Dietary antioxidants: Do they have a role to play in the ongoing fight against abnormal glucose metabolism?
Antoine Avignon, Marie Hokayem, Catherine Bisbal, Karen Lambert

To cite this version:
Antoine Avignon, Marie Hokayem, Catherine Bisbal, Karen Lambert. Dietary antioxidants: Do they have a role to play in the ongoing fight against abnormal glucose metabolism?. Nutrition, Elsevier, 2012, 10.1016/j.nut.2012.01.001. hal-02537052

HAL Id: hal-02537052
https://hal.umontpellier.fr/hal-02537052
Submitted on 8 Apr 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
Review

Dietary antioxidants: Do they have a role to play in the ongoing fight against abnormal glucose metabolism?

Antoine Avignon M.D., Ph.D., Marie Hokayem Ph.D. Student, Catherine Bisbal Ph.D., Karen Lambert Ph.D.

INSERM U1046, Physiologie & Médecine Expérimentale du Cœur et des Muscles, Montpellier, France
Université Montpellier I, Université Montpellier II, Montpellier, France
CHU Montpellier, Montpellier, France

A B S T R A C T

Overfeeding, an increased intake of saturated fatty acids, and sugary foods are key dietary changes that have occurred in recent decades in addition to the emergence of the obesity epidemic. In addition to an increase in energy storage as fat, these dietary changes are accompanied by an increase in mitochondrial macronutrient oxidation, leading to an excessive free radical production and, hence, oxidative stress. The latter has long been considered a central mechanism linking nutrient overload, insulin resistance, the metabolic syndrome, and diabetes. However, food, through fruit and vegetable consumption, also can be a great source of antioxidants that protect the body against oxidative damage and insulin resistance and thus help cope with the metabolic backlash of the energy-dense Westernized diet. Experimental data are in favor of the beneficial role conveyed by antioxidants in glucose metabolism, but clinical data in humans remain controversial. This review therefore aimed to sort out any underlying discrepancies and provide an overall clear view of the role of antioxidants in the ongoing fight against abnormal glucose metabolism.

Introduction

The obesogenic dietary changes of recent decades, the key aspects of which are increased fatty and sugary food intake in parallel to a decrease in fruit and vegetable consumption, have been proposed to play essential roles in the growing epidemic of chronic diseases afflicting developed and developing countries. These dietary changes combined with a sedentary lifestyle force the body to manage excess energy that must be metabolized. One of the expected actions is increased energy storage as fat, and other macronutrients undergo oxidation in the mitochondria, favoring an increased production of free radicals and oxidative stress, which has long been proposed as a unifying mechanism linking excessive nutrient intake, insulin resistance (IR), the metabolic syndrome, and diabetes. Beyond the quantitative aspects of food ingestion, quality is just as important in the development of oxidative stress; for example, different types of fatty acids have variable effects on the production of free radicals. Moreover, nutrition is a potent tool in regulating glucose metabolism, and it has been reported that food rich in antioxidants such as food in the Mediterranean diet might be protective [1,2].

We recently summarized the data linking oxidative stress to diet and to IR and the preventive role of antioxidants against these metabolic alterations [3]. In the present review, we present an update of recent discoveries in this rapidly evolving field, try to sort out any underlying controversies, and supply a global recapitulative view of the role of antioxidants in the ongoing fight against an abnormal glucose metabolism.

Oxidative stress and insulin resistance

Reactive oxygen species (ROS), or free radicals, are atoms or molecules characterized by an unpaired electron that allows the atoms or molecules to react with various molecules present at their site of formation (Table 1). They are produced within the cell by different mechanisms; the main source of superoxide anions is mitochondrial by the electron transport chain. Other precursors of endogenous superoxides include reduced nicotinamide adenine dinucleotide phosphate oxidase, xanthine oxidase, nitrite oxide synthase, the endoplasmic reticulum, an unfolded
protein response, and the lipid membrane, particularly arachidonic acid degradation. Oxidants are naturally neutralized or detoxified by antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase and by non-enzymatic antioxidants including glutathione, uric acid, reduced nicotinamide adenine dinucleotide phosphate, coenzyme Q, albumin, and bilirubin. This endogenous antioxidant system is supplemented by exogenous originating reducing compounds encompassing vitamin C, vitamin E, carotenoids, and polyphenols that play essential roles in many antioxidant mechanisms. When in excess, ROS interact with macromolecules and may lead to molecular alterations such as DNA fragmentation, lipid peroxidation, or protein carbonylation. However, ROS are also essential for many biological processes, including insulin-signaling transduction (Fig. 1) [3]; thus, they must not be seen as only toxic compounds. Then, “oxidative stress” results from an imbalance between the generation of ROS and the defense mechanisms against ROS (pro-oxidant–antioxidant imbalance). However, this remains an oversimplistic view of a highly complex process, with recent evidence suggesting that redox is considerably compartmentalized inside the cell. To this extent, the redox potential varies between subcellular compartments by differentially regulating redox-sensitive processes in distinctive parts of the cell, resulting in redox circuits that are kinetically limited and insulated from each other. For this reason, oxidative stress has been proposed to result from a disruption of these circuits (reviewed by Jones and Go [4]). Whatever its origin, oxidative stress leads to a toxic environment in which macromolecules are oxidatively damaged and cellular functions are altered.

Conversely, IR is defined as a decrease in insulin action, be it the cellular capture of glucose by muscle and fat cells or the inhibition of hepatic glucose production. It is found in numerous medical conditions, the most common being obesity, diabetes, and the metabolic syndrome, but also essential hypertension, polycystic ovary syndrome, hepatitis C, antiretroviral therapy for human immunodeficiency viral infection, and even *Helicobacter pylori* infection, among others. In addition to an insulin secretion defect, it represents one of the two core pathophysiologic mechanisms of type 2 diabetes (T2D). The determinants of IR are genetic and environmental, with the latter playing an important role in the development of IR dependent on a specific genetic background. In fact, the insulin-signaling cascade constitutes a complex signaling network; each step is interconnected with another. That is why the abrogation of one pathway can be compensated by another, allowing the ongoing propagation of the signal. Thus, understanding the mechanisms involved in IR is fairly difficult and thus far only fractional. The assumption that oxidative stress can be a mandatory mechanistic common denominator linking the many forms of IR has received increasing attention in recent years [5]. Indeed, be they in vitro or in vivo, the varied circumstances of IR are associated with oxidative stress and the diverse pharmacologic and transgenic approaches decreasing ROS levels, all improve insulin sensitivity to varying degrees [6]. More precisely, ROS production by the mitochondria and mitochondrial oxidative stress seem to be involved in IR [6]. These studies therefore suggest that mitochondrial superoxide production may be a unifying mechanism of IR and could well be the missing link between overeating and IR, as previously observed [7].

**Diet and oxidative stress**

In a recent review, we reported evidence showing that oxidative stress is a core mechanism connecting overfeeding to IR [3]. Briefly, in humans, obesity and more specifically visceral fat are associated with oxidative stress, which is reversed by weight loss. Multiple processes have been reported to be involved in obesity-associated oxidative stress, including a high metabolic load that exposes cells to an overload of nutrients with excessive mitochondrial oxidation and increased ROS generation, inflammation, endoplasmic reticulum stress, and endocrine dysregulations, mainly hyperinsulinemia. Beyond weight gain, the metabolic load per se is recognized as a decisive element of oxidative stress, because high-fat, high-carbohydrate meals increase oxidative stress and inflammation measured in peripheral blood cells [8] and in the skeletal muscles of lean volunteers [9]. Indeed, markers of oxidative stress are altered after eating a single high-fat, high-carbohydrate meal [9], specifically during the postprandial period, which is particularly sensitive in light of how postprandial hyperlipidemia, hyperglycemia, and hyperinsulinemia are associated with increased oxidative stress [10]. Supporting the importance of the postprandial period, in patients with T2D it has been shown that exenatide, a glucagon-like peptide-1 analog with marked effects on the postprandial period, which is particularly sensitive in light of how postprandial hyperlipidemia, hyperglycemia, and hyperinsulinemia are associated with increased oxidative stress [10].

Supporting the importance of the postprandial period, in patients with T2D it has been shown that exenatide, a glucagon-like peptide-1 analog with marked effects on the postprandial period, which is particularly sensitive in light of how postprandial hyperlipidemia, hyperglycemia, and hyperinsulinemia are associated with increased oxidative stress [10]. In regard to the significance of the postprandial period, one can easily imagine the consequences of a Westernized diet with its daily repeated snacking of energy-dense foods.

Any energy overload ensured by carbohydrates or lipids or even proteins leads to an increased ROS production and eventually oxidative stress [8]. However, the quality of macronutrients plays a decisive part, particularly when considering the nature of the fatty acids consumed. Experimental data have consistently shown that lipid toxicity is caused primarily by saturated fatty acids and involves oxidative stress, an effect that

---

**Table 1**

Major reactive species: origins and antioxidant defenses

<table>
<thead>
<tr>
<th>Reactive species</th>
<th>Cellular sources of reactive species</th>
<th>Antioxidative systems</th>
</tr>
</thead>
<tbody>
<tr>
<td>O₂</td>
<td>mitochondria; ETC, membrane and cytoplasm: NOX; endoplasmic reticulum: oxidative protein folding (PDI1 and ERO); peroxisome: β-oxidation of fatty acids; cytosolic oxidase (xanthine oxidase, lipooxygenase, cyclooxygenase)</td>
<td>Mn-SOD, CuZn-SOD, CuZn-SOD</td>
</tr>
<tr>
<td>H₂O₂</td>
<td>from O₂ in cytoplasm by CuZn-SOD</td>
<td>catalase</td>
</tr>
<tr>
<td>H₂O₂</td>
<td>from O₂ in mitochondria by Mn-SOD</td>
<td>GPx</td>
</tr>
<tr>
<td>OH</td>
<td>from H₂O₂ in cytoplasm by Cu²⁺, Fe²⁺ (Fenton reaction)</td>
<td>Trx/Prdx</td>
</tr>
<tr>
<td>RNS</td>
<td>NO from NO with O₂⁻</td>
<td>hydroxyl/carboxyl radicals</td>
</tr>
<tr>
<td>ONOO⁻</td>
<td>from NO with O₂⁻</td>
<td>hydroxyl/carboxyl radicals</td>
</tr>
</tbody>
</table>

CuZn-SOD, cytosolic superoxide dismutase; ERO, endoplasmic reticulum oxido-reductase; ETC, electron transport chain; GPx, glutathione peroxidase; H₂O₂, hydrogen peroxide; Mn-SOD, mitochondrial superoxide dismutase; NO, nitric oxide; NOS, nitric oxide synthase; NOX, reduced nicotinamide adenine dinucleotide phosphate oxidase; O₂⁻, superoxide anion; OH, hydroxyl radical; ONOO⁻, peroxynitrite; PDI1, protein disulfide isomerase-1; Prdx, peroxiredoxin; RNS, reactive nitrogen species; Trx, thioredoxin.
is much less evident or even absent for an unsaturated fat such as oleic acid. This has been confirmed by very recent work such as that by Michel et al. [14] on the involvement of small nucleolar RNAs in palmitate-induced oxidative stress and endoplasmic reticulum stress response with no effect of oleate, and the findings of Yuzefovych et al. [15] showing that palmitate but not oleate causes a significant increase in mitochondrial ROS production, which correlates with concomitant mitochondrial DNA damage, mitochondrial dysfunction, the induction of c-jun N-terminal kinase, apoptosis, and an inhibition of insulin signaling. Clinical studies on the subject remain sparse and the results remain controversial. Perez-Martinez et al. [16] showed that, after a diet rich in monounsaturated fatty acids, post-prandial lipid peroxidation levels, protein carbonyl concentrations, superoxide dismutase activity, and plasma hydrogen peroxide levels were lower compared with subjects adhering to a diet rich in saturated fatty acids. In contrast, in the LIPGENE study (a European multicentre dietary intervention study that investigates the effects of fat quality and quantity on risk factors associated with the metabolic syndrome), Petersson et al. [17] found no effect of dietary fat modification on oxidative stress and inflammatory markers. It has been established in several animal models that caloric restriction is one of the most effective ways to prolong life expectancy. The hypothesis regularly put forward for this effect is that it primarily involves a decrease of oxidative stress.

High-fat feeding and chronic fatty acid exposure also have been associated with increased ROS production in β-cells, leading to their dysfunction and cell death [18], and in hepatocytes, leading to hepatic IR [19]. Thus, the oxidative stress ignited by overnutrition can be found at all levels of the pathophysiological mechanisms of T2D (Fig. 2).

**Dietary antioxidants and glucose metabolism**

Excessive energy intake in the form of saturated fatty acids and high glycemic index foods can be a source of oxidative stress. Conversely, plant-derived foods contain hundreds of active antioxidant compounds, including ascorbic acid, tocopherols, carotenoids, and a wide range of phytochemicals such as phenolic acids and flavonoids. They modulate oxidative stress and protect against oxidative damage and its complications such as IR.

Many in vitro and animal studies have shown that a large range of dietary antioxidants, taken as extracts or as part of the food, have beneficial effects on glucose metabolism, including the prevention of T2D. Among them, polyphenols are the most studied and have shown very interesting results. To name only the most recent studies, queretin, one of the most common flavonoids in the human diet, has demonstrated favorable effects on glucose metabolism by attenuating tumor necrosis factor-α-mediated inflammation and IR in primary human adipocytes [20]; cyanidin-3-O-β-glucoside, a thoroughly investigated anthocyanin, has demonstrated insulin-like activities by peroxisome proliferator-activated receptor-γ activation in human omental adipocytes [21]; the citrus flavonoids hesperetin and naringenin have demonstrated a direct inhibition of tumor necrosis factor-α–stimulated release of fatty acid secretion in cultured adipocytes [22], increased glucose uptake by AMP-activated protein kinase (AMPK) in cultured skeletal muscle cells [23], and improved insulin
sensitivity by increasing tyrosine phosphorylation in fructose-fed rats [24]. Also, luteolin, a flavonoid, has been shown to increase insulin sensitivity by an activation of peroxisome proliferator-activated receptor-γ transcriptional activity in 3T3-L1 adipocytes [25] and caffeic acid phenethyl ester potently has been found to stimulate glucose uptake in cultured skeletal muscle cells through the AMPK pathway [26] and stimulate glucose uptake in insulin-resistant mouse hepatocytes, as has cinnamic acid [27]. One of the phenolic compounds whose effects have been largely studied on carbohydrate metabolism is unquestionably resveratrol, a phenolic compound of the stilbene family that, although exerting antioxidant properties, can interact directly with numerous metabolic pathways [28]. The studies by Lagouge et al. [29] and Baur et al. [30] showing how resveratrol improves energy balance, increases mitochondrial activity, and protects mice against diet-induced obesity and IR constitute important defending arguments on how this compound may play an important role in the prevention of metabolic diseases and diabetes. Its different actions on glucose metabolism are not restricted to its antioxidant capacity but rather are mediated through sirtuin 1 and thus associated with gene sequence silencing [20,31–34].

We therefore understand that experimental data are abundantly in favor of the central role of oxidative stress in IR, on the one hand, and of the protective effect of dietary antioxidants against these metabolic alterations, on the other hand. However, intervention studies directly assessing the effects of antioxidants on glucose metabolism in humans are rare and very few have used the insulin clamp technique, the gold standard method in assessing insulin sensitivity. The effects of α-lipoic acid and vitamin C and E supplementation alone or in association with other antioxidants have been evaluated using this method and have shown positive effects [3]. These positive results must be weighed against other studies with less favorable conclusions such as the recently published study by Yfantl et al. [35] indicating that the administration of antioxidants in the combined form of vitamin C (500 mg/d) and vitamin E (400 IU/d) during strenuous endurance training had no effect on the training-induced increase in insulin sensitivity. These data complement those of Ristow et al. [36] showing that supplementation with a combination of these same vitamins at high doses (1000 mg/d and 400 IU/d, respectively) may preclude the health-promoting effect of exercise on improved insulin sensitivity. However, these data are in conflict with the finding suggesting that high levels of vitamin C do not decrease the positive effects of exercise [37] and even decrease the risk of T2D [38]. Regarding polyphenols only, few studies have evaluated their effects on insulin sensitivity using the insulin clamp. In fact, only one study showed that cocoa consumption for 2 wk had no effect on the insulin sensitivity of hypertensive patients [39]. Very recently, the metabolic effects of resveratrol identified thus far in animals were confirmed in a clinical trial [40]. In this work, the investigators reported that a substantially high dose of 150 mg/d of trans-resveratrol (average daily intake in Europe of about 0.01–0.45 mg/d [41]) taken for 1 mo had favorable effects on glucose homeostasis in obese subjects by improving their homeostasis model assessment index, thus indicating favorable effects on insulin sensitivity. Unfortunately, the investigators could not determine if these effects resulted in an amelioration of whole-body insulin sensitivity when using the insulin clamp.

Epidemiologic studies have reported experimental data of diets rich in antioxidants such as vitamin C [38], vitamin E [42], α-tocopherol [43], or β-carotene [43] exhibiting beneficial effects on glucose metabolism and on diabetes prevention. Two meta-analyses examined the association between the intake of fruit, vegetables, and antioxidants and the risk of T2D. The major finding of the first study is that the consumption of antioxidants but not of fruits and vegetables was associated with a 13% decrease in the risk of T2D, mainly attributed to vitamin E [44], whereas in the second, the intake of green leafy vegetables was associated with a 14% decrease of the same risk [45].

The problem is that when reviewing clinical intervention trials evaluating the effects of antioxidant supplementation, it is hard to

**Fig. 2.** Environmental and genetic factors favoring oxidative stress and the mechanisms behind reactive oxidative stress-induced insulin resistance and type 2 diabetes mellitus. ROS, reactive oxygen species.
Main antioxidants, their natural sources, and recent evidence concerning their effects on glucose metabolism in humans

Table 2

<table>
<thead>
<tr>
<th>Antioxidant compounds</th>
<th>Type of study</th>
<th>Effects on glucose metabolism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyphenols</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flavonoids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flavonols: quercetin, myricetin, kaempferol (peas, carrot, broccoli, spinach, cauliflower, apple, plum, apricot, strawberries, tomatoes, black and green teas)</td>
<td>prospective epidemiological study</td>
<td>high intake of flavonols and flavones not associated with decrease in T2D, although a modest inverse association with apples and tea intake cannot be ruled out [49]; no effect epicatechin has no effect on IR [50]; no effect</td>
</tr>
<tr>
<td>Flavanols: catechins, epicatechin (cocoa, black chocolate, black and green teas)</td>
<td>randomized controlled trial</td>
<td>flavascid supplementation decreases thiobarbituric acid-reactive substances, plasma glucose, and HOMA-IR of overweight, hypertensive subjects with family history of diabetes [51]; positive effect supplement containing combination of antioxidants extracted from fruit, berries, and vegetables has no effect on blood glucose, HbA1c, insulin [52]; no effect</td>
</tr>
<tr>
<td>Anthocyanins: cyanidin, delphinidin, luteolinidin (berries, orange, eggplant, cherries, red grape, red wine)</td>
<td>randomized trial</td>
<td></td>
</tr>
<tr>
<td>Isoflavones: genistin, formononetin, coumestrol (Soy, black beans, alfalfa, peanuts)</td>
<td>randomized controlled trial</td>
<td></td>
</tr>
<tr>
<td>Flavanones: hesperidin, naringenin (orange, grapefruit, lemon, lime, tomato skin)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flavones: apigenin, luteolin, tangeritin (parsley, celery, sweet pepper)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenolic acids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydroxybenzoic acid derivatives: gallic acid, ellagic acid (black and green teases, red wine, berries, potatoes)</td>
<td>epidemiologic, cross-sectional study</td>
<td>green tea consumption associated with increased prevalence of diabetes mellitus in an Iranian population [53]; negative effect</td>
</tr>
<tr>
<td>Hydroxycinnamic acid derivatives: chlorogenic acid, caffeic acid, hydroxycinnamic acid (blueberries, coffee, kiwi fruit, apples, pears, red wine, broccoli, plums, cherries)</td>
<td>epidemiologic, cross-sectional study</td>
<td>coffee but not green tea consumption has beneficial effects on glycemic parameters (fasting plasma glucose, HOMA-IR, HOMA-B, plasma HbA1c) in multiethic Asian population [54]; positive effect of coffee, no effect of tea long-term consumption of oolong tea may be predictive factor for new-onset diabetes in a Japanese population [55]; negative effect</td>
</tr>
<tr>
<td>Vitamin E tocopherol (unheated vegetable oil: wheat germ oil, palm oil; cereal, almonds, hazelnuts, green vegetables, butter, milk, egg, avocado, oily fish, e.g., tuna)</td>
<td>epidemiologic, cross-sectional study</td>
<td>serum vitamin C but not vitamin E is inversely associated with HOMA-IR in an adult population [58]; positive effect</td>
</tr>
<tr>
<td>Vitamin C L-ascorbic acid (acarola, jujube, broccoli, Brussels sprouts, lychee)</td>
<td>randomized controlled trial</td>
<td>lipoic acid and vitamin E supplementation alone or in combination did not affect lipid profile or insulin sensitivity of patients with T2D [59]; no effect</td>
</tr>
<tr>
<td>Lipooic acid (spinach, broccoli)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carotenoids: β-cryptoxanthin, β-carotene, lutein (alfalfa, carrot, tomato, grapefruit, watermelon)</td>
<td>randomized controlled trial</td>
<td></td>
</tr>
</tbody>
</table>

CVD, cardiovascular disease; Hb, hemoglobin; HOMA-IR, homeostasis model assessment for insulin resistance; IR, insulin resistance; T2D, type 2 diabetes mellitus

pinpoint the positive effects, with all the larger intervention trials that evaluated the diabetes-preventive potential of antioxidant supplements consisting of antioxidant vitamins with or without trace elements reporting negative outcomes [46,47]. Moreover, one study has suggested an increased risk of diabetes in the group supplemented with α-tocopherol and/or β-carotene [48].

Thus, although experimental data on cellular and animal models seem quite clear on the role of oxidative stress in IR and on the positive effects of dietary antioxidants, human data show discrepancies. These discrepancies are found when comparing experimental data with human data, but also within human data. In this regard, it is interesting to note the results of tea consumption studies in humans. Several epidemiologic studies have shown how tea ingestion may be associated with an increased risk of diabetes. Conversely, other studies found no such effect, to the point where a meta-analysis suggested a protective effect of tea (Table 2) [34,40,47,49–59]. Several reasons can be given for these disparities. First, free radicals are necessary for the transduction of certain signals, including that of insulin, making their excessive neutralization deleterious. Second, the antioxidant capacity of dietary antioxidants may be modified by environmental conditions such as pH, the presence of metal ions, or their concentration. In fact, antioxidants can become pro-oxidants beyond certain concentrations [60]. Third, digestion metabolism leading to the production of specific potent metabolites and conjugated derivatives and the complexity of food matrix synergism may explain some of the differences found between in vivo and in vitro studies. Fourth, the efficiency of most natural products and/or diet supplements possessing antioxidant-like actions is not restricted to their antioxidative capacity, which can further add to the variability in response, depending on the model studied.
Conclusions

The involvement of ROS in the control of carbohydrate metabolism seems undeniable. Experimentally, the modulation of oxidative stress by antioxidants appears to have a positive outcome, but current intervention studies do not allow the recommendation of antioxidant supplementation for the sole purpose of preventing T2D. Most studies, however, have used supplements in the form of one or two vitamins associated or not with trace elements, although plants naturally contain a multitude of antioxidants. Among the countless compounds present in a particular plant food, it is often difficult to identify the one that plays the critical part; also, the overall total antioxidant capacity of the diet might be more important than the presence of any particular food [2]. Hence, the ideal antioxidant supplement for diabetes prevention will certainly be one that will be able to reproduce as closely as possible the innate combination of antioxidants found in plant foods even if we cannot exclude the fact that the beneficial effects of the latter might be synergistic with other compounds.

References


