Ischaemia and insulin, but not ischaemia and contraction, act synergistically in stimulating muscle glucose uptake in vivo in humans

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ABSTRACT

Ischaemia, like muscle contraction, has been reported to induce skeletal muscle glucose uptake in in vitro models. This stimulating effect appears independent of insulin and is probably mediated by activation of AMPK (AMP-activated protein kinase). In the present study, we hypothesized that in vivo in humans ischaemia- and insulin-induced glucose uptake are additive, and that the combined impact of ischaemia and contraction on glucose uptake is of a similar magnitude when each is applied separately. We assessed the effects of ischaemia with and without euglycaemic–hyperinsulinaemia (clamp; protocol 1) and with and without muscle contraction (protocol 2) on muscle FGU (forearm glucose uptake) in healthy subjects. Furthermore, we assessed the impact of ischaemia on FBF (forearm blood flow; plethysmography). In protocol 1, ischaemia increased FGU from 0.6 ± 0.1 at baseline to 5.5 ± 1.9 μmol·min⁻¹·dl⁻¹, and insulin increased FGU to 1.6 ± 0.3 μmol·min⁻¹·dl⁻¹ (P < 0.05 for both). The combination of ischaemia + insulin increased FGU to 15.5 ± 2.2 μmol·min⁻¹·dl⁻¹ (P < 0.05 compared with each stimulus alone). Maximal FBF obtained after ischaemia was similar with and without hyperinsulinaemia. In protocol 2, isometric contraction increased FGU from 0.3 ± 0.1 to 2.7 ± 0.8 μmol·min⁻¹·dl⁻¹ (P < 0.05), but FGU was not significantly different from ischaemia compared with ischaemia + contraction. However, combined ischaemia + contraction resulted in a greater increase in FBF. In summary, ischaemia and insulin independently stimulate skeletal muscle glucose uptake in vivo in humans, whereas ischaemia and contraction do not. The observed differential effects of these stimuli on glucose uptake appear to be unrelated to changes in muscle blood flow.

INTRODUCTION

Ischaemia [1], like muscle contraction [2], induces translocation of intracellular glucose transporters (GLUT4) to the plasma membrane and, subsequently, stimulates glucose uptake in skeletal muscle tissue. Both stimuli appear independent of the insulin signalling cascade [1,3–7]. The mechanism underlying ischaemia- and contraction-induced translocation of GLUT4 in skeletal muscle is only partly resolved, but may involve several kinases that sense and transduce signals relating to changes in the intracellular environment during contraction/ischaemia (i.e. higher Ca²⁺ and AMP concentrations) to other undefined proteins involved in GLUT4 movement [8]. Intracellular Ca²⁺ levels are related to the activity of the motor nerves, so proteins influenced by Ca²⁺, such

Key words: contraction, euglycaemic–hyperinsulinaemic clamp, forearm glucose uptake, forearm blood flow, ischaemia, muscle.

Abbreviations: AMPK, AMP-activated protein kinase; AS160, Akt substrate of 160 kDa; BP, blood pressure; CaM, calmodulin; FBF, forearm blood flow; FGU, forearm glucose uptake; GIR, glucose infusion rate; ΔGlucA-V, arteriovenous glucose difference; HR, heart rate; MAP, mean arterial pressure; SBP, systolic BP.

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as PKC (protein kinase C), CaM (calmodulin) and NOS (NO synthase), can be considered as feed-forward regulators. For example, Ihlemann et al. [9] demonstrated that inhibition of CaM resulted in a reduction in contraction-induced glucose uptake. In addition, other proteins are linked to glucose uptake, such as AMPK (AMP-activated protein kinase) [10–12]. AMPK is present in most mammalian cells and appears to act as a fuel gauge [13]. Low energy cellular status, such as ischaemia and contraction, but also hyperosmolarity and heat shock, increase the AMP/ATP ratio, thereby activating AMPK [14,15]. A recent human in vivo study [16], investigating the effects of endurance training, showed an increase in the phosphorylation of AS160 (Akt substrate of 160 kDa), which may be caused by changes in activity of AMPK acting upstream of AS160. As AMPK forms a potential pharmacological target for the treatment of Type 2 diabetes [17,18], to elucidate the pathways regulating glucose uptake in skeletal muscle tissue is of great clinical relevance to the development of interventional strategies to improve glycaemic control in Type 2 diabetes. Several in vitro studies have shown that the maximal effects of hypoxia and insulin on glucose transport are additive, whereas the effects of muscle contraction and hypoxia are not [1,14,19]. The latter implies that hypoxia and muscle contraction stimulate glucose transport via the same, insulin-independent, mechanism; however, in vitro studies suggest that the mechanisms responsible for ischaemia- and contraction-induced glucose uptake do not share common pathways and are, at least partly, additive [6,20,21]. Nevertheless, the latter has not been confirmed in an in vivo human model.

The present study aims to test the hypothesis that ischaemia- and insulin-induced glucose uptake are additive in vivo in healthy humans. Furthermore, we hypothesized that the combined effects of ischaemia and muscle contraction on glucose uptake are not different from the impact of either ischaemia or muscle contraction only. Therefore we conducted two experiments. In protocol 1, we investigated the effects of ischaemia in the presence and absence of a euglycaemic–hyperinsulinaemic condition on FGU (forearm glucose uptake) and FBF (forearm blood flow). In protocol 2, we assessed the impact of ischaemia, muscle contraction and a combination of both on FGU and FBF.

**MATERIALS AND METHODS**

**Subjects**

A total of 18 healthy subjects were studied [two groups of nine subjects, consisting of five men and 13 females; age, 21.1 ± 0.6 years; and BMI (body mass index), 21.8 ± 0.5 kg/m²; values are means ± S.D.). All subjects underwent a clinical screening and a resting ECG. All subjects were healthy, i.e. not suffering from any acute or chronic illness, and were not on any medication (except for oral contraceptives).

The research was performed in accordance with the Declaration of Helsinki (2000) of the World Medical Association. The local ethics committee approved the study, and all subjects gave written informed consent before participation.

**Protocol 1: impact of ischaemia, insulin and ischaemia + insulin on glucose uptake**

We studied the physiological response to ischaemia under normal physiological and euglycaemic–hyperinsulinaemic (clamp) conditions. First, the impact of 10 min of arterial occlusion on FGU and FBF was determined by inflating upper arm cuffs > 100 mmHg above SBP [systolic BP (blood pressure)]. After 40 min of rest [pilot studies revealed that after 40 min the effects of ischaemia (and contraction, see protocol 2) had totally disappeared; results not shown], a euglycaemic–hyperinsulinaemic clamp was started. After 95 min, the impact of 10 min arterial occlusion was studied during the euglycaemic–hyperinsulinaemic condition. We define ‘ischaemia’ as oxygen shortage of the muscle forearm without reactive hyperaemia or muscle necrosis. After 10 min of ischaemia, vasodilation will occur as a compensatory mechanism and an increase in glucose uptake can be a consequence. We based this definition on a previous study by Rongen et al. [22]. They also used the same experimental procedure (ischaemic exercise) as we have done and have extended this with the Annexin A5 scan.

**Protocol 2: impact of ischaemia, contraction and ischaemia + contraction on glucose uptake**

The effect of isometric contraction on FGU and FBF was determined over two 10-min periods, during which muscle contraction and relaxation were alternated (5 s at 30% of maximal hand grip power, followed by 5 s of intermittent rest). After 40 min of subsequent rest, arterial occlusion was applied for 10 min by inflating upper arm cuffs > 100 mmHg above SBP. After 40 min of rest, isometric contraction was repeated. After 10 min of contraction, contraction was combined with 10 min of arterial occlusion (or until exhaustion).

**Experimental procedure**

The experiments started at 08.00 hours after an overnight fast in a quiet temperature-controlled room (23–24°C). The subjects abstained from caffeine-containing foods and alcohol consumption for at least 24 h before the experiment. A 20-gauge Angiocath catheter (48 mm; Becton Dickinson) was inserted into the brachial artery of the non-dominant arm (experimental arm; the contralateral arm is the control arm) to obtain arterial blood samples and to allow continuous BP monitoring.
(Hewlett Packard). In both arms a 20-gauge Venflon catheter (32 mm; Becton Dickinson) was inserted retrogradely into a deep forearm vein for deep venous blood sampling, enabling measurement of forearm arteriovenous glucose differences ($\Delta$GlucA$-V$) in both arms. Blood samples were taken with an inflated wrist cuff for at least 5 min to exclude hand and skin flow to enter the deep venous system at the wrist. Bilateral FBF was measured by venous occlusion plethysmography [23,24]. In both protocols, FBF was measured immediately after the different stimuli (ischaemia and contraction) were applied. In protocol 1 (euglycaemic–hyperinsulinaemic clamp), an additional 20-gauge Venflon catheter (32 mm; Becton Dickinson) was inserted into a vein on the dorsal side of a foot for infusion of insulin and glucose (20 % glucose). After complete instrumentation, at least 30 min of rest was included to obtain a steady-state before baseline data (FBF, $\Delta$GlucA$-V$, plasma insulin level and haematocrit) were collected. During the euglycaemic–hyperinsulinaemic clamp (protocol 1), 50 units of insulin (Actrapid®; Novo Nordisk) was diluted in 47.5 ml of 0.9 % saline with the addition of 2 ml of blood (to avoid adherence of insulin to tubing) to a concentration of 1 unit/ml. Insulin was infused intravenously at a dose of 430 pmol$\cdot$min$^{-1}$$\cdot$kg$^{-1}$ (60 m-units$\cdot$m$^{-2}$$\cdot$min$^{-1}$). Subjects were clamped at fasting arterial glucose levels for 125 min. Euglycaemia was maintained by a variable infusion of 20 % glucose solution adjusted by arterial glucose measurements obtained at 5-min intervals.

**Laboratory measurements and analysis**

Plasma glucose levels were measured in duplicate using the glucose oxidation method (Beckman Glucose Analyser II). Plasma insulin was assessed by in-house RIA.

**Calculations and statistical analysis**

FBF, immediately obtained after 2 min of each intervention, was averaged to one value. FGU was calculated from $\Delta$GlucA$-V$ and FBF, assuming that whole-blood glucose = $[1 - (0.3 \times$ haematocrit)] $\times$ plasma blood glucose [25]:

$$\text{FGU} = \Delta\text{GlucA}_A - V \times [1 - (0.3 \times \text{haematocrit})] \times \text{FBF}$$

For calculation of whole-body glucose disposal ($M$ value), we used the GIR (glucose infusion rate) over the last 30 min of the euglycaemic–hyperinsulinaemic clamp before ischaemia was applied, divided by body weight ($\mu$mol$\cdot$min$^{-1}$$\cdot$kg$^{-1}$ of body weight).

Differences in means were tested using a paired Student’s $t$ test. $P<0.05$ was considered statistically significant. Results are expressed as means ± S.E.M., unless otherwise indicated.

**RESULTS**

**Baseline characteristics**

Baseline characteristics of the participants of both experiments did not differ. In protocol 1, one person was excluded from the study because she fainted during the experiment (see below).

**Impact of ischaemia, insulin and ischaemia + insulin on glucose uptake**

**Metabolic response to ischaemia**

$\Delta$GlucA$-V$ did not change after 10 min of ischaemia (Figure 1, middle panel), whereas FGU increased from 0.6 ± 0.1 to 5.5 ± 1.9 $\mu$mol$\cdot$min$^{-1}$$\cdot$dl$^{-1}$ of forearm tissue ($P = 0.03$ compared with baseline; Figure 1, bottom panel).

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Metabolic response to insulin
After 95 min of systemic hyperinsulinaemia, ΔGlucA–V increased in both arms (P < 0.05 compared with baseline for both, and P = not significant between the two sides). FGU increased in the experimental and control arms (to 1.6 ± 0.3 and 1.8 ± 0.4 μmol·min⁻¹·dl⁻¹ respectively; P = 0.01 compared with baseline for both) with no differences between the arms. During systemic insulin infusion, plasma insulin concentrations increased from 55 ± 10 pmol/l at baseline to 600 ± 34 and 551 ± 27 pmol/l after 95 and 125 min respectively. The GIR was 37.2 ± 2.8 μmol·min⁻¹·kg⁻¹ of body weight.

Metabolic response to ischaemia + insulin
Compared with insulin alone, ΔGlucA–V decreased significantly to 1.2 ± 0.3 mmol/l after 10 min of ischaemia + insulin. After ischaemia, in the experimental arm, ΔGlucA–V returned to baseline values (1.8 ± 0.3 mmol/l). FGU increased further to 15.6 ± 2.2 μmol·min⁻¹·dl⁻¹ after 10 min of ischaemia + insulin and was even synergistic when compared with the metabolic response to insulin and ischaemia separately (P = 0.0003 and P = 0.0007 respectively). In the control arm, FGU increased to 2.2 ± 0.4 μmol·min⁻¹·dl⁻¹ after 125 min of hyperinsulinaemia (P = 0.03).

Impact of ischaemia, insulin and ischaemia + insulin on blood flow
Haemodynamic response to ischaemia
After 10 min of ischaemia, FBF increased in the experimental arm to 14.8 ± 1.9 ml·min⁻¹·dl⁻¹ of forearm tissue (P = 0.0001; Figure 1, top panel). HR (heart rate) did not change (P = 0.7), but SBP decreased after 10 min of ischaemia (P = 0.0005; Table 1).

Haemodynamic response to insulin
After 95 min of hyperinsulinaemia, there was no significant increase in FBF when compared with baseline values (from 1.4 ± 0.3 to 1.6 ± 0.4 ml·min⁻¹·dl⁻¹; P = 0.3). HR increased from 58 ± 2 to 65 ± 2 beats/min (P = 0.01) and SBP increased from 123 ± 2 to 129 ± 1 mmHg (P = 0.001; Table 1). MAP (mean arterial pressure) did not change significantly (P = 0.3).

Haemodynamic response to ischaemia + insulin
After 10 min of ischaemia + insulin, FBF increased to 16.2 ± 2.1 ml·min⁻¹·dl⁻¹ (P < 0.001 compared with baseline), which did not differ from the vasodilator response to ischaemia alone. During the study, there was no significant increase in FBF in the control arm. HR was not affected by ischaemia under hyperinsulinaemic conditions (65 ± 2 to 68 ± 3 beats/min; P = 0.3), but SBP decreased during ischaemia + hyperinsulinaemia (129 ± 1 to 113 ± 3 mmHg; P < 0.001).

Impact of ischaemia, contraction and ischaemia + contraction on glucose uptake
Metabolic response to muscle contraction
Isometric contraction increased ΔGlucA–V in the experimental arm from 0.3 ± 0.1 to 0.6 ± 0.1 mmol/l (P < 0.05; Figure 2, middle panel) and increased FGU from 0.3 ± 0.1 to 2.7 ± 0.8 μmol·min⁻¹·dl⁻¹ (P = 0.01 compared with baseline; Figure 2, bottom panel).

Metabolic response to ischaemia
After 10 min of ischaemia, ΔGlucA–V remained unchanged, which was consistent with the findings in protocol 1. FGU increased from 0.5 ± 0.1 to 4.4 ± 0.8 μmol·min⁻¹·dl⁻¹ (P = 0.0008 compared with baseline). FGU after ischaemia was not significantly different compared with contraction alone (P = 0.1).

Metabolic response to ischaemia + muscle contraction
The combination of contraction and ischaemia did not significantly change ΔGlucA–V (0.4 ± 0.1 to 0.2 ± 0.1 mmol/l; P = not significant). There was no additive effect on FGU (from 0.6 ± 0.2 to 5.3 ± 1.6 μmol·min⁻¹·dl⁻¹; P = 0.4 and P = 0.1 respectively compared with ischaemia and contraction separately). The arterial glucose (5.1 ± 0.1 to 5.2 ± 0.1 mmol/l) and the insulin (36 ± 3 to 39 ± 3 pmol/l) levels did not differ significantly (t = 0 and 155 min respectively).

Impact of ischaemia, contraction and ischaemia + contraction on blood flow
Haemodynamic response to muscle contraction
Isometric contraction increased FBF in the experimental arm from 1.5 ± 0.2 to 5.1 ± 0.8 ml·min⁻¹·dl⁻¹

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>After ischaemia</th>
<th>Before insulin</th>
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<td>60 ± 3</td>
<td>58 ± 2</td>
<td>65 ± 2†</td>
<td>68 ± 2†</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>121 ± 2</td>
<td>109 ± 3†</td>
<td>123 ± 2</td>
<td>129 ± 1†</td>
<td>113 ± 3‡</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>66 ± 2</td>
<td>66 ± 2</td>
<td>67 ± 2</td>
<td>68 ± 2</td>
<td>67 ± 2</td>
</tr>
</tbody>
</table>

*P < 0.05 compared with baseline; †P < 0.05 compared with before insulin; ‡P < 0.05 compared with after 95 min of insulin. DBP, diastolic BP.
Table 2  Effect of ischaemia, contraction and the combination of ischaemia + contraction on HR, SBP and DBP

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>After contraction</th>
<th>Before ischaemia</th>
<th>After ischaemia</th>
<th>Before ischaemia + contraction</th>
<th>After ischaemia + contraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (beats/min)</td>
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<td>61 ± 2</td>
<td>60 ± 2</td>
<td>63 ± 2*</td>
<td>63 ± 2</td>
<td>75 ± 5†</td>
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<td>SBP (mmHg)</td>
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<td>127 ± 3</td>
<td>128 ± 4</td>
<td>116 ± 3*</td>
<td>132 ± 4</td>
<td>137 ± 6</td>
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<tr>
<td>DBP (mmHg)</td>
<td>68 ± 2</td>
<td>69 ± 2</td>
<td>68 ± 2</td>
<td>68 ± 2</td>
<td>71 ± 3</td>
<td>85 ± 3†</td>
</tr>
</tbody>
</table>

(∗P < 0.05 compared with before ischaemia, †P < 0.05 compared with before ischaemia + contraction, DBP, diastolic BP.

Figure 2  FBF (top panel), ΔGluc_A−V (middle panel) and FGU (bottom panel) during protocol 2

FBF, ΔGluc_A−V and FGU were measured before and after two bouts of contraction (C; 10 min each), before and after ischaemia (I), and after the combination of ischaemia + contraction. *P < 0.05. Significant levels for other time points are not indicated. exp arm, experimental arm; con arm, control arm; NS, not significant. Please note that the time schedule is not correct.

Haemodynamic response to ischaemia + muscle contraction

After the combination of contraction and ischaemia, there was a greater increase in FBF (maximal increase from 2.1 ± 0.3 to 25.3 ± 3.4 ml·min⁻¹·dl⁻¹; P < 0.001 compared with contraction or ischaemia alone). FBF in the control arm did not significantly change over the course of the experiment. HR increased from 63 ± 2 to 75 ± 5 beats/min and MAP increased from 92 ± 3 to 109 ± 4 mmHg (P < 0.05 compared with baseline for both).

Side effects

During the experiments with ischaemia and contraction (protocol 2), subjects reported no side effects, except for cramps in the forearm muscle during the combination of exercise and contraction. All subjects found the combination of ischaemia and contraction hard to carry out. Half of the participants were able to perform exercise during ischaemia for 8–10 min (men found this easier than women), the others found this more difficult, but could combine this for most of the time for at least 5–6 min. During the last 4–5 min, the subjects also contracted their muscles on a less regular basis. Thus, although the exercise stimulus may have been somewhat less intense, the participants still performed substantial muscle contraction during ischaemia and the exercise stimulus during ischaemia did not really differ from exercise only. In protocol 1, one person fainted during the euglycaemic–hyperinsulinaemic clamp as a result of pain at the site of the catheter in the experimental arm. This person was excluded from the study. Other subjects in protocol 1 reported no side effects at all.

DISCUSSION

The results from the present study show that ischaemia and insulin are synergistic in stimulating effects on FGU in vivo in human skeletal muscle, whereas ischaemia and muscle contraction are not. The observed differential effects of these stimuli on glucose uptake appear to be unrelated to changes in muscle blood flow.

These findings suggest that ischaemia and muscle contraction stimulate glucose uptake in muscle tissue through the same metabolic pathways and, as such, imply
that the pathways responsible for ischaemia-induced glucose uptake are at least partly different from the mechanism responsible for insulin-stimulated glucose uptake. The latter is supported by our observation that ischaemia-induced glucose uptake is of a similar magnitude compared with ischaemia + contraction-induced glucose uptake, and that the combined impact of insulin + ischaemia on glucose uptake is even greater than the sum of both separately. These findings appear consistent with earlier in vitro studies, which exposed isolated rat and human skeletal muscle to hypoxia. Cartee et al. [1] have shown that, in perfused rat hindlimb muscle, hypoxia increases the translocation of glucose transporters to the plasma membrane. Furthermore, they showed that the maximal effect of hypoxia and insulin on glucose transport was additive, whereas the effect of exercise and hypoxia was not. The latter provides evidence that hypoxia and exercise stimulate glucose transport via the same mechanism. Azevedo et al. [7] examined muscle tissue derived from lean subjects, obese subjects and obese patients with Type 2 diabetes and reported similar results. The authors showed that hypoxia in combination with insulin significantly stimulated glucose transport in all muscle tissue samples that were examined, suggesting that the glucose transport pathway is intact in muscle tissue in obese subjects as well as in obese patients with Type 2 diabetes when stimulated by hypoxia. More studies [14] have examined the effect of metabolic stressors on glucose transport and AMPK activation in rodent muscle. The glucose transport rates in the isolated muscle increased 5-fold above basal values during contraction and 8-fold during hypoxia. The latter was accompanied by a substantial activation of AMPK in skeletal muscle; however, insulin did not affect AMPK activity, but stimulated glucose transport 6.5-fold above baseline, again indicating that ischaemia- and muscle-contraction-stimulated glucose uptake are insulin-independent. In a previous human in vivo study [26], the effects of ischaemia on glucose uptake was measured by using muscle microdialysis in patients with Type 2 diabetes and healthy subjects. The authors showed that glucose uptake is efficiently activated by ischaemia in both healthy subjects and patients with Type 2 diabetes, and suggest that the activation of a pathway alternative to the insulin signalling cascade is not impaired [26]. The present findings are, to our knowledge, the first to show the differential effects of ischaemia, muscle contraction and/or hyperinsulinaemia on skeletal muscle in vivo in humans. Previous in vitro studies have suggested that part of the mechanism responsible for muscle-contraction-induced glucose uptake are different from ischaemia-stimulated glucose uptake and involve pathways independent of AMPK. The latter would imply that the impact of ischaemia and muscle contraction on glucose uptake may be, at least partly, additive [6,20,21]. Nielsen et al. [21] concluded that a role for AMPK in exercise-induced glucose uptake cannot be excluded, but that one or more additional pathways are involved in mediating glucose uptake in skeletal muscle tissue during exercise. So far, these speculations have not been studied further in an in vivo human model.

The molecular mechanism responsible for hypoxia- (and exercise-) stimulated glucose uptake in human skeletal muscles remains to be fully elucidated, but it appears evident that AMPK plays a central role in this process. A limitation of the present study is that we were unable to obtain muscle biopsies of the forearm, because of ethical and safety reasons, so we cannot prove whether ischaemia and/or contraction did or did not phosphorylate AMPK in healthy subjects. As AMPK activation has important stimulating effects on both fat and carbohydrate metabolism, it represents an interesting target for intervention strategies in the prevention and/or treatment of obesity and/or Type 2 diabetes [17,18]. Unravelling the exact physiological pathways responsible for the impact of ischaemia, contraction and/or insulin on fat and carbohydrate metabolism in liver and adipose tissue, as well as muscle tissue, in humans may prove to be of great clinical relevance in the treatment of chronic metabolic diseases.

Another (possible) limitation of our present study is that overactivity of the sympathetic activation can negatively influence glucose uptake. The subjects experienced no pain from protocol 1. Only the subjects in protocol 2 experienced some muscle cramps in their forearm during the combination of ischaemia + contraction (see the Side effects subsection of the Results). We have also shown that HR and BP increased during this period. Thus, with the exception of the combined exercise ischaemia protocol, pain-induced activation of the sympathetic nervous system is unlikely to have affected our results. Although less likely, it cannot be excluded that the pain associated with the combined exercise + ischaemia protocol might induce sympathetic activation, which in itself may then dampen subsequent glucose uptake stimulation.

Although our present study was mainly designed to investigate the metabolic effects of ischaemia to contraction/insulin in vivo in humans, our secondary goal was to explore the effects of ischaemia on FBF and its potential contribution to glucose uptake. In the present study, we have shown that ischaemia is a potent vasodilator, which is in accordance with previous findings [27–29]. Furthermore, exercise stimulates insulin-induced capillary recruitment in rodent skeletal muscle and, as such, augments glucose uptake [30]. The stimulatory effect of muscle contraction on blood flow has also been described in vivo in humans. A prolonged bout of exercise has been shown to increase FBF in healthy men under hyperinsulinaemic [31–33] as well as normoinsulinaemic [34–36] conditions. On the basis of the findings in protocol 1 that the increase in FGU is not preceded by an increase in FBF, which is also less visible in protocol 2 (see Figures 1 and 2), it appears that the increase in FGU is not
clearly correlated with an increase in FBF. This suggests that the differential stimulating effects on glucose uptake are unrelated to the changes in blood flow (measured with plethysmography). The latter is consistent with the idea that increased capillary recruitment is of greater importance than an increase in total blood flow to augment insulin/contraction-mediated glucose uptake [30,37,38]. However, in the present study, capillary recruitment was not assessed. Hyperinsulinemia was not associated with a significant increase in vasodilation. The latter is likely to be attributed to the relatively short period of insulin infusion, because insulin-induced vasodilation is slow in onset, taking at least 3 h to obtain its maximal effects and has a high inter-individual variability [39,40].

In the present study, we observed an increase in HR and SBP (but not in MAP) during insulin infusion, which is also well-described, although conflicting findings exist on this issue [41,42]. Strikingly, we observed a decrease in SBP after 10 min of ischaemia. This is probably the result of a false low intra-arterial BP in combination with an extreme high blood flow in the brachial artery after hypoxia.

We conclude that ischaemia and insulin independently stimulate glucose uptake in human skeletal muscle and that their stimulating effects on glucose uptake are synergistic. In contrast, contraction and ischaemia stimulate FGU to a similar extent, so their impact on glucose uptake is not additive. Therefore these findings suggest that ischaemia and contraction use similar physiological pathways to stimulate glucose uptake, and these are different from the stimulating effects of insulin. The differential effects on glucose uptake are not clearly related to the concomitant changes in blood flow. It will be of important clinical benefit to unravel further the mechanisms responsible for ischaemia-, contraction- and insulin-induced glucose uptake in vivo in humans.

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