Asymmetric increases in the intensity of the green autofluorescence of ischemic stroke patients' skin and fingernails: A novel diagnostic biomarker for ischemic stroke

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Abstract

Early diagnosis of stroke is critical for therapeutic efficacy. Our study was designed to test our hypothesis that altered autofluorescence (AF) may be a diagnostic biomarker for ischemic stroke. The major findings of our study include: First, the green AF intensity of acute ischemic stroke patients in their fingernails and certain regions of their skin is significantly higher than that of the healthy persons and Low-Risk persons as well as the High-Risk persons. Second, the AF increases of the acute ischemic stroke patients are asymmetrical: The AF intensity of their left hands is significantly different from that of their right hands. Third, the percentage of the people with one or more regions with increased green AF is 7.69%, 37.93%, 71.43% and 58.82% for the healthy persons and Low-Risk persons, High-Risk persons, acute ischemic stroke patients and ischemic stroke patients during recovery phase, respectively. Fourth, the number of the regions with increased AF intensity is highly positively correlated with the probability that the person examined is an ischemic stroke patient. Moreover, there is evidence suggesting that the AF increases in the fingernails and the skin of ischemic stroke patients may result from oxidative stress-induced alterations of keratins. Collectively, our study has indicated that asymmetric AF increases in ischemic stroke may become a novel diagnostic biomarker for the disease. The fingernails' AF in ischemic stroke may also become a predicative biomarker for the illness.

Keywords: Stroke; autofluorescence; asymmetry; diagnosis; disease prediction.

Introduction

Stroke is one of the leading causes of death and disability around the world. The only FDA-approved drug tissue plasminogen activator (tPA) for treating ischemic stroke has very limited use, mainly due to its short therapeutic window (15): The drug must be administered within 4.5 hours after the incidence of ischemic stroke, otherwise such major detrimental effects as hemorrhagic transformation ensue. However, it usually takes greater than 4.5 hrs for a stroke patient to get doctor's diagnosis. Therefore, early diagnosis has become a key factor that determines the therapeutic effectiveness for the disease. To solve this crucial problem, it becomes increasingly valuable to establish approaches that can conduct diagnosis at patients' homes non-invasively and efficiently, although it appears to be an exceedingly challenging task.

Human AF (AF) of skin or blood has shown great promise for non-invasive diagnosis of diabetes (13) and cancer (17). Our recent study has reported that UV-induced epidermal green AF, which is originated from UV-induced keratin 1 proteolysis, can be used as a novel biomarker for predicting UV-induced skin damage (9). Keratins, together with melanin, NADH and FAD, are major epidermal fluorophores (1,14). It is noteworthy that the AF of advanced glycation products (AGEs) in skin has profoundly different excitation wavelength compared with that of keratins' AF: The excitation wavelength for keratins' AF is 488 nm (9,14), while the excitation wavelength for AGEs' AF is 300 - 420 nm (10,11). Since detection of AF is non-invasive and relatively simple, it is of great clinical and scientific significance

to further investigate the potential of AF as biomarkers for diseases.

Our recent study has found that the oxidative stress induced by UVC is causative to the increased epidermal AF of mouse ears by inducing keratin 1 proteolysis (12). As we proposed in the article, since there is increased oxidative stress in the blood and urine of ischemic stroke patients (4,5,16) and animal models of cerebral ischemia (6), oxidative stress may also induce increases in the epidermal AF of the patients of ischemic stroke, which might become a novel biomarker for non-invasive and efficient diagnosis of ischemic stroke. Moreover, a large number of studies have indicated that oxidative stress plays a critical pathological role in ischemic brain injury (3,7,18). Therefore, it is necessary to determine if there are increases in the epidermal AF of ischemic stroke patients.

There has been little study on the AF of human fingernails. Since there is increased oxidative stress in the plasma of ischemic stroke patients (4,5,16) and animal models of cerebral ischemia (6), the oxidative stress may also enhance the AF of the nails' keratins of ischemic stroke patients. Thus, it is also warranted to determine if there are increases in the fingernails' AF of ischemic stroke patients. Moreover, it has been reported that there is increased oxidative stress in the plasma of genetic stroke-prone rats (6), which may also induce increased AF of fingernails and certain regions of the skin of the persons in high risk to have stroke. Therefore, increased AF of fingernails might also become a predictive biomarker for stroke.

In our current study, we tested our hypothesis that ischemic stroke patients may have altered AF of their fingernails and certain regions of their skin, which may

become both a novel diagnostic biomarker and a predictive biomarker for ischemic stroke. Our study has provided first evidence that there are asymmetric increases in green AF intensity of ischemic stroke patients' fingernails and certain regions of their skin, which may become a novel biomarker for both ischemic stroke diagnosis and prediction of ischemic stroke incidence.

Methods and materials

Human subjects

The study was conducted according to a protocol approved by the Ethics Committee of Shanghai Fifth People's Hospital, Fudan University. The human subjects in our study were divided into four groups: Group 1: The group of healthy persons and Low-Risk persons - the persons with only a mild level of hypertension; Group 2: The group of High-Risk persons - the persons who were clinically diagnosed as 'the patients in high risk to have ischemic stroke' by the neurologists of the Department of Neurology, Shanghai Fifth People's Hospital, Fudan University; Group 3: The group of acute ischemic stroke patients who had ischemic stroke within 7 days; and Group 4: The group of ischemic stroke patients in recovery phase, who have had ischemic stroke for at least one month. All of the human subjects of Group 3 were hospitalized patients of the Department of Neurology. All of the human subjects of Group 4 were the stroke patients in recovery phase who were examined in the Outpatient Clinic of the Department of Neurology. All of the human subjects of Group 2 were the High-Risk persons who were either hospitalized in the Department

of Neurology or examined in the Outpatient Clinic of the Department of Neurology. The age of Group 1, Group 2, Group 3 and Group 4 is 67.49±8.14, 68.91±8.53, 67.30±10.27, and 69.28±15.97 years of old, respectively.

Autofluorescence determination

A portable AF imaging equipment was used to detect the AF of the fingernails and certain regions of the skin of the human subjects. The excitation wavelength is 485 nm, and the emission wavelength is 500 – 550 nm. For all of the human subjects, the AF intensity in the following six regions on both hands, i.e., twelve regions in total, was determined, including the index fingernails, Ventroforefingers, Dorsal Antebrachium, Centremetacarpus, Ventribrachium, and dorsal side of index fingers.

Determinations of the AF spectrum of Fingernails

The fingernails' clippings of acute ischemic stroke patients and High-Risk persons were collected. The spectra of the fingernails' AF was determined by using a two-photon fluorescence microscope (A1 plus, Nikon Instech Co., Ltd., Tokyo, Japan). The excitation wavelength was 760 nm, and the emission wavelength ranged from 420 to 640 nm. The signal was detected each 10 nm. After the imaging, the images were analyzed automatically.

Statistical analyses

All data are presented as mean ± SEM. Data were assessed by one-way ANOVA, followed by Student – Newman - Keuls *post hoc* test, except where noted. *P* values less than 0.05 were considered statistically significant.

Results

1. The AF intensity of the fingernails and certain regions of the skin of acute stroke patients is significantly higher than that of healthy and Low-Risk persons as well as High-Risk persons

We determined the green AF intensities of two index fingernails and ten regions of the skin of the healthy persons and Low-Risk persons, High-Risk persons, acute stroke patients, and the stroke patients during recovery phase. Our study showed that in all of the regions examined, the AF intensity of acute ischemic stroke patients is significantly higher than that of the healthy persons and Low-Risk persons as well as the High-Risk persons (Figs. 1A – 1F). In the fingernails and the Ventroforefingers of both hands, as well as in the Centremetacarpus of the left hand, the AF intensity of acute ischemic stroke patients is significantly higher than that of the stroke patients during recovery phase (Figs. 1A – 1C). In the Ventroforefingers and dorsal side of the index fingers of both hands (Fig. 1B and Fig.1F), the index fingernails of the left hands (Fig. 1A), as well as the Centremetacarpus and Ventriantebrachium of the right hands (Fig. 1D and Fig. 1E), the AF intensity of the stroke patients during recovery phase is significantly higher than that of the healthy persons and Low-Risk persons. In contrast, there is no significant difference

between the AF intensity of the stroke patients during recovery phase and that of the High-Risk persons (Figs. 1A – 1F).

2. The AF asymmetry of the fingernails and certain regions of the skin of acute ischemic stroke patients is significantly higher than that of healthy and Low-Risk persons as well as High-Risk persons

We determined the 'asymmetry of the AF intensity', which is defined as 'a significant difference (in absolute value) between the same region at the left or right side of the body'. Our study showed that in nearly all of the regions examined, the AF asymmetry of acute ischemic stroke patients is significantly higher than that of the healthy persons and Low-Risk persons as well as the High-Risk persons (Figs. 2A – 2F), except that in the dorsal side of the index fingers, there is no significant difference between the AF asymmetry of the acute ischemic stroke patients and that of the High-Risk persons (Fig. 2F). In the Ventroforefingers and Dorsal Antebrachium of both hands, the AF asymmetry of acute ischemic stroke patients is significantly higher than that of the ischemic stroke patients at recovery phase (Fig. 2B and Fig. 2C). In contrast, only at the dorsal side of the index fingers, the AF asymmetry of the ischemic stroke patients at recovery phase is significantly higher than that of the healthy persons and Low-Risk persons (Figs. 2B and Fig. 2F). There was no significant difference between the AF asymmetry of the ischemic stroke patients at recovery phase and that of the High-Risk persons (Figs. 2A – 2F).

3. The healthy and Low-Risk group, High-Risk group, acute ischemic stroke group and ischemic stroke during recovery phase group have profound differences in the percentage of the people with one or more regions with increased AF

We found that the percentage of the people with one or more regions with increased green AF is 7.69%, 37.93%, 71.43% and 58.82% for the healthy persons and Low-Risk persons, High-Risk persons, acute ischemic stroke patients and the ischemic stroke patients during recovery phase, respectively (Fig. 3A). The percentage of the people with three or more regions with increased green AF is 2.56%, 8.05%, 48.98% and 30.77% for the healthy persons and Low-Risk persons, High-Risk persons, acute ischemic stroke patients and ischemic stroke patients during recovery phase, respectively (Fig. 3A). We also found that in all of the regions examined, higher percentage of the acute ischemic stroke patients have increased AF intensity, compared with the healthy persons and Low-Risk persons as well as the High-Risk persons (Fig. 3B).

4. The healthy and Low-Risk group, High-Risk group, acute ischemic stroke group and ischemic stroke during recovery phase group have profound differences in the percentage of the people with one or more regions with AF asymmetry

We found that the percentage of the people with one or more regions with AF asymmetry is 2.56%, 25.29%, 62.0% and 42.31% for the healthy persons and

Low-Risk persons, High-Risk persons, acute ischemic stroke patients and ischemic stroke patients during recovery phase, respectively (Fig. 4A). We also found that in all of the regions examined, higher percentage of the acute ischemic stroke patients have AF asymmetry, compared with the healthy persons and Low-Risk persons as well as the High-Risk persons (Fig. 4B).

Counting the sum of the number of the regions with increased AF and the number of the regions with AF asymmetry, we further found the percentage of the people with the sum of 3 or more is 2.56%, 16.09%, 54.17% and 36.54% for the healthy persons and Low-Risk persons, High-Risk persons, acute ischemic stroke patients and ischemic stroke patients during recovery phase, respectively (Fig. 5).

5. The number of the regions with increased AF intensity is highly positively correlated with probability that the examined subject is an ischemic stroke patient

We used SASS software to determine the relationship between the probability that the examined subject is an ischemic stroke patient and several potential risk factors of stroke such as diabetes and hypertension (Table 1). With the human subjects in our current study, the analysis did not show any significant association between the probability that the examined subject and any one of the risk factors examined (Table 1). We also used the software to determine the relationship between the number of the regions with increased AF intensity and the probability that the examined subject is an ischemic stroke patients, indicating strong positive

correlation between the number of the regions with increased AF intensity and the probability that the examined subject is an ischemic stroke patient (Table 2).

6. Determinations of the AF spectrum of the fingernails' clippings of acute ischemic stroke patients have suggested that the altered AF is originated from the keratins of their fingernails

By using a two-photon fluorescent microscope, we analyzed the AF spectrum of the fingernails' clippings of ischemic stroke patients and High-Risk persons. Our study indicates that the AF spectrum matches that of keratins (1,14) (Fig. 5). Since keratins are key components of fingernails (2), this observation has suggested that the increased fingernails' AF of ischemic stroke patients could result at least partially from the increased keratins' AF of the fingernails.

Discussion

The major findings of our study include: First, the AF intensity of acute ischemic stroke patients in their fingernails and certain regions of skin is significantly higher than that of the healthy persons and Low-Risk persons as well as the High-Risk persons. Second, there is significant AF asymmetry in the fingernails and certain regions of skin of acute ischemic stroke patients. Third, the percentage of the people with one or more regions with increased green AF is 5.13%, 36.78%, 76% and 61.54% for the healthy persons and Low-Risk persons, High-Risk persons, acute ischemic stroke patients and ischemic stroke patients during recovery phase,

respectively. Fourth, the number of the regions with increased AF is highly positively correlated with the probability that the person examined is a stroke patient. Moreover, our study on the AF spectrum of the fingernails' clippings of acute ischemic stroke patients has suggested that the increased AF is originated from their fingernails' keratins. Collectively, our study has indicated that acute ischemic stroke patients have significant asymmetric AF increases in their fingernails and certain regions of their skin, which may become a novel diagnostic biomarker for the disease.

One of the most important and challenging tasks for stroke treatment is to realize early diagnosis of stroke. Considering that the time for both traveling from patients' homes to hospitals and diagnosis is likely over 4.5 hrs, a key strategy to realize early diagnosis could be diagnosis at patients' homes non-invasively and efficiently, immediately after occurrence of stroke's symptoms. Our current study has provided the first evidence suggesting that stroke patients may be diagnosed at home by determining the AF of fingernails and certain regions of their skin, which has shown significant sensitivity and specificity in stroke diagnosis: First, the percentage of acute ischemic stroke patients with one or more regions with increased green AF is markedly higher than that of the healthy persons and Low-Risk persons as well as High-Risk persons. Second, there is significant AF asymmetry in the fingernails and certain regions of skin of acute ischemic stroke patients. Third, the number of the regions with increased AF is highly positively correlated with the probability that the person examined is a stroke patient. Based on these pieces of evidence, we proposed a novel criteria for stroke diagnosis – 'asymmetrical increases

in the AF of certain regions of skin and fingernails'. We expect that the sensitivity and specificity of our approach for diagnosis of ischemic stroke would be significantly enhanced in the future, with both increases in the regions examined and applications of artificial intelligence and big data science in our future studies.

A key question regarding our findings is: Why are there significant increases in the green AF in certain regions of skin of acute stroke patients? We have found that the oxidative stress induced by UVC mediates the increase in the epidermal green AF of mouse ears by inducing keratin 1 proteolysis (9). A number of studies have shown that there is increased oxidative stress in the blood and urine of ischemic stroke patients (4,5,16) and animal models of cerebral ischemia (6). Therefore, we propose that the oxidative stress may induce increases in the epidermal AF of the ischemic stroke patients by inducing keratin 1 proteolysis, which may become a novel biomarker for non-invasive and efficient diagnosis of ischemic stroke. Future studies are needed to further test the validity of this proposal.

Our study has also obtained the following highly interesting observation: Acute ischemic stroke patients have significantly greater AF asymmetry in the skin and fingernails than that of healthy and Low-Risk persons. To our knowledge, this is the first report regarding the asymmetry of AF of human body. We propose that this AF asymmetry may be accounted for by the following two potential mechanisms: First, the pathological changes in one side of the brains of the acute stroke patients can cause significant pathological alterations on one side of the patients' body, which is exhibited by the asymmetric increases in the AF intensity of their skin and fingernails;

and second, before the incidence of ischemic stroke, the patients had significant pathological alterations on one side of their body, which can be indicated by the asymmetric AF increases of their skin and nails. Future studies are necessary to test the validity of these two proposals, which may provide highly valuable information for understanding the pathogenesis of ischemic stroke.

Our current study found that acute stroke patients have significantly higher AF intensity in three regions of the skin, compared with that of the ischemic stroke patients during recovery phase. It has been reported that oxidative stress is higher in the plasma and urine of acute ischemic stroke patients up to the 7th day after the stroke incidence (16). Thus, we propose that the oxidative stress in the body of the acute stroke patients, which could be higher than that of the stroke patients during the recovery phase, may account for the differences in the AF intensity between these two groups of stroke patients.

Another key question regarding our findings is: Why are there significant increases in the green AF in the fingernails of acute stroke patients? The responses to this question include: First, our AF spectrum study on the fingernails has suggested that the AF results at least partially from the keratins in the fingernails. Keratins are well-established fluorophores (1,14), which are also the main components of human nails (2): It was reported that human nail plate is composed of approximately 10% - 20% soft epithelial keratins (48K/56K and 50K/58K keratin pairs), with the remainder being hard α-keratin (8); and second, our previous study has shown the oxidative stress induced by UV can produce increased epidermal green AF (9). Since there are

multiple disulfide bonds in α-keratin (2), it is reasonable to expect that oxidative stress may significantly affect both the structure and fluorescent properties of α-keratin, leading to increased AF of α-keratin. Based on these two pieces of information, we propose that the increased AF of the fingernails may result from alterations of fingernails' keratins, which are induced by oxidative stress or other stress signals. Future studies are needed to further test validity of this proposal.

Our study has found that the fingernails of acute stroke patients show significant and asymmetric increases in the AF, compared with healthy and Low-Risk persons as well as High-Risk persons. The growth of the fingernails should occur days or even months before our examination. Therefore, it is reasonable to propose that the asymmetric increases in the fingernails' AF should occur before the incidence of stroke, since the acute ischemic stroke patients had stroke only 1 - 7 days ago. Therefore, we propose that 'asymmetric increases in the fingernails' AF' may become a novel predicative biomarker for stroke incidence. The asymmetric increases in the AF may become a warning sign for stroke incidence, which requires medical intervention to prevent stroke incidence.

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Figure Legends:

Fig. 1. The AF intensity of the fingernails and certain regions of the skin of acute stroke patients is significantly higher than that of healthy and Low-Risk persons as well as High-Risk persons. (A) The green AF intensity in the index fingernails of both hands of acute ischemic stroke patients are significantly higher than that of the healthy persons and Low-Risk persons, the High-Risk persons and the stroke patients during recovery phase. (B) The green AF intensity in the Ventroforefingers of both hands of acute ischemic stroke patients is significantly higher than that of the healthy persons and Low-Risk persons, the High-Risk persons and the stroke patients during recovery phase. (C) The green AF intensity in the Dorsal Antebrachium of both hands of acute ischemic stroke patients are significantly higher than that of the healthy persons and Low-Risk persons as well as the High-Risk persons. (D) The green AF intensity in the Centremetacarpus of both hands of acute ischemic stroke patients are significantly higher than that of the healthy persons and Low-Risk persons as well as the High-Risk persons. (E) The green AF intensity in the Ventribrachium of both hands of acute ischemic stroke patients are significantly higher than that of the healthy persons and Low-Risk persons as well as the High-Risk persons. (F) The green AF intensity in the dorsal side of index fingers of both hands of acute ischemic stroke patients are significantly higher than that of the healthy persons and Low-Risk persons as well as the High-Risk persons. The number of subjects in the healthy and Low-Risk group, High-Risk group, acute ischemic stroke group, and ischemic stroke in recovery group is 39, 87, 47-50, and 52, respectively. *, $p < 0.05$; **, $p < 0.01$;

***, $p < 0.001$.

Fig. 2. The AF asymmetry of the fingernails and certain regions of the skin of acute ischemic stroke patients is significantly higher than that of healthy and Low-Risk persons as well as High-Risk persons. (A) The AF asymmetry in the index fingernails of acute ischemic stroke patients are significantly higher than that of the healthy persons and Low-Risk persons, the High-Risk persons and the stroke patients during recovery phase. (B) The AF asymmetry in the Ventroforefingers of acute ischemic stroke patients is significantly higher than that of the healthy persons and Low-Risk persons, the High-Risk persons and the stroke patients during recovery phase. (C) The AF asymmetry in the Dorsal Antebrachium of acute ischemic stroke patients is significantly higher than that of the healthy persons and Low-Risk persons as well as the High-Risk persons. (D) The AF asymmetry in the Centremetacarpus of acute ischemic stroke patients is significantly higher than that of the healthy persons and Low-Risk persons as well as the High-Risk persons. (E) The AF asymmetry in the Ventribrachium of acute ischemic stroke patients is significantly higher than that of the healthy persons and Low-Risk persons (t-test) as well as the High-Risk persons. (F) The AF asymmetry in the dorsal side of index fingers of acute ischemic stroke patients is significantly higher than that of the healthy persons and Low-Risk persons. The number of subjects in the healthy and Low-Risk group, High-Risk group, acute ischemic stroke group, and ischemic stroke in recovery group is 39, 87, 47-50, and 52, respectively. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Fig. 3. The healthy and Low-Risk group, High-Risk group, acute ischemic stroke group and ischemic stroke during recovery phase group have profound differences in the percentage of the people with one or more regions with increased AF. (A) The percentage of the people with one or more regions with increased green AF is 5.13%, 37.93%, 71.43% and 58.82% for the healthy persons and Low-Risk persons, High-Risk persons, acute ischemic stroke patients and ischemic stroke patients during recovery phase, respectively. The percentage of the people with 3 or more regions with increased green AF is 2.56%, 8.05%, 48.98% and 30.77% for the healthy persons and Low-Risk persons, High-Risk persons, acute ischemic stroke patients and ischemic stroke patients during recovery phase, respectively. (B) The percentage of the acute stroke patients with increased AF intensity in all regions examined is higher than that of healthy persons and Low-Risk persons as well as High-Risk persons. The number of subjects in the healthy and Low-Risk group, High-Risk group, acute ischemic stroke group, and ischemic stroke in recovery group is 39, 87, 47-50, and 52, respectively.

Fig. 4. The healthy and Low-Risk group, High-Risk group, acute ischemic stroke group and ischemic stroke during recovery phase group have profound differences in the percentage of the people with one or more regions with AF asymmetry. (A) The percentage of the people without any region with AF asymmetry is 97.44%, 74.71%, 38% and 57.69% for the healthy persons and Low-Risk persons, High-Risk persons, acute ischemic stroke patients and ischemic

stroke patients during recovery phase, respectively. The percentage of the people with 1 or more regions with AF asymmetry is 2.56%, 25.29%, 62.0% and 42.31% for the healthy persons and Low-Risk persons, High-Risk persons, acute ischemic stroke patients and ischemic stroke patients during recovery phase, respectively. (B) The percentage of the acute stroke patients with AF asymmetry in all regions examined is higher than that of healthy persons and Low-Risk persons as well as High-Risk persons. The number of subjects in the healthy and Low-Risk group, High-Risk group, acute ischemic stroke group, and ischemic stroke in recovery group is 39, 87, 47-50, and 52, respectively.

Fig. 6. The sum of the number of the regions with increased green AF and the number of the regions with AF asymmetry is 3 or greater for a majority of the acute ischemic stroke patients. Calculating the sum of the number of the regions with increased green AF and the number of the regions with AF asymmetry, the percentage of the people with the sum of 3 or greater is 2.56%, 16.09%, 54.17% and 36.54% for the healthy persons and Low-Risk persons, High-Risk persons, acute ischemic stroke patients and ischemic stroke patients during recovery phase, respectively. The number of subjects in the healthy and Low-Risk group, High-Risk group, acute ischemic stroke group, and ischemic stroke in recovery group is 39, 87, 47-50, and 52, respectively.

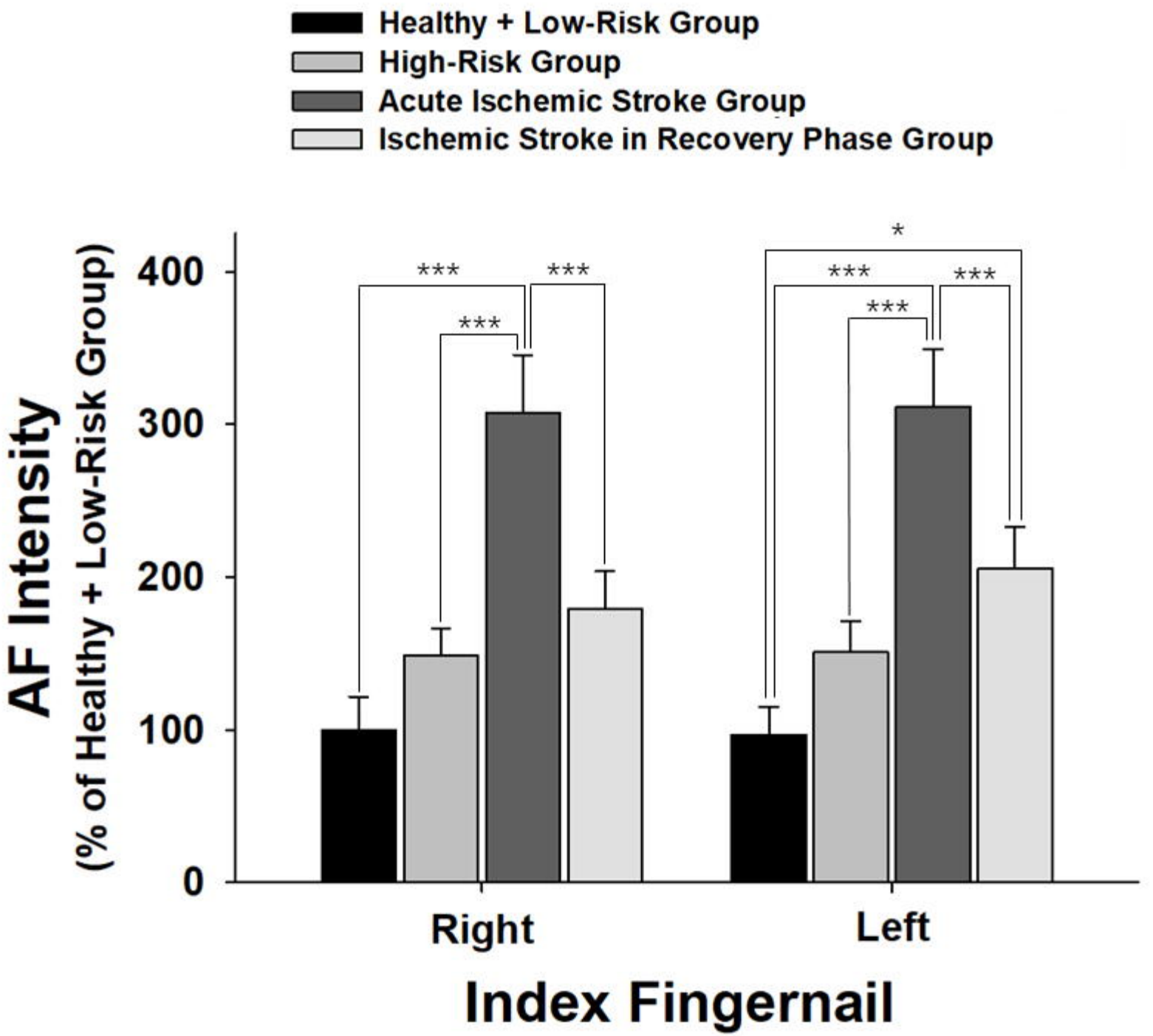
Fig. 6. Determinations of the AF spectrum of the fingernails' clippings of acute

ischemic stroke patients have suggested that the increased AF is originated from the keratins of their fingernails. The AF spectrum of the fingernails' clippings of ischemic stroke patients and High-Risk persons matches that of keratins. N =3.

Table legends:

Table 1. The probability that the examined subject is an ischemic stroke patient is not significantly correlated with multiple factors examined. We used SASS software to determine the relationship between the probability that the examined subject is an ischemic stroke patient and several potential risk factors of stroke such as diabetes and hypertension. The analysis did not show significant association between the probability that the examined subject and any one of the factors shown in the table. All of the subjects in the High-Risk group (N = 26) and the acute ischemic stroke group (N = 33) were hospitalized patients.

Table 2. The number of the regions with increased AF intensity is highly correlated with probability that the examined subject is an ischemic stroke patient. We used SASS software to determine the relationship between the number of the regions with increased AF intensity and the probability that the examined subject is an ischemic stroke patients, indicating strong positive correlation between the number of the regions with increased AF intensity and the probability that the examined subject is an ischemic stroke patient. All of the subjects in the High-Risk group (N = 26) and the acute ischemic stroke group (N = 33) were hospitalized patients.


Healthy + Low-Risk Group
High-Risk Group
Acute Ischemic Stroke Group
Ischemic Stroke in Recovery Phase Group
AF Intensity
(% of Healthy + Low-Risk Group)
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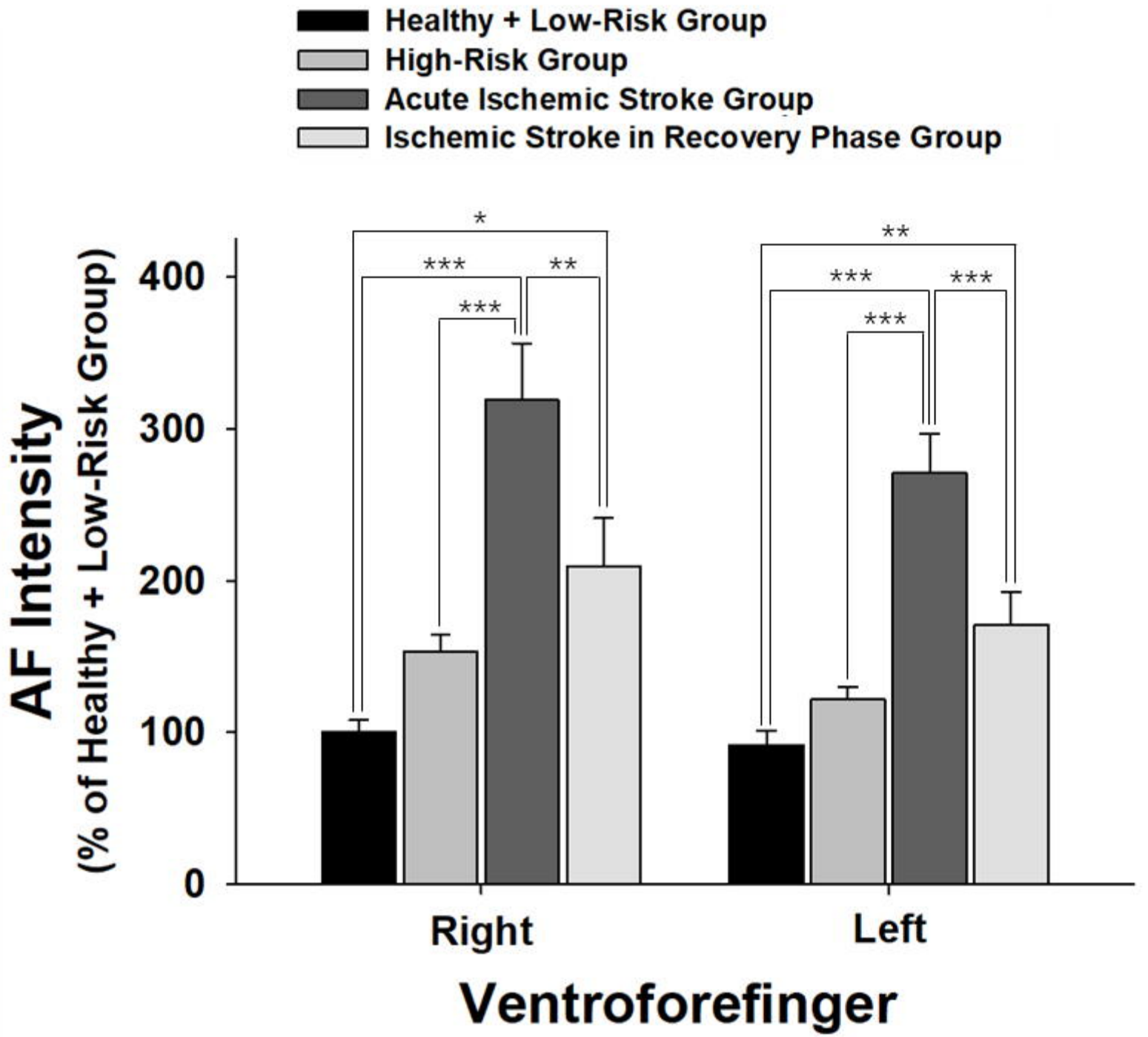
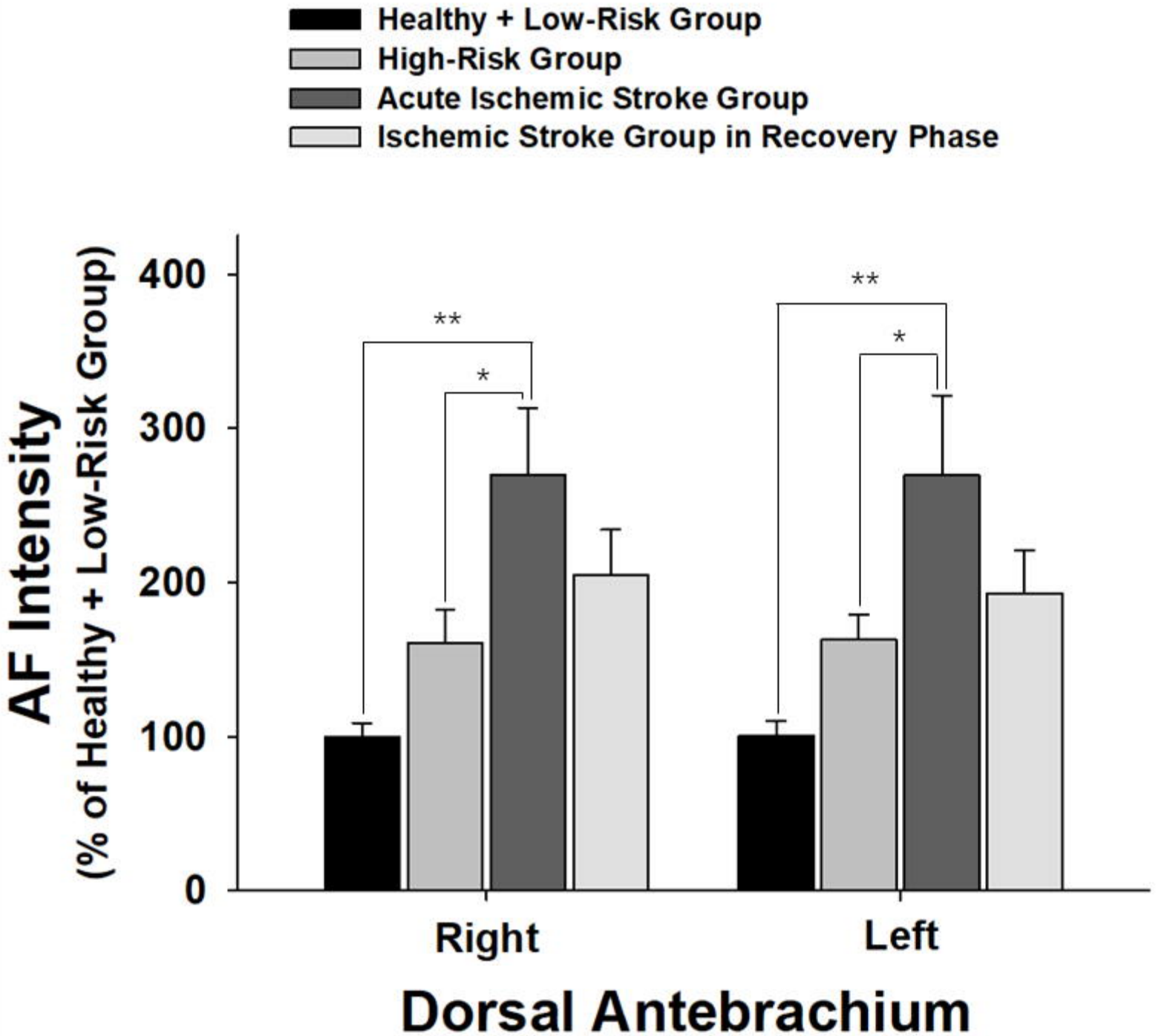
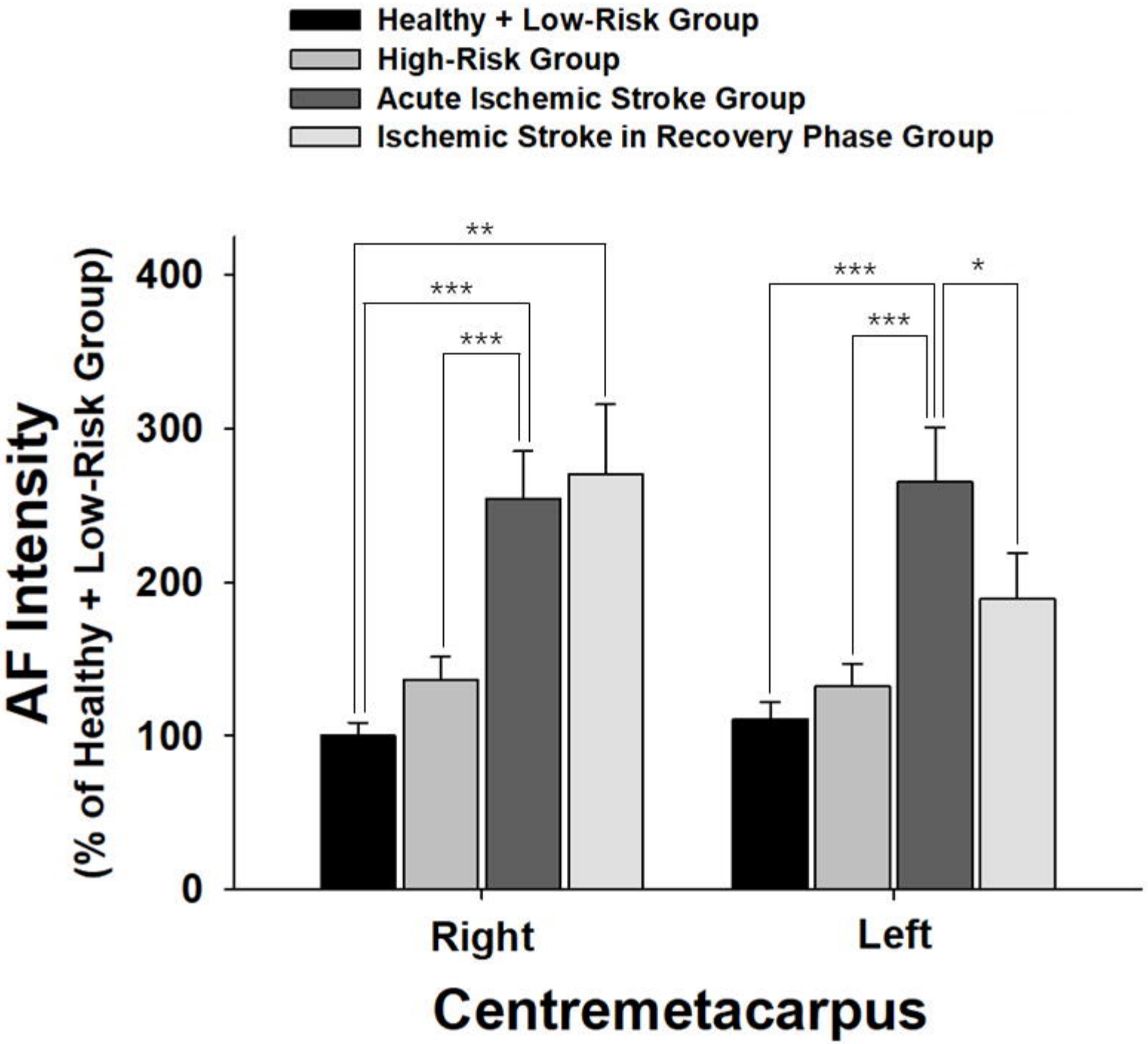
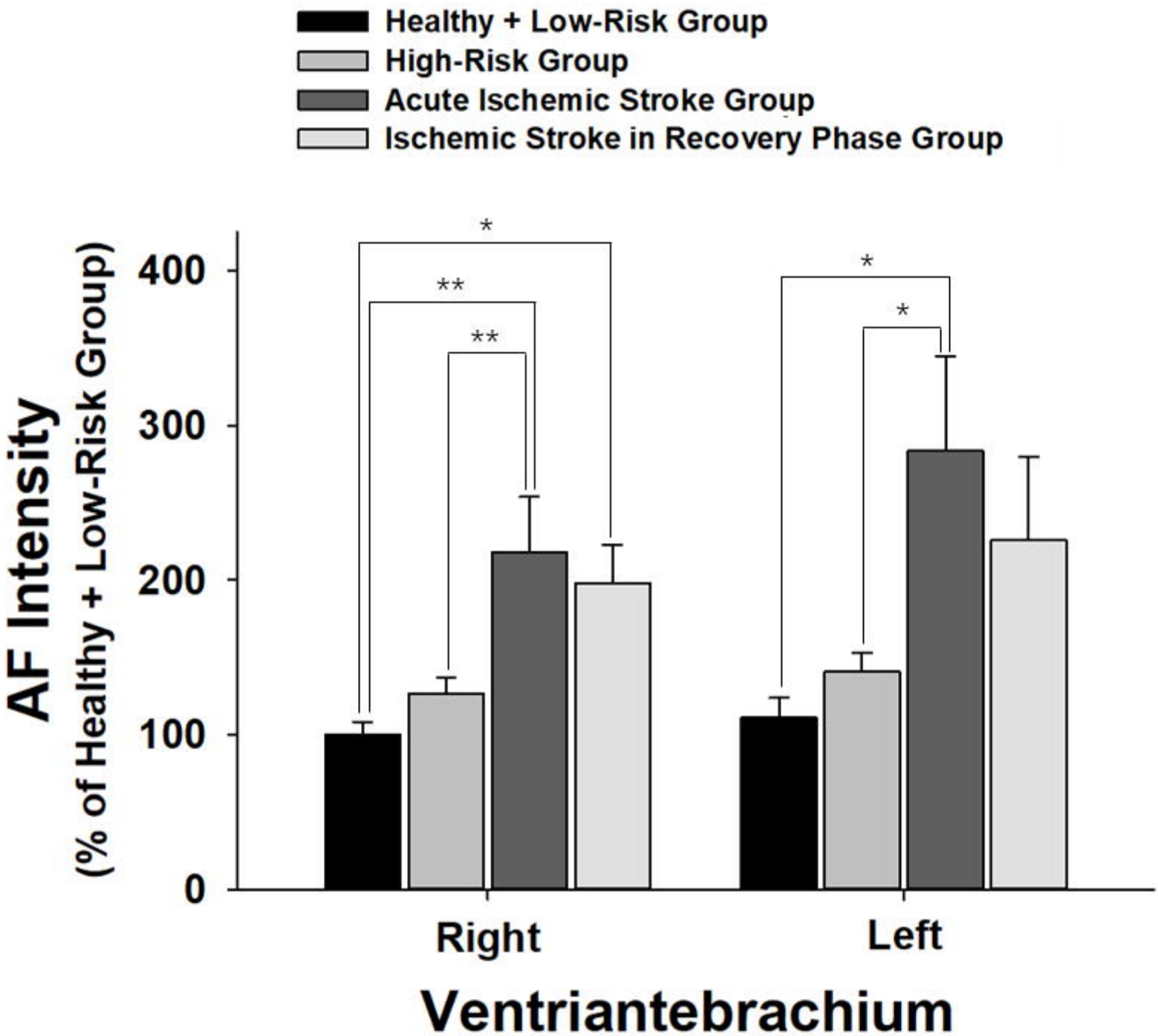
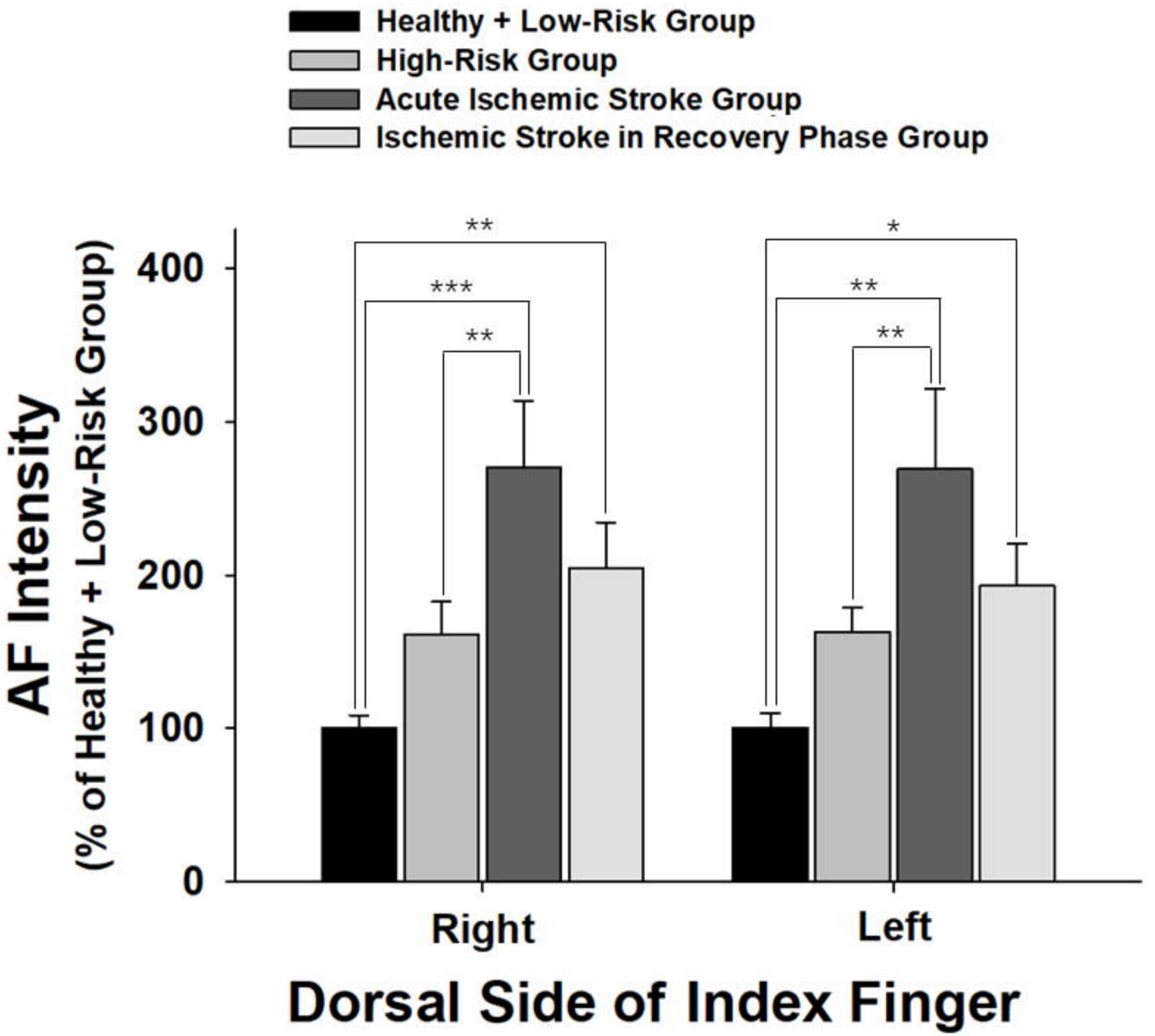
Index Fingernail

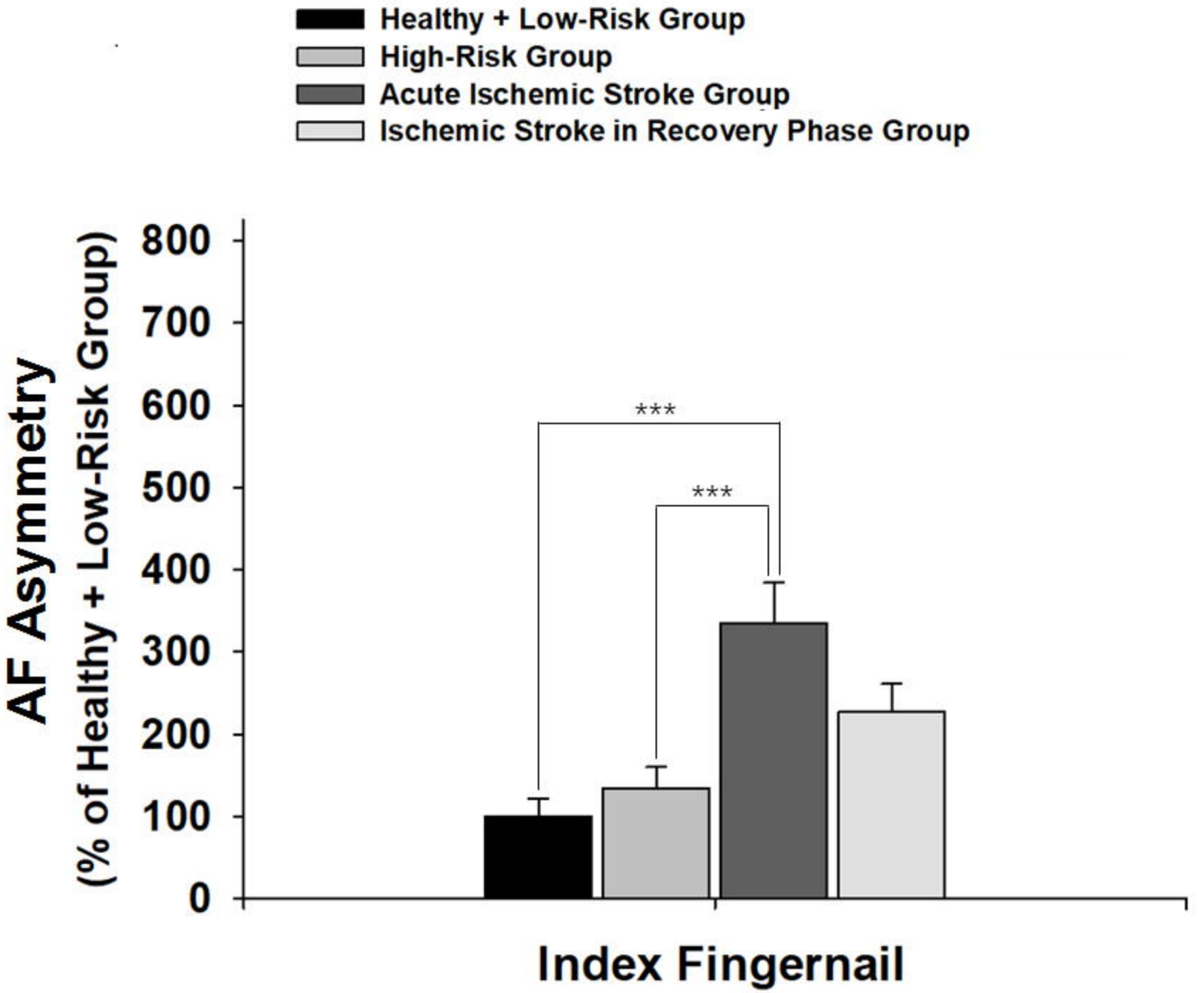

Healthy + Low-Risk Group
High-Risk Group
Acute Ischemic Stroke Group
Ischemic Stroke in Recovery Phase Group
AF Intensity
(% of Healthy + Low-Risk Group)
400
300
200
100
0
*
***
**
***
**
***
***
***
Right
Left
Ventroforefinger

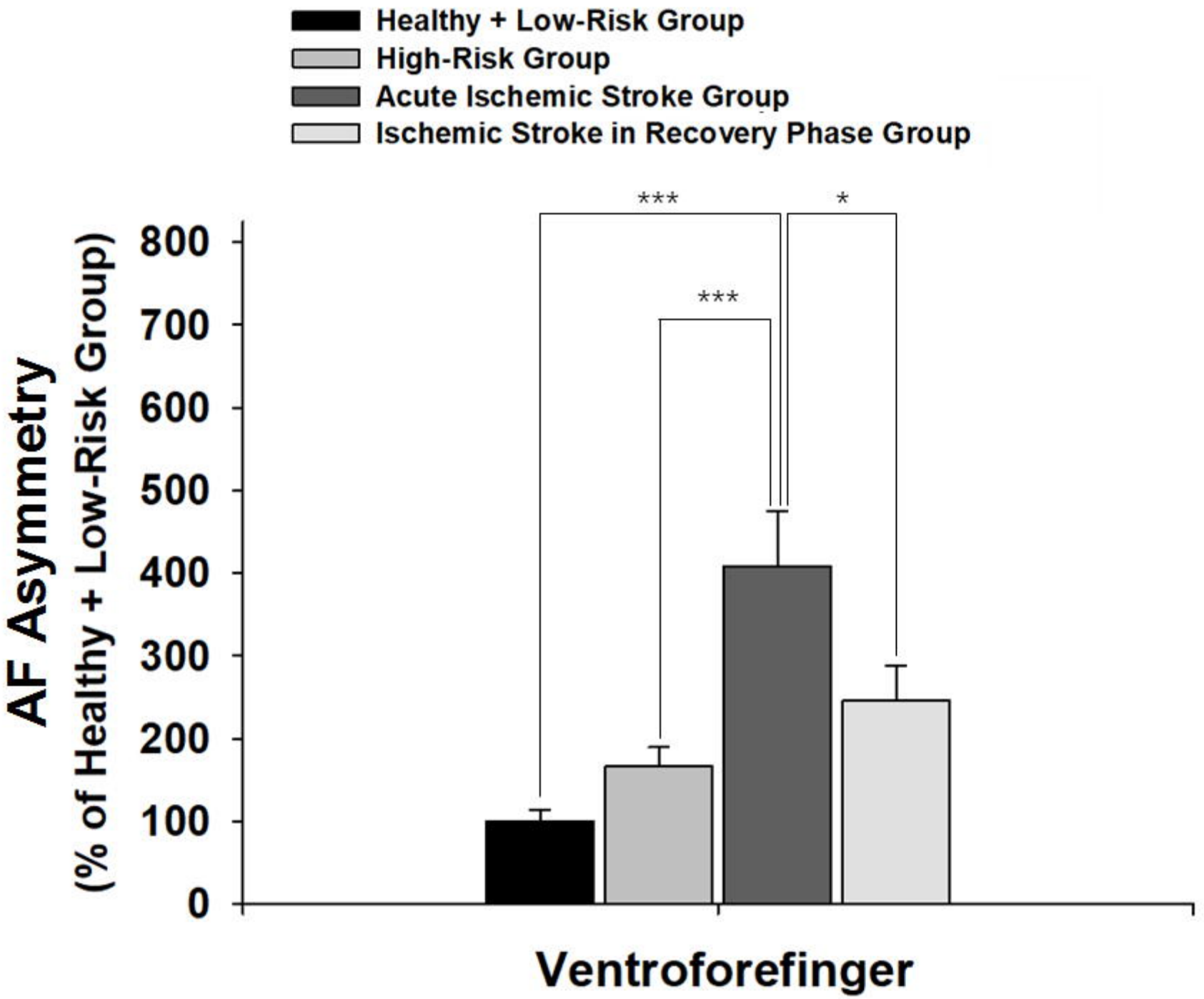

Healthy + Low-Risk Group
High-Risk Group
Acute Ischemic Stroke Group
Ischemic Stroke Group in Recovery Phase
AF Intensity
(% of Healthy + Low-Risk Group)
400
300
200
100
0
**
*
**
*
Right
Left
Dorsal Antebrachium

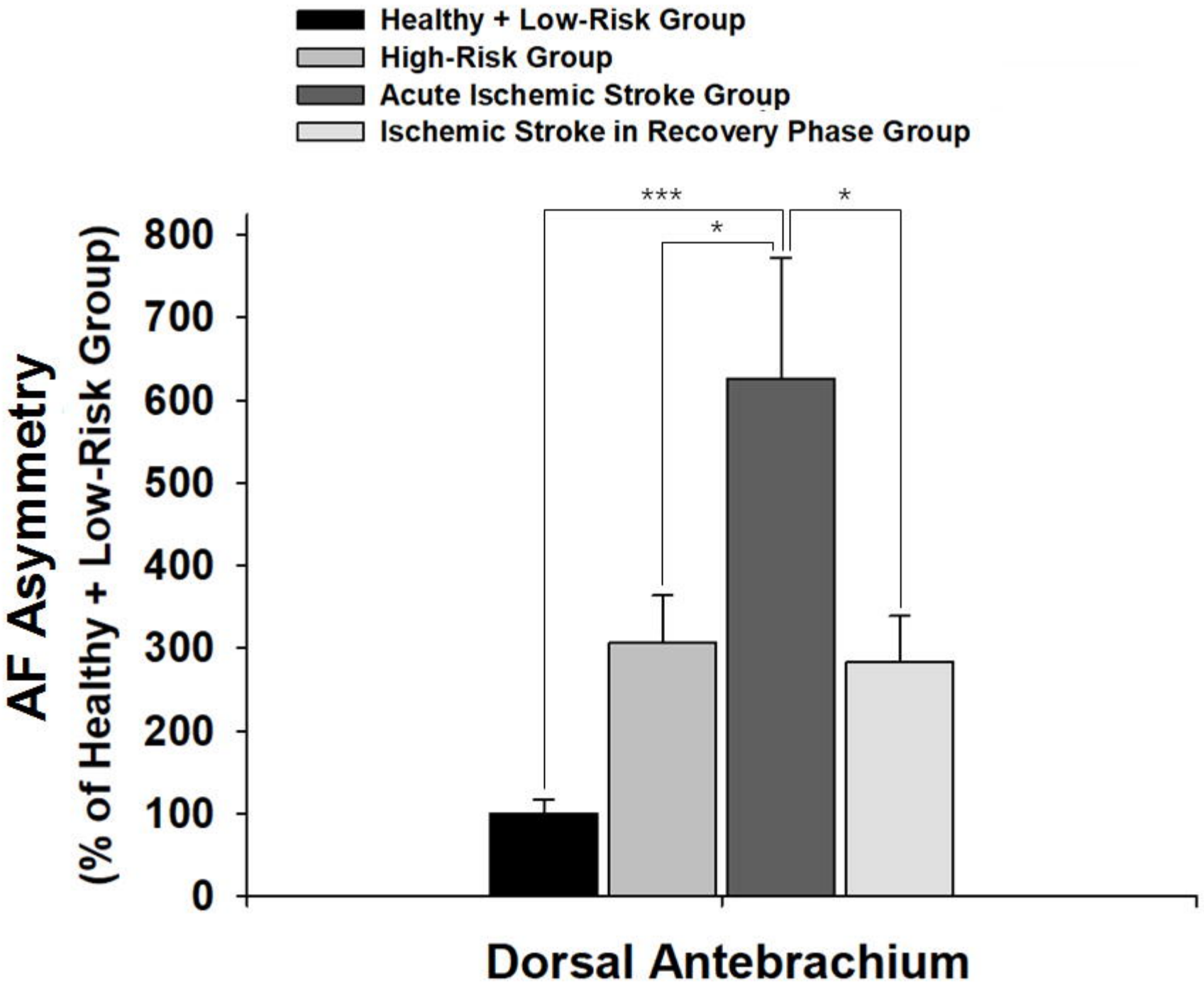

Healthy + Low-Risk Group
High-Risk Group
Acute Ischemic Stroke Group
Ischemic Stroke in Recovery Phase Group
AF Intensity
(% of Healthy + Low-Risk Group)
400
300
200
100
0
**
***
***
***
***
*
Right
Left
Centremetacarpus

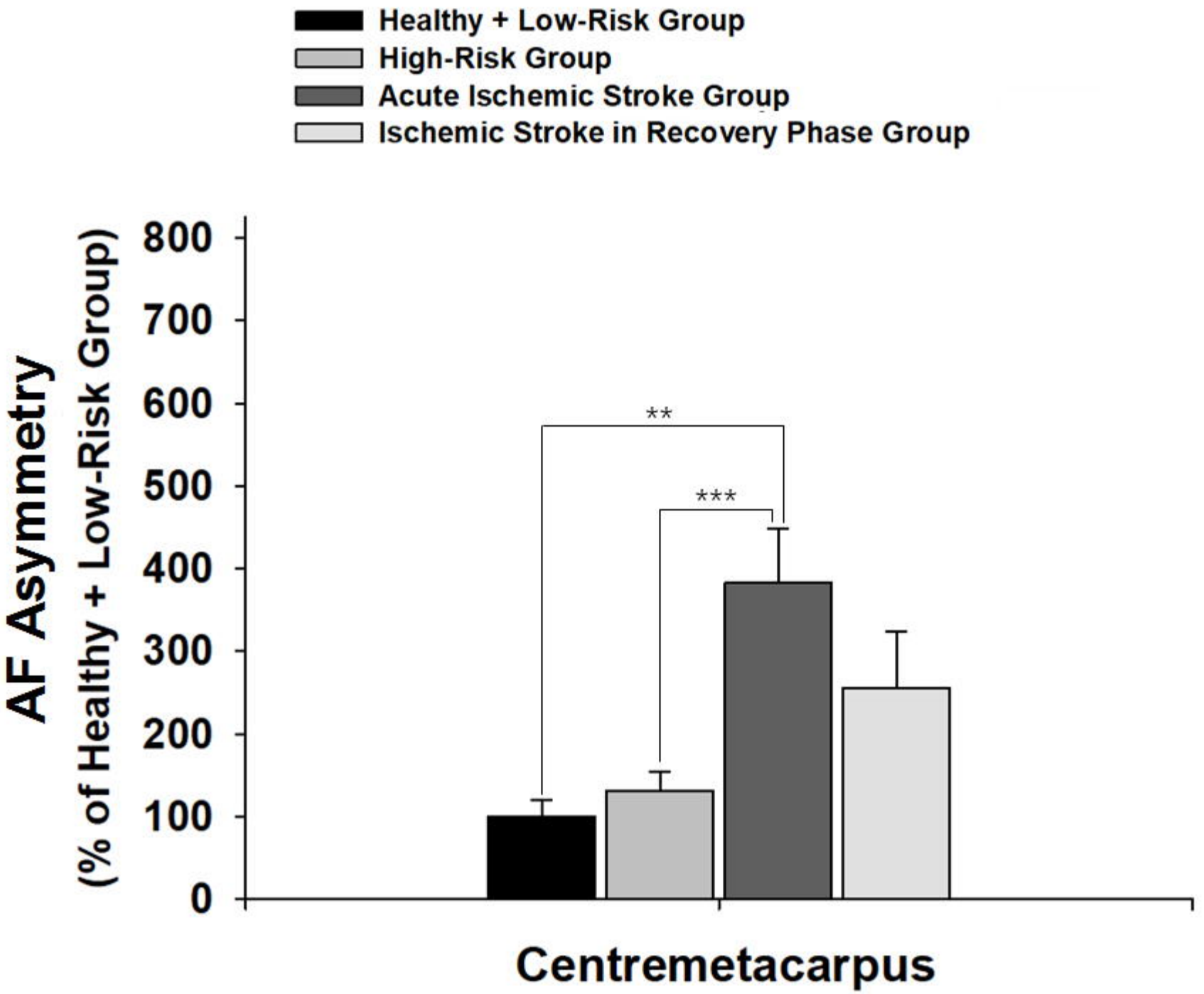

Healthy + Low-Risk Group
High-Risk Group
Acute Ischemic Stroke Group
Ischemic Stroke in Recovery Phase Group
AF Intensity
(% of Healthy + Low-Risk Group)
400
300
200
100
0
*
**
**
*
*
Right
Left
Ventriantebrachium

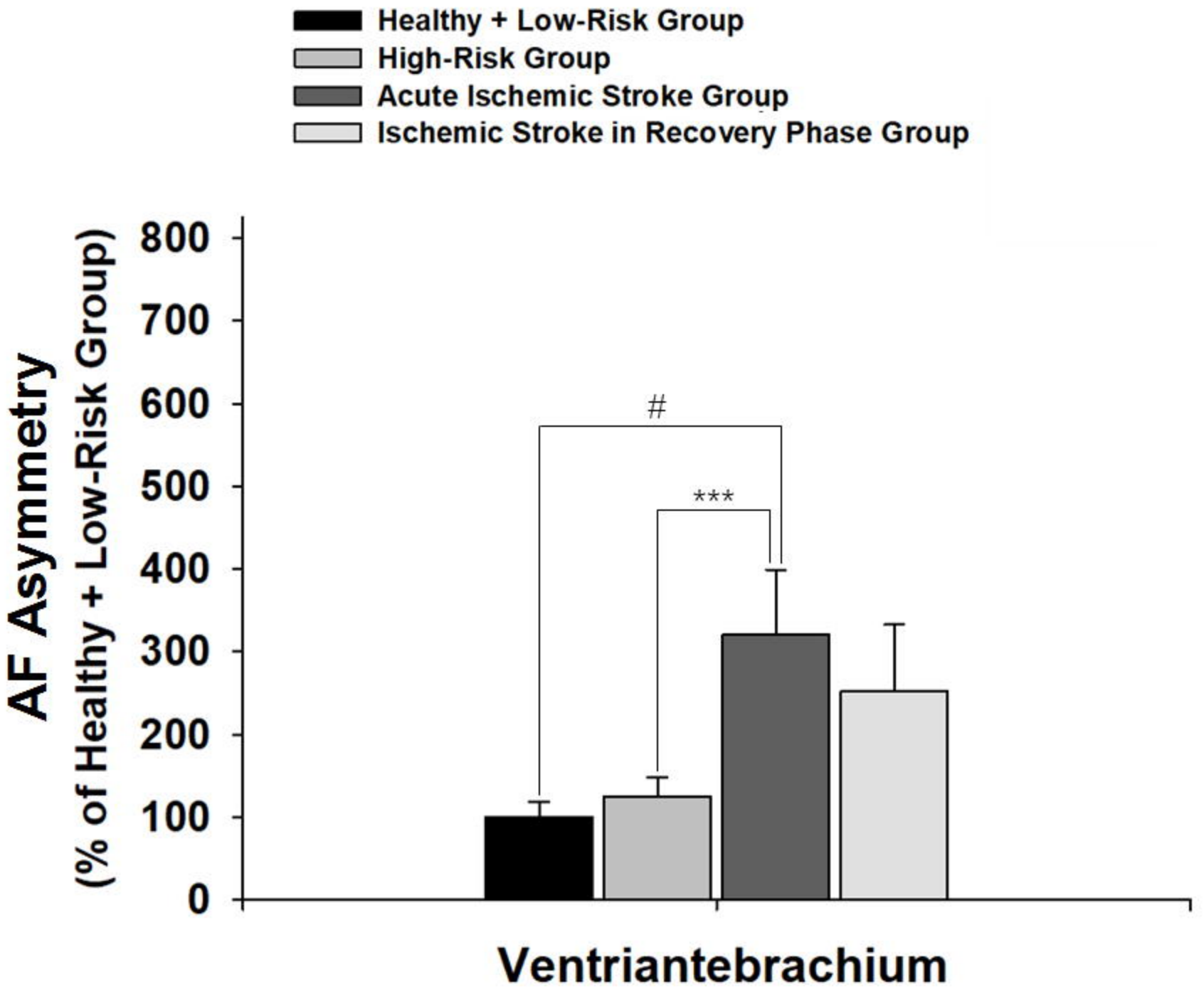

Healthy + Low-Risk Group
High-Risk Group
Acute Ischemic Stroke Group
Ischemic Stroke in Recovery Phase Group
AF Intensity
(% of Healthy + Low-Risk Group)
400
300
200
100
0
**
***
**
*
**
**
Right
Left
Dorsal Side of Index Finger

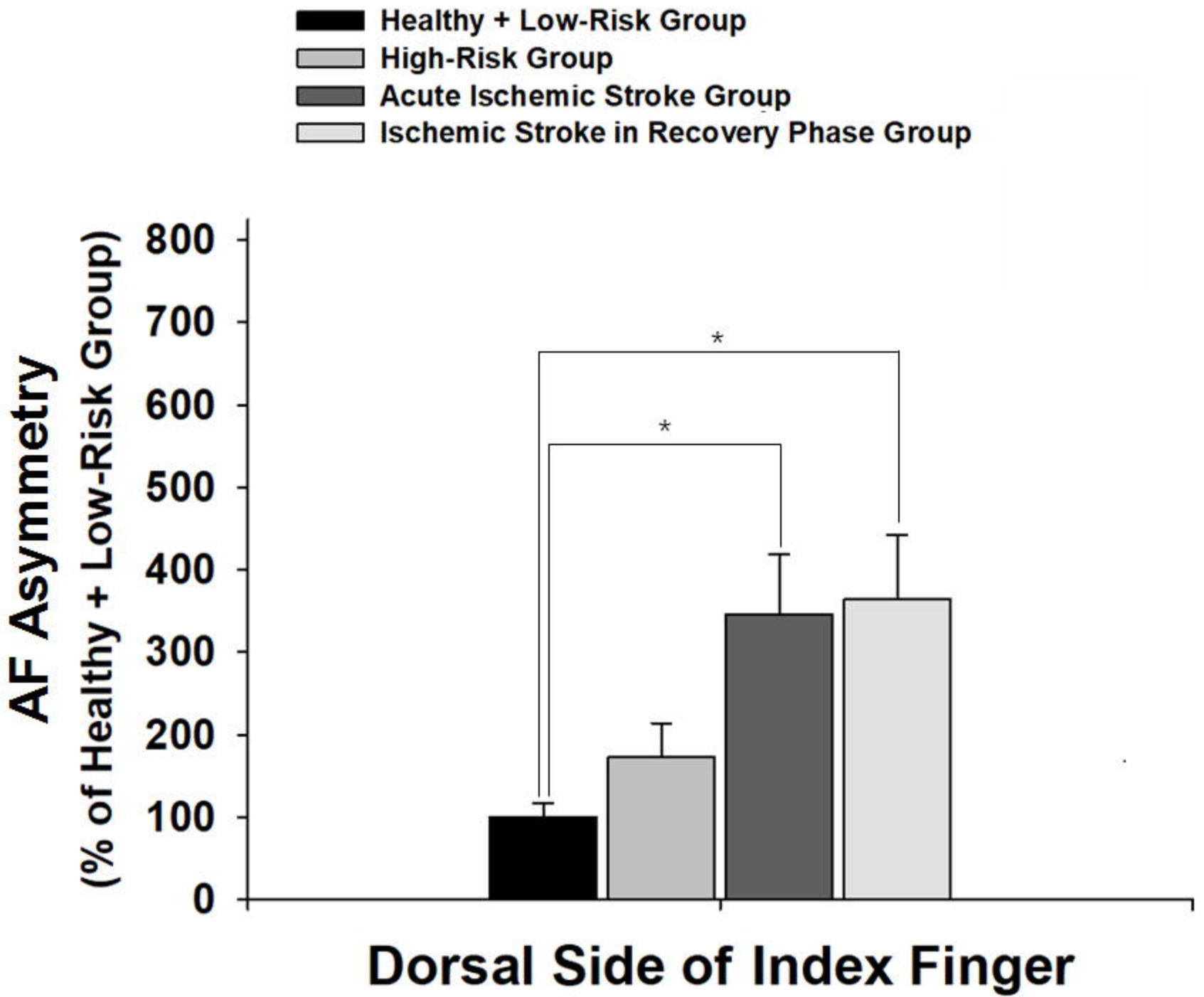

Healthy + Low-Risk Group
High-Risk Group
Acute Ischemic Stroke Group
Ischemic Stroke in Recovery Phase Group
AF Asymmetry
(% of Healthy + Low-Risk Group)
800
700
600
500
400
300
200
100
0
***
***
Index Fingernail

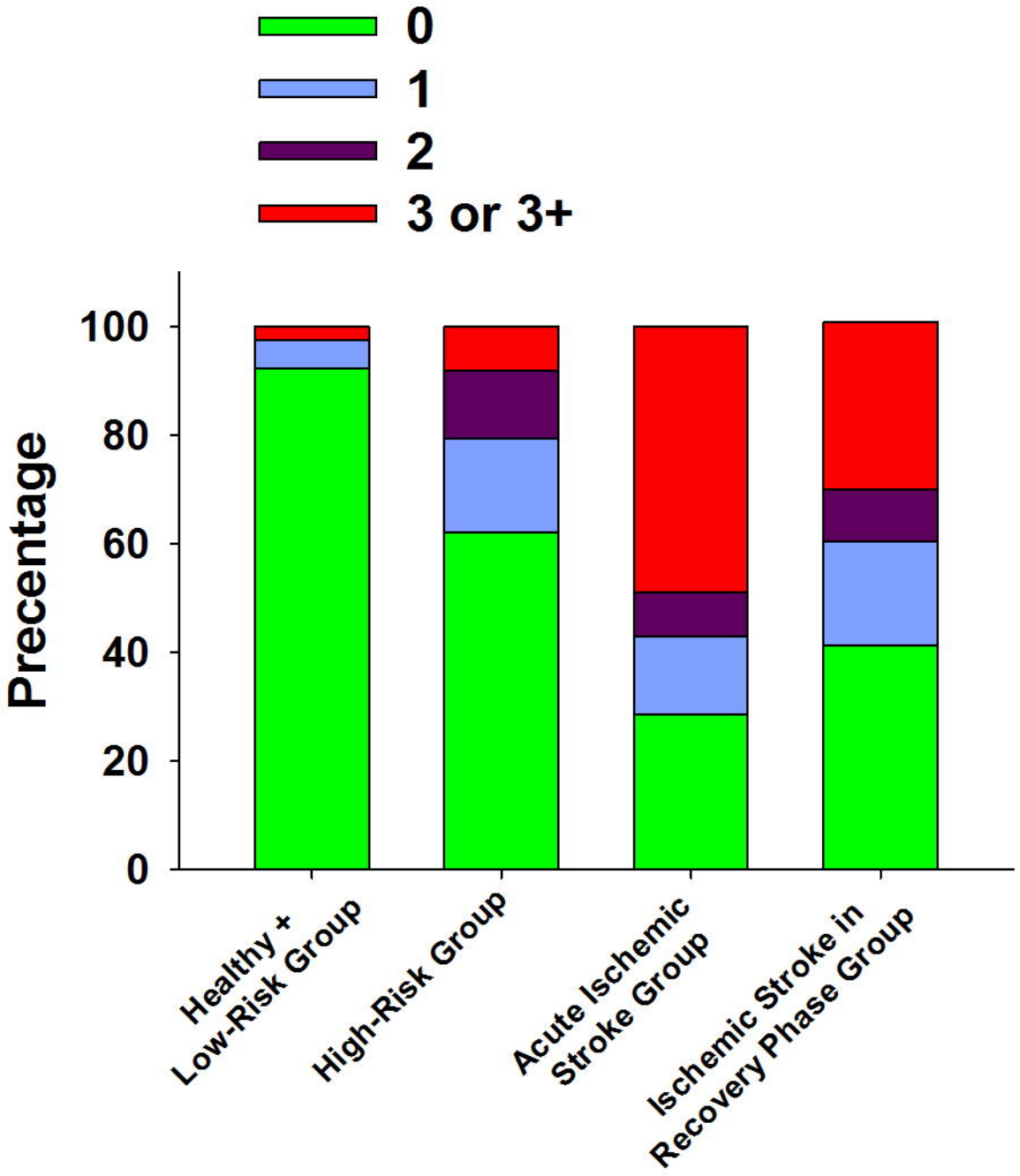

Healthy + Low-Risk Group
High-Risk Group
Acute Ischemic Stroke Group
Ischemic Stroke in Recovery Phase Group
***
*
***
AF Asymmetry
(% of Healthy + Low-Risk Group)
800
700
600
500
400
300
200
100
0
Ventroforefinger

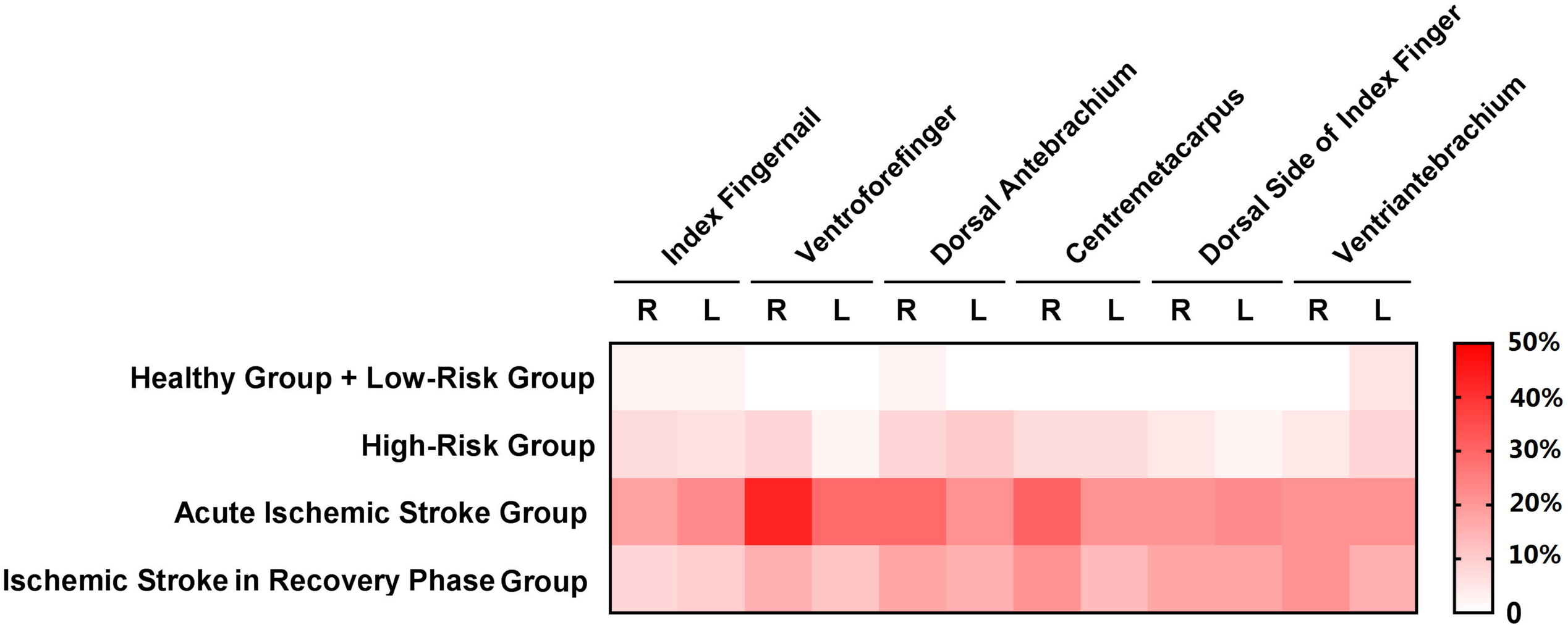

Healthy + Low-Risk Group
High-Risk Group
Acute Ischemic Stroke Group
Ischemic Stroke in Recovery Phase Group
***
*
*
AF Asymmetry
(% of Healthy + Low-Risk Group)
800
700
600
500
400
300
200
100
0
Dorsal Antebrachium

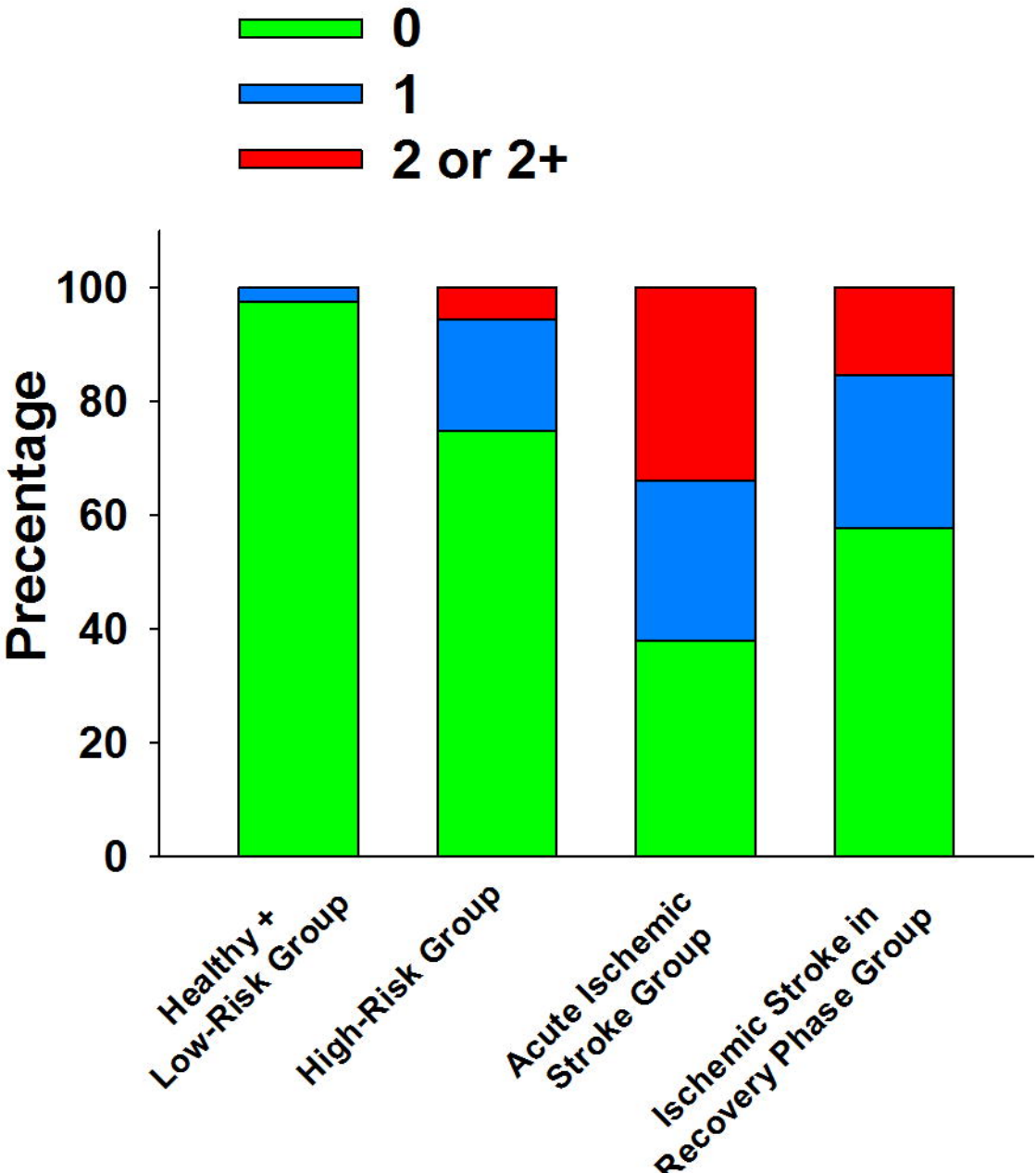

Healthy + Low-Risk Group
High-Risk Group
Acute Ischemic Stroke Group
Ischemic Stroke in Recovery Phase Group
AF Asymmetry
(% of Healthy + Low-Risk Group)
800
700
600
500
400
300
200
100
0
**
***
Centremetacarpus

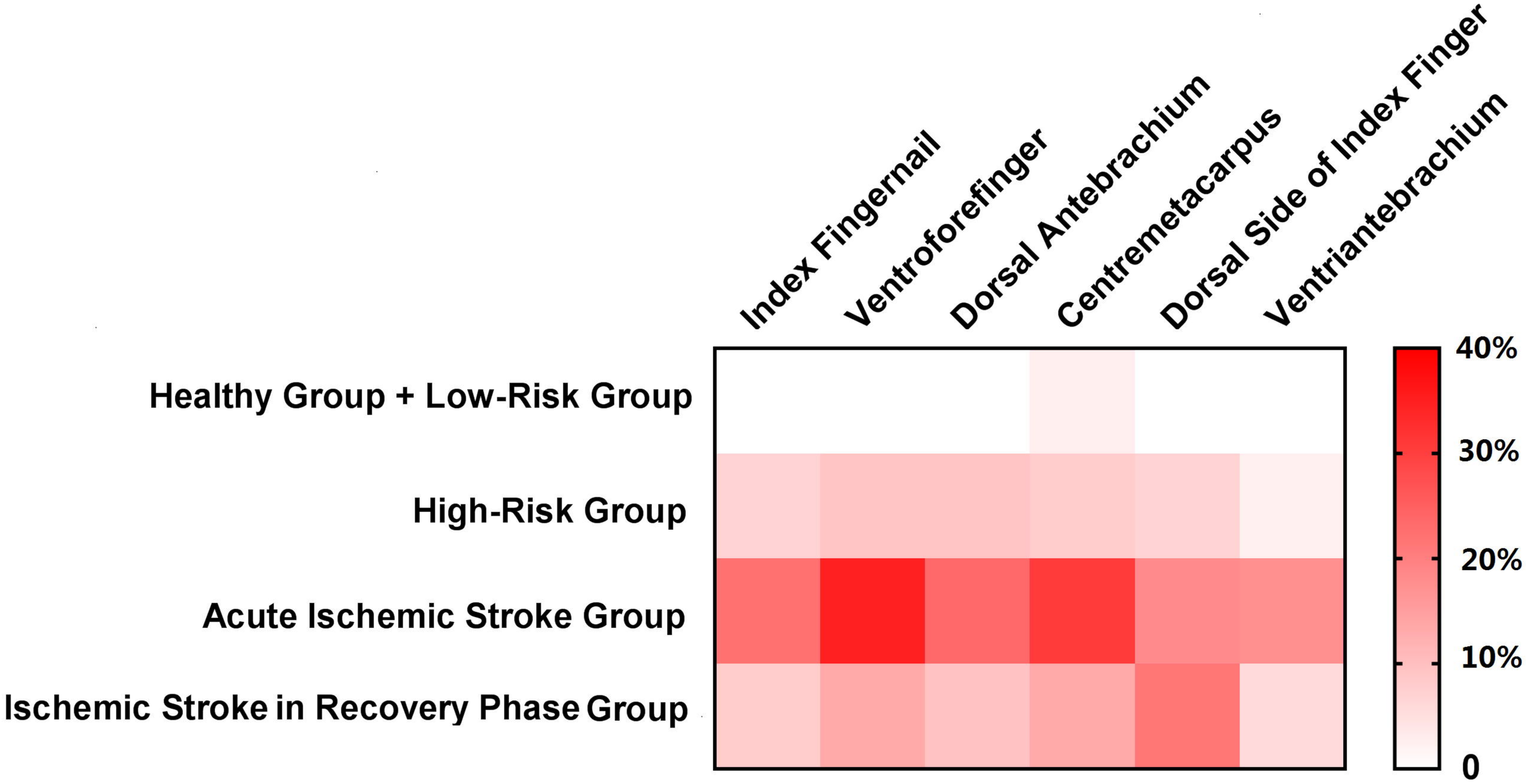

Healthy + Low-Risk Group
High-Risk Group
Acute Ischemic Stroke Group
Ischemic Stroke in Recovery Phase Group
AF Asymmetry
(% of Healthy + Low-Risk Group)
800
700
600
500
400
300
200
100
0
#
***
Ventriantebrachium

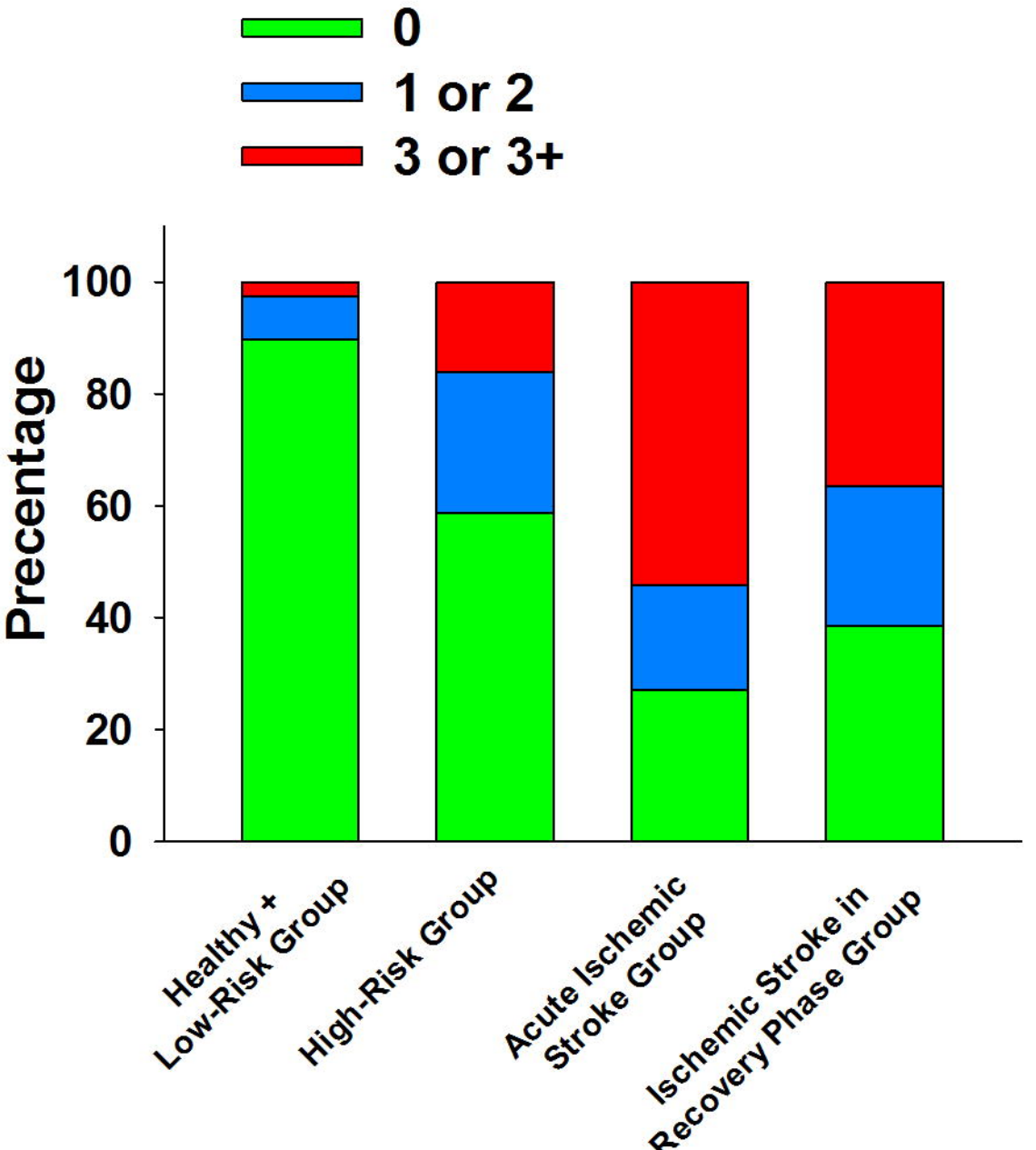

Healthy + Low-Risk Group
High-Risk Group
Acute Ischemic Stroke Group
Ischemic Stroke in Recovery Phase Group
AF Asymmetry
(% of Healthy + Low-Risk Group)
800
700
600
500
400
300
200
100
0
*
*
Dorsal Side of Index Finger

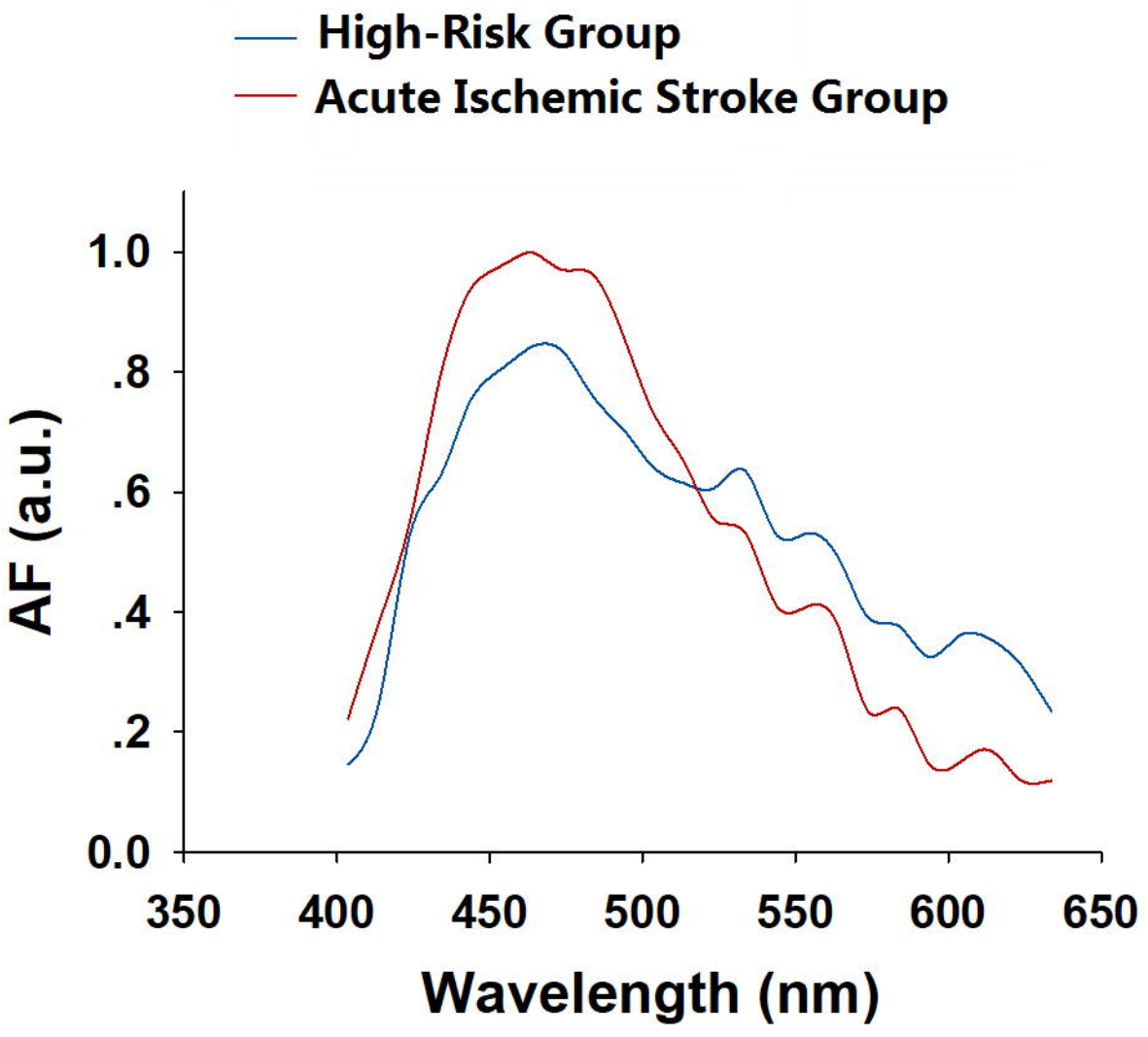

0
1
2
3 or 3+
Precentage
100
80
60
40
20
0
Healthy + Low-Risk Group
High-Risk Group
Acute Ischemic Stroke Group
Ischemic Stroke in Recovery Phase Group


Index Fingernail
Ventroforefinger
Dorsal Antebrachium
Centremetacarpus
Dorsal Side of Index Finger
Ventriantebrachium
R L R L R L R L R L R L
Healthy Group + Low-Risk Group
High-Risk Group
Acute Ischemic Stroke Group
Ischemic Stroke in Recovery Phase Group
50%
40%
30%
20%
10%
0


0
1
2 or 2+
Precentage
100
80
60
40
20
0
Healthy + Low-Risk Group
High-Risk Group
Acute Ischemic Stroke Group
Ischemic Stroke in Recovery Phase Group


Index Fingernail
Ventroforefinger
Dorsal Antebrachium
Centremetacarpus
Dorsal Side of Index Finger
Ventriantebrachium
Healthy Group + Low-Risk Group
High-Risk Group
Acute Ischemic Stroke Group
Ischemic Stroke in Recovery Phase Group
40%
30%
20%
10%
0


0
1 or 2
3 or 3+
Precentage
100
80
60
40
20
0
Healthy + Low-Risk Group
High-Risk Group
Acute Ischemic Stroke Group
Ischemic Stroke in Recovery Phase Group


High-Risk Group
Acute Ischemic Stroke Group
AF (a.u.)
1.0
.8
.6
.4
.2
0.0
350
400
450
500
550
600
650
Wavelength (nm)

Factors	High-Risk Group (n=20-26)	Actue Ischemic Stroke (n=30-33)	*P* value
Hypertension	22 (84.61)	26 (81.82)	0.5
Diabetes	9 (34.61)	10 (30.03)	0.366
INR	0.95 ± 0.01	0.95 ± 0.02	0.43
UA (µmol/L)	299.77 ± 16.57	306.48 ± 15.57	0.38
PLT (*10^9/L)	195.63 ± 11.36	187.53 ± 8.61	0.28
Heart Rate	74.58 ± 0.97	76.45 ± 1.3	0.13
HCY (µmol/L)	14 ± 0.88	15.22 ± 0.89	0.18
Urea (mmol/L)	5.45 ± 0.33	5.06 ± 0.2	0.17
Cr (µmol/L)	68.31 ± 3.05	73.3 ± 3.47	0.15
Glc (mmol/L)	5.65 ± 0.23	6.39 ± 0.47	0.11
LDL (mmol/L)	2.38 ± 0.19	2.72 ± 0.16	0.08
GHb (%)	6.1 ± 0.2	6.6 ± 0.3	0.08

Continuous Variables: Mean ± SEM; Categorical Variables: Number (Percentage%); INR: International Normalized Ratio; UA: Uric Acid; PLT: Platelet Count; HCY: Homocysteine;CR: Serum Creatinine; Glc: Glucose; LDL: Low Density Lipoprotein; GHb: Glycated Hemoglobin

Factors		OR (95% CI)	*P* value
AF of Ventroforefinger	R	1.065 (1.007,1.126)	0.029
	L	1.110 (1.016,1.214)	0.021
AF of Index Fingernail	R	1.108 (1.009,1.218)	0.032
	L	1.073 (1.001,1.150)	0.047
AF of Dorsal Side of Index Finger	R	1.272 (1.047,1.545)	0.016
	L	1.196 (1.012,1.413)	0.036
AF of Centremetacarpus	R	1.081 (0.988,1.184)	0.089
	L	1.045 (0.974,1.121)	0.216
AF of Ventriantebrachium	R	1.051 (0.986,1.120)	0.127
	L	1.032 (0.986,1.080)	0.181
AF of Dorsal Antebrachium	R	1.067 (0.986,1.153)	0.106
	L	1.040 (0.970,1.115)	0.275
Number of the Regions with Increased AF Intensity		3.068 (1.386,6.790)	0.006