adipose tissue. Hyperglycemia is required to increase TG stores in adipose tissue, via the uptake of circulating NEFA and promotion of *de* novo lipogenesis. These results suggest that the suppression of lipolysis and the expansion of adipose tissue are differently regulated, and that post-prandial hyperglycaemia may play a role in the pathogenesis of obesity.
*Disclosure:* **M.A. Guzzardi:** None.

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### Identifying microRNAs involved in the regulation of skeletal muscle mitochondrial function

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**Background and aims:** Type 2 diabetes mellitus is characterized by a reduced peripheral insulin sensitivity and a diminished mitochondrial capacity in skeletal muscle. Importantly, strategies that increase muscle mitochondrial capacity, such as caloric restriction and exercise, have been repeatedly demonstrated to parallel improvements in insulin resistance. Interestingly, previous research in cardiac tissue demonstrated that specific microRNAs could regulate PPARδ, a major transcription factor involved in regulating mitochondrial oxidative capacity. Based on these findings, we hypothesize that - also in skeletal muscle - specific microRNAs exist that regulate mitochondrial function.
**Materials and methods:** We conducted an unbiased, hypothesis free, high-throughput microRNA-silencing screen in C2C12 myoblasts, using a library containing >700 specific microRNA inhibitors. After 24 hours of microRNA silencing we investigated several read-out parameters for mitochondrial capacity, including ATP levels, redox potential, COX4, HSP60 and mitochondrial membrane potential. Subsequently, we aimed to confirm the identified candidate miRNAs in a validation screen in C2C12 myotubes, measuring the same 5 read-out parameters plus oxygen consumption rates, at both 24 and 48 hours post-microRNA silencing. The validation screen was followed by investigation of individual microRNAs.
**Results:** The screening in myoblasts resulted in 62 microRNA hits that changed at least one of the mitochondrial parameters. Subsequently, we aimed to validate these 62 candidates in differentiated C2C12 myotubes, which resemble the post-mitotic adult skeletal muscle more closely. This validation screen confirmed 45 candidate microRNAs that changed at least one of the read-outs in at least one of the timepoints. Next, we excluded microRNAs without a human homologue and investigated the 20 most prominent hits in more detail. To this end, we measured gene expression of 27 major mitochondrial genes, 24 and 48 hours post-silencing. This analysis revealed that silencing of miRNA-382 increased the expression of several genes involved in mitochondrial dynamics and -biogenesis. However, conventional microarray analysis in C2C12 myotubes with silenced microRNA-382, revealed a collective downregulation of mitochondrial ribosomal proteins and respiratory chain proteins. Interestingly, this effect was accompanied by an imbalance between mitochondrial proteins encoded by the nuclear and mitochondrial DNA ($p<0,01$). This so-called mitonuclear protein imbalance was accompanied by activation of the mitochondrial unfolded protein response (mtUPR), indicated by an induction of HSP60 protein ($p<0,05$).
**Conclusion:** In the present study, we identified 20 specific microRNAs as modulators of skeletal muscle mitochondrial metabolism. Furthermore, we highlighted microRNA-382 and showed that silencing of this microRNA leads to a collective downregulation of mitochondrial ribosomal protein expression, induces a mitonuclear protein imbalance and activates the mtUPR, previously associated with improved longevity.
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### New targets to control skeletal muscle inflammation: microRNAs regulated by adiponectin

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**Background and aims:** Low-grade pro-inflammatory state contributes to the metabolic syndrome (MS). Adiponectin (ApN), which is reduced in the MS, has emerged as a master regulator of inflammation/immunity. We wished to identify whether microRNAs (miRNAs) may mediate the anti-inflammatory action of ApN on skeletal muscle.
**Materials and methods:** miRNA expression profiling was performed in tibialis anterior muscles of ApN-knockout (ApN-KO) mice: one leg was electrotransferred with a plasmid coding for the ApN gene, while the contralateral leg received an empty plasmid and served as control. Mice were next challenged by lipopolysaccharide (LPS) to induce inflammation. Role of specific miRNAs was analyzed by gain-of or loss-of function approaches in C2C12 myotubes and in vivo by muscle electrotransfer. miRNA expression was also studied in human myotubes.
**Results:** Expression of miR-711 was up-regulated by muscle electrotransfer of ApN, which concomitantly reduced inflammation (TNFα, IL-1β) and oxidative stress (peroxiredoxin-3) markers. Likewise, in C2C12 cells, ApN treatment upregulated miR-711 expression. Transfection of miR-711 mimic reproduced the anti-inflammatory effects of ApN, while miR-711 blockade attenuated its protective effects. We found that miR-711 repressed the expression of 4 genes belonging to the Toll-like receptor-4 (TLR4) pathway. This pathway is activated by LPS and ultimately leads to stimulation of NF-kB, a pro-inflammatory transcription factor. As expected, NF-kB activity, measured via a luciferase reporter plasmid, was reduced by the miR mimic and enhanced by miR silencing. This protection against inflammation was recapitulated in ApN-KO mice by in vivo muscle electrotransfer of a plasmid coding for miR-711. Eventually, miR-711 expression was also upregulated in human myotubes after ApN treatment.
**Conclusion:** miR-711, which is up-regulated by ApN, represses TLR4 signaling and NF-kB, acting therefore as a major mediator of the anti-inflammatory action of ApN on muscle. This novel miRNA may open new therapeutic perspectives for the MS or other inflamed muscle conditions.
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