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The evolution of insulin resistance in muscle of the glucose infused rat

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ABSTRACT

Glucose infusion into rats causes skeletal muscle insulin resistance that initially occurs without changes in insulin signaling. The aim of the current study was to prolong glucose infusion and evaluate other events associated with the transition to muscle insulin resistance. Hyperglycemia was produced in rats by glucose infusion for 3, 5 and 8 h. The rate of infusion required to maintain hyperglycemia was reduced at 5 and 8 h. Glucose uptake into red quadriceps (RQ) and its incorporation into glycogen decreased between 3 and 5 h, further decreasing at 8 h. The earliest observed change in RQ was decreased AMPKα2 activity associated with large increases in muscle glycogen content at 3 h. Activation of the mTOR pathway occurred at 5 h. Akt phosphorylation (Ser^{473}) was decreased at 8 h compared to 3 and 5, although no decrease in phosphorylation of downstream GSK-3β (Ser^{9}) and AS160 (Thr^{642}) was observed. White quadriceps showed a similar but delayed pattern, with insulin resistance developing by 8 h and decreased AMPKα2 activity at 5 h. These results indicate that, in the presence of a nutrient overload, alterations in muscle insulin signaling occur, but after insulin resistance develops and appropriate changes in energy/nutrient sensing pathways occur.

Introduction

Hyperglycemia and insulin resistance are hallmarks of type 2 diabetes. It is also clear that hyperglycemia by itself can cause, and/or exacerbate, insulin resistance in multiple tissues [1]. Skeletal muscle is quantitatively the most important tissue target of glucose postprandially and accounts for 70–80% of insulin stimulated glucose uptake [2].

Early studies revealed that insulin resistance develops in skeletal muscle of rats infused with glucose at a high, but constant rate for 1–7 days [3–5] and that it occurs as early as day 1 of a glucose infusion [4]. Laybutt et al. [5] reported that skeletal muscle of these animals had an increased long chain acyl-CoA (LCACoA) content, as well as activation of PKCε, potentially implicating elevated diacylglycerol (DAG) levels as a pathogenic factor. They proposed that increased intramyocellular fatty acid metabolites may feed back onto the insulin signaling pathway to inhibit glucose uptake [5].

More recent studies in which variable rates of infusion were used to clamp blood glucose levels at ~11 mM revealed that insulin resistance develops at 5 h [6,7]. They also revealed that at this time point it was accompanied by increases in the concentrations of malonyl-CoA and DAG and decreased AMPK activity, providing further evidence of a link between fatty acid metabolites and the hyperglycemia-induced insulin resistant state [7]. However, a subsequent study revealed that the insulin resistance generated by an acute glucose infusion at 5 h is not associated with decreased phosphorylation of proteins in the insulin signaling pathway (insulin receptor, Akt and AS160). Furthermore, it occurred without changes in maximal insulin-stimulated glucose transport capacity [6]. These findings suggested that the insulin resistance caused by a hyperglycemic insult initially may be due to excess glycogen storage and metabolic feedback, rather than a defect in insulin signaling [6].

A lack of an insulin signaling defect in the setting of insulin resistance is not in keeping with current thinking, which is based on the concurrent presence of abnormalities in insulin signaling and insulin resistance in a variety of circumstances [8]. The present study has two aims. The first is to determine if insulin signaling

Abbreviations: ACC, acetyl CoA carboxylase; AMPK, 5′ adenosine monophosphate-activated protein kinase; AS160, Akt substrate of 160KDa; DAG, diacylglycerol; EDL, extensor digitorum longus, GSK-3β, glycogen synthase kinase 3 beta; IR, insulin receptor; IRS-1, insulin receptor substrate-1; LCACoA, long chain acyl CoA; mTOR, mammalian target of rapamycin; p70S6K, p70S6 kinase; R_d, whole body glucose disposal rate; R_g', glucose uptake; RQ, red quadriceps; WQ, white quadriceps.

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defects occur during a more prolonged exposure to hyperglycemia and if so whether they are associated with a further exacerbation of insulin resistance in skeletal muscle. The second is to identify other factors modified during a glucose infusion that could account for the decrease in muscle glucose uptake prior to changes in insulin signaling.

Materials and methods

Animals

All surgical and experimental procedures performed were approved by the Garvan Institute/St. Vincent's Hospital Animal Ethics Committee and were in accordance with the National Health and Medical Research Council of Australia's guidelines on animal experimentation.

Adult male Wistar rats (Animal Resources Centre, Perth, Australia) were communally housed in temperature controlled (22 ± 0.5 °C) 12 h light–dark cycle rooms. Rats were fed *ad libitum* a standard chow diet (Rat Maintenance Diet; Gordon Specialty Feeds, Sydney, Australia). After a 1 week acclimatization period, cannulae were inserted into both jugular veins [6]. Average body weight on the day of experiment was 319 ± 8 g.

Glucose infusion

Seven days after surgery, rats were randomly divided into treatment groups. After a basal blood sample was taken, a 50% (w/v) glucose infusion commenced. Rats were infused for either 0, 3, 5 or 8 h using a peristaltic roller pump (101U/R; Watson-Marlow, Falmouth, UK). Blood samples were taken every 30 min and the glucose infusion rate was altered to maintain blood glucose concentration at ~11 mM. Red blood cells from each sample were resuspended in heparinised saline and returned to the animal. In one cohort, 2-deoxy-D-[2,6-^{3}H]glucose and [U-^{14}C]glucose (Amersham Biosciences, Buckinghamshire, UK) were administered as an intravenous bolus in the last 30 min of the glucose infusion. Blood samples were taken 2, 5, 10, 15, 20 and 30 min after administration of the tracer bolus for estimation of tracer clearance and blood glucose. Animals were then euthanized with an overdose of pentobarbital sodium (Nembutal; Abbott Laboratories, Sydney, Australia) and tissues were rapidly removed, freeze-clamped, and stored at −80 °C for later analysis.

In the second cohort, rats were euthanized after the glucose infusion period ended (no tracer period). The soleus and EDL muscle from each leg was carefully dissected into 3 longitudinal strips from tendon to tendon with the use of a 25G needle and two strips of a similar weight from each leg were used. The third strip was immediately freeze-clamped (without further incubation) and stored at −80 °C for later immunoblotting analysis. Glucose transport was assayed in isolated muscles from one leg in sealed vials containing pregassed (95% O_2–5% CO_2) Krebs-Henseleit bicarbonate buffer supplemented with 4 mM sodium pyruvate, 8 mM mannitol, and 0.1% wt/vol BSA at 30 °C. Muscles were preincubated for 30 min and were then incubated with or without insulin for 30 min. Insulin-stimulated glucose transport capacity was then assayed for 15 min using [^{3}H]2DG (1 mM, 0.128 μCi/ml) in the presence or absence of 300 μU/ml insulin, a dose similar to that reached *in vivo* (Table 1) [6].

After the preincubation phase, the remaining muscle strips were transferred to buffer containing 5 mM glucose and 2 μCi of [U-^{14}C]glucose to monitor glucose incorporation into glycogen. The resulting glycogen concentration and [^{14}C]glucose incorporation into glycogen were measured as described previously [6,9].

Analytic methods

Blood and plasma glucose levels (YSI2300; Yellow Springs Instruments, Yellow Springs, OH), and plasma insulin (Linco, St. Louis, MO) were measured.

Plasma and tissue levels of ^{3}H- and ^{14}C-labelled tracers were measured to calculate whole body glucose disposal rate (R_d) and to estimate red (RQ) and white (WQ) quadriceps glucose uptake (R_g'), [^{14}C]glucose incorporation into glycogen and glycogen concentration. Assays and calculations are as previously described [9].

Long chain acyl-CoA (LCACoA) levels were measured as described by Antinozzi et al. [10]. Malonyl-CoA was assayed radioenzymatically by a modification of the method of McGarry et al. [11]. The measurement of DAG and ceramide content was determined as previously described [12]. Glucose metabolites were measured spectrophotometrically by standard enzymatic assays [13].

Western blot analysis

Protein extraction and immunoblots from RQ and WQ muscle homogenates were performed as previously described [6]. Antibodies raised against insulin receptor β were obtained from BD Biosciences (San Jose, CA), phospho-Tyr$^{1162/63}$ insulin receptor from BioSource International (Camarillo, CA), phospho-Tyr^{612} IRS-1 from Sigma–Aldrich (St. Louis, MO), Akt, phospho-Ser^{473} Akt, GSK-3β, phospho-Ser^{9} GSK-3β, IRS-1, phospho-IRS-1^{307} IRS-1, anti

Table 1
Blood, whole body and muscle parameters of basal and glucose infused animals.

	Basal	Time of glucose infusion		
		3 h	5 h	8 h
Whole body				
Glucose (mM)	4.4 ± 0.2	11.5 ± 0.5[a]	11.5 ± 0.2[a]	11.8 ± 0.3[a]
Insulin (mU/L)	36 ± 8	261 ± 44[a]	335 ± 24[a]	345 ± 39[a]
GIR (mg/min/kg)	N/A	63 ± 3	53 ± 2[b]	48 ± 1[b]
R_d (mg/min/kg)	12 ± 1	58 ± 3[a,c,d]	52 ± 1[a,b]	48 ± 1[a,b]
Red quadriceps				
R_g' (μmol/min/100 g)	8 ± 3	40 ± 2[a,c,d]	30 ± 2[a,b,d]	23 ± 1[a,b,c]
Glycogen content (μmol/g)	36 ± 3	111 ± 8[a,d]	122 ± 6[a,d]	154 ± 5[a,b,c]
Glycogen synthesis rate (μmol/100 g/min)	2 ± 1	24 ± 3[a,c,d]	16 ± 2[a,b,d]	8 ± 1[b,c]
White quadriceps				
R_g' (μmol/min/100 g)	5 ± 1	39 ± 4[a,d]	35 ± 2[a,d]	26 ± 2[a,b,c]
Glycogen content (μmol/g)	38 ± 3	70 ± 5[a,d]	71 ± 3[a,d]	94 ± 5[a,b,c]
Glycogen synthesis rate (μmol/100 g/min)	0.3 ± 0.1	4.6 ± 0.4[a]	4.2 ± 0.5[a]	3.1 ± 0.3[a]

Data are means ± SEM. [a]$P < 0.05$ vs basal, [b]$P < 0.05$ vs 3 h, [c]$P < 0.05$ vs 5 h, [d]$P < 0.05$ vs 8 h glucose infusion. $n = 4$–13. GIR, glucose infusion rate; R_d, whole body glucose disposal; R_g', glucose uptake into red or white quadriceps.