyl spermidine, and then converted in putrescine **(Fig. 1N)** detectable in the liver and in the plasma of WT, but less so *Sat1-/-*, mice. This biochemical reaction leading from spermidine to N1-acetyl spermidine (catalyzed by SAT1) and then to putrescine (catalyzed by acetylpolyamineoxidase, APAO) (Pegg, 2009) was stimulated by TETA in WT but not in *Sat1-/-* mice **(Fig. 1O-R)**. Commensurate with the increase in SAT1 activity (which can be expected to consume acetyl CoA) (Pegg, 2008), livers from TETA-treated WT mice exhibited a reduced acetyl CoA/CoA ratio **(Fig. 2A)**, as well as that of 3-hydroxybutyrate **(Fig. 2B)**, which usually correlates with acetyl CoA abundance (Perry et al., 2017)**.** Moreover, the level of N lysine acetylation, which is known to be *in equilibrium* with acetyl CoA (Pietrocola et al., 2015), was reduced in the liver of TETA-treated WT mice, as determined by means of a quantitative immunofluorescence assay **(Fig. 2C,D)**. N lysine deacetylation of cytoplasmic proteins can stimulate autophagy (Pietrocola et al., 2015), and, in WT mice, TETA indeed induced an increase in the autophagy-related variant II of the microtubule-associated protein 1A/1B light chain 3B (hereafter referred to as LC3), a lipidated variant of the protein that can be detected by its increased electrophoretic mobility, especially when the lysosomal destruction of LC3-II was blocked by the injection of leupeptin **(Fig. 2E,F)**. This TETA-induced reduction in acetyl CoA/CoA ratio, 3-hydroxybutyrate and N lysine acetylation, coupled to enhanced autophagic flux, was not detectable in *Sat1-/-* mice, meaning that it requires SAT1 function **(Fig. 2A-F)**. The SAT1-dependent, TETA-induced deacetylation of cellular proteins was also observed in the heart of mice **(Fig. S2A,B)**. In the nematode *Caenorhabditis elegans*, TETA induced an increase in autophagy detectable by measuring the abundance of LGG, an orthologue of LC3, **REF** fused to the fluorescent reporter XXX **(Fig. S2C,D)**. In accord with the fact that autophagy induction by non-toxic agents can increase lifespan (Rubinsztein et al., 2011);(Madeo et al., 2019), we observed that TETA caused a dose-dependent extension of longevity in *C. elegans* **(Fig. S2E)**. Altogether, these results support the contention that TETA acts as a classical Caloric Restriction Mimetic (CRM) (Madeo et al., 2014) inducing protein deacetylation, autophagy and positive health effects. In mice, the TETA-induced protein deacetylation and autophagy depend on SAT1 activation.

**SAT1-dependent anti-obese and anti-diabetic effects of TETA**. Continuous treatment with TETA of mice, from 7 weeks of age, did not affect weight gain in WT mice fed a normal chow, yet reduced weight gain of WT mice fed a high fat diet (HFD) or 30% sucrose in the drinking water **(Fig. 3A)**. TETA also reduced the weight gain of leptin-deficient (*ob*/*ob*) mice fed a normal diet **(Fig. 3A)**. Paralleling these anti-obesity effects, TETA ameliorated the glucose and insulin tolerance tests in obesogenic conditions **(Fig. 3B,C, Fig. S3A,B)**. In the context of HFD, TETA reduced adiposity without affecting lean mass **(Fig. 3D,E)**, reduced the diameter of visceral adipocytes **(Fig. 3F,G)** and ameliorated histological signs of hepatosteatosis **(Fig. 3H,I)**. At the biochemical level, TETA also prevented the HFD-induced increase in the plasma levels of, among others, leptin, gastrointestinal peptide (Gip), insulin, plasminogen activator inhibitor-1 (PAI-1), resistin and insulin growth factor binding protein (IGFBP3) **(Fig. 3J)**. TETA failed to affect food intake **(Fig. S3C,D)** and did not reduce the abundance of copper **(Fig. S3E)**, iron **(Fig. S3F)** and zinc **(Fig. S3G)** even in condition of HFD. Importantly, TETA lost its anti-obese effects in *Sat1-/-* (but not in partially autophagy-dependent *Atg4b*-/-) mice **(Fig. 4A)**. Under HFD, *Sat1-/-* (but not *Atg4b*-/-) mice also became resistant to the anti-diabetic effects of TETA **(Fig. 4B,C,** and **Fig 4D-E)**, failed to reduce theirfat mass **(Fig. 4F,G)** and neither ameliorated the histological correlates of visceral adiposity **(Fig. 4H,I** upper panels**)** nor those of hepatosteatosis **(Fig. 4J,K** upper panels**)**. However, these effects were preserved in *Atg4b*-/- mice **(Fig. 4H-K,** lower panels**)**. In conclusion, all favorable effects of TETA on metabolic health depended on SAT1. This is true for spermidine as well, since *Sat1-/-* mice fed a HFD failed to ameliorate their phenotype when treated with spermidine **(Fig. S4A-I)**. Similarly, the anti-obesity and hepatoprotective effects of spermidine, require autophagy induction, and hence are lost *Atg4-/-* mice **(Fig. S4J-P)**.

**Concluding remarks.** Although TETA is generally considered as a copper chelator, justifying its therapeutic use for the treatment of Wilson disease (Walshe, 1969), (Litwin et al., 2019), (Mohr and Weiss, 2019), the present report suggests that even prolonged treatment with TETA is unable to deplete copper and other heavy metals from the organs of mice in non-pathological conditions. Moreover, we demonstrated that TETA activates autophagy; this might suggest that TETA improves Wilson disease through alternative mechanisms including the induction of autophagy, which has been suggest to increase the turnover of copper-damaged mitochondria (Polishchuk et al., 2019). Interestingly, TETA, which is metabolized by SAT1, enhanced the activity of SAT1 in the mouse liver, though a mechanism that is difficult to be elucidated due to the absence of suitable SAT1-specific antibodies. It appears clear, however, that TETA does not increase the expression of the *Sat1* mRNA and that may stabilize the enzymatic activity of SAT1 protein. Irrespective of the precise (allosteric?) mechanism of SAT1 stabilization/activation by TETA, there are multiple signs that SAT1 is overactivated in TETA-treated mice, causing enhanced acetylation of spermidine with a commensurate consumption of acetyl CoA that results into N lysine deacetylation of cellular proteins and stimulation of autophagy. This TETA-triggered cascade of biochemical and cellular events is entirely lost in *Sat1-/-* mice.

Driven by the aforementioned observation that the autophagy inducer spermidine, a SAT1 substrate that is structurally related to TETA, can prevent obesity and diabetes (Fernandez et al., 2017), we investigated whether TETA might have similar beneficial effects on whole body metabolism. Indeed, TETA mitigated weight gain in obesogenic conditions (though without reducing food intake) and reduced obesity-related comorbidities such as diabetes and hepatosteatosis. In contrast to the pro-healthy and anti-diabetic effects of spermidine, which require autophagy induction (and hence are lost in partially autophagy deficient *Atg4b-/-* mice), the beneficial effects of TETA appeared to be autophagy-independent (since they persisted in *Atg4b-/-* animals). Nonetheless, both TETA and spermidine require SAT1 to mediate their anti-obese effects, meaning that both polyamines lose their anti-obesity, anti-diabetic and anti-steatotic properties in *Sat1-/-* mice. As a possible scenario, the exacerbated metabolism of spermidine by SAT1 (that is stimulated by TETA, which stabilizes SAT1, and perhaps also by spermidine when it is overabundant) may cause a futile cycle consuming acetyl CoA, thereby altering cellular metabolism and/or consuming energy to prevent lipo-anabolism and its consequences. Indeed, transgenic overexpression of SAT1 has previously been shown to yield a lean phenotype (Jell et al., 2007), arguing in favor of this conjecture. Moreover, SAT1 has been shown to contribute to differentiation of white into beige adipocytes (Yuan et al., 2018), suggesting yet another anti-obesity action for this enzyme.

In this context, TETA would be a new type of CRM not falling in any of the previously known categories (Madeo et al., 2014),(Madeo et al., 2019), namely (i) agents that deplete acetyl CoA by inhibiting its synthesis (examples: hydroxycitrate, SB-204990, two inhibitors of ATP citrate lyase), (ii) agents that inhibit acetyl transferases (examples: aspirin and spermidine that inhibit EP300) and (iii) agents that activate deacetylases (examples: resveratrol and nicotinamide that activate sirtuin-1). Indeed, TETA would be an agent that causes the overconsumption (rather than reduced generation) of acetyl CoA secondary to the activation of SAT1, thereby acting as a CRM. Given that TETA lacks any major toxicity (as indicated by mouse and worm experiments, as well as by its long-term use for the treatment of humans with Wilson disease) (Walshe, 1969), (Litwin et al., 2019), (Mohr and Weiss, 2019), it is tempting to speculate that this property may be taken advantage of for repurposing TETA for novel clinical applications including the prevention or treatment of obesity with its comorbidities.

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**Conflicts of interest:** GK and FM are co-founder of Samsara Therapeutics. GK and FP hold a patent protecting new medical use for TETA. FM is a co-founder of the Longevity Lab.

**Legends to Figures:**

**Fig. 1: Metabolic effects of TETA on mice.** **(A-F)** Wild-type (WT) C57Bl6 male mice were treated with TETA (3000 ppm dissolved in the drinking water) starting at 7 weeks of age. After 2 weeks of treatment, the organs (heart, liver and muscle) were removed for the determination of metals **(A-C)**, TETA and its mono- or di-acetylated metabolites **(D-F)** (n=7/8 mice/group). **(G-J)** Additionally, in the liver of mice, it was determined the endogenous polyamines (n=8 mice/group). **(K,L)** WT male mice were treated with TETA (3000 ppm) and, after 5 weeks, livers were removed and the hepatic SAT1 activity **(K)** (n=9/10 mice) and *Sat1* mRNA expression **(L)** (n=5/6 mice) were determined. **(M)** WT and *Sat1*-/- male mice were treated with TETA (3000 ppm) for 2 weeks. Livers were collected from mice to determine the ratio N1-acetyl spermidine over spermidine (n=6/8 mice/group). **(N)** Schematic representation of polyamines flux. **(O-R)** WT and *Sat1*-/- male mice were treated with TETA (3000 ppm) for 2 weeks. 13C-spermidine was injected intraperitoneally (*i.p.*, 50 mg/Kg) 3 hours before the recovery of livers and plasma for the mass spectrometric quantitation of 13C N1-acetyl spermidine **(O,P)** and 13C putrescine **(Q,R)** (n=4/5 mice/group). Results in **(A-K, M and O-R)** are displayed as box and whisker plots, which show mean, first, and third quartiles, and maximum and minimum values, or in **(L)** as scatter dot plot mean  S.E.M. Circles indicate each mouse used in the experiment. For statistical analyses in **(A-L, O-R)**, *p* values were calculated by two-tailed unpaired Student’s t test comparing TETA-treated to untreated mice (\*p < 0.05, \*\* p < 0.01, \*\*\*p < 0.001). For statistical analyses in **(M)**, *p* values were calculated by two-way ANOVA with Sidak all comparisons (\*\*\*p < 0.001). Ctrl: control; FC: fold change; ns: not significant; w.w: whole weight.

**Fig. 2: Acetyl CoA depletion, deacetylation and autophagy induction by TETA.** **(A,B)** WT or *Sat1*-/- male mice (7 weeks-old) were treated with TETA (3000 ppm in drinking water) for 2 weeks and livers were removed to determine the acetyl-CoA/CoA ratio **(A)** and the abundance of 3-hydroxybutyrate **(B)** by mass spectrometric metabolomics (n=6/8 mice/group). Results are displayed as box and whisker plots, which show mean, first and third quartiles, and maximum and minimum values. Circles indicate each mouse used in the experiment. *p* values were calculated by two-tailed unpaired Student’s t test comparing TETA-treated to untreated mice (\*\* p < 0.01). **(C,D)** TETA was injected *i.p.* (100 mg/Kg), in WT or *Sat1*-/- male mice to determine, 8 hours later, the level of N lysine acetylation of hepatic proteins by indirect immunofluorescence (representative images in **C**, quantitation in **D**) (n=3/4 mice/group). Bar size: XXX. Results are displayed as scatter dot plot mean  S.E.M. Circles indicate each mouse used in the experiment. Statistical comparisons were done by two-tailed unpaired Student’s t test comparing TETA-treated to untreated mice (\*p<0.05). **(E,F)** In the same experimental conditions described in **(C,D)**, the autophagy associated level of LC3 lipidation was evaluated in presence, or not, of leupeptin (*i.p*., 15 mg/Kg) 2 hours before recovery of livers (representative blots in **E**,quantitation in **F**) (n=3 mice/group; n=3 experiments). Results are displayed as scatter dot plot mean  S.E.M. Open circles indicate the mean of each experiment. Statistical comparisons were done by one-way ANOVA test (Tukey correction) (\*\*p<0.01). Ctrl: control; FC: fold change; ns: not significant.

**Fig. 3: Metabolic effects of TETA in the context of obesogenic diets or leptin deficiency. (A-C)** WT male mice receiving standard chow diet, a high-fat diet (HFD) or normal chow diet with 30% sucrose in the drinking water for at least 12 weeks in presence, or not, of TETA administration (3000 ppm in drinking water or daily *i.p.* injection 100 mg/Kg). Similarly, leptin-deficient *ob*/*ob* male mice were treated with TETA (3000 ppm), receiving a normal diet. Body weight was monitored weekly **(A)**, and glucose tolerance **(B)** or insulin tolerance **(C)** tests were performed after 5 to 6 weeks of treatment (n=4/10 mice/condition; here is reported one representative experiment of, at least, two independent experiments). Longitudinal statistical comparisons, for mice weight gain **(A)**, were performed by Wald test (\*\*p<0.01, \*\*\*p<0.001). *p* values in **(B**,**C)** were determined by two-tailed unpaired Student’s t test of areas under the curve **(Figure S3A,B)** (\*p < 0.05, \*\*p<0.01). **(D,E)** In addition, at 12 weeks of TETA treatment in HFD fed mice, body composition was determined by magnetic resonance imaging (representative images in **D** and quantitation in **E**)(n=8/9 mice/group). Results are displayed as box and whisker plots, which show mean, first and third quartiles, and maximum and minimum values. Circles indicate each mouse used in the experiment. Statistical comparisons were done by two-tailed unpaired Student’s t test comparing TETA-treated to untreated mice (\*p<0.05). **(F-I)** Moreover, visceral white adipose tissues (representative images in **F** and quantification in **G**) or livers (representative images in **H** and quantification in **I**) were removed and subjected to hematoxylin and eosin staining and image analysis for quantification of the surface of individual adipocytes **(G)** or the occupancy of the liver by lipid droplets **(I)** were performed (n= 8-10 mice per condition). Bar size: 200 M in **(F)**;50 M in **(H)**. Results are displayed as single bar mean  S.E.M **(G)**, or box and whisker plots **(I)**, which show mean, first and third quartiles, and maximum and minimum values. Circles indicate each mouse used in the experiment. Statistical comparisons in **(G)** were done by XXXXX or by two-tailed unpaired Student’s t test **(I)** comparing TETA-treated to untreated mice (\*\*p<0.01, \*\*\*p<0.001). **(J)** Additionally, at 12 weeks of treatments plasma was drawn and subjected to a multiplexed quantification of multiply hormones and cytokines, plotting a heat map (HFD versus normal diet) in otherwise untreated or TETA-treated mice (n=10 mice/condition). Statistical comparisons were done by Wilcoxon test (\*p<0.05; \*\*p<0.01, \*\*\*p<0.001). Ctrl: control; min: minutes; ns: not significant. Cytokines: ACTH: Adrenocorticotropic hormone; ADPN: Adiponectin; FSH: Follicle-stimulating hormone; G-CSF: Granulocyte colony-stimulating factor; GH: Growth hormone; Gip: Gastric inhibitory polypeptide; IGFBP1/2/3/6/7: Insulin-like growth factor-binding protein 1/2/3/6/7; IL-18/22/1a: Interleukin; IP-10: Interferon gamma-induced protein 10; KC: Keratinocyte chemoattractant; LH: Luteinizing hormone; LIX: lipopolysaccharide-inducible CXC chemokine; MCP-3: Monocyte chemotactic protein-3; PAI-1 total: Plasminogen activator inhibitor-1; TSH: Thyroid-stimulating hormone;

**Fig. 4: SAT1-dependent metabolic effects of TETA. (A)** Male mice with the indicated genotypes (WT, *Sat1*-/- or *Atg4*-/-) were subjected to a HFD diet in presence of TETA administration (3000 ppm in drinking water) for at least 10 weeks (n=7-10 mice/group). The body weight was monitored weekly and longitudinal statistical comparisons, for mice weight gain, were performed by Wald test (\*p<0.05, \*\*\*p<0.001). **(B-E)**Glucose tolerance tests **(B,D)** and insulin tolerance tests **(C,E)**, were performed after 5 to 6 weeks of treatment in *Sat1*-/- and *Atg4*-/- mice, respectively (n=5-8 mice/group). **(F,G)** After 11 weeks, *Sat1*-/- mice were subjected to magnetic resonance imaging, representative image in **(F)** and quantitation in **(G)** (n=9 mice/group). **(H-K)** Histological analysis of visceral white adipose tissue **(H,I)**, liver histopathology **(J,K)** were performed and quantifiedin *Sat1*-/- (upper panels) and *Atg4*-/- (lower panels) mice (n=5-12 mice/group). Representative images are shown in **(H,J)** and quantifications are reported in **(I,K)** respectively. Bar size: 200 M in **(H)**;50 M in **(K)**.Results **(G,K)** are displayed as box and whisker plots, which show mean, first and third quartiles, and maximum and minimum values; circles indicate each mouse used in the experiment. Data in **(I)** are shown as bar with mean ± SEM. Statistical comparisons in **(G,K)** were done by two-tailed unpaired Student’s t test comparing TETA-treated to untreated mice. Statistical comparisons in **(I,K)** were done by XXXXX. The HFD control group of *Sat1*-/- mice in **(A-C)** is shared with **Figure S4(A-C)­**;HFD control group of *Sat1*-/- mice in **(F)** is shared with the control group in **Figure S4D**,as well as the control group in **(H-K)** is shared with **Figure S4(F-I)**. Ctrl: control; AUC: area under the curve; min: minutes; ns: not significant.

**Legends to Supplemental figures**

**Figure S1. Related to Figure 1. (A-C)** Kaplan-Meier **(A)** of WT C57Bl6 male mice at 18-month-old (*late-in-life* feeding) treated for long-term oral administration (26 months) with TETA (3000 ppm in drinking water). After 4 weeks of treatment, the body weight **(B)** and food and water intake, as g/day per 30 g b.w, **(C)** were evaluated (n=24/30 mice/group). Results are shown as box and whisker plots, which show mean, first and third quartiles, and maximum and minimum values. In **(B,C)** circles indicate the mean values for each cage and statistical significance was determined by two-tailed unpaired Student’s t test comparing TETA-treated to untreated mice. **(D)** Molecular structure of spermidine and TETA. **(E)** WT male mice were treated for 2 weeks with oral administration of TETA (3000 ppm) or spermidine (Spd; 5 mM in drinking water) and liver was collected for metabolome analysis (n=8 mice/group). Results are depicted as heat map (versus chow diet mice control group) in otherwise TETA or Spd-treated mice. For the statistical analysis, it was performed a linear models on the normalized data for each metabolite; the output coefficients represent the difference between the average value of one condition and the average value of the control condition. *p*-values result from two-tailed unpaired Student’s t test applied on coefficients (\*p<0.05; \*\*p<0.01, \*\*\*p<0.001). Several metabolites for WT mice are reported in **Figure 1(D-I)** and **Figure 2B**. **(F)** WT and *Sat1*-/- mice were fed in HFD and treated or not with TETA (3000 ppm) for 12 weeks, livers were collected for the mRNA assay of *Sat1* (n=3/4 mice/group). Results are displayed as scatter dot plot mean with  S.E.M. Circles represent each mouse used in the experiment. Statistical significance was determined by one-way ANOVA non-corrected to non-treated group (\*p < 0.05, \*\* p < 0.01). **(G-I)** Liver lysate from non-treated *Sat1*-/- mice were used for the evaluation of the activity **(G,** and the relative AUC in **H)** and half-life **(I)** of human recombinant SAT1 in the presence or not of two different concentrations of TETA (0.5 mM and 5 mM) (n=3 mice/group). Results are displayed as single bar **(H)** or scatter dot plots **(I)** as mean ± S.E.M. Circles **(I)** represent each mouse used in the experiment. Statistical significance in **(G-I)** was determined by one-way ANOVA non-corrected to non-treated group (\*p < 0.05, \*\* p < 0.01, \*\*\*p < 0.001). AUC: area under the curve; Ctrl: control; b.w: body weight; FC: fold change; ns: not significant.

**Figure S2. Related to Figure 2. (A,B)** WT and *Sat1*-/- male mice were treated with TETA (*i.p.*, 100 mg/Kg) and, 8 hours later, the level of N lysine acetylation of heart proteins by indirect immunofluorescence was evaluated (representative images in **A**, quantitation in **B**) (n=3 mice/group). Results are presented as scatter dot plot mean with  S.E.M. Circles represent each mouse used in the experiment. Statistical significance was determined by two-tailed unpaired Student’s t test comparing TETA-treated to untreated mice (\*p < 0.05). **(C-D)** Representative confocal images **(C)** of LGG-1-expressing embryos treated with increased concentration of TETA (0.5 mM to 10 mM) compared to vehicle and quantification of LGG-1 puncta per embryo in **(D)** (n= XXXX). Bar size: XXX. Data are represented as scatter dot plot mean  S.E.M. Statistical significance was determined by XXXX comparing to control untreated group (\*\*\*p < 0.001). **(E)** Kaplan-Meier curves of *C. elegans* in presence or not, for XXX, with different concentrations of TETA (1mM, 2.5mM or 5mM) XXXXX . Log-rank (Mantel-Cox) test was performed (\*\*\*p < 0.001). Ctrl: control; FC: fold change; LGG: mCherry puncta (mCherry::LGG-1); ns: not significant.

**Figure S3. Related to Figure 3. (A,B)** AUC of glucose and insulin tolerance test, related to **Figure 3B,C**. Results are displayed as bar plot with mean  S.E.M. For statistical analyses, *p* values were calculated by two-tailed unpaired Student’s t test comparing TETA-treated to untreated mice. **(C,D)** WT male mice were treated for 12 weeks with TETA (3000 ppm in drinking water) and fed with chow or HFD diet. After 8 to 9 weeks of treatment food intake was measured daily for 1 week in metabolic cages (n=4/5 mice/group). **(E-G)** WT male mice were fed in HFD and treated with TETA (3000 ppm), after 2 weeks organs (heart, liver and muscle) were removed for the determination of heavy metals (n=7/8 mice/group). Results are displayed as box and whisker plots, which show mean, first and third quartiles, and maximum and minimum values. Circles represent each mouse used in the experiment. For statistical analyses, *p* values were calculated by two-tailed unpaired Student’s t test comparing TETA-treated to untreated mice (\*\*\*p < 0.001). AUC: area under the curve; w.w: whole weight; ns: not significant.

**Figure S4. Related to Figure 4. (A-I)** Male mice (7 weeks-old), with the indicated genotypes (WT or *Sat1*-/-) were subjected to a HFD diet and treated with Spd (*i.p.*, 50 mg/Kg, 3 times/week) for at least 11 weeks (n=8-11 mice/group). The body weight **(A)** was monitored weekly and glucose tolerance tests **(B)** and insulin tolerance tests **(C)** which their AUC, were performed after 5 to 6 weeks of treatment (in *Sat1*-/- mice). Moreover, after 11 weeks, mice were subjected to magnetic resonance imaging, representative image in **(D)** and quantitation in **(E)**, as well as to histological analysis of visceral white adipose tissue **(F,G)** and liver histopathology **(H,I)** (n=9-12 mice/group). Representative images are shown in **(F,H)** and quantifications are reported in **(G,I)** respectively. Bar size: 200 M in **(F)**;50 M in **(H)**. Results in **(E,I)** are displayed as box and whisker plots, which show mean, first and third quartiles, and maximum and minimum values. Circles indicate each mouse used in the experiment. Data in **(G)** are shown as bar means  S.E.M. Longitudinal statistical comparisons, for mice weight gain in **(A)**, was performed by Wald test (\*p<0.05, \*\*\*p<0.001). Statistical analysis in **(B,C,E,I)** were calculated by two-tailed unpaired Student’s t test comparing TETA-treated to untreated mice, and in **(G)** were performed by XXXXX. **(J-P)** Similarly,*Atg4b*-/- male mice (7 weeks-old) were subjected to the same treatment as in **(A-I)** for 10 weeks (n=8 mice/group), the body weight was monitored weekly **(J)** and after 5/6 weeks of treatment glucose tolerance tests **(K)** and insulin tolerance tests **(L)**, which their AUC, were performed. Additionally, histological analysis of visceral white adipose tissue (representative images in **M**, quantitation in **N**), and liver histopathology (representative images in **O**, quantitation in **P**)were performed. Bar size: 200 M in **(M)**;50 M in **(O)**. Data in **(N)** are displayed as single bar means  S.E.M. Results in **(P)** are displayed as box and whisker plots, which show mean, first and third quartiles, and maximum and minimum values. Circles indicate each mouse used in the experiment. For statistical analysis, longitudinal statistical comparisons for mice weight gain **(J)**, were performed by Wald test; statistical comparisons in **(K,L,P)** were done by two-tailed unpaired Student’s t test comparing TETA-treated to untreated mice, and in **(N)** were done by XXXXX. AUC: area under the curve; min: minutes; ns: not significant; Spd: spermidine.

**KEY RESOURCES TABLE**

|  |  |  |
| --- | --- | --- |
| **REAGENT or RESOURCE** | **SOURCE** | **IDENTIFIER** |
| **Antibodies** | | |
| MAP1LC3B | Cell Signaling Technologies | 2775S |
| ß-actin [AC-15] (HRP) | Abcam | ab49900, clone AC-15; RRID: AB\_867494 |
| Acetylated-Lysine | Cell Signaling Technologies | 9441S |
| Alexa Fluor® 568 anti-mouse | Invitrogen | A11031 |
| **Chemicals, Peptides, and Recombinant Proteins** | | |
| BSA | Euromedex | 04-100-812-E |
| Amersham ECL Prime | GE Healthcare | RPN2232 |
| DAPI Fluoromount-G | SouthernBiotech, | 0100-20 |
| Glucose | Sigma Aldrich | G8270 |
| Insulin | Lilly | HI0210 |
| Spermidine-(butyl-13C4) trihydrochloride | Sigma Aldrich |  |
| Sucrose | Merck Millipore | 1.07687.5000 |
| Spermidine | Sigma Aldrich | 740780-5MG |
| Triethylenetetramine dihydrochloride (TETA) | Sigma Aldrich | T5033-25G |
| Leupeptin | Euromedex | Sp042217 |
| Tween 20 | Sigma Aldrich | P9416 |
| Tris-buffered saline (TBS) | Euromedex | ET220 |
| 14C-acetyl-CoA | Moravek Inc. |  |
| 8-methylspermidine |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
| hematoxilin-eosin-saffranin |  |  |
| Paraffin |  |  |
| Formaldehyde solution | Sigma Aldrich | F8775 |
| OCT | Sakura Fine technical Co., Ltd |  |
| Bio-Rad protein assay dye reagent | Bio-Rad | 5000006 |
| QIAzol | QIAGEN | 74104 |
| TaqMan Gene Expression Master Mix | Applied Biosystems | 4369514 |
| Ponceau S Solution | Sigma Aldrich | P7170 |
| Immun-Blot PVDF Membrane | Biorad | 1620177 |
| **Critical Commercial Assays** | | |
| RNeasy Mini Kit | QIAGEN | 79306 |
| Complete® protease inhibitor cocktail | Roche Applied Science |  |
| **Experimental Models: Organisms/Strains** | | |
| C57BL/6JOlaHsd mice | ENVIGO, Harlan | ENVIGO, Harlan |
| C57BL/6JRj mice | Janvier Laboratories | Janvier Laboratories |
| *Atg4b-/-* C57BL/6 mice | Gift of Dr. Carlos Lopez-Otin, University of Oviedo, Spain |  |
| SATKO mice | Gift of Dr. Tuomo Keinänen, University of Kuopio |  |
| Ob/Ob (B6.V-Lepob/OlaHsd) mice | ENVIGO, Harlan | ENVIGO, Harlan |
| **Oligonucleotides: *Mice*** | | |
| *Sat1* | Thermo Fisher | Mm00485911\_g1 |
| *Ppia* | Thermo Fisher | Mm02342430\_g1 |
| **Software and Algorithms** |  |  |
| Inkscape |  |  |
| Microsoft Office | Microsoft |  |
| GraphPrism |  |  |
| Image J |  |  |
| R software | (http://www.r-project.org/) |  |
| Olyvia | v2.4-1 |  |
| **Others** | | |
| Accu-Chek Performa (glycemia) | Accu-Chek | 4702354 |
| High Fat Diet (HFD) | Safe (France) | 260 HF |
| Chow diet | Safe (France) | R04 |
| StepOnePlus Real-Time PCR System | Applied Biosystems |  |
| **Bacterial and Virus Strains** |  |  |
| **Deposited Data:**  **Scans of the original western blots before cropping** | | |

**Contact for Reagent and Resource Sharing**

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Guido Kroemer (kroemer@orange.fr).

**Experimental Model and Subject Details**

**Mouse experiments and tissue processing.** Wild-type C57BL/6 and Ob/Ob mice (Envigo, Gannat, France), *Atg4b-/-* C57BL/6 mice (gift of Dr. Carlos Lopez-Otin, University of Oviedo, Spain) and *Sat1*-/- mice (gift of Dr. Tuomo Keinänen, University of Kuopio, generated as described earlier (Niiranen et al., 2006) and breeding in Cordeliers Research Center), were bred and maintained according to the FELASA guidelines and local guidelines from the Animal Experimental Ethics Committee (#8216-2016121516216070v3, #2315-2015101617138161v1, #5333-2016050509281672v3, #5272-2016042112271931v2). Mice were housed in a specific pathogen–free conditions and in a temperature-controlled environment with 12 h light/dark cycles and received chow diet (R04, Safe, Augy, France) or high-fat diet (HFD; 260HF; Safe) and water *ad libitum*. Starting from 7-weeks of age, males mice (from the reported genetic strain) were divided in several groups fed with: (1) chow diet; (2) TETA (Triethylenetetramine dihydrochloride, Sigma T5033-25G), 3% weight/volume in the drinking water *ad libitum*; (3) HFD (20% protein, 36% lipids and 36.7% carbohydrate); (4) HFD and TETA; (5) chow diet + 30% sucrose in the drinking water *ad libitum*; (6) chow diet + TETA (daily intraperitoneally injection, *i.p.*, 100 mg/Kg) + 30% sucrose in the drinking water *ad libitum*. Mice were weight once a week, to monitor their weight gain, and subjected to metabolic tests around the fifth/sixth week of treatment. Then, based on the different settings, animals were sacrificed after 2-20 weeks of treatment by cervical dislocation and organs collected and processed. For the lifespan analysis, 18-month-old C57BL/6JRj mice (Janvier Laboratories), were divided in two groups and respectively treated, or not, with TETA 3% weight/volume in the drinking water ad libitum (*late-in-life* feeding), and they were monitored for 26 months. For the short-term autophagy induction studies, mice were treated with TETA (*i.p.,* 100 mg/Kg); 2 h before sacrifice mice were injected with Leupeptin (*i.p.,* 15 mg/Kg) and 8 h after TETA administration, mice were sacrificed. Tissues were snap-frozen in liquid nitrogen after extraction and homogenized in 2 cycles for 20 s at 5.500 rpm using a Precellys 24 tissue homogenator (Bertin Technologies, Montigny-le-Bretonneux, France) in 20 mM Tris buffer (pH 7.4) containing 150 mM NaCl, 1% Triton X-100, 10 mM EDTA and Complete® protease inhibitor cocktail (Roche Applied Science). Tissue extracts were centrifuged at 12.000 g at 4 ºC and supernatants were collected. Protein concentration in the supernatants was evaluated by the bicinchoninic acid technique (#23225, BCA protein assay kit). Then samples were subjected to western blot analysis. For acetyl lysine staining (AcLys), mice were sacrificed and the tissues collected and immediately fixed in 4% paraformaldehyde PBS solution. Fixed tissues were then kept 24 h in 15% sucrose solution and subsequently moved to a 30% sucrose solution for other 24 h. Tissues were embedded with tissue-TEK OCT compound (Sakura Fine technical Co., Ltd.) and frozen at -80 °C. 6-μm-thick sections were obtained from the frozen tissue samples by using a cryostat (CM3050S; Leica); it was cut 4 slices of tissue for each sample. The slices were then subjected to the immunohistochemistry assay.

**Immunohistochemistry.** Nonspecific binding sites were blocked with 5% bovine serum albumin in PBS, followed by incubation with primary antibodies, 1:200 in BSA 2% PBS solution (Acetylated-Lysine antibody, Cell Signaling Technology, 9441S), overnight at 4 °C. Later the slices were incubated with appropriate Alexa Fluor 568 anti-mouse (Invitrogen, A11031) 1:300 in BSA 2% PBS solution (Molecular Probes-Invitrogen, Eugene, OR, USA). Finally slice were mounted with DAPI Fluoromount-G (SouthernBiotech, 0100-20) and viewed under Axio Observer inverted fluorescence microscope (Carl Zeiss). The images were acquired with the Slide Scanner Zeiss Axio Scan Z1. For each of the three slices (one was used as negative control, it was not stained) of each organ, at least 10 pictures of three independent visual fields from at least three mice were acquired using Zen software. Acetylation intensity was evaluated by means of ImageJ software, calculating the corrected total cryosection fluorescence (CTCF) as “media of integrity density – (media of area of selected field X mean of fluorescence of background readings)”.

**Immunoblotting**. For immunoblotting, proteins extracts obtained by tissues lysis in radioimmunoprecipitation assay buffer (RIPA), were separated on 4-12% Bis-Tris acrylamide precast gels (Invitrogen) and electrotransferred to 0.2 μM polyvinylidene fluoride (PVDF) membranes (#1620177, Bio-Rad). Non-specific binding sites were saturated by incubating membranes for 1 h in 0.05% Tween 20 (#P9416, Sigma Aldrich) v:v in Tris-buffered saline (TBS) (#ET220, Euromedex) supplemented with 5% non-fat powdered milk (w:v in TBS), followed by an overnight incubation with primary antibodies specific for LC3B (#2775 Cell Signaling Technology). Membranes were cut in order to allow simultaneous detection of different molecular weight proteins. Equal protein loading was monitored by probing membranes with actin specific antibody (anti-beta Actin antibody [AC-15] (HRP), Abcam, ab49900). Membranes were developed with suitable horseradish peroxidase conjugates followed by chemiluminescence-based detection with the Amersham ECL Prime (#RPN2232, GE Healthcare) and the ImageQuant LAS 4000 software-assisted imager (GE Healthcare, Piscataway, NJ, USA). Quantification was performed by densitometry by means of ImageJ software (National Institutes of Health, Bethesda, Maryland, USA). Autophagy was quantified through evaluation of LC3-II/actin ratio according to (Klionsky et al., 2016).

**Histological analysis.** After the indicated treatment, mice were sacrificed by cervical dislocation and liver and visceral fat were immediately fixed in 4% paraformaldehyde PBS solution. Fixed samples were embedded in paraffin, and tissues were cut at 4µm and stained with stained with hematoxilin-eosin-saffranin (H&S). Each slide was examined using a Zeiss Axiophot microscope. Histological slides were acquired with a Virtual Slide microscope VS120-SL (Olympus, Tokyo, Japan), 20X air objective (0.75 NA). An in house algorithm was developed with ImageJ, in order to quantify the hepatic steatosis damage. The method calculates the proportion of steatosis in each sample by computing both the surfaces of the tissue and that occupied by the adipocytes. Tissue detection was performed at low resolution (image decimated 4 times) on the green component. The "Percentile" automatic segmentation was used followed by mathematical morphology operators (closing and then geodesic opening of size 3) in order to remove the artifacts. Calculation of the surface occupied by the adipocytes was carried out in two stages. Since the full-resolution image was large, it was processed in parts. It was therefore necessary first of all to calculate an optimal threshold for the adipocyte segmentation then to apply it to each piece of the original image. The calculation of the optimal segmentation threshold of the adipocytes was carried out on a detail, of 2000x2000 pixels, of the image at full resolution, taken in the center of the tissue. H&E color deconvolution was computed on this detail and the threshold parameters of the automatic thresholding "Triangle dark" applied to the second color component were stored. To process the whole image, H&E color deconvolution was applied to each piece of the original image and then the previously computed threshold parameters were applied to the second color component. Small artifacts were then removed by a size 4 geodesic opening. Close adipocytes were separated by means of a watershed. Finally, only adipocytes with a size between about 6 μm² and 400 μm² were taken into account. White cells adipose tissue size analysis was done with ImageJ and performed at low resolution (image decimated 4 times). To get the walls of cells, an automatic “Triangle” thresholding of each color component was done. To avoid artifacts, a Gaussian blurring of size 0.5 of each color component had been applied prior to the thresholding. The result of each thresholding was then combined (logical OR) to get the cell wall image. Big artifacts (normal tissue…) are removed thanks to mathematical morphology operators and size filter. Small artifacts (as broken parts of walls) are removed too by size filtering. For correct cell size analysis, big cells resulting from the gathering of several cells with broken walls have to be discarded. For that, a size filter can’t be used because “normal” big cells may have the same size that several small cells gathered. Watershed was used to characterize the gathered cells. Since these gathered cells usually keep remains of walls, watershed should split them. So only cells that had not been split by watershed were kept and taken into account for the size analysis. Finally to avoid artifacts, only adipocytes with a size in the range 1500 µm² to 30000µm² were kept. For the analysis of the adipocytes size distribution a Kolmogorov-Smirnov test was performed as following:

**Cytokines detection in plasma.** Plasma was harvested from blood collection tubes by centrifugation at 15.000 rpm for 30 min, and stored at −80 °C until use. Leptin (EZML-82K), ghrelin (EZRGRT-91), C-peptide and GIP (MMHMAG-44K), insulin (MMHMAG-44K, MADKMAG-71K), PAI-1 Total and resistin (MADKMAG-71K), IGFBP-1, IGFBP-2, IGFBP-3, IGFBP-6, and IGFBP-7 (MIGFBPMAG-43K), KC, eotaxin, G-CSF, IP-10, IL-1a and LIX (MCYTOMAG-70K), LH, FSH, TSH, ACTH and GH (MPTMAG-49K), and adiponectin (MADPNMAG-70K-01), IL-22 (MTH17MAG-47K) levels were measured using a mouse serum adipokine immunoassay kit (Cytokine multiplexing, Luminex assay), following the protocol provided by the manufacturers (Merck, Darmstadt, Germany and EMD Millipore, Temecula, CA, USA). The control, chow diet fed group, of this experiment is shared with the experiment reported in (Bravo-San Pedro et al., 2019).

**Tolerance Tests.** Mice were fasted for 6 h before glucose tolerance (GTT) and insulin resistance (ITT) tests. In GTT, non-anesthetized mice were injected *i.p.* glucose (2 g/Kg; Sigma Aldrich, G8270). In the ITT, mice were injected *i.p.* 0.75 U/Kg of insulin (Lilly, HI0210), prepared at 0.1 U/ml in advance. In both GTT and ITT, fasting plasma glucose levels were determined at time 0, and then after injection of glucose or insulin, glucose level were determined by glucometer (Accu-Chek Performa) in blood from tail vein, at specified time points.

**Analysis of whole-body composition.**  Non-invasive determination of lean tissue mass, fat mass and free fluid was performed on non-anesthetized mice using TD-NMR technology (minispec LF90II; Bruker BioSpin) in Centre de Recherche des Cordeliers. In other experiments, MRI was performed with a dedicated small-animal 4.7 Tesla MR system (Biospec 47/40 USR Bruker) using a quadrature transmit/receive body coil with a 7 cm inner diameter (Plateforme Imageries du Vivant, INSERM UMR 970). Mice were anesthetized with air and isoflurane (4% for induction and 1% during MRI). Experiments were performed with respiratory gating to avoid movement artefacts. We used a spin-echo 3D sequence of the entire mouse with the following parameters: TR/TE=750/65ms and 260 mm of resolution in the three dimensions, to bring out the signal of the fat of the mice.

**Food intake analysis.** Mice food intake was analyzed using metabolic cages. Mice were individually housed and acclimated to the metabolic cages for 24 h before experimental measurements. Subsequently the food consumption was measured daily for 5 days. The mice body weight has been measured before, during and after the experiments.

**Metabolomic analysis.**

***Sample preparation Tissue.*** About 30 mg of biological material for each condition are first weighted and solubilized into 1.5 mL polypropylene microcentrifuge tubes with ceramic beads with 1 mL of cold lysate buffer (MeOH/Water/Chloroform, 9/1/1, -20°C). They are then homogenized three times for 20 s at 5500 rpm using Precellys 24 tissue homogenator (Bertin Technologies, Montigny-le-Bretonneux, France), followed by a centrifugation (10 min at 15000 g, 4°C). Then upper phase of the supernatant is split in two parts: the first 270 µL used for the Gas Chromatography coupled by Mass Spectrometry (GC/MS) experiment, others 250 µL are used for the Ultra High Pressure Liquid Chromatography coupled by Mass Spectrometry (UHPLC/MS) experimentations. Concerning the GC-MS aliquots samples are evaporated and 50 µL of methoxyamine (20 mg/mL in pyridine) is added on dried extracts, then stored at room temperature in dark, during 16 hours. The day after, 80 µL of N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) is added and final derivatization occurred at 40°C during 30 minutes. Samples are then directly injected into GC-MS. Concerning the LC-MS aliquots, the collected supernatant is evaporated at 40°C in a pneumatically-assisted concentrator (Techne DB3, Staffordshire*,* UK). The LC-MS dried extracts are solubilized with 450 µL of MilliQ water. Samples are aliquoted (100 µL) for LC methods and backup. Biological samples and QC aliquots are kept at -80°C until injection or transferred in vials for direct analysis by UHPLC/MS. Concerning the rest of the supernatant and the pellet, 340 µl of methanol with 2% of sulfosalicylic acid (SSA) is added before vortex and centrifugation (10 min at 15000g, 4°C). 500 µl of the supernatant are transferred in a microtube and evaporated. The dried sample is spiked with 200 µl of MilliQ water before injection in UHPLC/MS of the polyamines method.

***Sample preparation plasma (lithium heparin).*** A volume of 50 µL of plasma is mixed with 500 µL a cold solvent mixture (MeOH/Water/Chloroform, 9/1/1, -20°C), into 1.5 mL eppendorf, vortexed and centrifugated (10 min at 15000 g, 4°C). Then upper phase of the supernatant is split in two parts: the first 220 µL used for the Gas Chromatography coupled by Mass Spectrometry (GC/MS) experiment, others 200 µL are used for the Ultra High Pressure Liquid Chromatography coupled by Mass Spectrometry (UHPLC/MS) experimentations. Concerning the GC-MS aliquots samples are evaporated and 50 µL of methoxyamine (20 mg/mL in pyridine) is added on dried extracts, then stored at room temperature in dark, during 16 hours. The day after, 80 µL of N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) is added and final derivatization occurred at 40°C during 30 minutes. Samples are then directly injected into GC-MS. Concerning the LC-MS aliquots, the collected supernatant is evaporated at 40°C in a pneumatically-assisted concentrator (Techne DB3, Staffordshire*,* UK). The LC-MS dried extracts are solubilized with 400 µL of MilliQ water. Samples are aliquoted (100 µL) for LC methods and backup. Biological samples and QC aliquots are kept at -80°C until injection or transferred in vials for direct analysis by UHPLC/MS. Concerning the rest of the supernatant and the pellet, 285 µl of methanol with 2% of sulfosalicylic acid (SSA) is added before vortex and centrifugation (10 min at 15000g, 4°C). 500 µl of the supernatant are transferred in a microtube and evaporated. The dried sample is spiked with 200 µl of MilliQ water before injection in UHPLC/MS of the polyamines method.

***Targeted analysis of CoAs and nucleoside phosphates by ion pairing ultra-high performance liquid chromatography (UHPLC) coupled to a Triple Quadrupole (QQQ) mass spectrometer.*** Targeted analysis is performed on a RRLC 1260 system (Agilent Technologies, Waldbronn, Germany) coupled to a Triple Quadrupole 6410 (Agilent Technologies) equipped with an electrospray source operating in positive mode. The gas temperature is set to 350°C with a gas flow of 12 L/min. The capillary voltage is set to 3.5 kV. 10 μL of sample are injected on a Column XDB-C18 (100 mm x 2.1 mm particle size 1.8 µm) from Agilent technologies, protected by a guard column XDB-C18 (5 mm × 2.1 mm particle size 1.8 μm) and heated at 40°C by a pelletier oven. The gradient mobile phase consists of water with 2 mM of DBAA (A) and acetonitrile (B). The flow rate isset to 0.2 mL/min, and gradient as follow: initial condition is90% phase A and 10% phase B, maintained during 4 min. Molecules are then eluted using a gradient from 10% to 95% phase B over 3 min. The column is washed using 95% mobile phase B for 3 minutes and equilibrated using 10% mobile phase B for 3 min. The autosampler is kept at 4°C. The collision gas is nitrogen. The scan mode used is the MRM for biological samples. Peak detection and integration of the analytes are performed using the Agilent Mass Hunter quantitative software (B.07.01).

***Widely-targeted analysis of intracellular metabolites gas chromatography (GC) coupled to a triple quadrupole (QQQ) mass spectrometer.*** The GC-MS/MS method is performed on a 7890B gas chromatography (Agilent Technologies, Waldbronn, Germany) coupled to a triple quadrupole 7000C (Agilent Technologies, Waldbronn, Germany) equipped with a High sensitivity electronic impact source (EI) operating in positive mode. The front inlet temperature is 250°C, the injection is performed in splitless mode. The transfer line and the ion-source temperature are 250°C and 230°C, respectively. The septum purge flow is fixed at 3 mL/min, the purge flow to split vent operated at 80 mL/min during 1 min and gas saver mode is set to 15 mL/min after 5 min. The helium gas flows through the column (J&W Scientific HP-5MS, 30m x 0.25 mm, i.d. 0.25 mm, 25µm d.f., Agilent Technologies Inc.) at 1 mL/min. Column temperature is held at 60°C for 1 min, then raises to 210°C (10°C/min), followed by a step to 230°C (5°C/min) and reaches 325°C (15°C/min), to be hold at this temperature for 5 min. The collision gas is nitrogen. The scan mode used is the MRM for biological samples. Peak detection and integration of the analytes are performed using the Agilent Mass Hunter quantitative software (B.07.01).

***Targeted analysis of bile acids by ion pairing ultra-high performance liquid chromatography (UHPLC) coupled to a Triple Quadrupole (QQQ) mass spectrometer.*** Targeted analysis is performed on a RRLC 1260 system (Agilent Technologies, Waldbronn, Germany) coupled to a Triple Quadrupole 6410 (Agilent Technologies) equipped with an electrospray source operating in positive mode. The gas temperature is set to 325°C with a gas flow of 12 L/min. The capillary voltage is set to 4.5 kV. 10 μL of sample are injected on a Column Poroshell 120 EC-C8 (100 mm x 2.1 mm particle size 2.7 µm) from Agilent technologies, protected by a guard column XDB-C18 (5 mm × 2.1 mm particle size 1.8 μm) and heated at 40°C by a pelletier oven. The gradient mobile phase consists of water with 0.2% of formic acid (A) and acetonitrile/isopropanol (1/1; v/v) (B) freshly made. The flow rate is set to 0.3 mL/min, and gradient as follow: initial condition is70% phase A and 30% phase B, maintained during 1.5 min. Molecules are then eluted using a gradient from 30% to 60% phase B over 9 min. The column is washed using 98% mobile phase B for 2 minutes and equilibrated using 30% mobile phase B for 2 min. After each injection, the needle is washed twice with isopropanol and thrice with water. The autosampler is kept at 4°C. The collision gas is nitrogen. The scan mode used is the MRM for biological samples. Peak detection and integration of the analytes are performed using the Agilent Mass Hunter quantitative software (B.07.01).

***Targeted analysis of polyamines by ion pairing ultra-high performance liquid chromatography (UHPLC) coupled to a Triple Quadrupole (QQQ) mass spectrometer.*** Targeted analysis is performed on a RRLC 1260 system (Agilent Technologies, Waldbronn, Germany) coupled to a Triple Quadrupole 6410 (Agilent Technologies) equipped with an electrospray source operating in positive mode. The gas temperature is set to 350°C with a gas flow of 12 L/min. The capillary voltage is set to 3.5 kV. 10 μL of sample are injected on a Column Kinetex C18 (150 mm x 2.1 mm particle size 2.6 µm) from Phenomenex, protected by a guard column C18 (5 mm × 2.1 mm) and heated at 40°C by a pelletier oven. The gradient mobile phase consists of water with 0, 1% of HFBA (A) and acetonitrile with 0,1% of HFBA (B) freshly made. The flow rate is set to 0.2 mL/min, and gradient as follow: initial condition is95% phase A and 5% phase B. Molecules are then eluted using a gradient from 5% to 40% phase B over 10 min. The column is washed using 90% mobile phase B for 2.5 minutes and equilibrated using 5% mobile phase B for 4 min. The autosampler is kept at 4°C. The collision gas is nitrogen. The scan mode used is the MRM for biological samples. Peak detection and integration of the analytes are performed using the Agilent Mass Hunter quantitative software (B.07.01).

***Untargeted analysis of intracellular metabolites by ultra-high performance liquid chromatography (UHPLC) coupled to a quadrupole-time of flight (QTOF) mass spectrometer.*** Profiling of intracellular metabolites is performed on a Liquid Chromatography (LC) 1260 system (Agilent Technologies, Waldbronn, Germany) coupled to a QTOF 6520 (Agilent Technologies) equipped with an electrospray source operating in both positive and negative mode, successively, and full scan mode from 50 to 1000 Da. The gas temperature is set to 350°C with a gas flow of 12 l/min. The capillary voltage is set to 3.5 kV, the fragmentor to 175 V and the skimmer to 65 V. Two reference masses are used to maintain the mass accuracy during analysis: m/z 121.050873 and m/z 922.009798 in positive mode and m/z 112.985587 and m/z 980.016375 in negative mode. 10 μL of sample are injected on a SB-Aq column (100 mm × 2.1 mm particle size 1.8 μm) from Agilent Technologies, protected by a guard column XDB-C18 (5 mm × 2.1 mm particle size 1.8μm) and heated at 40°C. The gradient mobile phase consists of water with 0.2% of acetic acid (A) and acetonitrile (B). The flow rate is set to 0.3mL/min. Initial condition is 98% phase A and 2% phase B. Molecules are then eluted using a gradient from 2% to 95% phase B in 7min. The column is washed using 95% mobile phase B for 3 minutes and equilibrated using 2% mobile phase B for 3min. The autosampler is kept at 4°C. Peak detection and integration of the analytes are performed using the Agilent Mass Hunter quantitative software (B.07.01).

***Untargeted analysis of intracellular metabolites by ultra-high performance liquid chromatography (UHPLC) coupled to a Q-Exactive mass spectrometer. Reversed phase acetonitrile method.*** Profiling of intracellular metabolites is performed on a Dionex Ultimate 3000 UHPLC system (Thermo Scientific) coupled to a Q-Exactive (Thermo Scientific) equipped with an electrospray source operating in both positive and negative mode and full scan mode from 100 to 1200 m/z. The Q-Exactive parameters are: sheath gas flow rate 55 au, auxiliary gas flow rate 15 au, spray voltage 3.3 kV, capillary temperature 300°C, S-Lens RF level 55 V. 10 μL of sample are injected on a SB-Aq column (100 mm × 2.1 mm particle size 1.8 μm) from Agilent Technologies, protected by a guard column XDB-C18 (5 mm × 2.1 mm particle size 1.8 μm) and heated at 40°C by a pelletier oven. The mass spectrometer is calibrated with sodium acetate solution dedicated to low mass calibration. The gradient mobile phase consists of water with 0.2% of acetic acid (A) and acetonitrile (B). The flow rate is set to 0.3 mL/min. Initial condition is 98% phase A and 2% phase B. Molecules are then eluted using a gradient from 2% to 95% phase B in 22 min. The column is washed using 95% mobile phase B for 2 minutes and equilibrated using 2% mobile phase B for 4 min. The autosampler is kept at 4°C. Peak detection and integration are performed using the Thermo Xcalibur quantitative software (3.1.).

***Quality control policy.*** A daily qualification of the instrumentation is set up with automatic tune and calibration processes. These qualifications are completed with double injections of standards mixes, at the beginning and at the end of the run, as for a blank extracted sample to control the background impurities. Mixtures are adapted for each chromatographic method. After the extraction, the pool of QC sample are used to passivate the column before the analysis with the proper biological matrix and re-injected during the batch to monitor and correct analytical bias occurring during the batch (m/z, retention time and sensitivity drifts) during post acquisition treatment signal. The following standards and reactive were from Sigma Aldrich: Acetonitrile (Isopropanol), Methanol, Chloroform, Acetic acid, Dibutylamine acetate concentrate (DBAA), Methoxyamine hydrochloride, N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA), Pyridine, Ammonium acetate, Sulfosalicylic acid, Heptafluorobutyric acid (HFBA). Ammonium carbonate was from VWR.

***Heatmap analyses.*** Itwasfirst normalized the data by dividing all the values by the average value for each metabolite. Using R software (R version 3.5.0), it was fitted linear models on the normalized data for each metabolite. The output coefficients represent the difference between the average value of one condition and the average value of the control condition. The *p*-values result from two-tailed unpaired Student’s t-test applied on coefficients.

**Analysis of SAT1 activity.** Tissues were homogenized using TissueLyser (30 Hz for 2-4 min, Qiagen) to an ice-cold buffer containing 25 mM Tris-HCl pH 7.4, 1 mM DTT, 0.1% Triton X-100 and 1x Complete EDTA-free protease inhibitor cocktail (Roche Diagnostics). Lysates were centrifuged at 12.000x g for 30 min at +4 C and the supernatant was used for SAT1 and SMOX activity assays. SAT1 activity was determined by an assay which measures the amount of incorporation of radioactivity from 14C-acetyl-CoA into 8-methylspermidine (8-MeSpd) in 10 min at +37 °C as described previously in (REF). A standard assay mixture contained 100 mM Tris-HCl pH 7.8, 1 mM semicarbazide, 5 mM EDTA, 3 mM 8-MeSpd (PMID: 21639123), and 50 nCi (50-60 mCi/mmol) of 14C-acetyl-CoA (Moravek Inc.) in a total volume of 100 μl. 8-MeSpd has higher affinity for SAT1 in comparison to spermidine (Km 78 ± 3 µM and Vmax of 7.35 ± 0.10 µmol/min/mg vs. Km 151 ± 15 µM and Vmax of 4.28 ± 0.13 µmol/min/mg). Furthermore, 8-MeSpd cannot be enzymatically acetylated at *N*8-position, thus allowing feasible SAT1-specific activity assay in crude enzyme preparations.

**SAT1 half-life.** XXXXXXXX

**Cu-chelation.**

**Gene expression analysis.** Total RNA from murine tissues was isolated by QIAzol (QIAGEN) trituration with Precellys 24 tissue homogenator (Bertin Technologies, Montigny-le-Bretonneux, France), followed by column purification with RNeasy Mini Kit (QIAGEN). Following the manufacturer’s instructions, Superscript III Reverse Transcriptase (Invitrogen) was used with random hexamers (Promega) for generation of cDNA. Quantitative PCR was performed on a StepOnePlus Real-Time PCR System (Applied Biosystems) using TaqMan Gene Expression Master Mix (Applied Biosystems) and the following TaqMan Gene Expression Assays: murine Sat1 (Mm00485911\_g1) purchased by ThermoFisher Scientific.

***C. elegans* experiments.** XXXX

**Statistical analysis.** Data are reported as the mean ± SEM, or Box and whisker plots (mean, first and third quartiles, and maximum and minimum values) as specified. For statistical analyses, *p* values were calculated by two-way ANOVA with Sidak all comparisons, one-way ANOVA non-corrected, one-way ANOVA with Tukey’s multiple comparisons test, Wald test (Enot et al., 2018) or two-tailed unpaired Student’s *t*-test. Differences were considered statistically significant when *p*-values are: \* (p<0.05), \*\* (p<0.01), \*\*\* (p<0.001).

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