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Effects of adrenaline on lactate, glucose, lipid and protein metabolism in the placebo controlled bilaterally perfused human leg

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Abstract

Aim: Adrenaline has widespread metabolic actions, including stimulation of lipolysis and induction of insulin resistance and hyperlactatemia. Systemic adrenaline administration, however, generates a very complex hormonal and metabolic scenario. No studies employing regional, placebo controlled and adrenaline infusion exist. Our study was designed to test the hypothesis that local placebo controlled leg perfusion with adrenaline directly increases local lactate release, stimulates lipolysis, induces insulin resistance and leaves protein metabolism unaffected.

Methods: We studied seven healthy volunteers with bilateral femoral vein and artery catheters during 3-h basal and 3-h hyperinsulinemic (0.6 mU kg^{-1} min^{-1}) euglycemic clamp conditions. One femoral artery was perfused with saline and the other with adrenaline (0.4 μg min m^{-2}). Lipid metabolism was quantified with [9,10-^{3}H] palmitate and amino acid metabolism with ^{15}N-phenylalanine and lactate and glucose by raw arterio-venous differences.

Results: Femoral vein plasma adrenaline increased ≈eightfold in the perfused leg with unaltered blood flows. Adrenaline perfusion significantly increased local leg lactate release from 0.01 to 0.25 mmol min^{-1} per leg, palmitate release in the basal state 11.5–16.9 μmol min^{-1} per leg and during the clamp 2.62–8.44 μmol min^{-1} per leg. Glucose uptake decreased during the clamp from ≈180 to 30 μmol min^{-1} per leg. Phenylalanine kinetics was not affected by adrenaline.

Conclusion: Adrenaline directly increases lactate release and lipolysis and inhibits insulin-stimulated glucose uptake in the perfused human leg. Adrenaline has no direct effects on peripheral amino acid metabolism. Adrenaline-induced lactate release from striated muscle may be an important mechanism underlying hyperlactatemia in the critically ill.

Keywords amino acid metabolism, adrenaline, glucose metabolism, lactate, lipid metabolism, regional metabolism.

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Introduction

Adrenaline has important metabolic actions, some of which are clear and more complex. It is clear that adrenaline effectively stimulates lipolysis and increases the concentrations of free fatty acid (FFA) (Baltzan *et al.* 1965) and leads to hyperglycaemia because of peripheral and hepatic insulin resistance (Deibert & Defronzo 1980). It is also clear that adrenaline generates hyperlactatemia (Laurent *et al.* 1998) possibly *via* stimulation of muscle lactate release. Hyperlactatemia has important clinical implications as high levels of lactate is a strong predictor of mortality in the critically ill. (Jansen *et al.* 2009, Khosravani *et al.* 2009, Nichol *et al.* 2010). Although being widely regarded as a catabolic hormone the observed effects of adrenaline on protein metabolism are much less coherent. Most studies report similar decreases in whole body leucine rates of appearance and disappearance (Miles *et al.* 1984, Castellino *et al.* 1990), i.e. an overall neutral impact, but one study also observed increased forearm leucine release (Kraenzlin *et al.* 1989). The picture is, however, complicated as adrenaline-induced hyperglycaemia, hyperinsulinemia and high levels of FFA may increase protein synthesis and decrease breakdown (Meek *et al.* 1998, Norrelund *et al.* 1998). Adrenaline lowers blood concentrations of amino acids probably because adrenaline increases the clearance of amino acids for gluconeogenesis (Goldstein *et al.* 1995) and low amino acid concentrations may *per se* increase protein breakdown and decrease protein synthesis (Giordano *et al.* 1995). Finally, adrenaline is a neurotransmitter with important actions at the hypothalamic level specifically and the CNS in general. These CNS effects may further modulate both metabolic and hormonal responses (Wortsman 2002). Intriguingly, it has also been shown that adrenaline increases CNS lactate uptake and thus changes cerebral metabolite consumption (Seifert *et al.* 2009). All of these widespread metabolic effects obviously add to the complexity of the metabolic scenario and some studies have used somatostatin and exogenous insulin in an attempt to circumvent some of these complexities. So perfusion studies without concurrent placebo control and studies employing systemic infusion have suggested that adrenaline acts directly in limb muscles to increase local lactate release, stimulate lipolysis, induce insulin resistance, while leaving protein metabolism unaffected. Our study was designed to test whether these assumptions are correct. Therefore, we designed a study to assess direct local effects of adrenaline infusing the active substance unilaterally in a leg utilizing simultaneous saline placebo control in the contra lateral femoral artery. The dose was chosen to ensure a high regional stimulation with minimal systemic effects.

Materials, methods and study design

Seven healthy, lean, male volunteers were included in the study (Table 1). The volunteers were investigated once during post-absorptive conditions (12-h fast). Vigorous physical exercise was not allowed 2 days before participating in the study. The volunteers were admitted to The Medical Research Laboratories at 7.30 and remained in bed throughout the day. The experiments were performed under thermo neutral conditions (21–23 °C). The study was approved by the local Ethics Committee (approval no. 20040144), listed at clinicaltrials.gov (NCT01116609) and all participants gave written informed consent before participating.

The study protocol is outlined in Fig. 1. The study day started with a 180-min basal period, henceforth referred to as 'basal', followed by a 180-min hyperinsulinemic euglycemic clamp referred to as 'clamp'. Randomization of adrenaline and placebo leg was carried out by simple draw (envelope).

The leg model

In local anaesthesia (lidocain 10 mg ml^{-1}, 'SAD', Copenhagen, Denmark), both femoral arteries and veins were cannulated percutaneously with double lumen 4 Fr. Catheters (BD Careflow™, Stockholm, Sweden) placed with the arterial catheter tip in the cranial direction, approximately at the level of the inguinal ligament and the venous catheter tip in the caudal direction with the tip distal to the merge of the great saphenous vein into the femoral vein. The double lumen catheters allowed simultaneous blood sampling and infusion in the arterial catheter with the cranial lumen used for sampling and the caudal lumen for indocyanine green (ICG) infusion. The exact location of the catheter tips were visualized by ultrasound (Vivid *e*; GE, Milwaukee, WI, USA). Because of the displacement of catheters during the clamp period one volunteer only supplied data to the basal period. Adrenaline (Adrenalin 1 mg ml^{-1}, 'SAD', Copenhagen, Denmark, 0.4 μg min^{-1} m^2) was infused in a single blind random choice femoral artery.

Albumin-bound [9,10-^{3}H] palmitate (GE, Buckinghamshire, UK) and phenylalanine (^{15}N-Phenylalanine; Cambridge Isotope Laboratories, Andover, MA, USA) were used as metabolite tracers. The chemical, isotopic and optical purity of the isotopes were tested before use. Solutions were prepared under sterile conditions and were shown to be free of bacteria and pyrogens before use.

Palmitate, isotonic saline, phenylalanine, insulin (Insulin Actrapid; Novo-Nordisk, Copenhagen, Denmark), amino acids (Glavamin®14 gN L^{-1}, Fresenius

Table 1 Volunteer characteristics

	Basal			Clamp		
	Placebo	Adrenaline	*P*-value	Placebo	Adrenaline	*P*-value
Age (years)	25 ± 3.4	–	–	–	–	–
Weight (kg)	82.7 ± 3	–	–	–	–	–
Body surface area (m^2)	2.04 ± 0.04	–	–	–	–	–
Leg blood flow ($mL \times min^{-1}$)	426 ± 85	416 ± 54	NS	369 ± 93	474 ± 107	NS
Adrenaline ($ng \times mL^{-1}$)	0.15 ± 0.01	1.2 ± 0.1	$P < 0.05$	0.27 ± 0.08	1.2 ± 0.1	$P < 0.05$
Glucose ($mmol \times L^{-1}$)	5.5 ± 0.08	5.5 ± 0.08	NS	5.2 ± 0.06	5.2 ± 0.06	NS
Insulin($pmol \times L^{-1}$)	49.9 ± 7.35	–	–	266 ± 24.5	–	$P < 0.01$

Body surface: calculated from the Dubois and Dubois formula; Leg blood flow: calculated from dye dilution (ICG) data; Adrenaline: venous concentrations; Glucose: arterial concentration; *P*-values placebo vs. adrenaline basal and clamp, Insulin basal vs. clamp arterial values.

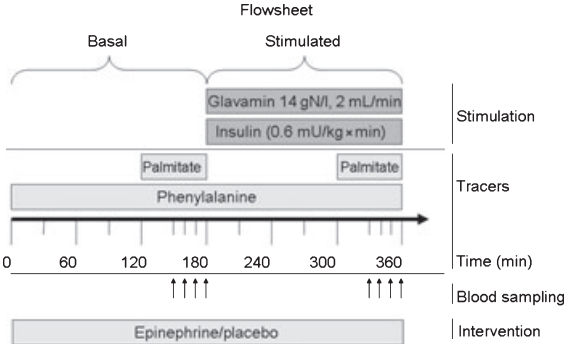

Figure 1 Study flowsheet. Study programme for all volunteers. Blood sampling; basal period: 150, 160, 170 and 180 min. Clamp period 330, 340, 350 and 360 min.

Kabi, Bad Homburg, Germany) and glucose were all infused in a cubital vein catheter during the experiments.

Blood flow of the legs was determined using continuous infusion of ICG. Samples from the femoral artery and vein were taken in triplicate at the end of each period for measurements of ICG (Jorfeldt & Rutberg 1990, Ott *et al.* 1993, Gjedsted *et al.* 2007) and haematocrit (Minutes: 160, 170 and 180 together with 340, 350, 360). Mean arterial and venous concentrations were used for calculations. ICG ~75 $\mu g\ min^{-1}$ (Akorn, Lake Forest, IL, USA) was infused into both arterial catheters from t = 70–120 min to 190–240 min. After priming the amino acid pool with bolus injection of ^{15}N-phenylalanine (0.7 $mg\ kg^{-1}$), continuous intravenous infusion of ^{15}N-phenylalanine (0.7 $mg\ kg^{-1}\ h^{-1}$) was started at t = *0* and maintained until termination of the study. At t = 180 min, insulin (Insulin Actrapid; Novo-Nordisk, Copenhagen, Denmark) was infused intravenously at a constant rate of 0.6 $mU\ kg^{-1}\ min^{-1}$ for 180 min (t = 180–360 min). Plasma glucose was clamped at 5 $mmol\ l^{-1}$ (DeFronzo *et al.* 1979). During the clamp plasma glucose was measured in duplicate every 10 min on a Beckman analyser (Beckman Instruments, Palo Alto, CA, USA). During the clamp period amino acids were infused (2 $mL\ min^{-1}$, Glavamin®14 $gN\ L^{-1}$, Fresenius Kabi) to avoid a decrease in amino acid levels and subsequent changes in insulin sensitivity (Flakoll *et al.* 1992).

[9,10-^{3}H] palmitate (0.3 $\mu Ci\ min^{-1}$) was infused from t = 120–180 min and again from t = 300–360 min. Blood samples for measurements of palmitate concentration and SA were drawn before the infusion and after 40, 50, and 60 min of the infusion period. Unless otherwise specified blood samples were obtained in triplicate from the femoral artery during the last 30 min of the basal and the clamp periods.

Analyses

We used a two-site immunoassay ELISA (DAKO, Glostrup, Denmark) (Andersen *et al.* 1993) to measure serum insulin. Plasma adrenaline concentrations were determined by a commercial ELISA kit (Adrenaline Research ELISA.EIA-4776. DRG Diagnostics, Marburg Germany). Serum FFA was determined by a colorimetric method employing a commercial kit (Wako Chemicals, Neuss, Germany). Enrichments of ^{15}N-phenylalanine were measured by gas chromatography–mass spectrometry (GC–MS) as their t-butyldimethylsilyl ether derivates under electron ionization conditions and concentration of phenylalanine were measured (for calculation of regional amino acid kinetics) using L-[$^2H_8$]phenylalanine as internal standard (Nair *et al.* 1995). Plasma palmitate concentration and SA were determined by HPLC (Miles *et al.* 1987) using [$^2H_{31}$] palmitate as internal standard (Jensen *et al.* 1988). ICG optical density was measured spectrophotometrically (Milton Roy Spectronic 601, Ivyland, PA, USA) at a wavelength of 800 and 900 nm.

Subsequently, blood flow was estimated by Fick's principle. Lactate concentrations were determined by an automated analyser (Cobas b221, Roche, Denmark) applying amperiometrics.

Phenylalanine and palmitate kinetics

In this study, phenylalanine balance (PheBal) was calculated as follows using Fick's principle:

$$\text{PheBal} = (\text{Phe}_a - \text{Phe}_v) \times F$$

in which Phe_a and Phe_v are arterial and venous phenylalanine concentrations and F is blood flow in the leg. Regional phenylalanine kinetics was calculated, using the equations described by Nair *et al.* (1995). The leg protein breakdown represented by phenylalanine rate of appearance ($R_a$phe) was calculated as follows: (Copeland & Nair 1994):

$$R_a\text{phe} = \text{Phe}_a \times ((\text{PheE}_a/\text{PheE}_v) - 1) \times F$$

in which PheE_a and PheE_v represent phenylalanine isotopic enrichment in arteries and veins. The local rate of disappearance, which represents the muscle protein synthesis rate, was calculated as:

$$R_d\text{phe} = \text{PheBal} + R_a\text{phe}.$$

Regional palmitate net balances (uptake and release) were estimated using blood flow and SA from arterial and venous samples and calculated as previously described (Nielsen *et al.* 2004). Plasma ^{15}N-phenylalanine enrichment and [9,10-^{3}H] palmitate-specific activity were in steady state at the time of sampling (data not shown).

Statistics

All results are given as mean ± SEM. All comparisons are *basal* placebo vs. adrenaline and *clamp* placebo vs. adrenaline unless otherwise specified. All comparisons were made by Student's two-tailed paired *t*-test. A *P*-value less than 0.05 was considered significant. Statistical analyses were performed using SPSS version 17.0 for Windows (SPSS, Chicago, IL, USA).

Results

The characteristics of the volunteers are given in Table 1.

Basal

Due to the nature of the model all arterial hormone and metabolite levels were similar except for adrenaline. Arterial glucose concentrations were 5.5 ± 0.2 mmol l^{-1} and blood flows were not different between the two legs during the basal period (Table 1). Venous levels of adrenaline were ≈eightfold elevated in the adrenaline perfused leg (Table 1) and adrenaline significantly increased net palmitate release (Table 2, Fig. 2). Basal glucose net balance was unaltered (Fig. 3, Table 2) as phenylalanine kinetics (Table 2). Net lactate release was significantly increased in the adrenaline perfused leg (Fig. 4, 0.01 ± 0.01 vs. 0.25 ± 0.1 mmol min^{-1}, $P < 0.05$).

Clamp

Arterial glucose concentrations were clamped at 5.2 ± 0.2 mmol L^{-1}. During the last 30 min of the clamp, we reached steady state glucose infusion rate. During the hyperinsulinemic euglycemic clamp leg blood flows were still similar (Table 1) and femoral venous levels of adrenaline remained significantly higher in the adrenaline perfused leg. Net palmitate release (Fig. 2, Table 2) was substantially decreased during insulin infusion, but remained threefold increased on the adrenaline perfused side ($P < 0.05$). Insulin stimulated glucose net balance was much lower in the adrenaline perfused leg (27 ± 16 vs. 184 ± 64 μmol · min^{-1} leg^{-1}; $P < 0.05$) (Table 2 and Fig. 3). Local leg phenylalanine metabolism and lactate balance were not affected by regional adrenaline infusion during the clamp. Net lactate release tended to be higher in the adrenaline perfused leg (0.02 ± 0.04 vs. −0.2 ± 0.01 mmol min^{-1} $P = 0.09$).

Discussion

Our study was designed to: (i) test the hypotheses that direct perfusion of the leg with adrenaline would increase lactate release, induce insulin resistance and increase lipolysis without having effects on protein metabolism and (ii) to establish a new method to assess direct local placebo controlled effects of metabolic agents in the human leg.

We accomplished 4–8 fold increases in femoral vein adrenaline concentrations, which are well within the physiological range. To our knowledge, this is the first placebo controlled study to show that adrenaline directly stimulates net lactate release and lipolysis (measured with labelled palmitate) and generates a state of insulin resistance in the perfused human leg.

Recently, the role of lactate in critical illness has attracted substantial interest (Jones & Puskarich 2009) and a whole series of studies have shown that blood lactate concentrations strongly predict mortality (Trzeciak *et al.* 2007, Jansen *et al.* 2009). It is often assumed that high lactate concentrations are because of local

Table 2 Regional metabolite balances

	Basal			Clamp		
	Placebo	Adrenaline	*P*-value	Placebo	Adrenaline	*P*-value
Net palmitate balance (μmol min^{-1} per leg^{-1})	1.69 ± 1.9	6.86 ± 2.2	NS	−1.51 ± 0.86	4.13 ± 1.5	$P < 0.05$
Net palmitate$_R$ (μmol min^{-1} per leg^{-1})	11.5 ± 1.8	16.9 ± 1.5	$P < 0.05$	2.62 ± 0.5	8.44 ± 1.6	$P < 0.05$
Net palmitate$_U$ (μmol min^{-1} per leg^{-1})	9.83 ± 2.3	10.0 ± 1.7	NS	4.1 ± 1.0	4.3 ± 0.7	NS
Phenylalanine balance (μmol min^{-1} per leg^{-1})	1.9 ± 0.3	1.7 ± 0.2	NS	4.0 ± 0.5	2.8 ± 0.9	NS
Phenylalanine$_{Ra}$ (μmol min^{-1} per leg^{-1})	5.1 ± 0.8	4.3 ± 0.6	NS	6.3 ± 1.1	5.4 ± 1.0	NS
Phenylalanine$_{Rd}$ (μmol min^{-1} per leg^{-1})	3.2 ± 0.6	2.6 ± 0.4	NS	10.3 ± 1.4	8.3 ± 1.9	NS
Glucose a-v balance (μmol min^{-1} per leg^{-1})	35 ± 18	9 ± 8	NS	184 ± 64	27 ± 16	$P < 0.05$

Net palmitate$_R$: Net palmitate release in leg calculated from tracer fractional uptake; Net palmitate$_U$: Net palmitate uptake in leg calculated from tracer fractional uptake; Palmitate balance: Palmitate a-v balance; Phenylalanine$_{Ra}$: phenylalanine rate of appearance in leg calculated from tracer fractional uptake; Phenylalanine$_{Rd}$: phenylalanine rate of disappearance in leg calculated from tracer fractional uptake; Phenylalanine balance: phenylalanine$_{Rd}$ – Phenylalanine$_{Ra}$; Glucose a-v balance: arterio-venous glucose difference, *P*-values: Placebo vs. adrenaline.

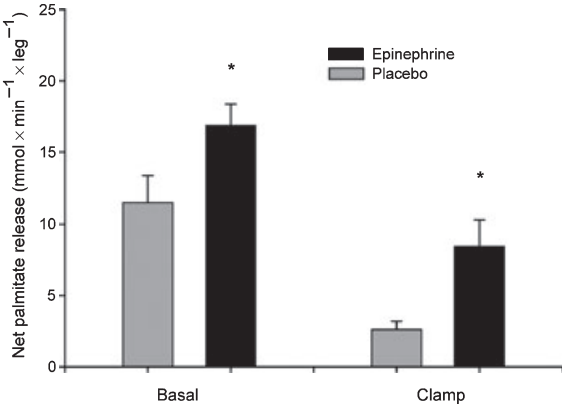

Figure 2 Palmitate balance. Palmitate balance calculated from simple a-v balance. Mean values from triplicate sampling at times 160, 170 and 180 min(basal) and 340, 350 and 360 min(clamp). *$P < 0.05$ adrenaline vs. placebo.

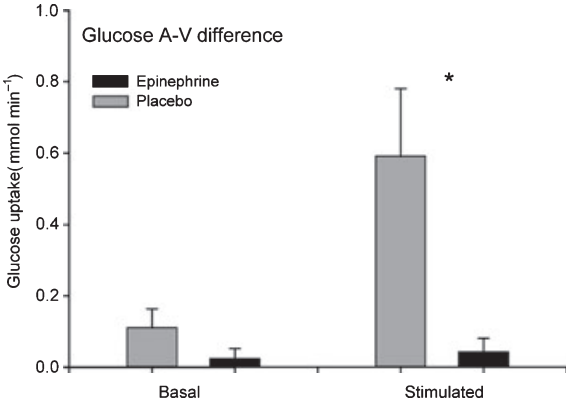

Figure 3 Glucose arterio-venous difference. Glucose raw a-v balances from mean values from triplicate sampling at times 160, 170 and 180 min(basal) and 340, 350 and 360 min (clamp). *$P < 0.05$ adrenaline vs. placebo.

tissue hypoperfusion and hypoxia. However, Fryburg *et al.* together with the original studies by Baltzan suggested increased forearm lactate release, during uncontrolled local adrenaline perfusion (Baltzan *et al.* 1965, Fryburg *et al.* 1995). Our results clearly show that adrenaline directly stimulates lactate release from the leg in the presence of normal blood flow and tissue oxygenation. During normal physiological conditions lactate production is an estimated rate of 1500 mmol per day (≈1 mmol min^{-1}), the production during critical illness is however very variable (Levy 2006). With an estimated lactate production of approx. 1 mmol · min^{-1} during low dose adrenaline infusion, adrenaline could play a significant role in lactic acidosis during critical illness.

In our study, the regional blood flow was unaltered by adrenaline. Adrenaline is in most studies described as

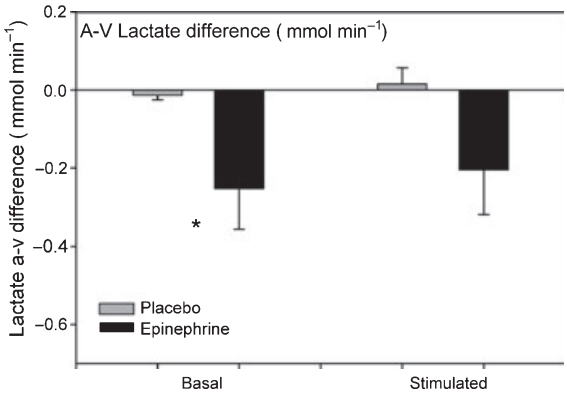

Figure 4 Lactate difference. Lactate raw a-v balances from mean values from triplicate sampling at times 160, 170 and 180 min(basal) and 340, 350 and 360 min(clamp). *$P < 0.05$ adrenaline vs. placebo.

an ino-dilatator resulting in increased cardiac output and skeletal muscle perfusion. In our study, however, we isolated the effect to the leg and did not see any effects on the blood flow. This could be due to the low dose infused or the lack of systemic effect i.e. an increase in cardiac output. Blood flow responses to intraarterial adrenaline infusion in the arm is highly variable (Baltzan *et al.* 1965).

It is unlikely that blood or interstitial glucose contribute to lactate formation as glucose arterio-venous differences were unaltered in the basal state. It is likely that the underlying mechanism involve stimulation of muscle glycogenolysis through β-adrenergic stimulation of glycogen phosphorylase activity. A number of human studies have shown that during exercise systemic adrenaline administration increases muscle glycogenolysis and glycogen phosphorylase activity (Febbraio *et al.* 1998, Kjaer *et al.* 2000, Watt *et al.* 2001). In addition, Qvisth *et al.* (2008) showed that local muscle lactate concentrations measured by microdialysis in humans increased during β-adrenergic stimulation and data by Hourani *et al.* (1990) suggest that β-stimulation increases muscle and hepatic lactate release. Thus, it appears that specific adrenergic stimulation of muscle glycogenolysis and lactate release constitutes an important evolutionary component of the fight-or-flight response liberating lactate fuel energy from resting muscle glycogen to the blood and utilization in exercising muscle and brain (Ahlborg & Felig 1982, Seifert *et al.* 2009).

As indicated above, there is contradictory data as regards the effect of adrenaline and catecholamines on protein metabolism. Virtually, all studies report decreases in blood amino acid concentrations after systemic adrenaline administration together with equally decreased amino acid breakdown and synthesis (Matthews *et al.* 1990, Ratheiser *et al.* 1999). Some of these effects could be caused by high levels of insulin inhibiting protein breakdown and thereby decreasing amino acid levels (Miles *et al.* 1984, Jansen *et al.* 2009). The low concentrations of amino acids *per se* tend to decrease protein synthesis and increase breakdown (Meek *et al.* 1998). Studies employing somatostatin and subsequent insulin administration to control insulin levels (Kraenzlin *et al.* 1989, Castellino *et al.* 1990) in general still have reported decreased amino acid concentrations; this decrease could be because of increased hepatic amino acid clearance caused by adrenaline (Goldstein *et al.* 1995). Using labelled phenylalanine to trace forearm protein metabolism Fryburg *et al.* (1995) perfused adrenaline into the brachial artery and observed decreased forearm protein breakdown and synthesis after 2 and 4 h and decreased net phenylalanine forearm balance after 4 h compatible with an 'anticatabolic' effect of adrenaline. This study protocol, however, was designed with no concurrent control arm leaving it susceptible to confounding. Our data clearly suggest that adrenaline has no direct effect on leg protein metabolism. In full agreement with a vast number of studies, we demonstrated that the adrenaline directly stimulated lipolysis and inhibited insulin stimulated glucose uptake. Please note that in the basal state, we also saw trends ($P > 0.05$) towards increased net palmitate release and decreased glucose uptake during adrenaline perfusion. Previous studies have shown that during exercise circulating adrenaline plays a central role in the stimulation of lipolysis, whereas the sympathetic nervous system is of less importance (Stallknecht *et al.* 2001). It remains, however, to be established whether local adrenaline-induced release of FFA influence regional glucose metabolism or *vice versa*.There are some limitations to the present study. Although adrenaline is quickly metabolized, spillover of adrenaline to the systemic circulation may have obscured some potential peripheral effects. It is also acknowledged that the present study does not offer a mechanistic explanation for the effects of adrenaline on lactate, palmitate and glucose metabolism. In future studies, the effect of adrenaline perfusion on insulin signalling in skeletal muscle biopsies can be investigated and established if adrenaline directly cause insulin resistance and secondarily lipolysis or *vice versa*.

In summary, our data show that adrenaline directly stimulates lactate release and lipolysis and inhibits insulin-stimulated glucose uptake, without affecting amino acid metabolism in the perfused human leg. This is compatible with the notion that adrenaline-induced lactate release from striated muscle may be a prime mechanism underlying hyperlactatemia in the critically ill. Our results also show that combined bilateral femoral arterio-venous catheterization with isotope dilution, hyperinsulinemic clamp technique and local adrenaline perfusion in healthy volunteers is an effective and safe human model for studying local effects for biological active substances with short half-lives. The technique could be supplemented with other methods, such as tissue biopsies and microdialysis.

Conflicts of interest

The authors report no conflicts of interest.

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