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## Diagnostic in Obesity and Complications

# Melanin and melanogenesis in adipose tissue: possible mechanisms for abating oxidative stress and inflammation?

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## Summary

Obesity has become a worldwide epidemic and can lead to multiple chronic diseases. Adipose tissue is increasingly thought to play an active role in obesity-related pathologies such as insulin resistance and non-alcoholic fatty liver disease. Obesity has been strongly associated with systemic inflammation and, to a lesser degree, with oxidative stress, although the causal relationships among these factors are unclear. A recent study demonstrating an expression of the components of the melanogenic pathway and the presence of melanin in visceral adipose has raised questions regarding the possible role of melanogenesis in adipose tissue. As this study also found larger amounts of melanin in the adipose tissue of obese patients relative to lean ones, we hypothesize that melanin, a pigment known for its antioxidant and anti-inflammatory properties, may scavenge reactive oxygen species and abate oxidative stress and inflammation in adipose tissue. This review considers the evidence to support such a hypothesis, and speculates on the role of melanin within adipocytes. Furthermore, we consider whether the α-melanocyte-stimulating hormone or its synthetic analogues could be used to stimulate melanin production in adipocytes, should the hypothesis be supported in future experiments.

**Keywords:** Melanin, metabolic syndrome, inflammation, obesity.

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## Introduction

In the USA and worldwide, obesity has become epidemic. The World Health Organization estimates that 300 million adults are clinically obese (1). In the USA, 127 million (66.3%) adults are overweight, 60 million (32.2%) are obese and 9 million (4.8%) are severely obese (2). A main contributor to the metabolic syndrome, obesity often leads to the development of several chronic conditions, including type 2 diabetes, cardiovascular disease and non-alcoholic fatty liver disease, all of which result in high medical costs (3,4). However, the effect of body weight on morbidity and mortality varies among individuals. A substantial number of morbidly obese patients with body mass index (BMI) >45 remain healthy and have normal sensitivity to insulin (5,6), while others may develop insulin resistance and type 2 diabetes although they are merely overweight (6). The variance among individuals is frequently attributed to gene–environment interactions (7–9); however, the molecular mechanisms underlying such interactions are not well understood.

Recently, obesity has become recognized as a state of chronic, systemic inflammation characterized in part by elevated serum levels of pro-inflammatory cytokines

(e.g. tumour necrosis factor-α [TNF-α], interleukin [IL]-6) and other inflammatory factors (e.g. C-reactive protein) and decreased levels of anti-inflammatory factors (e.g. adiponectin, IL-10) (10–12). Up-regulation of these secreted factors is due to activation of several inflammatory signalling pathways, some of which involve components that also contribute to insulin resistance (e.g. Jun N-terminal kinase; reviewed in (11)). Exactly how obesity, insulin resistance and inflammation are causally linked remains unknown, although some clues have been identified. For example, free fatty acids (FFA) can bind to innate immune receptors (e.g. toll-like receptor 4) in adipocytes, initiating the release of pro-inflammatory cytokines (13,14). Additionally, the release of pro-inflammatory cytokines and adipokines by adipocytes may be a reaction to hypoxic conditions caused by hypertrophy and hyperplasia of visceral adipose tissue (10,14). Cultured adipocytes exposed to hypoxic conditions increase their secretion of inflammatory adipokines such as IL-6, leptin and monocyte migration inhibitory factor, while the secretion of adiponectin, an anti-inflammatory adipokine, decreases (15). Average adipocyte size plays a role as well; Skurk *et al*. (16) reported that the secretion of pro-inflammatory cytokines IL-6 and IL-8 are significantly higher in hypertrophic adipocytes even after correction for cell surface area, whereas the secretion of the anti-inflammatory factors IL-10 and adiponectin are significantly lower or had no relationship to adipocyte size, respectively. White blood cells likely contribute to the inflammatory process as well. Although macrophages normally occur in adipose tissue, the extent of their infiltration is directly proportional to the degree of adiposity (17). Macrophages are thought to be responsible for most of the secretion of TNF-α and for some of the secretion of other inflammatory factors from adipose tissue (17–19). These, and other mechanisms allowing expanded adipose tissue to release inflammatory factors have significant health consequences; circulating inflammatory factors cause inflammation in distant organs and tissues (e.g. liver, bronchial lining and arterial wall), leading to progressing conditions such as insulin resistance and atherosclerosis (12).

Despite the strong relationship between obesity and inflammation, in a subset of morbidly obese individuals the adipose tissue remains relatively inert, secreting only low levels of pro-inflammatory cytokines and adipokines and resisting the development of the comorbidities of obesity, including the metabolic syndrome (5,20,21). Actually, in this group of 'healthy' obese individuals, loss of weight may adversely impact their favourable cardiometabolic profile (21). Currently, there is no explanation for this phenomenon.

Reactive oxygen species (ROS), as well as reactive nitrogen species, are argued to be the 'missing link' between obesity and inflammation (12). In patients diagnosed with the metabolic syndrome, systemic oxidative stress positively correlates with the accumulation of visceral fat (22). This correlation also was observed in non-diabetic human subjects and in obese mice, independent of hyperglycaemia (23). In the murine model systemic, oxidative stress was linked to adipose tissue, which expresses the NADPH oxidase complex, a producer of ROS, at significantly higher levels in obese mice relative to non-obese mice (23). Obese mice also expressed significantly lower levels of antioxidant enzymes such as superoxide dismutase and glutathione peroxidase. Furthermore, mean serum concentrations of α- and β-carotenes as well as the sum of five carotenoid concentrations are substantially lower in persons with the metabolic syndrome (after adjusting for age, sex, education, BMI status, alcohol intake, smoking, physical activity status and vitamin/mineral use) than persons without the syndrome (24).

Oxidative stress is associated with many of the components of the metabolic syndrome, leading to the concept that its amelioration may curtail the progression of metabolic disease complications. Importantly, in many cases both the metabolic syndrome and its underlying cause, resistance to insulin, could indeed be alleviated by direct application of antioxidants. For example, in C57BL/6J mice fed a high-fat diet, the antioxidative effect of pyridoxamine administration led to improvement in blood glucose levels after glucose injection, fasting hyperinsulinaemia and glucose transporter 4 translocation in skeletal muscle (25). In obese Zucker rats, a polyphenolic stilbene derivative with antioxidant properties, resveratrol, reduced plasma concentrations of triglycerides, total cholesterol, FFAs, insulin and leptin, lowered hepatic lipid content, and improved the inflammatory status by increasing the concentration of adiponectin and suppressing TNF-α production in visceral adipose tissue (26).

Given that the adipose tissue of obese individuals is under increased oxidative stress due to the presence of elevated levels of ROS, we surmise that it is the mechanisms by which adipose tissue attenuates the effects of ROS that varies among individuals rather than the levels of ROS. We further hypothesize that melanin and components of the melanogenic pathway, known for their anti-inflammatory and free radical scavenging properties and recently discovered in adipocytes (27) are responsible for alleviating the oxidative stress in adipose tissue, and that it is the differential expression of these pigments and proteins among individuals that explains why some succumb to the sequelae of obesity while others do not. While this hypothesis requires testing, our current understanding of the biological roles of melanin and its associated, melanogenic signalling factors, along with recent studies linking obesity with oxidative stress in adipose tissue, suggest that links between oxidative stress and melanogenesis in adipose are important avenues to

explore. Here, we summarize the premises for the hypothesis we set forth.

## Adipocytes are under oxidative stress

Exogenous and systemic-level sources of oxidative stress in obese individuals have been reviewed multiple times (28). However, ample evidence exists that adipose tissue also produces large amounts of ROS within its component adipocytes, in response to a number of extracellular and intracellular signals or events. Nutrients are one class of ROS promoting factors. Studies of murine models demonstrated that hyperglycaemia stimulates the production of ROS from mitochondria and the endoplasmic reticulum (reviewed in (12)). Similarly, cultured adipocytes significantly increased ROS production when incubated in the presence of linoleic, oleic and arachidonic fatty acids (26), known components of the Western diet. The increased levels of ROS from these sources are thought to participate in feedback loops that further amplify their production (12).

Hypoxia may be another trigger for ROS production in adipocytes. Early studies performed on growing rabbits demonstrated that blood flow declines with increasing accumulation of lipids in fat depots, and is inversely proportional to mean adipocyte size (29). Observational studies in humans have suggested a correlation between adiposity and hypoxia; for example, tissue hypoxia was observed in obese individuals undergoing surgery (30). Additionally, studies have confirmed hypoxia in the adipose tissue of obese mice (reviewed in (31)). Guzy *et al.* (32) demonstrated that the mitochondria of cells experiencing hypoxia produce ROS and release it to the cytosol in order to stabilize the hypoxia-responsive transcription factors, HIF-1 and HIF-2. As HIF-1 is functional in adipocytes cultured under hypoxic conditions (33), it is reasonable to expect that adipocytes of obese individuals produce excess ROS in response to hypoxia.

Although not well studied with regard to adipose tissue, lipid peroxidation is a known source of oxidative stress and cytotoxicity. In adipocytes, which store large amounts of lipids within fat vacuoles, this process may play a prominent role in ROS generation. Nathan (12) considers factors that may put adipocytes at greater risk for oxidative damage relative to other types of cells, especially in the obese. He argues that (i) hypertrophic adipocytes have decreased blood supply leading to hypoxia-induced mitochondrial production of ROS; and (ii) as obese individuals have relatively high proportions of necrotic adipose tissue, activated macrophages are drawn to the tissue and respond by producing oxidants and chemokines. The chemokines then attract more macrophages to the tissue, continuing the cycle. Nathan further argues that the relatively low volume of cytosol in adipocytes limits the availability of antioxidants, which are then consumed when adipocytes reach hypertrophy.

There are a number of mechanisms by which lipid peroxidation may increase oxidative stress and reduce cell viability in adipose tissue. Chen *et al.* (34) showed that the products of exogenous phospholipid oxidation are internalized by cells and associate with mitochondria, initiating apoptosis. Polyunsaturated fatty acids (PUFA), such as those found in the phospholipids of cell and organelle membranes, are susceptible to oxidative damage as the double bonds in these molecules are particularly reactive with ROS (35). Peroxidation of a PUFA can lead to the release of fatty aldehydes, which in turn may react with, and damage, components of nucleic acids and proteins. Moreover, the oxidation of FFAs in the proximity of a cell membrane, in combination with the oxidation of phospholipids, can compromise the integrity of the membrane, leading to cell lysis. In turn, cells undergoing apopototic and necrotic processes in response to the damage inflicted by ROS attract and activate macrophages, thus perpetuating the vicious cycle.

Taken together, these observations suggest that an abatement of ROS generation in adipose tissue might be a desirable trait.

## Melanogenesis and melanin in adipose tissue

In humans, melanin is produced in melanocytes, in retinal pigment epithelium cells, in some specialized cells of the inner ear and in the central nervous system. Melanin is responsible for the colouration of skin and hair, and is largely known for its ability to absorb energy from ultraviolet (UV) light, thereby reducing damage to DNA. Melanin also acts as an antioxidant that scavenges ROS such as hydroxyl radicals and superoxide anions (33). There are multiple forms of melanin, the most common of which are eumelanin and pheomelanin, responsible for brown/black and yellow/red colour phenotypes, respectively (36). For the purposes of this paper, 'melanin' will refer to eumelanin (unless otherwise noted) as this is the form most studied for its antioxidant activity (37,38) and the form identified in adipose tissue (27).

The pathway by which melanin is synthesized, known as melanogenesis, and the factors that regulate it were recently reviewed (39–41). Briefly, melanin is produced in a specialized, membrane-bound organelle called the melanosome. Melanosomes are very similar to lysosomes in terms of their protein content and are staged according to their internal structure and extent of melanin synthesis. In melanocytes, mature melanosomes are transported by motor proteins along microtubules to the tips of dendrites. From there, they are transferred to adjacent keratinocytes by a variety of mechanisms, including exocytosis.

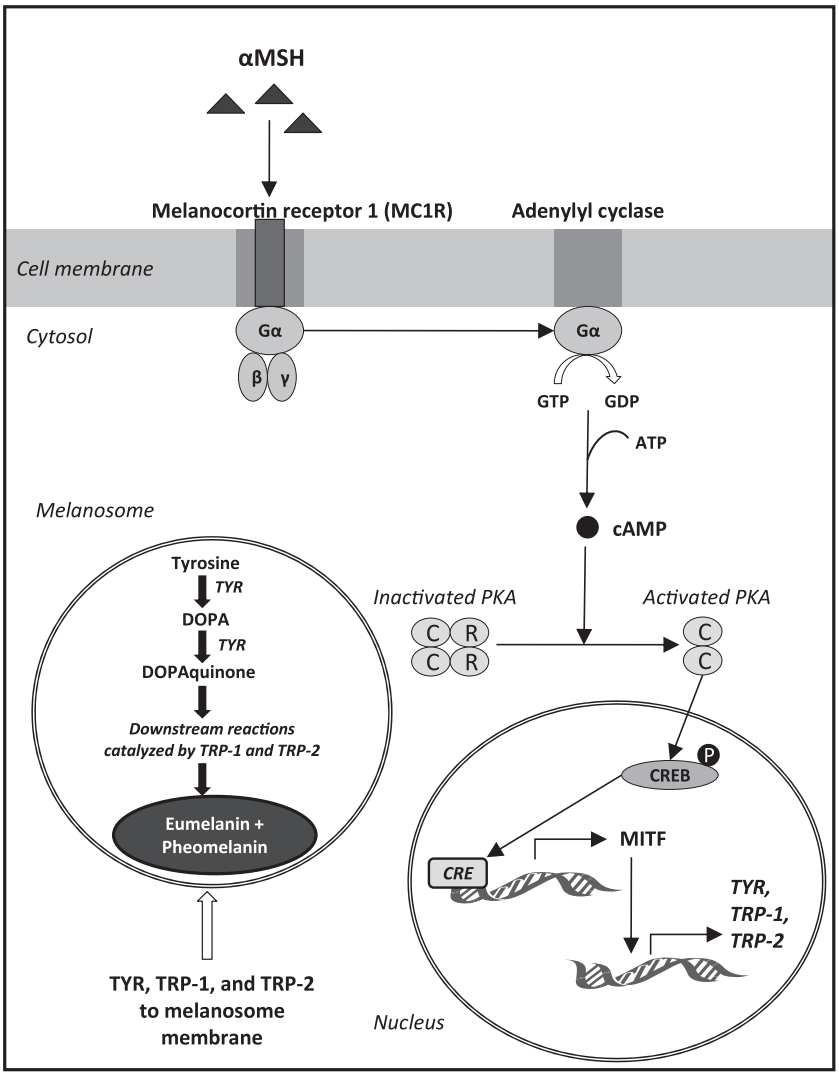


**Figure 1** Overview of the primary signalling pathway leading to melanin production in melanocytes. Melanocortin receptor 1 (MC1R) is one of several cognate receptors for α-melanocyte-stimulating hormone (α-MSH).

In simplified terms, the primary signalling pathway leading to melanin production in melanocytes is as follows: first, the extracellular ligand α-melanocyte-stimulating hormone (α-MSH) derived from the pro-opiomelanocortin (POMC) gene, binds one of several cognate receptors (e.g. MC1R) at the cell surface (Fig. 1). Collectively, these are known as the melanocortin receptors (MCRs), and five types have been cloned to date. The MCRs are members of the G-protein-coupled receptor superfamily; consequently, activation of these receptors leads to elevated cyclic adenosine monophosphate (cAMP) levels *via* stimulation of adenylate cyclase. Cyclic AMP, in turn, activates cAMP-dependent protein kinase, which phosphorylates the cAMP responsive element-binding protein (CREB) family of transcription factors in the nucleus. CREB, in turn, binds a cAMP responsive element in the promoter of the gene encoding microphthalmia-associated transcription factor, the product of which initiates the expression of melanogenic enzymes such as the melanosome membrane-spanning proteins, tyrosinase (TYR) and the TYR-related proteins, TRP-1 and TRP-2. In the melanosome, tyrosine is oxidized by TYR, forming the melanin precursor, dopaquinone. Dopaquinone is then processed into eumelanin in the absence of thiols, or pheomelanin in the presence of thiols (42). TRP-1 and TRP-2 are found near TYR in the melanosome membrane and have enzymatic activity that protects TYR from ROS-mediated oxidation (43).

A recent study demonstrated that the melanin biosynthesis pathway is functional in adipose tissue (27). Specifically, both melanin and the components of melanin biosynthesis were found in adipose tissue, and furthermore were expressed at much higher levels in samples from obese individuals. Melanin was identified in human adipose both by Fontana–Mason staining, which revealed melanin concentration along the periphery of adipocytes, and by

liquid chromatograpy (LC) with ultraviolet (UV) detection and mass-spectrometry (MS), which identified this melanin species as eumelanin. Likewise, *in situ* hybridization confirmed the presence of TYR transcripts in the periphery of adipocytes. Further experiments showed that transcript and protein components of the melanogenic pathway, namely TYR, TRP1, TRP2 and MC1-R were expressed in adipose tissue and were more highly expressed in obese individuals than in the non-obese. Together, these results suggest that the melanogenic pathway is functional in adipocytes and, for reasons as yet unknown, is hyperstimulated in the adipose tissue of the obese.

The results reported by Randhawa *et al*. (27) naturally lead to the question of why adipose tissue synthesizes melanin. On one hand, higher melanin expression in the adipose tissue of obese patients may suggest that melanogenesis is indeed stimulated by the same pro-adipogenic factors that lead to the gain of excessive weight. However, known stimulators of melanogenesis, e.g. α-MSH, induce weight loss in healthy human subjects (44), even though in this case peptide is acting centrally, through a direct access to hypothalamus. On the other hand, one may hypothesize that melanogenesis may play a compensatory role when stimulated in response to the gain of weight. The latter hypothesis is supported by the notion that both melanin and components of the melanogenic pathway have anti-inflammatory and antioxidant properties, and thus may help to delay the onset of obesity-related diseases linked to the increased inflammation and oxidative stress already known to accompany obesity. This hypothesis is summarized by Fig. 2. In the absence of melanin, dietary factors (e.g. FFA, high levels of glucose in the bloodstream), hypoxia and the peroxidation of cellular lipids (both a result of, and a source of ROS-mediated damage) generate a net excess of ROS in adipocytes, leading to apoptosis. Macrophages are then recruited to the tissue at an elevated rate, and, in combination with adipocytes undergoing oxidative stress, contribute to the release of pro-inflammatory cytokines and the deficiency of anti-inflammatory cytokines (Fig. 2a). In contrast, the presence of melanin provides a mechanism by which adipocytes are able to sequester excess ROS generated by dietary factors, hypoxia and lipid peroxidation, and as a result, improve cell viability and limit macrophage infiltration. Consequently, adipocytes release higher levels of anti-inflammatory adipokines and cytokines, while keeping pro-inflammatory cytokines at bay (Fig. 2b). Below, we put forth the case for the compensatory roles of melanin and melanogenic factors in adipose tissue, and further speculate that if our hypothesis is true, then it follows that agonists of melanin production such as α-MSH (or its synthetic analogues) could be considered for future testing as potentially therapeutic agents for the prevention of secondary consequences of obesity and the metabolic syndrome.

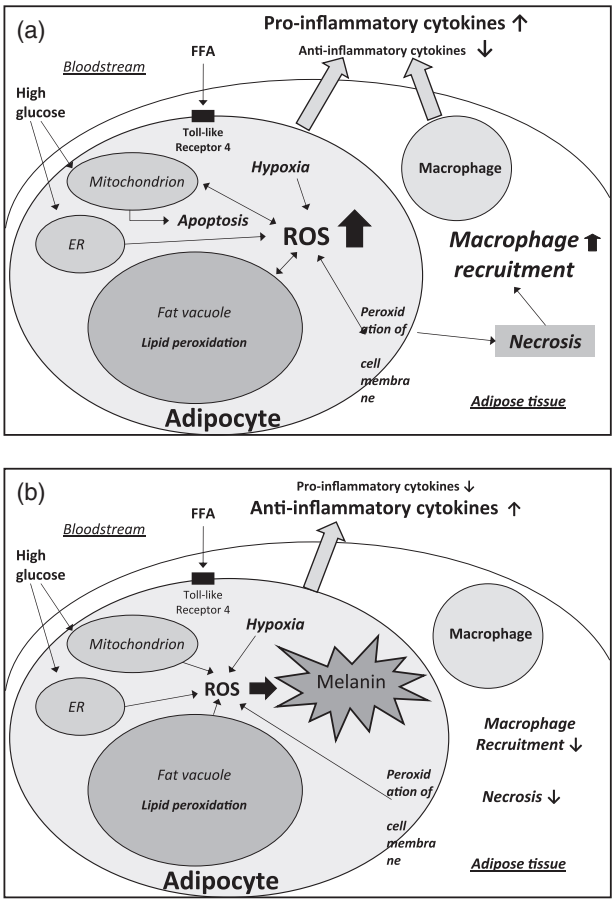


**Figure 2** (a) In the absence of melanin, dietary factors such as free fatty acids (FFA) and high levels of glucose in the bloodstream generate excess reactive oxygen species (ROS) in adipocytes, which in turn recruit macrophages to adipose tissue. The combined effect is the increased release of pro-inflammatory cytokines and decreased release of anti-inflammatory cytokines. (b) The production of melanin by adipocytes may serve to sequester excess ROS, thereby inhibiting the release of pro-inflammatory cytokines and stimulating the release of anti-inflammatory cytokines.

## Melanin has antioxidant and anti-inflammatory properties

As discussed previously, the primary forms of melanin in humans are eumelanin and pheomelanin. In the skin, both pigments absorb broadband UV and visible light and act as scavengers of free radicals and other oxidative species (45,46). In fact, melanin has been called the first line of defence against UV-generated free radicals (47). However, there are noted differences in the antioxidant capacity of melanin depending on the type under consideration. Eumelanin, the predominant form in humans, is photoprotective; in addition to its antioxidant behaviour, it minimizes damage to DNA by forming a cap structure around the nucleus (48,49). In contrast, iron-complexed pheomelanin can generate ROS in response to UV radiation, leading to caspase-independent apoptosis (50,51). UV radiation

also indirectly stimulates melanin production by inducing inflammatory responses such as the production of prostanoids, which in turn activate melanogenesis in melanocytes ($NO_2$) (52,53).

The role of melanin as a scavenger of free radicals is well established *in vitro* and *in vivo* (45,47,54–56). Early studies showed that melanin reacts with oxidative species such as singlet oxygen (57,58), hydroxyl radical (59) and the superoxide anion (59,60). Melanin also reacts with organic oxidants having the general structure RO· and ROO· (56), as well as with tryptophan and tyrosine radicals (Trp· and TyrO·, respectively), potent oxidizers such as the persulphate radical ($SO_4{\cdot}^-$), the peroxyl radical ($CCl_3O_2{\cdot}$) and the nitrogen dioxide radical? (54).

Melanin's role as an antioxidant has been largely confirmed by its ability to inhibit lipid peroxidation (51,56,61–64). For example, Krol and Liebler (51) showed that both eumelanin and pheomelanin inhibited UV-induced lipid peroxidation in liposomes in a concentration-dependent manner. As previously discussed, lipid peroxidation can induce mitochondria-mediated ROS production and can promote cell lysis by compromising membrane structure. Thus, by removing ROS, it is possible that melanin protects adipose tissue from these cytotoxic insults.

In addition to minimizing oxidative stress, melanin may also decrease the inflammatory response of adipose tissue by inhibiting the production of pro-inflammatory cytokines from infiltrating monocytes, and perhaps from adipocytes as well. Mohagheghpour *et al*. (64) demonstrated that synthetic analogues of melanin suppress the synthesis and secretion of TNF, IL-1β, IL-6 and IL-10 in peripheral blood monocytes. Interestingly, this effect was clearly observed even when melanin was administered to monocytes in the absence of the cytokine-stimulating agent, lipopolysaccharide (LPS). For example, in one experiment melanin at a dose of 100 μg $mL^{-1}$ reduced TNF secretion by 60–65% when cells were stimulated by LPS, and by 90% when cells were unstimulated. The suppression of cytokines by melanin was reversible, was similarly potent in the presence of non-LPS inducers of cytokine secretion, and was not limited to monocytes, as exemplified by its ability to suppress IL-6 release from human fibroblasts and endothelial cells. The mechanism by which melanin suppresses cytokine production is restricted to the post-translational stage, as it does not reduce cytokine mRNA levels nor does it interfere with the processing of cytokine precursors.

## Melanocortins and other melanogenesis-related proteins have antioxidant and anti-inflammatory properties

In addition to melanin, the melanocortins also are known for their roles as antioxidants and anti-inflammatory factors. 'Melanocortin' is the name given to the peptides derived from the POMC gene and reflects their ability to stimulate pigment and adrenyl cells; these include α-, β- and γ-MSH as well as adrenocorticotropic hormone (ACTH) (39,40). Studies of the anti-inflammatory properties of α-MSH are plentiful. Moreover, the peptide analogues of α-MSH show potential for use as pharmacotherapies against inflammatory diseases (discussed in the next section).

The biological functions of α-MSH were recently and extensively reviewed by Brzoska *et al*. (40) who list 62 and 40 studies providing *in vitro* and *in vivo* evidence, respectively, for its anti-inflammatory properties. *In vitro*, α-MSH reduces the expression or secretion of the pro-inflammatory cytokines interferon-γ, TNF-α, IL-1, IL-6, IL-8 and growth regulated oncogene-α (Gro-α), typically with the aid of mediating molecules such as IL-1β and in the presence of a pro-inflammatory stimulus (65–70). α-MSH has additional anti-inflammatory activities in cultured cells: it reduces the expression of the IL-8 receptor and non-cytokine pro-inflammatory mediators such as $NO_2^-$ and iNOS; it reduces inflammatory cell migration and the expression of cell adhesion molecules (e.g. ICAM-1); it increases the expression of the anti-inflammatory cytokine IL-10; and it inhibits activation of the NF-κB pro-inflammatory pathway (71–75). It also suppresses the chemotaxis of neutrophils, and a recent study showed that it does so by inhibiting the production of superoxide radicals from neutrophils (76). One study showed that α-MSH specifically prevented activation of the NF-κB pathway by $H_2O_2$ (77), underscoring α-MSH's role an antioxidant as well as an anti-inflammatory molecule.

*In vivo* studies have revealed systemic and organ-specific anti-inflammatory functions of α-MSH (40). α-MSH suppressed pyrogen-induced fever; counteracted organ fibrosis by reducing expression of collagen type-1; quenched allergic airway and ocular inflammation, and by mechanisms not completely elucidated, attenuated pancreatic inflammation (78–82). Systemic or subcutaneous injection of α-MSH decreased circulating levels of IL-1α and TNF-α and improved the survival rates of mice used as models of peritonitis/endotoxaemia (82,83). α-MSH also inhibited apoptosis in cultured human melanocytes, renal cells and dermal fibroblasts; and in studies of kidney disease and toxicity, it reduced organ damage in part by blocking apoptosis (84–89).

Other proteins in the melanogenic pathway are known to play a role in reducing ROS. For example, Valverde *et al*. (90) demonstrated that increasing melanocytic TYR activity in the presence of $O_2^-$ led to a decrease in that anion's concentrations, supporting the argument that $O_2^-$ acts as a substrate for TYR. Furthermore, melanocytes with increased TYR activity were equally resistant to the cytotoxic effects of $O_2^-$ as were keratinocytes, which otherwise are more resistant to $O_2^-$ than melanocytes.

## Melanocortins and melanocortin tripeptides are therapeutically promising for many inflammation-related disorders

It has been suggested that both α-MSH and its derivative tripeptides (e.g. KPV and its stereoisomer KPdV), which appear to have anti-inflammatory potency similar to, and in some cases exceeding α-MSH, may prove to be effective pharmacological treatments for inflammatory diseases (reviewed in (40,91)). For example, KPV suppresses synthesis of TNF-α, induces IL-10 expression and blocks the NF-κB pathway. It is therefore conceivable that administration of α-MSH or its analogues could halt the progression of inflammatory diseases such as rheumatoid arthritis and inflammatory bowel disease, which involve these cytokine-driven pathways in their pathogenesis. Consequently, Brzoska *et al*. argue that KPV, along with other α-MSH-derived peptides should be included in the suite of 'biologics' tested for their ability to target specific effector pathways. In addition to their anti-inflammatory actions, both α-MSH and KPV are also anti-microbial against the bacterial pathogens, *Staphylococcus aureus* and *Candida albicans*, due to their ability to raise intracellular cAMP levels. Thus, their use as pharmacological agents would likely be associated with reduced risk of infection. Systemic administration of α-MSH already is known to produce few side effects, possibly due to the swift degradation of α-MSH by serum proteases.

Brzoska *et al*. propose that although the pharmacokinetics of α-MSH-based tripeptides have not been tested, their administration may have several advantages over α-MSH (i) they are sufficiently small molecules to be cost-effectively produced on a commercial scale; (ii) they do not appear to elicit a melanogenic effect; (iii) they would likely provide a lower risk of infection relative to other anti-inflammatory drugs due to their anti-microbial activities; (iv) they appear to limited side effects based on preliminary toxicity data; and (v) they may be directed at localized targets due to their small molecular size.

## Retargeting synthetic melanocortins as candidates for preventive medicine in obesity-related disorders

According to the summarizing report by Brzoska *et al*. (40) many of the anti-inflammatory effects of α-MSH seen *in vitro* have been coupled with the detection of MC1-R. Given that adipose tissue expresses MCI-R (27,92) and, to a lesser extent, MC4-R and MC5-R (92), it is reasonable to hypothesize that α-MSH may have anti-inflammatory activity in adipose tissue. Currently, it is unknown whether circulating α-MSH plays any important role in the homeostasis of the peripheral tissues. In most people, the plasma levels of this peptide range from less than 10 to approximately 45 pg $mL^{-1}$ (93). Some studies have reported increased levels of α-MSH in the bloodstream of obese individuals relative to lean subjects, as well as positive correlations between plasma α-MSH levels and measures of adiposity (BMI, waist circumference, per cent body fat, visceral fat area) and measures of hormonal status (circulating leptin, insulin resistance) (94,95). The source of the increase in plasma α-MSH in obese subjects is unknown. Observed increases may indicate the compensatory role of this anorexigenic peptide in obesity or suggest possible peripheral resistance to its actions, similar to that of leptin. However, both of these hypotheses remain speculative, particularly given that other investigators found no relationship between circulating α-MSH concentrations and measures of adiposity, diet composition, or insulin sensitivity; nor were significant changes in plasma levels of α-MSH observed in obese subjects following weight loss (96–98).

We postulate that melanin is synthesized in adipose tissue, particularly in obese individuals, due to its antioxidant and anti-inflammatory qualities. It is tempting to speculate that an individual's propensity for melanin biosynthesis in adipose may be reflected in his ability to ward off secondary complications normally associated with the excessive accumulation of adipose mass. However, if these hypotheses hold true, then it follows that components of the melanogenic pathway, such as α-MSH, could be exploited to promote melanin biosynthesis in adipose tissue, thereby abating ROS production and inflammation and preventing the sequelae of obesity (Fig. 2). Importantly, synthetic analogues of α-MSH already are under investigation for other medical uses and passed Phase I studies for their safety; examples of already tested compounds include a linear peptide, melanotan I (MTI), and a cyclic truncated peptide, MTII, that have been tested clinically for studies on tanning of the skin (MTI) and for treatment of male erectile dysfunction (MTII/PT-141). However, it is important to note that questions were raised with regard to the possibility that α-MSH analogues might increase blood pressure (99). Another concern is that the stimulation of melanocyte proliferation might lead to melanoma; however, no tumours were reported in previous studies (91). There was, however, a report of rapidly growing new moles after self-administration of MTII in a male with a previous history of malignant melanoma (100). Moreover, there are no data concerning the safety of long-term administration of α-MSH analogues required for the preventive medicine approach; however, it is tempting to speculate that prolonged exposure to α-MSH analogues might lead to therapeutic effects at substantially lower concentrations that those required for short-term physiological effects (tanning, erectile dysfunction). We envision that low-dose, long-term stimulation of melanogenesis may become a useful avenue to explore in the prophylaxis of the

metabolic syndrome and other comorbidities in overweight and obese individuals.

## Conclusions

Obesity is a prevalent disorder that affects populations worldwide and can contribute to the development of chronic disease. On a systemic level, obesity is strongly associated with inflammation and there is some evidence that it correlates with oxidative stress. A recent study demonstrating that adipocytes express the pigment, eumelanin, as well as other proteins in the melanogenic pathway (27) elicits questions regarding the purpose of melanogenesis in adipose tissue. We hypothesize that adipose tissue in the obese is subject to elevated levels of oxidative stress, and speculate that melanin may play an important role in adipose tissue, particularly in obese individuals, due to its antioxidant and anti-inflammatory qualities. It is possible that an individual's propensity for melanin biosynthesis in adipose may be reflected in his ability to ward off secondary complications normally associated with the excessive accumulation of adipose mass. These hypotheses are as yet untested; however, if they are supported in future studies, then it follows that components of the melanogenic pathway, such as α-MSH, could be exploited to promote melanin biosynthesis in adipose tissue, thereby abating ROS production and inflammation and preventing the sequelae of obesity.

## Conflict of Interest Statement

No conflict of interest was declared.

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