Vitamin C Supplementation Decreases Insulin Glycation and Improves Glucose Homeostasis in Obese Hyperglycemic (*ob/ob*) Mice

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The effects of dietary vitamin C supplementation on glucose homeostasis and insulin glycation were examined in adult lean and obese hyperglycemic (*ob/ob*) mice. In lean mice, supplementation of the drinking water with vitamin C (25 g/L) for 14 days did not affect food intake, fluid intake, glycated hemoglobin, plasma glucose, or plasma insulin concentrations. Total pancreatic insulin content and the percentage of glycated pancreatic insulin were also similar to control lean mice. In *ob/ob* mice, vitamin C supplementation caused significant reductions by 26% to 48% in food intake and fluid intake, glycated hemoglobin, plasma glucose, and insulin concentrations compared with untreated control *ob/ob* mice. The total insulin content and the extent of insulin glycation in the pancreas of *ob/ob* mice were also significantly decreased by 42% to 45% after vitamin C supplementation. This change was accompanied by a significant 80% decrease in the percentage of glycated insulin in the circulation of vitamin C– supplemented *ob/ob* mice. These data demonstrate that vitamin C supplementation can decrease insulin glycation and ameliorate aspects of the obesity-diabetes syndrome in *ob/ob* mice.

VITAMIN C, the antiscorbutic vitamin, is found in a large variety of foods but particularly in fruits and vegetables. The 2 major forms, L-ascorbic acid and dehydro-L-ascorbic acid, are interconvertible through an oxidation-reduction system which represents the fundamental basis for many of the biomedical roles of the vitamin.[1]

Vitamin C is actively taken up in high concentration by secretory cells of the islets of Langerhans where it is believed to play a role in antioxidant defense[2] and as an electron donor in the post-translational enzymatic peptidyl alpha-amidation of islet peptides including amylin (IAPP) and pancreatic polypeptide (PP).[3,4] In diabetes mellitus, vitamin C metabolism is abnormal, and subjects have been shown to have low vitamin C and high dehydro-L-ascorbic acid concentrations in plasma.[4-10]

Vitamin C is also a potent inhibitor of protein glycation, which has the particular advantage of low inherent toxicity in humans even in megadoses.[11-13] Nonenzymic glycosylation or glycation of proteins has pronounced effects on the structure and function of proteins that contribute to the pathogenesis and longer-term complications of diabetes.[14-18] Substantial evidence indicates that circulating proteins including insulin can also be glycated[19,20] and that glycation results in decreased biological potency in vivo.[21-24] Glycation of insulin within the pancreatic beta cells[19,25] in type 2 diabetes therefore results in the secretion of an insulin molecule with decreased biological activity. This contributes to the cycle of events linking peripheral insulin resistance, hyperinsulinemia, and glucose intolerance.[9] By using inhibitors of glycation in vivo, it should be possible to investigate the contribution of glycated insulin to the glucose intolerance and hyperglycemia of type 2 diabetes. The present study investigated the effects of dietary vitamin C supplementation in spontaneously obese diabetic (*ob/ob*) mice, an animal model that displays hyperglycemia, hyperinsulinemia, glucose intolerance, and significant peripheral insulin resistance.[2]

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MATERIALS AND METHODS

Spontaneously obese hyperglycemic (*ob/ob*) mice and lean mice from the colony maintained at the University of Ulster were used at 20 to 23 weeks of age.[19] Mice received ad libitum normal drinking water or drinking water supplemented daily with 25 g/L vitamin C (Sigma Chemical Co, Poole, UK). All animals were housed in individual cages in an air-conditioned room (22 ± 2°C) with a 12-hour light-dark cycle. A standard pellet diet (RMBI diet, Pilsbury, UK) was provided ad libitum. This diet comprised 21.5% protein, 48% carbohydrates, and 3.5% fat (digestible energy, 14.2 MJ/kg) with added fiber, vitamins, and minerals.

During the study, food and fluid intake were monitored daily. Blood samples collected from the cut tip of the tail (days 0, 7, and 14) were used for the determination of glycated hemoglobin levels,[26] plasma glucose,[27] and insulin concentrations.[28] On day 14, all animals were killed by decapitation. The pancreas of each animal was excised, minced in acid ethanol (750 mL ethanol, 235 mL water, 15 mL concentrated hydrochloric acid), sonicated (3 × 30-second cycle) and insulin content determined.[28] Measurement of glycated and nonglycated insulin was performed using pancreatic extracts and, in *ob/ob* mice, terminal plasma samples. Glycated insulin in plasma of lean mice was below the detection limit of the assay employed. Glycated and nonglycated insulin were separated by boronate affinity chromatography as described in detail elsewhere.[20,26] The affinity chromatography columns (glycogel B) comprised of m-amino-phenyl boronic acid (Pierce and Warriner, Chester, UK). The nonglycated fraction was collected using 8 mL of wash buffer (250 mmol/L ammonium acetate, 50 mmol/L magnesium chloride, and 3 mmol/L sodium azide of pH 8.5). Glycated material was removed by addition of 3 mL elution buffer (200 mmol/L sorbitol, 50 mmol/L EDTA, 3 mmol/L sodium azide, and 100 mmol/L Tris buffer, pH 8.5). Insulin in both fractions was measured by radioimmunoassay[29] using an antibody that cross-reacts fully with glycated insulin.[26] It was checked that concentrations of vitamin C up to 1 mmol/L did not interfere with the method of glucose analysis.

Statistical Method

Groups of data are presented as means ± SEM. Statistical evaluation was performed using unpaired Student's *t* test. Differences were considered to be significant at $P < .05$.

Table 1. Effects of Dietary Vitamin C Supplementation on Body Weight, Food, and Fluid Intake of Lean and Obese (ob/ob) Mice

	Lean Mice		Obese Mice	
Parameter	Control	Vitamin C	Control	Vitamin C
Body weight (g)	45 ± 2	45 ± 2	93 ± 2	90 ± 4
Food intake (g/mouse/d)	6.8 ± 0.4	6.5 ± 0.8	10.8 ± 0.6	8.0 ± 0.4*
Fluid intake (mL/mouse/d)	13.3 ± 0.4	11.2 ± 0.2	18.4 ± 1.4	11.2 ± 0.6*

NOTE. Values are mean ± SEM for 5 animals averaged over the final 5 days of the 14-day study.

*$P < .01$ compared to respective control animals.

RESULTS

Treatment of lean mice with vitamin C in the drinking water had no effects on body weight, and food or fluid intake (Table 1.) Similarly, no significant differences were found in body weights of the vitamin C–treated *ob/ob* mice and untreated *ob/ob* controls (Table 1). However, vitamin C treatment of the *ob/ob* mice was associated with a significant reduction in the food ($P < .001$) and water ($P < .01$) intake averaged over the final 5 days of the study period (Table 1).

Investigation of plasma and pancreatic parameters revealed that vitamin C treatment of lean animals had no effect on plasma glucose, glycated hemoglobin, plasma insulin, pancreatic insulin content, or the extent of pancreatic insulin glycation (Table 2). Thus vitamin C did not inhibit glycation under conditions of normoglycemia. In contrast, vitamin C supplementation in *ob/ob* mice resulted in significantly decreased plasma glucose, glycated hemoglobin, and circulating insulin concentrations by day 7 (Fig 1). At 14 days, these changes were accompanied by substantial decreases in the percentage of glycated insulin in the pancreas and plasma of *ob/ob* mice (Fig 2). Vitamin C treatment also significantly decreased the pancreatic insulin content of the *ob/ob* animals (Fig 2).

DISCUSSION

Consistent with previous reports,[9,19] the severe hyperglycemia and hyperinsulinemia of adult *ob/ob* mice were accompanied by elevation of glycated hemoglobin and significant glycation of pancreatic insulin stores. Importantly, this study demonstrates increased concentrations of glycated insulin in the circulation of *ob/ob* mice. This corresponds with the increased content and secretion of glycated insulin from clonal beta cells maintained under hyperglycemic culture conditions in vitro.[25] Glycation of insulin is thought to follow from concentration-dependent GLUT-2–mediated glucose transport into the pancreatic beta cell, followed by metabolism to highly

Table 2. Effects of Dietary Vitamin C Supplementation for 14 Days on Plasma and Pancreatic Parameters in Lean Mice

Parameter	Control	Vitamin C
Plasma glucose (mmol/L)	7.5 ± 0.3	7.9 ± 0.5
Glycated hemoglobin (%)	4.2 ± 0.1	4.0 ± 0.1
Plasma insulin (ng/mL)	0.8 ± 0.1	0.7 ± 0.1
Total pancreatic insulin (μg/g wet weight)	160 ± 16	167 ± 17
Glycated pancreatic insulin (%)	5.9 ± 0.4	5.6 ± 0.5

NOTE. Values are mean ± SEM for 5 animals.

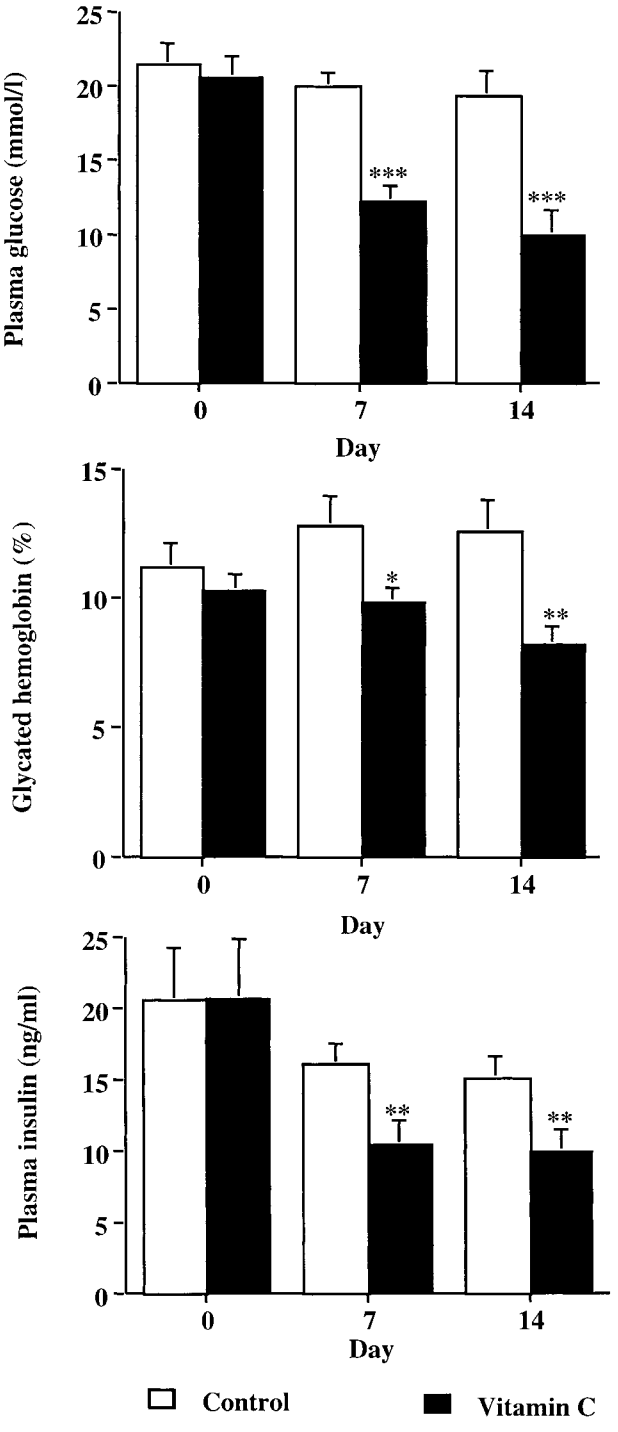

Fig 1. Time-dependent effects of dietary vitamin C supplementation on plasma glucose, glycated hemoglobin, and plasma insulin concentrations in obese mice. Values are the mean ± SEM of groups of 6 mice. *$P < .05$, **$P < .01$, and ***$P < .001$ compared to control animals at the same time.

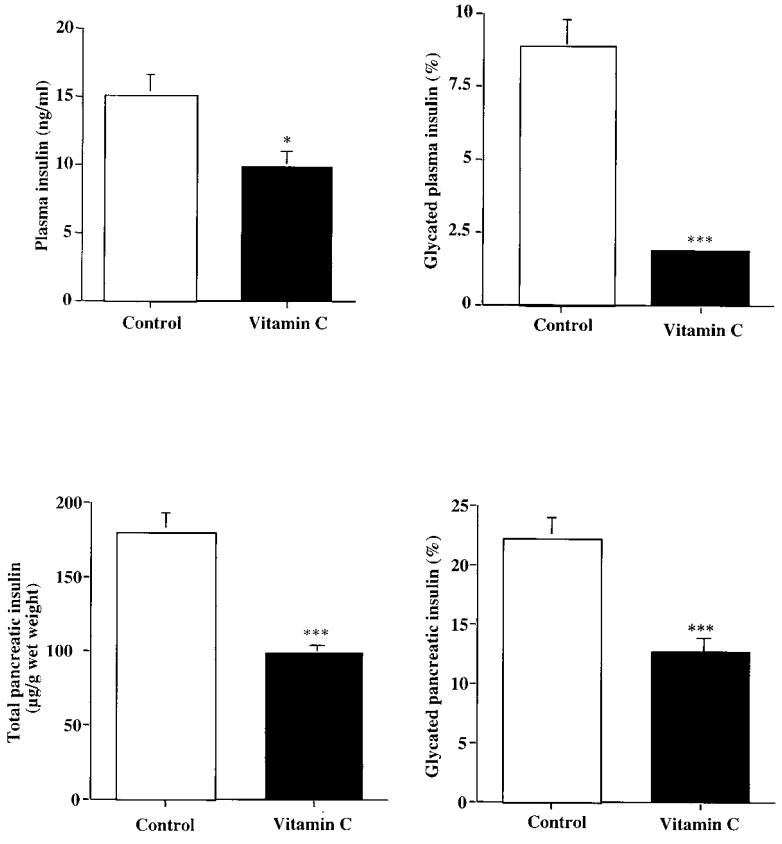

Fig 2. Effects of dietary vitamin C supplementation for 14 days on the extent of insulin glycation in the pancreas and plasma of obese mice. Values are the mean ± SEM of groups of 6 mice. *$P < .05$ and ***$P < .001$ compared to respective control animals.

reactive glucose-6-phosphate, which glycates proinsulin and insulin within the endoplasmic reticulum and secretory granules in the beta cell.[21] Secretion of glycated insulin contributes to glucose intolerane due to impaired ability to stimulate glucose transport and metabolism in peripheral tissues, including muscle and fat.[9,22,25]

This study has shown that dietary vitamin C supplementation ameliorates aspects of the obesity-diabetes syndrome in *ob/ob* mice. The beneficial effects on plasma glucose and glycated hemoglobin appear to be, at least partly, mediated by decreases in the levels of glycated insulin in the pancreas and most importantly in the circulation. This can be explained by the ability of beta cell to rapidly transport vitamin C and dehydroascorbic acid, which readily accumlate in the cytoplasm, mitochondria, endoplasmic reticulum, and secretory granules.[3,4] Indeed, we have previously demonstrated the ability of vitamin C to inhibit hyperglycemia-induced glycation of insulin in clonal beta cells maintained in tissue culture.[26]

It follows from the proposed sequence that the vitamin C–induced decrease in the proportion of glycated insulin in the circulation would result in greater biological potency of circulating insulin, thereby alleviating insulin resistance, increasing insulin-stimulated glucose uptake by peripheral tissues, and reducing plasma glucose. The resulting fall in plasma glucose concentration coupled with reduction of hyperphagia would in turn reduce the sustained stimulation of pancreatic beta cells to synthesise and secrete insulin. Consequently, insulin production would fall and lower levels of pancreatic and plasma insulin would be expected as was actually the case. In contrast, vitamin C–treated lean mice displayed no alterations of insulin glycation or any of the other parameters monitored. Although it is theoretically possible that vitamin C has a direct effect on glucose utilisation and pancreatic insulin content, this is ruled out by our findings in the vitamin C–treated lean mice. The decrease in the extent of hyperphagia during the final 5 days of the test is also likely to be important. This may be related to the improvement of insulin sensitivity,[29] although interference in the glycation and effectiveness of other regulatory peptides acting as satiety signals is also a theoretical possibility.

Several studies have demonstrated that diabetes is associated with abnormalities in plasma vitamin C levels and ascorbate status.[4-8] The loss of vitamin C in diabetes has been postulated to be due to the increased oxidative stress associated with the disease.[4] Benefits of vitamin C supplementation in humans with type 2 diabetes have been described.[29-31] Additionally, it has been proposed that dietary measures to increase plasma vitamin C may be an important strategy to counter diabetes.[32] Clearly, vitamin C has several important biological functions that may be of benefit in these situations. It is conceivable that one of these roles is the maintenance of the functional integrity of biologically active proteins, such as insulin by inhibition of glycation.

In conclusion, the current study has demonstrated that dietary vitamin C supplementation is associated with partial amelioration of type 2 diabetes syndromes in the obese hyper-

glycemic (*ob/ob*) mouse. It appears that inhibition of insulin glycation in pancreatic beta cells by vitamin C treatment is largely responsible for the improvement of insulin sensitivity and glycemic control. It is evident that further work is required to investigate such possibilities and whether naturally occurring inhibitors of glycation, such as vitamin C, may provide effective agents for the treatment of type 2 diabetes and associated complications.

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