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Research Article

Effect of exercise in combination with dietary nopal and zucchini on chronic and acute glucohomeostasis in genetically obese mice

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ABSTRACT

Objective: Type 2 Diabetes Mellitus (DM) is a major health problem for which folk medicinals such as nopal, or cactus stems from various species of Opuntia (Cactaceae), are often used as an inexpensive palliative dietary supplement. We sought to determine whether exercise and nopal, independently or interactively, could suppress the normal increase in blood glucose in genetically obese mice.

Methods: We tested the effects of nopal (experimental) and zucchini (control) supplemented diets, with and without exercise, on chronic and acute blood glucose levels in genetically obese (C57BL/6J*Lep*$^{ob/ob}$) mice, which mimic type 2 DM.

Results: A normal developmental increase in blood glucose was mitigated by nopal and exercise. A control vegetable, zucchini, was less effective in controlling blood glucose. Neither exercise nor vegetable supplementation suppressed the hyperglycemic response to an acute glucose challenge.

Conclusion: These results provide evidence that a nopal-supplemented diet combined with aerobic exercise mitigates chronic but not acute hyperglycemia in a genetically obese mouse model of type 2 DM.

Keywords: Hyperglycemia; Exercise; Diabetes Mellitus Type 2; Nopal

INTRODUCTION

Type 2 (adult onset, or non-insulin dependent) Diabetes Mellitus [DM], is a disorder of carbohydrate metabolism involving hyperglycemia that can lead to disabling or life-threatening conditions such as kidney dysfunction [1], peripheral neuropathy [2], retinopathy [3], and coronary artery disease [4]. Type 2 DM, is a particularly common correlate of obesity, and disproportionately afflicts those of Native American, Hispanic, African, and Asian ancestry [5]. It tends to be linked especially to poorer diets and the inability to obtain affordable medication. The traditional treatment for hyperglycemia is an appropriate combination of diet, exercise, and medication [6,7]. Folk medicinals are often used to supplement or replace these treatments in some patient populations [8,9].

Stems of the prickly pear cactus commonly known as nopal, including various species of Opuntia (Cactaceae), are thought to have a variety of beneficial physiological and health maintenance effects [10]. In Mexico and the Southwestern United States, nopal is frequently taken as a dietary supplement in the belief that it helps control hyperglycemia [9,11-13], both chronically and acutely. While some studies indicate that nopal may have hypoglycemic properties [10,14-17], these effects have not been observed in others [18,19]. Animal studies have likewise been inconclusive, with nopal appearing to be more effective in diabetic than in normoglycemic animals [20-26]. Exercise has long been part of the treatment regimen for obese and diabetic patients, in view of its beneficial effects on improved cardiovascular function, improved insulin binding and sensitivity [27], lower insulin requirements [28], improved blood glucose control [29], improved maximum oxygen uptake [30], and a decrease in stress that promotes a sense of general well-being [31]. Since exercise is an adjunct with dietary modifications, the combination of specific nopal dietary supplementation coupled with an aerobic exercise regimen warrants investigation. The combination of folk remedies such as nopal with exercise could play a major role in the management of obesity and type 2 DM, if their efficacy could be demonstrated.

In preliminary experiments we found dietary restriction to be effective in controlling the dramatic hyperglycemia of genetically obese C57Bl/6J*Lep*$^{ob/ob}$ mice (hereafter, ob/ob mice), suggesting that this animal could provide a reliable model for investigating the metabolic basis of obesity and type 2 DM. In this report, we provide the first evidence that nopal ingestion and exercise, independently and in combination, mitigate the rise in chronic blood glucose levels characteristic of the ob/ob mouse. We also present evidence, however, that the same treatment does not attenuate the rise in blood glucose in response to an acute glucose challenge.

Materials and Methods

Experimental Design and Animals

This study was designed to test the hypothesis that exercise and vegetable dietary supplements suppress both chronic and acute hyperglycemia in genetically obese mice. Forty-eight male C57Bl/6J*Lep*$^{ob/ob}$ mice were obtained from the Jackson Labs (Bar Harbor, ME) at 5 weeks of age. They were housed individually in polycarbonate cages with about one inch of soft bedding in a temperature-controlled animal room (~24^{O}C) under a constant (12L:12D) photoperiod.

Half the mice were exercised (E) by forced swimming trials for 20 min three times per week, while the other half were sedentary (S) in their home cages throughout the experiment. The diets consisted of lab chow alone (C) or lab chow in combination with either fresh zucchini (Z) or nopal (P) slices. A 2 X 3 factorial design therefore generated six groups (CE, ZE, PE, CS, ZS, PS) consisting of 8 mice each. Body weight and blood glucose levels were measured as dependent variables.

Animal procurement, housing, and experimental treatment were approved by the University of Texas at El Paso Institutional Animal Care and Use Committee, in accordance with the NIH Guide to the Use and Care of Animals.

Diet and Feeding Schedule

Animals were fed Purina^{R} Lab Chow and watered ad lib. Chow remaining was weighed at weekly intervals to the nearest 0.1 g for calculation of daily consumption. Fresh nopal, Opuntia sp., and zucchini, *Cucurbita pepo* (Cucurbitaceae), were obtained from a local market. Vegetable slices weighing ~ 2-3 g each were soaked in a fresh 0.1% saccharine solution for ~ 10 min to provide a non-caloric inducement for the mice to eat them and placed in the cages on top of the bedding daily. The following day, the remaining vegetables were weighed to the nearest 0.1 g, and corrected for dehydration based on loss of wet weight by control nopal and zucchini slices.

Exercise

Mice were exercised by being placed in a tank of warm

(32-35° C) water for a period of sustained swimming, three mornings (0900 – 1100) per week. Because of their obesity, mice were able simply to float for much of the time. This task was designed to moderately increase the activity level of these severely hypoactive animals without exhausting them or stressing them more than necessary, in accordance with principles of humane animal use, and to reasonably equate with levels of exercise in humans. Blood samples were not collected on exercise days. Sedentary mice were left in their home cages throughout the experiment.

Chronic Blood Glucose

Blood glucose levels were determined at the beginning (week 0) and end (week 3) of the experiment. Mice were fasted for 4h from 1000 to 1400. Blood samples were then collected from a cut at the distal end of the tail. One or two drops of blood were immediately assessed with ONE TOUCH^{R}glucose test strips in a reflectometer (Lifescan, Inc., Milpitas, CA) and read from the digital meter in mg/dl.

Acute Glucose Tolerance

Mice were tested for acute glucose tolerance at the beginning and end of the experiment. Between 1400 and 1430, mice received an intraperitoneal injection of a 32 mg/ml solution of glucose in a volume (ml) equal to 1.4% of their body weight in g. Basal blood samples were taken prior to glucose loading (0 min), then subsequent samples were collected at 15, 30, 60, and 90 minutes post-injection, from a cut at the distal end of the tail. Subsequent blood samples were obtained by sloughing off existing scab, or making a fresh cut if necessary. Blood glucose was then assessed as described above.

Statistical Analysis

Food consumption and the effects of activity level and diet on chronic blood glucose were tested by two-way ANOVA, and on glucose tolerance at specific time points by one-way ANOVA, with Tukey's correction for repeated measures to make individual pairwise comparisons.

Results

Overall Food Consumption

Exercised mice consumed 12% less total food than sedentary mice (Table 1). These differences were significant for activity level ($F = 23.9$, df = 1/42, $p = 0.0003$) but not quite significant ($F = 9.08$, df = 2/42, $p = 0.06$) among the different diets. Zucchini added 10% and nopal added 7% to the total mass of food consumed. Since the vegetables are very low in caloric content [20,32], the diets were essentially isocaloric within each activity group. Body weights of all mice at the end of the experiment varied insignificantly across treatment groups over a narrow range (45-46 g).

Effect of Exercise and Diet on Chronic Blood Glucose

Juvenile ob/ob mice show a normal developmental increase in chronic blood glucose levels with age. Figure 1 shows the change in chronic blood glucose levels over the three week duration of the experiment, by treatment group relative to the baseline levels of blood glucose for all mice at the start of the experiment. The normal increase in blood glucose was mitigated by both exercise ($F = 11.01$, df = 2/41, $p = 0.002$) and vegetable supplementation ($F = 5.04$, df = 2/41, $p = 0.011$). Lack of a significant interaction ($F = 0.72$, df = 2/41, $p = 0.49$) indicates that diet and exercise acted independently and additively. Of the two vegetables, nopal suppressed hyperglycemia more effectively than zucchini. The normal rise in blood glucose was reduced 42% ($p<0.02$) by nopal in sedentary mice and by 89% ($p<0.006$) in exercised mice, while reductions with zucchini were only 13% ($p >0.56$) in sedentary mice and 38% ($p >0.13$) in exercised mice.

Effect of Exercise and Diet on Response to An Acute Glucose Load

Neither exercise alone nor exercise combined with either zucchini or nopal were effective in reducing the rise in blood glucose following the glucose challenge. All groups showed a similar time course in absolute blood glucose levels over the 90 min period of the glucose challenge, though sedentary mice fed chow only (Group CS) were consistently higher than the others (Figure. 2A). The starting blood glucose baseline was also highest for this group, however. To normalize for the effect of different baseline glucose levels, the data were plotted after subtracting the zero time value (Fig. 2B). That plot reveals that no combination of diet and exercise was effective in mitigating the rise in blood glucose below that seen in the response of Group CS to a glucose challenge. Our data, therefore, provide no evidence for suppression of acute glucose tolerance by exercise or vegetable supplementation in these mice.

Discussion

Chronic Glucohomeostasis

Our hypothesis that chronic hyperglycemia in genetically obese mice would be mitigated by exercise and at least one of the vegetable supplements, nopal, was substantiated.

The effectiveness of exercise in mitigating the progressive hyperglycemia seen in ob/ob mice is consistent with clinical observations on the benefits of exercise in the management of obesity and type 2 DM [6]. We have shown that caloric restriction lowers chronic blood glucose levels in older ob/ob mice, but has little effect on normal juvenile mice at the same age as the animals in this study [unpublished data]. Since the exercised mice in this study consumed a small but significantly lower amount of food, the exercise effect may be due to lower caloric intake. Whether exercise acts through a direct metabolic effect or indirectly through appetite suppression or other mechanisms requires further study, but our results demonstrate that the ob/ob mouse should be a useful animal model for analyzing the mechanisms by which exercise helps control hyperglycemia.

The effectiveness of nopal in controlling blood glucose levels in genetically obese mice is likewise consistent with evidence for the efficacy of this treatment for chronic hyperglycemia in humans [17]. The suppression of blood glucose levels by nopal contrasted with the ineffectiveness of zucchini. This observation, plus the fact that all diets were essentially isocaloric, supports the possibility that nopal contains a pharmacologically active substance that acts at a cellular or systemic level to influence glucohomeostasis. The ob/ob mouse provides an animal model in which these mechanisms can be investigated as well.

Acute Glucohomeostasis

Our hypothesis that acute hyperglycemia following a glucose challenge in genetically obese mice would also be mitigated by exercise and vegetable supplements was not supported. Our failure to detect a suppression of glucose tolerance with a dietary supplement of nopal contrasts with reports of some animal studies [22-24], but not others [20,21]. Whether these differences are attributable to species-specific responses or the particular physiological characteristics of the ob/ob mouse must await further studies. The lack of clinical evidence for an acute hypoglycemic effect by nopal has been noted also in both the fasting state [19] and in response to a glucose challenge in fasting type 2 DM patients [15]. Until the relevant variables are better identified in both humans and animal models, it would be premature to generalize about the effect of nopal on acute glucose loads.

Clinical Relevance

We have shown that dietary supplements of nopal mitigate the progressive rise in blood glucose seen in one strain of genetically obese mice, particularly when combined with regular exercise. While the pathophysiology may well be different between ob/ob mice and human patients with Type 2 DM, these results substantiate the importance of including exercise as a component of the treatment regimen for obesity and type 2 DM, and provide scientific support for the efficacy of nopal in controlling chronic hyperglycemia, However, the hypoglycemic influence of exercise combined with nopal ingestion was restricted to chronic rather than acute effects in the ob/ob mice. Clinical studies with human patients are needed to determine whether the lack of nopal to mitigate an acute glucose challenge, as seen in *ob/ob* mice, is clinically relevant to humans.

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Table 1: Food consumption in sedentary and exercised obese mice with and without vegetable supplementation

Group	Activity	Food Consumed (g/day)		
		Chow	Zucchini	Nopal
CS	sedentary	6.31± 0.21	-	-
ZS	sedentary	7.11 ± 0.27	0.70 ± 0.08	-
PS	sedentary	6.73 ± 0.33	-	0.41 ± 0.03
CE	exercised	5.75 ± 0.18	-	-
ZE	exercised	6.33 ± 0.21	0.6 ± 0.09	-
PE	exercised	5.29 ± 0.44	-	0.43 ± 0.05

Mice were left in their home cages (sedentary) or given 20 min of aerobic exercise 3 times per week (exercised). Each activity group was fed lab chow alone, or chow supplemented with zucchini or nopal strips ad lib. Numbers represent means^{R} s.e.m. (n = 8) for dry lab chow and for vegetables after correction for loss of water by dehydration during the third and final week of the experiment. Mice in all groups varied in body weight over a very narrow range of 29-30 g at the start and 45-46 g at the end of the experiment.

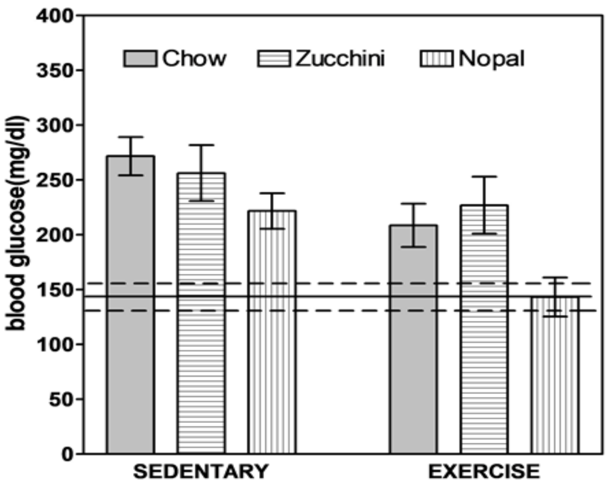

Figure 1 : Effect of activity and diet on chronic blood glucose levels in *ob/ob* mice. Histograms represent means ± s.e.m. for n = 7-8. Mice were left in their home cages without exercise (Sedentary) or given 20 min of aerobic swimming 3 times per week (Exercised). Each activity group was fed lab chow alone, or chow supplemented with zucchini or nopal strips ad lib. The horizontal lines represent the mean (solid) ± 95% confidence limits (dashed) for baseline blood glucose for all mice at the beginning of the 3 week experiment. Note the rise in chronic blood glucose in all mice except those that were exercised and fed nopal.

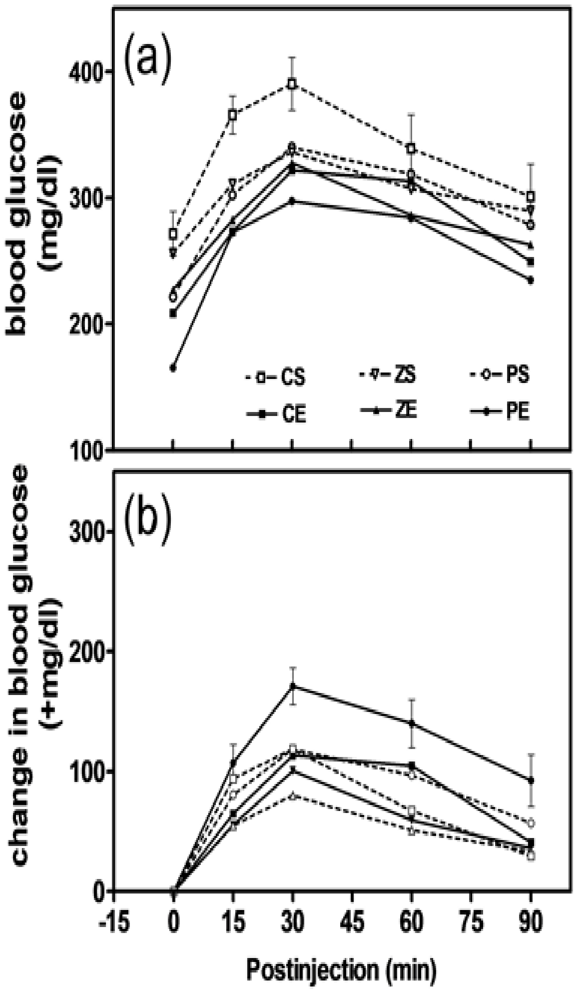

Figure 2: Effect of activity and diet on the time course of change in blood glucose following a glucose challenge . Mice were fed chow alone (C) or chow supplemented with Zucchini (Z) or nopal (P) for three weeks, and left sedentary (S) or exercised (E) 3 times/week as described under "Methods". After 3 weeks, a baseline level of blood glucose was measured (0 min), then all mice were injected intraperitoneally with a glucose solution (32 mg/ml in a volume [ml] equal to 1.4% of their body weight in g), and blood was sampled at 15, 30, 60, and 90 min post injection for measurement of blood glucose. Values indicated are means per treatment group (n = 7-8). To simplify the figure, s.e.m. is shown only for the topmost curve. (a) Absolute blood glucose levels. Group CS differed significantly ($p < .05$) from Groups CE, ZE, and PE at 15 min, and from Groups ZS and ZE at 30 min. No other pairwise comparisons were significant. (b) Blood glucose levels normalized by subtracting the baseline (0 min) value for each group.

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