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Effects of adrenaline on whole-body glucose metabolism and insulin-mediated regulation of glycogen synthase and PKB phosphorylation in human skeletal muscle

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Abstract

In the present study, we investigated the effect of adrenaline on insulin-mediated regulation of glucose and fat metabolism with focus on regulation of skeletal muscle PKB, GSK-3, and glycogen synthase (GS) phosphorylation. Ten healthy subjects (5 men and 5 women) received a 240-minute intravenous infusion of adrenaline (0.05 μg/[kg min]) or saline; after 120 minutes, a hyperinsulinemic-euglycemic clamp was added. Adrenaline infusion increased blood glucose concentration by approximately 50%, but the hyperinsulinemic clamp normalized blood glucose within 30 minutes. Glucose infusion rate during the last hour was approximately 60% lower during adrenaline infusion compared with saline (4.3 ± 0.5 vs 11.2 ± 0.6 mg/kg lean body mass per minute). Insulin increased PKB Ser^{473}, PKB Thr^{308}, and GSK-3β Ser^{9} phosphorylation in skeletal muscles; coinfusion of adrenaline did not influence insulin-stimulated PKB and GSK-3 phosphorylation. Adrenaline alone did not influence phosphorylation of PKB and GSK-3β. Insulin increased GS fractional activity and decreased GS Ser^{641} and Ser^{645,649,653,657} phosphorylation. In the presence of adrenaline, insulin did neither activate GS nor dephosphorylate GS Ser^{641}. Surprisingly, GS Ser^{7} phosphorylation was not influenced by adrenaline. Adrenaline increased plasma lactate concentration; and muscle glycogen content was reduced in skeletal muscle the day after adrenaline infusion, supporting that insulin does not stimulate glycogen synthesis in skeletal muscles when adrenaline is present. In conclusion, adrenaline did not influence basal or insulin-stimulated PKB and GSK-3β phosphorylation in muscles, but completely blocked insulin-mediated GS activation and Ser^{641} dephosphorylation. Still, insulin normalized adrenaline-mediated hyperglycemia.
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1. Introduction

Physical and mental stress is often accompanied by sympathetic nerve activation and release of catecholamines from the adrenal medulla. Catecholamines are known to have marked insulin-antagonistic effects. Thus, sympathetic activation induces insulin resistance measured by glucose utilization with the hyperinsulinemic-euglycemic clamp technique; and moreover, it impairs the antilipolytic action of insulin [1-4]. The autonomic innervation of fat tissue may affect insulin sensitivity partly via release of free fatty acid (FFA) as well as via altered gene expression of adipose tissue hormones such as resistin and leptin [5]. Such factors that are released into the circulation can, in turn, promote insulin resistance in major glucose-regulating tissues such as muscle and liver.

In clinical studies, sympathetic nerve activity in skeletal muscle has been positively associated to the amount of body

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fat; and it is also increased in obese subjects in the fasting state [6]. Offspring of type 2 diabetes mellitus patients were reported to have elevated resting muscle sympathetic nerve activity [7], and glucose regulation appears to be more sensitive to sympathetic stimulation in type 2 diabetes mellitus persons [8]. An elevated activity of the sympathetic nervous system related to mental stress factors, or a high ratio of sympathetic to parasympathetic activity, may contribute to the early development of type 2 diabetes mellitus as suggested by results in subjects with the metabolic syndrome or insulin resistance [9,10]. Thus, it is of relevance to further explore the mechanisms whereby sympathoadrenergic activation affects glucose turnover and potentially contributes to insulin resistance and development of type 2 diabetes mellitus.

Skeletal muscles account for the major part of insulin-mediated glucose disposal [11,12], and adrenaline impairs insulin action on whole-body glucose turnover in humans [13]. Insulin-stimulated glucose uptake is mediated via PKB, and impaired PKB phosphorylation and activation have been observed in insulin-resistant muscles [14,15]. The role of PKB in adrenaline-induced insulin resistance in humans is unclear, but it is well documented that insulin-stimulated glycogen synthesis and glycogen synthase (GS) activation are completely blocked by adrenaline [16,17]. Glycogen synthase is phosphorylated at 9 different sites that to various degrees regulate activity. Glycogen synthase Ser^{641} and Ser^{645}, which are phosphorylated by GSK-3, are strong regulators of GS activity [18]; but GS Ser^{7} phosphorylation has also been reported to participate in GS regulation during adrenaline stimulation in muscles [19-21]. Glycogen synthase phosphorylation has not been studied in human muscles in response to adrenaline.

The aim of the present study was to investigate the interaction between adrenaline and insulin in metabolic regulation in a protocol where a hyperinsulinemic-euglycemic clamp was initiated after 2-hour infusion of adrenaline or saline. The main purpose of the study was to investigate the effect of adrenaline on insulin-stimulated PKB, GSK-3, and GS phosphorylation in skeletal muscles. Finally, we performed an additional hyperinsulinemic-euglycemic clamp the day after infusion of adrenaline/saline to investigate whether adrenaline infusion for 4 hours influences insulin sensitivity the following day.

2. Materials and methods

2.1. Subjects

Ten healthy volunteers (5 men and 5 women) were recruited via advertisement. Anthropometric and metabolic characteristics are presented in Table 1. None of the subjects were on any regular medication, and a normal electrocardiographic result was a prerequisite for inclusion. Two of the subjects had a first-degree relative with type 2 diabetes mellitus. All subjects exercised regularly (2-3 times per week), and their level of physical activity was considered as medium. None of subjects used alcohol regularly. Five of the subjects used snuff tobacco regularly, and one subject was a smoker (<10 cigarettes per day). Male subjects had significantly lower serum concentration of high-density lipoprotein (HDL) compared with female subjects. Subjects refrained from vigorous exercise, tobacco, and alcohol for 48 hours and fasted overnight (from 10 PM) before all study occasions. Fat mass was estimated with the bioelectrical impedance method (Bodystat 1500; BODYSTAT, Douglas, Isle of Man, United Kingdom), and lean body mass was calculated. Body mass index and waist to hip ratio were calculated as described [10]. The study was performed at the Metabolic Unit, Umeå University Hospital, and was approved by the Ethics Committee of Umeå University. Subjects gave their informed consent to participate.

Table 1
Anthropometric and metabolic characteristics of the participants

	Male (n = 5)	Female (n = 5)	All (n = 10)
Age (y)	31 ± 3	30 ± 2	31 ± 2
BMI (kg/m^2)	26.6 ± 1.0	26.9 ± 1.1	26.7 ± 0.7
Waist to hip ratio	0.86 ± 0.02	0.80 ± 0.04	0.83 ± 0.02
Amount body fat (%)	19 ± 2*	32 ± 2	26 ± 3
Lean body mass (%)	81 ± 2*	68 ± 2	74 ± 3
Blood glucose (mmol/L)	5.0 ± 0.1	4.9 ± 0.2	5.0 ± 0.1
Serum insulin (mU/L)	6.1 ± 1.3	5.6 ± 0.6	5.9 ± 0.7
HOMA-IR index	8.2 ± 1.8	7.5 ± 1.1	7.8 ± 1
HDL cholesterol (mmol/L)	1.2 ± 0.1*	1.7 ± 0.1	1.4 ± 0.1
Serum triglycerides (mmol/L)	1.6 ± 0.3	0.9 ± 0.1	1.2 ± 0.2

Data are expressed as means ± SEM. BMI indicates body mass index; HOMA-IR, homeostasis model assessment of insulin resistance (Insulin [pmol/L] × glucose [mol/L] /22.5).

* $P < .01$ compared with female subjects.

2.2. Protocol

Subjects underwent 2 study protocols where adrenaline (A) or saline (S) was infused. Each protocol included hyperinsulinemic-euglycemic clamps on 2 successive days. On day 1 in the A protocol, the subjects reported to the laboratory at 7:00 AM and received an adrenaline infusion (see below) during 240 minutes starting at 8:00 AM. After 120 minutes of adrenaline infusion, a hyperinsulinemic-euglycemic clamp was added. Subjects returned to the hospital the following day at 9:00 AM (day 2) to perform another hyperinsulinemic-euglycemic clamp starting at 10:00 AM as the preceding day (Fig. 1). The S protocol was similar to the A protocol except that saline was infused instead of adrenaline on day 1. In both protocols, muscle biopsies were taken from musculus tibialis anterior at 3 occasions after local dermal anesthesia (Xylocaine; AstraZeneca, Södertälje, Sweden). The first biopsy was taken after 120 minutes of infusion of adrenaline (or saline), and the second biopsy was taken at the end of the hyperinsulinemic-euglycemic clamp with continued adrenaline or saline infusion. The third biopsy was taken at day 2 before the

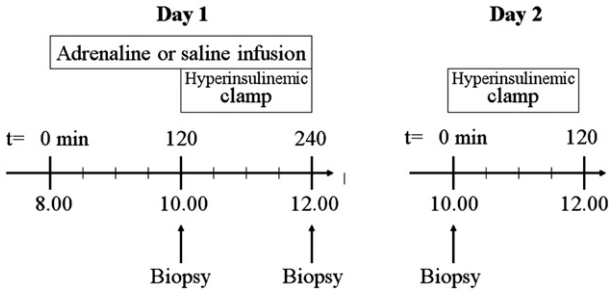

Fig. 1. Schematic illustration of the experimental protocol. At day 1, adrenaline or saline was infused for 240 minutes. After 120 minutes, a hyperinsulinemic-euglycemic clamp was added. An additional hyperinsulinemic clamp was performed the next day. Biopsies were taken as indicated in figure. Subjects performed the experiments with infusion of adrenaline and saline in randomized order separated by 10 to 30 days.

clamp. The muscle biopsies were taken from the middle part of musculus tibialis anterior through an approximately 0.5-cm incision of the skin and muscle fascia. Biopsies of 30 to 100 mg of muscle were removed with a biopsy forceps, frozen in liquid nitrogen, and stored at −70°C until analyses. One subject did not complete the biopsy protocol. The 2 protocols were separated by 10 to 30 days and performed in randomized order.

2.3. Adrenaline infusion and hyperinsulinemic-euglycemic clamp

Subjects rested in a comfortable bed at a room temperature of 24°C to 26°C. Polytetrafluoroethylene cannulaes were positioned in an antecubital vein of the right arm (for the glucose and insulin infusions), in the antecubital vein of the left arm (for the adrenaline/saline infusion), and in a distal vein of the left arm (for blood sampling). The left hand was put in a heating pad to obtain arterialized venous blood. Adrenaline was infused at a rate of 0.05 μg/(kg min) for 240 minutes. After 120 minutes of adrenaline infusion, a hyperinsulinemic-euglycemic clamp was performed as previously described [22] while the adrenaline infusion continued during the clamp. In brief, recombinant human insulin (Actrapid; Novo-Nordisk, Copenhagen, Denmark) was infused with a priming dose followed by a constant infusion rate of 56 mU/(m^2 min) for 110 minutes. In parallel, a 20% glucose infusion was started; and the infusion rate was adjusted to maintain a steady-state blood glucose level at 5.0 mmol/L. During the hyperinsulinemic-euglycemic clamp (120-240 minutes), blood glucose concentration was determined at 5-minute intervals for adjustment of glucose infusion rate. M-values were calculated by dividing the amount of glucose infused per minute during the last 60 minutes (steady state) of the clamp by lean body mass (LBM) (milligram per kilogram LBM per minute). The insulin sensitivity index (ISI) was calculated by dividing the M-value with the mean insulin concentration during the same period. The following day, an identical 120-minute hyperinsulinemic-euglycemic clamp was performed without adrenaline infusion starting at 10:00 AM. Blood samples, heart rate, and blood pressure measurements were obtained at 0, 15, 30, 60, 90, 120, 150, 180, 210, and 240 minutes during all clamps.

2.4. Adrenaline and noradrenaline

For analysis of adrenaline and noradrenaline, 3 mL of arterialized venous blood was added to precooled tubes containing 60 μL 0.2 mol/L glutathione/0.2 mol/L EGTA. The samples were kept on ice and centrifuged (10 minutes, 2000*g*, 4°C), and the plasma was stored at −70°C. Catecholamines were analyzed with high-performance liquid chromatography with electrochemical detection after alumina extraction as we have described [23].

2.5. Lactate and glycerol

For measurements of lactate, 50 μL plasma was deproteinized with 500 μL 0.4 mol/L perchloric acid; and concentration was measured fluorometrically [24]. For measurement of glycerol, 60 μL plasma was deproteinized with 600 μL 0.4 mol/L perchloric acid and centrifuged; and 500 μL of the supernatant was neutralized with 260 μL 0.7 mol/L KOH/0.2 mol/L $KHCO_3$. Glycerol was determined fluorometrically [24].

2.6. Additional blood chemistry

Blood glucose concentrations during the clamp were determined by HemoCue glucose system (HemoCue, Ängelholm, Sweden). Other serum and plasma samples collected during the infusion studies were centrifuged within 30 minutes and stored at −80°C until analyzed. Hemoglobin A_{1c} was measured by high-performance liquid chromatography (Integral 4000; BioRad, Anaheim, CA). Free fatty acids in plasma were analyzed with a commercial enzymatic kit (NEFA C; Wako Chemical, Richmond, VA). Plasma tumor necrosis factor (TNF)−α and interleukin (IL)-6 levels were determined with high-sensitivity commercial immunoassay kits (Quantikine HS Human TNFα Immunoassay and Quantikine HS Human IL-6, respectively; R&D System, Minneapolis, MN). Plasma adiponectin and leptin levels were determined using a commercial human adiponectin and leptin enzyme-linked immunosorbent assay kit of sandwich type (Linco Research, St Charles, MO). Serum analyses of insulin, C-peptide, and total and HDL cholesterol were made according to standard procedures at the Department of Clinical Chemistry, Umeå University Hospital.

2.7. Glycogen synthase activity

Muscle biopsies were freeze-dried, weighed, and homogenized before GS activity was measured with the filter paper method and D-[^{14}C]-uridine diphosphate glucose (200 mCi/mmol) from Perkin Elmer Life Sciences (Boston, MA), with the modifications that we have described previously [25]. The final concentration of uridine diphosphate glucose was 30 μmol/L with 0.5 μCi/mL [^{14}C]-uridine diphosphate glucose. Glycogen synthase activity was determined with 0.17 mmol/L

glucose 6-phosphate; and total activity, at a saturating concentration of glucose 6-phosphate (12 mmol/L). Fractional activity was calculated as the activity at 0.17 mmol/L glucose 6-phosphate in percentage of total activity.

2.8. Glycogen content

Muscles were freeze-dried and weighed. Glycogen was hydrolyzed in 1 mol/L HCl at 100°C for 2.5 hours, and glucose units were analyzed as described [26].

2.9. Western blotting

Muscle biopsies were homogenized on ice, and immunoblotting was performed as described previously [27]. Protein content was determined (DC Protein Assay; BioRad, Hercules, CA), and homogenates were diluted to 2 μg/μL. For Western blot, approximately 25 μg of protein was resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to Immobilon-P polyvinylidene difluoride membranes (Millipore,

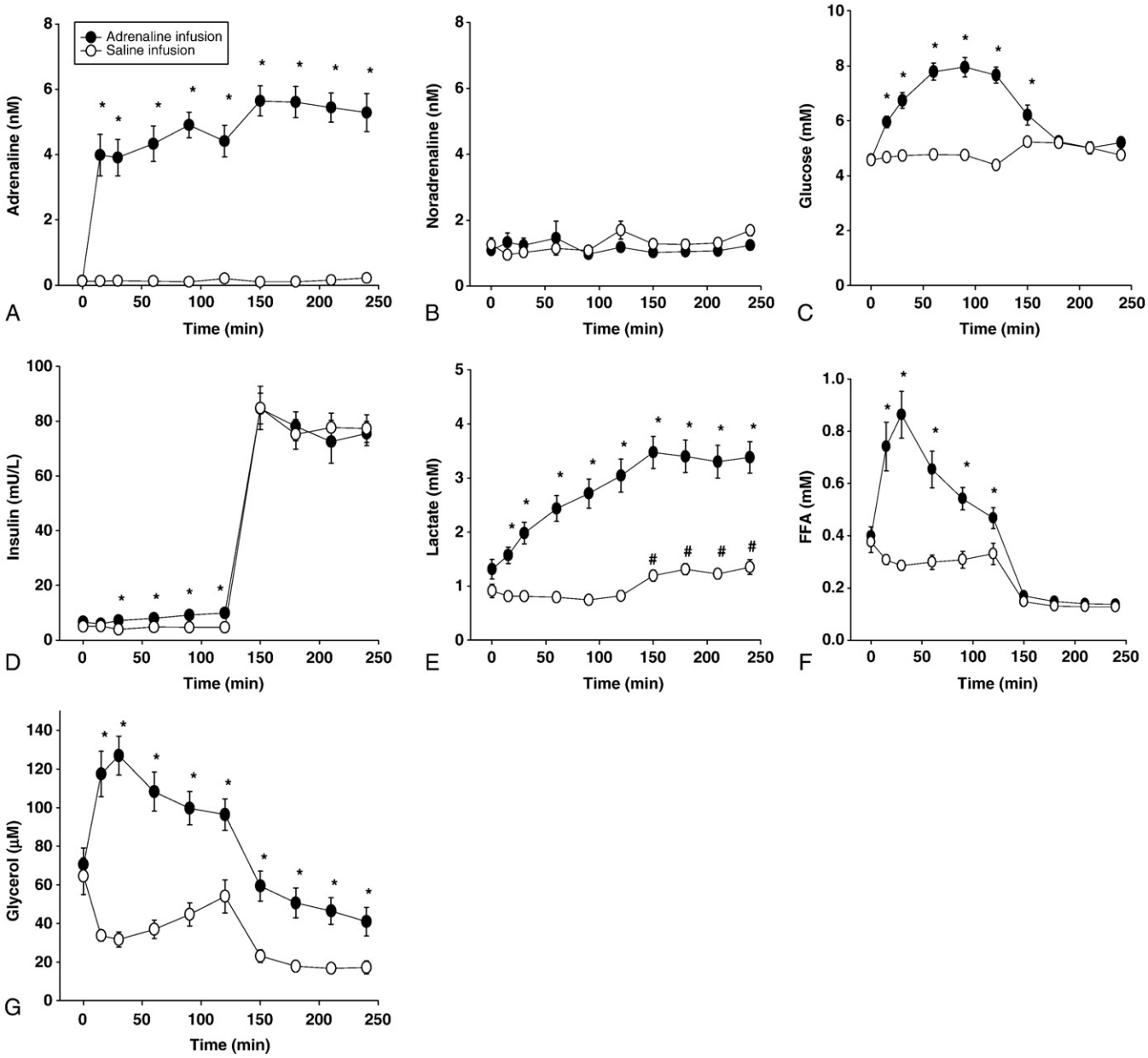

Fig. 2. Plasma concentrations of hormones and metabolites during infusion of adrenaline or saline in combination with a hyperinsulinemic clamp. Adrenaline or saline was infused for 240 minutes, and a hyperinsulinemic-euglycemic clamp was started after 120 minutes. Blood samples were taken after 0, 15, 30, 90, 120, 150, 180, 210, and 240 minutes of infusion and analyzed for adrenaline (A), noradrenaline (B), glucose (C), insulin (D), lactate (E), FFA (F), and glycerol (G) as described in "Materials and methods." *$P < .05$ compared with saline infusion. $^{\#}P < .05$ compared with before insulin infusion ($t = 0$). Data are mean ± SEM. n = 10 at each time point in all figures.

Billerica, MA) [28]. Membranes were probed with primary antibodies and appropriate secondary antibodies, and antibody binding was detected by enhanced chemiluminescence from Millipore and quantified as described [25].

2.10. Antibodies

Anti–phospho-glycogen synthase Ser^{7} was a gift from Grahame Hardie, and anti-glycogen synthase was a gift from Oluf Pedersen (Copenhagen, Denmark). Anti–phospho-glycogen synthase $Ser^{645,649,653,657}$ was from Oncogene (San Diego, CA). Anti–phospho-GSK-3α/β Ser^{21}/Ser^{9}, anti–phospho-PKB Thr^{308}, anti–phospho-glycogen synthase Ser^{641}, and anti-rabbit HRP-linked antibodies were from Cell Signaling (Beverly, MA). Anti–phospho-PKB (Ser^{473}), anti-mouse HRP-conjugate, and anti-sheep HRP-conjugate antibodies were from Upstate (Lake Placid, NY).

2.11. Statistics

Data are expressed as mean ± SEM. One- or 2-way analyses of variance were performed when appropriate. Student paired *t* test was used for comparisons between saline and adrenaline infusion, and unpaired *t* test was used to compare male and female subjects. Pearson correlation analysis was used to evaluate correlations. A P value < .05 was considered significant.

3. Results

3.1. Catecholamine levels

Adrenaline infusion increased plasma adrenaline concentration to about 4 to 5 nmol/L, and the concentration remained rather stable during the infusion period (Fig. 2A). This concentration agrees with other studies with similar infusion rate [29-31]. Adrenaline concentration remained low during saline infusion. The plasma level of noradrenaline was not influenced by adrenaline and insulin infusions (Fig. 2B). Plasma concentrations of adrenaline and noradrenaline were similar during the clamp study at day 2 whether adrenaline or saline was infused the preceding day (data not shown).

3.2. Cardiovascular responses

Adrenaline infusion increased heart rate by approximately 30% and systolic blood pressure by approximately 10%, whereas diastolic blood pressure decreased by approximately 30% (Table 2). The hyperinsulinemic-euglycemic clamp did not influence the adrenaline-mediated increase in heart rate and systolic blood pressure or the decrease in diastolic blood pressure. However, the hyperinsulinemic clamp decreased diastolic blood pressure in the saline protocol, whereas heart rate and systolic blood pressures did not change.

3.3. Whole-body glucose metabolism

Infusion of adrenaline increased blood glucose concentrations by approximately 50% compared with concentrations during saline infusion (P < .01, Fig. 2C). Glucose concentration decreased gradually to 5 mmol/L during the initial 30 minutes of the hyperinsulinemic-euglycemic clamp with coinfusion of adrenaline. During the last 60 minutes of the hyperinsulinemic clamp with adrenaline coinfusion, mean glucose infusion rate was 4.3 ± 0.5 mg/kg LBM per minute (Fig. 3). Glucose infusion rate was 11.2 ± 0.6 mg/kg LBM per minute during the hyperinsulinemic clamp with saline coinfusion (Fig. 3). Mean insulin concentrations during the hyperinsulinemic clamps were similar during adrenaline and saline infusion (Fig. 2D; mean: 76.7 ± 5.0 and 75.3 ± 5.5 mU/L, respectively). Insulin sensitivity index was lower during adrenaline infusion compared with saline coinfusion (6.3 ± 1.0 and 15.3 ± 1.4, respectively; P < .001). The adrenaline-induced reduction in M-value, when expressed in absolute numbers, was greatest in the subjects with highest insulin sensitivity; and the adrenaline-mediated absolute reduction in M-value correlated with M-value during saline infusion (r = 0.72, P < .02). However, when expressed as relative decrease in M-value, there was no

Table 2
Heart rate and systolic and diastolic blood pressure during saline and adrenaline infusion before and during a hyperinsulinemic-euglycemic clamp

	Saline			Adrenaline		
	0 min	120 min	240 min	0 min	120 min	240 min
Heart rate (beat/min)	60 ± 3	59 ± 1	63 ± 1	62 ± 3	77 ± 2†,‡	80 ± 2†,‡
Systolic blood pressure	108 ± 3	107 ± 1	110 ± 1	110 ± 3	117 ± 2†	118 ± 2*,‡
Diastolic blood pressure	71 ± 2	70 ± 1	66 ± 1‡	70 ± 4	49 ± 1†,‡	48 ± 2†,‡

Heart rate was measured before, during 120 minutes of adrenaline/saline infusion, and during a hyperinsulinemic-euglycemic clamp with coinfusion of adrenaline/saline (t = 240 minutes). Heart rate and blood pressure at 120 minutes are means of measurements at 15, 30, 60, 90, and 120 minutes. Heart rate and blood pressure at 240 minutes are means of measurements at 150, 180, 210, and 240 minutes. Data are expressed as means ± SEM. n = 10 in each group.

* P < .05 compared with saline infusion.

† P < .01 compared with saline infusion.

‡ P < .01 compared with 0 minute.

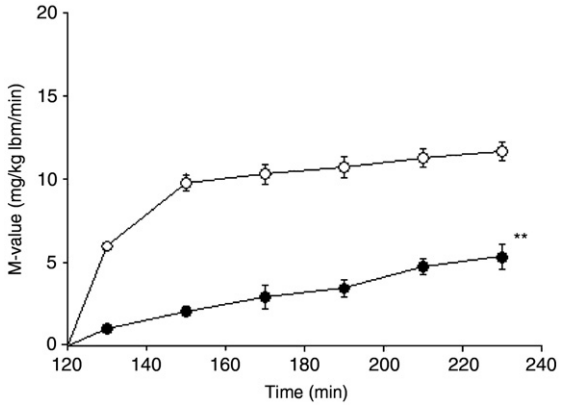

Fig. 3. Effect of adrenaline on glucose infusion rate during a hyperinsulinemic-euglycemic clamp. The hyperinsulinemic clamp was started after 120 minutes of adrenaline infusion, and adrenaline infusion was continued during the clamp. Glucose infusion rate during the hyperinsulinemic clamp when adrenaline (filled symbols) and saline (open symbols) were coinfused. Data are mean ± SEM. n = 10 at each time-point. $^{**}P < .05$ compared with M-value during saline infusion.

significant difference in this adrenaline effect across the range of insulin sensitivity ($r = 0.36$, $P = .30$).

Plasma lactate concentration increased gradually during adrenaline infusion and was approximately 3 mmol/L at 120 minutes ($P < .01$, Fig. 2E). During the hyperinsulinemic clamp with adrenaline coinfusion, lactate concentration stabilized at 3.5 mmol/L (Fig. 2E). Lactate concentration remained low during saline infusion, but increased by 20% during hyperinsulinemia ($P < .05$).

3.4. Lipid metabolism

During the first 30 minutes of adrenaline infusion, plasma glycerol and plasma FFA increased by approximately 100% ($P < .01$, Fig. 2F and G) and declined thereafter gradually during the continuing adrenaline infusion. During the hyperinsulinemic-euglycemic clamp, FFA concentration decreased to the same level whether adrenaline or saline was coinfused (Fig. 2F). Glycerol levels also decreased during the hyperinsulinemic clamp, but glycerol concentration remained higher during the adrenaline compared with saline infusion ($P < .01$,Fig. 2G).

3.5. Plasma insulin, C-peptide, and cytokines

Insulin concentrations increased by approximately 10% ($P < .01$) during the first 120 minutes of the adrenaline infusion compared with saline infusion (Fig. 1). In parallel with the increase of insulin, a significant increase in C-peptide was noticed during adrenaline infusion (from 0.71 ± 0.12 to 0.92 ± 0.12 nmol/L, $P < .05$). During the following hyperinsulinemic clamp, C-peptide decreased to 0.45 ± 0.09 nmol/L ($P < .05$). On the day of saline infusion, C-peptide concentration was 0.65 ± 0.07 nmol/L at baseline and 0.53 ± 0.07 nmol/L at the end of the insulin infusion. Adrenaline did not influence cortisol concentration (data not shown).

Adrenaline infusion did not influence plasma concentration of TNF-α, adiponectin, or leptin, but increased concentration of IL-6 (Table 3). The hyperinsulinemic-euglycemic clamp did not significantly increase IL-6 concentration further when adrenaline was coinfused. However, IL-6 concentration increased during the hyperinsulinemic clamp when saline was infused, but remained lower than when adrenaline was coinfused. The hyperinsulinemic clamp did not influence plasma concentration of TNF-α, adiponectin, or leptin (Table 3).

3.6. Skeletal muscle GS activity and phosphorylation

Basal GS fractional activity was low in skeletal muscles, and adrenaline infusion did not decrease fractional activity significantly (Fig. 4A). Glycogen synthase fractional activity increased during the hyperinsulinemic clamp when saline was infused. Adrenaline infusion completely blocked insulin-mediated GS activation (Fig. 4A). Adrenaline also prevented the insulin-mediated increase in GS I form (data not shown). Total GS activity was not changed significantly during the different treatments, and mean activity was 2.3 ± 0.2 mmol/kg dry weight per minute (n = 9).

Adrenaline infusion increased GS Ser^{641} phosphorylation, whereas phosphorylation of $Ser^{645,649,653,657}$ and Ser^{7} was not influenced (Fig. 3A-C). Insulin decreased GS Ser^{641} and $Ser^{645,649,653,657}$ phosphorylation, whereas Ser^{7}

Table 3
Plasma cytokines during saline or adrenaline infusion before and during a hyperinsulinemic-euglycemic clamp

	Saline			Adrenaline		
	0 min	120 min	240 min	0 min	120 min	240 min
TNF-α (pg/mL)	1.1 ± 0.1	1.5 ± 0.2	1.7 ± 0.2	1.3 ± 0.1	1.2 ± 0.2	1.4 ± 0.1
Adiponectin (μg/mL)	8.6 ± 1.2	7.7 ± 0.1	6.9 ± 0.8	8.6 ± 0.9	8.8 ± 0.9	8.8 ± 1.1
Leptin (ng/mL)	8.5 ± 2.2	7.6 ± 0.2	9.7 ± 3.1	9.9 ± 2.8	7.7 ± 0.2	8.3 ± 2.3
IL-6 (pg/mL)	0.7 ± 0.1	1.6 ± 0.4	2.0 ± 0.5†	0.7 ± 0.1	3.8 ± 0.5*,†	4.8 ± 0.7*,†

Blood samples were taken before infusion, after 120 minutes of infusion of adrenaline/saline, and at the end of the hyperinsulinemic clamp with coinfusion of adrenaline/saline ($t = 240$ minutes). Data are expressed as means ± SEM. n = 10 in each group.

* $P < .05$ compared with saline infusion.

† $P < .01$ compared with 0 minute.

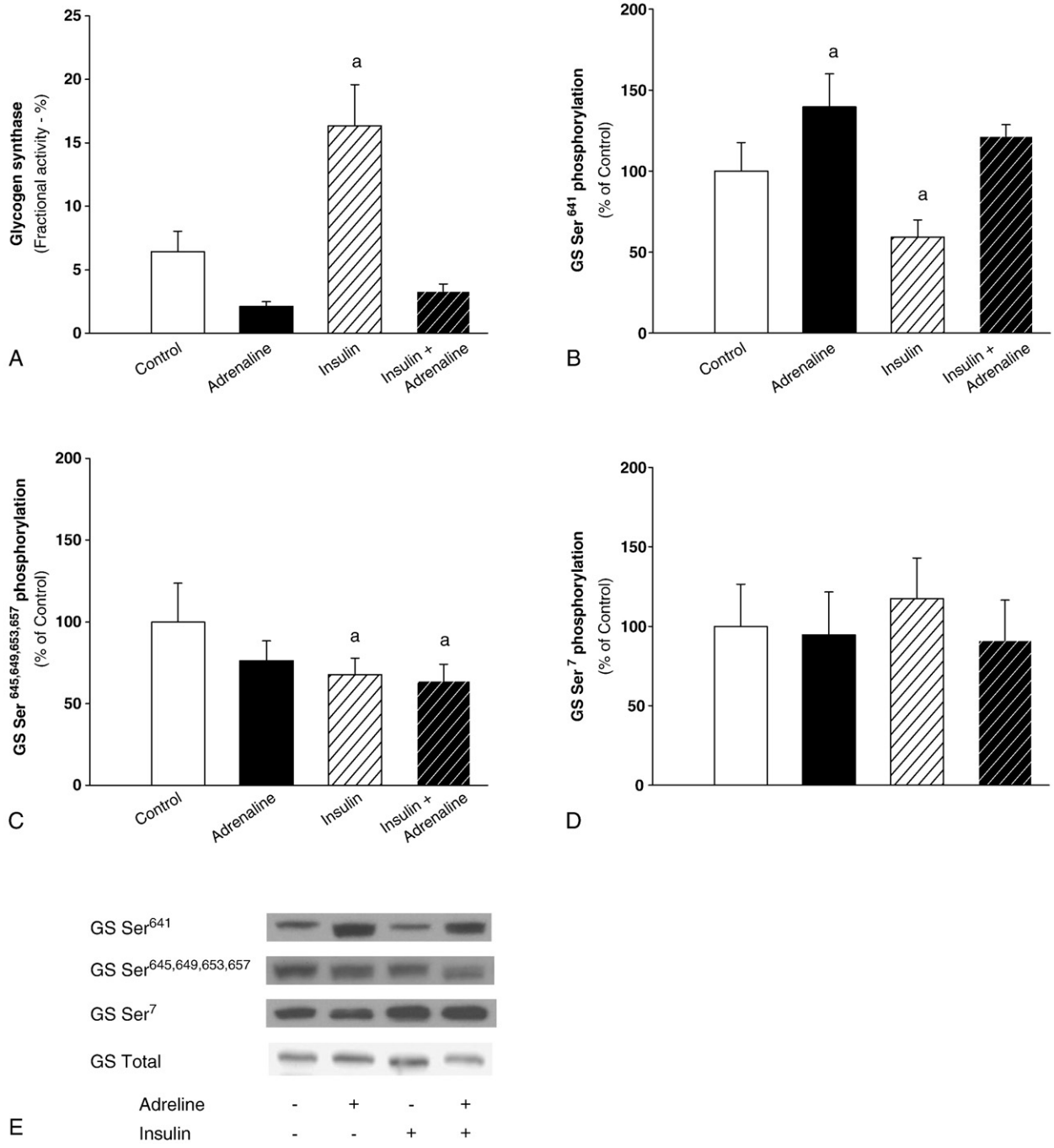

Fig. 4. Effect of adrenaline and insulin on GS fractional activity and GS phosphorylation. Biopsies were taken from musculus tibialis anterior after 120 minutes of infusion of adrenaline/saline and at the end of the hyperinsulinemic clamp (t = 240 minutes) where adrenaline/saline infusion was continued. A, Glycogen synthase fractional activity (percentage) in muscles after infusion of adrenaline or saline (control) alone or in combination with the hyperinsulinemic-euglycemic clamp (n = 8-9 in each group). B, Glycogen synthase Ser^{641} phosphorylation in muscles after infusion of adrenaline or saline (control) alone or in combination with the hyperinsulinemic-euglycemic clamp (n = 9 in each group). C, Glycogen synthase $Ser^{645,649,653,657}$ phosphorylation in muscles after infusion of adrenaline or saline (control) alone or in combination with the hyperinsulinemic-euglycemic clamp (n = 9 in each group). D, Glycogen synthase Ser^{7} phosphorylation in muscles after infusion of adrenaline or saline (control) alone or in combination with the hyperinsulinemic-euglycemic clamp (n = 9 in each group). E, Representative blots showing GS Ser^{641}, GS $Ser^{645,649,653,657}$, and GS Ser^{7} phosphorylation, and total GS expression. $^{a}P < .05$ compared with control.

phosphorylation was unchanged (Fig. 3B-C). Adrenaline prevented insulin-mediated Ser^{641} phosphorylation (Fig. 3B). However, insulin dephosphorylated $Ser^{645,649,653,657}$ normally in the presence of adrenaline (Fig. 3C).

3.7. PKB and GSK-3β phosphorylation

Phosphorylation of PKB at Ser^{473} and Thr^{308} was low when saline was infused, and adrenaline did not increase PKB

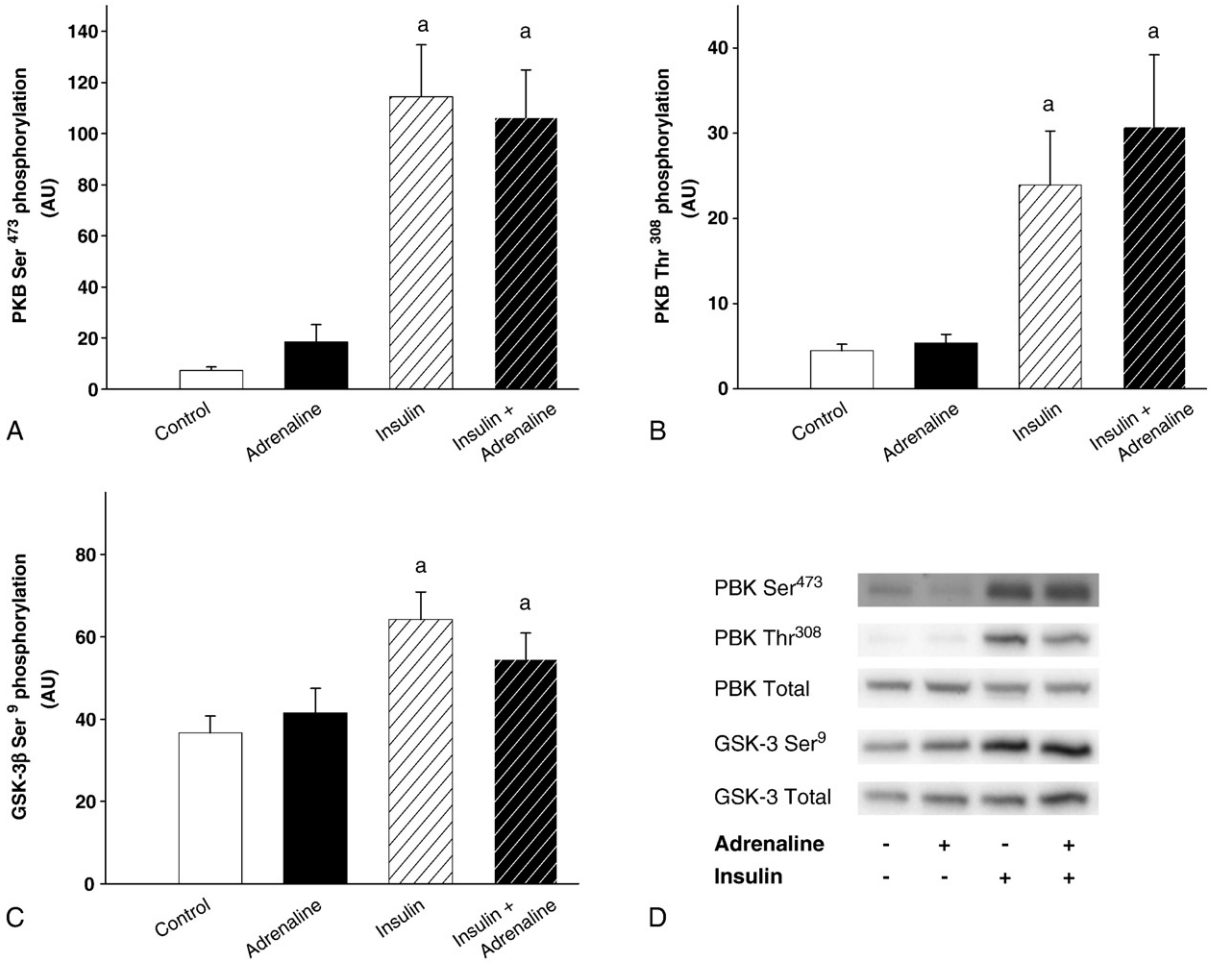

Fig. 5. Effect of adrenaline and insulin on PKB and GSK-3β phosphorylation in skeletal muscles. Biopsies were taken from musculus tibialis anterior after 120 minutes of infusion of adrenaline or saline and at the end of the hyperinsulinemic clamp with continuing infusion of adrenaline/saline (t = 240 minutes). A, PKB Ser^{473} phosphorylation. B, PKB Thr^{308} phosphorylation. C, GSK-3β Ser^{9} phosphorylation. D, Representative blots showing PKB and GSK-3 phosphorylation as well as total PKB and GSK-3β content in samples. n = 9 in each group; $^{a}P < .05$ compared with control.

Ser^{473} or Thr^{308} phosphorylation (Fig. 5). Insulin increased PKB Ser^{473} and Thr^{308} phosphorylation substantially (Fig. 5). Adrenaline did not alter insulin-stimulated PKB Ser^{473} and Thr^{308} phosphorylation. Muscles showed some GSK-3β Ser^{9} phosphorylation during saline infusion (Fig. 5C). Insulin increased GSK-3β Ser^{9} phosphorylation by about 75%. Adrenaline did not influence GSK-3β Ser^{9} phosphorylation during saline infusion or during insulin infusion (Fig. 5C).

3.8. Glycogen content and M-value at day 2

Glycogen content was significantly lower the day after infusion of adrenaline compared with saline, whereas synthase fractional activity was similar (Table 4). No sustained insulin resistance was observed 24 hours after adrenaline infusion. M-values at day 2 even tended (P = .14) to be higher the day after adrenaline infusion compared with saline infusion (Table 4). The average insulin levels during the clamp were, however, slightly higher after adrenaline infusion than after saline infusion (84 ± 1 vs 79 ± 1 mU/L, $P < .05$). Therefore, ISI was not altered by adrenaline infusion given the preceding day (data not shown).

4. Discussion

In the present study, we have investigated the mechanisms for adrenaline-induced insulin resistance in a protocol where adrenaline was infused for 4 hours in total: 2 hours

Table 4
Muscle glycogen content, GS fractional activity, and M-value on day 2 at 24 hours after adrenaline or saline infusion

Infusion previous day	Saline	Adrenaline	P value
Glycogen content (mmol/kg dry weight)	332.0 ± 27.1	243.8 ± 29.3	$P < .05$
GS FA (%)	4.8 ± 1.4%	9.8 ± 4.1%	$P = .27$
M-value (mg/[kg LBM min])	10.6 ± 0.5	12.0 ± 0.8	$P = .14$

Data are mean ± SEM. n = 10 for M-value. n = 8 in each group for glycogen content and GS fractional activity. FA indicates fractional activity.

before and during a 2-hour hyperinsulinemic-euglycemic clamp. Adrenaline-mediated hyperglycemia was normalized during the initial 30 minutes of insulin infusion. However, glucose infusion rate during the final hour of the clamp (euglycemia) with adrenaline coinfusion was reduced by approximately 60% compared with saline coinfusion. Adrenaline infusion completely blocked insulin-stimulated GS activation in skeletal muscles, which most likely occurred because GS Ser^{641} dephosphorylation was prevented. On the other hand, adrenaline did not influence insulin-stimulated PKB and GSK-3β phosphorylation. Moreover, glycogen content was lower in skeletal muscles the day after adrenaline infusion than the day after saline infusion, indicating that glucose disposal into skeletal muscles was inhibited during the hyperinsulinemic clamp when adrenaline was coinfused.

Adrenaline increased blood glucose concentration by approximately 50% as reported in other studies [1-3]. Blood glucose concentration, however, decreased to physiologic level within 30 minutes when insulin infusion was initiated. To the best of our knowledge, this is the first study where a hyperinsulinemic-euglycemic clamp has been started during adrenaline infusion; and we report that glucose concentration gradually decreased to physiologic concentration (5 mmol/L; ie, euglycemia) during 30 minutes. The glucose infusion rate during the hyperinsulinemic clamp with adrenaline coinfusion was reduced by more than 50%, which agrees with other studies [2,17,32,33]; and the glucose infusion rate of 4.2 mg/kg LBM per minute (≈3.1 mg/kg body weight per minute) during the last hour hardly exceeds previous reports of basal glucose disappearance rate (2-3 mg/[kg min]) in resting humans [13,33,34]. An adrenaline concentration of approximately 4 nmol/L has been reported to increase whole-body metabolic rate by about 20% [2,30,35] and more than doubles glucose oxidation [2]. However, coinfusion of insulin and adrenaline does not increase glucose oxidation more than adrenaline infusion alone [2]; therefore, insulin needs to increase glucose disposal or reduce glucose production to normalize blood glucose during adrenaline-mediated hyperglycemia.

It is well documented that adrenaline stimulates hepatic glucose release [13,36] and inhibits peripheral glucose uptake [4,13,32]. A high insulin level still reduces hepatic glucose production in the presence of adrenaline, although less effectively than when adrenaline is absent [4,13], whereas adrenaline causes a sustained inhibition of insulin-mediated glucose disposal [37]. Unfortunately, hepatic glucose production or peripheral glucose disposal was not measured in the present study; but insulin most likely reduced adrenaline-mediated hepatic glucose production, which contributed to normalization of blood glucose. In the present study, glucose infusion rate varied between 8.8 and 14.7 mg/kg LBM per minute during saline infusion; and adrenaline-mediated reduction in glucose infusion correlated with M-values, agreeing with Baron et al [33]. Normally, 70% to 90% of insulin-mediated glucose uptake occurs in skeletal muscles where glucose is incorporated into glycogen [11,12,38]; but the high physiologic concentration of adrenaline completely blocked insulin-stimulated GS activation as previously reported [17]. Inhibition of GS activation strongly reduces insulin-stimulated glycogen synthesis [16,39], and the fact that glycogen content was lower the day after adrenaline infusion supports that insulin-mediated glycogen synthesis was inhibited. However, previous studies have reported that insulin-stimulated glucose uptake is only reduced by 60% to 70% [32,33] and insulin-stimulated glycogen synthesis by 70% to 75% in skeletal muscles during adrenaline infusion [2]; and our data cannot exclude that a small amount of glycogen was synthesized, although adrenaline completely blocked insulin-stimulated GS activation.

This is the first study to investigate the effect of adrenaline on GS phosphorylation in human muscles. In the present study, adrenaline increased GS Ser^{641} phosphorylation and completely blocked insulin-mediated dephosphorylation; GS Ser^{641} phosphorylation therefore reflected GS fractional activity. In rat skeletal muscles, we have also seen that adrenaline prevents GS Ser^{641} dephosphorylation and that phosphorylation of this site reflects GS fractional activity [39,40]. On the other hand, adrenaline did not increase or prevent insulin-mediated dephosphorylation of GS $Ser^{645,649,653,657}$, which contrasts with our findings in rat muscles [39,40]. Glycogen synthase Ser^{7} phosphorylation has been reported to increase in rabbit and mice muscle exposed to adrenaline infusion [20,21] and in rat muscles exposed to high concentrations of adrenaline (Jensen, unpublished); but adrenaline infusion did not increase GS Ser^{7} phosphorylation. The molecular mechanisms for these differences are not clear and may simply arise from differences in adrenaline concentration. However, human muscles have been reported to express the glycogen targeting subunit of PP1 named *R3E* (gene *PPP1R3E*) that has low expression in rodent skeletal muscles; and regulation of GS activity may show some species-specific differences [41]. At least, our data show that a high physiologic concentration of adrenaline inhibits GS activity in human skeletal muscles via regulation of Ser^{641} phosphorylation rather than increasing GS Ser^{7} phosphorylation.

GSK-3 phosphorylates GS at Ser^{641}, Ser^{645}, Ser^{649}, and Ser^{653} and is the only known kinase to phosphorylate Ser^{641} in vivo [19]. In the present study, GSK-3β Ser^{9} phosphorylation was not regulated by adrenaline, which suggests that adrenaline regulates GS phosphorylation in human muscles via activation of protein phosphatase-1. Indeed, this interpretation is supported by the finding that adrenaline does not reduce GS activity in mice lacking the protein phosphatase-1 binding subunit R_{GL}/G_M [21]. On the other hand, regulation of GS by insulin requires GSK-3β phosphorylation in muscles [42]; and the insulin-mediated GSK-3β phosphorylation nicely reflected GS dephosphorylation at Ser^{641} and $Ser^{645,649,653,657}$. However, whereas adrenaline prevented insulin-stimulated GS Ser^{641} dephosphorylation, insulin

normally dephosphorylated GS $Ser^{645,649,653,657}$ in the presence of adrenaline despite no GS activation being observed. Therefore, our data support that GS Ser^{641} phosphorylation is the key phosphorylation site regulating activity during both insulin and adrenaline stimulation.

PKB Ser^{473} and Thr^{308} phosphorylation increased during insulin infusion as expected [43,44]. To the best of our knowledge, this is the first time effect of adrenaline on PKB phosphorylation has been studied in humans; and we report that adrenaline does not stimulate PKB Ser^{473} or Thr^{308} phosphorylation or influence insulin-stimulated PKB Ser^{473} and Thr^{308} phosphorylation. In rat skeletal muscles, we recently reported that adrenaline potentiated insulin-stimulated PKB phosphorylation at both Ser^{473} and Thr^{308}, and increased PKB activity [27,39]. There can be several reasons why we did not see a potentiation of insulin-stimulated PKB phosphorylation in humans. In the present study, muscles were exposed to a much lower adrenaline concentration compared with the rat muscles [27,39]; but we cannot exclude that adrenaline has different effects on PKB phosphorylation in humans and in rat muscles exposed to adrenaline in vitro.

Plasma lactate concentration increased gradually during adrenaline infusion, approaching 3 mmol/L after 120 minutes, agreeing with previous findings [2]. Adrenaline and β_2-adrenoceptor agonists stimulate lactate release from skeletal muscle [30,45,46], and the slow increase in lactate concentration was surprising in light of adrenaline's rapid activation of glycogen phosphorylase [47,48]. Skeletal muscles are the main contributor to systemic lactate appearance [30,46,49]. To the best our knowledge, lactate kinetic has not been studied in humans during adrenaline infusion or when adrenaline and insulin are coinfused. Lactate is a major energy substrate for the heart where lactate extraction correlates with plasma concentration [50], and lactate disappearance rate is about 1 to 2 mg/(kg min) in resting humans [49,51]. However, lactate disappearance rate can exceed glucose disappearance when lactate concentration is elevated to approximately 4 mmol/L by infusion [52]. Therefore, lactate was most likely actively metabolized throughout the adrenaline infusion with skeletal muscle being the major supplier of lactate.

The concentration of FFA increased during adrenaline infusion, and elevated FFA concentration for 4 hours has been reported to decrease insulin sensitivity and insulin signaling [53]. However, insulin decreased FFA level to similar levels during adrenaline infusion as compared with saline infusion, supporting that the 2-hour elevated FFA did not prevent insulin action in adipocytes. Indeed, insulin decreased glycerol less effectively when adrenaline was coinfused; and because the circulating concentration of glycerol more directly reflects lipolysis, we suggest that a physiologic insulin concentration does not completely block adrenaline-mediated lipolysis in humans, which agrees with data from adipocytes studied in vitro [54]. Insulin reduces adrenaline-mediated lipolysis because fat cells express the phosphodiesterase isoform PDE3B that becomes phosphorylated and activated by PKB [55]. In contrast, skeletal muscles express mainly PDE4 [56] that is not activated by insulin; and insulin does not influence adrenaline-stimulated glycogen phosphorylase activation [39].

Previous studies have reported that the physiologic threshold concentration for adrenaline-mediated response varies for different parameters. Threshold concentrations for adrenaline have been reported to be 0.3 to 0.7 nmol/L for heart rate and systolic and diastolic blood pressure, 0.5 to 0.8 nmol/L for increase in glycerol and FFA, and 0.8 to 1.1 nmol/L for effect on blood glucose and lactate [3,57,58]. In the present study, we have investigated the interaction between adrenaline and insulin in the regulation of these physiologic parameters. Infusion of adrenaline resulted in a sustained increase in heart rate and systolic blood pressure, whereas diastolic blood pressure decreased, which agrees with other studies [59]. Additional infusion of insulin did not influence any of the hemodynamic effects of adrenaline. On the other hand, insulin still decreased FFA and glycerol concentration when adrenaline was coinfused. The interaction in the regulation of carbohydrate metabolism seems more complex. Insulin normalized adrenaline-mediated hyperglycemia, whereas insulin-stimulated GS activation was completely prevented by adrenaline, and lactate concentration remained high. These data show the multifaceted interaction between adrenaline and insulin in the regulation of various physiologic effects.

The concentration of IL-6 in plasma increased during adrenaline infusion, which agrees with previous findings [60,61]. Adrenaline-stimulated IL-6 production has been reported to occur in adipose tissue [61]. However, other tissues, in particular muscle, cannot be excluded as sources of circulating IL-6 [62]. Although insulin counteracted adrenaline-mediated lipolysis and hyperglycemia, insulin infusion did not prevent adrenaline-stimulated IL-6 production. Instead, insulin increased IL-6 concentration further when adrenaline was coinfused; and insulin infusion per se slightly increased IL-6 production, agreeing with a recent study of ours [62]. Although it has been reported that IL-6 may cause insulin resistance [60], the metabolic role of IL-6 is still controversial; and it is not likely that adrenaline-stimulated IL-6 release explains the insulin resistance seen during adrenaline infusion. Our data also indicate that adrenaline did not cause insulin resistance via alterations in TNF-β, adiponectin, or leptin secretion.

The day after adrenaline infusion, M-value was normalized; and no sustained insulin resistance was observed. Intriguingly, adrenaline may have dual effects with respect to insulin resistance; and mice lacking β-adrenergic receptors that have lower diet-induced thermogenesis are prone to increase body weight [63], and treatment with β-blockade increases the risk for developing type 2 diabetes mellitus [64]. The increase in metabolic rate during adrenaline infusion is minimal (20%) compared with endurance exercise (10-fold), but adrenaline is a potent

activator of glycogen breakdown in skeletal muscle [65,66]. Interestingly, glycogen content in skeletal muscles was lower the day after adrenaline infusion when compared with saline infusion; but neither glucose infusion rate during the clamp nor insulin-stimulated GS activation was increased, although glycogen content is a strong regulator of insulin-stimulated glucose uptake in skeletal muscle [25,66]. Improved insulin sensitivity has been reported in humans after 1 or 2 weeks of terbutaline (β_2-agonist) treatment [67], but our data suggest that 4-hour adrenaline infusion is not sufficient to improve insulin sensitivity the following day even though glycogen content was reduced in skeletal muscles.

In conclusion, adrenaline did not influence basal or insulin-stimulated PKB or GSK-3β phosphorylation in human muscles. On the other hand, adrenaline completely blocked insulin-mediated GS activation most likely because adrenaline prevented GS Ser^{641} dephosphorylation. Still, insulin normalized adrenaline-mediated hyperglycemia within 30 minutes. Nonetheless, adrenaline induced marked insulin resistance that was partly explained by reduced glycogen storage in skeletal muscles.

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References

[1] Rizza RA, Cryer PE, Haymond MW, et al. Adrenergic mechanisms for the effects of epinephrine on glucose production and clearance in man. J Clin Invest 1980;65:682-9.
[2] Laurent D, Petersen KF, Russell RR, et al. Effect of epinephrine on muscle glycogenolysis and insulin-stimulated muscle glycogen synthesis in humans. Am J Physiol 1998;274:E130-8.
[3] Clutter WE, Bier DM, Shah SD, et al. Epinephrine plasma metabolic clearance rates and physiologic thresholds for metabolic and hemodynamic actions in man. J Clin Invest 1980;66:94-101.
[4] Deibert DC, DeFronzo RA. Epinephrine-induced insulin resistance in man. J Clin Invest 1980;65:717-21.
[5] Taube A, Eckardt K, Eckel J. Role of lipid-derived mediators in skeletal muscle insulin resistance. Am J Physiol Endocrinol Metab 2009.
[6] Scherrer U, Randin D, Tappy L, et al. Body fat and sympathetic nerve activity in healthy subjects. Circulation 1994;89:2634-40.
[7] Huggett RJ, Hogarth AJ, Mackintosh AF, et al. Sympathetic nerve hyperactivity in non-diabetic offspring of patients with type 2 diabetes mellitus. Diabetologia 2006;49:2741-4.
[8] Bruce DG, Chisholm DJ, Storlien LH, et al. The effects of sympathetic nervous system activation and psychological stress on glucose metabolism and blood pressure in subjects with type-2 (non–insulin-dependent) diabetes-mellitus. Diabetologia 1992;35:835-43.
[9] Brunner EJ, Hemingway H, Walker BR, et al. Adrenocortical, autonomic, and inflammatory causes of the metabolic syndrome: nested case-control study. Circulation 2002;106:2659-65.
[10] Lindmark S, Lonn L, Wiklund U, et al. Dysregulation of the autonomic nervous system can be a link between visceral adiposity and insulin resistance. Obes Res 2005;13:717-28.
[11] Shulman GI, Rothman DL, Jue T, et al. Quantification of muscle glycogen synthesis in normal subjects and subjects with non-insulin-dependent diabetes by 13C nuclear magnetic resonance spectroscopy. N Engl J Med 1990;322:223-8.
[12] DeFronzo RA, Gunnarsson R, Björkman O, et al. Effect of insulin on peripheral and splanchnic glucose metabolism in noninsulin-dependent (typeII) diabetes mellitus. J Clin Invest 1985;76:149-55.
[13] Lager I, Attvall S, Eriksson BM, et al. Studies on the insulin-antagonistic effect of catecholamines in normal man. Evidence for the importance of beta 2-receptors. Diabetologia 1986;29:409-16.
[14] Kurowski TG, Lin Y, Luo Z, et al. Hyperglycemia inhibits insulin activation of Akt/protein kinase B but not phosphatidylinositol 3-kinase in rat skeletal muscle. Diabetes 1999;48:658-63.
[15] Ruzzin J, Wagman AS, Jensen J. Glucocorticoids-induced insulin resistance: defects in insulin signalling and the effects of a selective glycogen synthase kinase-3 inhibitor. Diabetologia 2005;48: 2119-30.
[16] Franch J, Aslesen R, Jensen J. Regulation of glycogen synthesis in rat skeletal muscle after glycogen depleting contractile activity: effects of adrenaline on glycogen synthesis and activation of glycogen synthase and glycogen phosphorylase. Biochem J 1999;344:231-5.
[17] Raz I, Katz A, Spencer MK. Epinephrine inhibits insulin-mediated glycogenesis but enhances glycolysis in human skeletal muscle. Am J Physiol 1991;260:E430-5.
[18] Skurat AV, Roach PJ. Phosphorylation of sites 3a and 3b (Ser(640) and Ser(644)) in the control of rabbit muscle glycogen synthase. J Biol Chem 1995;270:12491-7.
[19] Cohen P. Dissection of the protein phosphorylation cascades involved in insulin and growth factor action. Biochem Soc Trans 1993;214: 555-67.
[20] Nakielny S, Campell DG, Cohen P. The molecular mechanism by which adrenaline inhibits glycogen synthesis. Eur J Biochem 1991; 199:713-22.
[21] Toole BJ, Cohen PT. The skeletal muscle-specific glycogen-targeted protein phosphatase 1 plays a major role in the regulation of glycogen metabolism by adrenaline in vivo. Cell Signal 2007;19:1044-55.
[22] Lindmark S, Wiklund U, Bjerle P, et al. Does the autonomic nervous system play a role in the development of insulin resistance? A study on heart rate variability in first-degree relatives of type 2 diabetes patients and control subjects. Diabet Med 2003;20:399-405.
[23] Jensen J, Ruzzin J, Jebens E, et al. Improved insulin-stimulated glucose uptake and glycogen synthase activation in rat skeletal muscles after adrenaline infusion: role of glycogen content and PKB phosphorylation. Acta Physiol Scand 2005;184:121-30.
[24] Passonneau JV, Lowry OH. Enzymatic analysis. A practical guide. Totowa: Human Press; 1993.
[25] Jensen J, Jebens E, Brennesvik EO, et al. Muscle glycogen inharmoniously regulates glycogen synthase activity, glucose uptake, and proximal insulin signaling. Am J Physiol Endocrinol Metab 2006; 290:E154-62.
[26] Aslesen R, Jensen J. Effects of epinephrine on glucose metabolism in contracting rat skeletal muscle. Am J Physiol 1998;275:E448-56.
[27] Brennesvik EO, Ktori C, Ruzzin J, et al. Adrenaline potentiates insulin-stimulated PKB activation via cAMP and Epac: implications for cross talk between insulin and adrenaline. Cell Signal 2005;17:1551-9.
[28] Lai YC, Stuenæs JT, Kuo CH, et al. Glycogen content and contraction regulate glycogen synthase phosphorylation and affinity for UDP-glucose in rat skeletal muscles. Am J Physiol Endocrinol Metab 2007; 293:E1622-9.
[29] Sherwin RS, Sacca L. Effect of epinephrine on glucose metabolism in humans: contribution of the liver. Am J Physiol 1984;247:E157-65.
[30] Simonsen L, Bulow J, Madsen J, et al. Thermogenic response to epinephrine in the forearm and abdominal subcutaneous adipose tissue. Am J Physiol 1992;263:E850-5.

[31] Stallknecht B, Simonsen L, Bulow J, et al. Effect of training on epinephrinestimulated lipolysis determined by microdialysis in human adipose tissue. Am J Physiol 1995;269:E1059-66.
[32] Bessey PQ, Brooks DC, Black PR, et al. Epinephrine acutely mediates skeletal muscle insulin resistance. Surgery 1983;94:172-9.
[33] Baron AD, Wallace P, Olefsky JM. In vivo regulation of non-insulin-mediated and insulin-mediated glucose uptake by epinephrine. J Clin Endocrinol Metab 1987;64:889-95.
[34] Miles JM, Nissen SL, Gerich JE, et al. Effects of epinephrine infusion on leucine and alanine kinetics in humans. Am J Physiol 1984;247: E166-72.
[35] Stob NR, Seals DR, Jensen J, et al. Increased thermogenic responsiveness to intravenous β-adrenergic stimulation in habitually exercising humans is not related to skeletal muscle β2-adrenergic receptor density. Exp Physiol 2007;92:823-30.
[36] Sacca L, Vigorito C, Cicala M, et al. Role of gluconeogenesis in epinephrine-stimulated hepatic glucose production in humans. Am J Physiol 1983;245:E294-302.
[37] Rizza R, Haymond M, Cryer P, et al. Differential effects of epinephrine on glucose production and disposal in man. Am J Physiol 1979;237: E356-62.
[38] Baron AD, Brechtel G, Wallace P, et al. Rates and tissue sites of non-insulin- and insulin-mediated glucose uptake in humans. Am J Physiol 1988;255:E769-74.
[39] Jensen J, Grønning-Wang LM, Jebens E, et al. Adrenaline potentiates insulin-stimulated PKB activation in the rat fast-twitch epitrochlearis muscle without affecting IRS-1 associated PI 3-kinase activity. Pflugers Arch-Eur J Physiol 2008;456:969-78.
[40] Jensen J, Brennesvik EO, Lai YC, et al. GSK-3 regulation in skeletal muscles by adrenaline and insulin: evidence that PKA and PKB regulate different pools of GSK-3. Cell Signal 2007;19:204-10.
[41] Munro S, Ceulemans H, Bollen M, et al. A novel glycogen-targeting subunit of protein phosphatase 1 that is regulated by insulin and shows differential tissue distribution in humans and rodents. FEBS J 2005; 272:1478-89.
[42] Bouskila M, Hirshman MF, Jensen J, et al. Insulin promotes glycogen synthesis in the absence of GSK3 phosphorylation in skeletal muscle. Am J Physiol Endocrinol Metab 2008;294:E28-35.
[43] Frøsig C, Rose AJ, Treebak JT, et al. Effects of endurance exercise training on insulin signaling in human skeletal muscle: interactions at the level of phosphatidylinositol 3-kinase, Akt, and AS160. Diabetes 2007;56:2093-102.
[44] Jessen N, Djurhuus CB, Jorgensen JO, et al. Evidence against a role for insulin signaling proteins PI 3-kinase and Akt in insulin resistance in human skeletal muscle induced by short-term GH infusion. Am J Physiol Endocrinol Metab 2005;288:E194-9.
[45] Simonsen L, Stefl B, Christensen NJ, et al. Thermogenic response to adrenaline during restricted blood flow in the forearm. Acta Physiol Scand 1999;166:31-8.
[46] Qvisth V, Hagstrom-Toft E, Moberg E, et al. Lactate release from adipose tissue and skeletal muscle in vivo: defective insulin regulation in insulin-resistant obese women. Am J Physiol Endocrinol Metab 2007;292:E709-14.
[47] Jensen J, Dahl HA. Adrenaline stimulated glycogen breakdown in rat epitrochlearis muscles: fibre type specificity and relation to phosphorylase transformation. Biochem Mol Biol Int 1995;35:145-54.
[48] Chasiotis D, Sahlin K, Hultman E. Regulation of glycogenolysis in human muscle in response to epinephrine infusion. J Appl Physiol 1983;54:45-50.
[49] Consoli A, Nurjhan N, Reilly Jr JJ, et al. Contribution of liver and skeletal muscle to alanine and lactate metabolism in humans. Am J Physiol 1990;259:E677-84.
[50] Gertz EW, Wisneski JA, Stanley WC, et al. Myocardial substrate utilization during exercise in humans. Dual carbon-labeled carbohydrate isotope experiments. J Clin Invest 1988;82:2017-25.
[51] Bergman BC, Wolfel EE, Butterfield GE, et al. Active muscle and whole body lactate kinetics after endurance training in men. J Appl Physiol 1999;87:1684-96.
[52] Miller BF, Fattor JA, Jacobs KA, et al. Lactate and glucose interactions during rest and exercise in men: effect of exogenous lactate infusion. J Physiol 2002;544:963-75.
[53] Dresner A, Laurent D, Marcucci M, et al. Effects of free fatty acids on glucose transport and IRS-1-associated phosphatidylinositol 3-kinase activity. J Clin Invest 1999;103:253-9.
[54] Desai KS, Li KC, Angel A. Bimodal effect of insulin on hormone-stimulated lipolysis: relation to intracellular 3′,5′-cyclic adenylic acid and free fatty acid levels. J Lipid Res 1973;14:647-55.
[55] Degerman E, Landstrom TR, Wijkander J, et al. Phosphorylation and activation of hormone-sensitive adipocyte phosphodiesterase type 3B. Methods 1998;14:43-53.
[56] Enoksson S, Degerman E, Hagstrom-Toft E, et al. Various phosphodiesterase subtypes mediate the in vivo antilipolytic effect of insulin on adipose tissue and skeletal muscle in man. Diabetologia 1998;41:560-8.
[57] Sjöström L, Schutz Y, Gudinchet F, et al. Epinephrine sensitivity with respect to metabolic rate and other variables in women. Am J Physiol 1983;245:E431-42.
[58] Galster AD, Clutter WE, Cryer PE, et al. Epinephrine plasma thresholds for lipolytic effects in man: measurements of fatty acid transport with [l-13C]palmitic acid. J Clin Invest 1981;67: 1729-38.
[59] Ratheiser KM, Brillon DJ, Campbell RG, et al. Epinephrine produces a prolonged elevation in metabolic rate in humans. Am J Clin Nutr 1998; 68:1046-52.
[60] Pedersen BK, Febbraio MA. Muscle as an endocrine organ: focus on muscle-derived interleukin-6. Physiol Rev 2008;88:1379-406.
[61] Keller P, Keller C, Robinson LE, et al. Epinephrine infusion increases adipose interleukin-6 gene expression and systemic levels in humans. J Appl Physiol 2004;97:1309-12.
[62] Ruge T, Lockton JA, Renstrom F, et al. Acute hyperinsulinemia raises plasma interleukin-6 in both nondiabetic and type 2 diabetes mellitus subjects, and this effect is inversely associated with body mass index. Metabolism 2009;58:860-6.
[63] Bachman ES, Dhillon H, Zhang CY, et al. β-AR signaling required for diet-induced thermogenesis and obesity resistance. Science 2002;297: 843-5.
[64] Pollare T, Lithell H, Berne C. Insulin resistance is a characteristic feature of primary hypertension independent of obesity. Metabolism 1990;39:167-74.
[65] Jensen J, Dahl HA, Opstad PK. Adrenaline-mediated glycogenolysis in different skeletal muscle fibre types in the anaesthetized rat. Acta Physiol Scand 1989;136:229-33.
[66] Jensen J, Aslesen R, Ivy JL, et al. Role of glycogen concentration and epinephrine on glucose uptake in rat epitrochlearis muscle. Am J Physiol 1997;272:E649-55.
[67] Scheidegger K, Robbins DC, Danforth EJ. Effects of chronic beta receptor stimulation on glucose metabolism. Diabetes 1984;33: 1144-9.