Available online at www.sciencedirect.com

ScienceDirect

Journal of Nutritional Biochemistry 40 (2017) 164–171

Journal of Nutritional Biochemistry

Effect of dry tomato peel supplementation on glucose tolerance, insulin resistance, and hepatic markers in mice fed high-saturated-fat/high-cholesterol diets

Sofiane Zidani[a], Amar Benakmoum[a,*], Ali Ammouche[b], Yasmine Benali[c], Anissa Bouhadef[c], Souheila Abbeddou[d]

[a]Food Technology Research Laboratory, University of M'hamed Bougara Boumerdes, Algeria
[b]Food Technology and Human Nutrition Department, École Nationale Supérieure d'Agronomie, Algiers, Algeria
[c]Bio-Pathology and Genetics Department, Pasteur Institute, Algiers, Algeria
[d]Program in International and Community Nutrition, Department of Nutrition, University of California, Davis, CA, 95616, USA

Received 22 May 2016; received in revised form 1 November 2016; accepted 7 November 2016

Abstract

Many studies have investigated the effect of crude tomato peel *in vivo*, but no studies have determined the dose-effect of dry tomato peel (DTP) on glucose intolerance, insulin resistance, and atherogenic dyslipidemia induced by a high-saturated-fat (HSF) diet *in vivo*. The aim of this study was to investigate the effects of different doses of DTP on the levels of oxidative stress in mice fed an HSF and cholesterol-rich diet for 12 weeks. The main outcomes are glucose and insulin tolerance, plasma lipids, and hepatic steatosis and inflammation. BALB/c male mice (n=40) (8 weeks old, weighing 22.2±1.0 g) were divided into four treatment groups (10 mice/group): (a) high-fat control diet (HF Ctrl), which contains sunflower oil as a sole source of fat; (b) HSF/high-cholesterol (HC) diet; (c) HSF/HC diet supplemented with 9% DTP and (d) HSF/HC diet supplemented with 17% DTP. The HSF/HC diet significantly increased body weight gain, adipose tissue weight, fasting plasma glucose, fasting plasma insulin and lipid peroxidation and caused the development of liver steatosis and inflammation. Supplementation with DTP increased plasma lycopene concentration and reduced the development of indicators of metabolic syndrome, with no consistent effect of the DTP dose. Hepatic steatosis and inflammation were not reversed with DTP supplementation. Among mice fed the HSF/HC diet, DTP supplementation appears to have a beneficial effect on insulin resistance, which confirms the antiatherogenic effect of DTP.

Keywords: Dry tomato peel; Lycopene; High-saturated-fat/high-cholesterol diet; Mice; Glucose tolerance; Insulin resistance; Type 2 diabetes

1. Introduction

A high intake of fruits and vegetables is associated with a low risk of cardiovascular diseases, diabetes and cancer, as indicated by prospective cohort studies [1]. Hence, an increased dietary intake of fruits and vegetables is recommended by the World Health Organization to promote good health throughout the entire life course [2]. Fruits and vegetables constitute a rich source of phytonutrients such as phenolic acids, carotenoids and flavonoids, with potent *in vitro* antioxidant activity. These phytochemicals have gained widespread attention because of their potential protective function against oxidative damage caused by reactive oxygen species (ROS) [3].

In recent decades, nutrition-related chronic diseases among people of all ages have become more common worldwide. In 2008, the global age-standardized prevalence of diabetes was 9.8% in men and 9.2% in women, up from 8.3% and 7.5% in 1980 [4]. Type 2 diabetes, which is diet related, accounts for 90% of all diabetes cases worldwide [5]. Oxidative stress has been proposed as one of the underlying factors that may lead to diabetes, and it has long been suggested as a unifying mechanism linking excessive nutrient intake, insulin resistance, metabolic syndrome and diabetes [6,7]. The key factors causing type 2 diabetes are a combination of high fatty and sugary food intake, low vegetable consumption and low physical activity [6].

Tomatoes and their main carotenoid constituent lycopene have received much attention in recent years because of their beneficial effect in the prevention of prostate cancer [8]. Lycopene is transported by low-density lipoproteins (LDLs), where it acts as an *in vivo* antioxidant, which perhaps indicates its potential biological significance in the human antioxidant defense system [9]. However, experiments testing the *in vivo* effect of extracted lycopene on lipid peroxidation *vs.* tomato powder, which provides the same amount of lycopene, showed that tomato powder had a significantly greater preventive effect against serum peroxidation products and seemed to lower liver triglycerides compared to both control and extracted lycopene treatments [10,11]. It has been hypothesized that the plethora of nutrients and phytochemicals in tomatoes, including folate, vitamin C and various other carotenoids and phytochemicals such as polyphenols, has antioxidant properties and, in combination with lycopene, may contribute more efficiently to the protection against oxidative stress than lycopene alone. However, other human clinical trials have shown antioxidant effects from supplementation

* Corresponding author. Tel.: +213 771457793.
E-mail address: benakmoum@gmail.com (A. Benakmoum).

http://dx.doi.org/10.1016/j.jnutbio.2016.11.001
0955-2863/

with purified lycopene (providing 6–17 mg lycopene/day) on cellular DNA in healthy human volunteers [11].

Given the high intake of saturated fat in modern societies, our objective was to simulate a high oxidative environment using a high-saturated-fat (HSF) and cholesterol-rich diet. The study's main objective was to test the effect of different doses of dry tomato peel (DTP) on glucose tolerance, insulin tolerance and resistance, and hepatic steatosis and inflammation in mice fed a HSF and cholesterol-rich diet.

2. Materials and methods

2.1. Animals, diets and experimental design

Forty BALB/c male mice (8 weeks old, with initial weight between 18 and 22 g/animal) were offered by the Research and Development Center Laboratories of SAIDAL (Algiers, Algeria). The mice were housed in plastic cages with stainless steel grids (10 mice/cage) in a temperature- and humidity-controlled environment (60% humidity and a temperature of 20°C–23°C) and a 12-h light/dark cycle. All of the experiments on mice were performed in strict accordance to the Algerian Pasteur Institute guidelines for the care and use of laboratory animals. The study protocol was approved by the scientific committee of the Institut Pasteur (Algeria).

Dried tomato peel was prepared as previously described [12]. Ripe tomatoes were purchased from the local market (Boumerdes, Algeria). The skin of the tomatoes was separated from the flesh using a sharp knife and was frozen immediately (the flesh was discarded). The tomato peel was then freeze-dried, placed in oxygen barrier bags and stored at −20°C until the feeding experiment started.

The mice were fed a standard chow diet for an adaptation period of 2 weeks. They were then divided into four treatment groups (10 mice/group). During the next 12 weeks, each group was fed one of the following diets: (a) high-fat control diet, which contains 15.0 g of sunflower oil as a sole source of fat (HF Ctrl); (b) HSF/high-cholesterol (HC) diet; (c) HSF/HC supplemented with 9% DTP (HSF/HC TP1) and finally (d) HSF/HC diet supplemented with 17% DTP (HSF/HC TP2). The HSF/HC diets were prepared by replacing two thirds of the sunflower oil with coconut oil (9.7, 8.8 and 8.1 g/100 g dry matter (DM) for the HSF/HC, HSF/HC TP1 and HSF/HC TP2 diets, respectively) and dietary cholesterol (1.2, 1.1 and 1.0 g/100 g DM for the HSF/HC, HSF/HC TP1 and HSF/HC TP2 diets, respectively). Dietary cholesterol was purchased from Alfa Aesar GMBH, Germany. Because casein is low in L-cysteine and/or L-cystine, diets were supplemented with 3 g methionine/kg DM diet [13]. Diets containing DTP were prepared by adding DTP to HSF/HC diets in two different proportions (9 and 17 g DTP/100 g DM of food providing, respectively, 46 and 84 mg of lycopene/kg of food) [14,15]. The diets were prepared in bulk, manually, by mixing the raw ingredients in the food technology laboratory (Boumerdes, Algeria). They were immediately stored at −20°C until use. The composition of each diet is shown in Table 1. Diets were offered to each group on a daily basis at 8 am. The mice had *ad libitum* access to feed and water during the following 12 weeks. Body weight and food intake were monitored weekly during this period. Body weight gain was calculated as the difference between the final weight (at 12 weeks) and the initial weight in grams. Daily food intake was calculated as the difference between the unconsumed food and the quantity served during the same week (in grams) divided by the number of days and the number of living animals per cage.

2.2. Oral glucose tolerance tests (OGTTs)

OGTT was performed 4, 8 and 12 weeks after access to the experimental diet was initiated. The mice were fasted overnight; then a glucose solution (0.2 g D-glucose/ml water) was administered by oral gavage (2 mg/g body weight) in the middle of the dark phase. Blood glucose levels were monitored using a glucometer (Accu-Chek, Roche Diagnostics, Germany) before the administration of glucose (0 min) and 15, 30, 60, 90 and 120 min after glucose administration.

2.3. Insulin tolerance test (ITT)

ITT was performed 4, 8 and 12 weeks after access to the experimental diet was initiated. The mice were fasted for 4 h in the middle of the dark phase; then the insulin solution (0.05 IU/ml saline) was administered by intraperitoneal injection (0.5 μU/g body weight). Blood glucose levels were monitored using a glucometer (Accu-Chek, Roche Diagnostics, Germany) before the administration of insulin (0 min) and 15, 30, 60, 90 and 120 min after insulin injection.

2.4. Collection of blood samples and organs

At the end of the experimental period (12 weeks after access to the experimental diet was initiated), the mice were fasted for 12 h and sacrificed by cervical dislocation. Blood was drawn into a heparin-coated tube; then plasma was obtained by centrifugation (1200g for 15 min at 4°C) and stored at −20°C until biochemical analyses were performed. The liver and all fat pads (parametrial, abdominal subcutaneous and renal) were removed, rinsed with physiological saline and weighed immediately. Two small pieces of the right lobe were fixed in 10% neutral formalin buffer (formaldehyde 4% weight/volume [w/v], 0.1 M phosphate buffer, pH 7.2) for histological analysis.

Table 1
Composition and nutritional value of the experimental diets (% of dry matter)

Contents (g/100 g)	Diet			
	HF Ctrl	HSF/HC	HSF/HCTP1	HSF/HCTP2
Defatted milk powder	46.5	46.6	42.3	38.8
Corn starch	28.2	27.0	24.5	22.5
Sunflower oil	15.0	5.2	4.7	4.3
Coconut oil	0	9.7	8.8	8.1
Cholesterol	0	1.2	1.1	1.0
Minerals[a]	4.0	4.0	3.6	3.3
Vitamins[b]	1.0	1.0	0.9	0.8
Fiber (agar)	5.0	5.0	4.6	4.2
DL-Methionine	0.3	0.3	0.3	0.3
DTP	0	0	9.1	16.7
Energy content (kcal/100 g)	419.2	427.0	388.1	355.7

[a] Contained per kg: Ca, 13.5 g; P, 9.5 g; Mg, 3.5 mg; K, 9.8 g; Na, 3.35 g; Fe, 154 mg; Al, 27.0 mg; Cu, 10.3 mg; Mn, 80 mg; Co, 2.0 mg; I, 1.0 mg; Zn, 75.7 mg.
[b] Contained per kg: vitamin A, 57,200 IU; vitamin D3, 8135 IU; vitamin E, 236 mg; vitamin K3, 2.6 mg; inositol, 8.3 g; vitamin B1, 74.8 mg; vitamin B2, 45.0 mg; vitamin C, 150 mg; vitamin B6, 18.1 mg; vitamin B12, 150 mg; biotin, 242 mg; niacin, 207.4 mg; choline, 0.55 g; folic acid, 4.5 mg.

2.5. Plasma, hepatic and biochemical analysis

Plasma total cholesterol, triglyceride (Tg), high-density lipoprotein cholesterol (HDL-C) and LDL cholesterol (LDL-C) concentrations were assayed by the colorimetric enzymatic method (Col. automate BioLis 24i, Boeki Machinery, Tokyo, Japan). Blood glucose concentration and insulin were measured using a commercial kit (BioLis, Boeki Machinery, Tokyo, Japan). Enzymatic assays for circulating free fatty acids (FFAs), alanine aminotransferase (ALT), bilirubin, aspartate aminotransferase (AST) and alkaline phosphatase (ALK) were also performed using a commercial kit (BioLis, Boeki Machinery, Tokyo, Japan). The homeostatic model assessment (HOMA-IR) was used to evaluate insulin resistance (fasting serum insulin (μU/ml) × fasting plasma glucose (mmol/L)/22.5) [16]. Lycopene concentration in plasma was measured by using high-performance liquid chromatography (HPLC; Alliance 2695 Waters Corporation, Milford, MI, USA) using the method described by Froescheis et al. [17].

2.6. Tissue preparation and histological examination

A piece of the liver was rapidly fixed in freshly prepared fixative solution (formaldehyde 4% w/v, 0.1 M phosphate buffer, pH 7.2). Fixed tissues were embedded in paraffin, and then 4-μm-thick sections were stained with hematoxylin–eosin and Masson's trichrome. The stained areas were viewed using an optical microscope (Axioscope, Germany). The steatosis and inflammation were semiquantitatively evaluated, and scores were blindly established from 0 to 4, where 0 refers to no hepatocyte injury or inflammation, 1 to 2 to mild focal injury, 3 to noticeable injury and 4 to severe hepatocyte injury.

2.7. Statistical analysis

Data were analyzed by analysis of variance using the general linear model (GLM) for all the outcomes at the end point and repeated measurement using the mixed procedure for weekly weight gain, OGTT and ITT. The fixed effect considered was the treatment group for the GLM and the treatment group, time and the interaction between these two factors for the mixed model. Table 2 displays means and standard errors of the mean (S.E.M.). Statistical analysis was performed using SAS (version 9.3; SAS Institute Inc., Cary, NC, USA). Group means were compared *post hoc* using the Tukey–Kramer test. Values were considered statistically significant at $P<.05$.

The reported results are presented on seven mice per group except for the baseline data. Three mice in each group died during the experiment and were not replaced.

3. Results

3.1. Effect of DTP supplementation on food intake, body weight gain, and liver and fat pad weights

Feeding the mice a HSF and cholesterol-rich diet led to significantly reduced food intake, regardless of DTP supplementation and dose (Table 2). Twelve weeks after starting the HSF and cholesterol-rich diet, there was a marked increase in mean body weight of HSF/HC mice