Glut4 is upregulated despite decreased insulin signaling during prolonged fasting in northern elephant seal pups

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Viscarra JA, Vázquez-Medina JP, Crocker DE, Ortiz RM. Glut4 is upregulated despite decreased insulin signaling during prolonged fasting in northern elephant seal pups. Am J Physiol Regul Integr Comp Physiol 300: R150–R154, 2011. First published October 27, 2010; doi:10.1152/ajpregu.00478.2010.—Postprandial cellular glucose uptake is dependent on an insulin-signaling cascade in muscle and adipose tissue, resulting in the translocation of the insulin-dependent glucose transporter 4 (Glut4) into the plasma membrane. Additionally, extended food deprivation is characterized by suppressed insulin signaling and decreased Glut4 expression. Northern elephant seals are adapted to prolonged fasts characterized by high levels of plasma glucose. To address the hypothesis that the fasting-induced decrease in insulin is associated with reduced insulin signaling in prolonged fasted seals, we compared the adipose protein levels of the cellular insulin-signaling pathway, Glut4 and plasma glucose, insulin, cortisol, and adiponectin concentrations between Early (n = 9; 2–3 wks postweaning) and Late (n = 8; 6–8 wks postweaning) fasted seals. Plasma adiponectin (230 ± 13 vs. 177 ± 11 ng/ml), insulin (2.7 ± 0.4 vs. 1.0 ± 0.1 μU/ml), and glucose (9.8 ± 0.5 vs. 8.0 ± 0.3 mM) decreased, while cortisol (124 ± 6 vs. 257 ± 30 nM) doubled with fasting. Glut4 increased (31%) with fasting despite the significant decreases in the cellular content of phosphorylated insulin receptor, insulin receptor substrate-1, and Akt2. Increased Glut4 may have contributed to the decrease in plasma glucose, but the decrease in insulin and insulin signaling suggests that Glut4 is not insulin-dependent in adipose tissue during prolonged fasting in elephant seals. The reduction of plasma glucose independent of insulin may make these animals an ideal model for the study of insulin resistance.

REMARKS

Glut4 expression is likely conserved in animals that are adapted to prolonged fasting. It is probable that there is increased Glut4 expression when fasting. However, the plasma glucose decreases and the insulin decreases. It is possible that the fasting increase in Glut4 expression and the decrease in insulin signaling make these animals a tractable model for the study of fasting-induced insulin resistance.

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The curves were constructed using Prism 5 Software (GraphPad Software, La Jolla, CA). All samples were analyzed in duplicate and run in a single assay with intra-assay, percent coefficients of variability of <10% for all assays. Blood glucose was measured on a clinical autoanalyzer (Roche Diagnostics, Somerville, NJ). Nonesterified fatty acids were determined by an enzymatic colorimetric method (Wako, Richmond, VA).

**RESULTS**

**Fasting decreases body mass.** Mean body mass decreased 18% (P < 0.001) over the sampling period during the postweaning fast in elephant seal pups (Early group: 112 ± 11 vs. 92 ± 5 kg).

**Concentrations of circulating parameters in fasting elephant seals.** There was an 18% decrease (P < 0.001) in mean plasma glucose (Early group: 9.8 ± 0.5 vs. Late group: 8.0 ± 0.3 mM) (Fig. 1) as well as a 63% decrease in plasma insulin (P < 0.01; 2.7 ± 0.4 vs. 1.0 ± 0.1 μU/ml) during prolonged fasting (Fig. 1). Mean plasma cortisol doubled (P < 0.001; 124 ± 6 vs. 257 ± 30 nM) late in the fast (Fig. 1), while plasma adiponectin decreased 23% (P < 0.001; 230 ± 13 vs. 177 ± 11 ng/ml) (Fig. 1). Mean plasma nonesterified fatty acids increased by 41% (0.65 ± 0.03 mM vs. 0.92 ± 0.05 mM) late in the fast.

**PPARγ and AMPK.** The expression of PPARγ was measured to determine the differentiation and insulin sensitivity status of adipocytes. AMPK was measured to evaluate the effect of fasting on the energy state of adipocytes. PPARγ content decreased (P < 0.001) 25% (Early group: 0.71 ± 0.03 vs. Late group: 0.53 ± 0.01) with fasting, while the phosphorylation of AMPK (p-AMPK-to-AMPK ratio) increased (P < 0.05) 17% (0.92 ± 0.03 vs. 1.08 ± 0.06) (Fig. 2).

**Insulin signaling pathway in adipose tissue is downregulated during prolonged fasting in elephant seals.** The expression of proteins involved in insulin signaling was measured in
adipose tissue to evaluate the effect of prolonged fasting on insulin-dependent glucose metabolism. Prolonged fasting decreased the (P < 0.01) ratio of p-IR to IR-β (Early group: 1.08 ± 0.02 vs. Late group: 0.66 ± 0.02), p-IRS-1 to IRS-1 (1.43 ± 0.05 vs. 0.48 ± 0.02), and p-Akt to Akt2 (P < 0.05; 0.58 ± 0.01 vs. 0.36 ± 0.03). The protein content of PI3-kinase decreased (P < 0.001) 38% (1.22 ± 0.03 vs. 0.76 vs. 0.02) with fasting (Fig. 3).

Glut4 content in adipose tissue increases with prolonged fasting in elephant seals. The expression of Glut4 was measured in the membrane-bound fraction of adipose tissue homogenate to evaluate its involvement in the clearance of circulating glucose. Despite the decrease in circulating insulin and proteins involved in insulin signaling, prolonged fasting increased (P < 0.01) adipose Glut4 content by 31% (Early group: 100 ± 4 vs. Late group: 131 ± 2%) (Fig. 4).

**DISCUSSION**

Despite the decrease in plasma insulin during the fast, plasma glucose also decreased with fasting. Glucocorticoids decrease insulin sensitivity in skeletal muscle and adipose tissue and stimulate hepatic gluconeogenesis (23, 29, 31), both of which can increase plasma insulin levels. However, the
Glut4 UPREGULATED DURING PROLONGED FASTING

To the best of our knowledge, the present study is the first to investigate the cellular insulin signaling pathway in a mammal that is adapted to a state of chronic hyperglycemia. Thus, the present study provides a unique perspective on mammalian insulin signaling as well as the initial description of a mammal, which not only tolerates chronically elevated glucose concentrations, but may actually require them to maintain proper function (5, 13). From an evolutionary perspective, the regulation of metabolic substrates during prolonged fasting (2–3 mo) are not well defined, so this study provides a more comprehensive understanding of the cellular mechanisms evolved by elephant seals to regulate carbohydrate. A recent study using the hibernating ground squirrel has shown increases in the expression of Glut4 in muscle during periods of reduced insulin secretion (i.e., torpor, hibernation) (36). Although fasting and hibernation result in different physiological responses, this suggests that the upregulation of Glut4 may be a shared trait between mammals that are adapted to prolonged food deprivation. Eventually, elucidation of the cellular and

Glucocorticoid-related increase in plasma insulin can be attenuated by a direct inhibitory effect of glucocorticoids on insulin release from pancreatic β-cells (1, 3, 11, 16, 22, 27, 30, 34). In the present study, the decrease in plasma insulin, which occurs concurrently with the increase in plasma cortisol, suggests that cortisol may have suppressed insulin secretion as has been reported previously (27).

Glucocorticoids play a critical adaptive role in regulating carbohydrate and lipid metabolism. During stressful conditions, such as prolonged food deprivation, elevated glucocorticoid release stimulates hepatic gluconeogenesis and increases the concentration of free fatty acids in plasma (12, 14, 22, 38). Both of these conditions counteract the actions of insulin in various tissues either by inhibiting the IR or by decreasing the Glut4-mediated uptake of glucose (29, 31). In the present study, fasting was associated with a twofold increase in plasma cortisol and an 18% decrease in plasma glucose, suggesting that glucose utilization is much greater than glucocorticoid-induced endogenous glucose production (5, 18, 32, 33). This decrease in plasma glucose in the presence of a 31% increase in adipose Glut4 corroborates the suggestion that tissue (i.e., adipose) glucose uptake is increased.

Glut4 protein was upregulated in the presence of reduced insulin signaling. Although we measured no significant changes in the total protein expression of IR, IRS-1, or Akt2, the significant decreases in their phosphorylated forms along with a decrease in PI3-kinase late in the fast is indicative of diminished insulin signaling via decreased phosphorylation. The decrease in insulin signaling was associated with a 63% decrease in plasma insulin, suggesting that adipose was responding to the decrease in stimulus (plasma insulin). Alternatively, the decrease in plasma glucose associated with decreased plasma insulin may suggest an increase in insulin sensitivity. However, the decrease in insulin and insulin signaling in the presence of a 23% decrease in plasma adiponectin would argue against that alternative and suggests that the elephant seal develops insulin resistance over the course of its fast (25). This is reinforced further by the decrease of PPARγ whose expression has been associated with insulin sensitivity in humans (6, 25). The decreases in PPARγ and plasma adiponectin also demonstrate that the adipocytes were mature (15), and so differentiation does not likely contribute to the observed increase in Glut4. Because it is not typically upregulated during an insulin-resistant state or with fasting, the increased expression of Glut4 in adipose tissue during prolonged fasting is a unique observation.

Additionally, AMPK has been shown to stimulate glucose uptake by increasing the translocation of Glut4 in adipocytes (39). Increased phosphorylation of AMPK (increased the ratio of p-AMPK to AMPK) late in the fast may have contributed to increased Glut4 translocation in adipose tissue that may have contributed to the decrease in plasma glucose. Thus, the upregulation of Glut4 in adipose tissue during prolonged fasting suggests that there is an insulin-independent increase in Glut4 expression and subsequent cellular glucose uptake, which may result from increased AMPK activity.

In conclusion, the parallel decreases in plasma glucose and insulin in conjunction with the increase in plasma cortisol suggest that insulin secretion is suppressed via increased cortisol. The decrease in intracellular adipose insulin signaling corresponds with a decrease in plasma insulin and plasma adiponectin, suggesting that there is a decrease in insulin sensitivity. This reduced insulin signaling, and likely reduced insulin sensitivity, is the consequence of reduced phosphorylation events. Despite the decrease in insulin signaling, the insulin-responsive glucose transporter, Glut4, is still upregulated in adipose tissue in prolong fasted seals. This suggests that Glut4 translocation is not insulin-dependent in this model and may rely instead on AMPK activity. Furthermore, the increase in adipose Glut4 coincides with the decrease in plasma glucose, suggesting that glucose uptake and utilization is increased in spite of suppressed insulin signaling. Despite the modest decrease in plasma glucose, the maintenance of high levels may be imperative to sustain increased glucose utilization by tissues to support many physiological functions during the fast and/or in anticipation of the ensuing diving life style following their extended fasts.

**Perspectives and Significance**

Fig. 4. Mean ± SE % change from control (Early group) of protein expression and a representative Western blot of adipose glucose transporter 4 (Glut4) from the Early (2–3 wk postweaning; n = 9) and Late (6–8 wk postweaning; n = 8) groups of prolong-fast ed seals. # Significant (P < 0.01) difference from Early group.
systemic mechanisms of glucose regulation in an adapted mammal has the potential to provide valuable insight to the therapeutic intervention in type 2 diabetes mellitus patients.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES


