Cortisol promotes endoplasmic glucose production via pyridine nucleotide redox

Zengmin Wang[1,2], Gail J Mick[2], Rongrong Xie[2,3], Xudong Wang[2], Xuemei Xie[2], Guimei Li[1] and Kenneth L McCormick[2]

[1]Department of Pediatrics, Shandong Provincial Hospital Affiliated to Shandong University, Jinan, Shandong, PR China
[2]Division of Pediatric Endocrinology, University of Alabama at Birmingham, Birmingham, Alabama, USA
[3]Department of Endocrinology, Children's Hospital of Soochow University, Suzhou, Jiangsu, PR China

Correspondence should be addressed to K McCormick or G Li
Emails
kmccormick@peds.uab.edu or gmlipeds@gmail.com

Abstract

Both increased adrenal and peripheral cortisol production, the latter governed by 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), contribute to the maintenance of fasting blood glucose. In the endoplasmic reticulum (ER), the pyridine nucleotide redox state (NADP/NADPH) is dictated by the concentration of glucose-6-phosphate (G6P) and the coordinated activities of two enzymes, hexose-6-phosphate dehydrogenase (H6PDH) and 11β-HSD1. However, luminal G6P may similarly serve as a substrate for hepatic glucose-6-phophatase (G6Pase). A tacit belief is that the G6P pool in the ER is equally accessible to both H6PDH and G6Pase. Based on our inhibition studies and kinetic analysis in isolated rat liver microsomes, these two aforesaid luminal enzymes do share the G6P pool in the ER, but not equally. Based on the kinetic modeling of G6P flux, the ER transporter for G6P (T_1) preferentially delivers this substrate to G6Pase; hence, the luminal enzymes do not share G6P equally. Moreover, cortisol, acting through 11β-HSD1, begets a more reduced pyridine redox ratio. By altering this luminal redox ratio, G6P flux through H6PDH is restrained, allowing more G6P for the competing enzyme G6Pase. And, at low G6P concentrations in the ER lumen, which occur during fasting, this acute cortisol-induced redox adjustment promotes glucose production. This reproducible cortisol-driven mechanism has been heretofore unrecognized.

Key Words
- rat liver microsomes
- cortisol
- pyridine nucleotides
- glucose

Journal of Endocrinology (2016) **229**, 25–36

Introduction

Circulating adrenal-derived cortisol, which generated intracellularly from cortisone in various tissues, contributes to maintain blood glucose homeostasis. Only the active 11-hydroxy derivatives (cortisol and corticosterone), and not their 11-oxosteroid counterparts, inhibit glucose utilization and accelerate hepatic gluconeogenesis, both of which may avert hypoglycemia.

Cortisol serves as a counter-regulatory hormone to insulin by stimulating the gene expression of phosphoenol pyruvate carboxy kinase (PEPCK) and glucose-6-phosphatase (G6Pase), two key rate-limiting enzymes of hepatic gluconeogenesis (Yabaluri & Bashyam 2010). This action is considered non-acute. Circulating cortisol is derived primarily from adrenal secretion, under the control of the hypothalamo–pituitary–adrenal (HPA) axis, and also from splanchnic production (as much as 25% of adrenal production), as for the latter non-adrenal source, 33% stems from the liver, the other 67% from

http://joe.endocrinology-journals.org
DOI: 10.1530/JOE-16-0006

Published by Bioscientifica Ltd.

visceral adipose tissue (Basu *et al.* 2004, Andrew *et al.* 2005). Few studies have confirmed that the local tissue generation of intracellular cortisol by 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) plays a role in glucose homeostasis, insulin resistance, and the metabolic syndrome (Rose & Herzig 2013).

The endoplasmic reticulum (ER) acts as a nutrient sensor in which several intraluminal biochemical pathways integrate carbohydrate and steroid metabolism (Csala *et al.* 2006). One such ER pathway is the terminal step of gluconeogenesis and glycogenolysis, namely *de novo* glucose production from the hydrolysis of glucose-6-phosphate (G6P) by G6Pase, an enzyme expressed mainly in the liver and kidneys. Heretofore, no direct acute effect of cortisol on ER glucose production has been described.

Total hepatic G6P concentrations in a 24 h-fasted rat decline to as low as 0.05–0.1 mM (Young 1966, Niewoehner *et al.* 1984, Kelmer-Bracht *et al.* 2003), whereas in the 48 h-fasted rat, the reported value is 0.068 mM (Nordlie *et al.* 1980). Limited to no information on the cellular G6P concentration in other fasted mammals has been reported, let alone the G6P concentration in the ER. After exposing rat liver microsomes to 0.2 mM exogenous G6P for 5 min, the estimated G6P concentration in microsomal vesicles was ≈0.18 mM (0.33 nmol in 1.65 μL); this luminal value increased with increasing added extra-vesicular G6P (Bánhegyi *et al.* 1997). Also, presumably, relevant to protracted fasting, it has been found that the ER content of G6P would decrease considerably with exceeding low total intracellular G6P.

During glucose production, G6P is catalyzed by G6Pase; yet, this intermediate metabolite also serves as the substrate for another luminal enzyme, tissue-specific hexose-6-phosphate dehydrogenase (H6PDH). This latter enzyme is spatially, and perhaps kinetically, linked to 11β-HSD1 (Sawada *et al.* 1981, Bánhegyi *et al.* 2004). H6PDH is a bifunctional enzyme exhibiting both hexose-6-phosphate dehydrogenase and 6-phosphogluconolactonase activities, in relation to the oxidative segment of the cytosolic pentose pathway. Although there is a wide distribution of tissues, the highest activity is found in the liver (Tanahashi & Hori 1980, Mandula *et al.* 1970, Blume *et al.* 1975, Barash *et al.* 1990). By generating NADPH, this enzyme governs the redox state of the luminal pyridine nucleotides, a pool that is kinetically, and possibly structurally, shared by 11β-HSD1. The luminal NADPH confers electrons to the oxo-reductase activity of 11β-HSD1 to such an extent that the net flux through the bidirectional 11β-HSD1 is dictated by the luminal NADPH/NADP redox milieu (Atanasov *et al.* 2004, Czegle *et al.* 2006). A high NADPH:NADP ratio, maintained by the facilitated transport of G6P and H6PDH activity, is a requisite for net 11β-HSD1 reductase activity, which, in turn, promotes cortisol production (Dzyakanchuk *et al.* 2009).

A tripartite kinetically interactive complex of G6P–H6PDH–11β-HSD1 in the ER-lumen exists (Marcolongo *et al.* 2007). It is unresolved as to whether substrate channeling exists between the G6P membrane transporter (T_1) and this complex. Do these two enzymes share a common G6P pool? This issue is addressed herein by two experimental approaches: (1) studies involving enzyme inhibition and (2) based on a mathematical construct incorporating Michaelis–Menten enzyme kinetics and calculating the measured reaction velocity ratios of these two enzymes.

An increase in intracellular G6P stimulates the oxo-reductase activity of 11β-HSD1 by altering the regulatory NADPH:NADP redox ratio (Walker *et al.* 2007, Dzyakanchuk *et al.* 2009). Conversely, NADPH may inhibit G6P oxidation by H6PDH by the law of mass action and uncompetitively with respect to G6P in rat liver microsomes (Oka *et al.* 1981) (Fig. 1). We propose an antithetical schema to the widely accepted route whereby nutrient (G6P) stimulates cortisol production in the ER. Based on this 'reverse' pathway, cortisol augments microsomal glucose production by adjusting the microsomal redox to a more reduced state, thereby curbing flux through H6PDH. Under extended fasting conditions, where the cytosolic and ER pools of G6P are reduced, the cortisol-induced restraint on flux through H6PDH provides additional G6P for G6Pase. This previously undescribed metabolic construct links a direct and acute redox effect of glucocorticoids on glucose production in the ER.

Materials and Methods

Materials

Cortisol, cortisone, corticosterone, 11-dehydrocorticosterone, G6P, NADP, vanadate, tungstate, alamethicin, and metyrapone were purchased from Sigma Chemical Co. [U-^{14}C]G6P (100 μCi/mmol) was purchased from American Radiolabeled Chemicals, Inc. (St. Louis, MO). All other reagents were of analytical grade.

Preparation of rat liver microsomes

Microsomes were prepared from the liver of overnight fasted rats according to the method as described by Raucy and Lasker (1991). Intactness of the microsomal

http://joe.endocrinology-journals.org
DOI: 10.1530/JOE-16-0006

Published by Bioscientifica Ltd.

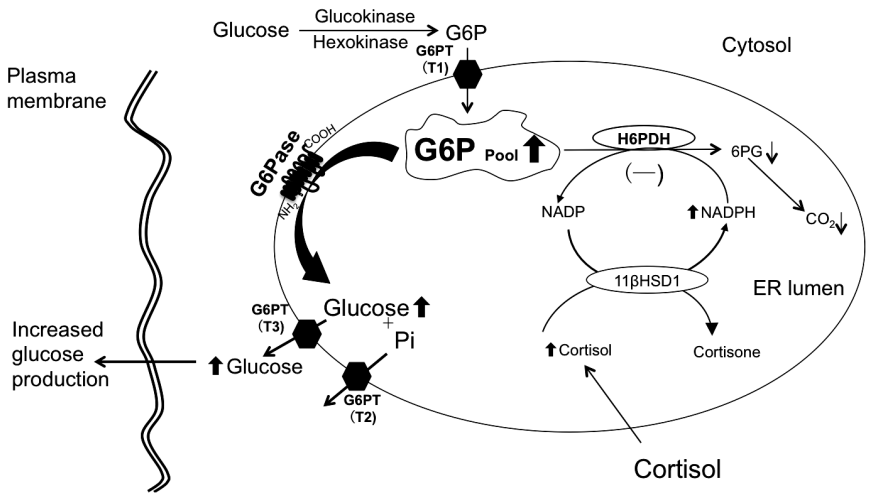


Figure 1
Illustration depicting luminal NADPH generation by cortisol and its effect on glucose production in the ER. By mass action, the increased NADPH alters the H6PDH kinetics in such a manner to increase the G6P pool. With glucose deprivation, and considering the nearly 1000-fold difference in the K_m values between G6Pase and H6PDH, and their competition for the shared substrate G6P, the increase in the luminal G6P pool due to a cortisol-induced increase in NADPH/NADP can augment glucose output.

membranes in all the preparations was verified by measuring latency of glucose dehydrogenase (>95%) (Bublitz & Steavenson 1988*b*). The aliquots were immediately frozen and maintained at −80°C until further processing.

Measurement of glucose production: G6Pase activity

G6Pase activity is based on the measurement of [^{14}C] glucose generated from [U-^{14}C]G6P, as described previously (Kitcher *et al.* 1978). Intact rat liver microsomes (0.5 mg protein/mL) were pre-incubated for 5 min with 100 μM cortisol or cortisone dissolved in DMSO, reactions were initiated upon addition of mixture containing 0.001, 0.01, 0.05, or 0.1 mM G6P plus [^{14}C]G6P (0.5 μCi/mL) with or without 5 mM NADP. Control experiment with no added steroid contained the identical amount of DMSO. Total reaction volume was 50 μL. After incubation of samples for 30 min at 37°C, the reactions were terminated by addition of 0.5 mL of 0.3 M-$ZnSO_4$ and 0.5 mL of $Ba(OH)_2$. The tubes were centrifuged (10,000 ***g***) for 2 min to separate the [^{14}C]glucose (supernatant) and [^{14}C]G6P (pellet) (Arion *et al.* 1975). Thereafter, a 0.5 mL portion of the clear supernatant was extracted to measure [^{14}C] glucose by liquid scintillation spectroscopy. Using this approach, more than 95% of glucose was recovered in the clear supernatant as assessed by spiking a reaction cocktail with a known amount of [^{14}C]glucose. The amount of [^{14}C] glucose in the blank (0 min) was subtracted from total [^{14}C]glucose after termination of the reaction. Glucose production is expressed as pmol/min/mg protein.

To reduce microsomal NADPH, the effect of cortisol on glucose production was evaluated in the presence of 1 mM metyrapone.

Measurement of microsomal CO_2 production: H6PDH activity

Microsomal H6PDH activity is based on the capture of $^{14}CO_2$ released from the conversion of [^{14}C]G6P to D-ribulose-5-phosphate (Hino & Minakami 1982a, Hino *et al.* 1987). Intact rat liver microsomes (0.5 mg protein/mL) were pre-incubated for 5 min in the presence of 100 μM cortisol or cortisone, reactions were initiated upon addition of mixture containing 0.001, 0.01, 0.05, or 0.1 mM G6P plus [^{14}C]G6P (0.5 μCi/mL) with or without 5 mM NADP. Total reaction volume was 50 μL. After incubation of samples for 30 min at 37°C, 20 μL perchloric acid (60%) was added to terminate the reaction. The $^{14}CO_2$ released was collected in center vials containing hyamine after sitting overnight. CO_2 formation is expressed as pmol/min/mg protein. All cpm of CO_2 were adjusted by the percentage of yield based on measured $^{14}CO_2$ in hyamine and a theoretical $^{14}CO_2$ release from a known amount of radiolabeled G6P in a reaction with excess NADP, glucose-6-phosphate dehydrogenase, and 6-phosphogluconate dehydrogenase.

To explore the effect of a specific G6Pase inhibitors on H6PDH activity, vanadate or tungstate was added to the reactions. Intact rat liver microsomes (0.5 mg protein/mL) were pre-incubated with 50 μM inhibitor for 5 min at 37°C. The reaction was initiated by adding the substrate

http://joe.endocrinology-journals.org
DOI: 10.1530/JOE-16-0006

Published by Bioscientifica Ltd.

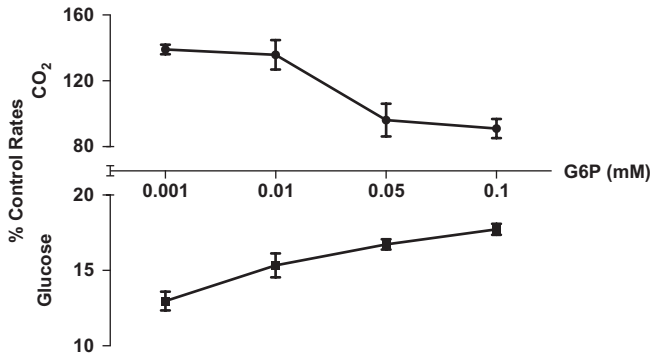


Figure 2
Glucose production and CO_2 release in intact rat liver microsomes exposed to vanadate. Intact rat liver microsomes (0.5 mg of protein/mL) were pre-treated with 50 μM vanadate for 5 min. The reaction was initiated by addition of 5 mM NADP plus various final concentration of G6P (0.001, 0.01, 0.05, and 0.1 mM) and terminated after 30 min. Vanadate inhibited glucose production in concert with an increase in CO_2. Data are presented as mean ± S.E.M. (n = 3) with 100% representing control (no vanadate). Note that the origins of the Y-axes are not set at 0.

mixture consisting of [^{14}C]G6P (0.5 μCi/mL) plus NADP (5 mM) and increasing G6P concentrations (0.001, 0.01, 0.05, and 0.1 mM) in a final volume of 50 μL after incubating at 37°C for 30 min. Reactions were terminated as described above.

Measurement of microsomal NADPH production

Intact rat liver microsomes (1 mg protein/mL) permeabilized with the pore-forming compound alamethicin (0.1 mg/mg microsomal protein) to ensure free access of the cofactor to the ER lumen (Piccirella *et al.* 2006). Thereafter, pore-formed microsomes were pre-incubated with the presence of 100 μM cortisol or cortisone for 5 min at room temperature. Reactions of the total 20 μL system were started with addition of 1 mM NADP (final concentrations) and stopped by freezing three times on solid CO_2 after 1–2 min reaction.

A sensitive micro-method was adopted for NADPH measurement based on the conversion of [U-^{14}C]-α-ketoglutarate to ^{14}C-labeled glutamate in a reaction catalyzed by excess glutamate dehydrogenase, as described previously (Sener & Malaisse 1990). NADPH production also measured upon the addition of 1 mM metyrapone. In order to see the effect of lower concentration of corticosterone on NADPH production, cortisol or cortisone was replaced by corticosterone (1.5 μM) or 11-dehydrocorticosterone (1.5 μM) respectively. The results were determined based on a standard curve in μmol/L (Fig. 3A).

Statistical analysis

The cpm basal (time = 0 min) levels in the measurements of glucose, CO_2, and NADPH were subtracted from all the subsequent time points. Data are presented as mean ± S.E.M. The significance of differences between groups was determined by Student's t-test and analysis of variance. $P < 0.05$ was considered significant. All statistical analyses were performed with GraphPad Prism software (San Diego, CA, USA).

Results

Vanadate effect on G6P pool of the intact hepatic ER

Previous studies have demonstrated that both vanadate and tungstate are specific competitive inhibitors of G6Pase in native hepatic microsomes (Singh *et al.* 1981, Foster *et al.* 1998). Vanadate is a competitive inhibitor of G6Pase in untreated microsomes with K_i of 5.6 μM (Singh *et al.* 1981). After the exposure of microsomes to 50 μM vanadate at varying G6P (substrate) concentrations of 0.001, 0.01, 0.05, and 0.1 mM, glucose production was inhibited compared with control by 87, 83.8, 83.3, and 82.3% respectively. Notably, in the presence of vanadate, as the G6P concentration was increased, there was a parallel increase in glucose production; antithetically, at the same time, this was accompanied by a decreasing trend of CO_2 release compared with control (Fig. 2). For example, at the aforementioned G6P concentration, the increase in CO_2 production versus control progressively waned: 139, 135.8, 110.8, and 103.6% respectively. In summary, the data infer that both G6Pase and H6PDH share a common luminal pool of G6P (Fig. 2). Tungstate experiments displayed nearly identical results as those of vanadate (data not shown).

Cortisol promotes luminal NADPH generation in rat liver microsomes

Microsomal membranes were permeabilized by 0.1 mg/mg microsomal protein alamethicin to facilitate the entry of NADP. Upon the addition of 100 μM cortisol and excess 1 mM NADP, a robust increase in NADPH content was detected in 2 min compared with control (no steroids), whereas no significant difference was noted with cortisone. The respective NADPH production rates were 15.31 ± 0.66 (cortisol) vs 0.78 ± 0.03 (cortisone) nmol/min/mg protein, confirming a copious increase in total NADPH due to the 11β-HSD1 reduction of cortisol ($P < 0.001$), and also significant compared with

http://joe.endocrinology-journals.org
DOI: 10.1530/JOE-16-0006

Published by Bioscientifica Ltd.

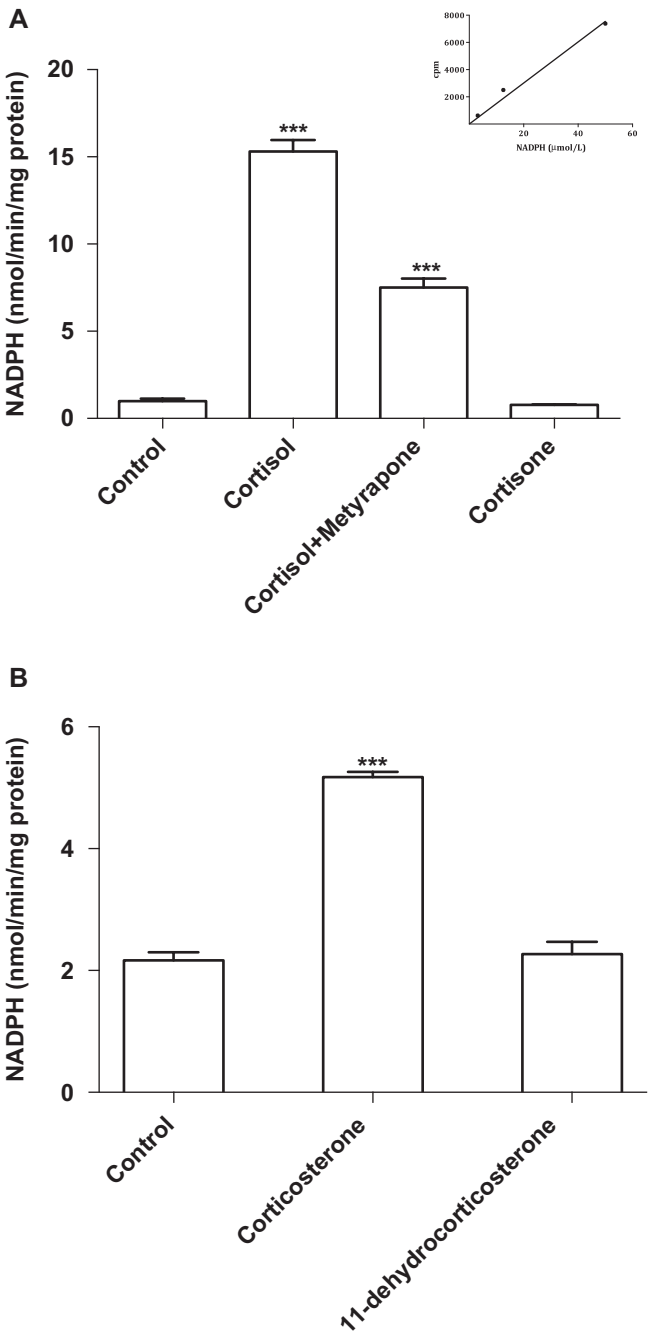


Figure 3
Effect of glucocorticoids on NADPH production in permeabilized rat liver microsomes. Alamethicin permeabilized rat liver microsomes (1 mg protein/mL) were treated for 5 min in the presence of 100 μM cortisol or cortisone. The reaction mixture did not contain G6P. Reactions were initiated by addition of 1 mM NADP and terminated after 2 min. (A) Cortisol increased NADPH production significantly compared with control and cortisone, and this effect was inhibited by metyrapone. The insert depicts a standard curve for NADPH. (B) Low concentrations of corticosterone (1.5 μM) significantly increased NADPH production, whereas 11-dehydrocorticosterone was ineffective. Data are expressed as mean ± S.E.M. of three experiments; ***$P < 0.001$.

control (0.99 ± 0.14, $P < 0.001$) (Fig. 3A). A similar increase in NADPH was found when using intact microsomes in a longer reaction (data not shown). Metyrapone inhibited NADPH production by 50.9%, which decreased to 7.51 ± 0.52 nmol/min/mg protein ($P < 0.001$) (Fig. 3A). In addition, low concentration of corticosterone (1.5 μM) still can stimulate NADPH production (5.18 ± 0.09 nmol/min/mg protein) compared with control (2.17 ± 0.13 nmol/min/mg protein, $P < 0.001$) and 11-dehydrocorticosterone (2.27 ± 0.20 nmol/min/mg protein, $P < 0.001$) respectively (Fig. 3B).

Both a high concentration of cortisol (100 μM) and a low concentration of corticosterone (1.5 μM) promoted NADPH production. Although the concentration of cortisol was approximately 66.7-fold greater than corticosterone, NADPH production was only about threefold higher (15.29 ± 0.64 vs 5.17 ± 0.08 nmol/min/mg protein).

Cortisol stimulates glucose production and inhibits CO_2 generation in rat liver microsomes

The production of glucose and CO_2 in intact rat liver microsomes (0.5 mg protein/mL) was measured with increasing G6P concentrations of 0.001, 0.01, 0.05, or 0.1 mM. In response to either added cortisol or cortisone (both at 100 μM) without NADP, cortisol increased glucose production by 48, 22, 4.7, and 10.4%, respectively, at each of the four above-mentioned G6P concentrations. Cortisol significantly augmented glucose production when compared with cortisone, an effect that was more apparent at the lowest G6P concentration. For example, G6P at 0.001 mM resulted in a significant difference ($P < 0.01$) in glucose production between cortisol and cortisone (42.9 ± 1.8 vs 29 ± 1.1 pmol/min/mg protein). However, at the higher concentration of 0.05 mM G6P, these respective values were 2152 ± 11.1 and 2028 ± 7.5 respectively (Fig. 4A). Compared with cortisone, cortisol caused a significant reduction in CO_2 formation at a G6P concentration of 0.001 and 0.01 mM. At higher G6P concentration, this difference between cortisol vs cortisone became smaller (Fig. 4B). Of note, these observations were near identical even at much lower steroid concentrations (10 μM, data not shown).

To deplete intraluminal NADPH, metyrapone (1 mM) was added to the microsomes. In support of our tenet, the metyrapone nullified the cortisol effect on glucose production at 0.01 mM G6P. Upon addition of 1 mM metyrapone, the difference in glucose production between cortisol and cortisone was no longer significant (Fig. 5).

http://joe.endocrinology-journals.org
DOI: 10.1530/JOE-16-0006

Published by Bioscientifica Ltd.

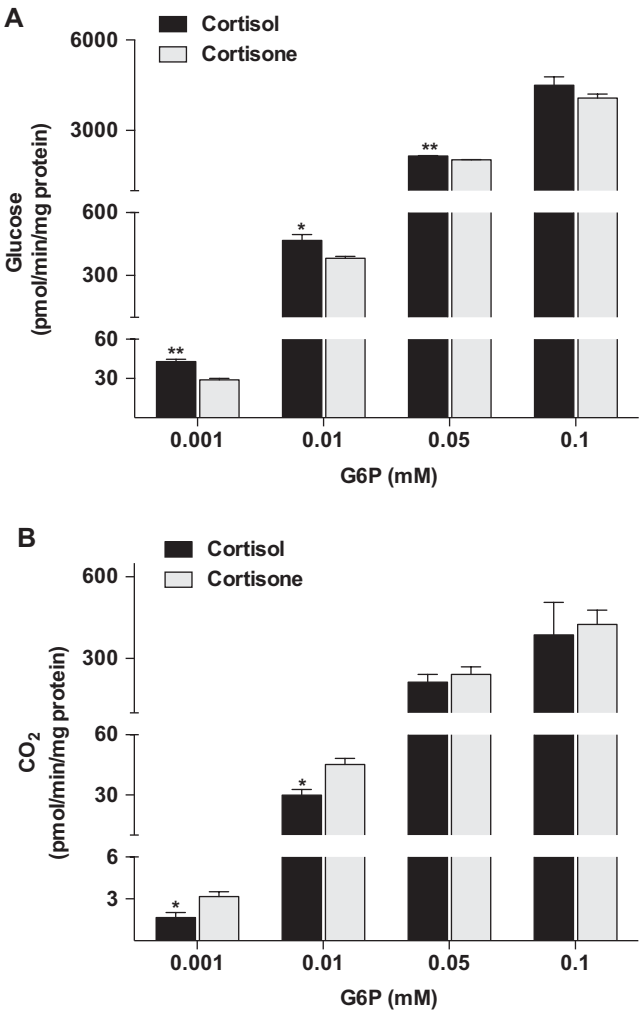


Figure 4
Cortisol and cortisone modify microsomal [^{14}C]glucose and $^{14}CO_2$ production. Intact liver microsomes (0.5 mg protein/mL) were pre-incubated with 100 μM cortisol or cortisone for 5 min, without NADP. Reaction mixture was composed of either 0.001, 0.01, 0.05, or 0.1 mM G6P. Reactions were conducted in a total volume of 50 μL and terminated after 30 min. Data are expressed as mean ± S.E.M. (n = 3~7), $^{**}P < 0.01$, $^{*}P < 0.05$ vs cortisone.

When replaced high concentrations of cortisol (100 μM) or cortisone (100 μM) by low concentrations of corticosterone (1.5 μM) or 11-dehydrocorticosterone (1.5 μM) in the reaction with 0.01 mM G6P, a significant difference was still observed between these two corticosteroids (207.9±1.7 vs 179.3±2.5 pmol/min/mg protein, $P<0.001$); however, CO_2 formation was not significant (Fig. 6).

Reaction rate ratios of glucose and CO_2 production

The velocity rates of glucose and CO_2 production in liver microsomes with cortisol and cortisone are presented

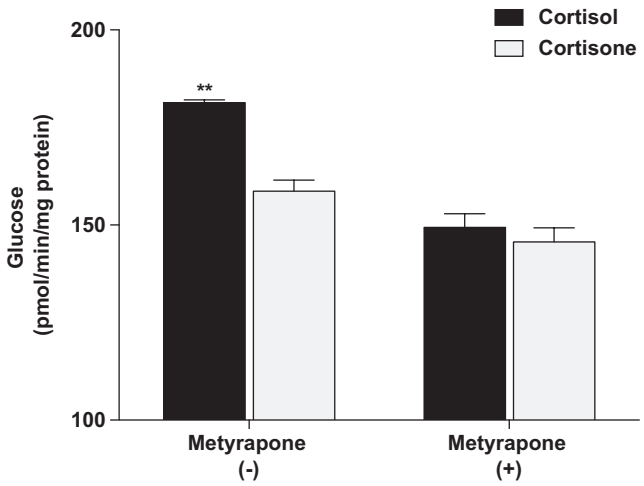


Figure 5
Stimulation of glucose production by cortisol is attenuated by metyrapone. Intact liver microsomes (0.5 mg protein/mL) were pre-incubated with 1 mM metyrapone. Thereafter, 100 μM cortisol or cortisone was added for 5 min-incubation. Reactions were initiated by addition of 0.01 mM G6P. After 30 min, reactions were terminated as described in Methods section. Before addition of metyrapone, cortisol increased glucose production by 14.3% compared with cortisone, while this stimulation was negated after addition of metyrapone. Data are represented as mean ± S.E.M. (n = 3), $^{**}P < 0.01$. Note that the origins of the Y-axes are not set at 0.

in Table 1. Using an extra-microsomal G6P substrate concentration [S_1] of 0.05 mM, the intra-microsomal concentration is approximate the same value (Bánhegyi *et al.* 1997). After overnight starvation, in rat liver microsomes, the measured ratio of V^G_{max}/V^H_{max} was found to be ≈16 (Bublitz & Steavenson 1988*a*). If the K_m for G6P is 1.7 mM for intact microsomes, the theoretical glucose:CO_2 production ratio at 0.05 mM G6P is ≈0.5–far less than the observed ratio of 10.1 (Table 1). One possible explanation is that the luminal G6P pool is not equally shared between G6Pase and H6PDH, possibly due to a more direct channeling of G6P to G6Pase by the T_1 transporter.

When NADP was added at 5 mM, there were a striking increase in CO_2 formation with 100 μM cortisol and cortisone. However, there was still a significant difference in CO_2 formation between cortisol and cortisone (Fig. 7A and B).

Discussion

The redox state maintained by the NADPH:NADP ratio plays a regulatory role in the metabolic function of the ER (Mandl *et al.* 2009). As the recognition of the inter-conversion of cortisol↔cortisone by intracellular 11β-HSD1, there has been considerable clinical interest in

http://joe.endocrinology-journals.org
DOI: 10.1530/JOE-16-0006

Published by Bioscientifica Ltd.

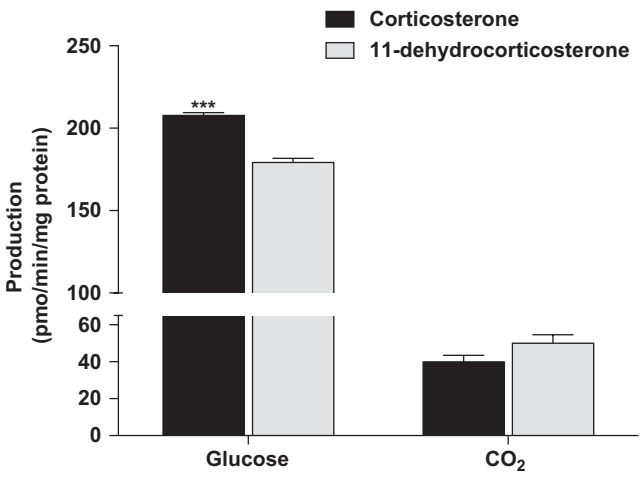


Figure 6
Corticosterone and 11-dehydrocorticosterone modify microsomal [^{14}C] glucose and $^{14}CO_2$ production. Intact liver microsomes (0.5 mg protein/mL) were pre-incubated with 1.5 μM corticosterone or 11-dehydrocorticosterone for 5 min, without NADP. Reactions were initiated by addition of 0.01 mM G6P in a total volume of 50 μL and terminated after 30 min. Data are expressed as mean ± S.E.M. (n = 4), ***P < 0.001 vs cortisone.

the paracrine and endocrine role of the enzyme in obesity and carbohydrate metabolism. Insofar, as the net flux of this bidirectional enzyme is determined by the luminal NADPH:NADP ratio, any shift of this ratio will disturb the equilibrium of intraluminal H6PDH. Because G6P can increase this pyridine nucleotide redox ratio, thereby promoting cortisol production, we reasoned that (under certain conditions) the reverse cascade may allow this 11-hydroxy steroid to directly and promptly augment ER glucose production.

First, however, experimental evidence must be adduced that the two ER enzymes (G6Pase and H6PDH) match for a common substrate (G6P). And if shared, is the G6P pool equally accessible to both enzymes? Vanadate is a potent competitive inhibitor for the phosphohydrolase activity of G6P (Singh *et al.* 1981). Depending on the G6P substrate concentration, a fixed concentration of vanadate inhibited to varying degrees microsomal glucose production with a concomitant increase in CO_2 release; an observation that supports the tenet that both enzymes compete for intraluminal G6P. In summary, luminal G6P appears to be shared between G6Pase and H6PDH.

Not unexpectedly, there is a wide disparity in the reported V_{max} and K_m values for G6Pase and H6PDH. Numerous factors account for the inconsistencies: assay conditions, temperature, pH, intact vs disrupted microsomes, crude vs. purified preparations, presence of detergents, etc. Moreover, the rat species, age, gender, or nutritional status (fed vs fasting) are inconsistent in these kinetic studies. Also, isolated *in vitro* enzyme studies disregard possible enzyme–enzyme complexes or substrate channeling. Finally, insofar, as enzyme concentration may be a key determinant of the Michaelis–Menten constants, and considering that in vitro enzyme assays are under dilute conditions, this further undermines extrapolation to living organisms.

Assuming Michaelis–Menten enzyme kinetics and, secondly, that the luminal G6P concentration approximates the extra-microsomal concentration, the observed velocity ratio of glucose to CO_2 production exceeded the theoretical ratio (Appendix). This observation suggests that there is preferential delivery of the T_1 transported G6P to G6Pase, perhaps due to coupling of the two proteins (Berteloot *et al.* 1991, Lei *et al.* 1996, Xie *et al.* 2001). Thus, the luminal pool of G6P appears to be not equally shared by the two enzymes, G6Pase and H6PDH.

With regard to the physiologically relevant concentrations of G6P in these experiments, the reported hepatic concentrations range from 0.05 to 0.1 mM. These represent total intracellular concentrations with no consideration of cytosolic compartmentalization. As for ER luminal concentrations, with an extra-microsomal G6P concentration of 0.1 mM, the estimated intra-microsomal concentration is 0.022 mM (Foster *et al.* 1991). In rats, it is

Table 1 Reaction velocities for glucose and CO_2 production.

G6P (mM)	V-glucose (pmol/min/mg protein)			V-CO_2 (pmol/min/mg protein)			V-glucose/V-CO_2		
	Control	Cortisol	Cortisone	Control	Cortisol	Cortisone	Control	Cortisol	Cortisone
0.001	37.9 ± 2.3	42.9 ± 1.8	29 ± 1.1	1.5 ± 0.6	1.7 ± 0.4	3.2 ± 0.3	14.5 ± 5.7	29.1 ± 7.1	9.4 ± 1.4
0.01	429.8 ± 29.4	467 ± 28.5	382.4 ± 8.2	37.7 ± 3.7	30 ± 2.7	45.2 ± 3.0	11.9 ± 1.6	16.0 ± 2.4	8.5 ± 0.4
0.05	2098 ± 55.8	2152 ± 11.1	2028 ± 7.5	210.7 ± 22.7	212.8 ± 28.7	241.8 ± 27.4	10.1 ± 1.1	10.5 ± 1.5	8.6 ± 0.9
0.1	5095 ± 989.4	4557 ± 274.3	4140 ± 196.9	477 ± 55.6	386.5 ± 119.7	424.8 ± 53	10.5 ± 0.9	13.4 ± 3.1	9.8 ± 1.0

G6Pase and H6PDH activities (pmol/min/mg protein) were assessed in intact rat liver microsomes as described in the Methods section. Cortisol and cortisone concentrations were 100 μM; control represents no added steroid but in the presence of diluent DMSO for the steroids. NADP was not present in the reactions. Data are expressed as mean ± S.E.M. (n = 3~7). The final concentration of DMSO in each reaction vessel was less than 0.1%.

http://joe.endocrinology-journals.org
DOI: 10.1530/JOE-16-0006

Published by Bioscientifica Ltd.

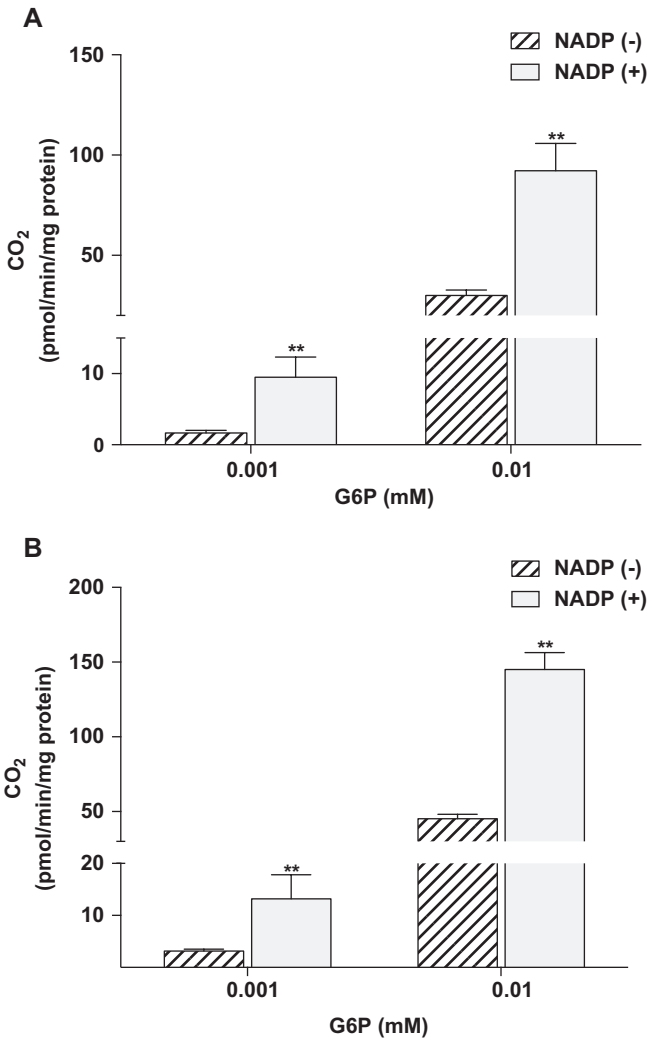


Figure 7
Effect of NADP on CO_2 formation. Intact liver microsomes (0.5 mg protein/mL) were pre-incubated with 100 μM cortisol (A) or cortisone (B) for 5 min, with or without NADP. Reactions were initiated by addition of 0.001 or 0.01 G6P. After addition of 5 mM NADP, there was a striking increase in CO_2 formation with 100 μM cortisol and cortisone. Regardless of the presence or absence of the nucleotide, there was still a significant difference in CO_2 formation between cortisol and cortisone. Data are expressed as mean ± S.E.M. ($n = 3$), $^{**}P < 0.01$.

unclear as to the hepatic intracellular G6P concentrations during prolonged fasting, let alone the corresponding ER luminal concentrations. Another consideration is that the cytosolic G6P pool itself may indeed be compartmentalized (Young 1966, Nordlie *et al.* 1980, Von Wilamowitz-Moellendorff *et al.* 2013). So the precise cytosolic G6P concentration abutting the ER is speculative.

Fasting results in a more oxidative state of the NADPH:NADP ratio in the ER lumen (Dzyakanchuk *et al.* 2009, Kereszturi *et al.* 2010). A diminished supply of intraluminal G6P, consequent to starvation or inhibition of G6PT, lowers the NADPH:NADP ratio, and cortisol synthesis will decline (Kereszturi *et al.* 2010). Serving as a substrate for 11β-HSD1, cortisone causes a concentration-dependent decrease in NADPH, whereas the opposite occurs with its hydroxy derivative, cortisol. For instance, in liver microsomes, the generation of NADPH by H6PDH was reduced more than 20% by 0.01 mM cortisone (968–741 pmol/min/mg protein) (Bánhegyi *et al.* 2004).

During fasting, cortisol production and circulating concentrations rise, without which hypoglycemia can ensue. And, even during glycogen depletion, nearly all hepatic glucose output ultimately stems from the terminal gluconeogenic enzymatic step that resides in the ER, namely G6Pase. And, in the ER lumen during fasting, the more severe the glucose deprivation, the more the NADPH:NADP redox ratio further declines (Dzyakanchuk *et al.* 2009, Kereszturi *et al.* 2010). For example, after 36 h of starvation, rat liver microsomes had a fivefold decrease in the luminal reducing:oxidizing ratio (NADPH:NADP), and in HEK-293 cells transfected with 11β-HSD1 and H6PDH, enzymes that govern the redox state of the ER, cortisol formation was attenuated significantly by glucose deprivation (from 100 to 10 mg/100mL). Reduced extracellular glucose lowers intracellular G6P which, in turn, reduces the NADPH:NADP redox ratio. Hence, because fasting lowers the NADPH:NADP redox ratio, and because cortisol acts to counteract this reduction, it is plausible that this hormone may mitigate severe hypoglycemia through this straightforward redox effect. Considering the Michaelis–Menten constants for G6Pase and H6PDH, the ability to do so depends on the intracellular G6P concentration. In the presence of 10 μM G6P for 10 min, cortisol production in rat liver microsomes increases nearly 40-fold consequent to increased NADPH production via H6PDH (Bánhegyi *et al.* 2004). Conversely, cortisone promotes G6P flux through H6PDH as this ketosteroid will deplete intraluminal endoplasmic NADPH via 11β-HSD1.

As an effective microsomal NADPH-depleting agent, metyrapone was found to reduce NADPH production by cortisol (Fig. 3A). Metyrapone appears to have multiple actions: competitive inhibitor of 11β-HSD1, an electron acceptor related to NADP, and can undergo reduction consuming NADPH (Sampath-Kumar *et al.* 1997, Marcolongo *et al.* 2008). These experiments in which the addition of metyrapone negated the difference in glucose production between cortisol and cortisone supports the role of NADPH in the schema depicted in Fig. 1.

In the 11β-HSD1 dehydrogenase reaction, the K_m for cortisol is 17 μM. However, to ensure that this substrate was unequivocally saturating, concentrations were set

http://joe.endocrinology-journals.org
DOI: 10.1530/JOE-16-0006

Published by Bioscientifica Ltd.

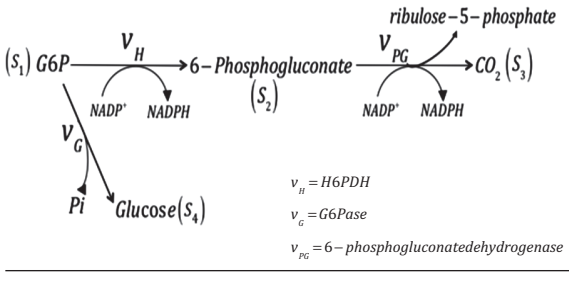
high, which were more than physiological. Yet, even when the cortisol concentration was reduced to 10 μM, similar results were obtained. Also salient is that the V_{max} and K_m for rat hepatic 11β-HSD1 in the dehydrogenase direction depends on the 11-hydroxysteroid substrate, which can be either cortisol or corticosterone (Lakshmi & Monder 1988). Notably, when corticosterone was present at a final concentration of 1.5 μM, which is in the physiological range for rats, similar percent increases in glucose production were observed (Lakshmi & Monder 1988)

The classic schema conjoins carbohydrate intake to peripheral cortisol synthesis: glucose →↑G6P →↑NADPH/NADP →11β-HSD1 →↑cortisol. Our studies, however, demonstrate that a diametric path holds true, namely cortisol reduces G6P flux through H6PDH by increasing luminal NADPH, thereby allowing more G6P for hydrolysis via G6Pase. In summary, at exceedingly low G6P concentrations during sustained fasting, cortisol can acutely augment microsomal glucose production by a direct redox action.

Appendix

Evidence can be adduced that intracellular G6P resides in several separate non-homogenous compartments, with distinct pools even within the cytosol (Christ & Jungermann 1987, Kalant *et al.* 1988, Seoane *et al.* 1996, Bandsma *et al.* 2001, Meijer 2002). After entry into the ER, it is uncertain as to whether the G6P exists in a single pool, accessible equally to two luminal enzymes, G6Pase and H6PDH. Insofar, as G6P transport and hydrolysis are coupled, it is unclear whether H6PDH has identical access to this substrate as does G6Pase. Based on our studies, the latter assumption is likely correct.

Kinetic considerations



The three endoplasmic enzymatic reactions are:

1. G6Pase: $G6P \xrightarrow{G6Pase} glucose + Pi$
2. H6PDH: $G6P + NADP^+ \xrightarrow{H6PDH} 6GP + NADPH$
3. 6-phosphogluconate dehydrogenase:
$$G6P + NADP^+ \xrightarrow{6-phosphogluconate\ dehydrogenase} CO_2 + NADPH + ribulose\text{-}5\text{-}phosphate$$

For the latter two enzymes, the general rate equation for two-substrate reactions under Michaels–Menten (MM) kinetics, wherein $A + B \rightarrow C + D$, is:

$$v = \frac{V_{max} \cdot [A] \cdot [B]}{K_m^B \cdot [A] + K_m^A \cdot [B] + [A] \cdot [B] + K_m^A \cdot K_m^B}$$

Where:

v = initial velocity

K_m^A = concentration of A for which $v = \frac{1}{2} V_{max}$, B is saturating

K_m^B = concentration of B for which $v = \frac{1}{2} V_{max}$, A is saturating

K_s^A = dissociation constant for $E + A \rightleftharpoons EA$

Regardless of the mechanism (ping-pong, ordered or random sequential), the above equation when B is saturating can be reduced to:

$$v = \frac{V_{max} \cdot [A]}{K_m^A + [A]}$$

In microsomes, if A and B represent in the above equation G6P and NADP, respectively, it is reasonable to assume that the above equation applies insofar as the NADP concentrations (B in the above equation) in intact rat liver microsomes is approximately 50 μM (Bublitz & Lawler 1987, Piccirella *et al.* 2006), far exceeding those of G6P (A in the above equation) under our experimental design. Furthermore, in our 30 min experiments, the microsomes were incubated with added NADP (5 mM).

After G6P enters the lumen, the two aforementioned enzymes produce glucose and CO_2 (plus other compounds). Luminal H6PHD has dual catalytic activity including G6P dehydrogenase and 6-phosphogluconolactonase activities. Its K_m for G6P is low (≈1.5 μM), far less than the corresponding value of 0.5–5.0 mM for G6Pase (Waddell & Burchell 1988, Waddell *et al.* 1990, Foster *et al.* 1991, Minassian *et al.* 1995, Kelmer-Bracht *et al.* 2003).

Reported Michaels–Menten constants:

K_m^B for $G6P \approx 0.5$–5.0 mM

(Waddell & Burchell 1988, Waddell *et al.* 1990, Foster *et al.* 1991, Minassian *et al.* 1995, Kelmer-Bracht *et al.* 2003),

http://joe.endocrinology-journals.org
DOI: 10.1530/JOE-16-0006

Published by Bioscientifica Ltd.

$$K_m^H \text{ for } G6P \approx \begin{matrix} 1.5\ \mu M \\ 1.9\ \mu M \end{matrix}$$

(Hino & Minakami 1982*b*, Senesi *et al*. 2010),

$$K_m^H \text{ for } NADP^+ \approx \begin{matrix} 0.14\ \mu M \\ 0.34\ \mu M \end{matrix}$$

(Hino & Minakami 1982*b*, Yl *et al*. 2009).

When the temporal production of CO_2 production is linear, the reactions are at steady state. Under our experimental conditions, using high concentrations of NADP, the two-substrate enzyme reactions (namely *v*H and *v*PG) reduce to single-substrate MM kinetics. Note: glucose efflux rates from microsomes (via the so-called T_3 transporter) are ignored as total glucose production is measured in our assay, both which exit the microsomes and any retained in the microsomes after G6P hydrolysis. Furthermore, the non-specific hydrolysis of G6P in liver microsomes is <3% of G6Pase; consequently, this component was not included in the kinetic analysis (Burchell & Burchell 1980):

$$v_H = V_{\max}^H \quad \text{because } [S_1] > K_m^H \tag{1}$$

$$v_{PG} = \frac{V_{\max} \cdot [NADP^+] \cdot [6PG]}{K_m^{PG} \cdot [NADP^+] + K_m^{NADP^+} \cdot [6PG] + [NADP^+] \cdot [6PG] + K_m^{PG} \cdot K_m^{NADP^+}} \tag{2}$$

$$v_G = \frac{V_{\max}^G \cdot [S_1]}{K_m^G} \quad \text{because } [S_1] \ K_m^G \tag{3}$$

Differential equations for these reactions:

1. $\frac{dS_1}{dt} = -V_{\max}^H - \frac{V_{\max}^G \cdot [S_1]}{K_m^G}$

2. $\frac{dS_2}{dt} = V_{\max}^H - v_{PG}$

3. $\frac{dS_3}{dt} = v_{PG}$

4. $\frac{dS_4}{dt} = \frac{V_{\max}^G \cdot [S_1]}{K_m^G}$

At steady state, $\frac{dS_2}{dt} = 0$

$$\therefore \quad V_{\max}^H = v_{PG} \tag{4}$$

glucose production $\frac{dS_4}{dt} = \left(\frac{V_{\max}^G \cdot [S_1]}{K_m^G}\right)$

CO_2 production $\frac{dS_3}{dt} = V_{\max}^H$ (after substituting from Eq. 4)

$$\therefore \frac{\text{glucose production } \frac{dS_4}{dt}}{CO_2 \text{ production } \frac{dS_3}{dt}} = \left(\frac{V_{\max}^G}{V_{\max}^H}\right) \cdot \left(\frac{[S_1]}{K_m^G}\right) \tag{5}$$

Using a G6P substrate concentration $[S_1]$ of 0.05 mM, the intra-microsomal concentration approximates the same value (Bánhegyi *et al*. 1997). And, after an overnight starvation, in rat liver microsomes, the ratio of $V_{\max}^G / V_{\max}^H$ is ≈16 (Bublitz & Steavenson 1988*a*). Using a K_m for G6P of 1.7 mM for intact microsomes, the theoretical glucose:CO_2 production ratio (Eq. 5) is ≈0.5: far less than the observed ratio of 10.1 (control). One possible explanation is that the luminal G6P pool is not equally shared between G6Pase and H6PDH, possibly due to a more direct efficient channeling of G6P to G6Pase by the T_1 transporter.

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.

Funding
This work was supported by Pediatric La Russa fund at the University of Alabama Birmingham and the China Scholarship Council.

References

Andrew R, Westerbacka J, Wahren J, Yki-Ja Rvinen H & Walker BR 2005 The contribution of visceral adipose tissue to splanchnic cortisol production in healthy humans. *Diabetes* **54** 1364–1370. (doi:10.2337/diabetes.54.5.1364)

Arion WJ, Wallin BK, Lange AJ & Ballas LM 1975 On the involvement of a glucose 6-phosphate transport system in the function of microsomal glucose 6-phosphatase. *Molecular and Cellular Biochemistry* **6** 75–83. (doi:10.1007/BF01732001)

Atanasov AG, Nashev LG, Schweizer RA, Frick C & Odermatt A 2004 Hexose-6-phosphate dehydrogenase determines the reaction direction of 11beta-hydroxysteroid dehydrogenase type 1 as an oxoreductase. *FEBS Letters* **571** 129–133. (doi:10.1016/j.febslet.2004.06.065)

Bánhegyi G, Marcolongo P, Fulceri R, Hinds C, Burchell A & Benedetti A 1997 Demonstration of a metabolically active glucose-6-phosphate pool in the lumen of liver microsomal vesicles. *Journal of Biological Chemistry* **272** 13584–13590. (doi:10.1074/jbc.272.21.13584)

Bánhegyi G, Benedetti A, Fulceri R & Senesi S 2004 Cooperativity between 11beta-hydroxysteroid dehydrogenase type 1 and hexose-6-phosphate dehydrogenase in the lumen of the endoplasmic reticulum. *Journal of Biological Chemistry* **279** 27017–27021. (doi:10.1074/jbc.M404159200)

Bandsma RH, Wiegman CH, Herling AW, Burger HJ, Ter Harmsel A, Meijer AJ, Romijn JA, Reijngoud D & Kuipers F 2001 Acute inhibition of glucose-6-phosphate translocator activity leads to increased de novo lipogenesis and development of hepatic steatosis without affecting VLDL production in rats. *Diabetes* **50** 2591–2597.

Barash V, Erlich T & Bashan N 1990 Microsomal hexose-6-phosphate and 6-phosphogluconate dehydrogenase in extrahepatic tissues: human

http://joe.endocrinology-journals.org
DOI: 10.1530/JOE-16-0006

Published by Bioscientifica Ltd.

placenta and pig kidney cortex. *Biochemistry International* **20** 267–274. (doi:10.2337/diabetes.50.11.2591)

Basu R, Singh RJ, Basu R, Chittilapilly EG, Johnson CM, Toffolo G, Cobelli C & Rizza RA 2004 Splanchnic cortisol production occurs in humans: evidence for conversion of cortisone to cortisol via the 11-beta hydroxysteroid dehydrogenase (11beta-hsd) type 1 pathway. *Diabetes* **53** 2051–2059. (doi:10.2337/diabetes.53.8.2051)

Berteloot A, Vidal H & Van De Werve G 1991 Rapid kinetics of liver microsomal glucose-6-phosphatase. Evidence for tight-coupling between glucose-6-phosphate transport and phosphohydrolase activity. *Journal of Biological Chemistry* **266** 5497–5507. (doi:10.1074/jbc.270.36.21092)

Blume KG, Schmidt GM, H. HH & Lohr GW 1975 Hexose-6-phosphate dehydrogenase from human tissues: an electrophoretic study in health and disease. *Experientia* **31** 496–498. (doi:10.1007/BF02026401)

Bublitz C & Lawler CA 1987 The levels of nicotinamide nucleotides in liver microsomes and their possible significance to function of hexose phosphate dehydrogenase. *Biochemical Journal* **245** 263–267. (doi:10.1042/bj2450263)

Bublitz C & Steavenson S 1988*a* The pentose phosphate pathwaiyn the endoplasmic reticulum. *Journal of Biological Chemistry* **263** 12849–12853.

Bublitz C & Steavenson S 1988*b* The use of glucose dehydrogenase to monitor the intergrity of microsomes. *Biochimica et Biophysica Acta* **965** 90–92. (doi:10.1016/0304-4165(88)90155-9

Burchell A & Burchell B 1980 Stabilization of partially-purified glucose 6-phosphatase by fluoride. Is enzyme inactivation caused by dephosphorylation? *FEBS Letters* **118** 180–184. (doi:10.1016/0014-5793(80)80214-6)

Christ B & Jungermann K 1987 Sub-compartmentation of the 'cytosolic' glucose 6-phosphate pool in cultured rat hepatocytes. *FEBS Letters* **221** 375–380. (doi:10.1016/0014-5793(87)80959-6)

Csala M, Banhegyi G & Benedetti A 2006 Endoplasmic reticulum: a metabolic compartment. *FEBS Letters* **580** 2160–2165. (doi:10.1016/j.febslet.2006.03.050)

Czegle I, Piccirella S, Senesi S, Csala M, Mandl J, Banhegyi G, Fulceri R & Benedetti A 2006 Cooperativity between 11beta-hydroxysteroid dehydrogenase type 1 and hexose-6-phosphate dehydrogenase is based on a common pyridine nucleotide pool in the lumen of the endoplasmic reticulum. *Molecular and Cellular Endocrinology* **248** 24–25. (doi:10.1016/j.mce.2005.10.018)

Dzyakanchuk AA, Balazs Z, Nashev LG, Amrein KE & Odermatt A 2009 11beta-hydroxysteroid dehydrogenase 1 reductase activity is dependent on a high ratio of NADPH/NADP(+) and is stimulated by extracellular glucose. *Molecular and Cellular Endocrinology* **301** 137–141. (doi:10.1016/j.mce.2008.08.009)

Foster JD, Nelson KL, Sukalski KA, Lucius RW & Nordlie RC 1991 Hysteretic behavior of the hepatic microsomal glucose-6-phosphatase system. *Biochimica et Biophysica Acta* **1118** 91–98. (doi:10.1016/0167-4838(91)90445-6)

Foster JD, Young SE, Brandt TD & Nordlie RC 1998 Tungstate- a potent inhibitor of multifunctional glucose-6-phosphatase. *Archives of Biochemistry and Biophysics* **354** 125–132. (doi:10.1006/abbi.1998.0695)

Hino Y, Ishio S & Minakami S 1987 Glucose-6-phosphate oxidation pathway in rat-liver microsomal vesicles. Stimulation under oxidative stress. *European Journal of Biochemistry* **165** 195–199. (doi:10.1111/j.1432-1033.1987.tb11211.x)

Hino Y & Minakami S 1982*a* Hexose-6-phosphate and 6-phosphogluconate dehydrogenases of rat liver microsomes involvement in NADPH and carbon dioxide generation in the luminal space of microsomal vesicles. *Journal of Biochemistry* **92** 547–557.

Hino Y & Minakami S 1982*b* Hexose-6-phosphate dehydrogenase of rat liver microsomes. *Journal of Biological Chemistry* **257** 2563–2568.

Kalant N, Parniak N & Lemieux M 1988 Compartmentation of glucose 6-phosphate in hepatocytes. *Biochemical Journal* **248** 927–931. (doi:10.1042/bj2480927)

Kelmer-Bracht AM, Santos CPB, Ishii-Iwamoto EL, Broetto-Biazon AC & Bracht A 2003 Kinetic properties of the glucose 6-phosphatase of the liver from arthritic rats. *Biochimica et Biophysica Acta* **1638** 50–56. (doi:10.1016/S0925-4439(03)00041-3)

Kereszturi É, Kálmán FS, Kardon T, Csala M & Bánhegyi G 2010 Decreased prereceptorial glucocorticoid activating capacity in starvation due to an oxidative shift of pyridine nucleotides in the endoplasmic reticulum. *FEBS Letters* **584** 4703–4708. (doi:10.1016/j.febslet.2010.10.053)

Kitcher SA, Siddle K & Luzio JP 1978 A method for the determination of glucose-6-phosphatase activity in rat liver with [U-14C]glucose 6-phosphate as substrate. *Analytical Biochemistry* **88** 29–36. (doi:10.1016/0003-2697(78)90395-0)

Lakshmi V & Monder C 1988 Purification and characterization of the corticosteroid 11 beta-dehydrogenase component of the rat liver 11 beta-hydroxysteroid dehydrogenase complex. *Endocrinology* **123** 2390–2398. (doi:10.1210/endo-123-5-2390)

Lei KJ, Chen H, Pan CJ, Ward JM, Mosinger BJ, Lee EJ, Westphal H, Mansfield BC & Chou JY 1996 Glucose-6-phosphatase dependent substrate transport in the glycogen storage disease type-1a mouse. *Nature Genetics* **13** 203–209. (doi:10.1038/ng0696-203)

Mandl J, Meszaros T, Ba´Nhegyi G, Hunyady L & Csala M 2009 Endoplasmic reticulum: nutrient sensor in physiology and pathology. *Trends in Endocrinology and Metabolism* **20** 194–201. (doi:10.1016/j.tem.2009.01.003)

Mandula B, Stivastava SK & Beutler E 1970 Hexose-6-phosphate dehydrogenase: distribution in rat tissue and effect of diet, age and steroids. *Archives of Biochemistry and Biophysics* **141** 155–161. (doi:10.1016/0003-9861(70)90118-9)

Marcolongo P, Piccirella S, Senesi S, Wunderlich L, Gerin I, Mandl J, Fulceri R, Banhegyi G & Benedetti A 2007 The glucose-6-phosphate transporter-hexose-6-phosphate dehydrogenase-11beta-hydroxysteroid dehydrogenase type 1 system of the adipose tissue. *Endocrinology* **148** 2487–2495. (doi:10.1210/en.2006-1472)

Marcolongo P, Senesi S, Gava B, Fulceri R, Sorrentino V, Margittai E, Lizak B, Csala M, Banhegyi G & Benedetti A 2008 Metyrapone prevents cortisone-induced preadipocyte differentiation by depleting luminal NADPH of the endoplasmic reticulum. *Biochemical Pharmacology* **76** 382–390. (doi:10.1016/j.bcp.2008.05.027)

Meijer A 2002 Glucose 6-phosphate compartmentation and the control of glycogen synthesis. *Journal of Hepatology* **37** 417–418. (doi:10.1016/S0168-8278(02)00166-6)

Minassian C, Daniele N, Bordet JC, Zitoun C & Mithieux G 1995 Liver glucose-6 phosphatase activity is inhibited by refeeding in rats. *Joural of Nutrition* **125** 2727–2732.

Niewoehner CB, Gilboe DP, Nuttall GA & Nuttall FQ 1984 Metabolic effects of oral fructose in the liver of fasted rats. *American Journal of Physiology* **247** 505–512.

Nordlie RC, Sukalski KA & Alvares FL 1980 Responses of glucose 6-phosphate levels to varied glucose loads in the isolated perfused rat liver. *Journal of Biological Chemistry* **255** 1834–1838.

Oka K, Takahashi T & Hori SH 1981 Differential effects of the NADPH/NADP+ ratio on the activities of hexose-6-phosphate dehydrogenase and glucose-6-phosphate dehydrogenase. *Biochimica et Biophysica Acta* **662** 318–325.

Piccirella S, Czegle I, Lizák B, Margittai E, Senesi S, Papp E, Csala M, Fulceri R, Csermely P, Mandl J *et al*. 2006 Uncoupled redox systems in the lumen of the endoplasmic reticulum. Pyridine nucleotides stay reduced in an oxidative environment. *Journal of Biological Chemistry* **281** 4671–4677. (doi:10.1074/jbc.M509406200)

Raucy JL & Lasker JM 1991 Isolation of P450 enzymes from human liver. *Methods in Enzymology* **206** 577–587. (doi:10.1016/0076-6879(91)06127-O)

http://joe.endocrinology-journals.org
DOI: 10.1530/JOE-16-0006

Published by Bioscientifica Ltd.

Rose AJ & Herzig S 2013 Metabolic control through glucocorticoid hormones: an update. *Molecular and Cellular Endocrinology* **380** 65–78. (doi:10.1016/j.mce.2013.03.007)

Sampath-Kumar R, Yu M, Khalil MW & Yang K 1997 Metyrapone is a competitive inhibitor of 11beta-hydroxysteroid dehydrogenase type 1 reductase. *Journal of Steroid Biochemistry and Molecular Biology* **62** 195–199. (doi:10.1016/S0960-0760(97)00027-7)

Sawada H, Hara A, Hayashibara M, Nakayama T, Usui S & Saeki T 1981 Microsomal reductase for aromatic aldehydes and ketones in guinea pig liver. Purification, characterization, and functional relationship to hexose-6-phosphate dehydrogenase. *Journal of Biochemistry* **90** 1077–1085.

Sener A & Malaisse WJ 1990 A sensitive radioisotopic method for the measurement of NAD(P)H: its application to the assay of metabolites and enzymatic activities. *Analytical Biochemistry* **186** 236–242. (doi:10.1016/0003-2697(90)90073-I)

Senesi S, Csala M, Marcolongo P, Fulceri R, Mandl J, Banhegyi G & Benedetti A 2010 Hexose-6-phosphate dehydrogenase in the endoplasmic reticulum. *Biological Chemistry* **391** 1–8. (doi:10.1515/BC.2009.146)

Seoane J, GóMez-Foix AM, O'doherty RM, GóMez-Ara C, Newgard CB & Guinovart JJ 1996 Glucose 6-phosphate produced by glucokinase, but not hexokinase I, promotes the activation of hepatic glycogen synthase. *Journal of Biological Chemistry* **271** 23756–23760. (doi:10.1074/jbc.271.39.23756)

Singh J, Nordlie RC & Jorgenson RA 1981 Vanadate: a potent inhibitor of multifunctional glucose-6-phosphatase. *Biochimica et Biophysica Acta* **678** 477–482.

Tanahashi K & Hori SH 1980 Immunohistochemical localization of hexose 6-phosphate dehydrogenase in various organs of the rat. *Journal of Histochemistry and Cytochemistry* **28** 1175–1182. (doi:10.1177/28.11.7430614)

Von Wilamowitz-Moellendorff A, Hunter RW, García-Rocha M, Kang L, López-Soldado I, Lantier L, Patel K, Peggie MW, Martínez-Pons C, Voss M *et al*. 2013 Glucose-6-phosphate-mediated activation of liver glycogen synthase plays a key role in hepatic glycogen synthesis. *Diabetes* **62** 4070–4082. (doi:10.2337/db13-0880)

Waddell ID & Burchell A 1988 The microsomal glucose-6-phosphatase enzyme of pancreatic islets. *Biochemical Journal* **255** 471–476. (doi:10.1042/bj2550471)

Waddell ID, Gibb L & Burchell A 1990 Calcium activates glucose-6-phosphatase in intact rat hepatic microsomes. *Biochemical Journal* **267** 549–551. (doi:10.1042/bj2670549)

Walker EA, Ahmed A, Lavery GG, Tomlinson JW, Kim SY, Cooper MS, Ride JP, Hughes BA, Shackleton CH, Mckiernan P *et al*. 2007 11beta-hydroxysterod dehydrogenase type 1 regulation by intracellular G6P provides evidence for a novel link between glucose metabolism and hypothalamo-pituitary-adrenal axis function. *Journal of Biological Chemistry* **282** 27030–27036. (doi:10.1074/jbc.M704144200)

Xie W, Van De Werve G & Berteloot A 2001 An integrated view of the kinetics of glucose and phosphate transport, and of glucose 6-phosphate transport and hydrolysis in intact rat liver microsomes. *Journal of Membrane Biology* **179** 113–126.

Yabaluri N & Bashyam MD 2010 Hormonal regulation of gluconeogenic gene transcription in the liver. *Journal of Biosciences* **35** 473–484. (doi:10.1007/s12038-010-0052-0)

Young DA 1966 Hepatic adenosine triphophate, glucose 6-phosphate, and uridine diphosphoglucose levels in fasted, adrenalectomized, and cortisol-treated rats. *Archives of Biochemistry and biophysics* **114** 309–313. (doi:10.1016/0003-9861(66)90339-0)

Zhang YL, Zhong X, Gjoka Z, Li Y, Stochaj W, Stahl M, Kriz R, Tobin JF, Erbe D & Suri V 2009 H6PDH interacts directly with 11beta-HSD1: implications for determining the directionality of glucocorticoid catalysis. *Archives of Biochemistry and Biophysics* **483** 45–54. (doi:10.1016/j.abb.2008.12.004)

Received in final form 5 February 2016
Accepted 9 February 2016
Accepted Preprint published online 9 February 2016

http://joe.endocrinology-journals.org
DOI: 10.1530/JOE-16-0006

Published by Bioscientifica Ltd.