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Review

The Effects of Anabolic-Androgenic Steroids on Gene Expression in Skeletal Muscle: A Systematic Review

LUKE M. PELTON†, STEPHEN A. MARIS‡, and JOSHUA LOSEKE†

Department of Exercise Science and Athletic Training, Springfield College, Springfield, MA, USA

†Denotes graduate student author, ‡Denotes professional author

ABSTRACT

International Journal of Exercise Science 16(3): 53-82, 2023. Anabolic-androgenic steroids (AAS) act via androgen receptor (AR) interaction to induce muscle protein synthesis. This process is achieved via altered gene expression via the Notch, Wnt, and Numb pathways and their interactions at the AR, manifesting in key skeletal muscle (SM) phenotypes such as morphology, ion conductance, and functionality. This review aims to report on the effects of AAS administration on gene expression in SM. Peer-reviewed empirical studies evaluating AAS administration on SM phenotypes and gene expression were considered for inclusion. The following databases were searched using a data range of Jan 2000-November 2020: MEDLINE Complete, Academic Search Complete, APA PsycInfo, SPORTDiscus, CINAHL Plus, Cochrane Central Register of Controlled Trials, Rehabilitation & Sports Medicine Source, GreenFILE, and APA PsycArticles. Potential risks of bias were assessed via a modified PEDro Scale. Twenty-nine peer-reviewed titles were included. All studies consisted of either human or rodent subjects and included an AAS dosing protocol, investigated SM phenotypes, and measured gene expression as an outcome variable. Studies investigated the effects of eight AAS compounds across a total of 88 different genes in SM. The most commonly identified genes increased by AAS were IGF, MYOG, and MyoD. There was a general lack of standardized dosing and AAS variety. Future studies should attempt to incorporate multiple AAS compounds and their effects on key SM gene expression.

KEY WORDS: Endocrinology, epigenetics, reproductive health

INTRODUCTION

The complexity of skeletal muscle (SM) tissue is directly characterized by the diversity of factors impacting its growth and development (8). SM hypertrophy may be induced by mechanotransduction, the activation of insulin-like growth factor-1 (IGF1) signaling, and downstream phosphatidylinositol 3-kinase (PI3k) signaling (16). Additional anabolic signaling may be induced by circulating steroid sex hormones such as the androgen testosterone. Testosterone produces myotropic effects directly via binding and activation of androgen receptors (AR), ligand-inducible transcription factors localized to the cytoplasmic compartment of target cells which regulate target gene transcription in the muscle cell nucleus resulting in

enhanced muscle protein synthesis (MPS; 8, 10, 19). Further, testosterone may also undergo either 5α-reduction to dihydrotestosterone (DHT) or aromatization to estradiol (E2), both of which mediate signaling pathways responsible for promoting muscle growth (5).

The signaling proteins Notch, Wnt, and Numb govern many of these myogenic events surrounding growth and development. Notch mediates cell proliferation, cell fates, apoptosis, cellular migration and adhesion, and stem cell maintenance during development and homeostasis (12, 17). These events are mediated by Notch's conduction of intercellular communication via interaction with ligands on neighboring cells (12). In the canonical Notch pathway, the binding of ligands to the Notch receptor causes the translocation of the Notch intracellular domain (NICD) to the cytoplasm, wherein it undergoes sequential enzymatic cleavage and translocates to the cell nucleus (12, 21). Here, it binds to a CSL transcription factor forming a bound complex which then activates the transcription of target genes such as Hes and Hey (12, 21). As it is critical to satellite cell activation and proliferation, this pathway plays an integral role in tissue morphogenesis and the repair of SM (21, 22). Androgens are able to modulate the activities of Notch (22).

Wnt regulates cell fate determination, cell proliferation, stem cell proliferation, tissue homeostasis, and cell polarity, making it a key regulator of myogenic differentiation and myogenic stem cell fate in adults (12, 17, 22, 24). Wnt acts directly on muscle stem cells to control the progression of the myogenic lineage and thereby promoting muscle development and tissue repair (22). In the canonical Wnt signaling pathway, the Wnt ligand binds to Fz and LRP wherein it activates downstream signal transduction β-catenin, an integral transcription regulator of growth and development, to induce the expression of target genes (12).

The frequent collaboration of the signaling proteins Notch and Wnt and their associated pathways are critical to overall mammalian development, representing the major pathway governing the regulation of numerous cellular processes and developmental and homeostatic events from zygote to complex organisms (12, 17, 22). A proposed factor of this collaborative cross-talk is the signaling protein Numb (22). Numb belongs to a broader family of proteins (which includes Numb and Numb-Like, or NumbL) with important roles in a large variety of cellular processes including cell adhesion and ubiquitination (13). Of particular note is Numb's role in asymmetric cell division, which facilitates differentiation of myogenic lineage stem cells (13, 22). Numb also inhibits the Notch pathway via interaction with NICD and is linked to the Wnt pathway via polyubiquitination of β-catenin, causing its degradation (13, 22). This decline in Notch signaling is necessary for the differentiation of progenitor cells into fusion component myoblasts (21).

Androgen signaling at the AR contributes to the regulation of body composition (22). The administration of androgens and their analogs promotes differentiation of the mesenchymal stem cells into the myogenic lineage (22). Crosstalk between Wnt and AR has been demonstrated (22). This is partly facilitated by the co-localization of the AR and β-catenin in the cell nucleus; further, β-catenin preferentially binds to the AR over several other steroid hormone receptors

including those of estrogen, progesterone, and glucocorticoids (22). B-catenin's role as a coactivator of the AR causes a forced overexpression of β-catenin to augment AR-mediated transcription (22). This signaling through the AR has also been observed to interact with Notch signaling (22). Androgens increase Notch in SM, which further increases Notch signaling (22). Conversely, the synthetic androgen analog Nandrolone Decanoate (ND) has been observed to reduce Notch activity while increasing Numb activity, highlighting the discrepancies of androgens on target outcomes (22).

The breakdown of these growth processes may result in atrophy, the loss of SM. Atrophy occurs due to multiple factors including age, disease, and reductions in anabolic stimuli, including unloading due to conditions such as microgravity, immobilization, or spinal cord injury (2, 21, 38, 40). These conditions often act via the ubiquitin-proteasome system, the Ca^{++}-dependent calpains, and the lysosomal cathepsins pathway, which in combination lead to muscle protein breakdown and eventual atrophy (3). Conditions of atrophy are marked by increased transcription and expression of genes such as atrogin1 and MuRF-1 (39). Reductions in anabolic agents such as testosterone that occur with age and lifestyle, known as "andropause," may heavily contribute to reduced muscle mass and strength, especially in males (14, 42). Hypogonadal men have been reported to exhibit three-fold increases in mortality and five-fold increases in the risk of cancer-related death (14). The rescue of hypogonadism/andropause is often accomplished via the administration of exogenous testosterone or its pharmacological congeners, collectively known as Anabolic-Androgenic Steroids (AAS; 14, 39).

AAS are synthetic exogenous hormonal compounds that mimic or enhance the physiological action of naturally occurring steroid hormones such as testosterone (1, 29). When taken in supra-physiologic doses, such as is seen in both amateur and professional athletes such as bodybuilders, AAS can greatly aid in increasing muscle hypertrophy and force production by increasing the rate of MPS and thus recovery from heavy resistance training (20, 29). The anabolic effects of AAS are mediated largely in part by altered regulation of the transcription of target genes responsible for the binding of DNA required to stimulate MPS activation by SM ARs, much in the same manner as endogenous androgens (20, 29). Further, AAS administration has been observed to have a dose-dependent effect, with larger doses resulting in greater anabolic effects (16).

Previous research has identified AAS to be efficacious countermeasures against muscle wasting in various disease states or unloading conditions, as well as an extremely effective anabolic agent when taken in supra-physiologic doses (3, 29). To understand the specific pathways associated with these changes, many studies have attempted to identify key genes involved in SM phenotypes and their interactions with the administration of AAS. However, limited reviews have been completed to summarize the results of these studies to highlight which genes have been identified. Thus, the aims of this review are to 1) analyze and summarize the current research about the effects of AAS administration on gene expression in SM, and 2) provide a summary of the genes identified that are most heavily impacted by AAS administration.

METHODS

This review followed the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) guidelines (26). This research was carried out fully in accordance with the ethical standards of the International Journal of Exercise Science (28). Studies pertaining to gene expression, AAS use, and SM were considered for inclusion. The following databases were included in the literature search process: Medline Complete, Academic Search Complete, APA PsychInfo, SPORTDiscus, CINAHL Plus, Cochrane Central Register of Controlled Trials, Rehabilitation & Sports Medicine Source, GreenFILE, and APA PsycArticles. The search strategy used the following keywords: "gene expression," "anabolic steroids," and "muscle." No language restrictions were imposed. Studies were included if they were peer-reviewed and published between January 2000 and November 2020. Search Strategies may be found in Table 1. Reviews and their references were reviewed for relevant publications.

Table 1. Search strategies

Concept	**Keyword:**	**Databases:**
1. Candidate Genes	Gene Expression	MEDLINE complete, Academinc Search Complete, APA PsychoInfo, SPORTDiscus, CINAHL Plus, Cochrane Central Register of Controlled Trials, Rehabilitation & Sports Medicine Source, GreenFILE, APA PsycArticles
2. Anabolic Steroid Use	Anabolic steroids	MEDLINE complete, Academinc Search Complete, APA PsychoInfo, SPORTDiscus, CINAHL Plus, Cochrane Central Register of Controlled Trials, Rehabilitation & Sports Medicine Source, GreenFILE, APA PsycArticles
3. Muscle Hypertrophy and Strength	Muscle	MEDLINE complete, Academinc Search Complete, APA PsychoInfo, SPORTDiscus, CINAHL Plus, Cochrane Central Register of Controlled Trials, Rehabilitation & Sports Medicine Source, GreenFILE, APA PsycArticles

Searches combined with AND: 1 AND 2 AND 3

RESULTS

Study Selection

One hundred and nineteen titles were generated from database searches; 38 redundant titles were removed. Of the remaining 81 titles, 53 titles were removed from consideration due to lack of topic relevance or status as a review, or due to not meeting the stated inclusion criteria. Twenty-eight titles met all inclusion criteria; one additional title was added from a previously removed review. A complete summation of inclusion and exclusion criteria may be found in Table 2. The systematic review process may be found in a PRISMA diagram in Figure 1. Studies were assessed for risk of biases via a modified PEDro scale.

Table 2. Inclusion Criteria

Inclusion Criteria	Reason for Exclusion
(1) Study included adult human or rodent subjects	Subject contained children or other animal subjects (e.g. cattle, pigs, rainbow trout)
(2) Study involved administration of AAS (testosterone congeners)	Study did not contain AAS administration, or administered SARMs, ecdysteroids, or prohormes
(3) Study must involve gene expression as a measured variable	Study measured non-genetic factors
(4) Study outcomes included at least one measured muscular phenotype	Study measured changes to bone, other hormones, or non-muscle related phenotypes
(5) Study must be an original peer-reviewed empirical study.	Study was a review, meta-analysis, or not peer-reviewed
(6) Study must be published between January 2000 and November 2020	Study was published before 2000

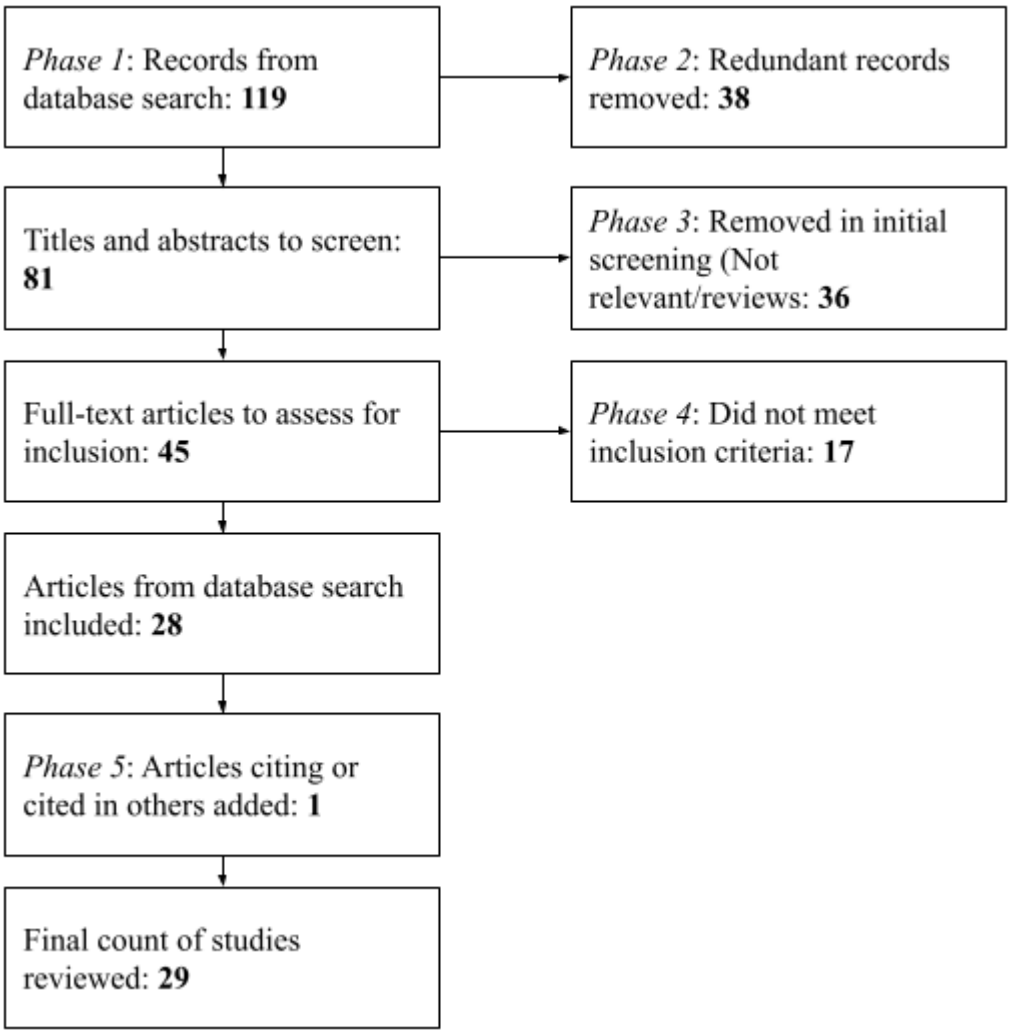

Figure 1. PRISMA Diagram of Study Inclusion

Study Characteristics

Studies included a total of 900 subjects: of these, 698 were rodent subjects (mutSOD1 and NGt mice, C57BL/6 mice, Wistar rats, Sprague-Dawley rats, Fisher 344xF1 Brown Norway rats) and 182 were human. Six rodent studies did not disclose the number of subjects. All human studies utilized male subjects. Three rodent studies utilized cell culture models (mouse C2C12 myoblasts, rat L6 cells). Descriptive characteristics of all subjects may be found in Table 3.

Table 3. Descriptive Characteristics of Subjects

Reference	Sample Size	Humans		Rodents		Age
		Men	Women	Mice	Rats	
Camerino (2015)	60-80	x	x	60-80	x	Adult
Dalbo (2017)	40-44	x	x	x	40-44	?
Diel (2008	?	x	x	C2C12 myoblasts	24	?
Diel (2007)	18	x	x	x	18	?
Dubois (2015)	32	x	x	32	x	12 weeks
Friedel (2005)	18	x	x	x	24	?
Fu (2012)	?	x	x	x	L6 cells	x
Galbiati (2012)	16	x	x	16 matched mutSOD1 and NTg mice	x	50-52 days
Gharahdaghi (2019)	18	18	x	x	x	"older"
Goncalves (2019)	?	x	x	Female C57BL/6 mice	x	12 weeks
Ikeda (2013)	12	x	x	x	12 male Wistar rats	8 weeks
Labrie (2005)	198	x	x	Male C57BL6 mice	x	11-12 weeks
Lee (2003)	43	x	x	x	Male Sprague-Dawley rats	?

Lewis (2007)	40	40	x	x	x	?
Liu (2011)	?	x	x	x	Male Wistar Rats	?
Liu (2013)	?	x	x	C2C12 Mouse cells	x	x
McClung (2005)	22	x	x	x	Fisher 344x F1Brown Norway rats	4 months
Mosler (2012)	?	x	x	x	Male Wistar Rats	?
Piovesan (2013)	100	x	x	x	Male Wistar Rats	Adult
Qin (2010)	?	x	x	x	Male Wistar-Hannover Rats	?
Reitzner (2019)	30	x	x	x	Male Wistar Rats	Adult
Santos (2012)	?	x	x	Normal Mice	x	?
Sinha-Hakim (2002)	61	61	x	x	x	18-35
Thompson (2006)	41	x	x	x	Male Fisher 344xF1 Brown Norway Rats	4-24 months
White (2009)	18-24	x	x	C57BL/6 Mice	x	8 weeks
White (2013)	?	x	x	C57BL/6 Mice; C2C12 myoblasts	x	8 weeks
Wyce (2010)	x	Human skeletal myoblasts	x	x	x	x

Ye (2014)	40	x	x	x		Male Fisher 344xF1 Brown Norway Rats	10 months
Zhao (2007)	?	x	x	x		Male Wistar Rats	?

Studies consisted of 64 experimental groups treated with AAS and 44 non-AAS experimental and control groups. Intervention periods lasted an average of 44 days. The most commonly administered AAS were nandrolone decanoate (ND) (3, 11, 19, 21, 22, 25, 30, 31, 33, 36, 38, 39, 43), testosterone esters (TE) (5, 6, 9, 10, 14, 15, 20, 35, 39), and dihydrotestosterone (DHT; 6-9, 18, 41). Tetrahydrogestrinone (THG) was administered in three studies (6, 9, 18); trenbolone esters (TREN) and methandienone (METH) were administered in two studies each (5, 27, 32, 42); desoxymethyltestosterone (DMT) and metenolone (MT) were administered in one study each (7, 16). The average ND dose was 5.15 mg/kg body mass with 6 mg/kg being the most common dose. TE doses ranged from 7 mg/week to 600 mg/week. The average DHT dose was 2.02 mg/kg. The average THG dose was 0.75 mg/kg. The average TREN dose was 1 mg/week. The average METH dose was 2.53 mg/kg. DMT was dosed at 1 mg/kg. MT was dosed at 20 mg/kg. Dosing protocols ranged from once every two weeks to daily. A complete description of all AAS variables may be found in Table 4.

Table 4. AAS Protocols

Reference	AAS groups	Non-AAS groups	Intervention Length	AAS	Dose	Dose Protocol
Camerino (2015)	1	3	4 weeks	ND	5mg/kg	1x/day, 6d/week
Dalbo (2017)	2	2	29 days	T, TrE	T: 7mg/week; TRE: 1 mg/week	1x/week
Diel (2008	2	2	7 days	THG	1 mg/kg	1x/day/12 days
Diel (2007)	2	x	12 days	TP, DMT	TP: 1 mg/kg; DMT: 1mg/kg	1x/day
Dubois (2015)	1	3	2 weeks	DHT	7 mg/kg	1x/day/2 weeks
Friedel (2005)	2	2	12 days	THG, TP	THG and TP: 1 mg/kg	1x/day/12 days

Fu (2012)	?	?	?	T	10^{-11} to 10^{-5} M	?
Galbiati (2012)	2	2	85-120 days	ND	10 mg/kg	1x/week
Gharahdaghi (2019)	1	1	6 weeks	TI	250 mg	1x/2weeks
Goncalves (2019)	4	?	12 weeks	TC	10 or 20mg/kg	every 48 hours
Ikeda (2013)	1	1	2 days	MT	20 mg/kg	1x
Labrie (2005)	18	2	7 days	THG, DHT	THG: 0.5 mg; DHT: 0.1 mg	?
Lee (2003)	2	5	3-7 days	ND	6 mg/kg	1x
Lewis (2007)	2	2	10 weeks	TE	100 mg	1x/week
Liu (2011)	?	?	56 days	ND	ND: 0.75 mg/kg; T: 2.8 mg/kg	ND: 1x/week; T: 1x/day
Liu (2013)	x	x	48 hours	ND	?	?
McClung (2005)	2	2	3 days	ND	6 mg/kg	1x/week
Mosler (2012)	1	1	3 weeks	METH	0.5 mg/kg	1x/day
Piovesan (2013)	2	2	3 weeks	ND	5mg/kg	2x/week
Qin (2010)	2	0	35 days	ND	?	?
Reitzner (2019)	2	2	6 weeks	METH	5mg/kg	Daily
Santos (2012)	?	?	?	ND	?	?
Sinha-Hakim (2002)	5	0	20 weeks	TE	25, 50, 125, 300, 600 mg	1x/week
Thompson (2006)	2	2	24 days	ND	6mg/kg	1x/week

White (2009)	3	4	14 days/42 days	ND	6mg/kg	1x/week
White (2013)	1	2	1 week	ND, T	6mg/kg	1x/week
Wyce (2010)	x	x	8 hours	DHT	30 nM	1x
Ye (2014)	2	2	42 days	TE, TrE	TE: 7.0mg; TRE: 1.0 mg	1x/week
Zhao (2007)	2	2	3-56 days	ND, T	0.75/2.8 mg/kg/week	continuous

Note: DHT = Dihydrotestosterone; DMT = Desoxymethyltestosterone; MT = Metenolone; METH = Methandienone; ND = Nandrolone Decanoate; T = Testosterone; TC = Testosterone Cypionate; TE = Testosterone Enanthate; THG = Tetrahydrogestrinone; TI = Testosterone Isocaproate; TrE = Trenbolone Enanthate

Muscle phenotypic changes in rodent subjects were measured in the gastrocnemius (3, 6, 7, 8, 11, 16, 18, 22, 27, 31, 32, 36, 39, 43), soleus (3, 8, 19, 25, 27, 32, 36), levator ani (5-7, 9, 18, 27, 42), tibialis anterior (15, 30, 38), extensor digitorum longus (3, 8), plantaris (19), and quadriceps (33). Additionally, two rodent studies utilized murine C6C12 cells in vitro (10, 22). Muscle phenotypic changes in human subjects were measured in the vastus lateralis in three studies (14, 20, 35); additionally, one study utilized human skeletal myoblasts in vitro (41). A complete listing of phenotypes, intervention variables, and genes investigated may be found in Table 5.

Table 5. Muscular Phenotypes and Gene Identification

Reference	Muscle Phenotype	Specific Muscular Variables	Muscles Investigated	Non-AAS Treatment Conditions	Exercise Intervention	Tissue Sampling	Target Genes	Gene extraction method
Camerino (2015)	Morphology, ions, function	Total protein content; ion channel resting conductances; resting cytosolic [CA+]; isometric contraction	Soleus, gastrocnemius, extensor digitorum longus	2 week hindlimb unloading	None	Anesthitized pre-euthanasia	mTOR, Eif2ak3, PGC1a, Map1lc3a, Ctsl, Fbxo32, Trim63, Myh7, Myh2, Myh4, Myh1, Notch, MyoD, Myf5, MYOG, Pax7, HPRT, Actb, B2m	RT-PCR

Friedel (2005)	Dubois (2015)	Diel (2007)	Diel (2008	Dalbo (2017)
Gene expression, endocrine	Morphology, gene expression	Morphology, gene expression	Signaling, enzymes, gene expression	Morphology, signaling, enzymes
AR-dependent gene expression in AR yeast; THG binding to all steroid hormone receptors (AR, PR, MR, GR)	Muscle weight; IGF-1 expression; % of AR positive cells corresponding to fibroblasts	Muscle mass, gene expression	Creatine kinase activity, myostatin signaling/expression	Myostatin signaling; effects of aromatisation/5a-reduction; muscle growth
Levator ani	Gastrocnemius, soleus, extensor digitorum longus, levator ani	Gastrocnemius, levator ani	Gastrocnemius	Levator ani
ORX	ORX, sham	ORX	None	ORX, sham
none	None	None	None	None
Post-euthanasia	Post-euthanasia	Post-euthanasia	Pre-obtained myoblasts	Post-euthanasia
Tat	Amd1, MSTN	Tat, MSTN, IGF1	MSTN, Notch, Delta, Sox8, Sox9, Pax7	MSTN, FSTN, ActrIIB, Mighty, Fibrillarin
RT-PCR	RT-PCR	RT-PCR	RT-PCR	RT-PCR

Goncalves (2019)	Gharahdaghi (2019)	Galbiati (2012)	Fu (2012)
Morphology, gene expression	Function, morphology, signaling, enzymes	Neuromuscular disease, gene expression, enzymes	Morphology, ions
SM cell size, metabolic gene expression in SM	1RM, isometric knee extension torque, Muscle architecture via ultrasound, DXA muscle mass, fibre type specific CSA, protein-bound alanine, MPS, muscle RNA/DNA/protein content, anabolic/catabolic signalling, mitochondrial CS activity	ALS, gene expression, SOD1 levels	AR, myogenin, MHC protein levels, Ca+ levels
Tibialis anterior	Vastus lateralis	Gastrocnemius	C6C12 cells
Normolipidic or hyperlipidic diet	Resistance exercise	None	None
None	3 sets of 8-10 reps of 6 exercises (leg ext, leg curl, leg press, pull down, shoulder press, bench press) 3x/week for 6 weeks	None	None
Post-euthanasia	Under rested conditions from VL	Post-euthanasia	Pre-obtained L6 cells
Glut-4, CPT-1	RPL12A, AR, SRD5A1, AKR1C3, HSD17B3, IGF1, MHC1, MHCIIa, MYOG, Myf6, C-Myc, MEOX-2, C-met, Pax7, PGC1a, Tfam	SOD1, MYOG, MyoD, FBXO32, TGFB1	HPRT, MYOG, MHC, PCNA, AR
RT-PCR	RT-PCR	RT-PCR	RT-PCR

Lee (2003)	Labrie (2005)					Ikeda (2013)
Morphology, gene expression	Gene expression					Gene expression
AR protein and mRNA expression at the onset of functional overload	Changes of expression of all genes in the genome to analyze in vivo alterations of genomic profiles in androgenic-sensitive tissues					Expression of myogenic transcription and muscle growth factors
Soleus, plantaris	Gastrocnemius, levator ani					Gastrocnemius
Functional overload via surgical ablation, sham	GDX					Stretching protocol
None	None					Right gastrocnemius stretching, 15x per minute for 15 minutes
Pre-euthanasia	Post-euthanasia					Post-euthanasia
AR, ACTA1	Fos, DUSP1, PDK4, MyoD					MGF, MyoD, MYOG, G3PDh
Semiquantitative RT-PCR	RNA extraction and microarray analysis					RT-PCR

McClung (2005)	Liu (2013)	Liu (2011)	Lewis (2007)		
Morphology	Signaling	Signaling	Endocrine, morphology, function		
Overload-induced muscle growth	Numb, Wnt, Notch signaling	Notch signaling in denervated SM	Muscle IGF-1, lean mass/strength		
Soleus	C6C12 cells	Gastrocnemius	Vastus lateralis		
Overload via surgical ablation, sham	None	Left sciatic nerve transection	Resistance exercise		
None	None	None	10 weeks of 45 minutes of 3 sets of seated leg press, seated leg curl, seated leg extension, standing calf raise, seated ankle dorsiflexion from 60-80% 1RM, 3x/week		
Pre-euthanasia	Pre-obtained C2C12 mouse cells	Not stated	Pre-and post-intervention from right VL		
MyoD, IGF1	Numb	Hey1, Numb	MHC, IGF1, IGF2, IGFBP, MyoD, MYOG, MSTN, MAFbx, GAPDH		
Semiquantitative RT-PCR	RT-PCR	RT-PCR	RT-PCR		

Sidha-Hakim (2002)	Santos (2012)	Reitzner (2019)	Qin (2010)	Piovesan (2013)	Mosler (2012)
Morphology	Morphology	Morphology, gene expression	Gene expression	Morphology	Signaling, endocrine
Increases in muscle fiber area and number	Muscle regeneration	Hypertrophy from exercise, physical parameters, histological characteristics, gene expression	Gene expression over time in denervated muscle	Skeletal muscle repair process	Effects on androgen signaling via HPT axis, MSTN levels
Vastus lateralis	Quadriceps	Gastrocnemius, soleus	Gastrocnemius	Tibialis anterior	Gastrocnemius, soleus, levator ani
Standardized nutritional intake at 36 kcal/kg/day, 1.2 g	Flutamide	Exercise intervention	Sciatic nerve transection	Surgical exposure of TA, sham	None
None	None	2-4 20 minute training sessions at s 25 degree incline treadmill	None	None	10-15 min/day ofmotor-driven treadmill running,
Pre- and post-treatment	Unknown	Post-euthanasia	Post-euthanasia	Post-euthanasia	Post-euthanasia
MHC1, MHC2a, MHC2b, MHC2x	MYOG, MyoD, Myf5, MSTN	MSTN, FSTN, ACVR2B, Smad3, Smad7, IL-6, IL-10, TNFa, AR, Pax7, MyoD, IGF1, MHCe, MYOG	THBS1, GAL, TPM3, ADAMTS1, SERPINE1, NR4A, ATF3, BCL, BHLHB3, BTG2, TCF4, TLE1,	GAPDH, MYOG	AR, FSTN, MSTN, MyoD, Smad7
RT-PCR	RT-PCR	RT-PCR	RT-PCR	RT-PCR	RT-PCR

Zhao (2007)	Ye (2014)	Wyce (2010)	White (2013)	White (2009)	Thompson (2006)
Morphology	Gene expression	Signaling, morphology, function	Signaling, morphology	Morphology	Signaling, morphology
Muscle atrophy after nerve transection	Expression of anabolic and catabolic SM genes	AR-mechanism to increase muscle mass and strength	Grip testing, Akt/mTORC1/FoxO3a in SM, muscle turnover	Muscle regeneration after bupivacaine	Inflammatory response to overload-induced SM hypertrophy
Gastrocnemius	Levator ani	Human skeletal myoblasts	Gastrocnemius	Tibialis anterior	Gastrocnemius, soleus
L sciatic nerve transection, sham	ORX, sham	None	Castration, sham	Castration, bupivacaine injection	Bilateral surgical ablation, sham
None	None	None	None	None	None
Unknown	Post-euthanasia	Pre-obtained human skeletal myoblasts	Pre-euthanasia	Pre-euthanasia	Post-euthanasia
MAFbx, MuRF1, MSTN	IGF1, MGF, AR, FBXO32, MuRF1, GR, WISP-2	FBXO32, MYC, SMARCC1, ACTA1, AR	FBXO32, MuRF1, IGF1, REDD1, PGC1a, MHCIIa, MHCIIb	IGF1, ACTA1, MyoD, CCND1	TNFa, IL-6, IL-8, ACTA1
RT-PCR	RT-PCR	RT-PCR	RT-PCR	RT-PCR	Semiquantitative RT-PCR

Note: 1RM = One repetition maximum; ACTA1 = Skeletal alpha-actin; ACTB = Actin beta; ACTRIIb = Activin receptor type-2B; ACVR2b = Activin A receptor type 2B; ADAMTS1 = ADAM metallopeptidase with thrombospondin type 1 motif 1; AKR1C3 = Aldo-keto reductase family 1 member C3; ALS = amyotrophic lateral sclerosis; AMD1 = Adenosylmethionine decarboxylase 1; AR = Androgen receptor; ATF3 = Activating transcription factor 3; B2M = Beta-2-microglobulin; BCL = B-cell lymphoma; BHLHB3 = Class E basic helix-loop-helix protein 41; BTG2 = BTG anti-proliferation factor 2; Ca+ = Calcium; CCND1 = Cyclin D1; C-MET = Tyrosine-protein kinase

Met; C-MYC = MYC proto-oncogene; CPT-1 = Carnitine palmitoyltransferase I; CS = Citrate synthase; CSA = Cross-sectional area; CTSL = Cathepsin L; DNA = Deoxyribonucleic acid; DUSP1 = Dual specificity phosphatase 1; DXA= Dual-energy X-ray absorptiometry; Eif2ak3/PERK = Eukaryotic translation initiation factor 2 alpha kinase 3; FBXO32 = F-box protein 32; Fos = Fos proto-oncogene, AP-1 transcription factor subunit; FSTN = Follistatin; GAL = Galanin and GMAP prepropeptide; G3PHd = Glyceraldehyde 3-phosphate dehydrogenase; GAPDH = Glyceraldehyde 3-phosphate dehydrogenase; GDX = Gonadectomy; GLUT4 = Glucose transporter type 4; GR = Glucocorticoid receptor; HEY1 = Hes related family BHLH transcription factor with YRPW motif 1; HPRT = Hypoxanthine phosphoribosyltransferase; HPT = Hypothalamic-pituitary; HSD17B3 = 17-beta hydroxysteroid dehydrogenase 3; IGF-1/IGF1 = Insulin-like growth factor 1; IGF2 = Insulin-like growth factor 2; IGFBP = IGF binding protein; IL-6 = Interleukin-6; IL-8 = Interleukin-8; IL-10 = Interleukin-10; L = Left; MAFbx = Muscle atrophy F-box; MAP1LC3A = microtubule associated protein 1 light chain 3 alpha; MEOX-2 = Mesenchyme homeobox 2; MGF = Mechano-growth factor; MHC = Myosin heavy chain; MHC1/MYH1 = Myosin heavy chain 1; MHCIIa/MCH2a = Myosin heavy chain type IIa; MCHIIb/MCH2b = Myosin heavy chain type IIb; MCH2x = Myosin heavy chain type IIx; MHCe = Embyonal myosin heavy chain; MPS = Muscle protein synthesis; MR = Mineralocorticoid receptor; MSTN = Myostatin; mTOR = Mechanistic target of rapamycin; MuRF1 = Muscle RING-finger protein-1; MYC = MYC proto-oncogene, BHLH transcription factor; MYF5 = Myogenic factor 5; MYF6 = Myogenic factor 6; MYH 2 = Myosin heavy chain 2; MYH4 = Myosin heavy chain 4; MYH7 = Myosin heavy chain 7; MYOD = Myoblast determination protein 1; MYOG = Myogenin; NR4A = Nuclear orphan receptor 4a; ORX = Orchiectomy; PAX7 = Paired box 7; PCNA = Proliferating cell nuclear antigen; PDK4 = Pyruvate dehydrogenase kinase 4; PGC1a = peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PR = Progesterone receptor; REDD1 = Regulated in development and DNA damage responses 1; RNA = Ribonucleic acid; RPL12A = Ribosomal 60S subunit protein L12A; RT-PCR = Reverse transcription polymerase chain reaction; SERPINE1 = Serpin family E member 1; SFRS10 = Transformer-2 protein homolog beta; SMAD3 = SMAD family member 3; SMAD7 = Mothers against decapentaplegic homolog 7; SMARCC1 = SWI/SNF related, matrix associated, actin dependent regulator of chromatin subfamily C member 1; SOD1 = Superoxide dismutase 1; SOX8 = SRY-box transcription factor 8; SOX9 = SRY-box transcription factor 9; SRD5A1 = Steroid 5 alpha-reductase 1; TA = Tibialis anterior; TAT = Tyrosine aminotransferase; TCF4 = Transcription factor 4; TFAM = Transcription factor A, mitochondrial; TGFB1 = Transforming growth factor-beta; THBS1 = Thrombospondin 1; THG = Tetrahydrogestrinone; TLE1 = TLE family member 1, transcriptional corepressor; TNFa = Tumor necrosis factor alpha; TPM3 = Tropomyosin 3; TRIM63 = Tripartite motif containing 63; UBE2E2 = Ubiquitin conjugating enzyme E2 E2; USP12 = Ubiquitin specific peptidase 12; VL = Vastus lateralis; WISP-2 = WNT1-inducible-signaling pathway protein 2

Effects of AAS on Gene Expression

A summary of all study results may be found in Table 6.

Nandrolone Decanoate: MYOG expression increased due to ND administration in both normal mice and male Wistar rats, with MYOG expression significantly elevated at 56 days in a sham group of male Wistar rats (33, 43). However, ND administration did not modify the effects of mutSOD1 on MyoD, MYOG, or Atrogin-1 expression in matched mutSOD1 and NTg mice (11). ND administration combined with surgical-induced ablation attenuated an overload induction of MYOG mRNA 440% greater than a sham condition in four-month-old male Fisher 344x F1 Brown Norway rats; however, ND administration alone was 440% less than ND + exercise training (25). ND significantly increased MYOG mRNA expression post-injury in adult male Wistar rats (30). ND significantly re-established MyoD expression in response to a hindlimb unloading condition in adult mice (3). Similarly, ND induced a significant increase in MyoD mRNA expression seven days post-injury in adult male Wistar rats and a main effect to increase

after 14 days in eight-week-old male C57BL/6 mice (30, 38). However, ND administration had no consistent effect on MyoD abundance in four-month-old male Fisher 344x F1 Brown Norway rats or expression in a normal condition not treated with flutamide in normal mice (25, 33).

ND administration resulted in no significant modification to MSTN, mTOR, or PERK expression in normal adult mice, with no effect on MSTN occurring after denervation in male Wistar rats (3, 33, 43). ND had no effect on IGF1 mRNA expression with or without exercise-induced overload in four-month-old male Fisher 344x F1 Brown Norway rats (25). However, injured muscle receiving ND for 14 days increased muscle IGF1 mRNA expression fivefold above control conditions and significantly greater than ND treatment without injury in eight-week-old male C57BL/6 mice (38). Similarly, ND increased IGF1 mRNA expression fivefold compared to a castrated control group of eight-week-old male C57BL/6 mice and 2.4-fold as compared to a sham group (39). ND administration increased AR mRNA expression by 50% with three days of overload stretching in male Sprague-Dawley rats compared to a control (19). ND administration resulted in a significant reduction of the oxidative isoforms of MHC types 1 and 2 and a non-significant increase in the fast glycolytic isoform type 2b in adult mice (3). ND did not restore mRNA expression of MHC isoforms in response to hindlimb unloading in adult mice (3).

ND significantly re-established Notch1 expression in response to a hindlimb unloading condition in adult mice (3). Myf5 expression increased due to ND administration (33). ND significantly reduced MuRF-1 and REDD1 expression by 60% and 37%, respectively, in eight-week-old male C57BL/6 mice, with significant reductions in MuRF1 expression at days 35 and 56 post-denervation in male Wistar rats (39, 43). However, ND did not affect MuRF1 expression in sham denervated muscle of male Wistar rats (43). ND administration resulted in a significant reduction in the expression of PGC1a in adult mice (3). ND did not change ACTA1 mRNA with functional overload in male Sprague-Dawley rats (19). However, there was an interaction at 14 days between ND and injury to increase ACTA1 mRNA expression fourfold above a control condition and significantly greater than ND treatment without injury in eight-week-old male C57BL/6 mice (38). Trends for a main effect were present for ND to increase ACTA1 mRNA expression at 42 days in eight-week-old male C57BL/6 mice (38).

ND induced a significant increase in Numb expression in hindlimb denervated subjects compared to sham or control groups in male Wistar rats and C2C12 mouse cells (21, 22). However, this increase in expression by ND was reduced after 56 days in C2C12 mouse cells (22). Significant effects of ND administration and time were observed with significantly elevated MAFbx expression at 31, 35, and 56 days after muscle denervation in male Wistar rats; expression tended to be reduced at day 35 and was significantly reduced at day 56 (43). ND administration significantly attenuated IL1 expression in an overload condition only in 25-month male Fisher 344xF1 Brown Norway rat muscle as compared to five-month muscle (36). IL6 mRNA abundance was significantly higher in a ND + overload condition as compared to control but was attenuated by ND in both five- and 25-month overloaded male Fisher 344xF1 Brown Norway rat muscle (36). ND administration had a main effect on TNFα mRNA

abundance in five-month male Fisher 344xF1 Brown Norway rat muscle only but did not attenuate overload-induced TNFα induction (36).
ND did not restore atrogin-1 levels compared to a control group of adult mice and reduced its expression by 69% compared to a sham group of eight-week-old male C57BL/6 mice (3, 39). There was a main effect of ND to increase Cyclin D1 mRNA expression after 14 days of recovery post-injury in eight-week-old male C57BL/6 mice, but no effect was present after 42 days (38). ND caused the expression of HBS1, GAL, ADAMTS1, SERPINE1, NR4A1-3, ATF3, BTG2, TCF4, and USP 12 to be downregulated at seven days post-administration in male Wistar-Hannover rats but upregulated at 35 days post-administration (31). TPM, BCL6, TLE1, and UBE2E2 were downregulated at both seven- and 35-days post-administration in male Wistar-Hannover rats (31). BHLHB3 and SFRS10 expression was upregulated at seven days post-administration in male Wistar-Hannover rats but downregulated at 35 days post-administration (31). ND affected overall TGFB1 mRNA levels in 7.5-week-old matched mutSOD1 and NTg mice (11). ND restored eIF2 expression in adult mice (3).

Testosterone Esters: TE significantly increased the number of rat L6 cells expressing MYOG and significantly increased MYOG expression levels in adult human males as compared to a placebo (10, 14, 20). However, TE brought about no significant changes in MyoD or MSTN mRNA abundances in adult human males (20). Between-treatment effects of MSTN were 64% lower with TE administration in rats compared to a sham control (5). TE significantly stimulated IGF1 and MSTN expression in both rats and older male humans, with IGF1 expression reaching values fivefold higher in a normal condition of 10-month-old male fisher 344xF1 Brown Norway rats compared to orchiectomized (ORX) groups (7, 14, 42). TE increased the expression of AR in older male humans (14). TE significantly increased the number of rat L6 cells expressing MHC (10). However, TE did not significantly affect the total concentrations of MHC isotype mRNA in 18–35-year-old human males relative to other groups (35). TE increased the expression of Myf-6 in older male humans (14). TE administration had no significant impact on FSTN expression in rats compared to other groups (5). TE administration prevented increases in MuRF-1 mRNA expression in ORX 10-month-old male fisher 344xF1 Brown Norway rats (42). TE brought about no significant changes MAFbx mRNA abundances in male humans (20).

Expression of ActrIIb was 76% lower with TE administration compared to a sham control (5). Between-treatment effects were found in Mighty expression 23% higher with TE administration in rats compared to a sham control (5). TE increased GLUT4 and CPT1 expression when dosed at 20 mg/kg in 12-week old female C57BL6 mice as compared to 10 mg/kg or a control condition (15). TE administration prevented increases in atrogin-1 mRNA expression in ORX 10-month-old male fisher 344xF1 Brown Norway rats (42). TE administration in ORX 10-month-old male fisher 344xF1 Brown Norway rats induced WISP2 expression threefold higher than ORX+TREN or ORX-only groups and seven-fold higher than a sham control (42). A trend indicated that TE treatment may prevent an ORX-induced increase in GR mRNA expression in 10-month-old male fisher 344xF1 Brown Norway rats (42). TE increased expression of Srd5a1, HSD17B3, AKRIC3, C-Myc, MEOX02, and C-met in older human males (14).

Dihydrotestosterone: DHT stimulated both MSTN in rats and MyoD expression in 11–12-week-old male C57BL6 mouse muscle (9, 18). DHT caused MSTN to have a biphasic response significantly different than a control in C2C12 myoblasts; expression was very low two days post-administration, increased strongly at four days post, decreased at six days post, and finally increased at eight days post (6). Treatment of C2C12 cells with DHT and THG resulted in strong MSTN mRNA expression four and seven days post-administration (6). DHT was unable to induce MSTN expression in 12-week old AR knockout mice but was able to reverse an ORX-instigated decrease in MSTN expression (8). DHT administration reversed decreased IGF1 levels in 12-week old ORX mice (8). DHT stimulated the expression of Notch in C2C12 myoblasts (6). Expression of Pax7 following DHT administration in C2C12 myoblasts was low two days post-administration but increased to peak values four days post-administration (6).

DHT repressed the expression of Delta and Sox9 in C2C12 myoblasts (6). DHT was able to reverse an ORX-instigated decrease in Amd1 expression in 12-week-old mice (8). However, DHT was unable to induce Amd1 expression in 12-week old AR knockout mice (8). DHT administration brought about a response in both ARE 1 and 2 expressions in 12-week old mice (8). DHT stimulated the expression of Sox8 in C2C12 myoblasts (6).

Tetrahydrogestrinone: THG stimulated MyoD expression in 11-12-week-old male C57BL6 mouse muscle (18). Treatment of C2C12 cells with THG and DHT resulted in strong MSTN mRNA expression four- and seven-days post-administration (6). THG induced AR-dependent gene activity in a dose-dependent manner in rats (9).

Trenbolone: Between-treatment effects of MSTN were 69% lower with TREN administration in rats compared to a sham control (5). IGF1 mRNA expression was fivefold higher in ORX+TREN groups of 10-month-old male fisher 344xF1 Brown Norway rats as compared to ORX only (42). TREN administration did not affect AR mRNA expression in 10-month-old male fisher 344xF1 Brown Norway rats as compared to other groups (42). TREN administration had no significant impact on FSTN expression in rats compared to other groups (5). TREN administration prevented increases in MuRF1 mRNA expression in ORX 10-month-old male fisher 344xF1 Brown Norway rats (42). Expression of ActrIIb was 70% lower with TREN administration in rats compared to a sham control (5). Between-treatment effects were found in Mighty expression 31% higher with TREN administration in rats compared to a sham control (5). TREN administration prevented increases in atrogin-1 mRNA expression in ORX 10-month-old male fisher 344xF1 Brown Norway rats, with the reduction in atrogin-1 mRNA greater following TREN administration compared to TE (42). TREN did not affect WISP expression in 10-month-old male fisher 344xF1 Brown Norway rats as compared to other groups (42). TREN prevented an ORX-induced increase in GR mRNA expression with GR expression 50% lower than in a TE+ORX group of 10-month-old male fisher 344xF1 Brown Norway rats (42).

Methandienone: METH administration induced no change in MYOG expression in adult male Wistar rats (32). METH administration reversed training-induced reductions in MyoD mRNA expression and induced significant upregulation in MyoD mRNA expression in adult male

Wistar rats compared to other conditions (27, 32). METH administration significantly upregulated MSTN; however, MSTN was significantly downregulated in groups exposed to METH and exercise conditions in adult male Wistar rats (27, 32). METH administration induced significant upregulation in IGF1 mRNA expression in adult male Wistar rats compared to other conditions (27). AR expression was significantly downregulated with METH administration with or without exercise in adult male Wistar rats (27, 32).

The expression of MHC mRNA was significantly downregulated in adult male Wistar rats only when METH administration was combined with an exercise condition (27). METH administration induced no change in Pax7 expression in adult male Wistar rats (27). METH administration significantly upregulated FSTN in adult male Wistar rats (27, 32). FSTN and Smad7 were only affected after training when combined with METH administration in adult male Wistar rats (27, 32). FSTN was significantly downregulated in groups exposed to METH and exercise conditions in adult male Wistar rats (27). METH administration reversed training-induced reductions in Smad7 expression (27). IL6 mRNA expression was significantly downregulated with METH administration and exercise in adult male Wistar rats (32). METH administration did not affect IL10 expression in adult male Wistar rats (32). Expression of TNFa mRNA was significantly downregulated only when METH administration was combined with an exercise condition in adult male Wistar rats (32).

Desoxymethyltestosterone: DMT significantly stimulated MSTN and IGF1 expression in rats (7).

Metenolone: MT administration caused no significant differences in MYOG or MyoD mRNA expression in eight-week-old male Wistar rats as compared to a control (16).

Table 6. Study Results

Reference	Results
Camerino (2015)	ND resulted in significant reduction of oxidative isoforms of MHC types 1/2a, non-significant increase in fast glycolytic type 2b; ND did not restore mRNA expression of MHC in response to HU; significant reduction in PGC1a; no significant modification to mTOR or PERK; ND did not restore atrogene levels compared to a control; ND significantly re-established MyoD and Notch1 expression; ND restored EIF2ak3
Dalbo (2017)	Between-treatment effects of MSTN 64/69% lower in T/TrE compared to sham; ActrIIb 76/70% lower in T/TrE than sham; between-treatment effects of Mighty 23/31% higher in T/TrE than sham; no differences in FSTN between conditions; MSTN protein levels higher in T/TrE than sham (197/209%)
Diel (2007)	TP/DMT stimulated IGF-1/MSTN expression in the gastroc (statistically significant)

Diel (2008)	Expression of all genes affected by DHT (stimulation of Notch, Sox8, MSTN; repression of Delta, Sox9); Mstn expression followed a biphasic pattern of very low 2 days post, increasing strongly at day 4, decreasing at day 6, increasing at day 8 significantly different to control; treatment of C2C12 with DHT+THG resulted in strong MSTN mRNA expression on days 4 and 7; DHT and expression of Pax7 was low at day 2 but increased to maximum at day 4
Dubois (2015)	ORX decrease in AMD1 and MSTN reversed by DHT; DHT unable to induce AMD1 or MSTN expression in knockout mice; DHT reversed decreased IGF-1Ea levels in ORX mice
Friedel (2005)	THG induces AR dependent gene activity in a dose-dependent manner; EC50 of THG (3×10−8 M) to activate the reporter gene expression was about ten times lower than the EC50 of the reference substance DHT (3×10−9 M)
Fu (2012)	AR mRNA not detected in L6 cells; TE induced differentiation promoted most at 10^{-6} M (sig. increased number of cells expressing MYOG and MHC)
Galbiati (2012)	ND did not modify massive effects of mutSOD1 on MyoD, MYOG, atrogin-1 expression; AAS affects overall TGFB1 mRNA levels;
Gharahdaghi (2019)	T genes increased w/EX: AR (0.71), Srd5a1 (0.7), HSD17B3 (0.33), AKRIC3 (0.14), IGF-1Ea and IGF-1Ec (0.63/0.83), MHCIIA (0.33), MYOG (0.53), C-Myc (0.28), Myf-6 (0.92), MEOX02 (0.19), C-met (0.89)
Goncalves (2019)	GLUT4 and CPT1 gene expression increased in groups SD+AS20 and HFD, and more upregulated in HFD+AS10 and HFD+AS20 compared to other groups
Ikeda (2013)	No significant differences in MyoD or MYOG mRNA expression between stretch and unstretched sides or between treatment and control conditions
Labrie (2005)	MyoD is stimulated in the levator ani by both THG and DHT;
Lee (2003)	7 days of functional OL significantly increased AR mRNA 430%; ND treatment did not change AR mRNA concentration; ND-treated AR mRNA increased 50% with 3 days of functional OL group compared to controls; ACTA1 mRNA was not changed by 3 and 7 days of functional OL with or without ND treatment
Lewis (2007)	No sig. change observed for MSTN or MyoD mRNA abundances; MYOG increase was significantly greater in treatment group compared to placebo; no statistically significant achieved in MAFbx mRNA abundance in TR groups compared to other groups
Liu (2011)	Numb mRNA levels were not significantly different when comparing sham-DN and DN-vehicle groups; Numb mRNA expression significantly increased in DN-ND group by nearly 2 fold at 7 days and more than 2 fold at 35 days compared to sham or vehicle DN groups; induction of Numb mRNA by ND was reduced at 56 days

Liu (2013)	ND induced a significant increase in Numb mRNA starting at 16 hours and reached peak at 48 hours after treatment; Numb mRNA declined to basal levels after
McClung (2005)	ND alone did not alter MyoD mRNA abundance, but combo with TR attenuated OL induction of MYOG mRNA; OL and ND had sig, interaction on myog mRNA; OL induced MYOG mRNA 440% above sham and 440% above DN alone; OL had a significant main effect on IGF-1 mRNA (inc. 90%); no sig. interaction or alteration by ND with or without OL
Mosler (2012)	In gastroc: MSTN, Smad7, MyoD, AR not significantly different, FSTN distinctly decreased with Me administration only compared to training control group; MSTN/FSTN had distinct decrease in expression by more than 50%, MyoD and AR showed slow but significant decrease in expression, Smad7 showed no sig. different with Me + EX compared to control; In soleus: MSTN showed distinct increase in mRNA expression more than 50%, FSTN/Smad7/MyoD showed no sig. difference to control, AR showed slow but sig. decrease with Me only; MSTN showed very strong increase in mRNA expression, FSTN/Smad7 showed distinct increase by more than 50%, MyoD showed no sig. difference, AR showed slow but sig. increase with Me + EX compared to control
Piovesan (2013)	Significant increase in MyoD mRNA in treatment group 7 days after injury; MYOG mRNA significantly increased in ND group
Qin (2010)	THBS1: downregulated at 7 days, upregulated at 35; GAL: down at 7, up at 35; TPM3: down both; ADAMTS1: down 7, up 35; SERPINE1: down 7, up 35; NR4A: down 7, up 35; ATF3: down 7, up 35; BCL6: down both; BHLHB3: up 7, down 35; BTG2: down 7, up 35; TCF4: down 7, up 35; TLE1: down both; SFRS10: up 7, down 35; UBE2E2: down both; USP12: down 7, up 35
Reitzner (2019)	MSTN and FSTN significantly upregulated in METH group; MSTN and FSTN significantly downregulated in METH+EX group compared to other groups; ACVR2B sig. downregulated compared to control following EX only; Smad3 sig. downregulated in all groups; Smad7 sig. downregulated in METH+EX group; IL6 sig. downregulated in EX and METH+EX; IL10 not changed in any group; TNFa sig. downregulated in METH+EX only; AR sig. downregulated in METH and METH+EX; IGF1 and MyoD expression was sig. upregulated only in response to METH; Pax7 no change; MHC sig. downregulated in EX and METH+EX; MYOG not changed
Santos (2012)	No statistical sig. diffs between groups for MSTN; MyoD, MYOG, Myf5 increased due to ND
Sinha-Hakim (2002)	Concentrations of MHC isotype mRNA did not change significantly relative to any group
Thompson (2006)	ND administration sig. attenuated IL1 expression only in 25 month soleus muscle; ND had no effect on OL-induced IL1 mRNA expression in 5 month soleus; 3-way interaction of age, functional OL, and ND was not significant for IL6 or TNFa; significant 2-way interactions were found for IL6 mRNA abundance in 5 and 25 month soleus; ND administration sig. attenuated IL6 mRNA abundance in OL 5 and 25 month soleus muscle; IL6 mRNA abundance was sig. greater in OL+ND group than in Control; main effect of ND administration on TNFa mRNA abundance in 5 but not 25 month soleus; ND administration did not attenuate OL TNFa induction

White (2009)	Interaction at 14 days between ND and injury to increase ACTA1 mRNA expression; injured muscle receiving ND for 14 days increased ACTA1 mRNA fourfold above control; ND induced ACTA1 expression sig. greater than ND treatment without injury; trend for main effect of ND to increase ACTA1 at 42 days; Injured muscle receiving ND for 14 days increased muscle IGF1 mRNA exp. 5fold above controls, sig. greater than ND treatment without injury; main effect at 42 days of ND to increase IGF1 mRNA; main effect of ND admin to increase MyoD mRNA after 14 days of rec.; main effect of ND to increase CyclinD1 mRNA expression after 14 days of recovery; ND had no effect on CyclinD1 after 42 days
White (2013)	ND significantly reduced MuRF1 mRNA expression by 60%, atrogin-1 gene expression by 69%, and REDD1 expression by 37%; ND increased IGF1 mRNA 5fold to castrated and 2.4 fold to sham
Ye (2014)	MuRF1 and atrogin-1 mRNA expression in LABC of ORX 3 and 3 fold higher than shams after 42 days; TE+TrE prevented this increase resulting in values not different from shams; reduction in atrogin-1 mRNA greater following TRE than TE; IGF1 expression 5 fold higher in LABC muscle of ORX+TE and ORX+TrE compared to ORX; No difference in WISP2 gene expression present between sham, ORX, and ORX+TrE groups; WISP2 expression 3 fold higher in ORX and TE compared with ORX and ORX+TrE, 7 fold higher than sham; TrE prevented ORX-induced increase in GR mRNA expression and a trend indicated that TE treatment may also prevent; GR expression 50% lower in ORX+TrE group than ORX+TE; AR expression not different
Zhao (2007)	ND had no effect on MSTN expression after DN; significantly elevated MYOG expression at 56 days in sham; MAFbx expression sig. elevated in DN muscle at 31, 35, or 56 days; significant effects of ND and time observed; MAFbx expression tended to be reduced at day 35 and was significantly reduced at day 56; ND administration on MuRF1 expression yielded significant two-way effects wihtout a significant effect of time after DN; ND did not effect MuRF1 expression at day 31 but sig. reduced expression of MuRF at days 35 and 56; ND had no effect on MuRF1 expression in sham-DN muscle

Note: AAS = Anabolic-androgenic steroids; ACTA1 = Skeletal alpha-actin; ACTRIIb = Activin receptor type-2B; ACVR2b = Activin A receptor type 2B; ADAMTS1 = ADAM metallopeptidase with thrombospondin type 1 motif 1; AKR1C3 = Aldo-keto reductase family 1 member C3; AMD1 = Adenosylmethionine decarboxylase 1; AR = Androgen receptor; AS10 = 10mg dose of anabolic steroids; AS20 = 20mg dose of anabolic steroids; ATF3 = Activating transcription factor 3; BCL6 = B-cell lymphoma-6; BHLHB3 = Class E basic helix-loop-helix protein 41; BTG2 = BTG anti-proliferation factor 2; C-MET = Tyrosine-protein kinase Met; C-MYC = MYC proto-oncogene; CPT-1 = Carnitine palmitoyltransferase I; DHT = Dihydrotestosterone; DMT = Desoxymethyltestosterone; DN = Denervated; EC50 = half maximal effective concentration; EIF2ak3/PERK = Eukaryotic translation initiation factor 2 alpha kinase 3; EX = Exercise; FSTN = Follistatin; GAL = Galanin and GMAP prepropeptide; GLUT4 = Glucose transporter type 4; GR = Glucocorticoid receptor; HFD = Hyperlipidic diet; HSD17B3 = 17-beta hydroxysteroid dehydrogenase 3; HU = Hindlimb unloading; IGF-1 Insulin-like growth factor 1; IGF-1Ea = Insulin-like growth factor Ea propeptide; IGF-1Ec = Insulin-like growth factor Ec propeptide; IL1 = Interleukin-1; IL6 = Interleukin-6; IL10 = Interleukin-10; LABC = Levator ani; MAFbx = Muscle atrophy F-box; MEOX-2 = Mesenchyme homeobox 2; METH = Methandienone; MHC = Myosin heavy chain; MHCIIa = Myosin heavy chain type IIa; mRNA = Messenger ribonucleic acid; MSTN = Myostatin; mTOR = Mechanistic target of rapamycin; MuRF1 = Muscle RING-finger protein-1; mutSOD1 = Mutant superoxide dismutase 1; MYF5 = Myogenic factor 5; MYF6 = Myogenic factor 6; MyoD = Myoblast determination protein 1; MYOG = Myogenin; ND = Nandrolone decanoate; NR4A = Nuclear orphan receptor 4a; OL= Overload; ORX = Orchiectomy; PAX7 = Paired box 7; PGC1a = peroxisome proliferator-

activated receptor gamma coactivator 1-alpha; REDD1 = Regulated in development and DNA damage responses 1; SD = standard diet; SERPINE1 = Serpin family E member 1; SFRS10 = Transformer-2 protein homolog beta; SMAD3 = SMAD family member 3; SMAD7 = Mothers against decapentaplegic homolog 7; SOX8 = SRY-box transcription factor 8; SOX9 = SRY-box transcription factor 9; SRD5A1 = Steroid 5 alpha-reductase 1; T = Testosterone; TCF4 = Transcription factor 4; TE = Testosterone enanthate; TGFB1 = Transforming growth factor-beta; THBS1 = Thrombospondin 1; THG = Tetrahydrogestrinone; TLE1 = TLE family member 1, transcriptional corepressor; TNFa = Tumor necrosis factor alpha; TP = Testosterone propionate; TPM3 = Tropomyosin 3; TR = Training; TrE = Trenbolone Enanthate; UBE2E2 = Ubiquitin conjugating enzyme E2 E2; USP12 = Ubiquitin specific peptidase 12; WISP-2 = WNT1-inducible-signaling pathway protein 2

DISCUSSION

The purpose of this review was to systematically identify and appraise the existing peer-reviewed literature that has measured the effects of AAS administration on gene expression in SM. Twenty-eight titles were included in this review; within those studies, 23 common genes were identified. The most commonly reported increases in gene expression with AAS administration occurred in MYOG, MyoD, and IGF. MYOG expression was increased by ND and TE (33, 43). No change was seen with METH or MT administration (16, 30). MyoD expression was increased by ND, DHT, THG, and METH; no change was seen with TE or MT administration (3, 16, 18, 20, 25, 27, 30, 32, 33, 38). IGF was increased by ND, TE, DHT, TREN, METH, and DMT (7, 8, 14, 32, 38, 39, 42). However, one ND study did find no effect on IGF with or without exercise (25). Notch and Myf-5 expressions were increased by ND administration; additionally, Notch was increased by DHT and Myf-6 by TE (3, 6, 14, 33). Though the AR gene was reported to be increased by ND, TE, and THG, it was decreased by METH in two studies, and had no change by TREN (9, 14, 19, 27, 32, 42). MSTN also showed no clear pattern of behavior; while it was increased in two studies by both THG and DMT, it was decreased by TREN in one study and showed inconclusive patterns as a result of TE, DHT, or METH administration across seven studies (5-8, 14, 20, 27, 32, 33, 42, 43). MHC isoform expression and FSTN were inconclusively affected (3, 5, 10, 27, 32, 35). MuRF-1 was affected relatively negatively, with its expression decreased or prevented from increasing (39, 42, 43).

A major strength of the current research is the number of titles located analyzing the effects of AAS on key SM genes. Within those titles, 88 different genes were identified for analysis. Many of the genes analyzed are responsible for both anabolic and catabolic SM processes. For example, three of the four most analyzed genes (MYOG, MyoD, IGF) are all integral to SM cell differentiation and proliferation, processes involved in SM anabolism (14, 42). In particular, IGF1 and its pathway have been observed to be integral in mediating the hypertrophic influences of AAS (20). In contrast, the third most commonly investigated gene, MSTN, and its downstream signaling agent Smad are suppressors of myogenesis and satellite cell activity (32). This investigation into both anabolic and catabolic gene expression allows for a unique insight into all the processes that regulate overall SM integrity. Additionally, the investigation into AR expression under AAS administration provides a key insight into the AAS mechanism of action. As stated previously, the anabolic effects of AAS are mediated largely in part by the activation

of AR in SM. AR regulate the transcription of target genes responsible for the binding of DNA required to stimulate MPS (20, 29).

To that end, different androgen-AR signaling pathways have been proposed, including the "classical" pathway involving androgen traversing of the cell membrane, binding to the intracellular AR inducing AR dimerization, and the traversing of the dimer into the cell nucleus where it binds to androgen response elements and activates gene transcription (10). It has also been observed that the androgen-AR complex upregulates AR-mediated transcription via c-SRC kinase interaction-driven phosphorylation (10). Separate from the AR, androgens may bind to steroid-hormone binding globulin (SHBG), activating the SHBG receptor and increasing protein kinase A (PKA) activity (10). This increased PKA activity influences subsequent AR-mediated transcription via an alteration of either androgen membrane binding sites or by membrane Ars (mARs) in SM cells (10). The mARs are functionally separate from the intracellular Ars, while still presenting the necessary characteristics of being saturable and androgen-selective of a binding site (10). These non-genomic signaling pathways rely on an increase in intracellular calcium levels via G protein-coupled receptors and the subsequent activation of signal transduction cascades modulating key transcription factors (10). Further, AR expression has been observed to differ in the presence or absence of mechanical stimulation, such as is present during resistance training (19). These differing pathways may help explain the discrepancies of different AAS effects on AR gene expression and may offer insight for future research aimed at clarifying relationships.

The current research does include several limitations. First, there was a lack of standardized dosing for the major AAS compounds studied. The most-reported dosing was 6 mg/kg for ND, used in five titles (3, 19, 25, 36, 38). As AAS have been reported to have a dose-dependent effect, standardization of dosing protocols should be prioritized. For example, the Md dosing protocol of 0.5 mg/kg BW/day used by Mosler and colleagues was based on previously identified doses meant to simulate those of an AAS-abusing athlete (27). Second, studies administered only seven AAS compounds. Previous survey research has identified over 18 commonly used AAS compounds (29). This suggests that there is the possibility of unknown effects on gene expression in SM. Within this review, only eight of the 28 included titles compared the effects of multiple compounds against each other (5, 6, 7, 9, 18, 38, 42, 43). Many genes were analyzed by only one study and thus under the effects of one AAS compound. It is therefore unknown how these genes may be affected under the influence of additional AAS compounds. Finally, only five of the included titles utilized any sort of exercise intervention (14, 16, 20, 27, 32).

Future research should attempt to incorporate an abundance of AAS compounds with standardized dosing protocols, and it may be beneficial for researchers to compare multiple compounds as they affect the expression of several key genes. Furthermore, there should be a marked increase in the inclusion of different exercise conditions in these studies. Such studies could prove beneficial to understanding combined exercise and AAS countermeasures against muscle-wasting conditions such as sarcopenia, hypogonadism, cachexia, and microgravity, as

well as their ever-present role in exercise and athletic performance and physical SM aesthetics in amateur and professional physique athletes. Finally, a more thorough understanding of proper dosing protocols may help prevent unnecessary deaths resulting from AAS misuse and abuse as has been highly prevalent in recent years (37). Conclusion: AAS compounds yield a variety of effects on SM gene expression in both anabolic and catabolic genes. Trends indicate that expression of the anabolic factors MYOG, MyoD, and IGF are largely increased by AAS administration. Future research should aim to establish standardized dosing protocols on human subjects, as well as determine the effects of different dosages on absolute gene expression.

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