EXERCISE

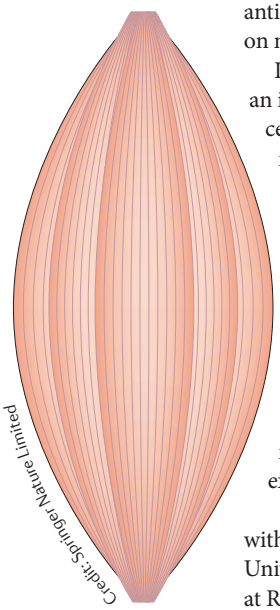
Exercise exerts anti-inflammatory effects on muscle via the JAK–STAT pathway

> “the authors used an in vitro 3D human muscle cell culture model … to simulate the effects of exercise on muscle”

Inflammation can be a double-edged sword when it comes to muscle homeostasis. Some inflammation is necessary for normal muscle healing and repair processes, but chronic inflammation, such as occurs in rheumatoid arthritis (RA), has detrimental effects on muscle. Exercise is known to have general anti-inflammatory effects in chronic inflammatory disease, but understanding what is going on in the muscle itself has been challenging.

“Previously, several human studies have suggested that exercise can be beneficial in the setting of chronic inflammation, which, in addition to other negative effects, induces muscle wasting and loss of contractile strength. What has not been clear from these studies is which cells, tissues or organs help with this anti-inflammatory response, and through what mechanisms,” explains Nenad Bursac, corresponding author of a new study investigating the anti-inflammatory effects of exercise on muscle.

In their study, the authors used an in vitro 3D human muscle cell culture model, known as a myobundle, to simulate the effects of exercise on muscle. This model was developed in the Bursac lab to mimic the contractile responses of normal muscle tissue in response to electrical stimulation and can be produced from human pluripotent stem cells or from primary myogenic cells, enabling muscle cell responses to be examined in isolation.



Credit: Springer Nature Limited

“Some 3 or 4 years ago, along with other investigators at Duke University, we started to look at RA as a prototypical chronic inflammatory disease and decided to explore if myobundles can be used to mimic effects of chronic inflammation on human skeletal muscle and if simulated exercise in this model system would induce anti-inflammatory effects,” says Bursac.

To mimic inflammation, the researchers used the pro-inflammatory cytokine IFNγ, which is known to contribute to muscle wasting in chronic inflammatory diseases. In response to IFNγ, myobundles lost some strength and ability to contract; effects that could be prevented by the application of intermittent electrical stimulation to mimic exercise. The secretory output of the myobundles also changed in response to IFNγ treatment and was characterized by increased output of pro-inflammatory cytokines and chemokines that could, again, mostly be prevented by electrical stimulation.

Mechanistically, IFNγ induced activation of the Janus kinase (JAK)–signal transducer and activator of transcription (STAT) pathway in myobundles. This activation could be partially reduced by electrical stimulation, suggesting a new mechanism for the anti-inflammatory effects of exercise, and could also be completely abrogated by the use of JAK inhibitors.

But what is the relevance of these results for RA? Gabriel Herrero-Beaumont, an expert in inflammatory rheumatic diseases, urges caution in untangling the effects of acute versus chronic inflammation.

“The time schedule and pro-inflammatory stimuli employed in this study might be more associated with an acute response than with a chronic injury, as occurs in RA sarcopenia,” suggests Herrero-Beaumont. “However, it is difficult to differentiate blocking homeostatic processes, such as healing minor muscle injuries, from chronic pathophysiological activation, such as that observed in RA, when both are based on JAK–STAT pathway activation. Therefore, the results of this study could introduce us to the concept of ‘dyshomeostasis’ and the dual effect of JAK–STAT and its inhibition in different clinical settings.”

Looking towards the future, Herrero-Beaumont advocates for studies that will advance our understanding of muscle atrophy in RA and how it might be managed, particularly given that JAK inhibitors are already used to treat patients with RA. “It would also be interesting to know whether exercise or electrical stimulation can have an additive anti-inflammatory effect on muscle regeneration in patients with RA with partial response to therapy,” he adds.

“We are interested in finding out if the presence of other relevant cell types, including inflammatory cells such as macrophages or T cells, can modulate this response to exercise and if specific regimes of exercise, such as those mimicking resistance versus endurance training, are particularly beneficial as an anti-inflammatory defence,” states Bursac. “We hope that homing in on specific molecular mechanisms could eventually allow us to identify novel anti-inflammatory targets for potential pharmacotherapy.”

Joanna Clarke

ORIGINAL ARTICLE Chen, Z. et al. Exercise mimetics and JAK inhibition attenuate IFN-γ–induced wasting in engineered human skeletal muscle. *Sci. Adv.* **7**, eabd9502 (2021)

OSTEOARTHRITIS

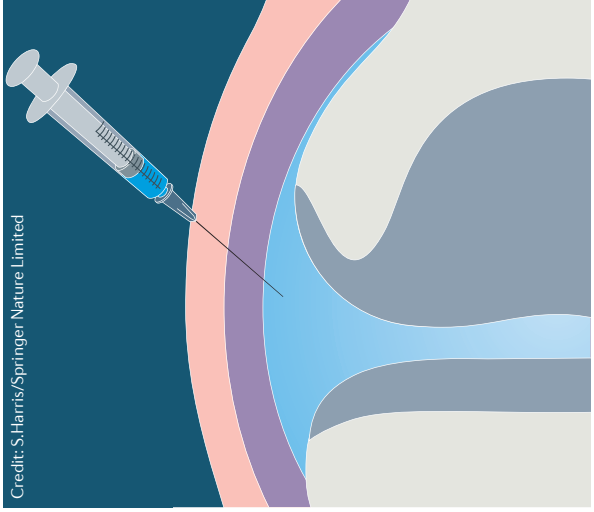
Targeting the EGFR pathway shows promise for OA

The epidermal growth factor receptor (EGFR) pathway is important for the maintenance of articular cartilage and is implicated as a potential treatment target in osteoarthritis (OA). However, studies of the effects of EGFR inhibition in models of OA have had conflicting results. New results published in *Science Translational Medicine* provide convincing evidence that enhancing activation of this pathway in the cartilage is a promising therapeutic strategy.

"Previously, our lab found that EGFR deficiency or inactivation accelerated OA progression in mice, and thus we proposed that its activation could be used to treat OA," explains Ling Qin, co-corresponding author on the new study.

To look at the effect of EGFR overactivation in cartilage, Qin and colleagues developed two mouse models with cartilage-specific overexpression of an EGFR ligand, heparin binding EGF-like growth factor (HBEGF). In both mouse models, overactivation of the EGFR pathway protected against cartilage degeneration and other pathological changes associated with OA following surgical destabilization of the medial meniscus. Notably, treatment of the mice with an EGFR inhibitor (gefitinib) reversed these protective effects.

EGFR ligands are rapidly cleared from the joint because of their small size and also have a short lifespan in the circulation, limiting their use as a therapy. To get around this issue, Zhiliang Cheng, another co-corresponding author on the study, designed polymeric micellar nanoparticles conjugated to transforming growth factor-α (TGFα), a potent EGFR ligand, to deliver this ligand to the joints while enabling prolonged retention and high cartilage penetration.



> "overactivation of the EGFR pathway protected against cartilage degeneration"

Similar to EGFR overactivation, intra-articular delivery of these TGFα-conjugated nanoparticles in the knees of mice attenuated the development of surgery-induced OA. "We are currently collaborating with other scientists to carry this project on into large animal models of OA," says Qin. "If the TGFα-conjugated nanoparticles work in large animals, we then plan to initiate a human clinical trial."

Jessica McHugh

ORIGINAL ARTICLE Wei, Y. et al. Targeting cartilage EGFR pathway for osteoarthritis treatment. *Sci. Transl Med.* **13**, eabb3946 (2021)

GENETICS

New insights into RA genetics from GWAS meta-analysis

Nearly 110 susceptibility loci for rheumatoid arthritis (RA) have been identified in population-based genetic association studies; however, these variants account for only a small proportion of RA heritability, suggesting that a large number of common variants with modest effects on RA susceptibility remain unidentified. Now, a genome-wide meta-analysis with the large sample sizes required to detect such signals has unveiled 11 novel susceptibility loci and provides new insights into the genetic architecture and biology of RA.

The meta-analysis integrated summary association data from genome-wide association studies (GWAS) in Korean, Japanese and European cohorts comprising 22,628 patients with RA and 288,644 controls in total. The variants in the 11 novel RA-associated loci (*DGUOK-AS1*, *DAP*, *BAD*, *TPCN2*, *LOC107984408*, *LOC105369698*, *IQGAP1*, *PRKCB*, *ZNF689*, *C20orf181* and *SMC1B*) increased the explained proportion of genetic susceptibility for RA, accounting for 6.9% of the non-MHC single-nucleotide polymorphism-based heritability in the East Asian populations and 1.8% of that in Europeans. The study also replicated 71 known non-MHC RA-risk loci. Across the East Asian and European groups, 90 distinct association signals were identified.

> "The variants in the 11 novel RA-associated loci … increased the explained proportion of genetic susceptibility for RA"

The researchers then catalogued the findings to provide further insights. "Employing cutting-edge post-GWAS approaches, we could prioritize the most likely causal variants, genes and RA variant-implicating features (tissues, pathways and transcription factors)," reports co-corresponding author Kwangwoo Kim. "For example, we identified plausible RA-relevant genes in the susceptibility loci that included targets of drugs approved for RA treatments and potentially repurposable drugs approved for other indications."

"We also re-emphasized the importance of $CD4^+$ T cell activation and non-immune organs such as the small intestine in RA pathogenesis," adds co-corresponding author Sang-Cheol Bae.

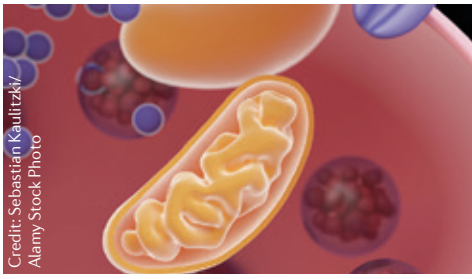
The researchers are now working on further studies using genomic, transcriptomic and epigenomic data from $CD4^+$ T cells at bulk and single-cell resolution to help understand how RA-associated variants shape RA-specific differential expression in $CD4^+$ T cells on a genome-wide scale. "With such insights into the biology of RA, we could devise sophisticated biomarkers or drug targets at an individual level," says Kim.

Sarah Onuora

ORIGINAL ARTICLE Ha, E. et al. Large-scale meta-analysis across East Asian and European populations updated genetic architecture and variant-driven biology of rheumatoid arthritis, identifying 11 novel susceptibility loci. *Ann. Rheum. Dis.* https://doi.org/10.1136/annrheumdis-2020-219065 (2020)

IMMUNOMETABOLISM

Metabolic defect causes invasive phenotype in RA T cells



Credit: Sebastian Kaulitzki/ Alamy Stock Photo

T cells from patients with rheumatoid arthritis (RA) have a metabolic signature that is indicative of a defect in their mitochondria. The results of a new study published in *Cell Metabolism* reveal not only what this defect is, but also how it contributes to the tissue-invasive nature of T cells in RA.

"We began with the quantification of mitochondrial metabolic intermediates in RA T cells and realized that the cells have abundant α-ketoglutarate but lack succinate. This suggested a break in the mitochondrial tricarboxylic acid (TCA) cycle (also known as the Krebs cycle)," explains corresponding author Cornelia Weyand.

This break in the TCA cycle was caused by reduced amounts of the enzymes that convert α-ketoglutarate into succinyl-CoA and then into succinate in T cells from patients with RA compared with T cells from patients with other rheumatic diseases. Transfecting RA T cells to overexpress these enzymes was able to reduce their tissue-invasive behaviour when adoptively transferred into NSG mice with engrafted human synovial tissue, suggesting a direct link between tissue invasiveness and metabolism.

> "the TCA cycle reverses in RA T cells, leading to excess production of acetyl-CoA"

To investigate how disruption of the TCA cycle might be linked to cell invasiveness, the researchers studied the mitochondrial metabolites produced by RA T cells. They found that the TCA cycle reverses in RA T cells, leading to excess production of acetyl-CoA, which is used by the cell to post-translationally modify proteins.

Interestingly, the excess acetyl-CoA in RA T cells seemed to affect the acetylation of microtubules in the cytoskeleton, causing mitochondria to shift towards the nucleus and the cells to form uropods, thereby priming them for migration. Blocking the acetylation of microtubules in RA T cells by gene knockdown reduced their ability to infiltrate synovial tissue in human synovium chimeric mice, thereby reducing synovitis.

"Our focus has now turned to the opportunity to utilize this metabolic abnormality to re-engineer the patients' T cells," says Weyand. "We have set a program in place to systematically identify small molecules and metabolites that can turn the directionality of the TCA cycle and thus control cellular acetyl-CoA concentrations."

Joanna Clarke

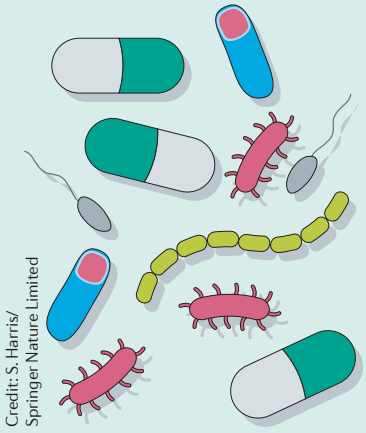
ORIGINAL ARTICLE Wu, B. et al. Succinyl-CoA ligase deficiency in pro-inflammatory and tissue-invasive T cells. *Cell Metab.* **32**, 967–980 (2020)

PHARMACOLOGY

Gut microbiome could predict drug response in RA

Evidence is emerging that various drugs are either metabolized by the human gut microbiota or are dependent on it for their efficacy.

The findings of a new study demonstrate for the first time that oral methotrexate can be metabolized by the human gut microbiota and suggest that the gut microbiome could have value in predicting response to the drug, which is well known to vary in patients with rheumatoid arthritis (RA).



Credit: S. Harris/ Springer Nature Limited

"Our analysis revealed that in patients with new-onset, treatment naive RA there are significant associations between the baseline abundance of gut bacterial taxa (and their genes) with future clinical response to methotrexate," reports Carles Ubeda, co-corresponding author of the study. "Notably, these included gene orthologues related to purine and methotrexate metabolism." The differences between the methotrexate responders ($n = 16$) and non-responders ($n = 10$) in microbial diversity in fecal samples were determined using 16S RNA gene sequencing and shotgun metagenomics sequencing.

"Using machine learning applied to the metagenomic data, we then developed a microbiome-based model that can predict lack of response to methotrexate in patients with RA," adds co-corresponding author Jose Scher. In an independent cohort of patients with new-onset RA ($n = 21$), the model correctly classified 80% of the patients as responders or non-responders.

> "the gut microbiome could have value in predicting response to [methotrexate]"

The findings also suggest a mechanistic link between microbiome features, methotrexate metabolism and clinical response. In ex vivo studies, methotrexate was depleted during incubation with fecal samples from patients with new-onset, treatment-naive RA. "Using metabolomic platforms, we found that methotrexate levels remaining after ex vivo incubation with these samples correlated with the magnitude of future clinical response, suggesting a possible direct effect of the gut microbiome on methotrexate metabolism and treatment outcomes," says Ubeda.

Sarah Onuora

ORIGINAL ARTICLE Artacho, A. et al. The pre-treatment gut microbiome is associated with lack of response to methotrexate in new onset rheumatoid arthritis. *Arthritis Rheumatol.* https://doi.org/10.1002/art.41622 (2020)
RELATED ARTICLE Scher, J. U. et al. Pharmacomicrobiomics in inflammatory arthritis: gut microbiome as modulator of therapeutic response. *Nat. Rev. Rheumatol.* **16**, 282–292 (2020)

OSTEOARTHRITIS

Adipose tissue triggers OA

Obesity is one of the primary risk factors for osteoarthritis (OA), although the exact contribution of obesity to the onset of OA is unknown. Increased biomechanical loading of joints, low-grade systemic inflammation and metabolic changes have all been suggested as potential mechanisms of obesity-related OA, but none has yet been proven as causal.

"Our group has been studying the interrelationship between obesity and OA for nearly two decades, and we now understand that changes in biomechanical loading due to the increased body weight that occurs with obesity do not solely account for the severity of OA," states Farshid Guilak, corresponding author of a new study into the role of adipose tissue in obesity-related OA.

"Several studies have implicated inflammatory signalling from fat, rather than changes in weight, in the pathogenesis of obesity-related OA," explains Guilak. "To address this question directly, we studied OA in a lipodystrophic mouse that is genetically modified to lack fat cells; in this manner, we could directly examine the effects of diet, body fat and other parameters on OA severity."

Lipodystrophic mice displayed several characteristics associated with obesity-related OA, such as bone sclerosis, muscle weakness and metabolic dysfunction, but were protected from developing OA spontaneously or following surgical destabilization of the medial meniscus (DMM). This protection was unchanged by feeding the mice a high-fat diet, which increases susceptibility to DMM-induced OA in wild-type mice. Notably, lipodystrophic mice still developed synovitis in response to DMM surgery, but had reduced pain compared with wild-type mice, suggesting that these features might be separable.

Crucially, subcutaneously implanting adipose tissue into the lipodystrophic mice restored their susceptibility to DMM-induced OA, implicating adipose tissue itself in the pathogenesis of OA.

> "implanting adipose tissue into the lipodystrophic mice restored their susceptibility to DMM-induced OA"

Credit: S.Harris/Springer Nature Limited

"An important conclusion of this study is that in many cases, OA may have a systemic component, with evidence of signalling between (remote) adipose tissue and joint cartilage," states first author Kelsey Collins. "Our goal is to determine which signals from fat are responsible for reversing the protection from OA. Fat–cartilage crosstalk and signalling could lead to novel therapeutic opportunities for OA, as well as for other musculoskeletal and metabolic diseases involving adipose dysfunction."

Joanna Clarke

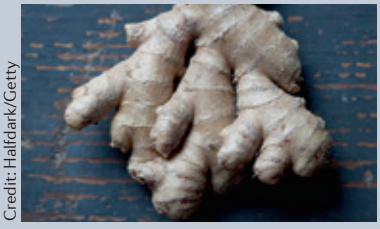
ORIGINAL ARTICLE Collins, K. H. et al. Adipose tissue is a critical regulator of osteoarthritis. *Proc. Natl Acad. Sci. USA* **118**, e2021096118 (2021)

AUTOIMMUNITY

Getting to the root of the anti-inflammatory effects of ginger

Ginger has a long history of medical use and is thought to contain anti-inflammatory and anti-oxidant compounds that could be of particular benefit for individuals with autoimmune or inflammatory diseases. A new study published in *JCI Insight* has found a protective role for the most abundant bioactive component of ginger root, 6-gingerol, in models of systemic lupus erythematosus (SLE) and antiphospholipid syndrome (APS).

> various gingerols, including 6-gingerol, could suppress NETosis in response to different stimuli



Credit: Halfdark/Getty

"Our work over the past several years has been exploring the role of pathways that increase neutrophil cAMP in suppressing NETosis (a form of neutrophil cell death implicated in various autoimmune diseases)," explains corresponding author Jason Knight. Evidence from the literature suggested that gingerols could antagonize the activity of phosphodiesterases (enzymes involved in the inactivation of cAMP), piquing his group's interest. "We wanted to focus on the mechanism and especially the role of gingerols in suppressing neutrophils," says Knight.

They first investigated the effects of gingerols on neutrophils in vitro. Notably, various gingerols, including 6-gingerol, could suppress NETosis in response to different stimuli (including stimuli relevant to SLE and APS). Further analysis suggested that gingerols could mitigate NETosis by suppressing reactive oxygen species formation through a mechanism partially dependent on inhibition of phosphodiesterase activity, resulting in increased intracellular levels of cAMP and increased activity of the cAMP-dependent kinase, protein kinase A.

Notably, in both a mouse model of SLE (Toll-like receptor 7 agonist-treated mice) and a mouse model of APS (an electrolytic model of venous thrombosis), administration of 6-ginger resulted in robust suppression of disease-relevant NETosis and other disease phenotypes (such as autoantibody development and thrombosis).

"It is hard to imagine gingerols being used as primary therapy for a highly active rheumatic disease. But could they help maintain remission? Or perhaps prevent disease in predisposed individuals?" asks Knight. "In my opinion, we need to be very systematic and start with a smaller mechanistic study asking whether we can confirm the same neutrophil phenotypes in humans. If successful, then a larger study with disease-relevant end points could probably be justified."

Jessica McHugh

ORIGINAL ARTICLE Ali, R. A. et al. Anti-neutrophil properties of natural gingerols in models of lupus. *JCI Insight* https://doi.org/10.1172/jci.insight.138385 (2020)