Pharmacological Research 146 (2019) 104332

Contents lists available at ScienceDirect

Pharmacological Research

journal homepage: www.elsevier.com/locate/yphrs

The beneficial effects of N-acetyl cysteine (NAC) against obesity associated complications: A systematic review of pre-clinical studies

Phiwayinkosi V. Dludla[a,b,*], Sithandiwe E. Mazibuko-Mbeje[b,c], Tawanda M. Nyambuya[d,f], Vuyolwethu Mxinwa[d], Luca Tiano[a], Fabio Marcheggiani[a], Ilenia Cirilli[a], Johan Louw[b,e], Bongani B. Nkambule[d]

[a] Department of Life and Environmental Sciences, Polytechnic University of Marche, Ancona, 60131, Italy
[b] Biomedical Research and Innovation Platform, South African Medical Research Council, Tygerberg, 7505, South Africa
[c] Division of Medical Physiology, Faculty of Health Sciences, Stellenbosch University, Tygerberg, 7505, South Africa
[d] School of Laboratory Medicine and Medical Sciences, College of Health Sciences, University of KwaZulu-Natal, Durban, 4000, South Africa
[e] Department of Biochemistry and Microbiology, University of Zululand, KwaDlangezwa, 3886, South Africa
[f] Department of Health Sciences, Faculty of Health and Applied Sciences, Namibia University of Science and Technology, Windhoek 9000, Namibia

ARTICLE INFO

Keywords:
N-acetyl cysteine
Therapeutic target
Obesity
Metabolic complications
Inflammation
Oxidative stress

ABSTRACT

Excessive adiposity in an obese state is known to drive the onset of metabolic dysregulations, mostly involving chronic immune activation and oxidative stress. Prolonged inflammation and oxidative stress have been linked to impaired adipose tissue function and the development of the metabolic syndrome. Currently available therapies offer minimal prophylactic effects, while substantial experimental evidence supports the ameliorative effects of N-acetylcysteine (NAC) against various metabolic complications associated with obesity. The current review provides a comprehensive synthesis of studies published in major search engines such as PubMed, Cochrane library, Embase, and Google Scholar assessing the therapeutic effect of NAC against obesity associated complications. Overwhelming literature included in this review supports the ameliorative effects of NAC against such complications in both in vitro and in vivo models of obesity. In addition to attenuating an abnormal pro-inflammatory response and limiting oxidative damage, NAC could inhibit lipid accumulation by targeting adipogenic transcription factors such as peroxisome proliferator-activated receptor gamma (PPARγ) and CCAAT/enhancer binding protein beta (C/EBPβ), and improve insulin sensitivity through augmenting phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT) pathway. Although necessary evidence informing on its optimal dose and its comparative effect with other well-studied pharmacological compounds is demonstrated, it is clear that future investigations are required to confirm the therapeutic effect of NAC in obese human subjects.

1. Introduction

Metabolic complications such as obesity have become a global epidemic, which is responsible for the rapid rise in noncommunicable diseases related mortality. According to the World Health Organization, obesity is one of noticeable and modifiable risk factor but remains amongst the most neglected public health challenges [1]. While noncommunicable diseases are responsible for up to 71% of all global mortalities [2], 39% of adults aged 18 years and over were reported to be overweight in 2016, whereas 13% were obese [3]. Although other factors such as genetic predisposition are implicated [4], overnutrition and physical inactivity which is driven by a sedentary lifestyle are components that have contributed significantly to the rise of obesity [5]. Moreover, obese individuals are known to be more susceptible to a

Abbreviations: AKT, protein kinase B; AMPK, 5′AMP-activated protein kinase; ATM, ataxia-telangiectasia mutated; C/EBP, CCAAT/enhancer binding protein; EGCG, epigallocatechin gallate; ERK1/2, extracellular signal-regulated kinases 1/2; GSH, glutathione; IL, interleukin; IRS, insulin receptor substrate; JNK, c-Jun N-terminal kinase; MCP-1, monocyte chemoattractant protein-1; MnTBAP, Mn(III)tetrakis(4-benzoic acid)porphyrin chloride; NAC, n-acetyl cysteine; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; PAI-1, plasminogen activator inhibitor-1; PGC1α, peroxisome proliferator-activated receptor gamma coactivator alpha; PI3K, phosphoinositide 3-kinase; PPAR, peroxisome proliferator-activated receptor; PRISMA, Preferred Reporting Items for Systematic reviews and Meta-Analysis; PROSPERO, Prospective Register of Systemic Reviews; ROS, reactive oxygen species; SIRT-1, NAD-dependent deacetylase; SOD, superoxide dismutase; SREBP-1, sterol regulatory element binding protein 1; TNFα, tumor necrosis factor alpha; UCP, uncoupling protein

* Corresponding author at: Department of Life and Environmental Sciences, Polytechnic University of Marche, Ancona, 60131, Italy.
E-mail address: pdludla@mrc.ac.za (P.V. Dludla).

https://doi.org/10.1016/j.phrs.2019.104332
Received 26 March 2019; Received in revised form 13 June 2019; Accepted 25 June 2019
Available online 26 June 2019
1043-6618/ © 2019 Elsevier Ltd. All rights reserved.

number of fatal illnesses, including diabetes mellitus, musculoskeletal disorders, cardiovascular diseases and some cancers [3]. The precise molecular mechanisms that are involved in the pathogenesis of obesity and its consequences are complex. Nonetheless, a persistent link has been acknowledged between increased adipogenesis, chronic inflammation and generation of oxidative stress in an obese state [6,7]. Hence, an increased demand for novel pharmacological compounds that not only target reduction of adipose tissue mass, but also modulate metabolic pathways that may be altered in an obese state such as those involved in inflammation and oxidative stress.

N-acetylcysteine (NAC) is a thiol-containing mucolytic agent that is increasingly explored for its anti-inflammatory and antioxidant properties [8,9]. NAC is listed under World Health Organization's List of Essential Medicines [10], while its known safety profile makes it an attractive agent for the treatment of a broad scope of diseases. Interestingly, accumulative experimental evidence supports the therapeutic benefits of NAC treatment in obesity associated complications. Several studies have shown that in addition to reducing cellular lipid accumulation by interfering with adipogenic transcription factors such as peroxisome proliferator-activated receptor gamma (PPARγ) and sterol regulatory element binding protein (SREBP-1) [11–13], NAC enhances the intracellular antioxidant levels of adipocytes leading to reduced inflammation and oxidative damage [14]. Its ability to enhance endogenous levels of the reduced form of glutathione (GSH), makes NAC one of the most important antioxidants to protect against oxidative stress, and also increases its therapeutic potential to prevent various metabolic conditions. Evidently, several systematic reviews have been published on the effects of NAC in the management of various conditions, including addiction [15] and mental disorders [16]. Except for our recent review on its potential against diabetes associated cardiovascular complications [17], very limited information has been systematically scrutinized to better inform on the therapeutic benefits of NAC usage in controlling metabolic complications. Thus, this review systematically assessed available studies reporting on the therapeutic potential of NAC in protecting against obesity and its associated complications.

2. Methods

This systematic review was prepared in accordance with the Preferred Reporting Items for Systematic reviews and Meta-Analysis (PRISMA) guidelines [18]. This is part of a big project assessing published studies for the ameliorative effects of NAC against various metabolic complications, and it was registered with International Prospective Register of Systemic Reviews (PROSPERO): CRD42017055851. Thus, the guidelines followed are modified from an already published studies [19–21]. This encompasses the following: literature search strategy, study selection, data extraction and data synthesis.

2.1. Search strategy

Two investigators (PVD and BBN), with assistance from an experienced librarian, identified articles through a systematic search of the PubMed, Cochrane library, Scopus, Embase, and Google Scholar databases up to December 2018. Any disagreements were resolved by consulting the third reviewer (SEM-M). The search strategy was modified for each database through the use of keywords and medical subject heading (MeSH) terms such as N-acetyl cysteine, obesity, metabolic syndrome, inflammation, and oxidative stress, including conforming synonyms and related terms for each item. No language restrictions were applied.

2.2. Study selection

This review included all experimental in vitro and in vivo studies reporting on the effect of NAC as monotherapy or in combination with other agents in managing obesity-associated complications. In vitro studies encompassed those investigating the direct effect of NAC on experimental models of cultured immortal adipocytes, or primary isolated fat cells from obese rodents or human subjects. In vivo studies were on isolated adipocytes from obese rodents or humans that were treated with NAC. Ongoing studies as well as review articles were screened for primary findings, while reviews, editorials, books, and letters were excluded.

2.3. Data extraction

Two investigators (PVD and BBN) independently reviewed all applicable articles and selected suitable studies. Discrepancies or doubts were resolved by reference to a third reviewer (SEM-M). The main outcome of the study was to establish whether the use of NAC intervention can hinder excess adiposity in experimental models or decrease body weight of obese subjects. The second outcome was to assess whether NAC treatment can ameliorate or influence the obesity associated complications such as adipogenesis, inflammation, and oxidative stress. Other points of interest were the effect of NAC as a monotherapy or in combination with other therapies. Briefly, extracted data items included author details such as name and year of publication, experimental model used, NAC dose and intervention period, comparative/combination therapy, experimental outcome, and the proposed mechanism of action if reported by each study. The software, EndNote version 10 (Clarivate Analytics, Philadelphia, USA) was used to manage extracted information, also to remove any duplicates.

3. Results

3.1. Characteristic features of included studies

A total of 129 records were retrieved through combined literature search of different search engines that was run until December 2018. The detailed flow diagram of included and excluded studies is represented in Fig. 1. While eight studies were excluded due to lack of full text accessibility, as much as 18 studies were eliminated because were reviews or did not report on the effect of NAC in an obese state. Consequently, 93 documents involving in vitro and in vivo experimental models of obesity either pre- or post-treated with NAC were included. For a comprehensive understanding on the impact on mitigating obesity associated complications, the below sections give an overview on the therapeutic potential of NAC in relation to its use as a monotherapy, whether in comparison to other drug agents, as well as its influence in vitro or in vivo settings.

3.2. NAC demonstrates an ameliorative effect against obesity associated complications in cultured adipocytes

Although a vast number of included studies tested the efficacy of NAC against obesity associated complications in various in vitro models, only those reporting on the use of NAC as a monotherapy without comparison to any other therapeutic agent are presented in Table 1. Here, up to 20 studies strongly supported the ameliorative effect of NAC against obesity linked complications, including lipid accumulation, insulin resistance, inflammation and oxidative stress-induced damage in cultured adipocytes [11,12,22–39]. For instance, inhibition of fat accumulation in cultured of adipocytes with NAC treatment was strongly linked with the suppression of metallothionein levels [26,37], as well as reduction of various adipogenic related markers such as aminoacylase-1, heat shock protein 70, transketolase and perilipin [32]. In relation to its reversal of insulin resistance, NAC treated adipocytes displayed improved insulin stimulated glucose uptake [28,38], mainly through the modulation of the well-studied proteins involved in insulin signaling pathway, including phosphoinositide 3-kinase (PI3K) activity and insulin receptor substrate 1 (IRS-1) [27].

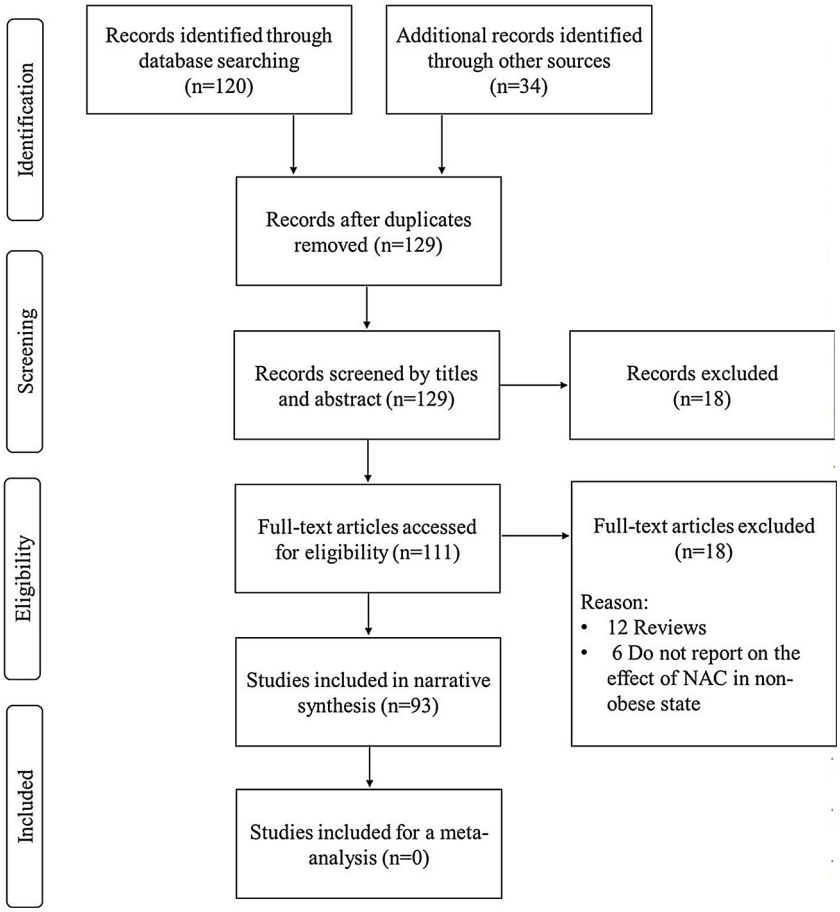


Fig. 1. A flow diagram showing study selection and inclusion criteria.

Certainly, one of the most tested hypothesis during the pathogenesis of obesity is the link between enhanced fat accumulation in an insulin resistant state and the initiation of abnormal inflammation response that may exacerbate oxidative damage. Beside its effect in blocking lipid accumulation and ameliorating insulin resistance, additional in vitro evidence showed that NAC can target and diminish an abnormal inflammatory state by reducing levels of plasminogen activator inhibitor-1 (PAI-1), tumor necrosis factor alpha (TNFα), interleukin (IL)-8, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), and monocyte chemoattractant protein-1 (MCP-1) [24,25,30,34,39]. Consistently, improvement of major intracellular antioxidants such as GSH by NAC could mitigate toxic ROS and inflammatory mediators like c-Jun N-terminal kinase (JNK) and extracellular signal-regulated kinases 1/2 (ERK1/2) [35], as well as adipogenic transcription factors including CCAAT/enhancer binding protein beta (C/EBPβ) and peroxisome proliferator activated receptor gamma (PPARγ) expression [11,12,36].

Other mechanisms implicated in the inhibitory effect of excess lipid accumulation and other obesity associated complications of NAC in cultured adipocytes extend to reducing the levels of apolipoprotein E, perilipin, angiotensinogen, and leptin [22,23,29,31,33]. The latter is one of the prominent hormones secreted by the adipose tissue for regulation of energy expenditure. Also evident from studies summarized in Table 1 was that 3T3-L1 cells were the prominent model used assess the therapeutic effects of NAC. Currently, 3T3-L1 adipocytes remain one the key models used to screen different pharmacological compounds for their therapeutic effect against obesity [40]. On the other hand, the doses between 1–20 mM were dominantly employed during various treatment times with NAC, whereas optimal time of exposure was mainly from an hour to up to five days during differentiation period.

3.3. NAC demonstrates a comparative effect to well-known pharmacological therapies in ameliorating obesity associated complications in cultured adipocytes

Various compounds including natural products, well-known or commonly employed antioxidants and inhibitors, along with anti-diabetic drugs have been used in combination or as a comparative therapy to NAC in testing its protective effects against obesity associated complications in cultured adipocytes (Table 2). Consistent to data presented in Table 1 on its use as a monotherapy, the inhibitory effect of lipid accumulation, amelioration of insulin resistance, and attenuation of an abnormal inflammatory response linked to generation of oxidative stress are the mechanisms linked with the therapeutic effect of NAC when used in combination or comparison to other drug agents such as diverse natural products (Table 2).

There is a general interest in the use of natural products or combining these compounds with available approved drug therapies to combat diseases and improve human health. Natural product-derived compounds that were used in combination or comparison to NAC in included studies were amentoflavone, apocynin, carnosic acid, curcumin, cyaniding 3-glucoside, epigallocatechin gallate (EGCG) and rutin have displayed similar potential to NAC in ameliorating obesity linked complications (Table 2). When used with compounds like amentoflavone, apocynin, curcumin and rutin, NAC was able to inhibit adipocyte differentiation by regulating adiponectin and genes involved in energy expenditure such as C/EBP, PPARs and NAD-dependent deacetylase (SIRT-1) [41–43]. Moreover, NAC in this experimental model exhibited strong an antioxidant effects [44,45]. From data presented in Table 3, NAC clearly displayed greater efficiency in blocking lipid accumulation when combined with green tea polyphenol, EGCG

Table 1
An overview of studies reporting on the therapeutic effect of n-acetylcysteine (NAC) to protect against obesity associated complications using cultured adipocytes.

Author, year	Experimental model, dose used, and intervention period	Experimental outcome and proposed mechanism
Unno et al., 2004 [22]	3T3-L1 adipocytes and isolated mouse epididymal adipocytes treated with 300 μM NAC for 4 days	Protected against glycolaldehyde-modified bovine serum albumin (BSA)- induced reduction in leptin expression
Horiuchi et al., 2005 [23]	3T3-L1 adipocytes treated with 300 μM NAC for 4 days	Inhibited glycolaldehyde-modified BSA induced reduction in leptin concentration
Araki et al., 2006 [24]	3T3-L1 adipocytes treated with 20 mM of NAC for 24 hours	Attenuated tumor necrosis factor alpha (TNFα)-mediated activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and improved the adverse changes in the levels of interleukin (IL)-6, plasminogen activator inhibitor-1 (PAI-1) and adiponectin
Kamigaki et al., 2006 [25]	3T3-L1 adipocytes were pre-treated with 20 mM NAC for 1 hour	Prevented oxidative stress induced atherogenic changes in adipokine gene expression by reducing PAI-1 levels
Kim et al., 2006 [26]	3T3-L1 pre-adipocytes treated with 20 mM NAC for the entire differentiation period	Inhibited adipocyte differentiation by repressing metallothionein-II levels
Jia and Wu, 2007 [27]	3T3-L1 pre-adipocytes treated with 600 μM NAC for the entire differentiation period	Reversed insulin resistance by blocking methylglyoxal-induced impairment of phosphoinositide 3-kinase (PI3K) activity and insulin receptor substrate 1 (IRS-1) phosphorylation
Unoki et al., 2007 [28]	3T3-L1 adipocytes treated 10 mM NAC for 8 hours	Reversed advanced glycation end products (AGEs) induced insulin resistance by promoting glucose uptake and blocking monocyte chemoattractant protein-1 (MCP-1) expression
Espiritu et al., 2008 [29]	3T3-L1 adipocytes treated with 20 mM NAC	Reversed oxidative stress-induced reduction of apolipoprotein E expression
Sun et al., 2009 [30]	3T3-L1 adipocytes treated with 1 mM NAC for 24 hours	Attenuated high glucose-induced increase in expression and secretion of interleukin 18 (IL-18), MCP-1 and PAI-1, while reducing adiponectin expression
Okada et al., 2010 [31]	3T3-L1 adipocytes treated with 10 mM for 10 days	Effectively reversed oxidative stress-induced dysregulation of angiotensinogen levels
Calzadilla et al., 2011 [11]	3T3-L1 pre-adipocytes treated with 5 or 10 μM NAC during differentiation	Enhanced glutathione (GSH) content and inhibited both adipogenic transcription factors CCAAT/enhancer binding protein beta (C/EBPβ) and peroxisomal proliferator activated receptor gamma (PPARγ) expression
Calzadilla et a., 2013 [32]	3T3-L1 pre-adipocytes treated with 10 μM NAC during differentiation process	Inhibited fat accumulation and reduced the expression of obesity-related proteins, including monoamine oxidase A, heat shock protein 70 (HSP70), aminoacylase -1 (ACY-1) and transketolase
Chang et al., 2013 [12]	3T3-L1 pre-adipocytes treated with 10 mM NAC and assessed after various times, including 12 and 30 minutes, 18 and 48 hours, as well as 4 days	Inhibited reactive oxygen species (ROS) formation and suppressed adipogenesis by reducing the expression of C/EBPβ and PPARγ
Lee et al., 2013 [33]	3T3-L1 pre-adipocytes treated with 500 μM BSA-bound free fatty acids with or without 10 mM NAC during differentiation period	Inhibited adipocyte differentiation by significantly reducing free fatty acid-induced perilipin expression
Tada et al., 2013 [34]	3T3-L1 and C3H/10 T 1/2-clone 8 (C3H) adipocytes treated with 10 mM NAC for 3 hours	Inhibited hydrogen peroxide induced oxidative stress and B cell activating factor expression in cultured adipocytes, including reduction of MCP-1
Posa et al., 2014 [35]	3T3-L1 adipocytes pre-treated with 2 mM NAC before exposure to hyperinsulinemia	Suppressed ROS, c-Jun N-terminal kinase (JNK) phosphorylation and partially downregulated extracellular signal-regulated kinases 1/2 (ERK1/2) phosphorylation, but had no effect on restoring insulin-stimulated glucose uptake in adipocytes
Liu et al., 2015 [36]	3T3-L1 adipocytes treated with 1 mM NAC for 48 hours	Ameliorated tamoxifen-induced ROS and suppressed apoptotic and autophagic markers, concomitant with reversal of adipocyte dedifferentiation. It also significantly restored PPARγ levels
Kadota et al., 2017 [37]	3T3-L1 adipocytes treated with 1 mM NAC for 1 hour	Suppressed lipid accumulation and ROS production that was enhanced by metallothionein gene silencing
Olson et al., 2017 [38]	3T3-L1 adipocytes pre-treated with 50 μM NAC for 24 hours	Improved insulin stimulated glucose uptake and eliminated any detectable oxidation of Rictor in the absence of peroxiredoxin 3
Andreeva et al., 2018 [39]	Adipose-derived stromal cells pre-treated with 5 mM NAC for 1 hour	Abolished interferon-gamma induced ROS elevation. Further enhanced IL-6, while suppressing IL-8 and vascular endothelial growth factor production

by reducing PPARγ expression and lowering ROS production [46]. EGCG [47,48], as well as cyaniding-3-glucoside [49] and insulin-like growth factor 1 [50] could perform better than NAC in improving insulin sensitivity by stimulating glucose uptake or influencing associated genes insulin growth factor 1/IRS-1. This was also concomitant with blockade of inflammation-oxidative stress associated destructive damage by interfering with ROS production and activation of JNK/TNFα induced signaling.

Furthermore, a large number of studies have also reported on the comparative effects of NAC with antioxidants such as ascorbic acid, superoxide dismutase (SOD), GSH, Mn(III)tetrakis(4-benzoic acid)porphyrin chloride (MnTBAP), coenzyme q10, vitamin E, and diphenyleneiodonium in cultured adipocytes [51–59]. Except for the negative effect in preventing β-adrenergic induced ROS production [60], the aforementioned studies supported the use of NAC in hindering lipid accumulation via regulation of PPARγ/C/EBP expression, ameliorating insulin resistance and associated inflammation-oxidative stress damage by activating protein kinase B (AKT) or suppressing ROS production and JNK/ERK1/2 signaling. However, alpha-lipoate performed better than NAC in promoting glucose uptake in adipocytes pre-incubated with this compound for 18 h [61]. Of further interest from studies presented in Table 2 were the complementing findings showing that NAC can target specific molecules or pathways such as 5′ AMP-activated protein kinase (AMPK), JNK, autophagy, p38 mitogen-activated protein kinase (MAPK) and NF-κB, in the process alleviating obesity associated complications, reversing insulin resistance, inflammation and oxidative stress [62–72]. Some of the studied pharmacological inhibitors and activators included compound C, SP600125, LY294002, BAY 11–7085, SB203580, U0125, pargyline, Mito-TEMPO and MDL72527.

More data presented on Table 2 also showed an interest in the comparative use of NAC with insulin sensitizing agents that belong to thiazolidinedione class of antidiabetic drugs such as troglitazone [73] and rosiglitazone [74,75] in evaluating its therapeutic effects against obesity associated complications. Data presented supported an equal or greater efficacy for NAC when compared to these antidiabetic drugs in reducing lipid accumulation, improving insulin sensitivity, and blocking ROS production in cultured adipocytes. Moreover, similar

Table 2
An overview of studies reporting on the therapeutic effect of n-acetylcysteine (NAC) in comparison to other therapies to protect the adipose tissue against obesity associated complications in cultured adipocytes.

Author, year	Experimental model, dose used, and intervention period	Comparative/ combination therapy	Experimental outcome and proposed mechanism
Krieger-Brauer and Kather, 1995 [51]	3T3-L1 pre-adipocytes treated with 1.25 and 2.5 mM NAC for the entire differentiation period	Ascorbic and dehydroascorbic acid a dose of 100 μM was used for the entire differentiation period	All compounds suppressed the differentiation process partially by reducing triglyceride content
Fontana et al., 2001 [72]	3T3 F442A cells treated with 1 mM NAC for the entire differentiation period	Pargyline at 0.1 mM and semicarbazide at 1 mM were used for entire the differentiation period	Only NAC and semicarbazide suppressed the differentiation of lipoblasts by inhibiting protein synthesis and triacylglycerol accumulation
Greene et al., 2001 [61]	3T3-L1 adipocytes were pre-incubated in 1 to 5 mM NAC for 18 hours	Alpha-lipoate and dihydrolipoate, with each used at 0.1 to 0.5 mM for 18 -h pre-incubation	Alpha-lipoate and dihydrolipoate promoted glucose uptake, while NAC was ineffective
Lennon et al., 2002 [62]	Mouse adipocyte cell line (1B8) were pre-treated with 20 mM NAC for 15 minutes	15-Deoxy-Δ^{12-14}-prostaglandin J_2 (dPGJ2) at 15 μM dPGJ2 or ciglitazone 20 μM were used for 2 hours post NAC treatment	dPGJ2 and ciglitazone activated extracellular signal–regulated kinases, including c-Jun N-terminal kinase (JNK) and p38 MAP kinase, and NAC inhibited this effect
Ben-Romano et al., 2006 [52]	3T3-L1 pre-adipocytes treated with 10 mM NAC for 18 hours	Mn(III)tetrakis(4-benzoic acid)porphyrin chloride (MnTBAP) was used at 300 μmol/l for 18 hours	NAC prevented nelfinavir-induced oxidative stress, but had no effect in ameliorating insulin resistance or cleavage of poly (ADP-ribose) polymerase (PARP). MnTBAP had no effect on oxidative stress but ameliorated impairment in insulin-stimulated protein kinase B (AKT) activation and PARP cleavage
Guo et al., 2006 [73]	3T3-L1 adipocytes treated with 20 mM NAC for 24 hours	Troglitazone was used at 5 μM for 24 hours	Both compounds reversed octanoate linked increase in reactive oxygen species (ROS), a decrease in triglyceride synthesis, and reduction of lipogenic gene expression
Araki et al., 2007 [76]	3T3-L1 adipocytes pre-treated with 5-20 mM NAC for 24 hours	Pyrrolidine dithiocarbamate was used at 100 μM for 24 hours	Both compounds ameliorated lipopolysaccharide and tumor necrosis alpha (TNFα) induced nitric oxide production by inhibiting nitric oxide synthase and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB)
An et al., 2007 [70]	3T3-L1 adipocytes were treated with NAC	Compound C was used at 10 μM for 30 minutes	Both compounds suppressed nicotine enhanced 5' AMP-activated protein kinase (AMPK) phosphorylation in association with inhibition of fatty acid synthase
Moon et al., 2007 [46]	3T3-L1 adipocytes treated with 5 mM NAC for 12 hours	Epigallocatechin gallate (EGCG) was used at 100 μM for 12 hours	A combination of EGCG and NAC inhibited lipid accumulation by reducing peroxisome proliferator-activated receptor γ (PPARγ) expression and ROS
Ge et al., 2008 [54]	3T3-L1 adipocytes treated with 10 mM NAC for 8 hours	Superoxide dismutase was used at 1000 units/ml, catalase at 5000 units/ml, whilst diphenylene iodonium was used at 20 μM for 8 hours	NAC showed greater efficacy in reversing hyperinsulinemia induced insulin resistance and generation of ROS by improving glucose uptake, as well as inhibiting endoplasmic reticulum stress and JNK activation
Ge et al., 2008 [53]	3T3-L1 adipocytes treated with 10 mM NAC for 8 hours	Superoxide dismutase was used at 1000 units/ml, catalase at 5000 units/ml, whilst diphenylene iodonium was used at 20 μM for 8 hours	NAC showed greater efficacy in reversing chromium induced insulin resistance and generation of ROS by improving glucose uptake, as well as inhibiting endoplasmic reticulum stress and JNK activation
Gou et al., 2008 [49]	3T3-L1 adipocytes were pre-treated with 1 mM NAC for 12 hours	Cyanidin 3-glucoside was used at 10, 20, or 40 μM, while SP600125 (JNK inhibitor) was used at 0, 10, 20, and 40 μM for 12 hours	Cyanidin 3-glucoside presented greater efficacy than NAC in reducing ROS, the activation of JNK, and attenuation of hydrogen peroxide or TNFα induced insulin resistance
Lee et al., 2008 [74]	3T3-L1 adipocytes stimulated with NAC (limited information on dose and time of exposure)	Rosiglitazone was used at 10 μM for 18 hours in 3T3-L1	Both compounds improved glucose tolerance in mice, prevented ROS by enhancing glutathione peroxidase 3 expression and downregulated inducible nitric oxide synthase expression
Takahashi et al., 2008 [63]	3T3-L1 adipocytes were treated with 10 mM NAC for 24 hours	SP600125, JIP-1 peptide, BAY 11-7085 and BMS-345541 were used at 10 μmol/L, 5 μmol/L, 20 μmol/L, and 25 μmol/L, respectively for 24 hours	At varying degree, all compounds suppressed palmitate induced chemoattractant protein-1 (MCP-1) secretion and presented limited effect on influencing adiponectin levels
Wang et al., 2008 [55]	Porcine adipose-tissue-derived stem cells were treated with 2 mM NAC in growth media for approximately 1 week	L-ascorbic acid 2-phosphate was used at 0.2 mM in growth media for approximately 1 week	Improved the lifespan and proliferative potential of cells
Wada et al., 2009 [56]	3T3-L1 adipocytes were pre-treated with 1 mM NAC for 30 minutes	Coenzyme Q10 pre-treatment was applied for 30 minutes	Both compounds promoted glucose uptake and prevented aldosterone-induced degradation of insulin receptor substrate 1 and 2 (IRS1 and IRS2)
Wang et al., 2009 [47]	3T3-L1 pre-adipocytes were treated with 5 to 10 mM NAC in presence of catechins for 8 hours	Epicatechin, EGCG, epigallocatechin, and epigallocatechin-3-gallate were used at 50 μM, while L-buthionine-[*S,R*]-sulfoximine was used at 40 μM for 8 hours	EGCG showed greater efficacy than other catechins in increasing ROS and reducing GSH content in cells. NAC was able to ameliorate this effect
Fukuoka et al., 2010 [50]	3T3-L1 adipocytes treated with 10 mM NAC for 1 hour	Rotenone, thenoyltrifluoroacetone, and oxypurinol were used at 100 μM, while diphenyleneiodonium was used at 10 μM for 1 hour	NAC presented a better effect in reversing insulin growth factor 1 induced impairment of insulin sensitivity via modulation of IRS1
Hallenborg et al., 2010 [75]	Mouse embryonic fibroblasts and 3T3-L1 pre-adipocytes treated with 5 mM NAC during differentiation period	Rosiglitazone, hepoxilin A3, and hepoxilin B were used at 27 nM, 11 μM, and 4 μM, respectively, during the differentiation period	NAC showed greater efficacy in inhibiting adipogenesis during differentiation by reducing

(*continued on next page*)

Table 2 (*continued*)

Author, year	Experimental model, dose used, and intervention period	Comparative/ combination therapy	Experimental outcome and proposed mechanism
			CCAAT/enhancer binding protein β (C/EBPβ) mRNA expression
Molgat et al., 2010 [57]	3T3-L1 pre-adipocytes were treated with 10 mM NAC for 2 hours	Diphenyleneiodonium was used at 10 μM for 30 minutes	Both compounds inhibited macrophage-conditioned medium (MacCM)-induced induced pro-survival signaling through the modulation of AKT and extracellular signal-regulated kinases 1/2 (ERK1/2) pathways
Kanda et al., 2011 [41]	Mesenchymal stem cells pre-treated with or without 5 mM NAC during differentiation	Apocynin was used at 1 μM, while alluprinol and L-NAME were used at 50 μM during differentiation	NAC blocked adipocyte differentiation and inhibited transcriptional activation of cAMP response element-binding protein (CREB), and the expression of dominant-negative mutants of CREB inhibited adipocyte differentiation
Kim et al., 2011 [58]	Adipose-derived stem cells treated with 01 and 1 mM NAC for 48–72 hours	Protein kinase B (Akt; LY294002) and ERK1/2 (U0126) inhibitors were used at 5 mM, while diphenyleneiodonium was used at 100-500 nM	NAC and diphenyleneiodonium reversed hypoxia-induced proliferation and migration of cells. This included reduction of ROS and phosphorylation of platelet-derived growth factor receptor-β as well as activation of ERK and AKT signal pathways
Hirata et al., 2012 [77]	3T3-L1 adipocytes treated with 10 m mol/L NAC for 24 hours	Corticosterone and cortisol were used at 10^{-7} mol/L, eplerenone was used at 10^{-5} mol/L, while RU486 was used at 10^{-5} mol/L for 24 hours	Glucocorticoids dysregulated mRNA levels of various genes related to adipocytokines and the increase of intracellular ROS. Such changes were rectified by mineralocorticoid receptor blockade, not by RU486. NAC reduced ROS by blocking lipid peroxidation products
Ishii et al., 2012 [64]	3T3-L1 pre-adipocytes were treated with MDL72527 and NAC at 5 μM and 5 mM, respectively a day prior induction of differentiation	Spermidine synthase inhibitor trans-4-methylcyclohexylamine (MCHA) and N-(3-aminopropyl)-cyclohexylamine (APCHA) were used 3 days prior to treatment with differentiation medium	NAC and MDL72527, an acetylpolyamine oxidase and spermine oxidase inhibitor prevented the promoting effect of APCHA on adipogenesis by inhibiting triglyceride accumulation
Nagasaki et al., 2012 [43]	3T3-L1 cells possessing a secretory Gaussia luciferase (GLuc) gene under the control of nuclear factor-kappa B (NF-κB) response element in co-culture with RAW macrophages treated with 10 mM NAC for 24 hours	Carnosic acid, EGCG and ursolic acid were used at 10 μM for 8 hours	All treatments suppressed GLuc secretion and up-regulated adiponectin secretion
Takahashi et al., 2013 [65]	3T3-L1 adipocytes exposed to palmitate were treated with 0.5 mmol/l NAC for 24 hours	LY294002 was used at 50 μmol/l, PD98059 at 20 μmol/l, SP600125 at 10 μmol/l, SB203580 at 10 μmol/l, and Wortmannin at 10 nmol/l for 24 hours	NAC and PI3K inhibitor LY294002 significantly inhibited vascular endothelial growth factor (VEGF) release and secretion. NAC also diminished augmented Akt phosphorylation. In contrast, increased MCP-1 release was suppressed with JNK inhibitor SP600125 and p38 mitogen-activated protein kinase (MAPK) inhibitor SB203580
Xiao et al., 2013 [66]	3T3-L1 pre-adipocytes were treated with 1 mM NAC for 24 hours	Except for SP600125 which was used at 10 μM, all other protein kinase inhibitors, including LY294002, SB203580 and U0126 were used at 20 μM for 24 hours	NAC significantly blocked chicoric acid induced cell death by partially modulating Akt and MAPK signaling pathways. Whereas the use of protein kinase inhibitors revealed that PI3K/AKT signaling pathway interplayed with MAPK signaling pathways
Cremers et al., 2014 [44]	Adipose-derived mesenchymal stem cells (ASCs) were routinely pre-treated with 6 mM NAC for 24 hours	Curcumin pre-treatment was used at 10 μM for 24 hours	Both compounds prevented hydrogen peroxide induced cytotoxicity
Ku et al., 2014 [48]	3T3-L1 adipocytes treated with 10 mM NAC for 1 hour	EGCG was used at 20 μM for 2 hours	NAC alone had no effect on glucose uptake but did not alter the effects of EGCG to suppress insulin-like growth factor stimulation of glucose uptake through inhibition of the glucose transporter (GLUT)4 translocation, but not through alterations of the GLUT1 pathway
Kim et al., 2015 [59]	3T3-L1 pre-adipocytes were treated with NAC-allyl-isothiocyanate (NAC-AITC) conjugate at a concentration of 50, 100 and 200 μM during differentiation	Glutathione-AITC (GSH-AITC) conjugate was used at a concentration of 50, 100 and 200 μM during differentiation	Both mixtures effectively inhibited adipogenic differentiation and suppressed expression of PPAR-γ, C/EBPα, and fatty acid snythase. These mixtures further suppressed oleic acid-induced lipid accumulation and lipogenesis in hepatocytes
Chen et al., 2016 [45]	3T3-L1 pre-adipocytes treated with 100 μM NAC treated during differentiation period	Amentoflavone was used at 10 μg/ml during differentiation	Both compounds inhibited ROS, cell proliferation and limited lipid formation
Kim et al., 2017 [67]	3T3-L1 adipocytes pre-treated with 10 mM NAC for 30 minutes	Mdivi-1, an inhibitor of the mitochondrial fission protein Drp1 was used at 50 μM for 30 minutes, whereas Mito-TEMPO, mitochondria-targeted ROS scavenger was used at 200 μM for 30 minutes	NAC and Mito-TEMPO inhibited ROS production, lipid accumulation, the expression of adipogenic and mitochondrial dynamics-related proteins, and mitochondrial fission during adipogenesis. While Mdivi-1 blocked mitochondrial fission but did not inhibit ROS production, lipid accumulation, or the expression of adipogenic and mitochondrial dynamics-related proteins, with the exception of phosphorylated Drp1 (Ser616)
Soto et al., 2017 [68]	3T3-L1 adipocytes treated with 0.01 or 1 mM NAC during differentiation	UO126 and SP600125, inhibitors for ERK1/2 and JNK1/2 were both used at 10 μM prior to NAC treatment	NAC inhibited cellular triglyceride and mitochondrial protein expression by preventing

(*continued on next page*)

Table 2 (*continued*)

Author, year	Experimental model, dose used, and intervention period	Comparative/ combination therapy	Experimental outcome and proposed mechanism
			both mitotic clonal expansion and kinase phosphorylation
Li et al., 2018 [69]	Adipose tissue-derived stem cells pre-treated with NAC (5 mM) for 2 hours before exposure to high glucose for 24 hours	SP600125 (JNK Inhibitor) was used at 20 μg/ml for 2 hours before exposure for 24 hours	Both compounds effectively regulated autophagy to ameliorate high glucose/ROS-mediated apoptosis
Peris et al., 2018 [60]	3T3-L1 adipocytes treated with 1 mM NAC for 48 hours	Cells were exposed to vitamin E and glutathione ethyl ester at 50 and 5 mM, respectively	Antioxidants did not prevent this ROS increase *in vitro*, instead enhanced β-Adrenergic stimulation–induced mitochondrial ROS production
Raffaele et al., 2018 [42]	Bone marrow adipose tissue cells treated with NAC at 1, 10 and 100 mM during different culture conditions	Rutin was used at 50 μM	NAC reduced lipid droplets in cells and enhanced adiponectin, PPARγ, heme oxygenase (HO)-1, and NAD-dependent deacetylase (SIRT-1). Further, increased beta-oxidation markers such as PPARα and PPARδ mRNA levels
Shin et al., 2018 [71]	3T3-L1 adipocytes treated with 10 mM NAC for 4 hours	Neuropeptide Y (NPY)/ neuropeptide Y1 receptor (NPY1R) antagonist (BIBO3304, 1 μM), NPY2R antagonist (BIIE0246, 1 μM), and NPY5R antagonist (CGP71683 hydrochloride, 1 μM) were added during cell differentiation	NAC and NPYR antagonists showed anti-adipogenic effects by reducing the ROS levels, indicating that pentraxin 3 mediates adipogenesis through NPY-dependent ROS production

outcome was observed in other studies that tested the comparative effects of NAC with anti-inflammatory agents such as pyrrolidine [76], including corticosterone hormones [77]. These studies reported that NAC could limit lipopolysaccharide and TNFα induced nitric oxide production by inhibiting nitric oxide synthase and NF-κB, while reducing excess ROS production. Nevertheless, an overview of data summarized in Table 2 suggest that NAC can offer comparative effects some well-known antioxidants, anti-inflammatory agents against obesity and its associated complications. However, there are limited studies testing its combinational effect with these therapies, which could further inform on its capability to manage obesity complications.

3.4. NAC demonstrates ameliorative effects against obesity associated complications in rodent models

A systematic search of literature revealed up to 20 studies reporting on the ameliorative effect of NAC as a monotherapy, without any comparative agent, against obesity related complications in various rodent models (Table 3). Included studies show that treatment of primary adipocytes or adipose tissue from obese and non-obese rodents is among predominant models used to assess the therapeutic effects of NAC. For example, one of the earliest studies published on the therapeutic effect of NAC showed that this antioxidant was able to prevent PAI-1 induced pro-inflammatory response in primary adipocytes from rats infused with high glucose [78]. Studies on primary adipocytes or adipose tissue support the rationale that NAC attenuates insulin resistance by modulating PI3K/IRS pathway, inhibit lipid accumulation by acting on MAPK and genes involved in energy expenditure like PPARγ, peroxisome proliferator-activated receptor gamma coactivator alpha (PGC1α) and uncoupling protein (UCP)3 [26,27,34,79–86]. Here, the improved effect of NAC was linked to its capacity to control the levels of energy controlling hormones secreted by the adipose tissue, leptin and adiponectin. Moreover, treatment with NAC was strongly associated with attenuation of oxidative stress, mostly related with reduction of apolipoprotein E levels, suppression of ROS levels and enhancement of intracellular antioxidants [29,87].

Evidence presented on Table 3 also showed that only a few studies investigated the effect of NAC on gene specific models of obesity. Such animal models included obese (*ob/ob*) and type 2 diabetic (*db/db*) mice, which are leptin resistant animals that become spontaneously obese with continuous eating [31,88]. Adipocytes from these mice showed reduction of lipid accumulation and improvement of markers of oxidative stress after treatment with NAC. Consistently, in adipocytes of hydroperoxides glutathione peroxidase, sphingomyelin synthase or adipose-specific spermidine/ spermine N1-acetyltransferase knockout mice [12,89,90], NAC was demonstrated to reduce lipid accumulation concomitant to normalizing raised serum lipid levels, and attenuated oxidative stress by suppressing ROS. Nonetheless, the overall findings presented in Table 3 showed that the use of NAC at various doses, mostly administered through drinking water, whether with acute or chronic treatment, hindered obesity associated complications in animals. Moreover, consistent with in vitro findings, NAC can improve insulin sensitivity, and modulate various genes and hormones involved in controlling energy expenditure.

3.5. NAC demonstrates comparative effect to well-known pharmacological therapies in ameliorating obesity associated complications in rodent models

When compared to in vitro findings displayed on Table 2, only few studies report on the therapeutic effect of NAC in comparison to other therapies in ameliorating obesity associated complication in rodent models (Table 4). Although other pharmacological agents such as pyruvate were used in comparison to NAC [91–94], those that were established ranged from known antioxidants such as vitamin E, natural products like crocetin, and antidiabetic agents included rosiglitazone and metformin [60,74,91,95]. Antioxidants such as vitamin E, MnTBAP and catalase were shown to comparatively induce browning of adipose tissue, regulate energy balance and suppress oxidative stress in adipocytes exposed to different stress conditions [46,60,96,97]. Rosiglitazone and metformin were shown to have similar potential to NAC in improving glucose tolerance in mice, while in adipocyte isolated from rodents, these compounds suppressed ROS and inflammation by enhancing intracellular antioxidants in addition to ameliorating inflammatory markers such as ataxia-telangiectasia mutated (ATM), NF-κB, IL-6, TNFα and MCP-1 [74,95].

Additional evidence demonstrated that NAC has a better effect in preventing free-fatty acid release in adipocytes from rats when used in conjugation with zinc [46]. While others support the notion that NAC can give comparative or better effect than crocetin, taurine and myriocin in preventing weight gain in rats, modulating adiponectin levels, or reducing inflammation, oxidative stress [98,99]. Furthermore, other studies focused on the use of compounds like vanadate and *L*-buthionine-*S,R*-sulfoximine to prove the modularity effect of NAC on specific pathways such as glucose transport, and prevention of oxidative stress [91,100]. The overall findings presented in Table 4 are consistent with in vitro findings on its inhibitory effects on lipid accumulation, and the

Table 3
An overview of studies reporting on the therapeutic effect of n-acetylcysteine (NAC) to protect against obesity associated complications in different rodent models.

Author, year	Experimental model, dose used, and intervention period	Experimental outcome and proposed mechanism
Lin et al., 2005 [78]	Primary adipocytes isolated from rats infused with 15 mM glucose and NAC for 6 hours	Prevented the plasminogen activator inhibitor-1 (PAI-1) induced pro-inflammatory response
Kim et al., 2006 [26]	Fat tissue isolated from Sprague-Dawley rats treated with 81, 163, 326, or 816 mg/kg/day NAC for 41 days	In addition to reducing the body weight, NAC inhibited the adipocyte differentiation by repressing metallothionein-II levels
Jia and Wu, 2007 [27]	Adipose tissue of Sprague-Dawley rats fed high fat diet (HFD) before treatment with 10 mg/kg/day of NAC	Reversed insulin resistance by blocking methylglyoxal-induced impairment of phosphoinositide 3-kinase (PI3K) activity and insulin receptor substrate 1 (IRS-1) phosphorylation
Espiritu et al., 2008 [29]	Primary adipocytes or adipose tissue from C57/BL6 mice treated with 20 mM NAC	Reversed oxidative stress induced reduction of apolipoprotein E expression
Okada et al., 2010 [31]	Primary adipocytes from *ob/ob* mice administered 150 mg/kg body weight NAC for 1 week	Effectively reversed oxidative stress induced dysregulation of angiotensinogen levels
Huh et al., 2012 [129]	Male Sprague-Dawley rats treated with 10 mmol/L NAC twice per day for 12 weeks	Improved adiponectin plasma and dialysate levels
Yan et al., 2012 [88]	Mesenchymal stem cells from *db/db* mice pre-treated with NAC	NAC reduced oxidative stress markers and inhibited the rate of adipocyte differentiation
Chang et al., 2013 [12]	Nonselenocysteine-containing phospholipid hydroperoxides glutathione peroxidase (NPGPx) knockout mice were treated with 2 mg/1 ml NAC in drinking water and was renewed twice weekly	Although had no effect on body weights and fasting glucose levels, NAC effectively prevented fat accumulation in mice lacking NPGPx
Tada et al., 2013 [34]	Male C57BL/6 J mice fed HFD were supplemented with 10 mg/ml NAC in drinking water for 24 weeks	NAC lowered B cell activating factor levels in both serum and visceral adipose tissue
Yano et al., 2013 [89]	Sphingomyelin synthase null mice postnatally administered 40 mM NAC in drinking water	NAC showed partial recovery of lipodystrophic phenotypes and normalized plasma triglyceride concentrations
Gillis et al., 2014 [130]	Inguinal fat grafts harvested from C57BL/6 mice treated with NAC	NAC improved fat graft retention
Wang et al., 2015 [80]	Female C57BL/6 J mice administered 0.1 g/kg/day NAC via intraperitoneal injection for 4 consecutive days. In addition, mice received 6 μM NAC in drinking water until date of termination	NAC enhanced antioxidant effects and inhibited adipogenesis by acting on PPARγ and adiponectin gene levels
Ma et al., 2016 [81]	Male C57BL/6 mice fed a HFD and treated with 2 g/l NAC in drinking water for 11 weeks	NAC suppressed weight gain and fat mass. NAC enhanced expression of genes involved in energy expenditure including type 2 Iodothyronine Deiodinase (DIO2), peroxisome proliferator-activated receptor gamma coactivator (PGC1α), PGC1β, cell death inducing DFFA like effector A (CIDEA), and uncoupling protein (UCP)3. NAC treatment also inhibited inflammation and oxidative stress, and enhances adiponectin gene expression in epididymal white adipose tissue
Pieralisi et al., 2016 [82]	Mouse embryonic fibroblasts prepared from CF-1 mouse embryos at day14 of gestation were treated with 0.01, 0.1, 1 and 5 mM NAC during differentiation period	NAC inhibited lipid accumulation and mitogen-activated protein kinases (MAPK) phosphorylation and further suppressed adipogenesis by blocking mitotic clonal expansion
da Silva et al., 2017 [83]	Male Wistar rats treated with 600 mg/l NAC in drinking water for 90 consecutive days	NAC improved insulin sensitivity, reduced urine thiobarbituric acid reactive substances (TBARS), adipose macrophage number and Itgam and Mrc mRNA and increased glucose transporter (GLUT)4 and peroxisome proliferator-activated receptor alpha (PPARα)
Williams et al., 2017 [84]	Female CD1 mice fed a HFD received NAC at 0.25, 0.5, 0.75 or 1 g/kg from ED0.5 throughout lactation	NAC normalized crown rump length in pups from HFD dams and delayed alveolarization
Berry et al., 2018 [85]	Female C57BL/6 J mice were fed HFD for 13 weeks (from 5-weeks of age until delivery) and were exposed to the NAC from 10-weeks	NAC reduced leptin levels, improved insulin sensitivity, increased glutathione synthesis, enhanced adiponectin concentrations, while reducing plasma corticosterone levels in response to restraint stress
Shen et al., 2018 [87]	Male C57B/L6 mice fed a HFD treated with NAC at 10 mM in drinking water for 3 and 6 months	Six-month NAC treatment performed better in increased motor activity, ameliorating mitochondrial and intracellular reactive oxygen species (ROS) production markers, including protein oxidative damage and inflammation
Yuan et al., 2018 [90]	Primary adipocytes from adipose-specific spermidine/ spermine N1-acetyltransferase knockout mice (SAT1-aKO) pre-treated with NAC at 100 μM NAC for 30 minutes	NAC abolished the elevated UCP1 induced by N1, N11-diethylnorspermine (DENSpm). NAC also reversed the elevated expression of CD14 and reduced hydrogen peroxide and ROS production
Zheng et al., 2019 [86]	C57BL/6 J mice fed HFD treated with NAC at 1 mg/ml in drinking water for 5 months	NAC reversed glucose intolerance, fasting glucose concentrations, and gains in body weight and plasma endotoxin. In addition, NAC modulated the metabolic pathways of intestinal bacteria, including lipopolysaccharide biosynthesis, oxidative stress, and bacterial motility

use of NAC compares well with other established compounds like metformin in ameliorating obesity associated complications in vivo. However, currently only a few studies have been done on this topic or looking at its combinational effects with these compounds.

3.6. Evidence from human systems reporting on the ameliorative effect of NAC against obesity associated complications

The majority of human studies presented in Table 5 employed human derived stem cells or adipocytes from obese subjects to evaluate the therapeutic effect of NAC against obesity and its associated complications. Initial evidence of NAC efficacy was presented in 2004, where in addition to reducing body fat mass, administration of 200 mg NAC in the form of capsules for eight weeks was linked to improved glucose tolerance and reversal of insulin resistance as measured by homeostasis model assessment for insulin resistance (HOMA-R) method in obese patients [101]. Following this, other studies showed that NAC as a monotherapy or in association with ascorbic acid can greatly enhance the replication of mesenchymal stem cells derived from human adipose tissue, as well as counteracting ceramide-induced ROS production and lowering apoptosis in these cells [102,103]. In human primary pre-adipocytes or adipose tissue-derived multilineage progenitor cells, NAC together with resveratrol, a natural phenol found in red wine, could inhibit lipolysis and ROS production, in part by suppressing fibroblast growth factor 2 [104]. Similarly, other studies on human adipose tissue derived cells, confirmed the inhibitory effect of NAC against adipocyte differentiation, inflammation, ROS production and cell death [71,80,105–110]. In part, some mechanisms involved

Table 4
An overview of studies reporting on the therapeutic effect of n-acetylcysteine (NAC) in comparison to other therapies to protect against obesity associated complications in different rodent models.

Author, year	Experimental model, dose used, and intervention period	Comparative/ combination therapy	Experimental outcome and proposed mechanism
Lu et al., 2001 [91]	Primary adipocytes isolated from Sprague-Dawley rats exposed to 10^{-7} M insulin and 15 mM glucose in culture before treatment with 30 mM NAC for 2 hours	Vanadate was used at 1 to 4000 mM for various time intervals, depending on the assay performed	Sensitivity of phosphotyrosine phosphatases inhibition and glucose transport to vanadate is regulated by cellular redox state, with NAC playing a role in reducing oxidative stress by enhancing glutathione (GSH) content
Uchida et al., 2004 [100]	Primary adipocytes isolated from Wistar-Imamichi rats treated with 1, 5 and 20 mM NAC for 24 hours	L-buthionine-*S,R*-sulfoximine was used at 10, 50 and 100 μM for 24 hours	L-buthionine-*S,R*-sulfoximine aggravated oxidative stress induced by advanced glycation end products (AGEs) and 4-hydroxynonenal (HNE), while NAC inhibited this effect
Adachi et al., 2007 [46]	Isolated Wistar rat adipocytes treated with 5×10^{-4} M zinc-NAC complex for 30 minutes	Zinc conjugates of L-cysteine, L-cysteine-methylester were used at a concentration of 10^{-4} and 10^{-3} M for 30 minutes	Zinc-NAC conjugate presented with better effect than other conjugates in preventing free fatty acid release from adipocytes
Xi et al., 2007 [131]	Primary adipocytes isolated from Wistar rats were treated with 10 mM NAC for 10 hours	Crocetin was used at 5 and 50 μM, diphenyleneiodonium at 20 μM, rotenone at 100 μM, whilst oxypurinol was used at 100 μM for 10 hours	NAC, crocetin and diphenyleneiodonium showed enhanced effect in blocking palmitate-induced insulin insensitivity and disordered tumor necrosis factor-alpha (TNFα) and adiponectin expression
Kim et al., 2008 [96]	Adipose tissue-derived stromal cells isolated from diabetic Sprague–Dawley rats treated with 1 mM NAC for 7 days	Catalase was used at 500 U/mL for 7 days	Both antioxidants prevented high glucose-induced inhibitory effect on proliferation and generation of ROS
Lee et al., 2008 [74]	Fat tissue from type 2 diabetic (*db/db*) mice intraperitoneally injected with NAC for 1 week	Rosiglitazone was used at 10 μM for 48 hours in 3T3-L1 adipocytes and at 5 mg/kg for 10 days in mice	Both compounds improved glucose tolerance in mice, prevented reactive oxygen species by enhancing glutathione peroxidase 3 (Gpx3) expression and downregulated inducible nitric oxide synthase expression
Wang et al., 2010 [92]	Adipocytes were isolated from male Sprague–Dawley rats treated with 10 or 20 mM NAC for 1 hour; and mice fed HFD before treated with 3 mg/ml NAC in drinking water for 10 days	Pyruvate was used a comparative therapy at 5 mmol/l in isolated adipocytes	Both compounds inhibited production of ROS and improved cellular respiration
Elshorbagy et al., 2013 [98]	Methionine-restricted male Fischer rats were maintained in diet containing 0.5% NAC for 12 weeks	Taurine supplementation in diet at 0.5% for 12 weeks	NAC raised serum total cysteine and partly or completely reversed methionine-restricted effects on weight, fat content, stearoyl-CoA desaturase-1 (SCD1) expression in liver and white adipose tissue, and estimated SCD1 activity. Taurine did not restore weight gain or hepatic SCD1 expression but further decreased adiposity
Ro et al., 2014 [93]	Primary pre-adipocytes isolated from the interscapular depot from wild type Sestrin2 or redox-inactive Sestrin2 mutant mice treated with 10 μM NAC after differentiation for 1 hour	p38 mitogen-activated protein kinases (MAPK) inhibitor, SB203580 was used at 10 μM for 1 hour	Both compounds inhibited ROS, p38 MAPK activation and uncoupling protein 1 expression in mouse brown adipose tissue upon cold exposure
Tian et al., 2014 [94]	Epididymal fat pads from B6 mice on high fat diet (HFD) or *db/db* mice treated with 1 mM NAC for 24 hours	NS-398 (a selective cyclooxygenase-2 inhibitor) was used at 10 μM for 24 hours	Reduced stimulation index of normal islets incubated with obese adipose tissue-conditioned medium
Chen et al., 2015 [95]	DNA polymerase η knockout mice treated with 1 mg/ml NAC in drinking water for 4 weeks	Metformin was used at 1.5 mg/ml and administered in drinking water for 4 weeks	Both compounds significantly reduced the amount of DNA damage, adipocyte hypertrophy and attenuated adipocyte senescence. Mechanisms proposed markers regulated include ataxia-telangiectasia mutated (ATM), tumor protein 53 (p53) and p21, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), interleukin-6 (IL-6), TNFα, and monocyte chemoattractant protein 1 (MCP-1)
Schneider et al., 2016 [97]	Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) knockout embryonic fibroblasts and white adipose tissue of Nrf2 knockout mice treated with NAC	Mn(III)tetrakis(4-benzoic acid) porphyrin chloride (MnTBAP), a cell-permeable superoxide dismutase mimetic	NAC and MnTBAP regulated energy balance by reducing UCP1 and oxygen consumption levels
Chimin et al., 2017 [99]	Mice with adipocyte raptor deletion treated with 300 mg/kg/daily NAC for 7 days	Ceramide synthesis inhibitor, myriocin was used at 0.5 mg/kg/daily for 7 days	NAC partially attenuated, whereas myriocin, completely blocked adipose tissue inflammation and nucleotide oligomerization domain-like receptor pyrin domain-containing 3 (NLRP3)-inflammationsome activation, but not hepatic steatosis and insulin resistance induced by adipocyte raptor deletion
Peris et al., 2018 [60]	Male C57/Bl6 mice administering NAC through drinking water at 1 g/l	Administration of vitamin E was at 0.5 g/kg enriched in food	At varying degree of efficacy, compounds treatment suppressed β-adrenergic stimulation induced browning of white adipose tissue and mitochondrial activity of brown adipose tissue in wild type mice

included preventing nuclear accumulation of apurinic apyrimidinic endonuclease/redox effector 1 (APEI), blocking NADPH oxidase activity, suppressing IL-8, and downregulating of miRNA-223. Anyhow, the overall findings summarized in Table 5, are consistent with those previously discussed in vitro, showing enhanced activity of NAC to reduce body weight or suppress adipogenesis, extend proliferative activity of adipocytes, and counteract oxidative stress. While medical uses of NAC are increasing [111], this review shows that very few studies have explored the molecular mechanisms involved in the proposed NAC effect, while also limited evidence was presented from actual humans treated with NAC as a therapy. Since aggravation of obesity-associated pathologies is related to insulin dysregulations and

Table 5
An overview of studies reporting on the therapeutic effect of n-acetylcysteine (NAC) to protect against associated complications in humans or human derived adipocytes.

Author, year	Experimental model, dose used, and intervention period	Comparative/ combination therapy	Experimental outcome and proposed mechanism
Hildebrandt et al., 2004 [101]	Body fat mass of obese patients receiving capsules with 200 mg NAC or placebo were administered at a total daily dose of 0.6 g for 8 weeks	Capsules with 333 mg creatine or placebo were given at a total daily dose of 2 g for 8 weeks	NAC reduced glucose tolerance and body fat mass but increased Homeostatic Model Assessment of Insulin Resistance (HOMA- R) index. Creatine reversed the NAC mediated increase in HOMA-R index without preventing the decrease in body fat
Lin et al., 2005 [102]	Mesenchymal stem cells from human adipose tissue supplemented with 2 mM NAC	L-ascorbic acid-2-phosphate (0.2 mM) supplemented in growth media	Both antioxidants greatly enhanced the growth rate and extended the lifespan of adipose-derived multipotential human mesenchymal stem cells
Park et al., 2011 [103]	Mesenchymal stem cells derived from human adipose tissue were pre-treated with 1 mM NAC for 15 minutes before exposure to 50 μM ceramide	None	NAC alleviated ceramide-induced ROS and apoptosis resulting in improved cell viability
Krawczyk et al., 2012 [104]	Human primary pre-adipocytes from subcutaneous fat tissue of subjects undergoing panniculectomy surgery were treated with 5 mM NAC for 15 minutes	Resveratrol was used at 100 mM, while diphenyliodonium (DPI) was used at 10 μM for 15 minutes	NAC, DPI and resveratrol reduced lipolysis and reactive oxygen species (ROS) production
Moriyama et al., 2012 [105]	Human adipose tissue-derived multilineage progenitor cells in neurite outgrowth in cells of the rat pheochromocytoma cell line (PC12) treated with 5 mM NAC for 2 hours	SB203580, U0126 and c-Jun N-terminal kinase (JNK) inhibitor II were used at 10 μM for 2 hours	NAC suppressed buthionine sulfoximine-induced bone morphogenetic protein 2 and fibroblast growth factor 2
Xiong et al., 2012 [106]	Human subcutaneous adipose tissue samples from seven healthy females were treated with 40-μg/ml NAC for 24 hours	None	NAC stimulated proliferation, inhibited cell death, and suppressed interleukin 8 (IL-8) production
Domenis et al., 2014 [107]	Human adipose tissue-derived multipotent adult stem cells were treated with 10 mM NAC during differentiation period	None	NAC partially prevented the nuclear accumulation of apurinic apyrimidinic endonuclease/redox effector factor 1 (APE1) as a result increased neuronal differentiation of cells
Gagnon et al., 2014 [108]	Human subcutaneous differentiated adipocytes from male and female overweight subjects undergoing elective abdominal surgery treated with 10 mmol/L NAC for 2 hours	None	NAC blocked thyroid-stimulating hormone induced pro-inflammatory response by inhibiting NADPH oxidase activity and IκB kinase β
Wang et al., 2015 [80]	Adipocyte differentiation of human of mesenchymal stem cells treated with 5 mM NAC during differentiation period	None	NAC reduced ROS production, limited lipid accumulation and inhibited adipocyte differentiation
Wang et al., 2016 [109]	Human adipose tissue-derived stem cells were pre-treated with 3 mM NAC for 20 hours	Ascorbic acid 2-phosphate was used at 0.2 mM for 20 hours	Both compounds suppressed advanced glycosylation end product-induced oxidative stress and apoptosis, possibly through downregulation of miR-223
Lee et al., 2018 [110]	Human adipose derived stem cells during a proliferation exposed to NAC at 0.01–50 mM	None	NAC promoted osteo-differentiation both when included in gelatin backbone or used directly in cells
Shin et al., 2018 [71]	Human mesenchymal stem cells isolated from human fat tissue	Neuropeptide Y (NPY)/ neuropeptide Y1 receptor (NPY1R) antagonist (BIBO3304, 1 μM), NPY2R antagonist (BIIE0246, 1 μM), and NPY5R antagonist (CGP71683 hydrochloride, 1 μM) were added during cell differentiation	NAC and NPYR antagonists showed anti-adipogenic effects by reducing the ROS levels, indicating that pentraxin 3 mediates adipogenesis through NPY-dependent ROS production

impaired adipose tissue vascularization [112–114], assessment of the protective effects of NAC against these consequences is also necessary in clinical settings, as demonstrated in rodent models [74,83].

4. Discussion

It is well acknowledged that excessive adiposity can aggravate an obese state, leading to the development of insulin resistance, an abnormal inflammatory response, and accelerated formation of toxic free radical species [115]. Significant reduction of intracellular antioxidant systems due to the aforementioned consequences can further contribute to the generation of oxidative stress, or reduction of adipose tissue vascularization, which may eventually damage cellular structures [116,117]. In fact, obese children with insulin resistance demonstrate enhanced levels of oxidative stress biomarkers such as lipoperoxides and oxidized GSH, occurring together with stunted antioxidant response after oral glucose loading [118]. Indeed, GSH is one of the most important and potent antioxidants in the human body that protects against oxidative stress, acting directly by neutralizing various free radical species or by donating electrons to maintain the reduced form of other essential antioxidant enzymes like glutaredoxin and glutathione peroxidase [119]. During the manifestation of obesity and associated metabolic syndrome, it remains controversial whether insulin resistance causes inflammation and oxidative stress, or the opposite process is it true [120,121]. Although precise molecular mechanisms explaining this phenomenon are complex, several pathways have been identified that link excess lipid buildup in the adipose tissue with reduced insulin sensitivity that may initiate and worsen the pro-inflammatory state and oxidative damage.

In recent years, adipose tissue has been increasingly recognized as a major endocrine organ, secreting various factors including hormones leptin and adiponectin that are essential in controlling energy metabolism. Opposing the protective effects of adiponectin [122], irregularly increased levels of leptin have been associated with weight gain and the development of insulin resistance [123]. In HFD fed mice, increased leptin concentrations from inflamed visceral fat are connected with oxidative stress and activation of cyclooxygenase-2 [94]. Other adipose tissue secreted factors that accelerate obesity associated complications include pro-inflammatory cytokines like TNFα. Cumulative research shows that TNFα can induced nitric oxide production, also

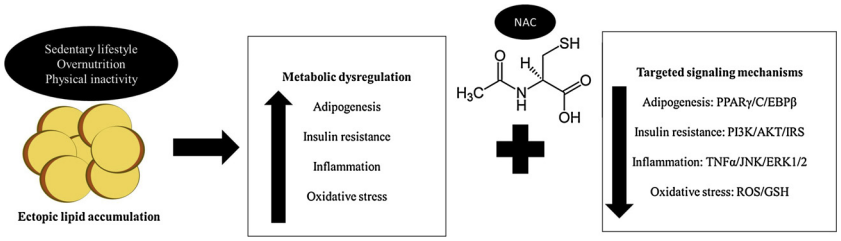


Fig. 2. An overview mechanisms linked with the protective effect of N-acetyl cysteine (NAC) against metabolic dysregulations associated with enhanced ectopic lipid accumulation, mostly driven by factors like sedentary lifestyle, overnutrition and physical inactivity. Briefly, an interconnected network of molecular mechanisms such as blocking peroxisome proliferator-activated receptor gamma (PPARγ) and CCAAT/enhancer binding protein beta (C/EBPβ), and improving insulin sensitivity through augmenting phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT) pathway. Consistently, NAC could induce anti-inflammatory properties by blocking an abnormal inflammatory response by interfering with secretion of pro-inflammatory cytokines such as tumor necrosis factor alpha (TNFα) and other pathways involving activation of c-Jun N-terminal kinase (JNK)/ extracellular signal-regulated kinases ½ (ERK1/2). Enhancement of intracellular antioxidants such as glutathione (GSH) is one of the major mechanisms by which NAC protects against reactive oxygen species (ROS) driven generation of oxidative stress. IRS, insulin receptor substrate 1.

causing chain activation of other damaging pro-inflammatory markers such as PAI-1, NF-κB and MCP-1 [24,30,76]. In addition to promoting adipogenesis, increased secretion and expression of these pro-inflammatory markers can target insulin signaling by impairing PI3K/AKT pathway, concomitantly causing excess production of ROS, partially mediated through the phosphorylation of JNK and ERK1/2 proteins [35,49]. The latter proteins, including MAPK and the energy sensing AMPK greatly participate in deciding the fate of cells under metabolic stress through their involvement in energy homeostasis, insulin signaling, inflammation and oxidative stress linked mechanisms, as reviewed elsewhere [124]. This suggests that molecular mechanisms involved in obesity associated complications remain diverse, but are somehow connected in driving insulin resistance, inflammation and oxidative stress linked damage.

Nonetheless, adipose tissue expansion in an obese state may also implicate regulation of other diverse molecular targets such as the nuclear receptor superfamily of ligand-activated transcription factors that drive the onset of obesity linked complications. For example, PPARγ is considered a master regulator of adipocyte differentiation, and its increased expression with C/EBPβ is linked with stimulation of adipocyte gene transcription program and drives adipogenesis [11]. Conversely, enhancing levels of GSH could block both adipogenic transcription factors PPARγ and C/EBPβ [11]. Consistently, inhibition of ROS formation by compounds that support GSH endogenous synthesis such as NAC could suppress adipogenesis by reducing the expression of PPARγ and C/EBPβ [12]. This may suggest that by mediating adipogenic genes like PPARs, NAC may be able to control lipid metabolism and other processes involved in mitochondrial biogenesis and antioxidant defenses.

Evidence presented on the review show that in addition to reducing body mass or inhibiting lipid accumulation, NAC can improve mitochondrial function in adipocytes by enhancing intracellular antioxidants [60,80]. Its therapeutic effects extend to improving insulin sensitivity via mainly improving PI3K/AKT signaling, blocking an abnormal inflammatory response by interfering with secretion of pro-inflammatory cytokines such as TNFα and other pathways involving activation of JNK/ERK1/2 [24,49,65]. Moreover, co-factors such as zinc may enhance the therapeutic effect of NAC in protecting against obesity associated complications [46]. Whereas majority of included studies summarized in Tables 1–5, through different experimental models, showed that NAC presents comparable effect when used in combination with other natural compounds like EGCG. Antioxidants and natural products co-treatment with NAC may support a combined synergistic effect although this data should be further inquired since only few studies tested the combined effect of NAC with other molecules. In particular, the major obstacle is the limited number of studies that have been done to assess the therapeutic effect of NAC in comparison or combination to well-known antidiabetic therapies. Some experimental evidence shows that NAC can comparatively improve glucose tolerance and block inflammation by mostly enhancing endogenous antioxidants when used with rosiglitazone or metformin [74,95], however more studies are necessary to validate these effects, especially using different obesity models. Taking into consideration that NAC affected a lot of molecular pathways including those involved in inflammation and metabolism, it could also be a viable drug target to assess its therapeutic value as an anti-inflammatory molecule for T1D clinical trials, as previously discussed with other therapies [125,126]. This could significantly enhance our understanding on whether the combination use of NAC with antidiabetic drugs is a viable approach to maintain glycemic levels and delay metabolic abnormalities. Nonetheless, overall summarized evidence highlights a strong potential of NAC to protect against obesity associated complications. This is consistent with others [127,128], assessing the use of antioxidants for management of obesity and its associated complications.

5. Concluding remarks

Obesity remains a serious health issue due to its epidemic prevalence and its association with the development of the metabolic syndrome. Rapid growth of obesity and associated commodities is driven by rapid urbanization that is accompanied by sedentary lifestyle and excess food intake. Several detrimental metabolic complications that aggravate an obese state include insulin resistance, inflammation and oxidative stress, and are driven by excessive adipocyte lipid accumulation. An interconnected network of molecular mechanisms such as enhancement of PPARγ and C/EBPβ adipogenetic transcription factors, impairment of PI3K/AKT insulin signaling pathway, as well as raised pro-inflammatory markers and depleted antioxidant systems all contribute to worsening of obesity associated complications (Fig. 2). This has caused more research to investigate novel therapies that could modulate the aforementioned molecular mechanisms to manage complications linked with an obese state. Although evidence presented on this review supports the role of NAC for such function, more research in obese human subjects is still necessary. The studies presented on this review are very heterogeneous in terms of experimental model used, mostly focusing on cultured cell lines, some deal with animal models, largely using ex vivo analysis, while very few have made use of proper in vivo clinical trials. Nonetheless, its enhanced bioavailability and safety profile makes NAC an interesting agent to further assess for its amelioration of obesity associated complications.

Acknowledgements

This work was supported in part by the Biomedical Research and Innovation Platform of the South African Medical Research Council (SAMRC) baseline funding and the National Research Foundation (Grant number:117829). While, PV Dludla was also partially supported as a Post-Doctoral Fellow by funding from Research Capacity Development Division of the SAMRC. The grant holders acknowledge that opinions, findings, and conclusions or recommendations expressed in any publication generated by the SAMRC or National Research Foundation supported research are those of the authors, and that the SAMRC accepts no liability whatsoever in this regard.

References

[1] Obesity, World Health Organization, 2019 Accessed at https://www.who.int/topics/obesity/en/ . Accessed on 22 January.
[2] Noncommunicable Diseases, World Health Organization, 2019 Accessed at https://www.who.int/news-room/fact-sheets/detail/noncommunicable-diseases . Accessed on 22 January.
[3] Global Status Report on Noncommunicable Diseases 2014, World Health Organization, 2019 Accessed at https://www.who.int/nmh/publications/ncd-status-report-2014/en/ . Accessed on 22 January.
[4] H. Choquet, D. Meyre, Genetics of obesity: what have we learned? Curr. Genomics 12 (2011) 169–179.
[5] A. Hruby, F.B. Hu, The epidemiology of obesity: a big picture, Pharmacoeconomics 33 (2015) 673–689.
[6] R. Monteiro, I. Azevedo, Chronic inflammation in obesity and the metabolic syndrome, Mediators Inflamm. 2010 (2010), https://doi.org/10.1155/2010/289645.
[7] P. Manna, S.K. Jain, Obesity, oxidative stress, adipose tissue dysfunction, and the associated health risks: causes and therapeutic strategies, Metab. Syndr. Relat. Disord. 13 (2015) 423–444.
[8] Q. Zhang, Y. Ju, Y. Ma, T. Wang, N-acetylcysteine improves oxidative stress and inflammatory response in patients with community acquired pneumonia: a randomized controlled trial, Medicine (Baltimore) 97 (2018) e13087.
[9] G. Hashemi, M. Mirjalili, Z. Basiri, A. Tahamoli-Roudsari, N. Kheiripour, M. Shahdoust, et al., Evaluating the effects of oral N-acetyl cysteine on inflammatory and oxidative stress biomarkers in rheumatoid arthritis, Curr. Rheumatol. Rev. (2018), https://doi.org/10.2174/1573403X14666180926100811.
[10] Essential Medicines and Health Products, World Health Organization, 2019 Accessed at https://www.who.int/medicines/en/ . Accessed on 01 Februarly.
[11] P. Calzadilla, D. Sapochnik, S. Cosentino, V. Diz, L. Dicelio, J.C. Calvo, et al., N-acetylcysteine reduces markers of differentiation in 3T3-L1 adipocytes, Int. J. Mol. Sci. 12 (2011) 6936–6951.
[12] Y.C. Chang, Y.H. Yu, J.Y. Shew, W.J. Lee, J.J. Hwang, Y.H. Chen, et al., Deficiency of NPGPx, an oxidative stress sensor, leads to obesity in mice and human, EMBO Mol. Med. 5 (2013) 1165–1179.
[13] L. Liu, P. Zou, L. Zheng, L.E. Linarelli, S. Amarell, A. Passaro, et al., Tamoxifen reduces fat mass by boosting reactive oxygen species, Cell Death Dis. 6 (2015) e1586.
[14] A. Talebi, J. Dehairs, F. Rambow, A. Rogiers, D. Nittner, R. Derua, et al., Sustained SREBP-1-dependent lipogenesis as a key mediator of resistance to BRAF-targeted therapy, Nat. Commun. 9 (2018) 2500.
[15] E.A. McClure, C.D. Gipson, R.J. Malcolm, P.W. Kalivas, K.M. Gray, Potential role of N-acetylcysteine in the management of substance use disorders, CNS Drugs 28 (2014) 95–106.
[16] W. Zheng, Q.E. Zhang, D.B. Cai, X.H. Yang, Y. Qiu, G.S. Ungvari, et al., N-acetylcysteine for major mental disorders: a systematic review and meta-analysis of randomized controlled trials, Acta Psychiatr. Scand. 137 (2018) 391–400.
[17] P.V. Dludla, S.C. Dias, N. Obonye, R. Johnson, J. Louw, B.B. Nkambule, A systematic review on the protective effect of N-acetyl cysteine against diabetes-associated cardiovascular complications, Am. J. Cardiovasc. Drugs 18 (2018) 283–298.
[18] L. Shamseer, D. Moher, M. Clarke, D. Ghersi, A. Liberati, M. Petticrew, et al., Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation, Bmj 350 (2015) g7647.
[19] P.V. Dludla, B.B. Nkambule, S.C. Dias, R. Johnson, Cardioprotective potential of N-acetyl cysteine against hyperglycaemia-induced oxidative damage: a protocol for a systematic review, Syst. Rev. 6 (2017) 96.
[20] The Joanna Briggs Institute, Joanna Briggs Institute reviewers' manual: 2016, edition, The Joanna Briggs Institute, Australia, 2016.
[21] J.P.T. Higgins, S. Green, Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [updated March 2011], The Cochrane Collaboration, 2011 Available from: http://handbook.cochrane.org/ . Accessed 07 November 2019.
[22] Y. Unno, M. Sakai, Y. Sakamoto, A. Kuniyasu, H. Nakayama, R. Nagai, et al., Advanced glycation end products-modified proteins and oxidized LDL mediate down-regulation of leptin in mouse adipocytes via CD36, Biochem. Biophys. Res. Commun. 325 (2004) 151–156.
[23] S. Horiuchi, Y. Unno, H. Usui, K. Shikata, K. Takaki, W. Koito, et al., Pathological roles of advanced glycation end product receptors SR-A and CD36, Ann. N. Y. Acad. Sci. 1043 (2005) 671–675.
[24] S. Araki, K. Dobashi, K. Kubo, Y. Yamamoto, K. Asayama, A. Shirahata, N-acetylcysteine attenuates TNF-alpha induced changes in secretion of interleukin-6, plasminogen activator inhibitor-1 and adiponectin from 3T3-L1 adipocytes, Life Sci. 79 (2006) 2405–2412.
[25] M. Kamigaki, S. Sakaue, I. Tsujino, H. Ohira, D. Ikeda, N. Itoh, et al., Oxidative stress provokes atherogenic changes in adipokine gene expression in 3T3-L1 adipocytes, Biochem. Biophys. Res. Commun. 339 (2006) 624–632.
[26] J.R. Kim, H.H. Ryu, H.J. Chung, J.H. Lee, S.W. Kim, W.H. Kwun, et al., Association of anti-obesity activity of N-acetylcysteine with metallothionein-II down-regulation, Exp. Mol. Med. 38 (2006) 162–172.
[27] X. Jia, L. Wu, Accumulation of endogenous methylglyoxal impaired insulin signaling in adipose tissue of fructose-fed rats, Mol. Cell. Biochem. 306 (2007) 133–139.
[28] H. Unoki, H. Bujo, S. Yamagishi, M. Takeuchi, T. Imaizumi, Y. Saito, Advanced glycation end products attenuate cellular insulin sensitivity by increasing the generation of intracellular reactive oxygen species in adipocytes, Diabetes Res. Clin. Pract. 76 (2007) 236–244.
[29] D.J. Espiritu, T. Mazzone, Oxidative stress regulates adipocyte apolipoprotein e and suppresses its expression in obesity, Diabetes 57 (2008) 2992–2998.
[30] J. Sun, Y. Xu, Z. Dai, Y. Sun, Intermittent high glucose stimulate MCP-l, IL-18, and PAI-1, but inhibit adiponectin expression and secretion in adipocytes dependent of ROS, Cell Biochem. Biophys. 55 (2009) 173–180.
[31] S. Okada, C. Kozuka, H. Masuzaki, S. Yasue, T. Ishii-Yonemoto, T. Tanaka, Adipose tissue-specific dysregulation of angiotensinogen by oxidative stress in obesity, Metabolism 59 (2010) 1241–1251.
[32] P. Calzadilla, M. Gómez-Serrano, E. García-Santos, A. Schiappacasse, Y. Abalde, J.C. Calvo, et al., N-acetylcysteine affects obesity-related protein expression in 3T3-L1 adipocytes, Redox Rep. 18 (2013) 210–218.
[33] R. Lee, I. Jung, M. Park, H. Ha, K.H. Yoo, Real-time monitoring of adipocyte differentiation using a capacitance sensor array, Lab Chip 13 (2013) 3410–3416.
[34] F. Tada, M. Abe, K. Kawasaki, T. Miyake, C. Shiyi, Y. Hiasa, et al., B cell activating factor in obesity is regulated by oxidative stress in adipocytes, J. Clin. Biochem. Nutr. 52 (2013) 120–127.
[35] J.K. Posa, S. Selvaraj, K.N. Sangeetha, S.K. Baskaran, B.S. Lakshmi, p53 mediates impaired insulin signaling in 3T3-L1 adipocytes during hyperinsulinemia, Cell Biol. Int. 38 (2014) 818–824.
[36] L. Liu, P. Zou, L. Zheng, L.E. Linarelli, S. Amarell, A. Passaro, et al., Tamoxifen reduces fat mass by boosting reactive oxygen species, Cell Death Dis. 6 (2015) e1586.
[37] Y. Kadota, Y. Toriuchi, Y. Aki, Y. Mizuno, T. Kawakami, T. Nakaya, et al., Metallothioneins regulate the adipogenic differentiation of 3T3-L1 cells via the insulin signaling pathway, PLoS One 12 (2017) e0176070.
[38] D.H. Olson, J.S. Burrill, J. Kuzmicic, W.S. Hahn, J.M. Park, D.H. Kim, et al., Down regulation of Peroxiredoxin-3 in 3T3-L1 adipocytes leads to oxidation of Rictor in the mammalian-target of rapamycin complex 2 (mTORC2), Biochem. Biophys. Res. Commun. 493 (2017) 1311–1317.
[39] E.R. Andreeva, O.O. Udartseva, O.V. Zhidkova, S.V. Buravkov, M.I. Ezdakova, L.B. Buravkova, IFN-gamma priming of adipose-derived stromal cells at "physiological" hypoxia, J. Cell. Physiol. 233 (2018) 1535–1547.
[40] P.V. Dludla, B. Jack, A. Viraragavan, C. Pheiffer, R. Johnson, J. Louw, et al., A dose-dependent effect of dimethyl sulfoxide on lipid content, cell viability and oxidative stress in 3T3-L1 adipocytes, Toxicol. Rep. 5 (2018) 1014–1020.
[41] Y. Kanda, T. Hinata, S.W. Kang, Y. Watanabe, Reactive oxygen species mediate adipocyte differentiation in mesenchymal stem cells, Life Sci. 89 (2011) 250–258.
[42] M. Raffaele, I. Barbagallo, M. Licari, G. Carota, G. Sferrazzo, M. Spampinato, et al., N-acetylcysteine (NAC) ameliorates lipid-related metabolic dysfunction in bone marrow stromal cells-derived adipocytes, Evid. Complement. Alternat. Med. 2018 (2018) 5310961.
[43] H. Nagasaki, T. Yoshimura, N. Aoki, Real-time monitoring of inflammation status in 3T3-L1 adipocytes possessing a secretory Gaussia luciferase gene under the control of nuclear factor-kappa B response element, Biochem. Biophys. Res. Commun. 420 (2012) 623–627.
[44] N.A. Cremers, D.M. Lundvig, S.C. van Dalen, R.F. Schelbergen, P.L. van Lent, W.A. Szarek, et al., Curcumin-induced heme oxygenase-1 expression prevents H2O2-induced cell death in wild type and heme oxygenase-2 knockout adipose-derived mesenchymal stem cells, Int. J. Mol. Sci. 15 (2014) 17974-1799.
[45] G. Chen, Y. Han, W. He, F. Liang, Amentoflavone protects against high fat-induced metabolic dysfunction: possible role of the regulation of adipogenic differentiation, Int. J. Mol. Med. 38 (2016) 1759–1767.
[46] Y. Adachi, Y. Yoshikawa, H. Sakurai, Antidiabetic zinc(II)-N-acetyl-L-cysteine complex: evaluations of in vitro insulinomimetic and in vivo blood glucose-lowering activities, Biofactors 29 (2007) 213–223.
[47] C.T. Wang, H.H. Chang, C.H. Hsiao, M.J. Lee, H.C. Ku, Y.J. Hu, et al., The effects of green tea (-)-epigallocatechin-3-gallate on reactive oxygen species in 3T3-L1 preadipocytes and adipocytes depend on the glutathione and 67 kDa laminin receptor pathways, Mol. Nutr. Food Res. 53 (2009) 349–360.
[48] H.C. Ku, Y.W. Tsuei, C.C. Kao, J.T. Weng, L.J. Shih, H.H. Chang, et al., Green tea (-)-epigallocatechin gallate suppresses IGF-I and IGF-II stimulation of 3T3-L1 adipocyte glucose uptake via the glucose transporter 4, but not glucose transporter 1 pathway, Gen. Comp. Endocrinol. 199 (2014) 46–55.
[49] H. Guo, W. Ling, Q. Wang, C. Liu, Y. Hu, M. Xia, Cyanidin 3-glucoside protects 3T3-L1 adipocytes against H2O2- or TNF-alpha-induced insulin resistance by inhibiting c-Jun NH2-terminal kinase activation, Biochem. Pharmacol. 75 (2008) 1393–1401.
[50] H. Fukuoka, K. Iida, H. Nishizawa, M. Imanaka, R. Takeno, G. Iguchi, et al., IGF-I stimulates reactive oxygen species (ROS) production and inhibits insulin-dependent glucose uptake via ROS in 3T3-L1 adipocytes, Growth Horm. IGF Res. 20 (2010) 212–219.
[51] H.I. Krieger-Brauer, H. Kather, Antagonistic effects of different members of the fibroblast and platelet-derived growth factor families on adipose conversion and NADPH-dependent H2O2 generation in 3T3 L1-cells, Biochem. J. 307 (1995) 549–556.
[52] R. Ben-Romano, A. Rudich, S. Etzion, R. Potashnik, E. Kagan, U. Greenbaum, et al., Nelfinavir induces adipocyte insulin resistance through the induction of oxidative stress: differential protective effect of antioxidant agents, Antivir. Ther. (Lond.) 11 (2006) 1051–1060.
[53] X. Ge, Z. Liu, W. Qi, X. Shi, Q. Zhai, Chromium (VI) induces insulin resistance in 3T3-L1 adipocytes through elevated reactive oxygen species generation, Free Radic. Res. 42 (2008) 554–563.
[54] X. Ge, Q. Yu, W. Qi, X. Shi, Q. Zhai, Chronic insulin treatment causes insulin

resistance in 3T3-L1 adipocytes through oxidative stress, Free Radic. Res. 42 (2008) 582–591.
[55] K.H. Wang, et al., Optimizing proliferation and characterization of multipotent stem cells from porcine adipose tissue, Biotechnol. Appl. Biochem. 51 (Pt 4) (2008) 159–166.
[56] K.H. Wang, A.P. Kao, H. Wangchen, F.Y. Wang, C.H. Chang, C.C. Chang, et al., Aldosterone inhibits insulin-induced glucose uptake by degradation of insulin receptor substrate (IRS) 1 and IRS2 via a reactive oxygen species-mediated pathway in 3T3-L1 adipocytes, Endocrinology 150 (2009) 1662–1669.
[57] A.S. Molgat, A. Gagnon, A. Sorisky, Macrophage-induced preadipocyte survival depends on signaling through Akt, ERK1/2, and reactive oxygen species, Exp. Cell Res. 317 (2011) 521–530.
[58] J.H. Kim, et al., The pivotal role of reactive oxygen species generation in the hypoxia-induced stimulation of adipose-derived stem cells, Stem Cells Dev. 20 (10) (2011) 1753–1761.
[59] J.H. Kim, S.H. Park, S.G. Park, J.S. Choi, Y. Xia, J.H. Sung, Pharmacokinetics, tissue distribution, and anti-lipogenic/adipogenic effects of allyl-isothiocyanate metabolites, PLoS One 10 (2015) e0132151.
[60] E. Peris, P. Micallef, A. Paul, V. Palsdottir, A. Enejder, M. Bauzá-Thorbrügge, et al., Antioxidant treatment induces reductive stress associated with mitochondrial dysfunction in adipocytes, J. Biol. Chem. 15 (2018) 2340–2352.
[61] E.L. Greene, B.A. Nelson, K.A. Robinson, M.G. Buse, Alpha-Lipoic acid prevents the development of glucose-induced insulin resistance in 3T3-L1 adipocytes and accelerates the decline in immunoreactive insulin during cell incubation, Metabolism 50 (2001) 1063–1069.
[62] A.M. Lennon, M. Ramaugé, A. Dessouroux, M. Pierre, MAP kinase cascades are activated in astrocytes and preadipocytes by 15-deoxy-Delta(12-14)-prostaglandin J(2) and the thiazolidinedione ciglitazone through peroxisome proliferator activator receptor gamma-independent mechanisms involving reactive oxygenated species, J. Biol. Chem. 277 (2002) 29681–29685.
[63] K. Takahashi, S. Yamaguchi, T. Shimoyama, H. Seki, K. Miyokawa, H. Katsuta, et al., JNK- and IkappaB-dependent pathways regulate MCP-1 but not adiponectin release from artificially hypertrophied 3T3-L1 adipocytes preloaded with palmitate in vitro, Am. J. Physiol. Endocrinol. Metab. 294 (2008) E898–909.
[64] I. Ishii, Y. Ikeguchi, H. Mano, M. Wada, A.E. Pegg, A. Shirahata, Polyamine metabolism is involved in adipogenesis of 3T3-L1 cells, Amino Acids 42 (2012) 619–626.
[65] K. Takahashi, K. Miyokawa-Gorin, K. Handa, A. Kitahara, R. Moriya, H. Onuma, et al., Endogenous oxidative stress, but not ER stress, induces hypoxia-independent VEGF120 release through PI3K-dependent pathways in 3T3-L1 adipocytes, Obesity Silver Spring (Silver Spring) 21 (2013) 1625–1634.
[66] H. Xiao, J. Wang, L. Yuan, C. Xiao, Y. Wang, X. Liu, Chicoric acid induces apoptosis in 3T3-L1 preadipocytes through ROS-mediated PI3K/Akt and MAPK signaling pathways, J. Agric. Food Chem. 61 (2013) 1509–1520.
[67] J.H. Kim, S.J. Park, B. Kim, Y.G. Choe, D.S. Lee, Insulin-stimulated lipid accumulation is inhibited by ROS-scavenging chemicals, but not by the Drp1 inhibitor Mdivi-1, PLoS One 12 (2017) e0185764.
[68] D. Soto, M. Gomez-Serrano, A. Pieralisi, J.C. Calvo, B. Peral, L.N. Guerra, N-acetylcysteine inhibits kinase phosphorylation during 3T3-L1 adipocyte differentiation, Redox Rep. 22 (2017) 265–271.
[69] Q. Li, Y. Yin, Y. Zheng, F. Chen, P. Jin, Inhibition of autophagy promoted high glucose/ROS-mediated apoptosis in ADSCs, Stem Cell Res. Ther. 9 (2018) 289.
[70] Z. An, H. Wang, P. Song, M. Zhang, X. Geng, M.H. Zou, Nicotine-induced activation of AMP-activated protein kinase inhibits fatty acid synthase in 3T3L1 adipocytes: a role for oxidant stress, J. Biol. Chem. 282 (2007) 26793–26801.
[71] M.K. Shin, B. Choi, E.Y. Kim, J.E. Park, E.S. Hwang, H.J. Lee, et al., Elevated pentraxin 3 in obese adipose tissue promotes adipogenic differentiation by activating neuropeptide Y signaling, Front. Immunol. 9 (2018) 1790.
[72] E. Fontana, J. Boucher, L. Marti, J.M. Lizcano, X. Testar, A. Zorzano, et al., Amine oxidase substrates mimic several of the insulin effects on adipocyte differentiation in 3T3 F442A cells, Biochem. J. 356 (2001) 769–777.
[73] W. Guo, W. Xie, J. Han, Modulation of adipocyte lipogenesis by octanoate: involvement of reactive oxygen species, Nutr. Metab. (Lond) 3 (2006) 30.
[74] Y.S. Lee, A.Y. Kim, J.W. Choi, M. Kim, S. Yasue, H.J. Son, et al., Dysregulation of adipose glutathione peroxidase 3 in obesity contributes to local and systemic oxidative stress, Mol. Endocrinol. 22 (2008) 2176–2189.
[75] P. Hallenborg, C. Jørgensen, R.K. Petersen, S. Feddersen, P. Araujo, P. Markt, et al., Epidermis-type lipoxygenase 3 regulates adipocyte differentiation and peroxisome proliferator-activated receptor gamma activity, Mol. Cell. Biol. 30 (2010) 4077–4091.
[76] S. Araki, K. Dobashi, K. Kubo, R. Kawagoe, Y. Yamamoto, A. Shirahata, N-acetylcysteine inhibits induction of nitric oxide synthase in 3T3-L1 adipocytes, J. UOEH 29 (2007) 417–429.
[77] A. Hirata, N. Maeda, H. Nakatsuji, A. Hiuge-Shimizu, T. Okada, T. Funahashi, et al., Contribution of glucocorticoid-mineralocorticoid receptor pathway on the obesity-related adipocyte dysfunction, Biochem. Biophys. Res. Commun. 419 (2012) 182–187.
[78] Y. Lin, A.H. Berg, P. Iyengar, T.K. Lam, A. Giacca, T.P. Combs, et al., The hyperglycemia-induced inflammatory response in adipocytes: the role of reactive oxygen species, J. Biol. Chem. 280 (2005) 4617–4626.
[79] J.A. Gillis, S. Gebremeskel, K. Phipps, P. Johnston, P. Hong, M. Bezuhly, N-acetylcysteine (NAC) improves free autologous fat graft survival: a preclinical study, Plast. Reconstr. Surg. 134 (Suppl. 1) (2014) 70–71.
[80] W. Wang, Y. Zhang, W. Lu, K. Liu, Mitochondrial reactive oxygen species regulate adipocyte differentiation of mesenchymal stem cells in hematopoietic stress induced by arabinosylcytosine, PLoS One 10 (2015) e0120629.
[81] Y. Ma, M. Gao, D. Liu, N-acetylcysteine protects mice from high fat diet-induced metabolic disorders, Pharm. Res. 33 (2016) 2033–2042.
[82] A. Pieralisi, C. Martini, D. Soto, M.C. Vila, J.C. Calvo, L.N. Guerra, N-acetylcysteine inhibits lipid accumulation in mouse embryonic adipocytes, Redox Biol. 9 (2016) 39–44.
[83] K.S. da Silva, P.R. Pinto, N.T. Fabre, D.J. Gomes, K. Thieme, L.S. Okuda, et al., N-acetylcysteine counteracts adipose tissue macrophage infiltration and insulin resistance elicited by advanced glycated albumin in healthy rats, Front. Physiol. 8 (2017) 723.
[84] L. Williams, M.J. Charron, R.S. Sellers, High post-natal mortality associated with defects in lung maturation and reduced adiposity in mice with gestational exposure to high fat and N-acetylcysteine, Res. Vet. Sci. 114 (2017) 262–265.
[85] A. Berry, V. Bellisario, P. Panetta, C. Raggi, M.C. Magnifico, M. Arese, et al., Administration of the antioxidant N-acetyl-cysteine in pregnant mice has long-term positive effects on metabolic and behavioral endpoints of male and female offspring prenatally exposed to a high-fat diet, Front. Behav. Neurosci. 12 (2018) 48.
[86] J. Zheng, X. Yuan, C. Zhang, P. Jia, S. Jiao, X. Zhao, et al., N-Acetylcysteine alleviates gut dysbiosis and glucose metabolic disorder in high-fat diet-fed mice, J. Diabetes 11 (2019) 32–45.
[87] F.C. Shen, S.W. Weng, C.F. Tsao, H.Y. Lin, C.S. Chang, C.Y. Lin, et al., Early intervention of N-acetylcysteine better improves insulin resistance in diet-induced obesity mice, Free Radic. Res. 52 (2018) 1296–1310.
[88] J. Yan, G. Tie, S. Wang, K.E. Messina, S. DiDato, S. Guo, et al., Type 2 diabetes restricts multipotency of mesenchymal stem cells and impairs their capacity to augment postischemic neovascularization in db/db mice, J. Am. Heart Assoc. 1 (2012) e002238.
[89] M. Yano, T. Yamamoto, N. Nishimura, T. Gotoh, K. Watanabe, K. Ikeda, et al., Increased oxidative stress impairs adipose tissue function in sphingomyelin synthase 1 null mice, PLoS One 8 (2013) e61380.
[90] F. Yuan, L. Zhang, Y. Cao, W. Gao, C. Zhao, Y. Fang, et al., Spermidine/spermine N1-acetyltransferase-mediated polyamine catabolism regulates beige adipocyte biogenesis, Metabolism 85 (2018) 298–304.
[91] B. Lu, D. Ennis, R. Lai, E. Bogdanovic, R. Nikolov, L. Salamon, et al., Enhanced sensitivity of insulin-resistant adipocytes to vanadate is associated with oxidative stress and decreased reduction of vanadate (+5) to vanadyl (+4), J. Biol. Chem. 276 (2001) 35589–35598.
[92] T. Wang, Y. Si, O.S. Shirihai, H. Si, V. Schultz, R.F. Corkey, et al., Respiration in adipocytes is inhibited by reactive oxygen species, Obesity (Silver Spring) 18 (2010) 1493–1502.
[93] S.H. Ro, M. Nam, I. Jang, H.W. Park, H. Park, I.A. Semple, et al., Sestrin2 inhibits uncoupling protein 1 expression through suppressing reactive oxygen species, Proc. Natl. Acad. Sci. U S A 111 (2014) 7849–7854.
[94] Y.F. Tian, W.C. Chang, C.H. Loh, P.S. Hsieh, Leptin-mediated inflammatory signaling crucially links visceral fat inflammation to obesity-associated beta-cell dysfunction, Life Sci. 116 (2014) 51–58.
[95] Y.W. Chen, R.A. Harris, Z. Hatahet, K.M. Chou, Ablation of XP-V gene causes adipose tissue senescence and metabolic abnormalities, Proc. Natl. Acad. Sci. U S A 112 (2015) E4556–E4564.
[96] H.K. Kim, Y.J. Kim, J.T. Kim, C.H. Kwon, Y.K. Kim, Y.C. Bae, et al., Alterations in the proangiogenic functions of adipose tissue-derived stromal cells isolated from diabetic rats, Stem Cells Dev. 17 (2008) 669–680.
[97] K. Schneider, J. Valdez, J. Nguyen, M. Vawter, B. Galke, T.W. Kurtz, et al., Increased energy expenditure, Ucp1 expression, and resistance to diet-induced obesity in mice lacking nuclear factor-erythroid-2-related transcription factor-2 (Nrf2), J. Biol. Chem. 291 (2016) 7754–7766.
[98] A.K. Elshorbagy, M. Valdivia-Garcia, D.A. Mattocks, J.D. Plummer, D.S. Orentreich, N. Orentreich, et al., Effect of taurine and N-acetylcysteine on methionine restriction-mediated adiposity resistance, Metabolism 62 (2013) 509–517.
[99] P. Chimin, M.L. Andrade, T. Belchior, V.A. Paschoal, J. Magdalon, A.S. Yamashita, et al., Adipocyte mTORC1 deficiency promotes adipose tissue inflammation and NLRP3 inflammasome activation via oxidative stress and de novo ceramide synthesis, J. Lipid Res. 58 (2017) 1797–1807.
[100] Y. Uchida, K. Ohba, T. Yoshioka, K. Irie, T. Muraki, Y. Maru, Cellular carbonyl stress enhances the expression of plasminogen activator inhibitor-1 in rat white adipocytes via reactive oxygen species-dependent pathway, J. Biol. Chem. 279 (2004) 4075–4083.
[101] W. Hildebrandt, A. Hamann, H. Krakowski-Roosen, R. Kinscherf, K. Dugi, R. Sauer, et al., Effect of thiol antioxidant on body fat and insulin reactivity, J. Mol. Med. 82 (2004) 336–344.
[102] T.M. Lin, J.L. Tsai, S.D. Lin, C.S. Lai, C.C. Chang, Accelerated growth and prolonged lifespan of adipose tissue-derived human mesenchymal stem cells in a medium using reduced calcium and antioxidants, Stem Cells Dev. 14 (2005) 92–102.
[103] J.Y. Park, M.J. Kim, Y.K. Kim, J.S. Woo, Ceramide induces apoptosis via caspase-dependent and caspase-independent pathways in mesenchymal stem cells derived from human adipose tissue, Arch. Toxicol. 85 (2011) 1057–1065.
[104] S.A. Krawczyk, J.F. Haller, T. Ferrante, R.A. Zoeller, B.E. Corkey, Reactive oxygen species facilitate translocation of hormone sensitive lipase to the lipid droplet during lipolysis in human differentiated adipocytes, PLoS One 7 (2012) e34904.
[105] M. Moriyama, H. Moriyama, A. Ueda, Y. Nishibata, H. Okura, A. Ichinose, et al., Human adipose tissue-derived multilineage progenitor cells exposed to oxidative stress induce neurite outgrowth in PC12 cells through p38 MAPK signaling, BMC Cell Biol. 13 (2012) 21.
[106] L. Xiong, J. Sun, C. Hirche, J. Yang, Y. Yang, Y. Xia, et al., In vitro N-acetyl-L-

cysteine promotes proliferation and suppresses interleukin-8 expression in adipose-derived stem cells, Aesthetic Plast. Surg. 36 (2012) 1260–1265.
[107] R. Domenis, N. Bergamin, G. Gianfranceschi, C. Vascotto, M. Romanello, S. Rigo, et al., The redox function of APE1 is involved in the differentiation process of stem cells toward a neuronal cell fate, PLoS One 9 (2014) e89232.
[108] A. Gagnon, M.L. Langille, S. Chaker, T.T. Antunes, J. Durand, A. Sorisky, TSH signaling pathways that regulate MCP-1 in human differentiated adipocytes, Metabolism 63 (2014) 812–821.
[109] Z. Wang, H. Li, R. Guo, Q. Wang, D. Zhang, Antioxidants inhibit advanced glycosylation end-product-induced apoptosis by downregulation of miR-223 in human adipose tissue-derived stem cells, Sci. Rep. 6 (2016) 23021.
[110] D. Lee, D.N. Heo, H.R. Nah, S.J. Lee, W.K. Ko, J.S. Lee, et al., Injectable hydrogel composite containing modified gold nanoparticles: implication in bone tissue regeneration, Int. J. Nanomedicine 13 (2018) 7019–7031.
[111] S. Šalamon, B. Kramar, T.P. Marolt, B. Poljšak, I. Milisav, Medical and dietary uses of n-acetylcysteine, Antioxidants Basel (Basel) 8 (2019), https://doi.org/10.3390/antiox8050111.
[112] R. Bassi, A. Trevisani, S. Tezza, M. Ben Nasr, F. Gatti, A. Vergani, et al., Regenerative therapies for diabetic microangiopathy, Exp. Diabetes Res. 2012 (2012) 916560.
[113] A. Petrelli, A. Maestroni, G.P. Fadini, D. Belloni, M. Venturini, M. Albiero, et al., Improved function of circulating angiogenic cells is evident in type 1 diabetic islet-transplanted patients, Am. J. Transplant. 10 (2010) 2690–2700.
[114] A. Guilherme, F. Henriques, A.H. Bedard, M.P. Czech, Molecular pathways linking adipose innervation to insulin action in obesity and diabetes mellitus, Nat. Rev. Endocrinol. 15 (2019) 207–225.
[115] P.V. Dludla, B.B. Nkambule, B. Jack, Z. Mkandla, T. Mutize, S. Silvestri, et al., Inflammation and oxidative stress in an obese state and the protective effects of gallic acid, Nutrients 11 (2018) 1.
[116] S. Le Lay, G. Simard, M.C. Martinez, R. Andriantsitohaina, Oxidative stress and metabolic pathologies: from an adipocentric point of view, Oxid. Med. Cell. Longev. 2014 (2014) 908539.
[117] L. Marseglia, S. Manti, G. D'Angelo, A. Nicotera, E. Parisi, G. Di Rosa, et al., Oxidative stress in obesity: a critical component in human diseases, Int. J. Mol. Sci. 16 (2014) 378–400.
[118] A.M. Lechuga-Sancho, D. Gallego-Andujar, P. Ruiz-Ocaña, F.M. Visiedo, A. Saez-Benito, M. Schwarz, et al., Obesity induced alterations in redox homeostasis and oxidative stress are present from an early age, PLoS One 13 (2018) e0191547.
[119] G. Wu, Y.Z. Fang, S. Yang, J.R. Lupton, N.D. Turner, Glutathione metabolism and its implications for health, J. Nutr. 134 (2004) 489–492.
[120] H. Urakawa, A. Katsuki, Y. Sumida, E.C. Gabazza, S. Murashima, K. Morioka, et al., Oxidative stress is associated with adiposity and insulin resistance in men, J. Clin. Endocrinol. Metab. 88 (2003) 4673–4676.
[121] S. Tangvarasittichai, Oxidative stress, insulin resistance, dyslipidemia and type 2 diabetes mellitus, World J. Diabetes 6 (2015) 456–480.
[122] Y. Wang, M. Zhou, K.S. Lam, A. Xu, Protective roles of adiponectin in obesity-related fatty liver diseases: mechanisms and therapeutic implications, Arq. Bras. Endocrinol. Metabol. 53 (2009) 201–212.
[123] M.G. Myers, R.L. Leibel, R.J. Seeley, M.W. Schwartz, Obesity and leptin resistance: distinguishing cause from effect, Trends Endocrinol. Metab. 21 (2010) 643–651.
[124] K.C. Nandipati, S. Subramanian, D.K. Agrawal, Protein kinases: mechanisms and downstream targets in inflammation-mediated obesity and insulin resistance, Mol. Cell. Biochem. 426 (2017) 27–45.
[125] M. Ben Nasr, A. Vergani, J. Avruch, L. Liu, E. Kefaloyianni, F. D'Addio, et al., Co-transplantation of autologous MSCs delays islet allograft rejection and generates a local immunoprivileged site, Acta Diabetol. 52 (2015) 917–927.
[126] M. Ben Nasr, F. D'Addio, V. Usuelli, S. Tezza, R. Abdi, P. Fiorina, The rise, fall, and resurgence of immunotherapy in type 1 diabetes, Pharmacol. Res. 98 (2015) 31–38.
[127] T. Tsuda, Recent progress in anti-obesity and anti-diabetes effect of berries, Antioxidants Basel (Basel) 5 (2016) 2.
[128] D. Abdali, S.E. Samson, A.K. Grover, How effective are antioxidant supplements in obesity and diabetes? Med. Princ. Pract. 24 (2015) 201–215.
[129] J.Y. Huh, E.Y. Seo, H.B. Lee, H. Ha, Glucose-based peritoneal dialysis solution suppresses adiponectin synthesis through oxidative stress in an experimental model of peritoneal dialysis, Perit. Dial. Int. 32 (2012) 20–28.
[130] J. Gillis, S. Gebremeskel, K.D. Phipps, L.A. MacNeil, C.J. Sinal, B. Johnston, et al., Effect of N-acetylcysteine on adipose-derived stem cell and autologous fat graft survival in a mouse model, Plast. Reconstr. Surg. 136 (2015) 179e–188e.
[131] L. Xi, Z. Qian, G. Xu, C. Zhou, S. Sun, Crocetin attenuates palmitate-induced insulin insensitivity and disordered tumor necrosis factor-alpha and adiponectin expression in rat adipocytes, Br. J. Pharmacol. 151 (2007) 610–617.