Nitric oxide (NO), which is the smallest chemical messenger molecule, has been shown to play a critical role in a wide variety of physiological functions, including neurotransmission, blood vessel tone, immune modulation, and cytotoxicity [1, 2]. NO is synthesized through the oxidation of L-arginine to L-citrulline by nitric oxide synthase (NOS). Three different isoforms of NOS have been determined: neuronal NOS (nNOS: NOS I) and endothelial NOS (eNOS: NOS III) are constitutively expressed, Ca\(^{2+}\)/calmodulin-dependent isoforms, whereas inducible NOS (iNOS: NOS II) is an inducible, Ca\(^{2+}\)/calmodulin-independent isoform.

Recently, white adipose tissue has been shown to express the iNOS and eNOS isoforms, indicating that this tissue is a potential source of NO production [3–5]. Moreover, Gaudiot et al. [4] reported that S-nitroso-N-acetyl-penicillamine (SNAP) and 1-propamine, 3-(2-hydroxy-2-nitroso-1-propylhydrazine) (PAPA-NONOate), significantly inhibited adipocyte lipolysis in response to isoproterenol in both groups. This inhibitory effect of SNAP, but not that of PAPA-NONOate, was greater in the adipocytes of trained rats than in those of the control rats. Thus it is possible that NO is involved in the regulation of lipolysis and that exercise training enhances the responsiveness of adipocytes to extracellular NO with the reduced production of nitrite/nitrate in adipocytes because of decreased activities of NOS’s. On the other hand, it is also possible that exercise increases either the activity or the protein expression of eNOS in adipose tissue. [Japanese Journal of Physiology, 52, 343–352, 2002]

Key words: exercise training, adipose tissue, adipocyte, lipolysis, nitric oxide.
fect on basal lipolysis, but it inhibited stimulated lipolysis, regardless of which stimulating agent was used, without altering cAMP production [4]. Anderson et al. [5] have shown that the inhibition of NO release by \( \text{N}^\text{G} \)-monomethyl L-arginine (L-NMMA) results in increased lipolysis *in vivo* and that the expression of iNOS and enzymatic NOS activity are also detected in human adipose tissue. Thus locally produced NO has been suggested to play a role in the physiological control of lipolysis [4, 5], and a recent study suggests that blunted lipolysis in obesity is due, at least in part, to increased gene expression and protein levels of eNOS in subcutaneous adipose tissue [6].

Exercise training has been shown to enhance the lipolysis of adipocytes in laboratory animals and humans [7–12]. Moreover, it has been shown to enhance NOS activity and to release NO in some tissues (for a review, see Balon [13]). Therefore NO could be involved in the control of lipolysis in adipose tissue and/or adipocytes from exercise-trained rats. Indeed, in preliminary experiments [14], we have shown that \( \text{N}^\text{G} \)-nitro-L-arginine methyl ester (L-NAME), one of the NOS inhibitors, increased the lipolysis in adipose tissue and that the inhibitory effect of SNAP on adipocyte lipolysis was significantly greater in adipocytes from exercise-trained rats than in those from control rats. However, the data obtained in that study are insufficient to establish the involvement of NO in the control of lipolysis in exercise-trained rats. Therefore in the present study, to clarify the role of NO on the control of lipolysis in exercise-trained adipose tissue and/or adipocytes, we performed some additional experiments concerning the effect of either L-NAME or NO donors on lipolysis in both adipocytes and adipose tissue. Moreover, we presented other important information on possible changes in NOS expression, NOS activity, and NO production in adipocytes and adipose tissue from exercise-trained rats.

**MATERIALS AND METHODS**

**Animal care and exercise training program.** Male Wistar rats (Japan SLC, Hamamatsu, Japan) with an initial body weight of 100–120 g were used for the experiments. The animals were randomly divided into two groups: sedentary (32 rats) and exercise-trained (32 rats). They were housed three to a cage in a room with the temperature controlled at 24°C and a 12:12-h light–dark cycle and given free access to food and water. The training rats were exercised on a rodent treadmill, set at a 5° incline, 5 d a week for 9 weeks according to the protocol of our previous work [7, 8]. The running time and speed were increased progressively until, after 6–7 weeks, the rats ran continuously for 90 min/d at 25–27 m/min. The trained rats were sacrificed about 48 h after the final exercise session. The sedentary rats were not subjected to treadmill running and were sacrificed. The animals in both groups were lightly anesthetized with pentobarbital (50 mg/kg body weight) and killed after overnight fasting. All experiments conducted in this study were approved by the Animal Care Committee of Graduate School of Science, Tokyo Metropolitan University.

**Incubation of adipose tissue segments.** The epididymal fat pads were removed. In the present study, because the several physiological responses (e.g., lipolytic responses, the productions of nitrate/nitrite, the activities of NOS’s, and the expressions of NOS’s) might be different between a thin distal portion of fat pad and a thick proximal portion of an epididymal fat pad, to compare each of the physiological responses in adipose tissue, we used only thin distal portions of the fat pads for all experiments employing adipose tissues. Thin distal portions of the fat pads from each animal were dissected into several segments weighing approximately 50 mg each and placed into plastic vials. The segments were minced in buffer A (Kreb’s-Ringer bicarbonate solution buffered with 10 mM HEPE, pH 7.4, containing 5.5 mM glucose and 2% (w/v) fatty-acid–free bovine serum albumin). They were then preincubated with or without 5 mM L-NAME for 30 min at 37°C in 1 ml of fresh buffer A containing adenosine deaminase (0.5 U/ml). Then, either isoproterenol (100 nM) or the buffer was added. After the next 30 min, the contents of each vial were immediately filtered, and 0.2 ml of an incubation medium was assayed for glycerol as an index of lipolysis. Glycerol was determined by use of a commercially available kit (Nitrate/Nitrite Fluorometric Assay Kit, Cayman Chemical, Ann Arbor, MI, USA).

**Preparation and incubation of isolated adipocytes.** Pools of isolated adipocytes were prepared by a modification of the method of Rodbell [15]. Fat pads were minced with scissors and placed in plastic vials in buffer A, as described above. Collagenase digestion was performed at 37°C in a water bath shaker for 30 min. As recommended by Honnor et al. [16], the medium contained 200 nM adenosine to limit glycerol production during collagenase incubation. After 30 min, the contents of each vial were immediately filtered and centrifuged for 1 min at 100 \(* g\). The
supernatant layer of cells was then washed three times with buffer A. Adipocytes (1–2×10⁶ cells) were incubated at 37°C for 30 min in plastic vials in a total volume of 1 ml of buffer A in the presence or absence of various agents. The incubation medium contained adenosine deaminase (0.5 U/ml). To examine the effects of either NO donors or L-NAME, adipocytes were preincubated with SNAP, PAPA-NONOate, or L-NAME for 30 min. Then 100 mM isoproterenol was added, and a cell-free incubation medium was assayed for glycerol and nitrate/nitrite, as described in the previous section.

**Western blot analysis.** Thin distal portions of the fat pads from each animal were dissected into several segments weighing 500 mg each and homogenized with 10 mM Tris-HCl, pH 7.5, and 0.25 M sucrose at 4°C. The homogenate was centrifuged at 1,000×g for 20 min, and the supernatant was removed. The supernatants were subjected to SDS-PAGE [17]. After electrophoresis, proteins were transferred to nitrocellulose membranes. Blots were blocked with 2.5% gelatine in a Tris-saline buffer containing 0.05% Tween 20, then incubated with the polyclonal eNOS or iNOS antibodies (Transduction Lab., Lexington, KY, USA). After being washed, the membranes were incubated for 1 h with antirabbit immunoglobulin G (1:10,000 dilution)–conjugated horseradish peroxidase in the Tris-saline buffer. The membranes were washed for 30 min, and the immunoreactive bands were detected by the enhanced chemiluminescence method. The membranes were scanned with a LightCapture scanner (ATTO Corporation, Tokyo, Japan), and the optical density of each specific band was analyzed with the CS Analyzer (ATTO Corporation).

**Nitric oxide synthase activity.** Thin distal portions (approximately 500 mg) of the fat pads from each animal and isolated adipocytes (approximately 10¹⁰ cells) were homogenized as described above. The homogenate was centrifuged at 1,000×g for 20 min, and the supernatant was used for experiments. NOS activity was measured by an examination of the conversion of [³H]arginine to [³H]citrulline, as described below. The reaction buffer contained 3 mM HEPES, 4 mM reduced nicotinamide adenine dinucleotide phosphate (NADPH), 20 mM tetrahydrobiopterin, 20 mM flavin adenine dinucleotide (FAD), 20 mM flavin mononucleotide (FMN), 1 mM dithiothreitol (DTT), 4 mM L-arginine, 1.0 mM CaCl₂, 30 mM calmodulin, and 2.0 mM/L-³H]arginine (Amersham, Arlington Heights, IL, USA). To determine iNOS activity, both Ca²⁺ and calmodulin were omitted, and 4 mM EGTA was added to the reaction buffer. The reaction was incubated at 37°C for 1 h, then terminated by the addition of a buffer containing 40 mM HEPES, pH 5.2, 2 mM EDTA, and 2 mM EGTA. The samples were then applied to 10 ml Dowex AG50WX-8 (Na⁺) columns (BioRad, Hercules, CA, USA) and eluted with 40 mM HEPES. The effluent was collected in scintillation vials and quantified by liquid scintillation spectroscopy with a Wallac LKB 1211 Rack Beta counter (Wallac, Helsinki, Finland). Constitutive NOS (cNOS) activity was calculated as total NOS activity minus Ca²⁺/calmodulin-independent NOS activity.

**RNA extraction and RT-PCR.** RNA extraction and RT-PCR analysis of NOS's (eNOS and iNOS) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNAs were performed via RT-PCR analysis. Total RNA was prepared from isolated adipocytes (approximately 10¹⁰ cells), using the RNeasy minikit (Qiagen, Valencia, CA, USA). First-strand cDNA was obtained by the incubation of 2 µg total RNA samples with reverse transcriptase (Superscript II, GIBCO BRL, Gaithersburg, MD, USA) in 18 µl of a reaction mixture. One microliter of the RT product was subjected to PCR using Taq DNA polymerase (Perkin-Elmer, Branchburg, NJ, USA). Twenty and 40 cycles of amplification were carried out for eNOS and iNOS, respectively, by use of the following conditions for each cycle: denaturing at 94°C for 1 min, annealing at 55°C for 2 min, and extension at 72°C for 3 min. The PCR products were electrophoresed in 1% agarose gels containing ethidium bromide. The primers used are described below.

- eNOS: 5’-GGAAAGGTTTCCCATCATTGC-3’
  5’-TCTGCAAGATGTCTTGAACG-3’
- iNOS: 5’-GAACAATTTCATCCGTGGC-3’
  5’-CGTAGAGATGGTCAGTTG-3’

**Expression of data and statistical methods.** The values represent the mean±SE. The significance of differences between means was assessed by the Scheffe test after an analysis of variance had been performed to establish that there were significant differences between the groups.

**RESULTS**

The final mean body weight (g) of the exercise-trained rats (313±8, N=32) was significantly less than that of the controls (385±18, N=32) (p<0.001). The weight of epididymal adipose tissues (mg) was also lower in exercise-trained rats (3,414±240, N=32) than in control rats (4,485±247, N=32) (p<0.01).

Figure 1 shows the effect of 5 mM L-NAME on lipolysis in adipose tissue segments (left panel) and in isolated adipocytes (right panel). In the absence of L-NAME, basal lipolysis in tissue segments was not dif-

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different between control rats and exercise-trained rats, whereas isoproterenol-stimulated lipolysis in tissue segments tended to be greater in exercise-trained rats than in control rats ($p = 0.10$). However, the incubation of adipose tissue segments with L-NAME significantly increased basal and isoproterenol-stimulated lipolysis. Under this condition, lipolysis in tissue segments was significantly greater in exercise-trained rats than in control rats, as reported in our preliminary experiments [14]. In contrast, L-NAME in isolated adipocytes had no effect on basal and isoproterenol-stimulated lipolysis in either rat group. D-NAME (5 mM) had no effect on lipolysis in either adipose tissue or adipocytes from either group (results not shown).

Table 1 shows the production of nitrate/nitrite from tissue segments and isolated adipocytes. In adipose tissue segments, the production of nitrate/nitrite with or without isoproterenol did not differ between control rats and exercise-trained rats. L-NAME significantly, but not completely, inhibited the production of nitrate/nitrite from tissue segments in both groups.
Under a basal condition, the inhibitory effect of L-NAME on nitrite/nitrate production tended to be greater in exercise-trained rats than in control rats ($p<0.14$). In accordance with this, the resultant production tended to be less in exercise-trained rats than in control rats ($p<0.12$). However, no statistical significance was found because of a somewhat large variance under this condition. In adipose tissue with isoproterenol, though isoproterenol did not alter nitrite/nitrate productions, the inhibitory effect of L-NAME on nitrite/nitrate production was significantly greater in exercise-trained rats than in control rats ($p<0.02$), resulting in a significantly smaller amount of the nitrite/nitrate in exercise-trained rats than in control rats ($p<0.02$).

In isolated adipocytes, however, the production of nitrite/nitrate was significantly less in exercise-trained rats than in control rats. Moreover, L-NAME and isoproterenol did not significantly alter the production of nitrite/nitrate from isolated adipocytes in either rat group.

Figure 2 shows $\text{Ca}^{2+}$/calmodulin-dependent and $\text{Ca}^{2+}$/calmodulin-independent NOS activities in tissue (left) and cellular crude extracts (right) from both conditioned rats. iNOS activity was estimated as NOS activity in the absence of $\text{Ca}^{2+}$ and calmodulin. Experimental conditions are described in MATERIALS AND METHODS. Open columns show the data from control rats ($N=8$), and filled columns show those from exercise-trained rats ($N=8$). The values are means±SE for five separate experiments. Two rats from each group were used for a single experiment. $^a p<0.05$ vs. iNOS; $^b p<0.05$ vs. control.

Figure 2 shows $\text{Ca}^{2+}$/calmodulin-dependent and $\text{Ca}^{2+}$/calmodulin-independent NOS activities in tissue extracts (left panel) and cellular crude extracts (right panel) from rats in both groups. iNOS activity, which is thought to be $\text{Ca}^{2+}$/calmodulin-independent NOS activity, was estimated as NOS activity in the absence of $\text{Ca}^{2+}$ and calmodulin. cNOS activity, which is thought to be $\text{Ca}^{2+}$/calmodulin-dependent NOS activity, was calculated as total NOS activity in the presence of $\text{Ca}^{2+}$ and calmodulin minus iNOS activity. Because it has been shown that nNOS is not present in rat [3] and human [6] adipose tissue, the cNOS activity determined in this study could represent eNOS activity. In tissue homogenates, iNOS activities did not differ between control and exercise-trained rats. However, eNOS activity was significantly greater in exercise-trained rats than in control rats. As a result, total NOS activity was significantly greater in exercise-trained rats than in control rats. In cellular homogenates, however, both eNOS and iNOS activities were less in exercise-trained rats than in control rats; this resulted in significantly less total activity of NOS in the exercise-trained rats.

In the extracts of adipocytes from both conditioned rats, eNOS activity was greater than iNOS activity: The mean activity ratio of eNOS/iNOS was 2.55 and 4.79 in control and exercise-trained rats, respectively. On the other hand, in the extracts of adipose tissue the activity ratio of eNOS to iNOS decreased to 0.42 and 1.45 in control and exercise-trained rats, respectively. Thus a different activity ratio of eNOS/iNOS was found between adipose tissue and cells and between groups.

As shown in Fig. 3, eNOS and iNOS were both detected in crude extracts of adipose tissue homogenates. To compare exercise-trained rats with control rats, we ran identical loading amounts of samples on the same gel. The expression of eNOS protein was greater in exercise-trained rats than in control rats. However, the expression of iNOS protein in exercise-trained rats was equivalent to that in control rats. We tried to examine the protein expressions of NOS’s in the homogenates of isolated adipocytes. But we did not succeed, even after increasing the protein in the homogenates of adipocytes to 20 $\mu$g. Because the available samples for experiments were limited in exercise-trained rats, the mRNA expressions of both eNOS and iNOS were examined in cellular extracts. As shown in Fig. 4, the expression of either eNOS or iNOS mRNA was not different between control and exercise-trained rats.
Figure 5 shows the effects of SNAP (0.5 mM) or PAPA-NONOate (0.5 mM) on lipolysis in isolated adipocytes from control and exercise-trained rats. Iso-
proterenol-stimulated lipolysis was significantly greater in adipocytes from exercise-trained rats than in those from control rats. Neither SNAP nor PAPA-NONOate significantly affected basal lipolysis in either group. However, both NO donors significantly inhibited iso-
proterenol-stimulated adipocyte lipolysis. This inhibitory effect by SNAP, which was determined as a percent inhibition of lipolysis because of a NO donor, was significantly greater in adipocytes from exercise-
trained rats than in those from control rats, and the decreases in glycerol releases as a result of SNAP were also greater in adipocytes from exercise-trained rats. PAPA-NONOate also inhibited adipocyte lipolysis. Exercise training, however, did not alter the percent inhibition of lipolysis because of PAPA-NONOate, but the decreases in glycerol releases because of PAPA-
NONOate were greater in adipocytes from exercise-trained rats than in those from control rats.

DISCUSSION

NOS isoforms are expressed in human and rodent adi-
pose tissues, and NO is suggested to regulate
adipocyte lipolysis [3–6]. Studies on human subcuta-
neous adipose tissue have suggested that eNOS pro-
tein originates primarily from adipocytes because of
the similar expression levels of eNOS mRNA in adi-
pose tissue and isolated adipocytes [6]. In rat adipose
tissue, the expression of either eNOS or iNOS protein
has also been detected in the extracts of tissue ho-
mogenates [3]. In this study, both eNOS and iNOS
proteins were detected in the extracts of adipose tissue
homogenates, but neither was detected in the extracts
of isolated adipocyte homogenates. An adequate inter-
pretation for the latter result remains unclear, but it
might be due to the small amounts of proteins (10–20
μg) that were loaded. However, the findings from this
study indicate that both eNOS and iNOS exist in
adipocytes themselves as well as in adipose tissue: (1)
NO productions were found in isolated adipocytes; (2)
Ca2+/calmodulin-dependent and Ca2+/calmodulin-in-
dependent NOS activities were also found in adipocyte
homogenates; and (3) eNOS and iNOS mRNA were
both found in the cellular extracts.

Elizalde et al. [6] showed in human subcutaneous
adipose tissue that eNOS protein was far more abun-
dant than iNOS protein. On the other hand, Ribiere et
al. [3] showed in rat adipose tissue that iNOS appears
to be the predominantly expressed NOS isoform. In
the present study, it was difficult to directly compare
the protein levels of eNOS with those of iNOS because the samples for the determination of eNOS and those of iNOS were run on a different gel. However, a comparison of eNOS activity with iNOS activity revealed that, in adipocytes, eNOS activity was greater than iNOS activity in both the control and exercise-trained rats. Moreover, exercise training significantly reduced the activities of both NOS’s. The latter result agrees with the finding, which shows that less nitrite/nitrate was produced from adipocytes in the exercise-trained rats than in the control rats. No alteration in eNOS mRNA or iNOS mRNA in exercise-trained rats indicates a possibility that exercise training alters the nature of both proteins of NOS’s within adipocytes.

However, the activity ratio of eNOS to iNOS in adipose tissue was different from that in adipocytes. In adipose tissue from control rats, iNOS activity was greater than eNOS activity. This result is in accordance with the finding by Ribiere et al. [3]. However, according to their study, most NOS activities in rat adipose tissue might originate from the adipocytes themselves. If this were so, the present finding that iNOS activity was significantly less than eNOS activ-

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**Fig. 5.** Effects of SNAP (0.5 mM) or PAPA-NONOate (0.5 mM) on 100 nM isoproterenol-stimulated adipocyte lipolysis from control (open columns, N=5) and exercise-trained rats (filled columns, N=5). The values are means±SE for five separate experiments. *a p<0.05 vs. without inhibitors; *b p<0.05 vs. control. w/o, without.
ity in adipocytes conflicts with the findings by Ribiere et al. [3]. We lack an adequate explanation for this discrepancy, but our present findings indicate that the adipose tissue used in this study may contain somewhat abundant iNOS derived from certain cell(s) other than adipocytes. On the other hand, eNOS activity in adipose tissue from exercise-trained rats was greater than iNOS activity, as with adipocytes. This result is in close agreement with the enhanced protein expression of eNOS, but not of iNOS, in adipose tissue from exercise-trained rats. Because the eNOS activity of adipocytes themselves was significantly reduced as a result of exercise training, the training may increase the activity of eNOS originated in certain cell(s) within adipose tissue, e.g., endothelial cells of vessels.

In our preliminary study [14], we found that the expressions of eNOS and iNOS in adipose tissue from exercise-trained rats were both equivalent to those from control rats. However, the present data indicate that in adipose tissue the expression of eNOS protein was greater in exercise-trained rats than in control rats. This discrepancy in the studies could be related to the differences in the portion of adipose tissue used in each study. That is, in our preliminary study, a thick proximal portion of the fat pad was used for the determinations of the protein expressions of NOS's; in this study, however, a thin distal portion of the fat pad was used. It is possible that adipose tissue is not homogeneous and that the population of blood vessels per mg unit of fat pad is different between a thin distal portion of a fat pad and a thick proximal portion of one.

As discussed above, in adipocytes from exercise-trained rats, the decreased activities of NOS's were accompanied by the reduced production of nitrite/nitrate. However, even though the activities of NOS's in the extracts of adipose tissues were enhanced by exercise training, the nitrite/nitrate production in adipose tissue from exercise-trained rats was equivalent to that from control rats. This discrepancy may be related with the differences between the experimental conditions for the measurement of NOS activities and that for the measurement of nitrite/nitrate production. The activities of NOS's determined in the extracts of either tissues or cells would reflect the maximal activities in the presence of superfluous Ca$^{2+}$/calmodulin, whereas a spontaneous production of nitrite/nitrate was determined in either intact tissues or intact cells. Consequently, the spontaneous production of nitrite/nitrate from intact samples might not represent the maximal production, and therefore the increases in the activities of NOS's in tissue extracts from the exercise-trained rats could not be reflected in the spontaneous production of nitrite/nitrate in adipose tissues. However, it seems that in isolated adipocytes from the exercise-trained rats, the reduced maximal activities of NOS's lead to a decrease in the production of nitrite/nitrate.

As shown in Table 1, L-NAME in both rat groups significantly inhibited the production of nitrite/nitrate from adipose tissue, but not from isolated adipocytes. This NOS inhibitor could also alter lipolysis in adipose tissue, but not in adipocytes. Gaudiot et al. [18] also showed a similar failure of L-NAME to affect NOS activity in adipocytes and suggested that it was due to either a weak uptake of L-NAME by adipocytes or a defective L-NAME bioactivation required for its inhibitory activity in adipocytes. On the basis of this suggestion, it is possible again that our tissue preparations were contaminated with eNOS and iNOS originating in certain cell(s) other than adipocytes and that L-NAME inhibits the generation of NO produced by such cell(s). Therefore the nitrite/nitrate production would still be found when adipose tissues from either group were incubated with L-NAME, and L-NAME could not alter lipolysis in isolated adipocytes. In this context, the enhanced inhibitory effect of L-NAME on nitrite/nitrate production in adipose tissue from exercise-trained rats would reflect the increase in the expression of eNOS in adipose tissue from exercise-trained rats. Whatever the source(s) of NO, however, the present finding, namely, that a pretreatment of tissue segments with L-NAME increased both basal lipolysis and isoproterenol-stimulated lipolysis, indicates that extracellular NO can inhibit the lipolytic capacity of adipocytes present within adipose tissue.

In a comparison of control and exercise-trained rats to test the effect of L-NAME on adipose tissue lipolysis in response to isoproterenol, the data suggest that the inhibitory effect of extracellular NO on stimulated lipolysis was significantly greater in the exercise-trained rats. This phenomenon is probably a result of these two possibilities: a greater accumulation of extracellular NO and a greater response of adipocytes, independently, to NO in exercise-trained rats. The former would be supported by the finding shown in Table 1. In adipose tissue, the resultant amount of nitrite/nitrate production after the incubation of adipocytes with L-NAME plus isoproterenol was significantly less in exercise-trained rats than in control rats. The latter, however, can clearly be substantiated by the data in Fig. 5. SNAP, a NO donor, significantly inhibited adipocyte lipolysis in response to isoproterenol in both groups, but this inhibitory effect was significantly greater in isolated adipocytes from exercise-trained rats than in those from control rats.

The exact mechanism(s) by which exercise training
enhances the action of extracellular NO on isoproterenol-stimulated lipolysis in adipocytes is at present unclear. A recent study suggests that SNAP, acting as a NO\(^+\) donor, interferes with the \(\beta\)-adrenergic signal transduction pathway upstream from the adenylate cyclase, but that PAPA-NONOate, acting as a NO\(^+\) donor, reduces hormone-sensitive lipase activity [4]. Therefore, the greater inhibitory effect of SNAP on stimulated lipolysis in exercise-trained rats strongly suggests that exercise training enhances the inhibitory effect of NO on the \(\beta\)-adrenergic signal transduction pathway upstream from the adenylate cyclase. However, the greater decrease in glycerol releases because of PAPA-NONOate in exercise-trained rats does not completely rule out the possibility that the inhibitory effect of NO on hormone-sensitive lipase activity is also enhanced, though the percent inhibition of lipolysis because of PAPA-NONOate did not differ between the groups.

It must be noted, however, that the increases in basal lipolysis of adipose tissue as a result of L-NAME require cautious interpretation because there are some conflicting data concerning the effect of NO on basal lipolysis in adipocytes. Andersson et al. [5] have shown that NO gas significantly inhibits basal and isoproterenol-stimulated lipolysis in isolated adipocytes, whereas Gaudiot et al. [4] have shown that NO gas inhibits adipocyte lipolysis in response to several lipolytic agonists, but fails to elicit any significant effect on basal adipocyte lipolysis. Moreover, Gaudiot et al. [4] have shown that SNAP increases basal adipocyte lipolysis through the S-nitrosylation-mediated inhibition of phosphodiesterase, but our data show that SNAP has no effect on basal lipolysis. Moreover, in contrast to our present finding, Gaudiot et al. [18] recently showed that L-NAME increases basal adipocyte lipolysis by its oxygen species’ scavenging property [19, 20], and they suggested that NO can act as an antioxidant to prevent prooxidative reactions linked to \(\text{H}_2\text{O}_2\). They also concluded that the endogenous production of NO within adipocytes might be required for lipolytic activity through NO antioxidant-related properties. Thus, the exact role of NO in regulating lipolysis, especially basal lipolysis, seems not so simple to explain. Further studies are needed to establish the exact role of NO in lipolysis \textit{in vitro} as well as \textit{in vivo} in laboratory animals and humans subjected to exercise.

In conclusion, isoproterenol-stimulated lipolysis was significantly greater in exercise-trained rats than in control rats when adipose tissue segments, but not isolated adipocytes, were treated with 5 mM L-NAME. On the other hand, the inhibitory effect of SNAP on isoproterenol-stimulated lipolysis was significantly greater in isolated adipocytes from exercise-trained rats than in those from control rats. Exercise training altered the activity and protein expression of eNOS, but not of iNOS in adipose tissue. However, nitrite/nitrate production of adipose tissue was not altered. On the other hand, the nitrite/nitrate production of isolated adipocytes was significantly reduced after exercise training. The activities of both eNOS and iNOS were also significantly reduced in adipocytes from exercise-trained rats. Thus, exercise training enhanced the responsiveness of adipocytes to extracellular NO with both a reduced production of nitrite/nitrate in adipocytes and increases in either the activity or protein expression of eNOS in adipose tissue.

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