Marked Improvement of Glucose Homeostasis in Diabetic *ob/ob* Mice Given Oral Vanadate

SONIA M. BRICHARD, CLIFFORD J. BAILEY, AND JEAN-CLAUDE HENQUIN

The trace element vanadium exerts insulinlike effects in vitro and decreases hyperglycemia in insulin-deficient animals. This study examined whether vanadate can improve glucose homeostasis in genetically obese hyperglycemic insulin-resistant *ob/ob* mice, which present metabolic abnormalities similar to those of human non-insulin-dependent diabetes. Sodium orthovanadate (0.3 mg/ml) was administered for 7 wk in H_2O. Vanadate treatment induced a fall in fed and fasted plasma glucose and insulin levels and improved tolerance to oral glucose; the stimulated glucose area was decreased by 65%, and an early peak of insulin secretion was restored. During an intravenous glucose tolerance test, the glucose disappearance rate was twofold higher in vanadate-treated mice, and the reappearance of a significant insulin response was also observed. Moreover, vanadate produced a twofold increase in hepatic glycogen content and prevented the exhaustion of pancreatic insulin stores. The hypoglycemic response to exogenous insulin was similar in control and treated mice. In vitro experiments showed that basal glucose oxidation by hemidiaphragms was 32% higher in vanadate-treated mice than in controls, although stimulation by insulin was similar in both groups. In conclusion, oral vanadate caused a marked and sustained improvement of glucose homeostasis in diabetic insulin-resistant mice by exerting an insulinlike effect on peripheral tissues and apparently preventing the exhaustion of pancreatic insulin stores. *Diabetes* 39:1326–32, 1990

Considerable interest has recently been shown concerning the effects of the trace element vanadium on carbohydrate metabolism (1). In vitro, vanadate increases glucose transport and metabolism in skeletal muscles (2–4) and adipocytes (2,3,5,6). On the other hand, its action on the liver is not clear. Vanadate has been shown to stimulate glycogen synthesis (2), activate glycolysis (7), and inhibit glucose-6-phosphatase (8). However, a glycogenolytic effect of the element has also been reported (9,10). The insulinlike effects of vanadate appear to result from enhanced phosphorylation of the insulin receptor by stimulation of the tyrosine kinase activity (11), direct vanadate esterification of certain tyrosine residues (12), or inhibition of phosphotyrosine phosphatase (13,14). Mechanisms distal to the receptor may also play a role (15,16).

In vivo, oral vanadate has little effect on plasma glucose levels in nondiabetic rats (17–22). In contrast, vanadate markedly decreased blood glucose concentrations in rats made insulin deficient and diabetic by streptozocin injection (STZ-D; 14,17,20–25) or partial pancreatectomy (26). Vanadate was also effective in insulin-resistant Zucker *fa/fa* rats (27). However, these animals are only mildly glucose intolerant. The aim of this work was therefore to investigate whether oral vanadate can improve glucose homeostasis in an animal model with severe insulin resistance and overt diabetes. We selected Aston *ob/ob* mice, which are characterized by their genetically transmitted obesity, hyperglycemia, and marked hyperinsulinemia (28).

RESEARCH DESIGN AND METHODS

Twenty-five obese hyperglycemic (*ob/ob*) mice (12–14 wk old, 70.3 ± 1.5 g) from the colony of Aston University (Birmingham, UK) were used in this study. The origin and the characteristics of Aston *ob/ob* mice have been described in detail elsewhere (28,29). The animals were housed in individual wire-bottomed cages that permitted accurate measurements of daily fluid intake. They received a standard pellet diet (AO_3, Usine d'Alimentation Rationnelle, Epinay/Orge, France) and were maintained at a constant temperature (21°C) on a fixed 12-h light-dark cycle.

From the Diabetology and Nutrition Unit, University of Louvain School of Medicine, Brussels, Belgium; and the Department of Pharmaceutical Sciences, Aston University, Birmingham, United Kingdom.

Address correspondence and reprint requests to Dr. J.-C. Henquin, Unité de Diabétologie et Nutrition, UCL 54.74, Avenue Hippocrate 54, B1200 Brussels, Belgium.

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The mice were divided into two experimental groups matched for initial body weight and fed plasma glucose. The control group (n = 15) did not receive vanadate, whereas the treated group (n = 10) received sodium orthovanadate (0.3 mg/ml) in drinking solutions. To partially overcome the initial aversion to vanadate and ensure long-term fluid intake by the mice, sodium orthovanadate (Janssen, Beerse, Belgium) was supplied in two different solutions, which were freshly prepared every 3rd day. The first solution was supplemented with 85 mM NaCl, known to reduce vanadate toxicity (17,30), and its pH was adjusted to 7 with citric acid; the second one was slightly flavored with cocoa. The drinking solutions of control mice contained the same concentration of NaCl and citric acid (neutralized with NaOH) or the same amount of cocoa.

Blood (100 μl) was sampled from the tail tip of fed animals between 0900 and 1000. An oral glucose tolerance test (OGTT) was conducted after 3 wk, an intravenous glucose tolerance test (IVGTT) after 4–5 wk, and an intraperitoneal insulin-hypoglycemia test after 6 wk of treatment. On the day before the test, food was removed at 1800, and NaCl solution without vanadate was given to all mice. The tests started at 0900. For the OGTT, glucose (40% in H_2O) was introduced directly into the stomach through a fine gastric catheter at a dose of 2 g/kg body wt. For the IVGTT, glucose (30% in H_2O) was injected into a tail vein at a dose of 1 g/kg body wt. For the insulin-hypoglycemia test, 60 μg/ml pork insulin (Actrapid MC, Novo, Copenhagen) was administered by injection at a dose of 600 μg/kg body wt i.p. Mice were wrapped in a towel to gently restrain them during injections and blood sampling.

After 7 wk of treatment, the animals were decapitated between 0900 and 1200, and blood was collected from trunk vessels. The pancreas and liver were frozen in liquid N_2. Hemidiaphragms were quickly removed, weighed, and placed in glass vials containing 2.5 ml of medium. Periovarian or epididymal adipose tissue was cut into small (~10-mg) pieces, and batches of five pieces were placed in glass vials also containing 2.5 ml of medium. The medium was a salt-balanced bicarbonate-buffered solution supplemented with 20 mg/ml bovine serum albumin, 5 mM HEPES, and 5 mM glucose. It was gassed with O_2/CO_2 (94:6), and pH was 7.4. The pieces of tissue were first preincubated for two 20-min periods. They were then incubated for 120 min at 37°C in 3 ml of medium supplemented with 0.5 μCi/ml [U-^{14}C]glucose (Amersham, Aylesbury, UK). Hemidiaphragms and pieces of adipose tissue from each animal were incubated either without (basal) or with insulin (2×10^{-7} M). The incubation vials were tightly sealed with rubber stoppers. At the end of the incubation, 0.5 ml of 1 M 10X Hyamine (Packard, Downers Grove, IL) was injected through the stopper into a small plastic tube inside the glass vial, and 0.3 ml of 3 M perchloric acid was injected into the incubation medium to stop the reaction and release CO_2. After a final incubation for 2 h, [^{14}C]CO_2 trapped in the Hyamine was counted in a liquid-scintillation counter.

Plasma glucose was measured on the day of sampling by a glucose oxidase method (Beckman glucose analyzer). Plasma samples were then kept frozen at −20°C until insulin measurement by a radioimmunoassay (RIA) with a dextran-coated–charcoal separation step (31) and rat insulin as standard (Novo). Qualitative measurements of glucosuria were performed with Tes-Tape strips (Lilly, Brussels). Pancreatic insulin and glucagon were extracted by homogenization and sonication of the tissue in acidified ethanol (32). Glucagon was measured by RIA with the 30K antiserum (33). Liver glycogen was measured after extraction with KOH, precipitation in ethanol, and hydrolysis with α-amyloglucosidase (Boehringer Mannheim, Mannheim, FRG) (34,35). Serum urea, creatinine, glutamic-oxaloacetic transaminase, and glutamic-pyruvic transaminase were measured with a Hitachi 717 automatic analyzer. Serum triglycerides were determined by a spectrophotometric method (Wako, Osaka, Japan), and serum vanadate was measured by atomic absorption spectrometry (36).

The glucose disappearance rate (K_g) during the IVGTT was calculated from the slope of the logarithm of the glucose levels between 5 and 30 min. The integrated glucose and insulin responses during the glucose tolerance tests were calculated as the areas under the curves and above basal values (for the stimulated responses) or above zero levels (for the total responses).

Results are given as means ± SE for the indicated number of mice. Student's t test for unpaired data was used to evaluate the statistical significance of differences between treated and control mice. Student's t test for paired data was used to assess the effect of insulin on glucose oxidation by muscle and adipose tissue in each animal. Differences were considered statistically significant at $P < 0.05$.

RESULTS

An OGTT was conducted in all mice after 3 wk of treatment. Afterward, 6 of the 15 controls died before the end of the study: 3 became cachectic (2 at 3 wk and 1 at 5.5 wk), 1 developed a severe infection (wk 4), 1 died for an unknown reason (wk 5.5), and 1 did not recover from hypoglycemia during the insulin test. Two of the 10 vanadate-treated mice died of hypoglycemia during the insulin test, and 1 was killed accidentally.

At the beginning of the study, the two groups of mice were matched for body weight: 71 ± 2 g in controls (n = 15) and 70 ± 3 g in vanadate-treated mice (n = 10). Thereafter, a small and similar decrease in body weight, which started before the dynamic tests, occurred in the two groups: 63 ± 2 and 65 ± 3 g in controls (n = 15) and vanadate-treated mice (n = 10), respectively, after 3 wk; 59 ± 3 and 64 ± 2 g in controls (n = 9) and vanadate-treated mice (n = 7), respectively, after 7 wk. The two groups of mice were also matched for fed plasma glucose levels before starting the treatment (Table 1). Retrospectively, it was found that they were also well matched for plasma insulin levels (Table 1).

In fed controls, plasma glucose levels remained elevated during the whole study period, whereas insulin levels decreased ($P < 0.05$), probably due to the progressive decompensation of the diabetes (29). Vanadate administration resulted in a 50% decrease in plasma glucose and insulin levels compared with controls (Table 1), but the insulin-glucose ratios remained similar in the two groups: 0.9 ± 0.2 in controls and 1.0 ± 0.2 in vanadate-treated mice. The decreases in glucose (43%) and insulin (50%) levels were already observed after 1 wk of treatment.

After an overnight fast, mean plasma glucose and insulin

TABLE 1
Effects of vanadate on plasma glucose and insulin levels in fed and fasted *ob/ob* mice

	Before treatment		During treatment	
	Control	Vanadate	Control	Vanadate
Fed				
Plasma glucose (mM)	21.0 ± 1.9	20.1 ± 2.3	24.2 ± 1.8	12.2 ± 2.4*
Plasma insulin (pM)	4429 ± 554	4239 ± 657	3183 ± 519	1678 ± 294†
Fasted				
Plasma glucose (mM)			16.1 ± 2.2	8.1 ± 1.3‡
Plasma insulin (pM)			779 ± 87	554 ± 87

Values are means ± SE for 15 control and 10 vanadate-treated mice. In fed animals, 2 samples were taken before the start of the study, and 3 samples were taken during treatment (after 1, 2.5, and 7 wk). In fasted animals, the samples were taken at time 0 of the 2 glucose tolerance tests. Values obtained were averaged for each mouse.
*$P < 0.001$, †$P < 0.05$, ‡$P < 0.02$, vs. control mice.

levels were again lower in vanadate-treated than control mice, but the difference did not achieve statistical significance ($0.05 < P < 0.10$) for insulin levels (Table 1). Fasted plasma insulin-glucose ratios remained similar in both groups: 0.4 ± 0.1 and 0.5 ± 0.1 in control and vanadate-treated mice, respectively.

The attenuation of hyperglycemia in vanadate-treated mice was accompanied by a threefold decrease in fluid consumption (8.4 ± 0.5 vs. 25.8 ± 6 ml/day in controls, $P < 0.02$). Their average vanadate intake was 39.8 ± 2.3 mg · kg^{-1} · day^{-1} (mean ± SE value of measurements made during the 4th wk).

Qualitative determinations of glucosuria were performed during the 7th wk and showed a positive result in eight of nine controls and three of seven vanadate-treated mice.

During the OGTT, plasma glucose concentrations rose to >30 mM and did not return to basal values after 120 min in controls (Fig. 1). In vanadate-treated mice, the increase in plasma glucose was smaller, and the return to basal values was faster. Stimulated (above-basal levels) and total (stimulated-plus-basal) glucose areas were reduced by 63 and 56%, respectively. The kinetics of the insulin response were different in the two groups (Fig. 1). No significant rise in plasma insulin levels was observed until 120 min in control mice, whereas an increase occurred at 30 min in vanadate-treated mice. However, the insulin areas were not modified by the treatment.

During the IVGTT, plasma glucose concentrations were consistently lower in vanadate-treated than control mice (Fig. 2). The K_g was more than two-fold higher ($P < 0.005$) in vanadate-treated mice (3.68 ± 0.29%/min) than in controls (1.51 ± 0.26%/min). The stimulated glucose areas were not statistically different in the two groups, but the total glucose area was 35% smaller in vanadate-treated than control mice ($P < 0.01$). Plasma insulin levels did not differ from baseline values at any time after glucose injection in controls. A lack of insulin response to intravenous glucose is characteristic of these animals (28,37). In contrast, plasma insulin levels increased significantly ($P < 0.05$) at 5 and 15 min in vanadate-treated mice. Hence, a stimulated insulin area was present only in vanadate-treated mice. Due to higher basal values in untreated mice, total insulin areas were similar in both groups (Fig. 2).

The intraperitoneal injection of insulin at a dose 60-fold higher than that usually administered to lean mice (28,37) caused a marked fall in plasma glucose levels in both groups of mice (Fig. 3). Not all mice were studied at the 1-h time point due to the death from hypoglycemia of one control mouse and two vanadate-treated mice. The fall in plasma glucose concentrations at 30 min, expressed as a percentage of baseline value, was similar in both groups.

Basal oxidation of glucose by hemidiaphragms was 32% higher ($P < 0.02$) in vanadate-treated mice than in controls, but the stimulation by a high concentration of insulin (2×10^{-7} M) was similar in both groups. Basal oxidation of glucose by white adipose tissue was not significantly different in control and vanadate-treated mice. The stimulatory effect of insulin, which was proportionately larger than on muscle, was also comparable in both groups (Fig. 4).

Vanadate treatment induced a two- to threefold increase in hepatic glycogen stores (Table 2). In the pancreas, insulin content and insulin concentrations were fourfold higher in vanadate-treated mice than in controls, whereas glucagon stores were not different (Table 2).

Seven weeks of treatment with vanadate did not alter kidney function or modify liver transaminase enzymes. Blood triglycerides also remained unchanged (Table 3). The serum

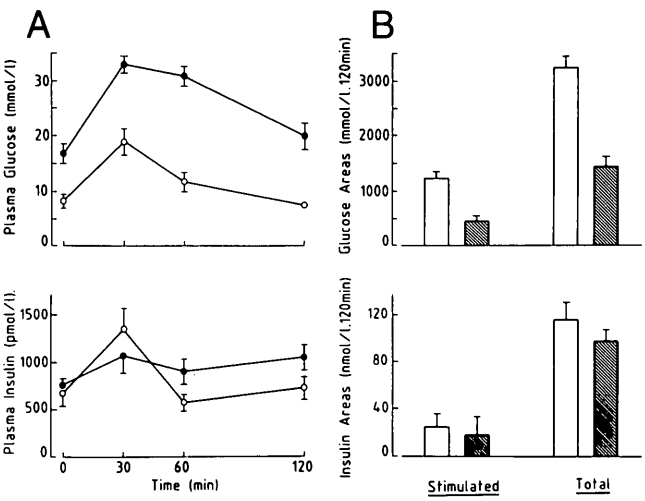

FIG. 1. Plasma glucose and insulin levels during oral glucose tolerance test (OGTT) in control (●; *open bars*) and vanadate-treated (○; *shaded bars*) *ob/ob* mice (*A*) and stimulated (above basal) and total (stimulated + basal) glucose and insulin areas during OGTT in 2 groups of mice (*B*). Test was performed after 3 wk of treatment. Values are means ± SE for 15 control and 10 vanadate-treated mice.

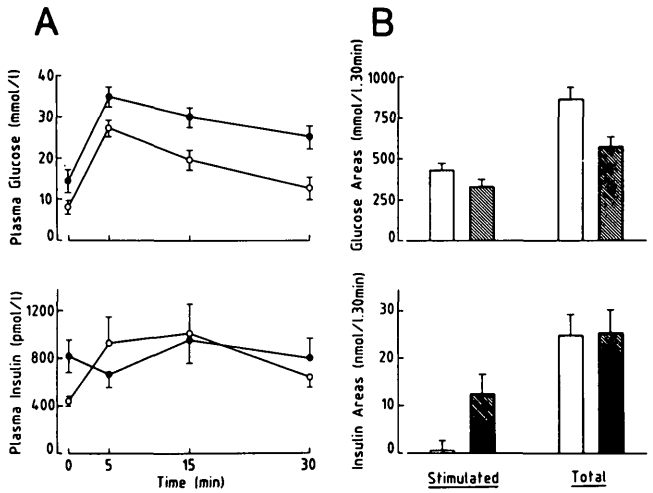

FIG. 2. Plasma glucose and insulin levels during intravenous glucose tolerance test (IVGTT) in control (●; *open bars*) and vanadate-treated (○; *shaded bars*) *ob/ob* mice (*A*) and stimulated (above basal) and total (stimulated + basal) glucose and insulin areas during IVGTT in 2 groups of mice (*B*). Test was performed after 4.5 wk of treatment. Values are means ± SE for 12 control and 10 vanadate-treated mice.

concentration of vanadate exceeded 26 μM in vanadate-treated mice, whereas only trace amounts were detected in controls.

DISCUSSION

This study demonstrates that vanadate exerts beneficial effects on glucose homeostasis in diabetic animals with severe insulin resistance. The hypoglycemic action of vanadate in *ob/ob* mice was marked and sustained and was observed during glucose tolerance tests and under basal conditions. It was associated with a decrease in fed plasma insulin levels, a faster insulin response to glucose loading, and a sparing effect on pancreatic insulin stores. Evidence was also found that a glycogenic action on the liver and an enhancement of glucose oxidation by muscles contribute to the blood glucose-lowering effect of vanadate.

The *ob/ob* mice from the Aston colony are usually moderately hyperglycemic; their fed plasma glucose levels plateau around 12–15 mM between 10 and 20 wk of age (28,29,37). The animals used in this study were thus more severely diabetic than usually observed in mice of similar genetic background and similar age. It is possible that small differences in the composition of the standard continental and English diets have contributed to this difference. Nevertheless, the earlier decompensation of diabetes observed in this experiment probably explains the slight decrease in body weight, the reduction in fed plasma insulin levels, and the relatively high mortality rate from diabetic complications noted in the control group.

In contrast, the small reduction in body weight and the marked fall in plasma insulin levels observed in vanadate-treated mice can certainly not be explained by a decompensation of the diabetic state, because blood glucose concentrations were also greatly decreased. The attenuation of hyperinsulinemia under vanadate treatment, also observed in obese insulin-resistant *fa/fa* rats (27), could contribute to the slight decrease in body weight (38). However, reduced food consumption may also be involved. Food consumption was not measured in this study, but it has been reported to fall in other animal models treated with vanadate (19,24,27). Because the degree of obesity may influence glucose homeostasis, it proved necessary to include a group of pair-fed animals in our previous study on the effects of vanadate in *fa/fa* rats (27). No such group was necessary with *ob/ob* mice, because the body weight was similar throughout the study in both treated and untreated animals. We cannot exclude, however, that the reasons for the similar small change in body weight are different—a worsening of diabetes in controls and the combination of a decrease in food consumption and plasma insulin levels in vanadate-treated mice.

An inhibition of intestinal glucose transport by vanadate is unlikely to play a role in the improvement of glucose homeostasis, because the beneficial influence of the element was detectable during IVGTT and OGTT and in fasted animals. Moreover, a recent study has demonstrated stimulation of intestinal glucose transport in rats chronically treated with vanadate (39). An accelerated kidney glucose loss cannot explain the hypoglycemic action of the element, because vanadate, unlike phloridzin (40), decreased glucosuria in *ob/ob* mice and in STZ-D rats (24).

No conclusive evidence could be obtained for an increase in the sensitivity to insulin by vanadate. First, insulin-induced hypoglycemia was similar in control and vanadate-treated mice. Admittedly, the dose of insulin may have been too high to reveal a possible change in insulin sensitivity. Second, fed and fasted plasma insulin-glucose ratios were not different in the two groups of mice despite the fall of glycemia during vanadate treatment. A decrease in this ratio might be expected if insulin sensitivity were increased by vanadate. Third, the stimulating effect of insulin on glucose oxidation in vitro was not more marked in tissues of vanadate-treated mice than of controls.

An insulinlike action of vanadate probably plays a major role in the improvement of glucose homeostasis in *ob/ob*

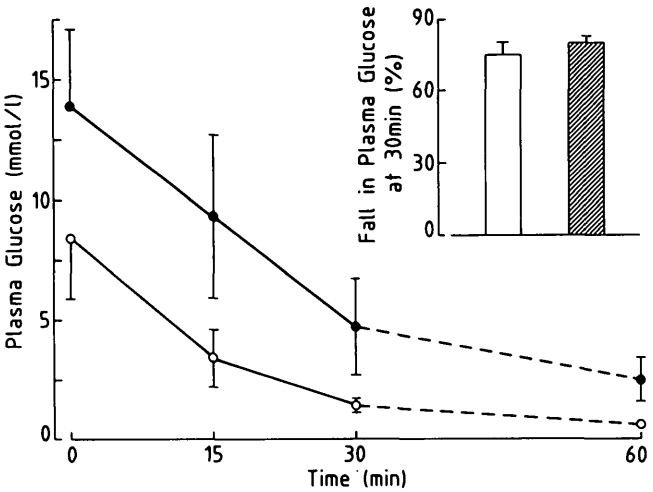

FIG. 3. Plasma glucose levels during intraperitoneal insulin hypoglycemia test in control (●; *open bar*) and vanadate-treated (○; *shaded bar*) *ob/ob* mice. *Inset*, fall in plasma glucose at 30 min, expressed as percentage of initial value (0 min) in 2 groups of mice. Test was performed after 6 wk of treatment. Values are means ± SE for 10 control and 8 vanadate-treated mice, respectively. In 7 control and 5 vanadate-treated mice, test was prolonged until 60 min (*dashed lines*).

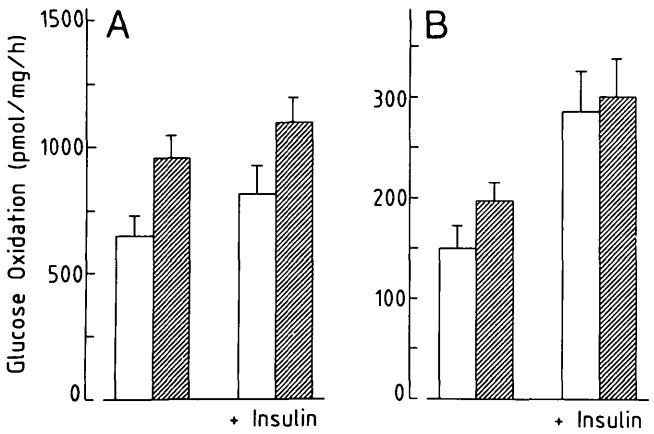

FIG. 4. Glucose oxidation by hemidiaphragms (*A*) and pieces of adipose tissue (*B*) obtained from control (*open bars*) and vanadate-treated (*shaded bars*) *ob/ob* mice. Tissue was incubated without or with insulin (2×10^{-7} M). Values are means ± SE for 9 control and 7 vanadate-treated mice.

mice. Many in vitro studies in which vanadate was added to the medium have clearly demonstrated an insulinlike effect on peripheral tissues from nondiabetic animals, including the diaphragm (2). Results of in vitro experiments with tissues of insulin-deficient rats previously given vanadate in vivo (19,22,23,26) are more spectacular and convincing than those obtained with similarly treated nondiabetic animals (18,19,22,23). A shift of the predominating gluconeogenic flux into a glycolytic flux (19,41) and a restoration of glycogen stores (19,24) were observed in the liver of STZ-D rats after vanadate treatment. Vanadate has also been reported to stimulate lipogenesis (23) and decrease lipolysis (22) in adipose tissue from these animals. Moreover, correction of chronic hyperglycemia with vanadate normalized glycogen synthase activity in skeletal muscle of pancreatectomized rats (26). Insulin resistance in skeletal muscle of *ob/ob* mice has been previously characterized as involving both receptor and postreceptor abnormalities (42,43). Because basal glucose oxidation by the diaphragm was increased in treated mice, it appears that vanadate is able to bypass the cellular defects of insulin resistance in these animals. This is of particular interest, because muscle is the major site of insulin-mediated glucose disposal (42).

The liver is another site of insulin resistance in *ob/ob* mice (44). In contrast to its lean littermate, the obese mouse does not decrease its gluconeogenic rate or activate its glycogenic pathway in response to a glucose load (44,45). The twofold increase in liver glycogen content measured in treated fed *ob/ob* mice suggests that vanadate overcomes the inability of the liver to store an appropriate fraction of the ingested glucose as glycogen. Interestingly, no effect of vanadate was noted in adipose tissues of *ob/ob* mice.

An effect of vanadate on β-cells must also be considered. In vitro, vanadate increases insulin release by nondiabetic rat (46) or mouse (A. Zhang, J.-C.H., unpublished observations) islets. However, this acute effect requires vanadate concentrations one or two orders of magnitude higher than those reached in the plasma of treated animals. Whether these lower concentrations of vanadate exert a direct chronic stimulatory effect on β-cell function in vivo has not been explored. However, if this were the case, we would expect a rise (or at least no change) in fed and fasted plasma insulin concentrations but not a marked decrease as observed in this study after 1 wk of treatment. The hypothesis of a stimulation of insulin release in vivo is also difficult to reconcile with the report that vanadate treatment lowers plasma insulin without changing plasma glucose in nondiabetic rats (21). Furthermore, it is unlikely that direct stimulation of β-cells by vanadate could be accompanied by a fourfold increase in insulin stores in *ob/ob* mice. We interpret this increase, unaccompanied by a change in glucagon reserves, as a sparing effect of vanadate. The insulinlike action of vanadate decreases the need for endogenous insulin, the stores of which are preserved. In addition, the decrease in blood glucose may attenuate the detrimental effect of protracted hyperglycemia on the insulin-releasing capacity of β-cells (47).

TABLE 2
Effects of vanadate treatment on liver glycogen and pancreatic insulin and glucagon stores in *ob/ob* mice

	Control	Vanadate
Liver		
Weight		
g	2.5 ± 0.1	3.3 ± 0.2*
% body wt	4.4 ± 0.3	5.1 ± 0.2
Glycogen		
mg	19.5 ± 5.3	57.2 ± 12.2†
mg/g liver	7.8 ± 2.1	17.2 ± 3.4‡
Pancreas		
Weight		
g	0.41 ± 0.02	0.37 ± 0.03
% body wt	0.72 ± 0.08	0.58 ± 0.04
Insulin		
nmol	9.3 ± 1.9	35.5 ± 7.4*
nmol/g pancreas	24.9 ± 5.4	104.7 ± 22.1*
Glucagon		
nmol	0.7 ± 0.1	0.8 ± 0.1
nmol/g pancreas	1.8 ± 0.1	2.3 ± 0.2

Values are means ± SE for liver in 9 control and 7 vanadate-treated mice and for pancreas in 10 control and 10 vanadate-treated mice. Pancreatic insulin and glucagon but not liver glycogen were measured in 4 mice that died before the end of the study.
*$P < 0.005$, †$P < 0.01$, ‡$P < 0.05$, vs. control mice.

TABLE 3
Effects of vanadate treatment on various plasma parameters in *ob/ob* mice

	Control	Vanadate
Urea (mM)	12.5 ± 0.5	9.0 ± 0.7*
Creatinine (μM)	20 ± 2	16 ± 3
GOT (IU/L)	145 ± 17	140 ± 20
GPT (IU/L)	66 ± 7	61 ± 10
Triglycerides (mg/dl)	181 ± 18	221 ± 57
Vanadate (μM)	0.12 ± 0.02	26.8 ± 3.4*

Values are means ± SE for 9 control and 7 vanadate-treated mice. Measurements were made on the plasma obtained when the mice were killed. GOT, glutamic-oxaloacetic transaminase; GPT, glutamic-pyruvic transaminase.
*$P < 0.001$ vs. control mice.

Both effects may thus explain the faster and sometimes larger insulin responses measured during glucose loads. This correction was particularly impressive after intravenous glucose and was reminiscent of observations made in non-insulin-dependent diabetic patients after lowering glucose

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