

Erythropoietin treatment leads to reduced blood glucose levels and body mass: insights from murine models

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Abstract

Erythropoietin (EPO) regulates proliferation and differentiation of erythroid precursor cells into erythrocytes. The last decade has revealed non-renal sites of EPO production and extrahematopoietic expression of the EPO receptor, thus suggesting that EPO has pleiotropic functions. Here, we addressed the interplay between EPO/glucose metabolism/body weight by employing a panel of relevant experimental murine models. The models focused on situations of increased EPO levels, including EPO-injected C57BL/6 and BALB/c mice, as well as transgenic mice (tg6) constitutively overexpressing human EPO, thus exposed to constantly high EPO serum levels. As experimental models

for diabetes and obesity, we employed protein Tyr phosphatase 1B (PTP1B) knockout mice associated with resistance to diabetes (PTP1B^{-/-}), and *ob/ob* mice susceptible to diabetes and obesity. The data presented herein demonstrate EPO-mediated decrease in blood glucose levels in all mice models tested. Moreover, in the *ob/ob* mice, we observed EPO-mediated attenuation of body weight gain and reduction of hemoglobin A1c. Taken together, our data bear significant clinical implications of EPO treatment in the management of a wide range of metabolic diseases, thus adding an important novel therapeutic potential to this pleiotropic hormone.

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Introduction

The hematopoietic growth factor erythropoietin (EPO) is produced in the kidney in response to hypoxia, and stimulates erythropoiesis in the bone marrow (Krantz 1991, Spivak *et al.* 1991). Recombinant human EPO (rHuEPO) is an effective treatment for anemia of various etiologies, including anemias associated with renal failure (Winearls *et al.* 1986, Eschbach *et al.* 1989) and cancer-related diseases (Spivak 1994, Mittelman 1996). The presence of EPO receptors (EPO-R) on cells other than erythroid progenitors, including endothelial, myocardial, neural, and retinal cells, suggests that EPO has other biological functions in addition to erythropoiesis, reviewed in Arcasoy (2008). Hence, such functions include neuroprotection (Brines *et al.* 2004, Cilloni *et al.* 2004), anti-neoplastic activity (Mittelman *et al.* 2001, Katz *et al.* 2005), and improvement in congestive heart failure (Silverberg *et al.* 2002, Fiordaliso *et al.* 2005, Camici *et al.* 2007). We (Prutchi-Sagiv *et al.* 2006a,b, 2008, Ghezzi & Mengozzi 2007, Katz *et al.* 2007, Lifshitz *et al.* 2009), as well as others (Ghezzi & Mengozzi 2007), have found EPO-associated improvement in immunological functions.

In that respect, we have been attracted by the reports of EPO effects on glucose metabolism in hemodialysis patients (Borissova *et al.* 1993, Allegra *et al.* 1996), which are associated with elevated insulin sensitivity (Spaia *et al.* 2000, Tuzcu *et al.* 2004). EPO effects were also documented on the metabolism of proteins and lipids; however, the latter are still controversial (reviewed in Allegra *et al.* (1997)).

The ability to control glucose, protein, and lipid metabolism is of paramount value in clinical practice and is especially relevant in subjects with metabolic syndrome. Metabolic syndrome is common, affecting more than 30% of adults in the United States (Feldeisen & Tucker 2007), and is referred to as a 'cluster of metabolic abnormalities', including obesity, hyperglycemia, dyslipidemia, and hypertension (Chew *et al.* 2006). In the 'modern' world, diet and lifestyle contribute to the high incidence of obesity which is linked to increased risk for diabetes, cancer, and cardiovascular diseases, which leads to a reduction in life expectancy (Kolonin *et al.* 2004). In the last few decades, the prevalence of obesity and diabetes has been increasing rapidly (reviewed in Keller (2006)). Consequently, the concern regarding obesity and its metabolic implications has directed intensive scientific effort

concerning the adipose tissue, which is a major endocrine and signaling organ involved in a wide range of physiological responses, reviewed in Rosen & Spiegelman (2006).

The positive effect of rHuEPO treatment on glucose metabolism in hemodialyzed patients set the scene for exploring critically the interplay between EPO/glucose metabolism/body weight in the absence of any clinical problems. We have thus employed a panel of relevant murine models to investigate this issue. The models focused on situations of increased EPO levels, including EPO-injected C57BL/6 and BALB/c mice, as well as transgenic mice constitutively overexpressing human EPO in an oxygen-independent manner (tg6; Ruschitzka *et al.* 2000, Vogel *et al.* 2003). EPO plasma levels in these mice are typically 10–12-fold higher than wt EPO levels (under normoxic conditions; Ruschitzka *et al.* 2000, Vogel *et al.* 2003). This elevation causes a doubling of their hematocrit from 40 to 80–90%, and up to 25% of the body weight is directed to the blood (Ruschitzka *et al.* 2000, Vogel *et al.* 2003). Expression of human EPO in these mice has been detected in the brain (where it most probably acts locally) as well as in the lung, from where it reaches circulation (Heinicke *et al.* 2006). As experimental models for diabetes and obesity, we employed phosphatase 1B (PTP1B or PTPN1 as listed in the MGI Database) knockout mice (Elchebly *et al.* 1999) associated with resistance to diabetes (PTP1B^{-/-}), and *ob/ob* mice susceptible to diabetes and obesity, reviewed in Houseknecht & Portocarrero (1998). Taken together, the data presented herein demonstrate EPO-associated decrease in blood glucose levels and attenuation of body weight gain, thus bearing significant clinical implications in the management of a wide range of metabolic diseases.

Materials and Methods

Mice

Female mice of the inbred strains BALB/c and C57BL/6, aged 8 and 12 weeks respectively, were obtained from the Tel-Aviv University Animal Breeding Center. Transgenic mice overexpressing HuEPO (tg6; Ruschitzka *et al.* 2000) aged 3–6 months were bred by us, and their wt littermates (C57BL/6) were used as controls. Male *ob/ob* mice (C57BL/6), aged 7 weeks, were obtained from Harlan (Jerusalem, Israel). Three-month-old male PTP1B^{-/-} mice (BALB/c) were previously described (Elchebly *et al.* 1999). Mice were maintained on a standard rodent chow diet (Koffolk, Tel Aviv, Israel). All procedures were approved by the animal care committees at the Sackler Faculty of Medicine Tel-Aviv University, Israel and at McGill University, Canada.

Injected materials

EPO in the form of EPREX, (rHuEPO, Epoetin alfa) was obtained from Cilag Ltd, Schaffhausen, Switzerland. Mice received three weekly injections (s.c.) of 180 U rHuEPO or

diluent (control) for the duration of the experiment. Glucose in the form of D-glucose was obtained from Sigma. Insulin in the form of Actrapid HM (ge) was obtained from Novo Nordisk, Kfar Saba, Israel.

Food consumption

Food consumption was measured for 4 weeks. Pre-weighed food was provided to the mice, the remaining food was measured subsequently, and the average food consumption per week was calculated for each mouse.

Metabolic assays

Serum glucose levels were determined by Accu-Chek Go glucometer (Roche). Hemoglobin A1c (HbA1c) concentration in the blood was measured with DCA2000 analyzer (Bayer). Serum insulin levels were measured using a commercial enzyme immunoassay kit (Mercodia, Uppsala, Sweden).

Glucose tolerance test For this test, mice deprived of food for over 4 h received an i.p. injection of glucose (2 g/kg body weight). Blood samples were then obtained from the tail for glucose determination at the indicated times.

Statistical analysis

Results are expressed as the mean \pm s.e.m. All results were analyzed by Student's unpaired two-tailed *t*-test. Values of $P < 0.05$ were considered statistically significant.

Results

We first examined, under non-fasting conditions, the effect of constitutively 10–12-fold elevated EPO serum levels on blood glucose level of EPO overexpressing transgenic mice (tg6; Ruschitzka *et al.* 2000). As shown in Fig. 1A, tg6 mice are hypoglycemic as compared to their normal (wt) littermates. Hence, in the fed state, tg6 mice displayed 49% less glucose level in their blood compared to the wt. This finding raised the question of whether short exposure to EPO may also affect the blood glucose levels. We thus compared the effect of rHuEPO treatment on blood glucose levels in murine models that are associated with diabetes and obesity (Fig. 1B and C), as well as wt mice (Fig. 1D and E). In these experiments, mice were injected with rHuEPO (180 U), three times weekly on alternating days. We found that after 1 week of treatment, all rHuEPO-treated mice had lower blood glucose levels, and these levels were further reduced during the second week of treatment. Moreover, this reduction was also observed within the group of rHuEPO-treated mice as compared to their initial blood glucose levels.

To investigate the effect of EPO on glucose clearance, we performed a glucose tolerance test (GTT) in all mice (Fig. 2).

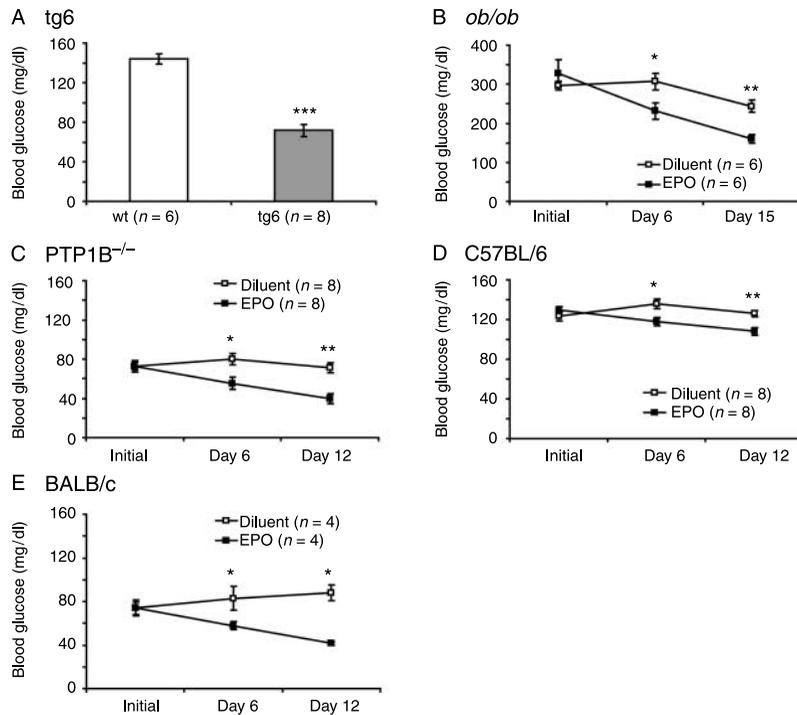


Figure 1 EPO-associated reduction in blood glucose levels. (A) Blood glucose was measured in 3-month-old tg6 mice and in their wt littermates. These measurements were under non-fasting conditions. (B–E) Murine models were injected with rHuEPO (180 U) or with diluent (control), three times weekly on alternating days, for a period of 2 weeks. Glucose measurements were performed before EPO injections (initial), and 1 and 2 weeks following EPO injections. These measurements were performed after 4 h of fasting. White and black squares represent diluent and EPO-injected mice respectively. (B) *ob/ob* mice, 8 weeks old. (C) PTP1B^{-/-} mice, 3 months old. (D) C57BL/6 mice, 3 months old. (E) BALB/c mice, 8 weeks old. The number of mice in each group is indicated. Values depict mean \pm S.E.M. * $P < 0.02$, ** $P < 0.003$, *** $P < 0.0001$.

In rHuEPO-treated mice, the test was performed during the third week of treatment. Administration of a bolus of glucose to tg6 and rHuEPO-treated mice resulted in a more rapid clearance of glucose than was observed in wt and the control group receiving just the diluent, instead of rHuEPO. In tg6 mice, a prominent hypoglycemia was evident 15 min following the glucose injection.

In order to determine whether tg6 mice are particularly sensitive to insulin, we measured serum insulin levels at fed state and performed intraperitoneal insulin tolerance test (Fig. 3). The results show that tg6 mice have lower serum insulin levels compared to their wt littermates (Fig. 3A). It thus appears that tg6 mice are more insulin sensitive, because they maintained lower glucose levels with significantly reduced insulin concentrations. This was further substantiated based on the results of the insulin tolerance test (ITT). The results indicated a hypoglycemic response in tg6 mice 15 min after i.p. insulin injection (0.5 U/Kg body weight), a state that was evident at all time points. In contrast, wt mice had a hyperglycemic response at 15 min, followed by a hypoglycemic response at the later time points (Fig. 3B).

Based on these data, we assessed the ability of EPO to affect the glucose metabolism using HbA1c measurements. This test was based on the fact that in patients with diabetes, HbA1c levels reflect the blood glucose levels during the preceding period of several months (Rohlfing *et al.* 2002). We thus measured HbA1c in tg6 and rHuEPO-treated *ob/ob* mice (Fig. 4). Due to the rapid half life of circulating erythrocytes in mice, the test was performed in *ob/ob* mice during the third week of rHuEPO treatment. HbA1c was reduced by 18 and 15% in tg6 and rHuEPO-treated *ob/ob* mice respectively, compared to their relevant control groups. Furthermore, the decrease in *ob/ob* mice was evident already after a short rHuEPO treatment.

We next questioned whether EPO has any effect on the body mass. We measured the body weight of tg6 mice as compared to their wt littermates. As shown in Fig. 5A, the body weight of tg6 mice was lower than that of wt mice in both males and females. To determine whether these weight differences result from a decrease in food intake, we estimated the amount of food consumed by the mice (Fig. 5B). The results showed that food intake was actually

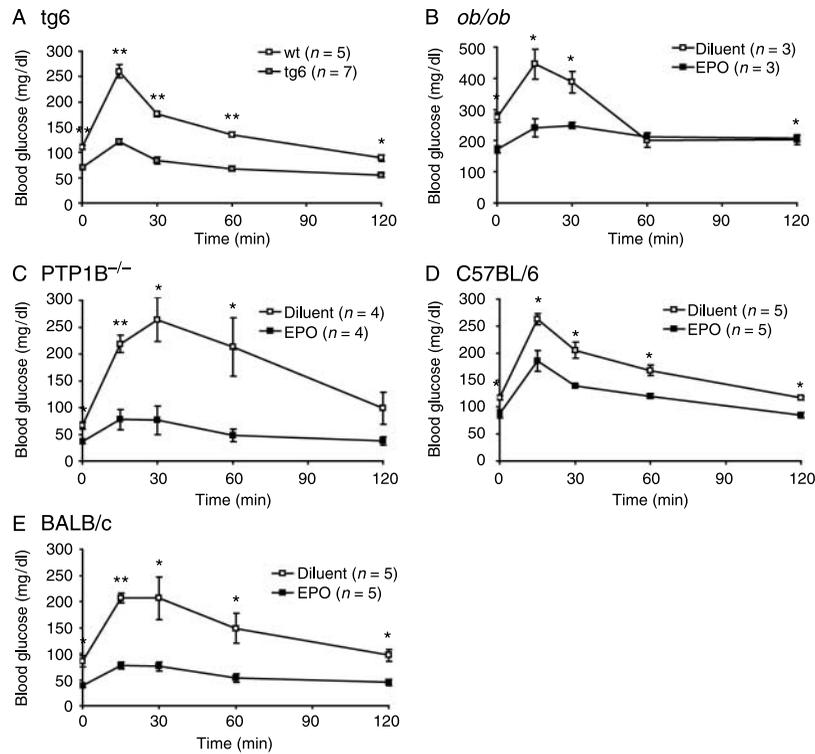


Figure 2 Improved glucose tolerance conferred by EPO. GTTs were performed in the following mouse strains: (A) tg6 and wt littermates (solid and open squares respectively); (B) *ob/ob*; (C) *PTP1B^{-/-}*; (D) C57BL/6; (E) BALB/c. For the rHuEPO- and diluent-treated mice, GTTs were performed after 2 weeks of rHuEPO or diluent injections. The number of mice in each group is indicated; solid and open squares indicate EPO- and diluent-injected mice respectively. Values depict mean \pm s.e.m. * $P < 0.03$, ** $P < 0.003$.

higher in tg6 mice than in wt mice. Hence, tg6 mice maintained low body weight, even when higher amounts of food were consumed. To further resolve the differences in body weight, we analyzed the weights of distinct tissues, including liver, muscle (gastrocnemius), epididymal fat, and spleen tissue (Fig. 6). Although histological examination of these organs revealed no overt differences, we found that tg6 mice had 42% less epididymal fat mass compared with their wt littermates. Differences in adipose tissues were also

noted in the subcutaneous skin fat; tg6 mice had very thin skin with a thin layer of subcutaneous fat. However, the blood lipids profile of the tg6 mice did not differ from those of the wt littermates (data not shown).

To evaluate the clinical significance of these EPO effects on the body mass, we examined whether rHuEPO treatment has any effect on the body mass in *ob/ob* mice. We thus measured the body weight gain and food consumption of *ob/ob* mice during 2 weeks of rHuEPO treatment (Fig. 7).

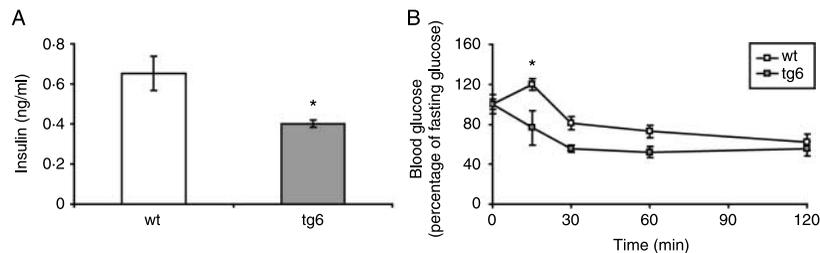


Figure 3 Lower insulin levels (A) and increased sensitivity to insulin (B) in tg6 mice. (A) Serum concentrations of insulin in fed tg6 mice and their wt littermates, $n = 5$ mice/group. * $P = 0.04$. (B) ITT was performed in tg6 mice and their wt littermates. Blood glucose values are expressed as a percentage of glucose concentration, prior to insulin administration. $n = 4$ Mice/group. Values depict mean \pm s.e.m. * $P = 0.05$.

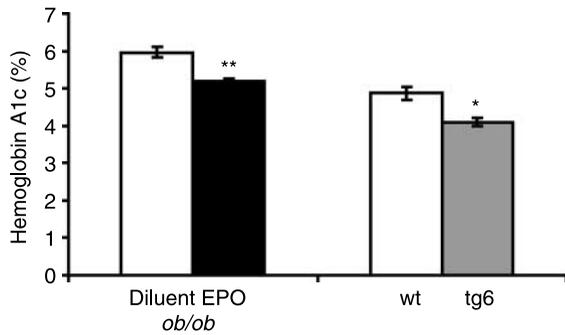


Figure 4 EPO-associated reduction in hemoglobin A1c. Groups of *ob/ob* mice were injected with rHuEPO or with diluent (control), three times weekly on alternating days for 3 weeks. HbA1c was measured in the sera of the *ob/ob* mice ($n=5$ and 6 for rHuEPO-treated and diluent-treated mice respectively) and in the tg6 mice and their wt littermates ($n=4$ and 3 tg6 and wt mice respectively). Values depict mean \pm S.E.M. * $P<0.03$, ** $P=0.001$.

The results indicated that rHuEPO-treated *ob/ob* mice gained less weight compared to the non-treated *ob/ob* mice. Hence, while non-treated *ob/ob* mice had 24% weight gain, the rHuEPO-treated *ob/ob* mice had only 17% weight gain ($P<0.004$), in spite of the fact that there was no difference in food intake between these groups.

Taken together, our data indicate that EPO has effects on glucose homeostasis, insulin sensitivity, and body mass, pointing to potential benefits to diabetic and obese patients.

Discussion

The present study deals with the emerging concept that EPO is a pleiotropic hormone, acting also beyond its role in erythropoiesis (Krantz 1991). Previous reports have documented an effect of EPO on reduction of blood glucose levels (Allegra *et al.* 1996, Cayla *et al.* 1999, Rasic-Milutinovic *et al.* 2008). Most of these studies were performed on patients suffering from diabetes, a condition linked to insulin resistance (Poithiwal *et al.* 2009). Long-term rHuEPO

therapy was found to improve glucose metabolism in maintenance hemodialysis patients, mainly by reduction of insulin resistance (Allegra *et al.* 1996). In rats, EPO treatment was also associated with lowered blood glucose levels, both in resting and under physical stress (Cayla *et al.* 1999).

In the current study, we explored the effect of EPO on glucose metabolism by employing a panel of murine experimental models. Each of these models enables a unique analysis of the *in vivo* effects of EPO on blood glucose and/or body weight. We measured these parameters under constant exposure to high EPO levels, as well as under short term exposure to EPO (EPO-injected mice). The transgenic murine model tg6 that overexpresses human EPO (Ruschitzka *et al.* 2000) was thus employed to determine the effects of long-term, constant exposure to high EPO levels. The EPO-injected mice included a) normal – wild-type C57BL/6 and BALB/c mice; b) PTP1B^{-/-} mice that are resistant to both diabetes and obesity (Elchebly *et al.* 1999, Klamann *et al.* 2000); c) *ob/ob* mice as a model for obesity and diabetes (Pelleymounter *et al.* 1995, Muzzin *et al.* 1996).

The results showed that the tg6 mice that are continuously exposed to EPO were hypoglycemic, even under non-fasting conditions. This result gained support from the experiments in which EPO was administered to mice for a period of 2 weeks. Moreover, the effect of EPO on the reduction of glucose levels was evident already 1 week following EPO administration in all the experimental models tested, including the PTP1B^{-/-} and *ob/ob* mice. This conclusion gained further support by the metabolic tests, namely GTT. EPO overexpression or administration led to improved glucose clearance in all the tested murine models. Finally, HbA1c measurements provide a surrogate marker for blood glucose levels during several months prior to the test and are routinely employed clinically for the management of diabetes (Rohlfing *et al.* 2002, Giugliano *et al.* 2008). In that respect, HbA1c levels were reduced in the rHuEPO-treated *ob/ob* mice, thus pointing to the clinical relevance of EPO treatment to improve glucose metabolism in diabetic subjects. The fact

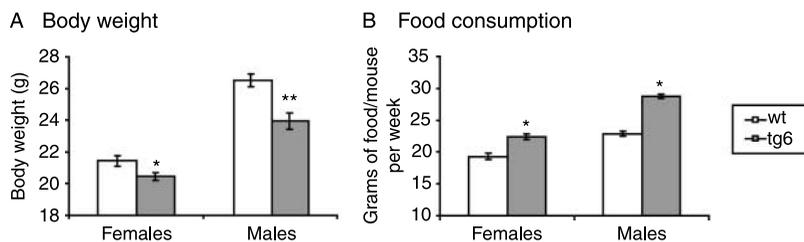


Figure 5 Reduced weight despite higher food consumption in tg6 mice. Body weight and food consumption of tg6 mice (4 months old), as compared to their wt littermates. (A) Body weight of female tg6 mice and their wt littermates ($n=17$ (tg6) and 19 (wt) mice/group) and of male tg6 mice and their wt littermates ($n=8$ (tg6) and 6 (wt) mice/group), * $P=0.02$, ** $P<0.004$. (B) Weekly food consumption of female and male tg6 mice and their wt littermates, as calculated over a period of 4 weeks. Results are expressed as grams of food/mouse per week. $n=5$ Mice/group. Values depict mean weekly consumption of food per mouse \pm S.E.M. * $P<0.004$.

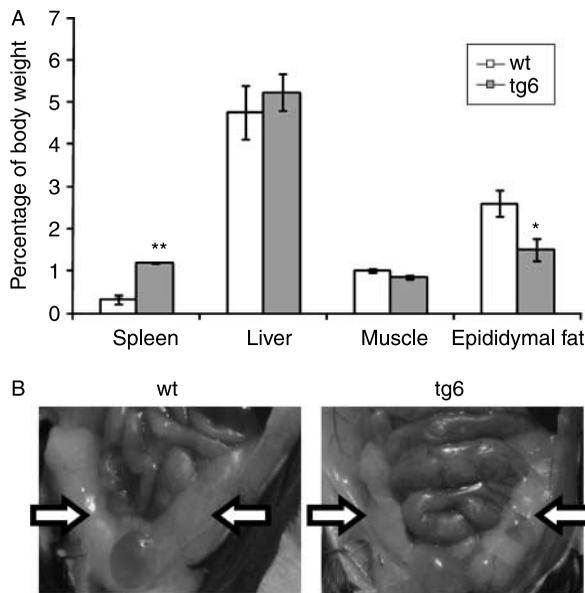


Figure 6 tg6 Mice have less epididymal fat tissue. (A) Spleens, livers, muscle (gastrocnemius), and epididymal fat were dissected from female tg6 mice and their wt littermates and were weighed. Organ weights were normalized to total body weight. $n=3$ Mice/group. Values depict mean \pm S.E.M., * $P=0.05$, ** $P=0.014$. (B) Photographs of mouse cavities; the arrows indicate the epididymal fat.

that the decrease in HbA1c was observed as early as 3 weeks following EPO administration could result from the rapid metabolism characteristic of the mice (Wickler & Gleeson 1993), thereby differing from the profile of HbA1c response in human subjects (Bloomgarden 2006). Notably, the decrease in HbA1c in the tg6 mice was in the same range as in the EPO-injected *ob/ob* mice, demonstrating that long-term as well as short-term exposures to EPO had a similar effect on this parameter, manifested in sustained levels of the HbA1c within the normal range.

What could be the mechanisms of EPO effects on blood glucose levels?

Lowered blood glucose associated with exposure to high EPO levels may result from an increase in the erythrocyte counts and their consequent uptake of glucose (Montel-Hagen *et al.* 2009). At least two adaptive mechanisms allow the tg6 mice to cope with this excessive erythrocytosis: highly elevated nitric oxide (NO) expression as well as a reduced erythrocyte life span, the latter keeping the erythrocytes young and flexible and thus, preventing the blood from becoming too viscous (Ruschitzka *et al.* 2000, Vogel *et al.* 2003, Bogdanova *et al.* 2007). Nevertheless, tg6 mice live only for about a year and die due to multiple organ degeneration (Heinicke *et al.* 2006).

Analysis of the ventilatory response in hypoxia revealed that both cerebral and circulating EPO enhanced ventilation in hypoxic tg6 male and female mice (Soliz *et al.* 2007, 2009). Interestingly, when measuring body temperature, oxygen consumption, and carbon dioxide production under normoxia and hypoxia conditions, neither of these parameters was changed compared to the wt control littermates. Nevertheless, since EPO-exposure associated reduction in body weight was observed, other possibilities cannot be excluded, including additional metabolic changes in the tg6 mice. These metabolic changes may be associated with increased energy expenditure, which could account for reduced body weight despite normal, or even increased, food intake.

Another possibility to be considered, although not necessarily mutually exclusive, is that EPO may also operate via increasing sensitivity to insulin. This is in line with our finding that the tg6 mice were more responsive to insulin and thus maintained lower glucose levels under lower levels of insulin, and insulin injection resulted actually in hypoglycemia (Fig. 3). EPO-associated increase in sensitivity to insulin was not evident in the *ob/ob* mice treated with EPO for 2–3 weeks (data not shown). Hence, it is possible that longer exposure to EPO is necessary to mediate this effect in these mice. The findings that pancreatic β -cells harbor functional EPO-Rs and that EPO acts directly on them (Fenjves *et al.* 2003) raise the possibility that EPO treatment may also affect insulin secretion by the pancreatic cells.

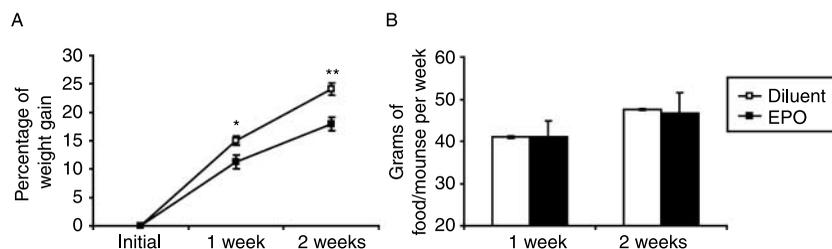


Figure 7 EPO administration attenuates weight gain in *ob/ob* mice. Weight gain (A) and food consumption (B) of *ob/ob* mice injected with EPO or with diluent (control). Body weight is presented as percentage change from initial body weight. Food consumption of individual *ob/ob* mice was measured for a period of 2 weeks. Results are expressed as grams of food/mouse per week. $n=6$ Mice/group. Values depict mean \pm S.E.M., * $P=0.03$, ** $P=0.003$.

The molecular mechanism by which EPO affects sensitivity to insulin is not yet resolved. EPO may operate via an increase in NO (Beleslin-Cokic *et al.* 2004), which is a powerful vasodilator as well as insulin sensitizer, reviewed in Marzo *et al.* (2008). Of note, tg6 mice express vast amounts of NO (Ruschitzka *et al.* 2000). In addition, the transcription factor *CEBP/α* (*Cebpa* as listed in the MGI Database) was found to decrease upon administration of EPO (Pinto *et al.* 2008). This transcription factor was found to be associated with energy homeostasis (Hackett *et al.* 1995). Thus, for example, *CEBP/α*^{-/-} mice do not display glycogen stores in the liver, they have elevated *Epo-R* mRNA in the liver, and they die of hypoglycemia shortly following birth (Wang *et al.* 1995, Zhang *et al.* 1997). Our observation that liver *CEBP/α* levels are reduced in the tg6 mice (data not shown) is in line with the possibility that the effects of EPO on glucose metabolism may also involve the reduction of this transcription factor.

Our results in the murine models led us to an insight into possible clinical applications. In that respect, we examined the effect of EPO treatment on the glucose levels and insulin consumption in a diabetic patient. Indeed, EPO treatment reduced the blood glucose levels and reduced the required doses of insulin (data not shown). These data are supported by a recent report on the effects of EPO on lowered insulin consumption and HbA1c in a diabetic patient with chronic kidney disease-induced anemia (Brown *et al.* 2009). These findings now call for an extensive clinical study on a larger scale of patients.

Concomitant with the effects of EPO on glucose levels and insulin sensitivity, we found that EPO administration or overexpression was associated with a decrease in body weight. This decrease is at least partially associated with reduced amount of fat tissue, especially evident in the leanness of the tg6 mice. Our results are in line with a recent publication demonstrating that targeted overexpression of EPO in muscle tissue of mice protected against diet induced obesity and increased metabolic parameters (Hojman *et al.* 2009). In line with this observation is our finding that EPO is associated with attenuated body weight gain in the *ob/ob* mice, despite similar food intake as the diluent-treated mice. The precise mechanism underlying the decreased body weight has yet to be elucidated. It does not appear to be related to nausea with or without reduced food intake, since this parameter was tested and ruled out. An increased energy consumption or expenditure is thus a possible mechanism, related to the main findings of this study, i.e. reduced blood glucose levels associated with EPO.

To gain insight into the molecular mediators, we questioned whether regulation of PTP1B could explain these EPO-mediated effects on glucose metabolism. Our choice to focus on PTP1B leans on the finding that it is a pivotal down-regulator of insulin signaling (Elchebly *et al.* 1999, Rondinone *et al.* 2002, Boute *et al.* 2003, Waring *et al.* 2003, Issad *et al.* 2005) and that PTP1B^{-/-} mice are resistant to obesity and diabetes (Elchebly *et al.* 1999, Klamann *et al.*

2000, Haj *et al.* 2005). Moreover, PTP1B was shown by us Cohen *et al.* (2004), and by others (Callero *et al.* 2007), to participate in downregulation of EPO signaling. Our present finding that EPO treatment culminated in reduction of blood glucose in the PTP1B^{-/-} mice thus rules out the possibility that this EPO action is mediated via PTP1B.

Various aspects of EPO action in rodent models have been documented, including prevention of diabetes-induced podocyte damage (Schiffer *et al.* 2008), enhancement of wound healing (Galeano *et al.* 2004), and promotion of vascular cell viability (Chong *et al.* 2007). Taken together, our findings single out EPO as a potential novel glucose regulator.

The current study exemplifies an exciting potential application for EPO as an adjunct anti-diabetic/obesity drug to reduce blood glucose levels and attenuate weight gain especially where these are associated with low levels of hemoglobin. Obviously, introducing EPO for these indications would require additional basic research as well as clinical trials. The idea that EPO acts on glucose metabolism places this hormone in a new and most important area, with significant clinical applications in diabetes and obesity. These are currently critical health issues at the forefront of medical and social interest.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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