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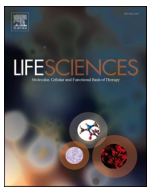

Review article

Role of hydrogen sulfide in the regulation of lipid metabolism: Implications on cardiovascular health

Flori Lorenzo, Piragine Eugenia, Calderone Vincenzo, Testai Lara *

Department of Pharmacy, University of Pisa, via Bonanno, 6-56120 Pisa, Italy

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ABSTRACT

The World Health Organization (WHO) defines obesity as an urgency for health and a social emergency. Today around 39 % of people is overweight, of these over 13 % is obese.

It is well-consolidated that the adipose cells are deputy to lipid storage under caloric excess; however, despite the classical idea that adipose tissue has exclusively a passive function, now it is known to be deeply involved in the regulation of systemic metabolism in physiological as well as under obesogenic conditions, with consequences on cardiovascular health. Beside two traditional types of adipose cells (white and brown), recently the beige one has been highlighted as the consequence of the healthy remodeling of white adipocytes, confirming their metabolic adaptability. In this direction, pharmacological, nutraceutical and nutrient-based approaches are addressed to positively influence inflammation and metabolism, thus contributing to reduce the obese-associated cardiovascular risk.

In this scenario, hydrogen sulfide emerges as a new mediator that may regulate crucial targets involved in the regulation of metabolism. The current evidence demonstrates that hydrogen sulfide may induce peroxisome proliferator activated receptor γ (PPARγ), a crucial mediator of adipogenesis, inhibit the phosphorylation of perlipin-1 (plin-1), a protein implicated in the lipolysis, and finally promote browning process, through the release of irisin from skeletal muscle. The results summarized in this review suggest an important role of hydrogen sulfide in the regulation of metabolism and in the prevention/treatment of obese-associated cardiovascular diseases and propose new insight on the putative mechanisms underlying the release of hydrogen sulfide or its biosynthesis, delineating a further exciting field of application.

1. Introduction

Obesity is a multifactorial pathology defined phenotypically by the abnormal and excessive deposition of visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT). In the specific, an accumulation of VAT is considered an unhealthy phenomenon, often accompanied by comorbidities that deeply impact both people's health and global health economics [1].

Type 2 diabetes (T2D), metabolic syndrome, cardiovascular diseases (CVDs), hypertension, dyslipidemia, non-alcoholic fatty liver disease, chronic kidney disease, and cancer are just some diseases related to obesity which contribute to the increased mortality of obese subjects [2]. It is also estimated that the burden of general health costs for obese people is about 30 % higher than that for normal weight people [3].

The World Health Organization (WHO) defines overweight when the body mass index (BMI, *i.e.* the ratio between body weight (Kg) and body height (m^2)) is equal to or greater than 25 and obesity when BMI is greater than 30. For children, the diagnosis is more complex, and age needs to be considered to distinguish between being overweight and obese.

WHO estimates dating to 2016 indicate that the prevalence of obesity worldwide increased almost three-fold in the last 40 years. In 2016, more than 1.9 billion adults over 18 were overweight, among them, over 650 million exceeded the threshold obesity. In particular, 39 % of men and 40 % of women were overweight, while 11 % of men and 15 % of women were obese.

This scenario spreads to the pediatric population as well. WHO estimated that nearly 38.2 million children under 5 were overweight or obese in 2019. The prevalence of overweight and obesity among children and adolescents aged 5–19 has also increased by about 14 % over

* Corresponding author.
E-mail addresses: lorenzo.flori@farm.unipi.it (F. Lorenzo), eugenia.piragine@unipi.it (P. Eugenia), vincenzo.calderone@unipi.it (C. Vincenzo), lara.testai@unipi.it (T. Lara).

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the past 40 years [4].

Furthermore, in the last years, due to the SARS-CoV-2 health emergency, the situation has worsened, becoming an epidemic within the pandemic. Indeed, a more sedentary lifestyle, often associated with reduced physical activity, and increased consumption of gratifying and palatable food (fatty and/or sugary foods), contributed to the increased incidence of overweight/obesity. On the other hand, obesity has emerged as one of the chronic diseases associated with more serious complications during the SARS-CoV-2 pandemic [5].

2. Role of adipose tissue in obesity

The adipose cells are distributed throughout the whole body (facial, cervical, supraclavicular, periarticular and epicardial, axillary, periaortic, paravertebral, subcutaneous, perirenal, visceral and retroperitoneal, inguinal, gonadal) and have different morphological and functional characteristics, taking part in the regulation of fat metabolism in physiological as well as under obesogenic conditions.

Generally, two different cell types are involved in lipid storage: the white and the brown adipocytes.

More recently a third adipocyte phenotype, defined as beige, and with middle characteristics between white and brown, has been described. However, beside this classification, mainly based on structural and functional differences and anatomic localization, several studies have provided comprehensive information on the presence of adipose tissue in many parts of the body, highlighting a huge complexity, especially in the regulation of cardiovascular system.

Indeed, beside the well-known white adipocytes located in the subcutaneous and visceral areas and the brown metabolically active adipocytes responsible for the thermogenesis, adipocytes have been also described around large blood vessels, such as the aorta and mesenteric arteries [6] (named as perivascular adipose tissue (PVAT)), and the myocardium (named as epicardial adipose tissue (EAT)) (Fig. 1).

2.1. White adipose tissue

White adipose tissue (WAT) plays a primary role in the control of energy and metabolic homeostasis. It is classified into SAT and VAT according to localization. WAT is the main organ involved in the accumulation, storage, and usage of lipid components. White adipocytes are large spheric cells in which about 90 % of the volume is occupied by triglyceride (TG) deposits, while mitochondria are minimal and uncoupling protein 1 (UCP1) expression is nearly lacking. However, its functions extend to an active involvement in energy production and paracrine modulation of the metabolic functionality of other tissues [7,8].

The ability of adipose tissue to manage the daily fat intake and a potential excess of calories is crucial in the adaptation process [9,10]. In this context, VAT has been generally considered to be linked to the development of metabolic disorders, whereas SAT has a protective function. Indeed, in obesity, the disproportionate caloric intake fails to be managed properly by the SAT leading to the deposition of ectopic fat in other tissues involved in metabolic homeostasis (VAT, liver, heart, and skeletal muscle) with the consequent onset of insulin-resistance [11,12].

Under prolonged conditions of positive energy balance, WAT reacts to lipid accumulation by triggering a hypertrophic program, increasing the size of the adipocytes; once it reaches a cut-off point, it triggers hyperplastic processes of cellular differentiation by forming new mature adipocytes from preadipocyte precursors and, within reasonable limits,

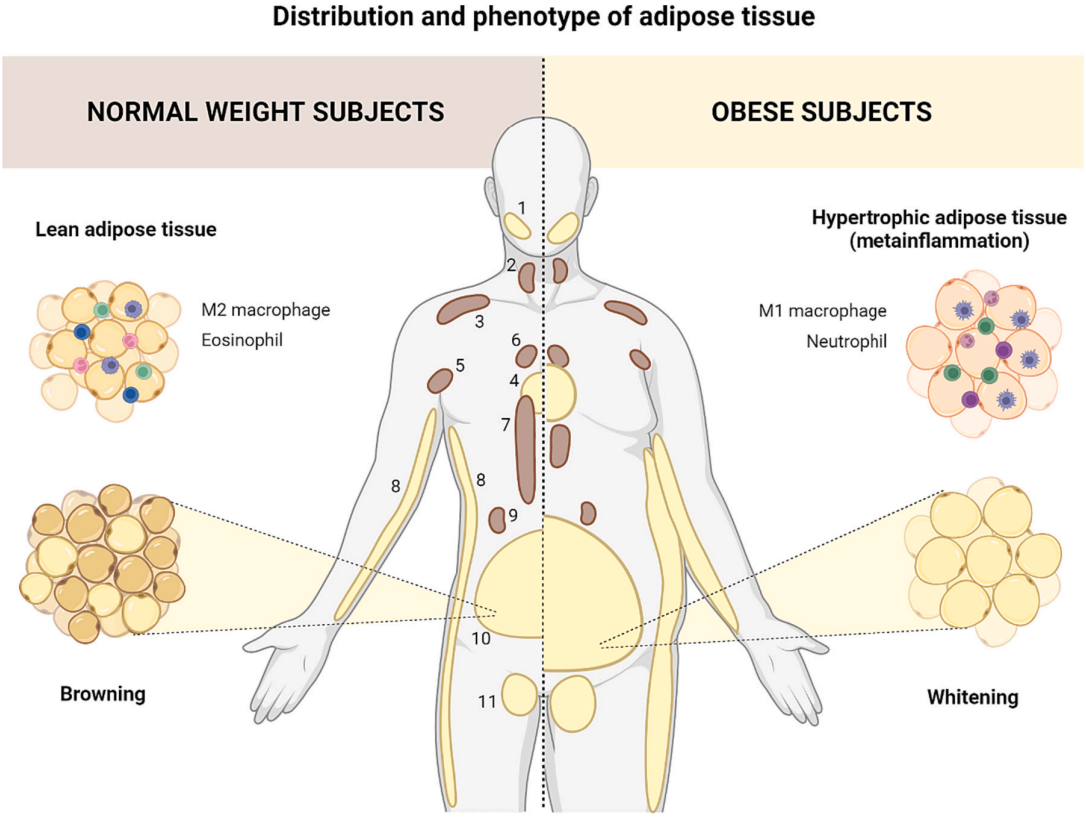

Fig. 1. Distribution and phenotype of adipose tissue. Differences between the healthy and altered phenotype characteristic of a metabolic imbalance such as obesity. Legend of the location of the adipose deposits: 1. facial, 2. cervical, 3. supraclavicular, 4. periarticular and epicardial, 5. axillary, 6. periaortic, 7. paravertebral, 8. subcutaneous, 9. visceral and retroperitoneal, 10. perirenal, 11. inguinal and gonadal.

leading to the development of a "healthy WAT" [7]. Once a threshold is exceeded, the hypertrophic response of the white adipocytes becomes overwhelming, and lipids accumulate causing excessive stress on the adipocytes which respond by triggering a mild chronic inflammation (meta-inflammation). This process is driven by immune cell infiltration; in particular, macrophages are the most abundant immune cells in the adipose tissue and the predominance of M1/M2 phenotype is indicative of a healthy or unhealthy condition of adipose tissue. Indeed, M1-like macrophages promote altered secretion of adipokines and pro-inflammatory cytokines, peripheral lipotoxicity, and consequent decreased insulin sensitivity [13,14]. As regard the adipokines, the expression and secretion of many pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1, also known as CCL2) and resistin are elevated in obesity, and each factor contributes to the activation of the chronic inflammatory state in adipose tissue through interaction with pro-inflammatory immune cells. Conversely, adiponectin expression, adipokine secreted by WAT, is closely correlated with the anti-inflammatory state of adipose tissue in rodents and humans (Fig. 1) [15]. Indeed, adiponectin is considered as a subclinical biomarker of obesity and its reduction, as well as a down-regulation of the specific receptors, are associated with insulin resistance in high-fat diet (HFD) fed subjects [16].

Interestingly, the exposition of WAT at healthy conditions, represented by cold exposition, physical exercise or some nutrients intake (including vegetables rich in polyphenols), drives a specific process named browning, because white adipocytes acquire morphological features similar to the brown ones, enriching itself of mitochondria and increasing the expression of UCP1. Moreover, it may contribute to change the phenotype of macrophages, shifting their polarization to M2, characterized by secretion of anti-inflammatory cytokines, including IL4 and IL10, and preservation of insulin sensitivity [17,18].

2.2. Brown adipose tissue

The brown adipose tissue (BAT) is a metabolically active organ, characterized by numerous tiny lipid drops (multilocular), a high density of mitochondria and elevated vascularization (similar to skeletal muscle). BAT is located mainly at inter-scapular level and utilized by mammals for cold-induced thermogenesis; indeed, in these adipocytes, UCP1, which is responsible for uncoupling of mitochondrial respiration from ATP synthesis and then associated to heat dissipation, is highly expressed [19,20]. BAT is positively associated with a healthy metabolism, being involved in thermogenesis and then in the utilization of lipidic depots to produce energy. However, BAT is also altered in obesity, and the meta-inflammatory process negatively impacts its functionality. Although BAT demonstrates greater resistance against inflammation and macrophage infiltration than WAT, a sufficiently prolonged HFD triggers an inflammatory process [21,22]. Inflammation affects insulin sensitivity in both mouse and human models, reducing glucose uptake which represents a critical substrate for thermogenic activity of BAT [23]. The thermogenic activity of BAT decreases in a mouse model of genetic obesity (ob/ob), in type 2 diabetic db/db mice and in diet-induced obesity, due to the reduction of UCP1 expression and the increase of pro-inflammatory markers, such as TNF-α and MCP-1 [23,24]. Such a type of process has been defined as whitening and has been also observed during aging (Fig. 1) [25].

2.3. Beige adipose tissue

Beige adipose tissue (BeAT) shares characteristics with WAT and BATin both morphology and function. The beige phenotype is defined as brown-like mainly due to the presence of UCP1 (missing in WAT and widely expressed in BAT) and the consequent energy dissipation capabilities [26]. However, this similarity from a functional point of view could be misleading regarding its location and lineage. BeAT is mainly localized in WAT deposits, sharing a common lineage. While brown adipocytes derive from progenitor cells common to myocytes, beige adipocytes develop from a lineage shared with white adipocytes [27,28]. Experimental evidence indicates that beige adipocytes, like the white ones, derive from preadipocyte cells, which in turn developed from perivascular and endothelial cells [29]. In addition to sharing the origin, WAT and BeAT show a peculiar plasticity. About this, the browning process can promote the *de novo* production of beige adipocytes from precursors located mainly in WAT depots [30,31]. Furthermore, it is interesting to underline how WAT and BeAT show their own plasticity capable of promoting a trans-differentiation mechanism affecting the mature white adipocytes themselves which, following appropriate stimuli, can acquire a beige phenotype. This plasticity is bidirectional and, under particular conditions, can lead beige adipocytes to acquire a white phenotype [32].

2.4. Adipose depots critical in cardiovascular health

2.4.1. Perivascular adipose tissue (PVAT)

PVAT has been described around the large vessels and performs functions of protection and mechanical regulation of blood vessel tone [33].

PVAT surrounding abdominal vessels, such as the abdominal aortic segment and mesenteric arteries in humans and mice, shows a predominantly white adipocyte phenotype with poor or completely absent UCP1 expression [34]. Interestingly, PVAT of rodent thoracic vessels has a thermogenic active adipocyte phenotype, with multilocular lipid distribution, high mitochondrial activity and UCP1 expression [35,36], suggesting two different functions based on the anatomic localization. However, experimental evidence supports the adaptive capacity of PVAT in the management of physiological balances and pathological alterations, such as inflammation and atherosclerosis, and its contribution in thermogenesis. In this regard, autopsy studies on Siberian adults and analyses on murine models of cold exposure demonstrate the induction of the browning process in both thoracic and abdominal/mediastinal PVAT by increasing the expression of UCP1 and peroxisome proliferator-activated receptor-gamma coactivator-1α (PGC-1α) [35,37]. In addition, the overexpression of the UCP1-driven mitochondrial membrane protein MitoNEET in PVAT prevented intravascular temperature drop during cold exposure and promoted increase of energy expenditure [38]. Conversely, the ablation of PVAT in ApoE-deficient mice with specific deletion of peroxisome proliferator activated receptor γ (PPARγ) promoted atherosclerotic lesions, and exposure to cold had no protective effect [35]. *Ex vivo* experiments on isolated rings of rat thoracic aorta and mesenchymal arteries highlighted the role of PVAT in vascular relaxation following stretch [39]. PVAT regulated and mitigated the effects of norepinephrine-induced adrenergic stimulation in isolated mesenteric arteries from mouse models *via* stimulation of β3-adrenergic receptors in the PVAT [40,41]. Nevertheless, obesity affects the PVAT-regulated vasorelaxant response to norepinephrine in the mesenteric arteries [42] by inhibiting the expression of vasodilatory mediators such as nitric oxide, angiotensin, and adiponectin and increasing the expression of vasoconstrictor agents such as angiotensin II [6,42,43].

Further experimental evidence demonstrates how obesity induces serious morphological and functional modifications of PVAT by altering buffering function in vascular contraction phenomena and predisposing to the development of obesity-related cardiovascular pathologies [44].

2.4.2. Epicardial adipose tissue (EAT)

EAT covers and holds part of the myocardial surface and is in direct touch with the coronary arteries allowing the transition of energy substrates [45].

EAT is mainly described as a WAT deposit even if it should be underlined the presence of genes and proteins typical of the thermogenic active phenotype, such as UCP1 and PRDM16. However, an interesting

study shows that EAT has a reduced expression of adipogenic markers, such as PPARγ, FABP4 and C/EBPα, and considerable levels of pro-inflammatory cytokines, indicating that the few clusters expressing UCP1 may derive from a legacy lost after the whitening post-birth process [46].

In pathological conditions, such as obesity or related pathologies (*i.e.*, atherosclerosis and hypertension), likewise the WAT, EAT is infiltrated by immune cells expressing pro-inflammatory genes (IL-1β, IL-6 and TNF-α) causing alteration in the production of cardioprotective polypeptides, such as adiponectin and adrenomedullin [47,48]. Interestingly, in recent studies, accumulation of EAT has been associated with left ventricular hypertrophy and diastolic dysfunction, typical of heart failure. However, high EAT volume is not necessarily associated with obesity and *vice versa*, although both conditions are correlated to heart failure. Probably, EAT may contribute to worsening the clinical condition and increase the mortality risk by infiltration or by mechanical compression, whereas obesity negatively acts on the cardiac performance exclusively through systemic ways [49].

Based on the achieved evidence, it is possible to summarize that WAT plays a negative role in metabolic disorders. It is well-known that the main role of white adipocytes is the storage of fatty acids (FAs) as TGs [50], and that the ability of adipocytes to promptly storage lipids prevents lipotoxicity in WAT and other ectopic tissues [51,52]. Moreover, an expansion of the number of white adipocytes is defined as adipogenesis and may improve insulin-sensitivity. Beside it, white adipocytes, under stimuli, may undergo lipolysis, characterized by hydrolysis of FAs and their release in the blood; such a phenomenon is associated to weight drop but it may worsen the condition, being FAs accumulated in ectopic tissues. Finally, white adipocytes may change their morphological and structural features, acquiring a brown-like phenotype. Therefore, pharmacological or nutraceutical strategies should be addressed to promote suitable modifications able to improve the WAT phenotype and positively impact on the systemic metabolism.

3. Role of H_2S in the regulation of adipose tissue and metabolism

Hydrogen sulfide (H_2S) is emerging as an endogenous mediator able to regulate crucial targets involved in the regulation of lipidic management.

H_2S is the third gasotransmitter of our organism, beside nitric oxide and carbon monoxide. It is mainly biosynthesized by two cytosolic enzymes, cystathionine γ-lyase (CSE) and cystathionine β-synthase (CBS), and one mitochondrial enzyme: 3-mercaptopyruvate sulfur-transferase (3MST), using a common substrate, the aminoacid L-cysteine [53]. However, additional/alternative biosynthetic ways have been described. H_2S can be generated by cysteinyl-tRNA-synthetase enzyme and by selenium-binding protein 1 (SELENBP1) [54]. In particular, this enzyme is able to regulate the conversion of organically bound sulfur to inorganic sulfur, although the potential role in the cellular function is not fully elucidated [54].

As regards degradation process, H_2S can follow different ways: first of all, H_2S is a reducing agent and so it is frequently consumed by several oxidant factors present in many tissues, but probably the main catabolic way for H_2S is represented by its oxidation at mitochondrial level, where it is rapidly converted to sulfite and sulfate species through the sequential action of quinone oxidoreductase, rhodanese and sulfur dioxygenase [55].

H_2S modulates a plethora of protein targets through persulfidation reactions. This is a direct post-translational modification of hydrosulfuryl groups of cysteine residues. In this regard, H_2S has been implicated in several pathways at adipose level related with adipogenesis, lipolysis and more recently browning, leading to discover the deep involvement of this gasotransmitter in the regulation of systemic metabolism (Fig. 2). The study of the potential effects and mechanisms of H_2S in isolated adipocytes and animal models of overweight/obesity has generally been performed with H_2S-releasing salts (*i.e.*, NaHS) or H_2S-releasing synthetic compounds (*i.e.*, GYY4137 and SG1002).

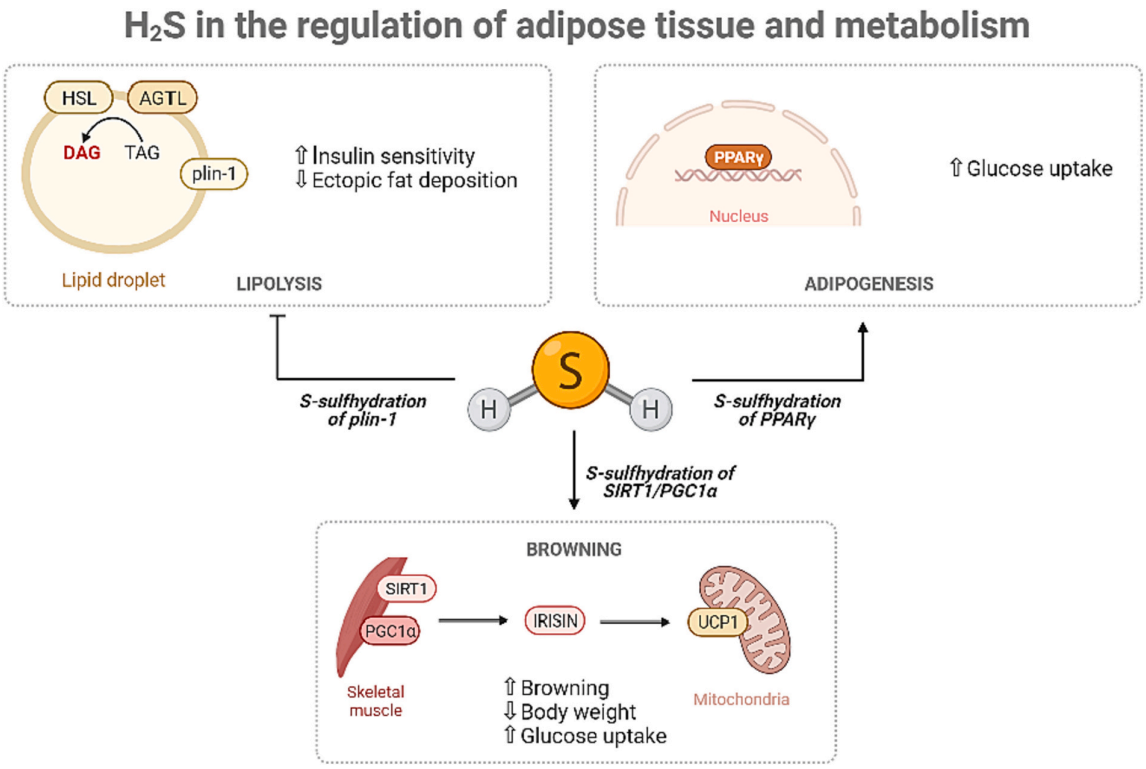

Fig. 2. Involvement of H_2S in the regulation of metabolic processes on adipose tissue. Modulation of lipolysis, adipogenesis and browning process *via* persulfidation.

Table 1
Main results of preclinical studies evaluating the potential role of H_2S-donors in obesity. Abbreviations: 3MST, 3-mercaptopyruvate sulfurtransferase; ADIPOQ, adiponectin-coding gene; aP2, adipocyte protein 2; ATGL, adipose triglyceride lipase; cAMP, cyclic adenosine monophosphate; Cd36, cluster of differentiation 36, also known as fatty acid translocase; CEBPα, CCAAT enhancer binding protein α; ChREBP, carbohydrate-responsive element-binding protein; CSE, cystathionine γ-lyase; DATS, diallyl disulfide; Dgat2, diacylglycerol O-acyltransferase 2; e.v., endovenously; eWAT, epididymal white adipose tissue; FABP4, fatty acid binding protein 4; FAS, fatty acid synthase; FFA, free fatty acid; FNDC5, fibronectin type III domain containing 5; HOMA-IR, homeostatic model assessment for insulin resistance; HSL, hormone-sensitive lipase; i.p., intraperitoneally; IRS-1, insulin receptor substrate-1; ISO, isoproterenol; iWAT, inguinal white adipose tissue; KFL15, Krüppel-like factor 15; LPL, lipoprotein lipase; NEFA, non esterified fatty acid; PDE, phosphodiesterase; p-ERK, phosphorilated extracellular signal-regulated kinase; PGC-1α, peroxisome proliferator-activated receptor γ coactivator-1α; plin-1, periliplin-1; PPARα/γ, peroxisome proliferator-activated receptor α/γ; PRDM16, PR domain-containing 16; SLC2A4, GLUT4-coding gene; SELENBP1, selenium-binding protein 1; SIRT1, sirtuin 1; SREBP, sterol regulatory-element binding protein; TG, triglyceride; Timm50, translocase of inner mitochondrial membrane 50; TIP47, tail-interacting protein of 47 kD; Tomm7, translocase of outer mithocondrial membrane; UCP1, uncoupling protein 1; VAT, visceral adipose tissue. Symbols: * 13 weeks for glucose and insulin tolerance tests; ** from obese patients; # gene expression; § protein expression; ↑ increased; ↓ decreased.

First author, year	Cell culture/ animal model	H_2S-donor (concentration or dosage)	Time	Main results (proposed mechanisms, if reported)
[70]	3 T3-L1 cells	GYY4137 or NaHS (both 50 μM)	2–7 days	↑ adipocyte differentiation (↑ aP2#,§, PPARγ#, CEBPα# and FABP4/aP2§); ↑ adipogenesis (FAS#, ChREBP# and SREBP1#); ↓ lipolysis (↓ plin-1#, HSL# and TIP47#); ↑ lipid accumulation
[72]	HFD-fed mice	GYY4137 (100 μmol/kg/day, i.p.)	4 weeks*	↑ energy storage and lipid accumulation in eWAT (↑ PPARγ#,§, PPARγ persulfidation at C139, plin-1#, LPL# and FABP4#; ↓ PDE activity); ↑ glucose tolerance and insulin sensitivity; ↓ serum TG levels
	3 T3-L1 cells	GYY4137 (100 μM)	3 days	↑ adipocyte differentiation, lipid accumulation and intracellular TG content (↑ plin-1§, PPARγ§#, PPARγ persulfidation at C139 and PPARγ DNA binding activity; ↓ PDE activity)
[74]	HFD-fed mice	DATS (40–80 mg/kg/day, by gavage)	12 weeks	↓ body weight gain and body fat rate; ↓ eWAT and iWAT weight; ↓ blood lipid levels; ↑ glucose tolerance and insulin sensitivity; ↓ lipid droplet size in eWAT and iWAT; ↑ lipolysis and FFA oxidation in WAT (↑ PGC-1α#,§, ATGL#,§, HSL#, UCP1#,§ and PPARα#); ↓ adipogenesis and lipogenesis in WAT (↓ PPARγ#,§, FABP4#,§, LPL#, Dgat2#, Cd36# and FAS#)
	3 T3-L1 cells	DATS (50 μM)	1–8 days	↓ lipid accumulation; ↓ adipogenesis and lipogenesis (↓ PPARγ§#, FABP4§#, LPL#, Dgat2# and Cd36#; ↓ binding of KLF15 to the promoter region of PPARγ)
[99]	HFD-fed mice	NaHS (2 mg/kg/day, i.p.)	12 weeks	↓ basal lypolisis and ISO-stimulated lypolisis in eWAT (↑ plin-1 persulfidation; ↓ p-HSL§)
	Isolated pre-adipocytes from eWAT	GYY4137 (1 mM)	1 hour	↓ basal lypolisis and ISO-stimulated lypolisis (↑ plin-1 persulfidation; ↓ p-plin-1§ and p-HSL§)
[100]	Isolated rat adipocytes	GYY4137 (1 mM)	1–4 h	↓ ISO-stimulated lypolisis (↓ p-plin-1§ and p-HSL§)
	HFD-fed mice	GYY4137 (200 mol/kg/day, i.p.)	13 weeks	↓ fasting blood glycerol levels and lipolysis in adipose tissue; ↓ fasting glucose and insulin levels; ↑ glucose tolerance, insulin sensitivity (↑ IRS-1§ in adipose tissue) and HOMA-IR index
[101]	Lean rats	Na_2S (100 mol/kg, e.v.)	15–60 min	↑ plasma glycerol and NEFA concentrations; ↑ cAMP levels in adipose tissue
[54]	SELENBP1 knock down 3 T3-L1 cells	GYY4137 (1–6 mM)	7 days	↑ lipid accumulation and bioenergetics
[115]	CSE knock down C2C12 cells	NaHS or Na_2S (both 20 μM)	6 h	↑ glucose metabolism (↑ glucose uptake) and irisin metabolism (↑ PGC-1α§ and FNDC5§ levels; ↑ irisin§ release by cultured myotubes)
[119]	3 T3-L1 cells	Allicin (1–100 ng/ml)	7 days	↑ lipid accumulation and browning process (↑ PRDM16#, PGC-1α#, UCP1#,§, KLF15§ and p-ERK; ↑ binding of KLF15 to the promoter region of UCP1)
	HFD-fed mice	Allicin (1 mg/kg/day, orally)	8 weeks	↓ body weight gain, iWAT mass and number of multilocular adipocytes in iWAT; ↑ browning of iWAT (↑ KLF15§, PRDM16#, PGC-1α#, UCP1#,§ and p-ERK§); ↑ O_2 consumption and heat production; ↓ respiratory exhcange ratio; ↓ serum TG and FFA levels

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Table 1 (*continued*)

First author, year	Cell culture/ animal model	H_2S-donor (concentration or dosage)	Time	Main results (proposed mechanisms, if reported)
[122]	Human VAT lysates**	GYY4137 (5–200 μM)	1–16 h	↑ adipogenesis (↑ADIPOQ#, FAS#, SLC2A4# and SIRT1#); ↑ sirtuin deacetylase and PPARγ transcriptional activities
[123]	3 T3-L1 cells	NaHS (30 μM)	4 days	↑ lipid accumulation and adipocyte differentiation; ↑ adipogenesis (↑ PPARγ# and PPARγ DNA binding activity *via* PPARγ persulfidation)
[125]	HFD-fed CSE knock out mice	NaHS (25 μmol/kg/ day, i.p.)	12 weeks	↓ body weight gain and relative mean adipocyte area; ↑ serum adiponectin; ↑ glucose tolerance and insulin sensitivity; ↓ serum insulin
[128]	3 T3-L1 cells	GYY4137 (1–6 mM)	6 days	↓ lipid accumulation
[129]	HFD-fed mice	SG1002 (40 mg/kg/day, orally)	10 weeks	↓ body weight gain and iWAT mass; ↓ adipocyte area in iWAT; ↑ O_2 consumption and metabolic rate; ↑ glucose tolerance and insulin sensitivity
	HFD-fed 3MST knock out mice	SG1002 (40 mg/kg/day, orally)	10 weeks	↓ body weight gain and iWAT mass; ↓ adipocyte area in iWAT; ↑ O_2 consumption and metabolic rate; ↑ glucose tolerance and insulin sensitivity; ↓ Tomm7# and Timm50# in iWAT

Table 1 shows the main findings of preclinical studies evaluating the potential role of H_2S-donors in obesity.

3.1. H_2S modulates the adipogenesis through persulfidation of PPARγ

Adipogenesis refers to as the process of differentiation of fibroblast-like mesenchymal precursors, located in the adipocyte district, towards a mature adipocyte phenotype. This process is characterized by a first phase of fibroblast shrinkage without morphological changes with characteristic expression of platelet-derived growth factor receptor-α (PDGFRα) and/or PDGFRβ leading to preadipocyte formation and a second phase of differentiation with arrest of cell growth in favor of a predominant lipid accumulation [56,57]. In cultured fibroblasts, bone morphogenic protein 2 (BMP2) and 4 (BMP4) bind to the transcription factor SMAD4 which in turn is able to promote adipocyte differentiation *in vitro* by stimulating the transcription of PPARγ [58,59].

PPARγ is essential for the adipocyte differentiation in culture and *in vivo* and, for this reason, it is identified as the main regulator of adipogenesis [60,61]. Indeed, PPARγ activates the transcription factor C/EBPα leading to the differentiation of cultured fibroblasts towards an adipocyte phenotype [62]. The synergistic action of PPARγ and C/EBP family proteins activates the transcription of genes characteristic of the mature white adipocyte phenotype, such as the adipose-specific gene (AP2), adiponectin, and the insulin receptor-1 (IRS-1) [63].

Other transcription factors, such as early growth response protein 1 (EGR1) and 2 (EGR2), modulate adipogenesis by regulating C/EBPβ transcription through the intermediate involvement of cAMP response element binding protein (CREB) [64].

Several studies on animal models have shown a close correlation between adipogenesis and preserved metabolic function. Indeed, adipogenesis allows the expansion of WAT in obese mice, limiting hypoxia, fibrosis, and chronic inflammation [65,66]. Interestingly, in several metabolically healthy obesity models, despite an increase in WAT mass, small white adipocytes are associated with preserved systemic metabolic health, if compared to the control group [67]. In another study, inhibition of adipogenesis in PDGFRβ+ perivascular cells, induced through PPARγ deletion, led to pathological and hypertrophic expansion of WAT after HFD [68]. Conversely, stimulation of adipogenesis in VAT, by PPARγ overexpression, reduced local inflammation and preserved adiponectin levels with a significant improvement in metabolic health in a condition of overnutrition without substantial changes in body weight or its adiposity [68].

In this context, selective PPARγ activators might represent a starting point to develop new agents for the treatment of obesity and various associated metabolic conditions, being characterized by improving of systemic metabolism in rodents and humans [69].

In the literature there is evidence that H_2S is a promoter of adipogenesis [70]. An *in vitro* investigation revealed an accumulation of TGs depots into the adipocytes and an enhancement of insulin sensitivity. Cai and colleagues elegantly demonstrated that H_2S persulfidated PPARγ at C139 residue. Such a post-translational modification may support nuclear accumulation of PPARγ, DNA binding and gene transcription, elevating glucose uptake and adipocyte differentiation. Parallelly, the authors also reported an inhibition of phosphodiesterase (PDE) enzyme, consistent with that in vascular smooth muscle cells [71] and with reduced insulin resistance [72].

Upstream of the master regulator of adipogenesis, PPARγ, a variety of targets have been identified, including the Kruppel-like factors (KLFs) [73]. It belongs to zinc finger protein, stabilized by a zinc ion bound to conserve cysteine and histidine residues. This family of proteins has been reported to regulate adipocyte development in several steps of the differentiation. Interestingly, diallyl trisulfide (DATS), one of the most recognized natural H_2S donors, showed to contain the body weight gain and lipid accumulation preventing the adipogenesis and lipogenesis acting on PPARγ, *via* KLF15 [74].

3.2. H_2S modulates the lipolysis through persulfidation of plin-1

Lipolysis is a dynamic process that can be required in different mechanisms of metabolic regulation and is involved in pathological alterations. It consists of the mobilization of TGs and conversion into FAs and glycerol. The released FAs can be oxidized to produce ATP and therefore energy for the organs and tissues. They can act as signaling molecules and, in adipocyte cells with a brown or brown-like phenotype, they promote the allosteric activation of non-shivering thermogenesis ensuring energy support for heat production [75,76].

Lipolysis is regulated by several hormonal factors, among which catecholamines, such as norepinephrine [77]. Through the involvement of adrenergic receptors β1, β2 and especially β3, they mediate non-shivering thermogenesis [78]. β-receptors cause the activation of adenylate cyclase, the increase of cyclic AMP (cAMP) and the activation of protein kinase A (PKA), able to modulate the phosphorylation of perlipin-1 (plin-1) [79,80].

Interestingly, plin-1 is an important protein involved in the lipolysis [81]. It is located on the surface of lipid drop and regulates the translocation of hormone sensitive lipase (HSL), the rate-limiting enzyme responsible for the hydrolysis of TGs [81–84]. Indeed, the phosphorylation of plin-1 promotes the translocation of HSL and stimulates lipolysis in adipocytes [85,86]. Plin-1 is also involved in protein-protein interaction with colipase alpha/beta hydrolase domain-containing

protein 5 (ABHD5), another protein implicated in this process. In fact, ABHD5 knockdown mice showed a reduction of both basal and induced lipolytic activity in adipocytes [87]. Granneman et al. identified ABHD5 ligands to stimulate lipolysis through the dissociation of ABHD5 from plin-1 [85]. Other actors seem to be involved in lipolysis, including patatin-like phospholipase domain containing-2 (PNPLA2)/adipocyte triglyceride lipase (ATGL) [88] and monoacylglycerol lipase (MGL) [89].

In pathological conditions, such as obesity, the excessive accumulation of TGs triggers lipotoxicity, due to ectopic lipid accumulation [90]. Moreover, the meta-inflammation state of WAT in obesity influences lipolysis. In particular, the release of pro-inflammatory cytokines promotes insulin resistance [91] and stimulates lipolysis through plin-1 phosphorylation [92,93]. Instead, conflicting results emerge in the relationships between insulin modulation and lipolysis in conditions of insulin resistance and T2D. In patients with T2D, insulin-mediated inhibition of lipolysis is believed to be impaired, thereby increasing plasma levels of FAs and ectopic accumulation of TGs. However, in chronically insulin-treated 3 T3-L1 cells and fat explants from insulin-resistant mice, only insulin-stimulated glucose uptake was decreased, while insulin suppression of lipolysis was intact [94]. In addition to impairing insulin signaling, adipocyte-derived FAs may also inhibit gluconeogenesis in the liver, where a hallmark of T2D is the loss of the inverse relationship between gluconeogenesis and lipogenesis.

To date, there are no specific pharmacological treatments capable of targeting lipolysis to reduce obesity and insulin resistance. A potential strategy is represented by stimulation of β3 receptors. Acute treatment of mice with β3 agonists stimulates lipolysis by doubling energy expenditure, reducing food intake, and increasing insulin levels approximately tenfold. Chronic administration of β3 agonists reduces WAT deposition and improves the insulin resistance in obese rodents [95,96]; nevertheless, this effect is not found in humans, probably due to cross reactivity with other β receptors. Furthermore, the positive effects of selective β3-agonists are observed at concentrations high enough to cause cardiac adverse effects [97,98]. A potential therapeutic target downstream of the β3 pathway might be ABHD5 protein.

Ding and co-workers in 2020 demonstrated that H_2S can directly persulfidate plin-1. Indeed, the H_2S donor GYY4137 induced the persulfidation of plin-1 in basal as well as in isoproterenol-stimulated conditions in adipocytes. A consequence of this post-translational modification is the failed phosphorylation of plin-1 and then the inhibition of lipolysis and the reduction of FAs release. Indeed, if plin-1 is phosphorylated, the translocation of phosphorylated HSL is promoted; on the contrary, plin-1 prevents the translocation of HSL and the subsequent lipolysis. Although such a mechanism is not associated to a reduction of the body weight (since under treatment with the H_2S donor the excess of energy storage is promoted in WAT), the insulin-resistance can be positively influenced and then it may be a way through which prevent the metabolic disorders [99]. Several evidence agrees findings of Ding et al. For example, in a previous study the specific inhibitor of CSE, DL-propargylglycine (PAG), promoted the time-dependent phosphorylation of HSL and plin-1, enhancing the adipocyte lipolysis. On the other hand, the H_2S precursor L-cysteine, as well as GYY4137, lowered the basal and isoproterenol-induced lipolysis. Therefore, the stimulation of H_2S/CSE system is associated to a reduced phosphorylation of plin-1 and HSL. Noteworthy, in HFD mice the supplementation with GYY4137 did not significantly influence the body weight and the visceral fat weight, but clearly improved the metabolic profile, reducing the fasting glucose and insulin levels and increasing the expression of IRS-1 receptor. In normal chow mice, H_2S donor was not able to influence the lipolysis. Very interestingly, PAG also up-regulated IRS-1 protein and 5′ adenosine monophosphate-activated protein kinase (AMPK) expression. Such a result, which seems to be in contrast with the findings about GYY4137, suggests a complex and differential regulation of the H_2S/CSE system, following various signaling pathways related to the metabolic condition [100].

Inconsistently with these findings, Beltowski and Wiorkowski reported that an H_2S administration was followed by a time-dependent increase of non-esterified fatty acids (NEFAs) and glycerol levels in the blood of lean rats. The authors tried to justify this discrepancy suggesting several hypotheses, including a different sensitivity to H_2S in relationship with dietary regimen. Indeed, under HFD the lipolysis rate probably is increased and the gasotransmitter can act for restoring the homeostasis. Interestingly, the H_2S-releasing salt Na_2S seems to stimulate the lipolysis through a cAMP-PKA-dependent pathway, since the specific inhibitor KT5720 abrogated its effects [101].

3.3. H_2S modulates the browning of white adipose tissue

A crucial trigger of the browning is irisin [18], a miokine mainly released from skeletal muscle after exercise and exposure to cold through the stimulation of PGC-1α, that in turn can be modulate by several protein kinases, including MAPK and AMPK, and by sirtuin-1 (SIRT1). Of note, recent experimental evidence points out that SIRT1 is the main responsible for PGC-1α deacetylation in skeletal muscle, whereas AMPK is responsible for the phosphorylation of PGC-1α and it is closely associated with the deacetylase activity of SIRT1 [102–104]. The release of irisin is driven by proteolytic cleavage of its precursor fibronectin type III domain-containing protein 5 (FNDC5) in the skeletal muscle. White adipocytes are a main target of circulating irisin, where the miokine may promote the UCP1-mediated thermogenesis [105,106]. UCP1 protein, located on the inner mitochondrial membrane, is responsible for the dissipation of the proton gradient (H^+) generated by the electron transport system promoting the oxidation of excess energy substrates.

Interestingly, the UCP1 stimulation is also regulated by other intracellular pathways, including the sympathetic activation of β3 receptors, that drive the thermogenic transcriptional response by phosphorylation of cAMP response element binding protein (CREB) and p38 MAPK. In this regard, p38 MAPK phosphorylates PGC-1α and transcription factor activating factor 2 (ATF2), which directly promotes transcription of the pgc1 α gene. The phosphorylated PGC-1α acts as a co-activator for PPARγ, inducing transcription of downstream thermogenic genes, including mainly UCP1 [107]. Moreover, natriuretic peptides, such as atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP), activate adipose tissue thermogenesis through cyclic GMP–dependent protein kinase (PKG) signaling [108].

In obesity condition, as well as in metabolic disorders, the browning process is deeply compromised. It has been probed that IL-1 affects WAT browning by reducing cAMP-mediated UCP1 gene expression [109] and cold-induced thermogenesis *in vivo* [110].

Although the stimulation of the browning in WAT is considered as an innovative strategy to improve the metabolic parameters, there are still no approved pharmacological treatments. β3 agonists are known to have positive effects on UCP1 expression and mitochondrial activity in WAT [98,111]. However, the clinical use of these drugs is hampered by the cardiovascular effects due to poor selectivity [112]. Mirabegron, a selective β3-agonist developed for the treatment of bladder overactivity, demonstrated positive effects on browning when administered in oral dose of 200 mg/day. Cardiovascular side effects are observed in this case as well [98].

In addition, triiodothyronine is involved in the regulation of metabolism through the activity on brain, liver, pancreas, skeletal muscle, WAT, and BAT [113]. Its administration induces UCP1 expression and promoted the browning process in humans [114].

Very recently, H_2S has been shown to regulate the glucose metabolism through stimulation of browning. Indeed, FNDC5/irisin pathway resulted impaired in skeletal muscle cells pre-treated with high levels of glucose and palmitate, to mimic diabetic conditions, but the administration of an H_2S donor improved the release of irisin and the expression of GLUT4 mRNA, suggesting positive effects on the glucose uptake. Similar results have been obtained *in vivo* on mice fed with a HFD for 16

weeks. Interestingly, the authors suggested that H_2S might activate PGC-1α, a transcription factor upstream to the FNDC5 hydrolysis. In this regard, they reported an upregulation of the expression, but not evidence of persulfidation was furnished [115]; therefore, it can not be excluded that H_2S indirectly regulates PCG-1α, through persulfidation of SIRT1 or AMPK, as already demonstrated in other conditions [53]. In fact, it is known that SIRT1 can activate PGC-1α through deacetylation and that AMPK can positively modulate it through phosphorylation [116,117]. On the other hand, in 2015 Untereiner and co-workers reported evidence of persulfidation of PGC-1α in hepatocytes isolated from CSE KO mice, whose modulation appeared to be involved in the gluconeogenesis [118].

A contribution of H_2S in the browning of WAT recently emerged from a study with allicin, another H_2S donor present the garlic. Interestingly, in pre-adipocytes allicin concentration-dependently increased the expression of PGC-1α, PRDM16 and UCP1, three specific genes typical of browning. Parallelly, an induction of the expression of KLF15 was also observed and since a depletion of KLF15 protein expression downregulated UCP1 expression, the authors concluded that KLF15 was essential and demonstrated that could promote the expression of UCP1 by binding to UCP1-promotor region. In HFD mice, the promotion of browning was confirmed; furthermore, allicin increased the energy expenditure and finally reduced the body weight *in vivo* [119].

The modulation of the irisin pathway by H_2S, demonstrated in the skeletal muscle, contributes to the greater release of irisin which, as a myokine, acts, among other things, on the WAT and on the pathways involved in the browning process. It has also been indicated that, from a quantitative point of view, WAT does not have high irisin production capacity, highlighting poor levels of FNDC5, unlike other tissues such as skeletal muscle are capable of releasing greater quantities of irisin. This experimental evidence assumes an indirect effect on adipose tissue and not attributed to H_2S as adipokine but to its effect on increasing in circulating irisin released by other tissues such as skeletal muscle [18].

4. Role of the H_2S system in metabolic disorders

4.1. Preclinical evidence

In 2017, Haj-Yasein and colleagues demonstrated that low-cysteine diet prevents the body mass gain in mice and, accordingly, the induction of PPARγ gene expression with a reduction of adipocyte differentiation. Such a behavior seems to be unique for cysteine since it is not observed with other essential aminoacids. Very interestingly, the supplementation with a PPARγ agonist, BRL-49653, partly reversed the inhibitory effect of low-cysteine diet, suggesting that a crucial role could be played by a downstream mediator. It is well-recognized that cysteine is the precursor of H_2S. In this regard, the authors of the paper tested specific inhibitors of CBS and CSE enzymes, observing a prevention in the adipogenesis differentiation; on the other hand, NaHS increased the expression of adipogenic markers (Pparg, plin-1, fabp-4 and scd-1 genes) in 3 T3-L1 cells [120].

Wu and coworkers recently observed that a dietary methionine restriction in mice fed with an HFD resulted in the upregulation of CSE expression (but not CBS) in the liver and, consequently, in an increase of endogenous H_2S production. Such an observation seems to be contradictory, since a dietary methionine restriction is inevitably correlated to a reduction of cysteine levels, that is the substrate of CSE enzyme and then precursor of H_2S. However, the authors focused on miR-328-3p, whose expression was positively influenced by the diet; therefore, they supposed that H_2S could be a final effector able to regulate the expression of genes involved in the maintenance/control of protein homeostasis and metabolic efficiency, including Nrf2 and FOXO3 [121].

Assuming that in obesity conditions the endogenous levels of H_2S are significantly reduced if compared to normal weight one, Comas and his colleagues isolated adipocytes from VAT explanted from obese people and observed that the exogenous treatment with the H_2S donor GYY4137 increased sulfide levels in tissue culture media, as well as the expression of specific adipogenic and insulin pathway-related markers (including ADIPOQ, FASN, SLC2A4). GYY4137 also increased SIRT1 and PPARγ transcriptional activities in adipose tissue lysates, suggesting that H_2S is implicated in the prevention of inflammation and preservation of insulin-sensitivity [122].

After this first evidence of the contribution of H_2S in the adipocyte differentiation, the importance of the gasotransmitter in obesity clearly emerged from the paper of Yang and colleagues. Indeed, they found CSE enzyme more expressed in the WAT, if compared to the CBS and MST; thus, they explored the correlation between CSE expression and adipocytes differentiation. The blockage of CSE, using PAG, the *si*-CSE-mediated knock-down or CSE-KO, significantly reduced the differentiation of pre-adipocyte 3 T3-L1 cells *in vitro* as well as in an *in vivo* model of HFD-induced obesity. In contrast, a CSE-overexpression promoted the lipid accumulation. They demonstrated that H_2S is the player responsible for the activation, by persulfidation, of PPARγ [123], confirming the previous discovery of Cai and collaborators, who for the first time demonstrated the persulfidation on the C139 site of PPARγ receptor [72].

CSE is highly expressed in the liver [124] and CSE-KO aggravated HFD-induced obesity and the insulin-resistance, through the hepatic gluconeogenesis and the reduction of IRS-1 expression. On the contrary, NaHS supplementation reversed it, at least in part, by persulfidation of IRS-1 receptor. Guo et al. observed that H_2S 25 μmol/kg/day, but not higher levels (50 μmol/kg/day), contributed to improve the insulin sensitivity and AMPK signaling pathways [125].

Zheng and colleagues furnished another interesting point of view, identifying the CBS enzyme in the paraventricular nucleus (PVN) as a fine regulator of the neuroendocrine hormone secretion. Indeed, CBS is located mainly in the central nervous system, where it is responsible for the biosynthesis of H_2S. Beside the increase of thyroxine and thyrotropin levels, they observed that when CBS was overexpressed in PVN, *via* lentivirus, the animals showed a lower food intake, a reduced body weight and fat mass gain, and an ameliorated metabolic profile. Accordingly to these findings, the administration of leptin, an adipose-derived hormone, enhanced the expression of CBS in central nervous system, through the nuclear translocation of FOXO3, leading to hypothesize the existence of an exciting axis between adipose tissue and brain, where the up-regulation of CBS is a critical step and its alterations may contribute to the pathogenesis of metabolic disorders [126].

More recently, a link between 3MST expression and obesity has been established in genetically obese (db/db) mice [127].

3MST has been proposed as an endogenous inhibitor of the process of adipocyte lipid uptake during the differentiation; in contrast, 3MST-KO mice showed a reduced expression of adipogenic, lipidogenic and insulin pathway-related genes. Moreover, the inhibition of 3MST resulted, *in vitro* as well as *in vivo*, in an increase of the adipocyte surface area and the number of lipidic drops, and in the acceleration of the adipogenesis [128]. Interestingly, Papapetropoulos and colleagues published a paper in which they explored, both in wild type and in 3MST-KO mice fed with HFD, the regulation of lipogenesis. The ablation of the enzyme increased reactive oxygen species (ROS) levels and to triggered – by HIFα activation – a downregulation of translocase of inner/outer mitochondrial membrane (in TIM/TOM complex), impairing the import of protein at mitochondrial level. Such an event has functional consequences, including the suppression of the Krebs cycle, oxidative phosphorylation and fatty acid oxidation, leading to lipid accumulation and increase of WAT mass. They highlighted the importance of 3MST/H_2S pathway in the mitochondrial biogenesis and in the maintenance of metabolic homeostasis [129]. Indeed, an obesogenic diet, rich in saturated fatty acids, is considered deleterious on mitochondrial function in various organs, and has dramatic repercussions on the overall energy production [130]. In this context, the central role of H_2S also emerged using a mitochondriotropic H_2S-donor molecule, AP39, endowed, in a metabolic disorder induced by HFD, with protective effects on the integrity of

mitochondrial DNA; furthermore, it can reduce the mitochondrial swelling, and preserve the potential of mitochondrial membrane [131]. Interestingly, Gao et al. also reported that the H_2S-donor allicin ameliorated the mitochondrial function and insulin-resistance in a model of obesity comorbid depressive-like behaviors [132]. In 2016, using a bioinformatic-based approach, elevated adipose expression of the *tst* gene correlated with markers of metabolic health across diverse mouse strains has been demonstrated. In the specific, *tst* gene is responsible for the transcription of rhodanese, enzyme implicated in the mitochondrial degradation of final by-products of H_2S, including sulfites [133]. Transgenic overexpression of *tst* in adipocytes protected mice from diet-induced obesity and insulin-resistance; conversely, *tst* gene deficiency markedly exacerbated diabetes; as well, pharmacological *tst* activation ameliorated diabetes in mice *in vivo* [134]. These results contribute to strengthen the idea that H_2S system finely regulate the metabolism.

Finally, Szabo and coworkers shed light to another putative mechanism by which it is possible to assure the endogenous production of H_2S. Indeed, they reported an increase of the SELENBP1 expression during the differentiation of pre-adipocytes similarly to the other enzymes correlated to the biosynthesis of H_2S, whereas in SELENBP1-silencing 3 T3-L1 cells they assessed a lower lipid accumulation. Interestingly, in response to SELENBP1 silencing, CSE, CBS and 3MST enzymes appeared downregulated in cells in which the adipogenesis was stimulated, and then the H_2S levels markedly fell [54].

Recent studies suggest that an endogenous source of H_2S is also represented by PVAT. Of note, under physiological conditions H_2S seems to be endogenously released from PVAT by CSE enzyme [135]. Based on the studies of Beltowski's group, in normal conditions H_2S plays anticontractile effects; instead in obesogenic conditions a time-dependent reduction of the release of H_2S was observed [136,137].

4.2. Preliminary evidence from clinical studies

The potential association between low plasma levels of H_2S and onset/progression of many pathological conditions, including metabolic syndrome and obesity, is emerging [138]. There are currently no clinical trials directly focused on the effects of H_2S-donors in the prevention and treatment of overweight/obesity, but many studies have investigated the possible role of sulfur amino acids on lipid metabolism. Epidemiological data have shown a positive correlation between plasma levels of cysteine, but not methionine, and fat mass [139]. Very recently, it has also been demonstrated that dietary sulfur amino acid restriction (SAAR) for 8 weeks leads to weight loss (~ 20 %), increased ketone bodies, and decreased leptin levels in overweight/obese patients compared with the control group (*i.e.*, patients receiving a diet rich in sulfur amino acids) [140]. These findings suggest, similarly to the above-reported pre-clinical results, that low levels of dietary sulfur amino acids, which could potentially reduce the biosynthesis of H_2S due to the lack of precursors, may be associated with a marked weight loss effect. In other words, from these preliminary results it is possible to deduce that H_2S could be "harmful" for lipid metabolism and body weight control. However, the direct role of sulfur amino acids in the regulation of adipose tissue function and metabolism, independently of H_2S production, can not be completely excluded. On the one hand, cysteine and homocysteine participate in the transsulfuration pathway, that is responsible for the endogenous production of the gasotransmitter H_2S. Other sulfur amino acids, such as taurine, do not take part directly in the transsulfuration pathway and, therefore, contribute less to the biosynthesis of H_2S. Further studies are needed to identify which sulfur amino acids are mainly responsible for the effects of SAAR in overweight/obese patients, also investigating their specific impact on the endogenous production of H_2S. The possible discrete metabolic effects of different sulfur amino acids were elegantly demonstrated by Nichenametla and colleagues, who showed the cysteine-specific properties of SAAR on lipid metabolism [141]. They found that cysteine restriction, but not methionine restriction, increases endogenous serine production; furthermore, they demonstrated that plasma serine concentrations are negatively correlated with plasma TGs and risk of metabolic syndrome in humans. These findings open a new, intriguing interpretation of current clinical data. In fact, it is possible to hypothesize that cysteine restriction favors serinogenesis, potentially reducing the biosynthesis of TGs and, thus, adipose tissue weight. Furthermore, cysteine restriction could enhance the biosynthesis of cysteine from methionine to counteract cysteine deficiency. This could lead to increased production of the intermediate homocysteine, a further precursor of H_2S, partially overcoming the possible reduction of the biosynthesis of H_2S under conditions of cysteine-restriction. In conclusion, the results of clinical studies are far from clarifying the role of H_2S in adipose tissue homeostasis but allow us to speculate that SAAR may perturb the transsulfuration pathway, thus potentially influencing H_2S biosynthesis to a different extent depending on the composition of the diet.

5. Conclusions

Taken together this evidence supports the idea that H_2S is a fine regulator of fat metabolism. Therefore, the intervention with promoters of its endogenous biosynthesis or with agents able to release H_2S may play an important role in the prevention/treatment of obese-associated CVDs However, a more exhaustive knowledge of the putative mechanisms underlying these pharmacological effects is needed, to have a full view of the role of H_2S and to plan an effective intervention to prevent the cardiovascular risk associated to fat metabolism alterations.

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Flori Lorenzo: Writing – original draft, Methodology. **Piragine Eugenia:** Writing – review & editing. **Calderone Vincenzo:** Writing – review & editing, Supervision. **Testai Lara:** Writing – original draft, Conceptualization.

Declaration of competing interest

The authors declare no conflict of interests, they didn't have any financial and personal relationships with other people or organizations that could inappropriately influence this work. Moreover, all authors approve the manuscript and its publication.

Data availability

Data will be made available on request.

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