Contribution of Nonesterified Fatty Acids to Mitogen-Activated Protein Kinase Activation in Human Skeletal Muscle During Endurance Exercise

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Mitogen-activated protein kinase (MAPK) pathways are activated in skeletal muscle during endurance exercise, but the upstream molecular events are incompletely resolved. As an increase in plasma nonesterified fatty acids (NEFA) is a common feature of long-lasting exercise, the authors tested the hypothesis that NEFA contribute to the activation of MAPK during endurance exercise. Acipimox was used before and during endurance exercise to prevent the elevation of plasma NEFA levels in healthy subjects and patients with diabetes. In 2 separate studies, healthy subjects cycled for 2 hr and patients with diabetes for 1 hr at 50% Wmax. In control conditions, plasma NEFA concentrations increased from 0.35 to 0.90 mM during exercise in healthy subjects and from 0.55 to 0.70 mM in patients with diabetes (p < .05). Phosphorylation states of extracellularly regulated kinase 1 and 2 (ERK1/2), p38, and c-Jun NH2-terminal kinases (JNK) were significantly increased after exercise in the vastus lateralis in both groups. Acipimox blocked the increase in plasma NEFA concentrations and almost completely repressed any rise in ERK1/2 and p38 but not in JNK. In conclusion, the data support a role for plasma NEFA in the activation of p38 and ERK1/2 in skeletal-muscle tissue of healthy and diabetic subjects during endurance exercise. Further investigation will be required to determine the molecular link between NEFA and MAPK activation during exercise in human skeletal muscle.

Keywords: p38 MAPK, ERK1/2, JNK, NF-κB, acipimox, cycling, TLR4, Type II diabetes

The mitogen-activated protein kinases (MAPKs) are a group of intracellular signaling proteins activated by multiple signals. They are critically involved in the transmission of information from outside the cell to the nucleus, where they regulate transcription. Extracellular-signal-regulated kinases (ERK) 1 and 2 (ERK1/2), p38 MAPK, and c-Jun NH2-terminal kinases (JNK) are three widely expressed MAPKs that regulate different cellular functions (Johnson & Lapadat, 2002). Endurance exercise is a well-known activator of MAPK, but the mechanisms by which ERK1/2, p38 MAPK, and JNK are activated during exercise remain incompletely resolved (Kramer & Goodyear, 2007). Increase in cytosolic calcium and subsequent calcium/calmodulin-dependent protein kinase II activation, as well as production of reactive oxygen species (ROS); acidification; increase in catecholamines, growth hormones, or cannabinoids; glycogen depletion; or mechanical stretch, has been proposed to participate in the activation of p38 MAPK during endurance exercise (Blair, Hajduch, Litherland, & Hundal, 1999; Chambers, Moylan, Smith, Goodyear, & Reid, 2009; Chan, McGee, Watt, Hargreaves, & Febbraio, 2004; Clerk, Fuller, Michael, & Sugden, 1998; Galbo, 1986; Li et al., 2005; Tedesco et al., 2010; Wretman et al., 2001; Wright, Geiger, Han, Jones, & Holloszy, 2007). In addition to those general mechanisms, MAPKs undergo member-specific regulations. ERK1/2 and p38 have been shown to be involved in exercise-induced signaling in human skeletal muscle, but the picture is less clear for JNK (Long, Widegren, & Zierath, 2004; Widegren et al., 1998; Yu, Blomstrand, Chibalini, Krook, & Zierath, 2001). Cycling markedly increased ERK1/2 phosphorylation, albeit only transiently, whereas similar exercise has been demonstrated to lead to a smaller but more persistent increase in p38 activation (Widegren et al., 1998). MAPK signaling seems to be regulated differentially according to the mode of contraction (concentric vs. eccentric), the intensity of the exercise, and the training status of the subjects (Widegren, Wretman, Lionikas, Hedin, & Henriksson, 2000).

Moderate to strenuous endurance exercise results in an increased extracellular level of nonesterified fatty acids (NEFAs; Stich et al., 2000), which contributes to energy expenditure depending on relative power output and duration of exercise (Kiens, 2006). This increment in NEFA availability is known to be less pronounced inactivité (Blair, Hajduch, Litherland, & Hundal, 1999; Chambers, Moylan, Smith, Goodyear, & Reid, 2009; Chan, McGee, Watt, Hargreaves, & Febbraio, 2004; Clerk, Fuller, Michael, & Sugden, 1998; Galbo, 1986; Li et al., 2005; Tedesco et al., 2010; Wretman et al., 2001; Wright, Geiger, Han, Jones, & Holloszy, 2007). In addition to those general mechanisms, MAPKs undergo member-specific regulations. ERK1/2 and p38 have been shown to be involved in exercise-induced signaling in human skeletal muscle, but the picture is less clear for JNK (Long, Widegren, & Zierath, 2004; Widegren et al., 1998; Yu, Blomstrand, Chibalini, Krook, & Zierath, 2001). Cycling markedly increased ERK1/2 phosphorylation, albeit only transiently, whereas similar exercise has been demonstrated to lead to a smaller but more persistent increase in p38 activation (Widegren et al., 1998). MAPK signaling seems to be regulated differentially according to the mode of contraction (concentric vs. eccentric), the intensity of the exercise, and the training status of the subjects (Widegren, Wretman, Lionikas, Hedin, & Henriksson, 2000).

Moderate to strenuous endurance exercise results in an increased extracellular level of nonesterified fatty acids (NEFAs; Stich et al., 2000), which contributes to energy expenditure depending on relative power output and duration of exercise (Kiens, 2006). This increment in NEFA availability is known to be less pronounced in
obese and diabetic people, mainly due to a lower sensitivity to catecholamines (Kanaley, Cryer, & Jensen, 1993). The contribution of NEFAs to energy production has been studied by using acipimox, a nicotinic acid analog able to prevent adipose-tissue lipolysis at rest and during exercise (O’Kane, Trinick, Tynan, Trimble, & Nicholls, 1992). A reduction in NEFA use during exercise after nicotinic acid administration increased the use of endogenous glycogen stores and intramuscular lipid in healthy trained (van Loon, Thomason-Hughes, et al., 2005) and Type II diabetic males (van Loon, Manders, et al., 2005).

Besides their role in energy supply for skeletal muscle, NEFAs have been suggested to play a role in activating signaling pathways that regulate gene expression (Tunstall, McAinch, Hargreaves, van Loon, & Cameron-Smith, 2007). It has been proposed that NEFAs could stimulate a family of membrane receptors called the Toll-like receptors (TLR; Senn, 2006; Shi et al., 2006), those most expressed in skeletal muscle being TLR2 and TLR4 (Reyna et al., 2008). On stimulation, TLR2 and TLR4 induce complex intracellular signaling leading to the activation of the MAPK family and transcription factors including nuclear factor-κB (NF-κB; Akira, Yamamoto, & Takeda, 2003). Using TLR2 and TLR4 KO mice, our group recently found that during endurance exercise, TLR2 and TLR4 mediated a signal linking the elevated plasma NEFA concentration to the activation of p38 MAPK and JNK in mouse skeletal muscle (Zbinden-Foncea, Raymackers, Deldicque, Renard, & Francaux, 2012). For the first time, a link was established between circulating NEFAs and stimulation of MAPKs via activation of TLR2/4 during endurance exercise in mice, but this remains to be confirmed in humans. We recently had the opportunity to analyze the activation of the MAPK pathways in remaining human muscle biopsies from previously published studies (van Loon, Manders, et al., 2005; van Loon, Thomason-Hughes, et al., 2005), where healthy subjects or diabetic patients received a placebo or acipimox, thereby blocking the rise in plasma NEFAs during exercise. Diabetic patients contributed to better understand the role of NEFAs in MAPK activation during exercise, as the increment in NEFA availability is known to be less in this population (Kanaley et al., 1993), although the resting concentration is higher. In the current study, we hypothesized that NEFAs contribute to MAPK activation in human skeletal muscle during endurance exercise.

**Material and Methods**

**Subjects**

Blood samples and muscle biopsies from 5 healthy subjects (van Loon, Thomason-Hughes, et al., 2005) and 10 diabetic patients (van Loon, Manders, et al., 2005) who had participated in a previous experiment were further analyzed. Subjects were informed about the nature and risks of the experimental procedures before their written informed consent was obtained. The two studies were approved by the medical ethical committee of the Academic Hospital Maastricht, and all procedures were carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association.

**Study 1.** Five healthy active male subjects (age 23 ± 1 years, height 1.79 ± 0.04 m, body weight 70 ± 3 kg, body-mass index 22.0 ± 0.9 kg/m², fat content 12.3% ± 0.8%, maximal power output [Wmax] 354 ± 11 W, 50% Wmax 177 ± 6 W, and maximal oxygen uptake capacity [VO2max] 61 ± 3 ml · kg⁻¹ · min⁻¹) participated in this study.

**Study 2.** Ten sedentary, overweight subjects (age 60 ± 2 years, height 1.79 ± 0.02 m, body weight 91 ± 3 kg, body-mass index 28.4 ± 1.0 kg/m², fat content 28.8% ± 1.8%, Wmax 200 ± 15 W, 50% Wmax 100 ± 6 W, and VO2max 32 ± 2 ml · kg⁻¹ · min⁻¹) who had been diagnosed with Type II diabetes over 5 years earlier participated in this study. All subjects were using oral blood-glucose-lowering medication (metformin with or without a sulphonylurea derivative). Type II diabetic status was verified with an oral glucose-tolerance test according to World Health Organization criteria (Alberti & Zimmet, 1998). In addition, insulin resistance was estimated using the homeostasis model assessment for insulin resistance index (Hosker et al., 1985). Medication was withheld for 24 hr before the experimental trials.

**Protocol**

All subjects maintained normal dietary and physical activity patterns throughout the experimental period. In addition, they filled out a food-intake diary for 2 days before the first exercise trial to keep their dietary intake as identical as possible before the other trials. The two trials were separated by a 1-week interval. The evening before each trial, subjects received the same standardized meal. The two experimental protocols are summarized in Figure 1 and have been detailed elsewhere (van Loon, Manders, et al., 2005; van Loon, Thomason-Hughes, et al., 2005).

**Study 1.** Briefly, a muscle biopsy was taken from the vastus lateralis muscle at rest (t = 0) after an overnight fast. Thereafter, a resting blood sample was taken and subsequent blood samples were collected every 15 or 30 min. Subjects performed 120 min of exercise (t = 90 to t = 210) on a cycle ergometer at a workload of 50% Wmax, as determined 1 week before the experimental trial during an incremental exhaustive exercise test. A capsule containing 250 mg acipimox (Nedios, Byk, Zwanenburg, The Netherlands) or placebo was randomly orally administered before (t = 0) and 75 min into the exercise session (t = 165). Immediately after cessation of exercise, a second muscle biopsy was taken (t = 210).

**Study 2.** After an overnight fast and 30 min of supine rest, a percutaneous muscle biopsy was taken from the vastus lateralis muscle (t = 0). A catheter was inserted into an antecubital vein for blood sampling, and an oral dose of 250 mg acipimox or placebo was randomly administered (t = 0). At t = 120 min, subjects started to exercise at 50%
W_{max} for a 60-min period. At t = 150, another dose of 250 mg acipimox or placebo was administered. Immediately after cessation of exercise, a second muscle biopsy was taken (t = 180).

**Analysis of Muscle Samples**

**Preparation of Muscle Lysates.** Muscle samples were ground using a pestle and homogenized in an ice-cold lysis buffer containing 20 mM Tris, pH 7.0; 270 mM sucrose; 5 mM EGTA; 1 mM EDTA, 1% Triton X-100; 1 mM sodium orthovanadate; 50 mM sodium β-glycerophosphate; 5 mM sodium pyrophosphate; 50 mM sodium fluoride; 1 mM 1,4-dithiothreitol; and a protease-inhibitor cocktail (Roche Applied Science, Vilvoorde, Belgium). Muscle homogenates were centrifuged at 10,000 g for 15 min at 4 °C. Protein concentration was determined using a DC protein assay kit (Bio-Rad Laboratories, Nazareth, Belgium) with bovine serum albumin as a standard. Muscle lysates were stored at −80 °C until subsequent analyses.

**Western Blotting.** The detailed procedure has been described in Deldicque et al. (2008). Briefly, protein lysates were combined with Laemmli and separated by SDS-PAGE. After electrophoretic separation at 40 mA, the proteins were transferred to PVDF membranes. Membranes were blocked for 60 min in TBST and 5% nonfat dried milk. Then, the membranes were incubated overnight at 4 °C in TBST containing 1% bovine serum albumin and one of the following antibodies: phospho-p38 Thr180/Tyr182, phospho-SAPK/JNK Thr183/Tyr185, phospho-ERK1/2 Thr202/Tyr204, IκBα, or GAPDH. All antibodies were obtained from Cell Signaling Technology (Leiden, The Netherlands) except GAPDH from Abcam (Cambridge, UK). Membranes were washed with TBST and incubated with the appropriate secondary antibody at room temperature for 60 min (Sigma, Bornem, Belgium). After additional washes, chemiluminescent detection was carried out using an ECL Western blotting kit (Amersham ECL Plus, GE Healthcare, Belgium). Bands were visualized on film, scanned, and quantified by densitometry. When several blots were needed to obtain values of the whole experiments, an internal control was used to minimize interblot variations due to incubation or exposure times. The internal control was a pool of remaining muscle biopsies from previous human studies of the laboratory. Results are reported as the ratio of the signal induced by the protein of interest divided by the signal induced by GAPDH. A value of 1.0 was assigned to the mean value of the preexercise samples, to which all other values from the respective condition were reported.

**Analysis of Blood Samples**

Plasma NEFA concentration was determined using a kit coupling enzymatic reaction and spectrophotometric detection (550 nm) of reaction end product (Wako, Neuss, Germany). The results we present constitute a subset of data already published (van Loon, Manders, et al., 2005; van Loon, Thomason-Hughes, et al., 2005). Means, SEMs, and statistics have been recalculated according to the new number of samples.

**Statistical Analysis**

A two-way ANOVA for repeated measures was used to test the interaction between time (NEFA) or exercise (MAPK) and treatment. Student-Newman-Keuls tests were used as post hoc tests. Student’s t test was used to
test differences in plasma NEFA concentrations between healthy subjects and diabetic patients at rest and at the end of the exercise. The significance threshold was set to $p < .05$. All results are presented as $M \pm SE$ of the mean.

**Results**

**Healthy Subjects**

A 2-hr cycling exercise induced a rise in plasma NEFA concentration from 0.35 to 0.90 mM immediately at the end of the exercise ($p < .001$) and to 1.35 mM 15 min after the end ($p < .001$) in subjects having received a placebo (Figure 2[A]). After 1 hr 15 min of exercise, plasma NEFA levels were significantly higher than pre-exercise values ($p < .05$). Acipimox administration before and during exercise prevented the rise in NEFA levels, resulting in lower values during exercise in the acipimox and during exercise prevented the rise in NEFA concentrations ($<0.1$ mM, $p < .05$; Figure 2[A]) than in the placebo conditions (0.35–0.9 mM).

With placebo, phosphorylations of p38 MAPK, ERK1/2, and JNK were 26- ($p < .05$, Figure 2[B]), 71- ($p < .05$, Figure 2[C]), and 3-fold ($p < .05$, Figure 2[D]) higher immediately after exercise compared with resting values. The exercise-induced increases in MAPKs were totally abolished after acipimox administration for p38 ($p < .05$, Figure 2[B]) and ERK1/2 ($p < .01$, Figure 2[C]) and partially repressed for JNK (Figure 2[D]). IκBα expression was not modified by exercise alone, although the combination of acipimox and exercise increased IκBα expression by about 50% compared with exercise in the placebo conditions ($p < .05$, Figure 2[E]).

**Diabetic Patients**

The ANOVA revealed a time effect and a Time × Treatment interaction for plasma NEFA concentrations of diabetic patients ($p < .001$). Resting plasma NEFA concentrations of diabetic patients (Figure 3[A]) were higher than those of healthy subjects (0.55 ± 0.05 vs. 0.35 ± 0.08, $p < .05$). At the end of the exercise, plasma NEFA concentrations were similar (0.70 ± 0.09 in diabetic patients vs. 0.90 ± 0.13 in healthy subjects, $p = .247$) despite a shorter exercise duration for diabetic patients (1 hr vs. 2 hr in healthy subjects). Compared with healthy subjects, the rise in NEFA concentrations was less pronounced and did not reach statistical significance when comparing immediately pre- and postexercise values. However, when taking the lowest preexercise value (the second one) as basal value, both the immediately postexercise and the 30-min-postexercise values were significantly higher ($p < .05$). Acipimox administration before and during exercise prevented the rise in NEFA concentrations, which remained lower throughout the trial in the acipimox (~0.15 mM, $p < .05$; Figure 3[A]) than the placebo conditions.

Phosphorylations of p38 MAPK, ERK1/2, and JNK were 5- ($p < .05$, Figure 3[B]), 14- ($p < .01$, Figure 3[C]), and 2-fold ($p < .05$, Figure 3[D]) higher immediately after exercise compared with resting values. The exercise-induced increases in p38 MAPK ($p < .05$, Figure 3[B]) and ERK1/2 ($p < .01$, Figure 3[C]) were totally abolished after acipimox administration. The increase in JNK phosphorylation after exercise was not repressed in acipimox conditions (Figure 3[D]). IκBα expression decreased after exercise in placebo conditions ($p < .05$, Figure 3[E]) but remained unchanged when acipimox was administered.

**Discussion**

Exercise has been linked to the activation of the MAPK pathway in skeletal muscle, although the mechanisms responsible for this activation are as yet unsolved. Increases in cytosolic calcium and subsequent calcium/calmodulin-dependent protein kinase II activation, as well as production of reactive oxygen species, increases in catecholamines, growth hormones or cannabinoids, glycogen depletion, and mechanical stretch, have been proposed to participate in the activation of p38 MAPK during endurance exercise (Blair et al., 1999; Chambers et al., 2009; Chan et al., 2004; Clerk et al., 1998; Galbo, 1986; Li et al., 2005; Tedesco et al., 2010; Wright et al., 2007). The current study identifies NEFAs as an additional trigger for MAPK phosphorylation in skeletal muscle during exercise.

Increases in NEFA levels after moderate to intense exercise have been regularly reported (Kiens, 2006; Watt, Holmes, et al., 2004; Wolfe, Klein, Carraro, & Weber, 1990), as well as the inhibitory effect of the nicotinic acid analog acipimox (Gautier et al., 1994). The results from the current study show, for the first time, that p38 MAPK and ERK1/2 activation after exercise are severely repressed in subjects having received acipimox. Compared with p38 MAPK and ERK1/2 activation after exercise are severely repressed in subjects having received acipimox. The lower response of JNK to cycling exercise may be related to the nature of the muscle contraction, as JNK phosphorylation has been shown to have a larger increase after eccentric than concentric contractions (Boppart et al., 1999).

With acipimox, some subjects showed higher phosphorylation levels of ERK1/2 or p38 MAPK after exercise versus basal, but the extent of such activation was ~10-fold smaller than with a placebo. Therefore, the increase in NEFAs should be considered an enhancer of MAPK phosphorylation in skeletal muscle rather than a mandatory event. Previous experiments performed on cell cultures and ex vivo muscle preparations reported that MAPKs can be activated by electrical, mechanical, or chemical conditions without the need of NEFAs (Hayashi, Hirshman, Dufresne, & Goodyear, 1999; Sheridan et al., 1999). It has also been shown that a 30-min one-legged exercise induced a 2.2-fold increase in p38 MAPK phosphorylation in muscles from both exercised and nonexercised legs (Widegren et al., 1998). This was not the case for ERK1/2, which presented an increased phosphorylation in the exercised leg only. Our results
Activation of MAPK by NEFA During Exercise

suggest that circulating molecules such as NEFAs may help maintain high levels of MAPK phosphorylation during exercise of a long duration.

Although we could not establish any correlation between individual values of plasma NEFAs and MAPK activation, there was a general trend to a less pronounced MAPK activation in the second study, in which the exercise-induced increase in NEFA concentrations was lower probably due to a shorter cycling protocol or the age or the pathological state of the subjects—patients...
with diabetes. In the current study, subjects in the diabetic group were older than those of the healthy group (60 vs. 23 years old on average). Age could have contributed to the difference in NEFA concentrations and MAPK activation. People with insulin resistance generally have higher resting NEFA levels and mobilize the same amount or slightly less fatty acids during exercise. The magnitude of the increase in plasma NEFA levels during exercise is thus less in these patients than in healthy people. The reduced lipolytic activity during exercise in patients with diabetes has been explained by a decreased sensitivity to catecholamines (Kanaley et al., 1993). Whereas no clear
correlation could be established between NEFA levels and MAPK activation during exercise, the previous observation reinforces the idea that increased plasma NEFA levels contribute to the stimulation of MAPKs during exercise. Whether the higher basal NEFA concentration in diabetic patients leads to a higher basal MAPK phosphorylation was beyond the scope of the current study—exercise-induced activation of MAPKs—and therefore was not investigated.

No muscle biopsy was taken between the first ingestion of acipimox and the beginning of the exercise, therefore making it difficult to draw conclusions on the effect of acipimox itself on the MAPK pathways. The effect of acipimox on MAPKs has never been studied before, but nicotinic acid has been shown to increase p38 MAPK and reduce ERK1/2 without altering JNK at rest (Watt, Southgate, Holmes, & Febbraio, 2004). Although acipimox is an analog of nicotinic acid (O’Kane et al., 1992), it is possible that its molecular mechanisms of action are not exactly the same, so the regulation of MAPKs may differ. Nevertheless, the effect of acipimox alone was not the purpose of the current study to ascertain, as we were mainly interested in the MAPK response to exercise in combination with acipimox ingestion. Only one study (Watt, Southgate, et al., 2004) has previously measured the phosphorylation state of p38, ERK1/2, and JNK after nicotinic acid ingestion and cycling. The results of that study do not argue about a regulation of MAPKs by NEFAs during exercise. Exercise, in combination with or without nicotinic acid, induced a MAPK-member-specific regulation rather than an overall increase in phosphorylation, suggesting that a general factor such as circulating NEFAs was not involved in MAPK regulation by exercise. The discrepancies between our results and those of Watt, Southgate, et al. (2004) may potentially be found in the following methodological issues. The protocol we used in the current study differed in the duration of exercise, 1 or 2 hr in the current study versus 3 hr. As MAPK activation is dependent on the duration of exercise, it is not unexpected to see different patterns of activation. After 3 hr of cycling, JNK phosphorylation was increased, whereas ERK1/2 was decreased and p38 remained unchanged (Watt, Southgate, et al., 2004). In another study of the same group (Watt, Holmes, et al., 2004), ERK1/2 increased after 90 min of cycling, indicating that MAPKs are very sensitive to the duration of exercise and the time of biopsy sampling. At the same time, exercise-induced increase in ERK1/2 was repressed by nicotinic acid ingestion (Watt, Holmes, et al., 2004). The increase in JNK found after 1 or 2 hr of exercise in our study or after 3 hr by Watt, Southgate, et al. (2004) was not repressed by either acipimox or by nicotinic acid, respectively. In summary, acipimox and nicotinic acid are able to repress exercise-induced increase in p38 MAPK and ERK1/2. As their most important common effect is to reduce plasma NEFA concentrations, it is highly possible that NEFAs contribute to the increase in p38 MAPK and ERK1/2 (but not JNK) during exercise. The specificity to p38 and ERK1/2 needs to be investigated further. It should be mentioned that nicotinic acid and acipimox are known to increase plasma epinephrine concentration, as well (O’Neill, Watt, Heigenhauser, & Spriet, 2004). However, MAPK regulation was shown not to follow the increase in plasma epinephrine concentrations due to nicotinic acid whether at rest or after exercise (Watt, Southgate, et al., 2004).

To further study how NEFAs could regulate MAPKs, we measured the expression of IκBα, based on our recently published study (Zbinden-Foncea et al., 2012), which reported a possible NEFA/TLR/MAPK signaling. IκBα is a well-known downstream target of TLR2/4 signaling (Akira et al., 2003) and regulator of the NF-κB pathway. However, we could not find any consistent regulation of IκBα by either exercise or acipimox. These results discard any role of the NF-κB in the current study, but it is still possible that NEFAs activate MAPKs through TLRs, independently of NF-κB. It is also possible that, contrary to what we found in mice, TLRs do not mediate the effect of NEFAs on MAPKs, but this clearly needs further investigation. Another potential candidate for mediating the effect of NEFAs on MAPK is the newly discovered hydroxycarboxylic acid receptor 2 (Hanson et al., 2010), as the currently known ligands for this receptor are nicotinic acid, fumaric acid esters, and 3-hydroxybutyric acid, all structurally close to fatty acids. The hydroxycarboxylic acid receptor 2 is expressed in white and brown adipose tissue, keratinocytes, and various immune cells (Hanson, Gille, & Offermanns, 2012). Activation of hydroxycarboxylic acid receptor 3 by 3-hydroxyoctanoate leads to activation of ERK1/2 in Chinese hamster ovary and human epidermoid cell lines (Zhou et al., 2012), but whether the activation of hydroxycarboxylic acid receptor 2 leads to the activation of the MAPK pathway has not been reported yet. The possible activation of hydroxycarboxylic acid receptor 2 by NEFAs and the downstream activation of MAPKs in skeletal muscle would be worth investigating.

In conclusion, our data support a role for plasma NEFAs in the activation of p38 MAPK and ERK1/2 in skeletal-muscle tissue of healthy and diabetic subjects during endurance exercise. Further investigation will now be required to determine the molecular link between NEFAs and MAPK activation during exercise in human skeletal muscle.

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References


ferential regulation of MAP kinase, p70(S6K), and Akt by contraction and insulin in rat skeletal muscle. The American Journal of Physiology, 276, E870–E878. PubMed


