supplements. However, other studies have shown beneficial effects of β-carotene in reducing cancer risk.[6] β-Carotene has been shown to have a number of other functions that are completely independent of its antioxidant role. It has been reported to influence the immune function[7, 8] and to enhance gap junction intercellular communication,[9] and indeed, other cancer preventive mechanisms for this molecule cannot be excluded.

Free radicals are chemical species with 1 or more unpaired electrons, such as the superoxide anion (O_2^-), hydroxyl radical (HO^-) and peroxyl radical (ROO^-). Highly reactive molecules which cause damage through free-radical generation include singlet O_2 and H_2O_2. Under normal physiological conditions, organisms can prevent free-radical damage by using protective mechanisms which involve superoxide dismutase (SOD; *EC* 1.11.19), catalase (CAT; *EC* 1.11.1.6), and glutathione peroxidase (GSH-Px; *EC* 1.11.1.9).

The liver has the greatest β-carotene storage capacity of any organ in the rat, although physiologic storage levels of β-carotene differ in their organs.[10]

Several lines of evidence suggest that important relationships exist between carotenoids and cancer.[11,12, 13] It has been suggested that carotenoids may act as anticarcinogenic agents by an antioxidant mechanism independent of their conversion into vitamin A.[14] However, few experimental studies have been conducted on the antioxidant properties of carotenoids in in vitro whole-cell systems. Most studies have focused on the antioxidant properties of β-carotene, and research has largely been conducted on liposomes,[15] lipoproteins,[16] and isolated membranes.[17]

Moreover, the products of β-carotene directly responsible for the prooxidant activity have not yet been completely identified. Improved knowledge of the prooxidant role of in vitro and in vivo carotenoids will assist in tests regarding their potential to influence biological processes in humans.[18] Moreover, the role of β-carotene in antioxidative efficacy is still controversial because of the antioxidative efficacy of β-carotene in vivo. Very little work has been conducted to investigate the effects of β-carotene supplementation in vivo on cell viability and the antioxidative system of primary rat hepatocytes.

In this study we examined the ability of β-carotene to protect against $FeCl_3$-induced oxidative stress in primary rat hepatocytes.

MATERIALS AND METHODS

Materials

Bovine serum albumin (BSA), collagenase, calcium chloride, dexamethasone, dimethyl sulfoxide (DMSO), glutathione reductase, N-2-hydroxy-ethyl-piperazine-N'2-ethanesufonic acid (HEPES), β-nicotinamide adenine dinucleotide, (β-NADH), β-nicotinamide adenine dinucleotide phosphate, (β-NADPH), Percoll, glutathione (GSH), sodium azide (NaN_3), sodium chloride, sodium phosphate, sodium selenite, α-tocopherol, trans-β-carotene, trypsin inhibitor, trypan blue, trichloroacetic acid, ferric chloride ($FeCl_3$), and lactate dehydrogenase (LDH) were purchased from Sigma Chemical (St Louis, MO, USA). D-glucose, insulin, L-15 medium, phenol red, sodium bicarbonate, sodium pyruvate, transferrin, fetal bovine serum (FBS), and penicillin/streptomycin were from Gibco Laboratory (Grand Island, NY, USA). All other chemicals used were of reagent grade.

Animals

Male Wistar rats (National Taiwan University Hospital Animal Center), weighing about 160 g, were housed individually in wire-bottomed cages, in a temperature-controlled room (22 °C) with a 12-h light: dark cycle and with free access to food and water. All animal experimental procedures followed the *Guide for the Care and Use of Laboratory Animals* from the National Science Council, Taiwan.[19] Rats were randomly divided into 2 groups of 6 rats each and fed for 6 wks. The 2 groups were fed AIN-76 diets with or without 0.1 g/kg β-carotene (Table 1). The contents of the diets were homogenized and then put into a plastic bag, tightly sealed, and refrigerated at 4 °C. Fresh diet and β-carotene were given once every 3 days, and the amount of intake was recorded daily. The body weight was recorded once a week. Food was withheld for 12 h be-