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„miRNA expression profile: a comparison between young and master strength athletes after resistance exercise"

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Isabella Rauchwerger BSc

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Abstract

This study was completed in cooperation with the Faculty of Sport Science, Ruhr University Bochum, Bochum, Germany. Data were collected as part of a previous study published by Schmidt and colleagues (2021). We aimed to assess recovery in strength-trained younger and older males in response to an acute bout of eccentric squat exercise and the effect of intensive resistance exercise on the expression of whole blood microRNAs (miRNAs). miRNAs expression profile of young (n=5; age, 25.2 ± 5.3 years) and master (n=5; age, 52.5 ± 3.9 years) strength-trained athletes were evaluated. The participants had at least one year of strength training experience and had a one-repetition half squat maximum of at least 120% of their body weight. In the context of Schmidt and colleagues' study, the participants conducted a standardised half squat protocol consisting of nine sets of eight repetitions and a final set until momentary muscular failure with 70% of the individual's one-repetition maximum. We analysed the miRNAs expression profile before and after the acute resistance exercise bout using miRNAs PCR arrays (MIHS-001ZA, Qiagen, Germany). In addition, a pathway enrichment analysis was conducted using experimentally validated target genes to assess possible miRNAs-target interactions. Nine differentially expressed miRNAs were identified, whereas an impairment of miRNAs regulation with aging was observed. Resistance exercise seemed to alter the expression of pro-angiogenic and -myogenic miRNAs in younger individuals. The divergent epigenetic response of the older age group to RE could help identify or explain the mechanisms underlying age-related anabolic resistance.

Zusammenfassung

Diese Studie wurde in Zusammenarbeit mit der Fakultät für Sportwissenschaft der Ruhr-Universität Bochum, Bochum, Deutschland, durchgeführt. Die Daten wurden im Rahmen einer früheren Studie erhoben, die von Schmidt und Kollegen (2021) veröffentlicht wurde. Ziel dieser Kooperation war es, die Erholung bei jüngeren und älteren männlichen krafttrainierten Männern nach einer akuten exzentrischen Kniebeuge und die Auswirkungen eines intensiven Widerstandstrainings auf die Expression von microRNAs (miRNAs) im Vollblut zu untersuchen. Das miRNA Expressionsprofil junger (n=5; Alter, 25,2 ± 5,3 Jahre) und älterer (n=5; Alter, 52,5 ± 3,9 Jahre) Sportler wurde ausgewertet. Die Teilnehmer verfügten über mindestens ein Jahr Erfahrung im Krafttraining und verfügten über ein Einwiederholungsmaximum von mindestens 120 % ihres Körpergewichts in der halben Kniebeuge. Im Rahmen der Studie von Schmidt und Kollegen (2021) führten die Teilnehmer ein standardisiertes Protokoll für halbe Kniebeugen durch, das aus neun Sätzen mit je acht Wiederholungen und einem abschließenden Ausbelastungssatz bis zum momentanen Muskelversagen mit 70% des individuellen Einwiederholungsmaximum bestand. Wir analysierten das Expressionsprofil der miRNAs vor und nach einer akuten Krafttrainingseinheit mit Hilfe von miRNAs PCR-Arrays (MIHS-001ZA, Qiagen, Deutschland). Darüber hinaus wurde eine Analyse der Anreicherung von Signalwegen mit experimentell validierten Zielgenen durchgeführt, um mögliche miRNAs-Ziel-Interaktionen zu erproben. Es wurden neun differentiell exprimierte miRNAs identifiziert, wobei eine Beeinträchtigung der miRNAs-Regulierung mit zunehmendem Alter beobachtet wurde. Widerstandstraining schien die Expression von pro-angiogenen und -myogenen miRNAs bei jüngeren Personen zu modellieren. Die inhibierte epigenetische Reaktion der älteren Teilnehmer auf Krafttrainingseinheit könnte zur Identifizierung oder Erklärung der Mechanismen beitragen, die der altersbedingten anabolen Resistenz zugrunde liegen.

Acknowledgements

This work was conducted in cooperation with the Faculty of Sport Science, Ruhr University Bochum, Bochum, Germany. Data were collected as part of a previous study published by Schmidt and colleagues (2021), which aimed to assess recovery in younger and older individuals in response to resistance exertion. I want to thank Alexander Ferrauti for this opportunity.

But firstly, I would like to express my deepest gratitude to Barbara, for this endeavor would not have been possible without her. I would never in a million years have imagined I would be involved in scientific research the way she has made it possible for me and many other students for that matter. Barbara, you have made one of my dreams come true and helped me find myself through this process. I will always be grateful to have encountered you and will always appreciate all those years working beside you – to many more years working together! I'd also like to extend my gratitude to my sister, Cami. I could not have undertaken this journey without you. Thank you for all your help, support, and guidance. Kevin, thank you for always being in my corner and pushing me when I get lost in details and uncertainty. And finally, thank you to Karin for helping me in the laboratory.

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List of abbreviations

Abbreviation	Definition
1RM	one-repetition maximum
AGO	argonaute proteins
Akt	serine/threonine kinase
AMPK	5' AMP-activated protein kinase
ANOVA	Analysis of variance
APP	amyloid beta precursor protein
ATM	serine/threonine kinase
ATP	Adenosine 5′-triphosphate
BCL2	B-cell lymphoma 2, apoptosis regulator
BDNF	brain derived neurotrophic factor
BLa	blood lactate
BMI	body mass. Index
CADM1	cell adhesion molecule 1
C2C12	immortalized mouse myoblast cell line
CCB1	cyclin B1
CCD1	carotenoid cleavage dioxygenase 1
CCD2	carotenoid cleavage dioxygenase 2
CCD25A	cell division cycle 25A
CCND1	cyclin D1
CCNE1	cyclin E1
CD56+	marker of natural killer cells
CDK1	cyclin-dependent kinase 1
CDK2	cyclin-dependent kinase 2
CDK6	cyclin-dependent kinase 6
CFTR	cystic fibrosis transmembrane conductance regulator
CHEK1	checkpoint kinase 1
CHEK2	checkpoint kinase 2
CK	creatine kinase
CMJ	countermovement jump
COPD	chronic obstructive pulmonary disease
CRKL	CRK like proto-oncogene, adaptor protein
CRP	c-reactive protein
C_T	cycle threshlod
CWI	cold-water immersion
DE	differentially expressed
DICER1	dicer 1, ribonuclease III
DM	dermatomyositis
DMD	Duchenne muscular dystrophy
DMT2	diabetes mellitus type 2

DNA	deoxyribonucleic acid
DNMT1	DNA methyltransferase 1
EE	endurance exercise
EFNA2	protein-tyrosine kinase ephrin A3
EZH2	enhancer of zeste 2 polycomb repressive complex 2 subunit
FC	fold change
FGF1	fibroblast growth factor 1
FHL1	half LIM domains protein 1
FOXO1	forkhead box O1
FOXO3	forkhead box O3
FR	fold regulation
FSHD	facioscapulohumeral muscular dystrophy
GDF3	growth differentiation factor-3
GO	Gene Ontology
GO BP	Gene Ontology, biological processes
GO CC	Gene Ontology, cellular components
GO MF	Gene Ontology, molecular functions
GSK3	glycogen synthase kinase-3
HIF1A	hypoxia inducible factor 1 subunit α
ICAW	intensive care-acquired weakness
ICU	intensive care unit
IGF-1	insulin-like growth factor-1
IL	interleukin
IRS-1	insulin receptor substrate 1
JAK-STAT	janus kinase - signal transducer and activator of transcription proteins
KEGG	Kyoto Encyclopaedia of Genes and Genomes
LGMD2A	limb-girdle muscular dystrophies with mutated calpain-3, type 2A
LGMD2B	limb-girdle muscular dystrophies with mutated dysferlin, type 2B
LHCN-M2 cells	human myoblast cell line
LVEF	left ventricular ejection fraction
M1	inflammatory macrophages
M2	regenerative cells
MAPK	mitogen-activated protein kinase
Master	master athletes
MED	myocyte-specific enhancer factor 2D
MEF2A	myocyte enhancer factor 2A
MEF2C	myocyte enhancer factor 2C
MET	mesenchymal epithelial transition
MIENTURNET	MicroRNA enrichment turned network
miRPathDB	miRNA Pathway Dictionary Database
miRNA	microRNA
c-miRNA	circulating microRNA
pre-miRNA	precursor microRNA

pri-miRNA	primary microRNA
MITF	melanocyte inducing transcription factor)
MMR	mixed-method recovery
MRF4	myogenic regulatory factor 4
MTOR	mechanistic target of rapamycin kinase
MVIC	Maximal voluntary isometric contraction
MYB	myoblastosis proto-oncogene, transcription factor
Myf5	myogenic factor 5
MyH3	myosin heavy chain 3
MyHC	myosin heavy chain
MyoD	myogenic differentiation
MyoG	myogenin
NF-κb	nuclear factor kappa-light-chain-enhancer of activated B cells
NM	nemaline myopathy
NOTCH1	notch receptor 1
ORA	over-representation analysis
PACT	protein kinase R-activating protein
PAX3	paired box protein 3
PAX7	paired box protein 7
PBMC	peripheral blood mononuclear cells
PCR	polymerase chain reaction
PGC-1α	proliferator-activated receptor-gamma coactivator 1 α
PHLPP1	PH domain and leucin rich repeat protein phosphatase 1
PI3k	phosphoinositide 3-kinases
PIK	phosphoinositide-3-kinase regulatory subunit 2
PLAG1	pleiomorphic adenoma gene 1
PM	polymyositis
PPC	perceived physical performance capability
PR	passive recovery
PTEN	phosphate and tensin homolog
PTGS2	prostaglandin-endoperoxide synthase 2
RE	resistance exercise
RECK	reversion inducing cysteine rich protein with kazal motifs
RPE	rate of perceived exhaustion
RT-qPCR	real-time quantitative polymerase chain reaction
RTF	resting twitch force
RUNX1	runt-related transcription factor 1
RNA	ribonucleic acid
m-RNA	messenger RNA
SD	standard deviation
SMAD3	mothers against decapentaplegic homolog 3
SMAD4	mothers against decapentaplegic homolog 4
SPRED1	sprouty related protein 1

STAT	Transducer and Activator of Transcription
STAT3	Transducer and Activator of Transcription 3
TGF-β	transforming growth factor-β
TGFBR	transforming growth factor-β receptor
TH	threshold
TL_{CO}%	transfer factor of the lung for carbon monoxide
TNF-α	tumour necrosis factor-α
TRBP	transactivation-response RNA-binding protein
VEGF	vascular endothelial growth factor
VEGFA	vascular endothelial growth factor A
VEGFR1	vascular endothelial growth factor receptor 1
VEGFR2	vascular endothelial growth factor receptor 2
WEE1	WEE1 G2 checkpoint kinase
WHO	World Health Organisation
XIST	x inactive specific transcript
Young	young athletes
ZEB1	zinc finger e-box binding homeobox 1
ZEB2	zinc finger e-box binding homeobox 2

1 Introduction

Biological ageing is associated with impaired muscular regenerative capacity, higher amounts of fibrotic tissue, and intramuscular fat due to a decline in neuromuscular and neuroendocrine system function (Grounds, 1998; Sadeh, 1988). Signalling cascades involved in myogenic stem cell activation for muscle regeneration are negatively influenced by age (Carlson et al., 2008; Chakkalakal et al., 2012; Drummond et al., 2011). Hence, the elderly experience inhibited muscular repair after exercise stimuli compared to the younger population. Their diminished capacity of adaptation to anabolic stimulation, i.e., anabolic resistance, is believed to be a contributing factor to age-related metabolic and physical conditions such as sarcopenia. However, the specific underlying mechanisms contributing to anabolic resistance have not been fully characterised. Furthermore, regular exercise has multiple positive effects on the human body, leading to varying health and performance benefits through physiological changes on a cellular and molecular level even in aging individuals. Those exercise-induced adaptations occur with through the external stimuli altered gene expression, which is the underlying cause of health improvements attributed to physical activity. Like many physiological processes, exercise modifies the epigenetic landscape and thereby the response to exercise within the body.

One group of molecules discovered at the end of the last decade has sprung the interest of many researchers as regulators of gene expression, small non-coding RNAs. Endogenous small non-coding RNAs are substantial regulators of gene expression found in most eukaryotes, microRNAs (miRNAs) being the largest and most diverse subgroup. miRNAs are short, ~22 nucleotide-long, single-stranded RNA molecules that play a substantial role in post-transcriptional gene regulation, modulating the complex system of cellular processes (Valinezhad Orang et al., 2014). The number of discovered miRNAs increases yearly – currently, in the leading online database miRBase (http://mirbase.org, Release 22.1), more than 2600 mature homo sapiens miRNAs (hsa-miRNAs) are documented (Kozomara et al., 2019). miRNAs serve as post-transcriptional regulators of targeted messenger RNA (mRNA) by repressing its translation or degrading it (Huntzinger & Izaurralde, 2011). Several studies described the role of miRNAs as mediators of signalling pathways involved in the adaptive response to exercise (Aránega et al., 2021; Domańska-Senderowska et al., 2019). Currently, a plausible relationship between whole blood miRNA expression profiles and signalling pathways activity, and thus their potential as biological markers of varying physiological states, are being studied by researchers. Margolis and Rivas (2018) have

proposed miRNAs to play a substantial role in a reduced muscle plasticity, ergo anabolic resistance.

1.1 miRNA biogenesis

miRNAs' biogenesis (Figure 1) starts in the nucleus with the transcription of miRNA genes into capped and polyadenylated primary miRNA (pri-miRNA) hairpin-formed transcripts by RNA polymerase II or III, commonly derived from the introns of protein-coding or introns or exons of non-coding genes (Choudhuri, 2010; Lee et al., 2004; Rodriguez et al., 2004). The microprocessor complex, compounded of RNase III Drosha enzyme and RNA-binding protein DGCR8, binds to the pri-miRNA stem-loop and processes it into ~70 nucleotide-long precursor miRNA (pre-miRNA) (Finnegan & Pasquinelli, 2013). pre-miRNA is exported to the cytoplasm by a complex of exportin 5 and GTPase RNA. There RNase III Dicer and RNA-binding protein TRBP (transactivation-response RNA-binding protein) or PACT (protein kinase R-activating protein) process pre-miRNA further, and a ~22 nucleotide-long, a double-stranded molecule is left behind (Chendrimada et al., 2005; Makarova et al., 2016). Of that duplex, one strand, referred to as the passenger strand, is believed to degrade, and the other strand, so-called mature miRNA, loads into an AGO protein, creating the core of the miRNA-induced silencing complex (miRISC), enabling it to interact with its target mRNA and resulting in regulation of gene expression (Choudhuri, 2010; Makarova et al., 2016). As a result, miRNAs generally function as mediators of post-transcriptional gene silencing by repressing the synthesis of complementary mRNAs or facilitating the decay of their half-live via cleavage (Huntzinger & Izaurralde, 2011; Vasudevan et al., 2007). Due to the partial complementarity of miRNAs with their target mRNAs, miRNAs can have multiple target mRNAs, and mRNAs can have various binding sites for the same or different miRNAs too (Bartel, 2009; Friedman et al., 2009; Makarova et al., 2014, 2016). Moreover, miRNAs can be found inside or outside cells, differentiating between intracellular and extracellular RNAs, and expressed by specific tissues/organs (Lagos-Quintana et al., 2002; Makarova et al., 2016). Extracellular, also called circulating miRNAs (c-miRNAs), can be observed in most bodily fluids, i.e., saliva, urine, blood plasma, and serum. c-miRNAs can be carried by extracellular vesicles, like microvesicles and exosomes, as well as in high-density lipoprotein particles or exists solely as a complex with AGO proteins in the bloodstream (Turchinovich et al., 2011; Vickers et al., 2011). miRNAs can likewise activate translation or transcription depending on the abundance and

localisation of miRNAs in the cell (O'Brien et al., 2018). They could potentially play an important role in mediating cell-cell communication, though further research into their biological significance is needed.

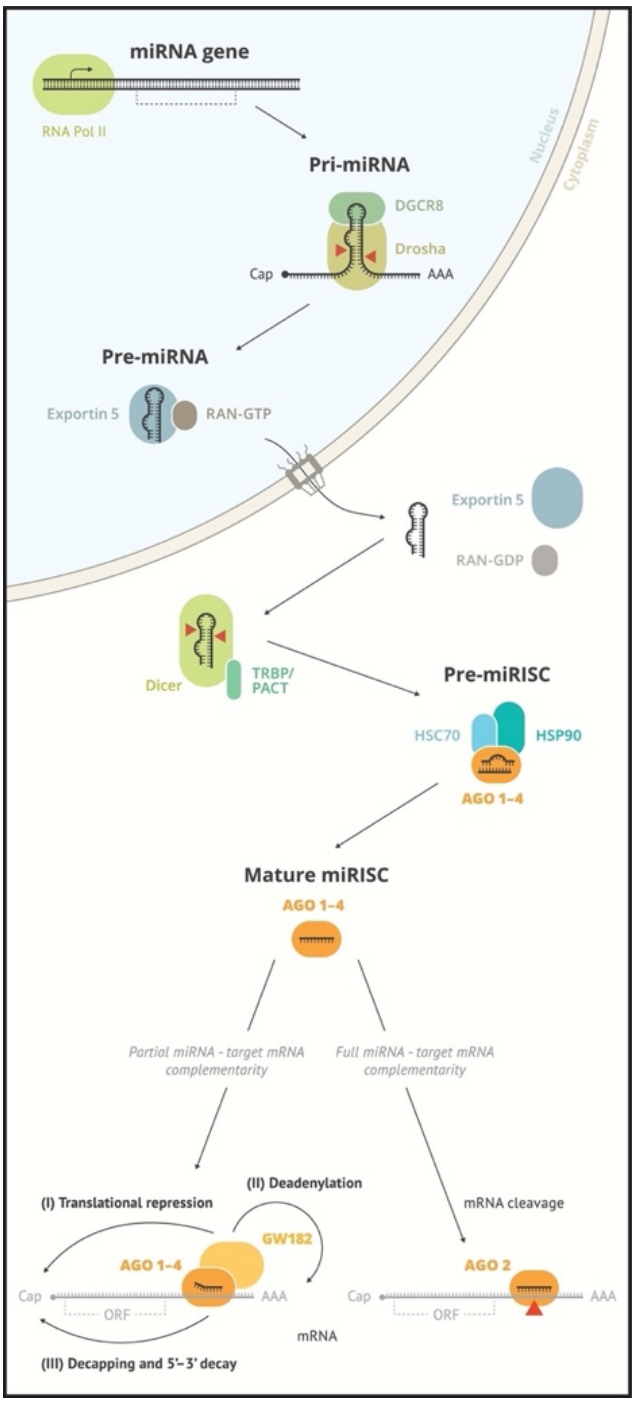


Figure 1: miRNA biogenesis and functions

Note. This figure illustrates the biogenesis of miRNA and its functions. Pri-miRNA, primary miRNA; pre-miRNA, precursor miRNA; miRISC, miRNA-induced silencing complex. Reprinted form "Intracellular and extracellular microRNA: An update on localisation and biological role". J. A. Makarova et al., 2016, *Progress in histochemistry and cytochemistry, 51*(3-4), https://doi.org/10.1016/j.proghi.2016.06.001, pg. 35. Copyright 2016 by Elsevier. Reprinted with permission.

1.2 Skeletal muscle regeneration

Exercise is a delaying and preventive tool for various diseases and physiological conditions by influencing several biological systems in the human body and inducing systemic adaptation (Forcina et al., 2020; Ruegsegger & Booth, 2018). Note that the effects of exercise vary with activity type and intensity and the subjects' sex, health, fitness, and developmental state. Physical activity affects tissue homeostasis and cellular metabolism, stimulating pleiotropic cellular adaptation by activating numerous signalling pathways by releasing growth factors, immune cells, and cytokines. Those molecules activate downstream cascades like mitogen-activated protein kinase (MAPK), mechanistic target of rapamycin (mTOR), which then, in turn, activate or repress varying transcriptional and translational responses and regulate gene expression (Hoffman et al., 2015). Often, resistance exercise causes direct muscle tissue damage provoking a regenerative response and activating inflammatory cells and signalling molecules like cytokines and myokines. Strength training, i.e., stimulates muscle protein synthesis, induces muscle hypertrophy, and increases myofiber size and force production, partially through the activation of the phosphoinositide 3-kinases-Serine/threonine kinase–mTOR (PI3k-Akt-mTOR) signalling cascade (Baar, 2006; Carroll et al., 2019; Hawley, 2009; Roberts et al., 2020). Exercise-induced muscle tissue damage repair involves crosstalk between muscle cells and immune cells. It is composed of an inflammatory response followed by a regenerative phase. The inflammatory reaction starts with disrupted myofibers releasing damage-associated molecular signals and activating immune cells, such as leukocytes, e.g., neutrophils, eosinophils, and macrophages – the latter is involved in the pro-and anti-inflammatory response of this process (Wosczyna & Rando, 2018). As an initial response, local leukocytes excrete inflammatory cytokines, such as interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF-α), with which they activate further circulating leukocytes and stimulate activation and proliferation of skeletal muscle stem cells, known as satellite cells (S.-E. Chen et al., 2007; Serrano et al., 2008). Infiltrating granulocytes then cleanse the damaged tissue through phagocytosis and promote the infiltration of macrophages. Subsequently, pro-inflammatory macrophages (M1) and T-cells modulate the environment of the injury and excrete inflammatory cytokines, such as IL-6, IL-1β, and TNF-α, stimulating proliferation of satellite cells and delaying differentiation (Arnold et al., 2007; Dort et al., 2019; Spadaro et al., 2017; Zhang et al., 2013). M1 shift into regenerative cells (M2), which release anti-inflammatory cytokines and growth factors, i.e., IGF-1, transforming growth factor-beta

(TGF-β), IL-4, IL-10, and growth differentiation factor-3 (GDF3), repressing the inflammatory response, promoting the differentiation of myoblasts, and enabling myogenesis and hypertrophy (Arnold et al., 2007; Chazaud, 2020; Dort et al., 2019; Varga et al., 2016). Next, the regenerative phase starts; in brief: satellite cells go from a quiescent to an activated state with the help of the pro-inflammatory signals released by the muscle tissue and leukocytes. They then migrate to the damaged tissue and begin to proliferate to so-called myoblasts, which secrete myogenic signalling molecules such as paired box protein 7 (PAX7), myogenic factor 5 (Myf5), and myogenic differentiation (MyoD) (Dumont et al., 2015). As a result, myoblasts differentiate into mature myocytes and fuse into new myotubes or with damaged tissue, accompanied by the expression of myogenin (MyoG), myosin heavy chain (MyHC) and myogenic regulatory factor 4 (MRF4), and downregulation of PAX7 and Myof5 (Dumont et al., 2015; Relaix & Zammit, 2012; Wosczyna & Rando, 2018). Exercise-induced transcriptional, translational, and post-transcriptional mediators regulate many molecular and cellular response processes by activating or repressing the expression of explicit genes and inducing physiological adaptations. Figure 2 illustrates the interaction between muscle and immune cells in the regenerative process of skeletal muscle.

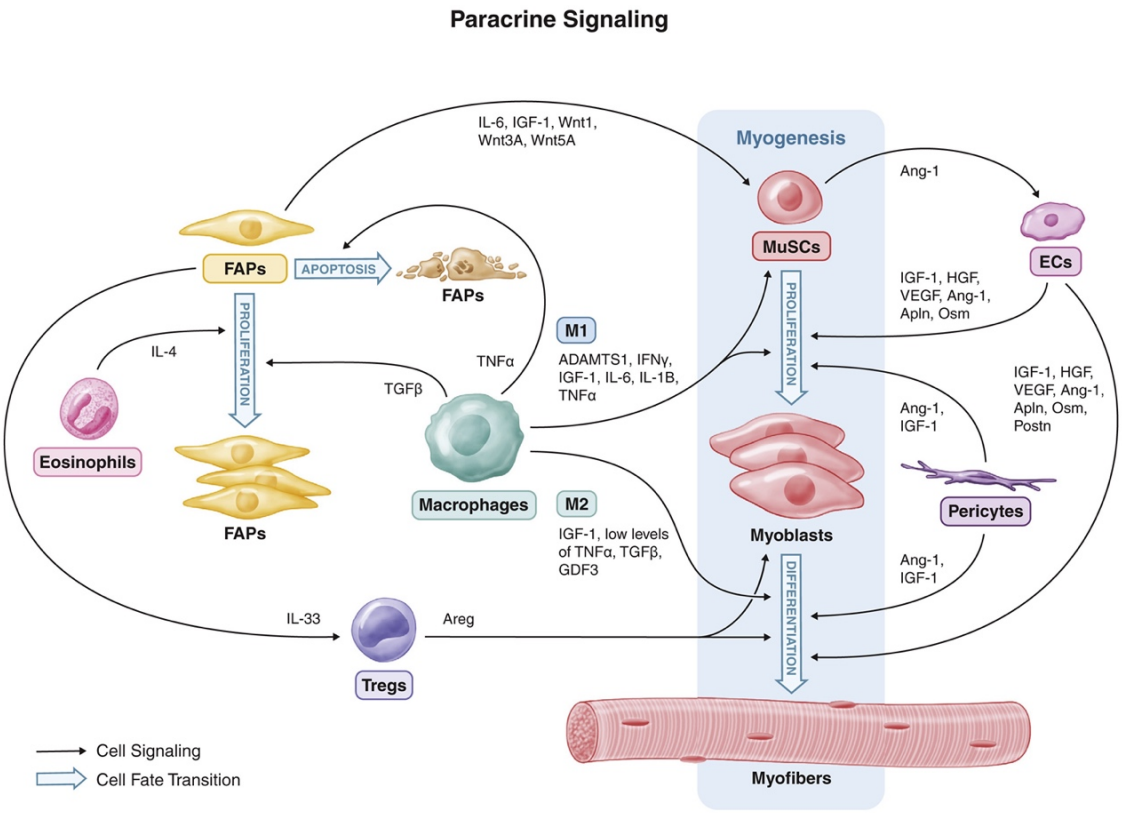


Figure 2: Crosstalk between muscle cells and immune cells during muscle regeneration

Note. This figure illustrates the interaction between immune cells and muscle cells during the regenerative process of skeletal muscle. M1 and M2, macrophages; FAPs, fibroadipogenic progenitors; MuSCs, muscle stem cells; Tregs, regulatory T cells; ECs, endothelial cells; Apln, Apelin; Postn, Periostin; Osm, Oncostatin M. Reprinted form "A Muscle Stem Cell Support Group: Coordinated Cellular Responses in Muscle Regeneration.". M. N. Wosczyna, & T. A. Rando, 2018, D*evelopmental cell*, *46*(2), https://doi.org/10.1016/j.devcel.2018.06.018, pg. 19. Copyright 2018 by Elsevier. Reprinted with permission.

miRNA expression and physical exercise

Exercise disrupts cellular homeostasis, inducing various transcriptional responses that mediate myogenic, regulatory, and metabolic gene expression. The influence of physical exertion on c-miRNAs and tissue-specific miRNAs, like muscle-specific miRNAs (myomiRs), is widely explored and has been thoroughly validated (Baggish et al., 2014; Banzet et al., 2013; Cui et al., 2017; D'Souza et al., 2017a; Kirby & McCarthy, 2013, 2013; Mooren et al., 2014; Sawada et al., 2013; Silva et al., 2020; Uhlemann et al., 2014). Altered miRNA expression after exercise has been also reported in other tissue, such as, cardiac, bone, adipose, and nervous tissue, and other organs like liver (dos Santos et al., 2022). In addition, miRNA expression is associated with the immunological response to exercise, and

miRNA expression levels show a relationship with the regulation of exercise-responsive genes in immune cells (Bi et al., 2009; Makarova et al., 2014; Wilhelmsen et al., 2021).

Exercise seems to regulate the expression of miRNAs involved in biological processes such as angiogenesis, protein synthesis, myogenesis, apoptosis, and cellular metabolism (dos Santos et al., 2022, pp. 8–9). Strength training appears to be lacking research when it comes to miRNA expression, especially in the elderly population. A few studies reporting about the effect of RE on miRNA expression had varying results partially due to divergent analysis methods, sample size, probe type, and RE protocols (Drummond et al., 2008; Margolis et al., 2016; Mueller et al., 2011; Rivas et al., 2014; Zacharewicz et al., 2014). Nevertheless, most of them reported a dysregulation of miRNA expression in older individuals when compared to younger ones.

1.3 Ageing

Biological ageing is linked to decreased regulatory processes of skeletal muscle, restricting muscle size, function, and power output (Arthur & Cooley, 2012; Borges et al., 2016; Gomes et al., 2017; Young, 1997). With advancing age, satellite cells' function decays, muscle tissues' regulatory and regenerative capacity declines, and typical age-related rapid loss in muscle mass occurs elderly. Perturbation of the regenerative ability of stem cells might be related to changes in regulatory networks mediating satellite cell function associated with ageing. Such functional alterations could derive from genetic and epigenetic changes over time. Satellite cells are less active in muscular regeneration and therefore produce fewer myocytes. With age, the Notch signalling pathway reduces, and Wtn signalling increases, resulting in diminished proliferation and differentiation of satellite cells in injury responses (Brack et al., 2007; Carlson et al., 2008; Carosio et al., 2011; Chakkalakal et al., 2012; Voog & Jones, 2010). Cellular senescence, irreversible cell cycle arrest, is caused by changes in molecular regulatory mechanisms due to stress or telomere erosion (F.-J. Liu et al., 2012). This process increases with ageeing, resulting in a decline in tissue function due to provoked changes in cell structure, function, and behaviour.

Additionally, the reduced vascularisation of skeletal muscle observed with increasing age limits the transport of essential nutrients and factors, e.g., hormones and growth factors, which could inhibit muscular growth and remodelling (Joanisse et al., 2017). This has been hypothesised as a contributing factor to the so-called anabolic resistance in older adults, which intends to explain the blunted molecular and physiological response to anabolic

stimulation, like RE, with advancing age. However, there is still little literature on anabolic resistance in physically active and strength-trained individuals. The accumulation of senescence tissue is a fundamental factor of biological ageing (Smith-Vikos & Slack, 2012). Immunosenescence also plays a pivotal role in age-related recovery decay affecting cellular response, muscle mass, function, and physical performance. In addition, it affects the immune system by reducing its efficiency, partially due to thymic shrinking and dysfunction (Montoya-Ortiz, 2013). Hence, immune-cell populations suffer functional dysregulation with age resulting in a higher pro-inflammatory basal state in the older population. As a result, the older population shows dysregulated crucial regulatory pathways for muscle repair and function, like muscle protein synthesis, and the regenerative and adaptive ability of the organism is impaired.

miRNA expression and ageing

One of the most significant contributing factors to age-related muscle atrophy is the so-called anabolic resistance. This phenomenon is believed to be the result of a blunted muscle protein synthetic response to anabolic stimuli such as food intake and physical activity and increased inhibition of muscle protein breakdown with advancing age (Brook et al., 2016b; Fry et al., 2011; Kumar et al., 2009, 2012; Sheffield-Moore et al., 2005). However, the cause of this imbalance in protein turnover with increasing age still needs to be fully understood.

The effect of ageing on miRNA expression profiles has been studied mostly on non-human organisms. Studies like Bates et al. (2009), Hamrick et al., (2010), and Soriano-Arroquia et al., 2016 have used rodents as models to compare miRNA expression between age groups. In the review of Bates and colleagues (2009), they described a dysregulation of miRNAs with a tendency of upregulation with ageing. The upregulation of miRNAs would indicate an inhibition of their target mRNAs, leading to attenuated physiological responses to stimuli and altered basal state of older individuals.

1.4 Research question

As post-transcriptional mRNA mediators, miRNAs function as regulators of signalling pathways crucial for numerous physiological processes, such as inflammation, apoptosis, cell proliferation, differentiation, and angiogenesis (Aránega et al., 2021; Chilton et al., 2014; Da Silva et al., 2012; Makarova et al., 2014; Tonevitsky et al., 2013; Zacharewicz et al., 2014). In the last few decades, researchers have studied the post-transcriptional regulatory potential of miRNAs in different physiological conditions. Given their regulatory role, miRNAs have been proposed as potential biomarkers for diseases such as cancer, and neuromuscular and muscular diseases (Aránega et al., 2021; Mercken et al., 2013).

As mentioned above, these small non-coding molecules have been reported to be differentially expressed with acute and chronic exercise and with ageing. These relationships could broaden our understanding of molecular processes that influence the regenerative and adaptive response of the human body at varying ages. Nevertheless, most of the existing scientific evidence derives from experiments performed in vitro or on experimental animals, focussing on myomiRs in skeletal muscle or on c-miRNAs from plasma or serum (Bi et al., 2009; Cheung et al., 2012; Hamrick et al., 2010; Kirby & McCarthy, 2013; Murach et al., 2022; Smith-Vikos & Slack, 2012). With few exceptions, there is a lack of research on the expression of whole blood miRNAs in the elderly population, especially in master athletes. A comprehensive analysis of whole-blood miRNAs' expression in trained men over 50 before and after eccentric exercise could provide a new perspective on the underlying acute processes in trained and aged organisms. This study aimed to examine whether the physiological processes after eccentric squat exercise varies between age groups in resistance-trained athletes by profiling whole blood intracellular miRNA expression in both groups with a miRNA array followed by a broad functional and network analysis.

2 Methods

This study was conducted in cooperation with the Faculty of Sport Science, Ruhr University Bochum, Bochum, Germany. Data were collected as part of a previous study published by Schmidt and colleagues (2021), which aimed to assess recovery in younger and older individuals in response to resistance exertion.

2.1 Ethics statement

Schmidt et al. (2021) study was ethically approved by the ethics committee from the institution of research athletics committee from the Faculty of Sport Science, Ruhr University Bochum, Bochum, Germany. Furthermore, each volunteer provided informed written consent after a preliminary health examination.

2.2 Participants

Sixteen resistance-trained male athletes participated in the previously named study evaluating age-related differences in recovery from eccentric exercise (Schmidt et al., 2021). The participants were divided by age groups, eight had a mean age of 22.1 ± 2.1 years (mean ± SD) (Young), and the remaining eight were master athletes (age: 52.4 ± 3.5 years) (Master). All participants were in good health and had no major health risk factors. The inclusion criteria consisted of a training frequency of no less than two strength training days per week for at least one year and a minimal half-squat one-repetition maximum (1RM) of 120% of the individual's body weight (Schmidt et al., 2021, p. 2).

2.3 Eccentric exercise study design

After giving their informed consent, the participants completed two exercise protocols, separated by a two-week washout period. The exercise session was followed by either the mixed-method recovery (MMR) intervention (a combination of cold-water immersion (CWI) and compression tights) or the control condition (passive recovery (PR), meaning the participants avoided any recovery methods). The participants were separated into age groups and randomly assigned to either MMR or PR for the first exercise protocol. Subsequently, the recovery interventions were interchanged. Athletes had to avoid strenuous exercise 96 hours before each training session, arrive hydrated, and consume a carbohydrate-rich meal pre- and post-training. The exact study design has been previously described (Schmidt et al., 2021).

2.3.1 Measurements

The participants' anthropometric measurements and estimated half-squat 1RM were assessed as part of the preliminary examination. Using a stadiometer and a biometrical impedance analysis (InBody Deutschland, Eschborn, Germany) the participant's height, weight, and body composition was measured (Schmidt et al., 2021, p. 4). Furthermore, the following values were determined as previously described by Schmidt et al. (2021). Maximal voluntary isometric contraction (MVIC) force in the half-squat (MVICsq) and leg press (MVIClp), countermovement jump (CMJ) height, resting twitch force (RTF) of the knee extensors, capillary whole-blood samples from the earlobe and analysed for blood lactate (BLa), and venous creatine kinase (CK) and c-reactive protein (CRP). Perceived muscle soreness was determined using the numeric pain scale (NPS). All variables, with exception of BLa were measured at pre-and post-training (post), 24, 48, and 72 hours. Venous blood samples were taken at the named time points, respectively.

2.3.2 Exercise test protocol

The standardised exercise protocol was previously described by Schmidt et al. (2021). In brief, before each training session, a ten-minute-long standardised dynamic warm-up protocol was performed. The warm-up protocol entailed dynamic movements, core stability exercises, and five repetitions at 50 and 70% of the individuals' 1RM half-squat, respectively. The training session consisted of using a Smith machine with a guided barbell (Technogym, Cesena, Italy), with which half-squats (at a 90° knee angle) were completed for nine sets of eight repetitions with an additional final loading set until momentary muscular failure. The intensity of the half-squats was selected at 70% of the participant's estimated 1RM. Each repetition was executed with a cadence of four seconds in eccentric mode and two seconds in concentric mode. As a result, the total time under tension for all ten sets was approximately 480s, with a rest time between sets of three minutes. All training sessions were supervised by researchers, who recorded the participants' number of completed repetitions in the final set, their heart rate, BLa, and perceived rate of exertion.

2.4 miRNA analysis study design

The samples referenced in the following section were drawn during Schmidt and colleagues (2021) study, which were sent to the molecular biology laboratory at the sports science department at the University of Vienna. The details of RNA extraction from whole blood and miRNA analysis using quantitative polymerase chain reaction are described in detail in the upcoming segments.

2.4.1 Blood sampling

Blood samples were collected using PAXgene Blood RNA Tubes (762165, Qiagen, PreAnalytiX GmbH, Switzerland) before (pre) and right after each training session (post), as well as 24, and 48 hours later. These specialised tubes stabilised whole blood miRNAs in, i.e., B cells, T cells, neutrophils, and monocytes.

2.4.2 RNA isolation

Total RNA, including miRNA, was purified, and then isolated using the PAXgene Blood miRNA Kit (763134, Qiagen, Germany) following manufacturer's protocol. The tubes contained a blood cell lysing reagent, which preserved the gene expression profile by stabilising intracellular RNA, thus preventing RNA degradation. Samples were then centrifuged for ten minutes to remove supernatant, the remaining pellet was washed with RNase-free water, and samples were resuspended using Buffer BM1. Next, buffer BM2 and proteinase K were mixed into the samples by vortexing, and the mix was incubated for ten minutes at 55°C to promote digestion. After centrifugation, the supernatant was removed without disturbing the pellet from the PAXgene Shredder spin column and transferred into a microcentrifuge tube, where isopropanol was added. Next, buffer BM2 and proteinase K were mixed into the samples by vortexing, and the mix was incubated for ten minutes at 55°C to promote digestion. DNase I incubation mix was added to remove the remaining genomic DNA, and samples were washed using Buffer BM3 and BM4. Lastly, pure RNA samples were eluted by adding 40 or 80 µl of Buffer BR5 and incubated for five minutes at 65°C. RNA quality (absorption at 260 nm) and quality (260/280 ratio) was analysed after RNA isolation and before miRNA expression analysis using a spectrophotometer (NanoDrop 1000, Thermo Fisher Scientific, Germany). Samples were stored at -80°C in Buffer BR5 for approximately two months until further analysis.

2.4.3 miRNA PCR Array

The expression profile of whole blood miRNAs was determined using a two-step real-time quantitative polymerase chain reaction (RT-qPCR) profiling using miScript miRNA PCR arrays (Qiagen, Germany), which consisted of miScript II RT Kit (218160), miScript SYBR Green PCR Kit (218073), and miScript miRNA PCR Array (MIHS-001ZA) (Qiagen, Germany).

First, isolated RNA single samples was reverse transcribed into complementary DNA (cDNA). 250 ng mature miRNAs from previously purified and isolated total RNA samples were polyadenylated with poly(A) polymerase and selectively reverse-transcribed into cDNA in 20 µl reaction mix using reverse transcriptase and miScript HiSpec Buffer according to manufacturers' instructions using miScript II RT Kit (Qiagen, Germany). Then, with the help of miScript Nucleics Mix, each sample was oligo-dT-primed, enabling the amplification of miRNAs in RT-qPCR. Subsequently, the RT Master Mix was prepared, containing 4 µl of miScript HiSpec Buffer, and 2 µl of miScript Nucleics Mix and miScript Reverse Transcriptase Mix, respectively, for each reaction. Next, RNA from each sample was mixed with RNase-free water, ensuing a concentration of 250 ng/12 µl of mixture per tube and 8 µl of RT Master Mix were added. After obtaining cDNA from total RNA, the samples were stored at a temperature of -80°C until further analysis.

Five participants from Young and five from Master from the PR group were selected based on RNA quality. Those participants' cDNA samples were pooled and utilised to determine miRNAs' profile expression. The samples from baseline (pre) and after the exercise protocol (post) were pooled separately for each age group.

miRNA PCR array plates were purchased from Qiagen (Human miFinder miScript miRNA PCR Array; MIHS-001ZA, 96-well; Qiagen, Germany). These plates profile 84 abundantly expressed miRNAs each. Figure 3 depicts the miScript miRNA PCR Array layout for a 96-well plate. Two separate miRNA PCR arrays were conducted for baseline and post, respectively. The analysis was performed using the cDNA pooled samples from each age group and point in time.

	1	2	3	4	5	6	7	8	9	10	11	12
A	hsa-miR-142-5p	hsa-miR-9-5p	hsa-miR-150-5p	hsa-miR-27b-3p	hsa-miR-101-3p	hsa-let-7d-5p	hsa-miR-103a-3p	hsa-miR-16-5p	hsa-miR-26a-5p	hsa-miR-32-5p	hsa-miR-26b-5p	hsa-let-7g-5p
B	miR-30c-5p	hsa-miR-96-5p	hsa-miR-185-5p	hsa-miR-142-3p	hsa-miR-24-3p	hsa-miR-155-5p	hsa-miR-146a-5p	hsa-miR-425-5p	hsa-miR-181b-5p	hsa-miR-302b-3p	hsa-miR-30b-5p	hsa-miR-21-5p
C	miR-30e-5p	hsa-miR-200c-3p	hsa-miR-15b-5p	hsa-miR-223-3p	hsa-miR-194-5p	hsa-miR-210-3p	hsa-miR-15a-5p	hsa-miR-181a-5p	hsa-miR-125b-5p	hsa-miR-99a-5p	hsa-miR-28-5p	hsa-miR-320a
D	miR-125a-5p	hsa-miR-29b-3p	hsa-miR-29a-3p	hsa-miR-141-3p	hsa-miR-19a-3p	hsa-miR-18a-5p	hsa-miR-374a-5p	hsa-miR-423-5p	hsa-let-7a-5p	hsa-miR-124-3p	hsa-miR-92a-3p	hsa-miR-23a-3p
E	hsa-miR-25-3p	hsa-let-7e-5p	hsa-miR-376c-3p	hsa-miR-126-3p	hsa-miR-144-3p	hsa-miR-424-5p	hsa-miR-30a-5p	hsa-miR-23b-3p	hsa-miR-151a-5p	hsa-miR-195-5p	hsa-miR-143-3p	hsa-miR-30d-5p
F	hsa-miR-191-5p	hsa-let-7i-5p	hsa-miR-302a-3p	hsa-miR-222-3p	hsa-let-7b-5p	hsa-miR-19b-3p	hsa-miR-17-5p	hsa-miR-93-5p	hsa-miR-186-5p	hsa-miR-196b-5p	hsa-miR-27a-3p	hsa-miR-22-3p
G	hsa-miR-130a-3p	hsa-let-7c-5p	hsa-miR-29c-3p	hsa-miR-140-3p	hsa-miR-128-3p	hsa-let-7f-5p	hsa-miR-122-5p	hsa-miR-20a-5p	hsa-miR-106b-5p	hsa-miR-7-5p	hsa-miR-100-5p	hsa-miR-302c-3p
H	cel-miR-39-3p	cel-miR-39-3p	SNORD61	SNORD68	SNORD72	SNORD95	SNORD96A	RNU6-6P	miRTC	miRTC	PPC	PPC

Purple: C. elegans miR-39 miScript Primer Assay; Orange: snoRNA/ snRNA miScript PCR Control; Yellow: Reverse transcription Control; Green: Positive PCR Control

Figure 3: *miScript miRNA PCR Array MIHS-001ZA layout*

Note. This figure illustrates the layout of the PCR array MIHS-001ZA (cat. no. 331221). SNORD61, -68, -72, -95, -96A, RNU6-6P were used as a normalisation control for the array data. miRTCs are miRTC miScript Primer Assays replicates, and PPCs are replicates of positive PCR controls. Adapted from "miScript® miRNA PCR Array Handbook, miScript II RT Kit; miScript SYBR® Green PCR Kit; miScript miRNA PCR Arrays; miScript miRNA QC PCR Array. For SYBR Green-based, real-time PCR; profiling of microRNAs using pathway-focused; arrays, HC arrays, and miRNome arrays". 2018, Qiagen, Germany, pg. 17. Copyright 2015 by Qiagen.

Due to the housekeeping genes and performance markers already included in each plate, this array facilitates data analysis and RT, as well as PCR performance assessment. cDNA, in a concentration of 1 ng/µl, of five participants from each age group, PR group, and each respective time point were thawed on ice. The miRNA expression profile was assessed according to the manufacturer's protocol, "Real-Time PCR for Mature miRNA Expression Profiling" (Qiagen, Germany). In brief, the protocol consists of miScript SYBR Green PCR Kit and miScript miRNA PCR Array. cDNA synthesis reaction was diluted with RNase-free water, followed by preparing the PCR reaction mix according to the manufacturer's instructions. First, 20.83 µl of cDNA premix of each sample, summing into a total of 104.12 µl cDNA, were mixed with 99.2 µl of RNase-free, and the PCR reaction mix, consisting of 275 µl of miScript Universal Primer, 1375 µl of QuantiTect SYBR Green PCR Master Mix, was added. No extra exogenous spike-in controls were added to the mixture, and 25 µl of the reaction mix was pipped into each well. RT-qPCR amplifications were conducted using a 7500 Real-Time PCR System device (Applied Biosystems 7500, 4351105). The real-time cycler was programmed according to the miScript miRNA PCR Array Handbook (Qiagen, Germany), with an initial activation step of 15 minutes at 95°C,

followed by 40 3-step cycles of 15 seconds at 94°C, 30 seconds at 55°C, and 34 seconds at 70°C each cycle, respectively (Figure 4).The procedure was repeated for all four plates.

Step	Time	Temperature	Additional comments
PCR **Initial activation step**	15 min	95°C	HotStarTaq DNA Polymerase is activated by this heating step.
3-step cycling:*†‡			
Denaturation	15 s	94°C	
Annealing	30 s	55°C	
Extension§	30 s	70°C	Perform fluorescence data collection.
Cycle number	40 cycles		Cycle number depends on the amount of template cDNA and abundance of the target.

Figure 4: Cycling conditions for Real Time PCR

Note. The figure above illustrates the exact cycling steps for real time PCR. Reprinted from "miScript® miRNA PCR Array Handbook, miScript II RT Kit; miScript SYBR® Green PCR Kit; miScript miRNA PCR Arrays; miScript miRNA QC PCR Array. For SYBR Green-based, real-time PCR; profiling of microRNAs using pathway-focused; arrays, HC arrays, and miRNome arrays". 2018, Qiagen, Germany, pg. 39. Copyright 2015 by Qiagen.

2.4.4 Analysis of whole blood miRNA expression

A background correction was performed before exporting the raw PCR data. With the assumption that housekeeping genes should only differ slightly between groups and time points, the differences between the pre-and post-values in each group were manually modified, changing each array's fluorescence threshold (TH). The TH of each array was adjusted to close the gap between values (Young athletes: pre TH = 0.004 and post TH = 0.007; Master athletes: pre TH = 0.018 and post TH = 0.025). As a result, new values of housekeeping genes were generated for each array, which were then used as reference values for normalisation. The miScript miRNA PCR Array Data Analysis Excel sheet from Qiagen (PROM-13391_miScript miRNA PCR Array Data Analysis 1810_1118_WW) (version 5.0, 2018) was used to identify differentially expressed (DE) miRNAs after resistance exercise in Young and Master, as well as at baseline between age groups. Relative miRNA expression ($2^{-\Delta\Delta CT}$ values) was obtained using the $\Delta\Delta C_T$ method (= average ΔC_T (sample of interest) – average ΔC_T (reference sample)) while using the housekeeping gene controls included in the array (five snoRNAs (SNORD61, -95, -96A, -68, -21 assay) and one snRNA (RNU6-2 assay)) as normalisation controls for relative

quantification. Only entities with a C_T value below 30 were selected for further data analysis for each age group. Fold change (FC) was calculated using the $2^{-\Delta\Delta CT}$ method by dividing the normalised gene expression in the test sample ($2^{-\Delta CT}$ (test sample)) with the normalised gene expression in the control sample ($2^{-\Delta CT}$ (control sample)). Fold regulation (FR) was also calculated; if FC >1, FR corresponds to the same value and indicates an upregulation. If FC <1, it indicates downregulation, and the FR is equivalent to the inverse of FC. A 1.5- or 0.67-fold expression difference was interpreted as relevant up- and downregulation, respectively. miRNA expression profile changes were assessed before and after eccentric squat exercise in each age group. Furthermore, the baseline miRNA expression values between Young and Master were assessed.

2.4.5 miRNA target analysis

Target and pathway analysis was performed for selected sets of differently regulated miRNAs using several different bioinformatic web-based tools to determine potential gene targets and signalling pathways. GeneTrail 3 (https://genetrail.bioinf.uni-sb.de), a web service that included a collection of numerous biological processes and signalling pathways for humans, amongst 11 other organisms, provided a miRNomics comprehensive enrichment and network analysis (Gerstner et al., 2020). This web tool was used for miRNA-gene target interaction analysis and miRNA functional enrichment analyses. Figure 5 depicts GeneTrail 3's workflow. First, targets were filtered based on experimentally validated with high confidence. Analyses were performed through an over-representation analysis (ORA) based on the hypergeometric distribution, which assesses if a miRNA target has more target genes than expected by chance using the miRNomics tool in GeneTrail 3. Experimentally validated gene targets were previously assessed using luciferase reporter assay, western blot, and qRT-PCR as interaction analyses. Protein coding genes targeted by at least one of the listed miRNAs with strong evidence were assessed using miRTarBase (release 7.0) (Chou et al., 2018). To further annotate the DE miRNAs, a functional enrichment analysis was performed using miRPathDB 2.0 (miRNA Pathway Dictionary Database, release 2.0; https://mpd.bioinf.uni-sb.de) in GeneTrail 3, which identifies statistically significant over-represented validated target genes in functional annotations and signalling pathways (Kehl et al., 2020). Pathway enrichment analysis is performed using functional annotations from Gene Ontology for biological processes (BPs), cellular components (CCs), and molecular functions (MFs), as well as signalling pathways from Kyoto Encyclopaedia of Genes and Genomes (KEGG), Reactome, and Wikipathways. Additionally, miRNA target gene

networks were portrayed with the help of MIENTURNET (MicroRNA enrichment turned network) (http://userver.bio.uniroma1.it/apps/mienturnet/) using miRTarBase (release 7.0). This web service can assess various miRNA-gene target interactions in multiple cellular processes and expresses computationally predicted (TargetScan, release 7.3) and or experimentally validated (miRTarBase, release 7.0) human target gene interactions of a user-inserted list of miRNAs (Licursi et al., 2019).

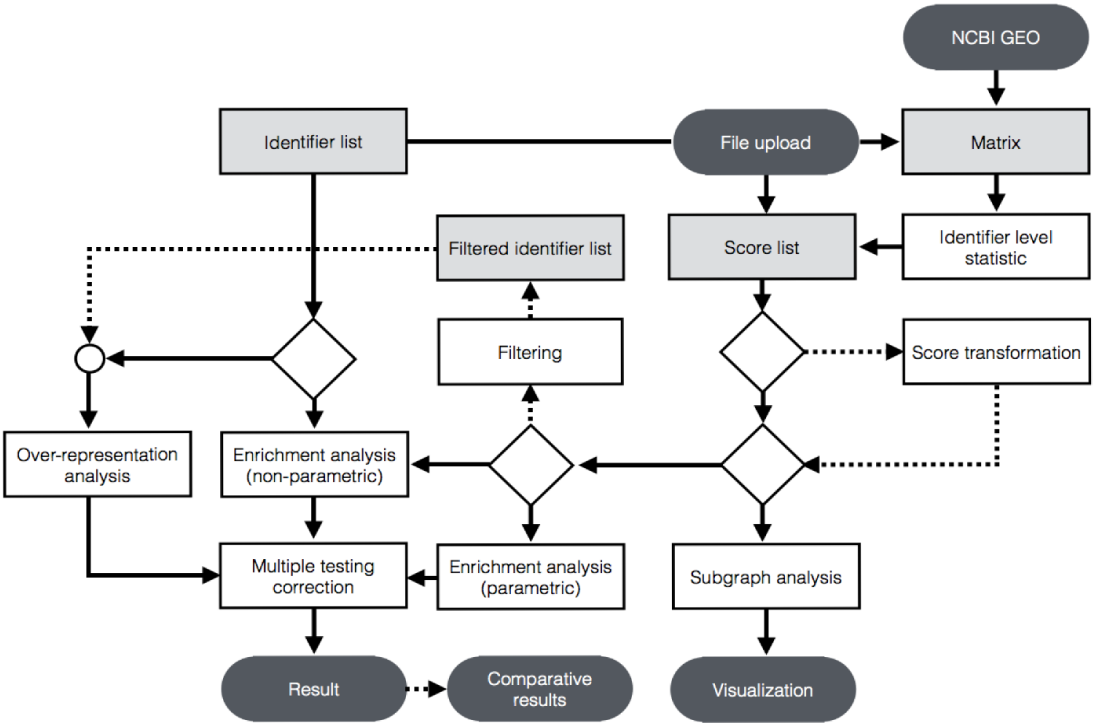


Figure 5: Flowchart of the web tool Gene Trail 3

Note. This figure illustrates the flowchart of GeneTrail 3. First, a file with a list of miRNAs or mRNAs is uploaded as input. Then, the tool performs an over-representation analysis using the selected databases and categories with user-modified statistics. This process can be performed to search for genes targeted by miRNAs or as a target enrichment analysis. Round boxes, start/end; gray background, input file; white background processing steps; diamonds, decision nodes. Reprinted from "Multi-omics enrichment analysis using the GeneTrail2 web service". D. Stöckel et al., 2016, *Bioinformatics (Oxford, England), 32(10)*, https://doi.org/10.1093/bioinformatics/btv770, pg. 1504. Copyright 2016 by Oxford University Press. Reprinted with permission.

2.5 Statistical data evaluation

Data are reported as mean ± standard deviation unless otherwise stated. Statistical data analysis was performed using Microsoft Excel version 16.58 for Mac (Microsoft Corporation, Redmond, WA, United States) and IBM SPSS Statistics version 27 for Mac (IBM Corp, Armonk, NY, United States). The significance level α was set at 0.05. The statistical data evaluation in this study is a sub-analysis of the data from Schmidt et al. (2021). This study utilizes the data of n = 10 participants (Young n = 5 and Master n = 5) of the total 16 participants of Schmidt and colleague's (2021) study.

First, an independent-samples t-test was run to determine if there were differences in anthropometric measures between Young and Master. If the assumption of homogeneity of variances was violated, a Mann Whitney U Test was performed to determine the differences between the age groups. The normality of the values was assessed by Shapiro-Wilk's test, and the homogeneity of variances was assessed using Levene's test for equality of variances. Second, a two-way mixed ANOVA was run using time as a within-subject factor (baseline and immediately after RE) and age as between subject-factor (young and master athletes) with the values of BLa, venous CK, cortisol, testosterone, and CRP, as well as CMJ, MVIC in the half squat and leg press, RTF, and muscle soreness. There was no need to test the assumption of sphericity with Mauchly's test of sphericity because there only were two levels of a repeated measure factor.

Additionally, simple main effect analyses of time and group were performed when the two-way mixed ANOVA showed a significant interaction between RE and age group on any of the variables. Lastly, we conducted pathway enrichment using two-sided ORA, and all *p*-values were adjusted separately using a false discovery rate (Benjamin-Hochberg method, *q*-value) for all databases. Pathway enrichment was performed using target genes with a *q*-value < 0.05 and mediated by at least two miRNAs. Annotations and signalling pathways were considered significantly enriched when it showed a *q* value < 0.05.

3 Results

3.1 Anthropometric characteristics

The participants' baseline anthropometric and physiological characteristics are shown in Table 1. The statistical analyses assessed that the age groups did not differ in lean muscle mass or lean muscle mass in the legs or any other parameter except for mean age and circulating testosterone concentration. However, there was a significant age difference ($t(8) = -9.218$, $p < 0.001$) and basal testosterone concentration ($t(6) = 3.950$, $p = 0.008$) between age groups, with a mean difference of 5.37 (95% CI, 2.04 to 8.70). Both younger and master athletes had, on average, a normal body-mass-index (BMI) according to the World Health Organisation (WHO) (mean ± standard deviation, 24.2 ± 1.7 and 24.7 ± 2.5 $kg \times m^{-2}$, respectively).

Table 1: Participants' anthropometric characteristics

Variable	Young athletes n = 5	Master athletes n = 5	Differences
	Means ± SD		*p*-value
Age [years]	25.2 ± 5.3	52.2 ± 3.9***	< 0.001
Height [cm]	177.6 ± 3.7	179.0 ± 8.7	0.746
Weight [kg]	76.5 ± 7.9	79.2 ± 10.6	0.662
BMI [$kg \times m^{-2}$]	24.2 ± 1.7	24.7 ± 2.5	0.711
Fat [%]	14.2 ± 3.1	13.0 ± 2.8	0.553
Lean muscle mass [kg]	37.5 ± 3.2	37.5 ± 5.2	0.994
Lean muscle legs [kg]	19.5 ± 1.7	19.7 ± 3.3	0.916
$1RM_{est}$ [kg]	103.6 ± 6.4	107.4 ± 14.6	0.608
$1RM_{est}$ [%]	136.8 ± 13.3	137.0 ± 23.4	0.987

Values are given in mean ± SD. BMI, body mass index; 1RMest, estimated one-repetition maximum
**** p < 0.001 difference between age groups*

Note. This table shows the anthropometric data of the young and master athletes pooled for PCR array profiling at baseline and post-eccentric squat exercise. Adapted from "Recovery From Eccentric Squat Exercise in Resistance-Trained Young and Master Athletes With Similar Maximum Strength: Combining Cold Water Immersion and Compression." by J. Schmidt et al., 2021, *Frontiers in physiology*, *12*, 665204, pg. 3. Copyright 2021 by Schmidt, Ferrauti, Kellmann, Beaudouin, Pfeiffer, Volk, Wambach, Bruder and Wiewelhove.

3.2 Eccentric squat exercise

According to a Mann-Whitney U Tests, participants' physical performance during the standardised squat exercise protocol did not differ significantly (Table 2).

Table 2: Eccentric squat exercise performance

Variable	Young athletes	Master athletes	Differences
	Means ± SD		*p*-value
Repetitions in set 10 [n]	10 ± 3	10 ± 3	0.690
Total repetitions [n]	81 ± 5	82 ± 3	0.548
Weight loaded [kg]	72.50 ± 3.95	76.11 ± 10.03	0.548
Workload [N]	5852.0 ± 592.4	6245.6 ± 915.0	0.841
RPE [1-10]	8.2 ± 0.8	7.4 ± 1.8	0.690

Values are given in mean ± SD. Young (n=5), Master (n=5), RPE, rate of perceived exertion

Note. This table shows the data of the young and master athletes pooled for PCR array profiling at baseline and post-eccentric squat exercise. Data from "Recovery From Eccentric Squat Exercise in Resistance-Trained Young and Master Athletes With Similar Maximum Strength: Combining Cold Water Immersion and Compression." by J. Schmidt et al., 2021, *Frontiers in physiology*, *12*, 665204, pg. 3. Copyright 2021 by Schmidt, Ferrauti, Kellmann, Beaudouin, Pfeiffer, Volk, Wambach, Bruder and Wiewelhove.

The total number of repetitions (mean ± SD, *p*-value) (Young: 81 ± 5 repetitions; Master: 81 ± 4 repetitions, $p > 0.05$) and the workload (Young: 5852.0 ± 592.4 N; Master: 6245.6 ± 915.0 N, $p > 0.05$) were not statistically significant different between age groups. The older athletes showed a slightly higher tendency in both variables. However, Master reported a lower rate of perceived exertion (RPE) (7.4 ± 1.8 points) than Young (8.2 ± 0.8 points), with no statistically significant difference between groups ($p > 0.05$). Figure 6 illustrates the number of total repetitions, weight on the bar, and the stated RPE post squat session separated by age group.

3.3 Biomarkers and performance measures

We analysed the values of fatigue-related biomarkers from all participants included in the miRNA profiling procedure. The samples of the ten participants measured at baseline and right after eccentric squat exercise, i.e., blood lactate concentration, the concentration of

venous CK, and CRP, were included in the analysis. It is important to note that cortisol and testosterone concentration was only measured in eight of the ten participants (four young and four master athletes). The two-way mixed ANOVAs were run separately respectively. For statistically significant results, simple main effect analyses were performed to assess the exact effect of the two factors, time (pre and post) and age (Y and M), on the respective biomarker.

3.3.1 Biomarkers

Of the six biomarkers measured, only one showed a statistically significant interaction between time and age. In Addition, two variables (BLa and CK) showed a significant main effect of time, and one variable showed a significant effect of group. The results are illustrated in Table 3.

Table 3: Effect of resistance exercise on fatigue-related biomarkers

Variable	Group	Time		*p*-value[2]
		pre	post	
BLa [mmol×l^{-1}]	Young	0.86 ± 0.26	9.83 ± 2.71***	< 0.001
	Master	1.34 ± 0.16$^{\#}$	5.37 ± 2.04*$^{,\#}$	0.015
p-value[1]		0.019	0.039	
CK [U×l^{-1}]	Young	162.6 ± 83.6	173.3 ± 63.7**	0.003
	Master	218.5 ± 106.1	213.5 ± 76.1*	0.012
p-value[1]		0.846	0.942	
CRP [mg×l^{-1}]	Young	1.2 ± 1.9	0.4 ± 0.3	0.487
	Master	1.2 ± 1.9	0.4 ± 0.3	0.341
p-value[1]		0.053	0.775	
Cortisol [µg×dl^{-1}]	Young	15.11 ± 3.61	10.42 ± 1.49	0.746
	Master	12.63 ± 4.72$^{\#\#}$	13.89 ± 7.01	0.080
p-value[1]		0.008	0.443	
Testosterone [pg×ml^{-1}]	Young	13.60 ± 2.39	8.23 ± 1.29	0.050
	Master	13.08 ± 1.91	11.47 ± 3.42	1.000
p-value[1]		0.427	0.447	

Values are given in mean ± SD. BLa, blood lactate concentration; CK, venous creatine kinase concentration; CRP, c-reactive protein.

[1]p-value from main effect of group (Young vs Master) at each time point

[2] p-value from main effect of time (pre vs post resistance exercise) for each group

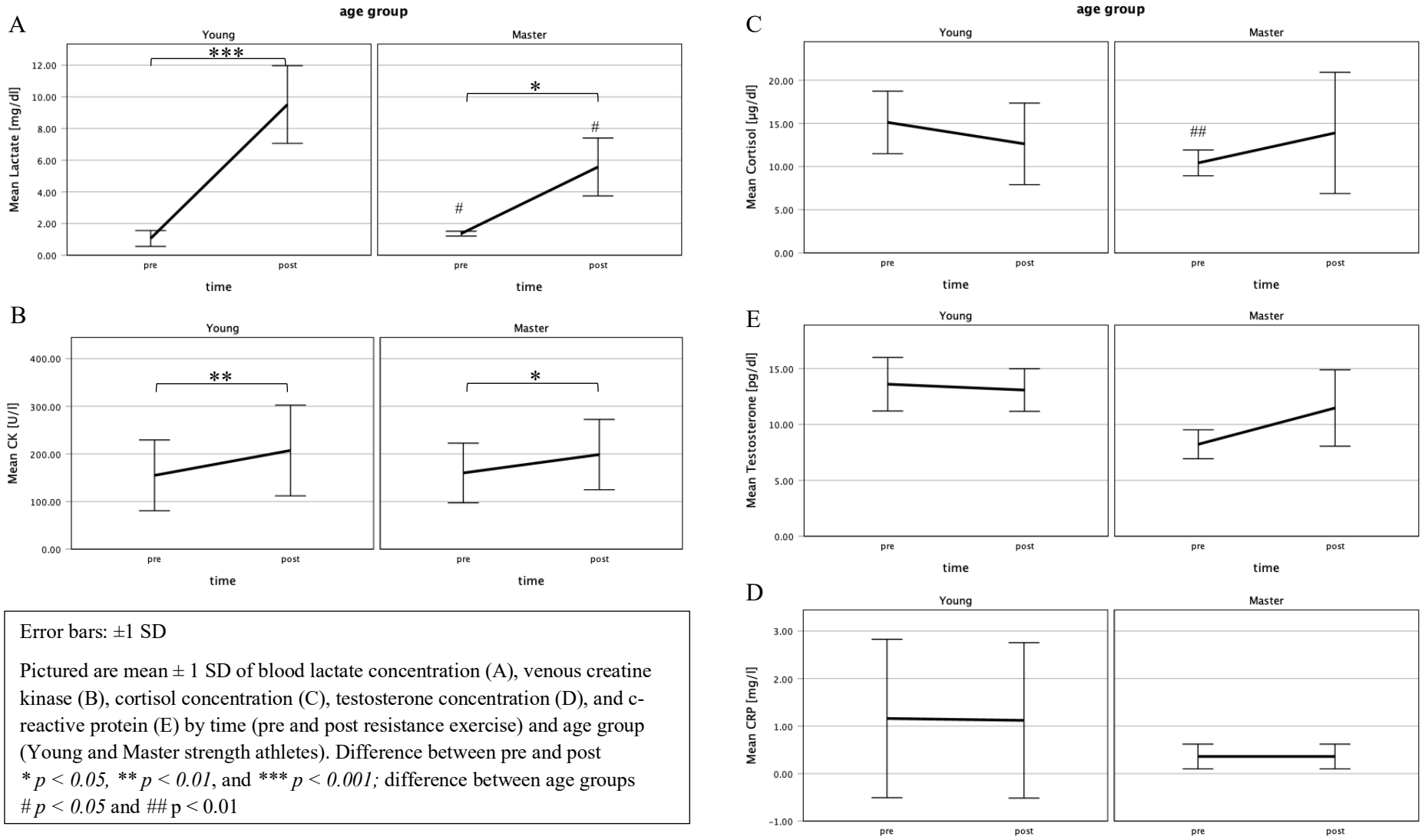
**,# p < 0.05, **,## p < 0.01, and *** p < 0.001*

Note. This table shows the biomarker data of the young and master athletes pooled for PCR array profiling at baseline and post-eccentric squat exercise. Adapted from "Recovery From Eccentric Squat Exercise in Resistance-Trained Young and Master Athletes With Similar Maximum Strength: Combining Cold Water Immersion and Compression." by J. Schmidt et al., 2021, *Frontiers in physiology*, *12*, 665204, pg. 6. Copyright 2021 by Schmidt, Ferrauti, Kellmann, Beaudouin, Pfeiffer, Volk, Wambach, Bruder and Wiewelhove.

There was a significant interaction between time and age on BLa concentration ($p = 0.018$). Basal BLa did not differ significantly between groups ($p > 0.05$). BLa increased post RE in both age groups in comparison to basal values (mean difference (95% CI), *p*-value) (Young: 8.46 (95% CI, 5.23 to 11.69) mmol×l^{-1}, $p = 0.002$ and Master: 4.21 (95% CI, 1.92 to 6.50) mmol×l^{-1}, $p = 0.007$)) and showed a statistically significant difference between age groups with Young having higher values than Master (mean difference (95% CI), *p*-value) (3.94 (95% CI, 0.79 to 7.10) mmol×l^{-1}, $p = 0.020$).

CK did not show a statistically significant interaction between time and age group ($p > 0.05$). The main effect analysis showed a statistically significant difference in mean venous CK concentration with time. For younger and master athletes, venous. CK concentration increased post RE (45.45 (95% CI, 30.48 to 60.42) U×l^{-1}, $p = 0.002$).

Furthermore, the main effect of group assessed a statistically significant difference in basal testosterone levels between Young and Master ($p = 0.035$). The younger participants had a higher basal Testosterone level than the older individuals (mean ± SD) (13.60 ± 2.39 pg×ml^{-1} and 8.23 ± 1.29 pg×ml^{-1}, respectively). No other significant interaction between time and age group was observed and no additional main effect of time or group were detected. Figure 6 depicts the mean ± 1SD of the fatigue-related biomarkers measured before and after RE. In addition, the statistical results are illustrated in Table 4.



Error bars: ±1 SD

Pictured are mean ± 1 SD of blood lactate concentration (A), venous creatine kinase (B), cortisol concentration (C), testosterone concentration (D), and c-reactive protein (E) by time (pre and post resistance exercise) and age group (Young and Master strength athletes). Difference between pre and post * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$; difference between age groups # $p < 0.05$ and ## $p < 0.01$

Figure 6: Simple line mean graphs of biomarkers by time and age group

Table 4: Statistical indicators of time and age interaction on fatigue-related biomarkers

Measure	Resistance exercise x age group interaction			Time				Age group			
	F	*p*	*η2*		*F*	*p*	*η2*		*F*	*p*	*η2*
Blood lactate [mmol×l^{-1}]	8.869	0.018*	0.526	Y	*52.786*	*0.002***	*0.930*	pre	*1.730*	*0.225*	*0.178*
				M	*25.991*	*0.007***	*0.867*	post	*8.305*	*0.020**	*0.509*
CK venous [U×l^{-1}]	1.114	0.322	0.122		49.023	<0.001***	0.860		0.001	0.972	0.000
CRP [mg×l^{-1}]	2.667	0.141	0.250		2.667	0.141	0.250		1.088	0.327	0.120
Cortisol [µg×dl^{-1}]	1.572	0.257	0.208		0.043	0.842	0.007		0.561	0.482	0.085
Testosterone [pg×ml^{-1}]	2.991	0.134	0.333		1.562	0.258	0.207		7.365	0.035*	0.551

Blood lactate, concentration of blood lactate; CK, creatine kinase; CRP, C-reactive protein; pre, baseline; post, after resistance exercise; Y, young athletes; M, master athletes

Values in italic represent simple main effect analyses of group and time, respectively

** $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$*

Note. This table shows the biomarkers' data of the young and master athletes pooled for PCR array profiling at baseline and post eccentric squat exercise. Adapted from "Recovery From Eccentric Squat Exercise in Resistance-Trained Young and Master Athletes With Similar Maximum Strength: Combining Cold Water Immersion and Compression." by J. Schmidt et al., 2021, *Frontiers in physiology*, *12*, 665204, pg. 3. Copyright 2021 by Schmidt, Ferrauti, Kellmann, Beaudouin, Pfeiffer, Volk, Wambach, Bruder and Wiewelhove.

3.3.2 Performance tests

To assess the physiological effect of the test protocol on the participants, parameters such as, CMJ height, MVIC power in the half squat and leg press, and RTF force were measured at baseline and immediately after RE. Additionally, the subjective 11-point numerical pain rating scale determined muscle soreness at both time points. Table 5 lists the results of the performance test in Young and Master before and after RE.

Table 5: Effect of resistance exercise on physical performance measurements

Variable	Group	Time		*p*-value[2]
		pre	post	
CMJ [cm]	Young	40.40 ± 4.59	28.82 ± 5.63***	< 0.001
	Master	33.60 ± 4.52$^{\#\#}$	25.56 ± 4.95**$^{,\#}$	0.006
p-value[1]		0.007	0.027	
MVICsq [N]	Young	1236.8 ± 228.0	917.6 ± 141.0***	< 0.001
	Master	993.6 ± 232.9$^{\#}$	818.6 ± 176.4*	0.039
p-value[1]		0.029	0.217	
MVIClp [N]	Young	1901.2 ± 300.4	1703.8 ± 388.3*	0.010
	Master	1741.6 ± 339.4	1574.0 ± 494.1*	0.025
p-value[1]		0.395	0.549	
RTF [N]	Young	132.6 ± 35.7	91.0 ± 36.9**	0.001
	Master	66.2 ± 25.6	55.4 ± 29.3*	0.029
p-value[1]		0.107	0.553	
NPS [0-10]	Young	0.4 ± 0.5	806 ± 0.5***	< 0.001
	Master	6.8 ± 1.8	3.6 ± 2.4*$^{,\#}$	0.020
p-value[1]		0.580	0.044	

Values are given in mean ± SD. BLa, blood lactate concentration; CK, venous creatine kinase concentration; CRP, c-reactive protein.

[1] p-value from main effect of group (Young vs Master) at each time point

[2] p-value from main effect of time (pre vs post resistance exercise) for each group

**,# p < 0.05, **,## p < 0.01, and *** p < 0.001*

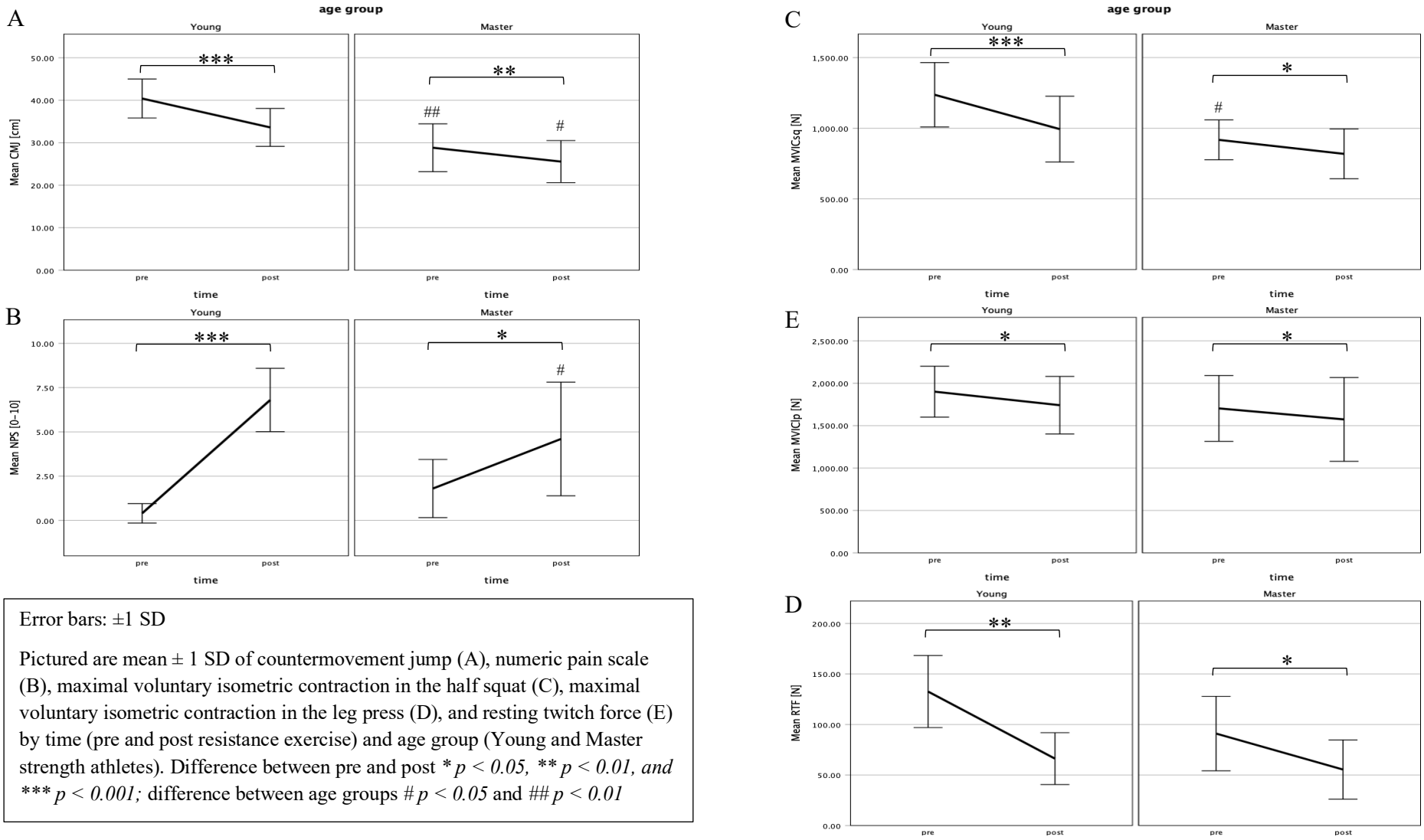
Note. This table shows the biomarker data of the young and master athletes pooled for PCR array profiling at baseline and post-eccentric squat exercise. Adapted from "Recovery From Eccentric Squat Exercise in Resistance-Trained Young and Master Athletes With Similar Maximum Strength: Combining Cold Water Immersion and Compression." by J. Schmidt et al., 2021, *Frontiers in physiology*, *12*, 665204, pg. 6. Copyright 2021 by Schmidt, Ferrauti, Kellmann, Beaudouin, Pfeiffer, Volk, Wambach, Bruder and Wiewelhove.

A two-way mixed ANOVA revealed a statistically significant interaction between age and RE on CMJ height ($p = 0.023$), MVIC output in the half squat ($p = 0.035$) and, marginally but surprisingly, muscle soreness ($p = 0.049$). Analyses of simple main effects of time and age groups were run on those three variables, respectively.

CMJ height decreased significantly between time points in Young (mean difference (95% CI), *p*-value) (6.8 (95% CI, 4.7 to 8.9) cm, $p = 0.003$). For Master, only CMJ height diminished significantly between both time points (3.3 (95% CI, 1.2 to 5.3) cm, $p = 0.008$) and MVIC force production in the half squat did not change significantly but was on the verge of significance with 99.0 (95% CI, 6.4 to 191.6) N ($p = 0.051$). At baseline, CMJ height was statistically significantly greater in younger athletes compared to master athletes (11.6 (95% CI, 4.1 to 19.1) cm, $p = 0.007$) and significantly greater post RE (8.0 (95% CI, 1.2 to 15.0) cm, $p = 0.027$).

As for MVIC in the half squat, the force production of the younger athletes decreased significantly between both measuring points (243.2 (95% CI, 150.7 to 335.8) N, $p = 0.005$) and did not change significantly in Master when with time. A simple main effect of group showed that the MVCIsq force production was only statistically significantly different at baseline (319.2 (95% CI, 42.8 to 595.6) N, $p = 0.029$) and not after eccentric squat exercise.

Regarding muscle soreness, there was an unexpected interaction between RE and age group on subjective pain perception ($p = 0.049$). The simple main effect of time analysis showed a statistically significant increase of perceived pain between baseline and right after RE in Young (6.4 (95% CI, 4.0 to 8.8) points, $p = 0.001$) but not in Master. As a result, this induced a significant difference of points in the pain rating scale post RE between younger and older athletes with a mean difference of 3.2 (95% CI, 0.1 to 6.3) points ($p = 0.044$) between age groups at post. Figure 7 depicts the mean ± 1SD of the fatigue-related biomarkers measured before and after RE. Additionally, the exact statistical results can be found in Table 6.



Error bars: ±1 SD

Pictured are mean ± 1 SD of countermovement jump (A), numeric pain scale (B), maximal voluntary isometric contraction in the half squat (C), maximal voluntary isometric contraction in the leg press (D), and resting twitch force (E) by time (pre and post resistance exercise) and age group (Young and Master strength athletes). Difference between pre and post * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$; difference between age groups # $p < 0.05$ and ## $p < 0.01$

Figure 7: Simple line mean graphs of physical performance tests by time and age group

Table 6: Statistical indicators of time and age interaction on physical performance measurements

Measure	Resistance exercise x age group interaction			Time				Age group			
	F	*p*	*η2*		*F*	*p*	*η2*		*F*	*p*	*η2*
CMJ [cm]	7.864	0.023*	0.496	Y	*39.828***	*0.003*	*0.909*	pre	*12.706***	*0.007*	*0.614*
				M	*24.567***	*0.008*	*0.806*	post	*7.295**	*0.027*	*0.477*
MVIC half squat [N]	6.454	0.035*	0.447	Y	*30.624***	*0.005*	*0.884*	pre	*7.093**	*0.029*	*0.470*
				M	*7.596*	*0.051*	*0.655*	post	*1.794*	*0.217*	*0.183*
MVIC leg press [N]	0.198	0.668	0.024		18.647**	0.003	0.700		0.565	0.474	0.066
RTF [N]	2.626	0.144	0.247		28.802**	0.001	0.783		2.116	0.184	0.209
Muscle soreness [0-10]	5.352	0.049*	0.401	Y	*62.061***	*0.001*	*0.939*	pre	*0.333*	*0.580*	*0.040*
				M	*6.000*	*0.070*	*0.600*	post	*5.689**	*0.044*	*0.416*

CMJ, counter movement jump; MVIC half squat, maximal voluntary isometric contraction in the half squat; MVIC leg press, maximal voluntary isometric contraction in the leg press; RTF, resting twitch force; pre, baseline; post, after resistance exercise; Y, young athletes; M, master athletes
Values in italic represent simple main effect analyses of group and time, respectively
* $p < 0.05$, and ** $p < 0.01$

Note. This table shows the performance measurement data of the young and master athletes pooled for PCR array profiling at baseline and post eccentric squat exercise. Adapted from "Recovery From Eccentric Squat Exercise in Resistance-Trained Young and Master Athletes With Similar Maximum Strength: Combining Cold Water Immersion and Compression." by J. Schmidt et al., 2021, Frontiers in physiology, 12, 665204, pg. 3. Copyright 2021 by Schmidt, Ferrauti, Kellmann, Beaudouin, Pfeiffer, Volk, Wambach, Bruder and Wiewelhove.

3.4 Whole blood miRNA expression

Whole blood from five young and five master strength-trained athletes were pooled respectively and subjected to miRNA PCR Array to assess miRNA expression at baseline and after eccentric squat exercise until failure. Using RT-qPCR, the expression of 84 intracellular miRNAs from a pool of five participants from each age group at baseline and post-RE from the PR group were profiled separately. A total of 83 miRNAs were detected at both points in the younger group pooled samples. The same 83 miRNAs were also detected at baseline in the pooled samples of the master athletes, but only 82 miRNAs were determined post exercise. In the pooled samples of the younger athletes, eight miRNAs showed C_T >30 at baseline (miR-9-5p, -141-3p, -124-3p, -302a-3p, -122-5p, and -302c-3p), and three further at post (miR-32-5p, -376c-3p, -243-3p) showed C_T values >30 at both time points. As for the pooled samples of the master athletes, ten miRNAs showed a C_T >30, miR-39-3p was not detected at post, and miR-141-3p was not detected at baseline. miRNAs with C_T >30 and miRNAs not determined at any and or only at one point in time are depicted in Table 7. miR-302b-3p could not be detected at any point in time in the pooled samples. miR-9-5p, 32-5p, 96-5p, 124-3p, 376c-3p, 143-3p, and 302c-3p had elevated C_T values at both baseline and post-exercise in Young and or Master.

Table 7: List of whole blood miRNAs not detected and or with C_T >30

miRNA ID	Well	Young		Master	
		pre	post	pre	post
hsa-miR-9-5p	A02	33.42	36.17	34.78	37.38
hsa-miR-32-5p	A10	<30	33.07	34.66	34.23
hsa-miR-96-5p	B02	<30	<30	30.09	30.14
hsa-miR-302b-3p	B10	not detected	not detected	not detected	not detected
hsa-miR-141-3p	D04	32.20	39.58	not detected	39.99
hsa-miR-124-3p	D10	32.43	32.09	34.68	35.10
hsa-miR-376c-3p	E03	<30	31.14	32.04	32.66
hsa-miR-143-3p	E11	<30	30.11	31.50	30.47
hsa-miR-302a-3p	F03	35.02	33.25	36.18	37.86

hsa-miR-122-5p	G07	31.68	38.77	32.19	32.10
hsa-miR-302c-3p	G12	32.27	32.29	36.06	39.62
cel-miR-39-3p	H01	36.29	35.61	not detected	not detected
cel-miR-39-3p	H02	35.6	36.6	31.17	not detected

pre, baseline; post, after eccentric squat exercise

Note. This table lists miRNAs that could not be detected and or appeared to have C_T values above 30 with their respective values, either in Young or Master athletes before (pre), directly after (post) physical exertion, or at both points in time.

3.4.1 Identification of differently expressed (DE) miRNAs using PCR Array

The PCR array data profiling the master athletes' pooled samples showed surprisingly only three DE miRNAs. Two miRNAs (miR-210-3p and miR-15a-5p) were upregulated and did not exceed a two-fold expression difference (FC = 1.5 and 1.8, respectively) (Figure 8). The other miRNA (miR-144-3p) was downregulated (FC = 0.57). The exact data is presented in Table A1 (Appendix A). Furthermore, 34 miRNAs were not-meaningfully upregulated (FC < 1.5), two miRNAs did not change post RE (miR-16-5p and miR-186-5p), and the remaining 34 miRNAs were not-meaningfully downregulated (FC > 0.5).

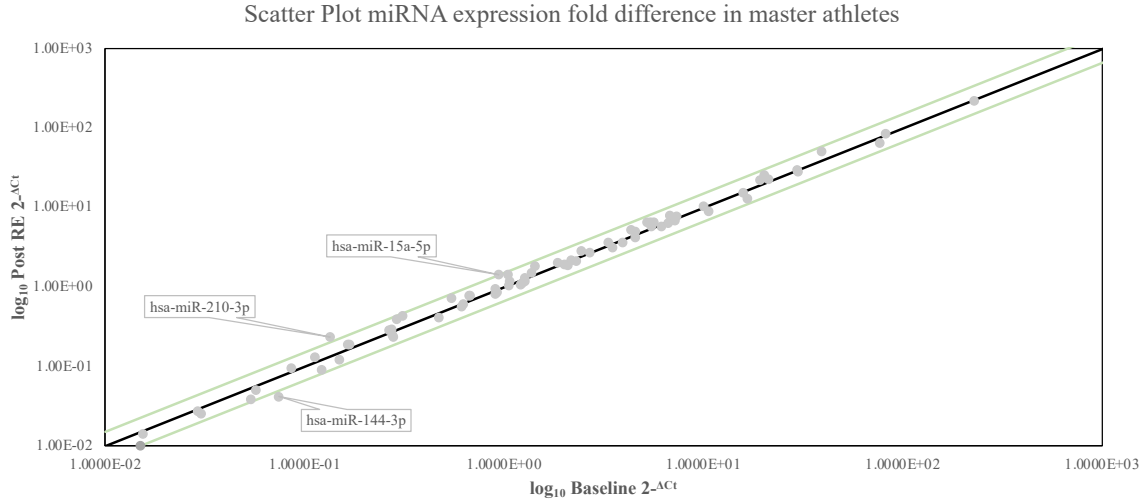


Figure 8:Fold Difference scatter plot of DE miRNAs in master athletes post RE

Note. This figure illustrates the fold changes ($2^{-\Delta Ct}$) of the miRNAs determined through PCR Array between baseline and immediately after eccentric squat exercise in master athletes. Only detected miRNAs with $C_T < 30$ are depicted in this figure. The black line indicates a fold change of 1 and the green lines indicate the pre-defined threshold of 1.5. miRNAs crossing the threshold are labelled with their respective ID. DE, differentially expressed; RE, resistance exercise.

Fifty-nine miRNAs were downregulated in the younger group (FC <0.67), pictured in Figure 9. Of those DE, a total of 45 miRNAs had a significant fold change (FC <0.5). The three DE miRNAs in Master are also regulated in Young – miR-210-3p and -15a-5p are upregulated in master but downregulated in younger athletes. The younger group also exhibits an overall grosser FR value in those three miRNAs (FR <-2) than the older group (FR >-2; <2).

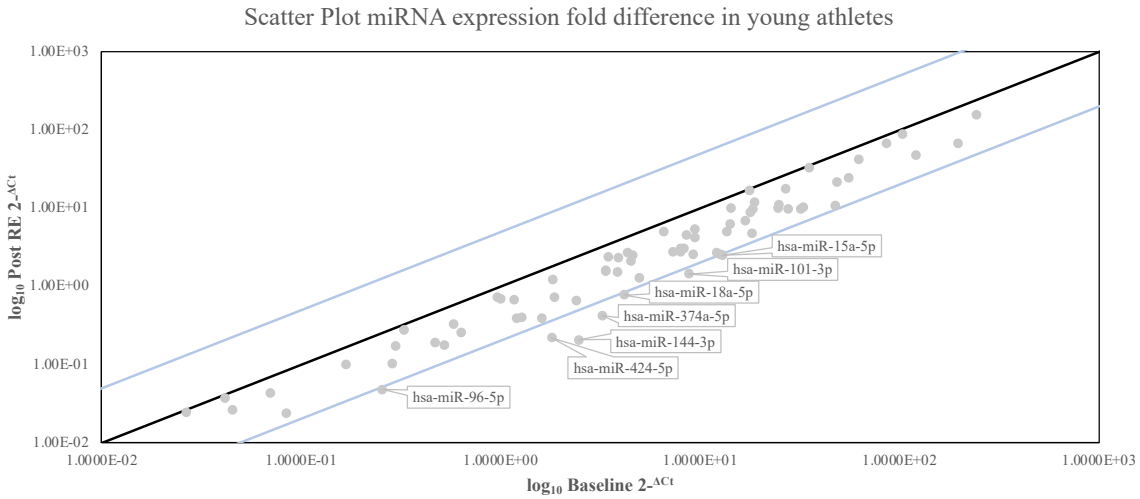


Figure 9: Fold Difference scatter plot of DE miRNAs in in young athletes post RE

Note. This figure illustrates the fold changes ($2^{-\Delta Ct}$) of the miRNAs determined through PCR Array between baseline and immediately after eccentric squat exercise in young athletes. Only detected miRNAs with C_T <30 are depicted in this figure. The black line indicates a fold change of 1, and the blue lines indicate the pre-defined threshold of 0.2. miRNAs crossing the threshold are labelled with their respective ID. DE, differentially expressed; RE, resistance exercise.

We obtained a set of seven miRNAs (miR-15a-5p, -18a-5p, 96-5p, -101-3p, -144-3p, -374a-5p, -424-5p) with a FR ≤-5 in Young. Only three miRNAs with FR ≥1.5 or ≤-1.5 could be identified in the master athletes' group (miR-15a-5p, -210-3p, and hsa-miR-144-3p), which were added to the analysis due to the low amount of DE miRNAs in Master. Table 8 depicts the selected miRNA sets with detailed data.

Table 8: Differentially expressed whole blood miRNAs in both age groups (baseline vs. directly after RE)

		Young				Master			
		2^-ΔC$_T$		change	reg.	2^-ΔC$_T$		change	reg.
miRNA ID	Well	post	pre	post/pre	post/pre	post	pre	post/pre	post/pre
hsa-miR-101-3p	A05	1.4E+00	8.8E+00	0.16	-6.12 ↓	7.3E-01	5.4E-01	1.34	1.34
hsa-miR-96-5p	B02	4.8E-02	2.6E-01	0.19	-5.33 ↓	1.2E-02	1.1E-02	1.02	1.02
hsa-miR-210-3p	C06	1.9E-01	4.7E-01	0.41	-2.45	2.3E-01	1.3E-01	1.74	1.74 ↑
hsa-miR-15a-5p	C07	2.5E+00	1.3E+01	0.19	-5.18 ↓	1.4E+00	9.4E-01	1.51	1.51 ↑
hsa-miR-18a-5p	D06	7.7E-01	4.2E+00	0.19	-5.40 ↓	4.3E-01	3.1E-01	1.39	1.39
hsa-miR-374a-5p	D07	4.2E-01	3.2E+00	0.13	-7.75 ↓	1.2E-01	1.5E-01	0.80	-1.24
hsa-miR-144-3p	E05	2.1E-01	2.5E+00	0.08	-11.90 ↓	4.2E-02	7.4E-02	0.57	-1.76 ↓
hsa-miR-424-5p	E06	2.2E-01	1.8E+00	0.12	-8.24 ↓	1.3E-01	1.1E-01	1.17	1.17

pre, baseline; post, after eccentric squat exercise; ↑, up-regulated; ↓, down-regulated

2^(- ΔC$_T$) *is the normalised gene expression in the respective sample ((post) or (pre))*

change *Fold change (2^(- ΔΔC$_T$)), is the quotient between ((2^(- ΔC$_T$)) post) and ((2^(- ΔC$_T$) pre). Fold change values greater than one indicate an up-regulation and fold change values less than one indicate a down-regulation*

reg. *Fold Regulation represents fold change results in a biologically meaningful way. Is there an up-regulation, the fold regulation value is equal to the fold change. Is there a down-regulation, the fold regulation value is the negative inverse of the fold change.*

Note. This table is a segment of the miScript miRNA PCR Array Data Analysis results with the excel sheet "PROM-13391" from Qiagen. This table shows two sets of miRNAs that appeared to have a substantial disparity (≥5 & ≤-5-fold regulation in Young; ≥1.5 & ≤-1.5-fold regulation in Master) between baseline (pre) and right after the intervention (post) in Young and Master, respectively.

Basal and post eccentric squat exercise differentially expressed whole blood miRNAs between age groups

When profiling the basal miRNA expression between age groups, the array data analysis showed a significant expression difference in 62 of the 73 detected miRNAs. Of those 62 miRNAs, two showed a positive fold expression change (hsa-miR-125b-5p, -100-5p, FC = 3.86 and 6.19, respectively), indicating that the younger athletes' values were higher than the values of the master athletes at baseline. The remaining 60 miRNA basal expression levels appeared to be higher in the older than in the younger group. Forty-nine of those 60 downregulated miRNAs had a FC <0.50 (Figure 10). After eccentric squat exercise, 31 miRNAs indicated a disparity between young and master athletes' expression values. miR-125b-5p, -100-5p remained upregulated after eccentric squat exercise (FC = 4.62 and 8.87, respectively). The remaining 29 miRNAs showed a negative expression change, indicating that the older group had higher miRNA expression values than the younger group at post. Thirty-one miRNAs are differently regulated at both points in time in both age groups. The basal values showed a greater discrepancy than the values at post. The exact data of both groups are shown in Table A2 (Appendix).

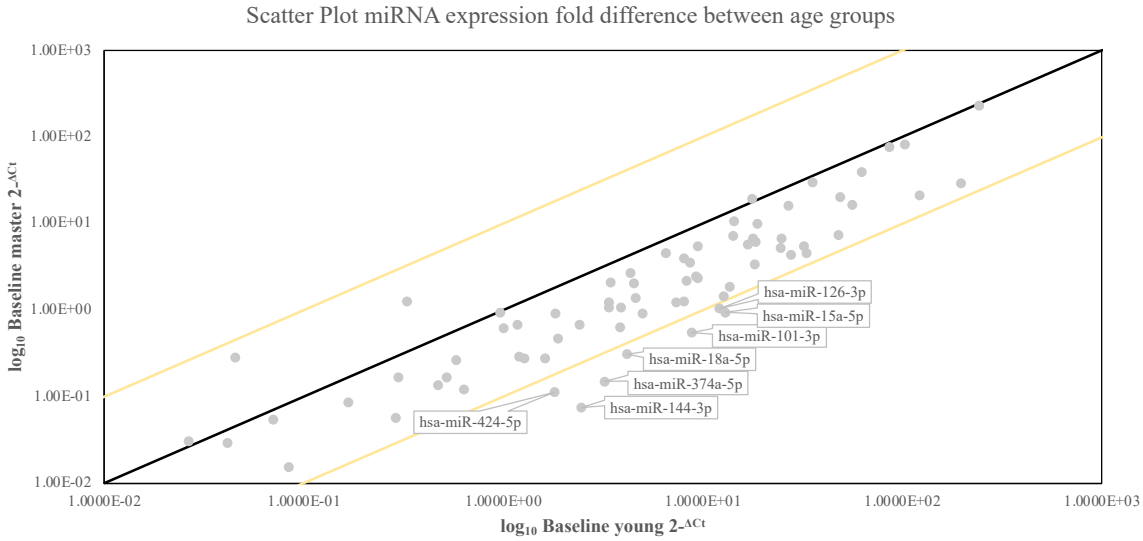


Figure 10: Fold difference scatter plot of DE miRNAs at baseline between age groups

Note. This figure illustrates the fold changes ($2^{-\Delta Ct}$) of the miRNAs determined through PCR Array between young and master athletes at baseline. Only detected miRNAs with C_T <30 are depicted in this figure. The black line indicates a fold change of 1, and the yellow lines indicate the pre-defined threshold of 0.1. miRNAs crossing the threshold are labelled with their respective ID. DE, differentially expressed.

Furthermore, seven miRNAs with basal FR ≤-10 between age groups were selected (miR-15a-5p, -18a-5p, -101-3p, 126-3p, -144-3p, -374a-5p, -424-5p). Specified data is listed in Table 9. Each miRNA set was then function analysed using GeneTrail 3.

Table 9: Whole blood miRNAs' basal expression (Young vs. Master)

		pre			
		$2^{-\Delta C_T}$		Fold change	Fold regulation
miRNA ID	Well	Master	Young	Master / Young	Master / Young
hsa-miR-101-3p	A05	5.4E-01	8.8E+00	0.06	-16.22 ↓
hsa-miR-15a-5p	C07	9.4E-01	1.3E+01	0.07	-13.74 ↓
hsa-miR-18a-5p	D06	3.1E-01	4.2E+00	0.07	-13.45 ↓
hsa-miR-374a-5p	D07	1.5E-01	3.2E+00	0.05	-21.56↓
hsa-miR-126-3p	E04	1.0E+00	1.2E+01	0.09	-11.63 ↓
hsa-miR-144-3p	E05	7.4E-02	2.5E+00	0.03	-33.36 ↓
hsa-miR-424-5p	E06	1.1E-01	1.8E+00	0.06	-16.00 ↓

pre, baseline; post, after eccentric squat exercise; ↑, up-regulated; ↓, down-regulated

2^(- ΔC$_T$) *is the normalised gene expression in the respective sample ((post) or (pre))*

Fold change *(2^(- ΔΔC$_T$)), is the quotient between ((2^(- ΔC$_T$)) post) and ((2^(- ΔC$_T$) pre). Fold change values greater than one indicate an up-regulation and fold change values less than one indicate a down-regulation*

Fold regulation *represents fold change results in a biologically meaningful way. Is there an up-regulation, the fold regulation value is equal to the fold change. Is there a down-regulation, the fold regulation value is the negative inverse of the fold change.*

Note. The table above is a segment of the miScript miRNA PCR Array Data Analysis results with the excel sheet "PROM-13391" from Qiagen. This table shows a set of miRNAs that appeared to have a substantial disparity (≤-10-fold regulation) between Young and Master at baseline (pre).

3.5 Predicted miRNA targets

DE miRNAs could mediate the physiological response to RE by targeting varying genes and signalling pathways relevant to exercise adaptation. From the results of the miScript miRNA PCR Array Data Analysis Excel sheet from Qiagen, DE miRNAs in the younger group (FC <0.20) and in the older group (FC >1.5 or <0.6) were selected for further functional analysis. The miRNAs used for further analysis are listed in Table 10.

Table 10: miRNAs used in analysis

microRNA name	miRBase accession	microRNA mature sequence
hsa-miR-15a-5p	MIMAT0000068	UAGCAGCACAUAAUGGUUUGUG
hsa-miR-18a-5p	MIMAT0000072	UAAGGUGCAUCUAGUGCAGAUAG
hsa-miR-96-5p	MIMAT0000095	UUUGGCACUAGCACAUUUUUGCU
hsa-miR-101-3p	MIMAT0000099	UACAGUACUGUGAUAACUGAA
hsa-miR-126-3p	MIMAT0000445	UCGUACCGUGAGUAAUAAUGCG
hsa-miR-144-3p	MIMAT0000436	UACAGUAUAGAUGAUGUACU
hsa-miR-210-3p	MIMAT0000267	CUGUGCGUGUGACAGCGGCUGA
hsa-miR-374a-5p	MIMAT0000727	UUAUAAUACAACCUGAUAAGUG
hsa-miR-424-5p	MIMAT0001341	CAGCAGCAAUUCAUGUUUUGAA

Note. This table illustrates the miRNAs' mature sequence and miRBase accession used for enrichment analysis.

3.5.1 Gene targets

A list of the equivalent miRBase ID of each miRNA set was inserted as input in GeneTrail 3 to assess the target genes of DE miRNAs in Young. Subsequently, an over-representation statistical analysis of miRNA-target interactions was performed using experimentally validated targets from miRTarBase (Gene (NCBI): all protein coding genes; Targets: miRNA targets, miRTarBase validated targets with strong evidence). The seven DE miRNAs in Young had 174 validated target genes, of which 30 were targeted by more than two miRNAs, leaving 144 unique target genes (q-values <0.001). Of the 30 genes, cyclin D1 (CCND1) was targeted by four miRNAs (miR-15a-5p, -101-3p, -374a-5p, -424-5p, q-values <0.001), three genes were targeted by three miRNAs amyloid beta precursor protein (APP), miR-15a-5p, -101-3p, -144-3p, q-values <0.001; serine/threonine kinase (ATM), miR-18a-5p, -101-3p, -374a-5p, q-values <0.001; zinc finger e-box binding homeobox 1 (ZEB1), miR-96-5p, -101-3p, -144-3p, q-values <0.001). The remaining 26 genes were targeted by two miRNAs (q-values <0.001), detailed data is shown in Table B1 (Appendix B). A miRNA-target interaction network was also carried out in MIENTURNET, only using experimentally strong validated targets (Figure 11).

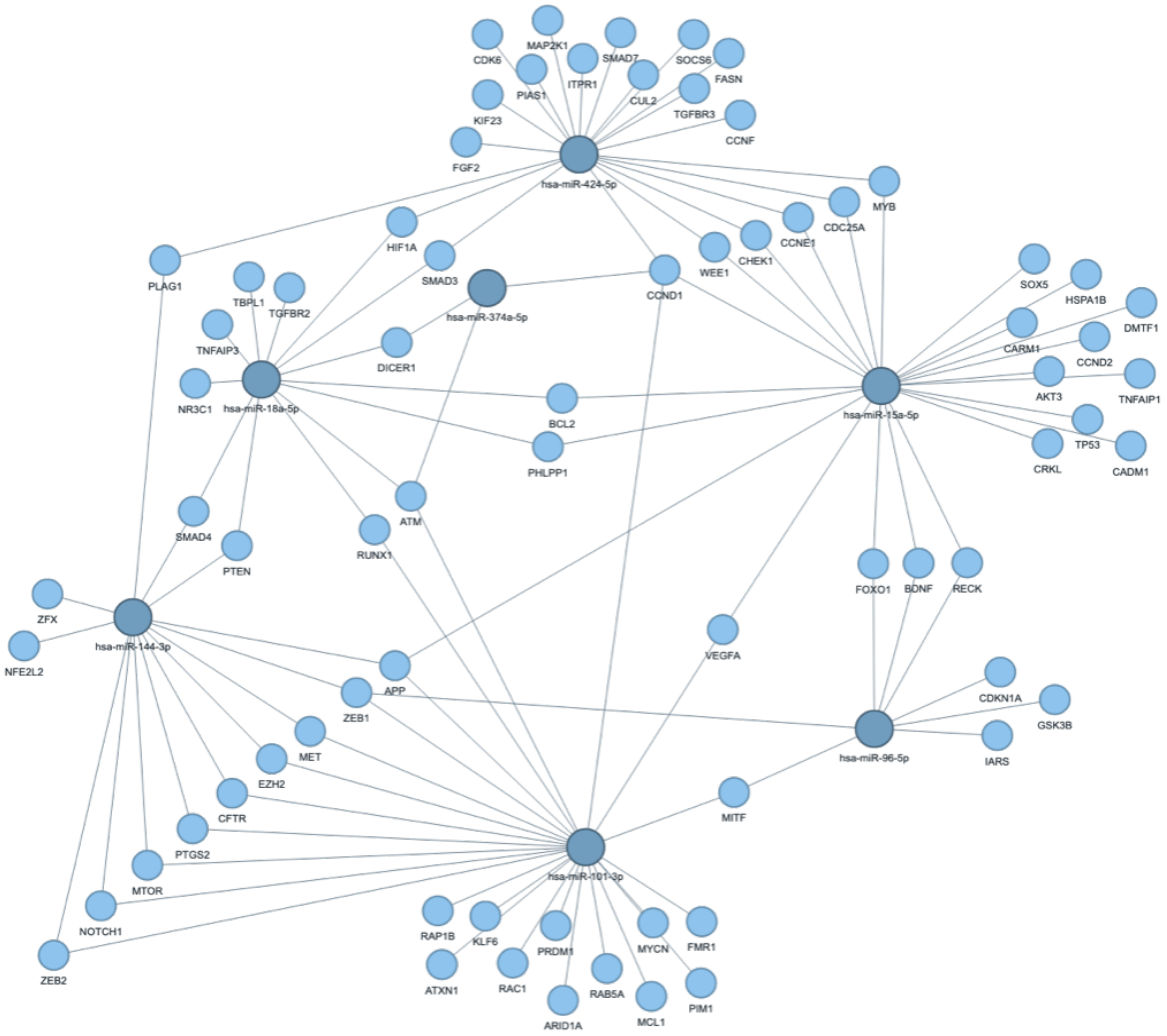


Figure 11: miRNA target interaction network (Young)

Note. This figure illustrates the miRNA target interaction network between deregulated miRNAS after eccentric exercise in younger athletes and their target genes (hsa-miR-15a-5p, -18a-5p, 96-5p, -101-3p, -144-3p, -374a-5p, and -424-5p; CCND1 (cyclin D1), APP (amyloid beta precursor protein), ATM (serine/threonine kinase), ZEB1 (zinc finger e-box binding homeobox 1), CCNE1 (cyclin E1), BCL2 (B-cell lymphoma 2, apoptosis regulator), BDNF (brain derived neurotrophic factor), CDC25A (cell division cycle 25A), CFTR (cystic fibrosis transmembrane conductance regulator), CHEK1 (checkpoint kinase 1), DICER1 (dicer 1, ribonuclease III), EZH2 (enhancer of zeste 2 polycomb repressive complex 2 subunit), FOXO1 (forkhead box O1), HIF1A (hypoxia inducible factor 1 subunit alpha), MET (mesenchymal epithelial transition), MITF (melanocyte inducing transcription factor), MTOR (mechanistic target of rapamycin kinase), MYB (myoblastosis proto-oncogene, transcription factor), NOTCH1 (notch receptor 1), PHLPP1 (PH domain and leucin rich repeat protein phosphatase 1), PLAG1 (pleiomorphic adenoma gene 1), PTEN (phosphate and tensin homolog), PTGS2 (prostaglandin-endoperoxide synthase 2), RECK (reversion inducing cysteine rich protein with kazal motifs), RUNX1 (runt-related transcription factor 1), SMAD3 (mothers against decapentaplegic homolog 3), SMAD4 (mothers against decapentaplegic homolog 4), VEGFA (vascular endothelial growth factor A), WEE1 (WEE1 G2 checkpoint kinase), ZEB2 (zinc finger e-box binding homeobox 2). miRNAs are depicted as big dark circles (source nodes), and the target genes are smaller brighter circles (target nodes) individually connected by strings.

In silico analysis of DE miRNAs in Master resulted in 110 genes identified as targets. APP, BDNF (brain-derived neurotrophic factor), and XIST (x inactive specific transcript) were targeted by two of the three miRNAs (miR-15a-5p, -144-3p; miR-15a-5p, -210-3p; miR-210-3p, -144-3p, respectively) (*q*-values <0.001). The remaining 107 genes were only targeted by one single miRNA (*q*-values <0.001). Detailed data is found in Table B2 (Appendix B). An additional target interaction network was carried out in MIENTURNET, again only including experimentally validated targets, with a minimum of two miRNA-target interactions (Figure 12).

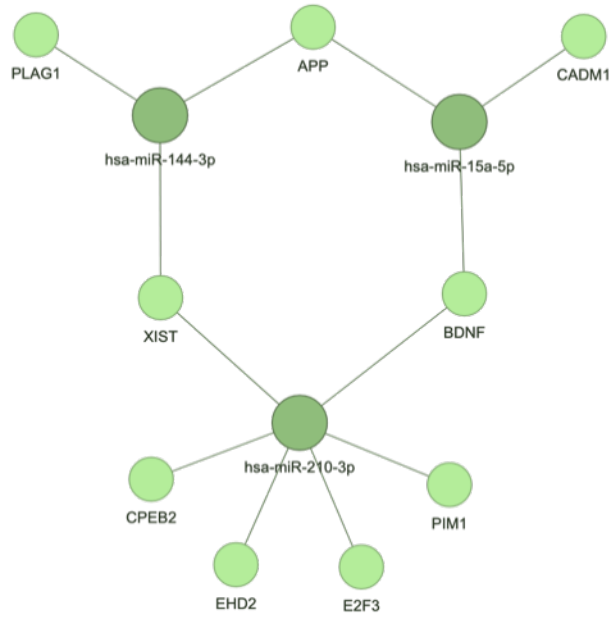


Figure 12: miRNA target interaction network (Master)

Note. This figure illustrates the miRNA target interaction network between deregulated miRNAs after eccentric exercise in master athletes and their target genes (hsa-miR-15a-5p, -144-3p, and -210-3p; (APP (amyloid beta precursor protein), BDNF (brain derived neurotrophic factor), XIST (X inactive specific transcript)). miRNAs are depicted as big dark circles (source nodes), and the target genes are smaller brighter circles (target nodes) individually connected by strings.

Lastly, there were 187 validated genes identified as miRNA targets of the DE miRNAs at baseline. Detailed data is included in Table B3 (Appendix B). The same four miRNAs targeted CCND1 as in the first miRNA set (miR-15a-5p, -101-3p, -374a-5p, -424-5p) (*q*-values <0.001), APP, ATM (serine/threonine kinase), BCL2 (B-cell lymphoma 2), EZH2 (enhancer of zeste 2 polycomb repressive complex 2 subunit), and VEGFA (vascular endothelial growth factor A) were targeted by three of the seven miRNAs (miR-15a-5p, -101-3p, -144-3p; miR-18a-5p, -101-3p, -374a-5p; miR-15a-5p, -18a-5p, -126-3p; miR-101-

3p, -126-3p, -144-3p; miR-15a-5p, -101-3p, 126-3p, respectively) (q-values <0.001). Further, 24 genes were targeted by two miRNAs (q-values <0.001). MIENTURNET was used to depict the interaction network between miRNAs and target genes, depicted in Figure 13.

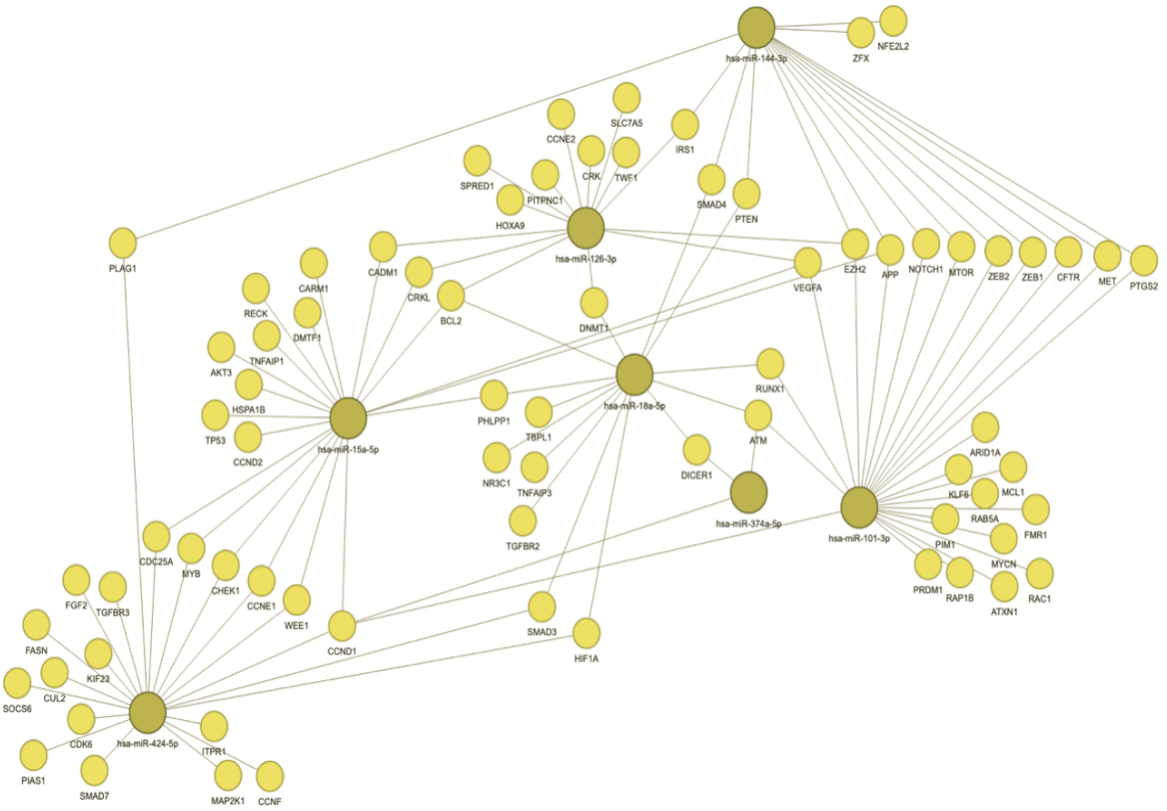


Figure 13: miRNA target interaction network (baseline)

Note. This figure illustrates the miRNA target interaction network between deregulated miRNAs at baseline when comparing Young and Master, and their target genes (hsa-miR-15a-5p, -18a-5p, -101-3p, 126-3p, -144-3p, -374a-5p, -424-5p; CCND1 (cyclin D1), APP (amyloid beta precursor protein), ATM (serine/threonine kinase), BCL2 (B-cell lymphoma 2, apoptosis regulator), EZH2 (enhancer of zeste 2 polycomb repressive complex 2 subunit), VEGFA (vascular endothelial growth factor A), CADM1 (cell adhesion molecule 1), CCNE1 (cyclin E1), CDC25A (cell division cycle 25A), CFTR (cystic fibrosis transmembrane conductance regulator), CHEK1 (checkpoint kinase 1), CRKL (CRK like proto-oncogene, adaptor protein), DICER1 (dicer 1, ribonuclease III), DNMT1 (DNA methyltransferase 1), HIF1A (hypoxia inducible factor 1 subunit alpha), IRS-1 (insulin receptor substrate 1), MET (mesenchymal epithelial transition), MTOR (mechanistic target of rapamycin kinase), MYB (myoblastosis proto-oncogene, transcription factor), NOTCH1 (notch receptor 1), PHLPP1 (PH domain and leucin rich repeat protein phosphatase 1), PLAG1 (pleiomorphic adenoma gene 1), PTEN (phosphate and tensin homolog), PTGS2 (prostaglandin-endoperoxide synthase 2), RUNX1 (runt-related transcription factor 1), SMAD3 (mothers against decapentaplegic homolog 3), SMAD4 (mothers against decapentaplegic homolog 4), WEE1 (WEE1 G2 checkpoint kinase), ZEB1 (zinc finger e-box binding homeobox 1), ZEB2 (zinc finger e-box binding homeobox 2). miRNAs are depicted as big dark circles (source nodes), and the target genes are smaller brighter circles (target nodes) individually connected by strings.

Overall, more protein-coding target genes were identified in Young than in Master due to the lower number of DE miRNAs in master athletes. Nevertheless, our results show that there appears to be a decreased transcriptional response to RE in men above 50 years of age than in men in their mid-twenties. The three genes enriched by two DE miRNAs in master athletes, APP, BDNF, and XIST, seem to be part of canonical biological processes not necessarily related to a response to stimuli such as RE. Genes targeted by DE miRNAs of young strength-athletes in response to eccentric squat exercise and at baseline, such as MTOR, NOTCH1, VEGFA, BCL2, RECK, HIF1A, MITF, FOXO1, insulin receptor substrate 1 (IRS1), transforming growth factor-β receptor 2/3 (TGFBR2/3) and SMAD3/4, known to be involved in the response of stimulatory events and in regulating cellular metabolism, growth, and adaptation.

3.5.2 Functional enrichment

All DE miRNAs were functionally enriched through ORAs using GeneTrail 3 miRNomics function (https://genetrail.bioinf.uni-sb.de/index?pipeline=mirna). Genes targeted by at least two DE miRNAs in the younger group appeared to enrich 482 functional annotations from GO (GO BP 442; GO MF 35; GO CC 5) (q-values <0.05) and 142 signalling pathways (KEGG 24; Reactome 59; Wikipathways 59) (q-values <0.05). As for the older group, 99 functional annotations appeared to be targeted by at least two DE miRNAs (GO BP 78; CO MF 3; GO CC 0) (q-values <0.05), as well as 18 signalling pathways (KEGG 3; Reactome 6; Wikipathways 9) (q-values <0.05). A total of 572 functional annotations from GO (GO BP532; GO MF 38; GO CC 5) and 186 signalling pathways (KEGG 41; Reactome 72; Wikipathways 73) were targeted by at least two of the seven basal DE miRNAs when comparing age groups (q-values < 0.05). A list of all overrepresented annotations in all three GO categories (BP, MF, and CC) and signalling pathways (KEGG, Reactome, and Wikipathways). The top KEGG pathways modulated by DE miRNAs in young and old athletes and at baseline are illustrated in Figure 14. The top enriched Reactome and Wikipathways pathways are illustrated in Figure D1 and 2 (Appendix D), respectively. Overall, DE miRNAs in young athletes after eccentric squat exercise collectively target several functional annotations and pathways related to immune response, metabolic processes, and cell growth, especially muscle tissue development. The older group regulated similar pathways to the young group, emphasising immunological pathways more. Basal DE miRNAs appeared to target analogous functional annotations and pathways to Young with

some additional metabolic process pathways. Generally, signalling pathways involved in exercise response were identified to be enriched through the miRNA-gene network. To name a few, mTOR signalling pathway, PI3K-Akt signalling pathway, Wnt signalling pathway, Notch signalling pathway, MAPK signalling pathway, p53 signalling pathway, HIF-1 signalling pathway, TNF signalling pathway, T-cell receptor signalling pathway, NFκB signalling pathway, AMPK signalling pathway, STAT3 signalling pathway, JAK-STAT signalling pathway, and p38 MAPK signalling pathway. Further details and the exact functional annotation and signalling pathways enriched by the DE miRNAs are provided in Table C1-18 (Appendix C).

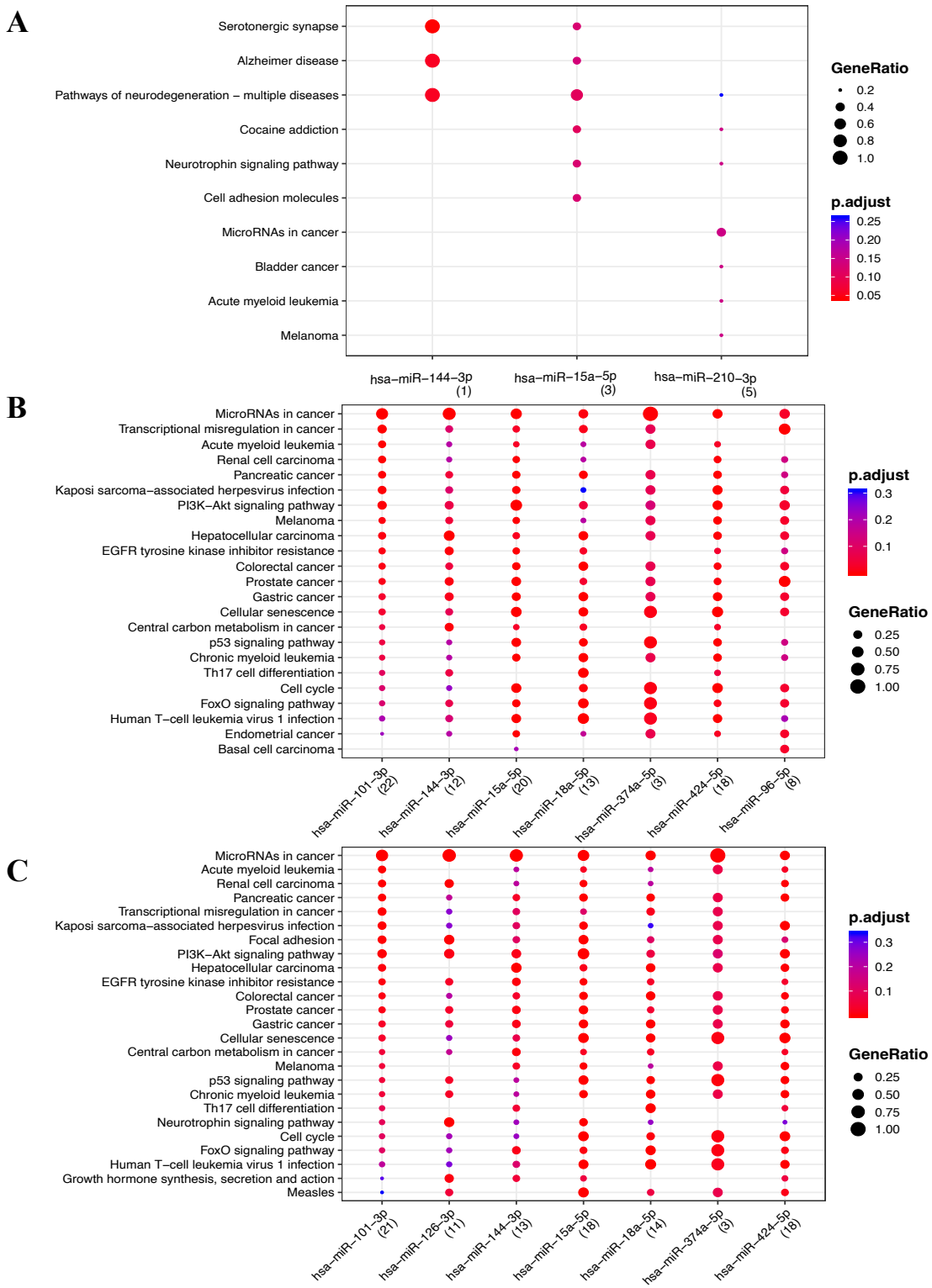


Figure 14: KEGG pathways modulated by DE miRNAs

Note. This figure illustrates the top KEGG pathways mediated by DE miRNAs in (A) Young, (B) Master, and (C) at baseline between younger and older athletes. Colours represent the adjusted *p*-value, and the number of genes regulating the signalling pathway indicates size. Other figures from the databases Reactome and Wikipathways can be found in Appendix.

4 Discussion

Ageing has been associated with decreased muscle mass and muscle strength and function. This natural decline in physical performance can be partially explained by muscular, neural, hormonal, and metabolic changes with age. The negative effect emerges around the age of 50 and is magnified after the seventh decade of life (Ganse & Degens, 2021; Janssen et al., 2000; Reaburn & Dascombe, 2009). The exact influence of human senescence on attenuated physical performance has yet to be understood entirely. It appears that decrease in strength and power capacities are partially caused by, e.g., a attenuated rate of force production and conduction, impaired cellular crosstalk, structural changes of skeletal muscles, neurological and vascular alteration, and diminished regenerative capacity (Borges et al., 2016; Brook et al., 2016a; Faulkner et al., 2007; Fell & Williams, 2008; Gries & Trappe, 2022). Some, if not almost all, of these factors can benefit from regular physical activity and a balanced and healthy diet. Systematic strength training has been shown to positively influence muscular strength and power in sedentary older individuals and young and older athletes (Distefano & Goodpaster, 2018; Tieland et al., 2018). Strength and power master athletes have been reported to have similar strength and power values to healthy untrained young individuals (Borges et al., 2016; Mckendry et al., 2018).

Nevertheless, RE does not seem to prevent age-related loss of muscle mass and its function but rather to lessen its decay/depletion in an exercise-mode-specific manner (Borges et al., 2016; Kundert et al., 2019; Mckendry et al., 2018). Thus, master athletes could help shine a light on the contributing factors to muscle atrophy, decreased muscle performance, and the resulting physical limitations that come with biological ageing. One of the most significant contributing factors to muscle atrophy is the so-called anabolic resistance. This phenomenon is believed to be the result of a blunted muscle protein synthetic response to anabolic stimuli, e.g., physical activity and food intake, and increased inhibition of muscle protein breakdown with advancing age (Brook et al., 2016b; Fry et al., 2011; Kumar et al., 2009, 2012; Sheffield-Moore et al., 2005). However, the cause of this imbalance in protein turnover with increasing age still needs to be fully understood.

In this study, we aimed to assess the effect of intensive eccentric squat exercise on the expression of circulating miRNAs in whole blood in younger and older strength-trained males. We identified nine DE miRNAs using microRNA PCR arrays with a 1.5-fold expression change between baseline and after physical exertion. Whole blood, compared to

other circulating bodily fluids, is a medium with the highest amount of miRNA detectable (Cho, 2011). A possible explanation is that whole blood contains more cells, such as leukocytes and erythrocytes, and is one of the body's main mediums for cell-cell communication. Hence, making it an excellent medium for research on health and longevity-promoting exercise in adults of all ages and sexes.

4.1 Acute effect of intensive squat exercise in strength-trained athletes

Master athletes generally show greater strength, muscle mass, and aerobic and anaerobic capacity than healthy, age-matched controls. However, the physical performance of master athletes seems to decrease with age, suggesting that structural and molecular changes in the human system cannot be prevented entirely despite countermeasures, e.g., regular exercise.

The effect of an acute bout of RE was measured using biomarkers, i.e., BLa, and through performance tests such as CMJ height and MVIC force. As expected, basal testosterone concentration differed between age groups, with the younger group showing a significantly greater value than the master group. Ageing has been associated with a decline in mean circulating testosterone levels with a plateau around the 40^{th} decade (Kanabar et al., 2022; Kelsey et al., 2014). Some studies have concluded that older adults show an increase in variability of testosterone concentration with increasing age rather than an age-related progressive linear reduction (Kelsey et al., 2014). Moreover, mean testosterone can be negatively influenced by obesity, impaired glucose control, and varying diseases (Kanabar et al., 2022). Low testosterone concentration in men has been associated with depression, low cognitive function, and low bone density and muscle mass (Kaufman et al., 2012, p. 345). Literature has contradicting findings regarding the effect of physical activity and regular exercise on testosterone production and circulatory concentration in sedentary older individuals (Barbosa et al., 2021; Häkkinen et al., 2000; Kraemer et al., 1998). However, it has been proposed that lifelong exercise and regular physical activity help mitigate the age-related decrease in testosterone (Kraemer et al., 1998). Our results support previous findings and ratify that older men, even physically active and strength-trained ones, show a lower basal circulating testosterone concentration than their younger counterparts.

Furthermore, the age-related loss of muscular strength and power capacity seems greater than muscle atrophy, indicating a decrease in muscle quality before quantity (Goodpaster et al., 2006). Older individuals seem to have a greater amount of non-contractile tissue and a lower amount of contractile tissue in muscles resulting in reduced muscle quality and, thus,

a decrease in muscle function, power, and force development (Distefano & Goodpaster, 2018; Wilkinson et al., 2018). The underlying mechanisms causing age-related muscular strength and function loss have not been fully elucidated. A possible explanation is the age-related impairment of neuromuscular activity coupled with a loss of motoneuron firing rate and their regenerative capacity, degeneration of neuromuscular junctions, and decline in nerve conduction velocity (Jang & Van Remmen, 2011; Lepore et al., 2019; Wu et al., 2020, 2021). The neurological decay is not necessarily accompanied by muscle mass loss as is evident in master athletes but could lead to greater fibre atrophy with time in non-physically active individuals (Deschenes et al., 2010; Drey et al., 2016; Wilkinson et al., 2018). Correspondingly, the specific force production capacity is lower with age, especially for fast-twitch muscle fibres. Thus, explosive movements are reduced, as shown in older people's progressive decline in power output (Alvero-Cruz et al., 2021; Mckendry et al., 2018). Our results demonstrate that the loss of power-generating capacity, illustrated by the statistically significant difference in CMJ height and MVIC force at baseline between age groups, probably occurs before the loss of muscle strength and muscle mass, as represented by similar 1RM and muscle mass values in our sample. Potentially showing a causal relationship between these parameters in master athletes and nonathletic individuals (but more accelerated and facilitated by physical inactivity). The age-related neuromuscular changes have been reported to attribute to a shift towards slow fibre type grouping in aged skeletal muscle and a loss of fast fibre size and thus force production (Alvero-Cruz et al., 2021; Mckendry et al., 2018). Greater atrophy of fast twitch fibres (type II fibres) and higher amounts of innervated slow twitch fibres (type I fibres) is observed in both master athletes of varying exercise modes and sedentary healthy older individuals (Distefano & Goodpaster, 2018; Reaburn & Dascombe, 2009). Therefore, this leads to reduced force development capacity and a change in skeletal muscle metabolism for energy production. Type I fibres primarily use oxidative metabolism, and type II fibres use glycolytic metabolism, and thus ageing could lead towards a more oxidative muscle profile. Single muscle fibre size or type distribution was not analysed in this study. Hence, it can only be assumed that the older participants could potentially have fewer type II fibres than more type I fibres resulting in a reduced glycolysis rate and lactic acid formation. Nonetheless, the fibre distribution could be reflected in the lower BLa concentration after intensive eccentric squat exercise in master athletes, while at the same time having a similar amount of muscle mass in lower extremities as younger athletes. Additionally, some have reported a reduced glycolytic enzyme activity involved in anaerobic energy production, e. g., lactate dehydrogenase and hexokinase in

older individuals (Reaburn & Dascombe, 2009; Tieland et al., 2018). Studies on age-related decreased enzyme activity are scarce, and further research is needed. Our observations support the findings of an age-related decrease in peak BLa concentration in master athletes in running and swimming (Benelli et al., 2007; Korhonen et al., 2005; Marcell et al., 2003; Reaburn & Dascombe, 2009). Further, it must be noted that the master athletes in our sample did not rate their perceived pain significantly higher between baseline and right after RE (pre: 0.6 ± 0.5 points; post: 3.6 ± 2.4 points), as was the case in younger athletes (pre: 0.4 ± 0.5 points; post: 6.8 ± 2.4 points). Even though the RPE of the test protocol did not differ between age groups. This, combined with the fact that the MVIC force produced in the half squat did not decrease significantly after RE in Master and a reduced peek BLa concentration post RE, could indicate that the master athletes were more cautious during the test protocol due to fear of injury. It has been hypothesised that functional measures like MVIC could be a more accurate method to identify muscle damage due to the high variability of parameters such as BLa and CK (Warren et al., 1999). Possibly indicating that they either did not perform the last set until true momentary muscle failure or older individuals tend to be more aware of their limitations, resulting in lower performance. Nonetheless, Ganse et al. (2014) and Walsh et al. (2013) reported a low risk of injury in master athletes at sports events, e.g., the European Veteran Athletics Championships and The World Masters Games, which could be associated with guarded actions in fear of injury.

4.2 Differentially expressed miRNA after eccentric squat exercise in young and master strength-trained athletes

The influence of physical exertion on c-miRNAs and tissue-specific miRNAs has been previously established (Baggish et al., 2014; Banzet et al., 2013; Cui et al., 2017; D'Souza et al., 2017a; Kirby & McCarthy, 2013, 2013; Mooren et al., 2014; Sawada et al., 2013; Silva et al., 2020; Uhlemann et al., 2014). Altered miRNA expression after exercise has also been reported in other tissues (dos Santos et al., 2022). In addition, miRNA expression is associated with the immunological response to exercise and with the regulation of exercise-responsive genes mediated by immune cells (Bi et al., 2009; Makarova et al., 2014; Radom-Aizik et al., 2010, 2012, 2013; Wilhelmsen et al., 2021). c-miRNAs and intracellular miRNAs have been suggested to work as cell-to-cell communicators due to their location in peripheral blood, which means miRNAs function as vessels of crosstalk between tissues and

organs within the body. Thus, making miRNAs from whole blood after an acute exercise bout is an exciting research medium. The miRNA expression profile observed in this study probably stems from activated and non-activated immune cells communicating with the other tissues, like skeletal muscle, as a response to an acute bout of RE. Since RE affects the immunological response, we will describe the literature assessing the function of the DE miRNAs found in this study and focusing on the role of those miRNAs in skeletal muscle.

Numerous studies have assessed the effect of acute exercise on miRNAs in blood or skeletal muscle after varying exercise modes. The current state of knowledge of each DE miRNA of our results will be discussed in this section. In this section of the manuscript the mature miRNAs miR-101-3p, miR-126-3p, miR-144-3p, miR-210-3p, miR-15a-5p, miR-18a-5p, miR-96-5p, miR-374a-5p, and miR-424-5p, will be referred to as: miR-101, miR-126, miR-144, miR-210, miR-15a, miR-18a, miR-96, miR-374a, and miR-424. Each miRNA will be discussed in this section separately.

miR-126

Exercise adaptation triggers blood vessels' adaptation, inducing vessels' remodelling and de novo angiogenesis, among many other things. Angiogenesis is triggered by chemical or mechanical stimuli, such as tissue damage, cellular growth or proliferation, and hypoxia. RE appears to induce angiogenesis as a supportive system of hypertrophy and does not induce an increase in blood vessels (Tieland et al., 2018). This regenerative process appears to be regulated by specific miRNAs, such as miR-126, which can be found in endothelial cells, skeletal muscle, and peripheral blood mononuclear cells (PBMCs) (Radom-Aizik et al., 2010, 2012, act, 2013; Tarnowski et al., 2022; van Solingen et al., 2009). miR-126 appears to regulate the expression of the pro-angiogenic gene vascular endothelial growth factor (VEGF) through suppression of the VEGF suppressors sprouty related protein 1 (SPRED1) and phosphoinositide-3-kinase regulatory subunit 2 (PIK3R2) (dos Santos et al., 2022). These proteins are cell differentiation and proliferation mediators involved in MAPK and PI3K/Akt signalling pathways. A study looking at the influence of long-term RE (12 weeks) in combination with cold-water immersion or active recovery in young, healthy males determined that the expression of miR-126 did not change with either intervention method in skeletal muscle (D'Souza et al., 2018a). Participants of the cold-water immersion group additionally showed an large increase in the number of capillaries per muscle fibre and a

higher expression of the anti-angiogenic gene SPRED1 and the pro-angiogenic gene VEGF1 when compared to the active recovery group. They concluded that miR-126 targets VEGF1 in transcription but not translation because SPRED1 protein levels did not show an association with the expression of this miRNA. Note that miR-126 has been linked as "endothelial- and angiogenesis specific" due to its differentiated regulation after aerobic exercise (D'Souza et al., 2018b; Lew et al., 2020; Ma et al., 2013; Naderi et al., 2018; Uhlemann et al., 2014). miR-126 does not merely play an essential role in angiogenesis. However, it is also a tissue growth regulator with a notable amount of blood vessels, such as skeletal muscle, heart, and brain (Rivas et al., 2014). One study looking at the basal expression of miR-126 in skeletal muscle reported it to be lower in elite Norwegian powerlifters compared to healthy age-matched controls (D'Souza et al., 2017a). A separate study by Rivas et al. (2014) concluded miR-126 to be lower expressed in older participants' muscles than in younger adults at baseline.

Interestingly, these findings do not align with our results. The younger individuals showed a lower basal expression of miR-126 measured from whole blood compared to the strength and muscle mass matched older athletes. *In vitro* analysis has reported C2C12 proliferating myoblasts transfected with antisense miR-126 to show changed protein expression of two crucial muscle growth regulators, insulin receptor substrate 1 (IRS-1) and FOXO, which were increased and decreased, respectively (Rivas et al., 2014). miR-126 mimics showed a reduced expression of Myf5 and MyoD protein expression compared to controls. Younger men have higher metabolic and mitogenic activity at baseline than older individuals. Astonishingly, there are several mixed results regarding the effect of RE on the expression of miR-126 in skeletal muscle biopsies. Most studies investigated this miRNAs' expression in muscle tissue of healthy young individuals followed by RE and revealed an upregulation (Davidsen et al., 2011; D'Souza et al., 2017b; Ogasawara et al., 2016).

It should be noted that the named studies implemented diverging resistance training test protocols and collected the samples at different time points after the RE stimulation. A furthert study looking at the expression of miR-126 in plasma also reported a statistically non-significant higher expression after RE in healthy young adults (Uhlemann et al., 2014). On the contrary, Rivas et al. (2014) indicated miR-126 to be downregulated in muscle tissue after strength training in younger individuals. This observation aligns with our findings described in the previous section. Implicating miR-126 plays a role in skeletal muscle remodelling, adaptation, and regeneration induced by physical activity. Further analysis in

C2C12 myotubes transfected with an antisense oligonucleotide to inhibit miR-126 treated with IGF-1 as a simulation of anabolic stimulus showed a higher expression of the pro-myogenic proteins MyoD, MyoG, IRS, Akt, FOXO1, TNF, and rpS6 (Margolis & Rivas, 2018; Rivas et al., 2014). They identified a downstream effect of miR-126 on essential muscle protein synthesis and breakdown regulators. Thus, concluding miR-126 to be a potential mediator of exercise adaptation in response to growth stimulation induced by RE.

miR-144

There is, to our knowledge, little literature on miR-144 in human skeletal muscle and RE. Most studies evaluating the effect of exercise on the expression of miR-144 use EE as the primary intervention method. Nonetheless, most of them reveal downregulation of miR-144 in skeletal muscle, heart muscle, blood leukocytes, and serum in non-human and human models (Fernandez et al., 2020; Keller et al., 2011; H.-A. Kim et al., 2018; Ma et al., 2013; Souza et al., 2015). These findings align with our results, where older and younger resistance-trained males showed downregulation of this miRNA after RE. miR-144 has been linked to multiple biological processes; it has been reported to mediate the expression of genes linked to muscle tissue regeneration, such as RUNX1 and PAX3, as well as to negatively regulate the expression of PTEN, activating the PI3K/Akt/mTOR signalling pathway as a result (Keller et al., 2011; Sanchis-Gomar et al., 2021). Other authors have assessed pro-myogenic IRS-1 and SMAD4 to be directly negatively targeted by miR-144 (Improta Caria et al., 2018; Karolina et al., 2011; Massart et al., 2016; Yang et al., 2017; Zhu & Leung, 2015). The exact relationship between PTEN and miR-144 has yet to be assessed thoroughly. Nevertheless, it has been established that PTEN inhibits IRS-1, thus positioning miR-144 in an interesting conundrum in which more research is needed. As for the possible age-related dysregulation of this miRNA observed in our data, two studies evaluating the basal expression of older non-human animal models reported similar results. According to those studies, miR-144 was downregulated in skeletal muscle biopsies of younger mice and upregulated in muscle biopsies of older rhesus monkeys (J. Y. Kim et al., 2014; Mercken et al., 2013). Studies on mammalian models could have some ageing characteristics in common with human ageing. There needs to be more understanding of the role of miR-144 on ageing muscle and RE as such. This miRNA also modulates genes related to glycogen formation IRS-1, FOXO1, and (glycogen synthase kinase-3 β) GSK3-β, which demonstrates a possible effect of miR-144 on metabolic pathways (Improta Caria et al., 2018; Massart et al., 2016; Mercken et al., 2013). Diabetes mellitus type 2 patients' muscle biopsies showed a basal

overexpression of miR-144 and a relative downregulation after aerobic exercise, which indicates a possible regulation of skeletal muscle insulin sensitivity (Improta Caria et al., 2018). Furthermore, some studies also reported miR-144 to be overexpressed in patients with diabetes mellitus type 2 (DMT2) and impaired fasting glycemia, proposing miR-144 as a potential biomarker for metabolic diseases like DMT2 when compared to controls (Karolina et al., 2011; Yang et al., 2017).

miR-15a

miR-15a appears to inhibit angiogenesis within the skeletal muscle by directly inhibiting the expression of VEGF, a pro-angiogenic protein (Sun et al., 2013). At the same time, overexpression of this miRNA also regulates important cell cycle proteins, such as carotenoid cleavage dioxygenase 1 (CCD1) and carotenoid cleavage dioxygenase 2 (CCD2), which are necessary for cell migration and coordination of mitotic cell phases (Bandi et al., 2009; Bandi & Vassella, 2011). Which was corroborated in humans by D'Souza et al. (2018a) when analysing the expression miR-15a in the muscle of young, healthy males followed by 12 weeks of resistance training. Angiogenesis normally occurs proportionally to hypertrophy. However, as a recovery method post RE, cold water immersion further promotes angiogenesis by decreasing miR-15a and elevating VEGF protein expression in the muscle (D'Souza et al., 2018a). They observed an increase in muscle fibres and the number of capillaries per muscle fibre, followed by RE in the active recovery group but a higher vascularisation of muscle tissue in the cold-water immersion group. Furthermore, miR-15a has been shown to inversely regulate IRS-1, an essential mediator of the IGF signalling pathway, and myogenic factors such as MyoD, MyoG, and MyHC (Z. Li et al., 2019). The overexpression of this miRNA in embryonic fibroblast cell of chickens lead to a reduction in the number of myoblasts and muscle size. Per our findings, D'Souza et al. (2017b) reported miR-15a to be downregulated two and four hours after a bout of RE in muscle cells and plasma of young and healthy men. A similar result was presented by Lew et al. (2020) in the heart tissue of mice after EE. miR-15a also seems to be upregulated in skeletal muscle biopsies of older adults (>60 years of age) after RE (D'Souza et al., 2019). The immediate ingestion of whey protein supplementation after the exercise bout appeared to inhibit the elevation of miR-15a's expression compared to the placebo group. D'Souza and colleagues (2019) concluded that a high whey protein supplementation after RE induced a physiological response similar to one of the young, healthy adults. Some studies have reported an upregulation of miR-15a in young adults' blood samples and immune cells after

aerobic exercise (Chilton et al., 2014; D'Souza et al., 2018b; Radom-Aizik et al., 2012, 2013). This is congruent with the observation made by Margolis & Silva (2018) where they commented that some miRNAs appear to be mostly downregulated following RE and upregulated after EE:

> *Divergent response of in [sic] miRNA expression to various exercise modes appears to be due, at least in part, to miRNA being sensitive to alteration in the rate of muscle protein synthesis, with miR-206 and miR-499 expression reported to be inversely associated with muscle protein synthesis during exercise (19). Given that miRNA function through negative inhibition, concurrent reductions in miRNA expression with increased muscle protein synthesis rates suggest a potential feed-forward mechanism, where acute anabolic stimulation downregulates the inhibition of miRNA to initiate training adaptions through enhanced translation of proteins regulating skeletal muscle anabolism (Figure 2).15/02/2023 09:01:00*

miR-18a

miR-18a is part of the highly conserved polycistronic miR-17~92 cluster and plays an important role in many cellular processes. This cluster encodes six miRNAs (miR-17, -18a, -19a, - 19b, -20a, and -92a) which share distinct seed regions and are involved in the proliferation and apoptosis of many cells (MacFarlane & Murphy, 2010; Ranji et al., 2013; Xiao & Rajewsky, 2009). This miRNA cluster has been identified as an oncogene in many types of cancer (Concepcion et al., 2012). Despite the individual miRNAs of this cluster overlapping in their function, they are expressed at varying levels. Little is known about the influence of RE on the expression of this miRNA cluster. Overexpression of miR-18a has been reported to negatively regulate skeletal muscle protein synthesis and influence cell differentiation and proliferation by targeting cell cycle genes like CCND1, cyclin-dependent kinase 6 (CDK6), and fibroblast growth factor 1 (FGF1) in mice and bovine skeletal muscle (Kong et al., 2019; C. Liu et al., 2017, 2018; Meng et al., 2022). C. Liu and colleagues (2017) reported that miR-18a directly reversely modulates the expression of IGF-1. Consequently, overexpression of this miRNA induces atrophy of myotubes upregulating atrophy markers and reducing the expression of Akt, FOXO3, myocyte-specific enhancer factor 2D

(MED2D), myosin heavy chain 3 (MyH3), mediating the PI3K/Akt signalling pathway as a result. This miRNA cluster family seems to target the TGF-β signalling pathway in cancer cells, miR-18a alone directly targeting SMAD2 and SMAD4, which regulate cell proliferation, differentiation and apoptosis (Concepcion et al., 2012). As we also observed, Ogasawara et al. (2016) reported that miR-18a is downregulated, followed by RE in muscle biopsies on healthy young men. Another study showed similar results in peripheral blood neutrophils after cycling (Radom-Aizik et al., 2010). During C2C12 myoblast differentiation and post-natal skeletal muscle growth, miR-18a seems downregulated, facilitating myotube fusion and myogenic differentiation, as indicated by several studies *in vitro* (C. Liu et al., 2017, 2018; Meng et al., 2022). It needs to be noted that there is a need for further research regarding the role of miR-18a in response to anabolic stimulation. Contrary to our findings, one study investigating the effect of RE in young and old adult men indicated that serum miR-18a was upregulated in the younger group and downregulated in the older group (Margolis et al., 2016). The varying results could also be due to differences in participants, samples, and exercise protocols. It could be argued that Margolis et al.'s (2016) protocol did not induce as much physical exertion as the one used for this study, causing a different physiological response. One study investigating the expression of miR-18a in rhesus monkeys' muscle biopsies describes an overexpression in older than younger monkeys (Mercken et al., 2013). This effect was revoked after caloric restriction. Additionally, Eisenberg et al. (2017) reported that patients with muscular diseases, e.g., facioscapulohumeral muscular dystrophy (FSHD), limb-girdle muscular dystrophies with mutated calpain-3, type 2A (LGMD2A), limb-girdle muscular dystrophies with mutated dysferlin, type 2B (LGMD2B), and nemaline myopathy (NM) show overexpression of miR-18a in skeletal muscle. Ageing muscle and muscular diseases and disorders both endure a loss of muscle quantity and quality, leading to muscle wasting resulting from declining muscle tissue regulatory and regenerative capacities.

miR-210

On account of miR-210 being pro-angiogenic and identified in cardiovascular diseases and tumours, most studies focused its role on the cardiovascular system and cancer. miR-210 influences mitochondrial metabolism, DNA repair, angiogenesis, apoptosis, cell cycle, and many more cellular processes (Devlin et al., 2011). It has been consistently reported that miR-210 is notably expressed during hypoxic states in several cell cultures (Alaiti et al., 2012; Besnier et al., 2019; Fasanaro et al., 2008). The overexpression of this miRNA induces

and promotes angiogenesis and the formation of capillary structures by promoting the expression of the pro-angiogenic protein VEGF and targeting the anti-angiogenic receptor protein-tyrosine kinase ephrin A3 (EFNA3) (Alaiti et al., 2012; Peng & Croce, 2016). Hypoxia represses mitochondrial respiration and induces glycolysis. This process is believed to be regulated by hypoxia-inducible factors (HIFs), which in turn has been directly associated with positively regulating miR-210, forming a feet-forward loop (Chan et al., 2009; Devlin et al., 2011; Fasanaro et al., 2008). miR-210 has been recognised to regulate the electron transport chain in mitochondrial metabolism, enhancing glycolysis and reducing ATP (Chan et al., 2009). Furthermore, miR-210 modulates skeletal muscle protein synthesis, promoting the signalling pathway PI3K/Akt (Zhuang et al., 2022). To our knowledge, there has been little to no research regarding miR-210 and RE, except for D'Souza and colleagues (2017b). miR-210 has been reported to be slightly upregulated post RE in skeletal muscle biopsies and plasma in healthy young men (D'Souza et al., 2017b). According to further studies, miR-210 was not differently expressed directly after RE in serum and plasma samples (Baggish et al., 2011; Ramos et al., 2018; Sawada et al., 2013). Contradictory to those results, serum miR-210 has been reported to be downregulated immediately after exhaustive exercise in competitive basketball players (Y. Li et al., 2018). EE seems to have no direct effect on the expression of this miRNA in plasma (Baggish et al., 2011; Ramos et al., 2018). The expression of miR-210 in biopsies of patients with muscular disorders such as FSHD, LHMD2A, LGMD2B, NM, as well as Duchenne muscular dystrophy (DMD), dermatomyositis (DM), polymyositis (PM), and Miyoshi myopathy was higher when compared to controls (Eisenberg et al., 2007). However, He et al. (2020) described serum miR-210 as downregulated in older adults diagnosed with sarcopenia. It appears that the expression of this miRNA is highly sensitive to the physiological state of the sample and the medium its expression is read on. Pro-inflammatory cytokines, i.e., IL-6, TNFα, and IL-4, are negatively regulated by miR-210 (Kopriva et al., 2013; Qi et al., 2012; Tahamtan et al., 2018). Some of those are partially regulated through the inhibition of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κb). Older individuals show a higher level of pro-inflammatory cytokines (Wyczalkowska-Tomasik et al., 2016). This could explain our results, in which older individuals showed a higher basal expression of miR-210 than younger athletes. Additionally, the discrepancy in the literature could be a product of varying factors such as exercise modus, age, and sampling medium. It has been established that miR-210 plays an important role in angiogenesis and cellular metabolism. Still, the mechanisms

behind it and its exact role in exercise and RE per se have not been expounded yet, and further research is needed.

miR-101

It appears that miR-101 is involved in skeletal muscle differentiation. D. Li et al. (2015), on the one hand, reported miR-101 to be upregulated during satellite cell and myoblast differentiation, promoting the formation of myotubes while inducing the expression of MyoG but reducing the expression of MyoD in goats. Their results were not statistically significant and could thus demonstrate that this miRNA plays a role in myogenesis but might not be a crucial element in this process. On the other hand, another study demonstrated that miR-101 negatively regulates myoblast differentiation in mice C2C12 cells by directly targeting proteins (myocyte enhancer factor 2A; MEF2A) involved in the pro-myogenic signalling pathway p38/MAPK (S. Liu et al., 2021). The overexpression of this miRNA inhibits myogenesis and thus reduces myotube formation. Several studies investigating the expression of miR-101 in whole blood and or skeletal muscle after EE reported it down and upregulated, respectively (Keller et al., 2011; Makarova et al., 2014). One study looked at the expression of miR-101, among other miRNAs, in skeletal muscle biopsies before and after RE in adults but did not report any specific details (Rivas et al., 2014). The differences could explain these inconsistent results between *in vitro* and *in vivo* studies and that these studies have been performed on divergent animal models. Our results indicate that younger individuals show a lower basal expression of miR-101 than older individuals and that RE downregulates the expression in young and upregulates the expression in master athletes. This aligns with S. Liu et al. (2021) findings, showing that age-related anabolic resistance could induce overexpression of miR-101 and inhibit myogenesis.

miR-374a

miR-374a has been mostly studied in disease models such as cancer. It has been described as regulating cellular proliferation and apoptosis in carcinogenesis and targeting the pro-angiogenic Akt, VEGF, PTEN, and Wnt signalling (Bian et al., 2019). Overexpression of miR-374a increased cell size in neonatal rat ventricular myocytes and downregulated the expression of vascular endothelial growth factor receptor 1 (VEGFR1) compared to controls (Lee et al., 2017). They concluded that miR-374a plays a pro-hypertrophic role in cardiomyocytes by inhibiting the hypertrophy regressive molecule VEGFR1. Nonetheless, Lee et al. (2017) only studied the expression of VEGFR1 and did not investigate the

influence of miR-374a on the hypertrophy-enhancing molecule vascular endothelial growth factor receptor 2 (VEGFR2). This could reveal if miR-374a is directly involved in muscle tissue formation or only plays a passive role as an inhibitor of contra-myogenic molecules. In CD56+ myoblasts from young individuals, miR-374a was reported to be upregulated during differentiation but not regulated during proliferation by Dmitriev et al. (2013). They suggested that miR-374a could affect myoblasts differentiation but did not analyse the specific mRNAs or signalling pathways targeted by this miRNA. The scarce information on miR-374a prevents us from interpreting the results of our study.

miR-96

miR-96 is part of the miR-183 cluster, a miRNA family of three nearly identical mature miRNA seed sequences (miR-183, miR-96, and miR-182). This miRNA family appears to be a highly conserved cluster and can be found in organisms like chimpanzees, mice, fish, and humans (Dambal et al., 2015). They target distinct mRNAs with similar functional roles due to the minor sequence differences between the three miRNAs. They are involved in biological processes such as apoptosis (targeting FOXO1), DNA repair (targeting CHEK2), and metabolism (targeting IRS-1) (Dambal et al., 2015). miR-96, when overexpressed, seems to work as anti-apoptotic in cancer by negatively regulating FOXO1. FOXO1 plays a crucial role in apoptosis and other biological processes within the cell cycle (Long et al., 2011). miR-96 appears to regulate cell proliferation through inversely regulating FOXO3a in breast cancer cells and tissue (Jung & Suh, 2015; Lin et al., 2010). Lin and colleagues (2010) reported miR-96 to be overexpressed in breast cancer cell lines and tissue, which induced the proliferation of breast cancer cells and the expression of the proliferative marker Ki-67. The inhibition of miR-96 resulted in a higher expression of FOXO3, p21, and p27, a lower expression of CCND1, and increased cellular growth (Lin et al., 2010). According to a review by Dambal et al. (2015), miR-96 is overexpressed in other cancer cell types like prostate, lung, liver, brain, and colorectal. In murine C2C12 cells, Nguyen et al. (2020) reported that miR-96 promotes proliferation but inhibits the differentiation of myoblasts. They noted that the overexpression of miR-96 induced the expression of proliferative genes like CCND1 and cyclin B1 (CCB1) and simultaneously suppressed the expression of four and a half LIM domains protein 1 (FHL1). Consequently, myogenic factors mediated by FHL1, such as MyoD, MyoG, MyHC, and myocyte enhancer factor 2C (MEF2C), were downregulated in myoblasts, inhibiting their differentiation (Nguyen et al., 2020). Interestingly, others have reported miR-96 knockdown mice not to show any deviation in

myofiber amount or the expression of Myf5 and MyoD compared to controls. The inhibition of miR-96 in mice led to an enriched muscle oxidative phenotype with a higher content of type I fibres and upregulation of expression of FOXO1 and proliferator-activated receptor-gamma coactivator (PGC-1α) (Wang et al., 2021). They revealed that miR-96 plays an important role in glucose homeostasis due to its inhibition causing a higher basal glucose level and lower glucose tolerance in mice. mir-96 appears to be upregulated in human immune cells after EE (Chilton et al., 2014; Radom-Aizik et al., 2010). However, the precise targets and the possible molecular processes mediated by miR-96 were not further analysed.

miR-424

miR-424 is a member of the miR-15/107 family cluster composed of miR-15a-3p, -15b-5p, -16-5p, -103a-3p, -108-3p, -195-5p, -424-5p, -497-5p, -503-5p, -646-5p. As in most miRNA families, the members resemble some of their functions but also play distinct roles in different biological processes. miR-424 regulates the cell cycle, muscle differentiation, and macrophage-specific differentiation. Wang et al. (2019) concluded that this miRNA is involved in physiological stress response, i.e., hypoxia and ischemia. Just like its family member, miR-15, miR-424 is a pro-angiogenic miRNA which inversely regulates the expression of the VEGF signalling via HIF1α in endothelial cells (Chamorro-Jorganes et al., 2012). It must be noted that the literature on miR-424 seems to be conflicting.

On the one hand, some report miR-424 to be involved in skeletal muscle differentiation and suppressing proliferation (X. Chen et al., 2022; Ge & Chen, 2011; Sarkar et al., 2010). On the other hand, however, studies have reported miR-424 to be overexpressed in muscle wasting and catabolic processes (Chamorro-Jorganes et al., 2012; Connolly et al., 2018; Fochi et al., 2020; van de Worp et al., 2020; Yanai et al., 2020). While most of the studies reporting miR-424 to be myogenic-inducing only showed their results in cell cultures and non-human animal models, such as C2C12 myocytes, human embryonic kidney and osteosarcoma cells, leukemic human cells, and skeletal muscle biopsies of Xuhuai goats. Studies investigating miRNA expression in patients with rapid muscle loss have shown miR-424 overexpressed in muscle biopsies. Connolly and colleagues (2018) performed *in vitro* and *in vivo* analyses examining the role of miR-424 in myogenesis. They described this miRNA to target cell division cycle 25A (CDC25A), a mitosis-inducing and CDK1/2 inhibiting protein, which others have corroborated (X. Chen et al., 2022; Sarkar et al., 2010).

Additionally, miR-424 appears to regulate the synthesis of ribosomal RNA (rRNA) inversely and, as a result, mediate the capacity of protein synthesis, as shown in LHCN-M2 cells (Connolly et al., 2018). The overexpression of miR-424 resulted in a decrease in fibre diameter in mice's muscles, and patients with a greater rate of muscle loss showed a higher expression than controls. Sarcopenic and cachectic patients, patients with intensive care-acquired weakness (ICAW), and chronic obstructive pulmonary disease (COPD) patients have all been reported to have a higher basal expression of miR-424 when compared to healthy age-matched controls. This could indicate a possible contribution of protein synthesis inhibition through the expression of miR-424. The expression of this miRNA also showed an association with markers of disease severity in COPD (transfer factor of the lung for carbon monoxide (TL_{CO}%)), in patients undergoing aortic surgery (left ventricular ejection fraction (LVEF%)), and in ICAW (days spent in the ICU) (Connolly et al., 2018). Basal miR-424-5p expression in skeletal muscle was associated with muscle loss in patients undergoing aortic surgery, (Fochi et al., 2020). IGF-1 and its receptor being a direct target of miR-424 could indicate that this miRNA contributes to the anabolic signalling response (Connolly et al., 2018). This miRNA seems to be also regulated in a feed-forward loop by TGF-β signalling and therefore modulates the expression of varying SMAD-proteins. This goes per our results, in which anabolic stimulus, eccentric squat exercise, induced an upregulation and downregulation in master and young athletes – indicating that the increased expression of miR-424 in master athletes induced the suppression of cell proliferation and rRNA synthesis and, as a result, reduced anabolism and increased catabolism in older athletes. This could partially explain the inhibited anabolic response reported in older individuals.

As Sapp et al. (2017) mentioned in their review, it should be acknowledged that there is a need for a standardised protocol for studying miRNAs in general. Especially standardised protocols accounting for each medium, e. g., saliva, serum, plasma, muscle biopsies, and whole blood, would be needed to ensure reproducibility. Additionally, the system used for miRNA extraction and analysis should be considered, as the results and the accuracy might vary depending on the implemented methodology.

The absence and sometimes opposite RE-induced regulation of miRNAs in master athletes could indicate and contribute to the blunted exercise physiological reaction observed with ageing. The diminished capacity of adaptation to anabolic stimuli of the skeletal muscle, i.e., anabolic resistance, is believed to be a contributing factor to age-related metabolic and

physical conditions such as sarcopenia. Older individuals' skeletal muscle cells and stem cells seem to respond differently (or not to respond) to the same relative stimulus as younger muscle does, contributing to its impaired repair and remodelling after exertion. However, the detailed molecular and cellular mechanisms causal to the diminished capacity for ageing skeletal muscle to respond to anabolic stimulation are yet to be elucidated.

5 Conclusion

With a worldwide increasing ageing population, it is important to understand the underlying mechanisms of senescence in the human body. As the exact processes responsible for biological ageing are yet not completely understood and physically active older individuals seem to abate some of these processes, master athletes could contribute to a better understanding of the effect of biological ageing on muscle characteristics and physical performance. miRNA expression is altered by anabolic stimulations such as RE, resulting in increased muscle protein synthesis and cell growth and regeneration. However, a divergent miRNA profile expression and dysregulation after an acute bout of RE as well as chronic RE have been reported in older individuals compared to young (Camera et al., 2016; dos Santos et al., 2022; Drummond, 2010; Margolis et al., 2016; Rivas et al., 2014; Russell & Lamon, 2015; Telles et al., 2021; Zacharewicz et al., 2014). These variations in miRNA expression could potentially shed light on some of the underlying molecular mechanisms inhibiting muscular and cellular response to anabolic stimuli, resulting in anabolic resistance in older individuals (Margolis & Rivas, 2018). Our study identified nine DE whole blood miRNAs followed by RE in strength-trained young and master athletes. Since miRNAs function as mediators of post-transcriptional gene silencing by repressing the synthesis of complementary mRNAs, downregulation of miRNAs could indicate a feed-forward control of induced translation of proteins involved in physiological adaptation followed by RE.

Furthermore, some miRNAs might induce intracellular signalling changes and inhibit or induce upstream or downstream proteins involved in anabolic or catabolic signalling pathways, possibly providing insight into the molecular processes involved in exercise response and adaptation. The feed-forward control could help explain that all nine DE miRNAs were downregulated in Young. *In silico* analysis indicated these miRNAs to be linked to genes and pathways known to mediate skeletal muscle modulation (myogenesis)

and physiological adaptation to exercise (angiogenesis, regeneration, cellular differentiation, and proliferation). The data obtained through network and overexpression analysis suggest that the identified DE miRNAs regulate signalling proteins involved in muscle protein synthesis and breakdown, such as mTOR and FOXO1. Seven of the nine DE miRNAs were downregulated at baseline in the younger group compared to master athletes.

Interestingly, some DE miRNAs belong to family clusters, e. g., miR-15/107 and miR-17~92 clusters. Both miRNA families mediate myogenesis at different cell cycle steps and muscle protein synthesis. On the contrary, the miRNA expression profile followed by RE differed in many ways in the master athletes. There were only three DE whole blood miRNAs post RE in master athletes, of which two had the opposite regulation to the younger group. The two miRNAs, miR-210 and miR-15a, mediate angiogenesis. The dysregulation of those miRNAs could be likely involved in the impaired vascular system of older individuals, even physically active ones. Thus, resulting in a reduced capillary density of skeletal muscles and compromising the transport of fundamental components, i.e., hormones, growth factors, nutrients, and oxygen (Distefano & Goodpaster, 2018; Ganse & Degens, 2021). It is hypothesised that miRNAs function partially as cell-cell communicators, and our findings could help identify their role in the molecular response or adaptation to an acute bout of RE. Thus, the divergent miRNA expression profile could help explain the blunted or delayed molecular and physiological response to anabolic stimulation in older individuals, leading to a decline in muscle mass and performance with time. However, there is little research on the exact function of most DE miRNAs found in this study on exercise and or ageing. Even though an exact interaction between mRNAs and miRNA expression could not be assessed in our sample, it could be the subject of further research.

One limiting factor of the present study is the low sample size. With such a sample size, each extreme value is likely to distort any variable's mean values. In addition, to assess the expression profile of whole blood miRNAs, samples of Young were pooled as well as of Master, resulting in extreme values and even outliers being predominantly assessed rather than the true mean expression of any given miRNA and obtaining more false discoveries than anticipated. Therefore, the results of this study need validation via miRNA PCR assays using single samples and higher sample size.

Furthermore, the sample population could also have been one possible limiting factor of this study. This study aimed to assess DE miRNAs in strength trained male athletes; to accomplish this, males with at least one year of strength training experience and a 1RM of

120% of their body weight were selected. On the one hand, the exact training years of the individuals were not assessed, leaving many questions unanswered regarding how many years they have been strength training and how many and what sports they have engaged with previously. This is especially important when it comes to long-term exercisers. Additionally, it was not mentioned if the participants solely performed RE or were recreationally active in other exercise forms one year before the study. On the other hand, the Master athletes seemed to have a very high half squat 1RM but underperformed in the other physical performance test compared to younger athletes. Thus, it is important to note that the older participants of this study were possibly highly well-trained in the half squat, or the younger participants were under-trained in the half squat compared to Master. Hence, it is surprising that the 1RM was not age-adjusted when defining the inclusion criteria for the study subjects performed by Schmidt and colleagues (2021).

Lastly, it seems like the master athletes could have performed the exercise protocol differently than Young. Despite total repetitions not being significantly different between age groups, older participants reported a lower RPE and NPS than younger ones. Indicating that Master under-performed in the last set, which was meant to be performed until exhaustion, due to a) higher awareness of potential injury risk, b) being customed to the training volume, or c) the 1RMest not being necessarily accurate.

In summary, our findings illustrate a differing change in miRNA expression following intense RE between younger and older strength-trained athletes. We identified these miRNAs as possible mediators of physiological adaptation to anabolic stimulation and indicated an impairment of miRNA regulation with age in strength-trained master athletes simultaneously. Additionally, different expression profiles of basal miRNAs between age groups were identified, potentially noting a basal dysregulation of miRNAs and reflecting the underlying physiological state of ageing individuals. The divergent epigenetic response of the older age group to RE could help identify or explain the mechanisms underlying age-related anabolic resistance.

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Appendix A

Table A1: miRNAs significantly up- or down-regulated in each age group, a baseline and post physical activity values comparison

		Young						Master					
		AVG ΔC_T		$2^{-\Delta C_T}$		Fold change	Fold up- or down-regulation	AVG ΔC_T		$2^{-\Delta C_T}$		Fold change	Fold up- or down-regulation
miRNA ID	Well	post-0	pre	post-0	pre	post-0/pre	post-0/pre	post-0	pre	post-0	pre	post-0/pre	post-0/pre
hsa-miR-142-5p	A01	1.96	0.66	2.6E-01	6.3E-01	**0.41**	**-2.47 ↓**	3.47	3.04	9.0E-02	1.2E-01	0.74	-1.35
hsa-miR-27b-3p	A04	3.30	1.80	1.0E-01	2.9E-01	**0.35**	**-2.83 ↓**	4.30	4.13	5.1E-02	5.7E-02	0.89	-1.13
hsa-miR-101-3p	A05	-0.53	-3.14	1.4E+00	8.8E+00	**0.16**	**-6.12 ↓**	0.45	0.88	7.3E-01	5.4E-01	1.34	1.34
hsa-let-7d-5p	A06	-1.33	-2.20	2.5E+00	4.6E+00	0.55	-1.83	-0.58	-0.46	1.5E+00	1.4E+00	1.08	1.08
hsa-miR-103a-3p	A07	-3.34	-4.62	1.0E+01	2.5E+01	**0.41**	**-2.43 ↓**	-2.72	-2.38	6.6E+00	5.2E+00	1.26	1.26
hsa-miR-16-5p	A08	-6.08	-7.61	6.8E+01	2.0E+02	**0.35**	**-2.89 ↓**	-4.89	-4.88	3.0E+01	2.9E+01	1.00	1.00
hsa-miR-26a-5p	A09	-3.29	-4.20	9.8E+00	1.8E+01	0.53	-1.88	-2.52	-2.62	5.7E+00	6.2E+00	0.93	-1.08
hsa-miR-26b-5p	A11	-1.48	-2.87	2.8E+00	7.3E+00	**0.38**	**-2.63 ↓**	-0.10	-0.28	1.1E+00	1.2E+00	0.88	-1.14
hsa-let-7g-5p	A12	-0.60	-1.94	1.5E+00	3.8E+00	**0.39**	**-2.54 ↓**	0.71	0.68	6.1E-01	6.2E-01	0.98	-1.02
hsa-miR-96-5p	B02	4.38	1.97	4.8E-02	2.6E-01	**0.19**	**-5.33 ↓**	6.42	6.46	1.2E-02	1.1E-02	1.02	1.02
hsa-miR-185-5p	B03	-4.45	-5.60	2.2E+01	4.9E+01	**0.45**	**-2.22 ↓**	-4.69	-4.33	2.6E+01	2.0E+01	1.28	1.28
hsa-miR-142-3p	B04	-2.24	-4.19	4.7E+00	1.8E+01	**0.26**	**-3.87 ↓**	-1.85	-1.74	3.6E+00	3.3E+00	1.08	1.08
hsa-miR-24-3p	B05	-0.66	-1.75	1.6E+00	3.4E+00	**0.47**	**-2.13 ↓**	-0.23	-0.10	1.2E+00	1.1E+00	1.09	1.09
hsa-miR-146a-5p	B07	2.53	1.75	1.7E-01	3.0E-01	0.58	-1.72	2.39	2.58	1.9E-01	1.7E-01	1.14	1.14
hsa-miR-181b-5p	B09	1.60	0.79	3.3E-01	5.8E-01	0.57	-1.76	1.79	1.92	2.9E-01	2.6E-01	1.09	1.09
hsa-miR-30b-5p	B11	-3.57	-4.23	1.2E+01	1.9E+01	**0.63**	**-1.58 ↓**	-3.37	-3.32	1.0E+01	1.0E+01	1.03	1.03

hsa-miR-21-5p	B12	-1.38	-3.66	2.6E+00	1.3E+01	**0.21**	**-4.87 ↓**	-0.89	-0.52	1.8E+00	1.4E+00	1.29	1.29
hsa-miR-30e-5p	C01	-2.17	-3.10	4.5E+00	8.6E+00	0.52	-1.91	-1.67	-1.81	3.2E+00	3.5E+00	0.90	-1.11
hsa-miR-15b-5p	C03	-4.62	-5.80	2.5E+01	5.6E+01	**0.44**	**-2.27 ↓**	-3.70	-4.05	1.3E+01	1.7E+01	0.78	-1.28
hsa-miR-194-5p	C05	-1.62	-3.05	3.1E+00	8.3E+00	**0.37**	**-2.70 ↓**	-1.11	-1.12	2.2E+00	2.2E+00	0.99	-1.01
hsa-miR-210-3p	C06	2.38	1.09	1.9E-01	4.7E-01	**0.41**	**-2.45 ↓**	2.10	2.90	2.3E-01	1.3E-01	1.74	1.74
hsa-miR-15a-5p	C07	-1.32	-3.69	2.5E+00	1.3E+01	**0.19**	**-5.18 ↓**	-0.51	0.09	1.4E+00	9.4E-01	1.51	1.51
hsa-miR-181a-5p	C08	2.49	0.94	1.8E-01	5.2E-01	**0.34**	**-2.93 ↓**	2.39	2.60	1.9E-01	1.7E-01	1.15	1.15
hsa-miR-99a-5p	C10	4.52	3.84	4.4E-02	7.0E-02	0.62	-1.61	4.71	4.22	3.8E-02	5.4E-02	0.71	-1.41
hsa-miR-28-5p	C11	3.33	2.58	9.9E-02	1.7E-01	0.59	-1.69	3.39	3.55	9.5E-02	8.5E-02	1.11	1.11
hsa-miR-320a	C12	-1.62	-3.00	3.1E+00	8.0E+00	**0.38**	**-2.61 ↓**	-1.86	-1.98	3.6E+00	3.9E+00	0.92	-1.09
hsa-miR-29b-3p	D02	0.59	-1.26	6.6E-01	2.4E+00	**0.28**	**-3.61 ↓**	0.36	0.58	7.8E-01	6.7E-01	1.16	1.16
hsa-miR-29a-3p	D03	1.36	-0.69	3.9E-01	1.6E+00	**0.24**	**-4.15 ↓**	1.78	1.87	2.9E-01	2.7E-01	1.06	1.06
hsa-miR-19a-3p	D05	-3.43	-5.57	1.1E+01	4.8E+01	**0.23**	**-4.42 ↓**	-2.96	-2.87	7.8E+00	7.3E+00	1.06	1.06
hsa-miR-18a-5p	D06	0.37	-2.06	7.7E-01	4.2E+00	**0.19**	**-5.40 ↓**	1.21	1.69	4.3E-01	3.1E-01	1.39	1.39
hsa-miR-374a-5p	D07	1.26	-1.69	4.2E-01	3.2E+00	**0.13**	**-7.75 ↓**	3.05	2.74	1.2E-01	1.5E-01	0.80	-1.24
hsa-miR-423-5p	D08	-1.08	-2.17	2.1E+00	4.5E+00	**0.47**	**-2.13 ↓**	-0.95	-1.01	1.9E+00	2.0E+00	0.96	-1.05
hsa-let-7a-5p	D09	-3.47	-4.63	1.1E+01	2.5E+01	**0.45**	**-2.24 ↓**	-2.67	-2.74	6.3E+00	6.7E+00	0.95	-1.05
hsa-miR-92a-3p	D11	-7.29	-7.92	1.6E+02	2.4E+02	0.64	-1.55	-7.79	-7.83	2.2E+02	2.3E+02	0.97	-1.03
hsa-let-7e-5p	E02	-1.23	-1.96	2.3E+00	3.9E+00	0.60	-1.66	-0.06	-0.08	1.04E+0	1.06E+0	0.98	-1.02
hsa-miR-126-3p	E04	-1.43	-3.60	2.7E+00	1.2E+01	**0.22**	**-4.51 ↓**	-0.52	-0.06	1.4E+00	1.0E+00	1.37	1.37
hsa-miR-144-3p	E05	2.27	-1.30	2.1E-01	2.5E+00	**0.08**	**-11.90 ↓**	4.57	3.76	4.2E-02	7.4E-02	0.57	-1.76
hsa-miR-424-5p	E06	2.19	-0.85	2.2E-01	1.8E+00	**0.12**	**-8.24 ↓**	2.92	3.15	1.3E-01	1.1E-01	1.17	1.17
hsa-miR-30a-5p	E07	-2.65	-3.82	6.3E+00	1.4E+01	**0.44**	**-2.26 ↓**	-2.80	-2.85	6.9E+00	7.2E+00	0.96	-1.04
hsa-miR-195-5p	E10	-5.59	-6.92	4.8E+01	1.2E+02	**0.40**	**-2.52 ↓**	-4.52	-4.41	2.3E+01	2.1E+01	1.08	1.08
hsa-miR-30d-5p	E12	-2.43	-3.23	5.4E+00	9.4E+00	0.57	-1.75	-2.53	-2.46	5.8E+00	5.5E+00	1.05	1.05

hsa-let-7i-5p	F02	-2.09	-3.24	4.3E+00	9.5E+00	**0.45**	**-2.22 ↓**	-1.10	-1.21	2.1E+00	2.3E+00	0.92	-1.08
hsa-miR-222-3p	F04	0.55	-0.23	6.8E-01	1.2E+00	0.58	-1.72	0.37	0.57	7.7E-01	6.7E-01	1.14	1.14
hsa-let-7b-5p	F05	-1.38	-3.20	2.6E+00	9.2E+00	**0.28**	**-3.54 ↓**	-1.50	-1.29	2.8E+00	2.4E+00	1.15	1.15
hsa-miR-19b-3p	F06	-3.31	-5.00	9.9E+00	3.2E+01	**0.31**	**-3.23 ↓**	-2.70	-2.44	6.5E+00	5.4E+00	1.19	1.19
hsa-miR-17-5p	F07	-2.32	-3.76	5.0E+00	1.4E+01	**0.37**	**-2.72 ↓**	-1.01	-0.90	2.0E+00	1.9E+00	1.08	1.08
hsa-miR-93-5p	F08	-3.16	-4.16	8.9E+00	1.8E+01	0.50	-2.00	-3.01	-2.76	8.0E+00	6.8E+00	1.19	1.19
hsa-miR-186-5p	F09	-1.42	-2.12	2.7E+00	4.4E+00	0.61	-1.63	-1.43	-1.43	2.7E+00	2.7E+00	1.00	-1.00
hsa-miR-196b-5p	F10	5.38	3.57	2.4E-02	8.4E-02	**0.28**	**-3.51 ↓**	6.14	6.02	1.4E-02	1.5E-02	0.92	-1.09
hsa-miR-27a-3p	F11	1.31	-0.35	4.0E-01	1.3E+00	**0.32**	**-3.17 ↓**	2.02	1.86	2.5E-01	2.8E-01	0.89	-1.12
hsa-miR-22-3p	F12	-2.78	-4.07	6.9E+00	1.7E+01	**0.41**	**-2.45 ↓**	-2.71	-2.50	6.5E+00	5.7E+00	1.15	1.15
hsa-miR-130a-3p	G01	-0.68	-1.75	1.6E+00	3.4E+00	**0.48**	**-2.10 ↓**	-0.18	-0.30	1.1E+00	1.2E+00	0.92	-1.09
hsa-let-7c-5p	G02	0.45	-0.90	7.3E-01	1.9E+00	**0.39**	**-2.56 ↓**	1.28	1.09	4.1E-01	4.7E-01	0.87	-1.14
hsa-miR-29c-3p	G03	-0.37	-2.31	1.3E+00	5.0E+00	**0.26**	**-3.85 ↓**	0.08	0.15	9.4E-01	9.0E-01	1.05	1.05
hsa-let-7f-5p	G06	-1.46	-3.00	2.7E+00	8.0E+00	**0.34**	**-2.91 ↓**	-0.24	-0.34	1.2E+00	1.3E+00	0.93	-1.08
hsa-miR-20a-5p	G08	-3.38	-5.04	1.0E+01	3.3E+01	**0.32**	**-3.17 ↓**	-2.33	-2.19	5.0E+00	4.6E+00	1.10	1.10
hsa-miR-106b-5p	G09	-3.29	-4.79	9.8E+00	2.8E+01	**0.35**	**-2.83 ↓**	-2.41	-2.12	5.3E+00	4.4E+00	1.22	1.22
hsa-miR-7-5p	G10	1.36	-0.26	3.9E-01	1.2E+00	**0.32**	**-3.08 ↓**	1.36	1.79	3.9E-01	2.9E-01	1.34	1.34
hsa-miR-100-5p	G11	5.24	4.47	2.6E-02	4.5E-02	0.59	-1.71	2.09	1.84	2.3E-01	2.8E-01	0.84	-1.19

Fold change values greater than 2 are indicated in in **red and bold (↑)** and fold change values less than 0.5 and fold regulation values less than -2 are indicated in **blue and bold (↓)**. miRNAs up-or down-regulated in both groups are highlighted in grey.

Legend:

AVG ΔC$_T$, is the difference between the C$_T$ value of the gene of interest (GOI) and the average C$_T$ value of the housekeeping genes (HKG). meaning (C$_T$ (GOI) – AVG C$_T$ (HKG))

2^(- ΔC$_T$), is the normalized gene expression in the respective sample (post-0) or (pre)

Fold Change (2^(- ΔΔC$_T$)),.is the quotient between ((2^(- ΔC$_T$)) post-0) and ((2^(- ΔC$_T$) pre). Fold change values greater than one indicate an up-regulation and fold change values less than one indicate a down-regulation

Fold Regulation, represents fold change results in a biologically meaningful way. Is there an up-regulation. the fold regulation value is equal to the fold change. Is there a down-regulation. the fold regulation value is the negative inverse of the fold change.

Note. This table is a section of the miScript miRNA PCR Array Data Analysis results (excel sheet "PROM-13391", Qiagen). It shows miRNAs' pre and post-exercise expression values and the difference between baseline (pre) and after eccentric squat exercise (post-0) in each age group. The data exclusively shows miRNAs with CT values <30 and were therefore reasonably detected in both samples and with a fold change value >1.5 or <0.67, respectively.

Table A2: miRNAs significantly up- or down-regulated before and after eccentric exercise, an age group comparison

		pre						post-0					
		AVG ΔCt		2^-ΔCt		Fold Change	Fold Up- or Down-Regulation	AVG ΔCt		2^-ΔCt		Fold Change	Fold Up- or Down-Regulation
miRNA ID	Well	Master	Young	Master	Young	Master/ Young	Master/ Young	Master	Young	Master	Young	Master/ Young	Master/ Young
hsa-miR-142-5p	A01	3.04	0.66	1.2E-01	6.3E-01	**0.19**	**-5.21 ↓**	3.47	1.96	9.0E-02	2.6E-01	**0.35**	**-2.85 ↓**
hsa-miR-150-5p	A03	-4.26	-4.14	1.9E+01	1.8E+01	1.09	1.09	-4.49	-4.08	2.2E+01	1.7E+01	1.33	1.33
hsa-miR-27b-3p	A04	4.13	1.80	5.7E-02	2.9E-01	**0.20**	**-5.03 ↓**	4.30	3.30	5.1E-02	1.0E-01	**0.50**	**-2.00 ↓**
hsa-miR-101-3p	A05	0.88	-3.14	5.4E-01	8.8E+00	**0.06**	**-16.22 ↓**	0.45	-0.53	7.3E-01	1.4E+00	0.51	-1.97
hsa-let-7d-5p	A06	-0.46	-2.20	1.4E+00	4.6E+00	**0.30**	**-3.34 ↓**	-0.58	-1.33	1.5E+00	2.5E+00	0.59	-1.68
hsa-miR-103a-3p	A07	-2.38	-4.62	5.2E+00	2.5E+01	**0.21**	**-4.72 ↓**	-2.72	-3.34	6.6E+00	1.0E+01	0.65	-1.54
hsa-miR-16-5p	A08	-4.88	-7.61	2.9E+01	2.0E+02	**0.15**	**-6.63 ↓**	-4.89	-6.08	3.0E+01	6.8E+01	**0.44**	**-2.28 ↓**
hsa-miR-26a-5p	A09	-2.62	-4.20	6.2E+00	1.8E+01	**0.33**	**-2.99 ↓**	-2.52	-3.29	5.7E+00	9.8E+00	0.59	-1.71
hsa-miR-26b-5p	A11	-0.28	-2.87	1.2E+00	7.3E+00	**0.17**	**-6.02 ↓**	-0.10	-1.48	1.1E+00	2.8E+00	**0.38**	**-2.61 ↓**
hsa-let-7g-5p	A12	0.68	-1.94	6.2E-01	3.8E+00	**0.16**	**-6.15 ↓**	0.71	-0.60	6.1E-01	1.5E+00	**0.40**	**-2.48 ↓**
hsa-miR-30c-5p	B01	-4.90	-5.14	3.0E+01	3.5E+01	0.85	-1.18	-4.84	-5.03	2.9E+01	3.3E+01	0.88	-1.14

hsa-miR-185-5p	B03	-4.33	-5.60	2.0E+01	4.9E+01	**0.41**	**-2.41 ↓**	-4.69	-4.45	2.6E+01	2.2E+01	1.18	1.18
hsa-miR-142-3p	B04	-1.74	-4.19	3.3E+00	1.8E+01	**0.18**	**-5.46 ↓**	-1.85	-2.24	3.6E+00	4.7E+00	0.76	-1.31
hsa-miR-24-3p	B05	-0.10	-1.75	1.1E+00	3.4E+00	**0.32**	**-3.14 ↓**	-0.23	-0.66	1.2E+00	1.6E+00	0.74	-1.35
hsa-miR-155-5p	B06	5.05	5.24	3.0E-02	2.6E-02	1.14	1.14	5.29	5.37	2.6E-02	2.4E-02	1.06	1.06
hsa-miR-146a-5p	B07	2.58	1.75	1.7E-01	3.0E-01	0.56	-1.78	2.39	2.53	1.9E-01	1.7E-01	1.10	1.10
hsa-miR-425-5p	B08	-3.99	-4.74	1.6E+01	2.7E+01	0.59	-1.68	-3.96	-4.16	1.6E+01	1.8E+01	0.87	-1.15
hsa-miR-181b-5p	B09	1.92	0.79	2.6E-01	5.8E-01	**0.46**	**-2.19 ↓**	1.79	1.60	2.9E-01	3.3E-01	0.88	-1.14
hsa-miR-30b-5p	B11	-3.32	-4.23	1.0E+01	1.9E+01	0.53	-1.88	-3.37	-3.57	1.0E+01	1.2E+01	0.87	-1.15
hsa-miR-21-5p	B12	-0.52	-3.66	1.4E+00	1.3E+01	**0.11**	**-8.82 ↓**	-0.89	-1.38	1.8E+00	2.6E+00	0.71	-1.41
hsa-miR-30e-5p	C01	-1.81	-3.10	3.5E+00	8.6E+00	**0.41**	**-2.45 ↓**	-1.67	-2.17	3.2E+00	4.5E+00	0.71	-1.42
hsa-miR-200c-3p	C02	5.11	4.59	2.9E-02	4.2E-02	0.70	-1.43	5.17	4.75	2.8E-02	3.7E-02	0.75	-1.34
hsa-miR-15b-5p	C03	-4.05	-5.80	1.7E+01	5.6E+01	**0.30**	**-3.36 ↓**	-3.70	-4.62	1.3E+01	2.5E+01	0.53	-1.89
hsa-miR-223-3p	C04	-6.36	-6.69	8.2E+01	1.0E+02	0.80	-1.26	-6.43	-6.48	8.6E+01	8.9E+01	0.96	-1.04
hsa-miR-194-5p	C05	-1.12	-3.05	2.2E+00	8.3E+00	**0.26**	**-3.81 ↓**	-1.11	-1.62	2.2E+00	3.1E+00	0.70	-1.43
hsa-miR-210-3p	C06	2.90	1.09	1.3E-01	4.7E-01	**0.29**	**-3.51 ↓**	2.10	2.38	2.3E-01	1.9E-01	1.21	1.21
hsa-miR-15a-5p	C07	0.09	-3.69	9.4E-01	1.3E+01	**0.07**	**-13.74 ↓**	-0.51	-1.32	1.4E+00	2.5E+00	0.57	-1.76
hsa-miR-181a-5p	C08	2.60	0.94	1.7E-01	5.2E-01	**0.32**	**-3.16 ↓**	2.39	2.49	1.9E-01	1.8E-01	1.07	1.07
hsa-miR-125b-5p	C09	-0.34	1.61	1.3E+00	3.3E-01	**3.86**	**3.86 ↑**	-0.37	1.84	1.3E+00	2.8E-01	**4.62**	**4.62 ↑**
hsa-miR-99a-5p	C10	4.22	3.84	5.4E-02	7.0E-02	0.77	-1.30	4.71	4.52	3.8E-02	4.4E-02	0.88	-1.14
hsa-miR-28-5p	C11	3.55	2.58	8.5E-02	1.7E-01	0.51	-1.96	3.39	3.33	9.5E-02	9.9E-02	0.96	-1.04
hsa-miR-320a	C12	-1.98	-3.00	3.9E+00	8.0E+00	**0.49**	**-2.03 ↓**	-1.86	-1.62	3.6E+00	3.1E+00	1.18	1.18
hsa-miR-125a-5p	D01	0.12	0.06	9.2E-01	9.6E-01	0.96	-1.04	0.23	0.45	8.5E-01	7.3E-01	1.16	1.16
hsa-miR-29b-3p	D02	0.58	-1.26	6.7E-01	2.4E+00	**0.28**	**-3.58 ↓**	0.36	0.59	7.8E-01	6.6E-01	1.17	1.17
hsa-miR-29a-3p	D03	1.87	-0.69	2.7E-01	1.6E+00	**0.17**	**-5.90 ↓**	1.78	1.36	2.9E-01	3.9E-01	0.75	-1.34
hsa-miR-19a-3p	D05	-2.87	-5.57	7.3E+00	4.8E+01	**0.15**	**-6.50 ↓**	-2.96	-3.43	7.8E+00	1.1E+01	0.72	-1.39

hsa-miR-18a-5p	D06	1.69	-2.06	3.1E-01	4.2E+00	**0.07**	**-13.45 ↓**	1.21	0.37	4.3E-01	7.7E-01	0.56	-1.79
hsa-miR-374a-5p	D07	2.74	-1.69	1.5E-01	3.2E+00	**0.05**	**-21.56 ↓**	3.05	1.26	1.2E-01	4.2E-01	**0.29**	**-3.46 ↓**
hsa-miR-423-5p	D08	-1.01	-2.17	2.0E+00	4.5E+00	**0.45**	**-2.23 ↓**	-0.95	-1.08	1.9E+00	2.1E+00	0.91	-1.10
hsa-let-7a-5p	D09	-2.74	-4.63	6.7E+00	2.5E+01	**0.27**	**-3.71 ↓**	-2.67	-3.47	6.3E+00	1.1E+01	0.57	-1.74
hsa-miR-92a-3p	D11	-7.83	-7.92	2.3E+02	2.4E+02	0.94	-1.06	-7.79	-7.29	2.2E+02	1.6E+02	1.41	1.41
hsa-miR-23a-3p	D12	-2.19	-2.71	4.6E+00	6.6E+00	0.70	-1.43	-2.07	-2.32	4.2E+00	5.0E+00	0.84	-1.19
hsa-miR-25-3p	E01	-5.29	-5.96	3.9E+01	6.2E+01	0.63	-1.59	-5.66	-5.41	5.0E+01	4.2E+01	1.19	1.19
hsa-let-7e-5p	E02	-0.08	-1.96	1.1E+00	3.9E+00	**0.27**	**-3.68 ↓**	-0.06	-1.23	1.0E+00	2.3E+00	**0.44**	**-2.25 ↓**
hsa-miR-126-3p	E04	-0.06	-3.60	1.0E+00	1.2E+01	**0.09**	**-11.63 ↓**	-0.52	-1.43	1.4E+00	2.7E+00	0.53	-1.88
hsa-miR-144-3p	E05	3.76	-1.30	7.4E-02	2.5E+00	**0.03**	**-33.36 ↓**	4.57	2.27	4.2E-02	2.1E-01	**0.20**	**-4.93 ↓**
hsa-miR-424-5p	E06	3.15	-0.85	1.1E-01	1.8E+00	**0.06**	**-16.00 ↓**	2.92	2.19	1.3E-01	2.2E-01	0.60	-1.66
hsa-miR-30a-5p	E07	-2.85	-3.82	7.2E+00	1.4E+01	0.51	-1.96	-2.80	-2.65	6.9E+00	6.3E+00	1.11	1.11
hsa-miR-23b-3p	E08	0.15	-0.87	9.0E-01	1.8E+00	**0.49**	**-2.03 ↓**	0.29	-0.30	8.2E-01	1.2E+00	0.66	-1.51
hsa-miR-151a-5p	E09	-1.06	-1.79	2.1E+00	3.5E+00	0.60	-1.66	-0.90	-1.26	1.9E+00	2.4E+00	0.78	-1.28
hsa-miR-195-5p	E10	-4.41	-6.92	2.1E+01	1.2E+02	**0.18**	**-5.70 ↓**	-4.52	-5.59	2.3E+01	4.8E+01	**0.48**	**-2.10 ↓**
hsa-miR-30d-5p	E12	-2.46	-3.23	5.5E+00	9.4E+00	0.59	-1.71	-2.53	-2.43	5.8E+00	5.4E+00	1.07	1.07
hsa-miR-191-5p	F01	-6.26	-6.42	7.7E+01	8.6E+01	0.90	-1.12	-6.03	-6.08	6.5E+01	6.8E+01	0.96	-1.04
hsa-let-7i-5p	F02	-1.21	-3.24	2.3E+00	9.5E+00	**0.24**	**-4.08 ↓**	-1.10	-2.09	2.1E+00	4.3E+00	0.50	-1.99
hsa-miR-222-3p	F04	0.57	-0.23	6.7E-01	1.2E+00	0.57	-1.74	0.37	0.55	7.7E-01	6.8E-01	1.13	1.13
hsa-let-7b-5p	F05	-1.29	-3.20	2.4E+00	9.2E+00	**0.27**	**-3.76 ↓**	-1.50	-1.38	2.8E+00	2.6E+00	1.09	1.09
hsa-miR-19b-3p	F06	-2.44	-5.00	5.4E+00	3.2E+01	**0.17**	**-5.90 ↓**	-2.70	-3.31	6.5E+00	9.9E+00	0.65	-1.53
hsa-miR-17-5p	F07	-0.90	-3.76	1.9E+00	1.4E+01	**0.14**	**-7.26 ↓**	-1.01	-2.32	2.0E+00	5.0E+00	**0.40**	**-2.48 ↓**
hsa-miR-93-5p	F08	-2.76	-4.16	6.8E+00	1.8E+01	**0.38**	**-2.64 ↓**	-3.01	-3.16	8.0E+00	8.9E+00	0.90	-1.11
hsa-miR-186-5p	F09	-1.43	-2.12	2.7E+00	4.4E+00	0.62	-1.61	-1.43	-1.42	2.7E+00	2.7E+00	1.01	1.01
hsa-miR-196b-5p	F10	6.02	3.57	1.5E-02	8.4E-02	**0.18**	**-5.46 ↓**	6.14	5.38	1.4E-02	2.4E-02	0.59	-1.70

hsa-miR-27a-3p	F11	1.86	-0.35	2.8E-01	1.3E+00	**0.22**	**-4.63 ↓**	2.02	1.31	2.5E-01	4.0E-01	0.61	-1.64
hsa-miR-22-3p	F12	-2.50	-4.07	5.7E+00	1.7E+01	**0.34**	**-2.97 ↓**	-2.71	-2.78	6.5E+00	6.9E+00	0.95	-1.05
hsa-miR-130a-3p	G01	-0.30	-1.75	1.2E+00	3.4E+00	**0.37**	**-2.73 ↓**	-0.18	-0.68	1.1E+00	1.6E+00	0.71	-1.42
hsa-let-7c-5p	G02	1.09	-0.90	4.7E-01	1.9E+00	**0.25**	**-3.97 ↓**	1.28	0.45	4.1E-01	7.3E-01	0.56	-1.78
hsa-miR-29c-3p	G03	0.15	-2.31	9.0E-01	5.0E+00	**0.18**	**-5.50 ↓**	0.08	-0.37	9.4E-01	1.3E+00	0.73	-1.37
hsa-miR-140-3p	G04	-3.41	-3.84	1.1E+01	1.4E+01	0.74	-1.35	-3.18	-3.34	9.0E+00	1.0E+01	0.89	-1.12
hsa-miR-128-3p	G05	0.71	0.00	6.1E-01	1.0E+00	0.61	-1.64	0.81	0.53	5.7E-01	6.9E-01	0.82	-1.22
hsa-let-7f-5p	G06	-0.34	-3.00	1.3E+00	8.0E+00	**0.16**	**-6.32 ↓**	-0.24	-1.46	1.2E+00	2.7E+00	**0.43**	**-2.33 ↓**
hsa-miR-20a-5p	G08	-2.19	-5.04	4.6E+00	3.3E+01	**0.14**	**-7.21 ↓**	-2.33	-3.38	5.0E+00	1.0E+01	**0.48**	**-2.07 ↓**
hsa-miR-106b-5p	G09	-2.12	-4.79	4.4E+00	2.8E+01	**0.16**	**-6.36 ↓**	-2.41	-3.29	5.3E+00	9.8E+00	0.54	-1.84
hsa-miR-7-5p	G10	1.79	-0.26	2.9E-01	1.2E+00	**0.24**	**-4.14 ↓**	1.36	1.36	3.9E-01	3.9E-01	1.00	-1.00
hsa-miR-100-5p	G11	1.84	4.47	2.8E-01	4.5E-02	**6.19**	**6.19 ↑**	2.09	5.24	2.3E-01	2.6E-02	**8.87**	**8.87 ↑**

Fold change values greater than 2 are indicated in **red and bold (↑)** and fold change values less than 0.5 and fold regulation values less than -2 are indicated in **blue and bold (↓)**. miRNAs with a fold change higher than 2 or less than 0.5 both at baseline (pre) and after eccentric squat exercise (post) are highlighted in grey.

Legend:

AVG ΔC_T, is the difference between the C_T value of the gene of interest (GOI) and the average C_T value of the housekeeping genes (HKG). meaning (C_T (GOI) – AVG C_T (HKG))

2^(- ΔC_T), is the normalized gene expression in the respective sample (post-0) or (pre)

Fold Change (2^(- $\Delta\Delta C_T$)), is the quotient between ((2^(- ΔC_T)) post-0) and ((2^(- ΔC_T) pre). Fold change values greater than one indicate an up-regulation and fold change values less than one indicate a down-regulation

Fold Regulation, represents fold change results in a biologically meaningful way. Is there an up-regulation. the fold regulation value is equal to the fold change. Is there a down-regulation. the fold regulation value is the negative inverse of the fold change.

Note. This table is a section of the miScript miRNA PCR Array Data Analysis results (excel sheet "PROM-13391", Qiagen). It shows miRNAs' baseline expression values and the difference between young and master athletes. The data exclusively shows miRNAs with CT values <30 and were therefore reasonably detected in both samples and with a fold change value >1.5 or <0.67, respectively.

Appendix B

Table B1: Enriched genes in young athletes

Type	Name	Hits	Expected score	*q*-value	Genes
enriched	hsa-miR-101-3p	50	0.467938	7.88E-075	APP, ATG4D, ATM, ATXN1, CCND1, CDH5, CDK8, CFTR, CPEB1, CTNNB1, DNMT3A, DUSP1, EED, EYA1, EZH2, FMR1, FOS, GRSF1, ITGA3, JAK2, KLF6, MCL1, MEIS1, MET, MITF, MTOR, MYCN, NLK, NOTCH1, PIK3CB, PIM1, PRDM1, PRKAB1, PTGER4, PTGS2, RAB5A, RAC1, RAP1B, RHOA, RUNX1, SNHG1, SOX9, SRF, STMN1, TET2, VEGFA, VEGFC, VHL, ZEB1, ZEB2
enriched	hsa-miR-15a-5p	43	0.397306	2.91E-064	AKT3, APP, BCL2, BDNF, BMI1, BRCA1, CADM1, CARM1, CCND1, CCND2, CCNE1, CDC25A, CDKN2B, CHEK1, CHUK, CLCN3, CRKL, CXCL10, DMTF1, FGF7, FOXO1, HMGA1, HMGA2, HSPA1B, IFNG, IL10RA, KLF4, MN1, MYB, PHLPP1, PURA, RECK, REPIN1, RET, SOX5, TNFAIP1, TP53, TSPYL2, UCP2, VEGFA, WEE1, WNT3A, YAP1
enriched	hsa-miR-424-5p	31	0.291358	1.15E-045	ANLN, ATF6, CCND1, CCND3, CCNE1, CCNF, CDC14A, CDC25A, CDK6, CDX2, CHEK1, CUL2, FASN, FGF2, FGFR1, HIF1A, KDM5B, KIF23, MAP2K1, MYB, NFIA, PLAG1, PTCH1, SIAH1, SMAD3, SMAD7, SOCS2, SOCS6, SPI1, TGFBR3, WEE1
enriched	hsa-miR-18a-5p	29	0.273700	1.06E-042	ATM, BCL2, BCL2L10, CDK19, DICER1, DNMT1, ESR1, FCGR2B, HIF1A, HSF2, IRF2, MEF2D, NEDD9, NEO1, NR1I2, NR3C1, PHLPP1, PIAS3, PTEN, RUNX1, SDC4, SMAD2, SMAD3, SMAD4, STK4, TBPL1, TGFBR2, TNFAIP3, TNFSF11
enriched	hsa-miR-96-5p	25	0.238384	9.87E-037	ADCY6, ALK, BDNF, CDKN1A, FOXO1, FOXO3, GDNF, GSK3B, KRAS, MITF, MTSS1, PRMT5, PTPN9, RAD51, RECK, REV1, SCARB1, SLC1A1, SLC6A6, SNAI2, SYCP1, TIMP1, TP53INP1, TRIB3, ZEB1
enriched	hsa-miR-144-3p	22	0.203067	1.70E-032	APP, CFTR, ETS1, EZH2, FGG, IRS1, MAP3K8, MET, MTOR, NFE2L2, NOTCH1, PBX3, PLAG1, PTEN, PTGS2, SMAD4, TGFB1, TTN, TUG1, XIST, ZEB1, ZEB2
enriched	hsa-miR-374a-5p	9	0.079461	1.12E-013	ATM, CCND1, CEBPB, DICER1, GADD45A, LDHA, SRCIN1, WIF1, WNT5A

Type, regulation direction; Name, microRNA; Hits, number of genes mediating by this miRNA; Expected score, expected number of hits; *q*-value, adjusted *p*-value.

Note. This table illustrates the results of hsa-miR-101-3p, hsa-miR-15a-5p, hsa-miR-18a-5p, hsa-miR-374a-5p, hsa-miR-96-3p, hsa-miR-144-3p, and hsa-miR-424-5p uploaded to GeneTrail 3 - miRNomics (Saarland University, Saarbrücken, Germany; https://genetrail.bioinf.uni-sb.de/). Targeted genes were identified using experimentally strong verified targets from miRTarBase 9.0.

Table B2: Enriched genes in master athletes

Type	Name	Hits	Expected score	*q*-value	Genes
enriched	hsa-miR-210-3p	50	0.290752	4.32E-087	AIFM3, ALDH5A1, ATG7, BDNF, BNIP3, BTK, CASP8AP2, COL4A2, CPEB2, DDAH1, DIMT1, E2F3, EFNA3, EHD2, FGFRL1, FOXN3, FOXP3, GPD1L, HIF1A, HIF3A, HOXA1, HOXA9, HSD17B1, IGFBP3, INPP5A, ISCU, KCMF1, LDHA, LDHB, MCM3, MNT, NCAM1, NDUFA4, NPTX1, P4HB, PIM1, PLK1, PTBP3, PTPN1, PTPN2, RAD52, SDHD, SH3BGRL, STMN1, TFRC, THSD7A, TP53I11, TWIST1, VMP1, XIST
enriched	hsa-miR-15a-5p	43	0.256546	5.67E-074	AKT3, APP, BCL2, BDNF, BMI1, BRCA1, CADM1, CARM1, CCND1, CCND2, CCNE1, CDC25A, CDKN2B, CHEK1, CHUK, CLCN3, CRKL, CXCL10, DMTF1, FGF7, FOXO1, HMGA1, HMGA2, HSPA1B, IFNG, IL10RA, KLF4, MN1, MYB, PHLPP1, PURA, RECK, REPIN1, RET, SOX5, TNFAIP1, TP53, TSPYL2, UCP2, VEGFA, WEE1, WNT3A, YAP1
enriched	hsa-miR-144-3p	22	0.131124	8.05E-037	APP, CFTR, ETS1, EZH2, FGG, IRS1, MAP3K8, MET, MTOR, NFE2L2, NOTCH1, PBX3, PLAG1, PTEN, PTGS2, SMAD4, TGFB1, TTN, TUG1, XIST, ZEB1, ZEB2

Type, regulation direction; Name, microRNA; Hits, number of genes mediating by this miRNA; Expected score, expected number of hits; *q*-value, adjusted *p*-value.

Note. This table illustrates the results of hsa-miR-210-3p, hsa-miR-15a-5p, and hsa-miR-144-3p uploaded to GeneTrail 3 - miRNomics (Saarland University, Saarbrücken, Germany; https://genetrail.bioinf.uni-sb.de/). Targeted genes were identified using experimentally strong verified targets from miRTarBase 9.0.

Table B3: Differentially enriched genes between age groups at baseline

Type	Name	Hits	Expected score	*q*-value	Genes
enriched	hsa-miR-101-3p	50	0.502699	4.87E-073	APP, ATG4D, ATM, ATXN1, CCND1, CDH5, CDK8, CFTR, CPEB1, CTNNB1, DNMT3A, DUSP1, EED, EYA1, EZH2, FMR1, FOS, GRSF1, ITGA3, JAK2, KLF6, MCL1, MEIS1, MET, MITF, MTOR, MYCN, NLK, NOTCH1, PIK3CB, PIM1, PRDM1, PRKAB1, PTGER4, PTGS2, RAB5A, RAC1, RAP1B, RHOA, RUNX1, SNHG1, SOX9, SRF, STMN1, TET2, VEGFA, VEGFC, VHL, ZEB1, ZEB2
enriched	hsa-miR-15a-5p	43	0.426820	9.28E-063	AKT3, APP, BCL2, BDNF, BMI1, BRCA1, CADM1, CARM1, CCND1, CCND2, CCNE1, CDC25A, CDKN2B, CHEK1, CHUK, CLCN3, CRKL, CXCL10, DMTF1, FGF7, FOXO1, HMGA1, HMGA2, HSPA1B, IFNG, IL10RA, KLF4, MN1, MYB, PHLPP1, PURA, RECK, REPIN1, RET, SOX5, TNFAIP1, TP53, TSPYL2, UCP2, VEGFA, WEE1, WNT3A, YAP1
enriched	hsa-miR-126-3p	40	0.417335	9.24E-058	ADAM9, ADGRE5, ADM, AKT1, BCL2, CADM1, CRK, CRKL, CXCL12, CXCR4, DNMT1, EGFL7, EZH2, FOXO3, HOXA9, IGFBP2, IRS1, KRAS, LRP6, MERTK, MMP7, NFKBIA, PGR, PIK3CG, PIK3R2, PITPNC1, PLK2, PTPN7, RGS3, RHOU, ROCK1, SIRT1, SLC7A5, SOX2, SPRED1, TCF4, TEK, TOM1, VCAM1, VEGFA
enriched	hsa-miR-424-5p	31	0.313001	9.51E-045	ANLN, ATF6, CCND1, CCND3, CCNE1, CCNF, CDC14A, CDC25A, CDK6, CDX2, CHEK1, CUL2, FASN, FGF2, FGFR1, HIF1A, KDM5B, KIF23, MAP2K1, MYB, NFIA, PLAG1, PTCH1, SIAH1, SMAD3, SMAD7, SOCS2, SOCS6, SPI1, TGFBR3, WEE1
enriched	hsa-miR-18a-5p	29	0.294032	7.88E-042	ATM, BCL2, BCL2L10, CDK19, DICER1, DNMT1, ESR1, FCGR2B, HIF1A, HSF2, IRF2, MEF2D, NEDD9, NEO1, NR1I2, NR3C1, PHLPP1, PIAS3, PTEN, RUNX1, SDC4, SMAD2, SMAD3, SMAD4, STK4, TBPL1, TGFBR2, TNFAIP3, TNFSF11
enriched	hsa-miR-144-3p	22	0.218152	8.92E-032	APP, CFTR, ETS1, EZH2, FGG, IRS1, MAP3K8, MET, MTOR, NFE2L2, NOTCH1, PBX3, PLAG1, PTEN, PTGS2, SMAD4, TGFB1, TTN, TUG1, XIST, ZEB1, ZEB2
enriched	hsa-miR-374a-5p	9	0.085364	1.94E-013	ATM, CCND1, CEBPB, DICER1, GADD45A, LDHA, SRCIN1, WIF1, WNT5A

Type, regulation direction; Name, microRNA; Hits, number of genes mediating by this miRNA; Expected score, expected number of hits; *q*-value, adjusted *p*-value.

Note. This table illustrates the results of hsa-miR-101-3p, hsa-miR-15a-5p, hsa-miR-18a-5p, hsa-miR-374a-5p, hsa-miR-126-3p, hsa-miR-144-3p, and hsa-miR-424-5p uploaded to GeneTrail 3 - miRNomics (Saarland University, Saarbrücken, Germany; https://genetrail.bioinf.uni-sb.de/). Targeted genes were identified using experimentally strong verified targets from miRTarBase 9.0.

Appendix C

Table C1: Enriched Gene Ontology Biological Process Annotation in young athletes

Type	Name	Hits	Expected score	*q*-value
enriched	hormone secretion	5	0.116306	1.27E-006
enriched	lymphocyte migration into lymphoid organs	5	0.113601	1.27E-006
enriched	positive chemotaxis	5	0.116306	1.27E-006
enriched	positive regulation of calcium ion import	5	0.121716	1.27E-006
enriched	positive regulation of metabolic process	5	0.116306	1.27E-006
enriched	positive regulation of response to wounding	5	0.116306	1.27E-006
enriched	receptor clustering	5	0.119011	1.27E-006
enriched	regulation of microtubule polymerization or depolymerization	5	0.119011	1.27E-006
enriched	regulation of skeletal muscle cell differentiation	5	0.119011	1.27E-006
enriched	NADH metabolic process	5	0.129830	1.43E-006
enriched	cellular extravasation	5	0.140649	1.43E-006
enriched	pancreas development	5	0.135240	1.43E-006
enriched	positive regulation of interleukin-17 production	5	0.127125	1.43E-006
enriched	protein catabolic process in the vacuole	5	0.140649	1.43E-006
enriched	receptor internalization	5	0.140649	1.43E-006
enriched	regulation of secretion	5	0.137944	1.43E-006
enriched	striated muscle tissue development	5	0.132535	1.43E-006
enriched	cellular amino acid catabolic process	5	0.148764	1.80E-006
enriched	regulation of dendrite development	5	0.154173	2.05E-006
enriched	positive regulation of cytosolic calcium ion concentration involved in phospholipase C-activating G protein-coupled signaling pathway	5	0.159583	2.22E-006
enriched	stress-activated protein kinase signaling cascade	5	0.159583	2.22E-006
enriched	macrophage activation involved in immune response	4	0.056801	2.22E-006
enriched	peptide transport	5	0.181221	3.88E-006
enriched	regulation of autophagy of mitochondrion	5	0.197450	5.70E-006
enriched	regulation of phospholipid biosynthetic process	4	0.073029	5.70E-006
enriched	actin polymerization or depolymerization	3	0.024343	1.72E-005
enriched	detection of mechanical stimulus	4	0.100077	1.97E-005
enriched	negative regulation of CD4-positive	4	0.105487	2.13E-005
enriched	negative regulation of mitotic metaphase/anaphase transition	4	0.105487	2.13E-005
enriched	negative regulation of smooth muscle cell apoptotic process	4	0.105487	2.13E-005
enriched	oocyte maturation	3	0.027048	2.13E-005
enriched	positive regulation of receptor signaling pathway via JAK-STAT	4	0.110896	2.54E-005
enriched	senescence-associated heterochromatin focus assembly	3	0.029753	2.66E-005
enriched	axon extension involved in axon guidance	4	0.116306	2.90E-005
enriched	sensory organ development	3	0.032458	3.33E-005
enriched	cartilage condensation	4	0.127125	3.74E-005
enriched	dendritic spine organization	4	0.127125	3.74E-005
enriched	detection of chemical stimulus	4	0.127125	3.74E-005
enriched	Notch receptor processing	4	0.129830	3.87E-005

enriched	positive regulation of endothelial cell proliferation	3	0.035162	3.87E-005
enriched	fibroblast migration	4	0.132535	3.92E-005
enriched	regulation of astrocyte differentiation	4	0.132535	3.92E-005
enriched	regulation of intracellular steroid hormone receptor signaling pathway	4	0.132535	3.92E-005
enriched	T-helper 2 cell cytokine production	3	0.037867	4.28E-005
enriched	macrophage derived foam cell differentiation	3	0.037867	4.28E-005
enriched	negative regulation of signal transduction by p53 class mediator	4	0.137944	4.31E-005
enriched	fibrinolysis	3	0.040572	4.72E-005
enriched	regulation of calcium ion transport into cytosol	4	0.143354	4.72E-005
enriched	regulation of endothelial cell migration	4	0.143354	4.72E-005
enriched	regulation of vasoconstriction	3	0.040572	4.72E-005
enriched	smooth muscle cell migration	3	0.040572	4.72E-005
enriched	glycosaminoglycan metabolic process	4	0.148764	5.19E-005
enriched	embryonic eye morphogenesis	2	0.005410	5.23E-005
enriched	cardiac muscle cell contraction	3	0.043277	5.38E-005
enriched	positive regulation of execution phase of apoptosis	3	0.043277	5.38E-005
enriched	ammonium transport	4	0.154173	5.57E-005
enriched	forebrain neuron development	4	0.159583	5.88E-005
enriched	growth plate cartilage development	3	0.045982	5.88E-005
enriched	negative regulation of cold-induced thermogenesis	3	0.045982	5.88E-005
enriched	regulation of branching involved in ureteric bud morphogenesis	3	0.045982	5.88E-005
enriched	regulation of natural killer cell activation	4	0.159583	5.88E-005
enriched	caveolin-mediated endocytosis	4	0.162287	6.04E-005
enriched	membrane depolarization during action potential	4	0.162287	6.04E-005
enriched	positive regulation of peptide hormone secretion	4	0.164992	6.04E-005
enriched	regulation of miRNA metabolic process	4	0.164992	6.04E-005
enriched	regulation of natural killer cell differentiation	4	0.164992	6.04E-005
enriched	regulation of p38MAPK cascade	4	0.164992	6.04E-005
enriched	somatic hypermutation of immunoglobulin genes	4	0.164992	6.04E-005
enriched	response to tumor necrosis factor	3	0.048686	6.23E-005
enriched	positive regulation of canonical Wnt signaling pathway	4	0.167697	6.26E-005
enriched	positive regulation of natural killer cell activation	4	0.170402	6.59E-005
enriched	ephrin receptor signaling pathway	4	0.173107	6.64E-005
enriched	negative regulation of cardiocyte differentiation	4	0.173107	6.64E-005
enriched	positive regulation of pathway-restricted SMAD protein phosphorylation	4	0.173107	6.64E-005
enriched	protein demethylation	4	0.173107	6.64E-005
enriched	response to ethanol	4	0.175811	6.98E-005
enriched	positive regulation of p38MAPK cascade	4	0.178516	7.33E-005
enriched	positive regulation of regulated secretory pathway	3	0.054096	7.58E-005
enriched	regulation of bicellular tight junction assembly	3	0.054096	7.58E-005
enriched	membrane repolarization	3	0.056801	8.73E-005
enriched	positive regulation of ion transmembrane transporter activity	4	0.189335	8.83E-005
enriched	cellular process	2	0.008114	9.44E-005
enriched	cellular response to ionizing radiation	4	0.194745	9.44E-005
enriched	monoamine transport	2	0.008114	9.44E-005
enriched	organelle assembly	3	0.059505	9.44E-005
enriched	positive regulation of calcium ion transport into cytosol	4	0.194745	9.44E-005

enriched	positive regulation of cell proliferation involved in kidney development	2	0.008114	9.44E-005
enriched	triglyceride homeostasis	2	0.008114	9.44E-005
enriched	modulation of chemical synaptic transmission	3	0.062210	1.03E-004
enriched	synaptic vesicle exocytosis	3	0.062210	1.03E-004
enriched	synaptic vesicle endocytosis	4	0.202859	1.04E-004
enriched	cellular aromatic compound metabolic process	3	0.067620	1.31E-004
enriched	regulation of type I interferon-mediated signaling pathway	3	0.073029	1.64E-004
enriched	negative regulation of release of cytochrome c from mitochondria	2	0.010819	1.71E-004
enriched	protein deneddylation	2	0.010819	1.71E-004
enriched	regulation of circadian rhythm	2	0.010819	1.71E-004
enriched	regulation of glutamate secretion	2	0.010819	1.71E-004
enriched	neuron projection development	3	0.075734	1.72E-004
enriched	protein localization to organelle	3	0.075734	1.72E-004
enriched	regulation of binding	4	0.238022	1.79E-004
enriched	positive regulation of chromosome organization	3	0.078439	1.88E-004
enriched	enzyme linked receptor protein signaling pathway	3	0.081144	2.07E-004
enriched	cell differentiation	4	0.248841	2.07E-004
enriched	heart trabecula morphogenesis	3	0.086553	2.47E-004
enriched	extracellular matrix organization	2	0.013524	2.51E-004
enriched	negative regulation of immune response	2	0.013524	2.51E-004
enriched	negative regulation of intracellular protein transport	2	0.013524	2.51E-004
enriched	negative regulation of long-chain fatty acid import across plasma membrane	2	0.013524	2.51E-004
enriched	positive regulation of MAP kinase activity	2	0.013524	2.51E-004
enriched	positive regulation of dendritic cell differentiation	2	0.013524	2.51E-004
enriched	neurotrophin TRK receptor signaling pathway	3	0.089258	2.54E-004
enriched	skin development	3	0.091963	2.76E-004
enriched	morphogenesis of an epithelial sheet	3	0.094668	2.94E-004
enriched	negative regulation of bone remodeling	3	0.094668	2.94E-004
enriched	response to fatty acid	3	0.094668	2.94E-004
enriched	modification of morphology or physiology of other organism	3	0.097373	3.18E-004
enriched	membrane biogenesis	2	0.016229	3.36E-004
enriched	phosphatidylinositol phosphorylation	2	0.016229	3.36E-004
enriched	positive regulation of protein localization to nucleus	2	0.016229	3.36E-004
enriched	regulation of intrinsic apoptotic signaling pathway in response to DNA damage by p53 class mediator	2	0.016229	3.36E-004
enriched	response to cGMP	2	0.016229	3.36E-004
enriched	response to exogenous dsRNA	2	0.016229	3.36E-004
enriched	response to gravity	2	0.016229	3.36E-004
enriched	cartilage morphogenesis	3	0.102782	3.45E-004
enriched	cell recognition	3	0.102782	3.45E-004
enriched	regulation of macrophage chemotaxis	3	0.102782	3.45E-004
enriched	protein acetylation	3	0.105487	3.67E-004
enriched	regulation of animal organ morphogenesis	3	0.105487	3.67E-004
enriched	regulation of cholesterol biosynthetic process	3	0.110896	4.11E-004
enriched	regulation of protein complex disassembly	3	0.110896	4.11E-004
enriched	regulation of transcription by RNA polymerase II	3	0.110896	4.11E-004
enriched	regulation of transcription by RNA polymerase III	3	0.110896	4.11E-004

enriched	regulation of wound healing	3	0.110896	4.11E-004
enriched	blood vessel endothelial cell differentiation	2	0.018934	4.16E-004
enriched	cellular glucose homeostasis	2	0.018934	4.16E-004
enriched	granulocyte migration	2	0.018934	4.16E-004
enriched	negative regulation of myotube differentiation	2	0.018934	4.16E-004
enriched	positive regulation of phospholipase C activity	2	0.018934	4.16E-004
enriched	positive regulation of protein polyubiquitination	2	0.018934	4.16E-004
enriched	hair follicle morphogenesis	3	0.113601	4.17E-004
enriched	positive regulation of amyloid-beta formation	3	0.113601	4.17E-004
enriched	regulation of transcription by glucose	3	0.116306	4.39E-004
enriched	regulation of transcription from RNA polymerase II promoter in response to hypoxia	3	0.116306	4.39E-004
enriched	spindle organization	3	0.116306	4.39E-004
enriched	negative regulation of protein kinase B signaling	3	0.119011	4.64E-004
enriched	regulation of leukocyte migration	3	0.119011	4.64E-004
enriched	TNFSF11-mediated signaling pathway	2	0.021638	4.88E-004
enriched	camera-type eye development	2	0.021638	4.88E-004
enriched	endothelial cell-matrix adhesion	3	0.121716	4.88E-004
enriched	hepatocyte differentiation	2	0.021638	4.88E-004
enriched	negative regulation of protein transport	2	0.021638	4.88E-004
enriched	positive regulation of extracellular matrix organization	2	0.021638	4.88E-004
enriched	positive regulation of macrophage migration	2	0.021638	4.88E-004
enriched	regulation of CD4-positive	2	0.021638	4.88E-004
enriched	regulation of aspartic-type endopeptidase activity involved in amyloid precursor protein catabolic process	2	0.021638	4.88E-004
enriched	regulation of fever generation	3	0.121716	4.88E-004
enriched	regulation of mismatch repair	2	0.021638	4.88E-004
enriched	regulation of protein serine/threonine kinase activity	2	0.021638	4.88E-004
enriched	negative regulation of lipid catabolic process	3	0.127125	5.19E-004
enriched	response to calcium ion	3	0.129830	5.50E-004
enriched	T-helper cell differentiation	2	0.024343	5.65E-004
enriched	aorta morphogenesis	2	0.024343	5.65E-004
enriched	cellular detoxification	3	0.135240	5.65E-004
enriched	cellular response to molecule of bacterial origin	2	0.024343	5.65E-004
enriched	entrainment of circadian clock	3	0.135240	5.65E-004
enriched	isoprenoid metabolic process	2	0.024343	5.65E-004
enriched	monocyte differentiation	3	0.132535	5.65E-004
enriched	negative regulation of astrocyte differentiation	2	0.024343	5.65E-004
enriched	negative regulation of cardiac muscle cell apoptotic process	3	0.132535	5.65E-004
enriched	negative regulation of endocytosis	3	0.132535	5.65E-004
enriched	negative regulation of lymphocyte proliferation	2	0.024343	5.65E-004
enriched	positive regulation of binding	2	0.024343	5.65E-004
enriched	positive regulation of dendrite morphogenesis	2	0.024343	5.65E-004
enriched	positive regulation of mast cell degranulation	2	0.024343	5.65E-004
enriched	positive regulation of melanocyte differentiation	2	0.024343	5.65E-004
enriched	regulation of skeletal muscle satellite cell proliferation	2	0.024343	5.65E-004
enriched	cation transport	3	0.137944	5.90E-004
enriched	response to Thyroglobulin triiodothyronine	3	0.137944	5.90E-004
enriched	response to X-ray	3	0.137944	5.90E-004
enriched	ERBB signaling pathway	3	0.140649	6.15E-004

enriched	positive regulation of aspartic-type endopeptidase activity involved in amyloid precursor protein catabolic process	3	0.140649	6.15E-004
enriched	regulation of attachment of spindle microtubules to kinetochore	3	0.140649	6.15E-004
enriched	leukocyte proliferation	2	0.027048	6.57E-004
enriched	planar cell polarity pathway	2	0.027048	6.57E-004
enriched	regulation of establishment of endothelial barrier	2	0.027048	6.57E-004
enriched	regulation of low-density lipoprotein particle receptor biosynthetic process	2	0.027048	6.57E-004
enriched	regulation of proton transport	2	0.027048	6.57E-004
enriched	skeletal muscle fiber development	2	0.027048	6.57E-004
enriched	negative regulation of blood vessel diameter	3	0.146059	6.63E-004
enriched	cartilage development	3	0.148764	6.97E-004
enriched	modulation by host of viral transcription	3	0.151468	7.28E-004
enriched	regulation of apoptotic process	3	0.151468	7.28E-004
enriched	chromatin silencing at rDNA	2	0.029753	7.46E-004
enriched	membrane invagination	2	0.029753	7.46E-004
enriched	mitral valve morphogenesis	2	0.029753	7.46E-004
enriched	positive regulation of epithelial cell migration	2	0.029753	7.46E-004
enriched	positive regulation of vascular endothelial cell proliferation	2	0.029753	7.46E-004
enriched	regulation of blood pressure	2	0.029753	7.46E-004
enriched	regulation of mRNA 3'-end processing	2	0.029753	7.46E-004
enriched	regulation of macrophage colony-stimulating factor production	2	0.029753	7.46E-004
enriched	regulation of programmed cell death	2	0.029753	7.46E-004
enriched	response to immobilization stress	2	0.029753	7.46E-004
enriched	regulation of cGMP-mediated signaling	3	0.156878	7.60E-004
enriched	regulation of oxidative stress-induced neuron intrinsic apoptotic signaling pathway	3	0.156878	7.60E-004
enriched	via spliceosome	3	0.162287	8.37E-004
enriched	RNA secondary structure unwinding	2	0.032458	8.44E-004
enriched	dendritic spine morphogenesis	2	0.032458	8.44E-004
enriched	dentate gyrus development	2	0.032458	8.44E-004
enriched	endocardium morphogenesis	2	0.032458	8.44E-004
enriched	mitotic G2/M transition checkpoint	2	0.032458	8.44E-004
enriched	regulation of natural killer cell mediated immunity	2	0.032458	8.44E-004
enriched	regulation of serotonin secretion	2	0.032458	8.44E-004
enriched	response to erythropoietin	2	0.032458	8.44E-004
enriched	spindle assembly	2	0.032458	8.44E-004
enriched	alternative mRNA splicing	3	0.167697	8.80E-004
enriched	RNA processing	2	0.035162	9.35E-004
enriched	heterochronic	2	0.035162	9.35E-004
enriched	isotype switching	2	0.035162	9.35E-004
enriched	negative regulation of cardiac muscle cell proliferation	2	0.035162	9.35E-004
enriched	negative regulation of gene expression	2	0.035162	9.35E-004
enriched	positive regulation of acute inflammatory response	2	0.035162	9.35E-004
enriched	positive regulation of neuron apoptotic process	2	0.035162	9.35E-004
enriched	regulation of bone resorption	2	0.035162	9.35E-004
enriched	regulation of cardiac muscle tissue development	2	0.035162	9.35E-004
enriched	regulation of macrophage activation	2	0.035162	9.35E-004

enriched	regulation of meiotic cell cycle	2	0.035162	9.35E-004
enriched	regulation of synaptic vesicle exocytosis	2	0.035162	9.35E-004
enriched	signal transduction involved in mitotic G1 DNA damage checkpoint	2	0.035162	9.35E-004
enriched	proximal/distal pattern formation	3	0.175811	9.51E-004
enriched	spinal cord patterning	3	0.181221	1.04E-003
enriched	drug metabolic process	2	0.037867	1.07E-003
enriched	positive regulation of cellular senescence	2	0.037867	1.07E-003
enriched	regulation of extrinsic apoptotic signaling pathway	2	0.037867	1.07E-003
enriched	negative regulation of calcineurin-NFAT signaling cascade	3	0.189335	1.16E-003
enriched	histone H2A monoubiquitination	2	0.040572	1.20E-003
enriched	regulation of aerobic respiration	2	0.040572	1.20E-003
enriched	regulation of axonogenesis	2	0.040572	1.20E-003
enriched	regulation of developmental pigmentation	2	0.040572	1.20E-003
enriched	synaptic vesicle recycling	3	0.192040	1.20E-003
enriched	autonomic nervous system development	2	0.043277	1.30E-003
enriched	cell-cell adhesion mediated by integrin	2	0.043277	1.30E-003
enriched	chemotaxis	2	0.043277	1.30E-003
enriched	collateral sprouting	2	0.043277	1.30E-003
enriched	embryonic digestive tract morphogenesis	2	0.043277	1.30E-003
enriched	endoderm development	2	0.043277	1.30E-003
enriched	ion transmembrane transport	2	0.043277	1.30E-003
enriched	regulation of ceramide biosynthetic process	2	0.043277	1.30E-003
enriched	regulation of cysteine-type endopeptidase activity involved in apoptotic process	2	0.043277	1.30E-003
enriched	regulation of fibroblast migration	2	0.043277	1.30E-003
enriched	regulation of protein polyubiquitination	2	0.043277	1.30E-003
enriched	regulation of protein targeting	2	0.043277	1.30E-003
enriched	activation of innate immune response	2	0.045982	1.42E-003
enriched	apoptotic process involved in embryonic digit morphogenesis	2	0.045982	1.42E-003
enriched	cartilage development involved in endochondral bone morphogenesis	2	0.045982	1.42E-003
enriched	cell death in response to hydrogen peroxide	2	0.045982	1.42E-003
enriched	chemokine (C-X-C motif) ligand 12 signaling pathway	2	0.045982	1.42E-003
enriched	entrainment of circadian clock by photoperiod	2	0.045982	1.42E-003
enriched	negative regulation of autophagy of mitochondrion	2	0.045982	1.42E-003
enriched	negative regulation of mRNA splicing	2	0.045982	1.42E-003
enriched	positive regulation of telomere maintenance	2	0.045982	1.42E-003
enriched	ribonucleoprotein complex localization	2	0.045982	1.42E-003
enriched	5'-to lesion	2	0.048686	1.54E-003
enriched	macromolecule metabolic process	2	0.048686	1.54E-003
enriched	macrophage differentiation	2	0.048686	1.54E-003
enriched	negative regulation of cell adhesion mediated by integrin	2	0.048686	1.54E-003
enriched	negative regulation of smooth muscle cell differentiation	2	0.048686	1.54E-003
enriched	negative regulation of ubiquitin-protein transferase activity	2	0.048686	1.54E-003
enriched	positive regulation of macrophage derived foam cell differentiation	2	0.048686	1.54E-003
enriched	regulation of epidermis development	2	0.048686	1.54E-003

enriched	regulation of mast cell activation	2	0.048686	1.54E-003
enriched	body fluid secretion	2	0.051391	1.64E-003
enriched	cell adhesion involved in heart morphogenesis	2	0.051391	1.64E-003
enriched	cell communication by electrical coupling	2	0.051391	1.64E-003
enriched	endocardial cushion fusion	2	0.051391	1.64E-003
enriched	modulating synaptic transmission	2	0.051391	1.64E-003
enriched	negative regulation of mRNA catabolic process	2	0.051391	1.64E-003
enriched	negative regulation of receptor signaling pathway via JAK-STAT	2	0.051391	1.64E-003
enriched	positive regulation of apoptotic DNA fragmentation	2	0.051391	1.64E-003
enriched	positive regulation of phospholipase activity	2	0.051391	1.64E-003
enriched	preincision complex stabilization	2	0.051391	1.64E-003
enriched	response to vitamin D	2	0.051391	1.64E-003
enriched	response to water-immersion restraint stress	2	0.051391	1.64E-003
enriched	sulfur compound metabolic process	2	0.051391	1.64E-003
enriched	negative regulation of calcium ion-dependent exocytosis	2	0.054096	1.79E-003
enriched	positive regulation of protein oligomerization	2	0.054096	1.79E-003
enriched	regulation of blood vessel diameter by renin-angiotensin	2	0.054096	1.79E-003
enriched	response to cobalt ion	2	0.054096	1.79E-003
enriched	positive regulation of behavioral fear response	3	0.238022	1.85E-003
enriched	cell chemotaxis	2	0.056801	1.93E-003
enriched	cell projection organization	2	0.056801	1.93E-003
enriched	midbrain dopaminergic neuron differentiation	2	0.056801	1.93E-003
enriched	negative regulation of mononuclear cell proliferation	2	0.056801	1.93E-003
enriched	positive regulation of transforming growth factor beta receptor signaling pathway	2	0.056801	1.93E-003
enriched	regulation of skeletal muscle fiber development	2	0.056801	1.93E-003
enriched	regulation of viral genome replication	2	0.056801	1.93E-003
enriched	alpha-beta T cell differentiation	2	0.059505	2.05E-003
enriched	amyloid-beta clearance	2	0.059505	2.05E-003
enriched	execution phase of apoptosis	2	0.059505	2.05E-003
enriched	granulocyte macrophage colony-stimulating factor production	2	0.059505	2.05E-003
enriched	nervous system process	2	0.059505	2.05E-003
enriched	nucleotide biosynthetic process	2	0.059505	2.05E-003
enriched	positive regulation of lipid transport	2	0.059505	2.05E-003
enriched	positive regulation of monooxygenase activity	2	0.059505	2.05E-003
enriched	regulation of epidermal growth factor-activated receptor activity	2	0.059505	2.05E-003
enriched	carbon catabolite regulation of transcription	2	0.062210	2.16E-003
enriched	cellular response to oxygen-glucose deprivation	2	0.062210	2.16E-003
enriched	intrinsic apoptotic signaling pathway	2	0.062210	2.16E-003
enriched	membrane protein proteolysis	2	0.062210	2.16E-003
enriched	membrane raft assembly	2	0.062210	2.16E-003
enriched	membrane raft organization	2	0.062210	2.16E-003
enriched	one-carbon metabolic process	2	0.062210	2.16E-003
enriched	positive regulation of chondrocyte differentiation	2	0.062210	2.16E-003
enriched	regulation of long-term neuronal synaptic plasticity	2	0.062210	2.16E-003
enriched	regulation of monocyte differentiation	2	0.062210	2.16E-003
enriched	skeletal system development	2	0.062210	2.16E-003
enriched	telomere maintenance via telomerase	2	0.062210	2.16E-003
enriched	mitotic spindle organization	2	0.064915	2.33E-003

enriched	regulation of bone development	2	0.064915	2.33E-003
enriched	response to interferon-gamma	2	0.064915	2.33E-003
enriched	activation of immune response	2	0.067620	2.46E-003
enriched	cell population proliferation	2	0.067620	2.46E-003
enriched	cell proliferation in bone marrow	2	0.067620	2.46E-003
enriched	histone H3-K4 trimethylation	2	0.067620	2.46E-003
enriched	nephrogenic mesenchyme morphogenesis	2	0.067620	2.46E-003
enriched	otic vesicle morphogenesis	2	0.067620	2.46E-003
enriched	positive regulation of chemokine-mediated signaling pathway	2	0.067620	2.46E-003
enriched	positive regulation of release of cytochrome c from mitochondria	2	0.067620	2.46E-003
enriched	regulation of epithelial to mesenchymal transition	2	0.067620	2.46E-003
enriched	cell differentiation involved in metanephros development	2	0.070325	2.65E-003
enriched	mitochondrial electron transport	2	0.070325	2.65E-003
enriched	behavior	2	0.073029	2.81E-003
enriched	carbohydrate transport	2	0.073029	2.81E-003
enriched	regulation of astrocyte activation	2	0.073029	2.81E-003
enriched	regulation of dendritic cell differentiation	2	0.073029	2.81E-003
enriched	response to growth hormone	2	0.073029	2.81E-003
enriched	detection of abiotic stimulus	2	0.075734	3.00E-003
enriched	gonad development	2	0.075734	3.00E-003
enriched	positive regulation of T cell activation	2	0.075734	3.00E-003
enriched	extrinsic apoptotic signaling pathway via death domain receptors	2	0.078439	3.20E-003
enriched	hydrogen peroxide catabolic process	2	0.078439	3.20E-003
enriched	positive regulation of artery morphogenesis	2	0.081144	3.37E-003
enriched	positive regulation of cellular biosynthetic process	2	0.081144	3.37E-003
enriched	regulation of cell cycle	2	0.081144	3.37E-003
enriched	regulation of cellular response to insulin stimulus	2	0.081144	3.37E-003
enriched	telomere organization	2	0.081144	3.37E-003
enriched	glycoprotein metabolic process	2	0.083849	3.55E-003
enriched	long-chain fatty-acyl-CoA biosynthetic process	2	0.083849	3.55E-003
enriched	regulation of triglyceride biosynthetic process	2	0.083849	3.55E-003
enriched	release of sequestered calcium ion into cytosol by sarcoplasmic reticulum	2	0.083849	3.55E-003
enriched	triglyceride-rich lipoprotein particle clearance	2	0.083849	3.55E-003
enriched	formation of primary germ layer	2	0.086553	3.75E-003
enriched	morphogenesis of a branching epithelium	2	0.086553	3.75E-003
enriched	nephron development	2	0.086553	3.75E-003
enriched	Golgi organization	2	0.089258	3.90E-003
enriched	cellular response to acetylcholine	2	0.089258	3.90E-003
enriched	cellular response to estradiol stimulus	2	0.089258	3.90E-003
enriched	early endosome to late endosome transport	2	0.089258	3.90E-003
enriched	negative regulation of helicase activity	2	0.089258	3.90E-003
enriched	positive regulation of macroautophagy	2	0.089258	3.90E-003
enriched	regulation of T cell apoptotic process	2	0.089258	3.90E-003
enriched	response to vitamin	2	0.089258	3.90E-003
enriched	monocyte chemotaxis	2	0.091963	4.07E-003
enriched	regulation of Golgi inheritance	2	0.091963	4.07E-003
enriched	regulation of bone remodeling	2	0.091963	4.07E-003

enriched	regulation of protein transport	2	0.091963	4.07E-003
enriched	response to interferon-beta	2	0.091963	4.07E-003
enriched	signal transduction involved in DNA damage checkpoint	2	0.091963	4.07E-003
enriched	lactation	2	0.094668	4.25E-003
enriched	negative regulation of epithelial cell apoptotic process	2	0.094668	4.25E-003
enriched	nucleoside monophosphate phosphorylation	2	0.094668	4.25E-003
enriched	regulation of low-density lipoprotein particle clearance	2	0.094668	4.25E-003
enriched	response to antineoplastic agent	2	0.094668	4.25E-003
enriched	cellular response to steroid hormone stimulus	2	0.097373	4.47E-003
enriched	endochondral bone growth	2	0.097373	4.47E-003
enriched	DNA methylation involved in embryo development	2	0.100077	4.68E-003
enriched	adenylate cyclase-inhibiting G protein-coupled receptor signaling pathway	2	0.100077	4.68E-003
enriched	striated muscle contraction	2	0.100077	4.68E-003
enriched	cell communication by electrical coupling involved in cardiac conduction	2	0.102782	4.90E-003
enriched	cellular response to X-ray	2	0.102782	4.90E-003
enriched	positive regulation of apoptotic process involved in morphogenesis	2	0.102782	4.90E-003
enriched	Rac protein signal transduction	2	0.105487	5.13E-003
enriched	leukotriene metabolic process	2	0.105487	5.13E-003
enriched	cellular response to lipid	2	0.108192	5.32E-003
enriched	mitochondrial RNA metabolic process	2	0.108192	5.32E-003
enriched	negative regulation of transmembrane receptor protein serine/threonine kinase signaling pathway	2	0.108192	5.32E-003
enriched	regulation of chemotaxis	2	0.108192	5.32E-003
enriched	response to isolation stress	2	0.108192	5.32E-003
enriched	cellular response to amino acid starvation	2	0.110896	5.50E-003
enriched	coronary vasculature development	2	0.110896	5.50E-003
enriched	gastrulation	2	0.110896	5.50E-003
enriched	positive regulation of helicase activity	2	0.110896	5.50E-003
enriched	regulation of hematopoietic stem cell differentiation	2	0.110896	5.50E-003
enriched	response to estrogen	2	0.110896	5.50E-003
enriched	NADH to ubiquinone	2	0.113601	5.68E-003
enriched	cell dedifferentiation	2	0.113601	5.68E-003
enriched	energy derivation by oxidation of organic compounds	2	0.113601	5.68E-003
enriched	epicardium-derived cardiac fibroblast cell development	2	0.113601	5.68E-003
enriched	positive regulation of chromatin silencing	2	0.113601	5.68E-003
enriched	regulation of cell-matrix adhesion	2	0.113601	5.68E-003
enriched	positive regulation of hormone biosynthetic process	2	0.116306	5.88E-003
enriched	regulation of angiogenesis	2	0.116306	5.88E-003
enriched	regulation of ossification	2	0.116306	5.88E-003
enriched	regulation of viral transcription	2	0.116306	5.88E-003
enriched	response to growth factor	2	0.116306	5.88E-003
enriched	positive regulation of apoptotic signaling pathway	2	0.119011	6.09E-003
enriched	positive regulation of organ growth	2	0.119011	6.09E-003
enriched	regulation of glycogen metabolic process	2	0.119011	6.09E-003
enriched	regulation of protein catabolic process	2	0.119011	6.09E-003
enriched	acyl-CoA metabolic process	2	0.121716	6.32E-003
enriched	catabolic process	2	0.121716	6.32E-003
enriched	cellular response to dexamethasone stimulus	2	0.121716	6.32E-003

enriched	amide transport	2	0.124420	6.56E-003
enriched	negative regulation of lipid metabolic process	2	0.124420	6.56E-003
enriched	focal adhesion assembly	2	0.127125	6.81E-003
enriched	negative regulation of vasoconstriction	2	0.127125	6.81E-003
enriched	morphogenesis of a polarized epithelium	2	0.129830	7.07E-003
enriched	regulation of leukocyte mediated cytotoxicity	2	0.129830	7.07E-003
enriched	cellular response to laminar fluid shear stress	2	0.135240	7.62E-003
enriched	negative regulation of blood vessel endothelial cell migration	2	0.135240	7.62E-003
enriched	necroptotic signaling pathway	2	0.137944	7.86E-003
enriched	negative regulation of protein complex disassembly	2	0.137944	7.86E-003
enriched	positive regulation of adherens junction organization	2	0.137944	7.86E-003
enriched	regulation of behavioral fear response	2	0.143354	8.46E-003
enriched	antibacterial humoral response	2	0.148764	9.02E-003
enriched	positive regulation of interferon-gamma biosynthetic process	2	0.148764	9.02E-003
enriched	positive regulation of protein deacetylation	2	0.148764	9.02E-003
enriched	endothelial cell activation	2	0.151468	9.30E-003
enriched	positive regulation of axon extension involved in axon guidance	2	0.151468	9.30E-003
enriched	positive regulation of calcium ion transmembrane transporter activity	2	0.154173	9.58E-003
enriched	regulation of JNK cascade	2	0.154173	9.58E-003
enriched	regulation of bone mineralization	2	0.156878	9.89E-003
enriched	positive regulation of behavior	2	0.159583	1.02E-002
enriched	regulation of hormone biosynthetic process	2	0.159583	1.02E-002
enriched	positive regulation of type 2 immune response	2	0.162287	1.04E-002
enriched	regulation of bile acid metabolic process	2	0.162287	1.04E-002
enriched	response to epidermal growth factor	2	0.162287	1.04E-002
enriched	regulation of neurotransmitter receptor activity	2	0.164992	1.07E-002
enriched	response to ether	2	0.164992	1.07E-002
enriched	catecholamine biosynthetic process	2	0.167697	1.11E-002
enriched	membrane hyperpolarization	2	0.170402	1.14E-002
enriched	cell migration involved in endocardial cushion formation	2	0.181221	1.28E-002
enriched	regulation of appetite	2	0.183926	1.31E-002

Type, regulation direction; Name, Annotation name; Hits, number of miRNAs mediating this annotation; Expected score, expected number of hits; *q*-value, adjusted *p*-value.

Note. This table illustrates the results of hsa-miR-101-3p, hsa-miR-15a-5p, hsa-miR-18a-5p, hsa-miR-374a-5p, hsa-miR-96-3p, hsa-miR-144-3p, and hsa-miR-424-5p uploaded to GeneTrail 3 - miRNomics (Saarland University, Saarbrücken, Germany; https://genetrail.bioinf.uni-sb.de/). Relevant Gene Ontology (GO; http://geneontology.org/) annotations were identified using experimentally strong verified targets from miRTarBase 9.0.

Table C2: Enriched Gene Ontology Molecular Function Annotation in young athletes

Type	Name	Hits	Expected score	*q*-value
enriched	DNA binding	4	0.086553	7.51E-006
enriched	RNA cap binding	4	0.089258	7.51E-006

enriched	RNA helicase activity	4	0.094668	7.51E-006
enriched	RNA polymerase II CTD heptapeptide repeat kinase activity	4	0.094668	7.51E-006
enriched	protease binding	4	0.097373	7.51E-006
enriched	decanoate-CoA ligase activity	4	0.110896	7.88E-006
enriched	protein tyrosine kinase binding	4	0.113601	7.88E-006
enriched	signaling adaptor activity	4	0.110896	7.88E-006
enriched	signaling pattern recognition receptor activity	4	0.110896	7.88E-006
enriched	co-SMAD binding	3	0.037867	1.53E-005
enriched	neurotransmitter transporter activity	3	0.054096	4.31E-005
enriched	nuclear receptor binding	3	0.059505	5.33E-005
enriched	DBD domain binding	3	0.073029	9.29E-005
enriched	bending	3	0.091963	1.75E-004
enriched	DNA helicase activity	3	0.100077	2.11E-004
enriched	sialyltransferase activity	3	0.102782	2.15E-004
enriched	extracellular matrix constituent conferring elasticity	2	0.018934	2.22E-004
enriched	phosphatidylinositol-3	2	0.018934	2.22E-004
enriched	phosphoprotein binding	3	0.113601	2.22E-004
enriched	protein tyrosine kinase activity	3	0.108192	2.22E-004
enriched	signaling receptor activator activity	3	0.108192	2.22E-004
enriched	phosphatidylinositol 3-kinase activity	3	0.116306	2.27E-004
enriched	NF-kappaB binding	2	0.021638	2.45E-004
enriched	poly(G) binding	3	0.124420	2.45E-004
enriched	serine-type peptidase activity	2	0.021638	2.45E-004
enriched	ion channel activity	3	0.127125	2.52E-004
enriched	sodium ion transmembrane transporter activity	2	0.024343	2.90E-004
enriched	sodium channel activity	2	0.043277	9.24E-004
enriched	phosphatidylinositol-4-phosphate binding	2	0.054096	1.41E-003
enriched	Rho GTPase binding	2	0.056801	1.50E-003
enriched	hyaluronic acid binding	2	0.059505	1.59E-003
enriched	phosphatidylserine binding	2	0.062210	1.69E-003
enriched	endonuclease activity	2	0.081144	2.79E-003
enriched	core promoter binding	2	0.113601	5.13E-003
enriched	core promoter sequence-specific DNA binding	2	0.113601	5.13E-003

Type, regulation direction; Name, Annotation name; Hits, number of miRNAs mediating this annotation; Expected score, expected number of hits; q-value, adjusted *p*-value.

Note. This table illustrates the results of hsa-miR-101-3p, hsa-miR-15a-5p, hsa-miR-18a-5p, hsa-miR-374a-5p, hsa-miR-96-3p, hsa-miR-144-3p, and hsa-miR-424-5p uploaded to GeneTrail 3 - miRNomics (Saarland University, Saarbrücken, Germany; https://genetrail.bioinf.uni-sb.de/). Relevant Gene Ontology (GO; http://geneontology.org/) annotations were identified using experimentally strong verified targets from miRTarBase 9.0.

Table C3: Enriched Gene Ontology Cellular Components Annotation in young athletes

Type	Name	Hits	Expected score	*q*-value
enriched	nuclear chromosome	3	0.048686	4.57E-005
enriched	nuclear outer membrane-endoplasmic reticulum membrane network	3	0.059505	4.57E-005
enriched	nuclear periphery	2	0.027048	4.66E-004

enriched	intracellular component	2	0.070325	1.98E-003
enriched	sarcoplasmic reticulum	2	0.070325	1.98E-003

Type, regulation direction; Name, Annotation name; Hits, number of miRNAs mediating this annotation; Expected score, expected number of hits; *q*-value, adjusted *p*-value.

Note. This table illustrates the results of hsa-miR-101-3p, hsa-miR-15a-5p, hsa-miR-18a-5p, hsa-miR-374a-5p, hsa-miR-96-3p, hsa-miR-144-3p, and hsa-miR-424-5p uploaded to GeneTrail 3 - miRNomics (Saarland University, Saarbrücken, Germany; https://genetrail.bioinf.uni-sb.de/). Relevant Gene Ontology (GO; http://geneontology.org/) annotations were identified using experimentally strong verified targets from miRTarBase 9.0.

Table C4: Enriched Reactome pathways in young athletes

Type	Name	Hits	Expected score	*q*-value
enriched	Telomere Maintenance	5	0.173107	9.43E-006
enriched	GRB2:SOS provides linkage to MAPK signaling for Integrins	5	0.319165	7.26E-005
enriched	Pyruvate metabolism and Citric Acid (TCA) cycle	5	0.321870	7.26E-005
enriched	Sphingolipid de novo biosynthesis	3	0.054096	2.00E-004
enriched	FGFR2c ligand binding and activation	3	0.067620	3.21E-004
enriched	Signal attenuation	4	0.227202	3.30E-004
enriched	Activation of APC/C and APC/C:Cdc20 mediated degradation of mitotic proteins	2	0.013524	3.69E-004
enriched	Toll Like Receptor 7/8 (TLR7/8) Cascade	2	0.013524	3.69E-004
enriched	Transport of small molecules	2	0.013524	3.69E-004
enriched	Transport to the Golgi and subsequent modification	2	0.013524	3.69E-004
enriched	Toll Like Receptor 5 (TLR5) Cascade	3	0.091963	3.75E-004
enriched	Cellular response to heat stress	2	0.016229	3.95E-004
enriched	FOXO-mediated transcription	2	0.016229	3.95E-004
enriched	Phase 0 - rapid depolarisation	2	0.016229	3.95E-004
enriched	Defective Mismatch Repair Associated With MSH6	3	0.116306	5.27E-004
enriched	GRB2 events in EGFR signaling	4	0.327280	5.27E-004
enriched	G beta:gamma signalling through CDC42	2	0.024343	7.77E-004
enriched	G-protein beta:gamma signalling	2	0.027048	8.68E-004
enriched	Other semaphorin interactions	3	0.143354	8.68E-004
enriched	Interleukin-20 family signaling	3	0.159583	1.09E-003
enriched	Constitutive Signaling by Ligand-Responsive EGFR Cancer Variants	2	0.032458	1.10E-003
enriched	Regulation of RAS by GAPs	2	0.032458	1.10E-003
enriched	PCNA-Dependent Long Patch Base Excision Repair	3	0.173107	1.21E-003
enriched	AKT phosphorylates targets in the nucleus	2	0.037867	1.33E-003
enriched	recycling	2	0.037867	1.33E-003
enriched	G-protein activation	2	0.040572	1.47E-003
enriched	Diseases of glycosylation	2	0.043277	1.56E-003
enriched	PI3K/AKT Signaling in Cancer	2	0.043277	1.56E-003
enriched	AKT phosphorylates targets in the cytosol	2	0.048686	1.85E-003
enriched	Rho GTPase cycle	2	0.048686	1.85E-003
enriched	Costimulation by the CD28 family	3	0.221793	1.86E-003
enriched	Interleukin-10 signaling	3	0.238022	2.22E-003
enriched	RNA Polymerase I Promoter Clearance	2	0.056801	2.30E-003

enriched	Diseases associated with O-glycosylation of proteins	3	0.246136	2.31E-003
enriched	Pentose phosphate pathway	2	0.059505	2.38E-003
enriched	Ion homeostasis	2	0.062210	2.46E-003
enriched	Signaling by MET	2	0.062210	2.46E-003
enriched	G alpha (s) signalling events	2	0.067620	2.84E-003
enriched	Caspase activation via Death Receptors in the presence of ligand	2	0.070325	2.92E-003
enriched	Ras activation upon Ca2+ influx through NMDA receptor	2	0.070325	2.92E-003
enriched	Phospholipase C-mediated cascade; FGFR3	2	0.073029	2.93E-003
enriched	Regulation of the apoptosome activity	2	0.073029	2.93E-003
enriched	XIAP-regulated apoptotic response	3	0.286708	2.93E-003
enriched	Macroautophagy	2	0.078439	3.30E-003
enriched	Clathrin-mediated endocytosis	2	0.081144	3.38E-003
enriched	Elastic fibre formation	2	0.081144	3.38E-003
enriched	TRIF-mediated programmed cell death	2	0.083849	3.53E-003
enriched	FGFR2 mutant receptor activation	2	0.102782	5.17E-003
enriched	Constitutive Signaling by NOTCH1 HD+PEST Domain Mutants	2	0.116306	6.34E-003
enriched	G alpha (q) signalling events	2	0.116306	6.34E-003
enriched	Regulation of necroptotic cell death	2	0.124420	7.10E-003
enriched	Energy dependent regulation of mTOR by LKB1-AMPK	2	0.132535	7.88E-003
enriched	Constitutive Signaling by NOTCH1 HD Domain Mutants	2	0.135240	7.89E-003
enriched	G alpha (z) signalling events	2	0.135240	7.89E-003
enriched	Cell junction organization	2	0.137944	7.91E-003
enriched	Constitutive Signaling by NOTCH1 PEST Domain Mutants	2	0.137944	7.91E-003
enriched	Cargo recognition for clathrin-mediated endocytosis	2	0.143354	8.38E-003
enriched	Caspase activation via Dependence Receptors in the absence of ligand	2	0.154173	9.48E-003
enriched	Metabolism of water-soluble vitamins and cofactors	2	0.178516	1.24E-002

Type, regulation direction; Name, Annotation name; Hits, number of miRNAs mediating this annotation; Expected score, expected number of hits; *q*-value, adjusted *p*-value.

Note. This table illustrates the results of hsa-miR-101-3p, hsa-miR-15a-5p, hsa-miR-18a-5p, hsa-miR-374a-5p, hsa-miR-96-3p, hsa-miR-144-3p, and hsa-miR-424-5p uploaded to GeneTrail 3 - miRNomics (Saarland University, Saarbrücken, Germany; https://genetrail.bioinf.uni-sb.de/). Reactome (https://reactome.org/) pathways were identified using experimentally strong verified targets from miRTarBase 9.0.

Table C5:Enriched KEGG Pathway Analysis in young athletes

Type	Name	Hits	Expected score	*q*-value
enriched	Metabolism of xenobiotics by cytochrome P450	6	0.486862	1.66E-005
enriched	Cell adhesion molecules (CAMs)	4	0.229907	2.82E-004
enriched	Pathogenic Escherichia coli infection	5	0.478748	2.82E-004
enriched	Cocaine addiction	4	0.270479	3.23E-004
enriched	HIF-1 signaling pathway	4	0.265070	3.23E-004

enriched	Focal adhesion	4	0.321870	5.35E-004
enriched	Herpes simplex infection	3	0.129830	6.82E-004
enriched	Toxoplasmosis	3	0.167697	1.28E-003
enriched	mTOR signaling pathway	3	0.194745	1.78E-003
enriched	Cholinergic synapse	3	0.286708	4.98E-003
enriched	PPAR signaling pathway	3	0.313756	5.87E-003
enriched	TNF signaling pathway	2	0.097373	7.56E-003
enriched	Oxidative phosphorylation	3	0.373261	7.60E-003
enriched	Viral myocarditis	2	0.102782	7.60E-003
enriched	Adherens junction	2	0.127125	9.60E-003
enriched	Regulation of autophagy	2	0.124420	9.60E-003
enriched	Maturity onset diabetes of the young	2	0.162287	1.45E-002
enriched	Acute myeloid leukemia	2	0.170402	1.51E-002
enriched	Vibrio cholerae infection	2	0.197450	1.90E-002
enriched	Fc gamma R-mediated phagocytosis	2	0.251546	2.86E-002
enriched	Melanogenesis	2	0.292117	3.61E-002
enriched	Propanoate metabolism	2	0.343509	4.45E-002
enriched	Protein processing in endoplasmic reticulum	2	0.343509	4.45E-002
enriched	Hematopoietic cell lineage	2	0.362442	4.71E-002

Type, regulation direction; Name, Annotation name; Hits, number of miRNAs mediating this annotation; Expected score, expected number of hits; *q*-value, adjusted *p*-value.

Note. This table illustrates the results of hsa-miR-101-3p, hsa-miR-15a-5p, hsa-miR-18a-5p, hsa-miR-374a-5p, hsa-miR-96-3p, hsa-miR-144-3p, and hsa-miR-424-5p uploaded to GeneTrail 3 - miRNomics (Saarland University, Saarbrücken, Germany; https://genetrail.bioinf.uni-sb.de/). Relevant Kyoto Encyclopedia of Genes and Genomes (KEGG; https://www.genome.jp/kegg/brite.html) pathways were identified using experimentally strong verified targets from miRTarBase 9.0.

Table C6: Enriched Wikipathways pathways in young athletes

Type	Name	Hits	Expected score	*q*-value
enriched	Cells and Molecules involved in local acute inflammatory response	6	0.297527	2.06E-006
enriched	Cytosine methylation	6	0.348918	2.18E-006
enriched	Oxidation by Cytochrome P450	6	0.359737	2.18E-006
enriched	Inflammatory Response Pathway	6	0.381376	2.33E-006
enriched	Glucuronidation	4	0.081144	5.92E-006
enriched	HIF1A and PPARG regulation of glycolysis	5	0.232612	7.08E-006
enriched	Selective expression of chemokine receptors during T-cell polarization	5	0.354328	5.03E-005
enriched	AMP-activated Protein Kinase (AMPK) Signaling	3	0.062210	1.38E-004
enriched	Electron Transport Chain (OXPHOS system in mitochondria)	3	0.062210	1.38E-004
enriched	Cardiac Hypertrophic Response	4	0.232612	2.07E-004
enriched	Sudden Infant Death Syndrome (SIDS) Susceptibility Pathways	4	0.235317	2.07E-004
enriched	Amplification and Expansion of Oncogenic Pathways as Metastatic Traits	4	0.251546	2.25E-004
enriched	Endochondral Ossification	4	0.259660	2.25E-004
enriched	Energy Metabolism	4	0.259660	2.25E-004

enriched	Mammary gland development pathway - Involution (Stage 4 of 4)	3	0.081144	2.25E-004
enriched	Tumor suppressor activity of SMARCB1	3	0.094668	2.82E-004
enriched	H19 action Rb-E2F1 signaling and CDK-Beta-catenin activity	3	0.119011	5.31E-004
enriched	Bone Morphogenic Protein (BMP) Signalling and Regulation	4	0.354328	5.87E-004
enriched	Constitutive Androstane Receptor Pathway	3	0.129830	5.87E-004
enriched	Sleep regulation	3	0.129830	5.87E-004
enriched	Structural Pathway of Interleukin 1 (IL-1)	3	0.132535	5.95E-004
enriched	Nuclear Receptors Meta-Pathway	2	0.024343	6.00E-004
enriched	Wnt Signaling Pathway and Pluripotency	3	0.140649	6.49E-004
enriched	Serotonin and anxiety	3	0.156878	8.63E-004
enriched	Wnt Signaling	3	0.167697	1.01E-003
enriched	Ethanol metabolism resulting in production of ROS by CYP2E1	3	0.181221	1.22E-003
enriched	Follicle Stimulating Hormone (FSH) signaling pathway	3	0.189335	1.34E-003
enriched	Glutathione metabolism	2	0.043277	1.50E-003
enriched	Vitamins A and D - action mechanisms	3	0.200155	1.50E-003
enriched	Cytoplasmic Ribosomal Proteins	3	0.205564	1.54E-003
enriched	Inhibition of exosome biogenesis and secretion by Manumycin A in CRPC cells	3	0.235317	2.22E-003
enriched	Resistin as a regulator of inflammation	3	0.270479	3.23E-003
enriched	Adipogenesis	2	0.070325	3.24E-003
enriched	Canonical and Non-canonical Notch signaling	2	0.070325	3.24E-003
enriched	MET in type 1 papillary renal cell carcinoma	2	0.067620	3.24E-003
enriched	Type III interferon signaling	2	0.070325	3.24E-003
enriched	Glycogen Synthesis and Degradation	2	0.078439	3.92E-003
enriched	miR-222 in Exercise-Induced Cardiac Growth	2	0.086553	4.65E-003
enriched	Selenium Metabolism and Selenoproteins	2	0.089258	4.81E-003
enriched	T-Cell Receptor and Co-stimulatory Signaling	2	0.091963	4.98E-003
enriched	Endothelin Pathways	2	0.094668	5.02E-003
enriched	Parkin-Ubiquitin Proteasomal System pathway	2	0.094668	5.02E-003
enriched	IL1 and megakaryocytes in obesity	2	0.097373	5.07E-003
enriched	Sterol Regulatory Element-Binding Proteins (SREBP) signalling	2	0.097373	5.07E-003
enriched	LTF danger signal response pathway	2	0.108192	6.11E-003
enriched	One Carbon Metabolism	2	0.113601	6.53E-003
enriched	One carbon metabolism and related pathways	3	0.394900	6.53E-003
enriched	ATM Signaling Network in Development and Disease	2	0.119011	6.91E-003
enriched	LncRNA-mediated mechanisms of therapeutic resistance	2	0.124420	7.39E-003
enriched	Extracellular vesicles in the crosstalk of cardiac cells	2	0.127125	7.40E-003
enriched	Liver X Receptor Pathway	2	0.127125	7.40E-003
enriched	EPO Receptor Signaling	2	0.140649	8.68E-003
enriched	PI3K-Akt Signaling Pathway	2	0.140649	8.68E-003
enriched	Physiological and Pathological Hypertrophy of the Heart	2	0.167697	1.20E-002
enriched	RAC1/PAK1/p38/MMP2 Pathway	2	0.170402	1.21E-002
enriched	Interleukin-1 Induced Activation of NF-kappa-B	2	0.186631	1.42E-002
enriched	Envelope proteins and their potential roles in EDMD physiopathology	2	0.216383	1.85E-002
enriched	NRF2-ARE regulation	2	0.229907	2.05E-002
enriched	Fibrin Complement Receptor 3 Signaling Pathway	2	0.267774	2.68E-002

Type, regulation direction; Name, Annotation name; Hits, number of miRNAs mediating this annotation; Expected score, expected number of hits; *q*-value, adjusted *p*-value.

Note. This table illustrates the results of hsa-miR-101-3p, hsa-miR-15a-5p, hsa-miR-18a-5p, hsa-miR-374a-5p, hsa-miR-96-3p, hsa-miR-144-3p, and hsa-miR-424-5p uploaded to GeneTrail 3 - miRNomics (Saarland University, Saarbrücken, Germany; https://genetrail.bioinf.uni-sb.de/). Relevant Wikipathways (https://www.wikipathways.org) pathways were identified using experimentally strong verified targets from miRTarBase 9.0.

Table C7: Enriched Gene Ontology Biological Process Annotation in master athletes

Type	Name	Hits	Expected score	*q*-value
enriched	growth plate cartilage development	2	0.019706	1.74E-003
enriched	negative regulation of cold-induced thermogenesis	2	0.019706	1.74E-003
enriched	negative regulation of protein transport	2	0.009274	1.74E-003
enriched	otic vesicle morphogenesis	2	0.028980	1.74E-003
enriched	positive regulation of monooxygenase activity	2	0.025502	1.74E-003
enriched	positive regulation of regulated secretory pathway	2	0.023184	1.74E-003
enriched	regulation of bicellular tight junction assembly	2	0.023184	1.74E-003
enriched	regulation of mRNA 3'-end processing	2	0.012751	1.74E-003
enriched	regulation of monocyte differentiation	2	0.026662	1.74E-003
enriched	regulation of phospholipid biosynthetic process	2	0.031298	1.74E-003
enriched	regulation of protein targeting	2	0.018547	1.74E-003
enriched	regulation of type I interferon-mediated signaling pathway	2	0.031298	1.74E-003
enriched	response to growth hormone	2	0.031298	1.74E-003
enriched	skeletal system development	2	0.026662	1.74E-003
enriched	protein localization to organelle	2	0.032458	1.75E-003
enriched	NADH metabolic process	2	0.055641	1.76E-003
enriched	acyl-CoA metabolic process	2	0.052164	1.76E-003
enriched	axon extension involved in axon guidance	2	0.049845	1.76E-003
enriched	cartilage condensation	2	0.054482	1.76E-003
enriched	cellular detoxification	2	0.057960	1.76E-003
enriched	cellular extravasation	2	0.060278	1.76E-003
enriched	detection of chemical stimulus	2	0.054482	1.76E-003
enriched	fibroblast migration	2	0.056801	1.76E-003
enriched	hormone secretion	2	0.049845	1.76E-003
enriched	lymphocyte migration into lymphoid organs	2	0.048686	1.76E-003
enriched	pancreas development	2	0.057960	1.76E-003
enriched	positive chemotaxis	2	0.049845	1.76E-003
enriched	positive regulation of calcium ion import	2	0.052164	1.76E-003
enriched	positive regulation of interleukin-17 production	2	0.054482	1.76E-003
enriched	positive regulation of metabolic process	2	0.049845	1.76E-003
enriched	positive regulation of receptor signaling pathway via JAK-STAT	2	0.047527	1.76E-003
enriched	positive regulation of response to wounding	2	0.049845	1.76E-003
enriched	protein acetylation	2	0.045209	1.76E-003
enriched	protein catabolic process in the vacuole	2	0.060278	1.76E-003
enriched	receptor clustering	2	0.051005	1.76E-003

enriched	receptor internalization	2	0.060278	1.76E-003
enriched	regulation of angiogenesis	2	0.049845	1.76E-003
enriched	regulation of astrocyte differentiation	2	0.056801	1.76E-003
enriched	regulation of hematopoietic stem cell differentiation	2	0.047527	1.76E-003
enriched	regulation of intracellular steroid hormone receptor signaling pathway	2	0.056801	1.76E-003
enriched	regulation of leukocyte migration	2	0.051005	1.76E-003
enriched	regulation of macrophage chemotaxis	2	0.044050	1.76E-003
enriched	regulation of microtubule polymerization or depolymerization	2	0.051005	1.76E-003
enriched	regulation of ossification	2	0.049845	1.76E-003
enriched	regulation of protein catabolic process	2	0.051005	1.76E-003
enriched	regulation of protein complex disassembly	2	0.047527	1.76E-003
enriched	regulation of secretion	2	0.059119	1.76E-003
enriched	regulation of skeletal muscle cell differentiation	2	0.051005	1.76E-003
enriched	regulation of wound healing	2	0.047527	1.76E-003
enriched	response to fatty acid	2	0.040572	1.76E-003
enriched	signal transduction involved in DNA damage checkpoint	2	0.039413	1.76E-003
enriched	striated muscle tissue development	2	0.056801	1.76E-003
enriched	regulation of calcium ion transport into cytosol	2	0.061437	1.76E-003
enriched	regulation of endothelial cell migration	2	0.061437	1.76E-003
enriched	negative regulation of blood vessel diameter	2	0.062597	1.79E-003
enriched	cellular amino acid catabolic process	2	0.063756	1.83E-003
enriched	positive regulation of cytosolic calcium ion concentration involved in phospholipase C-activating G protein-coupled signaling pathway	2	0.068393	1.90E-003
enriched	regulation of cGMP-mediated signaling	2	0.067233	1.90E-003
enriched	regulation of dendrite development	2	0.066074	1.90E-003
enriched	regulation of natural killer cell activation	2	0.068393	1.90E-003
enriched	regulation of oxidative stress-induced neuron intrinsic apoptotic signaling pathway	2	0.067233	1.90E-003
enriched	stress-activated protein kinase signaling cascade	2	0.068393	1.90E-003
enriched	regulation of miRNA metabolic process	2	0.070711	1.94E-003
enriched	regulation of natural killer cell differentiation	2	0.070711	1.94E-003
enriched	regulation of p38MAPK cascade	2	0.070711	1.94E-003
enriched	positive regulation of canonical Wnt signaling pathway	2	0.071870	1.97E-003
enriched	positive regulation of pathway-restricted SMAD protein phosphorylation	2	0.074189	2.04E-003
enriched	protein demethylation	2	0.074189	2.04E-003
enriched	response to ethanol	2	0.075348	2.07E-003
enriched	positive regulation of p38MAPK cascade	2	0.076507	2.11E-003
enriched	peptide transport	2	0.077666	2.14E-003
enriched	positive regulation of ion transmembrane transporter activity	2	0.081144	2.30E-003
enriched	cellular response to ionizing radiation	2	0.083462	2.37E-003
enriched	positive regulation of calcium ion transport into cytosol	2	0.083462	2.37E-003
enriched	regulation of autophagy of mitochondrion	2	0.084621	2.40E-003
enriched	positive regulation of behavioral fear response	2	0.102009	3.40E-003
enriched	regulation of binding	2	0.102009	3.40E-003
enriched	cell differentiation	2	0.106646	3.66E-003

Type, regulation direction; Name, Annotation name; Hits, number of miRNAs mediating this annotation; Expected score, expected number of hits; *q*-value, adjusted *p*-value.

Note. This table illustrates the results of hsa-miR-210-3p, hsa-miR-15a-5p, and hsa-miR-144-3p uploaded to GeneTrail 3 - miRNomics (Saarland University, Saarbrücken, Germany; https://genetrail.bioinf.uni-sb.de/). Relevant Gene Ontology (GO; http://geneontology.org/) annotations were identified using experimentally strong verified targets from miRTarBase 9.0.

Table C8: Enriched Gene Ontology Molecular Function Annotation in master athletes

Type	Name	Hits	Expected score	*q*-value
enriched	ion channel activity	2	0.054482	9.58E-004
enriched	phosphatidylinositol 3-kinase activity	2	0.049845	9.58E-004
enriched	phosphoprotein binding	2	0.048686	9.58E-004

Type, regulation direction; Name, Annotation name; Hits, number of miRNAs mediating this annotation; Expected score, expected number of hits; *q*-value, adjusted *p*-value.

Note. This table illustrates the results of hsa-miR-210-3p, hsa-miR-15a-5p, and hsa-miR-144-3p uploaded to GeneTrail 3 - miRNomics (Saarland University, Saarbrücken, Germany; https://genetrail.bioinf.uni-sb.de/). Relevant Gene Ontology (GO; http://geneontology.org/) annotations were identified using experimentally strong verified targets from miRTarBase 9.0.

Table C9: Enriched Gene Ontology Cellular Component Annotation in master athletes

Type	Name	Hits	Expected score	*q*-value

No significant categories have been found

Type, regulation direction; Name, Annotation name; Hits, number of miRNAs mediating this annotation; Expected score, expected number of hits; q-value, adjusted *p*-value.

Note. This table illustrates the results of hsa-miR-210-3p, hsa-miR-15a-5p, and hsa-miR-144-3p uploaded to GeneTrail 3 - miRNomics (Saarland University, Saarbrücken, Germany; https://genetrail.bioinf.uni-sb.de/). Relevant Gene Ontology (GO; http://geneontology.org/) annotations were identified using experimentally strong verified targets from miRTarBase 9.0.

Table C10: Enriched Reactome pathways in master athletes

Type	Name	Hits	Expected score	q-value
enriched	Interleukin-20 family signaling	2	0.068393	2.67E-003
enriched	Other semaphorin interactions	2	0.061438	2.67E-003
enriched	PCNA-Dependent Long Patch Base Excision Repair	2	0.074189	2.67E-003
enriched	Telomere Maintenance	2	0.074189	2.67E-003
enriched	Costimulation by the CD28 family	2	0.095054	3.06E-003
enriched	Signal attenuation	2	0.097373	3.06E-003

Type, regulation direction; Name, Annotation name; Hits, number of miRNAs mediating this annotation; Expected score, expected number of hits; *q*-value, adjusted *p*-value.

Note. This table illustrates the results of hsa-miR-210-3p, hsa-miR-15a-5p, and hsa-miR-144-3p uploaded to GeneTrail 3 - miRNomics (Saarland University, Saarbrücken, Germany; https://genetrail.bioinf.uni-sb.de/). Reactome (https://reactome.org/) pathways were identified using experimentally strong verified targets from miRTarBase 9.0.

Table C11: Enriched KEGG Pathway Analysis in master athletes

Type	Name	Hits	Expected score	*q*-value
enriched	Focal adhesion	2	0.137944	1.38E-002
enriched	Metabolism of xenobiotics by cytochrome P450	2	0.208655	1.38E-002
enriched	Pathogenic Escherichia coli infection	2	0.205178	1.38E-002

Type, regulation direction; Name, Annotation name; Hits, number of miRNAs mediating this annotation; Expected score, expected number of hits; *q*-value, adjusted *p*-value.

Note. This table illustrates the results of hsa-miR-210-3p, hsa-miR-15a-5p, and hsa-miR-144-3p uploaded to GeneTrail 3 - miRNomics (Saarland University, Saarbrücken, Germany; https://genetrail.bioinf.uni-sb.de/). Relevant Kyoto Encyclopedia of Genes and Genomes (KEGG; https://www.genome.jp/kegg/brite.html) pathways were identified using experimentally strong verified targets from miRTarBase 9.0.

Table C12: Enriched Wikipathways pathways in master athletes

Type	Name	Hits	Expected score	*q*-value
enriched	Mammary gland development pathway - Involution (Stage 4 of 4)	2	0.034776	3.48E-003
enriched	Constitutive Androstane Receptor Pathway	2	0.055641	4.50E-003
enriched	Cells and Molecules involved in local acute inflammatory response	2	0.127512	8.53E-003
enriched	Cytosine methylation	2	0.149536	8.53E-003
enriched	HIF1A and PPARG regulation of glycolysis	2	0.099691	8.53E-003
enriched	Inflammatory Response Pathway	2	0.163447	8.53E-003
enriched	Oxidation by Cytochrome P450	2	0.154173	8.53E-003
enriched	Resistin as a regulator of inflammation	2	0.115920	8.53E-003
enriched	Selective expression of chemokine receptors during T-cell polarization	2	0.151855	8.53E-003

Type, regulation direction; Name, Annotation name; Hits, number of miRNAs mediating this annotation; Expected score, expected number of hits; *q*-value, adjusted *p*-value.

Note. This table illustrates the results of hsa-miR-210-3p, hsa-miR-15a-5p, and hsa-miR-144-3p uploaded to GeneTrail 3 - miRNomics (Saarland University, Saarbrücken, Germany; https://genetrail.bioinf.uni-sb.de/). Relevant Wikipathways (https://www.wikipathways.org) pathways were identified using experimentally strong verified targets from miRTarBase 9.0.

Table C13: Enriched Gene Ontology Biological Process Annotation at baseline

Type	Name	Hits	Expected score	*q*-value
enriched	cellular extravasation	6	0.140649	6.00E-008
enriched	pancreas development	6	0.135240	6.00E-008
enriched	protein catabolic process in the vacuole	6	0.140649	6.00E-008
enriched	peptide transport	6	0.181221	2.19E-007
enriched	detection of mechanical stimulus	5	0.100077	9.20E-007
enriched	fibrinolysis	4	0.040572	9.20E-007
enriched	hormone secretion	5	0.116306	9.20E-007
enriched	lymphocyte migration into lymphoid organs	5	0.113601	9.20E-007
enriched	positive chemotaxis	5	0.116306	9.20E-007
enriched	positive regulation of calcium ion import	5	0.121716	9.20E-007
enriched	positive regulation of metabolic process	5	0.116306	9.20E-007
enriched	positive regulation of response to wounding	5	0.116306	9.20E-007
enriched	receptor clustering	5	0.119011	9.20E-007
enriched	regulation of microtubule polymerization or depolymerization	5	0.119011	9.20E-007
enriched	regulation of skeletal muscle cell differentiation	5	0.119011	9.20E-007
enriched	dendritic spine organization	5	0.127125	9.61E-007
enriched	detection of chemical stimulus	5	0.127125	9.61E-007
enriched	positive regulation of interleukin-17 production	5	0.127125	9.61E-007
enriched	NADH metabolic process	5	0.129830	1.02E-006
enriched	striated muscle tissue development	5	0.132535	1.07E-006
enriched	regulation of secretion	5	0.137944	1.26E-006
enriched	receptor internalization	5	0.140649	1.31E-006
enriched	response to tumor necrosis factor	4	0.048686	1.31E-006
enriched	cellular amino acid catabolic process	5	0.148764	1.63E-006
enriched	regulation of bicellular tight junction assembly	4	0.054096	1.83E-006
enriched	regulation of dendrite development	5	0.154173	1.83E-006
enriched	forebrain neuron development	5	0.159583	1.87E-006
enriched	positive regulation of cytosolic calcium ion concentration involved in phospholipase C-activating G protein-coupled signaling pathway	5	0.159583	1.87E-006
enriched	regulation of natural killer cell activation	5	0.159583	1.87E-006
enriched	stress-activated protein kinase signaling cascade	5	0.159583	1.87E-006
enriched	macrophage activation involved in immune response	4	0.056801	1.90E-006
enriched	positive regulation of peptide hormone secretion	5	0.164992	1.90E-006
enriched	regulation of natural killer cell differentiation	5	0.164992	1.90E-006
enriched	regulation of p38MAPK cascade	5	0.164992	1.90E-006
enriched	somatic hypermutation of immunoglobulin genes	5	0.164992	1.90E-006
enriched	positive regulation of canonical Wnt signaling pathway	5	0.167697	2.01E-006
enriched	positive regulation of natural killer cell activation	5	0.170402	2.12E-006
enriched	modulation of chemical synaptic transmission	4	0.062210	2.17E-006
enriched	positive regulation of pathway-restricted SMAD protein phosphorylation	5	0.173107	2.17E-006
enriched	protein demethylation	5	0.173107	2.17E-006
enriched	response to ethanol	5	0.175811	2.25E-006
enriched	positive regulation of p38MAPK cascade	5	0.178516	2.37E-006
enriched	positive regulation of ion transmembrane transporter activity	5	0.189335	3.13E-006

enriched	cellular response to ionizing radiation	5	0.194745	3.45E-006
enriched	positive regulation of calcium ion transport into cytosol	5	0.194745	3.45E-006
enriched	regulation of autophagy of mitochondrion	5	0.197450	3.57E-006
enriched	regulation of phospholipid biosynthetic process	4	0.073029	3.57E-006
enriched	regulation of type I interferon-mediated signaling pathway	4	0.073029	3.57E-006
enriched	synaptic vesicle endocytosis	5	0.202859	3.90E-006
enriched	regulation of binding	5	0.238022	8.60E-006
enriched	actin polymerization or depolymerization	3	0.024343	1.04E-005
enriched	cell differentiation	5	0.248841	1.04E-005
enriched	regulation of macrophage chemotaxis	4	0.102782	1.35E-005
enriched	negative regulation of CD4-positive	4	0.105487	1.37E-005
enriched	negative regulation of mitotic metaphase/anaphase transition	4	0.105487	1.37E-005
enriched	negative regulation of smooth muscle cell apoptotic process	4	0.105487	1.37E-005
enriched	oocyte maturation	3	0.027048	1.37E-005
enriched	regulation of animal organ morphogenesis	4	0.105487	1.37E-005
enriched	positive regulation of receptor signaling pathway via JAK-STAT	4	0.110896	1.55E-005
enriched	regulation of protein complex disassembly	4	0.110896	1.55E-005
enriched	regulation of transcription by RNA polymerase II	4	0.110896	1.55E-005
enriched	regulation of transcription by RNA polymerase III	4	0.110896	1.55E-005
enriched	regulation of wound healing	4	0.110896	1.55E-005
enriched	regulation of blood pressure	3	0.029753	1.62E-005
enriched	senescence-associated heterochromatin focus assembly	3	0.029753	1.62E-005
enriched	axon extension involved in axon guidance	4	0.116306	1.75E-005
enriched	regulation of transcription by glucose	4	0.116306	1.75E-005
enriched	regulation of transcription from RNA polymerase II promoter in response to hypoxia	4	0.116306	1.75E-005
enriched	negative regulation of protein kinase B signaling	4	0.119011	1.89E-005
enriched	regulation of natural killer cell mediated immunity	3	0.032458	1.98E-005
enriched	sensory organ development	3	0.032458	1.98E-005
enriched	regulation of fever generation	4	0.121716	1.99E-005
enriched	cartilage condensation	4	0.127125	2.34E-005
enriched	Notch receptor processing	4	0.129830	2.42E-005
enriched	positive regulation of endothelial cell proliferation	3	0.035162	2.42E-005
enriched	regulation of meiotic cell cycle	3	0.035162	2.42E-005
enriched	response to calcium ion	4	0.129830	2.42E-005
enriched	fibroblast migration	4	0.132535	2.54E-005
enriched	regulation of astrocyte differentiation	4	0.132535	2.54E-005
enriched	regulation of intracellular steroid hormone receptor signaling pathway	4	0.132535	2.54E-005
enriched	cellular detoxification	4	0.135240	2.72E-005
enriched	T-helper 2 cell cytokine production	3	0.037867	2.74E-005
enriched	cation transport	4	0.137944	2.74E-005
enriched	macrophage derived foam cell differentiation	3	0.037867	2.74E-005
enriched	negative regulation of signal transduction by p53 class mediator	4	0.137944	2.74E-005
enriched	response to Thyroglobulin triiodothyronine	4	0.137944	2.74E-005

enriched	response to X-ray	4	0.137944	2.74E-005
enriched	positive regulation of aspartic-type endopeptidase activity involved in amyloid precursor protein catabolic process	4	0.140649	2.94E-005
enriched	regulation of calcium ion transport into cytosol	4	0.143354	3.10E-005
enriched	regulation of endothelial cell migration	4	0.143354	3.10E-005
enriched	regulation of vasoconstriction	3	0.040572	3.15E-005
enriched	smooth muscle cell migration	3	0.040572	3.15E-005
enriched	glycosaminoglycan metabolic process	4	0.148764	3.49E-005
enriched	embryonic eye morphogenesis	2	0.005410	3.55E-005
enriched	cardiac muscle cell contraction	3	0.043277	3.67E-005
enriched	modulation by host of viral transcription	4	0.151468	3.67E-005
enriched	positive regulation of execution phase of apoptosis	3	0.043277	3.67E-005
enriched	ammonium transport	4	0.154173	3.83E-005
enriched	regulation of cGMP-mediated signaling	4	0.156878	4.03E-005
enriched	regulation of oxidative stress-induced neuron intrinsic apoptotic signaling pathway	4	0.156878	4.03E-005
enriched	activation of innate immune response	3	0.045982	4.07E-005
enriched	cell death in response to hydrogen peroxide	3	0.045982	4.07E-005
enriched	growth plate cartilage development	3	0.045982	4.07E-005
enriched	negative regulation of cold-induced thermogenesis	3	0.045982	4.07E-005
enriched	regulation of branching involved in ureteric bud morphogenesis	3	0.045982	4.07E-005
enriched	ribonucleoprotein complex localization	3	0.045982	4.07E-005
enriched	caveolin-mediated endocytosis	4	0.162287	4.24E-005
enriched	membrane depolarization during action potential	4	0.162287	4.24E-005
enriched	via spliceosome	4	0.162287	4.24E-005
enriched	regulation of miRNA metabolic process	4	0.164992	4.49E-005
enriched	positive regulation of macrophage derived foam cell differentiation	3	0.048686	4.62E-005
enriched	regulation of mast cell activation	3	0.048686	4.62E-005
enriched	alternative mRNA splicing	4	0.167697	4.67E-005
enriched	ephrin receptor signaling pathway	4	0.173107	5.22E-005
enriched	negative regulation of cardiocyte differentiation	4	0.173107	5.22E-005
enriched	cell communication by electrical coupling	3	0.051391	5.25E-005
enriched	preincision complex stabilization	3	0.051391	5.25E-005
enriched	positive regulation of regulated secretory pathway	3	0.054096	6.06E-005
enriched	response to cobalt ion	3	0.054096	6.06E-005
enriched	cell projection organization	3	0.056801	6.95E-005
enriched	membrane repolarization	3	0.056801	6.95E-005
enriched	synaptic vesicle recycling	4	0.192040	7.47E-005
enriched	cellular process	2	0.008114	7.81E-005
enriched	monoamine transport	2	0.008114	7.81E-005
enriched	nervous system process	3	0.059505	7.81E-005
enriched	organelle assembly	3	0.059505	7.81E-005
enriched	positive regulation of cell proliferation involved in kidney development	2	0.008114	7.81E-005
enriched	triglyceride homeostasis	2	0.008114	7.81E-005
enriched	positive regulation of chondrocyte differentiation	3	0.062210	8.59E-005
enriched	synaptic vesicle exocytosis	3	0.062210	8.59E-005
enriched	regulation of bone development	3	0.064915	9.73E-005
enriched	activation of immune response	3	0.067620	1.06E-004

enriched	cell proliferation in bone marrow	3	0.067620	1.06E-004
enriched	cellular aromatic compound metabolic process	3	0.067620	1.06E-004
enriched	histone H3-K4 trimethylation	3	0.067620	1.06E-004
enriched	nephrogenic mesenchyme morphogenesis	3	0.067620	1.06E-004
enriched	positive regulation of chemokine-mediated signaling pathway	3	0.067620	1.06E-004
enriched	behavior	3	0.073029	1.32E-004
enriched	response to growth hormone	3	0.073029	1.32E-004
enriched	negative regulation of release of cytochrome c from mitochondria	2	0.010819	1.40E-004
enriched	protein deneddylation	2	0.010819	1.40E-004
enriched	regulation of circadian rhythm	2	0.010819	1.40E-004
enriched	regulation of glutamate secretion	2	0.010819	1.40E-004
enriched	neuron projection development	3	0.075734	1.41E-004
enriched	positive regulation of T cell activation	3	0.075734	1.41E-004
enriched	protein localization to organelle	3	0.075734	1.41E-004
enriched	positive regulation of behavioral fear response	4	0.238022	1.46E-004
enriched	extrinsic apoptotic signaling pathway via death domain receptors	3	0.078439	1.54E-004
enriched	positive regulation of chromosome organization	3	0.078439	1.54E-004
enriched	enzyme linked receptor protein signaling pathway	3	0.081144	1.66E-004
enriched	positive regulation of artery morphogenesis	3	0.081144	1.66E-004
enriched	positive regulation of cellular biosynthetic process	3	0.081144	1.66E-004
enriched	regulation of cellular response to insulin stimulus	3	0.081144	1.66E-004
enriched	glycoprotein metabolic process	3	0.083849	1.82E-004
enriched	heart trabecula morphogenesis	3	0.086553	1.98E-004
enriched	nephron development	3	0.086553	1.98E-004
enriched	Golgi organization	3	0.089258	2.00E-004
enriched	cellular response to acetylcholine	3	0.089258	2.00E-004
enriched	cellular response to estradiol stimulus	3	0.089258	2.00E-004
enriched	extracellular matrix organization	2	0.013524	2.00E-004
enriched	negative regulation of immune response	2	0.013524	2.00E-004
enriched	negative regulation of intracellular protein transport	2	0.013524	2.00E-004
enriched	negative regulation of long-chain fatty acid import across plasma membrane	2	0.013524	2.00E-004
enriched	neurotrophin TRK receptor signaling pathway	3	0.089258	2.00E-004
enriched	positive regulation of MAP kinase activity	2	0.013524	2.00E-004
enriched	positive regulation of dendritic cell differentiation	2	0.013524	2.00E-004
enriched	regulation of NMDA receptor activity	2	0.013524	2.00E-004
enriched	regulation of T cell apoptotic process	3	0.089258	2.00E-004
enriched	response to vitamin	3	0.089258	2.00E-004
enriched	tight junction organization	2	0.013524	2.00E-004
enriched	monocyte chemotaxis	3	0.091963	2.17E-004
enriched	skin development	3	0.091963	2.17E-004
enriched	morphogenesis of an epithelial sheet	3	0.094668	2.32E-004
enriched	negative regulation of bone remodeling	3	0.094668	2.32E-004
enriched	response to fatty acid	3	0.094668	2.32E-004
enriched	cellular response to steroid hormone stimulus	3	0.097373	2.49E-004
enriched	endochondral bone growth	3	0.097373	2.49E-004
enriched	modification of morphology or physiology of other organism	3	0.097373	2.49E-004
enriched	mRNA stabilization	2	0.016229	2.66E-004

enriched	membrane biogenesis	2	0.016229	2.66E-004
enriched	phosphatidylinositol phosphorylation	2	0.016229	2.66E-004
enriched	positive regulation of protein localization to nucleus	2	0.016229	2.66E-004
enriched	regulation of cell population proliferation	2	0.016229	2.66E-004
enriched	regulation of intrinsic apoptotic signaling pathway in response to DNA damage by p53 class mediator	2	0.016229	2.66E-004
enriched	response to cGMP	2	0.016229	2.66E-004
enriched	response to exogenous dsRNA	2	0.016229	2.66E-004
enriched	response to gravity	2	0.016229	2.66E-004
enriched	cartilage morphogenesis	3	0.102782	2.74E-004
enriched	cell communication by electrical coupling involved in cardiac conduction	3	0.102782	2.74E-004
enriched	cell recognition	3	0.102782	2.74E-004
enriched	cellular response to X-ray	3	0.102782	2.74E-004
enriched	leukotriene metabolic process	3	0.105487	2.93E-004
enriched	protein acetylation	3	0.105487	2.93E-004
enriched	negative regulation of transmembrane receptor protein serine/threonine kinase signaling pathway	3	0.108192	3.13E-004
enriched	regulation of chemotaxis	3	0.108192	3.13E-004
enriched	coronary vasculature development	3	0.110896	3.27E-004
enriched	gastrulation	3	0.110896	3.27E-004
enriched	positive regulation of helicase activity	3	0.110896	3.27E-004
enriched	regulation of cholesterol biosynthetic process	3	0.110896	3.27E-004
enriched	regulation of hematopoietic stem cell differentiation	3	0.110896	3.27E-004
enriched	response to estrogen	3	0.110896	3.27E-004
enriched	blood vessel endothelial cell differentiation	2	0.018934	3.31E-004
enriched	cell dedifferentiation	3	0.113601	3.31E-004
enriched	cellular glucose homeostasis	2	0.018934	3.31E-004
enriched	energy derivation by oxidation of organic compounds	3	0.113601	3.31E-004
enriched	epicardium-derived cardiac fibroblast cell development	3	0.113601	3.31E-004
enriched	extracellular structure organization	2	0.018934	3.31E-004
enriched	granulocyte migration	2	0.018934	3.31E-004
enriched	hair follicle morphogenesis	3	0.113601	3.31E-004
enriched	negative regulation of myotube differentiation	2	0.018934	3.31E-004
enriched	positive regulation of amyloid-beta formation	3	0.113601	3.31E-004
enriched	positive regulation of chromatin silencing	3	0.113601	3.31E-004
enriched	positive regulation of phospholipase C activity	2	0.018934	3.31E-004
enriched	positive regulation of protein polyubiquitination	2	0.018934	3.31E-004
enriched	positive regulation of hormone biosynthetic process	3	0.116306	3.47E-004
enriched	regulation of angiogenesis	3	0.116306	3.47E-004
enriched	regulation of ossification	3	0.116306	3.47E-004
enriched	regulation of viral transcription	3	0.116306	3.47E-004
enriched	spindle organization	3	0.116306	3.47E-004
enriched	positive regulation of organ growth	3	0.119011	3.65E-004
enriched	regulation of glycogen metabolic process	3	0.119011	3.65E-004
enriched	regulation of leukocyte migration	3	0.119011	3.65E-004
enriched	regulation of protein catabolic process	3	0.119011	3.65E-004
enriched	acyl-CoA metabolic process	3	0.121716	3.87E-004
enriched	endothelial cell-matrix adhesion	3	0.121716	3.87E-004
enriched	TNFSF11-mediated signaling pathway	2	0.021638	3.95E-004
enriched	camera-type eye development	2	0.021638	3.95E-004

enriched	hepatocyte differentiation	2	0.021638	3.95E-004
enriched	negative regulation of protein transport	2	0.021638	3.95E-004
enriched	positive regulation of extracellular matrix organization	2	0.021638	3.95E-004
enriched	positive regulation of macrophage migration	2	0.021638	3.95E-004
enriched	regulation of CD4-positive	2	0.021638	3.95E-004
enriched	regulation of aspartic-type endopeptidase activity involved in amyloid precursor protein catabolic process	2	0.021638	3.95E-004
enriched	regulation of mismatch repair	2	0.021638	3.95E-004
enriched	regulation of protein serine/threonine kinase activity	2	0.021638	3.95E-004
enriched	amide transport	3	0.124420	3.95E-004
enriched	focal adhesion assembly	3	0.127125	4.16E-004
enriched	negative regulation of lipid catabolic process	3	0.127125	4.16E-004
enriched	negative regulation of vasoconstriction	3	0.127125	4.16E-004
enriched	T-helper cell differentiation	2	0.024343	4.64E-004
enriched	aorta morphogenesis	2	0.024343	4.64E-004
enriched	cellular response to laminar fluid shear stress	3	0.135240	4.64E-004
enriched	cellular response to molecule of bacterial origin	2	0.024343	4.64E-004
enriched	entrainment of circadian clock	3	0.135240	4.64E-004
enriched	isoprenoid metabolic process	2	0.024343	4.64E-004
enriched	male gonad development	2	0.024343	4.64E-004
enriched	monocyte differentiation	3	0.132535	4.64E-004
enriched	negative regulation of astrocyte differentiation	2	0.024343	4.64E-004
enriched	negative regulation of cardiac muscle cell apoptotic process	3	0.132535	4.64E-004
enriched	negative regulation of endocytosis	3	0.132535	4.64E-004
enriched	negative regulation of lymphocyte proliferation	2	0.024343	4.64E-004
enriched	positive regulation of binding	2	0.024343	4.64E-004
enriched	positive regulation of cytoskeleton organization	2	0.024343	4.64E-004
enriched	positive regulation of dendrite morphogenesis	2	0.024343	4.64E-004
enriched	positive regulation of mast cell degranulation	2	0.024343	4.64E-004
enriched	positive regulation of melanocyte differentiation	2	0.024343	4.64E-004
enriched	regulation of protein import into nucleus	2	0.024343	4.64E-004
enriched	regulation of skeletal muscle satellite cell proliferation	2	0.024343	4.64E-004
enriched	negative regulation of protein complex disassembly	3	0.137944	4.89E-004
enriched	positive regulation of adherens junction organization	3	0.137944	4.89E-004
enriched	ERBB signaling pathway	3	0.140649	5.14E-004
enriched	regulation of attachment of spindle microtubules to kinetochore	3	0.140649	5.14E-004
enriched	regulation of behavioral fear response	3	0.143354	5.42E-004
enriched	cell-substrate junction assembly	2	0.027048	5.46E-004
enriched	cellular response to interleukin-1	2	0.027048	5.46E-004
enriched	leukocyte proliferation	2	0.027048	5.46E-004
enriched	planar cell polarity pathway	2	0.027048	5.46E-004
enriched	regulation of establishment of endothelial barrier	2	0.027048	5.46E-004
enriched	regulation of low-density lipoprotein particle receptor biosynthetic process	2	0.027048	5.46E-004
enriched	regulation of proton transport	2	0.027048	5.46E-004
enriched	response to dsRNA	2	0.027048	5.46E-004
enriched	skeletal muscle fiber development	2	0.027048	5.46E-004
enriched	negative regulation of blood vessel diameter	3	0.146059	5.52E-004
enriched	antibacterial humoral response	3	0.148764	5.75E-004

enriched	cartilage development	3	0.148764	5.75E-004
enriched	positive regulation of interferon-gamma biosynthetic process	3	0.148764	5.75E-004
enriched	positive regulation of protein deacetylation	3	0.148764	5.75E-004
enriched	endothelial cell activation	3	0.151468	6.00E-004
enriched	positive regulation of axon extension involved in axon guidance	3	0.151468	6.00E-004
enriched	regulation of apoptotic process	3	0.151468	6.00E-004
enriched	chromatin silencing at rDNA	2	0.029753	6.07E-004
enriched	glomerular visceral epithelial cell differentiation	2	0.029753	6.07E-004
enriched	hippo signaling	2	0.029753	6.07E-004
enriched	membrane invagination	2	0.029753	6.07E-004
enriched	mitral valve morphogenesis	2	0.029753	6.07E-004
enriched	ossification involved in bone remodeling	2	0.029753	6.07E-004
enriched	pathway-restricted SMAD protein phosphorylation	2	0.029753	6.07E-004
enriched	positive regulation of calcium ion transmembrane transporter activity	3	0.154173	6.07E-004
enriched	positive regulation of cardiac muscle cell differentiation	2	0.029753	6.07E-004
enriched	positive regulation of epithelial cell migration	2	0.029753	6.07E-004
enriched	positive regulation of vascular endothelial cell proliferation	2	0.029753	6.07E-004
enriched	regulation of JNK cascade	3	0.154173	6.07E-004
enriched	regulation of branching involved in prostate gland morphogenesis	2	0.029753	6.07E-004
enriched	regulation of endodeoxyribonuclease activity	2	0.029753	6.07E-004
enriched	regulation of endopeptidase activity	2	0.029753	6.07E-004
enriched	regulation of mRNA 3'-end processing	2	0.029753	6.07E-004
enriched	regulation of macrophage colony-stimulating factor production	2	0.029753	6.07E-004
enriched	regulation of programmed cell death	2	0.029753	6.07E-004
enriched	response to immobilization stress	2	0.029753	6.07E-004
enriched	regulation of bone mineralization	3	0.156878	6.22E-004
enriched	positive regulation of behavior	3	0.159583	6.51E-004
enriched	regulation of hormone biosynthetic process	3	0.159583	6.51E-004
enriched	positive regulation of type 2 immune response	3	0.162287	6.77E-004
enriched	regulation of bile acid metabolic process	3	0.162287	6.77E-004
enriched	response to epidermal growth factor	3	0.162287	6.77E-004
enriched	RNA secondary structure unwinding	2	0.032458	6.88E-004
enriched	dendritic spine morphogenesis	2	0.032458	6.88E-004
enriched	dentate gyrus development	2	0.032458	6.88E-004
enriched	endocardium morphogenesis	2	0.032458	6.88E-004
enriched	mitotic G2/M transition checkpoint	2	0.032458	6.88E-004
enriched	positive regulation of amyloid-beta clearance	2	0.032458	6.88E-004
enriched	regulation of neurotransmitter receptor activity	3	0.164992	6.88E-004
enriched	regulation of serotonin secretion	2	0.032458	6.88E-004
enriched	response to erythropoietin	2	0.032458	6.88E-004
enriched	response to ether	3	0.164992	6.88E-004
enriched	spindle assembly	2	0.032458	6.88E-004
enriched	catecholamine biosynthetic process	3	0.167697	7.19E-004
enriched	RNA processing	2	0.035162	7.66E-004
enriched	cardioblast differentiation	2	0.035162	7.66E-004

enriched	heterochronic	2	0.035162	7.66E-004
enriched	isotype switching	2	0.035162	7.66E-004
enriched	negative regulation of cardiac muscle cell proliferation	2	0.035162	7.66E-004
enriched	negative regulation of gene expression	2	0.035162	7.66E-004
enriched	positive regulation of acute inflammatory response	2	0.035162	7.66E-004
enriched	positive regulation of neuron apoptotic process	2	0.035162	7.66E-004
enriched	regulation of bone resorption	2	0.035162	7.66E-004
enriched	regulation of cardiac muscle tissue development	2	0.035162	7.66E-004
enriched	regulation of cytokine production involved in immune response	2	0.035162	7.66E-004
enriched	regulation of defense response to virus by host	2	0.035162	7.66E-004
enriched	regulation of histone H3-K9 acetylation	2	0.035162	7.66E-004
enriched	regulation of histone H4 acetylation	2	0.035162	7.66E-004
enriched	regulation of macrophage activation	2	0.035162	7.66E-004
enriched	regulation of synaptic vesicle exocytosis	2	0.035162	7.66E-004
enriched	signal transduction involved in mitotic G1 DNA damage checkpoint	2	0.035162	7.66E-004
enriched	tissue development	2	0.035162	7.66E-004
enriched	proximal/distal pattern formation	3	0.175811	7.80E-004
enriched	cell migration involved in endocardial cushion formation	3	0.181221	8.49E-004
enriched	spinal cord patterning	3	0.181221	8.49E-004
enriched	base-excision repair	2	0.037867	8.64E-004
enriched	drug metabolic process	2	0.037867	8.64E-004
enriched	hemoglobin biosynthetic process	2	0.037867	8.64E-004
enriched	positive regulation of cellular senescence	2	0.037867	8.64E-004
enriched	regulation of cytokine biosynthetic process	2	0.037867	8.64E-004
enriched	regulation of extrinsic apoptotic signaling pathway	2	0.037867	8.64E-004
enriched	rhythmic process	2	0.037867	8.64E-004
enriched	thyroid hormone generation	2	0.037867	8.64E-004
enriched	negative regulation of calcineurin-NFAT signaling cascade	3	0.189335	9.41E-004
enriched	fatty acid catabolic process	2	0.040572	9.73E-004
enriched	histone H2A monoubiquitination	2	0.040572	9.73E-004
enriched	lateral mesoderm development	2	0.040572	9.73E-004
enriched	regulation of aerobic respiration	2	0.040572	9.73E-004
enriched	regulation of axonogenesis	2	0.040572	9.73E-004
enriched	regulation of developmental pigmentation	2	0.040572	9.73E-004
enriched	signal transduction by p53 class mediator	2	0.040572	9.73E-004
enriched	autonomic nervous system development	2	0.043277	1.07E-003
enriched	cell-cell adhesion mediated by integrin	2	0.043277	1.07E-003
enriched	chemotaxis	2	0.043277	1.07E-003
enriched	collateral sprouting	2	0.043277	1.07E-003
enriched	embryonic digestive tract morphogenesis	2	0.043277	1.07E-003
enriched	endoderm development	2	0.043277	1.07E-003
enriched	ion transmembrane transport	2	0.043277	1.07E-003
enriched	nerve development	2	0.043277	1.07E-003
enriched	regulation of body fluid levels	2	0.043277	1.07E-003
enriched	regulation of ceramide biosynthetic process	2	0.043277	1.07E-003

Type, regulation direction; Name, Annotation name; Hits, number of miRNAs mediating this annotation; Expected score, expected number of hits; *q*-value, adjusted *p*-value.

Note. This table illustrates the results of hsa-miR-101-3p, hsa-miR-15a-5p, hsa-miR-18a-5p, hsa-miR-374a-5p, hsa-miR-126-3p, hsa-miR-144-3p, and hsa-miR-424-5p uploaded to GeneTrail 3 - miRNomics (Saarland University, Saarbrücken, Germany; https://genetrail.bioinf.uni-sb.de/). Relevant Gene Ontology (GO; http://geneontology.org/) annotations were identified using experimentally strong verified targets from miRTarBase 9.0

Table C14: Enriched Gene Ontology Molecular Function Annotation at baseline

Type	Name	Hits	Expected score	*q*-value
enriched	DNA binding	4	0.086553	7.10E-006
enriched	RNA cap binding	4	0.089258	7.10E-006
enriched	RNA helicase activity	4	0.094668	7.10E-006
enriched	RNA polymerase II CTD heptapeptide repeat kinase activity	4	0.094668	7.10E-006
enriched	decanoate-CoA ligase activity	4	0.110896	7.10E-006
enriched	phosphoprotein binding	4	0.113601	7.10E-006
enriched	protease binding	4	0.097373	7.10E-006
enriched	protein tyrosine kinase binding	4	0.113601	7.10E-006
enriched	signaling adaptor activity	4	0.110896	7.10E-006
enriched	signaling pattern recognition receptor activity	4	0.110896	7.10E-006
enriched	phosphatidylinositol 3-kinase activity	4	0.116306	7.11E-006
enriched	poly(G) binding	4	0.124420	8.59E-006
enriched	ion channel activity	4	0.127125	8.66E-006
enriched	co-SMAD binding	3	0.037867	1.09E-005
enriched	neurotransmitter transporter activity	3	0.054096	3.16E-005
enriched	nuclear receptor binding	3	0.059505	4.00E-005
enriched	DBD domain binding	3	0.073029	7.10E-005
enriched	bending	3	0.091963	1.36E-004
enriched	DNA helicase activity	3	0.100077	1.67E-004
enriched	sialyltransferase activity	3	0.102782	1.72E-004
enriched	protein tyrosine kinase activity	3	0.108192	1.83E-004
enriched	signaling receptor activator activity	3	0.108192	1.83E-004
enriched	extracellular matrix constituent conferring elasticity	2	0.018934	1.91E-004
enriched	phosphatidylinositol-3	2	0.018934	1.91E-004
enriched	NF-kappaB binding	2	0.021638	2.35E-004
enriched	serine-type peptidase activity	2	0.021638	2.35E-004
enriched	sodium ion transmembrane transporter activity	2	0.024343	2.90E-004
enriched	sodium channel activity	2	0.043277	9.24E-004
enriched	phosphatidylinositol-4-phosphate binding	2	0.054096	1.41E-003
enriched	Rho GTPase binding	2	0.056801	1.50E-003
enriched	hyaluronic acid binding	2	0.059505	1.59E-003
enriched	phosphatidylserine binding	2	0.062210	1.69E-003
enriched	endonuclease activity	2	0.081144	2.79E-003
enriched	core promoter binding	2	0.113601	5.13E-003
enriched	core promoter sequence-specific DNA binding	2	0.113601	5.13E-003

Type, regulation direction; Name, Annotation name; Hits, number of miRNAs mediating this annotation; Expected score, expected number of hits; *q*-value, adjusted *p*-value.

Note. This table illustrates the results of hsa-miR-101-3p, hsa-miR-15a-5p, hsa-miR-18a-5p, hsa-miR-374a-5p, hsa-miR-126-3p, hsa-miR-144-3p, and hsa-miR-424-5p uploaded to GeneTrail 3 - miRNomics (Saarland University, Saarbrücken, Germany; https://genetrail.bioinf.uni-sb.de/). Relevant Gene Ontology

(GO; http://geneontology.org/) annotations were identified using experimentally strong verified targets from miRTarBase 9.0.

Table C15: Enriched Gene Ontology Cellular Components Annotation at baseline

Type	Name	Hits	Expected score	*q*-value
enriched	nuclear chromosome	3	0.048686	4.57E-005
enriched	nuclear outer membrane-endoplasmic reticulum membrane network	3	0.059505	4.57E-005
enriched	nuclear periphery	2	0.027048	4.66E-004
enriched	intracellular component	2	0.070325	1.98E-003
enriched	sarcoplasmic reticulum	2	0.070325	1.98E-003

Type, regulation direction; Name, Annotation name; Hits, number of miRNAs mediating this annotation; Expected score, expected number of hits; *q*-value, adjusted *p*-value.

Note. This table illustrates the results of hsa-miR-101-3p, hsa-miR-15a-5p, hsa-miR-18a-5p, hsa-miR-374a-5p, hsa-miR-126-3p, hsa-miR-144-3p, and hsa-miR-424-5p uploaded to GeneTrail 3 - miRNomics (Saarland University, Saarbrücken, Germany; https://genetrail.bioinf.uni-sb.de/). Relevant Gene Ontology (GO; http://geneontology.org/) annotations were identified using experimentally strong verified targets from miRTarBase 9.0.

Table C16: Enriched Reactome pathways at baseline

Type	Name	Hits	Expected score	*q*-value
enriched	Ovarian tumor domain proteases	2	0.008114	3.38E-004
enriched	Pentose phosphate pathway	3	0.059505	3.38E-004
enriched	Sphingolipid de novo biosynthesis	3	0.054096	3.38E-004
enriched	Telomere Maintenance	4	0.173107	3.38E-004
enriched	Activation of APC/C and APC/C:Cdc20 mediated degradation of mitotic proteins	2	0.013524	4.21E-004
enriched	Cellular response to heat stress	2	0.016229	4.21E-004
enriched	Diseases associated with O-glycosylation of proteins	4	0.246136	4.21E-004
enriched	FOXO-mediated transcription	2	0.016229	4.21E-004
enriched	MAP2K and MAPK activation	2	0.016229	4.21E-004
enriched	Phase 0 - rapid depolarisation	2	0.016229	4.21E-004
enriched	Signal attenuation	4	0.227202	4.21E-004
enriched	Toll Like Receptor 5 (TLR5) Cascade	3	0.091963	4.21E-004
enriched	Toll Like Receptor 7/8 (TLR7/8) Cascade	2	0.013524	4.21E-004
enriched	Transport of small molecules	2	0.013524	4.21E-004
enriched	Transport to the Golgi and subsequent modification	2	0.013524	4.21E-004
enriched	XIAP-regulated apoptotic response	4	0.286708	4.21E-004
enriched	Defective Mismatch Repair Associated With MSH6	3	0.116306	5.14E-004
enriched	GRB2 events in EGFR signaling	4	0.327280	5.14E-004
enriched	GRB2:SOS provides linkage to MAPK signaling for Integrins	4	0.319165	5.14E-004

enriched	Pyruvate metabolism and Citric Acid (TCA) cycle	4	0.321870	5.14E-004
enriched	Energy dependent regulation of mTOR by LKB1-AMPK	3	0.132535	7.01E-004
enriched	G beta:gamma signalling through CDC42	2	0.024343	7.01E-004
enriched	PI3K Cascade	2	0.024343	7.01E-004
enriched	Other semaphorin interactions	3	0.143354	8.04E-004
enriched	G-protein beta:gamma signalling	2	0.027048	8.05E-004
enriched	Constitutive Signaling by Ligand-Responsive EGFR Cancer Variants	2	0.032458	1.09E-003
enriched	Regulation of RAS by GAPs	2	0.032458	1.09E-003
enriched	ERK/MAPK targets	2	0.035162	1.24E-003
enriched	AKT phosphorylates targets in the nucleus	2	0.037867	1.31E-003
enriched	Activation of ATR in response to replication stress	2	0.037867	1.31E-003
enriched	recycling	2	0.037867	1.31E-003
enriched	G-protein activation	2	0.040572	1.46E-003
enriched	Diseases of glycosylation	2	0.043277	1.57E-003
enriched	PI3K/AKT Signaling in Cancer	2	0.043277	1.57E-003
enriched	Rho GTPase cycle	2	0.048686	1.93E-003
enriched	Costimulation by the CD28 family	3	0.221793	1.96E-003
enriched	Signaling by Activin	2	0.051391	2.04E-003
enriched	Interleukin-10 signaling	3	0.238022	2.28E-003
enriched	RNA Polymerase I Promoter Clearance	2	0.056801	2.37E-003
enriched	Ion homeostasis	2	0.062210	2.71E-003
enriched	Signaling by MET	2	0.062210	2.71E-003
enriched	FGFR2c ligand binding and activation	2	0.067620	2.99E-003
enriched	G alpha (s) signalling events	2	0.067620	2.99E-003
enriched	HATs acetylate histones	2	0.067620	2.99E-003
enriched	Caspase activation via Death Receptors in the presence of ligand	2	0.070325	3.09E-003
enriched	Ras activation upon Ca2+ influx through NMDA receptor	2	0.070325	3.09E-003
enriched	Phospholipase C-mediated cascade; FGFR3	2	0.073029	3.20E-003
enriched	Regulation of the apoptosome activity	2	0.073029	3.20E-003
enriched	Signaling by NOTCH1	2	0.075734	3.37E-003
enriched	Macroautophagy	2	0.078439	3.47E-003
enriched	Trafficking and processing of endosomal TLR	2	0.078439	3.47E-003
enriched	Elastic fibre formation	2	0.081144	3.64E-003
enriched	TRIF-mediated programmed cell death	2	0.083849	3.82E-003
enriched	Constitutive Signaling by NOTCH1 HD+PEST Domain Mutants	2	0.116306	7.03E-003
enriched	G alpha (q) signalling events	2	0.116306	7.03E-003
enriched	Regulation of necroptotic cell death	2	0.124420	7.61E-003
enriched	Signaling by FGFR3 fusions in cancer	2	0.124420	7.61E-003
enriched	Signaling by FGFR4 in disease	2	0.124420	7.61E-003
enriched	Constitutive Signaling by NOTCH1 HD Domain Mutants	2	0.135240	8.52E-003
enriched	G alpha (z) signalling events	2	0.135240	8.52E-003
enriched	IL-6-type cytokine receptor ligand interactions	2	0.135240	8.52E-003
enriched	Cell junction organization	2	0.137944	8.58E-003
enriched	Constitutive Signaling by NOTCH1 PEST Domain Mutants	2	0.137944	8.58E-003
enriched	Cargo recognition for clathrin-mediated endocytosis	2	0.143354	9.10E-003

enriched	Caspase activation via Dependence Receptors in the absence of ligand	2	0.154173	1.03E-002
enriched	Interleukin-20 family signaling	2	0.159583	1.09E-002
enriched	PCNA-Dependent Long Patch Base Excision Repair	2	0.173107	1.24E-002
enriched	Signaling by Hippo	2	0.173107	1.24E-002
enriched	Metabolism of water-soluble vitamins and cofactors	2	0.178516	1.29E-002
enriched	Signaling by FGFR3 in disease	2	0.186631	1.39E-002
enriched	Degradation of GLI1 by the proteasome	2	0.194745	1.49E-002
enriched	EPHB-mediated forward signaling	2	0.197450	1.50E-002

Type, regulation direction; Name, Annotation name; Hits, number of miRNAs mediating this annotation; Expected score, expected number of hits; *q*-value, adjusted *p*-value.

Note. This table illustrates the results of hsa-miR-101-3p, hsa-miR-15a-5p, hsa-miR-18a-5p, hsa-miR-374a-5p, hsa-miR-126-3p, hsa-miR-144-3p, and hsa-miR-424-5p uploaded to GeneTrail 3 - miRNomics (Saarland University, Saarbrücken, Germany; https://genetrail.bioinf.uni-sb.de/). Reactome (https://reactome.org/) pathways were identified using experimentally strong verified targets from miRTarBase 9.0.

Table C17: Enriched KEGG Pathways at baseline

Type	Name	Hits	Expected score	*q*-value
enriched	Metabolism of xenobiotics by cytochrome P450	7	0.486862	2.89E-007
enriched	Pathogenic Escherichia coli infection	6	0.478748	1.28E-005
enriched	Cocaine addiction	5	0.270479	1.58E-005
enriched	HIF-1 signaling pathway	5	0.265070	1.58E-005
enriched	Focal adhesion	5	0.321870	3.03E-005
enriched	Cell adhesion molecules (CAMs)	4	0.229907	2.41E-004
enriched	Cholinergic synapse	4	0.286708	4.96E-004
enriched	PPAR signaling pathway	4	0.313756	6.20E-004
enriched	Regulation of autophagy	3	0.124420	7.98E-004
enriched	Herpes simplex infection	3	0.129830	8.16E-004
enriched	Oxidative phosphorylation	4	0.373261	8.91E-004
enriched	Acute myeloid leukemia	3	0.170402	1.32E-003
enriched	Maturity onset diabetes of the young	3	0.162287	1.32E-003
enriched	Toxoplasmosis	3	0.167697	1.32E-003
enriched	mTOR signaling pathway	3	0.194745	1.82E-003
enriched	B cell receptor signaling pathway	2	0.043277	1.89E-003
enriched	Morphine addiction	2	0.045982	2.02E-003
enriched	Leishmaniasis	2	0.054096	2.65E-003
enriched	Fc gamma R-mediated phagocytosis	3	0.251546	3.06E-003
enriched	Melanogenesis	3	0.292117	4.49E-003
enriched	Antigen processing and presentation	2	0.086553	5.58E-003
enriched	Toll-like receptor signaling pathway	2	0.086553	5.58E-003
enriched	Propanoate metabolism	3	0.343509	5.97E-003
enriched	Protein processing in endoplasmic reticulum	3	0.343509	5.97E-003
enriched	Hematopoietic cell lineage	3	0.362442	6.16E-003
enriched	Intestinal immune network for IgA production	2	0.102782	6.16E-003
enriched	TNF signaling pathway	2	0.097373	6.16E-003
enriched	Viral myocarditis	2	0.102782	6.16E-003
enriched	Ubiquitin mediated proteolysis	2	0.105487	6.26E-003

enriched	Adherens junction	2	0.127125	8.20E-003
enriched	Epithelial cell signaling in Helicobacter pylori infection	2	0.127125	8.20E-003
enriched	Wnt signaling pathway	2	0.127125	8.20E-003
enriched	Neuroactive ligand-receptor interaction	2	0.129830	8.28E-003
enriched	Progesterone-mediated oocyte maturation	2	0.140649	9.40E-003
enriched	RNA transport	2	0.143354	9.48E-003
enriched	Rap1 signaling pathway	2	0.151468	1.03E-002
enriched	Thyroid cancer	2	0.175811	1.33E-002
enriched	Vibrio cholerae infection	2	0.197450	1.62E-002
enriched	Non-alcoholic fatty liver disease (NAFLD)	2	0.210974	1.79E-002
enriched	Shigellosis	2	0.224498	1.97E-002
enriched	Gastric acid secretion	2	0.294822	3.21E-002

Type, regulation direction; Name, Annotation name; Hits, number of miRNAs mediating this annotation; Expected score, expected number of hits; *q*-value, adjusted *p*-value.

Note. This table illustrates the results of hsa -miR-101-3p, hsa-miR-15a-5p, hsa-miR-18a-5p, hsa-miR-374a-5p, hsa-miR-126-3p, hsa-miR-144-3p, and hsa-miR-424-5p uploaded to GeneTrail 3 - miRNomics (Saarland University, Saarbrücken, Germany; https://genetrail.bioinf.uni-sb.de/). Relevant Kyoto Encyclopedia of Genes and Genomes (KEGG; https://www.genome.jp/kegg/brite.html) pathways were identified using experimentally strong verified targets from miRTarBase 9.0.

Table C18: Enriched Wikipathways pathways at baseline

Type	Name	Hits	Expected score	*q*-value
enriched	Inflammatory Response Pathway	7	0.381376	4.51E-008
enriched	Oxidation by Cytochrome P450	7	0.359737	4.51E-008
enriched	Cytosine methylation	6	0.348918	2.24E-006
enriched	Glucuronidation	4	0.081144	9.16E-006
enriched	HIF1A and PPARG regulation of glycolysis	5	0.232612	1.05E-005
enriched	Cells and Molecules involved in local acute inflammatory response	5	0.297527	2.60E-005
enriched	H19 action Rb-E2F1 signaling and CDK-Beta-catenin activity	4	0.119011	2.60E-005
enriched	Bone Morphogenic Protein (BMP) Signalling and Regulation	5	0.354328	4.84E-005
enriched	Selective expression of chemokine receptors during T-cell polarization	5	0.354328	4.84E-005
enriched	AMP-activated Protein Kinase (AMPK) Signaling	3	0.062210	1.39E-004
enriched	Electron Transport Chain (OXPHOS system in mitochondria)	3	0.062210	1.39E-004
enriched	Adipogenesis	3	0.070325	1.87E-004
enriched	Cardiac Hypertrophic Response	4	0.232612	2.01E-004
enriched	Sudden Infant Death Syndrome (SIDS) Susceptibility Pathways	4	0.235317	2.01E-004
enriched	Amplification and Expansion of Oncogenic Pathways as Metastatic Traits	4	0.251546	2.30E-004
enriched	Mammary gland development pathway - Involution (Stage 4 of 4)	3	0.081144	2.30E-004
enriched	Endochondral Ossification	4	0.259660	2.32E-004

enriched	Energy Metabolism	4	0.259660	2.32E-004
enriched	Tumor suppressor activity of SMARCB1	3	0.094668	2.94E-004
enriched	Constitutive Androstane Receptor Pathway	3	0.129830	6.60E-004
enriched	Extracellular vesicles in the crosstalk of cardiac cells	3	0.127125	6.60E-004
enriched	Sleep regulation	3	0.129830	6.60E-004
enriched	Structural Pathway of Interleukin 1 (IL-1)	3	0.132535	6.72E-004
enriched	Nuclear Receptors Meta-Pathway	2	0.024343	6.81E-004
enriched	Wnt Signaling Pathway and Pluripotency	3	0.140649	7.39E-004
enriched	One carbon metabolism and related pathways	4	0.394900	8.37E-004
enriched	Alzheimers Disease	2	0.029753	9.15E-004
enriched	Serotonin and anxiety	3	0.156878	9.15E-004
enriched	Wnt Signaling	3	0.167697	1.08E-003
enriched	Ethanol metabolism resulting in production of ROS by CYP2E1	3	0.181221	1.31E-003
enriched	Interleukin-1 Induced Activation of NF-kappa-B	3	0.186631	1.39E-003
enriched	Follicle Stimulating Hormone (FSH) signaling pathway	3	0.189335	1.40E-003
enriched	Glutathione metabolism	2	0.043277	1.59E-003
enriched	Vitamins A and D - action mechanisms	3	0.200155	1.59E-003
enriched	Cytoplasmic Ribosomal Proteins	3	0.205564	1.63E-003
enriched	Inhibition of exosome biogenesis and secretion by Manumycin A in CRPC cells	3	0.235317	2.36E-003
enriched	Fibrin Complement Receptor 3 Signaling Pathway	3	0.267774	3.35E-003
enriched	Resistin as a regulator of inflammation	3	0.270479	3.36E-003
enriched	MET in type 1 papillary renal cell carcinoma	2	0.067620	3.42E-003
enriched	Canonical and Non-canonical Notch signaling	2	0.070325	3.52E-003
enriched	Type III interferon signaling	2	0.070325	3.52E-003
enriched	Phytochemical activity on NRF2 transcriptional activation	2	0.075734	3.99E-003
enriched	Glycogen Synthesis and Degradation	2	0.078439	4.18E-003
enriched	miR-222 in Exercise-Induced Cardiac Growth	2	0.086553	4.97E-003
enriched	Selenium Metabolism and Selenoproteins	2	0.089258	5.16E-003
enriched	T-Cell Receptor and Co-stimulatory Signaling	2	0.091963	5.36E-003
enriched	Endothelin Pathways	2	0.094668	5.41E-003
enriched	Estrogen Receptor Pathway	2	0.097373	5.41E-003
enriched	IL1 and megakaryocytes in obesity	2	0.097373	5.41E-003
enriched	Parkin-Ubiquitin Proteasomal System pathway	2	0.094668	5.41E-003
enriched	Sterol Regulatory Element-Binding Proteins (SREBP) signalling	2	0.097373	5.41E-003
enriched	Serotonin Receptor 2 and STAT3 Signaling	2	0.100077	5.61E-003
enriched	Preimplantation Embryo	2	0.102782	5.80E-003
enriched	Control of immune tolerance by vasoactive intestinal peptide	2	0.108192	6.07E-003
enriched	LTF danger signal response pathway	2	0.108192	6.07E-003
enriched	Ultraconserved region 339 modulation of tumor suppressor microRNAs in cancer	2	0.108192	6.07E-003
enriched	One Carbon Metabolism	2	0.113601	6.57E-003
enriched	ATM Signaling Network in Development and Disease	2	0.119011	7.07E-003
enriched	LncRNA-mediated mechanisms of therapeutic resistance	2	0.124420	7.46E-003
enriched	Photodynamic therapy-induced NF-kB survival signaling	2	0.124420	7.46E-003
enriched	Liver X Receptor Pathway	2	0.127125	7.53E-003

enriched	Metabolic reprogramming in colon cancer	2	0.127125	7.53E-003
enriched	EPO Receptor Signaling	2	0.140649	8.89E-003
enriched	PI3K-Akt Signaling Pathway	2	0.140649	8.89E-003
enriched	Hypothesized Pathways in Pathogenesis of Cardiovascular Disease	2	0.143354	9.09E-003
enriched	Drug Induction of Bile Acid Pathway	2	0.156878	1.07E-002
enriched	and Retinoic Acids Regulation of p27 Expression	2	0.164992	1.16E-002
enriched	Physiological and Pathological Hypertrophy of the Heart	2	0.167697	1.18E-002
enriched	RAC1/PAK1/p38/MMP2 Pathway	2	0.170402	1.20E-002
enriched	Transcription co-factors SKI and SKIL protein partners	2	0.189335	1.45E-002
enriched	Envelope proteins and their potential roles in EDMD physiopathology	2	0.216383	1.84E-002
enriched	NRF2-ARE regulation	2	0.229907	2.04E-002
enriched	Benzo(a)pyrene metabolism	2	0.238022	2.15E-002

Type, regulation direction; Name, Annotation name; Hits, number of miRNAs mediating this annotation; Expected score, expected number of hits; *q*-value, adjusted *p*-value.

Note. This table illustrates the results of hsa-miR-101-3p, hsa-miR-15a-5p, hsa-miR-18a-5p, hsa-miR-374a-5p, hsa-miR-126-3p, hsa-miR-144-3p, and hsa-miR-424-5p uploaded to GeneTrail 3 - miRNomics (Saarland University, Saarbrücken, Germany; https://genetrail.bioinf.uni-sb.de/). Relevant Wikipathways (https://www.wikipathways.org) pathways were identified using experimentally strong verified targets from miRTarBase 9.0.

Appendix D

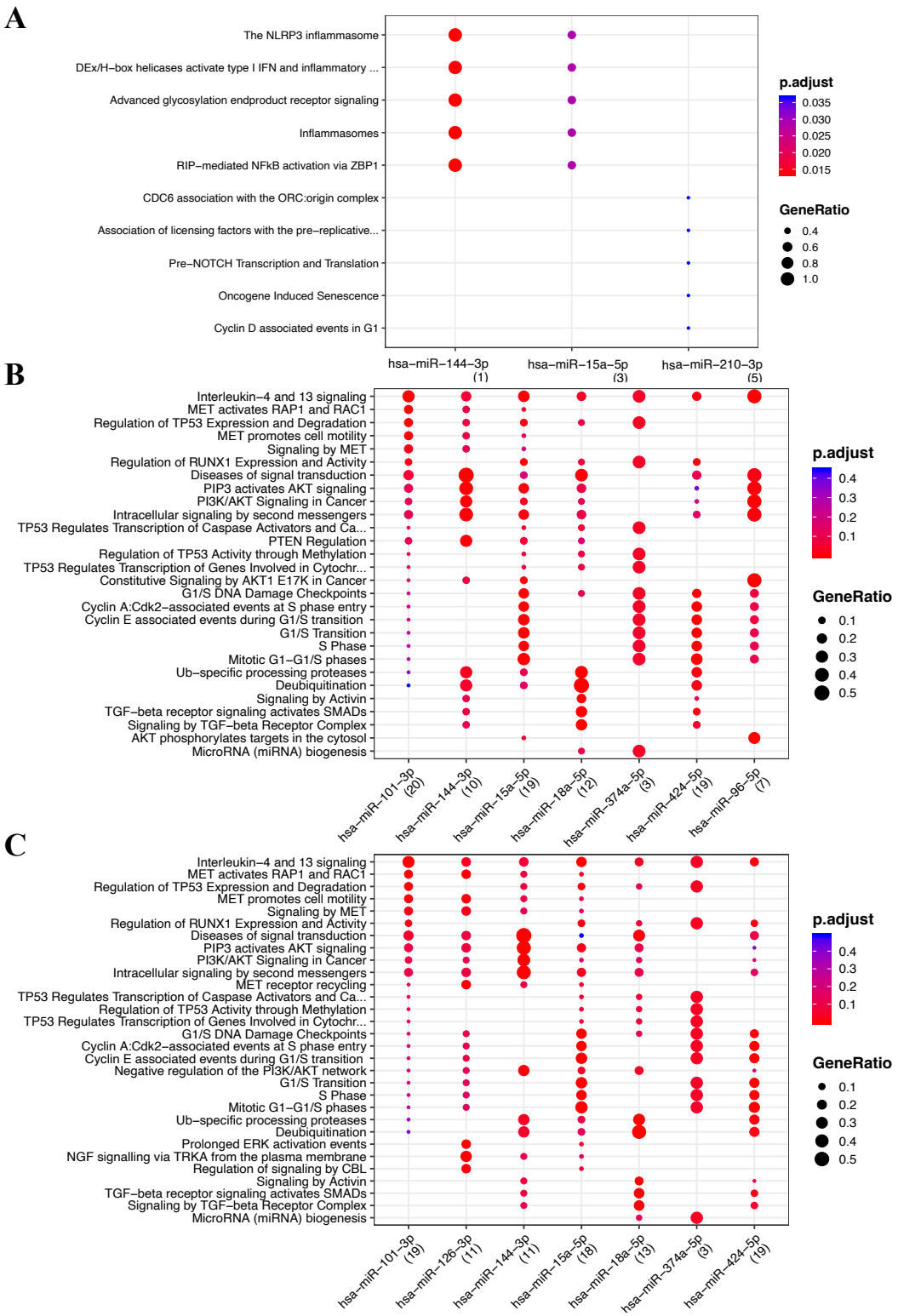

Figure D1: Reactome pathways modulated by DE miRNAs in young and old athletes, and at baseline

Note. This figure illustrates the top Reactome pathways mediated by DE miRNAs in (A) Young, (B) Master, and (C) at baseline between younger and older athletes. The adjusted *p*-value is represented by colours and the number of genes regulating the signalling pathway is indicated in size.

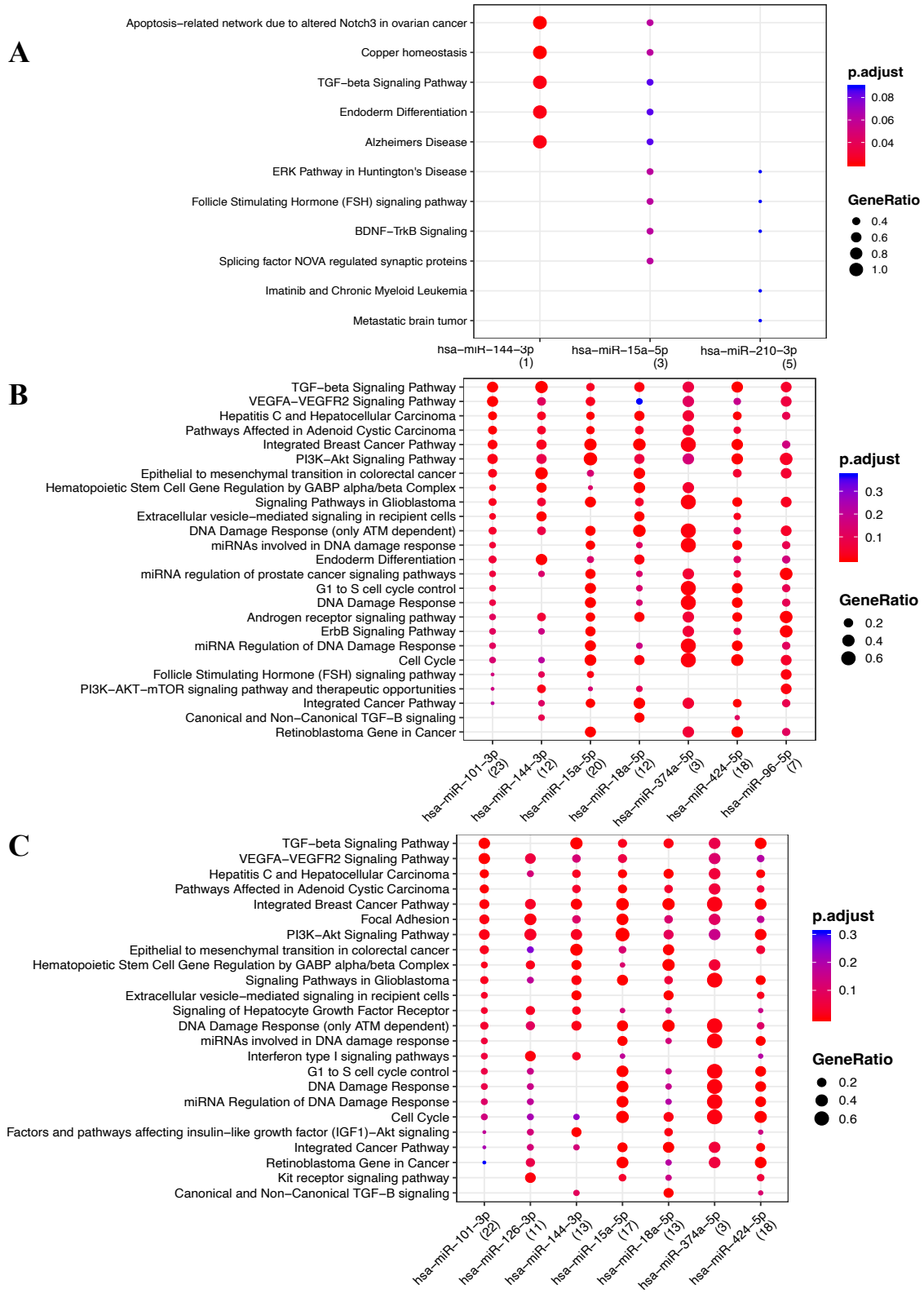

Figure D2: Wikipathways pathways modulated by DE miRNAs in young and old athletes, and at baseline

Note. This figure illustrates the top Wikipathways pathways mediated by DE miRNAs in (A) Young, (B) Master, and (C) at baseline between younger and older athletes. The adjusted *p*-value is represented by colours and the number of genes regulating the signalling pathway is indicated in size.