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Beneficial effects of quercetin–iron complexes on serum and tissue lipids and redox status in obese rats

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Abstract

Obesity is characterized by iron deficiency, carbohydrate and fat alterations as well as oxidative stress. Iron status monitoring is recommended because of the conventional oral iron preparations that frequently exacerbate the already present oxidative stress. Iron complexation by natural antioxidants can be exploited. We herein investigated the metabolic effects of quercetin (25 mg/kg/day), iron (2.5 mg Fe/kg/day) or quercetin–iron complexes (molar ratio 5:1; 25 mg/2.5 mg/kg/day) in animal models of obesity. Our results emphasized that obese rats displayed metabolic alterations that were worsened by iron supplementation. In contrast, quercetin used alone or as iron complex clearly prevented adipose fat accumulation and alleviated the hyperglycemia, hyperlipidemia, liver steatosis and oxidative stress. In addition, it induced a modulation of lipase activities in obese rats. Interestingly, quercetin–iron complexes showed enhanced beneficial effects such as a corrected iron deficiency in obese rats when compared to quercetin alone. In conclusion, antianemic, hypoglycemic, hypolipidemic and antioxidative effects of the quercetin–iron complexes shed a light on their beneficial use against obesity-related metabolic alterations.

Keywords: Quercetin; Iron; Obesity; Adipose tissue; Liver; Metabolism; Muscle

1. Introduction

Obesity is a major global health problem. Obesity arises from an imbalance in energy intake and expenditure that leads to adiposity with cell hypertrophy and hyperplasia [1], increasing the risk of type 2 diabetes, hypertension, heart disease, dyslipidemia, osteoarthritis, gynecological or respiratory problems, infections or cancer, in addition to social stigma [2]. Obesity is characterized by a state of chronic oxidative stress related to an overproduction of reactive oxygen species (ROS; e.g., hydrogen peroxide, peroxyl, superoxide anion and hydroxyl radicals) and a subsequent decrease in antioxidants levels such as superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase activities, vitamins and minerals [3,4]. This oxidative state is associated to metabolic abnormalities, including hyperinsulinemia, carbohydrate and lipid metabolism alterations, increased adipose tissue mass and triglyceride storage, elevated blood pressure and increased systemic inflammation [5].

Adipose tissue is not only a triglyceride storage organ but also currently recognized as an endocrine organ. It secretes cytokines and adipokines that contribute to inflammation and oxidative stress development [4]. Fat tissue is also a source of many hormones that can act either locally or systemically [6]. The increased oxidative stress in adipocytes might be a cause of obesity-associated metabolic syndrome [7]. Indeed, the adipose tissue role in contributing to obesity-associated cardiovascular and metabolic risks has gained much attention during the last years.

A relationship between obesity and iron deficiency has been previously demonstrated [8]. Serum iron levels of obese subjects were significantly lower than those of normal weight subjects [9]. Previous studies have investigated the association between body mass index and iron status in children [10]. Iron deficiency is also common in obese pregnancy associated to impaired maternal-to-fetal iron transfer [11]. Iron deficiency in obese people may be a result of low iron intake, reduced iron absorption, greater iron requirements and chronic low-grade inflammation state [8,9]. In addition, obesity can lead to chronic overexpression of hepcidin, an iron homeostatic regulator, consequent to an excess of fat mass and low-grade chronic inflammation [9]. Increased hepcidin levels may lead to poor iron status by inhibiting iron absorption and restricting iron bioavailability. In addition, the liver, which is also the key regulator of iron homeostasis, is characterized by

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lipid accumulation and oxidative stress for obese patients [8,12]. Close monitoring of the iron status with iron supplements is recommended in obese subjects [13]. However, iron supplementation in obese people remains a delicate question since providing free iron, a potent prooxidant electroactive metal ion, may further exacerbate the already current oxidative stress [14]. Indeed, conventional oral iron preparations, generally iron(II) salts such as iron(II) sulfate, frequently cause gastrointestinal side effects following ROS formation [i.e., in the presence of hydrogen peroxide, Fe(II) catalyzes deleterious hydroxyl radical formation through the Fenton reactions]. Thus, strong iron complexation by natural compounds derived from dietary constituents capable to (i) prevent oxidative stress triggered by Fe(II) and (ii) act as efficient antioxidant to alleviate the current pathological oxidative stress might be exploited in the development of novel iron supplements in obesity.

Today, growing interest is given to the association of specific components of human nutrition and oxidative stress. Flavonoids that are abundant in plant foods (e.g., vegetables and fruits) have recently captured the attention of the general public due to their demonstrated beneficial health effects. They potentially have protective roles against the pathogenesis of multiple diseases associated with oxidative stress such as cancer, coronary heart disease and atherosclerosis [15,16].

Among the vast family of polyphenols, quercetin is a flavonoid (3′,4′,3,5,7-pentahydroxyflavone bearing a catechol unit on the B-ring and being one of the most studied polyphenolic compound) found in a broad variety of fruits and vegetables including apple, citrus fruits, berries, bulbs, cereal grains, onions, cacao, legumes and tea. Quercetin has been shown to display several pharmacological properties, including antioxidant, antiinflammatory and hepatoprotective effects [17,18]. Quercetin is also able to decrease plasma cholesterol and hepatic lipids, ameliorate diabetes-induced oxidative stress and preserve pancreatic beta cell integrity [19]. Indeed, in obese Zucker rats, chronic administration of quercetin markedly improved dyslipidemia, hypertension and hyperinsulinemia and reduced body weight gain [20]. Metabolic alterations during the development of insulin resistance caused by high-fat feeding are also prevented by dietary quercetin [21].

The antioxidative activities of flavonoids are not only based on their free-radical scavenging capacities but also on their chelation properties of transition metal ions. Flavonoid metallic complexes revealed to possess potent biological activities in experiments with cells, tissues and animals, thus suggesting perspectives regarding their medical use [22].

The flavonoid–metal complexes were shown to be more effective free-radical scavengers than the free flavonoids alone. In particular, quercetin–iron complexes have been reported to exhibit high lipophilicity and anticancer activities [22].

Considering that obesity is associated to metabolic abnormalities, oxidative stress and iron deficiency, supplementation with quercetin–iron complexes rather than with quercetin alone would constitute an efficient and smart treatment to correct all these alterations. Although independent studies have shown that obesity is associated with increased oxidative stress, inflammation and iron deficiency and that quercetin–iron complexes exert antiinflammatory and antioxidant activities, there are, to the best of our knowledge, no reports in the literature of the beneficial effects of quercetin–iron complexes on obesity. Consequently, the importance to establish an animal model for investigation of this issue became crucial due to ethical and methodological limitations in human studies.

Experimental obesity can be mimicked by dietary manipulations, especially the so-called “cafeteria diet” that basically consists of a variety of snack-type foods, normally consumed by humans [23].

Herein, we have therefore evaluated the metabolic effects and the antioxidant potential of quercetin–iron complexes compared to quercetin or iron alone on obesity induced by “cafeteria diet” in rats. Concomitantly, we demonstrated that the deleterious effects of iron supplementation in obesity can be corrected by using quercetin–iron complexes.

2. Materials and methods

2.1. Dose selection and preparation of quercetin iron complex

Quercetin (quercetin dihydrate, 97%) and $FeSO_4$ were purchased from Alfa Aesar. All solutions were freshly prepared with ultrapure water before experiments and used immediately. Quercetin was dissolved in DMSO/0.9% normal saline so that the final amount of DMSO was less than 1%. $FeSO_4$ was dissolved in the same solvent (DMSO/0.9% normal saline). Quercetin–iron complexes were prepared by mixing aqueous stock solutions of quercetin and $FeSO_4$ with quercetin–iron molar ratio of 5:1. Under these conditions, the 5:1 quercetin–iron complex was found to be the most thermodynamically stable species with high lipophilicity [24,25]. UV–vis measurements were carried out at room temperature using a Lambda 45 model spectrophotometer (Perkin Elmer). The UV–vis spectra of the free quercetin and the quercetin–Fe(II) complex were measured at pH 7.4. Quercetin is characterized by an intense absorption band whose maximum is centered at 373 nm. Along the spectrophotometric titration of quercetin with Fe(II), the maximum absorption at 373 nm underwent a hypochromic shift while a new absorption band emerged at 425 nm and was attributed to the formation of quercetin–iron complexes.

For the *in vivo* experiment, the selected quercetin dose was 25 mg/kg while that of Fe(II) sulfate was 2.5 mg/kg. The dosage and administration of quercetin (25 mg/kg) were assessed based on reported studies showing beneficial metabolic effects at this dose [26,27]. To keep a large quercetin–iron molar ratio (equal to 5:1 in the present study), required for complex formation, the iron dose of 2.5 mg/kg was selected. In the previous studies, 1.5–3 mg/kg doses of iron ($FeSO_4$) were commonly used [28]. This iron dose is usually used for iron deficiency in humans [29].

2.2. Animals and experimental protocol

Male Wistar rats, 8 weeks old, weighing between 200 and 230 g and obtained from Animal Resource Centre (Algeria), were used in this study. All aspects of the experiments were conducted according to the guidelines provided by the ethical committee of the experimental animal care at Tlemcen University. Animals were housed in separate cages (2–3 per cage) at a constant temperature (25°C) and humidity (60±5%) with light regime identical to natural photoperiod (12-h:12-h light/dark cycle).

The rats were randomly divided into two groups of equal average body weight. The first group (control, C, $n=32$) was exposed to standard diet (330 kJ/100 g) composed of 25% of energy as protein, 65% of energy as carbohydrate and 10% of energy as lipids (ONAB; Algeria). The second group (obese, O, $n=32$) was fed with a cafeteria diet composed of dough, cheese, bacon, potato chips, biscuits and chocolate (in a proportion of 2:2:2:1:1:1, by weight) mixed with standard chow (w/w). The cafeteria diet (420 kJ/100 g) was composed of 23% of energy as protein, 35% of energy as carbohydrates and 42% of energy as lipids. We have previously shown that this cafeteria diet induced hyperphagia and obesity in rats [30,31]. Rats were exposed to the standard or the cafeteria diet for 6 weeks before starting the intragastric administration of quercetin and its iron complex. At the end of the 6th week, cafeteria-fed rats were significantly heavier than control-fed ones. Afterwards, the rats in each group (control or obese) were divided into four subgroups. The control and the obese groups (C or O, $n=8$) were gavaged with only DMSO/0.9% normal saline (1 ml per rat, with a final concentration of 0.5% DMSO). Quercetin (25 mg/kg/day) was provided to the CQ and OQ groups ($n=8$) by intragastric administration (1 ml in DMSO/0.9% normal saline/rat). In the CFe and OFe groups ($n=8$), rats were supplemented with $FeSO_4$ (2.5 mg Fe/kg/day) by gavage (1 ml in DMSO/0.9% normal saline/rat). The CQFe and OQFe groups ($n=8$) were exposed to intragastric administration of quercetin–iron complex (molar ratio 5:1; 25 mg/2.5 mg/kg/day). Rats were supplemented by quercetin, $FeSO_4$ or complexes via gavage for 8 weeks and had free access to their own diet throughout the entire experimental period.

2.3. Analysis of the total metal contents in the standard/cafeteria diets

About 0.5 g of the standard and cafeteria diets was introduced in a mixture of 8 ml of high-purity concentrated nitric acid (HNO_3) and 1 ml of hydrochloric acid (HCl) for 60 min to ensure complete dissolution of the samples. The samples were then diluted by a factor of about 40 with deionized water. The diluted digests were analyzed for concentrations of iron, copper and zinc on a Varian Inductively Coupled Plasma Optical Emission Spectrometer 735-ES.

2.4. Determination of total polyphenols using the Folin-Ciocalteau reagent

For the standardization of the spectrophotometric method using the Folin-Ciocalteau reagent, quercetin was used as the reference chemical standard. A quercetin stock solution was prepared at 211 μM in distilled water and was subjected to stepwise dilution. To 200 μl of a diluted solution of quercetin, 1 ml of freshly prepared Folin-Ciocalteau reagent was added and the mixture was left for reaction during 4 min. The volume (1.2 ml) was completed to 2 ml with anhydrous sodium carbonate (75 g/L) and left for reaction and equilibration for 2 additional hours. Absorption spectra (400–800 nm) were measured with a Varian CARY 50 and the absorbances at 765 nm were plotted as a function of the quercetin concentrations. Aliquots of 5 g of the standard and cafeteria diets were placed in