AMPK stimulation increases LCFA but not glucose clearance in cardiac muscle in vivo

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Shearer, Jane, Patrick T. Fueger, Jeffrey N. Rottman, Deanna P. Bracy, Paul H. Martin, and David H. Wasserman. AMPK stimulation increases LCFA but not glucose clearance in cardiac muscle in vivo. Am J Physiol Endocrinol Metab 287: E871–E877, 2004. First published July 20, 2004; doi:10.1152/ajpendo.00125.2004.—AMP-activated protein kinase (AMPK) independently increases glucose and long-chain fatty acid (LCFA) utilization in isolated cardiac muscle preparations. Recent studies indicate this may be due to AMPK-induced phosphorylation and activation of nitric oxide synthase (NOS). Given this, the aim of the present study was to assess the effects of AMPK stimulation by 5-aminomimidazole-4-carboxamide-1-β-d-ribofuranoside (AICAR; 10 mg·kg−1·min−1) on glucose and LCFA utilization in cardiac muscle and to determine the NOS dependence of any observed effects. Catheters were chronically implanted in a carotid artery and jugular vein of Sprague-Dawley rats. After 4 days of recovery, conscious, unrestrained rats were given either water or water containing 1 mg/ml nitro-L-arginine methyl ester (L-NAME) for 2.5 days. After an overnight fast, rats underwent one of four protocols: saline, AICAR, AICAR + l-NAME, or AICAR + Intralipid (20%:0.02 ml·kg−1·min−1). Glucose was clamped at −6.5 mM in all groups, and an intravenous bolus of 2-deoxy-[3H]glucose (eNOS-Ser1177) in the presence of Ca2+-calmodulin in both normal and ischemic hearts. In addition, Morrow et al. (37) demonstrate that AMPK activates and phosphorylates eNOS-Ser1177, resulting in increased NO production in aortic endothelial cells, whereas transfection of cells with dominant negative AMPK abrogates these effects. Together, these results clearly show NOS to be a target of AMPK.

Studies examining the effects of AMPK activation on substrate utilization suggest that AMPK concurrently increases both glucose and LCFA metabolism and that these effects may be due to increases in NOS (37, 40). Despite this, in vivo experiments assessing the simultaneous utilization of both substrates and their dependence on NO in cardiac muscle are lacking. Therefore, the aim of the present study was to examine the effects of AMPK stimulation by 5-aminomimidazole-4-carboxamide-1-β-d-ribofuranoside (AICAR) on glucose and LCFA clearance in cardiac muscle and to determine the dependence of any observed effects on NO. Our results show that unlike isolated cardiac muscle preparations (24, 40), AICAR stimulation results in increased LCFA but not glucose clearance. Furthermore, this effect was independent of NOS activation.

METHODS

Animals

Male Sprague-Dawley rats (Harlan Industries, Indianapolis, IN) were housed individually and maintained at 23°C on a 0600–1800
light cycle. Rats were fed standard chow ad libitum (Purina, Nestlé, St. Louis, MO) and given free access to water. The rats were housed under these conditions for ~1 wk, by which time their weights had reached ~300 g. After weight gain, rats were randomly divided into each of the experimental groups (n = 7/group). All procedures were approved by the Vanderbilt University Animal Care and Use Sub-committee and followed National Institutes of Health guidelines for the care and use of laboratory animals.

Surgical Procedures

Surgical procedures were performed as previously described for arterial and venous catheterizations (38). Briefly, animals were anesthetized with a 50:5:1 (vol/vol/vol) mixture of ketamine-rompun-acetopamlazine, and the left common carotid artery and right jugular vein were catheterized with PE50. Catheters were exteriorized and secured at the back of the neck, filled with heparinized saline (150 U/ml), and sealed with a stainless steel plug. Immediately post-surgery, each animal received 75 mg/kg of ampicillin subcutaneously to prevent infection. After surgery, animal weights and food intake were monitored daily, and only animals in which presurgery weight (>300 g) was restored were used for experiments.

Isotopic Analogs

Glucose and LCFA tracers employed in the present study were 2-deoxy-[1-14C]glucose (2-[14C]DG) and [1-13C]-15-(p-iiodophenyl)-3-β-3-methylpentadecanoic acid ([125I]BMIPP). 2-[14C]DG was purchased from New England Nuclear (Boston, MA). BMIPP was a kind gift from Oak Ridge International Laboratories (Oak Ridge, TN). Radio-iodination was performed according to the manufacturer’s suggested protocol. Briefly, BMIPP was heated in the presence of Na125I solution (740 MBq/200 µl), propionic acid, and copper (II) sulfate. Na2S2O3 was then added, and the organic-phase ether was extracted and sequentially back extracted with saturated NaHCO3 and water. After evaporation, the [125I]BMIPP was solubilized by use of sonication into ursodeoxycholic acid, with a final activity of 775 µCi/ml.

Nitro-L-arginine methyl ester

After 4 days of recovery, rats were given either drinking water (n = 21) or water containing 1 mg/ml nitro-L-arginine methyl ester (L-NAME) for 2.5 days (n = 7). Assessment of water consumption showed rats consumed 35–40 ml of water or ~120–160 mg L-NAME/kg·day−1. Previous studies have shown that this dose elicits a near-maximal but reversible inhibition of NO synthesis (2, 19, 47). This route of administration was chosen because L-NAME administration by acute intravenous injection results in increases in body temperature (6).

Experimental Procedures

Before the experiment, catheters were flushed with heparinized saline (10 ml) and connected to PE50 and Silastic tubing for infusions and sampling. Throughout the experimental protocol, rats were conscious and unrestrained. The experimental protocol consisted of continuous infusions of saline, AICAR (10 mg·kg−1·min−1), or AICAR + Intralipid (20%, 0.02 ml·kg−1·min−1), containing 100 U/ml heparin; Pharmacia & Upjohn, Uppsala, Sweden). All infusions lasted for 150 min. In those rats receiving AICAR, glucose (D2O) was infused at a rate variable to maintain glycemia at ~6.5 mM. Because the administration of AICAR resulted in a decline in plasma nonesterified fatty acids (NEFA), Intralipid was infused in one group of animals (AICAR + Intralipid) to maintain plasma NEFA levels and to ensure that the observed differences in LCFA uptake (Rf) were not concentration dependent. Accordingly, there were four experimental groups: saline (n = 7), AICAR (n = 7), AICAR + L-NAME (n = 7), and AICAR + Intralipid (n = 7). All infusions were performed for 90 min before steady-state sampling, where small arterial blood samples (20 µl) were obtained every 10 min throughout the experiment for the measurement of plasma glucose. This provided feedback, which was used to adjust glucose infusion rates needed to maintain euglycemia. After the equilibration period, rats received an intravenous bolus of 2-[14C]DG and [125I]BMIPP for the measurement of glucose and LCFA uptakes, respectively [time (t) = 95 min]. The time period from 90 to 150 min is referred to as the “experimental period” in RESULTS. At t = 0, 90, and 150 min, arterial blood samples (150 µl) were obtained for the measurement of glucose and NEFA. In addition, samples (50 µl) were obtained at 97, 100, 110, 120, 130, and 140 min for the measurement of 2-[14C]DG, [125I]BMIPP, glucose, NEFA, and/or insulin. To prevent declines in hematocrit, the erythrocytes taken before the isotopic analog infusion were washed in saline and reinfused shortly after each sample was taken. At t = 150 min, rats were anesthetized with pentobarbital sodium, and their hearts were rapidly excised, rinsed in saline to remove excess blood, freeze clamped in liquid nitrogen, and frozen at ~80°C until further analysis.

Plasma Analyses

Metabolites. Plasma glucose concentrations were measured by the glucose oxidase method, using an automated glucose analyzer (Beckman Instruments, Fullerton, CA), and immunoreactive insulin was measured in samples obtained at t = 0, 90, 120, and 150 min by use of a double-antibody method (36). NEFA concentrations were determined at t = 0, 90, 110, 130, and 150 min spectrophotometrically with the use of a kit obtained from Wako Chemicals (NEFA-C, Richmond, VA). In the AICAR + Intralipid group, administration of heparin with Intralipid would have resulted in increased in vitro lipolysis because of the presence of lipoprotein lipase. As such, samples used for NEFA analysis in this group were treated with 5 M NaCl as previously described (12, 17) before analysis.

Isotopic analogs. [125I]BMIPP and 2-[14C]DG were measured in the same plasma samples (20 µl) as previously described (39). Briefly, 20 µl of plasma were counted for [125I]BMIPP with the use of a Beckman Gamma-5500 counter (Beckman Instruments). After this, the plasma sample was deproteinized in 100 µl of Ba(OH)2 (0.06 N) and 100 µl of ZnSO4 (0.06 N) and subsequently centrifuged. Supernatant (100 µl) was then diluted in 900 µl of H2O. [3H]radioactivity was counted after addition of fluor (10 ml, Ultimate Gold; Packard Bioscience, Boston, MA) by use of a Packard Tri-Carb 2000TR liquid scintillation analyser (PerkinElmer, Boston, MA). The relationship between gamma radioactivity and beta emissions has been established in our laboratory for that specific counter. This relationship was used to correct [3H]radioactivity for beta emissions originating from [125I]radioactivity (39).

Plasma AICAR concentration. AICAR and 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranosyl 5′-monophosphate (ZMP) concentrations in cardiac muscle and plasma were determined by HPLC as previously described (1, 46). Briefly, 100 µl of plasma were extracted by the addition of HClO4 and neutralized by the addition of KH2PO4. Analysis was subsequently performed on the extracted plasma.

Cardiac Muscle Analyses

Isotopic analogs. Hearts were analyzed for accumulation of free 2-[14C]DG and phosphorylated 2-[14C]DG (2-[14C]DGPh) as previously described (16). Briefly, [125I]BMIPP and 2-[14C]DG were determined on 100 µg of tissue. After determination of tissue 125I radioactivity, tissue was homogenized in 2 ml of 0.5% perchloric acid and centrifuged for 20 min. Supernatants (1.5 ml) were then neutralized with the use of 5 M KOH, and 250 µl were counted after the addition of fluor. This fraction provided total radioactivity of 2-[14C]DG [free + phosphorylated (2-[14C]DGPh)]. In addition, 500 µl of supernatant were treated with 250 µl of Ba(OH)2 and 250 µl of ZnSO4 and centrifuged. After this, 500 µl of supernatant were diluted to 1 ml in distilled H2O.
before fluor (10 ml) was added and samples were counted. Treatment with Ba(OH)$_2$ (0.3 N) and ZnSO$_4$ (0.3 N) removed all but free 2-$^3$H[DG (34). Therefore, 2-$^3$H[DGP is calculated as the difference in radioactivity without (total) and with (free 2-$^3$H[DG] Ba(OH)$_2$ and ZnSO$_4$. This analytic approach allows the separation of 2-$^3$H[DGP from the fraction of 2-$^3$H[DG that is incorporated into glycogen, inclusion of which results in the underestimation of the metabolic indexes of glucose uptake (R$_g$) and metabolic (R$_g$) indexes were calculated in inclusion of which results in the underestimation of the metabolic indexes of glucose uptake (R$_g$) and metabolic (R$_g$) indexes were calculated in

Western blot analysis. Hearts were pulverized and powdered in liquid nitrogen, using a mortar and pestle. Resultant tissue was homogenized in ice-cold lysis buffer containing 10% glycerol, 20 mM Na-pyrophosphate, 150 mM NaCl, 50 mM HEPES (pH 7.5), 1% Nonidet P-40, 20 mM β-glycerophosphate, 10 mM NaF, 2mM EDTA (pH 8.0), 2mM phenylmethylsulfonyl fluoride, 1mM CaCl$_2$, 1mM MgCl$_2$, 10mM aprotinin, 10μg/ml leupeptin, 2mM Na$_2$VO$_3$, and 3mM benzamide. After centrifugation (1 h × 4,500 g), the pellets were discarded while supernatants were retained for protein determination using a Pierce bichinonic acid protein assay kit (Rockford, IL). Proteins (30 μg) were separated on SDS-PAGE gel and then transferred to a polyvinylidine di fluoride membrane. Membranes were blocked with rabbit phospho-ACC (ACC-Ser 79, which detects the equivalent ACC-Ser231 in heart) and AMPK phosphorylation (AMPK-Thr172, 1:1,000; Cell Signaling, Beverly, MA) and then incubated with α-rabbit-horseradish peroxidase (Pierce). Densitometry was performed using Lab Image software (Kapelan, Halle/Saale, Germany).

AICAR and ZMP concentrations. AICAR and ZMP concentrations in plasma and cardiac muscle were determined by HPLC as previously described (1, 46). Briefly, 100 mg of muscle were extracted by homogenization in HClO$_4$ before the supernatant was isolated and neutralized by the addition of KH$_2$PO$_4$.

Calculations

Indexes of glucose and LCFA uptake and clearance. Cardiac glucose clearance (K$_c$) and metabolic (R$_c$) indexes were calculated from the accumulation of 2-$^3$H[DGP in cardiac muscle and the integral of the plasma 2-$^3$H[DGP concentration (conc) after a 2-$^3$H[DG bolus (15, 20). The relationships are defined as

$$K_c = \frac{\text{conc}_{\text{pl}}(t) \int_0^t \text{conc}_{\text{pl}}(t) \text{d}t}{\text{conc}_{\text{pl}}(0)}$$

$$R_c = K_c \times [G]_c$$

The subscripts p and m refer to mean arterial plasma and total muscle concentrations from 90 to 120 min, respectively, and [G] is glucose concentration. The measurement of R$_c$ has been described earlier (15, 20). In an analogous manner, LCFA clearance (K$_c$) and metabolic (R$_c$) indexes were calculated after a $[^{125}]$BMIPP bolus from the accumulation of $[^{125}]$BMIPP in muscle and the integral of the plasma $[^{125}]$BMIPP concentration after the bolus

$$K_l = \frac{\text{conc}_{\text{pl}}(t) \int_0^t \text{conc}_{\text{pl}}(t) \text{d}t}{\text{conc}_{\text{pl}}(0)}$$

$$R_l = K_c \times [LCAF]_c$$

where [LCAF]$_c$ is the concentration of LCFA in plasma, ([$^{125}]$B-MIPP)$_m$ is the concentration of $[^{125}]$BMIPP in the cell, and ([$^{125}]$B-MIPP)$_p$ is the concentration of $[^{125}]$BMIPP in plasma. The measurement of R$_c$ and K$_c$ has been described earlier (9, 39).

Statistical analyses. A two-way repeated-measures analysis of variance (ANOVA) was performed to compare differences between groups over time. To establish differences within the ANOVA, Tukey’s post hoc test was used. Significance levels of P ≤ 0.05 were employed, and data are reported as means ± SE.

RESULTS

Animal Characteristics

Animal weights were 310 ± 9, 318 ± 11, 281 ± 8, and 304 ± 7 g for the saline, AICAR, AICAR + L-NAME, and AICAR + Intralipid groups, respectively. Rats in the AICAR + L-NAME group were slightly lighter (12%) compared with AICAR. This was not due to a treatment effect but rather to lower presurgery weights. Hematocrit values were not different among groups before (44 ± 0.4%, t = 0) or after the experimental protocol (42 ± 0.7%, t = 150 min). Plasma glucose levels were not different among groups before or during the experimental period, with mean values of 6.2 ± 0.2, 6.9 ± 0.2, 6.9 ± 0.2, and 6.9 ± 0.2 mM (90–150 min), respectively. Preexperimental (t = 0 min) plasma insulin values were not different with treatments, with mean values of 6.3 ± 0.7, 8.2 ± 0.5, 7.2 ± 0.9, and 9.0 ± 2.3 μU/ml for saline, AICAR, AICAR + L-NAME, and AICAR + Intralipid groups, respectively. During the experimental period, plasma insulin values were not different among saline, AICAR, and AICAR + L-NAME groups, with mean values of 7.6 ± 0.7, 5.8 ± 0.9, and 11.6 ± 2.1 μU/ml, respectively. However, in the AICAR + Intralipid group, plasma insulin was elevated during the experimental period (24.6 ± 3.1 μU/ml, P < 0.05). This was not unexpected, as previous studies have shown Intralipid infusion to increase plasma insulin levels because of a decrease in insulin clearance (3). Plasma NEFA in AICAR and AICAR + L-NAME rats were significantly lower at all time points in the experimental period compared with baseline values (t = 0; Fig. 1). NEFA values were lower in AICAR and AICAR + L-NAME compared with saline and AICAR + Intralipid groups in the experimental period at all time points measured. In AICAR + Intralipid, baseline NEFA (t = 0) were lower compared with 150 min. No differences were seen for glucose infusion rates in the experimental period (Table 1). All plasma measurements have been previously published (41), and they are included here to provide a frame of reference for the heart data.

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Fig. 1. Plasma nonesterified fatty acid (NEFA) concentrations at baseline and throughout the experimental period (90–150 min). All data represent means ± SE; n = 7 rats/treatment. L-NAME, nitro-L-arginine methyl ester; AICAR, 5-aminomidazole-4-carboxamide-1-β-o-ribosferonoside. *Significant difference (P < 0.05) from saline and AICAR + Intralipid. #Significant difference (P < 0.05) from 0 min within AICAR + Intralipid.
AICAR administration increased $K_f$ in cardiac muscle compared with saline (Fig. 2). Analysis of AICAR + L-NAME showed that this effect was not dependent on NOS, as there was no change from AICAR alone. In comparison, $R_f$ was not different among saline, AICAR, and AICAR + L-NAME because of AICAR-induced declines in NEFA. However, when NEFA levels were maintained in AICAR + Intralipid, $R_f$ was elevated, indicating that the effects of AICAR on $K_f$ were not dependent on the reduced concentrations in AICAR and AICAR + L-NAME. Results demonstrate that AICAR did not increase $K_g$ or $R_g$ in cardiac muscle compared with saline (Fig. 3). Furthermore, the addition of L-NAME to AICAR did not have an effect on either parameter. In contrast, AICAR + Intralipid resulted in increases in both $K_g$ and $R_g$ because of elevated plasma insulin concentrations with this treatment.

### Western Blot Analysis

The effects of AICAR on ACC-Ser$^{221}$ and AMPK-Thr$^{172}$ phosphorylation are shown in Fig. 4. Results demonstrate that the concentration of AICAR used in the present study was sufficient to activate AMPK.

### AICAR and ZMP Concentrations

Plasma AICAR in rats receiving AICAR was 13.7 ± 2.0 mM ($n = 9$), whereas the concentrations of AICAR in the rats receiving saline were undetectable. Heart AICAR concentrations were 2.3 ± 0.2, 3.7 ± 1.0, and 3.7 ± 0.2 μmol/g for AICAR ($n = 7$), AICAR + L-NAME ($n = 7$), and lipid ($n = 6$), respectively. ZMP concentrations for the above-mentioned groups were 5.8 ± 0.6, 3.7 ± 0.2, and 3.7 ± 0.4 μmol/g. In contrast, in rats receiving saline, both tissue AICAR and ZMP levels were undetectable. These measurements clearly demonstrate that 1) plasma AICAR levels were elevated in those rats receiving AICAR, 2) AICAR was readily taken up by the heart, and 3) AICAR was converted into ZMP in the heart.

### DISCUSSION

In cardiac muscle, substrate balance is integral to heart function in both normal and pathophysiological states as well as a potential target for therapeutic intervention (27, 42). A key protein mediating glucose and LCFA metabolism is AMPK.

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**Table 1. Arterial plasma glucose and glucose infusion rates at selected time points**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>90 min</th>
<th>110 min</th>
<th>130 min</th>
<th>150 min</th>
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<tbody>
<tr>
<td>Glucose, mM</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Saline</td>
<td>6.4±0.2</td>
<td>6.1±0.2</td>
<td>6.5±0.4</td>
<td>6.2±0.3</td>
</tr>
<tr>
<td>AICAR</td>
<td>6.9±0.2</td>
<td>6.9±0.3</td>
<td>6.6±0.2</td>
<td>6.9±0.3</td>
</tr>
<tr>
<td>AICAR + L-NAME</td>
<td>6.9±0.2</td>
<td>6.5±0.4</td>
<td>7.1±0.6</td>
<td>6.6±0.3</td>
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<tr>
<td>AICAR + Intralipid</td>
<td>7.1±0.3</td>
<td>7.3±0.3</td>
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Values are means ± SE; $n = 7$ rats/treatment. Arterial plasma glucose (in mM) and glucose infusion rates (GIR; in μmol/kg·min) at selected time points throughout the experimental protocol are shown. AICAR, 5-amino-imidazole-4-carboxamide-1-β-D-ribofuranoside; L-NAME, nitro-L-arginine methyl ester.
Activated in response to cellular stresses that deplete ATP, AMPK stimulation increases both glucose and LCFA metabolism. Recent studies suggest that this occurs by an NOS-mediated pathway, as increasing NO levels result in vasodilation and thus the potential for increased substrate delivery (7, 8, 37). Given this, the purpose of the present study was to assess the effects of the AMPK activation by AICAR on the uptake and clearance of glucose and LCFA in cardiac muscle as well as to determine the dependence of any observed effects on NO. Using isotopic analogs to simultaneously quantify glucose and LCFA kinetics, we show AICAR administration to increase LCFA but not glucose clearance in vivo. In addition, results demonstrate that this increase was not dependent on NO, as AICAR + L-NAME had no effect on either glucose or LCFA clearance compared with AICAR alone. The finding that AICAR did not increase glucose uptake was unexpected, considering previous studies have demonstrated that AICAR increases GLUT4 translocation, glucose uptake, and glycogenolysis in cardiac muscle (24, 40). Using quiescent isolated rat ventricular papillary muscles in vitro, Russell et al. (40) demonstrated that AICAR increases Rg and GLUT4 translocation, as measured by immunofluorescence. In the same study, the effects of an in vivo AICAR infusion were reported; however, only papillary muscles and not the entire cardiac muscle were examined for GLUT4 translocation by differential centrifugation and AMPK activation. This led the authors to conclude that AICAR stimulates cardiac glucose uptake and GLUT4 translocation both in vitro and in vivo.

Reasons for the discrepancy between these previously described findings and the present study may include the lack of LCFA in the quiescent in vitro preparations or specific differences between papillary and whole cardiac muscle metabolism. Although NEFA levels were suppressed by AICAR in the present study, results show that, when they are available (AICAR + Intralipid), they are preferentially utilized. Other plausible explanations include the differential metabolism of papillary muscles, which may be more characteristic of ischemia once isolated, or that AICAR results in GLUT4 translocation to specific subcellular locations rather than the entire surface of the membrane. In agreement with this latter hypothesis, Lemieux et al. (23) have shown that AICAR stimulation does not result in translocation of GLUT4 to the transverse tubules but rather the plasma membrane in skeletal muscle. Considering that the transverse tubules comprise >60% of skeletal muscle membranes and that the majority of GLUT4 translocates to this membrane fraction (10, 11), this finding was unexpected and led the authors to conclude that AMPK activation may selectively mobilize specific pools of GLUT4 to distinct areas of the membrane or that it enhances the activity of transporters already at the surface of the membrane. As well, LCFA clearance may have been elevated in this study, as it was performed in vivo, ensuring LCFA and oxygen are available to the stimulated tissue. In comparison, the lack of LCFA and blood flow in in vitro preparations where AMPK agonists metabolize metabolism may result in hypoxic conditions, which in itself could result in GLUT4 translocation. Finally, it could also be that a small fraction of GLUT4 transporters was mobilized to the plasma membrane in the present study, but that glucose uptake was not increased to a significant degree because of other limitations in glucose metabolism, such as glucose phosphorylation by hexokinase II (14).

Current evidence indicates that in vivo AICAR-induced stimulation of AMPK in cardiomyocytes results in fatty acid uptake by translocation of FAT/CD36 (30). In an analogous manner to GLUT4, FAT/CD36 translocates from contraction-inducible intracellular storage compartments to the sarcolemmal membrane and is thought to be a rate-determining step in LCFA uptake (29). In the present study, AMPK activation resulted in a large increase in cardiac LCFA uptake and clearance, likely due to enhanced FAT/CD36 translocation. These results are mirrored in a study by Makinde et al. (33), who showed that AMPK activation stimulates LCFA uptake in newborn rabbit hearts, demonstrating that, when substrate is available, cardiac muscle preferentially metabolizes LCFA in response to AICAR.

In the present study, Intralipid was infused to prevent AICAR-induced declines in LCFA. Because AICAR + Intralipid resulted in the same increase in Kf as AICAR alone, the effects of AICAR on Kf are not simply due to a LCFA concentration-dependent effect. However, the infusion of AICAR + Intralipid also resulted in elevations in plasma insulin due to reduced insulin clearance (3). As a result, Kf was elevated in AICAR + Intralipid beyond rates recorded during AICAR alone. This increase was anticipated for Rg, as the effects of AMPK and insulin on GLUT4 are additive and stimulate distinct metabolic pathways of glucose uptake (13,
22. Similarly, we cannot strictly rule out an effect of insulin on stimulation of LCFA uptake, as it induces translocation of FAT/CD36 (28). Studies in cardiomyocytes have shown LCFA uptake to increase by ~25% despite the presence of pharmacological concentrations of insulin (28). Therefore, it is possible that insulin may contribute, in part, to the increase in $K_t$ observed in the AICAR + Intralipid group. It is likely that this contribution is small, as in vivo studies assessing the effects of insulin on myocardial LCFA uptake by position emission tomography have shown insulin to have no effect on myocardial fractional LCFA extraction (31).

In summary, the activation of AMPK by AICAR results in the preferential clearance of LCFA and not glucose, provided that insulin levels are unchanged. Furthermore, the present study shows that this effect appears to be due to augmentation of fatty acid transport rather than NO-induced increases in substrate delivery or any other NO-mediated effect. These results also stress the importance of simultaneously assessing the interactions between glucose and LCFA utilization and clearance in metabolic studies.

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GRANTS

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