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Transferrin and iron induce insulin resistance of glucose transport in adipocytes

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Abstract

Normal serum can increase the rate of lipolysis in isolated adipocytes. Recently, we reported that the lipolytic effect of serum could be partly explained by effects of iron and transferrin. To further investigate these effects on fat cell metabolism, we have investigated effects of serum, iron, and transferrin on glucose transport in isolated rat adipocytes. Adipocytes were isolated by collagenase digestion of rat epididymal fat pads, and glucose transport was measured as uptake of [^{3}H]2-deoxyglucose, measured in the presence of 0 to 25 ng/mL insulin. Insulin stimulated glucose transport approximately 8- to 10-fold, with a half-maximally effective concentration (EC_{50}) of approximately 0.15 ng/mL. This was not affected by 45-minute treatment with normal human serum. However, when adipocytes were incubated with serum for 4 hours, cells became markedly insulin resistant. This was manifested as decrease in maximally stimulated glucose transport and a rightward shift in the dose-response curve. Both $FeS0_4$ (3 μg/mL) and transferrin (100 μg/mL) had similar, although less pronounced effects on insulin-stimulated glucose transport. Treatment of adipocytes with palmitic acid (120 μmol/L), representing the concentration of fatty acids released into the media after 4 hours of serum treatment, did not alter the effect of insulin on glucose transport. We conclude that transferrin and iron induce insulin resistance of glucose transport in adipocytes through a mechanism independent of fatty acids. These findings may further explain the association between body iron stores and risk of type 2 diabetes mellitus.

1. Introduction

Several reports published more than 30 years ago demonstrated that addition of normal serum to isolated rat adipocytes results in a prolonged increase in the rate of lipolysis [1-4]. Although the mechanism of this phenomenon has remained largely unknown, we recently reported that the effect of serum can be at least partly accounted for by stimulatory effects of transferrin and associated iron [5].

In the last few years there have been several reports of an association between total body iron stores and risk of development of type 2 diabetes mellitus [6-8]. Based on these findings, we have hypothesized that transferrin and iron increase the rate of adipocyte lipolysis, resulting in increased circulating free fatty acids (FFA) [5]. Such an increase in FFA would be expected to impair glucose uptake in muscle [9], increase hepatic glucose uptake [10,11], and impair beta-cell function [11,12]. Together, these effects would produce insulin resistance and raise the risk of type 2 diabetes mellitus.

In the course of our continuing studies of adipocyte metabolism, we found that serum also has pronounced effects on the insulin sensitivity and responsiveness of glucose transport. Here we report that, in addition to stimulating lipolysis, serum results in marked insulin resistance of adipocytes. Furthermore, the effect of serum on glucose transport, like its effect on lipolysis, can be at least partly accounted for by transferrin and associated iron. These findings may give further insight into the mechanism underlying the relationship between body iron stores and diabetes.

2. Experimental procedures

2.1. Materials

Bovine serum albumin was from Intergen (Purchase, NY); collagenase, type 2, was from Worthington (Freehold, NJ); human serum was from SeraCare Life Sciences (Oceanside, CA); and 2-deoxy-[^{3}H]glucose was from

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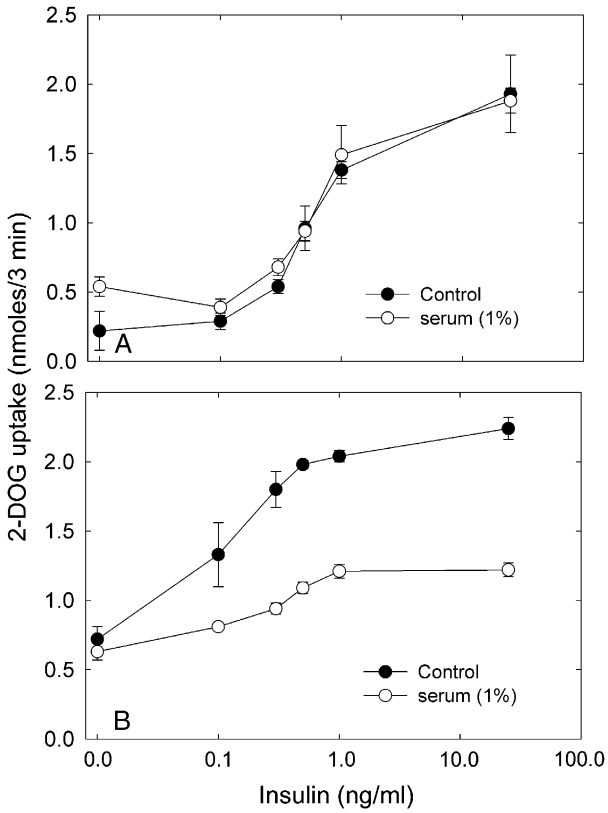

Fig. 1. Serum induces insulin resistance of glucose transport in adipocytes. In panel A, adipocytes were incubated with insulin as indicated, in the absence (●) or presence (○) of 1% human serum for 45 minutes, and then the rate of 2-deoxyglucose uptake was measured over the last 3 minutes. In panel B, adipocytes were incubated with or without serum for 4 hours and then washed and incubated with insulin for 45 minutes. Data are mean ± SE (n = 3).

Amersham (Piscataway, NJ). All other reagents were from Sigma (St Louis, MO) unless otherwise noted.

2.2. Animals

Male Sprague-Dawley rats were used for all experiments. Animals (180-240 g) were purchased from Harlan Sprague-Dawley (Indianapolis, IN). They were maintained on a 12-hour light-dark cycle and fed PROLAB RMH 1000, obtained from PMI Nutrition International (Brentwood, MO) and tap water ad libitum.

2.3. Adipocyte isolation

Animals were killed by carbon dioxide asphyxiation. Animal protocols were approved by the Bassett Healthcare's Institutional Animal Care and Use Committee. Adipocytes were isolated from epididymal fat pads as previously described [13,14]. Digestion was carried out at 37°C with constant shaking (140 cycles/min) for 26 minutes. Cells were filtered through nylon mesh (1 mm) and washed 3 times with glucose transport buffer (GTB) containing 137 mmol/L NaCl, 5 mmol/L KCl, 4.2 mmol/L $NaHCO_3$, 1.3 mmol/L $CaCl_2$, 0.5 mmol/L $MgCl_2$, 0.5 mmol/L $MgSO_4$, 0.5 mmol/L KH_2PO_4, 20 mmol/L HEPES (pH 7.6), plus 1% bovine serum albumin. All experiments were carried out in buffers equilibrated with room air.

2.4. Treatment of adipocytes

After isolation, adipocytes were resuspended 1:20 (wt/vol) in GTB and 1-mL aliquots were incubated with shaking (140 cycles/min) in 12 × 75-mm polypropylene tubes containing serum and/or other additions as indicated for 1 to 4 hours at 37°C.

For experiments on effects of fatty acids, palmitic acid was dissolved in ethanol at a concentration of 20 mmol/L and then added slowly with stirring to GTB, which contains 1% bovine serum albumin, to a final concentration of 120 μmol/L. This protocol results in a ratio of only approximately 0.8 mol of fatty acid to 1 mol of albumin, which can bind up to 6 mol of FFA.

2.5. Glucose transport assay

After cell culture, adipocytes were washed 3 times in 137 mmol/L NaCl, 5 mmol/L KCl, 4.2 mmol/L $NaHCO_3$, 1.3 mmol/L $CaC1_2$, 0.5 mmol/L KH_2PO_4, 0.5 mmol/L $MgCl_2$, 0.5 mmol/L $MgSO_4$, 20 mmol/L HEPES (pH 7.4), plus 1% bovine serum albumin. Uptake of 2-deoxy[^{3}H]glucose was used as an index of the rate of glucose transport as previously described [15].

3. Results

Several reports, including our own, have demonstrated that normal serum has a potent lipolytic effect on isolated adipocytes [1-5]. To determine whether serum affects other aspects of adipocyte metabolism, we investigated the effect of human serum on glucose transport in isolated rat adipocytes. Epididymal adipocytes were incubated for 45 minutes with or without serum (1%) in the presence of 0 to 25 ng/mL insulin,

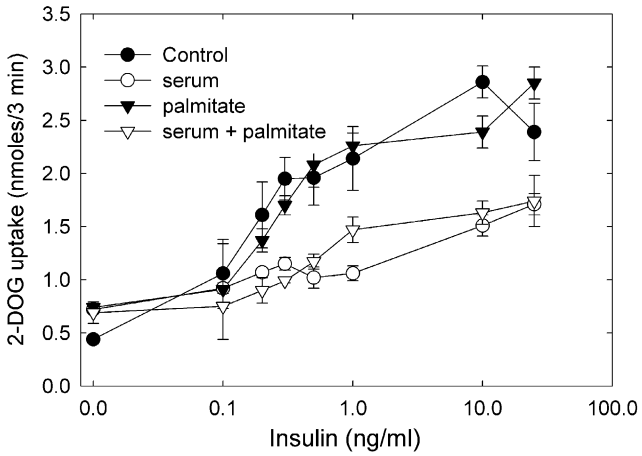

Fig. 2. The effect of serum is not due to FFA accumulation. Adipocytes were incubated for 4 hours with no additions (●), 1% human serum (○), 120μmol/L palmitate (▼), or 120 μmol/L palmitate plus 1% serum (▽). The cells were washed and then incubated with insulin at the concentrations indicated for 45 minutes, and glucose transport (2-deoxyglucose uptake) was measured over the final 3 minutes. Data are mean ± SE (n = 3).

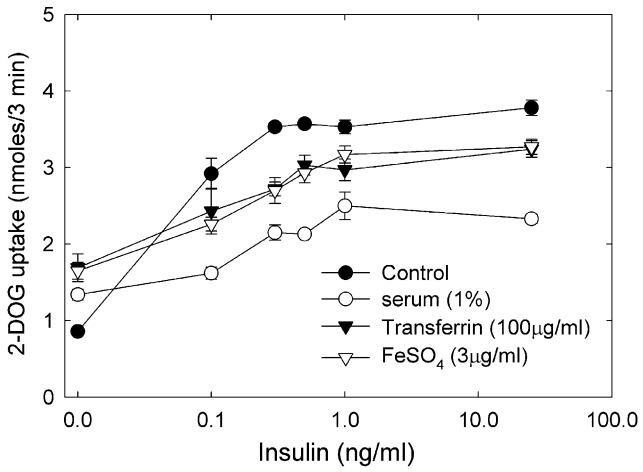

Fig. 3. Transferrin and iron partially account for the effect of serum on glucose transport. Adipocytes were incubated for 4 hours with no additions (●), 1% human serum (○), 100 μg/mL transferrin (▼), or 3 μg/mL $FeSO_4$ (▽). The cells were washed and then incubated with insulin at the concentrations indicated for 45 minutes, and glucose transport (2-deoxyglucose uptake) was measured over the final 3 minutes. Data are mean ± SE (n = 3).

and then the rate of glucose transport was determined. We have previously reported that 30 to 60 minutes of treatment of adipocytes with 1% serum results in a pronounced increase in the rate of lipolysis [5]. However, as shown in Fig. 1A, glucose transport was completely unaffected. In a second experimental approach, the cells were preincubated with 1% serum for 4 hours, washed, and then incubated with insulin (0-25 ng/mL) for 45 minutes. As shown in Fig. 1B, this prolonged treatment with serum treatment resulted in pronounced insulin resistance. The maximally stimulated rate of 2-deoxyglucose uptake in the serum-treated cells was almost 50% lower than in control cells, indicating that serum produced marked insulin resistance in adipocytes.

Fatty acids are known to induce insulin resistance, and so we hypothesized that the effect of serum was secondary to its lipolytic effect. To investigate this, we first determined the fatty acid concentration achieved after a 4-hour incubation of adipocytes with 1% serum and found this to be approximately 120 μmol/L (data not shown). We then incubated adipocytes in the absence or presence of 120 μmol/L palmitic acid for 4 hours and determined the effect of insulin on glucose transport as before (Fig. 2). This experiment confirmed that serum produced marked insulin resistance of glucose transport. However, palmitic acid did not affect glucose transport, either in the absence or presence of serum. This finding demonstrates that the effect of serum on glucose transport is not secondary to increased fatty acid release from the cells.

We have demonstrated that the lipolytic effect of serum can be largely accounted for by the presence of transferrin that, at concentrations equivalent to those found in serum, increases the rate of adipocyte lipolysis [5]. Additional experiments using iron salts suggested that transferrin's lipolytic activity is related to its function as an iron transporter [5]. Therefore, we determined the effect of transferrin and iron on glucose transport (Fig. 3). Adipocytes were incubated with transferrin at a concentration equivalent to what would be expected in 1% serum (100 μg/mL) or iron (3 μg/mL $FeSO_4$) for 4 hours, and then the effect of insulin on glucose transport was determined as before. Both iron and transferrin blunted the effect of insulin to a similar extent. Each was approximately half as effective as was serum. Therefore, transferrin and iron could partly explain the effect of serum on glucose transport.

4. Discussion

In recent years, a number of reports have demonstrated a relationship between serum and total body iron reserves, insulin resistance, and risk of type 2 diabetes mellitus. For example, findings from the nurses health study revealed a relationship between total body iron stores and risk of type 2 diabetes mellitus in women [8]. Conversely, blood letting has been shown to improve insulin resistance and beta-cell function in high-ferritin diabetes [6]. The relationship between iron metabolism and diabetes has recently been reviewed [7].

Although the mechanism underlying the relationship between iron and diabetes is largely unknown, we have recently reported that iron and transferrin can affect adipocyte metabolism [5]. Serum has been known for many years to contain factors that increase the rate of adipocyte lipolysis [1-4]. Although our studies primarily used human serum, we have found that rat and bovine serum are also approximately equally as effective (unpublished findings), and Curtis-Prior [3] reported that rat serum stimulates lipolysis in rat adipocytes. We have determined that iron and transferrin account for approximately 50% of the stimulatory activity of serum [5]. Based upon this, we proposed that increased total body iron leads to increased rates of adipocyte lipolysis and hence increased circulating FFA and insulin resistance. In the present report we have demonstrated that serum can also have a direct inhibitory effect on the sensitivity and responsiveness to insulin of adipocyte glucose transport.

The effect of serum on glucose transport differs from that on lipolysis in that the effect is slow to develop. The stimulatory effect on lipolysis was evident within 30 minutes [5]. By contrast, there was no effect of serum on glucose transport after a 45-minute treatment, and the effect became apparent only after a 4-hour incubation. This raised the possibility that the effect is secondary to the lipolytic effect of serum, resulting from FFA accumulation in the incubation medium. However, the effect was not replicated by added palmitate, and so this explanation seems unlikely.

Another possible explanation for the findings is that serum or its components cause release of cytokines such as tumor necrosis factor α or interleukin 6, which in turn antagonize insulin action in adipocytes. This could be from adipocytes themselves or from contaminating cells such as macrophages. However, this explanation is unlikely for

2 reasons. First, the effect on lipolysis that we have reported was very rapid [5], whereas effects of tumor necrosis factor α on lipolysis are slow to develop [16,17]. Second, cells in the primary adipocyte system are quite dilute, and it is unlikely that cytokine concentrations would reach a sufficient concentration in the duration of these experiments. Nevertheless, this possibility deserves further investigation.

Adipocyte glucose uptake and metabolism accounts for only a very small percentage of total body glucose metabolism [18]. Therefore, it is likely that the effect of iron and other serum-associated factors on lipolysis is more important than direct effects on adipocyte glucose transport [19]. However, it is possible that these mechanisms are also operative in skeletal muscle. We presented evidence that the effect of iron on lipolysis is mediated through a prooxidant mechanism [5], and oxidative stress is known to induce insulin resistance of muscle glucose transport. Therefore, it is likely that the effects we report here are operative in skeletal muscle and so could make a significant contribution to insulin resistance.

Acknowledgment

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