Contrasting effects of exercise, AICAR, and increased fatty acid supply on in vivo and skeletal muscle glucose metabolism

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Rantzau C, Christopher M, Alford FP. Contrasting effects of exercise, AICAR, and increased fatty acid supply on in vivo and skeletal muscle glucose metabolism. J Appl Physiol 104: 363–370, 2008. First published November 21, 2007; doi:10.1152/japplphysiol.00500.2007.—The increased energy required for acute moderate exercise by skeletal muscle (SkM) is derived equally from enhanced fatty acid (FA) oxidation and glucose oxidation. Availability of FA also influences contracting SkM metabolic responses. Whole body glucose turnover and SkM glucose metabolic responses were determined in paired dog studies during 1) a 30-min moderate exercise (maximal oxygen consumption of ~60%) test vs. a 60-min low-dose 5-aminoimidazole-4-carboxamide-1-β-β-ribofuranoside (AICAR) infusion, 2) a 150-min AICAR infusion vs. modest elevation of FA induced by a 150-min combined intralipid-heparin (IL/hep) infusion, and 3) an acute exercise test performed with vs. without IL/hep. The exercise responses differed from those observed with AICAR: plasma FA and glycerol rose sharply with exercise, whereas FA fell and glycerol was unchanged with AICAR; glucose turnover and glycolytic flux doubled with exercise but rose only by 50% with AICAR; SkM glucose-6-phosphate rose and glycogen content decreased with exercise, whereas no changes occurred with AICAR. The metabolic responses to AICAR vs. IL/hep differed: glycolytic flux was stimulated by AICAR but suppressed by IL/hep, and no changes in glucose turnover occurred with IL/hep. Glucose turnover responses to exercise were similar in the IL/hep and non-IL/hep, but SkM lactate and glycolgen concentrations rose with IL/hep vs. that shown with exercise alone. In conclusion, the metabolic responses to acute exercise are not mimicked by a single dose of AICAR or altered by short-term enhancement of fatty acid supply.

hepatic glucose production; glucose disposal; metabolic clearance rate of glucose; glycolytic flux; muscle biopsy; dog

with short-term moderate exercise [maximal oxygen consumption (V\textsubscript{O\textsubscript{2max}}) of ~55–65%], total energy expenditure in skeletal muscle (SkM; i.e., ATP production) increases ~10- to 12-fold from the resting state. This increased energy debt is met primarily by enhanced fatty acid (FA) oxidation (FA\textsubscript{ox}) (derived from plasma, as supplied from adipocyte depot and intramuscular fat lipolysis) and glucose rate of disappearance (R\textsubscript{d}) and oxidation (G\textsubscript{ox}) (derived from plasma glucose and SkM glycogen) (39, 49). However, in exercising SkM, the increased energy demand has the potential to outstrip its energy supply and become “metabolically” stressed (24, 51). AMP-activated protein kinase (AMPK) is an intracellular “metabolic sensor,” which may be activated during times of “stress” (24, 51) during moderate to severe exercise, in humans (19, 48, 52), rats (21, 36), and dogs (8).

Stimulation of SkM AMPK activity by exercise is postulated to be a significant factor in the enhanced SkM metabolic responses to exercise (29), including direct AMPK stimulation of insulin-independent GLUT4-mediated glucose uptake and G\textsubscript{ox} (3, 21, 26, 28) and promotion of FA\textsubscript{ox} (28, 30, 46). The latter is mediated by deactivation of acetyl-CoA carboxylase (ACC\textbeta) by AMPK-activated site-specific phosphorylation of Ser\textsuperscript{277} on ACC\textbeta (ACC\textbeta-pSer\textsuperscript{277}) (7, 28, 32, 46). This leads to reduced intracellular malonyl CoA and hence activation of SkM carnitine palmitoyltransferase-1, which facilitates long-chain FA entry into mitochondria for oxidation and ATP generation (28). Activation of endogenous AMPK involves both covalent [via phosphorylation of Thr\textsuperscript{172} by AMPK (24, 51, 53)] and allosteric [via rising AMP-to-ATP ratio (24, 51)] mechanisms, which lead to an increase of measured endogenous SkM ACC\textbeta-pSer\textsuperscript{277} (7, 28, 32, 46), as previously discussed (5, 6, 8, 9). Of the two AMPK catalytic subunits (α\textsubscript{i} and α\textsubscript{2}), only the α\textsubscript{2}-subunit is activated by moderately intense exercise (19, 48, 52), whereas the α\textsubscript{i}-subunit can be activated by extreme exercise (7).

Endogenous AMPK is also activated pharmacologically and nonspecifically by 5-aminoimidazole-4-carboxamide-1-β-β-ribofuranoside (AICAR), which is phosphorylated in vivo to the AMP analog AICAR monophosphate (ZMP) (12). AICAR activates AMPK-α\textsubscript{2} by specific phosphorylation of Thr\textsuperscript{172}, which then promotes a similar metabolic cascade in SkM as outlined above for exercise (3, 28, 30, 45, 46). Because of evidence that a similar activation of the AMPK metabolic cascade occurs in SkM by AICAR, the suggestion has arisen that in vivo administration of AICAR may simulate the metabolic benefits gained from exercise, especially in diabetic subjects (51). However, raised levels of ZMP can also allosterically directly activate other enzymes, such as glycogen phosphorylase (independent of AMPK activation) in cardiac muscle (4, 27) and liver (42).

Despite the apparent similarities between exercise and AICAR’s metabolic actions in SkM, important differences have emerged between exercise and AICAR-induced changes in SkM G\textsubscript{ox} and FA\textsubscript{ox}. In particular, with increasing intensities of exercise, G\textsubscript{ox} continues to progressively rise, whereas FA\textsubscript{ox} reaches a peak at V\textsubscript{O\textsubscript{2max}} of ~70% and thereafter declines (39, 49), despite the steady rise in AMPK-α\textsubscript{2} activity through all intensities of exercise (14). Furthermore, uncertainties exist as to whether exercise and AICAR-stimulated AMPK activation have the same metabolic responses in SkM. For example, initial studies failed to demonstrate increased AICAR-induced

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G_ox in SkM, despite enhanced glucose uptake (23). A more recent study has now clearly demonstrated increased AICAR-induced AMPK activation and G_ox (45, 46). In addition, it is likely that AICAR-induced activation of AMPK does not result in significant increase of endogenous glycogenolysis (2) and lipolysis, (1, 46), which stands in stark contrast to the impact of exercise on glycogenolysis (22) and intramuscular lipolysis (16). Finally, the relative contributions of the exercise-induced activation of AMPK vs. the direct effects of the exercise itself on G_ox and FA_ox in working SkM are unknown (29, 35).

Availability of FA may also influence the rate of G_ox and glycogenolysis and FA_ox in exercising SkM (20, 40) or in perfused hindlimb sciatric nerve-stimulated contracting SkM (37). In the latter study, inhibition of glucose uptake and glycolysis and glycogenolysis occur during exercise in the presence of excess FA supply (37). In humans, thigh SkM glucose uptake (20) and whole body G_ox (40) induced by exercise during an intralipid-heparin (IL/hep) infusion are also reduced, but glycogen utilization is either unaffected (20) or reduced (40). Similarly, discrepancies have been observed for FA_ox rates during in vivo exercise-induced contractions and IL/hep infusions in SkM from humans (20, 40) and during in vitro electrically stimulated contracting SkM in rats (46), with FA_ox being either increased (40, 46) or unchanged (20). These differences between data obtained from the separate studies may reflect the species studied, the use of isolated muscle preparations (in which there would be an absence of blood flow and hormonal effects) vs. whole body, and the actual levels of plasma FA employed (20, 37, 40, 46).

Thus experiments directly comparing downstream in vivo glucose turnover and SkM metabolism in the same individual after 1) short-term moderate exercise vs. AICAR infusion, 2) the same moderate exercise load performed in either the presence or absence of excess FA supply, and 3) AICAR infusion vs. an IL/hep-enhanced FA supply [both of which increase in vitro SkM FA_ox (45)] are lacking. To this end, we have compared, in paired experiments in the same dog, hepatic glucose production (HGP), R_a, metabolic clearance rate of glucose (MCR_g), and glycolytic flux [derived from plasma glucose (GF_exog)] after 1) a 30-min moderate intensity exercise (V_o2max of ~60%) test, 2) a 150-min low-dose AICAR infusion, 3) a 150-min physiological IL/hep infusion alone, and 4) the same exercise load performed during the final 30 min of the physiological IL/hep infusion. Paired SkM biopsies were taken for each experiment. In particular, we wanted to determine how the metabolic responses of short-term moderate exercise and AICAR infusion are different, how an excess supply of FA compares with the metabolic responses of AICAR infusion, and how an excess supply of FA during exercise impacts on in vivo glucose and SkM metabolism.

**Materials and Methods**

**Animals**

The studies were performed on six normal male dogs of mixed breed [20.4 ± 1.6 kg body wt (means ± SE)], with permission of the Experimental and Surgical Research Ethics Committee (St. Vincent’s Hospital, Melbourne, Victoria, Australia). Surgical preparation, the exercising program, dietary intake, health monitoring and training of dogs, and blood sampling schedules on day of experiments were as previously detailed (8–11). Dogs were acclimatized to the treadmill stepwise exercise program for 2–3 wk before formal experiments (8).

**Experiments**

A minimum of 10 days was allowed between all studies. After the overnight 15-h fast (with free access to water) and rest period of at least 30 min, fasting blood samples were taken for measurements of plasma glucose, insulin, free fatty acid (FFA), glycerol, glucagon, lactate, [3-^3H]glucose, and H_2O. The experiments described below were performed on dogs in random order.

**Experiment 1: total body water content.** A standard 120-min intravenous glucose tolerance test was performed, commencing with an intravenous bolus of 50% glucose (0.3 g/kg) and 50 μCi highly purified tritiated water (H_2O; NEN Life Science Products, Boston, MA) given over 30 s, as previously described (8–11), to determine total body water content (15).

**Experiment 2: exercise studies.** These experiments involved a 180-min euglycemic-basal insulinenic period, using a primed (20 μCi), continuous infusion (10 μCi/h) of highly purified [3-^3H]glucose (NEM Life Science Products) (8–11) followed by the 30-min exercise test, performed from minute 150 to minute 180 (8). Additional saline (3–4 ml/min) was infused to ensure adequate hydration of the dogs during the exercise test, which was performed over 30 min on a treadmill, with stepwise increases in speed (67–133 m/min) and gradients (12.5 to 22.5% slope), representing a final exercise load of ~60% V_o2max for dogs (8). The [3-^3H]glucose infusion rate was also increased stepwise by 3.0-, 2.0-, and 1.5-fold consecutively, to minimize the change in specific activity obtained from exercise-induced increments in SkM glucose uptake (8, 18).

**Experiment 3: baseline + AICAR infusion.** This study involved a 150-min baseline equilibration period, consisting of a primed (20 μCi), continuous infusion (10 μCi/h) of [3-^3H]glucose, followed by a 150-min infusion of AICAR (Toronto Research Chemicals, Toronto, Canada), given as a 30-s priming dose of 7.5 mg/kg AICAR dissolved in 10 ml of saline followed by continuous infusion (1.5–2.0 mg·kg^{-1}·min^{-1}) (9). The [3-^3H]glucose infusion, which occurred in parallel with the AICAR infusion, used a separate infusion pump, as previously detailed by our laboratory (9). The peak metabolic effect of this AICAR infusion protocol is reached by 60 min (9, 28). With this protocol in six dogs, SkM ATP (8.81 ± 0.04) and AMP (0.03 ± 0.00) are unchanged, whereas ZMP (0.00 to 0.24 ± 0.04) and AICAR (0.00 to 0.12 ± 0.04 μmol/g wet wt) concentrations are raised (9).

**Experiment 4: IL/hep infusion (with and without exercise) studies.** The standard [3-^3H]glucose clamp was combined with hepatic (30-s priming bolus of 6 IU/kg followed by a continuous infusion of 12 IU·kg^{-1}·h^{-1}) and 20% IL (Fresenius Medical Care, Sydney, NSW, Australia) (5 min priming bolus of 6.6 ml/kg followed by a continuous infusion of 1.35 ml·kg^{-1}·h^{-1}) for the duration of each study. The aim of this protocol was to increase plasma FFA by 1.5- to 2.0-fold. On one day (random order), the 30-min exercise test was performed from minute 150 to minute 180; on the other day, the FA infusion was continued without exercise to 180 min.

**Metabolite and Glucose Turnover Measurements**

Plasma levels of glucose, total insulin, FFA, glycerol, glucagon, and lactate and specific activities of plasma [3-^3H]glucose and H_2O were measured as previously described (8–11). The mean coefficients of variation obtained for plasma glucose and total insulin levels and [3-^3H]glucose-specific activities during the steady-state periods of experiments 2, 3, and 4 were similar to those obtained in our earlier studies (8–11). The average rates of total glucose appearance, R_a, and MCR_g were calculated from minute 120 to minute 150 pre-exercise and pre-AICAR infusion period and during the 30-min exercise period (minute 150 to 180, with or without IL/hep), employing plasma [3-^3H]glucose-specific activities and using either steady-state equations or, when not at steady state, Steele’s nonsteady-state equations (47). A glucose pool fraction of 0.65 and a glucose volume of distribution of 200 ml/kg were employed for these calculations (8–11). The measurement of the rate of in vivo glycolysis (GF_{exo}) from
extracellularly derived glucose was estimated during steady state from the linear generation of plasma \(^3\)H\(_2\)O from the \([3-\)\(^3\)H\(]\)glucose (15) using the formula previously detailed from our laboratory (8–11). During exercise, GF\(_{\text{exog}}\) was calculated as \(R_d\) (exercise) \(- GF_{\text{exog}}\) (basal), as previously described (8, 10, 39, 49).

**SkM Biopsy Studies**

Before and at the completion of both exercise studies (with and without IL/hep) and the AICAR and IL/hep infusions, SkM biopsy samples (4–5 passes) were taken from the thigh (vastus lateralis). Rapid induction of anesthesia was accomplished with a 4.2-mg/kg iv bolus followed by a 1.8-mg/kg iv titration of Diprivan (propofol, 2,6-disopropylphenol; Astra Zeneca, North Ryde, Australia), as previously described (8–10). Each SkM biopsy sample was immediately frozen in liquid nitrogen (within 5 s of biopsy) and stored at \(-80^\circ\)C until assayed (8, 9). For each dog, the opposite back leg was used for the subsequent muscle biopsy procedure. SkM concentrations of intracellular glucose, glucose-6-phosphate (G6P; in mmol/kg dry wt), glycogen (mmol glucose residues/kg dry wt), and lactate were determined (8, 9) with a fluorometric enzyme-coupled assay, as modified previously (8–10, 43, 44). AMPK-\(\alpha_1\) and AMPK-\(\alpha_2\) activities and concentrations of ACC\(_\beta\)-pSer\(^{221}\) (human sequence), ATP, AMP, ZMP, and AICAR were measured in SkM, as previously described (7–9).

**Statistical Analysis**

Values are presented as means \(\pm\) SE. Within-group and between-group responses for paired data were compared with Student’s \(t\)-test. Computations were carried out with Minitab II program (release 13; Minitab).

**RESULTS**

**Exercise vs. AICAR Infusion**

With 30 min of moderate exercise, plasma glucose, insulin [4.1 \(\pm\) 1.0 (before) vs. 3.0 \(\pm\) 0.5 mU/l (after)], and glucagon [75 \(\pm\) 17 (before) vs. 93 \(\pm\) 21 ng/l (after); \(P = \) not significant (NS)] were unchanged; however, plasma FFA, lactate, and glycerol all rose significantly (\(P > 0.01\)) (Fig. 1). In contrast, with the 60-min AICAR infusion, plasma glucose fell (\(P = 0.05\)), insulin and glucagon were unchanged ([insulin = 4.7 \(\pm\) 0.5 (before) vs. 6.1 \(\pm\) 0.8 mU/l (after), \(P = \) NS; glucagon = 53 \(\pm\) 11 (before) vs. 68 \(\pm\) 11 ng/l (after), \(P = \) NS]), plasma FFA decreased significantly (\(P < 0.01\), with no change in glycerol, and lactate rose sharply (\(P < 0.01\)) (Fig. 1). Thus the incremental responses of FFA and glycerol to exercise and AICAR were significantly different and in the opposite directions (Fig. 1). There was a greater than twofold increase (\(P < 0.02–0.01\)) in HGP, \(R_d\), MCR\(_g\), and GF\(_{\text{exog}}\) after the 30-min moderate exercise test (Fig. 2). In contrast, although these parameters also increased after the 60-min AICAR infusion (\(P < 0.02\)), the responses were considerably less (30–40%) and significantly (\(P < 0.01\)) smaller for the AICAR than for the exercise responses (Fig. 2). The metabolic responses to AICAR at 150 min were similar to those obtained at 60 min, with only a minor attenuation of MCR\(_g\) by 150 min. SkM substrate concentrations of intracellular glucose and lactate did not change with exercise alone, whereas G6P significantly increased (change of 1.0 \(\pm\) 0.3 mmol/l; \(P = 0.05\)) and glycerol decreased (change of \(-35 \pm 36\) mmol/kg dry wt; \(P = 0.06\)). In contrast, during AICAR infusion, a decrease in intracellular SkM glucose (change of \(-0.20 \pm 0.09\) mmol/l; \(P = 0.06\) was noted, but no significant changes occurred in G6P, lactate, or glyceron. However, the absolute decrease of the glycogen concentration was significantly different for exercise compared with that for AICAR (change of \(-35 \pm 36\) vs. 9 \(\pm\) 36 mmol/kg dry wt; \(P = 0.06\)). There were no changes in AMPK-\(\alpha_1\) and AMPK-\(\alpha_2\) activities with exercise or AICAR infusion (data not shown), but ACC\(_\beta\)-pSer\(^{221}\) was elevated by \(-35\%\) [from basal level of 1.05 \(\pm\) 0.02 to 1.38 \(\pm\) 0.15 (\(P \leq 0.05\) with exercise and to 1.32 \(\pm\) 0.15 (arbitrary units) (\(P = 0.06\) with AICAR).
**IL/Hep Infusion vs. Saline and AICAR Infusions**

The 150-min IL/hep infusion alone raised plasma FFA by \(-60\%\) and glycerol by \(-80\%\) \((P < 0.05–0.01)\) (Table 1). This resulted in a small but significant fall in fasting glucose \((P \leq 0.05)\), with no changes in insulin or glucagon (Table 1). There were also no changes induced by the IL/hep infusion in HGP, RA, and MCRg, but GFexog fell significantly \((P < 0.05)\) by \(-20\%\) (Table 1). The effects of increasing FA supply compared with the AICAR infusion at 150 min were different (Table 1). Plasma glucose did not change; however, in the IL/hep group, the change in plasma lactate was markedly reduced \((P < 0.001)\) and, as expected, FFA and glycerol were raised, compared with the responses observed in the AICAR studies \((P \leq 0.05–0.01)\) (Table 1). The modest significant increments observed for HGP, RA, MCRg, and GFexog with AICAR were not observed in the IL/hep studies (Table 1). There was no relationship between the changes in plasma glucagon and HGP with the combined AICAR and IL/hep groups \((r = 0.40, P = 0.22)\). After the IL/hep infusion, there were no changes \((\Delta)\) in intramuscular substrates \((\Delta)\) glucose = \(-0.05 \pm 0.27\) mmol/l, \(\Delta G6P = 0.7 \pm 0.7\) mmol/kg dry wt, \(\Delta Lactate = 0.51 \pm 0.50\) mmol/l, \(\Delta Glycogen = 15 \pm 33\) mmol/kg dry wt, and \(\Delta ACCβ-pSer^{221} = 0.03 \pm 0.05\) arbitrary units).

**Exercise With or Without IL/Hep Infusion**

The exercise-induced responses of plasma substrates in the presence or absence of excess FA supply were similar for

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**Table 1. Comparison of the absolute and incremental effects of 60-min and 150-min AICAR infusion and 150-min saline or IL/heparin infusion on plasma substrates and hormones, glucose turnover, and skeletal muscle substrates in 6 resting normal dogs**

<table>
<thead>
<tr>
<th></th>
<th>AICAR Infusion</th>
<th>IL/Hep Infusion</th>
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<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After 60 min</td>
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<tr>
<td><strong>Plasma</strong></td>
<td></td>
<td></td>
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<tr>
<td>Glucose, mmol/l</td>
<td>5.0±0.1</td>
<td>4.7±0.3(^*)</td>
</tr>
<tr>
<td>Insulin, mIU/l</td>
<td>4.4±0.6</td>
<td>5.0±0.6</td>
</tr>
<tr>
<td>Glucagon, ng/l</td>
<td>53±11</td>
<td>75±10</td>
</tr>
<tr>
<td>Lactate, mmol/l</td>
<td>0.6±0.1</td>
<td>1.1±0.1(^*)</td>
</tr>
<tr>
<td>FFA, mmol/l</td>
<td>0.68±0.10</td>
<td>0.44±0.08(^a)</td>
</tr>
<tr>
<td>Glycerol, mmol/l</td>
<td>97±8</td>
<td>85±8</td>
</tr>
<tr>
<td><strong>Glucose turnover</strong></td>
<td></td>
<td></td>
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<tr>
<td>HGP, (\mu)mol·kg(^{-1})·min(^{-1})</td>
<td>12.7±0.9</td>
<td>16.0±1.1(^b)</td>
</tr>
<tr>
<td>RA, (\mu)mol·kg(^{-1})·min(^{-1})</td>
<td>12.9±0.8</td>
<td>16.7±1.2(^a)</td>
</tr>
<tr>
<td>MCRg, ml·kg(^{-1})·min(^{-1})</td>
<td>2.5±0.1</td>
<td>3.6±0.4(^a)</td>
</tr>
<tr>
<td>GFexog, (\mu)mol·kg(^{-1})·min(^{-1})</td>
<td>10.4±0.8</td>
<td>11.9±0.5</td>
</tr>
</tbody>
</table>

Values are means ± SE. \(\Delta\), incremental change; AICAR, 5-aminooimidazole-4-carboxamide-1-β-n-ribofuranoside; IL/hep, combined intralipid-heparin; HGP, hepatic glucose production; RA, glucose rate of disappearance; MCRg, metabolic clearance rate of glucose; GFexog, glycolytic flux. \(^*P < 0.05, ^{**}P < 0.02, ^{***}P < 0.01,\) and \(^{****}P < 0.001\) within groups; \(^\dagger P < 0.05, ^{\dagger\dagger} P < 0.1,\) and \(^{\dagger\dagger\dagger} P < 0.01\) within groups; \(^\ddagger P < 0.05, ^{\ddagger \ddagger} P < 0.02, ^{\ddagger \ddagger\ddagger} P < 0.01,\) and \(^{\ddagger \ddagger \ddagger} P < 0.001\) between groups.
plasma glucose, glycerol, lactate, insulin, and glucagon. No exercise-induced increments for plasma FA were observed in the IL/hep group, since the FFA levels were already raised preexercise above those seen postexercise for the non-IL/hep group (Fig. 3). On the background of the lipid-induced lower basal GFexog (Table 1), the glucose turnover responses to exercise were similar for the IL/hep and non-IL/hep groups for HGP, Rd, MCRg, and GFexog (Fig. 4). Only small responses in the SkM substrates with exercise performed with and without IL/hep infusion were noted [G6P = 0.3 ± 0.1 vs. 0.0 ± 0.2 mmol/l, ΔG6P = 0.8 ± 0.6 vs. 1.0 ± 0.3 mmol/l, Δlactate = 2.6 ± 1.1 vs. 0.0 ± 0.3 mmol/l (P = 0.07), Δglycogen = 30 ± 37 vs. −35 ± 36 mmol/kg dry wt (P = 0.09), and ΔACCβ-pSer221 = 0.29 ± 0.06 vs. 0.23 ± 0.15 arbitrary units, respectively].

DISCUSSION

Three main questions are addressed in these acute in vivo studies: 1) what are the differences between the downstream metabolic responses of moderate 30-min exercise and 60-min AICAR infusion, 2) what is the metabolic impact of a modest elevation of FA (with its accompanying increase in basal SkM FA uptake and oxidation) (45) compared with that of AICAR, and 3) does an increased supply of FA during exercise alter the downstream metabolic response to moderate exercise? Em-
ploying the same animals for all studies, we found that the exercise-induced metabolic responses differed from the responses observed with AICAR. The AICAR and IL/hep infusion metabolic responses also differed, with GFexog rising with AICAR but falling with IL/hep. There was no in vivo metabolic impact on glucose turnover or SkM metabolism with the exercise performed with or without a concurrent IL/hep infusion.

First, considering the exercise- and AICAR-induced metabolic responses, we noticed that plasma FFA and glycerol rose sharply with exercise; however, with acute AICAR, FFA fell. Glycerol levels were unchanged. These latter metabolic responses to AICAR are consistent with inhibition of lipolysis within the adipocyte (12) and SkM (1, 45) and enhanced removal and oxidation of FA (28, 30, 32). As expected, HGP, Rd,M C Rg, and AICAR infusion (3, 5, 6, 9, 33), although responses were about threefold greater with exercise. Furthermore, GFexog only increased significantly with exercise (Fig. 2). However, it should be noted that the positive responses of HGP to exercise and AICAR are due to different mechanisms. With exercise, HGP is stimulated by a combination of substrate, hormonal, and neurogenic stimuli (39, 50). On the other hand, the AICAR-induced increase in HGP is due to direct allosteric stimulation by ZMP of hepatic glycogenolysis, as discussed by Comacho et al. (5). The exercise-induced changes in SkM (increased G6P and decreased glycerogen concentration) were not observed in the AICAR-treated animals, but ACCβ-pSer221 concentrations rose similarly in both groups. These SkM data, combined with the marked increases of HGP, Rd,M C Rg, and particularly GFexog in the exercise studies (vs. AICAR) are consistent with a greatly increased exercise-induced uptake and flux of glucose metabolites down the glycolytic pathway (39, 49). The combined increase of glucose uptake and its metabolic flux and the increased SkM glycogenolysis during exercise led to the observed elevation of SkM G6P. However, it is important to note that the sources of the increased ATP production with exercise and with AICAR are different. With exercise, the increased SkM ATP supply is from oxidation of glucose [derived from plasma glucose and SkM glycogenolysis (39, 49)] and FAox [derived from peripheral and SkM lipolysis (39, 49)]. In contrast, with AICAR, Gox and FAox are solely derived from oxidized plasma substrates (28, 30, 32). This is supported by recent observations obtained in isolated electrically stimulated rat soleus muscle, in which the addition of AICAR to the perfusion medium failed to further enhance the contraction-induced SkM endogenous FAox (46). In fact, the endogenous SkM triacyl glycerol hydrolysis was blunted (46). Thus the known beneficial effects of exercise on triacyl glycerol hydrolysis are unlikely to occur with AICAR infusion alone.

The second issue addressed in our studies was whether the metabolic responses to enhanced intracellular FAox, induced by either the 150-min AICAR or IL/hep infusions, were similar. We noted that, after both AICAR and IL/hep infusion, glucose turnover (HGP, Rd,M C Rg) were similar, but GFexog was suppressed during the IL/hep infusion (Table 1). These data support the in vitro observations in isolated muscle that AICAR-induced FAox does not suppress Gox (45), whereas increased FAox induced by excess FA supply does suppress GFexog, due to the Randle effect (34). SkM ACCβ-pSer221 rose only in the AICAR group, whereas intracellular glucose was significantly decreased with AICAR but was unchanged with IL/hep. Together, these data reflect that AICAR increases both Gox and FAox (45), in contrast to the potential inhibition of intracellular glucose metabolism during IL/hep infusion (31, 34). These data are supported by a recent study that used resting perfused rat soleus muscle strips, exposed to AICAR alone vs. low- and high-FFA concentrations. With AICAR alone, SkM exogenous FAox increases only 35%, although Gox increases two- to threefold; on the other hand, in response to high FFA concentrations, SkM exogenous FAox was enhanced two- to threefold and Gox was unchanged (45).

The third issue examined was, given that resting in vivo and in vitro SkM metabolic states induced by IL/hep infusion favor FAox rather than Gox (31, 4), whether the metabolic responses to moderate acute exercise with or without prior exposure to increased FA supply are altered. Earlier studies of the effect of raised FA supply on acute moderate exercise-induced glucose turnover are confusing, probably reflecting the different species and protocols employed (20, 37, 40, 46). In humans, glucose uptake, both whole body (40) and across leg thigh muscle (20), is either reduced (40) or unchanged (20, 31), whereas glycogen sparing during exercise and IL/hep infusion is generally increased (13, 20, 31, 40). However, these apparent contradictions in data probably reflect different intensities of workloads employed in these studies. When one only includes human studies in which acute (30–60 min) moderate exercise (V˙O2max of ~50–70%) are employed during the IL/hep infusion, more uniform metabolic responses are observed. Glucose uptake is unaltered, glycogen sparing occurs, Gox is decreased, FAox is increased, and pyruvate dehydrogenase activity is partially reduced during combined IL/hep infusion and moderate exercise (13, 31).

In the present study, with moderate acute exercise, the exercise-induced responses of glucose turnover (HGP, Rd,M C Rg, and GFexog) were similar in the IL/hep and non-IL/hep exercise studies (Fig. 4), despite the reduced resting GFexog in the IL/hep-infused dogs. SkM lactate oxidation is markedly increased with exercise (25); therefore, the incremental increase of SKM lactate noted in our IL/hep-infused exercising dogs is consistent with a partial inhibition of pyruvate dehydrogenase in exercised SkM with IL/hep infusion (17, 31). However, the exercise-induced rise in SkM lactate in the IL/hep-infused dogs was of insufficient magnitude to be reflected in the measured in vivo GFexog rate during exercise. We also noted some sparing of SkM glycogen during exercise and IL/hep infusion, compared with that shown with exercise alone. Thus the present studies are in general agreement with the earlier studies in humans (13, 20, 31).

Finally, two other issues need clarification. First, in our studies, we compared the metabolic responses of 30-min exercise to 60-min AICAR infusion and 150-min AICAR infusion to 150-min physiological elevation of plasma FFA. To match the acute response to exercise with the acute metabolic response to AICAR infusion, we noted in our earlier AICAR infusion studies (9) that the near-maximal metabolic response to the 150-min AICAR infusion occurred by 60 min (see Table 1). It is also well established that the acute downstream metabolic responses of increased plasma FFA on in vivo glucose transport and oxidation are reached by 150 min (38). Thus the comparisons between exercise and AICAR and IL/
hep and AICAR were reasonably matched in time in the present study. Whether this reflects a true “metabolic” matching cannot be resolved at this time. Second, the present ACCβ-pSer\(^{21}\) data confirm our and other workers’ reports of increased ACCβ-pSer\(^{21}\) in liver (5, 6) and in SkM (9) (consistent with activation of endogenous AMPK activity) utilizing the dose of AICAR used in the present study. Furthermore, the downstream in vivo metabolic responses of glucose turnover observed in the present study with AICAR and exercise closely match those of published studies in dogs (5, 6, 8–10, 33). Therefore, it seems reasonable that the in vivo and SkM metabolic responses observed here are due to direct stimulation by exercise or the AICAR infusion.

In conclusion, the metabolic and SkM responses to moderate acute exercise are unique with respect to in vivo changes in glucose turnover and SkM metabolism and FA metabolism. In particular, endogenous glycogenolysis and lipid hydrolysis are observed only with exercise and not with an acute AICAR infusion, and the stimulation of glucose turnover by exercise is more intense than results shown with AICAR (albeit by different mechanisms). These favorable exercise-induced responses in glucose turnover are not significantly altered by the presence of excessive FA supply, although some limitations of SkM Gox and glycogen sparing are observed. Thus a single dose of AICAR does not mimic the complex metabolic responses observed with acute moderate exercise, nor does a short-term increase in FA supply alter these responses to exercise.

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