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The individual and combined effects of the angiotensin-converting enzyme gene insertion/deletion polymorphism and endurance training status on muscle oxygen saturation markers during incremental cycle ergometer test

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Abstract

Introduction: The angiotensin-converting enzyme gene insertion/deletion polymorphism (ACE I/D genotype) has been one of the first and most investigated possible gene marker for physical performance. However, studies investigating the association between the ACE I/D genotype and peak oxygen uptake (VO_{2peak}) were not conclusive (Papadimitriou et al., 2018; Rankinen et al., 2000; Sonna et al., 2001). On the contrary, studies which have focused on skeletal muscle had more success (Valdivieso et al., 2017; Vaughan et al., 2016; Vaughan, Huber-Abel, Graber, Hoppeler, & Flück, 2013; Zhang et al., 2003). Muscle monitors based on near-infrared spectroscopy (NIRS) offer the possibility of non-invasive measurement of skeletal muscle oxygenation during exercise. Therefore, the aim of this study was to find out whether there are individual and combined effects of the ACE I/D genotype and endurance training status on markers of muscle oxygen saturation (SmO_2) during ramp test.

Methods: 21 non-specific trained subjects completed an incremental cycle ergometer test (ramp test) and were genotyped for the ACE I/D genotype. During ramp test VO_{2peak} was measured with a breath-by-breath system. SmO_2 of the *m. vastus lateralis* was measured using a NIRS device. The individual and combined effects of the ACE I/D genotype and endurance training status on SmO_2 markers during ramp test were analysed using two factor analysis of variance (ANOVA).

Results: ACE I/D genotype did not have a significant effect on any of the SmO_2 markers during ramp test. Endurance training status had a significant effect on the markers $SmO_{2\ min}$ (p = 0.017) and $\Delta_{deoxygenation}$ (p = 0.033) and a highly significant effect on the marker $\Delta_{1/2\ reoxygenation}$ (p = 0.007). In addition, there was a significant interaction effect between the ACE I/D genotype and training status on the marker $slope_{deoxygenation}$ (p = 0.028).

Discussion and Conclusion: Possible confounders, such as gender and training status, may have influenced a potential individual effect of the ACE I/D genotype. The observed greater muscle deoxygenation and reoxygenation in trained subjects could indicate an increased oxidative capacity of the *m. vastus lateralis*. The highly significant steeper $slope_{deoxygenation}$ for trained subjects only found within the I/I genotype could indicate a higher percentage of slow-twitch type I muscle fibres and thus a higher mitochondrial density. Based on these results it could be speculated that carriers of the I/I genotype could result in greater adaptability in a longitudinal training study. This pilot study may serve to obtain insightful data into the possibility of developing specific training programs *ad hoc* for different patient characteristics.

1 Introduction

Physical performance can be seen as a result from the interaction of nature with nurture. In the context of physical performance, the term nature can be further described as genetics, while the term nurture describes environmental factors such as physical training and nutrition. The human genetic material, also known as genome, consists of deoxyribonucleic acid (DNA) and is located in the nucleus of the cell as well as in the mitochondria (Roth, 2007). Minor DNA sequence variations contribute to the phenotypic differences, such as anatomy, physiology and disease predisposition (Roth & Thomis, 2011). An important goal in the field of exercise genomics is to identify potential gene candidates that may have an influence on physical performance and exercise adaptation. Up to 2009, there are 214 autosomal, seven gonosomal and 18 mitochondrial candidate genes that have been shown to influence physical performance (Bray et al., 2009). Among them, sequence variations within the angiotensin-converting enzyme (ACE) gene have been one of the first and most investigated gene markers for physical performance. The ACE gene contains a DNA sequence variation in form of an insertion/deletion (I/D) polymorphism. An I/D polymorphism is characterized by the presence or absence of an entire fragment of a DNA sequence (Roth & Thomis, 2011). Within the ACE gene, there is a gene variant in which a 287 base pair long Alu repeat DNA fragment is present (I allele) and there is a gene variant in which this DNA fragment is absent (D allele). Accordingly, there are three possible ACE I/D genotypes: I/I genotype, I/D genotype and D/D genotype.

1.1 The ACE I/D genotype distribution

There is evidence that the distribution of the ACE I/D genotype is associated with ethnic origin. In Europe, 80 healthy Caucasians (38 males and 42 females) were found to have an allele frequency of 0.4 for the I allele and 0.6 for the D allele (Rigat et al., 1990). In other words, 40 out of 100 European Caucasians carried the 287 base pair long Alu repeat DNA fragment while 60 out of 100 Europeans did not carry this insertion. According to Barley et al. (1994), the frequency ratio for Caucasian Europeans is 1:2:1 for the I/I, I/D and D/D genotype, respectively. Interestingly, the authors found that black Nigerians showed a tendency towards a higher frequency of the D allele, while Samoan Polynesians and Yanomami Indians showed a much higher frequency of the I allele. In addition, Foy et al. (1996) found a lower frequency of the D allele in 305 Pima Indians from Arizona compared to 80 Caucasian Americans. Furthermore, Barley et al. (1996) determined the ACE I/D genotype in a total of 320

subjects including 210 Caucasian Europeans and 110 black Afro-Caribbean. The distribution of the ACE I/D genotype in the Caucasian Europeans again corresponded to a ratio of approximately 1:2:1 for the I/I, I/D and D/D genotype, respectively. In comparison, a significantly higher frequency of the D allele was found in Afro-Caribbean people. In the United Kingdom, Sagnella et al. (1999) determined the ACE I/D genotype of a total of 1577 women and men. The investigated population consisted of 462 Caucasians, 462 Africans and 442 South Asians. The ACE I/D genotype frequency of the Caucasians and Africans was similar. However, the frequency of the I/I genotype was significantly higher in the South Asians.

1.2 The ACE I/D genotype and ACE concentration in the blood serum

It has been shown that the ACE I/D genotype correlates with the ACE concentration in blood serum. There is again evidence that this depends on ethnic origin. For example, Rigat et al. (1990) found that the ACE I/D genotype explained 47 % of the total phenotypic variance of blood serum ACE levels in 80 healthy Caucasians and that carriers of the I/I genotype showed the lowest and carriers of the D/D genotype showed the highest ACE concentration in blood serum (I/I: 299.3 ± 49.0 $\mu g \cdot L^{-1}$, I/D: 392.6 ± 66.8 $\mu g \cdot L^{-1}$, D/D: 494.1 ± 88.3 $\mu g \cdot L^{-1}$). Furthermore, in a study by Agerholm-Larsen, Tybjaerg-Hansen, Schnohr, and Nordestgaard (1999), in which a much larger study population was investigated (n = 869), the ACE I/D genotype explained 30-40 % of the total variation of ACE activity in blood serum in Scandinavian Caucasians. On the contrary, in Kenyans (Scott et al., 2005) the ACE I/D genotype explained 13 % and in Pima Indians (Foy et al., 1996) only 6.5 % of ACE activity in the blood serum. Furthermore, neither African Americans (Bloem, Manatunga, & Pratt, 1996) nor black South Africans (Payne et al., 2007) have shown any correlation between ACE I/D genotype and ACE activity in blood serum. According to Woods (2009) it can therefore be assumed that there are ethnic differences in the transcriptional regulation of ACE. Nevertheless, the ACE I/D genotype is an important and consistent marker for ACE activity in blood serum in different Caucasian populations, with the ACE concentration being consistent lowest in carriers of the I/I genotype, intermediate in carriers of the I/D genotype and highest in carriers of the D/D genotype (Woods, 2009).

1.3 ACE within the renin-angiotensin system

ACE plays an important role within the renin-angiotensin system. The renin-angiotensin system is an important control system for the blood pressure and fluid balance of the body (Sparks, Crowley, Gurley, Mirotsou, & Coffman, 2014). Within the renin-angiotensin system,

ACE produces the vasoactive Angiotensin II by cleaving 2 amino acids from the c-terminus of the inactive Angiotensin I (Corvol, Williams, & Soubrier, 1995). Angiotensin II further binds to specific angiotensin receptors to trigger a broad range of biological actions including vasoconstriction (A. Jones & Woods, 2003; Sparks et al., 2014). ACE also catalyses the inactivation of bradykinin, which is a potent vasodilator (A. Jones & Woods, 2003). In summary, ACE simultaneously produces the potent vasoconstrictor Angiotensin II and inactivates the potent vasodilator bradykinin (A. Jones & Woods, 2003).

1.4 The ACE I/D genotype and its associations with physical performance

Over the last 20 years, many studies have been published showing an association between the ACE I/D genotype and human physical performance. In general, the I allele has been associated with increased endurance performance, while the D allele has been associated with increased strength and power performance (Puthucheary et al., 2011).

One of the first studies showing an association between the ACE I/D genotype and physical performance was a study by Montgomery et al. (1997). The authors investigated whether there was an association between the ACE I/D genotype and the left ventricular hypertrophy in Caucasian military recruits in response to training. They found that a higher left ventricular hypertrophy resulted in carriers of the D/D genotype than in carriers of the I/I genotype. In addition, Montgomery et al. (1998) investigated the association between the ACE I/D genotype and physical performance in two parallel experiments. In the first experiment, they investigated 33 British elite mountaineers which were able to ascend beyond 7’000 m without using supplementary oxygen. They found, that among the 15 climbers who had successfully ascended beyond 8’000 m without using supplementary oxygen, none was carrier of the D/D genotype. Furthermore, they stated that the top performer was carrier of the I/I genotype. In a second experiment, they determined the ACE I/D genotype in 123 Caucasian British army recruits of which 78 completed an identical ten-week basic training program. Before and after the training period they assessed the maximum duration in which the recruits could perform repetitive elbow flexion while holding a 15 kg barbell. Pre-training performance was not dependent on the ACE I/D genotype. After the basic training program the exercise duration improved significantly for the recruits with the I/I and I/D genotype. This was not the case for the carriers of the D/D genotype. After these initial studies, there were many other studies, especially cross-sectional studies, which investigated the association between the ACE I/D genotype and physical performance.

1.4.1 Cross sectional studies and the ACE I/D genotype. Gayagay et al. (1998) investigated the ACE I/D genotype frequency of 64 Australian national rowers. The authors found an excess of the I allele in the rowers group when compared with a normal population. In addition, Myerson et al. (1999) examined the ACE I/D genotype distribution in 91 British Olympic-standard runners. The investigated group included runners competing in distances ranging from 100 m (sprinters) to 100 km (ultramarathon runners). In this case the authors found that the I allele frequency increased with running distance. In a study by Alvarez et al. (2000) the ACE I/D genotype was determined in 60 professional athletes (25 cyclists, 20 long-distance runners, and 15 handball players) and in 400 control participants. It could be shown that the I allele occurred in a significantly higher frequency in the group of elite athletes compared to the control group. Similarly, Woods et al. (2001) determined the ACE I/D genotype in 56 elite Caucasian swimmers from the European and Commonwealth championships and in 47 non-elite swimmers from an American college standard team. The authors compared the determined allele frequencies with several control groups. They found that there was a significant excess of the D allele in the elite swimmers when compared to the control group. Furthermore, this association remained in the short distance swimmers (< 400 m) but not in the swimmers competing over longer distances (> 400 m). Moreover, Nazarov et al. (2001) determined the ACE I/D genotype in 217 Russian athletes (swimmers, skiers, triathletes and track-and-field participants). In this case, the authors divided the athletes in distinct groups based on the physical performance (outstanding or average) and the duration of the event (short, middle or long distance). They found an excess of the D allele in the outstanding short distance athletes and an excess of the I allele in the outstanding middle distance athletes group. Collins et al. (2004) determined the ACE I/D genotype in 447 triathletes and in 199 male control subjects. The examined triathletes had completed the South African Ironman Triathlon either in 2000 or 2001. The authors found that there was a significantly higher I allele frequency in the 100 fastest triathletes compared to the 166 South Africa-born control subjects. Tsianos et al. (2004) hypothesized that the I allele frequency is elevated in swimmers competing over longer distances. To investigate this they formed two groups. The first group consisted of short long-distance swimmers (5-10 km) and the second group of long long-distance swimmers (25 km). It could be shown that there was a significant difference in the ACE I/D genotype frequency between the two groups. Juffer et al. (2009) determined the ACE I/D genotype in 54 professional footballers. The results of the football players were compared with those of 52 male elite endurance athletes and those of 123 inactive healthy control subjects. The authors found a higher frequency of the I/D genotype and a lower fre-

quency of the I/I genotype among footballers compared to the elite endurance athletes. Wang et al. (2013) investigated whether the ACE I/D genotype is associated with the elite swimmer status. The cohort consisted of 200 elite Caucasian swimmers from Europe, the Commonwealth, Russia and America and 326 elite swimmers of Japanese and Taiwanese origin. The authors found that the ACE I/D genotype was associated with the swimmer status in the group of Caucasian. The D allele was overrepresented in the short and medium distance swimmers. In addition, the ACE I/D genotype was associated with the swimmer status in the group of East Asian countries. In this group, however, the I allele was overrepresented among the short-distance swimmers. In addition, Papadimitriou et al. (2016) have shown that the ACE I/D genotype may influences sprint performance. The authors collected the personal best times in the disciplines 100, 200 and 400 m of elite sprinters from ten different countries (Caucasian and sprinters of African origin). They found that Caucasian sprinters with the D/D genotype performed better on average over 200 and 400 m than sprinters with the I/I genotype.

1.4.2 No association between ACE I/D genotype and physical performance. It is noteworthy to mention that there are also studies that have not found an association between the ACE I/D genotype and physical performance. For example, Taylor, Mamotte, Fallon, and van Bockxmeer (1999) compared the ACE I/D genotype frequency of 120 Caucasian athletes from different national teams of Australia with a control group of healthy subjects. The authors only included sports, which demand a high level of aerobic fitness such as cycling, track and field, swimming and rowing. In this study, they found no significant difference between the investigated groups. In addition, Karjalainen et al. (1999) investigated whether the left ventricular mass in athletes was associated with genes that encode renin-angiotensin system components. The authors measured the left ventricular mass and determined the ACE I/D genotype of 50 male and 30 female elite endurance athletes. They found no association between the ACE I/D genotype and left ventricular mass. Furthermore, Rankinen et al. (2000) compared the ACE I/D genotype of 192 male endurance athletes who had a peak oxygen uptake (VO_{2peak}) $\geq$ 75 $mL \cdot min^{-1} \cdot kg^{-1}$ with 189 inactive male control subjects. Both the genotype frequency and the allele frequency were not significantly different between the two investigated groups. Further analyses showed no overrepresentation of the I allele in athletes with the highest VO_{2peak} (> 80 $mL \cdot min^{-1} \cdot kg^{-1}$). This finding is supported by the results of the study by Sonna et al. (2001), in which the authors determined the ACE I/D genotype in 85 female and 62 male US Army recruits. In addition, they determined the VO_{2peak} before and after eight

weeks of basic training as well as standardized measurements of muscular endurance (sit-ups and push-ups) and a two-mile run within the Army Physical Fitness Test. The VO_{2peak} of the three ACE I/D genotype groups (I/I vs. I/D vs. D/D) did not differ significantly either before or after the basic training. Subjects carrying the I/I genotype achieved better results in Army Physical Fitness Test, but not to a significant extent. Similarly, Woods et al. (2002) found no association between the VO_{2peak} of 58 army recruits and the ACE I/D genotype. In this study, the authors tested the VO_{2peak} before and after 11 weeks of identical training. There was no significant difference between the I/I genotype and the D/D genotype before and after training. Therefore, the authors concluded that associations between ACE I/D polymorphism and increased endurance performance are not due to differences in the cardiorespiratory response to training. Interestingly, Interestingly, Bueno et al. (2016) investigated the influence of the ACE I/D genotype on markers of aerobic fitness. The authors examined the VO_{2peak}, aerobic capacity and running efficiency of 150 physically active young men. The VO_{2peak} and the aerobic capacity were measured using an incremental treadmill test. In order to determine running efficiency, two constant speed tests were carried out. None of the cardiorespiratory variables showed a significant association. Only a tendency towards higher VO_{2peak} in the I/I genotype compared to the I/D and DD genotype could be shown. A recent study by Papadimitriou et al. (2018) also found no association between the ACE I/D genotype and running times of 698 Caucasian endurance athletes from six countries (United Kingdom, Italy, Poland, Greece, Russia and Australia). The authors collected a total of 1064 personal best times in the disciplines 1’500, 3’000, 5’000 m and marathon. They did not find any association with the ACE I/D genotype in any of the disciplines.

The studies listed above show that the lack of association to the ACE I/D genotype is often associated with the measurement of whole-body VO_{2peak}. On the contrary, studies which have focused on skeletal muscle had more success in finding associations to the ACE I/D genotype.

1.4.3 Skeletal muscle studies and the ACE I/D genotype. Williams et al. (2000) investigated the efficiency of muscular contraction in 58 male Caucasian recruits before and after eleven-week of a primarily aerobic training program. In order to measure the efficiency of muscular contraction, the authors measured the steady-state VO_2 and the respiratory exchange ratio while the subjects were pedalling on a bicycle ergometer with a constant pedal cadence of 60 rpm for three minutes on three successive external power output stages (40, 60 and 80 W). Delta efficiency was defined as the percentage of the ratio of the change in the amount of work done per minute to the change in the energy expended per minute. Before the training,

delta efficiency was independent of genotype. After the training, however, delta efficiency was strongly genotype-dependent, whereby delta efficiency improved significantly only in carriers of the I/I genotype. The authors hypothesized that the relationship between the I/I genotype and the improved efficiency of exercising muscles is probably related to an increase in slow-twitch type I muscle fibres. Interestingly, Zhang et al. (2003) investigated the association between the ACE I/D genotype and skeletal muscle fibre types in 41 untrained healthy subjects. The authors found that carriers of the I/I genotype had a significantly higher percentage of slow-twitch type I fibres than carriers of the D/D genotype. Other authors who investigated the association between the ACE I/D genotype and the cellular and molecular properties of the skeletal muscle were also successful. For example, Vaughan et al. (2013) found that carriers of the I/I and I/D genotype showed a significantly higher mitochondrial density, intracellular lipid volume and a stronger expression of genes that are important in the metabolism of fatty acids as an adaption to endurance exercise when compared to carriers of the D/D genotype. In addition, Vaughan et al. (2016) investigated the association between the ACE I/D genotype and the changes of exercise-induced capillarisation of the skeletal muscle. The study examined 52 non-specifically trained Caucasian men who had either completed a one-legged cycling test until volitional fatigue and or participated in a marathon. The authors found that the capillary-to-fibre ratio in the *m. vastus lateralis* was higher for carriers of the I/I and I/D genotype than for carriers of the D/D genotype. Recently, Valdivieso et al. (2017) investigated the influence of ACE I/D genotype, endurance training status and its interaction on metabolic and angiogenic adaptations in the *m. vastus lateralis*. The authors examined 34 endurance trained (VO_{2peak} > 50 mL·min^{-1}·kg^{-1}) and 18 untrained (VO_{2peak} < 50 mL·min^{-1}·kg^{-1}) male Caucasians who had performed a one-legged cycling test until volitional fatigue. They found that the capillary density and the capillary-to-fibre ratio were dependent on the ACE I/D genotype. Furthermore, interaction effects of the ACE I/D genotype and endurance training status on the cross-sectional area (CSA) and mean cross-sectional area (MCSA) of slow-twitch type I fibres of the *m. vastus lateralis* were found. Untrained carriers of the I allele had a larger CSA than untrained non-carriers of the I allele. Furthermore, trained I allele carriers showed a larger slow-twitch type I MCSA than trained non-carriers of the I allele.

Based on these results, it could be concluded that the influence of ACE I/D genotype is more likely to be found on a skeletal muscle level. In order to answer the research questions concerning cellular and molecular skeletal muscle characteristics, the invasive intervention of the muscle biopsy is necessary. Since this is a cost-intensive and invasive procedure, this intervention is difficult to implement, especially in professional sports. Fortunately, there are also non-invasive techniques to investigate skeletal muscle characteristics. Interestingly, muscle monitors based on near-infrared spectroscopy (NIRS) offer the possibility of non-invasive measurement of skeletal muscle oxygenation during exercise.

1.5 The use of NIRS to study skeletal muscle oxygenation

NIRS for monitoring muscle tissue oxygenation was first described by Jobsis (1977). Since then, NIRS has been used to investigate muscle oxidative metabolism at rest and during different types of exercise (Ferrari, Muthalib, & Quaresima, 2011). A simple NIRS device consists of a light source in the near-infrared (650-1000 nm) range, which emits light of minimum two different wavelengths and a detector (S. Jones, Chiesa, Chaturvedi, & Hughes, 2016). NIRS is based on the principle that absorption of near-infrared light differs from oxygenated and deoxygenated haemoglobin and myoglobin (S. Jones et al., 2016). Therefore, muscle oxygen monitors using the NIRS technique offer the possibility of non-invasive measurement of muscle oxygen saturation (SmO_2). SmO_2 represents the dynamic balance between O_2 supply and O_2 consumption of the examined muscle (Ferrari et al., 2011). NIRS devices have been used to measure muscle deoxygenation during exercise as well as the reoxygenation kinetics after exercise. The muscle deoxygenation during exercise is mainly dependent on the O_2 consumption of mitochondria (Muthalib, Millet, Quaresima, & Nosaka, 2010). The reoxygenation kinetics has been shown to correlate with muscle oxidative capacity (Puente-Maestu et al., 2003)

During an incremental cycle ergometer test (ramp test) it could be shown that the SmO_2 decreased progressively and reached its physiological minimum when VO_{2peak} was reached (Belardinelli, Barstow, Porszasz, & Wasserman, 1995). Moreover, it has been shown that gender (Murias, Keir, Spencer, & Paterson, 2013), endurance training status (Boone, Koppo, Barstow, & Bouckaert, 2009) and different training modalities (Jacobs et al., 2013; Takagi et al., 2016) influences muscle deoxygenation during ramp test. In addition, studies have shown that the reoxygenation kinetics depends on the endurance training status (Ding et al., 2001) and changes with training (Puente-Maestu et al., 2003).

In the context of the ACE I/D genotype, it has been shown that the D/D genotype is associated with an impairment of peripheral tissue oxygenation in patients with chronic obstructive pulmonary disease (COPD) (Kanazawa, Otsuka, Hirata, & Yoshikawa, 2002). However, to date, to my knowledge, no study has investigated the individual and combined effects of the ACE I/D genotype and endurance training status on SmO_2 markers during a ramp test in healthy subjects using a non-invasive NIRS device.

1.6 Objective

The aim of this study is to find out whether there are individual and combined effects of the ACE I/D genotype and endurance training status on SmO_2 markers during a ramp test. On account of this, the following specific research questions were formulated:

a) Is there an effect of the ACE I/D genotype on SmO_2 markers during a ramp test?

b) Is there an effect of the endurance training status on SmO_2 markers during a ramp test

c) Is there an interaction effect between the ACE I/D genotype and the endurance training status on SmO_2 markers during a ramp test?

2 Methods

2.1 Study design

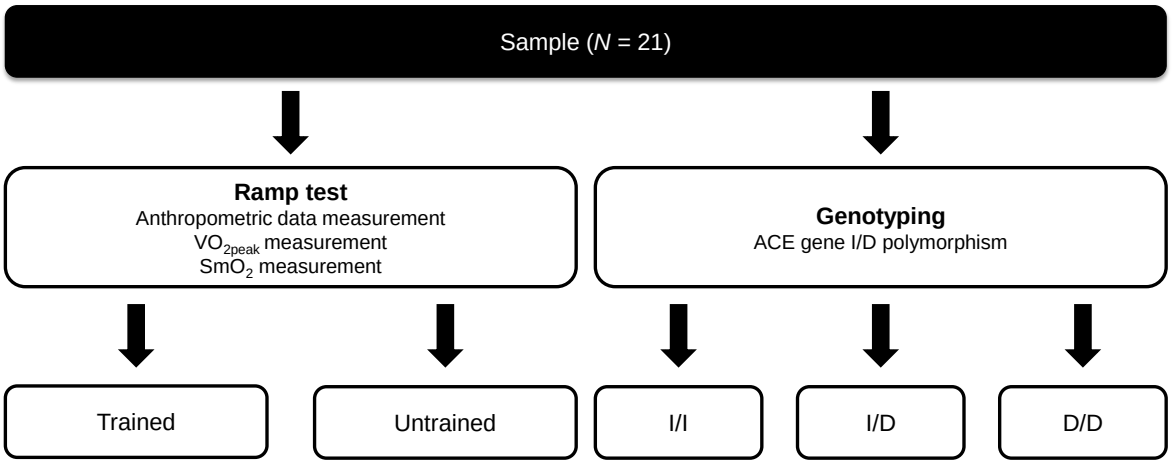

Figure 1. Study design. All subjects (*N* = 21) performed a ramp test and were genotyped for the ACE gene I/D polymorphism. For statistical analysis subjects were grouped according to endurance training status and ACE I/D genotype. Ramp test = incremental cycle ergometer test, VO_{2peak} = peak oxygen uptake, SmO_2 = muscle oxygen saturation, Trained = VO_{2peak} > 50 mL·min^{-1}·kg^{-1}, Untrained = VO_{2peak} < 50 mL·min^{-1}·kg^{-1}, ACE = angiotensin-converting enzyme, I/D = insertion/deletion.

The present study was a cross-sectional study in which the subjects performed a ramp test and were genotyped for the ACE gene I/D polymorphism (Figure 1). Prior to the ramp test, the anthropometric data (weight and height) of the subjects were collected. The ramp test was used to measure whole-body VO_{2peak} and the SmO_2 of the knee extensor muscle *m. vastus lateralis*. VO_{2peak} was used to differentiate between those who were endurance trained (Trained: VO_{2peak} > 50 mL·min^{-1}·kg^{-1}) and those who were endurance untrained (Untrained: VO_{2peak} < 50 mL·min^{-1}·kg^{-1}) as described by Valdivieso et al. (2017). Genotyping was performed retrospectively.

2.2 Subjects

21 non-specific trained women and men were recruited for the present study (Table 1). The inclusion criteria were as follows: a) healthy; b) cardiovascular unobtrusive; c) at the age of 20-70 years. After being informed about the objective and the protocol of the study, all subjects signed a declaration of consent (see Appendix). In order to ensure that they were physically healthy to pass the test, they completed a health questionnaire (see Appendix). In addition, a resting electrocardiography measurement was performed and checked by a physician for cardiovascular abnormalities. The study has been approved by the Ethics Committee of the Canton of Zurich. All investigations were conducted in accordance with the ethical standards of the Declaration of Helsinki of 1964.

Table 1
Anthropometric data of the subjects (N = 21)

Age in [years]	**Weight in [kg]**	**Height in [m]**	**BMI in [kg/m^2]**
Female (*n* = 9)			
25.67 ± 4.67	63.50 ± 7.66	1.67 ± 0.06	22.71 ± 1.99
Male (*n* = 12)			
28.08 ± 4.17	76.84 ± 5.32	1.80 ± 0.05	23.82 ± 2.17

Note. Anthropometric data are presented as mean ± standard deviation.

2.3 Ramp test

The ramp test was performed on an electrically braked cycle ergometer (ergoselect 200, ergoline, Bitz, Germany) in an air-conditioned laboratory (Figure 2). To determine the VO_{2peak} the gas exchange was measured with a breath-by-breath system (meta control 3000, Cortex, Leibzig, Germany). All Subjects performed the test in an upright sitting position on a self-paced pedal cadence in the range of 70 to 100 rpm. The ramp test protocol corresponded to a modified version of the protocol of Whipp, Davis, Torres, and Wasserman (1981). After a three-minute pre-rest period, in which the subjects had to sit on the cycle ergometer without moving the pedals, the initial power (75 W for women and 100 W for men) was increased every 20 s (18 $W \cdot min^{-1}$ for women and 30 $W \cdot min^{-1}$ for men). The test was stopped if volitional fatigue was reached or until the target pedal cadence could not be longer maintained. After the test stoppage, there was an additional eight-minute post-rest period, in which the subjects had to sit on the cycle ergometer without moving the pedals.

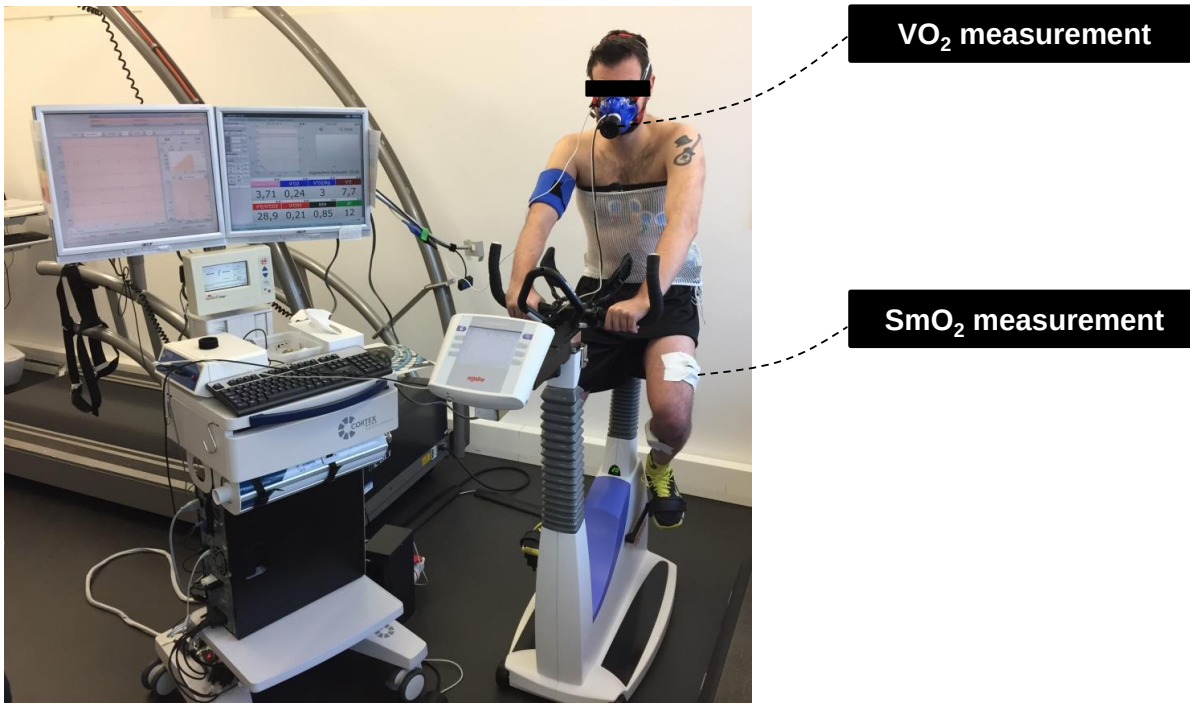

Figure 2. Incremental cycle ergometer test (ramp test) equipment. VO_2 = oxygen uptake, SmO_2 = muscle oxygen saturation.

2.4 NIRS measurement

A muscle oxygen monitor (Moxy, Fortiori Design LLC, Minnesota, United States), based on the technology of NIRS, was used to measure SmO_2 non-invasively. The Moxy Monitor uses four different light sources covering wavelengths in the range from 630 to 850 nm and the Beer-Lambert law to perform quantitative measurements of SmO_2 (Fortiori Design, 2015). SmO_2 refers to the percentage of haemoglobin and myoglobin that have bound oxygen in capillaries of the investigated muscle (Fortiori Design, 2015). SmO_2 is calculated with the following formula:

$$SmO_2 = \left(\frac{Oxygenated\ haemoglobin + myoglobin}{Total\ amount\ of\ haemoglobin + myoglobin}\right) \cdot 100$$

The Moxy Monitor was placed on the *m. vastus lateralis* of the left leg of the subjects. The sensor was placed 10 cm above the upper lateral point of the patella along the axis of the leg (Figure 3). Prior to the placement, the skin site was shaved using a disposable razor (Gallant, Dynarex, Orangeburg, United States) and cleaned with an alcohol swab (Webcol™, Covidien™, Dublin, Ireland). The sensor attachment was carried out using an attachment tape (Moxy Adhesive Attachments, Fortiori Design LLC, Minnesota, United States). In order to protect the NIRS device from ambient light it was covered with an adhesive non-woven fabric (Hypafix®, BSN medical, Hamburg, Gerrmany).

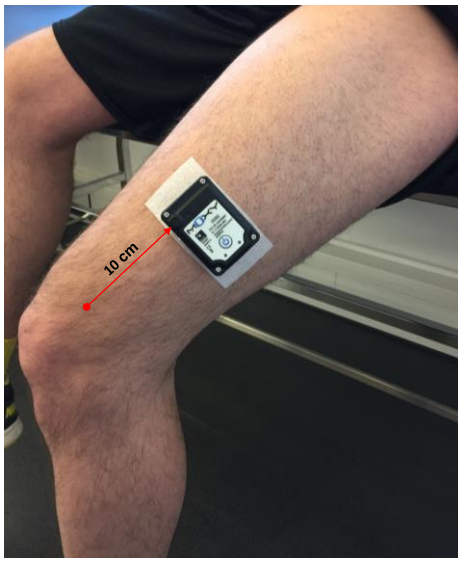

Figure 3. Moxy monitor placement. The sensor was placed 10 cm above the upper lateral point of the patella along the axis of the leg.

2.5 Genotyping

Genomic DNA (gDNA) was collected by buccal swabs. The subjects were told not to consume any food or to drink in the 30 min prior to gDNA collection to avoid contamination of the sample. To collect the gDNA sample, the buccal swab was firmly scraped against the inside of each cheek six times. After collection the buccal swab was air-dried in a laboratory fume cupboard (Secuflow 1500, Waldner, Wangen, Germany) for 2 hours and stored at + 4 °C.

gDNA extraction procedure was performed according to a commercially available protocol (QIAamp® DNA Mini Kit, Qiagen, Hilden, Germany). The cotton swab was separated from the stick with scissors, placed in a 2 mL micro centrifuge tube and 400 µL of phosphate-buffered saline buffer solution was added. Degradation of ribonucleic acid (RNA) was performed by adding 4 µL of RNase A stock solution (100 $mg \cdot mL^{-1}$) to solution. Lysis was further carried out by adding 20 µL QIAGEN® proteinase K stock solution and 400 µL Buffer AL. The lysis mix was mixed immediately for 15 s and incubated at 56 °C for 10 min using a digital dry bath (AccuBlock™, Labnet International, Edison, United States). After incubation 400 µL of ethanol (96-100 %) was added to the sample and mixed again. The mixture was then applied to the QIAamp Mini spin column and placed in a 2 mL collection tube. To bind gDNA, the closed columns were centrifuged at 8000 rpm for 1 min using an air-cooled micro centrifuge (Prism™, Labnet International, Edison, United States). The QIAamp Mini spin column was then placed in a clean 2 mL collection tube and the tube containing the filtrate was discarded. The remaining mixture was added to the QIAamp Mini spin column and again centrifuged at 8000 rpm for 1 min. Washing was carried out in two steps. First, 500 µL AW1 buffed was added and centrifuged at 8000 rpm for 1 min. Second, 500 µL of AW2 buffer was added and centrifuged at full speed (14’000 rpm) for 3 min. In order to elute the DNA, 75 µL AE buffer was added, incubated at room temperature (20 °C) for 1 min, and then centrifuged at 8000 rpm for 1 min. This step was performed twice to increase the DNA yield. The sample (150 µL elute containing the gDNA) was then stored at + 4 °C for the genotyping step.

ACE I/D gene polymorphism genotyping was carried out through polymerase chain reaction (PCR) followed by high resolution melt (HRM) analysis using a real-time PCR system (Eco™, illumina®, San Diego, United States). The reaction mix included for one sample 1 µL distilled H_2O, 1 µL of $MgCl_2$ (25 mmol), 5 µL of KAPA HRM FAST Master Mix (2x) and 1 µL of the I or D allel-specific primer mix (2 µmol). The primer mix for the detection of the 66 bp amplicon, which is specific to the I allele, contained the primer ACE2 (5'-tgggattacaggcgtgatacag-3') and the primer ACE3 (5'atttcagagctggaataaaatt-3'). The primer mix for the detection of the 83 bp amplicon, which is specific to the D allele, contained primers ACE1 (5'-catcctttctcccatttctc-3') and ACE3 (5'atttcagagctggaataaaatt-3'). 8 µL of the reaction mix were pipetted into each well of the 48 well-plate and 2 µL of the prepared gDNA solution was added. The plate was then sealed to prevent evaporation. To remove any bubbles the plate was centrifuged. PCR and HRM were performed with the Eco™ machine settings shown in Table 2. Genotype analysis was carried out using a genetic variation analysis software (EcoStudy Version 5.0, illumina®, San Diego, United States).

Table 2
Eco™ machine settings

Step	**Temperature**	**Duration**	**Cycle**
Enzyme activation	95 °C	3 min	Hold
Denaturation	95 °C	5 s	40
Annealing-Extension	60 °C	30 s	
HRM Denaturation	95 °C	1 min	Hold
HRM	55 °C	15 s	-
HRM	95 °C	15 s	-

Note. Eco™ machine settings. HRM = high resolution melt.

2.6 Data processing

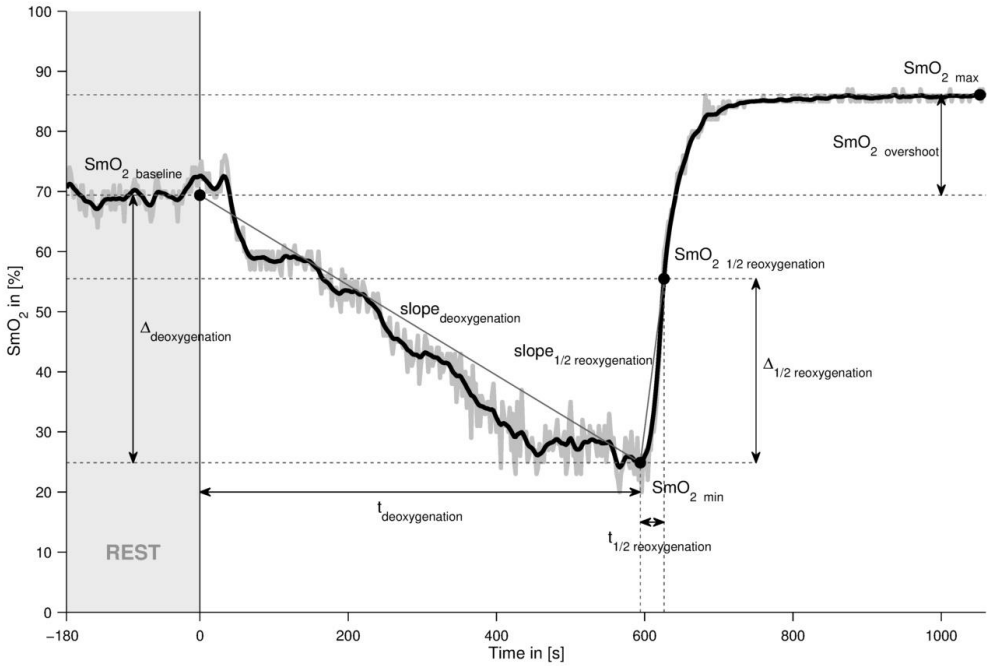

Figure 4. Representative example of the SmO_2 course during the ramp test including raw data, processed data and different markers. SmO_2 = muscle oxygen saturation, t = time, min = minimum, max = maximum.

A representative example of the SmO_2 course during the ramp test including raw data, processed data and different markers is shown in Figure 4. Data pre-processing and analysis was performed using a data processing program (MATLAB 2015a, The Mathworks, Natick, United States). SmO_2 data was filtered using a 2nd order zero-phase shift Butterworth low-pass filter with a cut-off frequency of 0.03 Hz. Data extraction was performed based on the description of NIRS signal interpretation by Ferrari et al. (2011). $SmO_{2\ baseline}$ represented the mean value of the three-minute pre-rest period. The minimum SmO_2 value during the ramp test ($SmO_{2\ min}$) was extracted by taking the last local minimum of the filtered SmO_2 prior to reoxygenation. $\Delta_{deoxygenation}$ was the difference between $SmO_{2\ baseline}$ and $SmO_{2\ min}$. $t_{deoxygenation}$ was the time from the beginning of the ramp test until $SmO_{2\ min}$ was reached. $slope_{deoxygenation}$ was calculated using $\Delta_{deoxygenation}$ over $t_{deoxygenation}$. $SmO_{2\ max}$ was defined as the highest value achieved within the period between the start of reoxygenation and test termination. $SmO_{2\ 1/2\ reoxygenation}$ was defined as 50 % of the difference between $SmO_{2\ max}$ and $SmO_{2\ min}$. $\Delta_{1/2\ reoxygenation}$ was the difference between $SmO_{2\ 1/2\ reoxygenation}$ and $SmO_{2\ min}$. $t_{1/2\ reoxygenation}$ was defined as the time between $SmO_{2\ min}$ and $SmO_{2\ 1/2\ reoxygenation}$. $Slope_{1/2\ reoxygenation}$ was calculated using $\Delta_{1/2\ reoxygenation}$ over $t_{1/2\ reoxygenation}$. $SmO_{2\ overshoot}$ represented the difference between $SmO_{2\ max}$ and $SmO_{2\ baseline}$.

2.7 Statistical analysis

An online calculator from Rodriguez, Gaunt, and Day (2009) was used in order to determine whether the observed genotype frequency is consistent with the Hardy-Weinberg equilibrium (HWE). To determine genotype-specific differences in SmO_2 markers during the ramp test, a codominant genetic model was selected (I/I vs. I/D vs. D/D). The statistical analysis was performed using statistical software (SPSS Statistics 22, IBM, Armonk, United States). To examine the variance homogeneity Levene test was carried out. Two factor analysis of variance (ANOVA) was used to investigate the individual and combined effects of the ACE I/D genotype and endurance training status on SmO_2 markers during ramp test. Least significant difference (LSD) of Fisher was used as a post hoc test. The significance levels were determined as follows: $p > 0.05$ = not significant, $p < 0.05$ = significant and $p < 0.01$ = highly significant. The effect size *d* was calculated according to Cohen (1992) and defined as follows: $d = 0.20$ = small effect, $d = 0.50$ = medium effect, $d = 0.80$ = large effect. All data are presented as mean ± standard deviation.

3 Results

3.1 No individual effect of the ACE I/D genotype on SmO_2 during ramp test

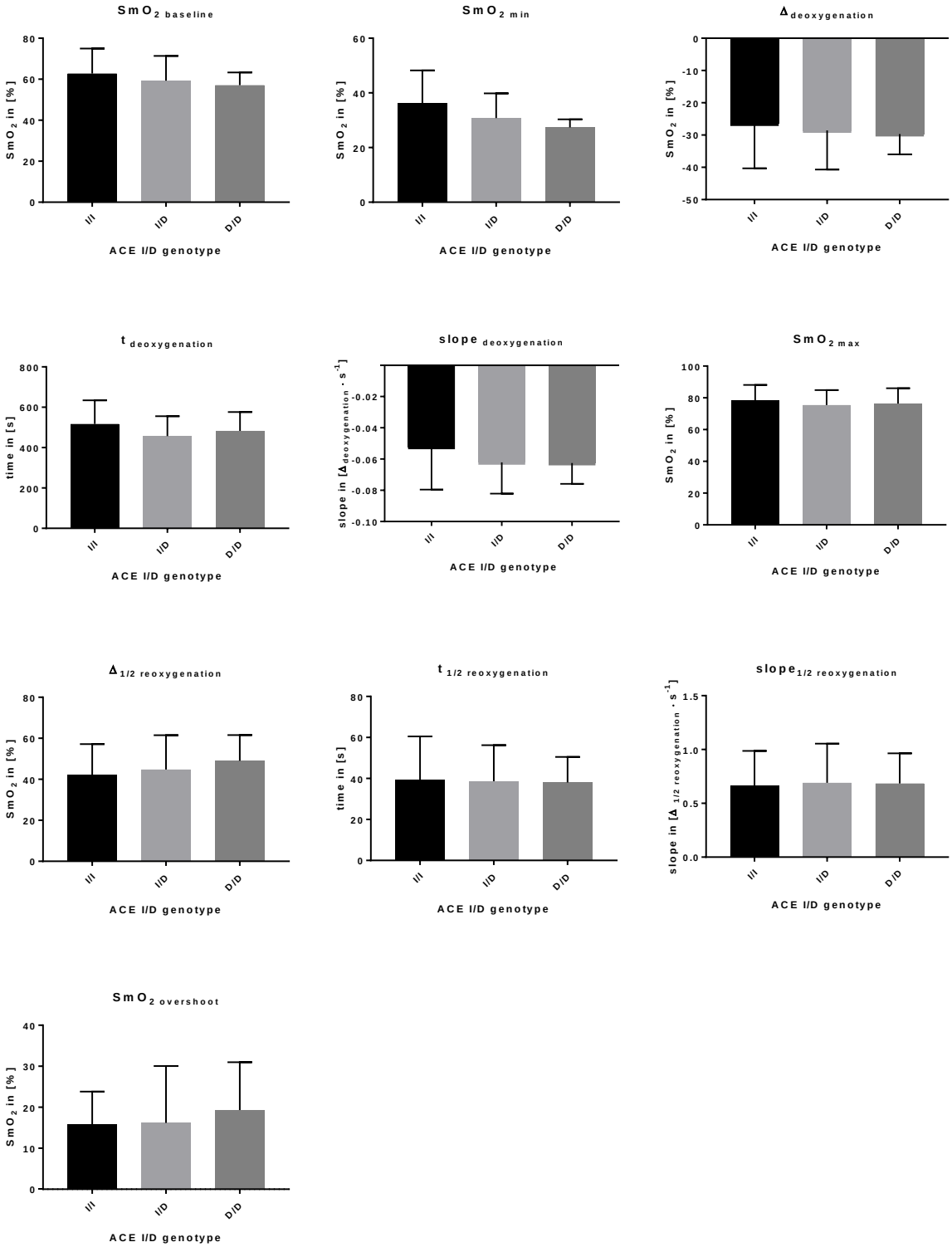

Figure 5. No individual effect of the ACE I/D genotype on SmO_2 markers during ramp test. Bar graphs represent mean ± standard deviation of extracted SmO_2 markers grouped by the ACE I/D genotype. Details of SmO_2 marker extraction are presented in section 2.6 Data processing. SmO_2 = muscle oxygen saturation, ACE = angiotensin-converting enzyme, I/D = insertion/deletion. I/I (*n* = 8), I/D (*n* = 10), D/D (*n* = 3).

ACE I/D genotype-grouped mean ± standard deviation values of the extracted SmO_2 markers are shown in Figure 5. The ACE I/D genotype alone did not have a significant effect on any of the extracted SmO_2 markers during ramp test. Individual *F* and *p* values are shown in Table 3 (see Appendix).

3.2 Effects of the endurance training status on SmO_2 during ramp test

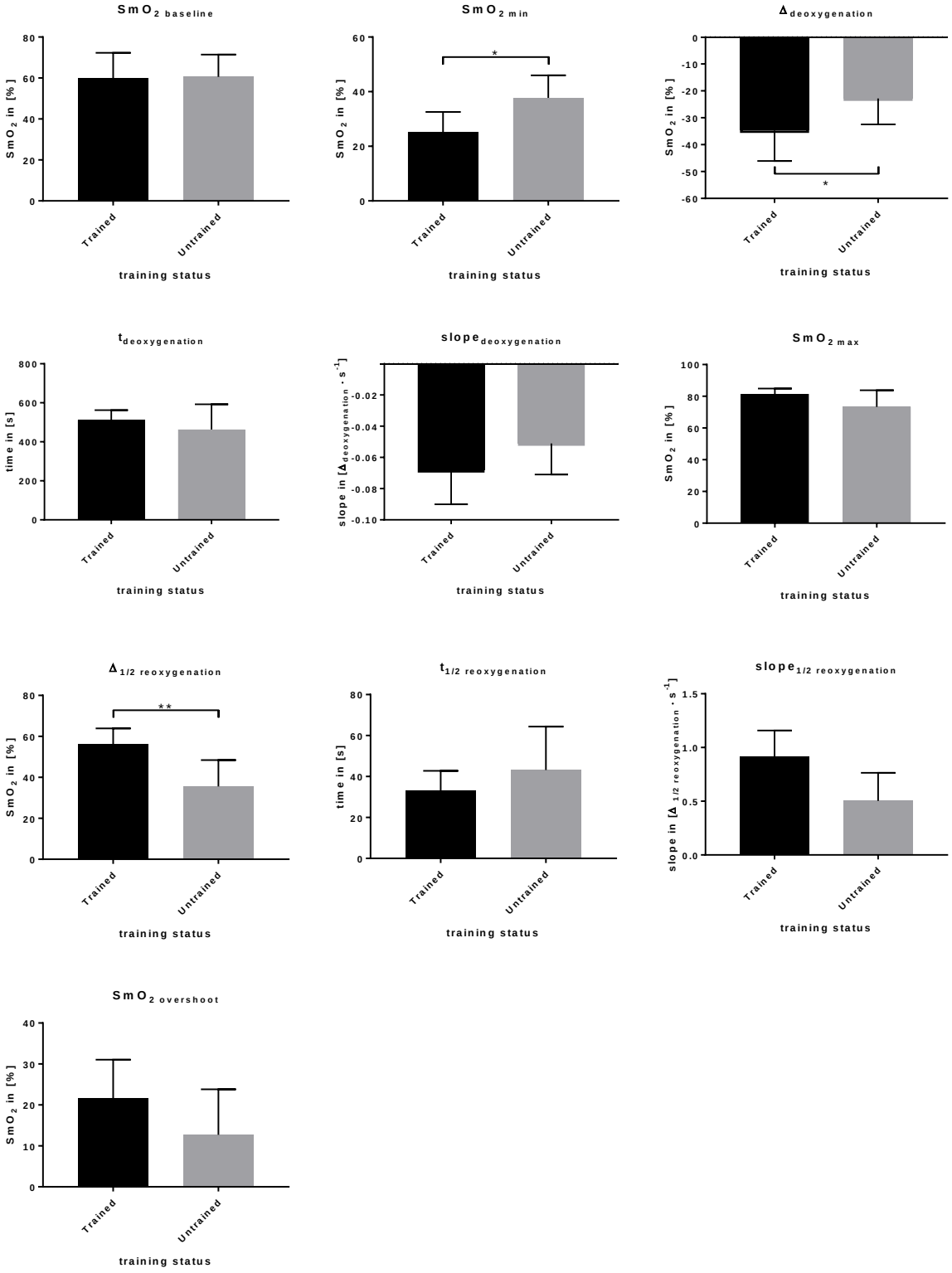

Figure 6. Effects of the endurance training status on SmO_2 markers during ramp test. Bar graphs represent mean ± standard deviation of extracted SmO_2 markers grouped by the endurance training status. Details of SmO_2 marker extraction are presented in section 2.6 Data processing. SmO_2 = muscle oxygen saturation, Trained = VO_{2peak} > 50 mL·min^{-1}·kg^{-1}, Untrained = VO_{2peak} < 50 mL·min^{-1}·kg^{-1}. Trained (n = 9), Untrained (n = 12). * indicates $p < 0.05$, ** indicates $p < 0.01$.

Endurance training status-grouped mean ± standard deviation values of the extracted SmO_2 markers are shown in Figure 6. There was a significant effect of the endurance training status on the marker $SmO_{2\ min}$ ($p = 0.017$) and $\Delta_{deoxygenation}$ ($p = 0.033$). The $SmO_{2\ min}$ was on average lower for the trained subjects compared to untrained subjects (25.24 ± 6.94 vs. 37.74 ± 7.86 %, respectively). $\Delta_{deoxygenation}$ was on average higher in trained than in untrained subjects (-34.72 ± 10.70 vs. -22.81 ± 9.25 %, respectively). In addition, there was a highly significant effect of the endurance training status on the marker $\Delta_{1/2\ reoxygenation}$ ($p = 0.007$). Trained subjects showed a higher $\Delta_{1/2\ reoxygenation}$ than untrained subjects (56.22 ± 7.22 vs. 35.54 ± 12.36 %, respectively).The effect sizes for the markers $SmO_{2\ min}$ ($d = 0.70$) and $\Delta_{deoxygenation}$ ($d = 0.61$) are to be classified as medium and for $\Delta_{1/2\ reoxygenation}$ ($d = 0.80$) as large (Cohen, 1992). In all other SmO_2 markers no significant effect of the endurance training status was found.

3.3 Interaction effect between the ACE I/D genotype and endurance training status

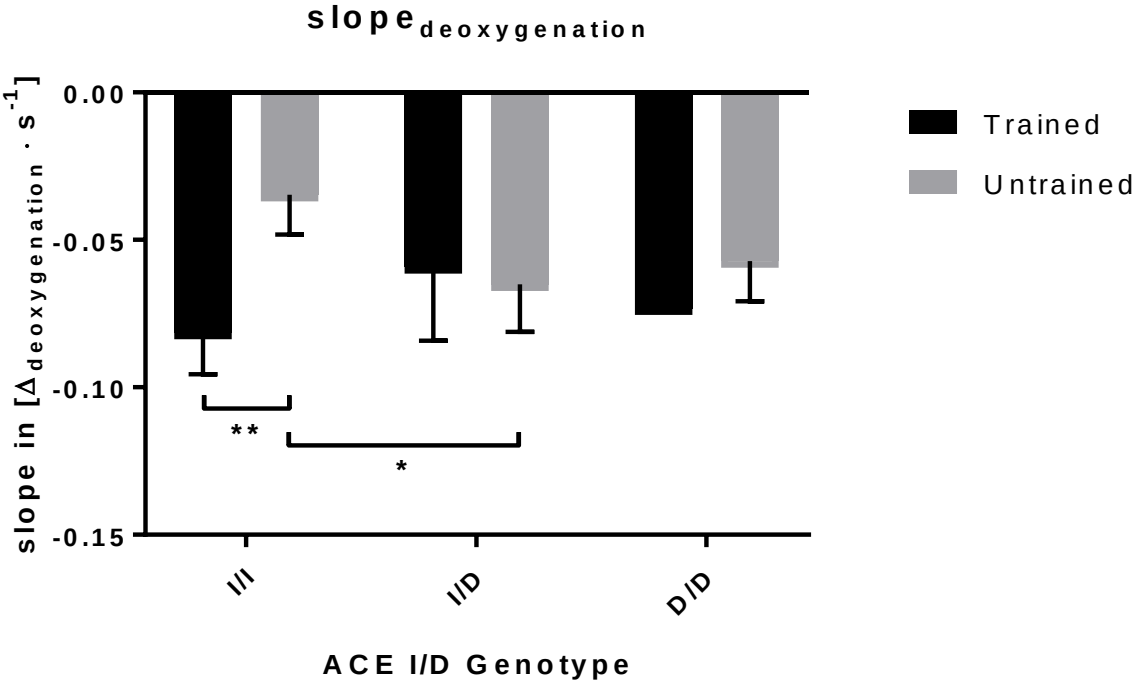

Figure 7. Interaction effect between the ACE I/D genotype and endurance training status on $slope_{deoxygenation}$. Bar graphs represent mean ± standard deviation of the marker $slope_{deoxygenation}$ grouped by the ACE I/D genotype and endurance training status. Only the SmO_2 marker is shown in which a significant interaction effect of ACE I/D genotype and endurance training status was found. Details of SmO_2 marker extraction are presented in section 2.6 Data processing. SmO_2 = muscle oxygen saturation, Trained = VO_{2peak} > 50 $mL \cdot min^{-1} \cdot kg^{-1}$, Untrained = VO_{2peak} < 50 $mL \cdot min^{-1} \cdot kg^{-1}$. * indicates $p < 0.05$, ** indicates $p < 0.01$.

Mean ± standard deviation values of the marker $slope_{deoxygenation}$ grouped by ACE I/D genotype and endurance training status are shown in Figure 7. There was a significant interaction effect between ACE I/D genotype and endurance training status on the marker $slope_{deoxygenation}$ (p = 0.028). The effect size (d = 0.78) is to be classified as medium according to Cohen (1992). Pairwise comparisons revealed that trained carriers of the I/I genotype showed a highly significant steeper $slope_{deoxygenation}$ (p = 0.003) than untrained carriers of the I/I genotype (-0.081 ± 0.012 vs. -0.035 ± 0.012 $\Delta_{deoxygenation} \cdot s^{-1}$, respectively). In addition, untrained carriers of the I/D genotype showed a significantly steeper $slope_{deoxygenation}$ (p = 0.017) than untrained carriers of the I/I genotype (-0.065 ± 0.014 vs. -0.035 ± 0.012 $\Delta_{deoxygenation} \cdot s^{-1}$, respectively). In all other SmO_2 markers no significant interaction effect between ACE I/D genotype and endurance training status was found.

4 Discussion

In the present study, the individual and combined effects of the ACE I/D genotype and endurance training status on SmO_2 markers during ramp test were investigated by a cross-sectional observation. It could be shown that the ACE I/D genotype individually had no significant effect on any of the extracted SmO_2 markers. The endurance training status, on contrary, had a significant effect on $SmO_{2\ min}$ and $\Delta_{deoxygenation}$ as well as a highly significant effect on $\Delta_{1/2\ reoxygenation}$. Interestingly, a significant interaction between the ACE I/D genotype and endurance training status on the $slope_{deoxygenation}$ was observed.

4.1 No individual effect of the ACE I/D genotype on SmO_2 during ramp test

The ACE I/D genotype did not have a significant effect on any of the SmO_2 markers. This result is in contrast to studies which were able to identify associations between the ACE I/D genotype and cellular and molecular skeletal muscle characteristics. As it has already been shown that the ACE I/D genotype is associated with the percentage of slow-twitch type I fibres (Zhang et al., 2003), mitochondrial density (Vaughan et al., 2013) and capillary density as well as capillary-to-fibre ratio (Valdivieso et al., 2017; Vaughan et al., 2016), it could be assumed that genotype-specific differences in the SmO_2 markers measured with a non-invasive NIRS could be found. One of the reasons for this result could be the sample heterogeneity. In the present study, the investigated sample was non-homogenous in terms of gender and endurance training status. According to Woods (2009), one of the main reasons for negative studies regarding the association of the ACE I/D genotype with physical performance is the inclusion of subjects from different sporting disciplines which results in a heterogeneous sample.

> Homogeneity is desirable in these genetic performance studies as the *ACE* gene is only one of many genetic factors affecting performance, and the addition of other (non-genetic factors) such as age, sex and sporting discipline, which may themselves interact with genotype, often results in too great a variance in phenotype to be able to discern the role of genotype. (Puthucheary et al., 2011, p. 436)

The assumption that the lack of the ACE I/D genotype effect occurred due to the heterogeneous sample is supported by studies that have shown that both gender and endurance training status influence SmO_2 during ramp test. For example, Murias et al. (2013) found gender-specific differences in the temporal profile of muscle deoxygenation during a ramp test. Compared to men, young women showed a weaker ability to transport O_2 to the working muscle.

In addition, it could be shown that endurance training status influences muscle deoxygenation during ramp test. In this context, Boone et al. (2009) found out that the aerobic fitness status influences muscle deoxygenation during ramp test. The authors compared the *m. vastus lateralis* deoxygenation of 10 highly trained cyclists with 11 physically active students. They found that the sigmoid pattern of deoxygenation in highly trained cyclists is shifted to the right compared to physically active students. According to Valdivieso et al. (2017) there are indications that regular intensive endurance training and its associated local metabolic effects may partly override the influence of the ACE I/D genotype on aerobic muscle performance. Therefore, gender and endurance training status can be considered as possible confounders and these factors may have eliminated a possible individual effect of the ACE I/D genotype on SmO_2 markers during ramp test.

In contrast to the present study, Kanazawa et al. (2002) found an association between the ACE I/D genotype and tissue oxygenation in 39 patients with COPD. The ratio between the change in oxygen supply to the increase in oxygen uptake was significantly lower in patients with the D/D genotype than in those with the I/I genotype. The authors concluded that the D/D genotype may be associated with an impairment of peripheral tissue oxygenation. However, it can be stated that the investigated sample was a specific pathological sample (COPD patients) with specific muscular characteristics and thus may not be reflective of healthy subjects.

4.2 Effects of the endurance training status on SmO_2 during ramp test

The endurance training status had a significant effect on the markers $SmO_{2\ min}$ ($p = 0.017$) and $\Delta_{deoxygenation}$ ($p = 0.033$). During the ramp test, the trained group achieved on average a lower $SmO_{2\ min}$ value than the untrained group (25.24 ± 6.94 vs. 37.74 ± 7.86 %, respectively). This resulted in a higher value for the $\Delta_{deoxygenation}$ marker for the trained compared to the untrained group (-34.72 ± 10.70 vs. -22.81 ± 9.25 %, respectively). Since the $SmO_{2\ baseline}$ value between trained and untrained subjects did not differ significantly, it can be concluded that there was a greater O_2 extraction of the *m. vastus lateralis* in endurance trained subjects compared to untrained subjects. This result is supported by longitudinal studies which have previously shown that physical training (and thus the endurance training status) can affect muscle deoxygenation in healthy and diseased subjects. For example, Jacobs et al. (2013) investigated the muscle deoxygenation before and after six sessions of high-intensity interval training (HIIT) in healthy subjects. The authors showed that an increase in muscle deoxygenation during ramp test resulted after the training phase. In addition, Takagi et al. (2016) investigated the muscle

deoxygenation before and after a twelve-week aerobic training period in post-myocardial infarction patients. The authors found that aerobic training led to an increased deoxygenation of the *m. vastus lateralis* during ramp test. Furthermore, Okushima et al. (2016) found that a greater VO_{2peak} is correlated with greater skeletal muscle deoxygenation amplitude during ramp test.

In addition, there was a highly significant effect of the endurance training status on the marker $\Delta_{1/2\ reoxygenation}$ ($p = 0.007$). It could be shown that trained subjects had a higher $\Delta_{1/2\ reoxygenation}$ than untrained subjects (56.22 ± 7.22 vs. 35.54 ± 12.36 %, respectively). This result is supported by previous studies which have shown that both endurance training status and training can influence the reoxygenation kinetics after exercise. For example, Ding et al. (2001) compared the reoxygenation kinetics after an incremental cycle ergometer test of 18 male elite athletes with 8 healthy young men. The authors found that elite athletes showed a significant higher half recovery increment of SmO_2 compared to the healthy control subjects. In addition, Puente-Maestu et al. (2003) investigated the effects of a six-week high work cycle ergometer training in COPD patients. The authors investigated the muscle deoxygenation and reoxygenation via NIRS and the change of oxidative enzymes via muscle biopsies. They found that training increased the speed of the *m. vastus lateralis* reoxygenation after exercise and that this improvement correlated with the change in several oxidative enzymes. Therefore, the higher $\Delta_{1/2\ reoxygenation}$ found in trained subjects could indicate a greater muscle oxidative capacity.

4.3 Interaction effect between the ACE I/D genotype and endurance training status

There was a significant interaction effect between the ACE I/D genotype and endurance training status on the marker $slope_{deoxygenation}$ ($p = 0.028$). Pairwise comparisons revealed that only within the I/I genotype there was a highly significant difference in the $slope_{deoxygention}$ ($p = 0.003$) between endurance trained and untrained subjects (-0.081 ± 0.012 vs. -0.035 ± 0.012 $\Delta_{deoxygenation} \cdot s^{-1}$, respectively). Trained carriers of the I/I genotype had a highly significant steeper $slope_{deoxygenation}$ than untrained carriers of the I/I genotype. Within the I/D and the D/D genotype there was no significant difference. Therefore, it could be concluded that carriers of the I/I genotype may have greater potential to improve in skeletal muscle O_2 extraction per unit time with training in a hypothetical training scenario. This result seems supported by the study by Williams et al. (2000), which showed that only subjects with the I/I genotype were able to significantly improve the efficiency of skeletal muscle contractions after training com-

pared to the I/D and D/D genotype. The authors concluded that this may be associated with an increase in slow-twitch type I muscle fibres. Since the deoxygenation of the muscle depends mainly on the O_2 extraction by the mitochondria (Muthalib et al., 2010), it can be speculated that the trained carriers of the I/I genotype had a higher percentage of slow-twitch type I muscle fibres and thus a higher mitochondrial density. In previous studies, it has been shown that the I/I genotype is associated with a higher percentage of slow-twitch type I muscle fibres (Zhang et al., 2003) and a higher mitochondrial density (Vaughan et al., 2013) in the *m. vastus lateralis*. Therefore, the present study was, to some extent, able to support these cellular and molecular components of the skeletal muscle by showing that only within the the I/I genotype endurance trained subjects had a steeper $slope_{deoxygenation}$ than untrained subjects. This was not the first study showing an interaction effect between the ACE I/D genotype and endurance training status on skeletal muscle components. Valdivieso et al. (2017) also found interaction effects between the ACE I/D genotype and endurance training status on the CSA and MCSA of slow-twitch type I fibres of *m. vastus lateralis* and therefore on components which were related to the skeletal muscle O_2 extraction. They could show that untrained carriers of the I allele had a larger CSA than untrained carriers of the D/D genotype. Furthermore, trained carriers of the I allele had a larger MCSA than trained carriers of the D/D genotype. In the present study, it was also possible to show a genotype-specific difference within the endurance untrained subjects. Untrained carriers of the I/D genotype showed a significantly steeper $slope_{deoxygenation}$ ($p = 0.017$) than untrained carriers of the I/I genotype (-0.065 ± 0.014 vs. -0.035 ± 0.012 $\Delta_{deoxygenation} \cdot s^{-1}$, respectively). This result is slightly in contrast to the study by Valdivieso et al. (2017). Due to the fact that carriers of the D/D genotype were associated with a smaller CSA of slow-twitch type I muscle fibres, it could have been assumed that the $slope_{deoxygenation}$ during ramp test is less steep in untrained carriers of the I/D genotype than in untrained carriers of the I/I genotype.

4.4 Limitations

One of the potential limitations of the present study was the small sample size ($N = 21$). Since a codominant model (I/I vs. I/D vs. D/D) was selected for the statistical analysis, the consequence was that the group of the D/D genotype counted only three subjects. In order to improve this limitation, further subjects would have to be included in the study until the desired number of subjects, based on a power analysis, is reached for the D/D genotype. Another pos-

sibility would be to choose another genetic model. In the present study, a recessive model (I/I vs. I/D + D/D) could have been chosen instead of a codominant model (I/I vs. I/D vs. D/D).
A further limitation was that the adipose tissue thickness was not measured above the sensor placement for the NIRS measurement. Several studies have shown that adipose tissue can influence the NIRS signal (Homma, Fukunaga, & Kagaya, 1996; Niemeijer et al., 2017; Van Beekvelt, Borghuis, Van Engelen, Wevers, & Colier, 2001). Since the group studied was a heterogeneous group in terms of gender and endurance training status, it can be assumed that adipose tissue thickness varied between subjects in the area of NIRS sensor placement. A simple way to measure adipose tissue thickness would have been to use a skinfold caliper. A far more precise but also more cost intensive method would have been to measure the adipose tissue thickness with an ultrasound device.
Furthermore, the model of NIRS data processing could be refined. As described in the methods, a linear slope was calculated for the $slope_{deoxygenation}$. However, studies have shown that the deoxygenation of the muscle during a ramp test is not linear. Belardinelli et al. (1995), for example, showed that muscle deoxygenation first reaches a plateau before reaching VO_{2peak}. Further authors described a sigmoid curve of muscle deoxygenation during ramp test (Boone et al., 2009). By refining the model of NIRS data processing, differences in muscle deoxygenation could be found more sensitively.

4.5 Outlook

In order to increase the possibility of identifying further effects and to increase the power of the study, further subjects should be included in the study. In a further step, the established scientific research questions could be answered by means of a longitudinal training intervention study. It would be interesting to investigate whether exercise-induced changes of SmO_2 markers during ramp test are ACE I/D genotype dependent. In a final step, muscle biopsies could be collected to investigate the associations between the non-invasive measured SmO_2 markers and cellular and molecular components of the skeletal muscle.

5 Conclusion

The present study has shown that in a heterogeneous sample the ACE I/D genotype did not have a significant effect on any of the SmO_2 markers during ramp test. The endurance training status, on contrary, had a significant effect on $SmO_{2\ min}$ and $\Delta_{deoxygenation}$ as well as a highly significant effect on $\Delta_{1/2\ reoxygenation}$. Endurance trained subjects showed a greater $\Delta_{deoxygenation}$ as well as a greater $\Delta_{1/2\ reoxygenation}$ within the reoxygenation kinetics, indicating an increased oxidative capacity of the *m. vastus lateralis*. Interestingly, a significant interaction effect between the ACE I/D genotype and endurance training status on the $slope_{deoxygenation}$ was observed. Trained carriers of the I/I genotype had a highly significant steeper $slope_{deoxygenation}$ than untrained carriers of the I/I genotype. Within carriers of the I/D and the D/D genotype there was no significant difference. The highly significant steeper $slope_{deoxygenation}$ for trained subjects only found within the I/I genotype could indicate a higher percentage of slow-twitch type I muscle fibres and thus a higher mitochondrial density. Based on these results it could be speculated that carriers of the I/I genotype could result in greater adaptability in a longitudinal training study. However, this was not the aim of the present master thesis as it was a cross-sectional observation. This pilot study may serve to obtain insightful data into the possibility of developing specific training programs *ad hoc* for different patient characteristics.

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Der Balgrist

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Beantworten Sie die unten stehenden Fragen nach bestem Wissen und Gewissen und mit etwas „gesundem“ Menschenverstand.

Hat Ihnen jemals ein Arzt gesagt, Sie hätten “etwas am Herzen“ und Ihnen Bewegung und Sport nur unter ärztlicher Kontrolle empfohlen?	Ja ☐	Nein ☐
Haben Sie zurzeit Schmerzen in der Brust in Ruhe oder bei körperlicher Belastung (bei Anstrengung)?	Ja ☐	Nein ☐
Haben Sie Probleme mit der Atmung in Ruhe oder bei körperlicher Belastung?	Ja ☐	Nein ☐
Sind Sie schon wegen Schwindel gestürzt oder haben Sie schon jemals das Bewusstsein verloren?	Ja ☐	Nein ☐
Haben Sie Knochen- oder Gelenkprobleme, die sich unter körperlicher Belastung verschlechtern könnten?	Ja ☐	Nein ☐
Hat Ihnen ein Arzt ein Medikament gegen hohen Blutdruck oder wegen eines Herzproblems oder Atemproblems verschrieben?	Ja ☐	Nein ☐
Kennen Sie irgendeinen weiteren Grund, warum Sie nicht körperlich / sportlich aktiv sein sollten?	Ja ☐ Welche(n):	Nein ☐

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Wir empfehlen Ihnen dringend zur besseren Risikoeinschätzung für Herzkreislauferkrankungen die jährliche Bestimmung ihrer Blutfettwerte.

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V3/26.07.17

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- Bitte lesen Sie dieses Formular sorgfältig durch.
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Titel der Studie:	Beeinflusst der ACE Genotyp die Trainingsanpassung an ein kardiovaskuläres Rehabilitationsprogramm?
verantwortliche Institution (Sponsor):	Uniklinik Balgrist, Forchstrasse 340, 8008 Zürich
Ort der Durchführung:	Move>Med, Uniklinik Balgrist, und Universitätsspital Zürich
Leiter / Leiterin der Studie:	Prof. Martin Flück
Teilnehmerin/Teilnehmer	Name: ..... Vorname: ..... Geburtsdatum: ..... ☐ weiblich ☐ männlich

- Ich wurde vom unterzeichnenden Arzt mündlich und schriftlich über den Zweck, den Ablauf der Studie, über die zu erwartenden Wirkungen, über mögliche Vor- und Nachteile sowie über eventuelle Risiken informiert.
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Table 3

Significance map of the two factor analysis of variance (ANOVA) results

	ACE I/D		Training Status		ACE I/D * Training Status	
	***F* value**	***p* value**	***F* value**	***p* value**	***p* value**	***p* value**
$SmO_{2\ baseline}$	0.284	0.757	0.133	0.721	0.739	0.494
$SmO_{2\ min}$	0.951	0.408	7.262	0.017*	1.365	0.285
$\Delta_{deoxygenation}$	0.085	0.919	5.499	0.033*	1.883	0.186
$t_{deoxygenation}$	0.647	0.537	0.631	0.439	0.872	0.438
$slope_{deoxygenation}$	0.194	0.826	4.132	0.060	4.585	0.028*
$SmO_{2\ max}$	0.396	0.680	3.272	0.091	0.112	0.894
$\Delta_{1/2\ reoxygenation}$	0.289	0.753	9.611	0.007**	0.359	0.704
$t_{1/2\ reoxygenation}$	0.047	0.954	0.104	0.752	1.163	0.339
$slope_{1/2\ reoxygenation}$	0.091	0.913	4.285	0.056	2.572	0.110
$SmO_{2\ overshoot}$	0.105	0.901	1.251	0.281	1.504	0.254

Note. Significance map of the two factor analysis of variance (ANOVA) results. Details of SmO_2 marker extraction are presented in section 2.6 Data processing. ACE = angiotensin-converting enzyme, I/D = insertion/deletion. * indicates $p < 0.05$, ** indicates $p < 0.01$.

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