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Purpose of review

Recent evidence suggests that oxidative stress is a cornerstone of the metabolic mechanisms by which overfeeding leads to insulin resistance. This review is an update of the most recent arguments in favor of this theory and of the possible role of antioxidants.

Recent findings

Reactive oxidative species (ROS) are produced by multiple pathways within the cell and are essential for many cellular functions. ROS production is balanced by enzymatic and nonenzymatic antioxidant systems. The perturbation of the pro-oxidant/antioxidant balance can lead to increased oxidative damage of macromolecules, a phenomenon known as 'oxidative stress'. ROS are involved both in insulin signal transduction and in insulin resistance when produced in excess. Overfeeding, saturated fatty acids, and obesity play a key role in the excessive production of ROS. However, a diet rich in fruits and vegetables, and therefore antioxidants, has demonstrated beneficial effects against oxidative damages and insulin resistance.

Summary

Experimental data are in favor of a beneficial role of antioxidants in glucose metabolism, but clinical data in humans are more controversial. Even if a diet rich in fruits and vegetables could provide an optimal mix of antioxidants, it remains unclear whether supplementation with antioxidants alone can reproduce the same effect.

Keywords

antioxidants, glucose metabolism, insulin resistance, oxidative stress

Introduction

Excess body weight and type 2 diabetes (T2D) are at the forefront of the growing epidemic of chronic diseases. They are at the core of a great number of cardiovascular disorders that still represent the leading cause of mortality in most developed countries. Apart from their impact on health and well being, these conditions have a considerable economic cost (http://www.cdc.gov/nchs/FASTATS/lcod.htm). Unfortunately, the burden of obesity and its associated complications is not limited to economically developed countries and is increasingly affecting economically developing countries [1].

Diet and nutrition are key factors in the regulation of glucose metabolism. On the one hand, chronic overnutrition is associated with insulin resistance. On the other hand, nutrition itself is a tool in regulating glucose metabolism, as it has been shown that some types of foods like the Mediterranean diet might be protective [2]. The wealth of fruits and vegetables included in this diet, and consequently the supply of antioxidants, may be essential for its beneficial effects.

The objective of this review is to summarize recent evidence suggesting that oxidative stress is at the core of the mechanisms implicated in chronic overfeeding leading to insulin resistance and to consider the possible preventive role of antioxidants in these metabolic changes.

Reactive oxygen species and oxidative stress

Reactive oxidative species (ROS), or free radicals, are atoms or molecules that have an unpaired electron that allows them to react with various molecules at the site of formation. They can contain both nitrogen and oxygen or only oxygen atoms (Table 1) and are produced by various physiological mechanisms. ROS are essential in several biochemical processes, including cellular differentiation, growth arrestment, apoptosis, immunity, and defense against microorganisms and intracellular messaging, including insulin signal transduction [3••] (Figs 1 and 2).

Several target proteins of ROS, such as protein kinase C (PKC), play an important role in cellular metabolism and signal transduction [4]. For example, ROS are essential

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Table 1 Principal reactive oxidative species

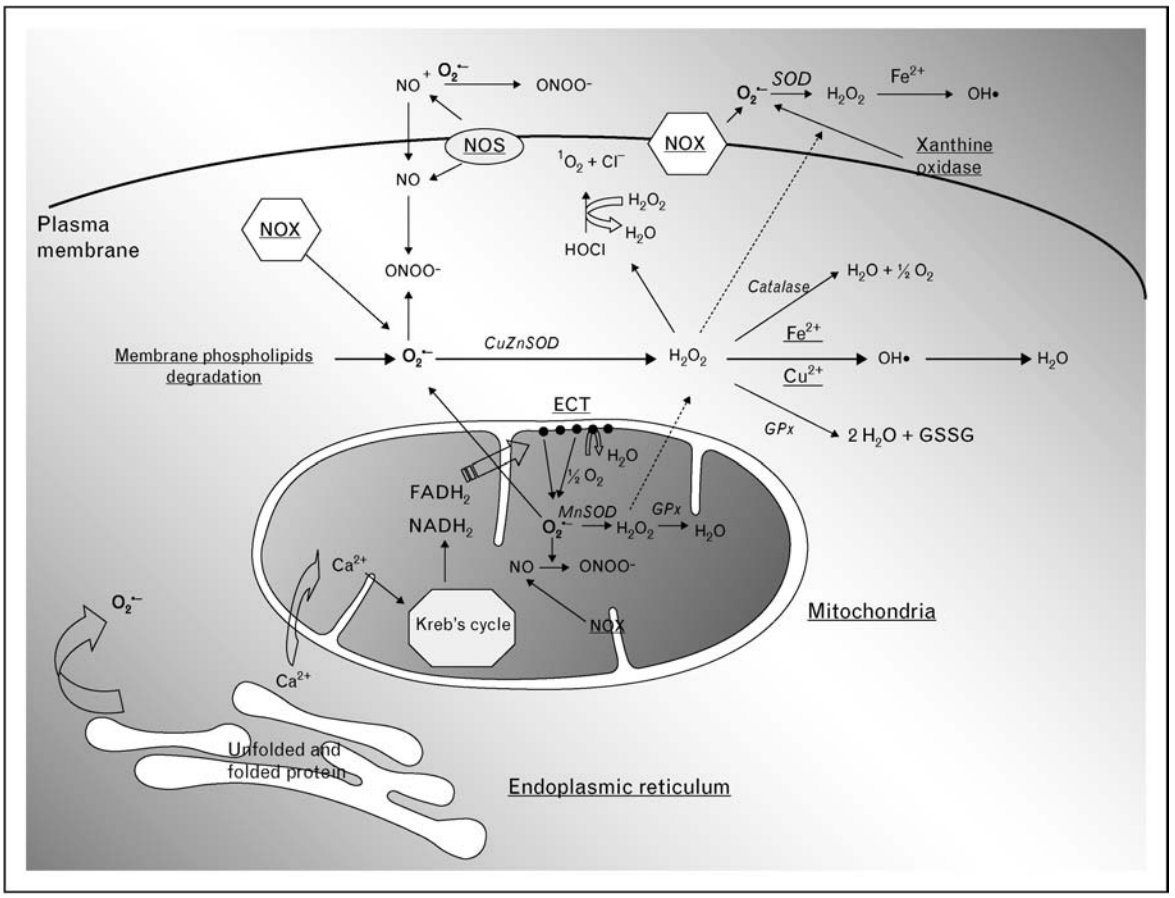
Name	Formula
Reactive oxygen species	
Singlet oxygen	$^1O_2$
Superoxide radical	$O_2^{\bullet -}$
Hydroxyl radical	$^{\bullet}OH$
Hydrogen peroxide	H_2O_2
Peroxide radical (lipid peroxide)	$ROO^{\bullet}$
Alkoxyl radical	$RO^{\bullet}$
Hypochlorous acid	HOCl
Reactive nitrogen species	
Peroxynitrite radical	$ONOO^{\bullet}$
Nitrogen dioxide radical	$NO_2^{\bullet}$
Alkyl peroxynitrite	ROONO

regulators of insulin sensitivity and glucose homeostasis *in vivo* via tyrosine phosphorylation-dependent signaling and reversible inhibition of protein tyrosine phosphatases (PTPs) such as PTB1B and PTEN (phosphatase with tensin homology) [5,6•]. According to the study by Loh *et al.* [6•], in-vivo production of H_2O_2, a process involving enhanced PTEN oxidation, also appears to be essential to maintain insulin sensitivity.

Therefore, ROS have a role in normal metabolism and 'oxidative stress' is an imbalance between the production of ROS and the defense systems against ROS (pro-oxidant–antioxidant balance). This imbalance can lead to a toxic state in which macromolecules (i.e., lipids, proteins, and/or DNA) are oxidatively damaged and cellular function is altered. Hence, although ROS are short-lived molecules, they leave detectable traces of modified oxidative products such as nitrated tyrosines and protein carbonyls as an indicator of protein oxidation. Isoprostanes, malondialdehyde, and 4-hydroxyl-2-nonenol are remnant signs of lipid peroxidation. In addition, the oxidized base, 8-hydroxy-2′-deoxyguanosine (8-OH-dG), is a marker of DNA oxidation.

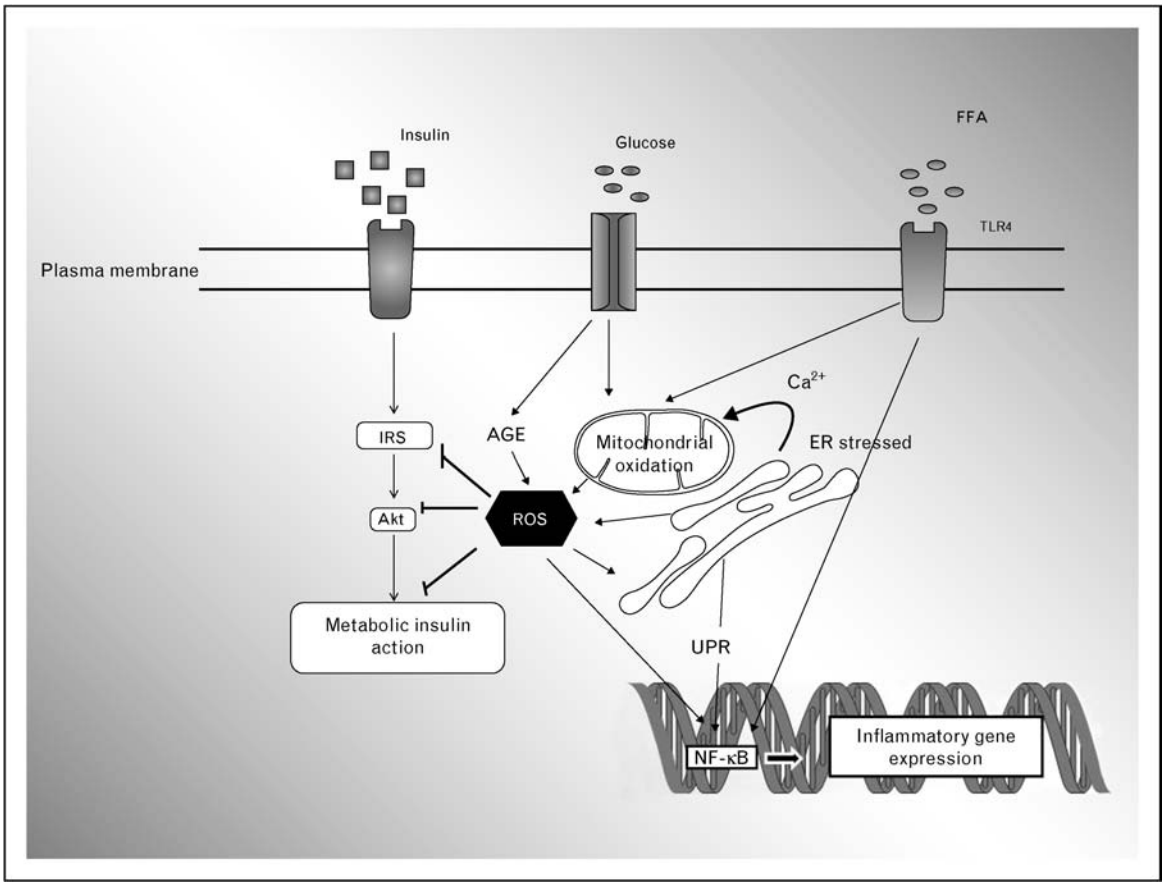
The fight against free radicals involves the trapping of free radicals in nonenzymatic systems such as vitamin C,

Figure 1 Intracellular sources of reactive oxidative species



The main producers of reactive oxidative species (ROS) are the electron chain transporters (ECTs) NADPH oxidase (NOX), xanthine oxidase, and nitrite oxide synthase (NOS), which are underlined in this figure. The endoplasmic reticulum participates in ROS production during protein folding. There is also an exacerbation in ROS production during the unfolded protein response. Another potential source of ROS is the lipid membrane, particularly arachidonic acid degradation. The enzymatic antioxidant system is represented in bold and italics: superoxide dismutase (SOD) activated with manganese (MnSOD), copper, and zinc (CuZnSOD), catalase, glutathione peroxidase (GPx).

Figure 2 From reactive oxidative species production to consequences on insulin signaling



Reactive oxidative species (ROS) produced by one pathway (see Fig. 1) can inhibit insulin pathways at several levels. An increase in substrate availability [glucose and free fatty acids (FFAs)] can participate in ROS accumulation via advanced glycation end products (AGE) and mitochondrial alteration. Moreover, ROS and FFAs can modulate NF-κB activation, inducing inflammatory gene activation. Direct implications of inflammation cannot be ruled out, because FFAs can directly activate innate immunity via Toll-like receptor 4 (TLR4). ER, endoplasmic reticulum.

vitamin E, carotenoids, reduced glutathione, and polyphenols. It also involves enzymatic systems including, but not limited to, superoxide dismutases (SODs), catalase (CAT), and glutathione peroxidase (GPX). The antioxidant trace elements are cofactors of these enzymes: copper, zinc, and manganese for mitochondrial SOD and selenium for glutathione peroxidase [7].

From oxidative stress to insulin resistance

Insulin resistance is defined as a reduction in the action of insulin both in the cellular capture of glucose by muscle and fat cells and in the inhibition of hepatic glucose production. It is observed in more than 80% of obese patients and in almost all patients with a metabolic syndrome and/or T2D. In the case of T2D, insulin resistance appears several decades before the clinical manifestation of the disease and represents, alongside the insulin secretion defect, one of its two core pathophysiological mechanisms. The determinants of obesity-associated insulin resistance are both genetic and environmental; the environment plays an important role in the development of insulin resistance dependent upon a given genetic background. Despite scientific efforts, the initiating mechanisms of insulin resistance are only partially known, and oxidative stress represents only one hypothesis among many others [3••].

Nevertheless, pathways leading to insulin resistance are multiple, including intracellular ceramide accumulation, activation of kinases such as c-Jun N-terminal kinase (JNK), or inhibition of kinases such as kappa B kinase (IKK-b), mitogen-activated protein kinase (p38-MAPK) or protein kinase C (PKC) isoforms. In recent years, the role of inflammation and of endoplasmic reticulum stress has received increasing attention [8••]. In fact, the insulin signaling cascade constitutes a complex signaling network, each step being interconnected with others. The abrogation of one pathway can be compensated by another, thus allowing the propagation of the signal

to the next step. Consequently, insulin resistance requires the alteration of several pathways before it becomes clinically evident.

In addition to obesity and metabolic syndromes, insulin resistance is encountered in situations as diverse as acute stress, pregnancy, hepatitis C, HIV protease inhibitor therapy, acromegaly and anti-inflammatory corticosteroid treatment. Thus, it appears logical to question whether one of the pathways could be an obligatory mechanistic common denominator linking these different forms of insulin resistance, an idea discussed by Houstis *et al.* [9]. In this study, they performed a genomic analysis of two very different cellular models of insulin resistance, one induced by treatment with the cytokine tumor-necrosis factor-α and the other with the glucocorticoid dexamethasone. Gene expression analysis suggested that ROS levels were increased in both models, which was confirmed through measures of the cellular redox state. Furthermore, different pharmacological and transgenic approaches that decrease ROS levels all ameliorated insulin resistance to varying degrees. One of these treatments was tested in obese insulin resistance mice and was shown to improve insulin sensitivity and glucose homeostasis. These results demonstrated that increased ROS levels are important triggers for creating insulin resistance in settings as different as inflammation and anti-inflammatory treatment with corticosteroids. More recently, Hoehn *et al.* [10••] compared four different models of insulin resistance including chronic treatment with insulin, corticosteroids, proinflammatory cytokines, or lipids in both muscle and adipose cell lines. The study showed a direct correlation between mitochondrial oxidative stress and insulin resistance in all models. Once again, both pharmacologic and genetic strategies that override mitochondrial $O_2^{\bullet -}$ reversed or prevented the onset of insulin resistance both *in vitro* and *in vivo*. Moreover, selective induction of $O_2^{\bullet -}$ using the mitochondrial complex III antagonist, antimycin A, rapidly induced insulin resistance [10••]. Mitochondrial $O_2^{\bullet -}$ could, thus, be a unifying element of insulin resistance, acting principally as a nutrient sensor in key metabolic tissues to regulate nutrient intake in accord with the energy oversupply.

From overnutrition to oxidative stress

An association between obesity and oxidative stress that is improved after weight loss was highlighted in humans during the past decade and confirmed in recent studies [11•–13•], some of which suggest that it could be mostly visceral fat that is responsible for this relationship [12•]. The possible processes involved in obesity-associated oxidative stress are multiple and include the following: a high metabolic load that exposes cells to an overload of nutrients with excessive mitochondrial oxidation and enhanced ROS generation, inflammatory states associated with obesity, endoplasmic reticulum stress, and endocrine dysregulation such as high levels of insulin, which have been shown to be associated with increased ROS production (Figs. 1 and 2). For more details on this subject, the reader may refer to the review by Bashan *et al.* [3••]. Interestingly, regardless of weight gain, increased fat intake is capable of shifting the cellular redox environment to a more oxidized state in the skeletal muscle of both rodents and humans, as shown by Anderson *et al.* [14••]. Mitochondrial production of hydrogen peroxide in the skeletal muscle of lean volunteers is doubled within 4 h after a high-fat meal (65% fat), a phenomenon also observed after some 5 days of a high-fat diet. These effects are also observed in rodents in association with insulin resistance and are prevented by pharmacological and genetic strategies to control mitochondrial oxidative stress. Hence, the oversupply of substrates has a direct effect on muscle cell function and involves an imbalance in the redox environment, with the mitochondria playing a central role. Other studies show that NADPH oxidase is also involved in the increased production of ROS in response to saturated fatty acids in muscle cells [15•]. Therefore, it is the metabolic burden of fat intake that appears to be responsible for oxidative stress and insulin resistance, regardless of whether obesity is present. In addition, saturated fatty acids regulate chemotactic factors expressed in cultured adipose cells by a mechanism that involves ROS generation and innate immunity via a Toll-like receptor 4 (TLR4)-dependent pathway [16••]. If such data were to be confirmed *in vivo* in humans, they would highlight the importance of dietary fat and oxidative stress in macrophage accumulation in adipose tissue, a hallmark of obesity implicated in systemic inflammation and insulin resistance.

The role of fatty acids cannot be confined to the obese population. Some people have a low expandability of their adipose tissue, meaning their adipocytes have a reduced capacity to accommodate an increase of fat storage. Even in the absence of excess body weight, these individuals are particularly sensitive to dietary fat [17•]. The inability of adipocytes to expand sufficiently to store lipids in excess leads to cellular hypertrophy; the oxygen supply of these hypertrophic adipocytes is reduced, inducing hypoxia and, in turn, an increase in inflammatory cytokine production, macrophage accumulation, lipolysis and apoptosis [17•,18•].

Finally, the finding in animal experiments that the boost in ROS production precedes both the increase in inflammatory markers and the onset of insulin resistance favors a causal role of oxidative stress in these disorders [10••,19•]. Therefore, many arguments demonstrate that oxidative stress plays a central role in the initiating mechanisms of peripheral insulin resistance. High-fat

feeding and chronic fatty acid exposure are also associated with increased ROS production, both in the β-cells, leading to dysfunction and cell death [20•], and in the liver, leading to hepatic insulin resistance [21•]. Thus, oxidative stress induced by overnutrition could be involved at all levels of the pathophysiological mechanisms of T2D.

Dietary antioxidants and glucose metabolism

In a number of the works we have mentioned so far, we have seen that the use of pharmacological and transgenic antioxidant approaches in cell culture or animals was able to reverse or prevent insulin resistance induced by fatty acids or overfeeding [9,10••,14••,15•]. Many experimental and animal studies show that dietary antioxidants, taken either as extracts or as a part of the food itself, also have beneficial effects on glucose metabolism. An incomplete list of the antioxidants with the most recent references supporting their effect on glucose metabolism is presented in Table 2 [22,23•,24–28,29•,30,31•,32,33,34••,35••,36,37,38•,39••,40••]. However, it is important to note that their benefits are not necessarily found at all levels of glucose metabolism. For example, dietary quercetin was found to have no effect on hepatic insulin resistance of mice subjected to a high-fat diet [41•], whereas it decreased inflammatory markers in parallel with a transient increase in energy expenditure [42•].

However, interventional studies that have directly assessed the effects of antioxidants on glucose metabolism in humans are rare. Very few have used the insulin clamp, the gold standard method used to evaluate insulin sensitivity. Only the effects of supplementation with alpha lipoic acid (ALA), or with vitamins C and E alone or in association, on insulin sensitivity have been studied using this method, and each has shown positive effects [33,37,43]. Nevertheless, the published evidence is ambiguous, as apparent in the recent study by Ristow *et al.* [44•] showing that supplementation with a combination of vitamins C and E at high doses may preclude the health-promoting effect of exercise on improved insulin sensitivity. However, high levels of vitamin C, associated with a healthy diet rich in fruits and vegetables, have a preventive effect on T2D [38•] and do not reduce the positive effect of exercise [45•]. Regarding the polyphenols, only one study has evaluated the effects of cocoa consumption on insulin sensitivity using the insulin clamp in essential hypertensive patients and did not detect any effect [46].

If antioxidants are beneficial to glucose metabolism, it seems logical to expect a preventive effect on the occurrence of T2D, which has been confirmed by almost all epidemiological studies. A meta-analysis of previously published studies confirms the protective effect of vitamin E and carotene consumption on the occurrence of T2D [22], an effect that could be mediated by an improvement in insulin sensitivity, as recently confirmed both by the Insulin Resistance and Atherosclerosis (ARIC) Study [34••] and the Uppsala Longitudinal Study of Adult Men (ULSAM) [35••]. Nevertheless, all larger intervention trials evaluating the diabetes-preventive potential of antioxidant supplements have been unable to find any positive effects of supplementation, with the most recently published study being the Women's Antioxidant Cardiovascular Study (WACS) and the SU.VI.MAX study (SUpplementation en VItamines et

Table 2 Main dietary source of antioxidant compounds with demonstrated activities on glucose metabolism, type 2 diabetes, or metabolic syndrome

Antioxidant compound [22,23•]	Main dietary sources [24,25]
Polyphenols	
Flavonoids [26–28]	
Flavonols: quercetin, myricetin, kampeferol, rutin	Peas, carrot, broccoli, spinach, cauliflower, apple, plum, apricot, strawberries, aloe vera (leaves), tomatoes, black and green tea, asparagus
Flavanols: catechins, epicatechin	Cocoa, black chocolate, black and green tea
Anthocyanins: cyaniding, delphinidin, luteolinidin	Blackberries, raspberries, blueberries, orange, eggplant, cherries, red grape, red wine, purple corn
Isoflavones: genistin, formononetin, coumestrol	Soy, blackbeans, red clover, alfalfa, peanut
Flavanones: hesperidin, naringenin	Orange, grapefruit, lemon, lime, tomato (skin)
Flavones: apigenin, luteolin, tangeritin	Parsley, celery, sweet pepper
Phenolic acids [23•,29•]	
Hydroxybenzoic acid derivatives: gallic acid, ellagic acid	Black and green tea, red wine, berries, potatoes
Hydroxycinnamic acid derivatives: chlorogenic acid, caffeic acid, hydrocinnamic acid	Blueberries, coffee, kiwi fruit, apples, pears, red wine, broccoli, plums, cherries
Stilbenoids	
Transresveratrol [30,31•,32]	Skin of red grapes, cranberries, blueberries, bilberries
Vitamin E: tocopherol [33,34••,35••,36]	Unheated vegetable oil: wheat germ oil, palm oil, cereals, almonds, hazelnuts, green vegetables (spinach), butter, milk, egg, avocado, oily fish (tuna)
Vitamin C: L-ascorbic acid [37,38•]	Kakadu, camu camu, acerola, jujube, broccoli, Brussels sprout, lychee
Lipoic acid [39••]	Spinach, broccoli
Carotenoids: β-cryptoxanthin, β-carotene, lutein [35••,40••]	Alfalfa, carrot, tomato, grapefruit, watermelon

Minéraux AntioXydants) [47••,48••]. Moreover, in a systematic review analyzing the effects of β-carotene, vitamins A and E, vitamin C, and selenium, on all-cause mortality in adults included in primary and secondary prevention trials, Bjelakovic *et al.* [49] did not find convincing evidence that these antioxidants supplementations had beneficial effects on diabetes prevention. In fact, supplementation with β-carotene, vitamin A, or vitamin E seemed to increase the risk of death. Data on phenolic compounds are much rarer and provide mixed results. The analysis of data from the 38 000 women of the Women's Health Study (WHS) does not show any link between flavonoid consumption and diabetes risk [26,27], but tea and coffee consumption is associated with a diabetes-protective effect that could be related to their polyphenol content [23•,29•].

Antioxidants: beyond the antioxidant effect

Although we see that the experimental data are overwhelmingly in favor of a beneficial role of antioxidants on glucose metabolism, clinical data in humans are less convincing. Several explanations can be given. As previously mentioned, excess ROS can negatively interfere with insulin signal transduction, but their presence in small quantities is ultimately essential for insulin action [3••]. An excess of antioxidants may prove deleterious, which might help explain the results of some studies. Second, antioxidants, when they have reacted with ROS, become pro-oxidants and must interact with other antioxidants. A chain reaction involving different antioxidants must be put in place until a product is achieved that does not have enough reducing power to interact with the macromolecular compounds in the cell [50]. Thus, perhaps a diet rich in fruits and vegetables could provide an optimal mix of antioxidants, but it is unclear whether supplementation with some antioxidants alone can reproduce the same effects. In addition, it cannot be excluded that the beneficial effects of fruits and vegetables on the prevention of diabetes may be due to other bioactive compounds than the antioxidants themselves.

Finally, the effects of most molecules are not solely confined to their antioxidant properties, which may ultimately prove to be of secondary importance. One of the most striking examples is resveratrol, a phenolic compound of the stilbene family that has antioxidant properties and is capable of interacting directly with numerous metabolic pathways [31•]. At high doses, resveratrol improves the insulin sensitivity of mice fed a high-fat diet [30], prevents diet-induced obesity and alleviates obesity-related insulin resistance [32]. These effects are most likely mediated via SIRT1, a protein deacetylase that plays a role in the extension of lifespan and in chromatin remodeling. Its mechanism of action is associated with gene silencing rather than direct antioxidant action [51]. Similarly, vitamin E may not be confined to its antioxidant effects, it participates directly in controlling the expression of certain genes [36].

Conclusion

Experimental studies in cell cultures and animals have shown that ROS may play a central role in the mechanisms linking excess food and saturated fat intake and altered glucose metabolism. Epidemiologic studies have shown that a diet rich in fruits and vegetables has beneficial effects on insulin sensitivity and diabetes prevention, which has been attributed to their antioxidant properties. However, human studies hardly support a beneficial effect of antioxidants supplementation on glucose metabolism, suggesting that specific combinations of antioxidants may be necessary to observe these effects. It is also possible that the health benefits of such diets might not be solely attributed to their antioxidant capacity but could involve synergistic mechanisms between the different molecules. Further studies are needed to clarify the potential role of antioxidants in the improvement or prevention of abnormal glucose metabolism.

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