Vitamin C protects against hypoxia, inflammation, and ER stress in primary human preadipocytes and adipocytes

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ABSTRACT

Dysregulation of adipose tissue involves increased cellular hypoxia, ER stress, and inflammation, and altered adipokine production. This study aimed to investigate the effects of Vitamin C supplementation on these processes in primary human preadipocytes and adipocytes.

Treatment of preadipocytes and adipocytes with the proinflammatory cytokine TNFα and palmitic acid (PA) mimicked the obesogenic milieu, significantly increased markers of hypoxia, ER stress, and inflammation, and reduced secretion of high molecular weight (HMW) adiponectin. Importantly, Vitamin C abolished TNFα + PA induced hypoxia and significantly reduced the increases in ER stress and inflammation in both cell types. Vitamin C also significantly increased the secretion of HMW adiponectin from adipocytes.

These findings indicate that Vitamin C can reduce obesity-associated cellular stress and thus provide a rationale for future investigations.

ARTICLE INFO

Keywords:
Ascorbic acid
Cytokines
Adipokines
Adiponectin
Obesity

1. Introduction

The adipocyte is the central cell type of adipose tissue and represents a primary determinant of metabolic homeostasis (Scherer, 2019). It secretes an array of structural and biologically active factors including components of the extracellular matrix, lipid species, and peptide hormones termed adipokines. Collectively, these factors regulate local and systemic insulin sensitivity, inflammation, and metabolism. From an evolutionary perspective, adipose tissue has evolved to promote survival during cycles of feast and famine. However, in the obesogenic 21st century environment, where there is typically chronic positive energy supply, adipose tissue expansion gives rise to dysregulation of function which underpins the development of obesity-related diseases including type 2 diabetes, atherosclerosis, and cardiovascular disease (Kahn et al., 2019; Ghaben and Scherer, 2019; White and Ravussin, 2018; Haczyzyli et al., 2018). Such adipocyte dysregulation is characterised by increased cellular stress at multiple levels (Scherer, 2019): Hypoxia, ER stress, and increased inflammation have all been demonstrated to contribute (Trayhurn, 2013; Lawler et al., 2016; Sun et al., 2013; Jiao et al., 2011; Lopez-Domenech et al., 2019; Lee et al., 2014; Hotamisligil, 2017).

A principal feature of obesity-associated adipocyte dysregulation is the altered production and secretion of adipokines (Unamuno et al., 2018). For example, hypoxia (Chen et al., 2006; Jiang et al., 2012), ER stress (Zhou et al., 2010; Mondal et al., 2012) and inflammation (He et al., 2015; Zulian et al., 2011) have all been shown to reduce the production of adiponectin, a key adipokine with pleiotropic beneficial anti-diabetic, anti-inflammatory and cardioprotective functions, contributing to the progression of obesity-related diseases (Scherer, 2019; Straub and Scherer, 2019; Wang and Scherer, 2016; Maeda et al., 2020). Adiponectin production involves a process of multimerisation to drive formation of the most biologically active HMW multimers prior to secretion that is dependent on several post-translational modifications (PTMs) (Richards et al., 2006; Wang et al., 2006; Simpson and Whitehead, 2010). The enzymes responsible for a subset of these PTMs, namely the prolyl- and lysyl-hydroxylases, belong to the

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https://doi.org/10.1016/j.mce.2022.111740
Received 20 December 2021; Received in revised form 22 July 2022; Accepted 28 July 2022
Available online 4 August 2022
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2-oxoglutarate-dependent dioxygenases which require Vitamin C as a co-factor (Myllyla et al., 1984). Based on this, we previously investigated the effects of Vitamin C on adiponectin production and demonstrated that supplementation with Vitamin C increased adiponectin multimerisation and secretion from human SGBS adipocytes (Rose et al., 2010).

In addition to its vital role as a co-factor for dioxygenase enzymes, Vitamin C also serves as a crucial water-soluble antioxidant (Traber and Stevens, 2011). Humans and higher primates are unable to synthesise Vitamin C due to a mutation in the L-glucuronolactone oxidase (GULO) enzyme (Yang, 2013) making them dependent on dietary intake to maintain sufficient levels. Perhaps surprisingly, Vitamin C insufficiency or hypovitaminosis (typically defined as a plasma concentration <23 μM) has been estimated to affect 10–20% of adults in high income countries with even higher prevalence in low-income countries (Rowe and Carr, 2020). Furthermore, there is evidence to suggest that obese subjects may have an increased requirement for Vitamin C (Wilson et al., 2017), that low Vitamin C levels may predispose individuals to the development of type 2 diabetes (Wilson et al., 2017; Harding et al., 2008) and that Vitamin C supplementation may have positive effects on glycaemic control and insulin sensitivity (Tabatabaei-Malazy et al., 2014; Dakhale et al., 2011; Mason et al., 2016, 2019; Ashor et al., 2017; He et al., 2021).

In the current study we aimed to investigate the effects of Vitamin C supplementation on markers of hypoxia, ER stress and inflammation in primary human preadipocytes and adipocytes. We included both preadipocytes and mature adipocytes as the former represent an important pool of precursor cells that can be recruited and differentiated into the latter, a process termed adipocyte hyperplasia (Hammarstedt et al., 2018). This process represents a key part of healthy adipose tissue expansion and is impaired by local stressors including inflammatory cytokines produced by the preadipocytes and adipocytes themselves (Hammarstedt et al., 2018; Jiang et al., 2019). We also extended our characterisation of the effects of Vitamin C on adiponectin production (Rose et al., 2010). Supplementation with Vitamin C was performed under control conditions or with co-treatment or pre-treatment of a TNFα-PA cocktail to simulate obesity-induced stress and enable us to determine the efficacy of Vitamin C to prevent or reverse such effects.

2. Materials and methods

2.1. Isolation, culture, differentiation, and treatment of primary human preadipocytes and adipocytes

Primary human preadipocytes (preadipocytes) were isolated from subcutaneous adipose tissue from three subjects (female, age <40 years old, BMI <28, metabolically healthy and no insulin resistance, diabetes or cardiovascular diseases) and cultured as described (Newell et al., 2006). All patients had given their written informed consent and the protocol was approved by the Research Ethics Committees of the University of Queensland, Princess Alexandra Hospital, and Mater Adults Hospital. Preadipocytes were maintained and differentiated into adipocytes eventually as previously described (Newell et al., 2006; Widberg et al., 2003). All patients had given their written informed consent and the protocol was approved by the Research Ethics Committees of the University of Queensland, Princess Alexandra Hospital, and Mater Adults Hospital. Preadipocytes were maintained and differentiated into adipocytes eventually as previously described (Newell et al., 2006; Widberg et al., 2003).

2.2. Measurement of gene expression by semi-quantitative real-time reverse Transcriptase-PCR (sqRT-PCR)

Gene expression was measured by sqRT-PCR and standardized against the expression of cyclophilin (using the 2−ΔΔCt method) essentially as previously described (Newell et al., 2006; Widberg et al., 2003). Briefly, total RNA was extracted using the RNeasy Mini Kit (Qiagen Pty Ltd., Vic, Australia). cDNA was synthesized from 1 μg total RNA using a cDNA synthesis kit (Bioline, NSW, Australia) and sqRT-PCR was performed using the SYBR Hi-ROX kit (Bioline) on a 7900HT Fast real-time PCR system (Ambion Life Technologies). All primers were from Origene and primer sequences are listed in Supp Table 2.

2.3. Measurement of intracellular and secreted proteins by ELISA

Measurement of intracellular hypoxia inducible factor-1α (HIF1α) protein, and secreted levels of IL6 protein, and total and HMW adiponectin were all determined by ELISA (R&D, Minnesota, USA). Measurement of HIF1α protein was performed in preference to gene expression as HIF1α is regulated predominantly at the protein level, via regulation of protein stability, with Vitamin C increasing prolyl-hydroxylation, leading to ubiquitination and proteasomal degradation (Marszen et al., 2004; Vissers et al., 2007). Cells were lysed at the appropriate time in lysis buffer consisting of 50 mM Tris (pH 7.4), 150 mM NaCl, 10% (w/v) glycerol, 10 mM EDTA, 1 mM MgCl2, 20 mM β-glycerophosphosphate, 30 mM NaF, 1% Triton X-100, 25 mg/ml leupeptin, 25 mg/ml pepstatin, and 3 mg/ml aprotinin. After being incubated on ice for 20 min, cell lysates were centrifuged at 12,000 g for 30 min at 4 °C. Protein concentration of cell lysates was determined by BCA Protein Assay. Levels of total and HMW adiponectin secreted from adipocytes in 24 h were determined in parallel to the measurement of ADIPOQ gene expression to investigate the distinct responses of these parameters, as per our previous observations (Rose et al., 2010; Yang et al., 2015). Measurement of secreted IL6 protein was performed to extend and support observations made at the level of gene expression (Yang et al., 2015).

2.4. Statistical analysis

All data are expressed as means ± SEM and are representative of at least three independent experiments. Data analysis was performed using GraphPad Prism version 9.2.0 (GraphPad Software, San Diego, CA). Methods of statistical analysis are indicated in the text. Differences between groups were analyzed by two-way ANOVA followed by Tukey’s multiple comparisons test. Statistical significance was defined as an adjusted p value of <0.05 (p < 0.05). Student’s t-test was performed to investigate differences between preadipocytes and adipocytes with statistical significance defined as p < 0.05.

3. Results

3.1. Vitamin C prevents obesity-associated cell stress in primary human preadipocytes

To recapitulate obesity-associated cellular stress in vitro, we treated preadipocytes with a combination of the pro-inflammatory cytokine TNFα (10 ng/ml) and the saturated fatty acid palmitate (300 μM) (TNFα-PA) for 24 h. This treatment led to an increase in: (i) hypoxia, as indicated by the significant 2–3-fold increases in hypoxia inducible factor-1α (HIF1α) protein as well as the gene expression of downstream effectors VEGFA and GLUT1 (Fig. 1A–B); (ii) ER stress, with expression of sXBP1, CHOP and GRP78 mRNA significantly increased 6–20 fold (Fig. 1C), and; (iii) increased expression of inflammatory genes IL6, MCP1 and TNFα (Fig. 1D). Treatment of control cells with physiological levels of Vitamin C (100 μM) was without effect on any of these parameters (Fig. 1A–D). In marked contrast, co-treatment of TNFα-PA-
treated preadipocytes with Vitamin C prevented the increase in hypoxia (Fig. 1A–B), significantly reduced the induction of ER stress (Fig. 1C) and ameliorated the increased expression of inflammatory genes (Fig. 1D).

3.2. Vitamin C prevents obesity-associated cell stress in primary human adipocytes

Adipocytes, derived from preadipocytes differentiated in vitro (Fig. 2A), treated with TNFα+PA as above showed a significant increase in all markers of hypoxia, ER stress and inflammation (Fig. 2B–E). Co-treatment with Vitamin C completely abrogated the induction of markers of hypoxia by TNFα+PA, reducing them to levels below those in control (untreated) cells (Fig. 2B–C). Vitamin C co-treatment also prevented the effects of TNFα+PA on ER-stress markers, such that these were indistinguishable from those in control cells (Fig. 2D). In contrast, the effects on inflammatory gene expression were more modest, with Vitamin C co-treatment reducing the TNFα+PA-induced increase by 30–50% (Fig. 2E).

Collectively, these observations demonstrate that co-treatment with Vitamin C can reduce or prevent obesity-associated cell stress in both preadipocytes and adipocytes. We next went on to investigate whether

Fig. 1. – Vitamin C prevents obesity-associated cellular stress in primary human preadipocytes. Preadipocytes were treated for 24 h with Vitamin C (100 μM), a cocktail of TNFα+PA (10 ng/ml + 300 μM) or both as indicated. Graphs show the effects on (A) HIF1α protein; (B) VEGFA and GLUT1; (C) ER stress markers - sXBP1, CHOP and GRP78; (D) inflammatory chemokines - IL6, MCP1 and TNFα gene expression. Data represent means ± SEM of three independent experiments. Gene expression is presented as fold change, relative to the control which was given an arbitrary value of 1. Statistical analysis was performed by Two-way ANOVA followed by Tukey’s multiple comparisons. *p < 0.05, **p < 0.01, ***p < 0.005, ****p < 0.001 with the color of asterisk denoting the comparator. Numbers in parentheses are similarly color coded and show p values approaching or equal to 0.05.
Fig. 2. – Vitamin C prevents obesity-associated cellular stress in primary human adipocytes. (A) Representative images of cells taken at day 0, 7, 14 and 21 of differentiation showing increasing lipid accumulation. Fully differentiated adipocytes (day 21) were treated for 24 h with Vitamin C (100 μM), a cocktail of TNFα+PA (10 ng/ml + 300 μM) or both as indicated. Graphs show the effects on (B) HIF1α protein; (C) VEGFA and GLUT1; (D) ER stress markers - sXBP1, CHOP and GRP78; (E) inflammatory chemokines - IL6, MCP1 and TNFα gene expression. Data represent means ± SEM of three independent experiments. Gene expression is presented as fold change, relative to the control which was given an arbitrary value of 1. Statistical analysis was performed by Two-way ANOVA followed by Tukey’s multiple comparisons. *p < 0.05, **p < 0.01, ***p < 0.005, ****p < 0.001 with the color of asterisk denoting the comparator. Numbers in parentheses are similarly color coded and show p values approaching or equal to 0.05.
Vitamin C supplementation was able to reverse the induction of obesity-associated cell stress following pre-treatment of preadipocytes or adipocytes with TNFα-PA.

3.3. Vitamin C reverses obesity-associated cell stress in preadipocytes

Preadipocytes were pre-treated with TNFα-PA for 24 or 48 h prior to co-treatment with Vitamin C (in the continued presence of TNFα-PA) for an additional 24 or 48 h (see Supp. Table 1 for experimental design). This approach revealed a positive effect, with Vitamin C treatment for 24 and 48 h significantly reducing induction of HIF1α protein by 30–40% and VEGFA and GLUT1 mRNA by 40–80% (Fig. 3A–B). The effects of Vitamin C treatment on markers of ER stress were more restricted, significantly reversing the effects of TNFα-PA pre-treatment only on GRP78 induction, by 30–40% (Fig. 3C). Vitamin C treatment reversed the effects of TNFα-PA pre-treatment on induction of all three inflammatory genes, significantly reducing induction of IL6 and MCP1 by 20–35% at the latter timepoint (72 h with TNFα-PA and 48 h with Vitamin C), and TNFα induction by 50–60% at all timepoints (Fig. 3D). Vitamin C had similar effects on the levels of secreted IL6 protein, a representative marker of inflammation (Fig. 3E).

3.4. Vitamin C reverses obesity-associated cell stress in adipocytes

Next, we explored whether Vitamin C treatment was able to reverse the effects of pre-treatment with TNFα-PA in adipocytes. Vitamin C treatment completely reversed the effects of TNFα-PA on hypoxia, reducing HIF1α protein and VEGFA and GLUT1 mRNA to levels below those in control cells at all timepoints (Fig. 4A–B). Whilst the effect of TNFα-PA on markers of ER stress was again relatively modest, particularly after 48 h, treatment with Vitamin C significantly reversed the induction of GRP78 and sXBP1 at all timepoints (Fig. 4C). Unexpectedly, expression of CHOP was not increased following incubation with TNFα-PA for 48 or 72 h, decreasing significantly at the 48 h timepoint (Fig. 4C). Notwithstanding, treatment with Vitamin C significantly reduced the levels of CHOP mRNA at all timepoints (Fig. 4C). The effects of Vitamin C on inflammatory gene expression following TNFα-PA pre-treatment were mixed. Vitamin C treatment did not reverse the induction of any of the inflammatory genes at the 48 h timepoint, or MCP1 at the 72 h timepoint (Fig. 4D). In contrast, TNFα-PA-induced IL6 expression at 72 h was significantly reduced by Vitamin C treatment for 24 and 48 h, and this was mirrored at the level of secreted IL6 protein (Fig. 4E). Surprisingly, induction of TNFα was significantly increased under these conditions (Fig. 4D). The underlying mechanisms for this observation are unclear and warrant further investigation.

3.5. Differences between preadipocytes and adipocytes

There were some notable differences between the preadipocytes and adipocytes in both baseline characteristics and the response to treatments. Under basal conditions, HIF1α protein was 2-fold higher in adipocytes compared with preadipocytes (90 ± 8 vs 45 ± 3 pg/mg; n = 6/group; Student’s t-test; p = 0.0005) whilst expression of the genes analyzed was similar between cell types, with the exception of CHOP which was 2-fold higher in the adipocytes (see Supp Table 3). The timing and magnitude of the response to TNFα-PA treatment were also different. In the preadipocytes peak induction of HIF1α and IL6 proteins and expression of all genes, apart from CHOP, occurred transiently at 48 h compared with 72 h for the adipocytes (see Supp Table 4). Peak induction of CHOP occurred at 24 h in both cell types. In all cases, excluding TNFα, the peak fold-induction was greater in the pre-adipocytes (see Supp Table 4), suggesting higher sensitivity to the TNFα-PA treatment. Notwithstanding this, the ability of Vitamin C to prevent or reverse the effects of TNFα-PA on the hypoxia and ER stress markers was consistently greater in the adipocytes (see Supp Table 5). This difference was not apparent, or was even inverted, when comparing the changes in markers of inflammation, where the response to Vitamin C was generally more substantial in the preadipocytes than the adipocytes (see Supp Table 5).

3.6. Effect of Vitamin C on total and HMW adiponectin production in fully differentiated adipocytes

Having previously reported that treatment with Vitamin C increased secretion of HMW adiponectin from human SGBS adipocytes and HEK293 cells engineered to express human adiponectin (Rose et al., 2010), we next analyzed the effects of Vitamin C on adiponectin gene (ADIPOQ) expression and secretion of total and HMW adiponectin in control (untreated) adipocytes as well as adipocytes either co-treated or pre-treated with TNFα-PA (adiponectin is not expressed/secreted by preadipocytes hence the focus on adipocytes only). Treatment with TNFα-PA significantly reduced ADIPOQ mRNA levels in a time-dependent manner whilst Vitamin C had no significant effects (Fig 5A and D). In marked contrast, treatment with TNFα-PA had no effect on secretion of total adiponectin at 24 and 48 h but significantly reduced total adiponectin secretion at 72 h by 80% (Fig. 5B and E). Interestingly, whilst supplementation with Vitamin C alone for 24 h had no effect on secretion of total adiponectin when combined with TNFα-PA total adiponectin secretion was increased more than two-fold (Fig. 5B). Treatment with TNFα-PA consistently reduced the secretion of HMW adiponectin (by 40–50%) whereas supplementation with Vitamin C promoted increased secretion of HMW adiponectin under all conditions, increasing secretion 5-fold in the absence or presence of TNFα-PA for 24 h (co-treatment) and 3-2 fold after treatment with TNFα-PA for 48 and 72 h respectively (pre-treatment) (Fig. 5C and F).

4. Discussion

The beneficial effects of Vitamin C have been reported in a wide variety of tissues and cell types, evidencing the resolution of hypoxia and ER stress (Mason et al., 2016; Ji et al., 2012; Akhara et al., 2016; Martino et al., 2009; Chen et al., 2000; Gupta et al., 2016), as well as in studies detailing the positive effects of Vitamin C in the context of obesity in humans as well as in rodents and isolated mouse and rat adipocytes (reviewed in Garcia-Diaz et al., 2014). Perhaps surprisingly, given our increasing recognition of the importance of preadipocyte and adipocyte function to human health, similar investigations have not been performed in human preadipocytes and adipocytes. To begin to address this gap in our understanding in the current study we characterized the effects of Vitamin C on markers of cell stress in primary human preadipocytes and adipocytes under basal conditions and during co-treatment or following pre-treatment of a cocktail (TNFα-PA) designed to recapitulate aspects of the obese milieu. Under basal conditions, the only significant effects of Vitamin C were to reduce markers of hypoxia in adipocytes and increase secretion of HMW adiponectin. Treatment with TNFα-PA induced markers of hypoxia, ER stress and proinflammatory cytokines in both cell types whilst treatment with Vitamin C prevented or reversed these effects, albeit with varying efficacy.

In the current study we first demonstrated that human preadipocytes and adipocytes treated with a TNFα-PA cocktail exhibited increased hypoxia, oxidative and ER stress, and proinflammatory cytokine gene expression. Levels of HIF1α protein were consistently 2-fold higher in adipocytes compared with preadipocytes under basal conditions and Vitamin C treatment significantly reduced these such that they were indistinguishable, in the absence or presence of TNFα-PA. Vitamin C co-treatment also prevented induction of downstream effectors of HIF1α, VEGFA and GLUT1, with these effects also significantly more marked in the adipocytes. Previous work in mouse models of diet-induced obesity has demonstrated that pharmacologic or genetic inhibition of HIF1α results in reduced weight gain and significant metabolic improvements, including improved insulin sensitivity and glucose tolerance (Sun et al.,
Fig. 3. Vitamin C reverses obesity-associated cellular stress in primary human preadipocytes. Preadipocytes were preincubated with TNFα+PA (10 ng/ml + 300 μM) for either 24 h or 48 h and then incubated with Vitamin C (100 μM) in the continued presence of TNFα+PA for a further 24 or 48 h. Graphs show the effects on (A) HIF1α protein; (B) VEGF and GLUT1; (C) ER stress markers - sXBP1, CHOP and GRP78; (D) inflammatory chemokines - IL6, MCP1 and TNFα gene expression; (E) IL6 protein secretion. Data represent means ± SEM of three independent experiments. Gene expression is presented as fold change, relative to the control which was given an arbitrary value of 1. Statistical analysis was performed by Two-way ANOVA followed by Tukey’s multiple comparisons. *p < 0.05, **p < 0.01, ***p < 0.005, ****p < 0.001 with the color of asterisk denoting the comparator.
Fig. 4. – Vitamin C reverses obesity-associated cellular stress in primary human adipocytes. Fully differentiated adipocytes were preincubated with TNFα+PA (10 ng/ml + 300 μM) for either 24 h or 48 h and then incubated with Vitamin C (100 μM) in the continued presence of TNFα+PA for a further 24 or 48 h. Graphs show the effects on (A) HIF1α protein; (B) VEGFA and GLUT1; (C) ER stress markers - sXBP1, CHOP and GRP78; (D) inflammatory chemokines - IL6, MCP1 and TNFα gene expression; (E) IL6 protein secretion. Data represent means ± SEM of three independent experiments. Gene expression is presented as fold change, relative to the control which was given an arbitrary value of 1. Statistical analysis was performed by Two-way ANOVA followed by Tukey’s multiple comparisons. *p < 0.05, **p < 0.01, ***p < 0.005, ****p < 0.001 with the color of asterisk denoting the comparator.
At a molecular level, Vitamin C promotes destruction of HIF1α via a mechanism that involves increased prolyl-hydroxylation, which subsequently targets it for increased ubiquitination and proteasomal degradation (Marxsen et al., 2004; Visser et al., 2007). Collectively, these observations highlight that the effects of Vitamin C are far greater in cells challenged with TNFα+PA and this reflects, at least in part, a direct effect on HIF1α protein levels and demonstrate the potential beneficial effects of increasing Vitamin C in the continued presence of TNFα+PA for a further 24 or 48 h. Graphs show the effects on (D) ADIPOQ gene expression; (E) Total adiponectin secretion; (F) HMW adiponectin secretion. Data represent means ± SEM of three independent experiments. Gene expression is presented as fold change, relative to the control which was given an arbitrary value of 1. Statistical analysis was performed by Two-way ANOVA followed by Tukey’s multiple comparisons. *p < 0.05, **p < 0.01, ***p < 0.005, ****p < 0.001 with the color of asterisk denoting the comparator. Numbers in parentheses are similarly color coded and show p values approaching or equal to 0.05.

We next extended our investigations, in experiments where cells were pretreated with TNFα+PA prior to treatment with Vitamin C in the continued presence of TNFα+PA, demonstrating that Vitamin C was able to reverse the effects of TNFα+PA. As before, the most marked effect of Vitamin C treatment was observed in the adipocytes, where Vitamin C reduced HIF1α protein, and VEGFA and GLUT1 mRNA to levels below those observed in control cells under all conditions. Comparison of the responses in preadipocytes and adipocytes highlighted some differences, with the preadipocytes typically demonstrating a larger, faster, albeit more transient response to TNFα+PA than the adipocytes. This has implications when considering the potential importance of obesity-induced dysregulation of preadipocyte function, which would contribute to increased adipose tissue inflammation and reduced adipocyte hyperplasia, thereby leading to further dysregulation of adipose tissue (Hammarstedt et al., 2018; Jiang et al., 2019). Notwithstanding, our results demonstrate that Vitamin C can prevent, ameliorate, or reverse the negative effects of TNFα+PA in both primary human preadipocytes and adipocytes.

The impact of the various treatment regimens on adiponectin gene expression and secretion was largely consistent with our previous observations in SGBS cells (Rose et al., 2010; Yang et al., 2015). Intriguingly, Vitamin C was unable to either prevent or reverse the reduction in ADIPOQ expression upon co-treatment or pre-treatment with TNFα+PA, clearly dissociating this response from the hypoxia and ER stress responses, which were completely blocked or markedly attenuated. Despite this, the secretion of HMW adiponectin was significantly increased by Vitamin C under all conditions. Previous investigations highlighted the importance of several post-translational modifications (PTM) in the efficient multimerisation and secretion of HMW adiponectin (summarized in (Simpson and Whitehead, 2010)), with hydroxylation of conserved proline and lysine residues playing an essential role (Richards et al., 2006; Wang et al., 2006). Vitamin C serves as a co-factor for the prolyl- and lysyl-hydroxylases responsible for these modifications, driving a shift from the lower molecular weight trimers and hexamers to the HMW multimers, as visualised by sucrose gradient and western blot analysis (Rose et al., 2010). The magnitude of this effect
was striking in the current study, with HMW adiponectin making up to 80% of the total pool of secreted adiponectin following 24 h treatment with Vitamin C. The combined effect of co-treatment with TNFα+PA and Vitamin C on secreted total adiponectin was unexpected. One possible explanation is that the intracellular pool of adiponectin that is not typically secreted may be released following co-treatment due to changes in the processes involved in quality control and retention of adiponectin multimers such as thiol-mediated retention (Simpson and Whitehead, 2010; Heiker et al., 2010). Further studies are required to explore this possibility. The relatively modest induction of ER stress markers observed in adipocytes treated with TNFα+PA was typically reduced to baseline values by Vitamin C. Such resolution of ER stress may have contributed to the increase in adiponectin secretion.

In contrast to the marked effects on hypoxia, ER stress and adiponecin secretion in the adipocytes, the effect of Vitamin C on pro-inflammatory gene expression was more modest, indicating that the latter are unlikely to be downstream of the former. Previous work in murine inflammatory gene expression was more modest, indicating that the latter may be upstream and reduce the expression of inflammatory cytokines via a NOD1 pathway that was Nox1/4-dependent yet induced insulin resistance and lipolysis in a Nox1/4-independent manner (Sharma et al., 2021). These findings highlight the complexity of the pathways involved and the need for further investigations to define the molecular mechanisms that underpin the observations described in the current study.

In summary, we have shown that Vitamin C is able to ameliorate the combined effects of a pro-inflammatory cytokine and saturated fatty acid in primary human preadipocytes and adipocytes, reducing markers of hypoxia, ER stress and inflammation. These observations provide a foundation for future studies to establish the molecular details and, perhaps more importantly, a rationale to explore the potential therapeutic opportunities of Vitamin C supplementation in subjects with obesity-related diseases.

CRediT authorship contribution statement

Xiaoxin Luo: Funding acquisition, Conceptualization, Data curation, Formal analysis, Investigation, Writing - original draft, Writing - review & editing. Chaoxing Ng: Data curation, Investigation, Writing - review & editing. Jingjing He: Data curation, Investigation, Writing - review & editing. Mengliu Yang: Data curation, Investigation, Writing - review & editing. Xiao Luo: Writing - original draft, Writing - review & editing. Terence P. Herbert: Writing - original draft, Writing - review & editing. Jonathan P. Whitehead: Data curation, Conceptualization, Formal analysis, Funding acquisition, Project administration, Visualization, Writing - original draft, Writing - review & editing.

Declaration of competing interest

The authors declare that there is no duality of interest associated with this manuscript.

Data availability

Data will be made available on request.

Acknowledgements

This work was supported by funding from the National Natural Science Foundation of China (81874263 to XQO), a University of Queensland International Research Scholarship (to MY), and the Australian National Health and Medical Research Council (511104 & 102568625 to JPW).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mce.2022.111740.

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