BOSTON UNIVERSITY
DIVISION OF GRADUATE MEDICAL SCIENCES

Dissertation

A MURINE MODEL OF GLUCOCORTICOID MYOPATHY

ALLEVIATION USING ANDROGEN THERAPY

by

NICOLAE LUCIAN SANDOR
B.M./M.D., Universitatea de Medicina si Farmacie Carol Davila, 2002
B.S., Universitatea Bucuresti, 2005

Submitted in partial fulfillment of the

requirements for the degree of

Doctor of Philosophy

2015

Approved by

First Reader

Monty Montano, Ph.D.
Assistant Professor of Medicine

Second Reader

Shalender Bhasin, MD
Professor of Medicine

”Se questa non piace, non voglio più scrivere.”

Acknowledgments

I am deeply indebted to the members of my committee, Drs. Caroline Apovian, Shalender Bhasin, Isabel Dominguez, Konstantin Kandror, Monty Montano, and Carlo Serra, who helped me focus my rather diffuse initial plan. It was a privilege to receive insights from so many angles of expertise. I am grateful to the leaders of the graduate programs at Boston University School of Medicine, Drs. William Cruikshank, David Atkinson, and Mary Jo Murnane. I am also obliged to Mary Kathleen Deloge, who helped me deal with limiting conditions.

Congratulations are due to my spouse who, all these years, took a positive view, managed many of the household businesses, and published as many papers as me. Knowing her, I am certain hers will have better impact factors. I am also thanking my past mentors, Vasile Munteanu, Drs. Alex Babes, Gordon Reid, Anne Treisman and Assen Marintchev.

I am dedicating this work those who were taken away untimely during these years. My mother spent her life in the most important frontline, as an EMT. My first lab mate, the prodigious Iurie Barbu, MD, died before his defense. We spent long weekend afternoons, manually switching polarizers in the Cotroceni lab, and dark winter nights, moonlighting in the pediatric ER at the Alexandrescu Hospital. This world is poorer without them.

This work would not have existed, were it not for the kind financial support of the American nation, through the National Institute of Health and Boston University.

A MURINE MODEL OF GLUCOCORTICOID MYOPATHY ALLEVIATION USING ANDROGEN THERAPY

NICOLAE LUCIAN SANDOR

Boston University School/Division of Graduate Medical Sciences, 2015

Major Professor: Monty Montano, PhD, Research Assistant Professor of Medicine

Abstract

Glucocorticoids (GC) are used widely for the treatment of a large number of inflammatory conditions. Loss of muscle mass and muscle weakness are common complications of GC therapy. Androgen therapy has been suggested to reverse GC-associated muscle loss (GAML), but evidence of its effectiveness is lacking.

Here, I established a mouse model of GAML. Young adult male mice receiving 0.25 mg/kg/day dexamethasone (D) s.c. daily, for a week, lost 3% of their body weight. Through NMR lean body mass quantification and muscle dissection, a loss of more than 10% of their muscle mass was lost. More than half of the muscle loss was reversed by co-administration of 0.7 mg/kg/day testosterone (T). This is the first mouse model of GAML alleviation by T.

Intramuscular atrogene expression and proteasome catalytic activity were upregulated by D and suppressed by T co-administration. T co-administration caused intramuscular downregulation of atrogene-activating Foxo transcription factors. Intramuscular pro-autophagic REDD1 and Klf15 were repressed by T co-administration. T co-administration reduced the autophagosome-characteristic lipidated form of LC3B. Translation regulators 4E-BP, eIF3f and eIF2 did not change significantly as a result of androgen co-administration. Calpains activity and levels were unchanged by D and T.

C2C12 differentiated myotubes were used to determine the effects of T and D on protein synthesis and degradation. Myotube diameters were reduced by D, while T co-administration suppressed D effect. Protein degradation was increased by 24 hour D treatment. D-stimulated protein degradation was inhibited by proteasomal inhibitor MG132, and, to a lesser degree, by lysosome inhibitor chloroquine. T co-administration returned protein degradation to basal levels. Protein synthesis response to D and T did not correlate with the observed phenotypes.

In vivo, D reduced intramuscular IGF-I expression, an effect reversed by T co-administration. In C2C12, inhibition of IGF-1R signaling with picropodophyllin did not modify T effects.

In conclusion, T protective action in GAML is mainly anti-catabolic, through reversal of proteasome and autophagosome upregulation induced by D. T stimulates a potentially protective intramuscular IGF-I response. Different models are needed to determine the role of protein synthesis and of IGF-I in GAML.

Contents

Acknowledgments i

Abstract ii

Contents iv

List of Tables vii

List of Figures viii

Abbreviations ix

1 Clinical questions and evidence **1**

Cushing's syndrome 1

Glucocorticoid therapy 3

Hypercortisolism-induced muscle loss 9

Muscle protection with androgen therapy 18

Hypercortisolism-induced changes in endogenous androgens levels . . . 25

Molecular mechanisms of androgenic myoprotection in humans 30

2 BIOLOGICAL PREMISES **36**

Skeletal muscle histology 36

Preadult muscle development 39

Physiological muscle metabolism 42

Adult muscle remodeling 45

Hormonal control of muscle mass 47

Interaction of muscle mass and vascularization 50

Control of muscle mass through innervation 51

Animal models of glucocorticoid myopathy 56

Glucocorticoid stimulation of ubiquitin-proteasome system 60

Glucocorticoid-induced loss of sensitivity on the IRS - Akt axis 64

Glucocorticoid inhibition of mTOR 69

Glucocorticoid activation of Foxo transcriptional program 74
Glucocorticoid activation of myostatin 77
Glucocorticoid modulation of protein synthesis 79
The effects of glucocorticoids on autophagy 83
Glucocorticoids and other proteolytic systems 85
Alleviation of glucocorticoid myopathy by IGF-I 86
Alleviation of glucocorticoid myopathy by anabolic steroids 88

3 Hypotheses **91**

4 Methods **92**
Literature review 92
Ethical procedures 92
Animal studies 92
Cell culture studies 94
Immunofluorescence microscopy 94
In vivo studies 94
Metabolic measurements at organism level 94
Measurement of muscle protein synthesis and degradation 94
Enzymatic assays 94
Gene expression 94
Immunoblot 94

5 In vivo experiments **95**

6 In vitro findings **96**

7 Discussion **97**
Conclusions 97
Future directions 97

Bibliography 97

Curriculum vitae **191**

List of Tables

1.1 Pharmacological agents used in Cushing's disease of unidentified ectopic, or diffuse localization (reviewed in [1, 2]) 24

List of Figures

1.1 Changes in proteasome enzymatic activity following 6 months of T therapy (reproduced from [3] with permission). 33

Abbreviations

24HUC	24-hour urine cortisol
3MH	3-methylhistidine
4E-BP	eIF4E binding protein
4E-BP1	eIF4E binding protein 1
AAP	adrenal androgen precursor
AAS	anabolic androgenic steroids
ACTH	adrenocorticotropic hormone;
ActRIIB	activin A receptor, type IIB
AMPK	AMP-activated protein kinase
AMPK	adenosine monophosphate kinase
AP-1	activator protein 1
AR	androgen receptor
ATF4	activating transcription factor 4
Atg12	autophagy-related 12
BCAA	branched-chain amino acids

bFGF	basic fibroblast growth factor
C/EBP	CCAAT-enhancer-binding protein
COPD	chronic obstructive pulmonary disease
CS	Cushing's syndrome
CSA	cross-sectional area
CT	computed tomography
Dexa	dexamethasone
DHEA	dehydroepiandrosterone
DHEAS	dehydroepiandrosterone sulfate
DHT	dihydrotestosterone
EC50	half maximal effective concentration
eIF2	eukaryotic initiation factor 2
eIF2B	eukaryotic initiation factor 2B
eIF3-f	eukaryotic initiation factor 3f
eIF4E	eukaryotic translation initiation factor 4E
eNOS	endothelial nitric oxide synthase
FDA	Food and Drug Administration
FSH	follicle stimulating hormone
FSR	fractional synthesis rate
Gadd45	Growth Arrest and DNA Damage 45

GAML	GC-associated muscle loss
GC	glucocorticoid
GCN2	General Control Nonderepressible 2
GFP	green fluorescent protein
GH	growth hormone
GLUT	glucose transporter
GnRH	gonadotropin-releasing hormone
GR	glucocorticoid receptor
Grb2	Growth factor receptor-bound protein 2
GRE	glucocorticoid responsive-elements
GSK3	glycogen synthase kinase 3
HAT	histone acetyltransferase
HDAC	histone deacetylase
HIF 1a	hypoxia-induced factor 1a
HOMA-IR	homeostatic model assessment - insulin resistance
HPA	hypothalamic - pituitary - adrenal
IBMX	3-isobutyl-1-methylxanthine
IGF-1R	IGF-I receptor
IGF-I	insulin-like growth factor I
IGF2	insulin-like growth factor 2

IGFBP	IGF-I binding protein
IL-1	interleukin 1
IL-1RII	interleukin 1 receptor type II
IL-2	interleukin 2
IRS	insulin receptor substrate
IκBα	inhibitory κBα
KLF-15	kruppel-like factor-15
LC3	microtubule-associated protein 1 light chain 3
LH	luteinizing hormone
LKB1	Liver Kinase B1
MAFbx	muscle atrophy F-box
MAPK	mitogen-activated protein kinase
MAPK	mitogen-activated protein kinases
MEF2A	myocyte enhancer factor 2A
MNK2	mitogen-activated protein kinase–interacting kinase 2
MR	mineralocorticoid receptor
MRF	muscle regulatory factor
Mrf	myogenic regulatory factor
mTOR	Mechanistic Target of Rapamycin

mTORC1	mTOR complex 1
MuRF1	muscle RING finger 1
Myf	myogenic factor
MyHC	myosin heavy chain
NEFA	non-esterified fatty acids
NFAT	nuclear factor of activated T-cells
NMJ	neuro-muscular junction
OMIM	Online Mendelian Inheritance in Man
ORF	open reading frame
p70-S6K	ribosomal protein S6 kinase, 70 kDa
p90-RSK	ribosomal protein S6 kinase, 90kDa
PDK1	3-phosphoinositide-dependent protein kinase 1
PGC-1	PPARg coactivator 1
PI3K	phosphatidylinositol 3-kinase
PI3K	phosphatidylinositol 3-kinase
Pitx	paired-like homeodomain
PLC	phospholipase C
POMC	pro-opiomelanocortin
PPAR	peroxisome proliferator-activated receptor
PRAS40	proline-rich Akt substrate of 40-kDa

qRT-PCR	quantitative real-time polymerase chain reaction
REDD1	regulated in development and DNA damage responses-1
SGKL	serum and glucocorticoid-inducible kinase-like kinase
Shc	Src homology 2 domain containing
SOS	Son of Sevenless
Sos	Son of sevenless homolog
Sp1	SV40 promoter-specific 1
STAT	Signal Transducer and Activator of Transcription
T	testosterone
Tbx	T-box
TCF4	transcription factor 4
TGF-β	transforming growth factor-beta
Tp	testosterone propionate
TSC1	Tuberous Sclerosis Complex 1
TSC2	Tuberous Sclerosis Complex 2
ULK1	UNC-51-like kinase 1
VEGFR	VEGF receptor

1. CLINICAL QUESTIONS AND EVIDENCE

Cushing's syndrome

Through the detailed case series written by Harvey Cushing[4], the scientific and medical community became aware of an otherwise rare disease, which bears his name. Unlike the earlier and better-studied deficiencies of the thyroid and pancreas, pituitary defects were more variable in manifestation and therefore harder to unify in a single clinical entity. Even when macroscopic hypertrophy of pituitary were localized to a gland subdomain, it was unclear whether observed pathology could be attributed to a hypersecretion from the hypertrophied sector, or to a deficiency in the neighboring compressed structures. Similarly, pituitary extracts caused multiple, and even opposite effects, in animal models[5], suggestive of a mixture of hormones.

Among 50 cases described by Cushing, about five stood out due to the involvement of other glands. In each of them, and, to a lesser extent, in a few more cases, "hyperadrenalism" was blamed for asthenia, hyperpigmentation of skin, low blood pressure, and hypoglycemia. Histopathology tests localized the adrenal abnormalities to the zona fasciculata of the cortex. Cushing wrote that some of these abnormalities, reflect current adrenal hypoactivity, caused by exhaustion after preceding intense stimulation and hyperactivity.

Twenty years later, Cushing narrowed the focus in an updated case series of combined pituitary-adrenal pathology[6]. Cushing noted that basophile adenomata of the pituitary and hypertrophy of the adrenal glands often coexisted. Based on the curative effect of pituitary surgery, he hypothesized that the adrenal defect is secondary to the pituitary abnormality. In turn, he inferred that the adrenal changes mediate the disease phenotype, which includes obesity with ectopic adipose deposits, kyphosis, amenorrhoea / impotence, hypertrichosis,

lineae atrophicae, fatigability and weakness. Among these disease manifestations, muscle impairment was a serious, if variable, component. Cushing considered intense muscle loss the cause of death for one of these cases.

Cushing's work did little to elucidate mechanisms leading to the phenotype. The variability in pituitary changes between the cases he described meant that many scientists rejected his hypothesis of pituitary primacy. A group at the Mayo Clinic was actively pursuing the opposite hypothesis, with the adrenal as the primary site of impairment in adrenal-pituitary combined afflictions[7]. On the clinical side, it was noted that some of Cushing's patients lacked observable pituitary changes. Moreover, some of the Mayo patients were cured by adrenal surgery. From a theoretical perspective, the adrenal hypothesis was more tempting because the adrenal deficiency (termed Addison's disease) and its reversal by administration of adrenal cortex extracts were better known than pituitary pathology[8].

Today, we know that the truth was more nuanced. Oversecretion of the adrenal cortex hormones cortisol and / or corticosterone is termed hypercortisolism. One or more clinical signs listed by Cushing (see above) suggest to the practitioner the activation of the hypothalamic - pituitary - adrenal (HPA) axis. If concomitant hypercortisolism is confirmed by an increase of urine free cortisol measurements, or by the effacement of the evening trough in circulating cortisol, there is suspicion for Cushing's syndrome (CS)[9]. Some hypercortisolism cases, termed pseudo-Cushing's syndrome, are ascribed to causes outside the HPA axis, such as in depression, morbid obesity, uncontrolled diabetes mellitus, and sleep apnea (reviewed in [10]).In theory, pseudo-Cushing responds to suppression tests by low dose Dexa, while true CS does not. In practice, specificity is very low and sensitivity is 95%, so latest guidelines are recommending against suppression tests. Tumors may be less responsive in Dexa suppression and in ACTH stimulation tests than hyperplasias per PMID: 4342889

True CS cases are further classified based on the role of the adrenal-stimulating pituitary hormone corticotropin (adrenocorticotropic hormone; ACTH). In some CS patients, hypercortisolism is paralleled by an increase in ACTH. Their adrenals are usually responsive to further ACTH stimulation tests, indicating that previously intact adrenals underwent hyperplasia in response to a pathological overstimulation with ACTH. When attributed to the pituitary, such ACTH oversecretion, followed by secondary hypercortisolism, is termed Cushing's disease (reviewed in [11]). Cushing's disease remains a staple of physiology textbooks, because it provides an excellent didactic example of a hormone hierarchy.

The remainder of CS cases consists of hypercortisolism in spite of low ACTH. In primary hypercortisolism, ACTH is typically suppressed by negative feedback. Adrenal neoplasms are are the most frequent cause of primary hypercortisolism. Ectopic or diffuse unregulated sources of ACTH or cortisol may cause hypercortisolism. In recent decades, overdose with synthetic derivatives of cortisol became the most important cause of low-intensity CS (discussed in the next section).

Despite being caused by a diverse set of HPA pathologies, CS is quite invaiable in its ability to cause muscle impairment.

Glucocorticoid therapy

A series of serendipitous decisions brought impressive knowledge about CS of non-pituitary etiology (reviewed in[12]). First, during World War II, US intelligence learned that Germans were importing large quantities of adrenal glands from neutral Argentina. This reignited US government interest in corticoadrenal research, despite the lackluster results with earlier adrenal extracts. At the end of the war, only a few grams of pure adrenal steroids were manufactured, from endogenous sources and at a high cost. The second

opportunity was in the allocation of those scarce steroids. One of them, cortisone, made by Merck, was shared by a few clinical researchers, including Phillip Hench. Hench's request was based on his previous work on rheumatoid arthritis. He observed that rheumatoid arthritis was alleviated in jaundice, and hypothesized the existence of a steroidal "anti-rheumatoid factor". Third, Hench's choice of dose and route elicited an extraordinary reversal in arthritic pain and dysfunction. In 1949, after treating only five patients[13], impressive improvements in those cases triggered redirected corticosteroid research.

Previous work did describe multiple effects for adrenal extracts, but little was known about purer preparations, such as cortisone. In fact, adrenal research was discouraged prior to cortisone purification, because less pure extracts combined antagonistic hormones in variable doses, leading to the impression that they lack a defined pharmacological effect. However, even with purified cortisone, Hench saw a very diverse set of consequences for cortisone administration[14].

First, cortisone's action on metabolism was accessible even to the less sophisticated clinical measurements used 60 years ago. Patients receiving cortisone gained weight. Chronic cortisone therapy led to accumulation of adipose tissue, often in ectopic locations, such as the interscapular "buffalo hump". Cortisone also induced hyperglycemia which can induce glycosuria. For this reason, cortisone, and its endogenous and synthetic analogs, are grouped in the glucocorticoid (GC) family.

Hench and collaborators hypothesized that cortisone's protective action is not limited to rheumatoid arthritis. In his 1950 Nobel lecture, Hench envisaged a role for alleviation of most inflammatory diseases. GCs share the ability to reduce inflammation (reviewed in [15, 16]). Some of these anti-inflammatory effects, such as reduction in the number of circulating white blood cells, are ample and robust. The cellular and molecular anti-inflammatory mechanisms are still subject of active research.

The knowledge gap around the anti-inflammatory actions of GCs is in part caused by the immunology progress. Still, some questions remain open, and illustrate the convoluted ways in which GC signals are relayed in the cell. For example, GCs are often acting in a manner shared with all steroids, by binding and activating the glucocorticoid receptor (GR). Activated GR translocates from cytosol to the nucleus, where it dimerizes on specific DNA sequences, termed glucocorticoid responsive-elements (GRE) [17]. The classical effect of the GRE-GR interaction is increased transcription for neighboring target genes (transactivation), as it is the case in polymorphonucleate cells with interleukin 1 (IL-1) receptor type II (IL-1RII) [18], a decoy inhibitor of the pro-inflammatory IL-1. In other circumstances, the activated receptor inhibits transcription directly (transrepression), or by interfering with transcription factors. For example, in human T lymphocytes, GCs inhibit the transcription factor activator protein 1 (AP-1), thus causing a reduction in their ability to synthesize pro-inflammatory interleukin 2 (IL-2)[19]. GCs employ nongenomic mechanisms, such as mRNA stability and enzymatic activity modulations. In airway epithelia, GCs reduce the half-life of the mRNA for interleukin 8 (IL-8), the major chemoattractant for neutrophils[20]. Within minutes, GC administration induces vasodilation, through direct, non-genomic activation of phosphatidylinositol 3-kinase (PI3K) leading to activation of endothelial nitric oxide synthase (eNOS)[21].

Some GC effects may be limited to a range of doses, durations, and frequencies of administration. Moreover, the example of adrenalectomized rats re-supplemented with corticosterone, their most abundant endogenous GC, illustrates how, at times, the same GC can induce or repress the same cellular response, depending on the dose. A 5 mg / kg physiological dose enhances the immune skin delayed-type hypersensitivity, while a pharmacological 40 mg / kg dose yields the more typical GC immunosuppressive behavior [22]. This behavior, suggestive of a U (or inverted U) shaped response curve, is called hormesis or

biphasic response, and poses great challenges, both to the investigative scientist and to the clinician attempting to establish a therapeutic regimen.

In 1950, endogenous GCs corticosterone and cortisol, were synthesized at Merck[23], thus lowering the price and creating the opportunity for large-scale trials. The Empire Rheumatism Council organized a randomized trial comparing cortisone with acetylsalycilate, and concluded that there is no benefit in cortisone[24]. While participants receiving cortisone claimed an improvement in subjective well-being, they were afflicted more often with deleterious side effects, including edema and hypertension. In retrospect, a comparison between two palliative symptomatic therapies using cure-indicating outcomes was likely misinformative,

By the time this trial was reported, the chemists were providing more effective and safe synthetic GCs (reviewed in [25]). While synthesizing esters with a longer half-life, Scherring chemists introduced a double bond in the A ring of cortisone, thus discovering prednisone, the first widely used oral GC[26]. In addition to an improved ability to induce hyperglycemia, prednisone lost some of the cortisone's ability to cause edema. NIH researchers synthesized and characterized prednisone's active metabolite, prednisolone[27]. In a trial of prednisolone versus acetylsalycilate in rheumatoid arthritis, the GC provided better functional protection to the articulations[28].

At Squibb, insertion of a halogen atom was found to abolish GCs ability to induce hyperglycemia[29, 30]. In 1958, Merck chemists led by Arth modified cortisol with the unsaturated A ring (Δ^1), the fluoride addition at position 9α, and with a methyl group on the 16α position to obtain dexamethasone (Dexa) [31, 32]. Dexa is the most effective and specific therapeutic synthetic GC to date, with 170 times higher ability to inhibit the immune reaction to subcutaneous foreign bodies (granuloma) compared to cortisol.

Dexa is completely unable to cause edema and electrolyte imbalance.

Nevertheless, Dexa is 52 times more potent in suppressing endogenous GC secretion, and 35 times more potent in causing hyperglycemia[33, 34]. In animals, Dexa is 160 times more potent than cortisol in the inhibition of granuloma test of immune function[35]. These findings suggest that anti-inflammatory and hyperglycemic actions are intermediated by the same specific, Dexa-sensitive receptor, whereas electrolyte changes are caused by cortisol through a different pathway. Further supporting the idea of a unique Dexa receptor, efforts to synthesize steroids with anti-inflammatory action that do not interfere with metabolism have failed. Compounds such as A276575[36] and RU 24858[37] did not reach the human studies stage. Mapracorat[38] did nor progress beyond phase II clinical trials. Therefore, clinicians prescribing GCs in these five decades had to balance therapeutic benefit with metabolic side-effects.

Edema is an example of non-specific GC effect, caused by a less typical interaction of the hormone with the mineralocorticoid receptor (MR). The GC family spans tens of active principles and thousands of formulations, from weak GCs with lower specificity, such as cortisone, to strong, specific GCs, such as Dexa. The list of Food and Drug Administration (FDA)-approved indications for cortisone, Dexa, and prednisone is often changed, as better, more specific drugs are developed, and more precautions are added[39, 40, 41].

The trivial case for using GC therapy is in hormone replacement, such as in adrenocortical insufficiency (reviewed in [42]). GC therapy is suitable for acute immune or allergic conditions, such as seasonal rhinitis (reviewed in [42]). GCs are relatively safe in topical applications in dermatological conditions (pemphigus, psoriasis, most types of dermatitis; reviewed in [43]). Similarly, GCs are commonly used in eye inflammatory conditions[44, 45], such as diffuse posterior uveitis and optical neuritis.

As envisaged by Hench in his Nobel Lecture, GCs do not address disease causes and are recommended for temporary respite. For many autoimmune

diseases, there are more specific therapeutic alternatives, often addressing the cause of disease. In addition to the glucose metabolism disturbance, long term GC administration causes osteoporosis and muscle loss. On the balance of benefits and drawbacks, GCs are recommended for life-threatening or impairing immune reactions, such as in polymyositis (reviewed in [46]), severe sarcoidosis (reviewed in [47]), and disseminated pulmonary tuberculosis. Based on their ability to lower white blood cell count, GC are an important adjuvant in the palliative and even etiologic treatment of leukemias and lymphomas[48, 49, 50].

Even in chronic diseases, GCs are recommended for short-term alleviation of exacerbations. Short-term GC therapy is recommended for rheumatoid arthritis, gouty arthritis, psoriatic arthritis, ankylosing spondylitis, asthma[51, 52], ulcerative colitis[53, 54], and idiopathic nephrotic syndrome[55].

GCs have been used off-label in many other diseases. Most commonly, GCs are perceived by physicians as a fall-back therapeutic alternative for cerebral hypertensive conditions, despite sparse evidence for efficacy in specific conditions. Small trials suggest GCs reduce vasogenic cerebral edema[56] and prevent acute mountain sickness[57], while systematic reviews suggest they might in fact worsen outcomes for acute brain trauma victims[58]. A similar, paradoxical situation is seen in ongoing clinical research. As of 2015, the patent-free status of the GCs discourages trials for new indications, while their de facto standard-of-care status makes them a common comparator in clinical trials. The National Cancer Institute sponsors 311 ongoing clinical studies employing Dexa, mainly in the standard-of-care arm, thus providing a plethora of data which have been, and may still be, misconstrued as support for the use of GCs. Every day practice may drift further apart for the officially sanctioned label, thus providing new opportunities for unjustifiable overdose.

Due their widespread use, GCs are likely to cause covert iatrogenic CS in a large population, impairing muscle and quality of life to a certain and

understudied degree.

Hypercortisolism-induced muscle loss

Primary and secondary endogenous hypercortisolism are rare diseases (1-2 cases per million and year each[59]), despite a recent boost from incidental findings during imaging tests for other needs. The symptomatology is non-specific, meaning that, even today in the developed world, an average of 6 years pass from signs onset until diagnosis is made and treatment is initiated[60]. It is a life-threatening disease, with untreated patients having a median survival rate of 5 years after diagnosis[61]. Some of the changes occurring in Cushing's disease are irreversible, especially at the level of brain, bone, adipose tissue, and liver levels (reviewed in [62]). Even after surgical adjustments of the hyperactive pituitary, the quality of life for CS patients lags behind that of the unaffected population.

At presentation, about two thirds of Cushing's syndrome cases present with muscular complaints, with similar incidence among pituitary and adrenal conditions[63]. Among patients suspected of endogenous CS, one fifth are referred to the endocrinologist due to muscle weakness[64]. Two-fifths of those whose endogenous hypercortisolism is successfully corrected by surgery still complain of fatigue[65].

On the other hand, therapy-induced (iatrogenic) CS is common. The glut of GC indications and off-label uses makes them some of the most used drugs in the developed countries. Every year, about 1% of the Americans and British receive some form of GC[66, 67]. GCs are likely even more often prescribed in the developing world, due to affordability and lack of alternatives, poor access to health care not withstanding. Dexa and cortisol are the only drugs listed five times in the World Health Organization's List of Essential Medicines[68].

In most cases, the cause of iatrogenic CS can be identified by careful history taking and medication reviews. However, an increasing number of cases are not

as easily diagnosed, because the excess GC is not from prescribed medicine. In United States, FDA approved in 1979 over-the-counter sale of 0.5% hydrocortisone cream for itching and minor skin inflammation. In 1990, 1% hydrocortisone creams were also permitted[69]. Where regulated, over-the-counter GC creams rarely cause CS on their own, but may lower the threshold for CS in patients who are also prescribed oral GC. Unregulated, mislabeled, overdosing GC creams sold as skin bleaching products pose a great CS risk to patients from ethnic groups with darker skin. Half of the respondents in a Nigeria poll admitted using GC-based skin bleaching products[70]. In 2015, the Ivory Coast government made illegal skin bleaching products, due to worries about GC overdose side effects[71]. The side-effects of skin bleaching are well recognized by the sub-Saharan medical community. Paradoxically, CS caused by bleaching products may be less identifiable to practitioners who care for the African diaspora in the developed world, where bleaching is more frequent, due to improved financial access and social pressures[72, 73].

Other, less frequent causes of iatrogenic CS, include the interaction between low dose GC therapy and cytochrome P450 3A4 inhibitors, such as the antiretroviral ritonavir[74]. Other steroid drugs may interact with GR and cause CS when overdosed, as it is the case with the synthetic progestin megestrol acetate[75].

Due to its insidious and erratic symptomatology, iatrogenic CS is often diagnosed years after onset or completely unrecognized[60]. The incidence of iatrogenic CS is difficult to estimate, because there is no reporting requirement. In the developed world, iatrogenic CS could be as frequent as 1 case per thousand and year[76].

Signs of iatrogenic CS are as varied as those of Cushing's disease. In a cohort of patients receiving for three months more than $0.4\,\mathrm{mg/(kg\,d)}$ prednisone, the most common signs were development of ectopic adipose deposits (50%),

hyperphagia (47%), and muscle cramps (32%) [77]. In the same cohort, 15% complained of muscle weakness. Patients stated that the most distressing signs of hypercortisolism were, in order, body shape changes, neuropsychiatric disorders, muscle cramps, and hand tremor. Mastaglia estimated that, in 1982, the most common cause of iatrogenic muscle weakness was caused by GC[78].

There are differences between GC-induced cardiovascular changes, depending on the nature of the GC. Endogenous GC, such as cortisol, have hypertensive effects, while some synthetic GCs, Dexa included, lack such non-specific MR-dependent action. Nevertheless, excess exogenous and endogenous GC cause essentially the same disabling effects on muscle[79], indicating that muscle damage is mediated by GR. GCs do differ quantitatively in their ability to cause myopathy. Myopathy is invariably induced in two weeks by either $0.2\,\mathrm{mg/(kg\,d)}$ Dexa[80], or by $0.5\,\mathrm{mg/(kg\,d)}$ prednisone[81]. Based on other metabolic effects, it is likely that the catabolic potency ratio is even more tilted towards Dexa than the referenced studies indicate, but more detailed human pharmacodynamic studies are lacking.

In their 1958 case series, Muller and Kugelberg were the first to describe muscle changes associated with long-term Cushing's disease[82]. In their mixed, primary and secondary, endogenous hypercortisolic cohort, they found that complaints of muscle weakness were primarily focused on the thigh. Objective loss of muscle force was correlated with histopathological changes indicative of a muscle fiber defect, such as degenerated fibers, at times hyalinized or with loss of striation, muscle replacement with fat and connective tissue, and rare hypertrophic fibers. Through electromyography, they established that the number of motor units is unaffected. Together with lack of changes in reflexes, their work negated a neurological component of CS. Muller and Kugelberg noted faster extinction of the action potential, which may be caused by a reduction in the number of fibers, or by fiber atrophy[83]. Based on the evidence that GC is a

muscle fiber disease, they coined the phrase “steroid myopathy”. Similar electromyographic changes are induced by long-term GC therapy[84], making some authors reserve the term “steroid myopathy” to muscle complaints of iatrogenic etiology. In 1966, D’Agostino and Chiga, confirming histological fiber changes in a rabbit model of iatrogenic CS, formulated the more precise, yet less commonly used “glucocorticoid myopathy”[85]. Owing to the fact that steroid myopathy is not a standalone disease or syndrome, terminology has never been standardized. In the present work, where an experimental and objective angle is taken, through the use of an animal model, the condition of interest will be termed GC-associated muscle loss (GAML).

In exogenous CS, GC excess can be better quantified. In a population with neurological maladies receiving long-term Dexa, the threshold for manifest steroid myopathy appears to be 50 μg/(kg d)[86]. However, the most significant predictor of clinical GAML is total dose[80, 87]. When GAML develops, the amplitude of electromyographic changes (that is, the reduction in action potential duration) is proportional with the total GC dose[88]. These findings imply that steroid myopathy can be induced in shorter periods, if the GC dose is extremely high. Foye and colleagues drew a distinction between “classical” chronic steroid myopathy, induced “within weeks to years”, and acute steroid myopathy, induced in 5-7 days of high-dose GC[89]. However, their description of the two forms of GAML is almost identical, suggesting that the two clinical entities are overlapping to a great extend.

In a comparative study of patients receiving GC therapy for asthma, half of the patients receiving more than 0.2 mg/(kg d) prednisone exhibited a reduction in hip flexor strength of 2 SD or more, compared with health age- and sex-matched controls[81]. In a study of adults with brain or spine cancer, 60% of the participants experienced loss of iliopsoas muscle force in response to GC therapy for cerebral edema[80]. In a small cohort, 6 months of 0.16 mg/(kg d) prednisone

treatment was associated with a 20% reduction in thigh muscle force, compared to healthy controls[90]. In a post-hoc analysis of a chronic obstructive pulmonary disease (COPD) trial, the placebo arm was stratified in GC-treated and GC-naive groups[91]. The maximal inspiratory mouth pressure, a proxy measurement for respiratory muscle strength, was significantly better maintained over the 8 weeks of the trial in the GC-naive, compared to GC-treated participants. Such findings suggest that GC-induced weakness is caused by an objective muscle disorder, and negate the alternative, neuropsychiatric etiology.

Another investigative direction in the study of GC-induced muscle weakness focused on muscle mass and volume. Although correlated, muscle force and muscle mass are not completely reflecting each other. The most accessible proxy measurements of muscle mass, such as mid upper-arm or thigh circumference, are not sensitive enough in monitoring GC-induced muscle loss, even after subtracting skin fold, because GC stimulate intramuscular adipose deposits[92]. The advent of modern imaging allowed non-invasive muscle measurements. Chronic prednisone administration causes a 20% reduction in mid-thigh muscle area measured by computed tomography, and a 36% increase in the ratio of fat-to-muscle areas (CT)[93]. Psoas muscle area and density, measured by computed tomography, are inversely correlated with GC levels indicated by 24-hour urine cortisol (24HUC)[94].

In an early study of chronic hypercortisolism, it was found that all types of fibers are affected by GC[95]. In more recent ones, a type-specific effect was found. Women with CS have an increased proportion of type IIx (fast twitch, glycolytic) and a lower proportion of type I (slow twitch, oxidative) fibers in their vastus lateralis muscles[96]. Similar histological findings were made in renal transplant patients receiving 25 $\mathrm{mg}/(\mathrm{kg\,d})$ prednisone over three months[97]. In the latter, GC caused an increase in the cross-sectional area (CSA) of the type I and IIa (slow twitch, oxidative / glycolytic) fibers. Gains in the ratio fast-to-slow

twitch fibers are associated with insulin resistance[98]. Diameter increases in spite of loss of function and protein content have been explained by a disorganized intracellular structure. A more practical consequence of these findings is the dereliction of CSA measurements in GAML.

A set of muscle mononucleate cells, expressing the paired-box transcription factor Pax7, are presumed to support muscle development and regeneration, and are termed satellite cells (reviewed in [99]). There are no definitive studies describing the effect of GC in human satellite cells. Some or all satellite cells may be activated to proliferate, thus becoming myoblasts. Many in vitro assays use dividing cells from human muscle, at times assumed to be myoblasts. These human "myoblasts" do not proliferate in the absence of at least 1 μM ([100], and personal observation; data not shown). For comparison, maximum normal concentrations of endogenous cortisol in humans is 0.78 μM[101], that is, tens of times less potent. Therefore, it is impossible to conceive an experiment where these human myoblasts are subjected to meaningful manipulations of GC concentration. Moreover, it appears that GCs are vital for human muscle development and maintenance. This implies that cell lines which do not require GC, such as those surviving in serum-free media, may be less accurate models of muscle.

There are no published cases of increase in circulating myoglobin or creatin kinase in response to GC monotherapy, or as a consequence of Cushing's disease.There are some mentions of the opposite in Uptodate, but the rumor has no literature to support it. Not even anecdotal. The absence of such intramuscular protein from the blood flow suggests GC do not cause rhabdomyolysis, that is, loss of muscle through uncontrolled rapid membrane leakage.

From its first trial, GC therapy ability to induce a negative nitrogen balance, through an ample increase in urinary creatine and creatinine, was interpreted as evidence for stimulation of tissue protein breakdown[102]. As little as 20 μg/kg

cortisol infused over 8 hours increases by a quarter the rate of appearance of leucine into the bloodstream, suggestive of proteolysis upregulation[103]. Leucine's rate of appearance is even higher when the GC-induced hyperinsulinemia is prevented, indicating that whole-body experiments do not capture the amplitude of the GC-induced proteolysis[104]. More modern mass spectrometric methods revealed that a single dose of 1 mg/kg prednisolone cause increases in all blood amino acids, presumably due to mobilization from muscle sources[105]. The same acute treatment causes an increase in 3-methylhistidine (3MH), a degradation product specific to muscle actin and myosin[106]. Similar increases in 3MH are seen with control diet in chronic GC excess of endogenous or exogenous nature[95]. These findings demonstrate that GC-induced loss of muscle mass is mediated by stimulation of protein degradation.

The last three decades brought a better understanding of protein degradative pathways and of muscle atrophy. Two major proteolytic systems, the proteasome-ubiquitin system and the autophagosome (discussed in later sections), have been discovered and dissected. But only one published trial investigated the action of GC in human muscle biopsies, at a molecular level. It failed to find a significant change in mRNA of ubiquitin and the C3 subunit of the proteasome[107]. The result is unsurprising, given that the control of the proteasome system may be exercised in other, unprobed ways. In animal models, the genes most correlated with muscle loss, including GAML, are two E3 ubiquitin ligases, muscle atrophy F-box (MAFbx) and muscle RING finger 1 (MuRF1), but no published studies confirm or refute their modulation in humans (reviewed in [108]).

Recently, pharmacological inhibition of the proteasome become widely available. The first proteasome inhibitor, bortezomib, is recommended by the FDA for multiple myeloma and mantle cell lymphoma[109]. The second generation, irreversible proteasome blocker carfilzomib is also approved for

advanced myeloma therapy[110]. In the light of data from the animal models of muscle loss, these drugs should have been useful in cachexia, but, to date, no human trials investigated their ability to prevent muscle atrophy.

There are no trials comparing GC with the combination (GC + bortezomib). However, an indirect comparison can be made. In a trial for multiple myeloma, fatigue was a complaint of 32% of the participants receiving 40 mg Dexa, compared to 42% for bortezomib[111]. In another trial, addition of 20 mg Dexa to bortezomib lowered the rate of fatigue from 57% to 25%[112]. Taking into account the large variability between trials and the use of a atypical population, the comparison is of marginal use, but it does not appear that the combination (Dexa + bortezomib) is more muscle protective than Dexa alone. Clinical studies directly addressing this comparison in patients prescribed high-dose GC are recommended, given that the most commonly accepted hypothesis centers on the proteasome as main effector of GC-induced muscle loss. It is still more likely to find that bortezomib provides muscle protection. Proving a beneficial action of bortezomib in co-administration with GC will have major practice implications. Even proving the opposite, that bortezomib has no protective action, will be very valuable in better understanding and eventually preventing GC-induced muscle loss.

The inhibition of the other proteolytic system, the autophagosome, is also the focus of clinical studies. Starting with the inexpensive antimalarials chloroquine and hydroxychloroquine, autophagosome inhibitors are now the focus of phase II clinical studies in many cancers[113]. Interestingly, hydroxychloroquine is also recommended for rheumatoid arthritis, where it may be prescribed for up to six months[114]. Chronic hydroxychloroquine therapy is known to induce muscle weakness and sporadic myopathy, through a distinct, vacuolar mechanism. The hydroxychloroquine-induced myopathy is associated with an increase in autophagosomal markers in muscle, demonstrating autophagosome's importance

in muscle regulation[115]. In two separate case reports, co-administration of prednisone and hydroxychloroquine led to vacuolar myopathy, which could be caused by the choice of doses, or could be indicative of true epistasis[116, 117]. Potential benefits of anti-lysosomal co-therapy for the atrophying muscle remain the subject of speculation.

Another putative parallel mechanism for GC-induced loss of muscle is downregulation of protein synthesis. Few human trials measured directly the effect of GC on protein synthesis in healthy volunteers. Brillion and colleagues[104] found that a 80 mg cortisol infusion over 13 hours led to 8% increase in non-oxidative leucine uptake, indicating an upregulation of protein synthesis. However, using a 200 mg cortisol infusion in the same protocol failed to cause a detectable change in protein synthesis compared to placebo, suggesting hormesis may confound experiments. Moreover, this finding was never replicated. Löfberg and colleagues[107] found that three days of 65 mg / day prednisolone caused a non-significant 21% increase in protein synthesis rate and a statistically-significant 52% increase in the rate of protein degradation, based on the difference between arterial and venous levels of tritiated phenyl alanine at leg level. Short and colleagues employed fractional synthesis rate (FSR), which describes the time rate of enrichment in muscle tracer, normalized to the circulating tracer concentration. They concluded repeatedly that, in leg muscles, 35 mg / day prednisone for 6 days “has no effect on [...] muscle protein metabolism or muscle function”[118, 119] . Some of these studies may have been underpowered (sample size n = 6-7) or may be troubled by the use of a small dose, but their validity is confirmed by the fact that, in each case, the expected hyperglycemic response to GC was observed.

The hypothesis that GC cause muscle loss by inhibition of protein synthesis is still debated, due to a plethora of indirect evidence. In Löfberg’s study, biopsies revealed a prednisolone-induced loss of muscle polyribosomes, interpreted as

evidence for decrease in protein synthesis rate. Even in studies where GC failed to elicit reductions in protein synthesis, they inhibited translation-stimulating signals in muscle from anabolic factors such as insulin[120], branched chain amino acids[121], and exercise[122].

At a molecular level, it appears that Dexa inhibits anabolic signals centered on the Akt / mechanistic target of rapamycin (mTOR) axis. More evidence has been collected in animal models, and is discussed in a dedicated section. One study on humans described how Dexa inhibits branched chain amino acids' ability to induce phosphorylation of eIF4E binding protein 1 (4E-BP1) and p70-S6K[123]. Dexa lacked similar action on another translation regulators, eIF2α.

In addition to GC excess, muscle weakness is caused by GC withdrawal[124], and by GC deficiency, illustrated by the Addisonian crisis[125]. In both hyercortisolism and hypocortisolism, effects on human muscle remain understudied. Human studies concord that GC-induced loss of muscle force is an objective finding caused by an increased proteolytic activity. Indirect evidence indicate human GAML is associated with changes in protein synthesis. In the absence of other proven mitigating interventions, current guidelines suggest GC discontinuation if myopathy develops. Animal models have been essential for the study of GC-induced muscle loss, although they have been confounded by hormesis (discussed in future sections).

In conclusion, CS of various etiologies leads to an increase in muscle protein catabolism, and a reduction in muscle protein synthesis.

Muscle protection with androgen therapy

A series of historical circumstances brought anabolic androgenic steroids (AAS) in the attention of clinicians treating hypercortisolism in muscle. The same circumstances meant that utility of AAS therapy in steroid myopathy has never been fully explored.

Male hormones have been considered an efficacious anabolic therapy long before they were purified and tested. The effects of male castration, such as reductions in aggressiveness and muscle force, were discovered independently by many human civilizations, starting more than three thousand years ago. Castration is omnipresent in ancient mythology, and, more mundanely, in primitive farming. For almost as long, people perceived testis ingestion as a reversal of castration, thought to improve muscle force. Such perceptions were caused by the placebo effect alone, given that this testis active principle is almost completely degraded by liver.

Testis extract benefits received more attention starting around 1889, when Brown-Séquard published his theory about rejuvenating abilities of sperm. He thought that loss of sperm during aging or masturbation causes degradation in muscle and brain performance, and hypothesized that chemicals from sperm may pass into blood where they have "a most-essential use in giving strength to the nervous system and to other parts." Consequently, he injected himself with a combination of sperm and testis extracts, which led to self-reported improvements in physical and intellectual abilities[126]. He describes how, at the age of 72, a single injection enables him stand for hours, or write longer scientific papers. Later on, he describes how testis extracts appeared to alleviate "serious affections of any kind", including cachexia, pulmonary tuberculosis, cancer and leprosy ulcers[127]. Because the active principle in testis is made as needed, rather than stored in high-concentration depots, it is now obvious that these observations were the product of preconception.

The cultural context in which Brown-Séquard worked introduced multiple biases in his experiments and conclusions. His mistaken theses were constrained into rather low-quality experiments, which luckily provided useful, testable, and eventually proven scientific hypotheses. First, the logical conclusion for Brown-Séquard's theory would have been endorsement for semen therapy.

Instead, due to the semen taboo, Brown-Séquard and his disciples resorted to surrogate interventions, such as vasectomy, believed to preserve sperm in the body, and injections with testis extracts. The introduction of injections gave a new lease of life to the therapeutic use of organ extracts, called "organotherapy", which had been banished from the British Pharmacopoeia in 1788 after failing the test of oral administration. Some organotherapies were shams or even harmful. Yet a few of them provided evidence that specific parts of the body store or release into the blood stream chemicals, which subsequently induce changes in other specific parts of the body. This conjecture led the discovery of endocrine glands and the establishment of endocrinology as a science. In fact, androgen organotherapy provided the pattern to GC discovery.

Second, the Victorian era is an age of body rediscovery. Georgian pastimes, such as cock fighting, horse racing, or cricket, are replaced by more muscular sports, such as football, rugby, gymnastics, and swimming. Body building becomes fashionable, with the first professional competition selling out Royal Albert Hall in 1901. Brown-Séquard's promise of muscle without effort makes testis organotherapy a widespread, well-earning business. When Voronoff is barred from practicing in Paris and judged as fraudulent by the Royal Society of Medicine, he takes his testis transplant business to Algiers, where he receives patients from all over the world (reviewed in [128]). Private sponsorship led to investment in androgen research, but with a focus on commercial rather than clinical efficacy.

Finally, Brown-Séquard's era tolerated unscientific theories, which ignored the physical and intellectual ability of women. Brown-Séquard claimed that ovary extracts provide some benefits, but with "less power" than testis extracts[127]. Such conclusions stemmed from cultural biases rather than comparative experiments. In 1849, Berthold showed that, through testis implants, roosters regain male characteristics they lost through castration, such as aggressiveness,

libido, and larger combs[129]. With maintenance of secondary sex characteristics as its sole ability, Berthold's secreted agent was therefore androgenic. In contrast, Brown-Séquard claimed that his extract increases muscle force, without mentioning any virilizing side effects. Moreover, in 1935, Kochakian proved that urine-extracted "male hormone" stimulates muscle accretion in castrated dogs, that is, that it is anabolic[130]. While ultimately proven correct, the idea that "male hormones" were simultaneously androgenic, anabolic, and ergogenic was based on a cultural construct that confounded manliness and physical force, rather than the product of evidence.

The belief in an male-secreted ergogenic substance inspired many commercial enterprises to sponsor research in male endocrinology, through the decades where the evidence was confined to changes in the combs of roosters. These dark ages end in 1927, McGee and Koch extract a lipophilic virilizing mixture from rooster testis[131, 132]. A pure and even more androgenic chemical is extracted in 1935 from bull testis by Laqueur, working for Organon[133]. Laqueur names his discovery testosterone (T). Three months later, Butenandt and Ruzicka, sponsored by Schering and Ciba respectively, announced the development of manufacturing methods for synthetic testosterone, an achievement that brought them the 1939 Nobel Chemistry Prize (reviewed in [134]). At the University of Chicago, Kenyon tests T on four eunuchoid patients of testicular and pituitary etiology. Daily injections of 25 mg testosterone propionate (Tp) cause an doubling in prostate and penis size[135] after less than two weeks, thus establishing the efficacy of T replacement therapy in men with pathological decreases in circulating T. With few, narrow exceptions, this population was and remains the only generally accepted, FDA-approved indication for T therapy[136, 137, 138]. Recent T preparations are still recommended for some breast cancers, but this indication is limited to a few, unpredictable, cases.

Due to manufacturing costs, limited commercial target, and governments'

lack of interest, T therapy traversed a very long experimental stage, which could easily be called “the second dark age of androgens”. Only in 1953, FDA gives its first approval for an androgenic therapy, a T enanthate injection. Then, as now, FDA’s approval was based on T ability to restore normal levels of androgens, rather than other, more functional or curative, outcome[137]. But in 18 years of life as experimental drugs, androgenic steroids have been trialled in diverse diseases, including male functional impotence[139], unwanted lactation[140], uterine bleeding and dysmenorrhea[141], or osteoporosis[142]. These early studies share the extremely small sample size, and the scarcity of controls, blinding, and objective outcomes. For example, a study found that 14-35 injections of Tp (cumulative dose 255-455 mg) caused an improvement of acne in half of the male participants[143]. Such findings are at odds with more modern trials, where weekly i.m. androgen injection lead to an increase in absolute risk of acne by 15%, in healthy males[144], and are possibly explained by the variability in the androgen arm, small sample size (n = 12), lack of blinding, and early stopping in the placebo arm. Nevertheless, these trials are, in many cases, the only source of information about the action of T in the normogonadal population. For example, early trials of oral methyltestosterone revealed its hepatic toxicity, with the effect that, 50 years later, the development of oral androgenic therapies is still discouraged.

The second dark age of T were times of limited knowledge, and even more limited adherence to the principles of clinical research. Yet in these years, androgenic steroids first gained their reputation as ergogens. Kenyon noted in his studies on eunuchoid men that T injections helped them gain weight through protein accretion, as demonstrated by a reduction in urinary nitrogen. Other trials evidenced benefits from androgenic therapy in muscle-depleting conditions, including thyrotoxic myopathy[145] and muscular dystrophy[146]. By 1940, Kenyon confirmed that Tp caused nitrogen retention, caused by increased protein

accretion, even in healthy men and women[147]. Interestingly, in 1942, Samuels and colleagues state that T does not change grip strength in healthy males[148]. According to a meta-analysis[149] and my literature search, no other test of androgens' effect on muscle strength is published until 1968. Despite the lack of evidence, androgens are used as ergogens in healthy people, starting with Olympic athletes around 1954[150].

As exemplified by the ergogenic hypothesis, benefits of androgen therapy on men with T deficiency have been extrapolated by clinicians and theoreticians to other muscle-depleting conditions, and even to healthy humans. Naturally, one of the conditions associated with loss of muscle mass that clinicians hoped to improve was hypercortisolism. In 1941, Albright shows that the newly-discovered Tp, in 25 mg daily injections, was better than estradiol benzoate, progesterone, or vitamin D in restoring nitrogen balance in three cases of Cushing's disease[151]. Similarly, in 1950, the Mayo Clinic team who discovered cortisone remarked that, in one case, 25 mg Tp daily injections reduced urinary nitrogen losses caused by 200 mg cortisone administration[14]. Some of the aforementioned researchers publish similar case reports, sharing the small sample size and the use of surrogate outcomes[152]. These shortcomings do not prevent each investigator from subjective claims of improvements in physical function.

During the 1950's, AAS became part of the standard of care for endogenous hypercortisolism during the gap between diagnosis and curative surgery. However, this gap narrowed to a few weeks, due to improvements in differential diagnosis. Development of accurate cortisol assays allowed the measurement of its changes in response to Dexa, thus discriminating conditions where feedback mechanisms fail (mainly endocrine neoplasms) from cortisol-stimulating non-endocrine conditions. ACTH assays differentiate ACTH-independent cases (typically adrenal tumors) from the ACTH-dependent ones (usually localized in the pituitary). Modern imaging, including computed tomography of the adrenal

Class	Medications
ACTH inhibitors	• Subtype 5 somatostatin receptor agonists: pasireotide (FDA-approved)[153] • Dopamine D2 receptor blockers: cabergoline
11-β hydroxylase inhibitors	Metyrapone, mitotane, ketoconazole.
Inhibitor of 3 β-hydroxysteroid dehydrogenase	Trilostane (EMA-approved, FDA-withdrawn)[154]
Inhibitor of the cholesterol side-chain cleavage enzyme	Aminoglutethimide
GR antagonist	Mifepristone (FDA-approved)[155]

Table 1.1: Pharmacological agents used in Cushing's disease of unidentified ectopic, or diffuse localization (reviewed in [1, 2])

and magnetic resonance imaging of the pituitary, identify the target of surgery. AAS therapy is now confined to inoperable cases, including unidentified ectopic sources of ACTH or cortisone. Even these cases benefit from more targeted interventions (see1.1).

Similarly, the opportunities for AAS as adjuvant to GC therapy are very limited. Many of the diseases previously treated by high-dose GC are now treated with more specific drugs. As practitioners became more accustomed with the risks of GC therapy, doses and durations were reduced. With the exception of life-threatening conditions, typical GC prescriptions switched to lower-potency compounds, such as prednisone or even cortisol. In particular, practitioners became well aware of the issues of GC withdrawal syndrome, where adrenal atrophy is aggravated by some other, still undiscovered, component[124]. By mid-1970's, it became common advice that "prescriptions for [glucocorticoid]

steroids should not be refillable"[156]. By the time modern trials with AAS began, the incidence of overt hypercortisolism have been greatly reduced. Despite a potential epidemic of covert hypercortisolism, with deleterious effects of life quality and expectancy, the interest for studies on hypercortisolism has largely waned. Clinical studies investigating the benefits of AAS in hyepercortisolism are scarce and small-scale. For example, there are no significant-size clinical studies analyzing the effect of AAS on the muscle strength of the endogenous CS patient.

An unblinded trial observed AAS-induced increases in lean body mass and apendicular muscle mass, in men already receiving an average of 6 mg prednisone a day over 9 years[157]. Another, randomized, blinded, placebo-controlled trial by Crawford and colleagues tested the benefits of testosterone or nandrolone decanoate as an adjuvant to chronic GC therapy for diverse pathologies[158]. The exposure to GC was an average of 12 mg prednisone a day, over more than 8 years, and was already causing osteopenia, hyperlipidemia, hypercholesterolemia, and a reduction in quality of life compared to historical controls[159]. Such side effects could arguably be considered evidence for mild iatrogenic CS. After six months of 200 mg testosterone injections every other week, the AAS group had higher bone density, muscle mass and strength, and a better quality of life, compared to the placebo group. To date, Crawford's study is the best evidence for effectiveness of AAS as adjuvant in GC therapy.

In a subset of CS patients, androgen administration improves muscle mass, and, presumably, quality of life.

Hypercortisolism-induced changes in endogenous androgens levels

Muscle wasting is more common in males than in females with hypercortisolism[160]. The interaction of GC with endogenous AAS distinguishes male from female hypercortisolism, to the point that the two syndromes are qualitatively different.

The period when AAS were frequently used as therapy of CS or as adjuvant to high-dose GC pre-dates modern molecular biology and genomics. Therefore, there are no published human trials to describe in molecular terms the interaction of GCs and AAS at muscle level. Most of our knowledge is derived from animal models (discussed later). On the other hand, clinical observational studies of circulating biomarkers remain common, and reveal an interesting interaction between the two classes of steroids. More specifically, in many cases, hypercortisolism suppresses endogenous AAS. Because loss of endogenous AAS, also termed hypoandrogenism, is associated with loss of muscle mass and strength[161, 162], an AAS replacement strategy in hypoandrogenic hypercortisolism may be beneficial for the muscle.

A series of trials observed the effect of short-term (hours or days) hypercortisolism in healthy volunteers. Experimental acute hypercortisolism represses circulating levels of T, in a reversible manner, in males and, to a lesser degree, in females [163, 164]. The mechanisms through which hypercortisolism causes hypoandrogenism are still to be elucidated. Some studies suggest that acute hypercortisolism downregulates the pituitary-secreted, T-upregulating, luteinizing hormone (LH)[165, 166, 167]. Others counter that GC induce hypoandrogenism even when LH is unchanged[168]. Another hypothesis is that the negative feedback loop repressing ACTH in hypercortisolism has a side effect of androgen suppression[169]. A few groups have even hypothesized the existence of another, still unknown hormone, synthesized from ACTH precursor, pro-opiomelanocortin (POMC), with the ability to stimulate androstenedione synthesis and secretion[170, 171]. Certainly, GC-induced repression of POMC should also repress this unknown androgen-stimulating hormone, but its existence was never proven.

In males, chronic hypercortisolism is also associated with hypoandrogenism. Long term prednisone therapy reduces circulating T levels[172]. Similar

observations have been made in endogenous CS, where exposure is longer and, depending on etiology, ACTH is increased or decreased compared to normal. Some studies found that, in CS, LH and another gonad-stimulating pituitary hormone, follicle stimulating hormone (FSH) are lower than normal[173]. This has been explained as a CS-associated pituitary defect, with loss of LH response to stimulation by its hypothalamic regulator, gonadotropin-releasing hormone (GnRH)[173, 174]. Alternatively, others concluded that hypercortisolism impairs hypothalamic GnRH secretion[175]. Finally, a small study found that male asthma patients receiving long-term prednisone have lower circulating T levels despite increases in LH and FSH, and concluded that prednisone has an direct inhibitory action on the testes[176]. Despite disagreeing on the mechanism, all these studies agree that chronic hypercortisolism represses testicular androgen secretion.

AAS therapy does not change circulating cortisol levels[177], suggesting that a reverse effect probably does not exist. Similarly, it is possible that the direct effect of GC on AAS is only an artifact caused by pathological and pharmacological doses.

In both sexes, the most concentrated circulating steroids are dehydroepiandrosterone (DHEA) and its ester, DHEA sulfate (DHEAS), which originate from the adrenal and, to a lesser degree, from gonads. Their most important role appears to be that of precursors for synthesis, in glands and peripheral tissue, of androgens and estrogens. DHEA has some affinity for the AR, which suggested it may be an AAS. Recent studies indicate that, in human female tissue, DHEA may in fact be a partial agonist, hindering the action of T[178]. DHEA and DHEAS, now termed adrenal androgen precursors (AAP), are upregulated by ACTH, through increased synthesis of DHEA in the adrenal and rapid bidirectional interconversion[179, 180]. Therefore, Cushing's disease and other conditions associated with increases in ACTH will present with increases in AAPs, while primary hypercortisolism will be associated with ACTH repression

and consequent AAP decrease[181, 182, 170, 183]. Both types of hypercortisolism manifest GAML, despite opposite effects on AAPs, suggesting that AAPs changes are not mediating GAML.

In adult women, the regulation of AAS is more complex. During reproductive age and a few years afterwards, the main source of androgenic stimulation is the ovary[184], where T is an intermediate product in the synthesis of estrogens (reviewed in [185]). A feedback loop links LH and estrogens levels, with LH stimulating synthesis and secretion of estrogens from the developing and atretic follicles[186]. The reverse link is more complex, with estrogens inhibiting LH for most of the menstrual cycle[187], with the possible exception of ovulation. In the direct link, LH must stimulate ovarian T synthesis, but a reverse link, where T directly inhibits LH, is absent in women[188, 189]. Although measurement methods and normal ranges are still to be perfected, it appears that circulating T level in women are reflecting the menstruation-related cyclical interplay of estrogen and LH, rather than being independently controlled[190, 191].

This sexual dimorphism differentiates male and female AAS response to chronic hypercortisolism. Women with CS have lower muscle mass compared to general population [192]. Decreased libido, a sign of hypoandrogenism in both genders, is reported by 40% of female CS patients[63]. But, in contrast to males, females with CS have normal or even increased AAS synthesis and levels, compared to healthy controls [193, 194]. Four fifths of women with CS have menstrual irregularities, which has been attributed to hyperandrogenism, direct cortisol action, or depletion of LH or estradiol[175]. More than 75% of CS cases present with hirsutism, that is, male-patterned body and face hair growth in female patients, and a clear sign of hyperandrogenism[1, 63]. Women with CS-related hirsutism have androgen levels higher than healthy controls[195]. Per PMID 5922193, urinary androgens in female CS are repressed by Dexa. That could be a biphasic response. Other signs of hyperandrogenism, such as voice

changes or acne, are rare in female CS.

In infants, tumors causing CS are exceedingly rare. In pediatric Cushing's disease and adrenocortical carcinoma, AAP circulating levels are usually normal for the age[196, 197]. Virilization signs such as change in voice, penile or clitoridian overgrowth, and hirsutism are common [198]. Published studies do not describe muscle changes in these children, possibly due to difficulties in assessment.

In adult female and in pediatric CS, virilization, muscle catabolism, and circulating androgens changes are not correlated. These examples suggest that relative hyperandrogenism in some tissues may be paralleled by relative hypoandrogenism in others.PMID 14329633 implies Cushing causes polycytemia, which would be another sign of hyperandrogenism. Some old textbooks also clam the same. There is no evidence in that paper nor anywhere else. PMID 10409572 shows acute Dexa in healthy men has no effect on hematocrit. Nieman reviews do not mention any RBC effect in CS either.For example, it may be possible that, in some tissues, excess GC activates the androgen receptor (AR)[199], the nuclear receptor specific for AAS at physiological concentrations. Because short-term Dexa inhibits AR expression in women's muscle[200], it may be possible that GCs interfere with T signals in a tissue-specific manner.

Understanding causality in the case of simultaneous muscle loss and hirsutism is complicated by dose- and compound-dependent crossconversion of GCs to AAS and interference of GCs in AAS synthesis and degradation. It is unclear to what degree muscle loss in CS is influenced by the changes in endogenous AAP and AAS. Based on endogenous levels, it appears that AAS therapy may benefit men, but not women and children, with CS.

Interestingly, the Crawford and colleagues trial observed muscle protection by AAS as adjuvant to GC therapy although, at enrollment, these men had circulating T levels in the lower normal range[158]. This confirms that GC deleterious effects

are not solely caused by hypoandrogenism.

Hypercortisolism is associated with hypoandrogenism solely in adult males. Androgen therapy for muscle protection in CS is predicted to benefit them more than other populations.

Molecular mechanisms of androgenic myoprotection in humans

GAML is a phenomenon well-studied, with its molecular mechanisms dissected in human studies. In contrast, the effect of AAS in GAML was studied in a few case reports, marred by the absence of objective physical outcomes and of molecular analysis. More information can be gleaned from the effect of AAS in other muscle-depleting conditions.

Most commonly, studies of AAS on muscle are carried on men with lower than normal circulating T and / or associated symptoms, also called hypogonadal. In male primary hypogonadism, rates of cortisol synthesis and degradation are typically normal[201]. In this population, AAS therapy, even with low, "replacement" doses, causes an increase in muscle mass and force[202, 203]. The gain in muscle mass is caused mainly by an increase in protein synthesis, as evidenced by increased nonoxidative uptake of labeled leucine[203]. Moreover, T causes an increase in FSR of myosin heavy chain (MyHC), indicating that protein accretion is localized in the myotubes.

The referenced studies also measured leucine flux, a proxy for protein degradation, but failed to detect significant changes as a result to T therapy. The absence of a detectable change in leucine flux may be attributed to a true lack of effect on catabolism, or may be an artifact caused by the use of whole-body, rather than isolated muscle, methods.

Typical naturally-occurring male hypogonadism is usually associated with pleiotropic pathology, such as Klinefelter's syndrome, where deficient androgen synthesis may be complicated by other peripheral defects. For this reason, some

studies were conducted in males with iatrogenic hypogonadism, induced by administration of GnRH agonists, such as goserelin or leuprolide, which disrupts and eventually abolishes LH secretion. Leuprolide-induced hypoandrogenism causes loss of muscle mass in healthy volunteers and in prostate cancer patients[204, 205]. In the former, most of the muscle losses are reversed if exogenous T is co-administered. Chemical castration causes decreases in both protein synthesis and degradation[206], suggesting that, in some cases, such as restoration of physiological levels, T supplementation may be followed by a paradoxical increase in protein degradation.

The protective action of AAS therapy in iatrogenic hypoandrogenism is not affected by co-administration of an aromatase inhibitor such as anastrozole[207]. Aromatase converts T in estradiol. The continuing muscle protection when T cannot be converted to estrogens demonstrates that muscle protection is an intrinsic ability of T. A more plausible mediator is the anabolic hormone insulin-like growth factor I (IGF-I), whose muscle expression is decreased by iatrogenic hypogonadism[206], and by short-term, high-dose Dexa.

Another well-studies group comprises older men, whose T levels and muscle mass are naturally declining[208, 209]. An argument has been made about benefits of T replacement therapy in this population. Multiple clinical studies tested this hypothesis. In older men with low bioavailable T, muscle mass and strength is improved by 200 mg T every other week[210, 211]. As in hypogonadal men, muscle recovery can be localized to the contractile cells, as indicated by increases in the CSA of fast- and slow-twitching fibers[212]. No evidence of fiber type switching or fiber type-specific effects in response to AAS therapy has been seen. Instead, histological studies reveal that elderly treated with AAS have significantly more satellite cells[212].

T causes improvement in the net balance between protein synthesis and degradation at muscle level[213]. The cause of protein accretion is an increase in

protein synthesis, as shown by an augmentation of mixed-muscle FSR[214]. Interestingly, some of this newly accrued protein is extracellular matrix, as indicated by the upregulation of circulating N-terminal propeptide of type III procollagen[215].

Ferrando and colleagues made the case for an anti-catabolic action of AAS in older men[3]. However, their study differs in key aspects from the other studies and the medical practice. They tested a variable, moderate dose of T on normogonadal older men, with the goal of maintaining a physiological T level. Moderate T therapy caused an improvement in muscle mass, strength, and net protein balance. However, they failed to observe an improvement in protein FSR, and concluded that the net protein balance improvement must be caused by T-induced inhibition of protein degradation. The failure to detect protein synthesis rate changes indicates that, perhaps due to unusual treatments, this study yielded unusual outcomes, which cannot be extrapolated to other studies or populations.

In support of their hypothesis, Ferrando and colleagues showed a significant decrease in the rate of phenylalanine disappearance at muscle level and in the proteasomal enzymatic activity. However, the data they provide show that, on the contrary, proteasomal activity is not reduced by T therapy. Digitizing their plot indicates that six-months of placebo changed the normalized lactastatin-sensitive proteasome activity from 0.12 to 0.076 relative units, whereas six months of T changed it from 0.084 to 0.078 relative units, a likely non-significant set of changes. The same group found a similar pattern of anticatabolic action, in a short-term trial of T on men with severe burns, once again doubled by an apparent absence of the pro-anabolic component[216]. It may be possible that the protective action of T changes qualitatively, depending on the cumulative dose. Alternatively, the anticatabolic action may be more salient when T supplementation is given to the normogonadal. The hypothesis that T inhibits

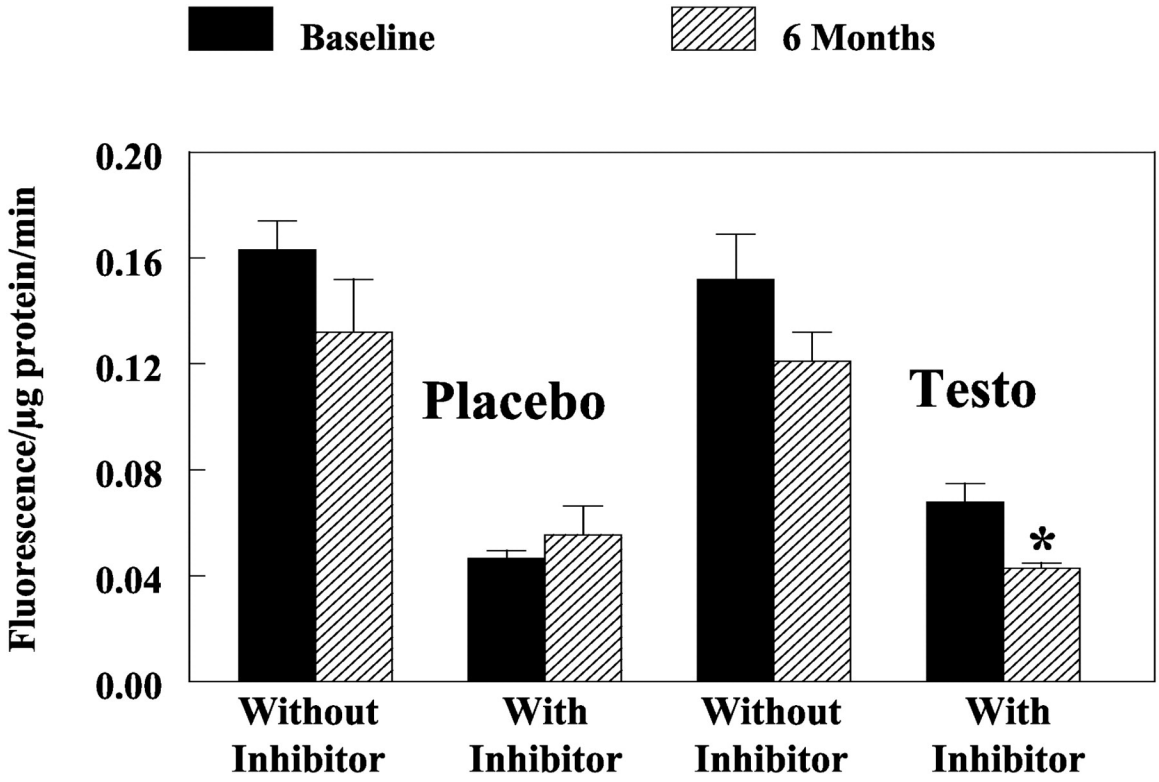

Figure 1.1: Changes in proteasome enzymatic activity following 6 months of T therapy (reproduced from [3] with permission).

protein degradation remains tempting, but better studies are needed.

In older men, T upregulated intramuscular and circulating levels of IGF-I[213, 217]. The protection of muscle force provided by T to the older hypogonadal men is not hindered by co-administration of finasteride, an inhibitor of 5α-reductase which causes the transformation of T to 5α-dihydrotestosterone (DHT)[218]. Similar lack of effect was seen with dutasteride, a less specific inhibitor of 5α-reductase, added to exogenous T, in a younger, possibly less hypoandrogenic cohort[219]. In human males, conversion to DHT is not required or T's regulation of muscle mass. Possibly more relevant, T upregulates IGF-I in the muscle and in the serum of the older men[213, 217].

While women's endogenous AAS levels are lower than men's, it is unclear if benefits of T therapy outweigh the deleterious effects[220, 221]. There is no FDA-approved T preparation for women. Therefore the action of T in women

losing muscle is yet to be investigated.

The best molecular observations on the action of T on muscle loss have been obtained from studies of HIV-positive men, who have significantly lower circulating T levels[222]. Some of the drugs used in HIV-AIDS, the anticachectic megestrol and the protease inhibitor ritonavir, may cause hypercortisolism, making it even more informative about the action of AAS in CS. AAS delays loss of muscle mass in AIDS wasting syndrome, leading to better quality of life[223]. Microarray analysis indicated that T-treated muscle upregulated, as expected, expression of genes from the IGF-I- and AR-stimulated signaling pathways[224]. Immunoblot confirmatory studies indicated that T caused the upregulation of a key component of the IGF-I signaling pathway, the protein kinase B, also known as Akt, in its Serine-473 phosphorylated, that is, activated form. Other genes upregulated by T are muscle development regulators, such as the myocyte enhancer factor 2A (MEF2A) and a host of macrophage-associated markers. In addition, T stimulated expression of genes from other pathways, including transcription factor 4 (TCF4) from the Wnt / β-catenin pathway, AMP kinase (AMPK), and the guanine nucleotide exchange factor Son of Sevenless (SOS), involved in the mitogen-activated protein kinase (MAPK) pathway. However, in the same study, at protein level, MAPK did not appear to be modulated by AAS therapy. The referenced microarray study failed to find a change in expression of the major muscle regulator myostatin[225, 226], or of the two E3 ligases typically associated with muscle loss, MAFbx and MuRF1[227, 228].

The histological and molecular findings from hypogonadal and HIV-positive males receiving AAS have been confirmed in many other pathologies that cause loss of muscle. AAS therapy improves muscle mass and strength in males with chronic kidney disease and liver cirrhosis[229, 230]. In men with COPD, 100 mg T enanthate injected weekly led to improvements in muscle mass and strength, potentially augmenting quality of life[231]. These improvements are caused by an

increase in fiber CSA, regardless of fiber type, and by an upregulation of the IGF-I mRNA isoform known as mechanogrowth factor or IGF-IEc[232]. In these men, MyHC upregulated by T in these men was of isoform 3, also known as embryonic MyHC. This is also one of the two MyHC isoforms upregulated by T in the HIV-positive men. In the COPD cohort, embryonic MyHC was found in thinnest fibers, possibly marking them as newly formed.

A cross-sectional study split a cohort of males with heart failure, without cachexia and with normal circulating cortisol, T and ACTH levels in two halves based on their cortisol level. I am rebranding CHF as HF, following the recent guidelines from PMID 16160202. The subgroup with lower circulating cortisol achieved a higher peak work rate, suggestive of GC-induced muscle damage[233]. Some randomized trial showed that heart failure patients improve their muscle force with AAS therapy[234, 235]. However, a series of recent studies found deleterious cardiovascular effects of AAS[236, 237], which will discourage the use of T in heart failure. In fact, many of the aforementioned conditions where AAS was used are marred by higher cardiovascular risks and frailty, pressing the need for alternative, equally efficient, anabolic adjuvants. To this end, a deeper understanding of AAS therapy at molecular level is required.

In various conditions that cause muscle loss, AAS benefits share a pattern including improved muscle mass and strength, fiber hypertrophy, tissue remodeling, and increased protein synthesis. In some conditions, AAS-driven muscle rehabilitation is associated with an increase in satellite cells and / or an inhibition of protein degradation. Putative molecular mediators known from animal models have not been confirmed in humans, with the invariant exception of the upregulation of IGF-I. Better clinical studies are required. Animal models and in vitro studies sketch the road ahead.

2. BIOLOGICAL PREMISES

Skeletal muscle histology

Muscles are specialized for their main ability, contractility. For mammalians, ability to move is vital for survival, meaning that a large portion of their bodies is muscle. In a cohort of 300 borderline overweight US Americans, skeletal muscle as a proportion of body weight was on average 41% for men and 31% for women[238]. Owing to the ability to measure individual muscles in any experiment, the scientific community has not been under pressure to develop accurate techniques that measure total skeletal muscle mass in mice. A proxy measure of murine skeletal muscle mass, lean body mass averaged 81% of total body weight in adult males (detailed in results section). Three fifths of the human body's protein is confined to the muscle contractile and support structures[239].

For the most part, the skeletal muscles confers the three-dimensional intricate conformation of the body, suggesting a complex, detailed organization, at least at macroscopic level. In contrast, at cell level, the relatively high specialization of the skeletal muscle leaves little space for diversity or inhomogeneity. Skeletal muscles are organized in anatomical units that may impose a force on two moving body segments (typically, bones), determining them to come closer to each other. This specificity of action is ensured by the presence of distinct, well-determined insertion points, to which skeletal muscles are attached by the means of tendons and aponeuroses (reviewed in [240]).

The tendons are dense connective tissue structure, which extend into the epimysium, a connective tissue sheath surrounding the muscle. In turn, epimysium emits connective septing structures termed perimysium, splitting muscles into subunits termed fascicles. At an even lower level, a thin, sparse connective structure called endomysium coats each polynucleate, elongated cell

(termed myofiber). Connective tissue supports the terminal branches of the nervous, circulatory and lymphatic systems. In addition, connective tissue inside the muscle provides mechanical anchoring between fibers, longitudinally, laterally and with the tendons. This is particularly true of perimysium, whose collagen content is 95%[241]. The collagen in muscle-associated connective tissue is mainly of types I and III (reticulin), with traces of type V collagen and fibronectin, while the most frequent proteoglycan is collagen I-binding decorin[242]. The external lamina is the equivalent of basement membranes in other tissues, an proteic structure surrounding each multinucleate, roughly tubular cell. The external lamina contains collagen IV, laminin, and heparan sulfates [243, 244]. Muscle mass changes should require remodeling of all these connective structures, with novel collagen synthesis possibly misleading measurements of protein synthesis in atrophying muscle. In addition to the contractile and connective components, muscles include vascular, nervous, adipose, blood and immune cells.

The myofiber is the histological base unit of contractile tissue. Another muscle-specific population of cells comprises the mononuclear, proliferating cells, called satellite cells. Satellite cells are nearly devoid of cytosol, sitting in close proximity to the fiber, under the external lamina. They can be identified by markers such as Pax7 and, on the membrane, CD34 (reviewed in [245]).

Each myofiber is a syncytium, that forms and grows by fusion with neighboring cells. The only way to acquire nuclei to the myofibers is fusion with surrounding proliferative mononucleate cells, or with neighboring myofibers. Cells that have the ability to undergo mitosis and to fuse with myofibers are typically designated myoblasts. In vivo, myoblasts are derived from a subset of satellite cells. Experimentally, α7 integrin is an effective marker for selecting proliferative precursors from muscle[246], although its sensitivity and specificity are yet to be established. Alternate sources of nuclei in the myofiber are subject of ongoing research, but their relative importance is expected to be minor at best

(reviewed in [247]). A transplant of seven satellite cells from an adult mouse is capable of yielding more than a hundred multinucleate myofibers, thus demonstrating former's ability to regenerate muscle[248]. The transformation of quiescent satellite cells to proliferating myoblast is regulated by the interplay of growth factors, external lamina, and contact with myofibers[249]. In vivo, myofiber nuclei are typically peripheral, while in vitro incomplete models often yield elongated, contractile cells, with central nuclei, akin to regenerating fibers. The latter are typically termed myotubes.

The proliferative niche can play an important role in muscle atrophy and recovery. However, muscle hypertrophy may occur without cell divisions. For example, the muscles of mice receiving clenbuterol and of rats undergoing eccentric training gain 20-30% muscle mass without apparent DNA changes[250, 251]. Quail muscles depleted of proliferating cells by irradiation still undergo hypertrophy in response to stretch-overload[252]. These example of amitotic hypertrophy demonstrate that, in some circumstances, the number of nuclei and the pace of transcription are not limiting factors in muscle growth.

A large majority of the myofiber cytosol is the contractile apparatus, in the shape of bundles of protein filaments termed myofibrils. Within myofibrils, myosin and actin filaments alternate, held together by multi-protein complexes containing titin. Myofibril proteins are about two thirds of the total myofiber protein[253]. Therefore, any myofiber size change with functional relevance should correlate with changes in myosin II and actin protein content. In rat muscles, three days of streptozotocin-induced acute diabetes causes intramuscular formation of a actin degradation fragment[254]. The apparent sensitivity of actin to muscle mass regulators poses a technical challenge to protein and even mRNA measurements, because traditionally actin is considered a housekeeping, unregulated, and rather constant protein, and is used in level normalization. If, in an atrophying muscle, some protein of interest is depleted

faster than actin, immunoblots will convey the certainty that the former is downregulated. But if the protein of interest is lost in a less preferential manner, at the same rate with actin, immunoblots normalized to actin will convey the mistaken appearance of constancy.

Similar issues govern the use of 3MH as a marker for myofibril protein catabolism. Given that the main correlate of urinary 3MH is muscle mass[255], 3MH measurement may lack sensitivity when used as a indicator for muscle catabolic rate. On one hand, increased catabolism is expected to cause increased 3MH output, but on the other, an atrophic muscle has less 3MH to release.

During experiments that perturb muscle mass equilibrium, the level of the regulators, of typical housekeeping proteins, and of non-myofiber proteins may fluctuate in manners that convolve their specific modulation with overall muscle protein kinetics. Investigation of recovery from muscle loss is burdened by the fact that it aims to dissect protein regulation, when the regulators are proteins themselves.

Preadult muscle development

Around puberty, muscle growth is associated with a massive shift of nuclei from the satellite cells to the myofibers[256]. Even earlier, de novo muscle development is remarkable for its accretion of new nuclei to the myofiber. Pre-adulthood muscle growth appears reliant on hyperplasia, that is, cell proliferation. Cell proliferation regulators are crucial in determination of muscle mass in before and soon after birth.

In utero, the mesoderm, which is the source of muscle progenitor cells, undergoes segmentation and differentiation to form somites, dermomyotomes, and eventually myotomes (reviewed in [257]). The latter contain the earliest cells expressing muscle regulatory factors (MRF). In animal models, the cells in epaxial and hypaxial muscle initiate the formation of trunk and limb muscles. The limb

muscle early progenitors express myogenic factor (Myf) 5, due to stimulation from the transcription factor Pax3[258, 259]. Myf5 is a strong inductor of the muscle transcriptional program and phenotype, with the ability to convert embryonic fibroblasts to myosin-containing syncitia[260]. Cranial muscle formation is coordinated in a partly different manner, through the transcription factors T-box (Tbx) 1 and paired-like homeodomain (Pitx) 2 (reviewed in [261]). Once this early stage is completed, later fetal muscle progenitors converge to a phenotype remarkable for the expression of the MRF MyoD, due to stimulation by the transcription factor Pax7[262]. MyoD knockout mice are normal, with Myf5 supplanting its absence[263]. In the Online Mendelian Inheritance in Man (OMIM) database, there is no reported case of human mutation of Myf5 or MyoD, further supporting the idea of duplicate function. Neither Myf5, nor MyoD induce muscle attributes. However, the expression of either will promote expression of another MRF, myogenin, which marks the transition form specification to differentiation. MRFs share structural features, and some are juxtaposed in the genome, making their study difficult.

Initiation of myogenin expression marks the transition from specification to differentiation stage. In cultured cells, myogenin expression is followed by p21 expression, which removes the muscle precursor from the cell cycle[264]. Subsequent changes include expression of muscle-specific enzymes and contractile proteins[265], of a fourth MRF, the myogenic regulatory factor (Mrf) 4[266], and, finally, acquisition of fusogenic abilities. In humans, by the seventh week of gestation, the initial wave of myoblast fusion slows down, and a second proliferative stage starts. The latter tapers off, leading to formation of secondary myotubes, within the same laminar sheath with a primary myotube. Around the seventeenth week, some secondary myotubes migrate to form independent centers of coalescence for a third set of myotubes[267].

In chick embryos, overexpression of IGF-I induces a rapid increase in the

ratio of myoblast to myofiber nuclei, while fiber density is unchanged[268]. In addition to the hyperplastic effect, IGF-I stimulates protein anabolism in prenatal muscle[269]. Defects in IGF-1R signaling determine low birth weight and subsequent growth retardation in humans and transgenic mice, although it is unclear this is due to the mitotic or to the anabolic deficiency[270, 271]. Moreover, the murine model may be marred by the formation of hybrid dimers between IGF-1R and insulin receptor, which is likely to impair insulin signaling as well.

In mice, phenotypic differences between myostatin-null and wild type develop by the second week of embryogenesis[272]. Experimental myostatin perturbations in chicken embryos alter the population of muscle precursor cells[273]. The few reports of viable human mutations in the myostatin gene concern newborns with unusual unusually large muscles, due to loss of function[274]. Nevertheless, myostatin defects appear to cause ampler changes in muscle mass after birth[275, 274]. The relative importance of myostatin in embryo muscle development is to be determined.

Immediately after birth, the number of satellite cells is much higher than in the adult, with a one magnitude order drops between birth and 10 years[276]. This decay carries on throughout the life time at slower rate. Perinatal Pax7 knockout reduces muscle ability to regenerate, while its genetic depletion in utero or at adulthood does not exhibit pathological traits[277], suggesting that juvenile muscle growth is distinct from muscle development at other ages. Overall, muscle formation in children appears qualitatively different from adult muscle hypertrophy and regeneration, and should be more susceptible to modulation via mitotic mechanisms. This may be underlie a difference between juvenile and adult in the atrophying effect of GC on muscle.

The most likely source of new myofiber nuclei are the adult satellite cells, derived from a subset of fetal $Pax3^{+}Pax7^{+}$ cells. Some cells, such as bone marrow stem cells and pericytes, have the ability to fuse with myotubes, and possibly

contribute to the muscle stem cell population[278, 279]. In adulthood, most limb and trunk satellite cells are $Pax7^+$, while head satellite cells are often $Pax3^+Pax7^-$[280]. However, in longitudinal studies, human muscle mass, as estimated by body potassium, decreases in men and and stagnates in women over the age of 30[281]. As evidenced by studies such as the New Mexico Elder Health Survey, 1993-1995, with aging, loss of muscle mass accelerates [282]. Any muscle growth mechanism, including that dependent on satellite cells, is likely less powerful in adulthood than in childhood. Indeed, after the age of 20, nuclei in human muscle maintain an almost constant length of telomeres, indicating that mitosis is a rare phenomenon in the adult muscle[283]. Moreover, the proportion of satellite cells with proliferating abilities decreases with age, as more of them approach the Hayflick limit[284].

Physiological muscle metabolism

Muscle is a major energy user in the body, using fat during rest and glucose during exercise (reviewed in [285, 286]). Because its capacity to synthesize fatty acid is negligible, muscle is a consumer and a minor store, but not a generator, of fatty acids. During fast, more than half of infused non-esterified fatty acids (NEFA) are taken up by muscle, with a higher rate of incorporation in type I oxidative muscle[287]. In contrast, after feeding, oversupply of NEFA is compensated mainly by increased uptake, in absolute terms, in the visceral adipose tissue, with minor contributions from other adipose tissue, liver, and muscle. After repeated exercise, muscle lipoprotein lipase expression is increased, indicative of an adaptive improvement in muscle ability to extract NEFA from circulating triglycerides[288]. Ongoing studies suggest that muscle oxidative (catabolic) uptake of NEFA is upregulated by peroxisome proliferator-activated receptor (PPAR) β/δ, which stimulates expression of the lipolysis rate-limiting enzyme, carnitine palmitoyltransferase I[289, 290]. This hypothesis is supported by fast-

and exercise-induced upregulation of muscle PPAR δ[291, 292]. Moreover, PPAR δ overexpression leads to increase in type I fibers and subsequent resistance to high-fat diet[289]. Organ-level studies are impaired by the existence of nontrivial intramuscular adipose tissue.

This work uses extensively the C2C12 cell line, an immortalized female mouse muscle progenitor line obtained from a muscle recovering after mechanical injury. Treatment of confluent C2C12 cells with Dexa and 3-isobutyl-1-methylxanthine (IBMX) causes their differentiation into adipocytes[293]. Some pre-adipocyte traits, such as upregulation of PPAR γ, Krüppel-like factor-15 (KLF-15), and CCAAT-enhancer-binding protein (C/EBP) β and δ ([294]; reviewed in [295]), may surface in cell culture experiments where muscle differentiation did not complete, and residual potential for adipogenesis remains (see, for example, [296]). An in vitro shift to a more adipose-like phenotype may be associated with diminished cell fusion ability, lower protein synthesis and lower mitochondrial content, which may be misinterpreted as muscle atrophy.

In humans, up to 90% of the glucose absorbed after a meal is removed from circulation by the skeletal muscles[297, 298], meaning that muscles should have a paramount role in the development of insulin resistance and eventually diabetes mellitus. After normal feeding, muscle builds polysaccharides reserves, in part because it can synthesize and deposit the largest glycogen stores in the body, but also because it cannot release glucose.

Muscle work is generated at such high rates, that most glucose is processed solely through glycolysis, in the cytosol, to the three-carbon pyruvate. Some of the pyruvate is further oxidized in the muscle, through the tricarboxylic acid cycle, but a significant amount is converted to lactate and released in the blood stream. As part of the Cori cycle, circulating lactate is reassembled into glucose by the liver, and re-released into the blood stream, for muscle use. A similar shuttling mechanism further enables muscle to rely on glycolysis, by transaminating excess

pyruvate to the amino acid alanine, similarly released in the blood. Circulating alanine is converted by liver to glucose. Pyruvate transamination requires the amino acid glutamate. Muscle uses glutamate for other metabolic processes, including the synthesis of non-essential amino acids, including proline and arginine. Therefore, at rest, human muscle uptakes significant amounts of glutamate, less serine, while releasing alanine, glutamine, and smaller amounts of the other amino acids[299]. Glutamine is synthesized in muscle in order to release the excess nitrogen yielded by amino acid release during protein degradation. Muscle's glutamine is then converted by the liver to urea and excreted.

Among the amino acids with a trend for release between meals, isoleucine, leucine, methionine, phenylalanine, threonine, and valine cannot be synthesized by humans. Their net release indicates that, at rest, basal level of protein degradation slightly surpass protein synthesis. Conversely, for a steady muscle mass, there must be net protein synthesis in the fed state. Feeding status is relayed to the muscle by a surge in insulin and, independently, by an increase in circulating levels of essential branched-chain amino acids (BCAA), that is, valine, isoleucine, and leucine. The signaling effects of BCAA are not completely understood. Some studies show that BCAA ingestion or infusion increase protein synthesis rate[300], while others claim that BCAA solely reduce protein degradation[301]. However, staying true to their energetic value, BCAA supplementation caused increases in insulin in all the referenced studies. It becomes difficult to extricate BCAA intrinsic effect from that of insulin. Moreover, the combination of insulin and BCAA is a potent synergistic anabolic stimulus in healthy human muscle[302]. One study investigated the action of BCAA at clamped normal insulin levels[303]. In young, healthy controls, BCAA alone were able to increase the fractional synthesis rate for myofibrilar protein. At the same time, BCAA caused an increase in phosphorylation of mTOR at Serine 2448. This posttranslational modification is caused by the ribosomal protein S6 kinase, 70

kDa (p70-S6K)[304]. Interestingly, p70-S6K is a substrate of mTOR complex 1 (mTORC1), with the latter considered an integrator of nutrients, energy, and growth factor signaling (reviewed in [305]). Indeed, in the same muscle, p70-S6K was activated, as indicated by an increase in its Thr 389 phosphorylated form. Another substrate of mTORC1, eukaryotic translation initiation factor 4E (eIF4E) binding protein (4E-BP), was hyperphosphorylated. The action of mTORC1 on 4E-BP is the canonical way in which the former stimulates protein synthesis, by abolishing the latter's ability to bind and inhibit the mandatory translation initiation factor eIF4E. Many of these anabolic markers may be paradoxically elevated in acute, non-starving atrophy settings, where BCAA sudden release increases their circulating levels.

In addition to their signaling roles, BCAA are also used by muscle as protein precursors, and as energetic substrates, when preferred energetic substrates are not available[306]. In studies which measure leucine disappearance from the bloodstream or culture medium, a distinction must be made between the leucine used in non-oxidative, anabolic reactions and the alpha-ketoisocaproate-forming, ergogenic usage.

The degree to which energetic needs modulate BCAA usage appears to differ between humans and rats, reducing the validity of animal models (reviewed in [307]). Amino acid uptake or release rates are perturbed by energy and hormonal factors, to a degree that is still to be measured in humans. An argument has been made for using phenylalanine as a tracer, because muscle catabolism is negligible, and because it has a lower insulin secretagogue effect[308, 309].

Adult muscle remodeling

With aging, muscle gradually shifts from a mitotic to a postmitotic profile, with muscle growth achieved increasingly through hypertrophy, that is, cell size growth. While the typical middle-aged or elderly adult is undergoing net loss of

muscle mass, the muscle is still adept at re-growing and remodeling under two common circumstances, exercise and injury.

Humans achieve muscle mass growth following exercise, although some forms of exercise are more suitable at increasing strength or resistance that mass per se. In animals, muscle growth is induced by muscle overload, or muscle unloading and reloading, which may be conceived as forms of aerobic exercise. In healthy volunteers, the acute response to exercise includes increased intramuscular expression of MRFs MyoD and myogenin, and increased circulating IGF-I and IL-6[310, 311, 312]. These signals are associated with increased proliferation of satellite cells and recruitment of neutrophils to the muscle[313, 314]. In the acute phase, the satellite cells colocalize with IGF-I[310]. The negative muscle regulator myostatin is not correlating with the phenotype, that is, it is not decreased by acute exercise[314, 315]. Acute exercise increases the fractional protein synthesis rate in muscle[316, 317]. Interestingly, a single bout of exercise during fast leads to increases circulating cortisol levels and the release of 3-MH, indicative of increased stimulation of protein degradation[318]. Concomitant amino acid feeding extinguishes GC and catabolic response to exercise. Variations in regimens of exercise and timing and composition of diet led to a plethora of studies. Just as most of the short-term exercise routines do not lead to muscle macroscopic changes, some studies describe an absence of changes at molecular level following short-term exercise (reviewed in [319]). An important future direction in exercise science is establishing what distinguishes an effective brief exercise routine from an ineffective one. In this context, GC may be interesting noninvasive markers.

In the long term, exercise increases fiber CSA, density of satellite cells, and the number of myofiber nuclei, while the level of intramuscular MyoD, IGF-I, myostatin slowly return to normal[320]. On the other hand, long-term exercise induces the expression of catabolic markers, such as the E3 ligases MAFbx and

MuRF1[321]. Taken together, these molecular findings indicate exercise causes muscle remodeling, which manifests as increased muscle turnover, with upregulation of both protein degradation and synthesis. Moreover, post-exercise muscle accretion combines hyperplasia and hypertrophy.

A similar biphasic response is yielded by injury. In the immediate stage after injury, the muscle is infiltrated by pro-inflammatory M1 macrophages, while at later stage, anti-inflammatory (M2) subclass dominates (reviewed in [322]). Although the studies are rather incomplete, it appears that, similar to exercise adaptations, injury triggers a burst of growth factors, probably including IGF-I, basic fibroblast growth factor (bFGF), and transforming growth factor-beta (TGF-β) ([323]). Most of the studies of regeneration provide circumstantial evidence, such as improved healing in the presence of a presumed mediator, rather than impaired healing in its absence. Still unidentified molecules from crushed muscle are able to cause myoblast hyperplasia, above the levels caused by stimulation with known growth factors[324].

Less than half of the C2C12 cells in their proliferating, undifferentiated form, express MyoD or Pax7[325]. Limited evidence suggest these myoblast-like cells do not express Pax3 either[326]. Therefore, the C2C12 line are an incomplete model of hyperplastic muscle accretion.

Generation and regeneration of muscle in common scenarios, such as development and adaptation, remain an object of study, due to their complexity. Variable importance of the immune cells, of MRFs and of IGF-I, and concurring redundancy forestall attempts to envisage a common pattern of muscle hypertrophy.

Hormonal control of muscle mass

The variability of muscle mass within population is reflective of the variable needs for muscle strength. Muscle mass and strength are adjusted to the needs of the

organism mainly through humoral mechanisms. The most relevant endocrine regulators of muscle mass, AAS, GC, and IGF-I, will be discussed in dedicated sections.

While anabolic role of IGF-I at muscle level is undisputed, insulin's effect is inconsistent, because it is easily altered by external factors. The acute phase of insulin response poses a conundrum, with some studies showing it stimulates protein synthesis in human muscle[327], while others demonstrating that its effect is limited to anti-catabolism[328]. In male rat muscle, 30 minutes in 30 nM insulin or IGF-I are equally able to stimulate protein synthesis and to inhibit protein degradation[329]. Both effects are above 10%, although the effect on protein degradation appears less ample and is marred by higher variability.

Insulin and IGF-I pathways overlap to a some degree. The liver is the main source of circulating IGF-I, under the pituitary stimulation with growth hormone (GH). However, auto- and paracrine secretions may full supplant the absence of hepatic IGF-I in adult conditional knockout mice[330]. In contrast, insulin is secreted solely by the pancreas. Our understanding of the regulation of insulin secretion is improving, dispelling the simplistic view that nutrients alone are its sole modulators (reviewed in [331]). Therefore, both IGF-I and insulin emerge as anabolic stimuli, responding to increased demand for macromolecules and / or increased supply of nutrients, with IGF-I embracing a more localized and insulin a systemic, integrative role. For both hormones, physiological concentrations are tens of times higher than the half maximal effective concentration (EC50) for their receptor, suggesting that physiological fluctuations cause marginal effects downstream[332, 333]. On the other hand, insulin has the ability to bind and activate IGF-I receptor (IGF-1R), with an EC50 about an order of magnitude lower than physiological insulinemia. The converse is true, with IGF-I being able to bind and activate insulin receptor (IR), isoforms A and B. There is a small, but real, potential for interference between insulin and IGF-I signals.

The levels of bioavailable IGF-I are under complex regulation (reviewed in [334, 335]). IGF-I may be sequestered by IGF-I binding proteins (IGFBP), which are secreted by muscle under IGF-I stimulation ([336]). The interaction with IGFBP may prevents IGF-I from interacting with receptor, or it may extend its circulating half-life by protecting it from degradation. Depending on the isoform and location of IGFBP, the interaction may result in extinction or amplification of the IGF-I signal. IGFBPs levels are modulated by insulin and their affinity is modified through competition by insulin-like growth factor 2 (IGF2). The latter can also stimulate IGF-1R, thus providing its own anabolic and pro-myogenic signals[337]. IGF2 is irreplaceable in fetal development[338], suggesting there might be distinct, unidentified receptors for this hormone family.

There is no consensus with regards to the ability of GH to stimulate muscular secretion of IGF-I. Multiple studies found an upregulation of its mRNA[339, 340], but protein data are lacking. Medium conditioned by GH-stimulated C2C12 cells fails to elicit hypertrophy in other C2C12 myotubes[341], suggesting that, at best, IGF-I has an intracellular autocrine action. In addition to the indirect effect mediated by hepatic and the putative muscular IGF-I, GH has an IGF-I-independent effect on muscle. For example, knockout of GH receptor impairs body growth further beyond IGF-1R knockout[342]. In the context of pituitary pathology associated with Cushing's disease, the associated GH perturbations may contribute to loss of muscle.

Hypothyroidism is often associated with muscle weakness and pseudohyertrophy[343]. Other hormones such as the parathormone, are likely to have small effects on muscle protein metabolism, essentially irrelevant outside their respective pathologies[344]. In conclusion, muscle mass homeostasis is under a tight, multifactorial hormonal control, whose study is complicated by significant redundancy. The absence of third-party organs, such as glands, from reductionist cell-culture may limit their ability to replicate in vivo phenomena.

Interaction of muscle mass and vascularization

Muscle vascularization is a modulator of muscle mass and contractility. Mice whose muscle VEGF-A secretion was genetically depleted still express a tenth of the muscle VEGF-A protein, but have only half of the capillaries per muscle fiber, compared to their $Cre^{-/-}$ siblings[345]. The muscle-restricted VEGF-A-depleted mice have 12% lighter gastrocnemii, although the muscle loss disappears when muscle mass is normalized to total body weight. The loss does not affect a specific fiber type more than the others. Therefore, the muscle depleted of VEGF-A is less able of endurance effort (80% shorter time to exhaustion on the inclined treadmill) and of brief anaerobic exercise (34% lower maximal running speed).

Conversely, murine muscles injected with VEGF-A-expressing retroviruses display a higher proportion of hypertrophic fibers than those expressing bacterial β-galactosidase[346]. Moreover, in the VEGF-A-overexpressing muscle, many of the fibers have central nuclei, a sign of increased fusion with myoblasts. In the murine C2C12 cell line, VEGF-A causes faster differentiation, into longer myotubes, with more nuclei per fiber, although mitotic rates are in fact diminished. These observations are consistent with direct anti-apoptotic and profusogenic effects.

It has been suggested that the most direct effect of VEGF-A depletion is partial segregation of muscle from blood-carried endocrine signals. For example, VEGF-A-depleted muscle has lower glucose uptake, a defect which is reversed by explantation [347]. In addition, vascularization defects may induce relative intramuscular hypoxia, which is an independent atrophying, pro-proteolytic factor[348].

The relative importance of VEGF overexpression during myogenesis is still open to debate, as muscle-restricted VEGF receptor (VEGFR) knockout animals were not studied yet. Multiple effects concur to obfuscate VEGF action in

hypertrophying muscle. First, VEGF promoter contains three binding sites for MyoD, meaning that growing muscle will express more VEGF[349]. Development of vasculature in growing muscle may be a physiologically meaningful way to ensure vasculature remains competent upon increase circulatory demands. Second, although the canonical upregulator of VEGF is hypoxia-induced factor 1α (HIF 1a), muscle VEGF is also stimulated by PPAR γ coactivator 1 (PGC-1)[350, 351]. Even in the absence of VEGF, PGC-1 coactivators facilitate mitochondria biosynthesis, leading to oxidative fiber hypertrophy and improvements in endurance capacity [352]. Therefore, it is difficult to distinguish VEGF-induced muscle changes from the common muscle remodeling program. Finally, VEGFR activation has multiple effects, including phosphorylation of Src family proteins[353], of phospholipase C (PLC) γ[354], and, indirectly, of regulatory subunits of phosphatidylinositol 3-kinases (PI3K)[355] and of the Signal Transducers and Activators of Transcription (STAT) STAT3 and STAT5[356]. Most of these VEGF effects overlap with the effects of many other muscle anabolic agents. Thus, it is unclear whether VEGF plays a mediating role in muscle hypertrophy, or it provides the homeostasis of the vasculature to fiber ratio.

Control of muscle mass through innervation

Progress made in the last few years distinguish denervation atrophy as one of the best studied models of muscle atrophy. Current understanding and future directions for the study of GAML are guided, to a large extend, by the data obtained in denervation experiments.

Limb and trunk muscles are controlled by the lower (alpha) motoneuron, located in the ventral horn of the spinal cord. Their main point of contact is a chemical synapse, the neuro-muscular junction (NMJ). In addition to the direct synaptic activity, muscles and lower motoneurons are involved in a mutually

beneficial life-long interaction. The loss of NMJ-mediated stimulation leads to rapid, sizeable loss of muscle mass, commonly seen in spinal cord injury. Experimentally, denervation of a hindlimb or hemidiaphragm is a relatively simple procedure, allowing the use of an animal as its own control. Experimental denervation is the most studied model of muscle atrophy, generating working hypotheses for the study of GAML.

In utero, experimental destruction of motoneurons with bungarotoxin abolishes formation of secondary myotubes, although it has limited effect on the formation of primary fibers[357]. Conversely, motoneuron precursors from the embryo spinal cord degenerate and die if they cannot engage in significant interactions with myofibers[358]. Eliminated motoneurons include neurons whose axons fail to reach myotubes and neurons which eventually fail to maintain contact with myotubes, due to synapse elimination. The latter is a selective, competitive process, whose molecular basis is still unknown, and which ensures injectivity of the correspondence between lower motoneurons and myofibers (reviewed in [359]). For a few days after birth, rat motoneurons go into a particularly sensitive state, when axotomy determines motoneuron death[360]. Thence, axotomy elicits the reprogramming of the neuron into a less differentiated state, followed by axonal regrowth (reviewed in [361, 362]). The re-establishment of contact between the motoneuron and muscle causes the reverse molecular changes, suggesting the existence of muscle-secreted neurotrophic factors. The ability of NMJ to regenerate or even preserve its optimal structure degrades with aging, although it is not clear whether NMJ cause or are caused by aging-related muscle loss[363]. The improvement in NMJ regeneration through exercise has been seen as an important path towards its understanding. Factors that improve muscle's ability to exercise or even mimic exercise, such as IGF-I or androgens, have been shown to benefit NMJ recovery or to slow down its degradation during aging[364, 365, 366].

The loss of contact with the lower motoneuron elicits similar changes in the muscle, with clinical relevance in the understanding and treatment of spinal cord injury and spinal muscular atrophy. Conceptually, denervation is distinct from disuse, such as that induced by damage to the upper motoneuron. Clinically, the latter manifests differently, through a syndrome termed pyramidal weakness, that mainly affect muscles opposing gravity[367]. In humans, after upper motoneuron damage, disuse response occurs in a few days after injury, and leads to exaggerated spasticity[368]. Understandably, literature does not describe any animal model of disuse by experimental damage to the upper motoneuron. In fact, literature contains multiple examples where “disuse” is taken to mean “absence of action potentials, due to denervation”, “lack of work, due to denervation, unloading, immobilization”, or even both[369]. Consequently, there are no experimental attempts to disentangle muscle-maintaining effects of work, of the NMJ transmitter, acetylcholine, and of any other musculotrophic neuron-released factor.

Experimental denervation causes rapid and ample loss of muscle mass. For example, three weeks after sciatic nerve removal, the tibialis anterior muscle halves in weight[370]. Fewer than one in 7,000 myofiber nuclei undergo apoptosis in this time[371], indicating that denervation and the fast recovery that follows renervation are more similar to the adult exercise remodeling paradigm, that to the proliferation-dependent regulation of the infantile muscle. Supporting the hypothesis on NMJ-independent trophic factors, denervation did cause massive and rapid apoptosis among stromal cells.

In a seminal study, Goldberg demonstrated that denervation leads to increased loss of prelabeled muscle protein, proving that denervation upregulates protein degradation[372]. Moreover, denervation increases urinary 3MH, indicating activation of myofibril catabolism[373]. The rate of muscle loss in denervated muscle is halved by the proteasome inhibitor bortezomib[374].

Denervation stimulates persistently proteasome enzymatic activity[375], and upregulates all the ubiquitin-proteasome pathway components, including ubiquitin, the E3 ligases MuRF1 and MAbx, and the proteasomal subunit A1 [376, 227]. The upregulation of the E3 ligases is induced by multiple independent transcription factors, including myogenin and the Foxo class[377, 378, 379, 380]. In the first few days of denervation, myogenin is induced by histone deacetylase (HDAC) 4[381]. After the first week, Foxo activation is attributed to the downregulation of its negative regulator, Akt[379, 382].

Because the mTOR inhibitor rapamycin prevents fiber hypertrophy that normally follows in vivo injections with a plasmid coding constitutively active Akt[382], the scientific community assumed, since the beginning of century, that denervation-induced Akt inactivation leads to loss of downstream mTOR-mediated effects (for example, [383, 384]). Recent studies contradicted this paradigm. In 2013, Quy and colleagues found that denervation increased Thr 389 phosphorylation and catalytic activity of p70-S6K, indicating that denervation causes in fact activation of mTORC1[385]. In 2014, Tang and colleagues proved that rapamycin, an inhibitor of mTORC1 which lacks intrinsic anabolic properties, abolishes denervation-induced loss of muscle mass[379]. These experiments prove that mTORC1 should be activated for denervation-associated muscle atrophy to proceed in its later stage. Tang showed that denervation causes phosphorylation of insulin receptor substrate (IRS) 1, proving that, in denervation, activated mTORC1 inhibits Akt through a p70-S6K - IRS 1-mediated negative feedback loop. Quy found that mTORC1 activation is lost upon proteasome inhibition with bortezomib. One can hypothesize the existence of an mTOR negative regulator which is specifically targeted by the ubiquitin-proteasome system during denervation. However, the late timing of the Akt / mTOR involvement suggests that the most plausible explanation places mTOR activation after the proteasome releases significant amounts of free amino

acids. It is therefore more likely that denervation-induced mTOR activation is caused by the proteasome-stimulated amino acid release.

Establishing the role for mTORC1 in denervation-induced loss of muscle mass is crucial. In conceptual frame before Quy and Tang experiments, mTORC1 was thought as being inactivated by denervation, thus downregulating on protein synthesis and removing a restriction on autophagy. This hypothesis was conceptually attractive, because it implied a co-operation between multiple anabolic and catabolic pathways, towards achieving muscle loss. In particular, denervation-induced autophagy appealed to the muscle biologists of the 2000's. For example, in a never-replicated experiment, one group claimed that the lysosomal inhibitor chloroquine prevents denervation-induced muscle loss[386]. Others showed that denervation upregulates lysosomal enzyme cathepsin L[387], while a third group claimed that denervation causes buildup of the autophagosomal marker LC3-II [388]. However, most experiments refuted the role of autophagy in GAML. The lysosomal inhibitors leupeptin, methylamine, and E64-c have minimal effects on the release of free tyrosine from denervated muscle[373]. GFP-LC3 transgenic mice exhibited a loss of autophagosomes in denervated muscles[385].

A similar debate surrounds protein synthesis regulation in denervation. In Goldberg's 1969 experiment, the specific activity of the remaining muscle protein was essentially the same as in control limbs. Increased or even unchanged protein synthesis rates would have caused a reduction in specific activity. Therefore, Goldberg concluded that denervation causes protein synthesis decreases, thus opening a debate that is still unsettled. Goldberg's observed reduction in protein synthesis rate is consistent with an indiscriminate loss of translation initiation and elongation factors, as much as with an active mechanism of protein synthesis cessation. Moreover, more recent functional studies, including some from Goldberg's group, found that denervation stimulates

translation[389, 385, 375, 390, 373]. Unlike the 1969 study, contemporary studies measured protein synthesis rate directly and over shorter time intervals (hours, rather than weeks). As in the case of autophagy, follow-up studies found molecular changes in regulators of translation, in some cases supporting a regulated, denervation-triggered mechanism of protein translation shutoff, in others refuting them. Both Tang and Quy found that denervation causes increased phosphorylation of 4E-BP, thus leading to stimulation of protein synthesis. Both also show that the effect on 4E-BP is prevented by rapamycin, demonstrating that protein synthesis is upregulated through an mTOR-dependent mechanism.

Given that rapamycin has essentially no effect on adult muscle mass, it may be that mTOR is not the crucial effector of denervation-induced muscle loss once thought. Recent studies show that its activation, and the downstream net anabolic effects it elicits through autophagy and translation, cannot explain the observed phenotype. Denervation-induced muscle loss is, to great extent, produced by the ubiquitin-proteasome system. Better experiments are needed in order to understand autophagy and of mTOR in denervation-induced muscle loss. The irreconcilable differences between functional and molecular studies of denervation epitomize similar predicaments in the study of muscle atrophy due to other etiologies, including the less-explored GAML.

Animal models of glucocorticoid myopathy

In the 75 years since the discovery of chronic steroid myopathy, scientists attempted, with variable success, to develop multiple animal models. In dogs, seven days of 0.44 mg/(kg d) cause increased nitrogen excretion, increased glutamine and alanine release from the hindlimb[391]. In horses, the action of Dexa on glycogen regulating pathways was replicated, but muscle mass was not measured[392].

A few species are particularly unsuitable for modeling GC myopathy. In cows,

GAML is undetectable at macroscopic level. Any putative change in myofibers is compensated by bovines' unusually rich intramuscular adipose component, further hypertrophied by Dexa[393]. In contrast to rodents, a putative mediator of GAML, myostatin, is downregulated in bulls' muscle by Dexa[394]. In rabbits, four days of 1 mg/(kg d) did not change muscle mass and reduced urinary 3MH, despite the upregulation of some catabolic markers[395]. Another article describes GAML in rabbits, and attributes it to, unusually, lysosome upregulation[396].

For large animals, cats, or dogs, practical and humane reasons prevailed, meaning that data about muscle mass changes are often lacking. For many of these species, the genome was not known, and specific antibodies are not manufactured, meaning that signaling pathways could not be dissected. Especially in the early years, studies did not record most relevant outcomes, such as muscle mass and / or force, putting into question their validity. In the era of genome sequencing, studies of GAML focused on mice and rats. Muscle mass in mice is smaller, making dissection harder and changes closer to detection threshold, compared to rats. In terms of glucose metabolism, mice are more GC-resistant than rats[397]. It is unclear whether this resistance extends to protein metabolism. The first study of mouse GAML was published in 1964[398]. Nevertheless, rats were preferred to mice for modeling steroid myopathy. In the last two decades, mice studies became more interesting, as genome manipulations were more easily induced in mouse than in rat.

Myofiber-restricted knockout of GR abolishes GAML, while having no effect on denervation atrophy[399]. In rat muscle, chronic Dexa treatment upregulates expression of the NMJ essential component, muscle-specific nicotinic acetylcholine receptor, and resistance to NMJ-specific non-depolarizing muscle relaxants[400]. If anything, GAML appears associated with an improved NMJ, suggesting that GAML is not mediated by the motoneuron. Similarly, there is no

evidence of satellite cell dysfunction in GAML. In one report, Dong and colleagues hypothesize a “glucocorticoid-induced satellite cell dysfunction”, but describe solely that Dexa reduces the number of satellite cells in a post-injury regenerating muscle[401]. In conclusion, GAML is the direct effect of Dexa on myofibers.

The perception that myotubes response to Dexa is a complete model of GAML inspired many reductionist in vitro models. Many published studies documents the effect of Dexa on the mouse cell line C2C12 and the rat cell line L6. However, these and other myogenic cell lines have significant limitations, which may cause divergence between in vitro models and the steroid myopathy they aim to describe. As mentioned earlier, in the case of primary cells, experiments with Dexa are outright impossible, because primary cell survival is GC-dependent. Moreover, Dexa has hyperplastic and hypertrophic effects on myogenic cell lines. Commonly tested doses of Dexa, in the range of tens of nM, have been shown to synergize with IGF-I [402] and even to act directly towards improved proliferation of L6 myoblasts[403, 404]. C2C12 fusion is more efficient when Dexa is added to IGF-I[91].

Until the end of the 1990s, Dexa was a common ingredient in myogenic culture media[405]. Even contemporary standard proliferation media, containing 10% fetal bovine serum, provide significant and unpredictable concentrations of GC, insulin, and IGF-I, impairing their study at physiological concentrations. In fusing C2C12 cells, IGF-I and Dexa synergize to amplify some Dexa catabolic signals, such as the expression of REgulated in Development and DNA damage responses-1 (REDD1)[91]. Conversely, the synergy with Dexa amplifies some of insulin and IGF-I effects, such as Akt phosphorylation on Ser 473[405]. In addition to myotubes, C2C12 may differentiate into adipose or osteoblast phenotypes. Whenever possible, this section will refer to in vivo studies. Evidence from in vitro studies on myogenic cell lines will be limited to studies where multi-nucleate myotubes were obtained and myoblasts were depleted.

With this caveat, in vitro studies have been vital in excluding third-party organs, such as the pancreas, from the analysis of GAML, at the time when genome manipulations were not available. Some molecular features of GAML have been initially described in cell culture, and confirmed in vivo later. Fully differentiated myotubes from L6 and C2C12 cell lines lose more than a quarter of their diameter when treated with 100 nM [406].

The effects of 7-day, 5 mg/(kg d) treatment on rats allow the classification of GCs in two subsets[407]. Members of the short-acting subset, including prednisolone and corticosterone, cause net gains in body weight, through increased adiposity, MR-mediated water retention, and a net neutral effect on muscle mass. In contrast, long-acting, specific GCs, such as Dexa, betamethasone, and triamcinolone, cause loss of body weight, and an even more rapid loss of muscle mass. The muscle mass loss is paralleled by reductions in maximal twitch and tetanic force[408]. GAML is an organized process, lacking the microscopic features of necrosis[409]. Although Dexa temporarily reduces food intake, possibly through stimulation of leptin secretion[410], pair-feeding experiments demonstrated that the GAML is not solely the effect of changes in appetite[411, 412]. In response to Dexa, rat myofibers undergo reductions in CSA, to an ampler degree in fast twitch fibers[413, 414]. Dexa-induced loss of muscle mass is present, although less manifest, in female rats, possibly because, in males, hypercortisolism is compounded by a reduction in circulating T levels[413].

The nitrogen imbalance induced by Dexa slows down by the third day, and is compensated around the seventh day of treatment[415, 416]. Given that later time points are marred by feedback mechanisms and by animal mortality, almost no published experiment on rats extends past two weeks. For an animal weighing 300 times less and aging 20 times faster than humans, the common 5-10 days experiments are comparable to chronic exposure in humans. Rats appear more resistant than humans to GAML, given that reported experiments start at about

0.5 mg/(kg d) Dexa, more than twice the muscle-imparing dose in humans.

The next sections are informed by experiments with chronic (5-10 days) Dexa on adult (not aged) male rats, which are the best model from human male steroid myopathy. The few exceptions, mainly transgenic mice and acute treatments, will be identified as such.

Glucocorticoid stimulation of ubiquitin-proteasome system

The loss of muscle in the Dexa-treated adult rat is the result of an increase in protein degradation. The 1969 Goldberg study on atrophy revealed that cortisone induced an even ampler upregulation of proteolysis than denervation[372]. His comparison of fast-twitch plantaris and slow-twitch soleus found that the latter did not exhibit lower muscle mass, not increased proteolysis. Goldberg found that cortisone increases in equal manner the degradation of older and newer proteins, and of myofibril and sarcoplasmic proteins. A search begun for an undiscriminating proteolytic machine stimulated by GC.

In rats receiving 0.5 mg/(kg d) Dexa for six days, epitrochlearis muscle proteolysis rate increased by 50%, while protein synthesis was essentially unchanged[411]. Dexa-stimulated proteolysis affects the contractile apparatus, as indicated by the doubling of urinary 3-MH output[417]. The upregulation of proteolysis occurs even when explants, rather than animals, are treated with Dexa, suggesting that GAML does not require extramuscular inputs[418].

In vivo GAML is best correlated with an upregulation of the ubiquitin-proteasome system. The increase in proteolytic rate is unchanged when explants are treated with the lysosome inhibitor methylamine and the lysosome / calpain inhibitor E-64. On the other hand, the Dexa-stimulated increase in proteolysis is abrogated when the explant is treated the proteasome inhibitor MG132[419], or depleted of ATP by a combination of the mitochondrial decoupling agent dinitrophenol and the unlysable glucose homologue,

2-deoxyglucose[420]. Proteasome chymotrypsin-like catalytic activity is doubled by Dexa[421]. Dexa upregulates proteasomal subunits such as C1, C2, C4, C5 [411, 419].

In cultured myotubes, the loss of diameter is reliably underlined by an 20% increase in protein degradation rate[422, 406]. Between 78% and 95% of the Dexa-induced proteolysis augmentation is lost, when proteasome inhibitors, such as beta-lactone or MG-132, are co-administered[422, 423]. Demonstrating proteasome's primacy, MG-132 has this overriding effect even co-administered as an addition to a cocktail of lysosome and calpain inhibitors[419]. Co-administration of dinitrophenol essentially abolishes Dexa-induced proteolysis[422]. GAML dependence on ATP reinforces the idea that GC-stimulated proteolysis takes place in the proteasome. In contrast, lysosomal inhibitors had no effect, while E-64 has minimal effect[422].

In L6 myotubes, Dexa causes increased expression of the ubiquitin gene UbC, through a putative SV40 promoter-specific 1 (Sp1) response element[424]. In vivo, upregulation of UbC was confirmed only for acute Dexa treatment[418] and in diabetic rats[425].

One of the most tempting hypotheses attributed a role to NF-κB, a mediator of cancer cachexia (reviewed in [426]). However, the few reports are contradictory. In L6 myotubes, Dexa stimulates acetylation and nuclear translocation of the translational activator p65[427], seemingly in consonance with cancer cachexia. But in contradiction to such parallels, Dexa was shown to inhibit NF-κB signals by upregulating inhibitory κBα (IκBα)[428]. Moreover, Dexa's inhibition of NF-κB was shown to be necessary for proteasomal subunit C3 upregulation in the same L6 cells[429].

Unbiased searches in atrophying mouse muscles revealed two upregulated genes, termed atrogenes, MAFbx and MuRF1[227]. Both are E3 ligases, pointing to an important role for the proteasome-ubiquitin system in muscle atrophy.

In contrast to other muscle atrophy models, GAML is associated with stronger reliance on MuRF1 than on MAFbx. While genetic depletion of either atrogene leads to muscle sparing in the denervation model[227], only MuRF1, but not MAFbx genetic depletion spares muscle treated with Dexa[430]. The incomplete sparing of the MuRF1 knockout may be caused by genetic redundancy, but may also be explained by a need for a second, co-operating gene.

While MuRF1 promoter includes a GC response element, no similar structure was found on MAFbx promoter[431, 432]. Indicating a lower amplitude and / or higher variability in MAFbx, unbiased searches in rat GAML failed to identify MAFbx as a target of Dexa[433].

The difference in amplitude of change may be related to the role that MAFbx plays in muscle restructuration. In C2C12 cells, MAFbx is upregulated by differentiation[434]. MAFbx is induced in muscle during hypertrophy from reloading[435]. Moreover, MAFbx knockout abolishes hypertrophy of loading[436]. In contrast to denervation atrophy, it appears that remodeling in GAML is minimal. Supporting this distinction, denervation is a stronger upregulator myogenin than Dexa[437]. Since MAFbx promoter is activated by myogenin [378], the moderate myogenin response in GAML may explain a less ample MAFbx upregulation.

Substrate specificity is also distinguishing the two ligases. The only two known MAFbx ubiquitination substrates are MyoD and the eukaryotic initiation factor 3f (eIF3-f), identified in C2C12 ([438, 439], reviewed in [108]). This suggests that MAFbx may be an initiator, neutralizing a few specific muscle protecting factors, belonging to multiple metabolic pathways. Because MAFbx neutralizes a translation initiation factor, it has been suggested that MAFbx relative importance is augmented in conditions where muscle loss relies more on loss of protein synthesis. In contrast, two-yeast hybrid experiments revealed two large classes of well-expressed putative MuRF1 substrates, myofibrilar

components, including titin, nebulin, troponin-I, troponin-T, myosin light chain 2, and components of ATP-generating machinery, including NADH dehydrogenase 1a, NADH-ubiquinone oxidoreductase, pyruvate dehydrogenase, and ATP synthase beta-subunit[440]. The loss of the latter, but not the former group, was confirmed in the transgenic mouse overexpressing MuRF1[441]. In vitro, MuRF1 ubiquitinates myosin heavy chains[442] and actin[443]. MuRF1 is therefore a more plausible effector of bulk protein degradation, as it is a better fit for the "undiscriminating" proteolytic machine predicated by Goldberg.

Studies on cultured myotubes confirm that GC induce the two atrogenes directly, without a third-party organ mediation[444]. The sparing of MuRF1 knockout mice revelats its central role in GAML[430]. A sizable body of indirect evidence suggests that the main positive regulator of atrogenes in GAML are FOXO transcription factors. These transcription factors are reliably upregulated by Dexa in muscle. In addition, Dexa inhibits their kinase, Akt, thus protecting them from ubiquitination and degradation. Atrogenes are also modulated by myostatin, through SMAD3 transcription factor, and by AMP-activated protein kinase (AMPK). Transgenic mice with a defective GR still exhibit upregulated atrogenes in denervation and fasting. Therefore, atrogenes integrate a multitude of GC-activated and GC-independent metabolic and hormonal signals (discussed in dedicated sections).

A series of less investigated, Foxo-independent factors lend support to Dexa's upregulation of atrogenes. The E3 ligase TRAF6, whose expression is increased by Dexa, appears necessary for GAML and atrogene upregulation[445]. In cultured cells, Dexa upregulates the nuclear cofactor p300[446]. Dexa also upregulates acetylation and nuclear translocation of C/EBP β, in a p300 dependent-manner[427]. Because atrogenes promoters contain putative binding sites for C/EBP transcription factors, p300 was hypothesized to be yet another mechanism by which GC stimulate atrogenes. Interestingly, p300 is a histone

acetyltransferase (HAT). Dexa increases HAT activity and reduces HDAC activity in muscle[447]. Because HDAC 3 and 6 are repressed by Dexa and because trichostatin A, an HDAC inhibitor, upregulates MAFbx, it has been hypothesized that Dexa acts by increased histone acetylation. The studies are interesting opening move in the field of epigenetics, and should be followed up with identification of specific chromatin loci and / or extranuclear proteins whose acetylation is modulated by Dexa. The importance of histone acetylation in in vivo GAML is still to be determined.

In conclusion, explant models suggest that GAML is, to a wide extend, the result of upregulation of the proteasome-ubiquitin system. While two important E3 ligases have been identified, the absence of specific anti-MAFbx and anti-MuRF1 antibodies prevents the study of their protein flux and their intracellular localization[108]. Judging by the significant residual atrophy after depletion or inhibition of the atrogenes, other mechanisms, within and outside the ubiquitin-proteasome system, are co-opted by Dexa in GAML. Next sections will describe the various pathways that collaborate to stimulate the ubiquitin-proteasome system. Each of them has proteasome-unrelated side effects, including activation of other proteolytic and anti-translational pathways.

Glucocorticoid-induced loss of sensitivity on the IRS - Akt axis

Surprisingly, the most comprehensive account on GC-induced changes on protein metabolic regulation comes from the study of glucose metabolism changes. Dexa causes systemic insulin resistance, manifested as uncompensated hyperglycemia[412]. On one hand, Dexa induces concerted catabolic changes which eventually converge to hyperglycemia, and consequent hyperinsulinemia. Together, these yield a higher index of homeostatic model assessment - insulin resistance (HOMA-IR)[448]. On the other, Dexa-induced hyperinsulinemia fails to trigger its typical anabolic program in muscle. For example, Dexa overrides

insulin to reduce muscle glucose uptake[449]. Dexa does not alter expression and activity of hexokinase, and of glucose transporter (GLUT) 4 expression[450], but inhibits GLUT4 recruitment to the cell membrane[449, 450]. Because translocation of GLUT4 in response to insulin is fundamentally dependent on Akt[451], its suppression by Dexa is evidence for Dexa-imposed Akt inactivation. But in normal muscle, hyperinsulinemia causes Akt activation. Therefore, there is a disconnection between between the circulating anabolic insulin signal and the catabolic state in the sarcoplasm.

Insulin or IGF-I, in concentrations close to the physiological levels, increase glucose uptake about 10-fold[449]. The fact that there is significant leeway for amplification of anabolic pathways demonstrates that, at basal state, muscle Akt-mediated signals are diminished. Unsurprisingly, investigations of muscle in basal state have been prone to fail to observe changes in Akt-related signals. Moreover, studies on the Akt pathway are conceptually challenged by the low basal activity. In order to prove causality by downstream inhibition, one must reverse that inhibition, and establish that the final effect is lost. Chemical reversal of inhibition is rarely an option. In the case of Akt, genetic reversal of inhibition, that is, overexpression studies, have minute chances of matching wildtype basal level. Most often, overexpression of IGF-I, IGF-1R, Akt, or other mediators of GAML overcompensates Dexa's effect, and causes overriding, possibly non-specific and irrelevant, hypertrophy.

In contrast, Dexa co-administration blunts muscle anabolic and anti-catabolic response to exogenous supplementation with either insulin or IGF-I, at the levels of protein and glucose metabolism[452, 329]. Typical reductions in metabolism insulin sensitivity are between 40% and 80%. Most of our knowledge about Dexa effect on the Akt axis is derived from studies of muscle stimulated with pharmacological doses of insulin or IGF-I, each with their own risks of inducing experimental artifacts.

Dexa extinguishes the insulin signal very early. By itself, Dexa treatment does not change basal levels of autophosphorylated insulin receptor in absolute or stoichiometric terms[448, 453, 454]. Similarly, Dexa alone does not change the basal level of phosphorylated IRS. However, in response to insulin, mice treated for 5 days with 1 mg/(kg d) Dexa exhibit only a third of the IRS 1 and 2 phosphorylation, compared to untreated animals [455]. The suppression of insulin sensitivity at IRS level has been attributed, based on observational studies, to an improved protection of IRS by calmodulin[456], to an inhibitory phosphorylation on another residue, possibly by PKC[457, 454], or to the upregulated phosphatase C1-Ten[458]. In C2C12 myotubes, Dexa may downregulate IRS through caveolin repression[459].

The notion that Dexa interferes with IGF-I signaling suggested that GAML also attenuates downstream mitogen-activated protein kinase (MAPK) response (reviewed in [460]). Activated receptors for growth factors, such as IGF-1R, phosphorylate and assemble a transduction complex, including Src homology 2 domain containing (Shc) and Growth factor receptor-bound protein 2 (Grb2). Dexa reduces insulin's ability to cause Shc phosphorylation and association with Grb2[461]. Paralleling the findings in sugar metabolism, Dexa has no detectable effect in basal state. Further downstream, the complex including Shc and Grb2 binds and activates Son of sevenless homolog (Sos), the GTPase exchange factor for Ras. Ras-GTP activates a cascade of kinases, including Raf, Mek, and Erk, eventually leading to cell proliferation. A report describes that Dexa upregulates phosphorylation of Mek and Erk in diabetic rats[425]. Others describe increased Erk phosphorylation in L6 myotubes during acute Dexa[462]. Nevertheless, no reports describe such changes in wild-type animals, which still undergo GAML. Measurements of changes in activation of another MAPK, p38, are contradictory[463, 464]. Together with the limited role of hyperplasia in adult muscle, available evidence suggests that MAPK cascades do not mediate GAML.

More to the point, absence of sizable changes in the MAPK cascades reinforces the idea that insulin and IGF-I signaling is extinguished at IRS level.

In addition to interference at IRS level, Dexa hinders the recruitment of PI3K to the membrane complex containing insulin receptor and IRS1[448, 455]. Mutagenic studies demonstrate that GR's transcriptional and DNA binding domains are required, in order for it to inhibit p70-S6K[465]. In the current model, p70-S6K is downstream of IRS and PI3K, suggesting that Dexa inhibitory action is a transcriptional effect. Two mechanisms have been found in cell culture models. One asserts that PI3K catalytic subunits are sequestered in the cytosol by an glut of its regulatory unit, p85 α[448, 466, 467], whose expression is upregulated in vivo by Dexa[433]. The other contends that activated GR binds p85 in a competitive manner, thus displacing it from IRS1[468]. This non-transcriptional effect has not been explored in GAML in vivo, but is supported by the unusual persistence of IRS1-PI3K complexes during diabetes in GR knockout mice muscle.

The formation of a membrane complex including the transmembrane receptor, IRS, p85 and, PI3K catalytic subunit, p110 (reviewed in [469]). The latter acts on the membrane lipids to synthesize 3-phosphoinositides. In muscle cell lines treated with Dexa in vitro, the depletion of membrane-bound PI3K and of 3-phosphoinositides leads to lower activation of 3-phosphoinositide-dependent protein kinase 1 (PDK1), and lower phosphorylation of the latter's substrate, Thr 308 on the activation loop of Akt. While Thr 308 phosphorylation is not detectable in rat muscle in basal state, its sensitivity to insulin is depressed by Dexa[470].

In other systems, the other relevant Akt phosphorylation site, Ser 473, is paralleling the changes in Thr 308. Even in GAML, Dexa lowers insulin's ability to upregulate Ser 473 phosphorylation[470, 455, 471, 399]. However, the effect of Dexa on Ser 473 phosphorylation in basal state is debated. Until about a decade

ago, Akt phoshporylation was identified by changes in electrophoretic mobility. With that method, in basal conditions, in rat muscle, Ser 473 phosphorylation is either undetectable or below the dynamic range of immunoblot[470, 455, 471, 399]. Even in cultured cells, Akt phosphorylation is too low to be quantified[472, 444, 473]. To establish whether Dexa has an effect on Ser 473, one had to use the insulin or IGF-I challenge. Lately, the use of phospho-specific antibodies brought about a series of reports where Ser 473 phosphorylation appeared measurable[474, 475, 476]. These reports also describe that Dexa achieves a reduction in Ser 473 phosphorylation in basal state. No mechanistic explanation was provided for basal state changes at Ser 473.

The relationship between Ser 473 and Thr 308 phosphorylation is a subject of ongoing research. Ser 473 phosphorylation is stimulated by growth factors, through mTOR complex 2, and through mTOR-independent, 3-phosphoinositide-stimulated mechanisms[477]. In vitro manipulations indicate that the two sites synergize for maximal Akt activation[478]. The interdependence between Ser 473 and Thr 308 is illustrated by the fact that non-mutagenic in vivo experiments rarely describe Akt activation without phosphorylation at both residues (reviewed in [479]). This correlation may be the result of an initial priming event, conditioning a non-limiting second phosphorylation.

Mutagenic abolition of Ser 473 phosphorylation abrogates Akt activity on substrates belonging to the FoxO class, but does not change Akt ability to phosphorylate tuberin (Tuberous Sclerosis Complex 2; TSC2) and glycogen synthase kinase 3 (GSK3)[480]. Such changes in substrate imply that Ser 473 controls specificity. Nevertheless, similar results would be seen if Ser 473 alters solely Akt activity, but FoxO substrates are scarcer compared to tuberin and GSK3. In either case, because Foxo is at the core of atrogene regulation in muscle atrophy, Ser 473 status has been of greater interest for GAML studies.

More evidence for Dexa-induced inhibition of Akt comes from its substrates,

such as Ser 9 on GSK3-β. Chronic Dexa reduces GSK3-β phosphorylation at Ser 9, thus leading to GSK3-β activation [470]. The activation of GSK3-β in GAML is confirmed by increased phosphorylation of its substrate glycogen synthase at Ser 645, 649, 653, 657, and decreases intramuscular glycogen synthesis rate[481, 482, 470]. Among the many substrates of GSK3-β, the subunit ε of the eukaryotic initiation factor 2B (eIF2B) may mediate an anti-anabolic effect[483]. In cultured myotubes, GSK3-β knockdown reduces Dexa ability to upregulate MAFbx[484]. While inactivation of GSK3-β is typically attributed to latent Akt inhibition, the Ser 9 site is also a target for ribosomal protein S6 kinase, 90kDa (p90-RSK), serum and glucocorticoid-inducible kinase-like kinase (SGKL), and p70-S6K[485, 486, 487]. The role of GSK3-β in muscle atrophy was briefly the subject of research at the turn of the century, in the context of a hypothesized atrophic mechanism involving calcineurin and nuclear factor of activated T-cells (NFAT). Since then, evidence that calcineurin is not involved in muscle anabolism[488] led to an unjustified neglect of GSK3-β.

In conclusion, the muscle response to GC is associated with signaling and glucose metabolism changes consistent with IRS and Akt loss of sensitivity to trophic factors such as insulin and IGF-I. Interestingly, the pathway appears largely unaffected by Dexa in basal conditions, when trophic factors are withheld. Because the model of GAML centered on Akt is derived from experiments with acute states, cell models, and intrinsic anabolic interventions, it is perfectible. The best studied downstream targets of Akt are mTOR and FOXO.

Glucocorticoid inhibition of mTOR

One of the most effective pathways for Akt to induce its anabolic program is through mTORC1 (reviewed in [489, 490, 491]). Its modulation in GAML is clearly proven by changes in its downstream targets. However, the mechanism by which mTOR is modulated by Dexa is not elucidated yet.

Cell models established that activated Akt phosphorylates and inhibits proline-rich Akt substrate of 40-kDa (PRAS40), a negative regulator of mTORC1[492]. Moreover, the same cell models showed that activated Akt phosphorylates TSC2, thus causing its sequestration with a cytosol partner, 14-3-3, and away from its transmembrane partner, hamartin (Tuberous Sclerosis Complex 1; TSC1)[493]. The destruction of TSC1-TSC2 complexes relieves the negative regulation from Rheb, a small GTPase, that can induce activation of mTORC1, manifested in amplified phosphorylation of mTORC1 substrates such as p70-S6K. Therefore, Dexa-induced repression of Akt should inhibit mTORC1. However, the only confirmation of this mechanism comes from the observation that, in L6 myotubes, Dexa stimulates the formation of TSC1-TSC2 complexes[494].

Based on upstream changes, the effect would be subtle, detectable only during hyperinsulinemia. For always-on, feeding-independent GAML to proceed, mTOR-independent are also required. It has been hypothesized that Akt-independent mechanisms contribute to mTOR suppression in GAML. A well-studied negative regulator of mTOR is the energy sensor AMPK[495, 496]. However, Dexa inhibits muscle AMPK phosphorylation and activity[471, 497], probably as a consequence of intramuscular ATP upregulation[498]. The surge in intramuscular ATP is caused by Dexa-induced improvements in mitochondrial function, exemplified by upregulation of cytochrome c oxidase expression and activity[499] and of Na(+)-K(+)-ATPase expression and maximal activity[500]. Chronic Dexa or corticosterone do not alter Liver Kinase B1 (LKB1)[471, 501], the main AMPK kinase, indicating that energy sensing employs other, unknown mediator. This body of evidence shows that in GAML, mTOR suppression does not employ AMPK. Moreover, GAML does not involve mitochondrial dysfunction.

Another putative mTORC1 inactivating mechanism is centered on the stress sensor REDD1. Dexa upregulates REDD1 expression in muscle[433].

Demonstrating its key role, genetic depletion of REDD1 abolishes GAML and Dexa-induced myotube atrophy[494, 502]. In cell culture, REDD1 interferes with 14-3-3 in order to release TSC2, restore TSC1-TSC2 complexes, and eventually inhibit mTORC1[503]. Moreover, REDD1 is in epistasis with AMPK[504], at times overriding its action on mTOR. Interestingly, a REDD1-based mechanism could explain the few observations on mTORC1 changes just as well as a Akt-centered model would.

One way to asses mTORC1 activation is to observe its enzymatic activity. In contrast to Akt, phosphorylation of mTORC1 substrates, 4E-BP (Thr 37/46) and p70-S6K (Thr 389), is measurable in rat muscles in basal state, with acute Dexa causing a reduction of their phosphorylation[505]. Chronic Dexa changes 4E-BP and p70-S6K phosphorylation in a pattern that mirrors Akt activation, with no detectable Dexa effect on their basal phosphorylation, and a Dexa-induced loss of sensitivity to insulin[506, 452]. Conversely, insulin sensitivity at 4E-BP and p70-S6K is enhanced by removal of endogenous GC, through adrenalectomy[507, 508].

Another way to gauge the role of mTORC1 is to use its inhibitor, rapamycin. One group reported that Dexa-induced decrement in protein synthesis is unmodified, in absolute terms, upon rapamycin co-administration[452], suggesting that mTOR signaling is dispensable. In a C2C12 microarray study, interference of IGF-I transcriptional program with PI3K inhibitor was virtually identical to the interference induced by rapamycin[509], suggesting that, on the contrary, mTOR is indispensable for PI3K effects. Most likely, both hypotheses are based on reductionist models that do not reflect true in vivo phenomena.

Diverging from in vivo studies, some studies showed that L6 and C2C12 myotubes exhibit Dexa-induced downregulation of p70-S6K Thr 389 and 4B-EP phosphorylation and activities, even in the absence of an anabolic stimulus [510, 433, 472]. It is unclear whether the difference between in vivo and in vitro

studies of mTORC1 substrates is caused by the use of different anabolic stimuli. Whereas in vivo studies use insulin to elicit an Akt response whose modulation by GC is measurable, in vitro studies commonly use IGF-I (for example, [444]). One reason could be the remarkable hyperplastic abilities of insulin[511], which may perturb experiments where proliferating myoblasts were not actively eliminated.

While Akt repression is consistent with the induction of the ubiquitin-proteasome system via FOXO, its side effect, mTOR repression, is expected to lead to simultaneous downregulation of protein synthesis via 4E-BP (discussed later). Discovery of novel mTORC1 substrates such as UNC-51-like kinase 1 (ULK1)[512] led to speculations about additional contributions to the GAML phenotype from autophagy. In vivo evidence for mTOR-mediated autophagy upregulation in GAML is indirect (discussed in a later section).

Based on p70-S6K divergence, it appears that GC causes Akt-mediated mTOR modulation, in contrast with denervation atrophy, where Akt is downstream of mTOR. However, an unreplicated recent study opens the perspective for a paradigm-overturning situation. In an acute study of Dexa on female rates, Britto and colleagues confirmed most of the current model[502]. Dexa repressed mTORC1 activity, as indicated by lower phosphorylation of its substrate 4E-BP1. The repression of mTORC1 was correlated with an reduced inactivation of the negative regulator PRAS40. In contrast, and unexpectedly, acute Dexa increased TSC2 phosphorylation, indicating that Dexa induces substrate selectivity changes in Akt. As discussed earlier, such Akt selectivity are attributed to dissociation between Ser 473 and Thr 308 phosphorylation state. Indeed, Britto and colleagues found that, after acute Dexa, phosphorylation at Ser 473 increases, while at Thr 308 it decreases. This is a rare, although not unique, account of Akt hyperphosphorylation in GAML. In each case, these findings may be explained by the particular conditions of the unique experiment. Here, proteolysis was not unaltered, indicating that acute Dexa is not a true model of GAML.

Britto and colleagues refined their model by observing the effect of Dexa in REDD1 knockout mice. REDD1 depletion abolishes GAML, although atrogenes are still upregulated. On the other hand, REDD1 depletion prevents Dexa's hypophosphorylating action on 4E-BP1 and p70-S6K. These findings place mTORC1 inhibition at the core of GAML. Britto and colleagues propose a model of GAML where Ser 473 hyperphosphorylation is caused by an mTORC1 feedback loop being severed by Dexa. Upstream of mTORC1, REDD1 knockout mice exhibit loss of phosphorylation on PRAS40 and Akt on Thr 308. While Akt remains upstream of mTOR and vital for GAML, Britto's model discards phosphorylation of Akt at Ser 473 and of TSC2 from the rapid REDD1-dependent response to Dexa. Multiple studies in transformed cells confirm the role of Thr 308 in REDD1 repressive action on mTOR, while proposing different intermediaries ([513]; reviewed in [514]).

The most robust inducer of REDD1 is hypoxia, through HIF transcription factors[514]. As described in the dedicated section, HIF1a also induces VEGF, which in turn has a myoprotective action. But in GAML, VEGF is downregulated[515], suggesting that HIF1a is not involved in REDD1 upregulation. The mechanism by which Dexa stimulates REDD1 remains to be studied. Candidates include ATF4 and NFAT, discussed in other sections of this chapter.

While it is certain that GAML is correlated with mTORC1 downregulation, it is still unclear how mTORC1 repression is achieved, and what is its relative importance. Based on the evidence published until now, the recent change of paradigm which placed p70-S6K at the forefront of denervation atrophy appears unlikely in GAML. For now, our understanding of GAML places Akt firmly upstream of mTORC1. Better transgenic models, a deeper understanding of mTOR pathways and more in vitro / recombinant experiments are required for a definitive conclusion.

Glucocorticoid activation of Foxo transcriptional program

The evidence for the role of Foxo in GAML is not as direct and compelling as that gathered in denervation atrophy. Given the partial redundancy between the three regulatable Foxo transcription factors, only a triple knockout will give the true measure of their relative importance. Nevertheless, an impressive body of indirect evidence supports their involvement in GAML.

The importance of Foxo transcription factors is supported intuitively by their role as integrators of multiple atrophy signaling pathways. Multiple mechanisms converge to induce Foxo upregulation in GAML.

First, chronic Dexa doubles Foxo expression[433]. Foxo1 promoter contains a GR response element[516]. In C2C12 myotubes, the response is biphasic, combining a short-lived ample and rapid increase, with a gentler long term augmentation[434]. Foxo promoters contain GR-binding sites[517]. In vitro, Foxo induction by GR is facilitated by the histone acetyl transferases p300 and CREB-binding protein, which are independently upregulated by Dexa[447]. As a consequence, GR knockout reduces expression of FOXO1 and FOXO3a in muscle[431].

Second, Foxo transcription factors are thought to be potentiated by Dexa through post-translational means involving the earlier described Akt pathway (reviewed in [518]). Akt phosphorylates Foxo1 at Tyr 32 and Ser 253, thus creating binding sites for 14-3-3. Phosphorylated Foxo transcription factors are exported from the nucleus, and eventually marked for ubiquitin-proteasome-mediated degradation. It is believed that the Dexa-induced impairment of Akt contributes to an increase in their activity in GAML. Given that phosphorylated Foxo is degraded and is elicited by a low-activity Akt pathway, Foxo phosphorylation changes may be undetectable in basal state. In C2C12 myotubes, Foxo phosphorylation downregulation in response to Dexa is more readily

detected[383, 519]. A few groups reported reductions in Foxo phosphorylation by chronic Dexa at basal state in vivo[520, 474, 521]. Two out of the three referenced studies also report an unusual activation of Akt in basal state, indicating that they may be reflective of special conditions (40 days treatment, the use of male levator ani, and so on). As mentioned in previous sections, an ongoing debate surrounds the effect of Dexa on Akt Ser 473, which alters its ability to phosphorylate FOXO. As with mTORC1, the Akt pathway may be more relevant in fed state.

Third, PGC nuclear cofactors may facilitate the Dexa-induced Foxo surge. Loss of sensitivity to insulin causes PGC-1 repression in muscle[522]. In particular, Dexa represses muscle PGC-1α[463, 521] and PGC-1β[523]. In cultured myotubes, PGC-1β overexpression and knockdown cause changes in the reverse direction for Foxo3a and atrogenes' mRNA. Because PGC-1α overexpression drives conversion of fast to slow twitch fibers[352], it has been speculated that differences in PGC-1α levels make different types of muscle fiber more or less sensitive to Dexa[384]. A whole higher level of regulation of is yet to be studied, with PGC-1α splicing dictating its role more. For example, isoform 1, and not 4, of PGC-1α is correlated with muscle hypertrophy[524].

Fourth, indirect evidence links Foxo1 to PPARβ/δ. Dexa stimulates simultaneously PPARβ/δ DNA binding and Foxo1 acetylation[525]. PPARβ/δ inhibition reduces GAML and, at the same time, prevents Dexa-stimulated acetylation of Foxo1. While the direct effects of this post-transcriptional modification is not elucidated (reviewed in [526, 527]), it was speculated that PPARβ/δ changes induce physiologically relevant alterations in Foxo1.

Finally, miR-182, whose expression is inhibited by Dexa, is a negative regulator of Foxo3[528]. Other mechanisms, such as AMPK, have been hypothesized, based on the latter's ability to upregulate atrogenes.

The net result of these mechanisms is that Dexa treatments upregulates nuclear Foxo[463]. More convincing evidence towards Foxo activation comes

from the concordance in its downstream effects. For example, Dexa increases muscle glutamine synthetase activity[529]. Glutamine synthetase promoter contains a FOXO response element. Dexa-induced expression of muscle glutamine synthetase is absent in Foxo1 knockout[530].

Manipulations of Foxo have supported their role in GAML. Transfection with dominant negative Foxo3A partially prevents diameter reduction in disuse of rat soleus and Dexa-treated C2C12 myotubes[519, 531].

Among the Foxo-induced genes, a special importance was given to the E3 ligases associated with muscle loss, MAFbx and MuRF1[444]. Both atrogenes contain FOXO-binding regions in their promoter[519, 532, 431]. These Foxo-responsive elements are in close proximity of SMAD3-binding elements, in a shared response element which facilitates synergistic interactions between the two classes of transcription factors[533]. MuRF1 promoter facilitates another synergistic interaction, between FOXO1 and GR[431]. A positive feedback loop links MuRF1 and FOXO1, as indicated by downregulation of p85 and FOXO1 in late GAML in the MuRF1 knockout[534].

In both atrogenes' promoter, a KLF-15 response element is located, near the FOXO binding site[535]. KLF-15 is a direct transcriptional target of GR, and interferes with mTOR signaling. A complex network is centered on KLF-15, which upregulates expression of FOXO transcription factors, and synergizes with them to upregulate atrogenes. Overall, atrogenes appear to be under a strong control of Foxo, favored by concomitant activation of synergistic factors. Indeed, dominant negative Foxo3A transfection in rat soleus leads to loss of atrogene upregulation[536]. In cultured myotubes, where transfection of constitutively active FOXO3a upregulates MAFbx[519], while knocking down FOXO1 causes a reduction in the atrogene response to GC[537].

A majority of the effectors described in this chapter appear to be under the control of Foxo pathway. In addition to atrogenes, Foxo targets include 4E-BP1,

cathepsin L, and the effector of denervation atrophy, Growth Arrest and DNA Damage 45 (Gadd45)[538].

Glucocorticoid activation of myostatin

In rodents, Dexa upregulates myostatin, a strong negative regulator of muscle mass, in the atrophying muscle[539]. In myostatin knockout mice, Dexa induces most of its transcriptional program, including modulations of IGF-I, MuRF1, MAFbx, FOXO3A, but Dexa-induced muscle loss does not occur [421]. These findings suggest that myostatin is an important mediator of Dexa.

Myostatin's promoter contains GR response element[539], a FOXO responsive element[540], and a CCAAT sequence[541], all known to be stimulated by Dexa. Myostatin expression is also upregulated by the histone methyltransferase SMYD 3, which in turn is upregulated in muscle by Dexa[542]. The stability of the myostatin transcript is improved by Dexa-exerted repression of its negative regulators, miR-27[543].

Glutamine supplementation reduces GAML and myostatin expression in muscle[544]. This finding suggests that, in the context of GAML, myostatin may double as a nutrient sensor.

Myostatin's effects are manifold, and not completely understood. For example, while myostatin inhibition in utero leads to doubling of muscle mass in adult animals[225], its overexpression in adult animals causes a more moderate effect[545], indicating that its effect is based on hyperplasia- and hypertrophy-based pathways of muscle growth. In explanted adult mouse myofibers, where proliferation has been minimized, expression of a dominant negative form of activin A receptor, type IIB (ActRIIB), the receptor for myostatin, causes an increase in CSA, which is halved by rapamycin[546]. Therefore, proliferation-independent mechanisms of myostatin-induced atrophy may include both mTOR-dependent and -independent pathways. The extensive,

multifactorial action of myostatin on muscle suggests that it may override any atrophic stimulus, in non-specific ways. Recent molecular studies endeavored to establish whether GAML-associated myostatin modulation is physiologically relevant, or only a side effect.

A series of experiments describe Akt inhibition in response to myostatin treatments. In vivo electroporation with myostatin-coding plasmids leads to 10% loss in tibialis anterior mass in the transfected muscle, in the absence of any changes at the level of atrogenes[547]. The investigators behind this study performed an analysis of the mTOR pathway, which found loss of phosphorylation on tuberin and p70-S6K, and concluded that myostatin affects protein metabolism. A simpler explanation would have taken into account the regeneration in the scarred electroporated muscle, which should have contributed a high number of proliferating cells. Another provoking study investigated human myotubes differentiated from cultured myoblasts. In this model, myostatin treatments lead to reductions in diameter and atrogene downregulation [548]. The authors of the latter report explain their observations by demonstrating that myostatin reduces myoblast fusion and differentiation. While interesting in context of muscle development and regeneration, such experiments cannot replicate adult GAML. Moreover, both reports describe even in the myostatin-free controls, an unusual Akt phosphorylation. Most of their observations would be consistent with the well-documented myostatin-induced impairment of muscle regeneration[549, 550].

The study on human myotubes[548] showed that exogenous myostatin downregulates muscle atrogenes. In contrast, GAML is characterized by an upregulation of atrogenes. Moreover, the upregulation of atrogenes in GAML is attributed, in part, to Akt inhibition. Therefore, a myostatin - Akt axis does not seem to underlie GAML.

Myostatin is particularly adept at modulating the fusion of myoblast to

myotubes[551]. Myostatin's action appears more relevant for processes where myoblasts or satellite cells are involved, such as muscle development and regeneration (reviewed in [552, 553]). But normal adult muscle maintenance involve to a small degree satellite cells. Loss of their contribution to muscle maintenance, which is supposedly a major effect of myostatin, may contribute to, but not command adult GAML. In contrast, there is no evidence that myostatin upregulates catabolic processes within GAML. Even in other models of atrophy, such as microgravity, myostatin upregulation occurs while 3MH excretion is unchanged[554]. Myostatin's relative importance for GAML remains unclear. The typical experiments, using qualitative alterations of myostatin, may override any endogenous mechanism, and yield non-specific anti-atrophic effects.

Glucocorticoid modulation of protein synthesis

Goldberg's[372] 1969 study on cortisone-induced atrophy of rat muscle brought one more important theme for the field of GAML. In that experiment, the specific activity, that is, the ratio between tracer and total protein, was the same in GC-treated muscle as in control animals, despite decreased muscle mass and decreased total tracer in the GC-treated group. Goldberg conjectured that an equal rate of protein synthesis in the two groups would have caused a faster dilution of the tracer in the GC-treated muscle. Failing to find that was interpreted as evidence for decreased protein synthesis in cortisone-treated muscle. This was an elegant way of bypassing the denominator effect from the typical experiments, where synthesis rate would be measured over hours, in muscles that already underwent atrophy in the prior days. In these cases, a loss in protein synthesis rate might be underestimated or even factored out, after normalization to a lower muscle mass.

As in human studies, the role of protein synthesis modulation during chronic hypercortisolism is still debated, because chronic Dexa effect is superimposed by

acute effects. A single 20 mg/(kg d) triamcinolone dose nearly halves incorporation of labeled precursors in rat muscle protein at 8 hours, yet has no effect after 24 hours[555]. Similar acute effects may be expected from feeding, which causes fluctuations of insulin and possibly of the Akt / mTOR / 4E-BP axis in muscle.

Variability in the time from feeding and treatment to biopsy is a significant source of experimental noise, which must contribute to inconsistencies in literature. For example, some of the most exhaustive studies of protein metabolism in GAML came from Grizard laboratory. In two studies, they found that protein synthesis rate was unchanged after 0.5 mg/(kg d) Dexa for 6 days, compared to pair-fed animals, in the epitrochlearis muscle[411, 556]. In two other studies, assaying other glycolytic muscles or quadrupling the Dexa dose, led to observable reductions in protein FSR[557, 452]. In cell cultures, Dexa inhibition of protein synthesis should be more readily detectable, given that, in vitro, Dexa affects 4E-BP phosphorylation directly, even in basal state[472]. However, studies are similarly equivocal. In the most glaring example, the same group, using the same methods, found that protein synthesis is “not altered” by Dexa in one experiment, and decreased in another[406, 558].

Even when detected, the amplitude of changes in protein synthesis in vitro[558, 559, 560] and in vivo[329] is lower than that on protein degradation, when Dexa is given at the dose that causes muscle loss. In contrast, doses of Dexa below the apparent EC50 are unable to change protein degradation rate, while still lowering protein synthesis rate[336].

In the MuRF1 knockout mouse, Dexa fails to change FSR[430], suggesting a temporal order, with proteolytic processes preceding and initiating changes in protein translation.

Suggestive data for protein synthesis modulation in GAML come from various molecular correlates. For example, acute GC administration causes a reduction in

the proportion of polyribosomes[561]. The proportion of polysomes recovers to basal level in less than 24 hours, when the GC is short-acting prednisone[407]. In contrast, polysome downregulation lasts about two days after Dexa. No published accounts describe polysome profile changes after longer GC exposure.

Dexa has a dual effect on 4E-BP, the inhibitor of protein synthesis that binds and inhibits the eukaryotic initiation eIF4E. First, Dexa upregulates 4E-BP mRNA[433]. It was long believed 4E-BP induction is direct, and based on a Foxo response element in 4E-BP promoter[562]. As of 2015, this response element is proven only in Drosophila. The only replication in mouse cultured cells was withdrawn during the preparation of this thesis, for unspecified reason. Second, Dexa may regulate 4E-BP through post-translational phosphorylation. As shown earlier, Dexa reduces mTOR-effected 4E-BP phosphorylation sensitivity to insulin, which is expected to cause reductions in protein synthesis rate mainly after feeding. The study of mTOR action is challenged by the existence of multiple phosphorylation sites, inability of antibodies to discern them, and inability of rapamycin to inhibit mTORC1 action on 4E-BP1[563].

Dexa upregulates mitogen-activated protein kinase–interacting kinase 2 (MNK2) expression[564]. Dexa reduces eIF4G Ser 1108 phosphorylation in wild type mice, but not in MNK2 knockout mice. This posttranslational modification correlates with nutrient availability, but its role in GAML or in general is not known (reviewed in [565]).

In C2C12 myotubes, GAML may repress protein synthesis by MAFbx-initiated neutralization of eIF3-f[439]. Overexpression of eIF3-f causes hypertrophy, and eIF3-F knockout induces atrophy, supporting the hypothesis that loss of an initiation factor causes reductions in the rate of protein synthesis. Moreover, further data from the same group shows that loss of eIF3-f impairs the ability of mTOR to bind and phosphorylate p70-S6K, suggesting that eIF3-f pro-anabolic action is more complex[566]. The role of eIF3-f in GAML has not been confirmed

in vivo.

Canonical control of protein synthesis includes translational derepression, a cytosol-based mechanism for sensing amino acid starvation (reviewed in [567]). Relative lack of amino acids enriches uncharged tRNA, which bind and activates General Control Nonderepressible 2 (GCN2)[568]. Activated GCN2 phosphorylates eukaryotic initiation factor 2 (eIF2) at Ser 51 on its α subunit, leading to the formation of an inactivating complex with eIF2B[569]. The inactivation of eIF2B, the guanine exchange factor for eIF2, leads to general translation shutdown[570]. The lack of eIF2-GTP complexes leads to start codon skipping, which, for transcripts including multiple open reading frames (ORF), determines extraordinary translation from downstream start ORFs. In eukaryotes, a physiologically relevant downstream ORF is Activating transcription factor 4 (ATF4), which upregulates genes involved in transport of essential amino acids and synthesis of non-essential amino acids, such as asparagine synthetase[571]. Overall, the translational derepression pathway is a mechanism for inhibiting protein synthesis, initiated by apparent depletion of free amino acids, and leading to ATF4 upregulation.

The study of translational derepression in mammalians is still in its beginnings. The ATF4 knockout mouse has normal weight, but exhibits some sparing from muscle atrophy in response to starvation[572]. ATF4 knockout mice exhibit a normal atrogene response to starvation, indicating that ATF4 is part of a novel atrophy pathway. The only study that measured chronic Dexa effect on phosphorylation of eIF2 reported negative results[123]. However, Dexa abrogated amino acid infusion ability to reduce eIF2 phosphorylation, a situation reminiscent of Dexa's action on Akt. In a fibroblastic cell line, Dexa upregulated ATF4 translation as long as insulin was withheld from the medium[573].

Unexpectedly, in C2C12 myotubes, ATF4 protein levels are upregulated by insulin in a rapamycin-dependent manner[573]. A plausible explanation is that

ATF4 is upregulated by apparent amino acid deficits, including cases when mTOR-stimulated protein translation depletes the free amino acid pool.

Changes in 4E-BP1 and perhaps some other pathways regulating protein synthesis are induced by Dexa in a manner consistent with GAML. The mild alterations of translation molecular markers correlate with the moderate loss in protein synthesis rate. While the overall effect is not negligible, its reduced amplitude and its downstream relation to MuRF1 indicate that anabolism adjustments play a complementary role to the activation of the ubiquitin-proteasome system in GAML.

The effects of glucocorticoids on autophagy

Proteasome inhibition does not abolish completely Dexa-activated proteolysis, indicating that some other catabolic mechanisms must be involved. Based on newly discovered role of mTOR as a major modulator of autophagy, the second most important effector of GAML was presumed to be autophagosome. Moreover, in L6 myotubes, 100 uM chloroquine or 200 mM E-64 reduce the rate at which Dexa amplifies proteolytic tracer release[422].

A series of molecular markers further support the idea that autophagy is upregulated by Dexa. In C2C12 myotubes, Dexa may induce the formation of double-membrane autophagic vesicles, although evidence is limited to unquantified micrographs[574].

Dexa reliably upregulates the family of lysosome proteases known as cathepsins. In vivo, Dexa doubles the lysosome proteases cathepsin L and D [411, 575, 434]. In L6 myotubes, Dexa upregulates cathepsin B[422].

A new modality for investigating autophagy hinges on one of the longest-living markers on its surface, microtubule-associated protein 1 light chain 3 (LC3). Transgenic mice with LC3 fused to green fluorescent protein (GFP) have been developed, and are regularly used for tracking autophagosomes. There are no

reports of this model being used in the study of GAML. Alternatively, endogenous LC3 is tracked through immunofluorescence microscopy. Some reports describe an accumulation of punctate LC3-containing structures in L6 myotubes treated with Dexa for 6 hours[462]. Another way of tracking LC3 is based on immunoblot. The autophagosome-associated form of LC3, termed LC3-II, is faster migrating during electrophoresis. Various indices, such as absolute amount of LC3-II or the ratio between LC3 electrophoretic forms, are used for estimating the number of autophagosomes (reviewed in XXX). LC3-II is enriched in C2C12 myotubes overexpressing LC3[574] and in L6 myotubes[462]. Similar to the microscopy experiments, these reports use acute Dexa treatments. Moreover, a time course reveals that in L6 myotubes, LC3-II peaks at 6 hours and is extinguished at 24 hours after Dexa administration[576]. It appears that in vitro Dexa causes a rapid accumulation of autophagosomes, which are dismantled at a lower pace, as they fuse with lysosomes, and their content is cleared by proteolysis.

In vivo, acute Dexa causes accumulation of LC3-II protein[502]. Recently, the enrichment of LCII-B after chronic Dexa has been reported[577]. The latter report is uncommon, and may be reflecting a short interval between last Dexa dose and sample collection.

In contrast with the limited body of evidence we have for autophagy upregulation, there is a significant amount of literature describing what would drive autophagy up in GAML. In C2C12 myotubes, overexpression of a constitutively active FoxO3 or chemical inhibition of Akt leads to increased lysosomal proteolysis[383]. As mentioned earlier, mTORC1 is a negative regulator of autophagy, whose inhibition in GAML is hypothesized to stimulate autophagy. Indeed, acute Dexa administration reduces ULK1 phosphorylation at Ser 575, the mTOR-specific, inhibitory site[502].

Other lines of evidence provide indirect support for autophagy upregulation in GAML. Acute Dexa downregulates p62, one of the shortest-lived markers on

the autophagosome, thus suggesting that autophagic flux is upregulated[502]. Acute Dexa increases expression of the mitophagy effector Bnip3[502, 462]. In C2C12, acute Dexa upregulates expression of lysosomal markers such as autophagy-related 12 (Atg12)[528].

Given the spatial segregation between lysosomes internal and outer space, autophagy must rely on a selective mechanism for any protein that it is processing. Therefore, it is likely that autophagy acts only on a few specific, perhaps limiting, proteins. For example, Dexa-induced depletion of sialidase Neu2 is prevented by 3-MA[574].

Autophagy may play a significant role in triggering and regulating GAML. Current evidence, based on transgenics and cell culture, is far from satisfactory. Given its limited amplitude in vivo, it is improbable that autophagy is responsible with bulk protein elimination.

Glucocorticoids and other proteolytic systems

In 1986, it was discovered that proteolysis in muscle is increased when explants are soaked in 2.5 mM calcium[578, 579]. The calcium-stimulated proteolysis subsides upon co-administration of leupeptin, a wide-spectrum protease inhibitor. The discovery of a class of calcium-dependent proteases, called calpains, suggested that they might be contributing to muscle atrophy. The calpain system includes μ-calpain, which is activated by micromolar concentrations of calcium, m-calpain, which is activated by millimolar concentrations of calcium, and their inhibitor, calpastatin (reviewed in [580]). Transgenic mice overexpressing calpastatin have 30% lower loss of muscle upon unloading[581], proving that the calpain system is important in some atrophy models.

There is limited evidence for calpains involvement in GAML, beyond the experiments from 1980's. In vivo, expression of the calcium-dependent protease m-calpain is trebled by Dexa[411]. In L6 myotubes, calpastatin overexpresion

halves Dexa-induced proteolysis[582]. Still in L6 myotubes, Dexa promotes store-operated calcium entry, the mechanism by which intracellular Ca concentration is increased when ER stores are depleted[583].

It has been speculated that activations of calpains is an initial step in GAML, allowing myofibril protein to interact with MuRF1[584]. Calpains are important for some atrophy models, but their role in GAML is understudied.

Many unbiased studies found that a family of proteased, metallothioneins, are upregulated in GAML[585]. However, they contribution to GAML has not been analyzed.

With the advent of new genome technology, enzymatic and functional studies that brought the proteasome in the center of GAML have been abandoned. The example of calpains illustrates how non-transcriptional events can contribute to GAML, and testifies to a blind spot in GAML research.

Alleviation of glucocorticoid myopathy by IGF-I

Upon finding that, in L6 myoblasts, Dexa-stimulated proteolysis is abated by co-administration of insulin, Ballard hypothesized in 1983 that Dexa acts indirectly, by depleting body's supply of IGF-I[586]. While the use of non-fusing myoblasts is certain to introduce confounding changes in proliferation, and the equivalence between IGF-I and insulin lacks subtlety, his hypothesis captured the attention of many investigators, despite going against another theme of interest in those days, the hyperplastic synergy between IGF-I and GC[587, 588]. As described in the sections on Akt and mTOR, most of the knowledge we have about it is derived from studies of anabolism, where muscle, rather than being analyzed in its basal state, is first stimulated with insulin or IGF-I. In a sense, we know more about the interaction between Dexa and IGF-I than we know about Dexa alone. Lately, the interpretation of such experiments shifted from mechanism-explaining to a therapeutic paradigm. In mice, electroporation of

IGF-I plasmid in tibialis protected solely the transformed fibers from Dexa-induced atrophy[589]. In Dexa-treated rats, co-administration of IGF-I reduces loss of muscle mass, fiber atrophy, and 3MH release[590], thus providing a blueprint for the ideal anti-GAML therapy.

Co-administration of IGF-I reverses upregulation of ubiquitin, and of proteasome subunits C2 C3, C8[591, 592], thus blunting one of the major effectors of GAML, the ubiquitin-proteasome system. But Dexa antagonizes IGF-I on many other downstream effects, such as glutamine synthesis[593].

In vivo, Dexa reduces muscle expression of IGF-I[594, 200], and possibly interferes by altering IGFBP secretion[433]. Therefore, GAML manifests as an absolute loss of IGF-I exacerbated by a downstream inhibition. Intuitively, IGF-I supplementation may provide GAML alleviation.

Despite the positive results seen in rodents (described above), very few studies analyzed molecular mechanisms by which IGF-I works in vivo. Because co-administration of GH cannot reverse muscle loss from Dexa or triamcinolone[595, 592], it is likely that, similar to Dexa, IGF-I action on muscle is cell-autonomous. Therefore, a series of studies dissected the molecular mechanisms by which IGF-I could alleviate cultured myotube atrophy. There, IGF-I alone improves protein synthesis, but has no effect on protein degradation[596]. Nevertheless, myotubes treated with Dexa, co-administration of IGF-I has an anti-proteolytic effect[596]. The reduction in proteolysis covers all domains, but, in acute settings, appears more effective in repressing lysosomal than proteasomal activity[597]. IGF-I co-administration reverses MAFbx and MuRF1 upregulation[488, 431], in a Foxo dependent manner. During co-administration, Dexa is still able to upregulate protein levels of Foxo transcription factors, but IGF-I appears to counter them by increasing their phosphorylation[444] and reducing its ability to bind MuRF1 promoter[431]. The IGF-I induced FOxo phosphorylation is consonant with upstream modulations of

the Akt axis. First, IGF-I co-administration in cells treated with Dexa causes myoprotective increases in phosphorylation of Akt, GSK-3β, p70-S6K and 4E-BP1[598, 599]. Second, studies with chemical inhibitors revealed that IGF-I protective effect is mediated by Akt and PI3K[598].

From the similarity between muscle protection conferred by IGF-I and by GSK-3β inhibitors, it was speculated that IGF-I acts by inhibiting GSK-3β[600, 475].

Muscle accretion is not completely conditioned by availability of IGF-I. Myostatin knockout mice have double muscle size, yet lower circulating IGF-I levels, compared to wild type[601]. Muscle growth and recovery is halved by rapamycin, indicating that other anabolic mediators are as important as the Akt / mTOR pathway [602, 382, 603].

Based on the current evidence, it appears that IGF-I alleviating action is not completely overlapping with the wide spectrum of atrophic actions of Dexa. Indeed, the putative mediator of muscle loss REDD1 is upregulated by acute insulin or IGF-I[510]. It is unclear whether such REDD1 upregulation in an anabolic context may refute the role of REDD1 in muscle loss, or whether, on the contrary, it explains the incomplete reversal of GAML by IGF-I.

As our understanding of GAML improved, studies on its alleviation by IGF-I are lagging. A large number of publications focus on the balance of GC and IGF-I on Akt in cultured cells. Given the reduced number of co-administration in vivo studies, our understanding of how IGF-I could alleviate glucocorticoid myopathy is incomplete.

Alleviation of glucocorticoid myopathy by anabolic steroids

Human studies demonstrated that AAS addition to chronic Dexa benefits male adult patients, by reducing their loss of muscle and improving their quality of lifes[158]. The idea of alleviating the CS by AAS therapy came two years after the

discovery of an affordable source of T. In the case of exogenous hypercortisolism, the same idea surfaced less than a year after the discovery of GC. Two years later, Courier and Marois report the first replication of the myoprotective effect in rats[604]. For a long time, during the second dark age of steroids, the combination of AAS and GC, with androgenic, but without anabolic potency, was investigated in the hope that it would show the way towards splitting the anabolic from the androgenic principle in T.

Another, related, theoretic question was the nature of the nuclear receptor. At the time when it was not clear how many species of nuclear receptors there are, precise measurements tried to find putative interference between the various steroids, with Dexa and T among the most studied compounds. In vitro studies have quantified androgens ability to interfere with the binding of Dexa to GR. The Kd for GR-Dexa association is below nanomolar range[605]. In vitro Dexa doses used in muscle atrophy experiments range in the tens of hundred nanomolar. Similar concentrations are likely in the blood of mice injected with the 0.5-1 g/kg doses described before. At a concentration of 2 pM in rat skeletal muscle[606], virtually all GR should be bound to Dexa. On the other hand, Ki for T competing with Dexa for binding to muscle protein extracts is 10 uM[607]. While the referenced report does not distinguish non-specific binding for T, Ki is tens or hundreds of times higher than typical T concentrations used in literature for biological reversal of GAML. T binding affinity to Dexa binding sites in rat muscle cytosol is less than 100 times lower than Dexa's affinity[608]. Therefore, barring allosteric effects, the direct competition between the two steroids remains only of theoretical importance.

The experiments in the 1980's and 1990's tested the interaction of AAS and GC on the diaphragm. While these studies could not have measured the yet-undiscovered mediators of GAML, they established that myoprotection provided by AAS manifests in both muscle mass and force[609, 610, 611].

The mechanism by which AAS accomplishes muscle protection remains unclear to date. One early study found T re-establishes the percentage of ribosomes that are involved in translation[612]. Two later studies on L6 and another on C2C12 myotubes stated that AAS cannot reverse the downregulation of protein synthesis induced by Dexa in L6[586, 613, 559]. The effects of T on protein degradation remain unexplored.

A few studies investigated the interaction of AAS and GC, by measuring the changes in one signal when the other is altered. An interaction at the level of receptors cannot be excluded. In skeletal muscle, GR mRNA and binding activity are increased upon castration[614, 615], suggesting a way by which castration causes muscle atrophy. Conversely, Dexa reduces the expression of AR in skeletal muscle[200]. Hypercortisolism reduces endogenous T levels in male rats, thus leading to ampler loss of muscle than in females, which experience T upregulation[413]. Sexual dimorphism in animal models confirm that males stand to benefit more from AAS therapy in GAML.

In the absence of direct interference, studies sought downstream effectors at which T could prevent Dexa's program. Myostatin promoter contains putative androgen responsive elements[539]. In intact animals and even in atrophic muscle after spinal cord injury, T does not alter myostatin[616, 617]. Myostatin changes in co-administration of AAS and GC has not been investigated. Given myostatin's limited role in GAML, it is unlikely myostatin repression could contribute to the ample alleviation provided by T.

The other effector of GAML is the Akt axis. In the era of immunoblot, three laboratories published studies of co-administration of AAS and GC. All three converged towards describing an reversal of Akt pathway inhibition.

T is not required for IGF-I upregulation after exercise[618].

T represses Dexa's activation of the MAFbx promoter[619].

3. HYPOTHESES

4. METHODS

Literature review

The introduction section was based on review of all literature indexed by PubMed. Search expressions included 'testosterone OR androgens', 'dexamethasone OR betamethasone OR triamcinolone OR prednisone OR prednisolone OR hydrocortisone OR cortisone OR triamcinolone OR fludrocortisone', 'Cushing', 'ribosome OR polysome OR lysosome OR autophagosome OR proteasome OR ligase OR cathepsin OR FOXO OR IGF1 OR calpain OR mTOR OR AMPK OR Akt'and combinations thereof. Relevant primary data were summarized.

Literature plots were digitized with WebPlotDigitizer, a web application created by Ankit Rohatgi[620].

Ethical procedures

All animal procedures have been described in protocols drafted by the author. The protocols were written in advance, submitted with Dr. Carlo Serra as principal investigator, and approved by the Institutional Animal Care and Use Committee at the Boston University School of Medicine and Harvard Medical Area Standing Committee on Animals.

Animal studies

Male, 6-8 week old (young adult), C57Bl/6J mice were purchased from The Jackson Laboratories (Bar Harbor, Maine). Mice were acclimated for 3-7 days between delivery and initiation of the experimental interventions. Before and during the experiments, mice were maintained in a temperature-controlled facility, at 21°C, with 12 hour light / 12 dark cycles. Mice were offered water and chow (Purina, Richmond, Indiana) ad libitum.

Every morning, for 1-7 days, mice were weighted, then injected subcutaneously every morning 200 µL corn-oil based solution, including 14 µL (?) ethanol, which delivered .

Mice were euthanized humanely by Euthasol® (pentobarbital sodium and phenytoin sodium solution;200 mg/kg pentobarbital) intraperitoneal, followed by quick cervical dislocation. Blood was collected immediately after death through thoracotomy and cardiac puncture, incubated 15 minutes at room temperature, centrifugated 15 minutes, at 10,000 RCF, 4°C. Levator ani, gastrocnemius (a mixed muscle; see PMID: 8847313, PMID: 10090572, PMID: 22013216), tibialis anterior, quadriceps, and triceps brachii muscles were collected, weighted in wet state, flash-frozen by submersion in liquid nitrogen, and stored at −80 °C.

Muscles were crushed under liquid nitrogen, using a mortar and pestle pre-chilled in liquid nitrogen, and muscle powder was stored at −80 °C. Small quantities of muscle powder (25 µg to 30 µg) were lyzed for enzymatic activity assays, immunoblot, or quantitative real-time polymerase chain reaction (qRT-PCR).

Cell culture studies

Immunofluorescence microscopy

In vivo studies

Metabolic measurements at organism level

Measurement of muscle protein synthesis and degradation

Enzymatic assays

Gene expression

Immunoblot

5. IN VIVO EXPERIMENTS

todo

6. IN VITRO FINDINGS

todo

7. DISCUSSION

todo

Conclusions

Future directions

todo

Bibliography

[1] J. Newell-Price, X. Bertagna, A. B. Grossman, and L. K. Nieman. Cushing's syndrome. *Lancet*, 367(9522):1605–1617, May 2006. PMID: 16698415.

[2] M. E. Molitch. Current approaches to the pharmacological management of Cushing's disease. *Molecular and Cellular Endocrinology*, October 2014. PMID: 25450859.

[3] A. A. Ferrando, M. Sheffield-Moore, D. Paddon-Jones, R. R. Wolfe, and R. J. Urban. Differential anabolic effects of testosterone and amino acid feeding in older men. *The Journal of Clinical Endocrinology and Metabolism*, 88(1):358–362, January 2003. PMID: 12519877.

[4] H. Cushing. *The pituitary body and its disorders, clinical states produced by disorders of the hypophysis cerebri*. J.B. Lippincott Co., Philadelphia & London, 1912.

[5] E. A. Schäfer and S. Vincent. The physiological effects of extracts of the pituitary body. *The Journal of Physiology*, 25(1):87–97, 1899.

[6] H. Cushing. The basophil adenomas of the pituitary body and their clinical manifestations (pituitary basophilism). *Bulletin of the Johns Hopkins Hospital*, L:137–195, 1932. PMID: 16353601.

[7] E. J. Kepler. Cushing's disease; a primary disorder of the adrenal cortices? *Annals of the New York Academy of Sciences*, 50(Art. 6):657–678, June 1949. PMID: 18148623.

[8] G. W. Thorn, R. P. Howard, and K. Emerson. Treatment of Addison's disease with desoxy-corticosterone acetate, a synthetic adrenal cortical hormone (preliminary report). *Journal of Clinical Investigation*, 18(4):449–467, July 1939. PMID: 16694679.

[9] L. K. Nieman, B. M. K. Biller, J. W. Findling, J. Newell-Price, M. O. Savage, P. M. Stewart, and V. M. Montori. The diagnosis of Cushing's syndrome: an endocrine society clinical practice guideline. *The Journal of Clinical Endocrinology and Metabolism*, 93(5):1526–1540, May 2008. PMID: 18334580.

[10] L. K. Nieman. Diagnostic tests for Cushing's syndrome. *Annals of the New York Academy of Sciences*, 970:112–118, September 2002. PMID: 12381546.

[11] L. F. Kirk, R. B. Hash, H. P. Katner, and T. Jones. Cushing's disease: clinical manifestations and diagnostic evaluation. *American Family Physician*, 62(5):1119–1127, 1133–1134, September 2000. PMID: 10997535.

[12] J. Glyn. The discovery and early use of cortisone. *Journal of the Royal Society of Medicine*, 91(10):513–517, October 1998. PMID: 10070369.

[13] P. S. Hench. The reversibility of certain rheumatic and non-rheumatic conditions by the use of cortisone or of the pituitary adrenocorticotropic hormone. In *Nobel Lectures, Physiology or Medicine 1942-1962*. Nobel Media AB, Amsterdam, 1964.

[14] R. G. Sprague, M. H. Power, H. L. Mason, A. Albert, D. R. Mathieson, P. S. Hench, E. C. Kendall, C. H. Slocumb, and H. F. Polley. Observations on the physiologic effects of cortisone and ACTH in man. *Archives of Internal*

Medicine, 85(2):199–258, February 1950. PMID: 15405892.

[15] A. R. Clark. Anti-inflammatory functions of glucocorticoid-induced genes. *Molecular and Cellular Endocrinology*, 275(1-2):79–97, September 2007. PMID: 17561338.

[16] A. E. Coutinho and K. E. Chapman. The anti-inflammatory and immunosuppressive effects of glucocorticoids, recent developments and mechanistic insights. *Molecular and Cellular Endocrinology*, 335(1):2–13, March 2011. PMID: 20398732.

[17] M. Truss, G. Chalepakis, and M. Beato. Contacts between steroid hormone receptors and thymines in DNA: an interference method. *Proceedings of the National Academy of Sciences of the United States of America*, 87(18):7180–7184, September 1990. PMID: 2169621.

[18] F. Re, M. Muzio, M. De Rossi, N. Polentarutti, J. G. Giri, A. Mantovani, and F. Colotta. The type II ”receptor” as a decoy target for interleukin 1 in polymorphonuclear leukocytes: characterization of induction by dexamethasone and ligand binding properties of the released decoy receptor. *The Journal of Experimental Medicine*, 179(2):739–743, February 1994. PMID: 8294881.

[19] F. Paliogianni, A. Raptis, S. S. Ahuja, S. M. Najjar, and D. T. Boumpas. Negative transcriptional regulation of human interleukin 2 (IL-2) gene by glucocorticoids through interference with nuclear transcription factors AP-1 and NF-AT. *Journal of Clinical Investigation*, 91(4):1481–1489, April 1993. PMID: 8473495.

[20] M. M. Chang, M. Juarez, D. M. Hyde, and R. Wu. Mechanism of dexamethasone-mediated interleukin-8 gene suppression in cultured airway epithelial cells. *American Journal of Physiology. Lung Cellular and Molecular Physiology*, 280(1):L107–115, January 2001. PMID: 11133500.

[21] A. Hafezi-Moghadam, T. Simoncini, Z. Yang, F. P. Limbourg, J.-C. Plumier, M. C. Rebsamen, C.-M. Hsieh, D.-S. Chui, K. L. Thomas, A. J. Prorock, V. E. Laubach, M. A. Moskowitz, B. A. French, K. Ley, and J. K. Liao. Acute cardiovascular protective effects of corticosteroids are mediated by non-transcriptional activation of endothelial nitric oxide synthase. *Nature Medicine*, 8(5):473–479, May 2002. PMID: 11984591.

[22] F. S. Dhabhar and B. S. McEwen. Enhancing versus suppressive effects of stress hormones on skin immune function. *Proceedings of the National Academy of Sciences of the United States of America*, 96(3):1059–1064, February 1999. PMID: 9927693.

[23] N. L. Wendler, R. P. Graber, R. E. Jones, and M. Tishler. Synthesis of 11-hydroxylated cortical steroids. 17(α)-hydroxycorticosterone. *Journal of the American Chemical Society*, 72(12):5793–5794, 1950.

[24] The Empire Rheumatism Council Sub-Committee. Multi-centre controlled trial of cortisone acetate and acetyl salicylic acid in the long-term treatment of rheumatoid arthritis: results of three years' treatment. *Annals of the Rheumatic Diseases*, 16(3):277–289, September 1957. PMID: 13470729.

[25] L. H. Sarett. Some aspects of the evolution of anti-inflammatory steroids. *Annals of the New York Academy of Sciences*, 82(4):802–808, 1959.

[26] H. L. Herzog, C. C. Payne, M. A. Jevnik, D. Gould, E. L. Shapiro, E. P. Oliveto, and E. B. Hershberg. 11-oxygenated steroids. XIII. synthesis and proof of structure of Δ-1,4-pregnadiene-17α,21-diol-3,11,20-trione and Δ-1,4-pregnadiene-11β,17α,21-triol-3,20-dione. *Journal of the American Chemical Society*, 77(18):4781–4784, 1955.

[27] J. J. Bunim, R. L. Black, A. J. Bollet, and M. M. Pechet. Metabolic effects of metacortandralone and metacortandracin. *Annals of the New York Academy of Sciences*, 61(2):358–368, 1955.

[28] Medical Research Council and Nuffield Foundation Report. A comparison of prednisolone with aspirin or other analgesics in the treatment of rheumatoid arthritis. *Annals of the Rheumatic Diseases*, 19(4):331–337, December 1960. PMID: 14447267.

[29] J. Fried, K. Florey, E. F. Sabo, J. E. Herz, A. R. Restivo, A. Borman, and F. M. Singer. Synthesis and biological activity of 1- and 6-dehydro-9α-halocorticoids. *Journal of the American Chemical Society*, 77(15):4181–4182, 1955.

[30] J. Fried and E. F. Sabo. Synthesis of 17α-hydroxycorticosterone and its 9α-halo derivatives from 11-epi-17α-hydroxycorticosterone. *Journal of the American Chemical Society*, 75(9):2273–2274, 1953.

[31] G. E. Arth, D. B. R. Johnston, J. Fried, W. W. Spooncer, D. R. Hoff, and L. H. Sarett. 16-methylated steroids. I. 16α-methylated analogs of cortisone, a new group of anti-inflammatory steroids. *Journal of the American Chemical Society*, 80(12):3160–3161, 1958.

[32] G. E. Arth, J. Fried, D. B. R. Johnston, D. R. Hoff, L. H. Sarett, R. H. Silber, H. C. Stoerk, and C. A. Winter. 16-methylated steroids. II. 16α-methyl analogs of cortisone, a new group of anti-inflammatory steroids. 9α-halo derivatives. *Journal of the American Chemical Society*, 80(12):3161–3163, 1958.

[33] T. F. Frawley, H. Kistler, and T. Shelley. Effects of anti-inflammatory steroids on carbohydrate metabolism, with emphasis on hypo-glycemic and diabetic states. *Annals of the New York Academy of Sciences*, 82(4):868–885, October 1959.

[34] A. W. Meikle and F. H. Tyler. Potency and duration of action of glucocorticoids. Effects of hydrocortisone, prednisone and dexamethasone on human pituitary-adrenal function. *The American Journal of Medicine*,

63(2):200–207, August 1977. PMID: 888843.

[35] R. H. Silber. The biology of anti-inflammatory steroids. *Annals of the New York Academy of Sciences*, 82(4):821–828, October 1959.

[36] C. W. Lin, M. Nakane, M. Stashko, D. Falls, J. Kuk, L. Miller, R. Huang, C. Tyree, J. N. Miner, J. Rosen, P. R. Kym, M. J. Coghlan, G. Carter, and B. C. Lane. trans-Activation and repression properties of the novel nonsteroid glucocorticoid receptor ligand 2,5-dihydro-9-hydroxy-10-methoxy-2,2,4-trimethyl-5-(1-methylcyclohexen-3-y1)-1H-[1]benzopyrano[3,4-f]quinoline (A276575) and its four stereoisomers. *Molecular Pharmacology*, 62(2):297–303, August 2002. PMID: 12130681.

[37] M. G. Belvisi, S. L. Wicks, C. H. Battram, S. E. Bottoms, J. E. Redford, P. Woodman, T. J. Brown, S. E. Webber, and M. L. Foster. Therapeutic benefit of a dissociated glucocorticoid and the relevance of in vitro separation of transrepression from transactivation activity. *Journal of Immunology (Baltimore, Md.: 1950)*, 166(3):1975–1982, February 2001. PMID: 11160246.

[38] M. Baiula, A. Bedini, J. Baldi, M. E. Cavet, P. Govoni, and S. Spampinato. Mapracorat, a selective glucocorticoid receptor agonist, causes apoptosis of eosinophils infiltrating the conjunctiva in late-phase experimental ocular allergy. *Drug Design, Development and Therapy*, 8:745–757, June 2014. PMID: 24959069.

[39] Merck & Co., Inc. Dexamethasone tablets [package insert], 2004.

[40] Pharmacia and Upjohn and Company. Prednisone tablets [package insert], 2007.

[41] West-ward Pharmaceutical Corp. Hydrocortisone tablets [package insert], 2008.

[42] G. Johannsson, A. Falorni, S. Skrtic, H. Lennernäs, M. Quinkler, J. P. Monson, and P. M. Stewart. Adrenal insufficiency: review of clinical outcomes with current glucocorticoid replacement therapy. *Clinical Endocrinology*, 82(1):2–11, January 2015. PMID: 25187037.

[43] B. Brazzini and N. Pimpinelli. New and established topical corticosteroids in dermatology: clinical pharmacology and therapeutic use. *American Journal of Clinical Dermatology*, 3(1):47–58, 2002. PMID: 11817968.

[44] J. B. Christoforidis, S. Chang, A. Jiang, J. Wang, and C. M. Cebulla. Systemic treatment of vitreous inflammation. *Mediators of Inflammation*, 2012, 2012. PMID: 23028205.

[45] D. Gordon and J. McLean. Effects of pituitary adrenocorticotropic hormone (ACTH) therapy in ophthalmologic conditions. *Journal of the American Medical Association*, 142(16):1271–1276, April 1950.

[46] I. Marie. Therapy of polymyositis and dermatomyositis. *Presse Médicale (Paris, France: 1983)*, 40(4 Pt 2):e257–270, April 2011. PMID: 21334847.

[47] O. J. Dempsey, E. W. Paterson, K. M. Kerr, and A. R. Denison. Sarcoidosis. *BMJ (Clinical research ed.)*, 339:b3206, 2009. PMID: 19717499.

[48] M. Crump, J. Kuruvilla, S. Couban, D. A. MacDonald, V. Kukreti, C. T. Kouroukis, M. Rubinger, R. Buckstein, K. R. Imrie, M. Federico, N. Di Renzo, K. Howson-Jan, T. Baetz, L. Kaizer, M. Voralia, H. J. Olney, A. R. Turner, J. Sussman, A. E. Hay, M. S. Djurfeldt, R. M. Meyer, B. E. Chen, and L. E. Shepherd. Randomized comparison of gemcitabine, dexamethasone, and cisplatin versus dexamethasone, cytarabine, and cisplatin chemotherapy before autologous stem-cell transplantation for relapsed and refractory aggressive lymphomas: NCIC-CTG LY.12. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology*, 32(31):3490–3496, November 2014. PMID: 25267740.

[49] C.-H. Pui and W. E. Evans. Treatment of acute lymphoblastic leukemia. *The New England Journal of Medicine*, 354(2):166–178, January 2006. PMID: 16407512.

[50] A. K. Stewart, S. V. Rajkumar, M. A. Dimopoulos, T. Masszi, I. Špička, A. Oriol, R. Hájek, L. Rosiñol, D. S. Siegel, G. G. Mihaylov, V. Goranova-Marinova, P. Rajnics, A. Suvorov, R. Niesvizky, A. J. Jakubowiak, J. F. San-Miguel, H. Ludwig, M. Wang, V. Maisnar, J. Minarik, W. I. Bensinger, M.-V. Mateos, D. Ben-Yehuda, V. Kukreti, N. Zojwalla, M. E. Tonda, X. Yang, B. Xing, P. Moreau, A. Palumbo, and ASPIRE Investigators. Carfilzomib, lenalidomide, and dexamethasone for relapsed multiple myeloma. *The New England Journal of Medicine*, 372(2):142–152, January 2015. PMID: 25482145.

[51] G. E. Keeney, M. P. Gray, A. K. Morrison, M. N. Levas, E. A. Kessler, G. D. Hill, M. H. Gorelick, and J. L. Jackson. Dexamethasone for acute asthma exacerbations in children: a meta-analysis. *Pediatrics*, 133(3):493–499, March 2014. PMID: 24515516.

[52] F. Qureshi, A. Zaritsky, and M. P. Poirier. Comparative efficacy of oral dexamethasone versus oral prednisone in acute pediatric asthma. *The Journal of Pediatrics*, 139(1):20–26, July 2001. PMID: 11445789.

[53] B. Crotty and D. P. Jewell. Drug therapy of ulcerative colitis. *British Journal of Clinical Pharmacology*, 34(3):189–198, September 1992. PMID: 1389944.

[54] W. Rosenberg, A. Ireland, and D. P. Jewell. High-dose methylprednisolone in the treatment of active ulcerative colitis. *Journal of Clinical Gastroenterology*, 12(1):40–41, February 1990. PMID: 2303687.

[55] D. Haack, K. Schärer, A. Asam-Tauscher, and P. Vecsei. Glucocorticoid receptors in idiopathic nephrotic syndrome. *Pediatric Nephrology (Berlin,*

Germany), 13(8):653–656, October 1999. PMID: 10502121.

[56] C. Kotsarini, P. D. Griffiths, I. D. Wilkinson, and N. Hoggard. A systematic review of the literature on the effects of dexamethasone on the brain from in vivo human-based studies: implications for physiological brain imaging of patients with intracranial tumors. *Neurosurgery*, 67(6):1799–1815; discussion 1815, December 2010. PMID: 21107211.

[57] B. D. Levine, K. Yoshimura, T. Kobayashi, M. Fukushima, T. Shibamoto, and G. Ueda. Dexamethasone in the treatment of acute mountain sickness. *The New England Journal of Medicine*, 321(25):1707–1713, December 1989. PMID: 2687688.

[58] P. Alderson and I. Roberts. Corticosteroids for acute traumatic brain injury. *The Cochrane Database of Systematic Reviews*, (1):CD000196, 2005. PMID: 15674869.

[59] J. Lindholm, S. Juul, J. O. Jørgensen, J. Astrup, P. Bjerre, U. Feldt-Rasmussen, C. Hagen, J. Jørgensen, M. Kosteljanetz, L. Kristensen, P. Laurberg, K. Schmidt, and J. Weeke. Incidence and late prognosis of Cushing's syndrome: a population-based study. *The Journal of Clinical Endocrinology and Metabolism*, 86(1):117–123, January 2001. PMID: 11231987.

[60] T. Psaras, M. Milian, V. Hattermann, T. Freiman, B. Gallwitz, and J. Honegger. Demographic factors and the presence of comorbidities do not promote early detection of Cushing's disease and acromegaly. *Experimental and Clinical Endocrinology & Diabetes: Official Journal, German Society of Endocrinology [and] German Diabetes Association*, 119(1):21–25, January 2011. PMID: 20827660.

[61] C. M. Plotz, A. I. Knowlton, and C. Ragan. The natural history of Cushing's syndrome. *The American Journal of Medicine*, 13(5):597–614, November

1952. PMID: 12996538.

[62] E. Valassi, I. Crespo, A. Santos, and S. M. Webb. Clinical consequences of Cushing's syndrome. *Pituitary*, 15(3):319–329, September 2012. PMID: 22527617.

[63] E. Valassi, A. Santos, M. Yaneva, M. Tóth, C. J. Strasburger, P. Chanson, J. A. H. Wass, O. Chabre, M. Pfeifer, R. A. Feelders, S. Tsagarakis, P. J. Trainer, H. Franz, K. Zopf, S. Zacharieva, S. W. J. Lamberts, A. Tabarin, S. M. Webb, and ERCUSYN Study Group. The European Registry on Cushing's syndrome: 2-year experience. Baseline demographic and clinical characteristics. *European Journal of Endocrinology / European Federation of Endocrine Societies*, 165(3):383–392, September 2011. PMID: 21715416.

[64] M. Muller, T. L. Mazzuco, M. Martinie, I. Bachelot, and O. Chabre. Diagnosis of Cushing's syndrome: A retrospective evaluation of clinical practice. *European Journal of Internal Medicine*, 17(5):334–338, August 2006. PMID: 16864008.

[65] J. R. Lindsay, T. Nansel, S. Baid, J. Gumowski, and L. K. Nieman. Long-term impaired quality of life in Cushing's syndrome despite initial improvement after surgical remission. *The Journal of Clinical Endocrinology and Metabolism*, 91(2):447–453, February 2006. PMID: 16278266.

[66] A. L. Skversky, J. Kumar, M. K. Abramowitz, F. J. Kaskel, and M. L. Melamed. Association of glucocorticoid use and low 25-hydroxyvitamin D levels: results from the National Health and Nutrition Examination Survey (NHANES): 2001-2006. *The Journal of Clinical Endocrinology and Metabolism*, 96(12):3838–3845, December 2011. PMID: 21956424.

[67] T. P. van Staa, H. G. Leufkens, L. Abenhaim, B. Begaud, B. Zhang, and

C. Cooper. Use of oral corticosteroids in the United Kingdom. *QJM: Monthly Journal of the Association of Physicians*, 93(2):105–111, February 2000. PMID: 10700481.

[68] World Health Organization. WHO Essential Medicines List, 2013.

[69] S. M. Ravis and W. H. Eaglstein. Topical hydrocortisone from prescription to over-the-counter sale: a past controversy: a cautionary tale. *Archives of Dermatology*, 143(3):413–415, March 2007. PMID: 17372109.

[70] S. B. Adebajo. An epidemiological survey of the use of cosmetic skin lightening cosmetics among traders in Lagos, Nigeria. *West African Journal of Medicine*, 21(1):51–55, March 2002. PMID: 12081345.

[71] A. France-Presse. Ivory Coast bans potentially deadly skin-whitening creams. *The Guardian*, May 2015.

[72] Y. M. Olumide, A. O. Akinkugbe, D. Altraide, T. Mohammed, N. Ahamefule, S. Ayanlowo, C. Onyekonwu, and N. Essen. Complications of chronic use of skin lightening cosmetics. *International Journal of Dermatology*, 47(4):344–353, April 2008. PMID: 18377596.

[73] J. N. Rozen, E. Alseddeeqi, and J. Rivera. Cosmetic agents causing endocrinopathy in an African immigrant. *Canadian Family Physician*, 58(2):169–171, February 2012. PMID: 22337740.

[74] M. M. Foisy, E. M. K. Yakiwchuk, I. Chiu, and A. E. Singh. Adrenal suppression and Cushing's syndrome secondary to an interaction between ritonavir and fluticasone: a review of the literature. *HIV medicine*, 9(6):389–396, July 2008. PMID: 18459946.

[75] M. Mann, E. Koller, A. Murgo, S. Malozowski, J. Bacsanyi, and M. Leinung. Glucocorticoidlike activity of megestrol. A summary of Food and Drug Administration experience and a review of the literature. *Archives of Internal Medicine*, 157(15):1651–1656, August 1997. PMID: 9250225.

[76] J. K. Prague, S. May, and B. C. Whitelaw. Cushing's syndrome. *BMJ (Clinical research ed.)*, 346:f945, 2013. PMID: 23535464.

[77] L. Fardet, A. Flahault, A. Kettaneh, K. P. Tiev, T. Généreau, C. Tolédano, C. Lebbé, and J. Cabane. Corticosteroid-induced clinical adverse events: frequency, risk factors and patient's opinion. *The British Journal of Dermatology*, 157(1):142–148, July 2007. PMID: 17501951.

[78] F. L. Mastaglia. Adverse effects of drugs on muscle. *Drugs*, 24(4):304–321, October 1982. PMID: 6128217.

[79] J. A. Douglass, D. V. Tuxen, M. Horne, C. D. Scheinkestel, M. Weinmann, D. Czarny, and G. Bowes. Myopathy in severe asthma. *The American Review of Respiratory Disease*, 146(2):517–519, August 1992. PMID: 1362636.

[80] T. T. Batchelor, L. P. Taylor, H. T. Thaler, J. B. Posner, and L. M. DeAngelis. Steroid myopathy in cancer patients. *Neurology*, 48(5):1234–1238, May 1997. PMID: 9153449.

[81] S. L. Bowyer, M. P. LaMothe, and J. R. Hollister. Steroid myopathy: incidence and detection in a population with asthma. *The Journal of Allergy and Clinical Immunology*, 76(2 Pt 1):234–242, August 1985. PMID: 4019954.

[82] R. Müller and E. Kugelberg. Myopathy in Cushing's syndrome. *Journal of Neurology, Neurosurgery, and Psychiatry*, 22(4):314–319, November 1959. PMID: 14425173.

[83] I. Rodriguez-Carreno, L. Gila-Useros, and A. Malanda-Trigueros. Motor unit action potential duration: measurement and significance. In I. M. Abud Ajeena, editor, *Advances in Clinical Neurophysiology*. InTech, October 2012.

[84] E. J. Dropcho and S. J. Soong. Steroid-induced weakness in patients with

primary brain tumors. *Neurology*, 41(8):1235–1239, August 1991. PMID: 1866012.

[85] A. N. D'Agostino and M. Chiga. Cortisone myopathy in rabbits. A light and electron microscopic study. *Neurology*, 16(3):257–263, March 1966. PMID: 5948610.

[86] C. J. Vecht, A. Hovestadt, H. B. Verbiest, J. J. van Vliet, and W. L. van Putten. Dose-effect relationship of dexamethasone on Karnofsky performance in metastatic brain tumors: a randomized study of doses of 4, 8, and 16 mg per day. *Neurology*, 44(4):675–680, April 1994. PMID: 8164824.

[87] C. D. Shee. Risk factors for hydrocortisone myopathy in acute severe asthma. *Respiratory Medicine*, 84(3):229–233, May 1990. PMID: 2218007.

[88] E. N. Coomes. Corticosteroid myopathy. *Annals of the Rheumatic Diseases*, 24(5):465–472, September 1965. PMID: 5834228.

[89] P. M. Foye, L. Rispoli, G. E. Hwang, and S. S. Lim. Corticosteroid-induced myopathy, December 2014.

[90] F. F. Horber, J. R. Scheidegger, B. E. Grünig, and F. J. Frey. Thigh muscle mass and function in patients treated with glucocorticoids. *European Journal of Clinical Investigation*, 15(6):302–307, December 1985. PMID: 3938399.

[91] N. A. Pansters, R. C. Langen, E. F. Wouters, and A. M. Schols. Synergistic stimulation of myogenesis by glucocorticoid and IGF-I signaling. *Journal of Applied Physiology (Bethesda, Md.: 1985)*, 114(9):1329–1339, May 2013. PMID: 22936724.

[92] F. F. Horber, H. Hoopeler, J. R. Scheidegger, B. E. Grünig, H. Howald, and F. J. Frey. Impact of physical training on the ultrastructure of midthigh

muscle in normal subjects and in patients treated with glucocorticoids. *Journal of Clinical Investigation*, 79(4):1181–1190, April 1987. PMID: 3558821.

[93] F. F. Horber, J. R. Scheidegger, B. E. Grünig, and F. J. Frey. Evidence that prednisone-induced myopathy is reversed by physical training. *The Journal of Clinical Endocrinology and Metabolism*, 61(1):83–88, July 1985. PMID: 3998075.

[94] B. S. Miller, K. M. Ignatoski, S. Daignault, C. Lindland, P. G. Gauger, G. M. Doherty, S. C. Wang, and University of Michigan Analytical Morphomics Group. A quantitative tool to assess degree of sarcopenia objectively in patients with hypercortisolism. *Surgery*, 150(6):1178–1185, December 2011. PMID: 22136838.

[95] A. A. Khaleeli, R. H. Edwards, K. Gohil, G. McPhail, M. J. Rennie, J. Round, and E. J. Ross. Corticosteroid myopathy: a clinical and pathological study. *Clinical Endocrinology*, 18(2):155–166, February 1983. PMID: 6851197.

[96] M. Rebuffé-Scrive, M. Krotkiewski, J. Elfverson, and P. Björntorp. Muscle and adipose tissue morphology and metabolism in Cushing's syndrome. *The Journal of Clinical Endocrinology and Metabolism*, 67(6):1122–1128, December 1988. PMID: 3142910.

[97] K. S. Topp, P. L. Painter, S. Walcott, J. B. Krasnoff, D. Adey, G. K. Sakkas, J. Taylor, K. McCormick, M. TeNyenhuis, M. Iofina, S. Tomlanovich, and P. Stock. Alterations in skeletal muscle structure are minimized with steroid withdrawal after renal transplantation. *Transplantation*, 76(4):667–673, August 2003. PMID: 12973106.

[98] J. A. Simoneau, S. R. Colberg, F. L. Thaete, and D. E. Kelley. Skeletal muscle glycolytic and oxidative enzyme capacities are determinants of insulin sensitivity and muscle composition in obese women. *FASEB*

journal: official publication of the Federation of American Societies for Experimental Biology, 9(2):273–278, February 1995. PMID: 7781930.

[99] F. Le Grand and M. A. Rudnicki. Skeletal muscle satellite cells and adult myogenesis. *Current opinion in cell biology*, 19(6):628–633, December 2007. PMID: 17996437.

[100] R. G. Ham, J. A. St Clair, C. Webster, and H. M. Blau. Improved media for normal human muscle satellite cells: serum-free clonal growth and enhanced growth with low serum. *In Vitro Cellular & Developmental Biology: Journal of the Tissue Culture Association*, 24(8):833–844, August 1988. PMID: 3045074.

[101] G. T. Griffing. Serum cortisol, March 2014.

[102] R. G. Sprague, M. H. Power, and H. L. Mason. Physiological effects of cortisone and pituitary adrenocorticotropic hormone (ACTH) in man. *Journal of the American Medical Association*, 144(16):1341–1347, December 1950. PMID: 14778744.

[103] P. S. Simmons, J. M. Miles, J. E. Gerich, and M. W. Haymond. Increased proteolysis. An effect of increases in plasma cortisol within the physiologic range. *Journal of Clinical Investigation*, 73(2):412–420, February 1984. PMID: 6365973.

[104] D. J. Brillon, B. Zheng, R. G. Campbell, and D. E. Matthews. Effect of cortisol on energy expenditure and amino acid metabolism in humans. *The American Journal of Physiology*, 268(3 Pt 1):E501–513, March 1995. PMID: 7900796.

[105] S. Ellero-Simatos, E. Szymańska, T. Rullmann, W. H. Dokter, R. Ramaker, R. Berger, T. M. van Iersel, A. K. Smilde, T. Hankemeier, and W. Alkema. Assessing the metabolic effects of prednisolone in healthy volunteers using urine metabolic profiling. *Genome Medicine*, 4(11):94, November 2012.

PMID: 23199229.

[106] M. Elia, A. Carter, S. Bacon, C. G. Winearls, and R. Smith. Clinical usefulness of urinary 3-methylhistidine excretion in indicating muscle protein breakdown. *British Medical Journal (Clinical research ed.)*, 282(6261):351–354, January 1981. PMID: 6780020.

[107] E. Löfberg, A. Gutierrez, J. Wernerman, B. Anderstam, W. E. Mitch, S. R. Price, J. Bergström, and A. Alvestrand. Effects of high doses of glucocorticoids on free amino acids, ribosomes and protein turnover in human muscle. *European Journal of Clinical Investigation*, 32(5):345–353, May 2002. PMID: 12027875.

[108] S. C. Bodine and L. M. Baehr. Skeletal muscle atrophy and the E3 ubiquitin ligases MuRF1 and MAFbx/atrogin-1. *American Journal of Physiology. Endocrinology and Metabolism*, 307(6):E469–484, September 2014. PMID: 25096180.

[109] Millenium Pharmaceuticals, Inc. Velcade (bortezomib) for injection [prescribing information], 2014.

[110] Onyx Pharmaceuticals, Inc. Kyprolis (carfilzomib) for injection [prescribing information], 2012.

[111] P. G. Richardson, P. Sonneveld, M. W. Schuster, D. Irwin, E. A. Stadtmauer, T. Facon, J.-L. Harousseau, D. Ben-Yehuda, S. Lonial, J.-F. San Miguel, J. D. Cavenagh, and K. C. Anderson. Safety and efficacy of bortezomib in high-risk and elderly patients with relapsed multiple myeloma. *British Journal of Haematology*, 137(5):429–435, June 2007. PMID: 17451408.

[112] S. Jagannath, P. G. Richardson, B. Barlogie, J. R. Berenson, S. Singhal, D. Irwin, G. Srkalovic, D. P. Schenkein, D. L. Esseltine, K. C. Anderson, and SUMMIT/CREST Investigators. Bortezomib in combination with dexamethasone for the treatment of patients with relapsed and/or

refractory multiple myeloma with less than optimal response to bortezomib alone. *Haematologica*, 91(7):929–934, July 2006. PMID: 16818280.

[113] R. K. Amaravadi, J. Lippincott-Schwartz, X.-M. Yin, W. A. Weiss, N. Takebe, W. Timmer, R. S. DiPaola, M. T. Lotze, and E. White. Principles and current strategies for targeting autophagy for cancer treatment. *Clinical cancer research : an official journal of the American Association for Cancer Research*, 17(4):654–666, February 2011. PMID: 21325294.

[114] Sanofi Aventis US, LLC. Plaquenil (hydroxychloroquine sulfate) tablets, U.S.P [prescribing information], 2006.

[115] H. S. Lee, B. H. Daniels, E. Salas, A. W. Bollen, J. Debnath, and M. Margeta. Clinical utility of LC3 and p62 immunohistochemistry in diagnosis of drug-induced autophagic vacuolar myopathies: a case-control study. *PLoS ONE*, 7(4), April 2012. PMID: 22558391.

[116] P. S. Ghosh, D. Swift, and A. G. Engel. Teaching NeuroImages: Hydroxychloroquine-induced vacuolar myopathy. *Neurology*, 80(23):e248–e249, June 2013. PMID: 23733558.

[117] A. Nucci, L. S. Queiroz, and A. M. Samara. Chloroquine neuromyopathy. *Clinical Neuropathology*, 15(5):256–258, October 1996. PMID: 8879864.

[118] K. R. Short, J. Nygren, M. L. Bigelow, and K. S. Nair. Effect of short-term prednisone use on blood flow, muscle protein metabolism, and function. *The Journal of Clinical Endocrinology and Metabolism*, 89(12):6198–6207, December 2004. PMID: 15579778.

[119] K. R. Short, M. L. Bigelow, and K. S. Nair. Short-term prednisone use antagonizes insulin's anabolic effect on muscle protein and glucose metabolism in young healthy people. *American Journal of Physiology. Endocrinology and Metabolism*, 297(6):E1260–E1268, December 2009. PMID: 19738036.

[120] R. J. Louard, R. Bhushan, R. A. Gelfand, E. J. Barrett, and R. S. Sherwin. Glucocorticoids antagonize insulin's antiproteolytic action on skeletal muscle in humans. *The Journal of Clinical Endocrinology and Metabolism*, 79(1):278–284, July 1994. PMID: 8027242.

[121] Z. Liu, L. A. Jahn, W. Long, D. A. Fryburg, L. Wei, and E. J. Barrett. Branched chain amino acids activate messenger ribonucleic acid translation regulatory proteins in human skeletal muscle, and glucocorticoids blunt this action. *The Journal of Clinical Endocrinology and Metabolism*, 86(5):2136–2143, May 2001. PMID: 11344218.

[122] D. R. Garrel, P. D. Delmas, C. Welsh, M. J. Arnaud, S. E. Hamilton, and M. M. Pugeat. Effects of moderate physical training on prednisone-induced protein wasting: a study of whole-body and bone protein metabolism. *Metabolism: Clinical and Experimental*, 37(3):257–262, March 1988. PMID: 3257806.

[123] Z. Liu, G. Li, S. R. Kimball, L. A. Jahn, and E. J. Barrett. Glucocorticoids modulate amino acid-induced translation initiation in human skeletal muscle. *American Journal of Physiology. Endocrinology and Metabolism*, 287(2):E275–281, August 2004. PMID: 15039148.

[124] T. T. Amatruda, D. R. Hollingsworth, N. D. D'esopo, G. V. Upton, and P. K. Bondy. A study of the mechanism of the steroid withdrawal syndrome. Evidence for integrity of the hypothalamic-pituitary-adrenal system. *The Journal of Clinical Endocrinology and Metabolism*, 20:339–354, March 1960. PMID: 13793101.

[125] F. Mor, P. Green, and A. J. Wysenbeek. Myopathy in Addison's disease. *Annals of the Rheumatic Diseases*, 46(1):81–83, January 1987. PMID: 3813679.

[126] C.-É. Brown-Séquard. Note on the effects produced on man by

subcutaneous injections of a liquid obtained from the testicles of animals. *The Lancet*, 134(3438):105–107, July 1889.

[127] C.-É. Brown-Séquard. On a new therapeutic method consisting in the use of organic liquids extracted from glands and other organs. *British Medical Journal*, 1(1693):1212–1214, June 1893. PMID: 20754269.

[128] E. Nieschlag and S. Nieschlag. Testosterone deficiency: a historical perspective. *Asian Journal of Andrology*, 16(2):161–168, 2014. PMID: 24435052.

[129] A. A. Berthold. Transplantation der Hoden. *Archiv für Anatomie, Physiologie und Wissenschaftliche Medicin*, pages 42–46, 1849.

[130] C. D. Kochakian and J. R. Murlin. The effect of male hormone on the protein and energy metabolism of castrate dogs. *The Journal of Nutrition*, 10(4):437 –459, October 1935. PMID: 0.

[131] T. F. Gallagher and F. C. Koch. The testicular hormone. *Journal of Biological Chemistry*, 84(2):495–500, November 1929.

[132] L. C. McGee, M. Juhn, and L. V. Domm. The development of secondary sex characters in capons by injections of extracts of bull testes. *American Journal of Physiology – Legacy Content*, 87(2):406–435, December 1928.

[133] K. David, E. Dingemanse, J. Freud, and E. Laqueur. Über krystallinisches männliches Hormon aus Hoden (Testosteron), wirksamer als aus Harn oder aus Cholesterin bereitetes Androsteron. *Hoppe-Seyler's Zeitschrift fur physiologische Chemie*, 233(5-6):281–283, 1935.

[134] J. M. Hoberman and C. E. Yesalis. The history of synthetic testosterone. *Scientific American*, 272(2):76–81, February 1995. PMID: 7817189.

[135] A. T. Kenyon, I. Sandiford, H. A. Bryan, K. Knowlton, and F. C. Koch. The effect of testosterone propionate on nitrogen, electrolyte, water and energy metabolism in eunuchoidism. *Endocrinology*, 23(2):135–153, August 1938.

[136] Auxilium Pharmaceuticals, Inc. Testim (testosterone gel) for topical use [prescribing information], 2014.

[137] Endo Pharmaceuticals Solutions Inc. Delatestryl (testosterone enanthate) injection [prescribing information], 2014.

[138] Unimed. Androgel, Physician's Package Insert, 2004.

[139] A. W. Spence. Testosterone propionate in functional impotence. *British Medical Journal*, 2(4160):411–413, September 1940. PMID: 20783311.

[140] R. Kurzrok and C. P. O'Connell. The inhibition of lactation during the puerperium by testosterone propionate. *Endocrinology*, 23(4):476–478, October 1938.

[141] E. F. E. Black. The use of testosterone propionate in gynæcology. *Canadian Medical Association Journal*, 47(2):124–128, August 1942. PMID: 20322517.

[142] E. C. Reifenstein and F. Albright. The metabolic effects of steroid hormones in osteoporosis. *Journal of Clinical Investigation*, 26:24–56, January 1947. PMID: 21424243.

[143] M. Molitch. The treatment of acne vulgaris with testosterone propionate. *Endocrinology*, 23(6):803–804, December 1938.

[144] E. Mommers, W. M. Kersemaekers, J. Elliesen, M. Kepers, D. Apter, H. M. Behre, J. Beynon, P. M. Bouloux, A. Costantino, H.-P. Gerbershagen, L. Grønlund, D. Heger-Mahn, I. Huhtaniemi, E. L. Koldewijn, C. Lange, S. Lindenberg, M. C. Meriggiola, E. Meuleman, P. F. A. Mulders, E. Nieschlag, A. Perheentupa, A. Solomon, L. Väisälä, F. C. Wu, and M. Zitzmann. Male hormonal contraception: a double-blind, placebo-controlled study. *The Journal of Clinical Endocrinology and Metabolism*, 93(7):2572–2580, July 2008. PMID: 18413423.

[145] L. W. Kinsell, S. Hertz, and E. C. Reifenstein. The effect of testosterone

compounds upon the nitrogen balance and creatine excretion in patients with thyrotoxicosis. *Journal of Clinical Investigation*, 23(6):880–890, November 1944. PMID: 16695173.

[146] F. H. Hesser, O. R. Langworthy, and S. A. Vest. Muscle strength in myotonia atrophica (dystrophia myotonica) improved by testosterone propionate. *Endocrinology*, 26(2):241–243, February 1940.

[147] A. T. Kenyon, K. Knowlton, I. Sandiford, F. C. Koch, and G. Lotwin. A comparative study of the metabolic effects of testosterone propionate in normal men and women and in eunuchoidism. *Endocrinology*, 26(1):26–45, January 1940.

[148] L. T. Samuels, A. F. Henschel, and A. Keys. Influence of methyl testosterone on muscular work and creatine metabolism in normal young men. *The Journal of Clinical Endocrinology & Metabolism*, 2(11):649–654, November 1942.

[149] J. D. Elashoff, A. D. Jacknow, S. G. Shain, and G. D. Braunstein. Effects of anabolic-androgenic steroids on muscular strength. *Annals of Internal Medicine*, 115(5):387–393, September 1991. PMID: 1830732.

[150] V. Cowart. Steroids in sports: after four decades, time to return these genies to bottle? *JAMA*, 257(4):421–423, 427, January 1987. PMID: 3795420.

[151] F. Albright, W. Parson, and E. Bloomberg. Cushing's syndrome interpreted as hyperadrenocorticism leading to hypergluconeogenesis: results of treatment with testosterone propionate. *The Journal of Clinical Endocrinology & Metabolism*, 1(5):375–384, May 1941.

[152] E. H. Keutmann and H. A. Friedman. Metabolic studies in Cushing's syndrome; treatment with various androgens and a 6-year follow-up. *The American Journal of Medicine*, 5(4):518–531, October 1948. PMID: 18886577.

[153] A. Colao, S. Petersenn, J. Newell-Price, J. W. Findling, F. Gu, M. Maldonado, U. Schoenherr, D. Mills, L. R. Salgado, B. M. K. Biller, and Pasireotide B2305 Study Group. A 12-month phase 3 study of pasireotide in Cushing's disease. *The New England Journal of Medicine*, 366(10):914–924, March 2012. PMID: 22397653.

[154] P. Komanicky, R. F. Spark, and J. C. Melby. Treatment of Cushing's syndrome with trilostane (WIN 24,540), an inhibitor of adrenal steroid biosynthesis. *The Journal of Clinical Endocrinology and Metabolism*, 47(5):1042–1051, November 1978. PMID: 233687.

[155] L. K. Nieman, G. P. Chrousos, C. Kellner, I. M. Spitz, B. C. Nisula, G. B. Cutler, G. R. Merriam, C. W. Bardin, and D. L. Loriaux. Successful treatment of Cushing's syndrome with the glucocorticoid antagonist RU 486. *The Journal of Clinical Endocrinology and Metabolism*, 61(3):536–540, September 1985. PMID: 2991327.

[156] R. K. Bergner and A. Bergner. Rational asthma therapy for the outpatient. *JAMA*, 235(3):288–293, January 1976. PMID: 811824.

[157] O. Ragnarsson, M. G. Burt, K. K. Y. Ho, and G. Johannsson. Effect of short-term GH and testosterone administration on body composition and glucose homoeostasis in men receiving chronic glucocorticoid therapy. *European Journal of Endocrinology / European Federation of Endocrine Societies*, 168(2):243–251, February 2013. PMID: 23169695.

[158] B. A. L. Crawford, P. Y. Liu, M. T. Kean, J. F. Bleasel, and D. J. Handelsman. Randomized placebo-controlled trial of androgen effects on muscle and bone in men requiring long-term systemic glucocorticoid treatment. *The Journal of Clinical Endocrinology and Metabolism*, 88(7):3167–3176, July 2003. PMID: 12843161.

[159] N. M. van Schoor, D. L. Knol, C. a. W. Glas, R. W. J. G. Ostelo, A. Leplège,

C. Cooper, O. Johnell, and P. Lips. Development of the Qualeffo-31, an osteoporosis-specific quality-of-life questionnaire. *Osteoporosis international: a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA*, 17(4):543–551, 2006. PMID: 16362146.

[160] F. Pecori Giraldi, M. Moro, F. Cavagnini, and Study Group on the Hypothalamo-Pituitary-Adrenal Axis of the Italian Society of Endocrinology. Gender-related differences in the presentation and course of Cushing's disease. *The Journal of Clinical Endocrinology and Metabolism*, 88(4):1554–1558, April 2003. PMID: 12679438.

[161] S. Volpato, L. Bianchi, A. Cherubini, F. Landi, M. Maggio, E. Savino, S. Bandinelli, G. P. Ceda, J. M. Guralnik, G. Zuliani, and L. Ferrucci. Prevalence and clinical correlates of sarcopenia in community-dwelling older people: application of the EWGSOP definition and diagnostic algorithm. *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences*, 69(4):438–446, April 2014. PMID: 24085400.

[162] E. Ramos, W. R. Frontera, A. Llopart, and D. Feliciano. Muscle strength and hormonal levels in adolescents: gender related differences. *International Journal of Sports Medicine*, 19(8):526–531, November 1998. PMID: 9877143.

[163] D. C. Cumming, M. E. Quigley, and S. S. Yen. Acute suppression of circulating testosterone levels by cortisol in men. *The Journal of Clinical Endocrinology and Metabolism*, 57(3):671–673, September 1983. PMID: 6348068.

[164] M. Fassnacht, N. Schlenz, S. B. Schneider, S. A. Wudy, B. Allolio, and W. Arlt. Beyond adrenal and ovarian androgen generation: Increased peripheral 5 alpha-reductase activity in women with polycystic ovary

syndrome. *The Journal of Clinical Endocrinology and Metabolism*, 88(6):2760–2766, June 2003. PMID: 12788885.

[165] A. Kamischke, D. E. Kemper, M. A. Castel, M. Lüthke, C. Rolf, H. M. Behre, H. Magnussen, and E. Nieschlag. Testosterone levels in men with chronic obstructive pulmonary disease with or without glucocorticoid therapy. *The European Respiratory Journal: Official Journal of the European Society for Clinical Respiratory Physiology*, 11(1):41–45, January 1998. PMID: 9543268.

[166] V. Cortés-Gallegos, A. J. Gallegos, N. B. Tovar, C. Cervantes, and A. Parra. Effect of paramethasone acetate on ovarian steroids and gonadotropins. I. Normal menstrual cycle. *The Journal of Clinical Endocrinology and Metabolism*, 41(2):215–220, August 1975. PMID: 1159039.

[167] J. M. Kuhn, D. Gay, J. P. Lemercier, M. Pugeat, A. Legrand, and L. M. Wolf. Fonction testiculaire au cours de la corticothérapie prolongée. *Presse Médicale (Paris, France: 1983)*, 15(12):559–562, March 1986. PMID: 2939405.

[168] G. Schaison, F. Durand, and I. Mowszowicz. Study of plasma androstenedione and testosterone levels in hypercorticism syndromes. *Annales D'endocrinologie*, 40(1):51–52, February 1979. PMID: 443735.

[169] R. Yehuda, J. A. Golier, S. L. Halligan, M. Meaney, and L. M. Bierer. The ACTH response to dexamethasone in PTSD. *The American Journal of Psychiatry*, 161(8):1397–1403, August 2004. PMID: 15285965.

[170] L. Barbetta, C. Dall'Asta, T. Re, P. Colombo, P. Travaglini, and B. Ambrosi. Androgen secretion in ectopic ACTH syndrome and in Cushing's disease: modifications before and after surgery. *Hormone and Metabolic Research*, 33(10):596–601, October 2001. PMID: 11607879.

[171] S. K. Cunningham and T. J. McKenna. Dissociation of adrenal androgen

and cortisol secretion in Cushing's syndrome. *Clinical Endocrinology*, 41(6):795–800, December 1994. PMID: 7889616.

[172] H. F. Martens, P. K. Sheets, J. S. Tenover, C. E. Dugowson, W. J. Bremner, and G. Starkebaum. Decreased testosterone levels in men with rheumatoid arthritis: effect of low dose prednisone therapy. *The Journal of Rheumatology*, 21(8):1427–1431, August 1994. PMID: 7983641.

[173] J. P. Luton, P. Thieblot, J. C. Valcke, J. A. Mahoudeau, and H. Bricaire. Reversible gonadotropin deficiency in male Cushing's disease. *The Journal of Clinical Endocrinology and Metabolism*, 45(3):488–495, September 1977. PMID: 198424.

[174] G. Boccuzzi, A. Angeli, D. Bisbocci, D. Fonzo, G. P. Giadano, and F. Ceresa. Effect of synthetic luteinizing hormone releasing hormone (LH-RH) on the release of gonadotropins in Cushing's disease. *The Journal of Clinical Endocrinology and Metabolism*, 40(5):892–895, May 1975. PMID: 1092712.

[175] J. Lado-Abeal, J. Rodriguez-Arnao, J. D. Newell-Price, L. A. Perry, A. B. Grossman, G. M. Besser, and P. J. Trainer. Menstrual abnormalities in women with Cushing's disease are correlated with hypercortisolemia rather than raised circulating androgen levels. *The Journal of Clinical Endocrinology and Metabolism*, 83(9):3083–3088, September 1998. PMID: 9745407.

[176] I. R. Reid, H. K. Ibbertson, J. T. France, and J. Pybus. Plasma testosterone concentrations in asthmatic men treated with glucocorticoids. *British Medical Journal (Clinical research ed.)*, 291(6495):574, August 1985. PMID: 2931151.

[177] N. Baume, Y. O. Schumacher, P.-E. Sottas, C. Bagutti, M. Cauderay, P. Mangin, and M. Saugy. Effect of multiple oral doses of androgenic

anabolic steroids on endurance performance and serum indices of physical stress in healthy male subjects. *European Journal of Applied Physiology*, 98(4):329–340, November 2006. PMID: 16896724.

[178] F. Chen, K. Knecht, E. Birzin, J. Fisher, H. Wilkinson, M. Mojena, C. T. Moreno, A. Schmidt, S.-i. Harada, L. P. Freedman, and A. A. Reszka. Direct agonist/antagonist functions of dehydroepiandrosterone. *Endocrinology*, 146(11):4568–4576, November 2005. PMID: 15994348.

[179] T. Jones, M. Groom, and K. Griffiths. Steroid biosynthesis by cultures of normal human adrenal tissue. *Biochemical and Biophysical Research Communications*, 38(2):355–361, January 1970. PMID: 4313933.

[180] J. L. Vaitukaitis, S. L. Dale, and J. C. Melby. Role of ACTH in the secretion of free dehydroepiandrosterone and its sulfate ester in man. *The Journal of Clinical Endocrinology and Metabolism*, 29(11):1443–1447, November 1969. PMID: 4310735.

[181] R. Kouyama, K. Hiraishi, T. Sugiyama, H. Izumiyama, T. Yoshimoto, T. Akashi, K. Kihara, K. Homma, H. Shibata, and Y. Hirata. Clinicopathological features, biochemical and molecular markers in 5 patients with adrenocortical carcinoma. *Endocrine Journal*, 58(7):527–534, 2011. PMID: 21521927.

[182] P. Monteleone, M. Luisi, V. Martiadis, C. Serritella, N. Longobardi, E. Casarosa, A. R. Genazzani, and M. Maj. Impaired reduction of enhanced levels of dehydroepiandrosterone by oral dexamethasone in anorexia nervosa. *Psychoneuroendocrinology*, 31(4):537–542, May 2006. PMID: 16229957.

[183] T. Yamaji, M. Ishibashi, H. Sekihara, A. Itabashi, and T. Yanaihara. Serum dehydroepiandrosterone sulfate in Cushing's syndrome. *The Journal of Clinical Endocrinology and Metabolism*, 59(6):1164–1168, December

1984. PMID: 6238041.

[184] H. L. Judd, G. E. Judd, W. E. Lucas, and S. S. Yen. Endocrine function of the postmenopausal ovary: concentration of androgens and estrogens in ovarian and peripheral vein blood. *The Journal of Clinical Endocrinology and Metabolism*, 39(6):1020–1024, December 1974. PMID: 4430702.

[185] H. G. Burger. Androgen production in women. *Fertility and Sterility*, 77 Suppl 4:S3–5, April 2002. PMID: 12007895.

[186] K. K. Miller, G. Sesmilo, A. Schiller, D. Schoenfeld, S. Burton, and A. Klibanski. Androgen deficiency in women with hypopituitarism. *The Journal of Clinical Endocrinology and Metabolism*, 86(2):561–567, February 2001. PMID: 11158009.

[187] S. S. C. Yen and C. C. Tsai. The effect of ovariectomy on gonadotropin release. *Journal of Clinical Investigation*, 50(5):1149–1153, May 1971. PMID: 5552412.

[188] B. Couzinet, G. Thomas, J. C. Thalabard, S. Brailly, and G. Schaison. Effects of a pure antiandrogen on gonadotropin secretion in normal women and in polycystic ovarian disease. *Fertility and Sterility*, 52(1):42–50, July 1989. PMID: 2744186.

[189] M. Y. Abdel-Rahman. Androgen Excess, April 2014.

[190] A. Salonia, M. Pontillo, R. E. Nappi, G. Zanni, F. Fabbri, M. Scavini, R. Daverio, A. Gallina, P. Rigatti, E. Bosi, P. A. Bonini, and F. Montorsi. Menstrual cycle-related changes in circulating androgens in healthy women with self-reported normal sexual function. *The Journal of Sexual Medicine*, 5(4):854–863, April 2008. PMID: 18371044.

[191] R. Guerrero, T. Aso, P. F. Brenner, Z. Cekan, B. M. Landgren, K. Hagenfeldt, and E. Diczfalusy. Studies on the pattern of circulating steroids in the normal menstrual cycle. I. Simultaneous assays of

progesterone, pregnenolone, dehydroepiandrosterone, testosterone, dihydrotestosterone, androstenedione, oestradiol and oestrone. *Acta Endocrinologica*, 81(1):133–149, January 1976. PMID: 128969.

[192] B. L. Wajchenberg, A. Bosco, M. M. Marone, S. Levin, M. Rocha, A. C. Lerário, M. Nery, J. Goldman, and B. Liberman. Estimation of body fat and lean tissue distribution by dual energy X-ray absorptiometry and abdominal body fat evaluation by computed tomography in Cushing's disease. *The Journal of Clinical Endocrinology and Metabolism*, 80(9):2791–2794, September 1995. PMID: 7673425.

[193] H. Vierhapper, P. Nowotny, and W. Waldhäusl. Production rates of testosterone in patients with Cushing's syndrome. *Metabolism: Clinical and Experimental*, 49(2):229–231, February 2000. PMID: 10690950.

[194] M. Luisi, F. Franchi, D. Drafta, and E. Stroe. Plasma steroid dynamics in Cushing's syndrome. *Annales D'endocrinologie*, 39(2):107–115, 1978. PMID: 686649.

[195] A. G. Smals, P. W. Kloppenborg, and T. J. Benraad. Plasma testosterone profiles in Cushing's syndrome. *The Journal of Clinical Endocrinology and Metabolism*, 45(2):240–245, August 1977. PMID: 195973.

[196] B. P. Hauffa, S. L. Kaplan, and M. M. Grumbach. Dissociation between plasma adrenal androgens and cortisol in Cushing's disease and ectopic ACTH-producing tumour: relation to adrenarche. *Lancet*, 1(8391):1373–1376, June 1984. PMID: 6145833.

[197] B. L. Wajchenberg, M. A. Albergaria Pereira, B. B. Medonca, A. C. Latronico, P. Campos Carneiro, V. A. Alves, M. C. Zerbini, B. Liberman, G. Carlos Gomes, and M. A. Kirschner. Adrenocortical carcinoma: clinical and laboratory observations. *Cancer*, 88(4):711–736, February 2000. PMID: 10679640.

[198] F. H. Tyler and C. D. West. Laboratory evaluation of disorders of the adrenal cortex. *The American Journal of Medicine*, 53(5):664–672, November 1972. PMID: 4342889.

[199] V. K. Arora, E. Schenkein, R. Murali, S. K. Subudhi, J. Wongvipat, M. D. Balbas, N. Shah, L. Cai, E. Efstathiou, C. Logothetis, D. Zheng, and C. L. Sawyers. Glucocorticoid receptor confers resistance to antiandrogens by bypassing androgen receptor blockade. *Cell*, 155(6):1309–1322, December 2013. PMID: 24315100.

[200] W. J. Inder, C. Jang, V. R. Obeyesekere, and F. P. Alford. Dexamethasone administration inhibits skeletal muscle expression of the androgen receptor and IGF-1–implications for steroid-induced myopathy. *Clinical Endocrinology*, 73(1):126–132, July 2010. PMID: 19681914.

[201] H. Vierhapper, P. Nowotny, and W. Waldhäusl. Production rates of cortisol in men with hypogonadism. *Metabolism: Clinical and Experimental*, 53(9):1174–1176, September 2004. PMID: 15334380.

[202] S. Bhasin, T. W. Storer, N. Berman, K. E. Yarasheski, B. Clevenger, J. Phillips, W. P. Lee, T. J. Bunnell, and R. Casaburi. Testosterone replacement increases fat-free mass and muscle size in hypogonadal men. *The Journal of Clinical Endocrinology and Metabolism*, 82(2):407–413, February 1997. PMID: 9024227.

[203] I. G. Brodsky, P. Balagopal, and K. S. Nair. Effects of testosterone replacement on muscle mass and muscle protein synthesis in hypogonadal men–a clinical research center study. *The Journal of Clinical Endocrinology and Metabolism*, 81(10):3469–3475, October 1996. PMID: 8855787.

[204] M. R. Smith, J. S. Finkelstein, F. J. McGovern, A. L. Zietman, M. A. Fallon, D. A. Schoenfeld, and P. W. Kantoff. Changes in body composition during

androgen deprivation therapy for prostate cancer. *The Journal of Clinical Endocrinology and Metabolism*, 87(2):599–603, February 2002. PMID: 11836291.

[205] R. S. Boxer, A. M. Kenny, R. Dowsett, and P. Taxel. The effect of 6 months of androgen deprivation therapy on muscle and fat mass in older men with localized prostate cancer. *The Aging Male: The Official Journal of the International Society for the Study of the Aging Male*, 8(3-4):207–212, December 2005. PMID: 16390748.

[206] N. Mauras, V. Hayes, S. Welch, A. Rini, K. Helgeson, M. Dokler, J. D. Veldhuis, and R. J. Urban. Testosterone deficiency in young men: marked alterations in whole body protein kinetics, strength, and adiposity. *The Journal of Clinical Endocrinology and Metabolism*, 83(6):1886–1892, June 1998. PMID: 9626114.

[207] J. S. Finkelstein, H. Lee, S.-A. M. Burnett-Bowie, J. C. Pallais, E. W. Yu, L. F. Borges, B. F. Jones, C. V. Barry, K. E. Wulczyn, B. J. Thomas, and B. Z. Leder. Gonadal steroids and body composition, strength, and sexual function in men. *The New England journal of medicine*, 369(11):1011–1022, September 2013. PMID: 24024838.

[208] I. H. Rosenberg. Summary comments. *The American Journal of Clinical Nutrition*, 50(5):1231–1233, November 1989.

[209] H. A. Feldman, C. Longcope, C. A. Derby, C. B. Johannes, A. B. Araujo, A. D. Coviello, W. J. Bremner, and J. B. McKinlay. Age trends in the level of serum testosterone and other hormones in middle-aged men: longitudinal results from the Massachusetts male aging study. *The Journal of Clinical Endocrinology and Metabolism*, 87(2):589–598, February 2002. PMID: 11836290.

[210] J. E. Morley, H. M. Perry, F. E. Kaiser, D. Kraenzle, J. Jensen, K. Houston,

M. Mattammal, and H. M. Perry. Effects of testosterone replacement therapy in old hypogonadal males: a preliminary study. *Journal of the American Geriatrics Society*, 41(2):149–152, February 1993. PMID: 8426037.

[211] R. Sih, J. E. Morley, F. E. Kaiser, H. M. Perry, P. Patrick, and C. Ross. Testosterone replacement in older hypogonadal men: a 12-month randomized controlled trial. *The Journal of Clinical Endocrinology and Metabolism*, 82(6):1661–1667, June 1997. PMID: 9177359.

[212] I. Sinha-Hikim, M. Cornford, H. Gaytan, M. L. Lee, and S. Bhasin. Effects of testosterone supplementation on skeletal muscle fiber hypertrophy and satellite cells in community-dwelling older men. *The Journal of Clinical Endocrinology and Metabolism*, 91(8):3024–3033, August 2006. PMID: 16705073.

[213] A. A. Ferrando, M. Sheffield-Moore, C. W. Yeckel, C. Gilkison, J. Jiang, A. Achacosa, S. A. Lieberman, K. Tipton, R. R. Wolfe, and R. J. Urban. Testosterone administration to older men improves muscle function: molecular and physiological mechanisms. *American Journal of Physiology. Endocrinology and Metabolism*, 282(3):E601–607, March 2002. PMID: 11832363.

[214] M. Sheffield-Moore, E. L. Dillon, S. L. Casperson, C. R. Gilkison, D. Paddon-Jones, W. J. Durham, J. J. Grady, and R. J. Urban. A randomized pilot study of monthly cycled testosterone replacement or continuous testosterone replacement versus placebo in older men. *The Journal of Clinical Endocrinology and Metabolism*, 96(11):E1831–E1837, November 2011. PMID: 21865352.

[215] S. Bhasin, E. J. He, M. Kawakubo, E. T. Schroeder, K. Yarasheski, G. J. Opiteck, A. Reicin, F. Chen, R. Lam, J. A. Tsou, C. Castaneda-Sceppa, E. F.

Binder, S. P. Azen, and F. R. Sattler. N-terminal propeptide of type III procollagen as a biomarker of anabolic response to recombinant human GH and testosterone. *The Journal of Clinical Endocrinology and Metabolism*, 94(11):4224–4233, November 2009. PMID: 19837911.

[216] A. A. Ferrando, M. Sheffield-Moore, S. E. Wolf, D. N. Herndon, and R. R. Wolfe. Testosterone administration in severe burns ameliorates muscle catabolism. *Critical Care Medicine*, 29(10):1936–1942, October 2001. PMID: 11588456.

[217] X. Huang, M. R. Blackman, K. Herreman, K. M. Pabst, S. M. Harman, and B. Caballero. Effects of growth hormone and/or sex steroid administration on whole-body protein turnover in healthy aged women and men. *Metabolism: Clinical and Experimental*, 54(9):1162–1167, September 2005. PMID: 16125527.

[218] S. E. Borst, J. F. Yarrow, C. F. Conover, U. Nseyo, J. R. Meuleman, J. A. Lipinska, R. W. Braith, D. T. Beck, J. S. Martin, M. Morrow, S. Roessner, L. A. Beggs, S. C. McCoy, D. F. Cannady, and J. J. Shuster. Musculoskeletal and prostate effects of combined testosterone and finasteride administration in older hypogonadal men: a randomized, controlled trial. *American Journal of Physiology. Endocrinology and Metabolism*, 306(4):E433–442, February 2014. PMID: 24326421.

[219] S. Bhasin, T. G. Travison, T. W. Storer, K. Lakshman, M. Kaushik, N. A. Mazer, A.-H. Ngyuen, M. N. Davda, H. Jara, A. Aakil, S. Anderson, P. E. Knapp, S. Hanka, N. Mohammed, P. Daou, R. Miciek, J. Ulloor, A. Zhang, B. Brooks, K. Orwoll, L. Hede-Brierley, R. Eder, A. Elmi, G. Bhasin, L. Collins, R. Singh, and S. Basaria. Effect of testosterone supplementation with and without a dual 5α-reductase inhibitor on fat-free mass in men with suppressed testosterone production: a randomized controlled trial. *JAMA: the journal of the American Medical Association*, 307(9):931–939,

March 2012. PMID: 22396515.

[220] A. M. Traish, R. J. Feeley, and A. T. Guay. Testosterone therapy in women with gynecological and sexual disorders: a triumph of clinical endocrinology from 1938 to 2008. *The Journal of Sexual Medicine*, 6(2):334–351, February 2009. PMID: 19138368.

[221] M. E. Wierman, W. Arlt, R. Basson, S. R. Davis, K. K. Miller, M. H. Murad, W. Rosner, and N. Santoro. Androgen therapy in women: a reappraisal: an Endocrine Society clinical practice guideline. *The Journal of Clinical Endocrinology and Metabolism*, 99(10):3489–3510, October 2014. PMID: 25279570.

[222] T. S. Croxson, W. E. Chapman, L. K. Miller, C. D. Levit, R. Senie, and B. Zumoff. Changes in the hypothalamic-pituitary-gonadal axis in human immunodeficiency virus-infected homosexual men. *The Journal of Clinical Endocrinology and Metabolism*, 68(2):317–321, February 1989. PMID: 2493026.

[223] S. Grinspoon, C. Corcoran, H. Askari, D. Schoenfeld, L. Wolf, B. Burrows, M. Walsh, D. Hayden, K. Parlman, E. Anderson, N. Basgoz, and A. Klibanski. Effects of androgen administration in men with the AIDS wasting syndrome. A randomized, double-blind, placebo-controlled trial. *Annals of Internal Medicine*, 129(1):18–26, July 1998. PMID: 9652995.

[224] M. Montano, J. N. Flanagan, L. Jiang, P. Sebastiani, M. Rarick, N. K. LeBrasseur, C. A. Morris, R. Jasuja, and S. Bhasin. Transcriptional profiling of testosterone-regulated genes in the skeletal muscle of human immunodeficiency virus-infected men experiencing weight loss. *The Journal of Clinical Endocrinology and Metabolism*, 92(7):2793–2802, July 2007. PMID: 17440010.

[225] A. C. McPherron, A. M. Lawler, and S. J. Lee. Regulation of skeletal muscle

mass in mice by a new TGF-beta superfamily member. *Nature*, 387(6628):83–90, May 1997. PMID: 9139826.

[226] N. F. Gonzalez-Cadavid, W. E. Taylor, K. Yarasheski, I. Sinha-Hikim, K. Ma, S. Ezzat, R. Shen, R. Lalani, S. Asa, M. Mamita, G. Nair, S. Arver, and S. Bhasin. Organization of the human myostatin gene and expression in healthy men and HIV-infected men with muscle wasting. *Proceedings of the National Academy of Sciences of the United States of America*, 95(25):14938–14943, December 1998. PMID: 9843994.

[227] S. C. Bodine, E. Latres, S. Baumhueter, V. K. Lai, L. Nunez, B. A. Clarke, W. T. Poueymirou, F. J. Panaro, E. Na, K. Dharmarajan, Z. Q. Pan, D. M. Valenzuela, T. M. DeChiara, T. N. Stitt, G. D. Yancopoulos, and D. J. Glass. Identification of ubiquitin ligases required for skeletal muscle atrophy. *Science (New York, N.Y.)*, 294(5547):1704–1708, November 2001. PMID: 11679633.

[228] T. Gustafsson, T. Osterlund, J. N. Flanagan, F. von Waldén, T. A. Trappe, R. M. Linnehan, and P. A. Tesch. Effects of 3 days unloading on molecular regulators of muscle size in humans. *Journal of Applied Physiology (Bethesda, Md.: 1985)*, 109(3):721–727, September 2010. PMID: 20538844.

[229] J. H. Macdonald, S. M. Marcora, M. M. Jibani, M. J. Kumwenda, W. Ahmed, and A. B. Lemmey. Nandrolone decanoate as anabolic therapy in chronic kidney disease: a randomized phase II dose-finding study. *Nephron. Clinical Practice*, 106(3):c125–135, 2007. PMID: 17522475.

[230] A. Yurci, M. Yucesoy, K. Unluhizarci, E. Torun, S. Gursoy, M. Baskol, K. Guven, and O. Ozbakir. Effects of testosterone gel treatment in hypogonadal men with liver cirrhosis. *Clinics and Research in Hepatology and Gastroenterology*, 35(12):845–854, December 2011. PMID: 22074639.

[231] R. Casaburi, S. Bhasin, L. Cosentino, J. Porszasz, A. Somfay, M. I. Lewis, M. Fournier, and T. W. Storer. Effects of testosterone and resistance training in men with chronic obstructive pulmonary disease. *American Journal of Respiratory and Critical Care Medicine*, 170(8):870–878, October 2004. PMID: 15271690.

[232] M. I. Lewis, M. Fournier, T. W. Storer, S. Bhasin, J. Porszasz, S.-G. Ren, X. Da, and R. Casaburi. Skeletal muscle adaptations to testosterone and resistance training in men with COPD. *Journal of Applied Physiology (Bethesda, Md.: 1985)*, 103(4):1299–1310, October 2007. PMID: 17673568.

[233] V. Agapitou, S. Dimopoulos, C. Kapelios, E. Karatzanos, C. Manetos, A. Georgantas, A. Ntalianis, J. Terrovitis, H. Karga, and S. Nanas. Hormonal imbalance in relation to exercise intolerance and ventilatory inefficiency in chronic heart failure. *The Journal of Heart and Lung Transplantation: The Official Publication of the International Society for Heart Transplantation*, 32(4):431–436, April 2013. PMID: 23402947.

[234] A. Mirdamadi, M. Garakyaraghi, A. Pourmoghaddas, A. Bahmani, H. Mahmoudi, and M. Gharipour. Beneficial effects of testosterone therapy on functional capacity, cardiovascular parameters, and quality of life in patients with congestive heart failure. *BioMed Research International*, 2014, 2014. PMID: 25110677.

[235] M. Stout, G. A. Tew, H. Doll, I. Zwierska, N. Woodroofe, K. S. Channer, and J. M. Saxton. Testosterone therapy during exercise rehabilitation in male patients with chronic heart failure who have low testosterone status: a double-blind randomized controlled feasibility study. *American Heart Journal*, 164(6):893–901, December 2012. PMID: 23194490.

[236] L. Xu, G. Freeman, B. J. Cowling, and C. M. Schooling. Testosterone therapy and cardiovascular events among men: a systematic review and

meta-analysis of placebo-controlled randomized trials. *BMC Medicine*, 11:108, April 2013. PMID: 23597181.

[237] S. Basaria, A. D. Coviello, T. G. Travison, T. W. Storer, W. R. Farwell, A. M. Jette, R. Eder, S. Tennstedt, J. Ulloor, A. Zhang, K. Choong, K. M. Lakshman, N. A. Mazer, R. Miciek, J. Krasnoff, A. Elmi, P. E. Knapp, B. Brooks, E. Appleman, S. Aggarwal, G. Bhasin, L. Hede-Brierley, A. Bhatia, L. Collins, N. LeBrasseur, L. D. Fiore, and S. Bhasin. Adverse events associated with testosterone administration. *The New England Journal of Medicine*, 363(2):109–122, July 2010. PMID: 20592293.

[238] Z. Wang, S. Zhu, J. Wang, R. N. Pierson, and S. B. Heymsfield. Whole-body skeletal muscle mass: development and validation of total-body potassium prediction models. *The American Journal of Clinical Nutrition*, 77(1):76–82, January 2003. PMID: 12499326.

[239] V. Santilli, A. Bernetti, M. Mangone, and M. Paoloni. Clinical definition of sarcopenia. *Clinical Cases in Mineral and Bone Metabolism*, 11(3):177–180, 2014. PMID: 25568649.

[240] A. L. Mescher and L. C. U. Junqueira. *Junqueira's basic histology: text and atlas*. 2013.

[241] N. Light and A. E. Champion. Characterization of muscle epimysium, perimysium and endomysium collagens. *The Biochemical journal*, 219(3):1017–1026, May 1984. PMID: 6743238.

[242] K. H. Eggen, A. Malmstrøm, and S. O. Kolset. Decorin and a large dermatan sulfate proteoglycan in bovine striated muscle. *Biochimica Et Biophysica Acta*, 1204(2):287–297, February 1994. PMID: 8142471.

[243] M. D. Grounds, L. Sorokin, and J. White. Strength at the extracellular matrix-muscle interface. *Scandinavian journal of medicine & science in sports*, 15(6):381–391, December 2005. PMID: 16293150.

[244] C. J. Mann, E. Perdiguero, Y. Kharraz, S. Aguilar, P. Pessina, A. L. Serrano, and P. Muñoz Cánoves. Aberrant repair and fibrosis development in skeletal muscle. *Skeletal Muscle*, 1:21, May 2011. PMID: 21798099.

[245] J. Scharner and P. S. Zammit. The muscle satellite cell at 50: the formative years. *Skeletal Muscle*, 1(1):28, 2011. PMID: 21849021.

[246] W. E. Blanco-Bose, C. C. Yao, R. H. Kramer, and H. M. Blau. Purification of mouse primary myoblasts based on alpha 7 integrin expression. *Experimental Cell Research*, 265(2):212–220, May 2001. PMID: 11302686.

[247] H. Yin, F. Price, and M. A. Rudnicki. Satellite cells and the muscle stem cell niche. *Physiological Reviews*, 93(1):23–67, January 2013. PMID: 23303905.

[248] C. A. Collins, I. Olsen, P. S. Zammit, L. Heslop, A. Petrie, T. A. Partridge, and J. E. Morgan. Stem cell function, self-renewal, and behavioral heterogeneity of cells from the adult muscle satellite cell niche. *Cell*, 122(2):289–301, July 2005. PMID: 16051152.

[249] R. Bischoff. Proliferation of muscle satellite cells on intact myofibers in culture. *Developmental Biology*, 115(1):129–139, May 1986. PMID: 3516758.

[250] A. K. Sharma, Y. B. Lee, and J. D. Murray. The response of transgenic mice to beta-adrenergic agonist administration is different from that of normal mice. *Journal of Animal Science*, 75(8):2092–2099, August 1997. PMID: 9263056.

[251] T. S. Wong and F. W. Booth. Protein metabolism in rat tibialis anterior muscle after stimulated chronic eccentric exercise. *Journal of Applied Physiology (Bethesda, Md.: 1985)*, 69(5):1718–1724, November 1990. PMID: 1703146.

[252] D. A. Lowe and S. E. Alway. Stretch-induced myogenin, MyoD, and MRF4

expression and acute hypertrophy in quail slow-tonic muscle are not dependent upon satellite cell proliferation. *Cell and Tissue Research*, 296(3):531–539, June 1999. PMID: 10370140.

[253] J. C. Waterlow, P. J. Garlick, and D. J. Millward. *Protein turnover in mammalian tissues and in the whole body*. North-Holland Pub. Co. ; Sole distributors for the U.S.A. and Canada, Elsevier North-Holland, Amsterdam; New York; New York, 1978.

[254] J. Du, X. Wang, C. Miereles, J. L. Bailey, R. Debigare, B. Zheng, S. R. Price, and W. E. Mitch. Activation of caspase-3 is an initial step triggering accelerated muscle proteolysis in catabolic conditions. *The Journal of Clinical Investigation*, 113(1):115–123, January 2004. PMID: 14702115.

[255] H. C. Lukaski, J. Mendez, E. R. Buskirk, and S. H. Cohn. Relationship between endogenous 3-methylhistidine excretion and body composition. *The American Journal of Physiology*, 240(3):E302–307, March 1981. PMID: 7212061.

[256] A. Neal, L. Boldrin, and J. E. Morgan. The Satellite Cell in Male and Female, Developing and Adult Mouse Muscle: Distinct Stem Cells for Growth and Regeneration. *PLoS ONE*, 7(5), May 2012. PMID: 22662253.

[257] F. Yusuf and B. Brand-Saberi. Myogenesis and muscle regeneration. *Histochemistry and Cell Biology*, 138(2):187–199, August 2012. PMID: 22644378.

[258] G. Daston, E. Lamar, M. Olivier, and M. Goulding. Pax-3 is necessary for migration but not differentiation of limb muscle precursors in the mouse. *Development (Cambridge, England)*, 122(3):1017–1027, March 1996. PMID: 8631247.

[259] T. Francetic and Q. Li. Skeletal myogenesis and Myf5 activation. *Transcription*, 2(3):109–114, 2011. PMID: 21922054.

[260] T. Braun, G. Buschhausen-Denker, E. Bober, E. Tannich, and H. H. Arnold. A novel human muscle factor related to but distinct from MyoD1 induces myogenic conversion in 10T1/2 fibroblasts. *The EMBO Journal*, 8(3):701–709, March 1989. PMID: 2721498.

[261] D. M. Noden and P. A. Trainor. Relations and interactions between cranial mesoderm and neural crest populations. *Journal of Anatomy*, 207(5):575–601, November 2005. PMID: 16313393.

[262] S. Tajbakhsh, D. Rocancourt, G. Cossu, and M. Buckingham. Redefining the genetic hierarchies controlling skeletal myogenesis: Pax-3 and Myf-5 act upstream of MyoD. *Cell*, 89(1):127–138, April 1997. PMID: 9094721.

[263] M. A. Rudnicki, P. N. Schnegelsberg, R. H. Stead, T. Braun, H. H. Arnold, and R. Jaenisch. MyoD or Myf-5 is required for the formation of skeletal muscle. *Cell*, 75(7):1351–1359, December 1993. PMID: 8269513.

[264] V. Andrés and K. Walsh. Myogenin expression, cell cycle withdrawal, and phenotypic differentiation are temporally separable events that precede cell fusion upon myogenesis. *The Journal of Cell Biology*, 132(4):657–666, February 1996. PMID: 8647896.

[265] D. A. Bergstrom and S. J. Tapscott. Molecular distinction between specification and differentiation in the myogenic basic helix-loop-helix transcription factor family. *Molecular and Cellular Biology*, 21(7):2404–2412, April 2001. PMID: 11259589.

[266] P. Hasty, A. Bradley, J. H. Morris, D. G. Edmondson, J. M. Venuti, E. N. Olson, and W. H. Klein. Muscle deficiency and neonatal death in mice with a targeted mutation in the myogenin gene. *Nature*, 364(6437):501–506, August 1993. PMID: 8393145.

[267] A. Draeger, A. G. Weeds, and R. B. Fitzsimons. Primary, secondary and tertiary myotubes in developing skeletal muscle: a new approach to the

analysis of human myogenesis. *Journal of the Neurological Sciences*, 81(1):19–43, October 1987. PMID: 2445924.

[268] P. J. Mitchell, S. E. Johnson, and K. Hannon. Insulin-like growth factor I stimulates myoblast expansion and myofiber development in the limb. *Developmental Dynamics: An Official Publication of the American Association of Anatomists*, 223(1):12–23, January 2002. PMID: 11803566.

[269] W. Shen, P. Wisniowski, L. Ahmed, D. W. Boyle, S. C. Denne, and E. A. Liechty. Protein anabolic effects of insulin and IGF-I in the ovine fetus. *American Journal of Physiology. Endocrinology and Metabolism*, 284(4):E748–756, April 2003. PMID: 12488244.

[270] A. M. Fernández, J. K. Kim, S. Yakar, J. Dupont, C. Hernandez-Sanchez, A. L. Castle, J. Filmore, G. I. Shulman, and D. Le Roith. Functional inactivation of the IGF-I and insulin receptors in skeletal muscle causes type 2 diabetes. *Genes & Development*, 15(15):1926–1934, August 2001. PMID: 11485987.

[271] M. J. Abuzzahab, A. Schneider, A. Goddard, F. Grigorescu, C. Lautier, E. Keller, W. Kiess, J. Klammt, J. Kratzsch, D. Osgood, R. Pfäffle, K. Raile, B. Seidel, R. J. Smith, S. D. Chernausek, and Intrauterine Growth Retardation (IUGR) Study Group. IGF-I receptor mutations resulting in intrauterine and postnatal growth retardation. *The New England Journal of Medicine*, 349(23):2211–2222, December 2003. PMID: 14657428.

[272] A. Matsakas, A. Otto, M. I. Elashry, S. C. Brown, and K. Patel. Altered primary and secondary myogenesis in the myostatin-null mouse. *Rejuvenation Research*, 13(6):717–727, December 2010. PMID: 21204650.

[273] M. Manceau, J. Gros, K. Savage, V. Thomé, A. McPherron, B. Paterson, and C. Marcelle. Myostatin promotes the terminal differentiation of embryonic muscle progenitors. *Genes & Development*, 22(5):668–681, March 2008.

PMID: 18316481.

[274] M. Schuelke, K. R. Wagner, L. E. Stolz, C. Hübner, T. Riebel, W. Kömen, T. Braun, J. F. Tobin, and S.-J. Lee. Myostatin mutation associated with gross muscle hypertrophy in a child. *The New England Journal of Medicine*, 350(26):2682–2688, June 2004. PMID: 15215484.

[275] J. Lin, H. B. Arnold, M. A. Della-Fera, M. J. Azain, D. L. Hartzell, and C. A. Baile. Myostatin knockout in mice increases myogenesis and decreases adipogenesis. *Biochemical and Biophysical Research Communications*, 291(3):701–706, March 2002. PMID: 11855847.

[276] L. B. Verdijk, T. Snijders, M. Drost, T. Delhaas, F. Kadi, and L. J. C. van Loon. Satellite cells in human skeletal muscle; from birth to old age. *Age*, 36(2):545–557, April 2014. PMID: 24122288.

[277] C. Lepper, S. J. Conway, and C.-M. Fan. Adult satellite cells and embryonic muscle progenitors have distinct genetic requirements. *Nature*, 460(7255):627–631, July 2009. PMID: 19554048.

[278] A. Dellavalle, G. Maroli, D. Covarello, E. Azzoni, A. Innocenzi, L. Perani, S. Antonini, R. Sambasivan, S. Brunelli, S. Tajbakhsh, and G. Cossu. Pericytes resident in postnatal skeletal muscle differentiate into muscle fibres and generate satellite cells. *Nature Communications*, 2:499, 2011. PMID: 21988915.

[279] M. A. LaBarge and H. M. Blau. Biological progression from adult bone marrow to mononucleate muscle stem cell to multinucleate muscle fiber in response to injury. *Cell*, 111(4):589–601, November 2002. PMID: 12437931.

[280] F. Relaix, D. Montarras, S. Zaffran, B. Gayraud-Morel, D. Rocancourt, S. Tajbakhsh, A. Mansouri, A. Cumano, and M. Buckingham. Pax3 and Pax7 have distinct and overlapping functions in adult muscle progenitor

cells. *The Journal of Cell Biology*, 172(1):91–102, January 2006. PMID: 16380438.

[281] G. B. Forbes and J. C. Reina. Adult lean body mass declines with age: some longitudinal observations. *Metabolism: Clinical and Experimental*, 19(9):653–663, September 1970. PMID: 5459997.

[282] R. N. Baumgartner, K. M. Koehler, D. Gallagher, L. Romero, S. B. Heymsfield, R. R. Ross, P. J. Garry, and R. D. Lindeman. Epidemiology of sarcopenia among the elderly in New Mexico. *American Journal of Epidemiology*, 147(8):755–763, April 1998. PMID: 9554417.

[283] S. Decary, V. Mouly, C. B. Hamida, A. Sautet, J. P. Barbet, and G. S. Butler-Browne. Replicative potential and telomere length in human skeletal muscle: implications for satellite cell-mediated gene therapy. *Human Gene Therapy*, 8(12):1429–1438, August 1997. PMID: 9287143.

[284] V. Renault, G. Piron-Hamelin, C. Forestier, S. DiDonna, S. Decary, F. Hentati, G. Saillant, G. S. Butler-Browne, and V. Mouly. Skeletal muscle regeneration and the mitotic clock. *Experimental Gerontology*, 35(6-7):711–719, September 2000. PMID: 11053661.

[285] J. M. Berg, J. L. Tymoczko, and L. Stryer. Each organ has a unique metabolic profile. In *Biochemistry*. W.H. Freeman, 2002.

[286] D. H. Wasserman. Four grams of glucose. *American Journal of Physiology - Endocrinology and Metabolism*, 296(1):E11–E21, January 2009. PMID: 18840763.

[287] M. Li and P. Björntorp. Triglyceride uptake in muscles in rats. *Obesity Research*, 3(5):419–426, September 1995. PMID: 8521161.

[288] R. L. Seip, K. Mair, T. G. Cole, and C. F. Semenkovich. Induction of human skeletal muscle lipoprotein lipase gene expression by short-term exercise is transient. *The American Journal of Physiology*, 272(2 Pt 1):E255–261,

February 1997. PMID: 9124332.

[289] Y.-X. Wang, C.-H. Lee, S. Tiep, R. T. Yu, J. Ham, H. Kang, and R. M. Evans. Peroxisome-proliferator-activated receptor delta activates fat metabolism to prevent obesity. *Cell*, 113(2):159–170, April 2003. PMID: 12705865.

[290] V. A. Narkar, M. Downes, R. T. Yu, E. Embler, Y.-X. Wang, E. Banayo, M. M. Mihaylova, M. C. Nelson, Y. Zou, H. Juguilon, H. Kang, R. Shaw, and R. M. Evans. AMPK and PPARδ agonists are exercise mimetics. *Cell*, 134(3):405–415, August 2008. PMID: 18674809.

[291] M. J. Watt, R. J. Southgate, A. G. Holmes, and M. A. Febbraio. Suppression of plasma free fatty acids upregulates peroxisome proliferator-activated receptor (PPAR) alpha and delta and PPAR coactivator 1alpha in human skeletal muscle, but not lipid regulatory genes. *Journal of Molecular Endocrinology*, 33(2):533–544, October 2004. PMID: 15525607.

[292] D. Holst, S. Luquet, V. Nogueira, K. Kristiansen, X. Leverve, and P. A. Grimaldi. Nutritional regulation and role of peroxisome proliferator-activated receptor delta in fatty acid catabolism in skeletal muscle. *Biochimica Et Biophysica Acta*, 1633(1):43–50, July 2003. PMID: 12842194.

[293] A. Mancini, O. El Bounkari, A.-F. Norrenbrock, M. Scherr, D. Schaefer, M. Eder, A. H. Banham, K. Pulford, L. Lyne, A. D. Whetton, and T. Tamura. FMIP controls the adipocyte lineage commitment of C2C12 cells by downmodulation of C/EBP α. *Oncogene*, 26(7):1020–1027, February 2007. PMID: 16909111.

[294] T. Mori, H. Sakaue, H. Iguchi, H. Gomi, Y. Okada, Y. Takashima, K. Nakamura, T. Nakamura, T. Yamauchi, N. Kubota, T. Kadowaki, Y. Matsuki, W. Ogawa, R. Hiramatsu, and M. Kasuga. Role of Krüppel-like factor 15 (KLF15) in transcriptional regulation of adipogenesis. *The*

Journal of Biological Chemistry, 280(13):12867–12875, April 2005. PMID: 15664998.

[295] A. G. Cristancho and M. A. Lazar. Forming functional fat: a growing understanding of adipocyte differentiation. *Nature Reviews. Molecular Cell Biology*, 12(11):722–734, November 2011. PMID: 21952300.

[296] Y. Itoigawa, K. N. Kishimoto, H. Okuno, H. Sano, K. Kaneko, and E. Itoi. Hypoxia induces adipogenic differentitation of myoblastic cell lines. *Biochemical and biophysical research communications*, 399(4):721–726, September 2010. PMID: 20692234.

[297] R. A. DeFronzo, E. Jacot, E. Jequier, E. Maeder, J. Wahren, and J. P. Felber. The effect of insulin on the disposal of intravenous glucose. Results from indirect calorimetry and hepatic and femoral venous catheterization. *Diabetes*, 30(12):1000–1007, December 1981. PMID: 7030826.

[298] L. D. Katz, M. G. Glickman, S. Rapoport, E. Ferrannini, and R. A. DeFronzo. Splanchnic and peripheral disposal of oral glucose in man. *Diabetes*, 32(7):675–679, July 1983. PMID: 6862113.

[299] R. A. Gelfand, M. G. Glickman, R. Jacob, R. S. Sherwin, and R. A. DeFronzo. Removal of infused amino acids by splanchnic and leg tissues in humans. *The American Journal of Physiology*, 250(4 Pt 1):E407–413, April 1986. PMID: 3963181.

[300] W. M. Bennet, A. A. Connacher, C. M. Scrimgeour, K. Smith, and M. J. Rennie. Increase in anterior tibialis muscle protein synthesis in healthy man during mixed amino acid infusion: studies of incorporation of [1-13C]leucine. *Clinical Science (London, England: 1979)*, 76(4):447–454, April 1989. PMID: 2714054.

[301] K. S. Nair, R. G. Schwartz, and S. Welle. Leucine as a regulator of whole body and skeletal muscle protein metabolism in humans. *The American*

Journal of Physiology, 263(5 Pt 1):E928–934, November 1992. PMID: 1443126.

[302] R. Barazzoni, K. R. Short, Y. Asmann, J. M. Coenen-Schimke, M. M. Robinson, and K. Sreekumaran Nair. Insulin fails to enhance mTOR phosphorylation, mitochondrial protein synthesis, and ATP production in human skeletal muscle without amino acid replacement. *American Journal of Physiology - Endocrinology and Metabolism*, 303(9):E1117–E1125, November 2012. PMID: 22967500.

[303] D. Cuthbertson, K. Smith, J. Babraj, G. Leese, T. Waddell, P. Atherton, H. Wackerhage, P. M. Taylor, and M. J. Rennie. Anabolic signaling deficits underlie amino acid resistance of wasting, aging muscle. *FASEB journal: official publication of the Federation of American Societies for Experimental Biology*, 19(3):422–424, March 2005. PMID: 15596483.

[304] G. G. Chiang and R. T. Abraham. Phosphorylation of mammalian target of rapamycin (mTOR) at Ser-2448 is mediated by p70S6 kinase. *The Journal of Biological Chemistry*, 280(27):25485–25490, July 2005. PMID: 15899889.

[305] M. Laplante and D. M. Sabatini. mTOR signaling in growth control and disease. *Cell*, 149(2):274–293, April 2012. PMID: 22500797.

[306] A. Suryawan, J. W. Hawes, R. A. Harris, Y. Shimomura, A. E. Jenkins, and S. M. Hutson. A molecular model of human branched-chain amino acid metabolism. *The American Journal of Clinical Nutrition*, 68(1):72–81, July 1998. PMID: 9665099.

[307] D. E. Matthews. Observations of Branched-Chain Amino Acid Administration in Humans. *The Journal of nutrition*, 135(6 Suppl):1580S–1584S, June 2005. PMID: 15930473.

[308] P. J. Garlick, M. A. McNurlan, and V. R. Preedy. A rapid and convenient

technique for measuring the rate of protein synthesis in tissues by injection of [3H]phenylalanine. *The Biochemical journal*, 192(2):719–723, November 1980. PMID: 6786283.

[309] L. A. Cynober. *Metabolic & Therapeutic Aspects of Amino Acids in Clinical Nutrition*. CRC Press, Hoboken, 2003.

[310] A. Grubb, S. Joanisse, D. R. Moore, L. M. Bellamy, C. J. Mitchell, S. M. Phillips, and G. Parise. IGF-1 colocalizes with muscle satellite cells following acute exercise in humans. *Applied Physiology, Nutrition, and Metabolism = Physiologie Appliquée, Nutrition Et Métabolisme*, 39(4):514–518, April 2014. PMID: 24669996.

[311] H. Ullum, P. M. Haahr, M. Diamant, J. Palmø, J. Halkjaer-Kristensen, and B. K. Pedersen. Bicycle exercise enhances plasma IL-6 but does not change IL-1 alpha, IL-1 beta, IL-6, or TNF-alpha pre-mRNA in BMNC. *Journal of Applied Physiology (Bethesda, Md.: 1985)*, 77(1):93–97, July 1994. PMID: 7961280.

[312] C. S. Bickel, J. Slade, E. Mahoney, F. Haddad, G. A. Dudley, and G. R. Adams. Time course of molecular responses of human skeletal muscle to acute bouts of resistance exercise. *Journal of Applied Physiology (Bethesda, Md.: 1985)*, 98(2):482–488, February 2005. PMID: 15465884.

[313] R. A. Fielding, T. J. Manfredi, W. Ding, M. A. Fiatarone, W. J. Evans, and J. G. Cannon. Acute phase response in exercise. III. Neutrophil and IL-1 beta accumulation in skeletal muscle. *The American Journal of Physiology*, 265(1 Pt 2):R166–172, July 1993. PMID: 8342683.

[314] T. Snijders, L. B. Verdijk, B. R. McKay, J. S. J. Smeets, J. van Kranenburg, B. B. B. Groen, G. Parise, P. Greenhaff, and L. J. C. van Loon. Acute dietary protein intake restriction is associated with changes in myostatin expression after a single bout of resistance exercise in healthy young men.

The Journal of Nutrition, 144(2):137–145, February 2014. PMID: 24306214.

[315] T. Schiffer, S. Geisler, B. Sperlich, and H. K. Strüder. MSTN mRNA after varying exercise modalities in humans. *International Journal of Sports Medicine*, 32(9):683–687, September 2011. PMID: 21695666.

[316] H. C. Dreyer, S. Fujita, E. L. Glynn, M. J. Drummond, E. Volpi, and B. B. Rasmussen. Resistance exercise increases leg muscle protein synthesis and mTOR signalling independent of sex. *Acta physiologica (Oxford, England)*, 199(1):71–81, May 2010. PMID: 20070283.

[317] M. P. Harber, A. R. Konopka, B. Jemiolo, S. W. Trappe, T. A. Trappe, and P. T. Reidy. Muscle protein synthesis and gene expression during recovery from aerobic exercise in the fasted and fed states. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, 299(5):R1254–1262, November 2010. PMID: 20720176.

[318] S. P. Bird, K. M. Tarpenning, and F. E. Marino. Liquid carbohydrate/essential amino acid ingestion during a short-term bout of resistance exercise suppresses myofibrillar protein degradation. *Metabolism: Clinical and Experimental*, 55(5):570–577, May 2006. PMID: 16631431.

[319] L. J. C. van Loon. Is there a need for protein ingestion during exercise? *Sports Medicine (Auckland, N.z.)*, 44(Suppl 1):105–111, 2014. PMID: 24791922.

[320] K. E. Hanssen, N. H. Kvamme, T. S. Nilsen, B. Rønnestad, I. K. Ambjørnsen, F. Norheim, F. Kadi, J. Hallèn, C. A. Drevon, and T. Raastad. The effect of strength training volume on satellite cells, myogenic regulatory factors, and growth factors. *Scandinavian Journal of Medicine & Science in Sports*, 23(6):728–739, December 2013. PMID: 22417199.

[321] R. J. Stefanetti, S. Lamon, M. Wallace, M. H. Vendelbo, A. P. Russell, and K. Vissing. Regulation of ubiquitin proteasome pathway molecular markers in response to endurance and resistance exercise and training. *Pflugers Archiv: European Journal of Physiology*, August 2014. PMID: 25104573.

[322] E. Rigamonti, P. Zordan, C. Sciorati, P. Rovere-Querini, and S. Brunelli. Macrophage plasticity in skeletal muscle repair. *BioMed Research International*, 2014, 2014. PMID: 24860823.

[323] T. A. Robertson, M. A. Maley, M. D. Grounds, and J. M. Papadimitriou. The role of macrophages in skeletal muscle regeneration with particular reference to chemotaxis. *Experimental Cell Research*, 207(2):321–331, August 1993. PMID: 8344384.

[324] K. L. Haugk, R. A. Roeder, M. J. Garber, and G. T. Schelling. Regulation of muscle cell proliferation by extracts from crushed muscle. *Journal of Animal Science*, 73(7):1972–1981, July 1995. PMID: 7592081.

[325] H. C. Olguin and B. B. Olwin. Pax-7 up-regulation inhibits myogenesis and cell cycle progression in satellite cells: a potential mechanism for self-renewal. *Developmental Biology*, 275(2):375–388, November 2004. PMID: 15501225.

[326] J. A. Epstein, P. Lam, L. Jepeal, R. L. Maas, and D. N. Shapiro. Pax3 inhibits myogenic differentiation of cultured myoblast cells. *The Journal of Biological Chemistry*, 270(20):11719–11722, May 1995. PMID: 7744814.

[327] G. Biolo, R. Y. Declan Fleming, and R. R. Wolfe. Physiologic hyperinsulinemia stimulates protein synthesis and enhances transport of selected amino acids in human skeletal muscle. *Journal of Clinical Investigation*, 95(2):811–819, February 1995. PMID: 7860765.

[328] L. S. Chow, R. C. Albright, M. L. Bigelow, G. Toffolo, C. Cobelli, and K. S. Nair. Mechanism of insulin's anabolic effect on muscle: measurements of

muscle protein synthesis and breakdown using aminoacyl-tRNA and other surrogate measures. *American Journal of Physiology. Endocrinology and Metabolism*, 291(4):E729–736, October 2006. PMID: 16705065.

[329] D. Dardevet, C. Sornet, I. Savary, E. Debras, P. Patureau-Mirand, and J. Grizard. Glucocorticoid effects on insulin- and IGF-I-regulated muscle protein metabolism during aging. *The Journal of Endocrinology*, 156(1):83–89, January 1998. PMID: 9496237.

[330] K. Sjögren, J.-L. Liu, K. Blad, S. Skrtic, O. Vidal, V. Wallenius, D. LeRoith, J. Törnell, O. G. P. Isaksson, J.-O. Jansson, and C. Ohlsson. Liver-derived insulin-like growth factor I (IGF-I) is the principal source of IGF-I in blood but is not required for postnatal body growth in mice. *Proceedings of the National Academy of Sciences of the United States of America*, 96(12):7088–7092, June 1999. PMID: 10359843.

[331] P. Rorsman and M. Braun. Regulation of insulin secretion in human pancreatic islets. *Annual Review of Physiology*, 75:155–179, 2013. PMID: 22974438.

[332] M. A. Soos, J. Whittaker, R. Lammers, A. Ullrich, and K. Siddle. Receptors for insulin and insulin-like growth factor-I can form hybrid dimers. Characterisation of hybrid receptors in transfected cells. *Biochemical Journal*, 270(2):383–390, September 1990. PMID: 1698059.

[333] G. Pandini, F. Frasca, R. Mineo, L. Sciacca, R. Vigneri, and A. Belfiore. Insulin/insulin-like growth factor I hybrid receptors have different biological characteristics depending on the insulin receptor isoform involved. *The Journal of Biological Chemistry*, 277(42):39684–39695, October 2002. PMID: 12138094.

[334] W. Chao and P. A. D'Amore. IGF2: Epigenetic regulation and role in development and disease. *Cytokine & growth factor reviews*,

19(2):111–120, April 2008. PMID: 18308616.

[335] C. P. Velloso. Regulation of muscle mass by growth hormone and IGF-I. *British Journal of Pharmacology*, 154(3):557–568, June 2008. PMID: 18500379.

[336] F. M. Tomas, S. E. Knowles, P. C. Owens, C. S. Chandler, G. L. Francis, L. C. Read, and F. J. Ballard. Insulin-like growth factor-I (IGF-I) and especially IGF-I variants are anabolic in dexamethasone-treated rats. *Biochemical Journal*, 282(Pt 1):91–97, February 1992. PMID: 1371669.

[337] E. M. Wilson, M. M. Hsieh, and P. Rotwein. Autocrine growth factor signaling by insulin-like growth factor-II mediates MyoD-stimulated myocyte maturation. *The Journal of Biological Chemistry*, 278(42):41109–41113, October 2003. PMID: 12941952.

[338] T. M. DeChiara, E. J. Robertson, and A. Efstratiadis. Parental imprinting of the mouse insulin-like growth factor II gene. *Cell*, 64(4):849–859, February 1991. PMID: 1997210.

[339] M. Imanaka, K. Iida, A. Murawaki, H. Nishizawa, H. Fukuoka, R. Takeno, Y. Takahashi, Y. Okimura, H. Kaji, and K. Chihara. Growth hormone stimulates mechano growth factor expression and activates myoblast transformation in C2C12 cells. *The Kobe Journal of Medical Sciences*, 54(1):E46–54, 2008. PMID: 18772608.

[340] E. Resmini, B. Morte, E. Sorianello, E. Gallardo, N. de Luna, I. Illa, A. Zorzano, J. Bernal, and S. M. Webb. Identification of novel GH-regulated genes in C2C12 cells. *Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et métabolisme*, 43(13):919–930, December 2011.

[341] A. Sotiropoulos, M. Ohanna, C. Kedzia, R. K. Menon, J. J. Kopchick, P. A. Kelly, and M. Pende. Growth hormone promotes skeletal muscle cell fusion

independent of insulin-like growth factor 1 up-regulation. *Proceedings of the National Academy of Sciences of the United States of America*, 103(19):7315–7320, May 2006. PMID: 16670201.

[342] F. Lupu, J. D. Terwilliger, K. Lee, G. V. Segre, and A. Efstratiadis. Roles of growth hormone and insulin-like growth factor 1 in mouse postnatal growth. *Developmental Biology*, 229(1):141–162, January 2001. PMID: 11133160.

[343] M. Mastropasqua, G. Spagna, V. Baldini, I. Tedesco, and A. Paggi. Hoffman's syndrome: muscle stiffness, pseudohypertrophy and hypothyroidism. *Hormone Research*, 59(2):105–108, 2003. PMID: 12589116.

[344] A. J. Garber. Effects of parathyroid hormone on skeletal muscle protein and amino acid metabolism in the rat. *Journal of Clinical Investigation*, 71(6):1806–1821, June 1983. PMID: 6306055.

[345] I. M. Olfert, R. A. Howlett, K. Tang, N. D. Dalton, Y. Gu, K. L. Peterson, P. D. Wagner, and E. C. Breen. Muscle-specific VEGF deficiency greatly reduces exercise endurance in mice. *The Journal of Physiology*, 587(Pt 8):1755–1767, April 2009. PMID: 19237429.

[346] N. Arsic, S. Zacchigna, L. Zentilin, G. Ramirez-Correa, L. Pattarini, A. Salvi, G. Sinagra, and M. Giacca. Vascular endothelial growth factor stimulates skeletal muscle regeneration in vivo. *Molecular Therapy: The Journal of the American Society of Gene Therapy*, 10(5):844–854, November 2004. PMID: 15509502.

[347] J. S. Bonner, L. Lantier, C. M. Hasenour, F. D. James, D. P. Bracy, and D. H. Wasserman. Muscle-specific vascular endothelial growth factor deletion induces muscle capillary rarefaction creating muscle insulin resistance. *Diabetes*, 62(2):572–580, February 2013. PMID: 23002035.

[348] C. C. de Theije, R. C. J. Langen, W. H. Lamers, H. R. Gosker, A. M. W. J. Schols, and S. E. Köhler. Differential sensitivity of oxidative and glycolytic muscles to hypoxia-induced muscle atrophy. *Journal of Applied Physiology (Bethesda, Md.: 1985)*, 118(2):200–211, January 2015. PMID: 25429096.

[349] B. A. Bryan, T. E. Walshe, D. C. Mitchell, J. S. Havumaki, M. Saint-Geniez, A. S. Maharaj, A. E. Maldonado, and P. A. D'Amore. Coordinated vascular endothelial growth factor expression and signaling during skeletal myogenic differentiation. *Molecular biology of the cell*, 19(3):994–1006, March 2008. PMID: 18094043.

[350] C. R. Vianna, M. Huntgeburth, R. Coppari, C. S. Choi, J. Lin, S. Krauss, G. Barbatelli, I. Tzameli, Y.-B. Kim, S. Cinti, G. I. Shulman, B. M. Spiegelman, and B. B. Lowell. Hypomorphic mutation in PGC1β causes mitochondrial dysfunction and liver insulin resistance. *Cell metabolism*, 4(6):453–464, December 2006. PMID: 17141629.

[351] G. C. Rowe, C. Jang, I. S. Patten, and Z. Arany. PGC-1beta regulates angiogenesis in skeletal muscle. *American journal of physiology. Endocrinology and metabolism*, 301(1):E155–163, July 2011. PMID: 21364124.

[352] J. Lin, H. Wu, P. T. Tarr, C.-Y. Zhang, Z. Wu, O. Boss, L. F. Michael, P. Puigserver, E. Isotani, E. N. Olson, B. B. Lowell, R. Bassel-Duby, and B. M. Spiegelman. Transcriptional co-activator PGC-1 alpha drives the formation of slow-twitch muscle fibres. *Nature*, 418(6899):797–801, August 2002. PMID: 12181572.

[353] J. Waltenberger, L. Claesson-Welsh, A. Siegbahn, M. Shibuya, and C. H. Heldin. Different signal transduction properties of KDR and Flt1, two receptors for vascular endothelial growth factor. *The Journal of Biological*

Chemistry, 269(43):26988–26995, October 1994. PMID: 7929439.

[354] L. Seetharam, N. Gotoh, Y. Maru, G. Neufeld, S. Yamaguchi, and M. Shibuya. A unique signal transduction from FLT tyrosine kinase, a receptor for vascular endothelial growth factor VEGF. *Oncogene*, 10(1):135–147, January 1995. PMID: 7824266.

[355] H. P. Gerber, A. McMurtrey, J. Kowalski, M. Yan, B. A. Keyt, V. Dixit, and N. Ferrara. Vascular endothelial growth factor regulates endothelial cell survival through the phosphatidylinositol 3'-kinase/Akt signal transduction pathway. Requirement for Flk-1/KDR activation. *The Journal of biological chemistry*, 273(46):30336–30343, November 1998. PMID: 9804796.

[356] E. I. Korpelainen, M. Kärkkäinen, Y. Gunji, M. Vikkula, and K. Alitalo. Endothelial receptor tyrosine kinases activate the STAT signaling pathway: mutant Tie-2 causing venous malformations signals a distinct STAT activation response. *Oncogene*, 18(1):1–8, January 1999. PMID: 9926914.

[357] A. J. Harris. Embryonic growth and innervation of rat skeletal muscles. I. Neural regulation of muscle fibre numbers. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, 293(1065):257–277, July 1981. PMID: 6116248.

[358] T. M. O'Connor and C. R. Wyttenbach. Cell death in the embryonic chick spinal cord. *The Journal of Cell Biology*, 60(2):448–459, February 1974. PMID: 4855905.

[359] M. Favero, A. Cangiano, and G. Busetto. Hebb-based rules of neural plasticity: are they ubiquitously important for the refinement of synaptic connections in development? *The Neuroscientist: A Review Journal Bringing Neurobiology, Neurology and Psychiatry*, 20(1):8–14, February 2014. PMID: 23753676.

[360] A. Burls, K. Subramaniam, M. B. Lowrie, and G. Vrbová. Absence of nerve -

muscle interaction influences the survival of developing motoneurons. *The European Journal of Neuroscience*, 3(3):216–221, 1991. PMID: 12106198.

[361] M. Kuno. Target dependence of motoneuronal survival: the current status. *Neuroscience Research*, 9(3):155–172, December 1990. PMID: 1963675.

[362] A. D. Grinnell. Dynamics of nerve-muscle interaction in developing and mature neuromuscular junctions. *Physiological Reviews*, 75(4):789–834, October 1995. PMID: 7480163.

[363] M. Gonzalez-Freire, R. de Cabo, S. A. Studenski, and L. Ferrucci. The neuromuscular junction: aging at the crossroad between nerves and muscle. *Frontiers in Aging Neuroscience*, 6, August 2014. PMID: 25157231.

[364] C. Serra, D. Lee, N. L. Sandor, G. Toraldo, H. Jang, R. Jasuja, and S. Bhasin. Characterization of the neuromuscular junction in castrated male mice. San Francisco, June 2013.

[365] H. Nishimune, J. A. Stanford, and Y. Mori. Role of exercise in maintaining the integrity of the neuromuscular junction. *Muscle & nerve*, 49(3):315–324, March 2014. PMID: 24122772.

[366] P. J. Apel, J. Jun, M. Callahan, C. N. Northam, T. B. Alton, W. E. Sonntag, and Z. Li. Effect of locally delivered IGF-1 on nerve regeneration during aging: an experimental study in rats. *Muscle & nerve*, 41(3):335–341, March 2010. PMID: 19802878.

[367] R. Thijs, N. Notermans, J. Wokke, Y. van der Graaf, and J. van Gijn. Distribution of muscle weakness of central and peripheral origin. *Journal of Neurology, Neurosurgery, and Psychiatry*, 65(5):794–796, November 1998. PMID: 9810962.

[368] D. Purves, G. J. Augustine, D. Fitzpatrick, L. C. Katz, A.-S. LaMantia, J. O. McNamara, and S. M. Williams. Damage to descending motor pathways: the upper motor neuron syndrome. In *Neuroscience*. Sinauer Associates,

Inc., Sunderland, United States, 2 edition, 2001.

[369] R. W. Jackman and S. C. Kandarian. The molecular basis of skeletal muscle atrophy. *American Journal of Physiology. Cell Physiology*, 287(4):C834–843, October 2004. PMID: 15355854.

[370] E. M. MacDonald, E. Andres-Mateos, R. Mejias, J. L. Simmers, R. Mi, J.-S. Park, S. Ying, A. Hoke, S.-J. Lee, and R. D. Cohn. Denervation atrophy is independent from Akt and mTOR activation and is not rescued by myostatin inhibition. *Disease Models & Mechanisms*, 7(4):471–481, April 2014. PMID: 24504412.

[371] J. C. Bruusgaard and K. Gundersen. In vivo time-lapse microscopy reveals no loss of murine myonuclei during weeks of muscle atrophy. *The Journal of Clinical Investigation*, 118(4):1450–1457, April 2008. PMID: 18317591.

[372] A. L. Goldberg. Protein turnover in skeletal muscle. II. Effects of denervation and cortisone on protein catabolism in skeletal muscle. *The Journal of Biological Chemistry*, 244(12):3223–3229, June 1969. PMID: 5792658.

[373] K. Furuno, M. N. Goodman, and A. L. Goldberg. Role of different proteolytic systems in the degradation of muscle proteins during denervation atrophy. *The Journal of Biological Chemistry*, 265(15):8550–8557, May 1990. PMID: 2187867.

[374] B. C. Beehler, P. G. Sleph, L. Benmassaoud, and G. J. Grover. Reduction of skeletal muscle atrophy by a proteasome inhibitor in a rat model of denervation. *Experimental Biology and Medicine (Maywood, N.J.)*, 231(3):335–341, March 2006. PMID: 16514182.

[375] A. V. Gomes, D. S. Waddell, R. Siu, M. Stein, S. Dewey, J. D. Furlow, and S. C. Bodine. Upregulation of proteasome activity in muscle RING finger 1-null mice following denervation. *The FASEB Journal*, 26(7):2986–2999,

July 2012. PMID: 22508689.

[376] R. Medina, S. S. Wing, and A. L. Goldberg. Increase in levels of polyubiquitin and proteasome mRNA in skeletal muscle during starvation and denervation atrophy. *Biochemical Journal*, 307(Pt 3):631–637, May 1995. PMID: 7741690.

[377] A.-K. Fjällström, K. Evertsson, M. Norrby, and S. Tågerud. Forkhead box O1 and muscle RING finger 1 protein expression in atrophic and hypertrophic denervated mouse skeletal muscle. *Journal of Molecular Signaling*, 9:9, September 2014. PMID: 25276226.

[378] V. Moresi, A. H. Williams, E. Meadows, J. M. Flynn, M. J. Potthoff, J. McAnally, J. M. Shelton, J. Backs, W. H. Klein, J. A. Richardson, R. Bassel-Duby, and E. N. Olson. Myogenin and class II HDACs control neurogenic muscle atrophy by inducing E3 ubiquitin ligases. *Cell*, 143(1):35–45, October 2010. PMID: 20887891.

[379] H. Tang, K. Inoki, M. Lee, E. Wright, A. Khuong, A. Khuong, S. Sugiarto, M. Garner, J. Paik, R. A. DePinho, D. Goldman, K.-L. Guan, and J. B. Shrager. mTORC1 promotes denervation-induced muscle atrophy through a mechanism involving the activation of FoxO and E3 ubiquitin ligases. *Science Signaling*, 7(314):ra18, February 2014. PMID: 24570486.

[380] P. C. D. Macpherson, X. Wang, and D. Goldman. Myogenin regulates denervation-dependent muscle atrophy in mouse soleus muscle. *Journal of cellular biochemistry*, 112(8):2149–2159, August 2011. PMID: 21465538.

[381] H. Tang, P. Macpherson, M. Marvin, E. Meadows, W. H. Klein, X.-J. Yang, and D. Goldman. A histone deacetylase 4/myogenin positive feedback loop coordinates denervation-dependent gene induction and suppression. *Molecular Biology of the Cell*, 20(4):1120–1131, February 2009. PMID: 19109424.

[382] S. C. Bodine, T. N. Stitt, M. Gonzalez, W. O. Kline, G. L. Stover, R. Bauerlein, E. Zlotchenko, A. Scrimgeour, J. C. Lawrence, D. J. Glass, and G. D. Yancopoulos. Akt/mTOR pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle atrophy in vivo. *Nature Cell Biology*, 3(11):1014–1019, November 2001. PMID: 11715023.

[383] J. Zhao, J. J. Brault, A. Schild, P. Cao, M. Sandri, S. Schiaffino, S. H. Lecker, and A. L. Goldberg. FoxO3 coordinately activates protein degradation by the autophagic/lysosomal and proteasomal pathways in atrophying muscle cells. *Cell metabolism*, 6(6):472–483, December 2007. PMID: 18054316.

[384] S. Schiaffino, K. A. Dyar, S. Ciciliot, B. Blaauw, and M. Sandri. Mechanisms regulating skeletal muscle growth and atrophy. *The FEBS journal*, 280(17):4294–4314, September 2013. PMID: 23517348.

[385] P. N. Quy, A. Kuma, P. Pierre, and N. Mizushima. Proteasome-dependent activation of mammalian target of rapamycin complex 1 (mTORC1) is essential for autophagy suppression and muscle remodeling following denervation. *The Journal of Biological Chemistry*, 288(2):1125–1134, January 2013. PMID: 23209294.

[386] J. Schwartz, L. Rath-Wolfson, D. Varon, E. Skutelsky, E. Gaton, and M. Wolman. Effects of various drugs on denervation changes in rat muscles. *Clinical Neuropathology*, 9(6):305–309, December 1990. PMID: 2286023.

[387] C. Mammucari, G. Milan, V. Romanello, E. Masiero, R. Rudolf, P. Del Piccolo, S. J. Burden, R. Di Lisi, C. Sandri, J. Zhao, A. L. Goldberg, S. Schiaffino, and M. Sandri. FoxO3 controls autophagy in skeletal muscle in vivo. *Cell metabolism*, 6(6):458–471, December 2007. PMID: 18054315.

[388] J.-S. Ju, A. S. Varadhachary, S. E. Miller, and C. C. Weihl. Quantitation of "autophagic flux" in mature skeletal muscle. *Autophagy*, 6(7):929–935,

October 2010. PMID: 20657169.

[389] D. A. P. Gonçalves, W. A. Silveira, E. C. Lira, F. A. Graça, S. Paula-Gomes, N. M. Zanon, I. C. Kettelhut, and L. C. C. Navegantes. Clenbuterol suppresses proteasomal and lysosomal proteolysis and atrophy-related genes in denervated rat soleus muscles independently of Akt. *American Journal of Physiology. Endocrinology and Metabolism*, 302(1):E123–133, January 2012. PMID: 21952035.

[390] S. K. Joshi, H. T. Kim, B. T. Feeley, and X. Liu. Differential ubiquitin-proteasome and autophagy signaling following rotator cuff tears and suprascapular nerve injury. *Journal of orthopaedic research : official publication of the Orthopaedic Research Society*, 32(1), January 2014. PMID: 24018537.

[391] F. Muhlbacher, C. R. Kapadia, M. F. Colpoys, R. J. Smith, and D. W. Wilmore. Effects of glucocorticoids on glutamine metabolism in skeletal muscle. *The American Journal of Physiology*, 247(1 Pt 1):E75–83, July 1984. PMID: 6146264.

[392] H. A. Tiley, R. J. Geor, and L. J. McCutcheon. Effects of dexamethasone administration on insulin resistance and components of insulin signaling and glucose metabolism in equine skeletal muscle. *American Journal of Veterinary Research*, 69(1):51–58, January 2008. PMID: 18167087.

[393] T. J. Corah, J. D. Tatum, J. B. Morgan, R. G. Mortimer, and G. C. Smith. Effects of a dexamethasone implant on deposition of intramuscular fat in genetically identical cattle. *Journal of Animal Science*, 73(11):3310–3316, November 1995. PMID: 8586589.

[394] L. Carraro, S. Ferraresso, B. Cardazzo, C. Romualdi, C. Montesissa, F. Gottardo, T. Patarnello, M. Castagnaro, and L. Bargelloni. Expression profiling of skeletal muscle in young bulls treated with steroidal growth

promoters. *Physiological Genomics*, 38(2):138–148, July 2009. PMID: 19383624.

[395] J. Y. Yeh, B. R. Ou, and N. E. Forsberg. Effects of dexamethasone on muscle protein homeostasis and on calpain and calpastatin activities and gene expression in rabbits. *The Journal of Endocrinology*, 141(2):209–217, May 1994. PMID: 7519233.

[396] A. F. Clark and P. J. Vignos. The role of proteases in experimental glucocorticoid myopathy. *Muscle & Nerve*, 4(3):219–222, June 1981. PMID: 6264287.

[397] A. O. P. Protzek, J. M. Costa-Júnior, L. F. Rezende, G. J. Santos, T. G. Araújo, J. F. Vettorazzi, F. Ortis, E. M. Carneiro, A. Rafacho, and A. C. Boschero. Augmented beta-cell function and mass in glucocorticoid-treated rodents are associated with increased islet Ir-beta/AKT/mTOR and decreased AMPK/ACC and AS160 signaling. *International Journal of Endocrinology*, 2014, 2014. PMID: 25313308.

[398] M. Rohdewald and H. Glasmacher. Über die antianabole Wirkung von Glucocorticoiden. I. Mitteilung: Untersuchungen am Skelettmuskel von Ratte und Maus. *Enzymologia*, 28:49–56, November 1964. PMID: 14238775.

[399] M. L. Watson, L. M. Baehr, H. M. Reichardt, J. P. Tuckermann, S. C. Bodine, and J. D. Furlow. A cell-autonomous role for the glucocorticoid receptor in skeletal muscle atrophy induced by systemic glucocorticoid exposure. *American journal of physiology. Endocrinology and metabolism*, 302(10):E1210–1220, May 2012. PMID: 22354783.

[400] D. Chen, M. R. Yang, L. N. Huang, Y. W. Qiu, and S. T. Li. Different magnitude of resistance to non-depolarizing muscle relaxants in dexamethasone-treated rat diaphragm associated with altered

acetylcholine receptor expression. *Genetics and molecular research: GMR*, 13(3):5892–5900, 2014. PMID: 25117347.

[401] Y. Dong, J. S. Pan, and L. Zhang. Myostatin suppression of Akirin1 mediates glucocorticoid-induced satellite cell dysfunction. *PloS One*, 8(3):e58554, 2013. PMID: 23516508.

[402] F. Giorgino and R. J. Smith. Dexamethasone enhances insulin-like growth factor-I effects on skeletal muscle cell proliferation. Role of specific intracellular signaling pathways. *The Journal of clinical investigation*, 96(3):1473–1483, September 1995. PMID: 7544807.

[403] J. R. Florini and S. B. Roberts. A serum-free medium for the growth of muscle cells in culture. *In vitro*, 15(12):983–992, December 1979. PMID: 94034.

[404] P. A. Whitson, C. A. Stuart, M. H. Huls, C. F. Sams, and N. M. Cintron. Dexamethasone effects on creatine kinase activity and insulin-like growth factor receptors in cultured muscle cells. *Journal of cellular physiology*, 140(1):8–17, July 1989. PMID: 2544617.

[405] A. Takahashi, Y. Kureishi, J. Yang, Z. Luo, K. Guo, D. Mukhopadhyay, Y. Ivashchenko, D. Branellec, and K. Walsh. Myogenic Akt signaling regulates blood vessel recruitment during myofiber growth. *Molecular and cellular biology*, 22(13):4803–4814, July 2002. PMID: 12052887.

[406] M. Menconi, P. Gonnella, V. Petkova, S. Lecker, and P.-O. Hasselgren. Dexamethasone and corticosterone induce similar, but not identical, muscle wasting responses in cultured L6 and C2C12 myotubes. *Journal of Cellular Biochemistry*, 105(2):353–364, October 2008. PMID: 18615595.

[407] G. R. Bullock, E. E. Carter, P. Elliott, R. F. Peters, P. Simpson, and A. M. White. Relative changes in the function of muscle ribosomes and mitochondria during the early phase of steroid-induced catabolism.

Biochemical Journal, 127(5):881–892, May 1972. PMID: 5076236.

[408] N. Alamdari, G. Toraldo, Z. Aversa, I. Smith, E. Castillero, G. Renaud, R. Qaisar, L. Larsson, R. Jasuja, and P.-O. Hasselgren. Loss of muscle strength during sepsis is in part regulated by glucocorticoids and is associated with reduced muscle fiber stiffness. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, 303(10):R1090–1099, November 2012. PMID: 23019215.

[409] S. Nava, G. Gayan-Ramirez, H. Rollier, A. Bisschop, R. Dom, V. de Bock, and M. Decramer. Effects of acute steroid administration on ventilatory and peripheral muscles in rats. *American Journal of Respiratory and Critical Care Medicine*, 153(6 Pt 1):1888–1896, June 1996. PMID: 8665051.

[410] F. Caldefie-Chézet, C. Moinard, R. Minet-Quinard, F. Gachon, L. Cynober, and M. Vasson. Dexamethasone treatment induces long-lasting hyperleptinemia and anorexia in old rats. *Metabolism: Clinical and Experimental*, 50(9):1054–1058, September 2001. PMID: 11555838.

[411] D. Dardevet, C. Sornet, D. Taillandier, I. Savary, D. Attaix, and J. Grizard. Sensitivity and protein turnover response to glucocorticoids are different in skeletal muscle from adult and old rats. Lack of regulation of the ubiquitin-proteasome proteolytic pathway in aging. *The Journal of Clinical Investigation*, 96(5):2113–2119, November 1995. PMID: 7593595.

[412] H. Nicastro, B. Gualano, W. M. A. M. de Moraes, V. de Salles Painelli, C. R. da Luz, A. dos Santos Costa, F. de Salvi Guimarães, A. Medeiros, P. C. Brum, and J. Lancha, Antonio Herbert. Effects of creatine supplementation on muscle wasting and glucose homeostasis in rats treated with dexamethasone. *Amino acids*, 42(5):1695–1701, May 2012. PMID: 21373767.

[413] D. J. Prezant, M. L. Karwa, B. Richner, D. Maggiore, E. I. Gentry, and

J. Cahill. Gender-specific effects of dexamethasone treatment on rat diaphragm structure and function. *Journal of Applied Physiology (Bethesda, Md.: 1985)*, 82(1):125–133, January 1997. PMID: 9029207.

[414] I. L. Baptista, W. J. Silva, G. G. Artioli, J. P. L. F. Guilherme, M. L. Leal, M. S. Aoki, E. H. Miyabara, and A. S. Moriscot. Leucine and HMB differentially modulate proteasome system in skeletal muscle under different sarcopenic conditions. *PloS One*, 8(10):e76752, 2013. PMID: 24124592.

[415] R. Minet-Quinard, C. Moinard, S. Walrand, F. Villié, B. Normand, M. P. Vasson, J. Chopineau, and L. Cynober. Induction of a catabolic state in rats by dexamethasone: dose or time dependency? *JPEN. Journal of parenteral and enteral nutrition*, 24(1):30–36, February 2000. PMID: 10638469.

[416] S. B. Bowes, N. C. Jackson, D. Papachristodoulou, A. M. Umpleby, and P. H. Sönksen. Effect of corticosterone on protein degradation in isolated rat soleus and extensor digitorum longus muscles. *The Journal of Endocrinology*, 148(3):501–507, March 1996. PMID: 8778228.

[417] T. Seene, M. Umnova, K. Alev, and A. Pehme. Effect of glucocorticoids on contractile apparatus of rat skeletal muscle. *Journal of Steroid Biochemistry*, 29(3):313–317, March 1988. PMID: 3282126.

[418] S. S. Wing and A. L. Goldberg. Glucocorticoids activate the ATP-ubiquitin-dependent proteolytic system in skeletal muscle during fasting. *The American Journal of Physiology*, 264(4 Pt 1):E668–676, April 1993. PMID: 7682781.

[419] L. Combaret, D. Taillandier, D. Dardevet, D. Béchet, C. Rallière, A. Claustre, J. Grizard, and D. Attaix. Glucocorticoids regulate mRNA levels for subunits of the 19 S regulatory complex of the 26 S proteasome in

fast-twitch skeletal muscles. *Biochemical Journal*, 378(Pt 1):239–246, February 2004. PMID: 14636157.

[420] G. Tiao, J. Fagan, V. Roegner, M. Lieberman, J. J. Wang, J. E. Fischer, and P. O. Hasselgren. Energy-ubiquitin-dependent muscle proteolysis during sepsis in rats is regulated by glucocorticoids. *Journal of Clinical Investigation*, 97(2):339–348, January 1996. PMID: 8567953.

[421] H. Gilson, O. Schakman, L. Combaret, P. Lause, L. Grobet, D. Attaix, J. M. Ketelslegers, and J. P. Thissen. Myostatin gene deletion prevents glucocorticoid-induced muscle atrophy. *Endocrinology*, 148(1):452–460, January 2007. PMID: 17038559.

[422] L. Wang, G. J. Luo, J. J. Wang, and P. O. Hasselgren. Dexamethasone stimulates proteasome- and calcium-dependent proteolysis in cultured L6 myotubes. *Shock (Augusta, Ga.)*, 10(4):298–306, October 1998. PMID: 9788663.

[423] M. G. Thompson, A. Thom, K. Partridge, K. Garden, G. P. Campbell, G. Calder, and R. M. Palmer. Stimulation of myofibrillar protein degradation and expression of mRNA encoding the ubiquitin-proteasome system in C(2)C(12) myotubes by dexamethasone: effect of the proteasome inhibitor MG-132. *Journal of Cellular Physiology*, 181(3):455–461, December 1999. PMID: 10528231.

[424] A. C. Marinovic, W. E. Mitch, and S. R. Price. Tools for evaluating ubiquitin (UbC) gene expression: characterization of the rat UbC promoter and use of an unique 3' mRNA sequence. *Biochemical and biophysical research communications*, 274(2):537–541, August 2000. PMID: 10913373.

[425] B. Zheng, S. Ohkawa, H. Li, T. K. Roberts-Wilson, and S. R. Price. FOXO3a mediates signaling crosstalk that coordinates ubiquitin and atrogin-1/MAFbx expression during glucocorticoid-induced skeletal muscle

atrophy. *FASEB journal: official publication of the Federation of American Societies for Experimental Biology*, 24(8):2660–2669, August 2010. PMID: 20371624.

[426] M. J. Tisdale. Catabolic mediators of cancer cachexia. *Current Opinion in Supportive and Palliative Care*, 2(4):256–261, December 2008. PMID: 19069310.

[427] W. Chamberlain, P. Gonnella, N. Alamdari, Z. Aversa, and P.-O. Hasselgren. Multiple muscle wasting-related transcription factors are acetylated in dexamethasone-treated muscle cells. *Biochemistry and cell biology = Biochimie et biologie cellulaire*, 90(2):200–208, April 2012. PMID: 22292478.

[428] G. Luo, X. Sun, E. Hungness, and P. O. Hasselgren. Heat shock protects L6 myotubes from catabolic effects of dexamethasone and prevents downregulation of NF-kappaB. *American journal of physiology. Regulatory, integrative and comparative physiology*, 281(4):R1193–1200, October 2001. PMID: 11557628.

[429] J. Du, W. E. Mitch, X. Wang, and S. R. Price. Glucocorticoids induce proteasome C3 subunit expression in L6 muscle cells by opposing the suppression of its transcription by NF-kappa B. *The Journal of Biological Chemistry*, 275(26):19661–19666, June 2000. PMID: 10867022.

[430] L. M. Baehr, J. D. Furlow, and S. C. Bodine. Muscle sparing in muscle RING finger 1 null mice: response to synthetic glucocorticoids. *The Journal of physiology*, 589(Pt 19):4759–4776, October 2011. PMID: 21807613.

[431] D. S. Waddell, L. M. Baehr, J. van den Brandt, S. A. Johnsen, H. M. Reichardt, J. D. Furlow, and S. C. Bodine. The glucocorticoid receptor and FOXO1 synergistically activate the skeletal muscle atrophy-associated MuRF1 gene. *American Journal of Physiology. Endocrinology and*

Metabolism, 295(4):E785–797, October 2008. PMID: 18612045.

[432] T. P. Braun and D. L. Marks. The regulation of muscle mass by endogenous glucocorticoids. *Frontiers in Physiology*, 6, February 2015. PMID: 25691871.

[433] Y. Wu, W. Zhao, J. Zhao, Y. Zhang, W. Qin, J. Pan, W. A. Bauman, R. D. Blitzer, and C. Cardozo. REDD1 is a major target of testosterone action in preventing dexamethasone-induced muscle loss. *Endocrinology*, 151(3):1050–1059, March 2010. PMID: 20032058.

[434] M. Nishimura, M. Mikura, K. Hirasaka, Y. Okumura, T. Nikawa, Y. Kawano, M. Nakayama, and M. Ikeda. Effects of dimethyl sulphoxide and dexamethasone on mRNA expression of myogenesis- and muscle proteolytic system-related genes in mouse myoblastic C2C12 cells. *Journal of biochemistry*, 144(6):717–724, December 2008. PMID: 18835828.

[435] L. Slimani, D. Micol, J. Amat, G. Delcros, B. Meunier, D. Taillandier, C. Polge, D. Béchet, D. Dardevet, B. Picard, D. Attaix, A. Listrat, and L. Combaret. The worsening of tibialis anterior muscle atrophy during recovery post-immobilization correlates with enhanced connective tissue area, proteolysis, and apoptosis. *American Journal of Physiology. Endocrinology and Metabolism*, 303(11):E1335–1347, December 2012. PMID: 23032683.

[436] L. M. Baehr, M. Tunzi, and S. C. Bodine. Muscle hypertrophy is associated with increases in proteasome activity that is independent of MuRF1 and MAFbx expression. *Frontiers in Physiology*, 5:69, 2014. PMID: 24600408.

[437] T. Mozaffar, F. Haddad, M. Zeng, L. Y. Zhang, G. R. Adams, and K. M. Baldwin. Molecular and cellular defects of skeletal muscle in an animal model of acute quadriplegic myopathy. *Muscle & Nerve*, 35(1):55–65, January 2007. PMID: 16967495.

[438] L. A. Tintignac, J. Lagirand, S. Batonnet, V. Sirri, M. P. Leibovitch, and S. A. Leibovitch. Degradation of MyoD mediated by the SCF (MAFbx) ubiquitin ligase. *The Journal of Biological Chemistry*, 280(4):2847–2856, January 2005. PMID: 15531760.

[439] J. Lagirand-Cantaloube, N. Offner, A. Csibi, M. P. Leibovitch, S. Batonnet-Pichon, L. A. Tintignac, C. T. Segura, and S. A. Leibovitch. The initiation factor eIF3-f is a major target for atrogin1/MAFbx function in skeletal muscle atrophy. *The EMBO journal*, 27(8):1266–1276, April 2008. PMID: 18354498.

[440] S. H. Witt, H. Granzier, C. C. Witt, and S. Labeit. MURF-1 and MURF-2 target a specific subset of myofibrillar proteins redundantly: towards understanding MURF-dependent muscle ubiquitination. *Journal of Molecular Biology*, 350(4):713–722, July 2005. PMID: 15967462.

[441] S. Hirner, C. Krohne, A. Schuster, S. Hoffmann, S. Witt, R. Erber, C. Sticht, A. Gasch, S. Labeit, and D. Labeit. MuRF1-dependent regulation of systemic carbohydrate metabolism as revealed from transgenic mouse studies. *Journal of Molecular Biology*, 379(4):666–677, June 2008. PMID: 18468620.

[442] B. A. Clarke, D. Drujan, M. S. Willis, L. O. Murphy, R. A. Corpina, E. Burova, S. V. Rakhilin, T. N. Stitt, C. Patterson, E. Latres, and D. J. Glass. The E3 Ligase MuRF1 degrades myosin heavy chain protein in dexamethasone-treated skeletal muscle. *Cell metabolism*, 6(5):376–385, November 2007. PMID: 17983583.

[443] C. Polge, A.-E. Heng, M. Jarzaguet, S. Ventadour, A. Claustre, L. Combaret, D. Béchet, M. Matondo, S. Uttenweiler-Joseph, B. Monsarrat, D. Attaix, and D. Taillandier. Muscle actin is polyubiquitinylated in vitro and in vivo and targeted for breakdown by the E3 ligase MuRF1. *FASEB journal: official*

publication of the Federation of American Societies for Experimental Biology, 25(11):3790–3802, November 2011. PMID: 21764995.

[444] T. N. Stitt, D. Drujan, B. A. Clarke, F. Panaro, Y. Timofeyva, W. O. Kline, M. Gonzalez, G. D. Yancopoulos, and D. J. Glass. The IGF-1/PI3K/Akt pathway prevents expression of muscle atrophy-induced ubiquitin ligases by inhibiting FOXO transcription factors. *Molecular Cell*, 14(3):395–403, May 2004. PMID: 15125842.

[445] H. Sun, Y. Gong, J. Qiu, Y. Chen, F. Ding, and Q. Zhao. TRAF6 inhibition rescues dexamethasone-induced muscle atrophy. *International Journal of Molecular Sciences*, 15(6):11126–11141, 2014. PMID: 24955790.

[446] H. Yang, M. J. Menconi, W. Wei, V. Petkova, and P.-O. Hasselgren. Dexamethasone upregulates the expression of the nuclear cofactor p300 and its interaction with C/EBPbeta in cultured myotubes. *Journal of Cellular Biochemistry*, 94(5):1058–1067, April 2005. PMID: 15669015.

[447] N. Alamdari, I. J. Smith, Z. Aversa, and P.-O. Hasselgren. Sepsis and glucocorticoids upregulate p300 and downregulate HDAC6 expression and activity in skeletal muscle. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, 299(2):R509–520, August 2010. PMID: 20538901.

[448] M. J. Saad, F. Folli, J. A. Kahn, and C. R. Kahn. Modulation of insulin receptor, insulin receptor substrate-1, and phosphatidylinositol 3-kinase in liver and muscle of dexamethasone-treated rats. *Journal of Clinical Investigation*, 92(4):2065–2072, October 1993. PMID: 7691892.

[449] S. P. Weinstein, T. Paquin, A. Pritsker, and R. S. Haber. Glucocorticoid-induced insulin resistance: dexamethasone inhibits the activation of glucose transport in rat skeletal muscle by both insulin- and non-insulin-related stimuli. *Diabetes*, 44(4):441–445, April 1995. PMID:

7698514.

[450] G. Dimitriadis, B. Leighton, M. Parry-Billings, S. Sasson, M. Young, U. Krause, S. Bevan, T. Piva, G. Wegener, and E. A. Newsholme. Effects of glucocorticoid excess on the sensitivity of glucose transport and metabolism to insulin in rat skeletal muscle. *Biochemical Journal*, 321(Pt 3):707–712, February 1997. PMID: 9032457.

[451] Q. Wang, R. Somwar, P. J. Bilan, Z. Liu, J. Jin, J. R. Woodgett, and A. Klip. Protein kinase B/Akt participates in GLUT4 translocation by insulin in L6 myoblasts. *Molecular and Cellular Biology*, 19(6):4008–4018, June 1999. PMID: 10330141.

[452] D. Dardevet, C. Sornet, and J. Grizard. Glucocorticoid-induced insulin resistance of protein synthesis is independent of the rapamycin-sensitive pathways in rat skeletal muscle. *The Journal of Endocrinology*, 162(1):77–85, July 1999. PMID: 10396023.

[453] C. Corporeau, C. L. Foll, M. Taouis, J.-P. Gouygou, J.-P. Bergé, and J. Delarue. Adipose tissue compensates for defect of phosphatidylinositol 3'-kinase induced in liver and muscle by dietary fish oil in fed rats. *American Journal of Physiology. Endocrinology and Metabolism*, 290(1):E78–E86, January 2006. PMID: 16339925.

[454] T. Ishizuka, M. Yamamoto, T. Nagashima, K. Kajita, O. Taniguchi, K. Yasuda, and K. Miura. Effect of dexamethasone and prednisolone on insulin-induced activation of protein kinase C in rat adipocytes and soleus muscles. *Metabolism: Clinical and Experimental*, 44(3):298–306, March 1995. PMID: 7885273.

[455] F. A. Rojas, A. E. Hirata, and M. J. A. Saad. Regulation of insulin receptor substrate-2 tyrosine phosphorylation in animal models of insulin resistance. *Endocrine*, 21(2):115–122, July 2003. PMID: 12897373.

[456] Z. Li, J. L. Joyal, and D. B. Sacks. Binding of IRS proteins to calmodulin is enhanced in insulin resistance. *Biochemistry*, 39(17):5089–5096, May 2000. PMID: 10819975.

[457] S. A. Morgan, M. Sherlock, L. L. Gathercole, G. G. Lavery, C. Lenaghan, I. J. Bujalska, D. Laber, A. Yu, G. Convey, R. Mayers, K. Hegyi, J. K. Sethi, P. M. Stewart, D. M. Smith, and J. W. Tomlinson. 11beta-hydroxysteroid dehydrogenase type 1 regulates glucocorticoid-induced insulin resistance in skeletal muscle. *Diabetes*, 58(11):2506–2515, November 2009. PMID: 19675138.

[458] A. Koh, M. N. Lee, Y. R. Yang, H. Jeong, J. Ghim, J. Noh, J. Kim, D. Ryu, S. Park, P. Song, S.-H. Koo, N. R. Leslie, P.-O. Berggren, J. H. Choi, P.-G. Suh, and S. H. Ryu. C1-Ten is a protein tyrosine phosphatase of insulin receptor substrate 1 (IRS-1), regulating IRS-1 stability and muscle atrophy. *Molecular and cellular biology*, 33(8):1608–1620, April 2013. PMID: 23401856.

[459] Y. H. Son, S.-J. Lee, K.-B. Lee, J.-H. Lee, E. M. Jeong, S. G. Chung, S.-C. Park, and I.-G. Kim. Dexamethasone downregulates caveolin-1 causing muscle atrophy via inhibited insulin signaling. *The Journal of Endocrinology*, 225(1):27–37, April 2015. PMID: 25688118.

[460] M. C. Mendoza, E. E. Er, and J. Blenis. The Ras-ERK and PI3K-mTOR Pathways: Cross-talk and Compensation. *Trends in biochemical sciences*, 36(6):320–328, June 2011. PMID: 21531565.

[461] E. V. Páez Espinosa, E. M. Rocha, L. A. Velloso, A. C. Boschero, and M. J. Saad. Insulin-induced tyrosine phosphorylation of Shc in liver, muscle and adipose tissue of insulin resistant rats. *Molecular and Cellular Endocrinology*, 156(1-2):121–129, October 1999. PMID: 10612430.

[462] M. D. Girón, J. D. Vílchez, S. Shreeram, R. Salto, M. Manzano, E. Cabrera,

N. Campos, N. K. Edens, R. Rueda, and J. M. López Pedrosa. Beta-hydroxy-beta-methylbutyrate (HMB) normalizes dexamethasone-induced autophagy-lysosomal pathway in skeletal muscle. *PLoS ONE*, 10(2), February 2015. PMID: 25658432.

[463] W. Qin, J. Pan, Y. Wu, W. A. Bauman, and C. Cardozo. Protection against dexamethasone-induced muscle atrophy is related to modulation by testosterone of FOXO1 and PGC-1α. *Biochemical and Biophysical Research Communications*, 403(3-4):473–478, December 2010. PMID: 21094144.

[464] J. M. McClung, A. R. Judge, S. K. Powers, and Z. Yan. p38 MAPK links oxidative stress to autophagy-related gene expression in cachectic muscle wasting. *American journal of physiology. Cell physiology*, 298(3):C542–549, March 2010. PMID: 19955483.

[465] O. J. Shah, J. A. Iniguez-Lluhi, A. Romanelli, S. R. Kimball, and L. S. Jefferson. The activated glucocorticoid receptor modulates presumptive autoregulation of ribosomal protein S6 protein kinase, p70 S6K. *The Journal of biological chemistry*, 277(4):2525–2533, January 2002. PMID: 11705993.

[466] F. Giorgino, M. T. Pedrini, L. Matera, and R. J. Smith. Specific increase in p85alpha expression in response to dexamethasone is associated with inhibition of insulin-like growth factor-I stimulated phosphatidylinositol 3-kinase activity in cultured muscle cells. *The Journal of Biological Chemistry*, 272(11):7455–7463, March 1997. PMID: 9054447.

[467] J. R. Singleton, B. L. Baker, and A. Thorburn. Dexamethasone inhibits insulin-like growth factor signaling and potentiates myoblast apoptosis. *Endocrinology*, 141(8):2945–2950, August 2000. PMID: 10919283.

[468] Z. Hu, H. Wang, I. H. Lee, J. Du, and W. E. Mitch. Endogenous glucocorticoids and impaired insulin signaling are both required to

stimulate muscle wasting under pathophysiological conditions in mice. *The Journal of Clinical Investigation*, 119(10):3059–3069, October 2009. PMID: 19759515.

[469] B. Geering, P. R. Cutillas, and B. Vanhaesebroeck. Regulation of class IA PI3Ks: is there a role for monomeric PI3K subunits? *Biochemical Society Transactions*, 35(Pt 2):199–203, April 2007. PMID: 17371237.

[470] J. Burén, Y. C. Lai, M. Lundgren, J. W. Eriksson, and J. Jensen. Insulin action and signalling in fat and muscle from dexamethasone-treated rats. *Archives of Biochemistry and Biophysics*, 474(1):91–101, June 2008. PMID: 18328801.

[471] D. B. Williams, Z. Wan, B. C. Frier, R. C. Bell, C. J. Field, and D. C. Wright. Dietary supplementation with vitamin E and C attenuates dexamethasone-induced glucose intolerance in rats. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, 302(1):R49–58, January 2012. PMID: 22031784.

[472] O. J. Shah, S. R. Kimball, and L. S. Jefferson. Glucocorticoids abate p70S6k and eIF4E function in L6 skeletal myoblasts. *American Journal of Physiology. Endocrinology and Metabolism*, 279(1):E74–E82, July 2000. PMID: 10893325.

[473] T. Gwag, K. Park, E. Kim, C. Son, J. Park, T. Nikawa, and I. Choi. Inhibition of C2C12 myotube atrophy by a novel HSP70 inducer, celastrol, via activation of Akt1 and ERK1/2 pathways. *Archives of biochemistry and biophysics*, 537(1):21–30, September 2013. PMID: 23810294.

[474] A. Fappi, T. S. Godoy, J. R. Maximino, V. R. Rizzato, J. d. C. Neves, G. Chadi, and E. Zanoteli. The effects of omega-3 fatty acid supplementation on dexamethasone-induced muscle atrophy. *BioMed Research International*, 2014:961438, 2014. PMID: 24982916.

[475] O. Schakman, S. Kalista, L. Bertrand, P. Lause, J. Verniers, J. M. Ketelslegers, and J. P. Thissen. Role of Akt/GSK-3beta/beta-catenin transduction pathway in the muscle anti-atrophy action of insulin-like growth factor-I in glucocorticoid-treated rats. *Endocrinology*, 149(8):3900–3908, August 2008. PMID: 18467435.

[476] J. E. Cho, M. Fournier, X. Da, and M. I. Lewis. Time course expression of Foxo transcription factors in skeletal muscle following corticosteroid administration. *Journal of Applied Physiology*, 108(1):137–145, January 2010. PMID: 19850732.

[477] D. D. Sarbassov, D. A. Guertin, S. M. Ali, and D. M. Sabatini. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science (New York, N.Y.)*, 307(5712):1098–1101, February 2005. PMID: 15718470.

[478] D. R. Alessi, M. Andjelkovic, B. Caudwell, P. Cron, N. Morrice, P. Cohen, and B. A. Hemmings. Mechanism of activation of protein kinase B by insulin and IGF-1. *The EMBO Journal*, 15(23):6541–6551, December 1996. PMID: 8978681.

[479] B. D. Manning and L. C. Cantley. Akt/PKB signaling: navigating downstream. *Cell*, 129(7):1261–1274, June 2007. PMID: 17604717.

[480] E. Jacinto, V. Facchinetti, D. Liu, N. Soto, S. Wei, S. Y. Jung, Q. Huang, J. Qin, and B. Su. SIN1/MIP1 maintains rictor-mTOR complex integrity and regulates Akt phosphorylation and substrate specificity. *Cell*, 127(1):125–137, October 2006. PMID: 16962653.

[481] J. Ruzzin and J. Jensen. Contraction activates glucose uptake and glycogen synthase normally in muscles from dexamethasone-treated rats. *American Journal of Physiology. Endocrinology and Metabolism*, 289(2):E241–250, August 2005. PMID: 15741240.

[482] L. Coderre, G. A. Vallega, P. F. Pilch, and S. R. Chipkin. Regulation of glycogen concentration and glycogen synthase activity in skeletal muscle of insulin-resistant rats. *Archives of Biochemistry and Biophysics*, 464(1):144–150, August 2007. PMID: 17509520.

[483] G. I. Welsh and C. G. Proud. Glycogen synthase kinase-3 is rapidly inactivated in response to insulin and phosphorylates eukaryotic initiation factor eIF-2B. *Biochemical Journal*, 294(Pt 3):625–629, September 1993. PMID: 8397507.

[484] K. J. P. Verhees, A. M. W. J. Schols, M. C. J. M. Kelders, C. M. H. Op den Kamp, J. L. J. van der Velden, and R. C. J. Langen. Glycogen synthase kinase-3β is required for the induction of skeletal muscle atrophy. *American Journal of Physiology. Cell Physiology*, 301(5):C995–C1007, November 2011. PMID: 21832246.

[485] H. H. Zhang, A. I. Lipovsky, C. C. Dibble, M. Sahin, and B. D. Manning. S6K1 regulates GSK3 under conditions of mTOR-dependent feedback inhibition of Akt. *Molecular cell*, 24(2):185–197, October 2006. PMID: 17052453.

[486] F. Dai, L. Yu, H. He, Y. Chen, J. Yu, Y. Yang, Y. Xu, W. Ling, and S. Zhao. Human serum and glucocorticoid-inducible kinase-like kinase (SGKL) phosphorylates glycogen syntheses kinase 3 beta (GSK-3beta) at serine-9 through direct interaction. *Biochemical and Biophysical Research Communications*, 293(4):1191–1196, May 2002. PMID: 12054501.

[487] S. Frame and P. Cohen. GSK3 takes centre stage more than 20 years after its discovery. *Biochemical Journal*, 359(Pt 1):1–16, October 2001. PMID: 11563964.

[488] J. M. Sacheck, A. Ohtsuka, S. C. McLary, and A. L. Goldberg. IGF-I stimulates muscle growth by suppressing protein breakdown and

expression of atrophy-related ubiquitin ligases, atrogin-1 and MuRF1. *American journal of physiology. Endocrinology and metabolism*, 287(4):E591–601, October 2004. PMID: 15100091.

[489] D. A. Guertin and D. M. Sabatini. Defining the role of mTOR in cancer. *Cancer Cell*, 12(1):9–22, July 2007. PMID: 17613433.

[490] M. Laplante and D. M. Sabatini. mTOR signaling at a glance. *Journal of Cell Science*, 122(20):3589–3594, October 2009. PMID: 19812304.

[491] K. Inoki, Y. Li, T. Zhu, J. Wu, and K.-L. Guan. TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. *Nature Cell Biology*, 4(9):648–657, September 2002. PMID: 12172553.

[492] Y. Sancak, C. C. Thoreen, T. R. Peterson, R. A. Lindquist, S. A. Kang, E. Spooner, S. A. Carr, and D. M. Sabatini. PRAS40 is an insulin-regulated inhibitor of the mTORC1 protein kinase. *Molecular Cell*, 25(6):903–915, March 2007. PMID: 17386266.

[493] S.-L. Cai, A. R. Tee, J. D. Short, J. M. Bergeron, J. Kim, J. Shen, R. Guo, C. L. Johnson, K. Kiguchi, and C. L. Walker. Activity of TSC2 is inhibited by AKT-mediated phosphorylation and membrane partitioning. *The Journal of Cell Biology*, 173(2):279–289, April 2006. PMID: 16636147.

[494] H. Wang, N. Kubica, L. W. Ellisen, L. S. Jefferson, and S. R. Kimball. Dexamethasone represses signaling through the mammalian target of rapamycin in muscle cells by enhancing expression of REDD1. *The Journal of Biological Chemistry*, 281(51):39128–39134, December 2006. PMID: 17074751.

[495] H.-J. Koh, J. Brandauer, and L. J. Goodyear. LKB1 and AMPK and the regulation of skeletal muscle metabolism. *Current opinion in clinical nutrition and metabolic care*, 11(3):227–232, May 2008. PMID: 18403917.

[496] C. Cantó and J. Auwerx. AMP-activated protein kinase and its downstream

transcriptional pathways. *Cellular and molecular life sciences : CMLS*, 67(20):3407–3423, October 2010. PMID: 20640476.

[497] S. L. U. Amaral, J. C. U. de Andrade Louzada, T. J. Dionisio, B. A. U. Viscelli, E. J. U. Dionisio, J. R. U. Bosqueiro, and A. M. U. Martuscelli. Opposite effects of exercise and dexamethasone on skeletal muscle glucose uptake: Role of AMPK and CaMKII. *FASEB Journal*, page 1, April 2010.

[498] J.-F. Dumas, G. Bielicki, J.-P. Renou, D. Roussel, P.-H. Ducluzeau, Y. Malthièry, G. Simard, and P. Ritz. Dexamethasone impairs muscle energetics, studied by (31)P NMR, in rats. *Diabetologia*, 48(2):328–335, February 2005. PMID: 15645207.

[499] K. Weber, P. Brück, Z. Mikes, J.-H. Küpper, M. Klingenspor, and R. J. Wiesner. Glucocorticoid hormone stimulates mitochondrial biogenesis specifically in skeletal muscle. *Endocrinology*, 143(1):177–184, January 2002. PMID: 11751607.

[500] N. Nordsborg, C. Goodmann, M. J. McKenna, and J. Bangsbo. Dexamethasone up-regulates skeletal muscle maximal Na+,K+ pump activity by muscle group specific mechanisms in humans. *The Journal of physiology*, 567(Pt 2):583–589, September 2005. PMID: 15975987.

[501] G. N. Nakken, D. L. Jacobs, D. M. Thomson, N. Fillmore, and W. W. Winder. Effects of excess corticosterone on LKB1 and AMPK signaling in rat skeletal muscle. *Journal of Applied Physiology*, 108(2):298–305, February 2010. PMID: 19959768.

[502] F. A. Britto, G. Begue, B. Rossano, A. Docquier, B. Vernus, C. Sar, A. Ferry, A. Bonnieu, V. Ollendorff, and F. B. Favier. REDD1 deletion prevents dexamethasone-induced skeletal muscle atrophy. *American Journal of Physiology. Endocrinology and Metabolism*, page ajpendo.00234.2014, October 2014. PMID: 25315696.

[503] M. P. DeYoung, P. Horak, A. Sofer, D. Sgroi, and L. W. Ellisen. Hypoxia regulates TSC1/2–mTOR signaling and tumor suppression through REDD1-mediated 14–3–3 shuttling. *Genes & Development*, 22(2):239–251, January 2008. PMID: 18198340.

[504] A. Sofer, K. Lei, C. M. Johannessen, and L. W. Ellisen. Regulation of mTOR and Cell Growth in Response to Energy Stress by REDD1. *Molecular and Cellular Biology*, 25(14):5834–5845, July 2005. PMID: 15988001.

[505] O. J. Shah, J. C. Anthony, S. R. Kimball, and L. S. Jefferson. Glucocorticoids oppose translational control by leucine in skeletal muscle. *American Journal of Physiology. Endocrinology and Metabolism*, 279(5):E1185–1190, November 2000. PMID: 11052975.

[506] W. Long, L. Wei, and E. J. Barrett. Dexamethasone inhibits the stimulation of muscle protein synthesis and PHAS-I and p70 S6-kinase phosphorylation. *American Journal of Physiology. Endocrinology and Metabolism*, 280(4):E570–575, April 2001. PMID: 11254463.

[507] W. Long, E. J. Barrett, L. Wei, and Z. Liu. Adrenalectomy enhances the insulin sensitivity of muscle protein synthesis. *American Journal of Physiology. Endocrinology and Metabolism*, 284(1):E102–109, January 2003. PMID: 12388148.

[508] Y. Wu, E. J. Barrett, W. Long, and Z. Liu. Glucocorticoids differentially modulate insulin-mediated protein and glycogen synthetic signaling downstream of protein kinase B in rat myocardium. *Endocrinology*, 145(3):1161–1166, March 2004. PMID: 14630710.

[509] E. Latres, A. R. Amini, A. A. Amini, J. Griffiths, F. J. Martin, Y. Wei, H. C. Lin, G. D. Yancopoulos, and D. J. Glass. Insulin-like growth factor-1 (IGF-1) inversely regulates atrophy-induced genes via the phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin

(PI3K/Akt/mTOR) pathway. *The Journal of biological chemistry*, 280(4):2737–2744, January 2005. PMID: 15550386.

[510] R. A. Frost, D. Huber, A. Pruznak, and C. H. Lang. Regulation of REDD1 by insulin-like growth factor-I in skeletal muscle and myotubes. *Journal of cellular biochemistry*, 108(5):1192–1202, December 2009. PMID: 19795384.

[511] R. Conejo and M. Lorenzo. Insulin signaling leading to proliferation, survival, and membrane ruffling in C2C12 myoblasts. *Journal of Cellular Physiology*, 187(1):96–108, April 2001. PMID: 11241354.

[512] N. Hosokawa, T. Hara, T. Kaizuka, C. Kishi, A. Takamura, Y. Miura, S.-i. Iemura, T. Natsume, K. Takehana, N. Yamada, J.-L. Guan, N. Oshiro, and N. Mizushima. Nutrient-dependent mTORC1 Association with the ULK1–Atg13–FIP200 Complex Required for Autophagy. *Molecular Biology of the Cell*, 20(7):1981–1991, April 2009. PMID: 19211835.

[513] M. D. Dennis, C. S. Coleman, A. Berg, L. S. Jefferson, and S. R. Kimball. REDD1 enhances protein phosphatase 2A-mediated dephosphorylation of Akt to repress mTORC1 signaling. *Science signaling*, 7(335):ra68, July 2014. PMID: 25056877.

[514] M. Canal, J. Romaní Aumedes, N. Martín Flores, V. Pérez Fernández, and C. Malagelada. RTP801/REDD1: a stress coping regulator that turns into a troublemaker in neurodegenerative disorders. *Frontiers in Cellular Neuroscience*, 8, October 2014. PMID: 25324725.

[515] M. Barel, O. A. B. Perez, V. A. Giozzet, A. Rafacho, J. R. Bosqueiro, and S. L. do Amaral. Exercise training prevents hyperinsulinemia, muscular glycogen loss and muscle atrophy induced by dexamethasone treatment. *European Journal of Applied Physiology*, 108(5):999–1007, March 2010. PMID: 19967395.

[516] W. Qin, J. Pan, Y. Qin, D. N. Lee, W. A. Bauman, and C. Cardozo. Identification of functional glucocorticoid response elements in the mouse FoxO1 promoter. *Biochemical and Biophysical Research Communications*, 450(2):979–983, July 2014. PMID: 24971545.

[517] N. Lützner, H. Kalbacher, A. Krones-Herzig, and F. Rösl. FOXO3 is a glucocorticoid receptor target and regulates LKB1 and its own expression based on cellular AMP levels via a positive autoregulatory loop. *PLoS ONE*, 7(7), July 2012. PMID: 22848740.

[518] D. R. Calnan and A. Brunet. The FoxO code. *Oncogene*, 27(16):2276–2288, April 2008. PMID: 18391970.

[519] M. Sandri, C. Sandri, A. Gilbert, C. Skurk, E. Calabria, A. Picard, K. Walsh, S. Schiaffino, S. H. Lecker, and A. L. Goldberg. Foxo transcription factors induce the atrophy-related ubiquitin ligase atrogin-1 and cause skeletal muscle atrophy. *Cell*, 117(3):399–412, April 2004. PMID: 15109499.

[520] A. Jones, D.-J. Hwang, R. Narayanan, D. D. Miller, and J. T. Dalton. Effects of a novel selective androgen receptor modulator on dexamethasone-induced and hypogonadism-induced muscle atrophy. *Endocrinology*, 151(8):3706–3719, August 2010. PMID: 20534726.

[521] S. R. Jesinkey, M. C. Korrapati, K. A. Rasbach, C. C. Beeson, and R. G. Schnellmann. Atomoxetine prevents dexamethasone-induced skeletal muscle atrophy in mice. *The Journal of Pharmacology and Experimental Therapeutics*, 351(3):663–673, December 2014. PMID: 25292181.

[522] C. Ling, P. Poulsen, E. Carlsson, M. Ridderstråle, P. Almgren, J. Wojtaszewski, H. Beck-Nielsen, L. Groop, and A. Vaag. Multiple environmental and genetic factors influence skeletal muscle PGC-1α and PGC-1β gene expression in twins. *Journal of Clinical Investigation*, 114(10):1518–1526, November 2004. PMID: 15546003.

[523] M. J. Menconi, Z. P. Arany, N. Alamdari, Z. Aversa, P. Gonnella, P. O'Neal, I. J. Smith, S. Tizio, and P.-O. Hasselgren. Sepsis and glucocorticoids downregulate the expression of the nuclear cofactor PGC-1beta in skeletal muscle. *American journal of physiology. Endocrinology and metabolism*, 299(4):E533–543, October 2010. PMID: 20647557.

[524] J. L. Ruas, J. P. White, R. R. Rao, S. Kleiner, K. T. Brannan, B. C. Harrison, N. P. Greene, J. Wu, J. L. Estall, B. A. Irving, I. R. Lanza, K. A. Rasbach, M. Okutsu, K. S. Nair, Z. Yan, L. A. Leinwand, and B. M. Spiegelman. A PGC-1alpha isoform induced by resistance training regulates skeletal muscle hypertrophy. *Cell*, 151(6):1319–1331, December 2012. PMID: 23217713.

[525] E. Castillero, N. Alamdari, Z. Aversa, A. Gurav, and P.-O. Hasselgren. PPARβ/δ regulates glucocorticoid- and sepsis-induced FOXO1 activation and muscle wasting. *PLoS ONE*, 8(3), March 2013. PMID: 23555761.

[526] H. Daitoku, J.-I. Sakamaki, and A. Fukamizu. Regulation of FoxO transcription factors by acetylation and protein-protein interactions. *Biochimica Et Biophysica Acta*, 1813(11):1954–1960, November 2011. PMID: 21396404.

[527] J. Nakae, M. Oki, and Y. Cao. The FoxO transcription factors and metabolic regulation. *FEBS letters*, 582(1):54–67, January 2008. PMID: 18022395.

[528] M. B. Hudson, J. A. Rahnert, B. Zheng, M. E. Woodworth-Hobbs, H. A. Franch, and S. R. Price. miR-182 attenuates atrophy-related gene expression by targeting FoxO3 in skeletal muscle. *American Journal of Physiology. Cell Physiology*, May 2014. PMID: 24871856.

[529] P. A. King, L. Goldstein, and E. A. Newsholme. Glutamine synthetase activity of muscle in acidosis. *Biochemical Journal*, 216(2):523–525, November 1983. PMID: 6140920.

[530] Y. Kamei, M. Hattori, Y. Hatazawa, T. Kasahara, M. Kanou, S. Kanai, X. Yuan, T. Suganami, W. H. Lamers, T. Kitamura, and Y. Ogawa. FOXO1 activates glutamine synthetase gene in mouse skeletal muscles through a region downstream of 3'-UTR: possible contribution to ammonia detoxification. *American Journal of Physiology. Endocrinology and Metabolism*, 307(6):E485–493, September 2014. PMID: 25074987.

[531] S. M. Senf, S. L. Dodd, and A. R. Judge. FOXO signaling is required for disuse muscle atrophy and is directly regulated by Hsp70. *American Journal of Physiology. Cell Physiology*, 298(1):C38–45, January 2010. PMID: 19864323.

[532] M. Sandri, J. Lin, C. Handschin, W. Yang, Z. P. Arany, S. H. Lecker, A. L. Goldberg, and B. M. Spiegelman. PGC-1alpha protects skeletal muscle from atrophy by suppressing FoxO3 action and atrophy-specific gene transcription. *Proceedings of the National Academy of Sciences of the United States of America*, 103(44):16260–16265, October 2006. PMID: 17053067.

[533] L. M. Bollinger, C. A. Witczak, J. A. Houmard, and J. J. Brault. SMAD3 augments FoxO3-induced MuRF-1 promoter activity in a DNA-binding-dependent manner. *American Journal of Physiology. Cell Physiology*, 307(3):C278–287, August 2014. PMID: 24920680.

[534] J. D. Furlow, M. L. Watson, D. S. Waddell, E. S. Neff, L. M. Baehr, A. P. Ross, and S. C. Bodine. Altered gene expression patterns in Muscle Ring Finger 1 null mice during denervation and dexamethasone-induced muscle atrophy. *Physiological genomics*, October 2013. PMID: 24130153.

[535] N. Shimizu, N. Yoshikawa, N. Ito, T. Maruyama, Y. Suzuki, S.-i. Takeda, J. Nakae, Y. Tagata, S. Nishitani, K. Takehana, M. Sano, K. Fukuda, M. Suematsu, C. Morimoto, and H. Tanaka. Crosstalk between

glucocorticoid receptor and nutritional sensor mTOR in skeletal muscle. *Cell Metabolism*, 13(2):170–182, February 2011. PMID: 21284984.

[536] S. M. Senf, S. L. Dodd, J. M. McClung, and A. R. Judge. Hsp70 overexpression inhibits NF-kappaB and Foxo3a transcriptional activities and prevents skeletal muscle atrophy. *FASEB journal: official publication of the Federation of American Societies for Experimental Biology*, 22(11):3836–3845, November 2008. PMID: 18644837.

[537] I. J. Smith, N. Alamdari, P. O'Neal, P. Gonnella, Z. Aversa, and P.-O. Hasselgren. Sepsis increases the expression and activity of the transcription factor Forkhead Box O 1 (FOXO1) in skeletal muscle by a glucocorticoid-dependent mechanism. *The international journal of biochemistry & cell biology*, 42(5):701–711, May 2010. PMID: 20079455.

[538] K. S. Bongers, D. K. Fox, S. M. Ebert, S. D. Kunkel, M. C. Dyle, S. A. Bullard, J. M. Dierdorff, and C. M. Adams. Skeletal muscle denervation causes skeletal muscle atrophy through a pathway that involves both Gadd45a and HDAC4. *American Journal of Physiology - Endocrinology and Metabolism*, 305(7):E907–E915, October 2013. PMID: 23941879.

[539] K. Ma, C. Mallidis, S. Bhasin, V. Mahabadi, J. Artaza, N. Gonzalez-Cadavid, J. Arias, and B. Salehian. Glucocorticoid-induced skeletal muscle atrophy is associated with upregulation of myostatin gene expression. *American Journal of Physiology. Endocrinology and Metabolism*, 285(2):E363–371, August 2003. PMID: 12721153.

[540] D. L. Allen and T. G. Unterman. Regulation of myostatin expression and myoblast differentiation by FoxO and SMAD transcription factors. *American Journal of Physiology. Cell Physiology*, 292(1):C188–199, January 2007. PMID: 16885393.

[541] D. L. Allen, A. S. Cleary, A. M. Hanson, S. F. Lindsay, and J. M. Reed.

CCAAT/enhancer binding protein-delta expression is increased in fast skeletal muscle by food deprivation and regulates myostatin transcription in vitro. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, 299(6):R1592–1601, December 2010. PMID: 20844262.

[542] V. Proserpio, R. Fittipaldi, J. G. Ryall, V. Sartorelli, and G. Caretti. The methyltransferase SMYD3 mediates the recruitment of transcriptional cofactors at the myostatin and c-Met genes and regulates skeletal muscle atrophy. *Genes & development*, 27(11):1299–1312, June 2013. PMID: 23752591.

[543] D. L. Allen and A. S. Loh. Posttranscriptional mechanisms involving microRNA-27a and b contribute to fast-specific and glucocorticoid-mediated myostatin expression in skeletal muscle. *American journal of physiology. Cell physiology*, 300(1):C124–137, January 2011. PMID: 20980549.

[544] B. Salehian, V. Mahabadi, J. Bilas, W. E. Taylor, and K. Ma. The effect of glutamine on prevention of glucocorticoid-induced skeletal muscle atrophy is associated with myostatin suppression. *Metabolism: clinical and experimental*, 55(9):1239–1247, September 2006. PMID: 16919545.

[545] T. A. Zimmers, M. V. Davies, L. G. Koniaris, P. Haynes, A. F. Esquela, K. N. Tomkinson, A. C. McPherron, N. M. Wolfman, and S.-J. Lee. Induction of cachexia in mice by systemically administered myostatin. *Science (New York, N.Y.)*, 296(5572):1486–1488, May 2002. PMID: 12029139.

[546] R. Sartori, G. Milan, M. Patron, C. Mammucari, B. Blaauw, R. Abraham, and M. Sandri. Smad2 and 3 transcription factors control muscle mass in adulthood. *American Journal of Physiology. Cell Physiology*, 296(6):C1248–1257, June 2009. PMID: 19357234.

[547] A. Amirouche, A.-C. Durieux, S. Banzet, N. Koulmann, R. Bonnefoy, C. Mouret, X. Bigard, A. Peinnequin, and D. Freyssenet. Down-regulation of Akt/mammalian target of rapamycin signaling pathway in response to myostatin overexpression in skeletal muscle. *Endocrinology*, 150(1):286–294, January 2009. PMID: 18801898.

[548] A. U. Trendelenburg, A. Meyer, D. Rohner, J. Boyle, S. Hatakeyama, and D. J. Glass. Myostatin reduces Akt/TORC1/p70S6K signaling, inhibiting myoblast differentiation and myotube size. *American Journal of Physiology. Cell Physiology*, 296(6):C1258–1270, June 2009. PMID: 19357233.

[549] S. McCroskery, M. Thomas, L. Maxwell, M. Sharma, and R. Kambadur. Myostatin negatively regulates satellite cell activation and self-renewal. *The Journal of Cell Biology*, 162(6):1135–1147, September 2003. PMID: 12963705.

[550] T. V. Cohen, H. D. Kollias, N. Liu, C. W. Ward, and K. R. Wagner. Genetic disruption of Smad7 impairs skeletal muscle growth and regeneration. *The Journal of Physiology*, April 2015. PMID: 25854148.

[551] M. Nozaki, Y. Li, J. Zhu, F. Ambrosio, K. Uehara, F. H. Fu, and J. Huard. Improved muscle healing after contusion injury by the inhibitory effect of suramin on myostatin, a negative regulator of muscle growth. *The American Journal of Sports Medicine*, 36(12):2354–2362, December 2008. PMID: 18725651.

[552] G. Carnac, B. Vernus, and A. Bonnieu. Myostatin in the pathophysiology of skeletal muscle. *Current Genomics*, 8(7):415–422, November 2007. PMID: 19412331.

[553] B. Elliott, D. Renshaw, S. Getting, and R. Mackenzie. The central role of myostatin in skeletal muscle and whole body homeostasis. *Acta*

Physiologica (Oxford, England), 205(3):324–340, July 2012. PMID: 22340904.

[554] R. Lalani, S. Bhasin, F. Byhower, R. Tarnuzzer, M. Grant, R. Shen, S. Asa, S. Ezzat, and N. F. Gonzalez-Cadavid. Myostatin and insulin-like growth factor-I and -II expression in the muscle of rats exposed to the microgravity environment of the NeuroLab space shuttle flight. *The Journal of Endocrinology*, 167(3):417–428, December 2000. PMID: 11115768.

[555] R. F. Peters, M. C. Richardson, M. Small, and A. M. White. Some biochemical effects of triamcinolone acetonide on rat liver and muscle. *Biochemical Journal*, 116(3):349–355, February 1970. PMID: 5435682.

[556] I. Rieu, C. Sornet, J. Grizard, and D. Dardevet. Glucocorticoid excess induces a prolonged leucine resistance on muscle protein synthesis in old rats. *Experimental Gerontology*, 39(9):1315–1321, September 2004. PMID: 15489054.

[557] I. Savary, E. Debras, D. Dardevet, C. Sornet, P. Capitan, J. Prugnaud, P. P. Mirand, and J. Grizard. Effect of glucocorticoid excess on skeletal muscle and heart protein synthesis in adult and old rats. *The British Journal of Nutrition*, 79(3):297–304, March 1998. PMID: 9577308.

[558] Z. Aversa, N. Alamdari, E. Castillero, M. Muscaritoli, F. Rossi Fanelli, and P.-O. Hasselgren. Beta-Hydroxy-beta-methylbutyrate (HMB) prevents dexamethasone-induced myotube atrophy. *Biochemical and biophysical research communications*, 423(4):739–743, July 2012. PMID: 22705301.

[559] M. M. Desler, S. J. Jones, C. W. Smith, and T. L. Woods. Effects of dexamethasone and anabolic agents on proliferation and protein synthesis and degradation in C2C12 myogenic cells. *Journal of animal science*, 74(6):1265–1273, June 1996. PMID: 8791198.

[560] K. L. Jones, J. Harty, M. J. Roeder, T. A. Winters, and W. J. Banz. In vitro

effects of soy phytoestrogens on rat L6 skeletal muscle cells. *Journal of medicinal food*, 8(3):327–331, 2005. PMID: 16176142.

[561] V. R. Young, S. C. Chen, and J. Macdonald. The sedimentation of rat skeletal-muscle ribosomes. Effect of hydrocortisone, insulin and diet. *Biochemical Journal*, 106(4):913–919, February 1968. PMID: 5637371.

[562] H. Bai, P. Kang, A. M. Hernandez, and M. Tatar. Activin signaling targeted by insulin/dFOXO regulates aging and muscle proteostasis in drosophila. *PLoS Genetics*, 9(11), November 2013. PMID: 24244197.

[563] M. Livingstone and M. Bidinosti. Rapamycin-insensitive mTORC1 activity controls eIF4E:4E-BP1 binding. *F1000Research*, 1, July 2012. PMID: 24358826.

[564] S.-I. Hu, M. Katz, S. Chin, X. Qi, J. Cruz, C. Ibebunjo, S. Zhao, A. Chen, and D. J. Glass. MNK2 inhibits eIF4G activation through a pathway involving serine-arginine-rich protein kinase in skeletal muscle. *Science signaling*, 5(211):ra14, February 2012. PMID: 22337810.

[565] T. C. Vary and C. J. Lynch. Nutrient signaling components controlling protein synthesis in striated muscle. *The Journal of Nutrition*, 137(8):1835–1843, August 2007. PMID: 17634251.

[566] A. Csibi, K. Cornille, M.-P. Leibovitch, A. Poupon, L. A. Tintignac, A. M. J. Sanchez, and S. A. Leibovitch. The translation regulatory subunit eIF3f controls the kinase-dependent mTOR signaling required for muscle differentiation and hypertrophy in mouse. *PloS One*, 5(2):e8994, 2010. PMID: 20126553.

[567] J. Gallinetti, E. Harputlugil, and J. R. Mitchell. Amino acid sensing in dietary-restriction-mediated longevity: roles of signal-transducing kinases GCN2 and TOR. *The Biochemical journal*, 449(1):1–10, January 2013. PMID: 23216249.

[568] J. J. Berlanga, J. Santoyo, and C. De Haro. Characterization of a mammalian homolog of the GCN2 eukaryotic initiation factor 2alpha kinase. *European journal of biochemistry / FEBS*, 265(2):754–762, October 1999. PMID: 10504407.

[569] M. Gross, M. Wing, C. Rundquist, and M. S. Rubino. Evidence that phosphorylation of eIF-2(alpha) prevents the eIF-2B-mediated dissociation of eIF-2 X GDP from the 60 S subunit of complete initiation complexes. *The Journal of Biological Chemistry*, 262(14):6899–6907, May 1987. PMID: 3646234.

[570] R. Sood, A. C. Porter, D. A. Olsen, D. R. Cavener, and R. C. Wek. A mammalian homologue of GCN2 protein kinase important for translational control by phosphorylation of eukaryotic initiation factor-2alpha. *Genetics*, 154(2):787–801, February 2000. PMID: 10655230.

[571] A. Gjymishka, N. Su, and M. S. Kilberg. Transcriptional induction of the human asparagine synthetase gene during the unfolded protein response does not require the ATF6 and IRE1/XBP1 arms of the pathway. *The Biochemical journal*, 417(3):695–703, February 2009. PMID: 18840095.

[572] S. M. Ebert, M. C. Dyle, S. D. Kunkel, S. A. Bullard, K. S. Bongers, D. K. Fox, J. M. Dierdorff, E. D. Foster, and C. M. Adams. Stress-induced skeletal muscle Gadd45a expression reprograms myonuclei and causes muscle atrophy. *The Journal of Biological Chemistry*, 287(33):27290–27301, August 2012. PMID: 22692209.

[573] C. M. Adams. Role of the transcription factor ATF4 in the anabolic actions of insulin and the anti-anabolic actions of glucocorticoids. *The Journal of Biological Chemistry*, 282(23):16744–16753, June 2007. PMID: 17430894.

[574] S. Rossi, E. Stoppani, W. Martinet, A. Bonetto, P. Costelli, R. Giuliani, F. Colombo, A. Preti, S. Marchesini, and A. Fanzani. The cytosolic sialidase

Neu2 is degraded by autophagy during myoblast atrophy. *Biochimica Et Biophysica Acta*, 1790(8):817–828, August 2009. PMID: 19371771.

[575] C. Deval, S. Mordier, C. Obled, D. Bechet, L. Combaret, D. Attaix, and M. Ferrara. Identification of cathepsin L as a differentially expressed message associated with skeletal muscle wasting. *The Biochemical Journal*, 360(Pt 1):143–150, November 2001. PMID: 11696001.

[576] R. Troncoso, F. Paredes, V. Parra, D. Gatica, C. Vásquez Trincado, C. Quiroga, R. Bravo-Sagua, C. López Crisosto, A. E. Rodriguez, A. P. Oyarzún, G. Kroemer, and S. Lavandero. Dexamethasone-induced autophagy mediates muscle atrophy through mitochondrial clearance. *Cell Cycle (Georgetown, Tex.)*, 13(14):2281–2295, July 2014. PMID: 24897381.

[577] D. Yamamoto, T. Maki, E. H. Herningtyas, N. Ikeshita, H. Shibahara, Y. Sugiyama, S. Nakanishi, K. Iida, G. Iguchi, Y. Takahashi, H. Kaji, K. Chihara, and Y. Okimura. Branched-chain amino acids protect against dexamethasone-induced soleus muscle atrophy in rats. *Muscle & Nerve*, 41(6):819–827, June 2010. PMID: 20169591.

[578] H. P. Rodemann, L. Waxman, and A. L. Goldberg. The stimulation of protein degradation in muscle by Ca2+ is mediated by prostaglandin E2 and does not require the calcium-activated protease. *The Journal of Biological Chemistry*, 257(15):8716–8723, August 1982. PMID: 6807980.

[579] K. Furuno and A. L. Goldberg. The activation of protein degradation in muscle by Ca2+ or muscle injury does not involve a lysosomal mechanism. *The Biochemical Journal*, 237(3):859–864, August 1986. PMID: 3099758.

[580] E. V. Kachaeva and B. S. Shenkman. Various jobs of proteolytic enzymes in skeletal muscle during unloading: facts and speculations. *Journal of biomedicine & biotechnology*, 2012:493618, 2012. PMID: 22496611.

[581] J. G. Tidball and M. J. Spencer. Expression of a calpastatin transgene slows

muscle wasting and obviates changes in myosin isoform expression during murine muscle disuse. *The Journal of Physiology*, 545(Pt 3):819–828, December 2002. PMID: 12482888.

[582] M. U. Fareed, A. R. Evenson, W. Wei, M. Menconi, V. Poylin, V. Petkova, B. Pignol, and P.-O. Hasselgren. Treatment of rats with calpain inhibitors prevents sepsis-induced muscle proteolysis independent of atrogin-1/MAFbx and MuRF1 expression. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, 290(6):R1589–1597, June 2006. PMID: 16455766.

[583] K. Itagaki, M. Menconi, B. Antoniu, Q. Zhang, P. Gonnella, D. Soybel, C. Hauser, and P.-O. Hasselgren. Dexamethasone stimulates store-operated calcium entry and protein degradation in cultured L6 myotubes through a phospholipase A(2)-dependent mechanism. *American journal of physiology. Cell physiology*, 298(5):C1127–1139, May 2010. PMID: 20107037.

[584] D. E. Goll, V. F. Thompson, H. Li, W. Wei, and J. Cong. The calpain system. *Physiological Reviews*, 83(3):731–801, July 2003. PMID: 12843408.

[585] S. H. Lecker, R. T. Jagoe, A. Gilbert, M. Gomes, V. Baracos, J. Bailey, S. R. Price, W. E. Mitch, and A. L. Goldberg. Multiple types of skeletal muscle atrophy involve a common program of changes in gene expression. *The FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology*, 18(1):39–51, January 2004. PMID: 14718385.

[586] F. J. Ballard and G. L. Francis. Effects of anabolic agents on protein breakdown in L6 myoblasts. *The Biochemical journal*, 210(1):243–249, January 1983. PMID: 6342615.

[587] D. Z. Ewton and J. R. Florini. Effects of the somatomedins and insulin on

myoblast differentiation in vitro. *Developmental Biology*, 86(1):31–39, August 1981. PMID: 6169566.

[588] P. Elsner, B. Quistorff, T. S. Hermann, J. Dich, and N. Grunnet. Regulation of glycogen accumulation in L6 myotubes cultured under optimized differentiation conditions. *The American journal of physiology*, 275(6 Pt 1):E925–933, December 1998. PMID: 9843733.

[589] O. Schakman, H. Gilson, V. de Coninck, P. Lause, J. Verniers, X. Havaux, J. M. Ketelslegers, and J. P. Thissen. Insulin-like growth factor-I gene transfer by electroporation prevents skeletal muscle atrophy in glucocorticoid-treated rats. *Endocrinology*, 146(4):1789–1797, April 2005. PMID: 15661854.

[590] F. Kanda, K. Takatani, S. Okuda, T. Matsushita, and K. Chihara. Preventive effects of insulinlike growth factor-I on steroid-induced muscle atrophy. *Muscle & Nerve*, 22(2):213–217, February 1999. PMID: 10024134.

[591] D. Chrysis, J. Zhang, and L. E. Underwood. Divergent regulation of proteasomes by insulin-like growth factor I and growth hormone in skeletal muscle of rats made catabolic with dexamethasone. *Growth hormone & IGF research: official journal of the Growth Hormone Research Society and the International IGF Research Society*, 12(6):434–441, December 2002. PMID: 12423629.

[592] D. Chrysis and L. E. Underwood. Regulation of components of the ubiquitin system by insulin-like growth factor I and growth hormone in skeletal muscle of rats made catabolic with dexamethasone. *Endocrinology*, 140(12):5635–5641, December 1999. PMID: 10579327.

[593] K. Kimura, F. Kanda, S. Okuda, and K. Chihara. Insulin-like growth factor 1 inhibits glucocorticoid-induced glutamine synthetase activity in cultured L6 rat skeletal muscle cells. *Neuroscience letters*, 302(2-3):154–156, April

2001. PMID: 11290410.

[594] G. Gayan-Ramirez, F. Vanderhoydonc, G. Verhoeven, and M. Decramer. Acute treatment with corticosteroids decreases IGF-1 and IGF-2 expression in the rat diaphragm and gastrocnemius. *American Journal of Respiratory and Critical Care Medicine*, 159(1):283–289, January 1999. PMID: 9872851.

[595] B. J. Petrof, S. B. Gottfried, J. Eby, J. Lamanca, and S. Levine. Growth hormone does not prevent corticosteroid-induced changes in rat diaphragm structure and function. *Journal of Applied Physiology (Bethesda, Md.: 1985)*, 79(5):1571–1577, November 1995. PMID: 8594016.

[596] L. S. Quinn, B. G. Anderson, and S. R. Plymate. Muscle-specific overexpression of the type 1 IGF receptor results in myoblast-independent muscle hypertrophy via PI3K, and not calcineurin, signaling. *American journal of physiology. Endocrinology and metabolism*, 293(6):E1538–1551, December 2007. PMID: 17940216.

[597] B.-G. Li, P.-O. Hasselgren, C.-H. Fang, and G. D. Warden. Insulin-like growth factor-I blocks dexamethasone-induced protein degradation in cultured myotubes by inhibiting multiple proteolytic pathways: 2002 ABA paper. *The Journal of burn care & rehabilitation*, 25(1):112–118, February 2004. PMID: 14726748.

[598] B.-G. Li, P.-O. Hasselgren, and C.-H. Fang. Insulin-like growth factor-I inhibits dexamethasone-induced proteolysis in cultured L6 myotubes through PI3K/Akt/GSK-3beta and PI3K/Akt/mTOR-dependent mechanisms. *The International Journal of Biochemistry & Cell Biology*, 37(10):2207–2216, October 2005. PMID: 15927518.

[599] O. J. Shah, S. R. Kimball, and L. S. Jefferson. Among translational effectors, p70S6k is uniquely sensitive to inhibition by glucocorticoids. *The*

Biochemical Journal, 347(Pt 2):389–397, April 2000. PMID: 10749668.

[600] A. R. Evenson, M. U. Fareed, M. J. Menconi, J. C. Mitchell, and P.-O. Hasselgren. GSK-3beta inhibitors reduce protein degradation in muscles from septic rats and in dexamethasone-treated myotubes. *The International Journal of Biochemistry & Cell Biology*, 37(10):2226–2238, October 2005. PMID: 16051512.

[601] N. G. Williams, J. P. Interlichia, M. F. Jackson, D. Hwang, P. Cohen, and B. D. Rodgers. Endocrine actions of myostatin: systemic regulation of the IGF and IGF binding protein axis. *Endocrinology*, 152(1):172–180, January 2011. PMID: 21147879.

[602] G. Pallafacchina, E. Calabria, A. L. Serrano, J. M. Kalhovde, and S. Schiaffino. A protein kinase B-dependent and rapamycin-sensitive pathway controls skeletal muscle growth but not fiber type specification. *Proceedings of the National Academy of Sciences of the United States of America*, 99(14):9213–9218, July 2002. PMID: 12084817.

[603] E. E. Spangenburg, D. Le Roith, C. W. Ward, and S. C. Bodine. A functional insulin-like growth factor receptor is not necessary for load-induced skeletal muscle hypertrophy. *The Journal of Physiology*, 586(Pt 1):283–291, January 2008. PMID: 17974583.

[604] R. Courrier and M. Marois. Relations entre la testostérone et la cortisone dans leurs effets sur certains organes récepteurs. *Comptes rendus hebdomadaires des séances de l'Académie des sciences*, 234(3):271, 1952.

[605] F. O. Levy, A. H. Ree, L. Eikvar, M. V. Govindan, T. Jahnsen, and V. Hansson. Glucocorticoid receptors and glucocorticoid effects in rat Sertoli cells. *Endocrinology*, 124(1):430–436, January 1989. PMID: 2909375.

[606] E. Dahlberg, M. Snochowski, and J. A. Gustafsson. Regulation of the

androgen and glucocorticoid receptors in rat and mouse skeletal muscle cytosol. *Endocrinology*, 108(4):1431–1440, April 1981. PMID: 6970661.

[607] M. Mayer and F. Rosen. Interaction of anabolic steroids with glucocorticoid receptor sites in rat muscle cytosol. *The American Journal of Physiology*, 229(5):1381–1386, November 1975. PMID: 173192.

[608] M. Snochowski, E. Dahlberg, and J. A. Gustafsson. Characterization and quantification of the androgen and glucocorticoid receptors in cytosol from rat skeletal muscle. *European journal of biochemistry / FEBS*, 111(2):603–616, October 1980. PMID: 6970125.

[609] G. T. Ferguson. Effects of cortisone and testosterone on diaphragmatic function and biochemistry in the rabbit. *Journal of Applied Physiology (Bethesda, Md.: 1985)*, 78(4):1459–1468, April 1995. PMID: 7615456.

[610] R. H. Van Balkom, P. N. Dekhuijzen, H. T. Folgering, J. H. Veerkamp, H. T. Van Moerkerk, J. A. Fransen, and C. L. Van Herwaarden. Anabolic steroids in part reverse glucocorticoid-induced alterations in rat diaphragm. *Journal of Applied Physiology (Bethesda, Md.: 1985)*, 84(5):1492–1499, May 1998. PMID: 9572790.

[611] J. M. Eason, S. L. Dodd, and S. K. Powers. Use of anabolic steroids to attenuate the effects of glucocorticoids on the rat diaphragm. *Physical Therapy*, 83(1):29–36, January 2003. PMID: 12495410.

[612] G. Bullock, A. M. White, and J. Worthington. The effects of catabolic and anabolic steroids on amino acid incorporation by skeletal-muscle ribosomes. *Biochemical Journal*, 108(3):417–425, July 1968. PMID: 5673936.

[613] R. A. Roeder, S. D. Thorpe, F. M. Byers, G. T. Schelling, and J. M. Gunn. Influence of anabolic agents on protein synthesis and degradation in muscle cells grown in culture. *Growth*, 50(4):485–495, 1986. PMID: 3596326.

[614] F. Ye, S. C. McCoy, H. H. Ross, J. A. Bernardo, A. W. Beharry, S. M. Senf, A. R. Judge, D. T. Beck, C. F. Conover, D. F. Cannady, B. K. Smith, J. F. Yarrow, and S. E. Borst. Transcriptional regulation of myotrophic actions by testosterone and trenbolone on androgen-responsive muscle. *Steroids*, 87:59–66, September 2014. PMID: 24928725.

[615] D. C. DuBois and R. R. Almon. Perineal muscles: possible androgen regulation of glucocorticoid receptor sites in the rat. *The Journal of Endocrinology*, 102(2):225–229, August 1984. PMID: 6747500.

[616] A. E. Rigamonti, L. Locatelli, S. G. Cella, S. M. Bonomo, M. Giunta, F. Molinari, A. Sartorio, and E. E. Müller. Muscle expressions of MGF, IGF-IEa, and myostatin in intact and hypophysectomized rats: effects of rhGH and testosterone alone or combined. *Hormone and Metabolic Research = Hormon- Und Stoffwechselforschung = Hormones Et Métabolisme*, 41(1):23–29, January 2009. PMID: 18841527.

[617] Y. Wu, J. Zhao, W. Zhao, J. Pan, W. A. Bauman, and C. P. Cardozo. Nandrolone normalizes determinants of muscle mass and fiber type after spinal cord injury. *Journal of Neurotrauma*, 29(8):1663–1675, May 2012. PMID: 22208735.

[618] T. Kvorning, M. Andersen, K. Brixen, P. Schjerling, C. Suetta, and K. Madsen. Suppression of testosterone does not blunt mRNA expression of myoD, myogenin, IGF, myostatin or androgen receptor post strength training in humans. *The Journal of Physiology*, 578(Pt 2):579–593, January 2007. PMID: 17095559.

[619] W. Zhao, J. Pan, Z. Zhao, Y. Wu, W. A. Bauman, and C. P. Cardozo. Testosterone protects against dexamethasone-induced muscle atrophy, protein degradation and MAFbx upregulation. *The Journal of steroid biochemistry and molecular biology*, 110(1-2):125–129, May 2008. PMID:

18436443.

[620] Ankit Rohatgi. WebPlotDigitizer, January 2015.

Curriculum vitae

Nicolae Lucian Sandor

Education

PhD candidate, Boston University School of Medicine, expected graduation 2015

BS (Licentiat in Biofizica), Universitatea Bucuresti, 2005

BM\DM (Doctor in Medicina), Universitatea de Medicina si Farmacie Carol Davila Bucuresti, 2002

Research experience

- 2012-2015: Department of Medicine, Boston University School of Medicine, P.I. Dr. Shalender Bhasin, under the supervision of Dr. Carlo Serra

Subject: Testosterone alleviating glucocorticoid-induced muscle loss.

- 2009-2012: Department of Biophysics, Boston University School of Medicine, P.I. Dr. Assen Marintchev

Subject: Interactions between translation initiation factors eIF1A and eIF5B

- 2005-2008: Center for the Study of Brain, Mind and Behavior, Princeton University, P.I. Dr. Anne Treisman, FRS

Subject: Brain mechanisms for statistical processing of visual scenes

- Summer 2004: Biology Department, Universitatea Bucuresti, P.I. Dr. Gordon Reid, under the supervision of Dr. Iurie Barbu

Subject: The mediation of thermoception by ionic membrane channels

- 2001-2002: Biophysics Department, Universitatea de Medicina si Farmacie Carol Davila Bucuresti, P.I. Dr. Dan Eremia, under the supervision of Dr. Eva Katona,

Subject: The effect of non-ionizing radiation of the mobility of the cell membrane lipids

Peer-reviewed publications

1. Serra C, Tangherlini F, Rudy S, Lee D, Toraldo G, Sandor NL, Zhang A, Jasuja R, Bhasin S. Testosterone improves the regeneration of old and young mouse skeletal muscle. J Gerontol A Biol Sci Med Sci. 2013 Jan;68(1).

2. Serra C, Sandor NL, Jang H, Lee D, Toraldo G, Guarneri T, Wong S, Zhang A, Guo W, Jasuja R, Bhasin S. The effects of testosterone deprivation and supplementation on proteasomal and autophagy activity in the skeletal muscle of the male mouse: differential effects on high-androgen responder and low-androgen responder muscle groups. Endocrinology. 2013 Dec;154(12).

3. Guo W, Bachman E, Vogel J, Li M, Peng L, Pencina K, Serra C, Sandor NL, Jasuja R, Montano M, Basaria S, Gassmann M, Bhasin S. The effects of short-term and long-term testosterone supplementation on blood viscosity and erythrocyte deformability in healthy adult mice. Endocrinology. 2015 Mar 16;en20141784. PMID: 25774550.

Other scientific communications

1. Sandor NL, Hendrickson E, Sandor D, Wagner G, Pestova TV, Marintchev A. Interplay Between Intra- And Intermolecular Interactions Involving Human eIF1A and eIF5B. Abstract presented at the 2010 Meeting of Translational Control, Sept. 2010, Cold Spring Harbor, NY.

2. Sandor NL, Lee D, Toraldo G, Zhang A, Jasuja R, Bhasin S, Serra C. The Role Of Testosterone On The Control Of Muscle Protein Synthesis And

Degradation. Abstract presented at the 2011 Evans Center Days, Nov. 2011, Boston, MA.

3. Serra C, Lee D, Sandor NL, Toraldo G, Jang H, Jasuja R, Bhasin S. Characterization of the neuromuscular junction in castrated male mice. Poster presented at ENDO2013, The Endocrine Society's 95th Annual Meeting & Expo, 2013, San Francisco, CA.

4. Sandor NL, Jasuja R, Serra C, Bhasin S. Testosterone alleviates glucocorticoid myopathy by inhibiting the proteolytic machinery. Poster presented at the 2013 Evans Center Days, Nov. 2013, Boston, MA.