Angiotensin-(1-7) participates in enhanced skeletal muscle insulin sensitivity after a bout of exercise

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Abstract

A single bout of exercise increases subsequent insulin-stimulated glucose uptake in skeletal muscle; however, it is unknown whether Angiotensin-(1-7) (Ang-(1-7)), a vasoactive peptide of the Renin-Angiotensin System (RAS), participates in this process. The aim of this study was to investigate the possible involvement of Ang-(1-7) in enhanced skeletal muscle insulin sensitivity after an exercise session. Male Wistar rats were forced to swim for 2.5 h. Two hours after exercise, insulin tolerance tests and 2-deoxyglucose uptake in isolated soleus muscle were assessed in the absence or presence of the selective Mas receptor (MasR, Ang-(1-7) receptor) antagonist A779. Ang II and Ang-(1-7) levels were quantified in plasma and soleus muscle by HPLC. The protein abundance of Angiotensin-Converting Enzyme (ACE), ACE2, Ang II Type 1 Receptor (AT1R), and MasR was measured in soleus muscle by Western blot. Prior exercise enhanced insulin tolerance and insulin-mediated 2-deoxyglucose disposal in soleus muscle. Interestingly, these insulin-sensitizing effects were abolished by A779. After exercise, the Ang-(1-7)/Ang II ratio decreased in plasma, while it increased in muscle. In addition, exercise reduced ACE expression, but it did not change the protein abundance of AT1R, ACE2, and MasR. These results suggest that Ang-(1-7) acting through MasR participates in enhanced insulin sensitivity of skeletal muscle after a bout of exercise.

Keywords: Angiotensin-(1-7), Exercise, Glucose uptake, Insulin sensitivity, Renin-Angiotensin System, Skeletal muscle.
Introduction

Exercise promotes diverse beneficial effects for health and is useful for the prevention and treatment of several diseases (1). Some of exercise-induced benefits have been attributed to improved insulin sensitivity in skeletal muscle. In addition to directly promote glucose transport, exercise enhances insulin-stimulated glucose uptake in muscle and improves whole-body insulin sensitivity, even in insulin-resistance states such as obesity and type 2 diabetes, in which insulin-mediated glucose disposal is markedly impaired (1, 2). Due to this insulin-sensitizing action, exercise interventions can also delay or prevent the onset of type 2 diabetes (3). Therefore, defining the mechanisms underlying intensified insulin action in skeletal muscle after exercise is important to develop better therapeutic strategies for the regulation of glucose homeostasis.

One exercise session can elevate subsequent insulin-stimulated glucose uptake in skeletal muscle (4, 5). This increase in post-exercise muscle insulin sensitivity: i) has been observed up to 48 h after a single bout of exercise in both human and rodent skeletal muscle (5, 6); ii) is limited to the prior working muscles (7); iii) involves both microvascular and metabolic actions (8); iv) can be explained by an increase in glucose transporter 4 (GLUT4) translocation to the muscle plasma membrane (9), and v) apparently requires enhanced distal insulin signaling (10, 11). Despite these findings, the mechanisms by which acute exercise improves muscle insulin sensitivity have not been fully elucidated.

The Renin-Angiotensin System (RAS) is a hormone system that regulates several biological functions, including blood pressure and fluid balance (12). RAS is constituted of the classical and nonclassical axes. The classical axis is formed by Angiotensin-Converting Enzyme (ACE), Angiotensin (Ang) II, and Ang II Type 1 Receptor (AT1R). The nonclassical axis, in contrast, comprises of ACE2, Angiotensin-(1-7) (Ang-(1-7)), and Mas Receptor (MasR). The activation of these pathways usually produces opposite biological effects (12).
In addition to modulating cardiovascular function, RAS has recently emerged as a key regulator of metabolic homeostasis (13). Several studies have documented that chronic activation of the classical RAS axis promotes insulin resistance. Transgenic rodents overexpressing Ang II developed hypertension, glucose intolerance, and insulin resistance (14). In addition, Ang II treatment decreased whole-body and skeletal muscle insulin-stimulated glucose uptake in rats (15). In support of these findings, the pharmacological blockade of RAS with ACE inhibitors or AT1R blockers improved insulin sensitivity in both animals and humans (14, 16) and, possibly due to this action, it reduced the incidence of type 2 diabetes (16). Contrariwise, it has been shown that stimulation of the nonclassical RAS axis improves insulin sensitivity. The increase in circulating Ang-(1-7) levels improved lipid and carbohydrate metabolism in rats (17). Further, Ang-(1-7) was able to reverse Ang II- or fructose-induced insulin resistance through restoring the decreased insulin signaling in skeletal muscle, adipose tissue, and liver from rats (18, 19). In contrast, ACE2 deletion exacerbated high-calorie diet-induced insulin resistance (20), and MasR deficiency produced a metabolic syndrome-like state in mice (21).

Interestingly, recent studies show that physical activity downregulates the ACE/Ang II/AT1R axis and, to the contrary, it upregulates the ACE2/Ang-(1-7)/MasR axis in several tissues from rodents and humans (22-26). The physiological implications of exercise training of the shift of RAS toward the nonclassical axis are beginning to be elucidated. Treadmill training attenuated hypertension through decreasing the content of Ang II and enhancing that of Ang-(1-7) in both aorta and rostral ventrolateral medulla from hypertensive rats (24, 25). Moreover, high intensity exercise prevented diet-induced obesity in part by reducing AT1R expression and augmenting MasR abundance in rat skeletal muscle (26). We have proposed a novel hypothesis to explain how acute exercise through stimulating the nonclassical RAS axis increases insulin sensitivity in skeletal muscle (27). Therefore, the objective of this study was to research the possible participation of Ang-(1-7) in enhanced muscle insulin sensitivity after a bout of exercise.
Materials and Methods

Animals

Male Wistar rats (weighing 200–250 g) were used for this study and were provided by our animal facility. The rats had free access to water and food, and were maintained under controlled conditions of light/dark cycles (12/12 h), temperature (22 ± 1°C), and humidity (50 ± 10%). All experimental procedures were conducted in accordance with Mexican Federal Regulations (NOM-062-ZOO-1999, Ministry of Agriculture), and were approved by our Institutional Ethics Committee.

Exercise protocol

Exercise consisted of subjecting the rats to swimming in a pool (dimensions: 75 cm in diameter x 45 cm in height; temp.: 34 ± 1°C). In order to familiarize animals with exercise, all rats swam 10 min/day for 2 consecutive days (Fig. 1A). On day 3, under non-fasting conditions, sedentary rats remained in their cages, while exercised rats were forced to swim for 2.5 h. The exercise protocol consisted of three 30-min sessions each with 5-min rest intervals followed by a fourth session of 60 min. After the first exercise session, extra weight corresponding to 2% of the body weight of each rat was placed at the base of the tail; the load was removed at the end of the exercise protocol (28). All rats had free access to water and food restriction during and 4 h after exercise.

Insulin tolerance tests

Insulin tolerance tests were performed 2 h post-exercise. Briefly, rats received an intraperitoneal (i.p.) injection of insulin (0.25 U/kg, HUMULIN® R; Eli Lilly Co., Indianapolis, IN, USA) and blood samples were taken from the tails of the rats 0, 30, 60, 90, and 120 min after insulin administration. Blood glucose was measured in samples using a glucometer (ACCU-CHEK®; M. Roche Diagnostics, Mannheim, Germany). Some rats were pretreated with deionized water (1 ml/kg, i.p.) or A779 (100 μg/kg, i.p., a selective MasR (Ang-(1-7) receptor) antagonist; Bachem Americas, Inc., Torrance, CA, USA), 15 min prior to insulin delivery. A779 has a high affinity constant for MasR (IC50 for Ang-(1-7)
binding = 0.3 nM) (29). In contrast, the binding affinity of A779 to the AT\(_1\) and AT\(_2\) receptors is negligible (30).

2-Deoxyglucose uptake assays

Two h after exercise, the rats were anesthetized with sodium pentobarbital (60 mg/kg, i.p.), and soleus muscles were isolated and incubated in Krebs–Henseleit buffer for 15 min in an atmosphere of 95% O\(_2\) and 5% CO\(_2\) at 37°C. Saline (10 \(\mu\)l/ml), insulin (0.06 \(\mu\)M), A779 (1 \(\mu\)M), or their combination were incorporated into the medium and the muscles were hatched for another 15 min. After this, 2-deoxyglucose (8 mM; Sigma-Aldrich Co., St. Louis, MO, USA) was added and the tissues were incubated for additional 30 min (31). At the end of this period, the muscles were rinsed with saline solution, frozen in liquid nitrogen, and stored at -80°C for later analysis. The concentration of 2-deoxyglucose-6-phosphate (2-DG6P, an indicator of glucose uptake) in muscles was determined by an enzymatic fluorometric assay as described (32).

Quantification of angiotensins

Independent groups of sedentary and post-exercised rats (0, 1, 2, and 4 h) were sacrificed. Plasma and soleus muscles were collected, frozen in liquid nitrogen, and stored at -80°C for later analysis. Ang II and Ang-(1-7) levels were quantified in obtained samples by HPLC as described (33, 34). Briefly, the samples were homogenized (1:1.5) in a cold mixture of methanol and 1 N HCl (40:1), and centrifuged at 13 000 rpm for 10 min at 4-8°C. The supernatants were filtered with Millex-GV syringe filter units (Millipore, Billerica, MA, USA), and diluted (1:10) with 0.1 N NaOH. The solution was filtered consecutively with a Sephadex G-25 cartridge (GE Healthcare Life Sciences, Issaquah, WA, USA), a Sep-Pak Classic C-18 solid phase extraction cartridge (Waters Corporation, Milford, MA, USA), and finally with a nitrocellulose membrane (0.22 \(\mu\)m) (Corning Incorporated, Corning, NY, USA). For the separation of Ang II, 20 \(\mu\)l of sample were injected into an Acquity HPLC System coupled to a UV-visible diode array detector (Waters Corporation, Milford, MA, USA), equipped with
an Acquity HPLC Peptide Separation Technology BEH300 C18 column (Waters Corporation, Milford, MA, USA). The samples were passed through a gradient from 0 to 50% of phase B at a flow of 0.2 ml/min for 30 min (Phase A: 0.02 % trifluoroacetic acid in water, phase B: 0.018 % trifluoroacetic acid in acetonitrile, previously degassed at 100 Hz for 15 min (Elma, Lab-Line, Urbana, IL, USA), with detection at 225 nm at 10°C. For the separation of Ang-(1-7), 40 µl of sample were mixed with 40 µl of water and 20 µl of fluorescamine (2 mg/ml of acetonitrile). The mixture was allowed to react 3 min at room temperature and 20 µl were injected into the HPLC system. The separation was performed by Acquity HPCL System coupled to a fluorescence detector (Waters Corporation, Milford, MA, USA), equipped with an Acquity HPLC Peptide Separation Technology BEH300 C18 column. For the mobile phase, a mixture (77:23) of dibasic sodium phosphate buffer (50 mM, pH 8) and acetonitrile was used. The mixture was degassed at 100 Hz for 15 min, and filtered with a nitrocellulose membrane (0.22 µm). The separation was carried out at a flow of 1 ml/min with detection at 390/470 nm of excitation/emission, respectively.

Western blot

Soleus muscles from sedentary and 2 h post-exercised rats were homogenized in phosphate buffer (pH 7.4) with protease inhibitors. Proteins were quantified by Bradford method (35). Thirty µg of proteins were separated using the standard SDS-PAGE procedure, and transferred onto PVDF membranes. These were blocked in PBS with 5% fat-free milk for 2 h at room temperature and under constant agitation. Then the membranes were incubated with diverse primary antibodies overnight at 4°C: mouse anti-AT1R (36), mouse anti-ACE (37), rabbit anti-ACE2 (38), and rabbit anti-MasR (39). Anti-β-actin was used as load control (40). The following day, the membranes were rinsed with PBS-Tween (0.1%) and immediately incubated with their corresponding secondary antibodies during 1 h at room temperature. Finally, the membranes were incubated with chemiluminescence reagent (Immobilon Western Millipore, MA, USA) and exposed on photographic films (Kodak, USA). The blots obtained were quantified by densitometry using ImageJ software (41).
Results are expressed as the fold induction of the protein/actin relationship in units of relative density.

Statistical analysis

SigmaPlot 12.0 software (42) was used to determine the sample size for each experiment and the corresponding statistical tests. Results are presented as the mean ± Standard Error of the Mean (SEM). Two-way Analysis of Variance (ANOVA) was employed to analyze the differences between groups, followed by the Student–Newman–Keuls (SNK) post hoc test. The unpaired Student t test was utilized to compare Western blot results. The Area Under the Curve (AUC) was calculated by the trapezoidal rule. Statistical significance was set at p<0.05.

Results

To assess whole-body insulin sensitivity after physical activity, the rats were subjected to insulin tolerance tests 2 h post-exercise. There was no significant baseline difference between blood glucose levels from sedentary and post-exercised rats (114.51 ± 1.93 vs 114.56 ± 1.43 mg/dl, respectively). As depicted in Fig. 1B, insulin administration transiently decreased circulating glucose levels in both sedentary and previously exercised rats; however, this effect was markedly increased post-exercise (PAUC=0.004, Figs. 1B and 1C). To explore the possible involvement of Ang-(1-7) in enhanced whole-body insulin sensitivity after exercise, the rats were treated with A779 15 min before performing insulin tolerance tests. In the sedentary group, A779 treatment did not modify the insulin-induced decrease in blood glucose levels compared to vehicle (deionized water) (Figs. 1B and 1C). Nevertheless, A779 prevented the insulin-sensitizing effect in the post-exercised group (PAUC=0.02, Figs. 1B and 1C).

Because skeletal muscle is the most important reservoir of insulin-stimulated glucose uptake in vivo (43), we investigated the local contribution of Ang-(1-7) in enhanced muscle insulin sensitivity after exercise. For this purpose, 2-deoxyglucose uptake was evaluated in isolated skeletal muscle. As
shown in Fig. 1D, insulin incubation increased 2-deoxyglucose uptake in soleus muscle from sedentary and post-exercised rats compared to vehicle (saline). However, this effect was significantly greater in muscles from previously exercised rats ($P=0.02$, Figs. 1D and 1E). A779 incubation did not modify saline- or insulin-stimulated 2-deoxyglucose uptake in soleus muscle from sedentary rats (Figs. 1D and 1E). Nonetheless, A779 abolished the insulin-induced increase in muscle glucose disposal of post-exercised rats ($P=0.003$, Figs. 1D and 1E).

To further explore the participation of Ang-(1-7) in the improvement of skeletal muscle insulin sensitivity following a bout of exercise, we measured systemic and local levels of Ang-(1-7). In plasma, Ang II and Ang-(1-7) concentrations were similar before exercise (Fig. 2A). However, after physical activity the levels of Ang II were higher than those of Ang-(1-7) ($P<0.05$, Fig. 2A). In comparison to baseline values, circulating levels of Ang II increased 1 and 2 h after exercise ($P=0.01$), whereas those of Ang-(1-7) decreased 1 h post-exercise ($P=0.04$, Fig. 2A). In soleus muscle, baseline Ang II content was greater than that of Ang-(1-7) ($P=0.02$, Fig. 2B). Nevertheless, Ang II and Ang-(1-7) levels were similar 2 and 4 h after exercise (Fig. 2B). Compared to baseline values, the content of Ang II decreased ($P=0.025$) while that of Ang-(1-7) increased ($P=0.04$) 4 h post-exercise (Fig. 2B).

Because skeletal muscle expresses a local RAS (44), we explored whether the exercise-induced increase in muscle Ang-(1-7) levels was related to expression changes of RAS components. Exercise significantly decreased the protein abundance of ACE in soleus muscle ($P=0.03$, Fig. 2C), but it did not change the expression of AT$_1$R, ACE2, and MasR (Figs. 2D-F).

**Discussion**

The main findings of this study show that after an exercise session in rats: i) the selective MasR antagonist A779 prevented the increase in whole-body and skeletal muscle insulin-stimulated glucose uptake; ii) ACE expression decreased in soleus muscle, and iii) muscle Ang-(1-7) content
increased. Taken together, these results suggest that Ang-(1-7) acting through MasR participates in enhanced skeletal muscle insulin sensitivity after a bout of exercise.

Prior exercise improves muscle insulin sensitivity through microvascular and metabolic actions (8, 45), but the mechanisms are unclear. Here, we demonstrated that Ang-(1-7) is necessary to enhance insulin-stimulated glucose uptake in muscle after acute exercise. Considering that skeletal muscle is the most important tissue for glucose disposal in vivo (43), and that vascular flow is absent in 2-deoxyglucose uptake assays, our findings suggest that exercise increases skeletal muscle insulin sensitivity mainly by the metabolic action of Ang-(1-7). However, we cannot rule out the possible involvement of Ang-(1-7) in the recruitment of muscle microvasculature after exercise.

Although in this study plasma Ang-(1-7) levels decreased after exercise, it was demonstrated that Ang-(1-7) infusion in rats improved insulin sensitivity by increasing insulin transport to and glucose disposal in skeletal muscle (46, 47), supporting the notion that Ang-(1-7) could modulate insulin-stimulated muscle microvascular perfusion after a bout of exercise (Fig. 3). Interestingly, we also found that circulating levels of Ang II increased post-exercise. In this context, it was reported that the pharmacological activation of Ang II Type 2 Receptor (AT₂R) increased microvascular perfusion, and insulin delivery and action in skeletal muscle (48). In contrast, AT₂R blockade abolished the insulin-induced increment in muscle microvascular perfusion and decreased insulin-stimulated glucose disposal (49). Thus, it is possible that in the present study Ang II via AT₂R could also enhance insulin-mediated microvascular perfusion in muscle after exercise (Fig. 3). In addition, it has been documented that Mas and AT₂ receptors form heterodimers and both functionally depend on each other (50). Further studies are necessary to clarify the possible involvement of Ang II and AT₂R in insulin-induced muscle microvascular perfusion after a bout of exercise.

The improvement in skeletal muscle insulin sensitivity after contraction and presumably exercise appears to depend on a yet unknown humoral factor present in serum (28, 51). Our results might suggest that Ang-(1-7) is responsible for this process; however, it is important to consider the
following: i) the experimental design in this study is not adequate to test this hypothesis; ii) Ang-(1-7) is a peptide (0.9 kDa vs. a >10-kDa unknown protein) (28), and iii) plasma levels of Ang-(1-7) did not increase during exercise. Therefore, we postulate that Ang-(1-7) is one of the effectors involved in increasing post-exercise skeletal muscle insulin sensitivity, but not the unidentified protein(s) that initiate(s) this event.

It has been suggested that the Kallikrein-Kinin System (KKS) may contribute to improving skeletal muscle insulin sensitivity after exercise (51-53). Pharmacological inhibition or the absence in plasma of the kallikrein enzyme, a protease that performs the synthesis of bradykinin, prevented the increase of insulin-stimulated glucose uptake after muscle contraction ex vivo (51). However, bradykinin does not enhance post-exercise muscle insulin sensitivity (51-53). Kallikrein enzyme also synthetizes renin (from prorenin) (54), the limiting-rate enzyme of the RAS. Nevertheless, no study has investigated the possible involvement of renin in the improved insulin action after exercise. Interestingly, renin has not been detected in muscle cells (44). In the present study, plasma Ang II levels raised after exercise, suggesting that circulating renin or its activity is augmented during physical activity as reported (55, 56). In addition, Ang-(1-7) synthesis was favored in soleus muscle, due in part to the reduced expression of ACE, the main enzyme for Ang-(1-7) degradation, and to alternate pathways for Ang-(1-7) generation (12). Considering the previous information, we hypothesize that the renin produced during exercise travels to skeletal muscle and favors the local synthesis of Ang-(1-7), which in turn increases insulin-stimulated glucose uptake (Fig. 3).

In this study, the molecular mechanisms by which exercise via Ang-(1-7) increased insulin-mediated glucose uptake in skeletal muscle were not investigated. However, several reports have demonstrated that Ang-(1-7) through MasR improves muscle insulin sensitivity by potentiating proximal (IRS-1, PI3K, Akt) and distal (AS160) insulin signaling (18, 19). Although the majority of evidence suggests that improvements in muscle insulin sensitivity following exercise are mediated by mechanisms downstream to the IRS-1/PI3K/Akt pathway (2, 57), some studies have reported
enhanced insulin-stimulated phosphorylation of Akt (10, 57, 58). Therefore, it is feasible that exercise improves skeletal muscle insulin sensitivity through amplifying proximal or distal insulin signaling induced by Ang-(1-7).

In conclusion, Ang-(1-7) acting through MasR participates in enhanced insulin sensitivity of skeletal muscle after a bout of exercise. This novel finding is important because it reveals a potential role of Ang-(1-7) in the comprehensive understanding of glucose metabolism after exercise.

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Legends for Figures

Figure 1.

Ang-(1-7) participates in enhanced skeletal muscle insulin sensitivity after a bout of exercise. (A) Experimental design. All rats were trained to swim 10 min/day for 2 consecutive days. On day 3, under non-fasting conditions, sedentary rats remained in their cages, while exercised rats were forced to swim for 2.5 h. After the first 30 min of exercise, extra weight (corresponding to 2% of the body weight) was placed at the base of the tail of each rat; the load was removed at the end of the exercise protocol. All rats had free access to water and food restriction during and 4 h after exercise. Insulin tolerance tests, 2-deoxyglucose uptake assays, and Western blot (WB) experiments were performed 2 h post-exercise. Angiotensins were quantified before and after exercise (0, 1, 2, and 4 h). In both insulin tolerance tests and 2-deoxyglucose uptake assays, A779 (a selective MasR (Ang-(1-7) receptor) antagonist) was administered or hatched 15 min prior to insulin delivery or 2-deoxyglucose incubation, respectively. (B) Effect of insulin administration (0.25 U/kg, i.p.) on blood glucose levels (decimals, △) from sedentary and 2 h post-exercised rats. Some rats were pretreated with deionized water (1 ml/kg, i.p.) or A779 (100 µg/kg, i.p.) 15 min prior to insulin delivery. (C) Areas under the curve (AUC) of the insulin tolerance tests performed in sedentary and previously exercised rats. (D) 2-Deoxyglucose uptake (measured as 2-Deoxyglucose-6-Phosphate (2-DG6P)) stimulated by saline (10 µl/ml) or insulin (0.06 µM) in isolated soleus muscles from sedentary and 2 h post-exercised rats. Some muscles were incubated with deionized water (10 µl/ml) or A779 (1 µM) 15 min before adding 2-deoxyglucose (8 mM). The tissues were hatched for 30 min. (E) Increments (△) of 2-deoxyglucose uptake stimulated by insulin or insulin + A779 in soleus muscles from sedentary and previously exercised rats. Results are expressed as mean ± SEM of 6-11 rats/assays per group. Two-way ANOVA followed by the SNK post hoc test was utilized to analyze the data. *P<0.05 vs. sedentary; †P<0.05 vs. post-exercised + A779; ‡P<0.05 vs. respective saline; §P<0.05
vs. respective saline + A779; \%P<0.05 vs. insulin + A779, post-exercised; /P<0.05 vs. insulin, sedentary.

Figure 2.

Exercise downregulates the ACE/Ang II/AT\textsubscript{1}R axis of RAS in rat skeletal muscle. Ang II and Ang-(1-7) levels in plasma (A) and soleus muscle (B) from sedentary (Sed) and post-exercised rats (0, 1, 2, and 4 h PEx). Representative immunoblots and densitometry analysis of protein expression of ACE (C),
AT1R (D), ACE2 (E), and MasR (F) in soleus muscle from sedentary and 2 h post-exercised rats. β-actin was used as load control. Western blot data are expressed as the fold induction of the protein/actin relationship in units of relative density. All results represent the mean ± SEM of 4-6 rats per group. Two-way ANOVA followed by the SNK post hoc test was employed to analyze angiotensin data. The unpaired Student t test was used to compare Western blot results. *P<0.05 vs. corresponding group of Ang-(1-7); #P<0.05 vs. Ang II, Sed; &P<0.05 vs. Ang-(1-7), Sed; $P<0.05 vs. sedentary.

Proposed mechanism for the participation of Ang-(1-7) in enhanced skeletal muscle insulin sensitivity after a bout of exercise. Sympathetic Nervous System (SNS) and Renin-Angiotensin System (RAS) are activated during exercise. Sympathetic discharge in both kidney and adipose tissue stimulates the release of renin into the systemic circulation. As result, the plasma levels of Ang II increase while those of Ang-(1-7) decrease. After exercise, circulating concentrations of Ang II and Ang-(1-7) (through AT1R and MasR, respectively) could facilitate transendothelial insulin transport to...
the skeletal muscle interstitium by increasing insulin signal transduction. On the other hand, the renin produced during exercise or in vitro contractions travels to the skeletal muscle and stimulates the local synthesis of angiotensin peptides. Because exercise decreases muscle ACE expression, the main enzyme for Ang II generation and Ang-(1-7) degradation, the synthesis of Ang II is reduced while that of Ang-(1-7) is raised by alternate pathways in muscle. It is feasible that the local elevation of Ang-(1-7) through MasR amplifies proximal (IRS-1, PI3K, Akt) or distal (AS160) insulin signaling. As a result, Ang-(1-7) enhances insulin-stimulated glucose uptake in skeletal muscle. Thus, Ang-(1-7) through microvascular and metabolic actions could improve skeletal muscle insulin sensitivity after a bout of exercise. Kallikrein-Kinin System (KKS) also is upregulated during exercise; however, evidence suggests that bradykinin (BK), a vasoactive protein of KKS, does not participate in this process.

ACE (Angiotensin-Converting Enzyme), Akt (Protein Kinase B), Ang II (Angiotensin II), Ang-(1-5) (Angiotensin-(1-5)), Ang-(1-7) (Angiotensin-(1-7)), Ang-(1-9) (Angiotensin-(1-9)), Aog (Angiotensinogen), AS160 (TBC1 domain family member 4), AT₂R (Ang II Type 2 Receptor), GLUT4 (Glucose Transporter 4), IR (Insulin Receptor), IRS (Insulin Receptor Substrate), MasR (Mas Receptor), NEP (Neutral Endopeptidase), PI3K (Phosphatidylinositol 3-kinase), (P)RR ((Pro)Renin Receptor). Dotted lines indicate the proposed mechanisms.
Figure 1.

A. Angiotensin measurement

- Training
- Exercise protocol:
  - Three 50-min swimming sessions, each with 5-min rest intervals, followed by a fourth 60-min session.

B. Blood glucose (mg/dl)

C. Insulin sensitivity tests

D. Insulin

E. G6PD

Figure 1.
Figure 2.
Figure 3