

Article

Effects of Low and High Maternal Protein Intake on Fetal Skeletal Muscle miRNAome in Sheep

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Simple Summary: Prenatal maternal feeding is critical for fetal development. miRNAs may impart unique metabolic and immunologic effects on developing fetuses. The fetal muscle miRNAome was found to be altered via maternal protein intake levels. oar-miR-3957-5p and miR-329a-3p were up-regulated in low- and standard-protein diets vs. high-protein diets. Many of the putative miRNA target genes and genetic pathways identified were involved in known muscle disease and immune function.



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Abstract: Prenatal maternal feeding plays an important role in fetal development and has the potential to induce long-lasting epigenetic modifications. MicroRNAs (miRNAs) are non-coding, single-stranded RNAs that serve as one epigenetic mechanism. Though miRNAs have crucial roles in fetal programming, growth, and development, there is limited data regarding the maternal diet and miRNA expression in sheep. Therefore, we analyzed high and low maternal dietary protein for miRNA expression in fetal *longissimus dorsi*. Pregnant ewes were fed an isoenergetic high-protein (HP, 160–270 g/day), low-protein (LP, 73–112 g/day), or standard-protein diet (SP, 119–198 g/day) during pregnancy. miRNA expression profiles were evaluated using the Affymetrix GeneChip miRNA 4.0 Array. Twelve up-regulated, differentially expressed miRNAs (DE miRNAs) were identified which are targeting 65 genes. The oar-3957-5p miRNA was highly up-regulated in the LP and SP compared to the HP. Previous transcriptome analysis identified that integrin and non-receptor protein tyrosine phosphatase genes targeted by miRNAs were detected in the current experiment. A total of 28 GO terms and 10 pathway-based gene sets were significantly ($p_{adj} < 0.05$) enriched in the target genes. Most genes targeted by the identified miRNAs are involved in immune and muscle disease pathways. Our study demonstrated that dietary protein intake during pregnancy affected fetal skeletal muscle epigenetics via miRNA expression.

Keywords: *Ovis aries*; nutrimiromics; dietary protein; prenatal life; gestation

1. Introduction

In mammals, maternal nutrition in pregnancy plays a critical role in intrauterine development, fetal survival, growth, and organogenesis [1,2]. Therefore, maternal under- or over-nutrition during gestation may cause low offspring growth and performance [3] with significant influence on the economic viability of the livestock industry [4,5].

Dietary nutrients, especially macronutrients, can influence the rate of gene transcription directly through interactions with the genomic regulatory elements or indirectly affecting and/or modulating important signaling pathways [6,7]. The understanding of how nutrition affects an individual's phenotype has greatly benefited from developments in molecular biology and genomics, and this area is called nutrigenomics. The term "epigenetics" describes heritable phenotypic alterations that occur without a change in the DNA sequence [8]. A few examples of epigenetic mechanisms are those that modify the chromatin structure, such as DNA methylation and histone modifications, as well as those that control the activity of proteins, like microRNAs (miRNAs). The term "Nutrimiomics" has been used to refer to the study of interactions between nutrients and food components with the organism's genome via modification of gene expression due to epigenetic processes related to miRNAs [9]. Due to an incomplete understanding of how nutrition affects fetal growth regulation mechanisms, intrauterine growth restriction (IUGR) persists as a significant issue in animal husbandry despite ongoing advancements in herd management and extensive research using contemporary analytical techniques into mammalian nutritional requirements [10–12]. An improperly balanced diet is the main culprit when it comes to livestock. It is interesting to note that intrauterine growth inhibition syndrome can result from either a high-protein or low-protein diet [13]. According to Meza-Herrera et al. [14], one of the primary causes of this occurrence is the delay in blastocyst development, brought on by the higher levels of hazardous protein breakdown products in the mother's bloodstream. A high-protein diet also raises the energy costs associated with hepatic gluconeogenesis and urine output, which limits the fetus's access to energy-rich substances. High-protein intake and elevated ammonia levels (up to 300 percent higher than the controls) in pregnant rats and sheep have been linked to reduced blastocyst development, impaired embryo metabolism, and fetal growth abnormalities [15,16]. When a pregnant woman consumes a high-protein diet, her amino acid profile shifts, and her concentrations of threonine, glutamine, glycine, alanine, and serine drop, which slows the formation of fetal tissue [17]. A high-protein diet was found to have an impact on the GH/JAK/STAT/IGF pathway in mice. This led to a reduction in placental growth hormone levels, which, in turn, prevented the fetus from gaining weight [18]. Reducing the amount of protein consumed through diet has the impact of decreasing the number of amino acids present in the mother's blood plasma, the fetus's blood plasma, and uterine fluid. According to Wu et al. [19], this ultimately results in the suppression of embryonic growth. Protein intake has a variety of nutritional and biological effects. Protein plays a vital role in the regulation of food intake, glucose and lipid metabolism, blood pressure, bone metabolism, and immunological function in addition to nutritional importance as a supply of amino acids for protein synthesis [20]. Proteins' physiological functions are influenced by their physico-chemical characteristics, amino acid make-up, and bioactive peptides encoded into their amino acid sequences [20]. Furthermore, research has been conducted on how proteins affect fetal and post-natal development [21]. To date, more experiments have focused on protein restriction compared to high protein regarding the maternal nutritional effects on the fetus and infant [22,23]. Blumfield and Collins [23] stated that an altered maternal protein intake during pregnancy might have detrimental effects on the offspring, and an optimal macronutrient ratio is crucial for the pre- and post-natal environments.

Additionally, protein content is one of the most expensive and limiting feed characteristics for livestock nutrition. As a feed ingredient, protein content is usually provided from human-edible ingredients like soybean, other oilseeds, and grains [24]. Thus, careful management of these limiting feedstuffs is critical for sustainable agriculture in the context of a growing world population and increasing livestock product demand [25]. Furthermore, balanced protein consumption may help to control nitrogen pollution to aid both environmental and economic sustainability [26].

Previous research has examined under-nutrition or over-nutrition conditions to test the effects of maternal nutrition on fetal development in livestock and model animals, including

the fetal programming of muscle and fat tissues [22,27,28]. However, few studies have examined the potential effects of maternal protein intake on the overall miRNA expression of fetal skeletal muscle. We have previously reported that maternal diets differing in protein content alter the fetal skeletal transcriptome, and that maternal high-protein diets, compared to standard-protein diets, trigger pathways related to the immune system and disease in sheep skeletal muscle [28]. On the other hand, the mechanisms by which these diets affect fetal miRNAome in sheep skeletal muscle are not known. The objective of this study was to investigate the impact of different maternal dietary protein levels during mid-to-late gestation on the total miRNA expression of fetal skeletal muscle in sheep, specifically to determine the consequences of the maternal dietary protein intake on fetuses throughout gestation on (1) the expression of whole genome skeletal muscle miRNAs; (2) biological pathways and gene ontology related to the miRNAome; and (3) the understanding of the connections between miRNAs and their targets.

2. Materials and Methods

2.1. Experimental Animals and Diets

Details of the ewes and diets were described previously [28]. Briefly, three distinct protein diets (standard-, high-, and low-protein) were used in the study to assess the effect of maternal dietary protein on the miRNAome of fetal skeletal muscle. Twelve Akkaraman ewes aged two were divided into three treatment groups at random, with four ewes in each group. Ewe and fetus specifications are presented in Table S1. After the empty ewes selected for the trial were brought from the pasture, an adaptation process was applied to get them accustomed to pen feeding. During the 15-day adaptation period, the ewe's daily dry matter (DM) feed consumption was assessed and compared with the US National Research Council (NRC) recommendations for ewes [29]. Following the adaptation period, synchronization and insemination were applied to the empty ewes. The ewes were fed a standard diet from day 0 to 30 of gestation according to the NRC recommendations [29]. Three alternative feed regimens with variable protein content were created, consisting of wheat straw, alfalfa, soybean meal, barley, and maize grain; alfalfa and soybean meal were the main protein sources, whereas barley and corn were the main sources of energy. Wheat straw and alfalfa were additional sources of cellulose and energy. The protein content of the diets was changed by substituting different amounts of soybean meal and alfalfa. During mid-to-late gestation (Day 31 to 105 of gestation), the ewes were fed the following diets: The standard-protein diet (SP) was a conventional diet with a NRC level of the nutritional content of feeds (the daily DM intake ranged from 1252 to 1862 g, crude protein ranged from 119 to 198 g, and kcal ME/kg ranged from 2506 to 3909 g); a low-protein diet (LP) (a daily DM intake of 1247–1726 g, 73–112 g crude protein, and 2520–3649 kcal ME/kg); and a high-protein diet (HP) (the daily DM intake ranged from 1252–1629, 160–271 g crude protein, and 2513–3880 kcal ME/kg). The chemical composition of the materials for the feed was ascertained in the feed analysis laboratory in accordance with AOAC (1990). Diets were made in an isoenergetic fashion. Specific information about the diets was reported previously [28].

2.2. Estrus Synchronization, Semen Preparation, and Artificial Insemination

During the annual mating season, estrus synchronization was achieved using progesterone-infused pessaries (EaziBreed CIDR; 1.38 mg progesterone; Zoetis, İstanbul, Türkiye) for 12 days, followed by 2 mL of PGF2 (5 mg/mL Dinoprost tromethamine; Dinolytic; Zoetis, İstanbul, Türkiye) treatment on day 12, and 400 IU of eCG (Folligon, 1000 IU/vial PMSG, MSD Animal Health İstanbul, Türkiye) at the time of pessary removal. The semen was extracted from four 1- to 3-year-old rams using the artificial vaginal procedure in the presence of estrus ewe. A phase-contrast microscope with a heated plate set at 37 °C was used to measure the motility of the artificial vaginal method-obtained sperm. For the insemination of ewes, the ejaculates of four rams with a motility of 70% or higher were combined. To achieve homogeneity, the appropriate ejaculates were blended based

on their spermatological motility. The density of the mixed ejaculates was determined using the hemocytometry method. To create 0.5 mL of semen containing 150×10^6 motile spermatozoa, the semen was diluted with Trisma base, consisting of d-fructose, citric acid, and skimmed milk [30] and kept in a 35 °C water bath for further use. Using diluted fresh semen, artificial insemination was carried out intravaginally 48 h following sponge removal.

2.3. Necropsy, Sample Collection, and Isolation of miRNA

On the 105th day of pregnancy, the dams had necropsies. A total of 14 fetuses were gathered from 12 dams. To avoid growth bias resulting from multiple births, twin birth fetuses were not included in the experiment. There were two twin pregnancies in the LP and SP groups, respectively. To make sure that each experimental group contained three fetuses, a fetus from the HP group was also removed. A sample of muscle tissue was taken from the fetal left *longissimus dorsi* muscles. Prior to miRNA extraction, all tissues were snap-frozen in liquid nitrogen and stored at −80 °C. Thirty milligrams of each skeletal muscle tissue were homogenized in QIAzol lysis reagent (Qiagen, Hilden, Germany) with liquid nitrogen using a homogenizer (Precellys Bertin, Montigny-le-Bretonneux, France). Using a miRNeasy mini kit (Qiagen, Hilden, Germany) in accordance with the manufacturer's instructions, total RNA containing miRNA was isolated from the tissue homogenates. The manufacturer's recommendations were followed for the on-column DNA digestion stage using the RNase-Free DNase Set (Qiagen, Hilden, Germany). With the help of a BioSpec-nano micro-volume UV–Vis spectrophotometer (Shimadzu Co., Kyoto, Japan), the purity and concentration of the total RNA were assessed.

2.4. Microarray Hybridization and Data Analysis

The miRNA profile was determined by the Affymetrix Microarray system using the GeneChip miRNA 4.0 Array (version 4.0.0.1567G) (Affymetrix, Santa Clara, CA, USA). The miRNA microarray contains 30,434 mature miRNAs from all organisms, miRBase set Release 20.0. Following the manufacturer's instructions, 500 ng of total RNA was labeled using an Affymetrix FlashTag Biotin HSR RNA Labeling Kit. Following hybridization, the chip arrays were washed and stained using AGCC Fluidics Control Software (version 1.4.1) on a Fluidics Station 450. After that, a high-resolution Affymetrix GeneChip Scanner 3000 was used to scan the array's fluorescence. The Affymetrix GeneChip Command Console program produced probe cell intensity files (*CEL files), which were imported into probe-level summary files (*CHP files) for data extraction. Software called the Expression Console and Transcriptome Analysis Console (version 4.0.2) were used to analyze these *CHP files. After image analysis, the data were normalized. The data were compared using tests, including the t-test, analysis of variance (ANOVA), and significance analysis of microarray after log₂ translation of the normalized signal intensities of the arrays. The identification of miRNA-targeted genes was performed using TargetScan [31] (<http://www.targetscan.org>; accessed on 8 January 2024), with the selection criteria of cumulative weighted value ≤ -0.75 for Targetscan. Genes targeted by multiple miRNAs were assembled from individual miRNA target lists. ConsensusPathDB [32] (<http://cpdb.molgen.mpg.de/>; accessed on 10 January 2024) was used to perform gene ontology (GO) annotation and enrichment analysis from three ontologies (molecular function, biological process, and cellular component) on the frequently identified target genes. The threshold for significance was set at $p < 0.01$ for both of these analyses. The predicted target gene lists were examined using the default collections of the KEGG, Reactome, and BioCarta (http://cgap.nci.nih.gov/Pathways/BioCarta_Pathways; accessed on 11 January 2024) databases.

3. Results

The expression profiles of miRNAs in the fetal *longissimus dorsi* muscle that were collected from ewes fed with the SP, HP, and LP were compared using the microarray

analysis. As presented in Table 1, 12 different miRNAs were differentially expressed (>2-fold, $p < 0.01$, FDR < 0.05) in the different diet groups, as per the Affymetrix GeneChip miRNA 4.0 Array. The chromosomal locations and mature miRNA sequences of these 12 prominent miRNAs identified using the Transcriptome Analysis Console commercial software (version 4.0.2) are shown in Table 1.

Table 1. The list of differentially expressed miRNAs with a fold change ≥ 2 and FDR = 0 in fetal skeletal muscles derived from HP, LP, and SP groups.

Transcript ID (Array Design)	miRBase ID	Fold Change	<i>p</i> -Value	Style	Mature Sequence	OAR
LP vs. HP						
oar-miR-3957-5p	MIMAT0019325	4.58	0.04	up	cucggagaguggagcugugggugu	18
oar-miR-329a-3p	MIMAT0019266	2.96	0.04	up	aacacaccugguuaacuuuuu	18
SP vs. HP						
oar-miR-3957-5p	MIMAT0019325	4.25	0.005	up	cucggagaguggagcugugggugu	18
oar-miR-432	MIMAT0001416	2.47	0.007	up	ucuuggaguaggucuuugggugg	18
oar-miR-200c	MIMAT0030044	2.24	0.009	up	uaauacugccgguaaugaugg	3
oar-miR-362	MIMAT0030060	2.24	0.01	up	aaucuuuggaaccuaggugugagu	X
oar-miR-409-3p	MIMAT0019328	2.2	0.01	up	cgaauuguugcucggugaaccccu	18
oar-let-7c	MIMAT0014964	2.18	0.01	up	ugagguaguagguuguaugguu	1
oar-miR-493-3p	MIMAT0019238	2.17	0.01	up	ugaaggucucacuguguccagg	18
oar-miR-181a	MIMAT0014973	2.12	0.02	up	aacauucaacgcugucggugagu	12
oar-miR-379-5p	MIMAT0019247	2.11	0.02	up	ugguagacuauuggaacguaggc	18
oar-miR-134-5p	MIMAT0019308	2.1	0.03	up	ugugacugguugaccagaggg	18
oar-miR-127	MIMAT0001415	2.04	0.04	up	aucggauccgucugagcuuggcu	18
SP vs. LP						
oar-miR-200c	MIMAT0030044	2.45	0.01	up	uaauacugccgguaaugaugg	3

We identified two significantly up-regulated miRNAs in the HP group compared to the SP group (Table 1). The two miRNAs were oar-miR-3957-5p and oar-miR-329a-3p. The fold change of those miRNAs was 4.58 and 2.96, respectively. The oar-miR-3957-5p miRNA was also up-regulated in the SP group compared to the HP group, with a fold change of 4.25. One of the remarkable results of our miRNAome analysis was the identification of nine miRNAs on OAR18, in which they clustered together in a tight locus (Figure S1). The oar-miR-200c miRNA showed up-regulation in the SP group compared to the LP group. The differentially expressed miRNAs were grouped using a hierarchical clustering technique, and the outcomes demonstrated that the various diet groups could be readily distinguished by the hierarchical grouping of all covered ovine miRNAs (Figure S2).

Next, we analyzed the target genes that may be regulated by these 12 miRNAs in two layers. In the first layer, we identified the genes targeted by multiple miRNAs (Table S2). The *in silico* analyses identified that 32 genes were found to be targeted by two miRNAs, and no mRNA was found to be targeted by three or more miRNAs (Table S2). In the second layer, a total of 65 genes were targeted by at least one of twelve miRNAs (Table S3). Hypergeometric distribution testing was used to examine the gene ontology (GO) of their anticipated target genes to investigate the roles of differentially regulated miRNAs among three distinct diet groups. The GO terms for the genes that were targeted by two miRNAs were identified as being related to 19 biological processes (BP) and six molecular functions (MF) (Table S4). The GO terms for the genes that were targeted by single miRNA were identified as being related to 20 biological processes, which were involved in the creation of five molecular functions and were expected to take part in three cellular components (CC) (Table S5).

The GO functional enrichment and pathway enrichment analyses were performed on 32 genes targeted by multiple miRNAs and separately on 65 genes targeted by at least

one miRNA (Tables S4 and S5). The GO terms showed that genes targeted by multiple miRNAs were mainly involved in BP lymphocyte chemotaxis, monocyte chemotaxis, the regulation of the nitrogen compound metabolic process, granulocyte chemotaxis, and other categories that control the immune system (Table S4). In terms of the MF, targeted mRNAs were involved in cytokine activity, cytokine receptor binding, and protein kinase regulator activity (Table S4). We conducted a pathway enrichment study using public pathway databases to better understand the roles of these projected target genes, and 31 pathways were found (Table S5). The pathway analysis showed that genes targeted by multiple miRNAs were significantly enriched in chemokine receptors bind chemokines, rheumatoid arthritis-*Homo sapiens* (human), viral protein interaction with cytokine and cytokine receptor-*Homo sapiens* (human), and the toll-like receptor signaling pathway-*Homo sapiens* (human) (Table S5).

For the GO functional enrichment analysis for the genes targeted by single mRNAs in terms of the BP, the primary categories for the functions were the cellular macromolecule biosynthetic process, macromolecule biosynthetic process, regulation of the biosynthetic process, and the cellular nitrogen compound biosynthetic process cell (Table S6). In terms of the MF, targeted mRNAs were involved in DNA binding, regulatory region nucleic acid binding, and metal ion binding (Table S6). In terms of the CC, targeted mRNAs were involved in the nuclear lumen, nucleus, and nucleoplasm (Table S6). The pathway analysis showed that genes targeted by single miRNAs were significantly enriched in rheumatoid arthritis-*Homo sapiens* (human), transferrin endocytosis and recycling, oncogene-induced senescence, and G1 to S cell-cycle control (Table S7).

4. Discussion

Proteins are vital for the structure, function, and regulation of the body's tissues and organs in all living organisms. In addition, they are the most expensive and often most limiting ingredients in livestock feed formulation [33]. Excess protein intake can increase nitrogen excretion, which can lead to unfavorable nitrate leakage into groundwater and emissions of ammonia into the atmosphere. [34]. Despite the importance of protein for all living organisms, ruminants use dietary protein for production rather inefficiently (as defined by g N in product/g N intake) [35]. This creates an opportunity to utilize technologies such as dynamically altering nutrient intakes via mid-infrared spectroscopy of milk composition [34,36]. Additionally, feedomics may assist in modifying nutrition for monetary and long-term gains. The key components of the feedomics study are nutrigenomics, nutrigenetics, and nutritional epigenetics, which aid in establishing a link between genetic variation and nutrient-driven epigenetic changes that are proposed as the main barrier to meeting nutritional demands [37]. Maternal nutrition is a major intrauterine environmental factor that alters the expression of the fetal genome in ways that may have lifelong consequences. Such fetal programming has been shown to be regulated by epigenetic modifications, including DNA methylation, histone modifications, as well as miRNAs [22].

In addition, we have identified that miR-3957-5p was up-regulated in both the LP and SP groups vs. the HP group (Table 1). Pokharel et al. [38] detected miR-3957-5p in the tissue biopsies of the corpus luteum and endometrium of pregnant ewes. Prior work demonstrated an abundance of miRNAs in packed form as extracellular vesicles to maintain feto-maternal communication in multiple mammals [39–41]. In the current study, together with miR-3957-5p, other miRNAs revealed a large cluster of miRNAs on OAR18 (Table 1), which may co-regulate different biological processes [42]. This is consistent with Pokharel et al. [38], who also detected similar miRNAs in the same clustered locus. This miRNA cluster is highly conserved among placental mammals and is known to be regulated by a maternally imprinted *DLK1-DIO3* region, which has been associated with severe muscle disease, which is caused by the impaired expression of dystrophin called Duchenne muscular dystrophy [43–45]. Additionally, two growth QTLs associated with sheep body weight [46] and average daily gain [47] were found to coincide with the miRNA cluster on OAR18. In the same experimental design subjected to the current study,

Sohel et al. [18] showed that a high-protein diet consumed by the mother predominantly changed the expression of mRNAs involved in myogenesis and immunity in the developing fetus's skeletal muscle. Both Shandilya et al. [48] and Sharma et al. [49,50] showed that sheep exposed to a bacterial lipopolysaccharide challenge exhibited differentially regulated miRNAs, such as miR-3957-5p, miR-329a-3p, and miR-379-5p (Table 1) which regulate biological processes such as stress and immune responses.

A critical layer of analysis involves miRNA targeting the same genes (Table S2). This is an established part of miRNA biology and a useful way to highlight important genes and pathways [51,52]. Multiple miRNAs regulating the same target gene are naturally cooperative since miRNAs act to inhibit targets; this heterotypic regulation is believed to offer extra regulatory specificity [53]. And especially, there is an ongoing debate over whether clustered miRNAs with different seed sequences co-evolved due to shared functions [53]. In the current study, two members of the miRNA cluster, which are oar-miR-3957-5p and oar-miR-432 (Table S2; Figure S1), are cooperatively targeting the calcium/calmodulin-dependent protein kinase IG (*CAMK1G*) and ras protein-specific guanine nucleotide releasing factor 1 (*RASGRF1*) genes. Calcium/calmodulin-dependent protein kinases are known to be key regulators of calcium signaling in health and disease [54], and it has been observed that T cells and B cells produce less IFN- γ when *CaMKIV* is inhibited or silent [55]. Furthermore, the experiments showed that in myoblasts and myotubes treated with IFN- γ , calcium/calmodulin-dependent protein kinase IV specifically suppresses the production of co-stimulatory molecules and pro-inflammatory cytokines/chemokines [56]. In terms of *RASGRF1*, it is an intrinsic key mediator for brain-derived neurotrophic factor (BDNF)-induced R-Ras activation and R-Ras-mediated axonal morphological regulation [57]. One of the nerve growth factor family members, BDNF, is primarily produced by the brain, and its main role involves synaptic modulation, neurogenesis, neuron survival, immune regulation, myocardial contraction, and angiogenesis in the brain [58]. On the other hand, previous experiments have shown that BDNF has a significant regulatory role in myogenic differentiation in skeletal muscle, which acts as a retrograde survival factor for motor neurons in the neuromuscular system [59,60]. Additionally, on the same miRNA cluster, oar-miR-379-5p and oar-miR-134-5p were found to be cooperatively targeting Glutamyl-TRNA Amidotransferase Subunit C (*GATC*) (Table S2). *GATC* was associated with mitochondrial respiratory dysfunction, which causes skeletal muscle atrophy in a state of uncontrolled inflammation and oxidative stress [61]. Multiple target analyses showed a complex network of mutual interactions between let-7c, miRNAs on various chromosomes, and mRNAs (Table S2). In the literature, the role of Let-7c and other Let-7 family members in myogenesis [62] and ovine fetal skeletal muscle development related to maternal obesity has been shown [63].

A second outcome involves the immune genes. Chemokine ligands (CCLs), *CCL3*, *CCL3L1*, and *CCL3L3* are all targeted by both oar-miR-432 and let-7c (Table S2). *CCL3* is critical for trophoblast invasion and the maintenance of pregnancy [64,65]. While others have studied dietary impacts on sheep, to our knowledge, this is the first demonstration that maternal diet protein intakes in the context of isoenergetic dietary formulations could impact *CCL3* and the maintenance of pregnancy. In addition, *IL7* is targeted by both oar-miR-432 and oar-miR-200c (Table S2). This is particularly striking because *IL7* influences the development of the offspring's T cell immunity [66,67]. These examples highlight the importance of maternal protein intake on multiple critical outcomes for fetal health and lifetime well-being.

Rheumatoid arthritis-*Homo sapiens* (human) pathways were identified in different protein intake groups among both layers of the target prediction (Tables S3 and S5). Rheumatoid arthritis (RA) is a chronic, inflammatory, systemic autoimmune disease that causes skeletal muscle loss and weakness in RA individuals [68,69]. Similarly, transferrin endocytosis and recycling pathways are associated with iron homeostasis, a key regulator of innate and adaptive immunity, and play a decisive role in inflammatory processes [70]. These findings were consistent with the findings of the transcriptome analysis [28], which

revealed that many detected genes are involved in the pathways related to the immune system and diseases.

5. Conclusions

The findings of our research suggested that maternal diets of different protein levels affected fetal miRNAome, which epigenetically affects the skeletal muscle transcriptome in vivo. The discovery of multiple miRNAs that target mRNAs is a significant development that may enable us to further understand the network that connects miRNAs and their targets. Since the maternal HP diet specifically changed the expression of the miRNAs involved in a number of pathways and biological processes that are unique to disease and immunity, these results might have provided insight into the reversible nature of epigenetic modifications. New studies are warranted for the expression levels of already detected fetal miRNAs in different environmental conditions, breeds, etc., in the offspring.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/ani14111594/s1>, Figure S1: miRNA cluster on OAR18; Figure S2: Heat map of the microarray miRNA expression profile in fetal skeletal muscle derived from high-, low-, and standard-protein groups; Table S1: Ewe and fetus specifications; Table S2: List of targeted mRNAs by single miRNA; Table S3: List of targeted mRNAs by multiple miRNA; Table S4: GO terms of 32 genes targeted by multiple miRNAs with ConsensusPathDB (category level 4) (adjusted $p < 0.01$); Table S5: Pathway analysis of 32 genes targeted by multiple miRNAs with ConsensusPathDB (adjusted $p < 0.01$); Table S6: GO terms of 65 genes with ConsensusPathDB (category level 4) (adjusted $p < 0.01$); Table S7: Pathway analysis of 65 genes with ConsensusPathDB (adjusted $p < 0.01$).

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Informed Consent Statement: Not applicable.

Data Availability Statement: All data generated and analyzed in this study are presented in the figures/tables and Supplementary Information documents.

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References

1. Ji, Y.; Wu, Z.; Dai, Z.; Wang, X.; Li, J.; Wang, B.; Wu, G. Fetal and Neonatal Programming of Postnatal Growth and Feed Efficiency in Swine. *J. Anim. Sci. Biotechnol.* **2017**, *8*, 42. [CrossRef]
2. Lin, Y.J.; Huang, L.T.; Tsai, C.C.; Sheen, J.M.; Tiao, M.M.; Yu, H.R.; Lin, I.C.; Tain, Y.L. Maternal High-fat Diet Sex-Specifically Alters Placental Morphology and Transcriptome in Rats: Assessment by Next-Generation Sequencing. *Placenta* **2019**, *78*, 44–53. [CrossRef]
3. Hoffman, M.L.; Reed, S.A.; Pillai, S.M.; Jones, A.K.; McFadden, K.K.; Zinn, S.A.; Govoni, K.E. Physiology and Endocrinology Symposium: The Effects of Poor Maternal Nutrition During Gestation on Offspring Postnatal Growth and Metabolism. *J. Anim. Sci.* **2017**, *95*, 2222–2232. [CrossRef]

4. Du, M.; Tong, J.; Zhao, J.; Underwood, K.R.; Zhu, M.; Ford, S.P.; Nathanielsz, P.W. Fetal Programming of Skeletal Muscle Development in Ruminant Animals. *J. Anim. Sci.* **2010**, *88*, E51–E60. [[CrossRef](#)]
5. Du, M.; Wang, B.; Fu, X.; Yang, Q.; Zhu, M.J. Fetal Programming in Meat Production. *Meat Sci.* **2015**, *109*, 40–47. [[CrossRef](#)] [[PubMed](#)]
6. Sohel, M.M.H. Macronutrient Modulation of mRNA and microRNA Function in Animals: A Review. *Anim. Nutr.* **2020**, *6*, 258–268. [[CrossRef](#)] [[PubMed](#)]
7. Sohel, M.M.H.; Konca, Y.; Cinar, M.U. Impacts of Macronutrients on Gene Expression: Recent Evidence to Understand Productive and Reproductive Performance of Livestock. *Turk. J. Agric. -Food Sci. Technol.* **2018**, *6*, 203–212. [[CrossRef](#)]
8. Ho, D.H.; Burggren, W.W. Epigenetics and Transgenerational Transfer: A Physiological Perspective. *J. Exp. Biol.* **2010**, *213*, 3–16. [[CrossRef](#)]
9. Quintanilha, B.J.; Reis, B.Z.; Duarte, G.B.S.; Cozzolino, S.M.; Rogero, M.M. Nutrimiomics: Role of microRNAs and Nutrition in Modulating Inflammation and Chronic Diseases. *Nutrients* **2017**, *9*, 1168. [[CrossRef](#)]
10. Wu, G.; Bazer, F.W.; Wallace, J.M.; Spencer, T.E. Board-Invited Review: Intrauterine Growth Retardation: Implications for the Animal Sciences. *J. Anim. Sci.* **2006**, *84*, 2316–2337. [[CrossRef](#)]
11. McMillen, I.C.; Robinson, J.S. Developmental Origins of the Metabolic Syndrome: Prediction, Plasticity, and Programming. *Physiol. Rev.* **2005**, *85*, 571–633. [[CrossRef](#)]
12. Murphy, V.E.; Smith, R.; Giles, W.B.; Clifton, V.L. Endocrine Regulation of Human Fetal Growth: The Role of the Mother, Placenta, and Fetus. *Endocr. Rev.* **2006**, *27*, 141–169. [[CrossRef](#)]
13. Mickiewicz, M.; Zabielski, R.; Grenier, B.; Le Normand, L.; Savary, G.; Holst, J.J.; Guilloteau, P. Structural and Functional Development of Small Intestine in Intrauterine Growth Retarded Porcine Offspring Born to Gilts Fed Diets with Differing Protein Ratios Throughout Pregnancy. *J. Physiol. Pharmacol.* **2012**, *63*, 225.
14. Meza-Herrera, C.A.; Ross, T.T.; Hallford, D.M.; Hawkins, D.E.; Gonzalez-Bulnes, A. High Periconceptional Protein Intake Modifies Uterine and Embryonic Relationships Increasing Early Pregnancy Losses and Embryo Growth Retardation in Sheep. *Reprod. Domest. Anim.* **2010**, *45*, 723–728. [[CrossRef](#)] [[PubMed](#)]
15. Lane, M.; Gardner, D.K. Ammonium Induces Aberrant Blastocyst Differentiation, Metabolism, pH Regulation, Gene Expression and Subsequently Alters Fetal Development in the Mouse. *Biol. Reprod.* **2003**, *69*, 1109–1117. [[CrossRef](#)] [[PubMed](#)]
16. Gardner, D.K.; Hewitt, E.; Linck, D. Diet Affects Embryo Imprinting and Fetal Development. *Hum. Reprod.* **2004**, *19*, i27.
17. Rehfeldt, C.; Lang, I.S.; Görs, S.; Hennig, U.; Kalbe, C.; Stabenow, B.; Metges, C.C. Limited and Excess Dietary Protein During Gestation Affects Growth and Compositional Traits in Gilts and Impairs Offspring Fetal Growth. *J. Anim. Sci.* **2011**, *89*, 329–341. [[CrossRef](#)]
18. Vanselow, J.; Kucia, M.; Langhammer, M.; Koczan, D.; Rehfeldt, C.; Metges, C.C. Hepatic Expression of the GH/JAK/STAT/IGF Pathway, Acute-Phase Response Signalling and Complement System are Affected in Mouse Offspring by Prenatal and Early Postnatal Exposure to Maternal High-Protein Diet. *Eur. J. Nutr.* **2011**, *50*, 611–623. [[CrossRef](#)] [[PubMed](#)]
19. Wu, G.; Pond, W.G.; Ott, T.; Bazer, F.W. Maternal Dietary Protein Deficiency Decreases Amino Acid Concentrations in Fetal Plasma and Allantoic Fluid of Pigs. *J. Nutr.* **1998**, *128*, 894–902. [[CrossRef](#)]
20. Jahan-Mihan, A.; Luhovyy, B.L.; Khoury, D.E.; Anderson, G.H. Dietary Proteins as Determinants of Metabolic and Physiologic Functions of the Gastrointestinal Tract. *Nutrients* **2011**, *3*, 574–603. [[CrossRef](#)]
21. Jahan-Mihan, A.; Rodriguez, J.; Christie, C.; Sadeghi, M.; Zerbe, T. The Role of Maternal Dietary Proteins in Development of Metabolic Syndrome in Offspring. *Nutrients* **2015**, *7*, 9185–9217. [[CrossRef](#)] [[PubMed](#)]
22. Meruvu, S.; Schutz, L.F.; Choudhury, M. Nutritional Influence on miRNA Epigenetic Regulation: Effect of Maternal Diet and miRNAs on the Fetal Metabolic Programming. In *Molecular Nutrition: Mother and Infant*, 1st ed.; Vinciguerra, M., Cordero Sanchez, P., Eds.; Academic Press: Cambridge, MA, USA, 2021; pp. 401–420.
23. Blumfield, M.L.; Collins, C.E. High-Protein Diets During Pregnancy: Healthful or Harmful for Offspring? *Am. J. Clin. Nutr.* **2014**, *100*, 993–995. [[CrossRef](#)] [[PubMed](#)]
24. Kim, S.W.; Less, J.F.; Wang, L.; Yan, T.; Kiron, V.; Kaushik, S.J.; Lei, X.G. Meeting Global Feed Protein Demand: Challenge, Opportunity, and Strategy. *Annu. Rev. Anim. Biosci.* **2019**, *7*, 221–243. [[CrossRef](#)] [[PubMed](#)]
25. Rauw, W.M.; Rydhmer, L.; Kyriazakis, I.; Øverland, M.; Gilbert, H.; Dekkers, J.C.; Gomez-Raya, L. Prospects for Sustainability of Pig Production in Relation to Climate Change and Novel Feed Resources. *J. Sci. Food Agric.* **2020**, *100*, 3575–3586. [[CrossRef](#)] [[PubMed](#)]
26. Almaraz, M.; Kuempel, C.D.; Salter, A.M.; Halpern, B.S. The Impact of Excessive Protein Consumption on Human Wastewater Nitrogen Loading of US Waters. *Front. Ecol. Environ.* **2022**, *20*, 452–458. [[CrossRef](#)]
27. Kizilaslan, M.; Arzik, Y.; Cinar, M.U.; Konca, Y. Genome-Wise Engineering of Ruminant Nutrition–Nutrigenomics: Applications, Challenges, and Future Perspectives—A Review. *Ann. Anim. Sci.* **2021**, *22*, 511–521. [[CrossRef](#)]
28. Sohel, M.M.H.; Akyuz, B.; Konca, Y.; Arslan, K.; Gurbulak, K.; Abay, M.; Cinar, M.U. Differential Protein Input in the Maternal Diet Alters the Skeletal Muscle Transcriptome in Fetal Sheep. *Mamm. Genom.* **2020**, *31*, 309–324. [[CrossRef](#)]
29. NRC: *Nutrient Requirements of Small Ruminants*; National Academy Press: Washington, DC, USA, 2007.
30. Paulenz, H.; Ådnøy, T.; Fossen, O.H.; Söderquist, L.; Andersen Berg, K. Effect of Deposition Site and Sperm Number on the Fertility of Sheep Inseminated with Liquid Semen. *Vet. Rec.* **2002**, *150*, 299–302. [[CrossRef](#)] [[PubMed](#)]

31. McGeary, S.E.; Lin, K.S.; Shi, C.Y.; Pham, T.M.; Bisaria, N.; Kelley, G.M.; Bartel, D.P. The Biochemical Basis of microRNA Targeting Efficacy. *Science* **2019**, *366*, eaav1741. [\[CrossRef\]](#)
32. Herwig, R.; Hardt, C.; Lienhard, M.; Kamburov, A. Analyzing and Interpreting Genome Data at the Network Level with ConsensusPathDB. *Nat. Protoc.* **2016**, *11*, 1889–1907. [\[CrossRef\]](#)
33. Parisi, G.; Tulli, F.; Fortina, R.; Marino, R.; Bani, P.; Dalle Zotte, A.; Danieli, P.P. Protein hunger of the feed sector: The alternatives offered by the plant world. *Ital. J. Anim. Sci.* **2020**, *19*, 1204–1225. [\[CrossRef\]](#)
34. Moorby, J.M.; Fraser, M.D. New Feeds and New Feeding Systems in Intensive and Semi-Intensive Forage-Fed Ruminant Livestock Systems. *Animal* **2021**, *15*, 100297.
35. Calsamiglia, S.; Ferret, A.; Reynolds, C.K.; Kristensen, N.B.; Van Vuuren, A.M. Strategies for Optimizing Nitrogen Use by Ruminants. *Animal* **2010**, *4*, 1184–1196. [\[CrossRef\]](#) [\[PubMed\]](#)
36. Gengler, N.; Soyeurt, H.; Dehareng, F.; Bastin, C.; Colinet, F.; Hammami, H.; Dardenne, P. Capitalizing on Fine Milk Composition for Breeding and Management of Dairy Cows. *J. Dairy Sci.* **2016**, *99*, 4071–4079. [\[CrossRef\]](#)
37. Preethi, K.A.; Sekar, D. Dietary microRNAs: Current Status and Perspective in Food Science. *J. Food Biochem.* **2021**, *45*, e13827. [\[CrossRef\]](#) [\[PubMed\]](#)
38. Pokharel, K.; Peippo, J.; Li, M.H.; Kantanen, J. Identification and Characterization of miRNAs During Early Pregnancy in Domestic Sheep. *Anim. Genet.* **2020**, *51*, 833–836. [\[CrossRef\]](#) [\[PubMed\]](#)
39. Kaczmarek, M.M.; Najmula, J.; Guzewska, M.M.; Przygodzka, E. miRNAs in the Peri-Implantation Period: Contribution to Embryo–Maternal Communication in Pigs. *Int. J. Mol. Sci.* **2020**, *21*, 2229. [\[CrossRef\]](#)
40. Morales-Prieto, D.M.; Favaro, R.R.; Markert, U.R. Placental miRNAs in Feto-Maternal Communication Mediated by Extracellular Vesicles. *Placenta* **2020**, *102*, 27–33. [\[CrossRef\]](#)
41. Salilew-Wondim, D.; Gebremedhn, S.; Hoelker, M.; Tholen, E.; Hailay, T.; Tesfaye, D. The Role of Micrnas in Mammalian Fertility: From Gametogenesis to Embryo Implantation. *Int. J. Mol. Sci.* **2020**, *21*, 585. [\[CrossRef\]](#)
42. Kabekkodu, S.P.; Shukla, V.; Varghese, V.K.; D'Souza, J.; Chakrabarty, S.; Satyamoorthy, K. Clustered miRNAs and Their Role in Biological Functions and Diseases. *Biol. Rev.* **2018**, *93*, 1955–1986. [\[CrossRef\]](#)
43. Hong, A.V.; Bourg, N.; Sanatine, P.; Poupiot, J.; Charton, K.; Gicquel, E.; Israeli, D. Dlk1-Dio3 Cluster miRNAs Regulate Mitochondrial Functions in the Dystrophic Muscle in Duchenne Muscular Dystrophy. *Life Sci. Alliance* **2023**, *6*, e202201506. [\[CrossRef\]](#) [\[PubMed\]](#)
44. Sanson, M.; Vu Hong, A.; Massourides, E.; Bourg, N.; Suel, L.; Amor, F.; Israeli, D. miR-379 Links Glucocorticoid Treatment with Mitochondrial Response in Duchenne Muscular Dystrophy. *Sci. Rep.* **2020**, *10*, 9139. [\[CrossRef\]](#) [\[PubMed\]](#)
45. Amor, F.; Vu Hong, A.; Corre, G.; Sanson, M.; Suel, L.; Blaie, S.; Israeli, D. Cholesterol Metabolism is a Potential Therapeutic Target in Duchenne Muscular Dystrophy. *J. Cachexia Sarcopenia Muscle* **2021**, *12*, 677–693. [\[CrossRef\]](#) [\[PubMed\]](#)
46. Walling, G.A.; Visscher, P.M.; Wilson, A.D.; McTeir, B.L.; Simm, G.; Bishop, S.C. Mapping of Quantitative Trait Loci for Growth and Carcass Traits in Commercial Sheep Populations. *J. Anim. Sci.* **2004**, *82*, 2234–2245. [\[CrossRef\]](#) [\[PubMed\]](#)
47. Zhang, L.; Liu, J.; Zhao, F.; Ren, H.; Xu, L.; Lu, J.; Du, L. Genome-Wide Association Studies for Growth and Meat Production Traits in Sheep. *PLoS ONE* **2013**, *8*, e66569. [\[CrossRef\]](#)
48. Shandilya, U.K.; Sharma, A.; Naylor, D.; Canovas, A.; Mallard, B.; Karrow, N.A. Expression Profile of miRNA from High, Middle, and Low Stress-Responding Sheep During Bacterial Endotoxin Challenge. *Animals* **2023**, *13*, 508. [\[CrossRef\]](#) [\[PubMed\]](#)
49. Sharma, A.; Shandilya, U.K.; Sullivan, T.; Naylor, D.; Canovas, A.; Mallard, B.A.; Karrow, N.A. Identification of Ovine Serum miRNAs Following Bacterial Lipopolysaccharide Challenge. *Int. J. Mol. Sci.* **2020**, *21*, 7920. [\[CrossRef\]](#) [\[PubMed\]](#)
50. Sharma, A.; Shandilya, U.K.; Sullivan, T.; Naylor, D.; Cánovas, A.; Mallard, B.; Karrow, N. PSXIV-20 Ovine Circulatory Markers Regulating the Acute-phase Response of Variable Stress Responding Sheep to LPS Challenge. *J. Anim. Sci.* **2021**, *99*, 494–495. [\[CrossRef\]](#)
51. Peter, M.E. Targeting of mRNAs by Multiple miRNAs: The Next Step. *Oncogene* **2010**, *29*, 2161–2164. [\[CrossRef\]](#)
52. Wu, S.; Huang, S.; Ding, J.; Zhao, Y.; Liang, L.; Liu, T.; He, X. Multiple microRNAs Modulate p21Cip1/Waf1 Expression by Directly Targeting Its 3' Untranslated Region. *Oncogene* **2010**, *29*, 2302–2308. [\[CrossRef\]](#)
53. DeVeale, B.; Swindlehurst-Chan, J.; Billeloch, R. The Roles of microRNAs in Mouse Development. *Nat. Rev. Genet.* **2021**, *22*, 307–323. [\[CrossRef\]](#) [\[PubMed\]](#)
54. Junho, C.V.C.; Caio-Silva, W.; Trentin-Sonoda, M.; Carneiro-Ramos, M.S. An Overview of the Role of Calcium/Calmodulin-Dependent Protein Kinase in Cardiorenal Syndrome. *Front. Physiol.* **2020**, *11*, 735. [\[CrossRef\]](#) [\[PubMed\]](#)
55. Ichinose, K.; Juang, Y.T.; Crispin, J.C.; Kis-Toth, K.; Tsokos, G.C. Suppression of Autoimmunity and Organ Pathology in Lupus-prone Mice Upon Inhibition of Calcium/Calmodulin-Dependent Protein Kinase Type IV. *Arthritis Rheum.* **2011**, *63*, 523–529. [\[CrossRef\]](#)
56. Gu, R.; Ding, M.; Shi, D.; Huang, T.; Guo, M.; Yu, L.; Hu, J.; Huang, W.; Liao, H. Calcium/Calmodulin-Dependent Protein Kinase IV Mediates IFN- γ -Induced Immune Behaviors in Skeletal Muscle Cells. *Cell. Physiol. Biochem.* **2018**, *46*, 351–364. [\[CrossRef\]](#) [\[PubMed\]](#)
57. Umeda, K.; Negishi, M.; Katoh, H. RasGRF1 Mediates Brain-Derived Neurotrophic Factor-Induced Axonal Growth in Primary Cultured Cortical Neurons. *Biochem. Biophys. Rep.* **2018**, *17*, 56–64. [\[CrossRef\]](#) [\[PubMed\]](#)
58. Rentería, I.; García-Suárez, P.C.; Fry, A.C.; Moncada-Jiménez, J.; Machado-Parra, J.P.; Antunes, B.M.; Jiménez-Maldonado, A. The Molecular Effects of BDNF Synthesis on Skeletal Muscle: A Mini-Review. *Front. Physiol.* **2022**, *13*, 934714. [\[CrossRef\]](#)

59. Mousavi, K.; Jasmin, B.J. BDNF is Expressed in Skeletal Muscle Satellite Cells and Inhibits Myogenic Differentiation. *J. Neurosci.* **2006**, *26*, 5739–5749. [[CrossRef](#)] [[PubMed](#)]
60. Mousavi, K.; Parry, D.J.; Jasmin, B.J. BDNF Rescues Myosin Heavy Chain IIB Muscle Fibers after Neonatal Nerve Injury. *Am. J. Physiol. Cell Physiol.* **2004**, *287*, C22–C29. [[CrossRef](#)] [[PubMed](#)]
61. Chen, X.; Ji, Y.; Liu, R.; Zhu, X.; Wang, K.; Yang, X.; Liu, B.; Gao, Z.; Huang, Y.; Shen, Y.; et al. Mitochondrial Dysfunction: Roles in Skeletal Muscle Atrophy. *J. Transl. Med.* **2023**, *21*, 503. [[CrossRef](#)]
62. Papaioannou, G.; Inloes, J.B.; Nakamura, Y.; Paltrinieri, E.; Kobayashi, T. let-7 and miR-140 microRNAs Coordinately Regulate Skeletal Development. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, E3291–E3300. [[CrossRef](#)]
63. Yan, X.; Huang, Y.; Zhao, J.X.; Rogers, C.J.; Zhu, M.J.; Ford, S.P.; Nathanielsz, P.W.; Du, M. Maternal Obesity Downregulates microRNA let-7g Expression, A Possible Mechanism for Enhanced Adipogenesis during Ovine Fetal Skeletal Muscle Development. *Int. J. Obes.* **2013**, *37*, 568–575. [[CrossRef](#)] [[PubMed](#)]
64. Jones, A.K.; Hoffman, M.L.; Pillai, S.M.; McFadden, K.K.; Govoni, K.E.; Zinn, S.A.; Reed, S.A. Gestational Restricted-and Over-feeding Promote Maternal and Offspring Inflammatory Responses That are Distinct and Dependent on Diet in Sheep. *Biol. Rep.* **2018**, *98*, 184–196. [[CrossRef](#)] [[PubMed](#)]
65. Zhang, S.; Ding, J.; Zhang, Y.; Liu, S.; Yang, J.; Yin, T. Regulation and Function of Chemokines at the Maternal–Fetal Interface. *Front. Cell Dev. Biol.* **2022**, *10*, 826053. [[CrossRef](#)] [[PubMed](#)]
66. Ngom, P.T.; Collinson, A.C.; Pido-Lopez, J.; Henson, S.M.; Prentice, A.M.; Aspinall, R. Improved Thymic Function in Exclusively Breastfed Infants Is Associated with Higher Interleukin 7 Concentrations in Their Mothers' Breast Milk. *Am. J. Clin. Nutr.* **2004**, *80*, 722–728. [[CrossRef](#)] [[PubMed](#)]
67. Aspinall, R.; Prentice, A.M.; Ngom, P.T. Interleukin 7 from Maternal Milk Crosses the Intestinal Barrier and Modulates T-Cell Development in Offspring. *PLoS ONE* **2011**, *6*, e20812. [[CrossRef](#)] [[PubMed](#)]
68. Bennett, J.L.; Pratt, A.G.; Dodds, R.; Sayer, A.A.; Isaacs, J.D. Rheumatoid Sarcopenia: Loss of Skeletal Muscle Strength and Mass in Rheumatoid Arthritis. *Nat. Rev. Rheumatol.* **2023**, *19*, 239–251. [[CrossRef](#)] [[PubMed](#)]
69. Steinz, M.M.; Santos-Alves, E.; Lanner, J.T. Skeletal Muscle Redox Signaling in Rheumatoid Arthritis. *Clin. Sci.* **2020**, *134*, 2835–2850. [[CrossRef](#)]
70. Rossatti, P.; Redpath, G.M.; Ziegler, L.; Samson, G.P.; Clamagirand, C.D.; Legler, D.F.; Rossy, J. Rapid Increase in Transferrin Receptor Recycling Promotes Adhesion during T Cell Activation. *BMC Biol.* **2011**, *20*, 189. [[CrossRef](#)]

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