Exercise Adds to Metformin and Acarbose Efficacy in db/db Mice

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Physical exercise is frequently recommended for the treatment of type 2 diabetes, whether as primary therapy with diet modification or as an adjunct to drug therapy. We hypothesized that mild exercise would enhance the glucose-lowering effects of 2 oral antihyperglycemic drugs, metformin and acarbose, in an animal model of type 2 diabetes. Eight-week-old male C57BL/Ks (db/db) mice were sorted into control and exercise groups and dosed daily for 4 weeks with vehicle, metformin (150 mg/kg/d), or acarbose (40 mg/kg/d). Exercise consisted of swimming (initially 5 min/d and ultimately 1 h/d for the last 2 weeks). Exercise, metformin, and acarbose independently reduced serum glucose concentrations 15% to 25% compared with the respective controls ($P < .0001$), but the effect on glucose concentration of combining drug therapy with exercise was no greater than the sum of the individual effects. Exercise training independently increased muscle glycogen (30%; $P < .05$) and liver glycogen (250%; $P < .05$) levels and slightly reduced serum high-density lipoprotein cholesterol (−8%; $P < .05$), whereas drug treatment had no effect on these variables. In addition, exercise but not drug treatment prevented the approximately 30% decline in serum insulin concentrations that occurred in the control animals ($P < .05$). Twenty-four hours after the last drug or exercise treatment, oral glucose tolerance and hemoglobin A1c were not significantly different between groups. Treatment also did not greatly affect triglyceride, glycerol, or total cholesterol concentrations. In conclusion, exercise and drug therapy independently decreased serum glucose in db/db mice, and these effects did not appear to be synergistic. In addition, exercise training maintained serum insulin concentrations and increased tissue glycogen storage. These results suggest that exercise has the potential to add to the efficacy of oral antihyperglycemic drugs.

EXERCISE is prescribed in the treatment of type 2 diabetes to assist in weight management and glucose control and has been shown to increase whole-body insulin sensitivity.[1] When used in combination with oral antihyperglycemic drug therapy, exercise therefore has the potential to improve the glucose-lowering actions of these drugs. The combination of exercise and sulfonylurea treatment has been shown to be more effective at decreasing glucose in severely diabetic rats than either treatment alone.[2] However, the combination of exercise with sulfonylurea treatment increases the risk of hypoglycemic episodes.[3] Therefore, exercise therapy has potentially greater value when used in combination with oral antidiabetic agents that have a low hypoglycemic risk. Therefore, as an initial step to test the utility of adding exercise therapy to oral antihyperglycemic therapy, we tested the effects of a mild swim training program on metformin and acarbose activity in an animal model for type 2 diabetes, the C57BL/Ks (db/db) mouse.[4]

MATERIALS AND METHODS

Animal Care

Eight-week-old male C57BL/Ks db/db mice were obtained from Jackson Labs, Bar Harbor, ME. Animals were housed 4 per cage under standard conditions of lighting (12:12-hour light:dark) and temperature (22 ± 3°C). Laboratory Rodent Diet (PMI Feeds Inc, Richmond, IN) and tap water were available to animals ad libitum except during exercise and before the oral glucose tolerance test.

Experimental Protocol

Animals were sorted into 6 treatment groups (n = 8 each) based on serum glucose concentrations (mean 32.8 ± 0.2 mmol/L per group). Each group was assigned 1 of 2 exercise treatments (control or exercise) and 1 of 3 drug treatments (vehicle, metformin, or acarbose). The exercise program consisted of swimming (see Exercise Protocol). The drug treatments were vehicle (0.25% carboxymethylcellulose [CMC]), metformin (150 mg/kg/d), or acarbose (40 mg/kg/d). Drug doses and sampling schedules were determined from pilot studies to show acute, reliable, and statistically significant reductions in serum glucose. Treatments were given for 28 consecutive days.

Metformin (Sigma, St Louis, MO) and acarbose (Precose, Bayer, Germany) were dissolved in 0.25% CMC and administered by oral gavage in a volume of 10 mL/kg. Exercised animals swam once daily at 9 AM, metformin-treated animals were dosed once daily at 10 AM, and acarbose-treated animals were dosed once daily at noon. Blood samples were taken weekly at 1 PM in the fed state by tail bleeding without anesthesia. Blood samples were centrifuged at 12,000 rpm for 4 minutes (Beckman microfuge; Beckman Instruments, Fullerton, CA), and serum was analyzed for glucose, hemoglobin A1c, triglyceride, glycerol, total and high-density lipoprotein (HDL) cholesterol (Sigma Diagnostics, St Louis, MO), and insulin (radioimmunoassay; Linco Research, Inc, St Charles, MO) concentrations. Body weight and food consumption per cage were measured weekly. At the end of the study, animals were fasted overnight and given an oral glucose tolerance test (OGTT). In an effort to reduce short-term treatment effects, animals were not exercised or dosed with drug on the day of the OGTT. For the OGTT, animals were given a 20% glucose solution (2.0 g glucose/kg) by oral gavage, and blood samples were taken for the measurement of serum glucose 0, 15, 30, 60, and 120 minutes postadministration. At the end of the OGTT, animals were euthanized by CO_2 asphyxiation and tissue samples were taken for the measurement of liver and muscle (gastrocnemius) glycogen[5] and muscle citrate synthase.[6] Coefficients of variation (within assay and between assay) for all assays were less than 15%.

Exercise Protocol

Each swimming tray (21 × 45 × 24 cm) contained tap water (10 cm deep) maintained between 24°C and 28°C. All exercised mice began swimming 5 min/d, and swimming duration was increased 5 min/d until a swimming time of 1 hour was reached (day 12). This daily duration was maintained for the rest of the study (days 12 through 28). Exercised animals swam together in groups of 12 and were immediately removed from the water if they did not stay on the surface. Of 24 total mice

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assigned to the swimming program, 23 animals successfully completed the 4-week exercise regimen.

Statistical Analysis

Treatments were compared using 2-way (exercise × drug) analysis of variance (ANOVA) and Fishers protected least significant difference (PLSD) post hoc test. A $P < .05$ was considered statistically significant. If a statistically significant difference was not observed between treatments, the data for those treatments were collapsed for the data analysis and presentation of results.

RESULTS

Body Weight and Food Consumption

Body weight at the start of the study was 36.7 ± 0.2 g for the study population. Body weight gain over the 4 weeks of the study was significantly lower in the exercise-trained groups (8.1 ± 0.3 *v* 9.1 ± 0.2 g; $P < .05$). Average food consumption was also slightly but significantly lower in the exercise-trained groups (5.6 ± 0.1 *v* 5.8 ± 0.1 g/animal/d; $P < .05$). Drug treatment had no effect on body weight or food consumption.

Exercise

Pilot work showed that a single 1-hour bout of swimming exercise in untrained db/db mice (n = 4) reduced gastrocnemius glycogen concentration 45% (13 ± 1 *v* 23 ± 1 μmol/g; $P < .01$) when measured immediately after exercise. However, at the end of the 4-week training period, gastrocnemius citrate synthase activity was not significantly different between the exercise and control groups (16.9 ± 0.4 *v* 16.0 ± 0.4 μmol/g/min), suggesting that the training stimulus was mild.

Glucose

Glucose concentrations are shown in Fig 1A through C. Exercise (Fig 1A) and drug (Fig 1B) treatments had immediate and significant main effects on glucose concentration ($P < .0001$), as well as a significant interaction effect (Fig 1C; $P < .05$). In short, exercise increased the glucose-lowering effects of both drug treatments. However, the interaction graph suggests that this effect was less dramatic in the acarbose-treated group.

Glycogen

Exercise-trained animals had significantly higher glycogen concentrations at the end of the study after the overnight fast and OGTT in the gastrocnemius muscle (16.9 ± 0.8 *v* 13.0 ± 0.9 μmol/g; $P < .01$) and in the liver (230 ± 14 *v* 90 ± 9 μmol/g; $P < .0001$). Drug treatment had no effect on glycogen levels.

Insulin

Insulin concentrations are shown in Fig 2. Insulin concentrations in the exercise-trained groups were significantly higher than in the control groups ($P < .0001$), primarily because of a 30% to 40% reduction in concentrations in the control groups

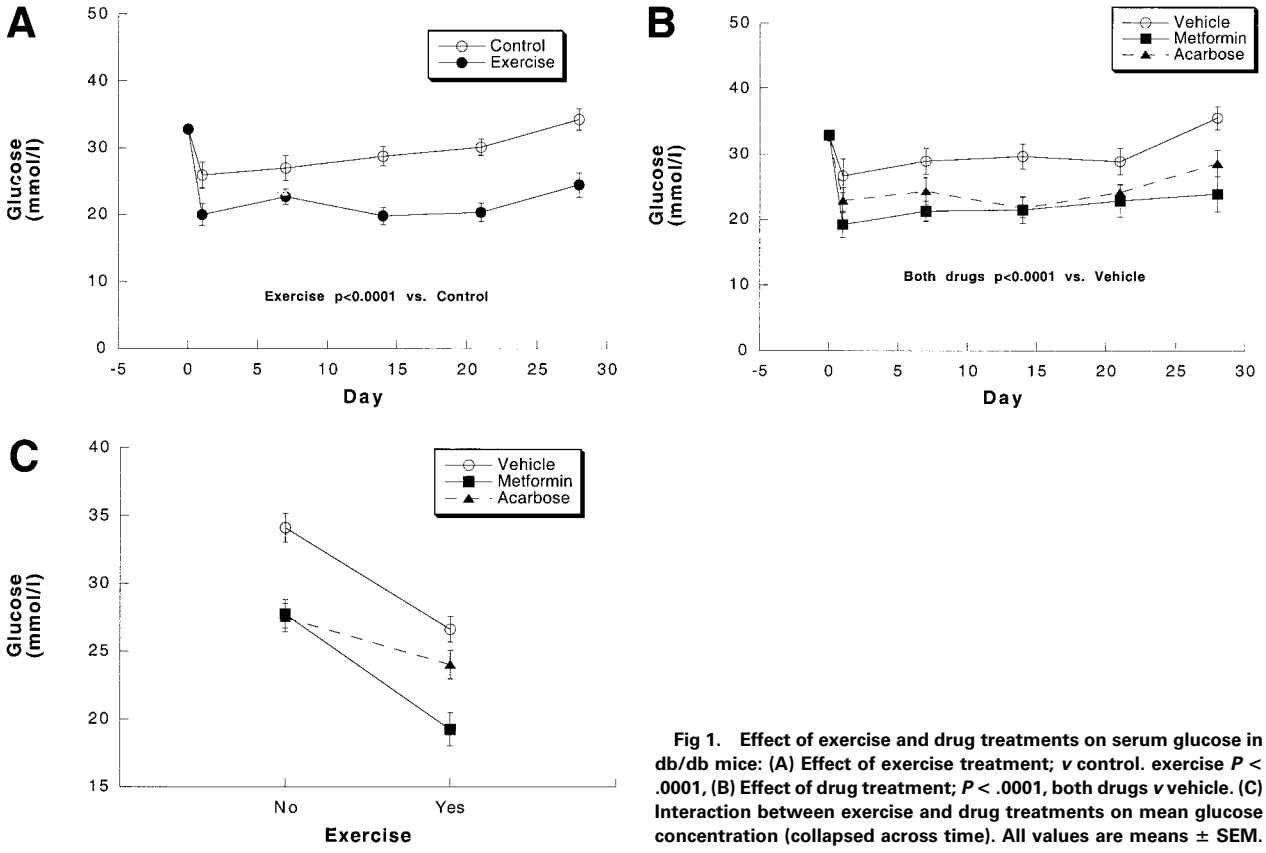

Fig 1. Effect of exercise and drug treatments on serum glucose in db/db mice: (A) Effect of exercise treatment; *v* control. exercise $P < .0001$, (B) Effect of drug treatment; $P < .0001$, both drugs *v* vehicle. (C) Interaction between exercise and drug treatments on mean glucose concentration (collapsed across time). All values are means ± SEM.

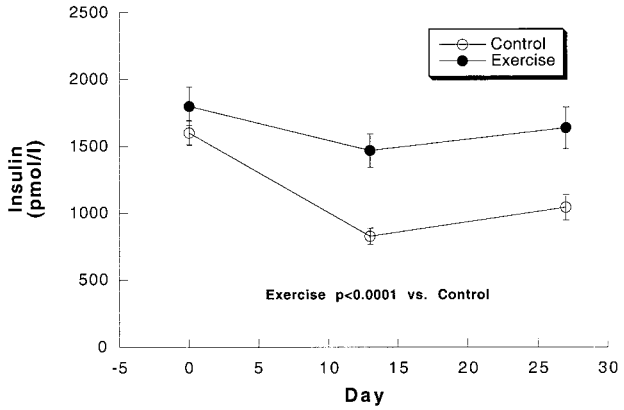

Fig 2. Effect of exercise treatment on serum insulin concentrations in db/db mice; $P < .0001$, exercise *v* control. All values are means ± SEM.

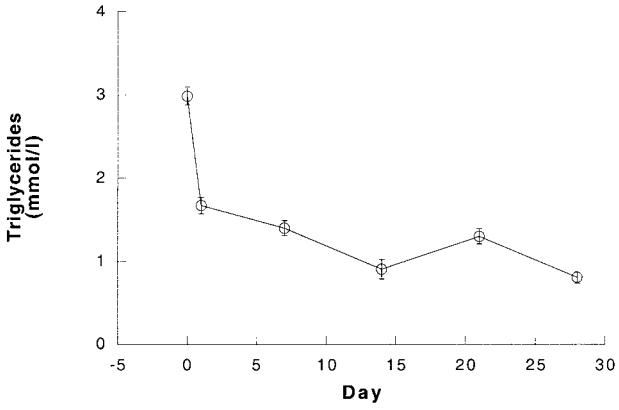

Fig 4. Triglyceride concentrations in the study population; there were no significant differences between groups. All values are means ± SEM.

over the course of the study. In contrast, drug treatment had no effect on insulin concentration.

Lipids

Neither exercise nor drug treatment affected triglyceride (average concentration, 1.54 ± 0.05 mmol/L), glycerol (average concentration, 4.89 ± 0.11 mmol/L), or total cholesterol (average concentration, 3.75 ± 0.08 mmol/L) concentrations. Exercise had a slight but statistically significant lowering effect on HDL cholesterol concentration (Fig 3; $P < .05$). Triglyceride concentrations (Fig 4) in the study population were approximately 2-fold higher at the pretreatment time point than at all subsequent time points.

Hemoglobin A1c and Oral Glucose Tolerance

Hemoglobin A1c and oral glucose tolerance were measured 24 hours after the final exercise or drug treatment. Hemoglobin A1c (average concentration, 2.4% ± 0.1%) was not significantly different between any of the treatments. Oral glucose tolerance (Fig 5) likewise was not significantly different between any of the treatments.

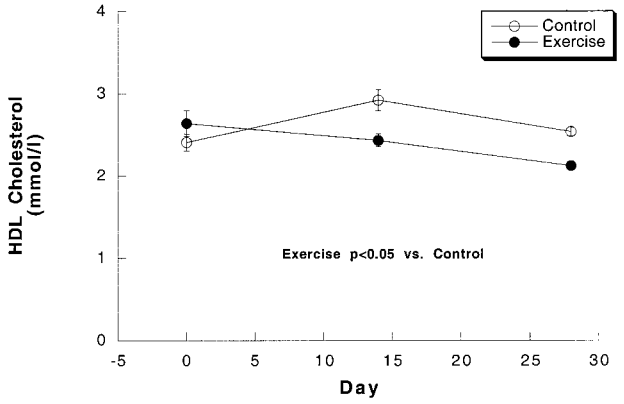

Fig 3. Effect of exercise treatment on serum HDL cholesterol concentrations in db/db mice; $P < 0.05$, exercise *v* control. All values are means ± SEM.

DISCUSSION AND CONCLUSIONS

To our knowledge, no previous study has assessed the effects of exercise training on glucose regulation in the db/db mouse. In addition, few studies have systematically assessed the glucoregulatory effects of exercise in combination with metformin or acarbose. The present study shows that exercise, metformin, and acarbose therapy independently reduce serum glucose concentrations in severely hyperglycemic db/db mice. Although the glucose-lowering effects of exercise in combination with drug therapy were greater than with either treatment alone, the combined effects of exercise and drug treatment were no greater than additive. These results suggest that the effects of exercise and drug therapy in the present study were independent and not synergistic. These results support previous observations that exercise decreases glucose concentrations in animal models of diabetes[7] and supplement previous findings that exercise can increase the glucose-lowering action of sulfonylureas.[2] In addition, because metformin and acarbose treatment present little risk of hypoglycemia,[8,9] the combination of exer-

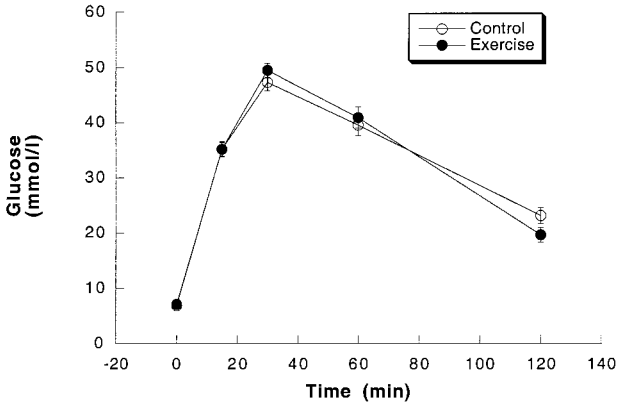

Fig 5. Effect of exercise treatment on oral glucose tolerance in db/db mice. Twenty-four hours after the last treatment and after an overnight fast, animals were orally dosed with glucose (2 g/kg). There were no significant differences between groups. All values are means ± SEM.

cise with these antihyperglycemic drugs probably presents a lower risk of hypoglycemia than the combination of exercise with sulfonylureas.[10] However, the glucose-lowering effect of exercise was less dramatic in combination with acarbose treatment than with metformin treatment.

A potential benefit of adding exercise to metformin or acarbose therapy is that exercise training has been shown to improve whole-body insulin sensitivity and glucose disposal.[1] Although the mechanism of action of metformin is not well characterized, it is believed to reduce hyperglycemia by inhibiting hepatic glucose output.[11] In contrast, acarbose lowers postmeal blood glucose levels by slowing disaccharide digestion in the intestine.[12] Therefore, the potential value of exercise therapy in combination with these treatments is that its mechanism of action for lowering blood glucose is different and thus complementary to the mechanisms of action of both drugs. In the present study, there is little evidence that the exercise or drug treatments improved long-term glucose control. Instead, it is likely that the primary benefit of each daily drug or exercise treatment was a short-term decrease in blood glucose concentration for several hours after treatment. This possibility is supported by the fact that reductions in plasma glucose concentration were apparent immediately after the onset of treatment but were not present 24 hours after cessation of treatment. In addition, the observation that animals in the pilot study could swim for 1 hour without training and that citrate synthase was not elevated in the exercised animals at the end of the study suggest that exercise intensity or duration was insufficient to produce training effects. Therefore, it is likely that the changes in glycogen and plasma glucose observed during the study were short-term results of the last exercise bout.

Nevertheless, the exercise program appeared to have beneficial effects, most notably the maintenance of serum insulin concentrations in exercise-treated animals (Fig 2). In untreated db/db mice, insulin concentrations increase rapidly during the first few weeks of life, then decline rapidly after 8 week of age as β cells are destroyed.[13] In the present study, exercise training prevented this decrease in insulin concentration, but antihyperglycemic drug therapy did not. This apparent protective effect of exercise on insulin secretory capacity supports human data linking the reductions in β cell damage with exercise training to reduced hyperglycemia.[14,15] However, the failure of drug therapy to "protect" the β cell in this study suggests that reductions in hyperglycemia alone were not sufficient to preserve insulin secretory capacity in the db/db mouse. In addition, the lack of improvement in glycosylated hemoglobin and oral glucose tolerance in exercise-treated animals suggests that this apparent retention of insulin secretory capacity was either a short-term effect of the last exercise bout or insufficient to improve long-term glucose control.

An additional potential benefit of exercise is suggested by the higher muscle and liver glycogen concentrations in exercised animals at the end of the study. In contrast, metformin or acarbose treatment in the absence of exercise had no effect on glycogen concentrations. This observation suggests that the daily use of glycogen during exercise enhanced glucose storage as glycogen in these tissues after exercise.

Lipid concentrations, including HDL cholesterol, in the present study were not influenced greatly by either exercise or antihyperglycemic drug therapy. In addition, triglyceride concentrations in the mice became markedly unstable with the onset of handling (Fig 4). These observations suggest that the exercise stimulus was insufficient to produce lipid changes and that db/db mice may not be a suitable animal model for lipid research. Therefore, although previous research suggests that exercise,[16] metformin,[17,18] and acarbose[19] each have beneficial effects on lipid regulation in type 2 diabetes, these effects could not be confirmed in the present study, nor could the potential benefit of combination therapy on lipid regulation be supported.

In conclusion, we found that a mild exercise program was an effective treatment for achieving short-term reductions in serum glucose concentrations in C57BL/Ks db/db mice whether used alone or in combination with metformin or acarbose. In addition, exercise had beneficial effects that metformin and acarbose therapy lacked. The mechanisms responsible for these benefits and the effect of such improvements in glucose and insulin regulation on morbidity and mortality in this animal model remain to be seen, and longer studies and systematic measurements of insulin sensitivity are needed. Nevertheless, these data suggest that exercise has the potential to add to the efficacy of oral antihyperglycemic drugs with a low risk of hypoglycemia.

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