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## Scientific Contributions

## Effects of Glutathione on Red Blood Cell Intracellular Magnesium

### Relation to Glucose Metabolism

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## ▶ Abstract

**Abstract**—Recent evidence suggests that the endogenous antioxidant glutathione may play a protective role in cardiovascular disease. To directly investigate the role of glutathione in the regulation of glucose metabolism in hypertension, we studied the acute effects of in vivo infusions of this antioxidant (alone or in combination with insulin) on whole body glucose disposal (WBGD) using euglycemic glucose clamp and the effects on total red blood cell intracellular magnesium (RBC-Mg) in hypertensive (n=20) and normotensive (n=30) subjects. The relationships among WBGD, circulating reduced/oxidized glutathione (GSH/GSSG) levels, and RBC-Mg in both groups were evaluated. The in vitro effects of glutathione (100  $\mu$ mol/L) on RBC free cytosolic magnesium ( $Mg_i$ ) were also studied. In vivo infusions of glutathione (15 mg/minx120 minutes) increased RBC-Mg in both normotensives and hypertensives (1.99 $\pm$ 0.02 to 2.13 $\pm$ 0.03 mmol/L,  $P$ <0.01, and 1.69 $\pm$ 0.03 to 1.81 $\pm$ 0.03 mmol/L,  $P$ <0.01, respectively). In vitro GSH but not GSSG increased  $Mg_i$  (179 $\pm$ 3 to 214 $\pm$ 5  $\mu$ mol/L,  $P$ <0.01). In basal conditions, RBC-Mg values were related to GSH/GSSG ratios ( $r$ =0.84,  $P$ <0.0001), and WBGD was directly, significantly, and independently related to both GSH/GSSG ratios ( $r$ =0.79,  $P$ <0.0001) and RBC-Mg ( $r$ =0.89,  $P$ <0.0001). This was also true when hypertensive and control groups were analyzed separately. On multivariate analysis, basal RBC-Mg ( $t$ =6.81,  $P$ <0.001), GSH/GSSG ( $t$ =3.67,  $P$ <0.02), and blood pressure ( $t$ =2.89,  $P$ <0.05) were each independent determinants of WBGD, with RBC-Mg explaining 31% of the variability of WBGD. These data demonstrate a direct action of glutathione both in vivo and in vitro to enhance intracellular magnesium and a clinical linkage between cellular magnesium, GSH/GSSG ratios, and tissue glucose metabolism.

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**Key Words:** glutathione • magnesium • hypertension, essential • glucose • insulin resistance • antioxidants

## ► Introduction

Glutathione in the reduced state (GSH) is present in human plasma and intracellularly, has antioxidant properties to inhibit free radical formation, and functions more generally as a redox buffer.<sup>1 2 3</sup> Recent evidence suggests that GSH may also be important in blood pressure and glucose homeostasis, consistent with the involvement of free radicals in both essential hypertension and diabetes mellitus.<sup>3 4 5 6</sup> Thus, glutathione infusions both lower blood pressure<sup>6</sup> and directly potentiate insulin secretion in subjects with insulin resistance and impaired glucose tolerance.<sup>3</sup> However, the mechanism(s) underlying the contribution of GSH to vascular tone and carbohydrate metabolism remains undefined.

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We have recently investigated ionic aspects of insulin resistance in diabetes and hypertension and have suggested that the depletion of intracellular free magnesium common to both conditions may help to explain their frequent clinical association,<sup>7 8 9 10 11 12</sup> especially because all kinases and other ATP-related enzymes and channels regulating insulin action<sup>11 12</sup> and vascular tone<sup>13 14</sup> are magnesium dependent. Because magnesium deficiency is also associated with increased free radical-dependent oxidative tissue damage<sup>15 16 17</sup> and because magnesium supplementation may lower blood pressure<sup>18 19 20</sup> and improve circulating glucose levels and tissue glucose oxidation in subjects with non-insulin-dependent diabetes mellitus (NIDDM),<sup>21</sup> we wondered whether changes in intracellular magnesium might mediate the relation of GSH to glucose metabolism.

Therefore, in normal and hypertensive subjects, we studied the direct effects of glutathione on total intracellular magnesium content in red blood cells (RBC-Mg) after in vivo infusions of glutathione alone or in

combination with insulin, as well as the relation of RBC-Mg to circulating levels of GSH and oxidized glutathione (GSSG) and to glucose clamp–derived indices of peripheral insulin action. The effects of glutathione on cytosolic free magnesium ( $Mg_i$ ) in vitro in red blood cells (RBCs) were as well evaluated to determine the direct effects of glutathione on  $Mg_i$ , independent of insulin or other circulating hormones and/or metabolic conditions.

## ► Methods

### In Vivo Experimental Protocol 1

After an overnight fast, 10 controls and 8 hypertensive subjects (a subset of individuals from protocol 2 below) underwent the following on different days: (1) saline infusion (120 minutes); (2) glutathione infusion (Boehringer) (15 mg/min×120 minutes); (3) insulin infusion with glucose clamp (Humulin R, Ely Lilly) (0.5 mU/kg per minute from 0 to 120 minutes and 1 mU/kg per minute from 121 to 240 minutes); and (4) infusion of glutathione and insulin (same doses as above). The tests were performed in a random order with at least 24-hour intervals. Blood samples for RBC-Mg and plasma glutathione concentration measurements were taken at baseline and at 120 and 240 minutes (end of each test).

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### In Vivo Experimental Protocol 2

After an overnight fast, a total of 50 subjects (20 hypertensives and 30 nondiabetic normotensive controls matched for age, body mass index [BMI], and gender ratio) (Table 1) underwent a 3-hour, 75-g oral glucose tolerance test (OGTT) between 9 AM and noon and euglycemic glucose clamp, in random order, with at least a 3-day interval between each test. Essential hypertension was diagnosed on the basis of outpatient blood pressure >150/90 mm Hg on at least 3 occasions and the absence of any history, physical examination, or laboratory evidence of secondary forms of hypertension. None of the subjects had a family history of diabetes, had significant renal dysfunction, or had taken medications for at least 3 weeks before the study. The protocol was approved by the Ethics Committee of our institutions, and it was conducted according to the guidelines of the Helsinki Declaration. Informed consent was obtained from each subject.

**View this table:** **Table 1.** Baseline Clinical Characteristic of the Study Subjects

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### Euglycemic Glucose Clamp and OGTT

Euglycemic glucose clamp was performed according to De Fronzo et al.<sup>22</sup> In brief, with a fixed insulin infusion rate (1 mU/kg per minute), the pump delivered a variable amount of glucose (as 30%) solution supplemented with 0.26 mmol/L KCl to maintain euglycemia and basal plasma potassium levels throughout the experiment, and blood samples were drawn for measurements of glucose and insulin at -20, -5, and 0 minutes and then every 20 minutes until the end of the test. Whole body glucose disposal (WBGD) was calculated during the final 60 minutes of the clamp as glucose infusion rate plus pool correction, in which the pool correction takes into account the change in the whole body glucose pool, as estimated from the change in plasma glucose concentration.<sup>22</sup> For OGTT, blood was obtained before and 60, 90, 120, and 180 minutes after oral glucose (75 g) administration. Plasma glucose was determined by the glucose-oxidase method (Beckman Auto-Analyzer), and serum insulin was measured with the use of standard radioimmunoassay techniques.

### Indirect Calorimetry

Indirect calorimetry was used at baseline and during the last 60 minutes of the glucose clamp to estimate the net rate of glucose and lipid oxidation and to calculate basal fat-free mass. The constants to calculate glucose and lipid oxidation from gas exchange data are those described in Reference 23<sup>23</sup>. A computerized open-circuit system was used to measure gas exchange through a 25-L polyvinylchloride plastic canopy (Deltatrac; Datas). Nonoxidative glucose metabolism was calculated as WBGD minus oxidative glucose metabolism calculated by indirect calorimetry.<sup>23</sup>

### Glutathione Measurements

Samples for plasma glutathione determinations were collected according to the techniques described by Beutler and Gelbart.<sup>2</sup> Plasma total glutathione, GSH, and GSSG levels were determined with the use of an enzymatic assay<sup>24</sup> that allows a recovery of GSH >90% and that has no appreciable interference with other thiols present in the plasma or in the reactive mixture.

### Total RBC-Mg Measurements

We used a method previously described in detail elsewhere.<sup>25</sup> Briefly, blood samples were collected into tubes containing heparin; erythrocytes were isolated by centrifugation and washed 3 times with saline solution and subsequently incubated for 90 minutes in a Krebs-Ringer buffer (NaCl<sub>2</sub> 2.5 mmol/L, MgCl<sub>2</sub> 1.2 mmol/L, and NaHCO<sub>3</sub> 20 mmol/L), continuously gassed with 95% O<sub>2</sub> and 5% CO (pH of 7.4 and 37°C). Cells were counted to normalize samples, lysed by the addition of deionized water, and centrifuged, and the supernatant was kept at -20°C until magnesium determinations in duplicates were made by atomic absorption spectrophotometry (Perkin-Elmer Co).

### In Vitro Experimental Protocol

Ten milliliters of heparinized blood was drawn from fasting normotensive, nondiabetic volunteers (n=10) (clinical data are shown in Table 1) at the Division of Endocrinology, Wayne State University Medical Center (Detroit, Mich) between 9 AM and noon. Samples were processed with the use of <sup>31</sup>P nuclear magnetic resonance (NMR) techniques for analysis of Mg<sub>i</sub> levels before (basal, time=0 minutes) and 30, 60, and 120 minutes after the in vitro addition (directly into the tube) of 100 μmol/L GSH or GSSG (Sigma). The method for <sup>31</sup>P NMR analysis of Mg<sub>i</sub> has been described in detail elsewhere.<sup>26</sup> In brief, 10 mL of heparinized blood was spun at 2000 rpm for 10 minutes, the plasma was discarded, and the remaining packed cell fraction was decanted into a 12-mm NMR tube. <sup>31</sup>P NMR spectra were recorded at 81 MHz and at 37°C for 30 minutes on an XL200 spectrometer (Varian Associates Inc) in the Fourier transform mode and with wide-band proton noise decoupling. Mg<sub>i</sub> was determined according to the following equation<sup>26</sup>:

$$Mg_i = K_d(MgATP) \times [(\phi)^{-1} - 1]$$

where  $K_d(MgATP)$  is the apparent dissociation constant for the reaction  $MgATP = Mg^{2+} + ATP = 40 \mu\text{mol/L}$  under physiological conditions at 37°C and at pH 7.2, and  $\phi = (ATP)_{\text{free}} / (ATP)_{\text{total}}$ , as determined from the chemical shift difference of the α- and β-phosphoryl group resonances of ATP in the <sup>31</sup>P NMR spectrum.

### Statistical Analysis

Data are expressed as mean±SEM. Differences between hypertensive patients and controls were assessed by unpaired *t* tests. One-way ANOVA for repeated measurements was used to compare time-dependent changes in Mg<sub>i</sub> values before and after the in vitro addition of GSH. Pearson's correlation coefficients were used to analyze the linear correlations between variables. Stepwise multivariate analysis was used to study the different contribution of basal plasma GSH/GSSG and RBC-Mg to WBGD. Differences were considered to be statistically significant for *P*<0.05.

## Results

### In Vivo Effect of Glutathione and Insulin Infusions on Total Glutathione Concentrations and on RBC-Mg

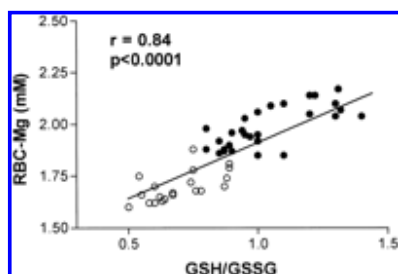
Baseline fasting plasma total glutathione concentrations were similar in normotensive and hypertensive subjects ( $0.91 \pm 0.15$  versus  $0.87 \pm 0.18$   $\mu\text{mol/L}$ ,  $P=\text{NS}$ ). At the end of glutathione infusion, plasma levels of total glutathione were also similar between the 2 groups ( $1.43 \pm 0.32$  versus  $1.49 \pm 0.24$   $\mu\text{mol/L}$ ,  $P=\text{NS}$ ). Insulin infusion did not affect plasma total glutathione concentrations when infused alone or with glutathione (data not shown). Glutathione infusion alone or with insulin at both concentrations significantly increased RBC-Mg in both groups. Saline infusion did not alter glutathione levels or RBC-Mg concentrations (Table 2). During insulin infusion, steady state plasma glucose ( $5.1 \pm 0.3$  versus  $4.9 \pm 0.4$   $\text{mmol/L}$ ,  $P=\text{NS}$ ) and insulin concentrations ( $218 \pm 35$  versus  $231 \pm 42$   $\text{pmol/L}$ ,  $P=\text{NS}$  at low insulin dose, and  $444 \pm 62$  versus  $484 \pm 72$   $\text{pmol/L}$ ,  $P=\text{NS}$ , at high insulin dose, respectively) were not different between controls and hypertensives. Despite similar metabolic conditions, the effects of insulin alone and insulin plus glutathione on RBC-Mg were stronger in controls than in hypertensives. Glutathione and high insulin infusion produced a significantly greater increase in RBC-Mg in controls than in hypertensives ( $28 \pm 3.1\%$  versus  $17 \pm 2.2\%$ , respectively,  $P < 0.03$ ) (Table 2).

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**View this table:** **Table 2.** In Vivo Effects of Glutathione and Insulin Infusions on Total RBC Intracellular Magnesium  
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### In Vivo Relationship Between Glutathione, Magnesium, and Insulin Action

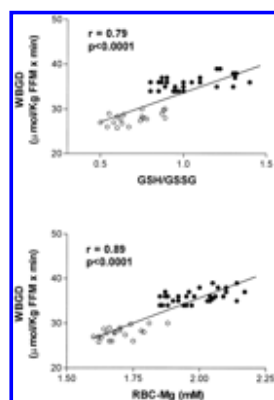
In addition to elevated blood pressure, hypertensive subjects had lower basal GSH/GSSG ratio ( $P < 0.005$ ), lower RBC-Mg ( $P < 0.01$ ), and higher fasting plasma insulin levels ( $P < 0.02$ ) than normotensive controls (Table 1). In all subjects, higher GSH/GSSG was associated with higher RBC-Mg ( $r = 0.84$ ,  $P < 0.0001$ ) (Figure 1). This relationship was also significant for each patient subgroup individually (hypertensives:  $r = 0.64$ ,  $P = 0.002$ ; normotensives:  $r = 0.62$ ,  $P < 0.001$ ). WBGD was significantly lower in hypertensives than in normotensive control subjects ( $P < 0.02$ , Table 1), despite the narrow range of plasma glucose concentrations (coefficient of variation,  $3.9 \pm 0.4\%$  versus  $4.3 \pm 0.3\%$ ,  $P = \text{NS}$ ) and equivalent steady state plasma insulin concentrations achieved ( $518 \pm 41$  versus  $561 \pm 53$   $\text{pmol/L}$ ,  $P = \text{NS}$ ). WBGD was significantly related to GSH/GSSG ratios ( $r = 0.79$ ,  $P < 0.0001$ ) and to RBC-Mg ( $r = 0.89$ ,  $P < 0.0001$ ) (Figure 2). These relations were also true when hypertensive and control groups were analyzed separately (hypertensives: WBGD versus GSH/GSSG,  $r = 0.60$ ; WBGD versus Mg,  $r = 0.56$ ; normotensives: WBGD versus GSH/GSSG,  $r = 0.47$ ; WBGD versus Mg,  $r = 0.50$ ; for all  $P < 0.01$ ).



**Figure 1.** Relation between glutathione and RBC-Mg. This relation was also significant when hypertensive  $\circ$  and normotensive  $\bullet$  subgroups were analyzed individually (normotensives:  $r = 0.62$ ,  $P < 0.001$ ; hypertensives:  $r = 0.64$ ,  $P = 0.002$ ).

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**Figure 2.** Relations of WBGD to glutathione and to RBC-Mg. These relations to WBGD were also true when hypertensive ○ and normotensive • subgroups were analyzed individually (normotensives: WBGD vs GSH/GSSG:  $r=0.47$ ,  $P<0.01$ ; WBGD vs RBC-Mg:  $r=0.50$ ,  $P<0.01$ ; hypertensives: WBGD vs GSH/GSSG:  $r=0.60$ ,  $P<0.01$ ; WBGD vs RBC-Mg:  $r=0.56$ ,  $P<0.01$ ). FFM indicates fat-free mass.

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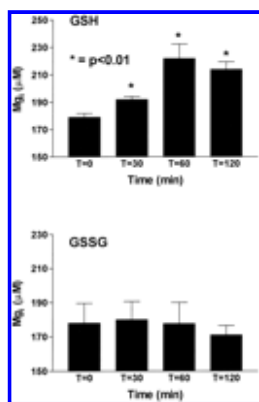
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Mechanistically, when analyzed during the last hour of the clamp procedure, hypertensive subjects exhibited a blunted stimulation of nonoxidative glucose metabolism ( $16.7 \pm 0.5$  versus  $25.8 \pm 0.4$   $\mu\text{mol/kg}$  per minute,  $P<0.05$ ) and a lesser inhibition of lipid oxidation ( $1.6 \pm 0.2$  versus  $5.8 \pm 0.4$  nmol/kg per minute,  $P<0.01$ ) compared with normotensive controls. By contrast, no differences were observed in basal oxidative glucose metabolism ( $7.3 \pm 0.3$  versus  $7.7 \pm 0.4$   $\mu\text{mol/kg}$  per minute,  $P=\text{NS}$ ) or lipid metabolism ( $10.1 \pm 0.3$  versus  $9.9 \pm 0.4$   $\mu\text{mol/kg}$  per minute,  $P=\text{NS}$ ) among the subgroups. Similarly, although basal oxidative glucose and lipid metabolism were not significantly correlated with basal plasma GSH/GSSG ratios or basal RBC-Mg, nonoxidative glucose metabolism was significantly correlated with both basal plasma GSH/GSSG (all subjects,  $r=0.45$ ,  $P<0.01$ ; controls,  $n=30$ ,  $r=0.45$ ,  $P<0.01$ ; hypertensives,  $n=20$ ,  $r=0.43$ ,  $P<0.05$ ) and RBC-Mg levels (all subjects,  $r=0.47$ ,  $P<0.01$ ; controls,  $n=30$ ,  $r=0.51$ ,  $P<0.005$ ; hypertensives,  $n=20$ ,  $r=0.57$ ,  $P<0.004$ ). These correlations were still significant after adjustment for age, gender, BMI, and mean arterial blood pressure.

Multivariate analysis allowed us to investigate the separate contribution of age, gender, BMI, mean arterial blood pressure, basal plasma GSH/GSSG, and basal RBC-Mg to WBGD. Together, these factors explained 62% of the variability of the dependent variable WBGD. In this model, basal RBC-Mg ( $t=6.81$ ,  $P<0.001$ ), basal plasma GSH/GSSG ( $t=3.67$ ,  $P<0.02$ ), and mean arterial blood pressure ( $t=2.89$ ,  $P<0.05$ ) were each significantly and independently associated with WBGD; RBC-Mg had the strongest association, accounting for 31% of the variability in WBGD.

### In Vitro Effects of Glutathione on $\text{Mg}_i$

In parallel with the in vivo results above, GSH significantly increased  $\text{Mg}_i$  levels in vitro. Basal  $\text{Mg}_i$  levels were  $179.0 \pm 2.8$   $\mu\text{mol/L}$ . Addition of GSH significantly increased  $\text{Mg}_i$  to  $192.0 \pm 2.2$ ,  $221.8 \pm 10.6$ , and  $213.9 \pm 5.4$   $\mu\text{mol/L}$  at 30, 60, and 120 minutes ( $P<0.01$  versus basal at all times) (Figure 3). The effect of GSH was specific because addition of an equal amount of oxidized glutathione, GSSG, did not significantly alter  $\text{Mg}_i$  levels ( $178.0 \pm 11.6$ ,  $180.0 \pm 10.6$ ,  $177.7 \pm 12.3$ , and  $171.1 \pm 5.4$   $\mu\text{mol/L}$  at 0, 30, 60, and 120 minutes,  $P=\text{NS}$  versus basal at all times) (Figure 3).



**Figure 3.** In vitro, direct effects of GSH and GSSG on  $Mg_i$ .

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## ► Discussion

Current interest in the protective role of endogenous antioxidant compounds such as glutathione in cardiovascular diseases led our group in the past to examine the effects of glutathione on glucose and insulin metabolism and on blood pressure in normotensive and hypertensive subjects.<sup>3 4 6</sup> Improved insulin secretion, peripheral insulin action, and decreased blood pressure were consistently observed after GSH infusions.<sup>3 4 6</sup> We have recently formulated an ionic hypothesis in which altered intracellular steady state concentrations of ions, such as magnesium, act as a final common pathway to regulate cellular metabolism in general and, in particular, cellular glucose homeostasis, insulin sensitivity, peripheral vascular tone, and blood pressure.<sup>7 8 9 10 11 12</sup> Thus, suppressed levels of  $Mg_i$  (1) directly promote cellular insulin resistance,<sup>27</sup> (2) are characteristic of insulin-resistant states, such as hypertension, and NIDDM,<sup>7 8 9 10 11 12 27 28</sup> and (3) contribute to the pressor effects of dietary salt loading in salt-sensitive hypertension.<sup>29</sup> Intracellular magnesium levels are lower in hypertensive than in normotensive subjects<sup>11 12 25 30</sup> and have been quantitatively and inversely related to systolic and diastolic blood pressure, to fasting blood glucose values, and to the hyperinsulinemic response to oral glucose loading.<sup>11 12</sup>

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Thus, we wondered to what extent the effects of glutathione on peripheral insulin action and blood pressure may be explained by interactions between glutathione and intracellular magnesium content. The present study is consistent with our hypothesis, demonstrating (1) the in vivo stimulation of total intracellular magnesium by glutathione infusions alone or in association with insulin; (2) the direct in vitro effects of glutathione in the reduced (GSH) but not oxidized (GSSG) state on free cytosolic magnesium, independently of insulin or other circulating hormones and/or metabolic conditions; (3) a similar direct relation between endogenous circulating GSH/GSSG ratios and RBC-Mg levels; and (4) the dependence of WBGD in vivo on both RBC-Mg and GSH/GSSG ratios. RBC-Mg levels were not only directly related to euglycemic clamp-derived values for WBGD but were the strongest determinant of WBGD on multivariate analysis. It is therefore reasonable to suggest not only a significant role for magnesium in glucose disposal but also that the link between glutathione levels and glucose and insulin metabolism may also, at least in part, derive from the ability of GSH to stimulate cellular magnesium concentrations.

Our results are also consistent with previous observations in the literature. Increased oxygen free radical production, which may contribute to several human diseases,<sup>31</sup> is associated with both low plasma GSH/GSSG

ratios<sup>4</sup> and with low intracellular magnesium concentrations,<sup>16</sup> and antioxidants drugs or nutrients such as  $\alpha$ -tocopherol,<sup>15 32 33</sup> probucol, and captopril<sup>33</sup> have been demonstrated to protect against magnesium deficiency-associated cerebral vascular damage<sup>32</sup> and magnesium deficiency-induced myocardial injury.<sup>15 17 33</sup> Conversely, prior magnesium depletion renders cells more sensitive to oxidative damage.<sup>15 17</sup> Furthermore, magnesium may itself possess antioxidant properties, scavenging oxygen radicals, possibly by affecting the rate of spontaneous dismutation of the superoxide ion.<sup>34</sup> Chronic hypomagnesemia results in excessive production of oxygen-derived free radicals,<sup>33</sup> supporting a role for magnesium in altering the threshold antioxidant capacity.

With regard to glucose metabolism, the mechanisms underlying the relationship between elevated plasma free radical concentration and poor insulin-mediated glucose uptake are still unclear,<sup>1 2 3 4 35</sup> but glutathione may improve glucose metabolism,<sup>3 4 36</sup> enhancing glucose-induced insulin secretion in aged patients with impaired glucose tolerance<sup>3</sup> and increasing insulin action in NIDDM patients.<sup>4</sup> Conversely, membrane-penetrating thiol oxidants impair insulin secretion.<sup>37</sup> Mechanistically, that magnesium exerts similar protective effects, that glutathione directly stimulates intracellular magnesium both in vivo and in vitro, and that there was such a close correspondence in vivo between GSH/GSSG ratios and RBC-Mg all suggest magnesium as one potential factor mediating the insulin-sensitizing effects of GSH reported. Accordingly, previous studies have indicated a role for magnesium in insulin action.<sup>7 8 9 10 11 12 21 25 26 27 38</sup> The ability of insulin to elevate cellular magnesium levels<sup>25 27 39</sup> and decreased magnesium responsiveness to insulin in cells from subjects with hypertension<sup>25 27</sup> has been demonstrated with the use of different techniques and cellular models. Furthermore, altered ionic actions of insulin in hypertension were linked with parallel alterations of insulin-mediated glucose uptake.<sup>25</sup> The insulin-induced changes in magnesium are directly proportional to the initial intracellular magnesium level, depleting normal cells of magnesium renders them "insulin resistant,"<sup>27</sup> and dietary-induced magnesium deficiency is as well associated with a decrease in insulin action.<sup>38</sup> These observations emphasize the potential contribution of altered cellular magnesium as an independent determinant of insulin action. It is noteworthy that in the present study glutathione was as potent as insulin in elevating intracellular magnesium. However, only a part of the influence of glutathione on WBGD may be mediated by magnesium, because on multivariate analysis a residual contribution of GSH/GSSG ratios to WBGD independent of RBC-Mg was observed. Thus, other mechanisms, such as a beneficial effect of plasma GSH on membrane fluidity,<sup>4</sup> may also be relevant.

Also consistent with the literature,<sup>7 8 12 40</sup> essential hypertensive subjects in the present study displayed a reduced glucose disposal together with a lower GSH/GSSG ratio compared with normotensive controls. An increased free radical activity and alterations of antioxidant status have been previously reported in essential and pediatric hypertension,<sup>5 41</sup> in women with pregnancy-induced hypertension,<sup>42</sup> and in other cardiovascular diseases.<sup>35 43</sup> Conversely, Ceriello et al<sup>6</sup> reported that glutathione infusion lowered blood pressure in hypertensive but not in diabetic hyperglycemic subjects.<sup>44</sup> Although the mechanisms of the vasodilatory action of this antioxidant have not been elucidated, these data are potentially explainable by the direct relation we observed in vivo between cellular magnesium status and circulating GSH/GSSG ratios, and the well-known, direct vasodilating actions of magnesium, which we observed to directly follow provision of glutathione. The effect of hyperglycemia on intracellular magnesium is the opposite of the effect of glutathione, with glucose decreasing intracellular free magnesium and increasing intracellular calcium.<sup>9 10 27</sup> This counteracting ionic action may also contribute to the absence of the hypotensive effect of glutathione observed in diabetic subjects with hyperglycemia.<sup>44</sup>

A possible caveat of the present study is that all the cellular data have been obtained in nonnucleated RBCs and may not apply to nucleated cells. However, our group has previously obtained similar ionic effects with insulin and glucose in nucleated and nonnucleated cells. Although RBC is not an insulin-sensitive tissue,



RBCs are insulin and glucose responsive in ionic terms. Thus, insulin has ionic effects, increasing intracellular calcium and/or magnesium in the same fashion in nucleated cells such as adipocytes,<sup>45</sup> vascular smooth muscle cells,<sup>46</sup> and lymphocytes<sup>47</sup> as well as in nonnucleated RBCs.<sup>25 48</sup> Glucose also stimulates intracellular calcium in both vascular smooth muscle cells<sup>10</sup> and RBCs.<sup>9</sup> In addition, our group has recent experience with <sup>31</sup>P NMR spectroscopic measurement of in situ intracellular free magnesium in the brain and in the muscle.<sup>49</sup> Not only were very high correlations found when magnesium was measured in insulin-sensitive (muscle) or in insulin-insensitive (brain or RBC) tissue, but the effect of glucose ingestion in vivo on intracellular magnesium with the use of <sup>31</sup>P NMR spectroscopy is similar in any of the tissues where magnesium was measured (skeletal muscle, brain, or RBC).

In conclusion, our data demonstrate for the first time a direct in vivo and in vitro action of glutathione to enhance intracellular magnesium content and show significant and independent positive relationships in vivo between intracellular magnesium content, GSH/GSSG ratios, and insulin-mediated glucose disposal. Thus, our data are consistent with a role of magnesium in mediating the effects of glutathione on peripheral insulin action.

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## ► References

- 1 Hwang C, Sinskey AJ, Lodish HF. Oxidized redox state of glutathione in the endoplasmic reticulum. *Science*. 1992;257:1496–1502. [[Abstract/Free Full Text](#)]
- 2 Beutler E, Gelbart T. Plasma glutathione in healthy and in patients with malignant disease. *J Lab Clin Med*. 1985;105:581–584. [[Medline](#)] [[Order article via Infotrieve](#)]
- 3 Paolisso G, Giugliano D, Pizza G, Gambardella A, Varricchio M, D'Onofrio F. Glutathione infusion potentiates glucose-induced insulin secretion in aged patients with impaired glucose tolerance. *Diabetes Care*. 1992;15:1–7. [[Abstract](#)]
- 4 Paolisso G, Di Maro G, Pizza G, D'Amore A, Sgambato S, Tesaro P, Varricchio M, D'Onofrio F. Plasma GSH/GSSH affects glucose homeostasis in healthy subjects and non-insulin-dependent diabetics. *Am J Physiol*. 1992;263:E435–E440. [[Abstract/Free Full Text](#)]
- 5 Sagar S, Kallo IJ, Kaul N, Ganguly NK, Sharma BK. Oxygen free radicals in essential hypertension. *Moll Cell Biochem*. 1992;111:103–108. [[Medline](#)] [[Order article via Infotrieve](#)]
- 6 Ceriello A, Giugliano D, Quatraro A, Lefebvre PJ. Antioxidants show an antihypertensive effect in diabetics and hypertensive subjects. *Clin Sci*. 1991;81:739–742. [[Medline](#)] [[Order article via Infotrieve](#)]
- 7 Barbagallo M, Resnick LM. Calcium and magnesium in the regulation of smooth muscle function and blood pressure: the ionic hypothesis of cardiovascular and metabolic diseases and vascular aging. In: Sowers JR, ed. *Endocrinology of the Vasculature*. Totowa, NJ: The Humana Press Inc; 1996:283–300.
- 8 Resnick LM, Gupta RK, Bhargava KK, Gruenspan H, Alderman MH, Laragh JH. Cellular ions in hypertension, diabetes and obesity: a nuclear magnetic resonance spectroscopic study. *Hypertension*. 1991;17:951–957. [[Abstract/Free Full Text](#)]
- 9 Resnick LM, Barbagallo M, Gupta RK, Laragh JH. Ionic basis of hypertension in diabetes mellitus: role of hyperglycemia. *Am J Hypertens*. 1993;6:413–417. [[Medline](#)] [[Order article via Infotrieve](#)]
- 10 Barbagallo M, Shan J, Pang PKT, Resnick LM. Glucose-induced alterations of cytosolic free calcium in

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cultured rat tail artery vascular smooth muscle cells. *J Clin Invest.* 1995;95:763–767.

11 Resnick LM, Gupta RK, Gruenspan H, Alderman MH, Laragh JH. Hypertension and peripheral insulin resistance: mediating role of intracellular free magnesium. *Am J Hypertens.* 1990;3:373–379.[\[Medline\]](#) [\[Order article via Infotrieve\]](#)

12 Paolisso G, Barbagallo M. Hypertension, diabetes mellitus, and insulin resistance: the role of intracellular magnesium. *Am J Hypertens.* 1997;10:346–355.[\[Medline\]](#) [\[Order article via Infotrieve\]](#)

13 Altura BM, Altura BT. Magnesium and contractions of arterial smooth muscle. *Microvasc Res.* 1974;7:145–155.[\[Medline\]](#) [\[Order article via Infotrieve\]](#)

14 Altura BM, Altura BT, Gebrewold A, Ising H, Gunter T. Magnesium deficiency and hypertension: correlation between magnesium deficient diets and microcirculatory changes *in situ*. *Science.* 1984;223:1315–1317.

15 Freedman AM, Mak IT, Stafford RE, Dickens BF, Cassidy MM, Muesing RA, Weglicki WB. Erythrocytes from magnesium-deficient hamsters display an enhanced susceptibility to oxidative stress. *Am J Physiol.* 1992;262(pt 1):C1371–C1375.

16 Rayssiguier Y, Durlach J, Gueux E, Rock E, Mazur A. Magnesium and ageing: experimental data: importance of oxidative damage. *Magnes Res.* 1993;6:369–378.[\[Medline\]](#) [\[Order article via Infotrieve\]](#)

17 Wu F, Altura BT, Gao J, Barbour RL, Altura BM. Ferrylmyoglobin formation induced by acute magnesium deficiency in perfused rat heart causes cardiac failure. *Biochim Biophys Acta.* 1994;1225:158–164.[\[Medline\]](#) [\[Order article via Infotrieve\]](#)

18 Widman L, Wester PO, Stegmayr BG, Wirell MP. The dose dependent reduction in blood pressure through administration of magnesium: a double blind placebo controlled trial. *Am J Hypertens.* 1993;6:41–45.[\[Medline\]](#) [\[Order article via Infotrieve\]](#)

19 Paolisso G, Gambardella A, Balbi V, Galzerano D, Verza M, Varricchio M, D'Onofrio F. Effects of magnesium and nifedipine on insulin action, substrate oxidation and blood pressure in aged subjects. *Am J Hypertens.* 1993;6:920–926.[\[Medline\]](#) [\[Order article via Infotrieve\]](#)

20 Altura BM, Altura BT, Gebrewold A, Ising H, Günther T. Noise-induced hypertension and magnesium in rats: relationship to microcirculation and calcium. *J Appl Physiol.* 1992;72:194–202.[\[Abstract/Free Full Text\]](#)

21 Paolisso G, Scheen AJ, Cozzolino D, Di Maro G, Varricchio M, D'Onofrio F, Lefebvre PJ. Changes in glucose turnover parameters and improvement of glucose oxidation after 4 week magnesium administration in elderly non-insulin dependent (type II) diabetic patients. *J Clin Endocrinol Metab.* 1994;78:1510–1514.[\[Abstract\]](#)

22 De Fronzo RA, Tobin JD, Anderson R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol.* 1979;237:E214–E223.[\[Abstract/Free Full Text\]](#)

23 Ferrannini E. The theoretical basis of indirect calorimetry: a review. *Metabolism.* 1988;37:287–301.[\[Medline\]](#) [\[Order article via Infotrieve\]](#)

24 Anderson ME. Determination of glutathione and glutathione sulfide in biological samples. In: Meister A, ed. *Methods in Enzymology*. New York, NY: Academic Press; 1985;113:548–557.

25 Paolisso G, Sgambato S, Giugliano D, Torella R, Varricchio M, Scheen AJ, D'Onofrio F, Lefebvre PJ. Impaired insulin-induced erythrocyte magnesium accumulation is correlated to impaired insulin-mediated glucose disposal in type 2 (non insulin-dependent) diabetic patients. *Diabetologia.* 1988;31:910–915.[\[Medline\]](#) [\[Order article via Infotrieve\]](#)

- 26 Resnick LM, Gupta RK, Sosa RE, Corbett ML, Laragh JH. Intracellular free magnesium in erythrocyte of essential hypertension. *Proc Natl Acad Sci U S A*. 1987;84:7663–7667. [[Abstract/Free Full Text](#)]
- 27 Barbagallo M, Gupta RK, Bardicef O, Bardicef M, Resnick LM. Altered ionic effects of insulin in hypertension: role of basal ion levels in determining cellular responsiveness. *J Clin Endocrinol Metab*. 1997;82:1761–1765. [[Abstract/Free Full Text](#)]
- 28 Altura BT, Altura BM. Ionized magnesium measurement in serum, plasma and whole blood in health and disease In: Smetana R, ed. *Advances in Magnesium Research*. London, England: John Libbey & Co; 1997:538–546.
- 29 Resnick LM, Gupta RK, Di Fabio B, Barbagallo M, Marion R, Laragh JH. Intracellular ionic consequences of dietary salt loading in essential hypertension. *J Clin Invest*. 1994;94:1269–1276.
- 30 Kisters K, Tepel M, Spieker C, Dietl KH, Barenbrock M, Rahn KH, Zidek W. Decreased cellular Mg<sup>2+</sup> concentrations in a subgroup of hypertensives: cell models for the pathogenesis of primary hypertension. *J Hum Hypertens*. 1997;11:367–372. [[Medline](#)] [[Order article via Infotrieve](#)]
- 31 Cross CE, Halliwell B, Borisch ET, Pryor WA, Ames BN, Saul RL, McCord JM, Harman D. Oxygen radicals and human diseases. *Ann Intern Med*. 1987;107:526–545.
- 32 Altura BM, Gebrewold A. Alpha-Tocopherol attenuates alcohol-induced cerebral vascular damage in rats: possible role of oxidants in alcohol brain pathology and stroke. *Neurosci Lett*. 1996;220:207–210. [[Medline](#)] [[Order article via Infotrieve](#)]
- 33 Weglicki WB, Bloom S, Cassidy MM, Freedman AM, Atrackchi AH, Dickens BF. Antioxidants and the cardiomyopathy of Mg-deficiency. *Am J Cardiovasc Pathol*. 1992;4:210–215. [[Medline](#)] [[Order article via Infotrieve](#)]
- 34 Afanas'ev IB, Suslova TB, Cheremisina ZP, Abramova Korkina LG. Study of antioxidant properties of metal aspartates. *Analyst*. 1995;120:859–862. [[Medline](#)] [[Order article via Infotrieve](#)]
- 35 Paolisso G, Giugliano G. Oxidative stress and insulin action: is there a relationship? *Diabeteologia*. 1996;39:357–363.
- 36 Ammon HPT, Klumpp S, Fub A, Verspol EJ, Jaecke H, Wendel A, Muller P. A possible role of plasma glutathione in glucose-mediated insulin secretion: *in vitro* and *in vivo* studies in rats. *Diabetologia*. 1989;32:797–800. [[Medline](#)] [[Order article via Infotrieve](#)]
- 37 Giugliano D, Ceriello A, Paolisso G. Diabetes mellitus, hypertension and cardiovascular disease: which role for oxidative stress? *Metabolism*. 1995;44:363–368. [[Medline](#)] [[Order article via Infotrieve](#)]
- 38 Nadler JL, Buchanan T, Natarajan R, Antonipillai I, Bergman R, Rude R. Magnesium deficiency produces insulin resistance and increased thromboxane synthesis. *Hypertension*. 1993;21:1024–1029. [[Abstract/Free Full Text](#)]
- 39 Hwang DL, Yen CF, Nadler JL. Insulin increases intracellular magnesium transport in human platelets. *J Clin Endocrinol Metab*. 1993;76:549–553. [[Abstract](#)]
- 40 Ferranini E, Buzzigoli G, Bonadonna R, Giorico MA, Oleggini M, Graziadei L, Pedrinelli R, Brandi L, Bevilacqua S. Insulin resistance in essential hypertension. *N Engl J Med*. 1987;317:350–357. [[Medline](#)] [[Order article via Infotrieve](#)]
- 41 Stojadinovic ND, Petronijevic MR, Paviaevio MH, Mrsulja BB, Kostij MM. Alteration of erythrocyte membrane Na, K, ATPase in children with borderline or essential hypertension. *Cell Biochem Funct*. 1996;14:79–87. [[Medline](#)] [[Order article via Infotrieve](#)]

- 42 Chen G, Wilson R, Cumming G, Walker JJ, Smith WE, McKillop JH. Intracellular and extracellular antioxidant buffering levels in erythrocytes from pregnancy-induced hypertension. *J Hum Hypertens*. 1994;8:37–42. [\[Medline\]](#) [\[Order article via Infotrieve\]](#)
- 43 Parik K, Allikmets K, Teesalu R, Zilmer M. Evidence for oxidative stress in essential hypertension: perspective for antioxidant therapy. *J Cardiovasc Risk*. 1996;3:49–54. [\[Medline\]](#) [\[Order article via Infotrieve\]](#)
- 44 Ceriello A, Motz E, Cavarape A, Lizzio S, Russo A, Quatraro A, Giugliano D. Hyperglycemia counterbalances the antihypertensive effects of glutathione in diabetic patients: evidence linking hypertension and glycemia through the oxidative stress in diabetes mellitus. *J Diabetes Complications*. 1997;11:250–255. [\[Medline\]](#) [\[Order article via Infotrieve\]](#)
- 45 Draznin B, Sussman KE, Eckel R, Kao M, Yost T, Sherman NA. Possible role of cytosolic free calcium concentration in mediating insulin resistance of obesity and hyperinsulinemia. *J Clin Invest*. 1988;82:1848–1852.
- 46 Kuriyama S, Nakamura K, Horiguchi M, Uchida H, Sakai O. Decreased insulin-sensitive calcium transport in cultured vascular smooth muscle cells from spontaneously hypertensive rats. *Am J Hypertens*. 1992;5:892–895. [\[Medline\]](#) [\[Order article via Infotrieve\]](#)
- 47 Barbagallo M, August PA, Resnick LM. Altered cellular calcium responsiveness to insulin in normal and preeclamptic pregnancy. *J Hypertens*. 1996;14:1081–1085. [\[Medline\]](#) [\[Order article via Infotrieve\]](#)
- 48 Barbagallo M, Gupta RK, Resnick LM. Cellular ionic effects of insulin in normal human erythrocytes: a nuclear magnetic resonance study. *Diabetologia*. 1993;36:146–149. [\[Medline\]](#) [\[Order article via Infotrieve\]](#)
- 49 Resnick LM, Bardicof O, Barbagallo M, Militianu D, Evelhock J. <sup>31</sup>P-NMR spectroscopic studies of oral glucose loading and in situ skeletal ion content in essential hypertension. *Hypertension*. 1995;26:552. Abstract.

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