ISSN 2472-1972

Angiotensin-(1-7) Participates in Enhanced Skeletal Muscle Insulin Sensitivity After a Bout of Exercise

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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, 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 hours. 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 (AT_1R), 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, whereas it increased in muscle. In addition, exercise reduced ACE expression, but it did not change the protein abundance of AT_1R, 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.

Key Words: angiotensin-(1-7), exercise, glucose uptake, insulin sensitivity, renin-angiotensin system, skeletal muscle

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]. Because of 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.

Abbreviations: ACE, angiotensin-converting enzyme; Ang, angiotensin; AT1R, angiotensin II type 1 receptor; AT2R, angiotensin II type 2 receptor; MasR, Mas receptor; RAS, renin-angiotensin system

Received 27 November 2019
Accepted 27 January 2020
First Published Online 31 January 2020
Corrected and Typeset 24 February 2020

One exercise session can elevate subsequent insulin-stimulated glucose uptake in skeletal muscle [4, 5]. This increase in postexercise muscle insulin sensitivity: (1) has been observed up to 48 hours after a single bout of exercise in both human and rodent skeletal muscle [5, 6]; (2) is limited to the prior working muscles [7]; (3) involves both microvascular and metabolic actions [8]; (4) can be explained by an increase in glucose transporter 4 translocation to the muscle plasma membrane [9]; and (5) 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 (AT_1R). 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 AT_1R blockers improved insulin sensitivity in both animals and humans [14, 16] and, possibly because of 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/AT_1R 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 AT_1R 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.

1. Materials and Methods

A. Animals

Male Wistar rats (weighing 200 to 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 hours), 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.

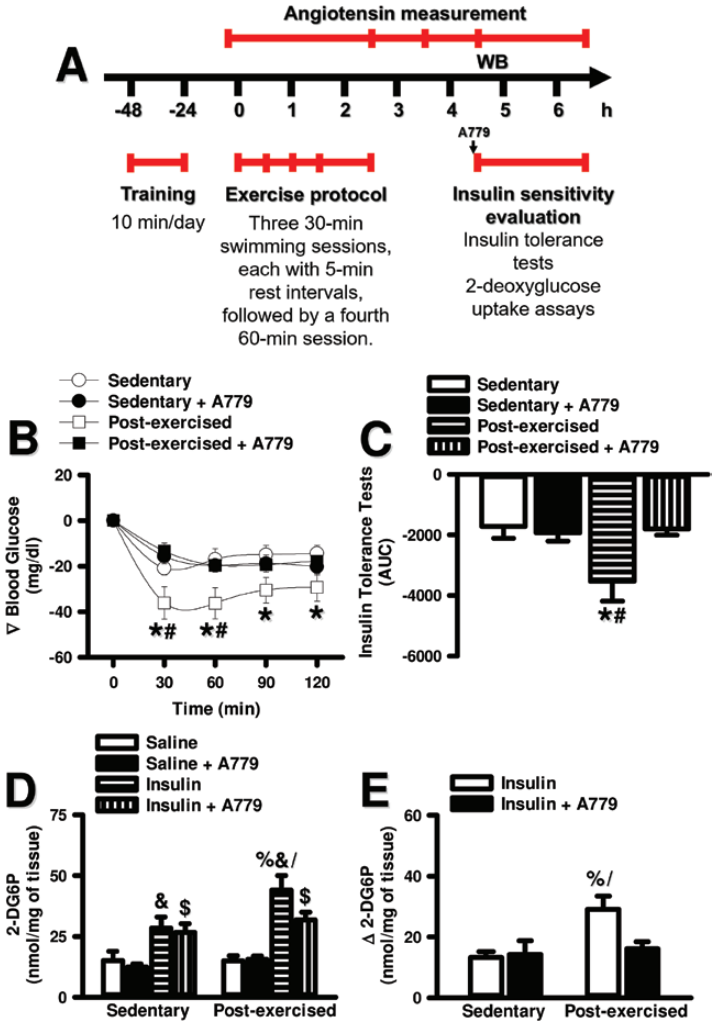

Figure 1. Ang-(1-7) participates in enhanced skeletal muscle insulin sensitivity after a bout of exercise. (A) Experimental design. All rats were trained to swim 10 minutes/day for 2 consecutive days. On day 3, under nonfasting conditions, sedentary rats remained in their cages, whereas exercised rats were forced to swim for 2.5 hours. After the first 30 minutes 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 hours after exercise. Insulin tolerance tests, 2-deoxyglucose uptake assays, and Western blot (WB) experiments were performed 2 hours postexercise. Angiotensins were quantified before and after exercise (0, 1, 2, and 4 hours). In both insulin tolerance tests and 2-deoxyglucose uptake assays, A779 (a selective Mas receptor [MasR, angiotensin 1-7 [Ang-(1-7)] receptor] antagonist) was administered or hatched 15 minutes before insulin delivery or 2-deoxyglucose incubation, respectively. (B) Effect of insulin administration (0.25 U/kg, IP) on blood glucose levels (decrements, ∇) from sedentary and 2-hours postexercised rats. Some rats were pretreated with deionized water (1 mL/kg, IP) or A779 (100 µg/kg, IP) 15 minutes before 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 hours postexercised rats. Some muscles were incubated with deionized water (10 µl/mL) or A779 (1 µM) 15 minutes before adding 2-deoxyglucose (8 mM). The tissues were hatched for 30 minutes. (E) Increments (Δ) of 2-deoxyglucose uptake stimulated by insulin or insulin + A779 in soleus muscles from sedentary and previously exercised rats. Results are expressed as the mean ± SEM of 6 to 11 rats/assays per group. Two-way ANOVA followed by the SNK *post hoc* test was used to analyze the data. $^{*}P < 0.05$ vs. sedentary; $^{\#}P < 0.05$ vs. postexercised + A779; $^{\&}P < 0.05$ vs. respective saline; $^{\$}P < 0.05$ vs. respective saline + A779; $^{\%}P < 0.05$ vs. insulin + A779, postexercised; $^{/}P < 0.05$ vs. insulin, sedentary.

B. Exercise Protocol

Exercise consisted of subjecting the rats to swimming in a pool (dimensions: 75 cm in diameter × 45 cm in height; temperature: 34 ± 1°C). To familiarize animals with exercise, all rats swam 10 minutes/day for 2 consecutive days (Fig. 1A). On day 3, under nonfasting conditions, sedentary rats remained in their cages, whereas exercised rats were forced to swim for 2.5 hours. The exercise protocol consisted of 3 30-minute sessions each with 5-minute rest intervals followed by a fourth session of 60 minutes. 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 hours after exercise.

C. Insulin Tolerance Tests

Insulin tolerance tests were performed 2 hours postexercise. Briefly, rats received an intraperitoneal (IP) injection of insulin (0.25 U/kg, HUMULIN R; Eli Lilly Co., Indianapolis, IN) and blood samples were taken from the tails of the rats 0, 30, 60, 90, and 120 minutes 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, IP) or A779 (100 µg/kg, IP, a selective MasR (Ang-(1-7) receptor) antagonist; Bachem Americas, Inc., Torrance, CA), 15 minutes before insulin delivery. A779 has a high affinity constant for MasR (IC_{50} 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].

D. 2-Deoxyglucose Uptake Assays

Two hours after exercise, the rats were anesthetized with sodium pentobarbital (60 mg/kg, IP), and soleus muscles were isolated and incubated in Krebs–Henseleit buffer for 15 minutes in an atmosphere of 95% O_2 and 5% CO_2 at 37°C. Saline (10 µL/mL), insulin (0.06 µM), A779 (1 µM), or their combination were incorporated into the medium and the muscles were hatched for another 15 minutes. After this, 2-deoxyglucose (8 mM; Sigma-Aldrich Co., St. Louis, MO) was added and the tissues were incubated for additional 30 minutes [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 (an indicator of glucose uptake) in muscles was determined by an enzymatic fluorometric assay as described [32].

E. Quantification of Angiotensins

Independent groups of sedentary and postexercised rats (0, 1, 2, and 4 hours) 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 HCI (40:1), and centrifuged at 13 000 rpm for 10 minutes at 4 to 8°C. The supernatants were filtered with Millex-GV syringe filter units (Millipore, Billerica, MA), 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), a Sep-Pak Classic C-18 solid phase extraction cartridge (Waters Corporation, Milford, MA), and finally with a nitrocellulose membrane (0.22 µm) (Corning Incorporated, Corning, NY). For the separation of Ang II, 20 µL of sample were injected into an Acquity HPLC System coupled to a UV-visible diode array detector (Waters Corporation), equipped with an Acquity HPLC Peptide Separation Technology BEH300 C18 column (Waters Corporation). The samples were passed through a gradient from 0 to 50% of phase B at a flow of 0.2 mL/min for 30 minutes (phase A: 0.02% trifluoroacetic acid in water, phase B: 0.018% trifluoroacetic acid

in acetonitrile, previously degassed at 100 Hz for 15 minutes (Elma, Lab-Line, Urbana, IL), 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 minutes 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), 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 minutes 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.

F. Western Blot

Soleus muscles from sedentary and 2-hours postexercised rats were homogenized in phosphate buffer (pH 7.4) with protease inhibitors. Proteins were quantified by Bradford method [35]. Thirty micrograms of proteins were separated using the standard SDS-PAGE procedure, and transferred onto Polyvinylidene difluoride membranes. These were blocked in PBS with 5% fat-free milk for 2 hours at room temperature and under constant agitation. Then the membranes were incubated with diverse primary antibodies overnight at 4°C: mouse anti-AT_1R [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 hour at room temperature. Finally, the membranes were incubated with chemiluminescence reagent (Immobilon Western, Millipore, MA) and exposed on photographic films (Kodak, Rochester, NY). 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.

G. 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 ± SEM. Two-way ANOVA was used to analyze the differences between groups, followed by the Student-Newman-Keuls (SNK) *post hoc* test. The unpaired Student *t* test was used to compare Western blot results. The area under the curve was calculated by the trapezoidal rule. Statistical significance was set at $P < 0.05$.

2. Results

To assess whole-body insulin sensitivity after physical activity, the rats were subjected to insulin tolerance tests 2 hours postexercise. There was no significant baseline difference between blood glucose levels from sedentary and postexercised 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 postexercise ($P_{AUC} = 0.004$, Fig. 1B and C). To explore the possible involvement of Ang-(1-7) in enhanced whole-body insulin sensitivity after exercise, the rats were treated with A779 15 minutes before performing insulin tolerance tests. In the sedentary group, A779 treatment did not modify the insulin-induced decrease in blood glucose levels compared with vehicle (deionized water) (Fig. 1B and C). Nevertheless, A779 prevented the insulin-sensitizing effect in the postexercised group ($P_{AUC} = 0.02$, Fig. 1B and C).

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 postexercised rats compared to vehicle (saline). However, this effect was significantly greater in muscles from previously exercised rats ($P = 0.02$, Fig. 1D and E). A779 incubation did not modify saline- or insulin-stimulated 2-deoxyglucose uptake in soleus muscle from sedentary rats (Fig. 1D and E). Nonetheless, A779 abolished the insulin-induced increase in muscle glucose disposal of postexercised rats ($P = 0.003$, Fig. 1D and E).

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 hours after exercise ($P = 0.01$), whereas those of Ang-(1-7) decreased 1 hour postexercise ($P = 0.04$, 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 hours after exercise (Fig. 2B). Compared with baseline values, the content of Ang II decreased ($P = 0.025$), whereas that of Ang-(1-7) increased ($P = 0.04$) 4 hours postexercise (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_1R, ACE2, and MasR (Fig. 2D–F).

3. Discussion

The main findings of this study show that after an exercise session in rats: (1) the selective MasR antagonist A779 prevented the increase in whole-body and skeletal muscle insulin-stimulated glucose uptake; (2) ACE expression decreased in soleus muscle; and (3) 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 (AT_2R) increased microvascular perfusion, and insulin delivery and action in skeletal muscle [48]. In contrast, AT_2R 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 AT_2R could also enhance insulin-mediated microvascular perfusion in muscle after exercise (Fig. 3). In addition, it has been documented that Mas and AT_2 receptors form heterodimers and both functionally depend on each other [50]. Further studies are necessary to clarify the possible involvement of Ang II and AT_2R in insulin-induced muscle microvascular perfusion after a bout of exercise.

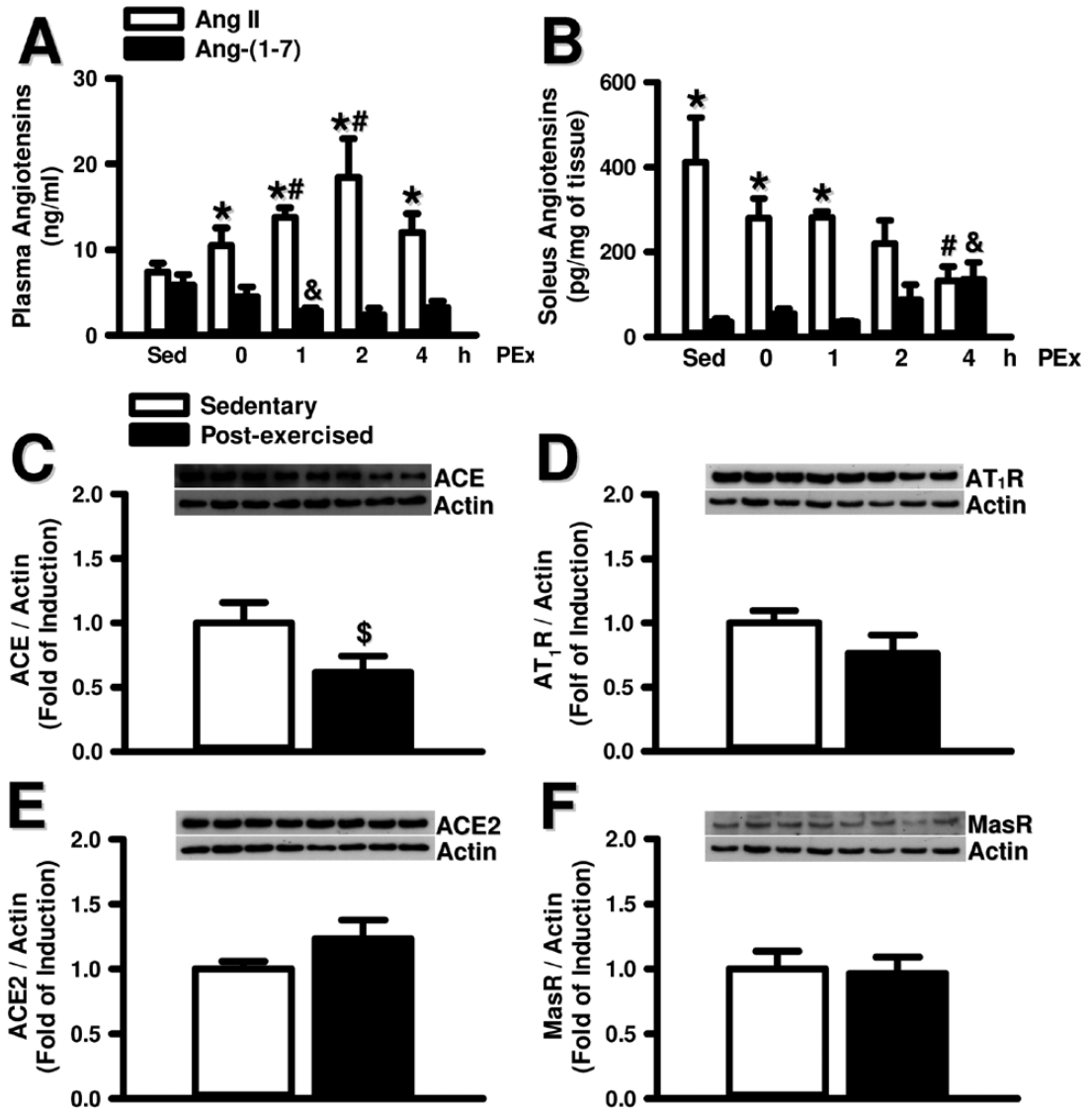

Figure 2 . Exercise downregulates the ACE/Ang II/AT_1R axis of RAS in rat skeletal muscle. Angiotensin II (Ang II) and Ang-(1-7) levels in (A) plasma and (B) soleus muscle from sedentary (Sed) and postexercised rats (0, 1, 2, and 4 hours PEx). Representative immunoblots and densitometry analysis of protein expression of (C) angiotensin-converting enzyme (ACE), (D) Ang II type 1 receptor (AT_1R), (E) ACE2, and (F) Mas Receptor (MasR) in soleus muscle from sedentary and 2-hour postexercised 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 to 6 rats per group. Two-way ANOVA followed by the SNK *post hoc* test was used 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.

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: (1) the experimental design in this study is not adequate to test this hypothesis; (2) Ang-(1-7) is a peptide (0.9-kDa vs. a >10-kDa unknown protein) [28]; and (3) plasma levels of Ang-(1-7) did not increase during exercise. Therefore, we postulate that Ang-(1-7) is 1 of the effectors involved in increasing postexercise skeletal muscle insulin sensitivity, but not the unidentified protein(s) that initiate(s) this event.

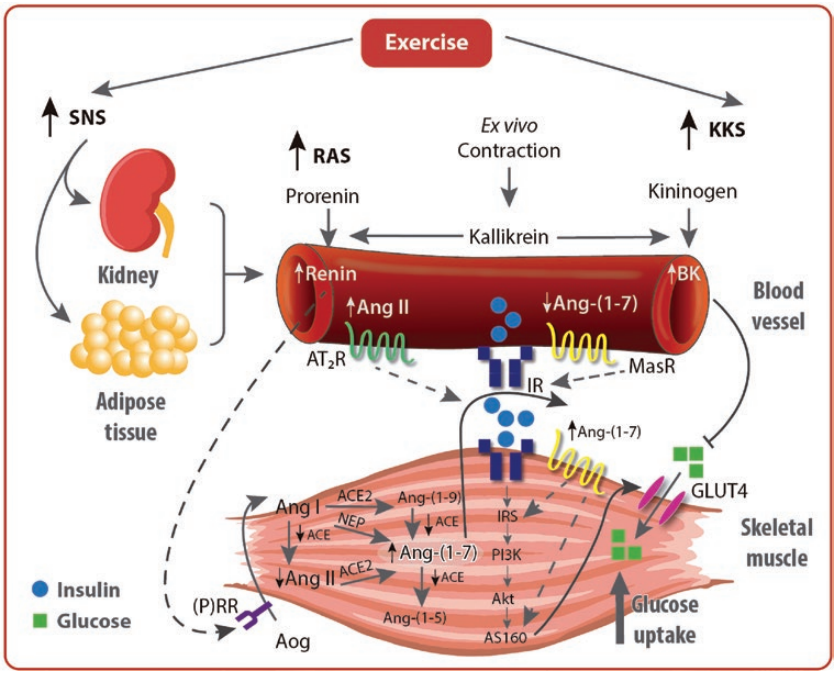

Figure 3. Proposed mechanism for the participation of angiotensin 1-7 (Ang-(1-7)) in enhanced skeletal muscle insulin sensitivity after a bout of exercise. Sympathetic nervous system (SNS) and renin-angiotensin system (RAS) are activated during exercise. Sympathetic discharge in both kidney and adipose tissue stimulates the release of renin into the systemic circulation. As result, the plasma levels of Ang II increase, whereas those of Ang-(1-7) decrease. After exercise, circulating concentrations of Ang II and Ang-(1-7) (through Ang II type 2 receptor [AT_2R] and Mas receptor [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, angiotensin; Aog, angiotensinogen; AS160, TBC1 domain family member 4; GLUT4, glucose transporter 4; IR, insulin receptor; IRS, insulin receptor substrate; NEP, neutral endopeptidase; PI3K, phosphatidylinositol 3-kinase; (P)RR, (pro)renin receptor. Dotted lines indicate the proposed mechanisms.

It has been suggested that the Kallikrein-Kinin System 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 postexercise 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, because 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.

Acknowledgments

We thank Maggie Brunner, MA, for the grammar and style correction of this manuscript.

Financial Support: This work was partially supported by PAPIIT-DGAPA, UNAM, México (grants IN226819 to I.A.G.-O. and IN223519 to R.V.-M.). O.E.-R. received a postdoctoral fellowship from DGAPA, UNAM, México.

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***Disclosure Summary*:** The authors have nothing to disclose.

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