UNIVERSITY OF TURIN

PhD in
EXPERIMENTAL MEDICINE AND THERAPY

SEMINARIO

CANCER CACHEXIA: MOLECULAR MECHANISMS, DIAGNOSIS AND TREATMENT

Dr. SILVIA BUSQUETS

Departament de Bioquímica i Biomedicina Molecular

Universitat de Barcelona

Barcelona, Spain

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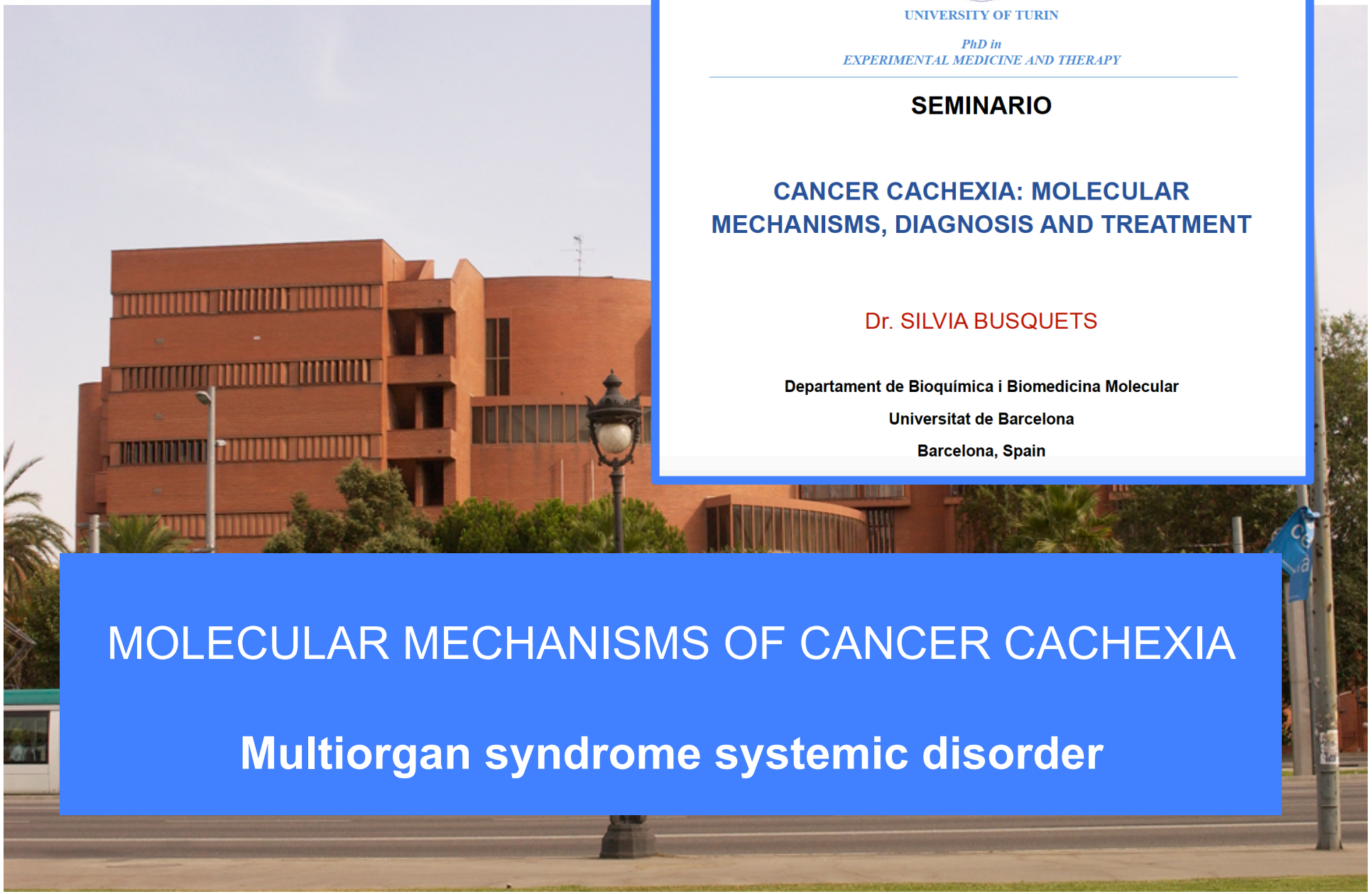
Dos Campus d'Excel·lència Internacional:

B:KC Barcelona Knowledge Campus

HUB Health Universitat de Barcelona Campus



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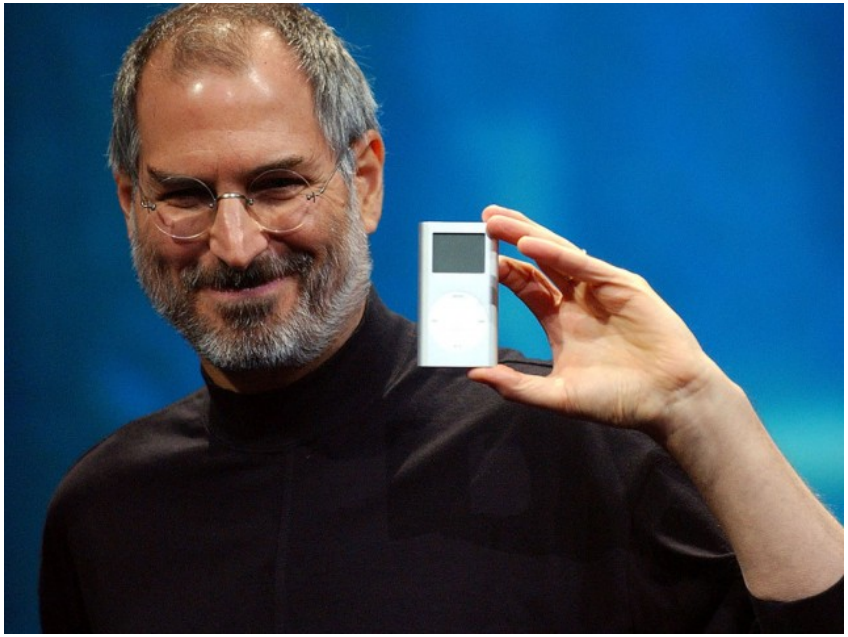
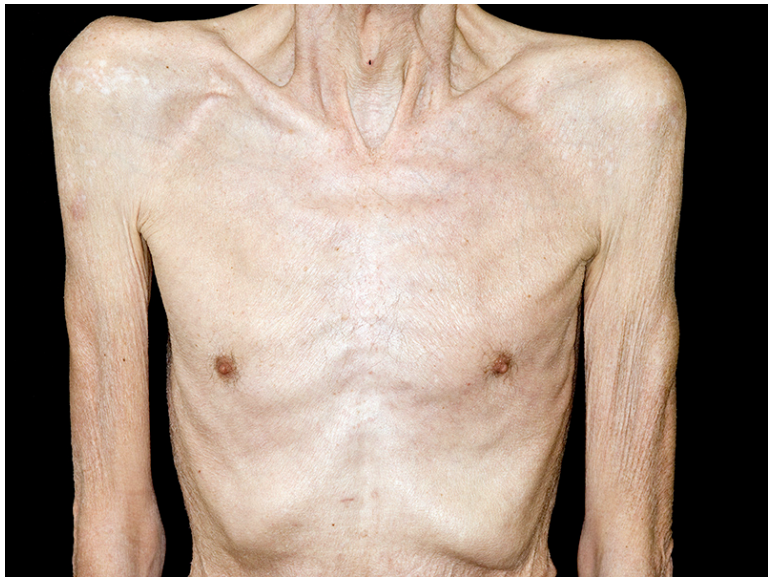
MOLECULAR MECHANISMS OF CANCER CACHEXIA

Multiorgan syndrome systemic disorder



Steve Jobs
1955 - 2011

CACHEXIA



κακοσ
kakos

ηεξισ
hexis

bad condition

Cachexia, is a complex metabolic syndrome associated with underlying illness and characterized by **loss of muscle with or without loss of fat mass**.

CACHEXIA

DISEASES ASSOCIATED WITH THE CACHECTIC SYNDROME

- Acquired immune deficiency syndrome (AIDS)
- Sepsis
- Severe burn
- Chronic obstructive pulmonary disease (COPD)
- Cardiovascular pathology
- Neuromuscular disease hyperthyroidism
- Muscle atrophy and/or distrophy
- **Cancer**
- ...

Cancer cachexia is a devastating, multifactorial and often irreversible syndrome that affects a high percentage of cancer patients, depending on the tumour type, and that leads to **substantial weight loss, primarily from loss of skeletal muscle and body fat.**

CACHEXIA

Table 2. The commonest malignancies in which cachexia develops as part of the clinical course.[6]

Malignancy	Patients with cachexia (%)
Gastric cancer	85
Pancreatic cancer	83
Non-small cell lung cancer	61
Small cell lung cancer	57
Prostate cancer	56
Colon cancer	54
Unfavourable non-Hodgkin's lymphoma	48
Sarcoma	40
Acute non-lymphocytic leukaemia	39
Breast cancer	36
Favourable non-Hodgkin's lymphoma	31

cachexia is directly responsible for the death of **at least 20%** of all cancer patients (cardiac/respiratory failure)

CME Palliative care

Cancer cachexia and fatigue

Grant D Stewart BSc(Hons) MBChB MRCS(Ed), Surgical Research Fellow

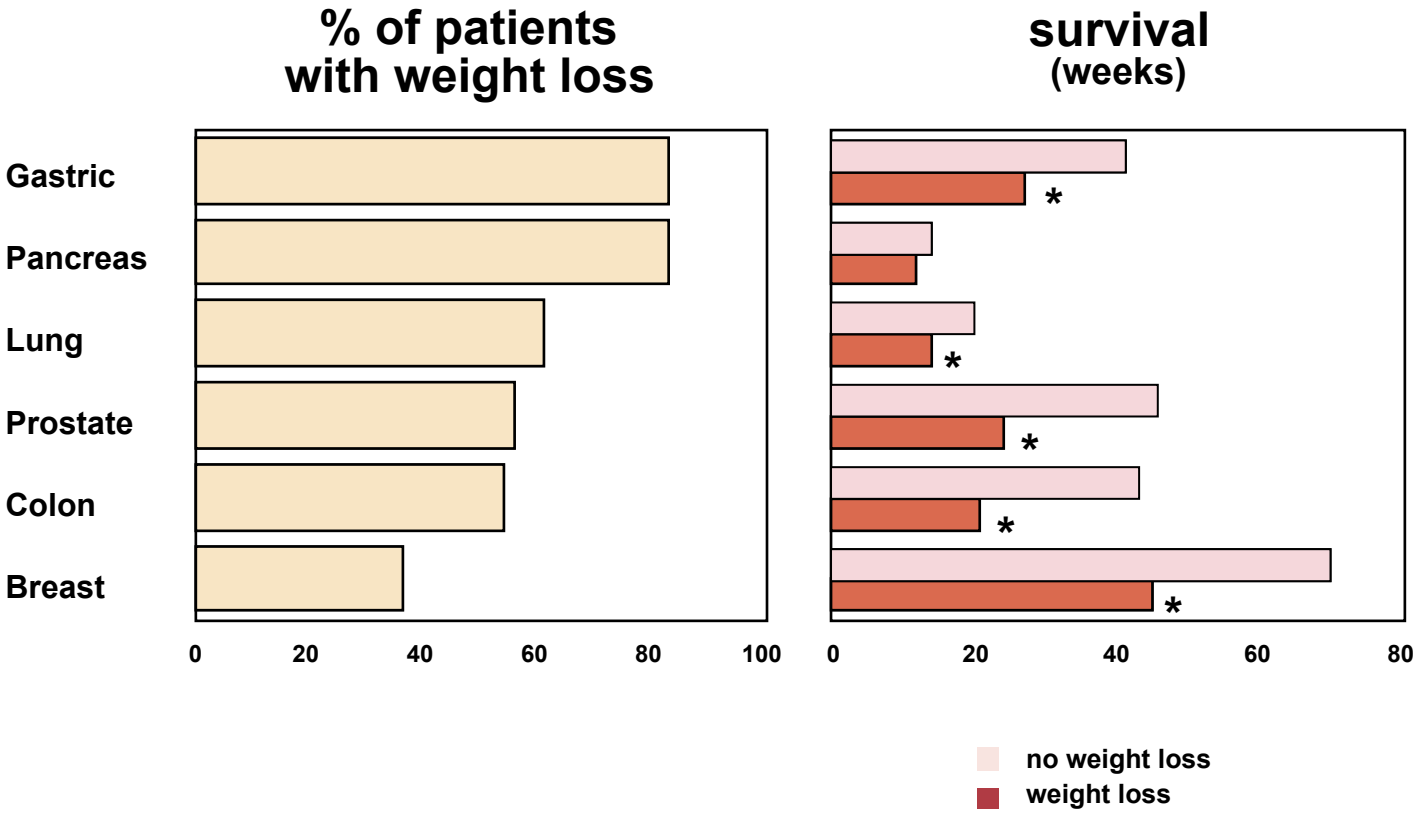
Richard JE Skipworth BSc(Hons) MBChB MRCS(Ed), Surgical Research Fellow

Kenneth CH Fearon MBChB(Hons) MD FRCS(Glas) FRCS(Ed) FRCS(Eng), Professor of Surgical Oncology

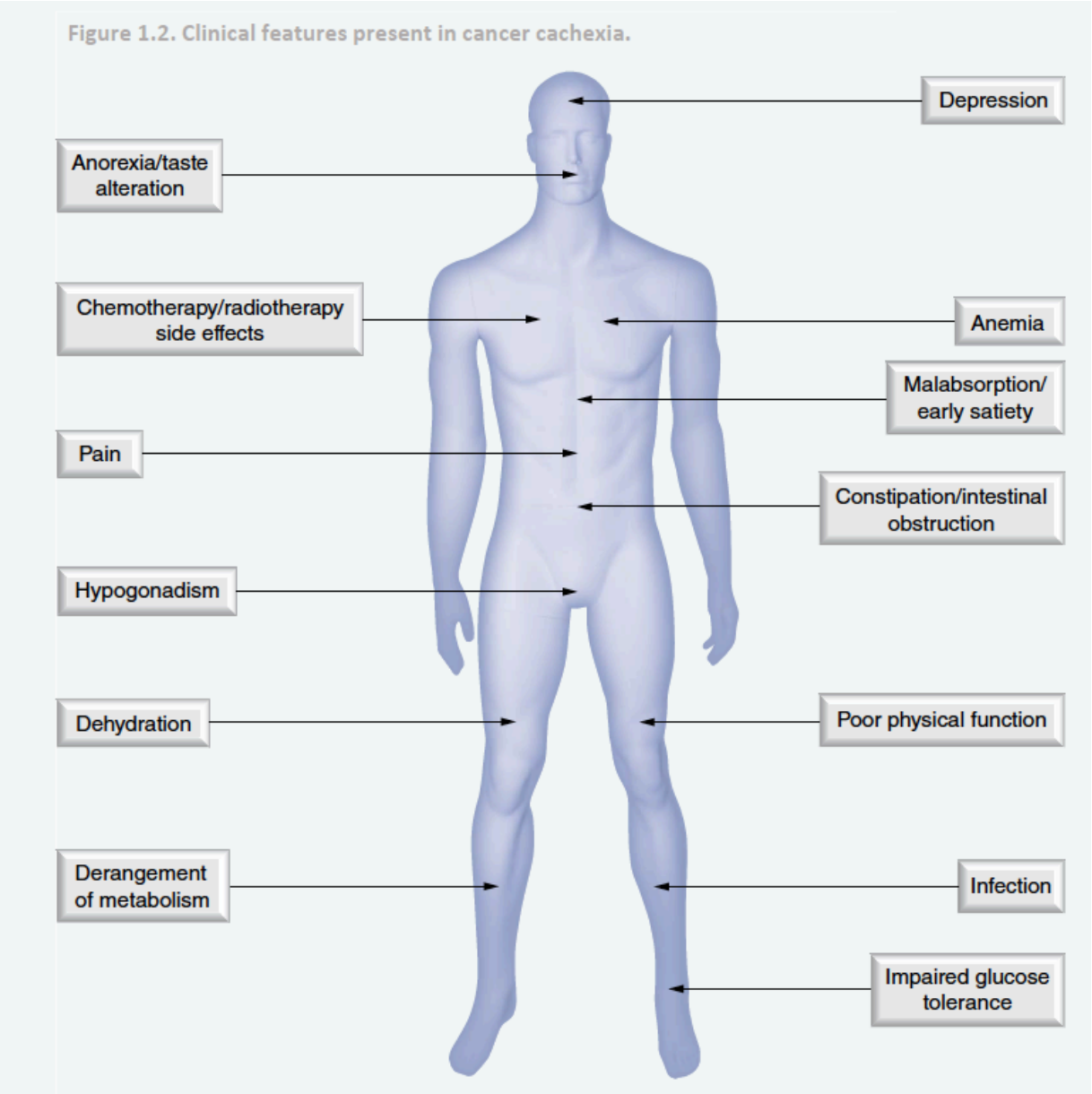
Department of Clinical and Surgical Sciences (Surgery), University of Edinburgh, Royal Infirmary, Edinburgh

Clin Med 2006;**6**:140–3

CANCER CACHEXIA

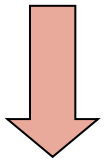


DeWys et al; Am.J.Med. 69: 491 (1980)



Neil Johns, Richard JE, Skipworth, Kenneth CH Fearon & James A Ross.
Prevalence and clinical features of cancer cachexia.
Cancer Cachexia. Future Medicine. 2012 doi:10.2217/EBO.12.147

CACHEXIA



Reduced survival time

Poor prognosis

Reduced reponse to therapy

Increased toxicity associated to treatment

Altered immune response

Reduced mobility

Increased risk of surgery complications

Reduced quality of life

Increased sanitary costs

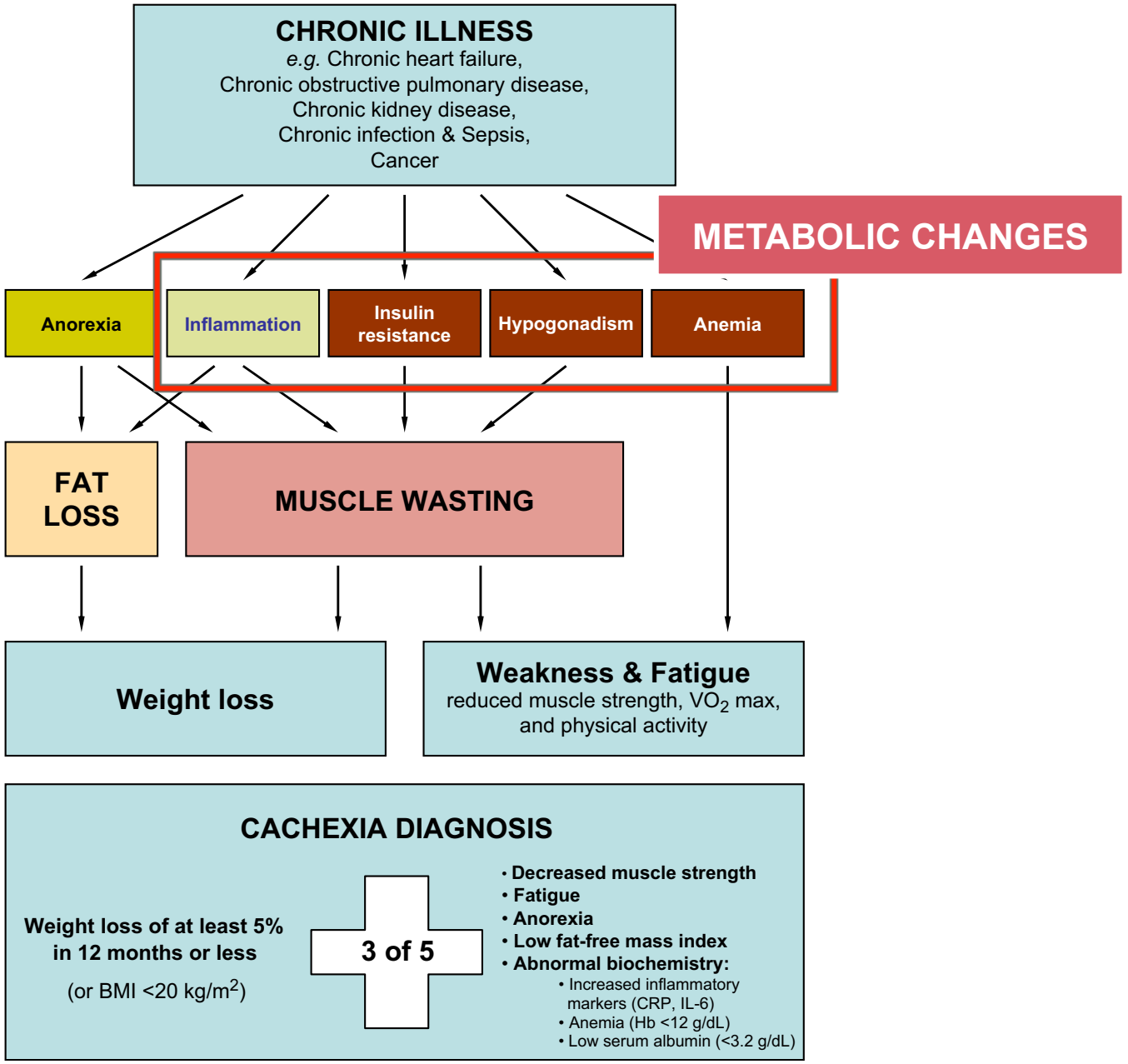
CONSENSUS DEFINITION OF CACHEXIA

Cachexia, is a complex metabolic syndrome associated with underlying illness and characterized by **loss of muscle with or without loss of fat mass**.

The prominent clinical feature of cachexia is
weight loss in adults or **growth failure in children.**

Anorexia, inflammation, insulin resistance and increased muscle protein breakdown
are frequently associated with cachexia.

Evans et al. Cachexia a new definition. Clinical Nutrition 2008, 27:793



Evans et al, Clinical Nutrition (2008) 27, 793-799

Molecular mechanisms

Cachexia is a **multifactorial syndrome** involving changes in several **metabolic pathways**, in many tissues and organs:

- Energy balance disorder
- Tumour-driven inflammation
- Muscle wasting and atrophy
- Adipose tisue wasting
- Multi-organ syndrome

Nature Reviews Cancer | AOP, published online 9 October 2014; doi:10.1038/nrc3829

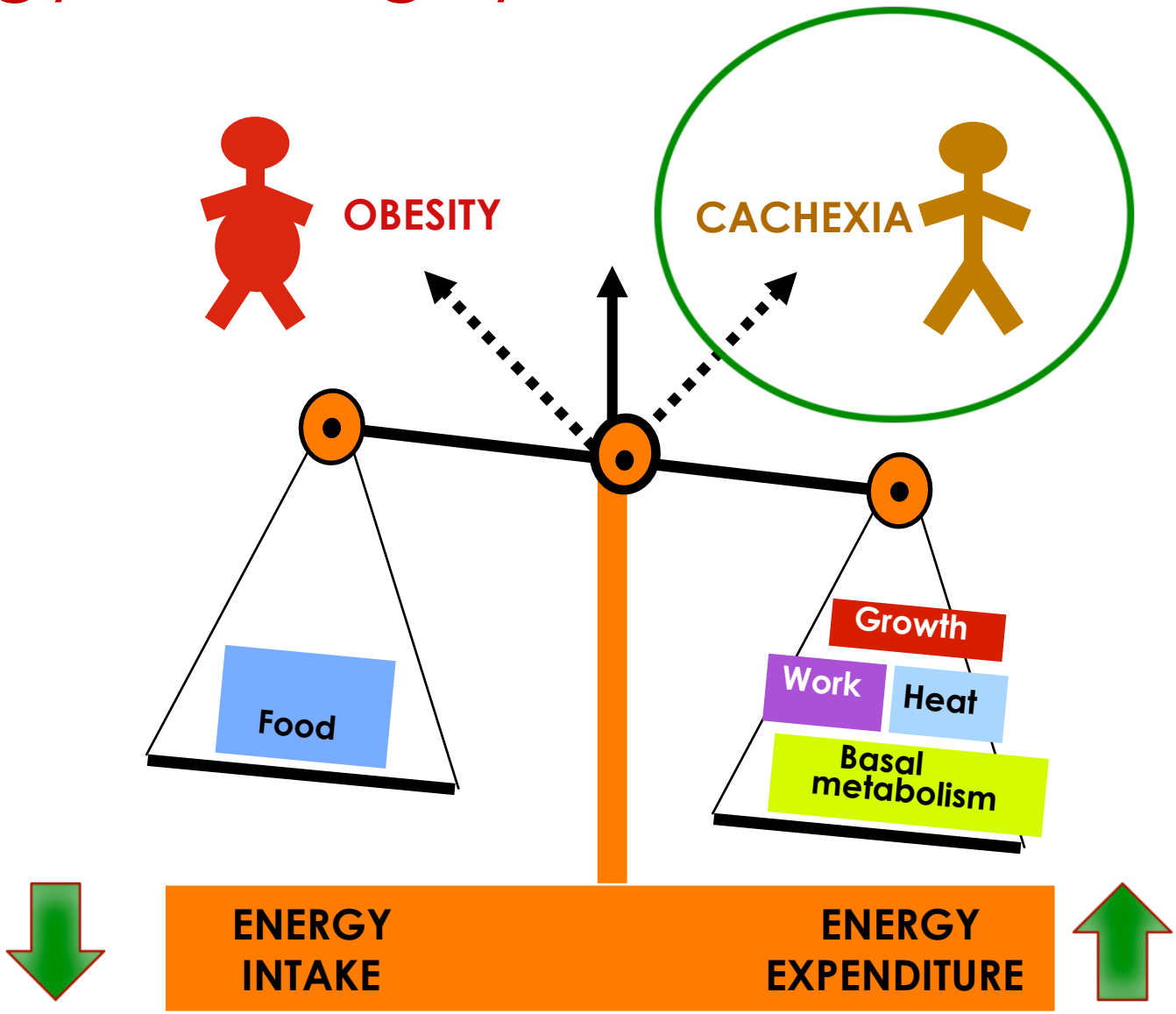
OPINION

Cancer cachexia: understanding the molecular basis

Josep M. Argilés, Silvia Busquets, Britta Stemmler and Francisco J. López-Soriano

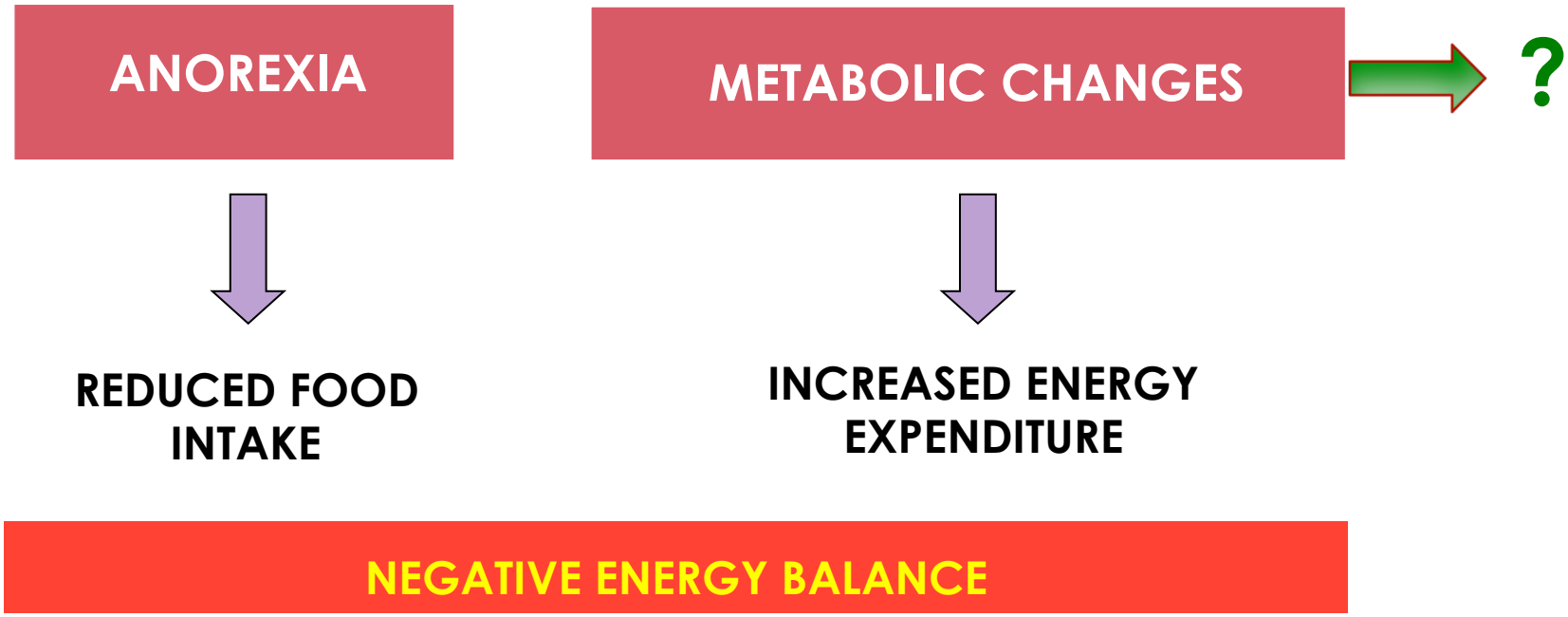
Abstract | Cancer cachexia is a devastating, multifactorial and often irreversible syndrome that affects around 50–80% of cancer patients, depending on the tumour type, and that leads to substantial weight loss, primarily from loss of skeletal muscle and body fat. Since cachexia may account for up to 20% of cancer deaths, understanding the underlying molecular mechanisms is essential. The occurrence of cachexia in cancer patients is dependent on the patient response to tumour progression, including the activation of the inflammatory response and energetic inefficiency involving the mitochondria. Interestingly, crosstalk between different cell types ultimately seems to result in muscle wasting. Some of the recent progress in understanding the molecular mechanisms of cachexia may lead to new therapeutic approaches.

Energy-wasting syndrome

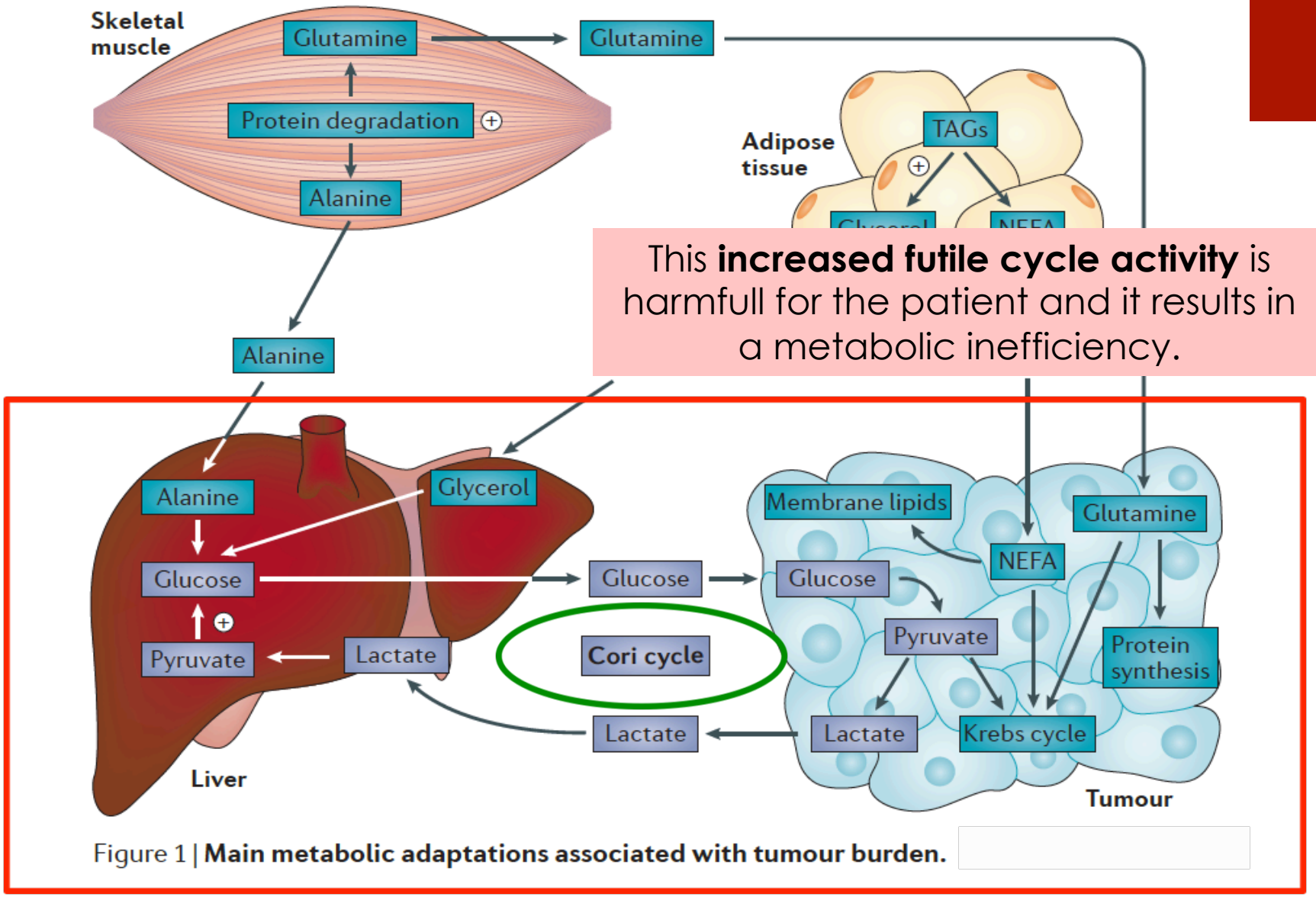


Energy-wasting syndrome

Cancer cachexia is basically a **problem of energy balance**



Energy-wasting syndrome



This **increased futile cycle activity** is harmfull for the patient and it results in a metabolic inefficiency.

Figure 1 | **Main metabolic adaptations associated with tumour burden.**

Cori cicle

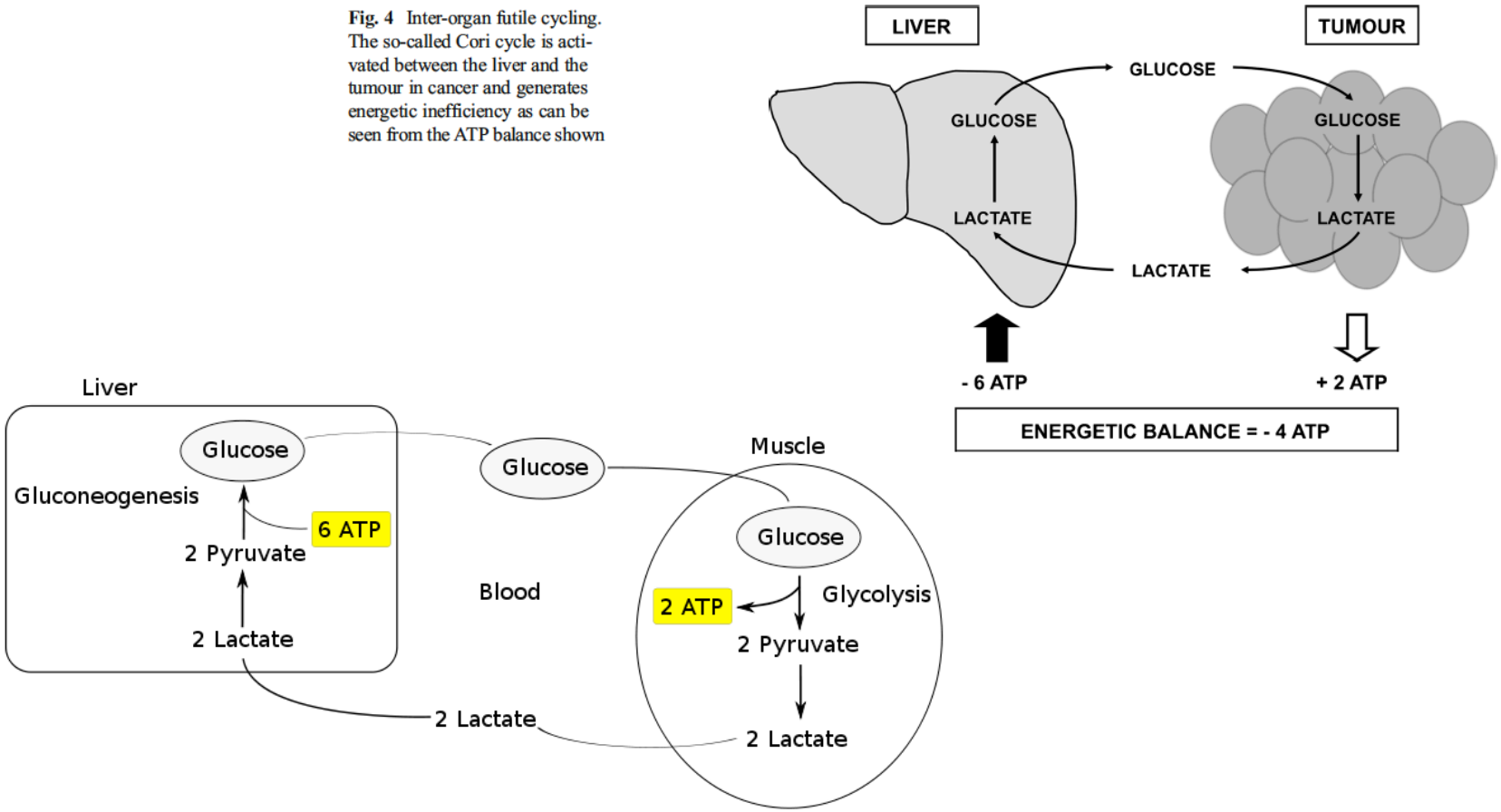
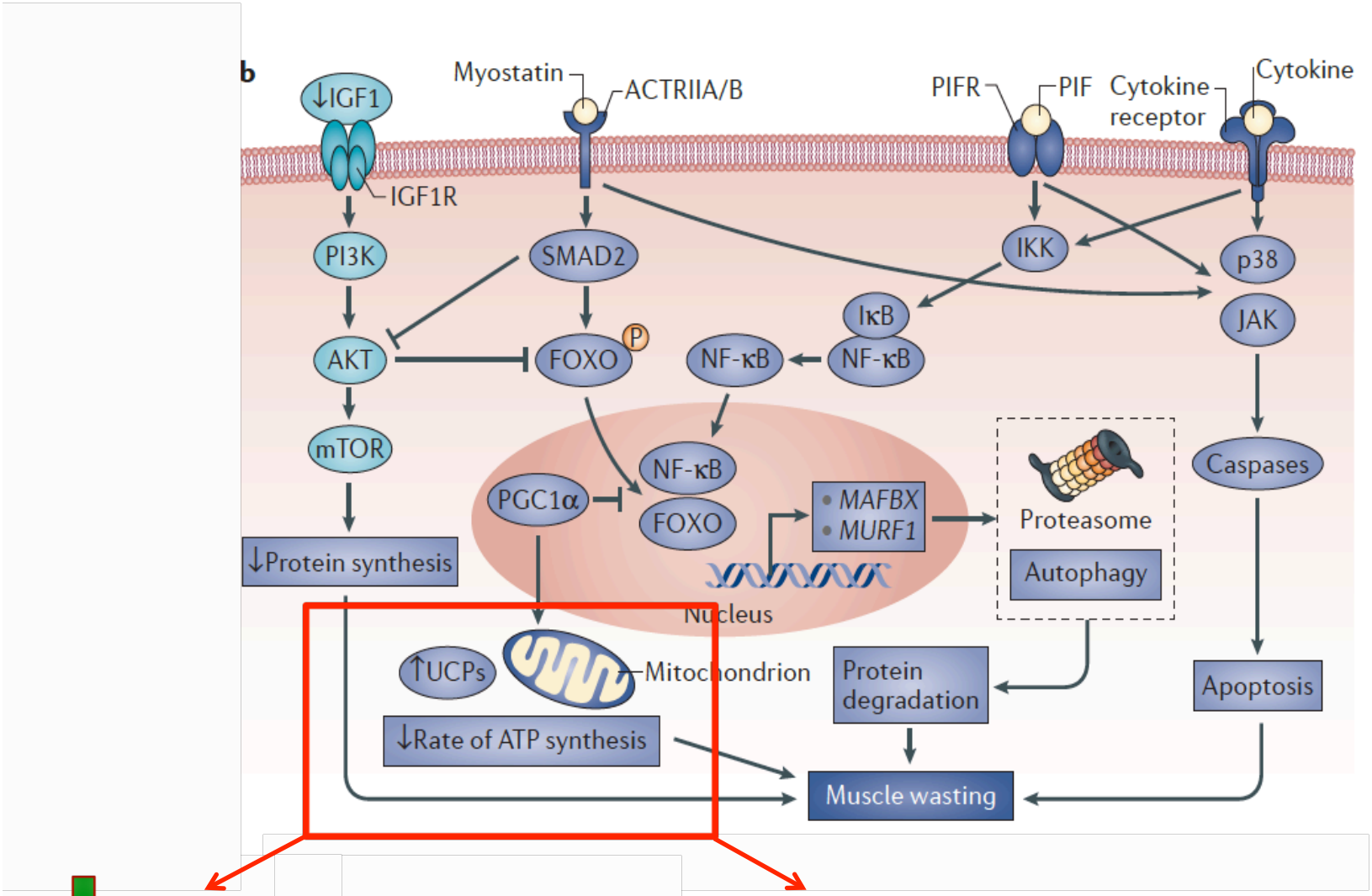


Fig. 4 Inter-organ futile cycling. The so-called Cori cycle is activated between the liver and the tumour in cancer and generates energetic inefficiency as can be seen from the ATP balance shown

Energy-wasting syndrome



MITOCHONDRIA

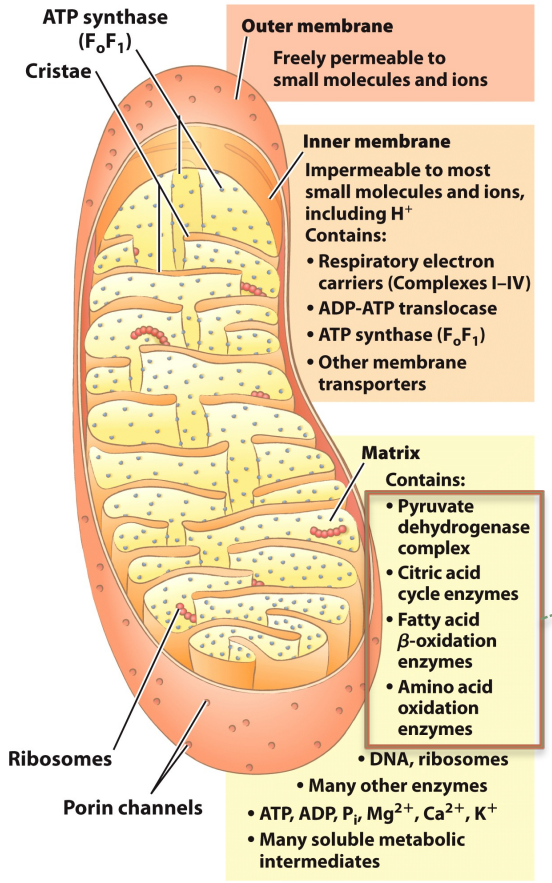


Figure 19-2a
Lehninger Principles of Biochemistry, Sixth Edition

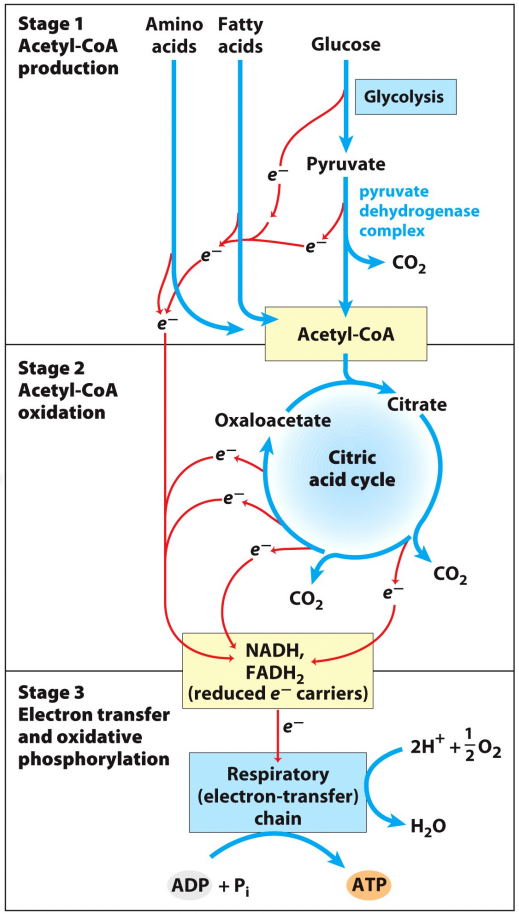
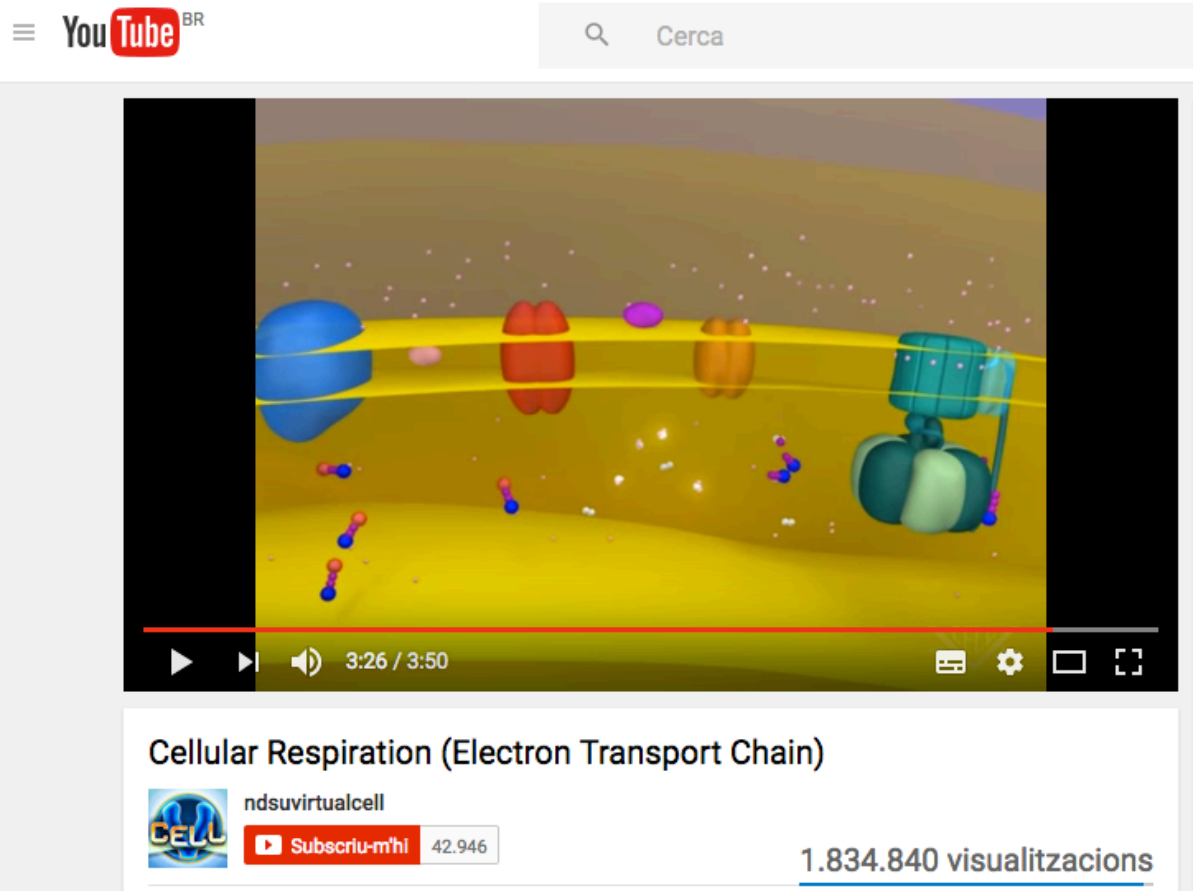


Figure 16-1
Lehninger Principles of Biochemistry, Sixth Edition

MITOCHONDRIA: Oxidative phosphorylation

https://www.youtube.com/watch?v=xbJonbzt5Kw
T=0:41



Thermogenesis: mitochondrial Uncoupling

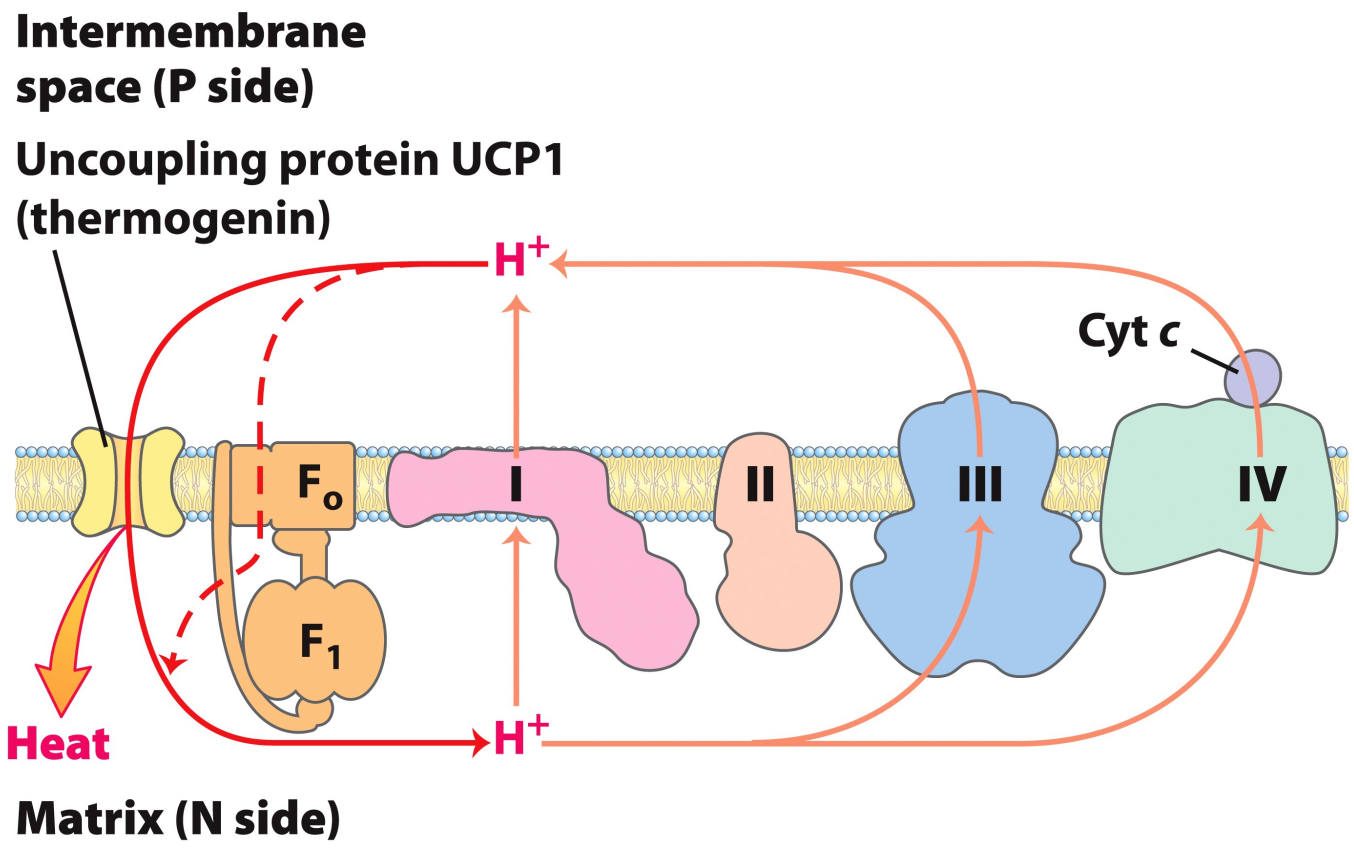
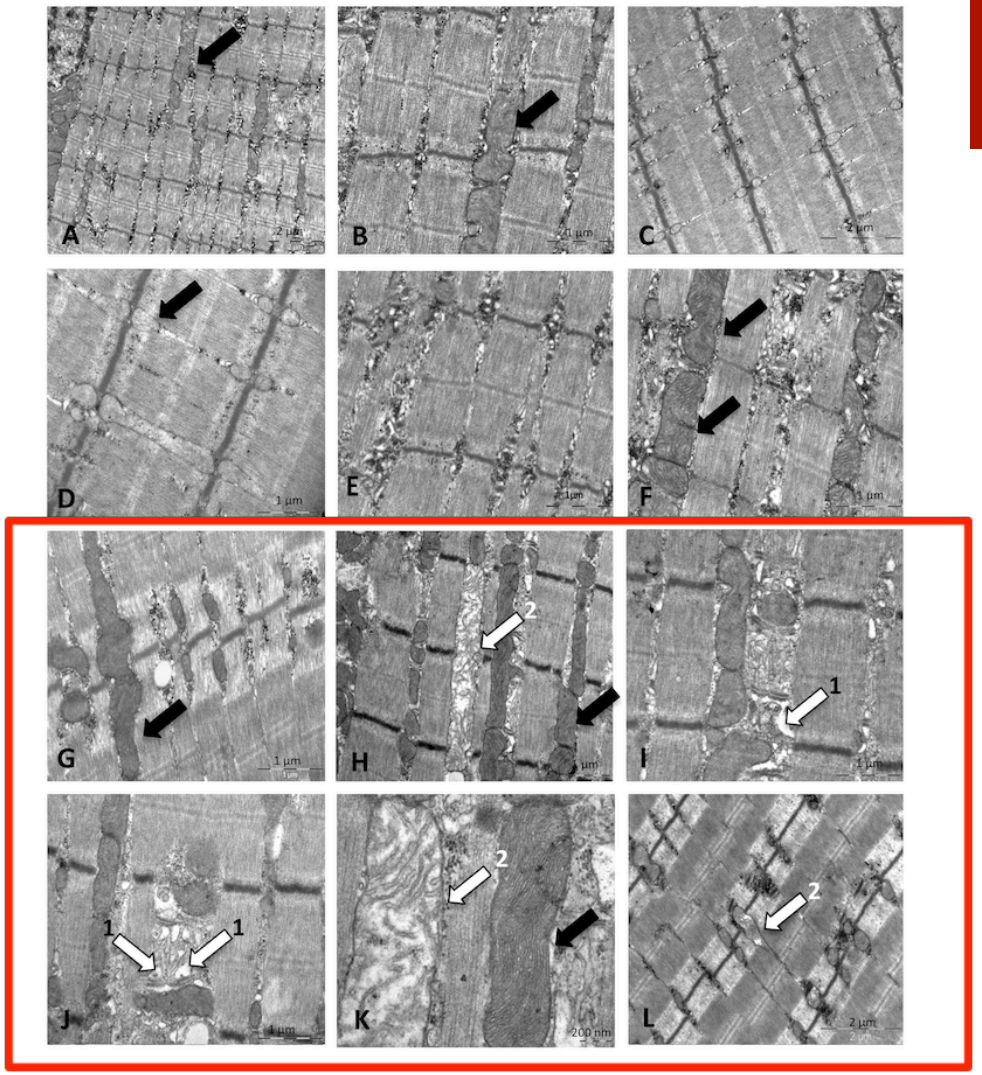


Figure 19-36
Lehninger Principles of Biochemistry, Sixth Edition

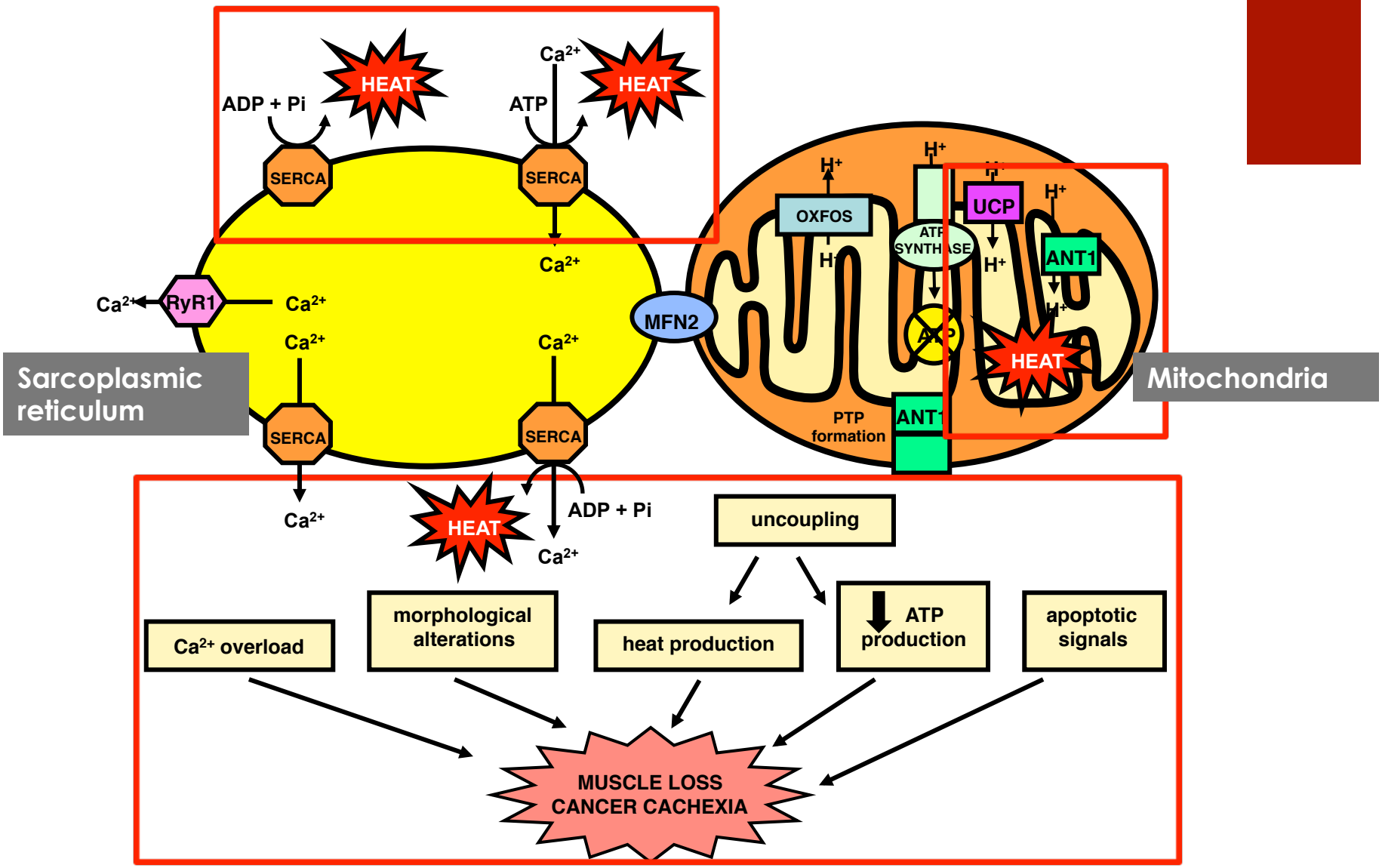
Energy-wasting syndrome

- Altered changes in mitochondrial morfology
- Decreased oxidative capacity
- Disrupted protein synthesis
- Changes in membrane fluidity
- Oxidatively modified mitochondrial proteins



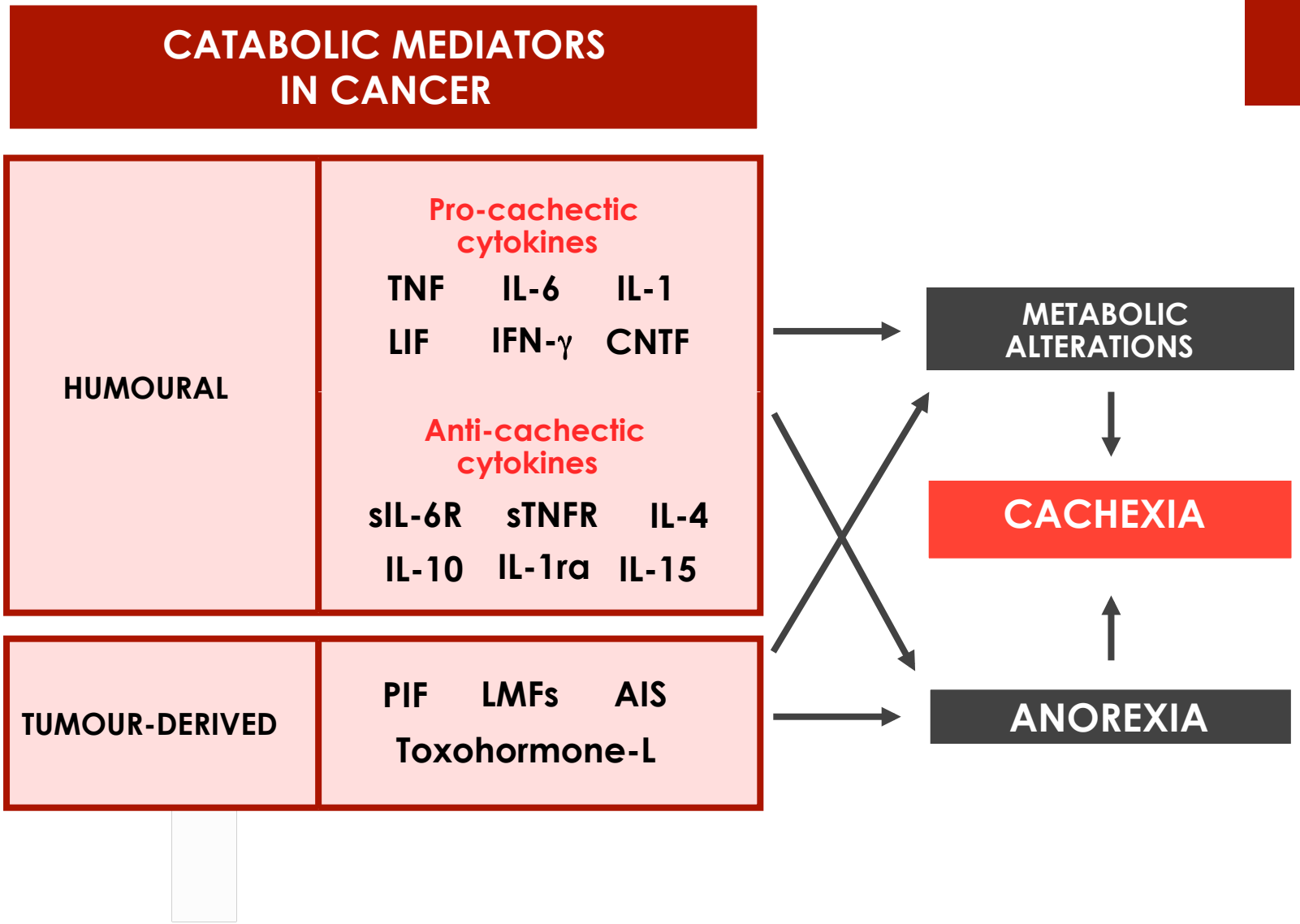
Fontes-Oliveira et al, BBA(2013) 1830, 2770-2778

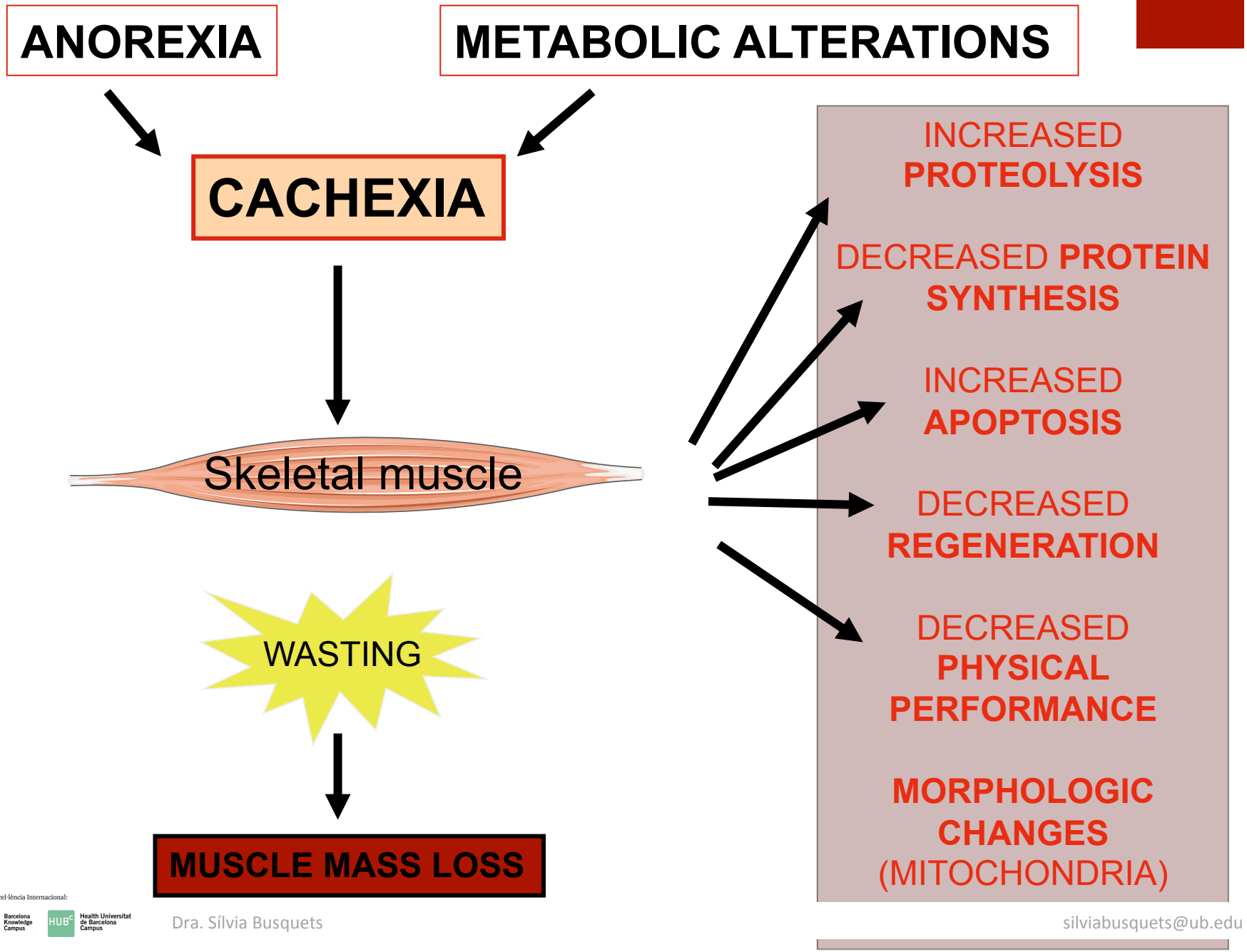
Energy-wasting syndrome



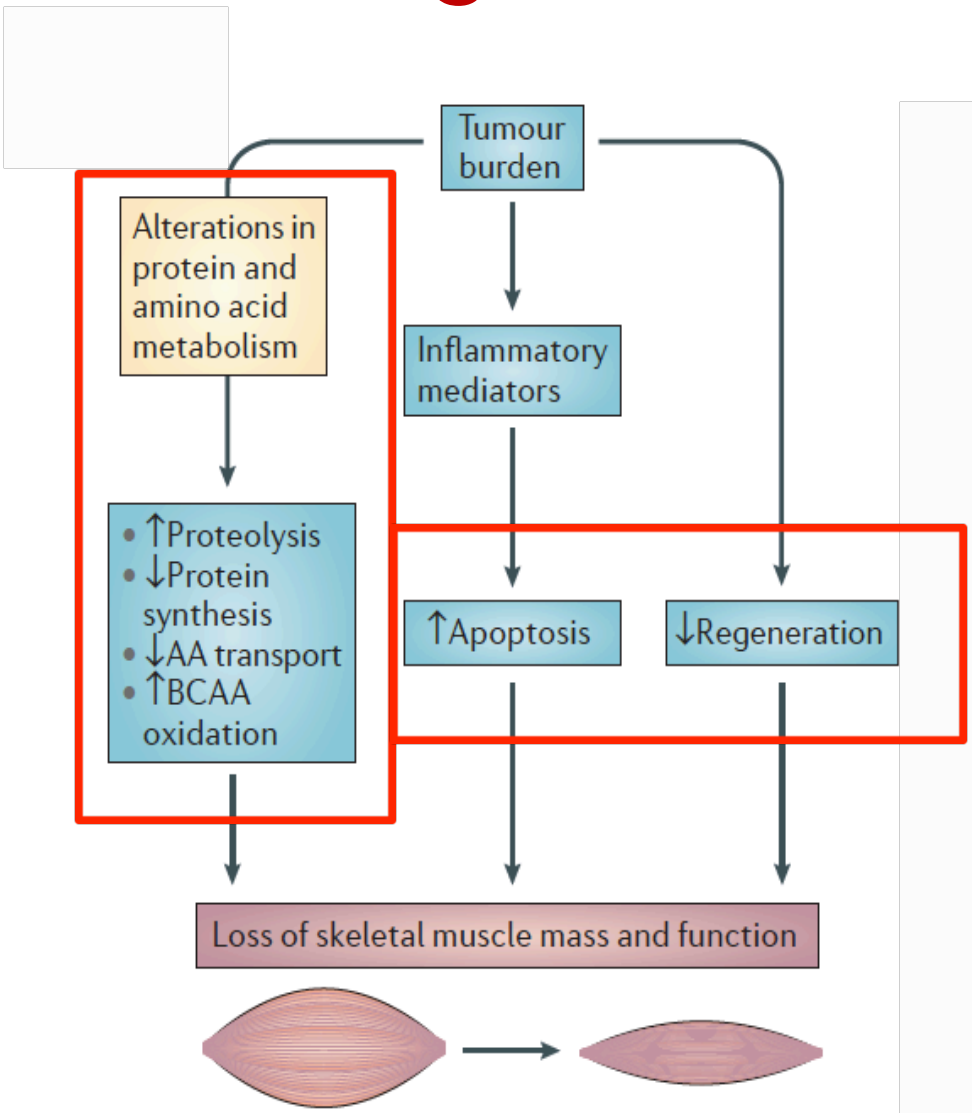
Fontes-Oliveira et al, BBA(2013) 1830, 2770-2778

Tumour-driven inflammation

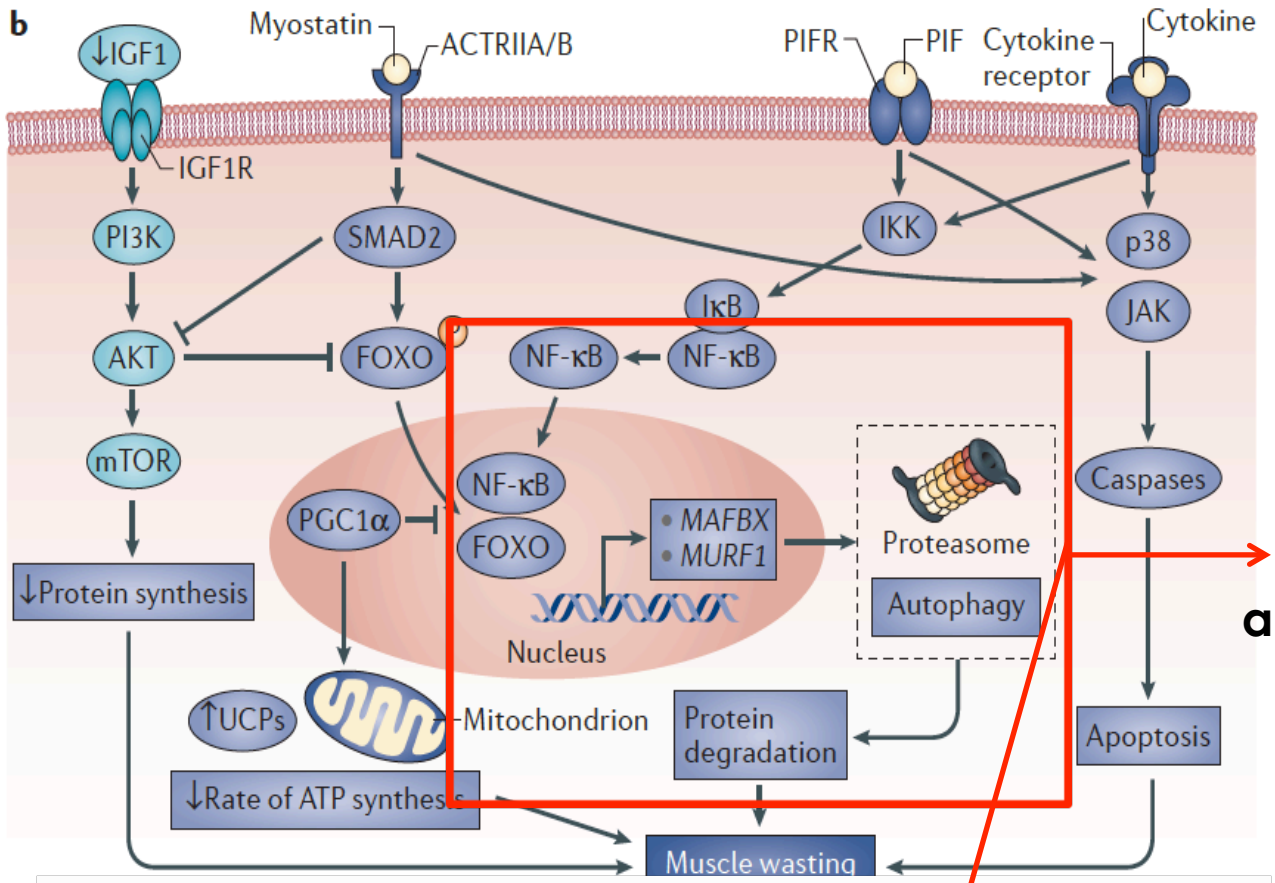



ANOREXIA
METABOLIC ALTERATIONS
CACHEXIA
Skeletal muscle
WASTING
MUSCLE MASS LOSS
INCREASED PROTEOLYSIS
DECREASED PROTEIN SYNTHESIS
INCREASED APOPTOSIS
DECREASED REGENERATION
DECREASED PHYSICAL PERFORMANCE
MORPHOLOGIC CHANGES (MITOCHONDRIA)

Muscle wasting



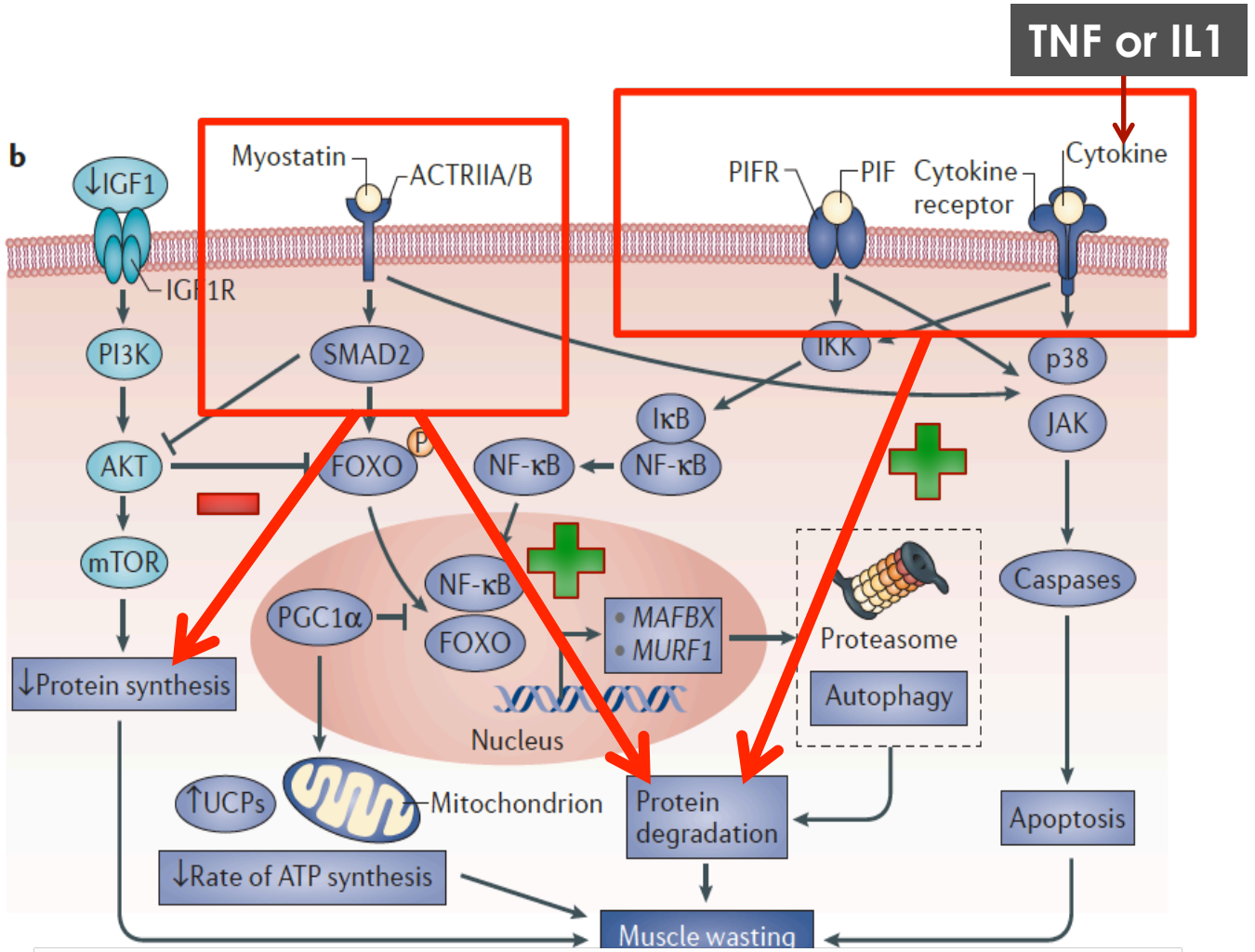
Muscle wasting



autophagy

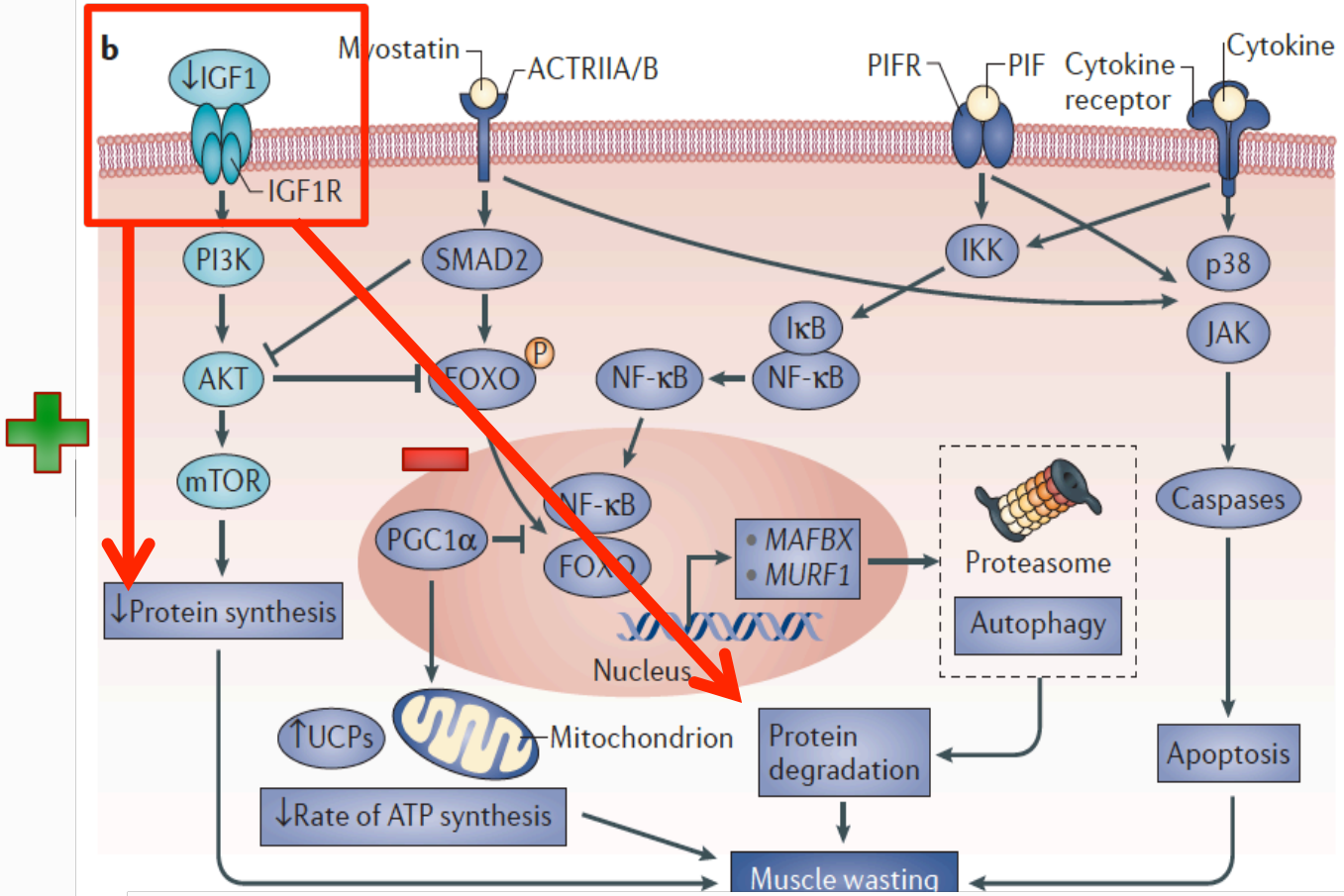
ubiquitin-dependent proteasome pathway

Muscle wasting

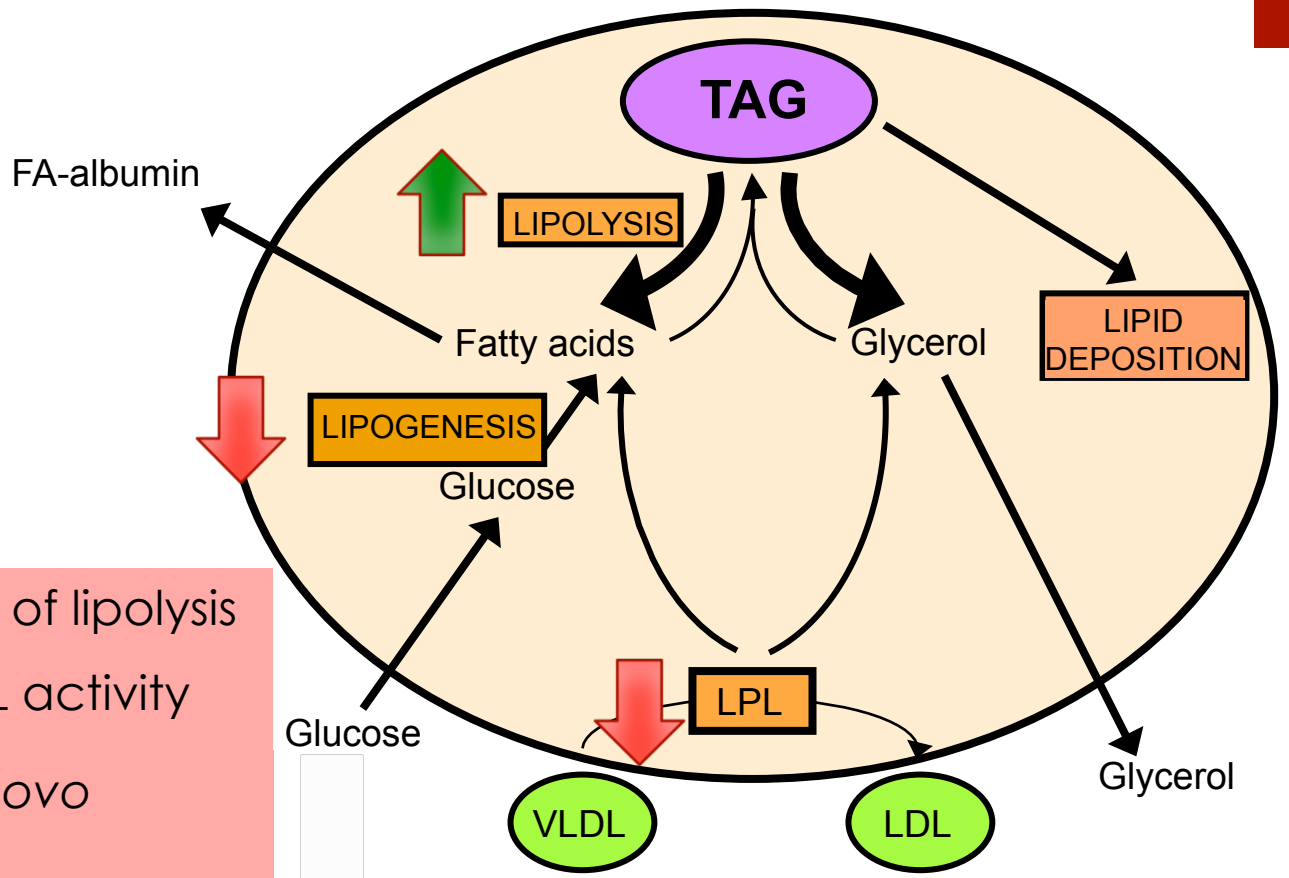


Muscle wasting

insulin-like growth factor 1



Adipose tissue wasting



1. Increased rate of lipolysis
2. Decreased LPL activity
3. Reduced *de novo* lipogenesis

Adipose tissue wasting

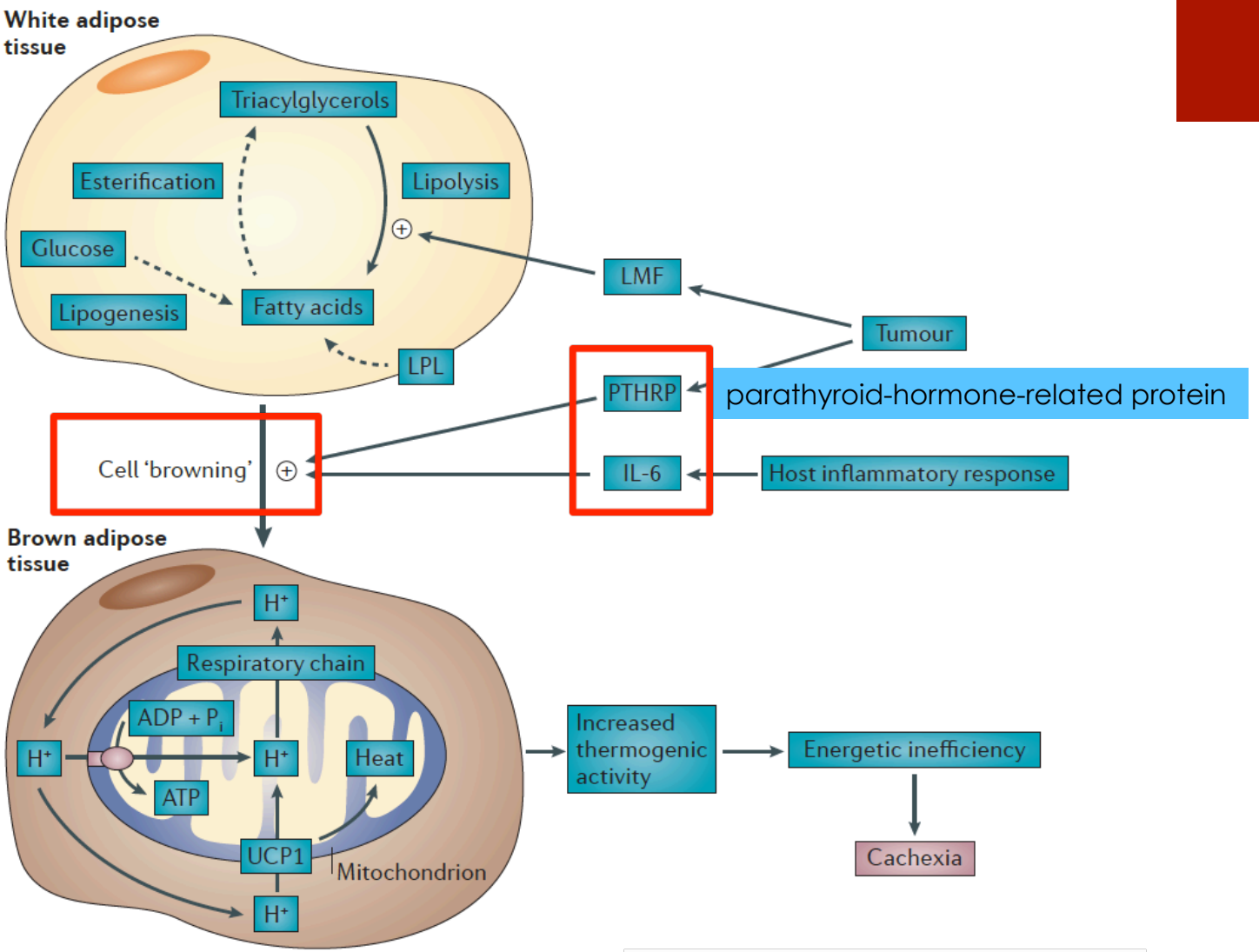
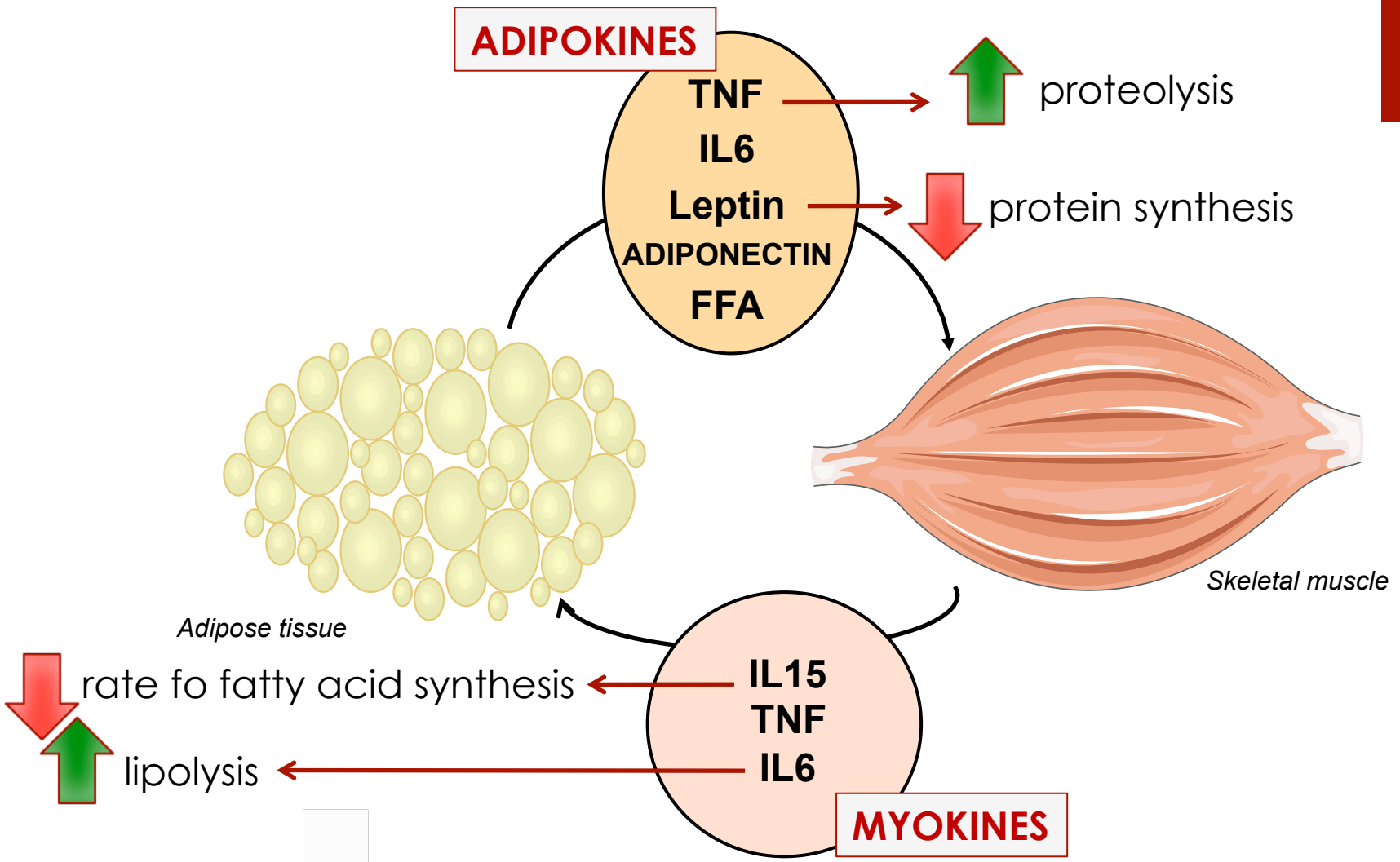


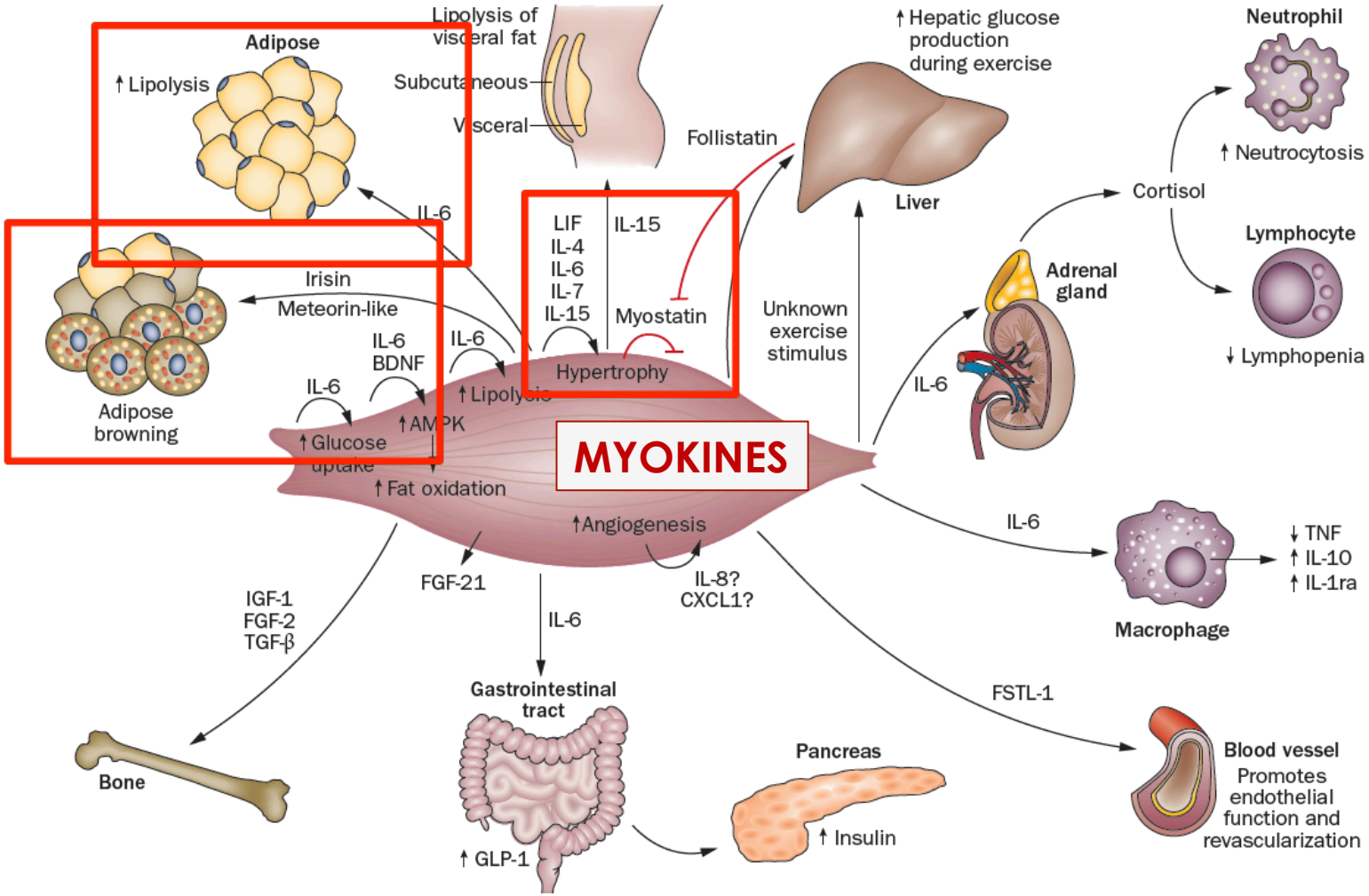
Figure 3 | **Browning of white adipose tissue in cachexia.**

Crosstalk between adipose tissue and muscle



Argilés et al. Med Res Rev, 25, No. 1, 49–65, 2005

Crosstalk between muscle and other tissues



Benatti et Pedersen, Nat. Rev. Rheumatol. 11, 86-97 (2015)

Multi-organ syndrome

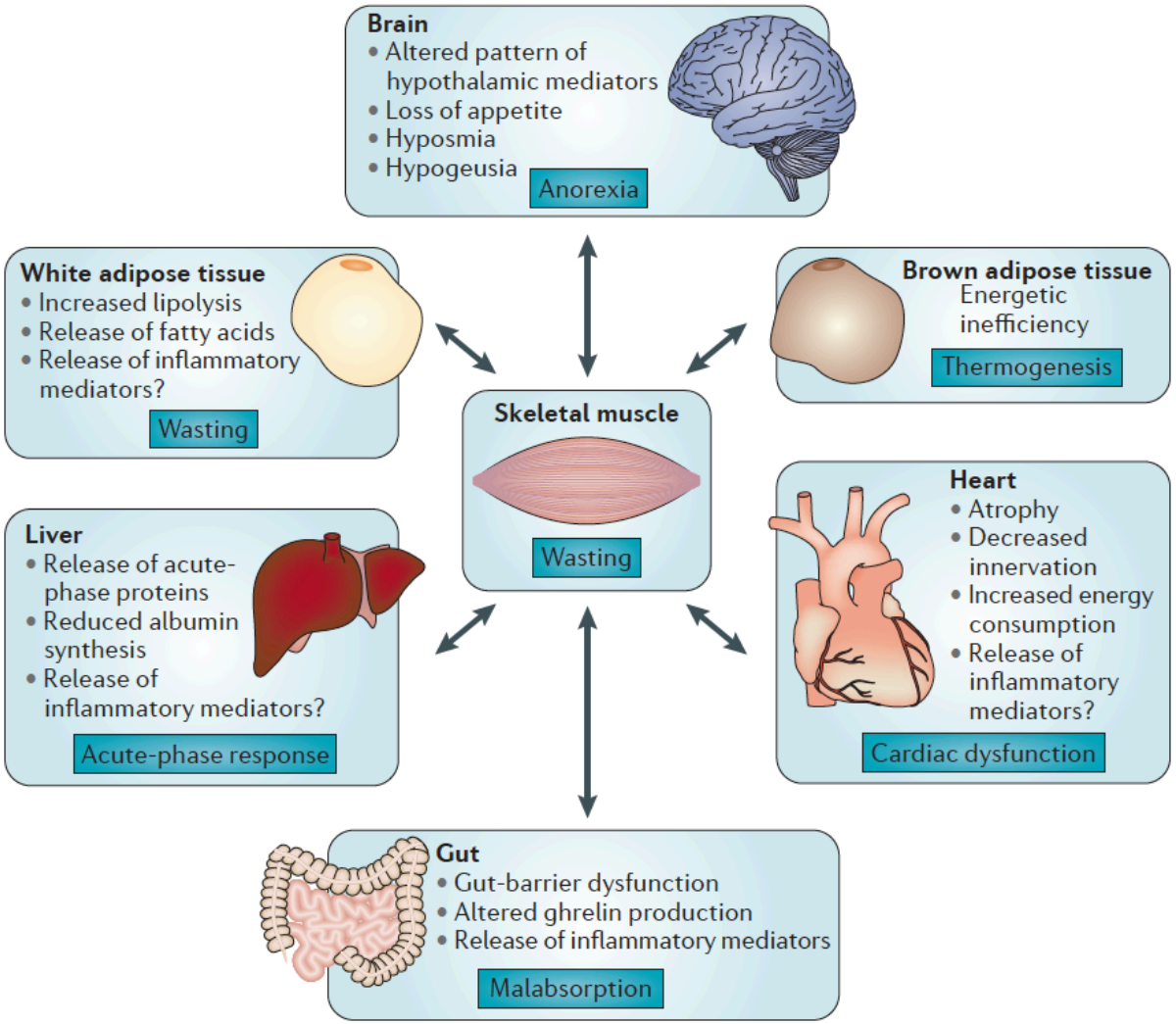


Figure 4 | **Cachexia as a multi-organ syndrome.**

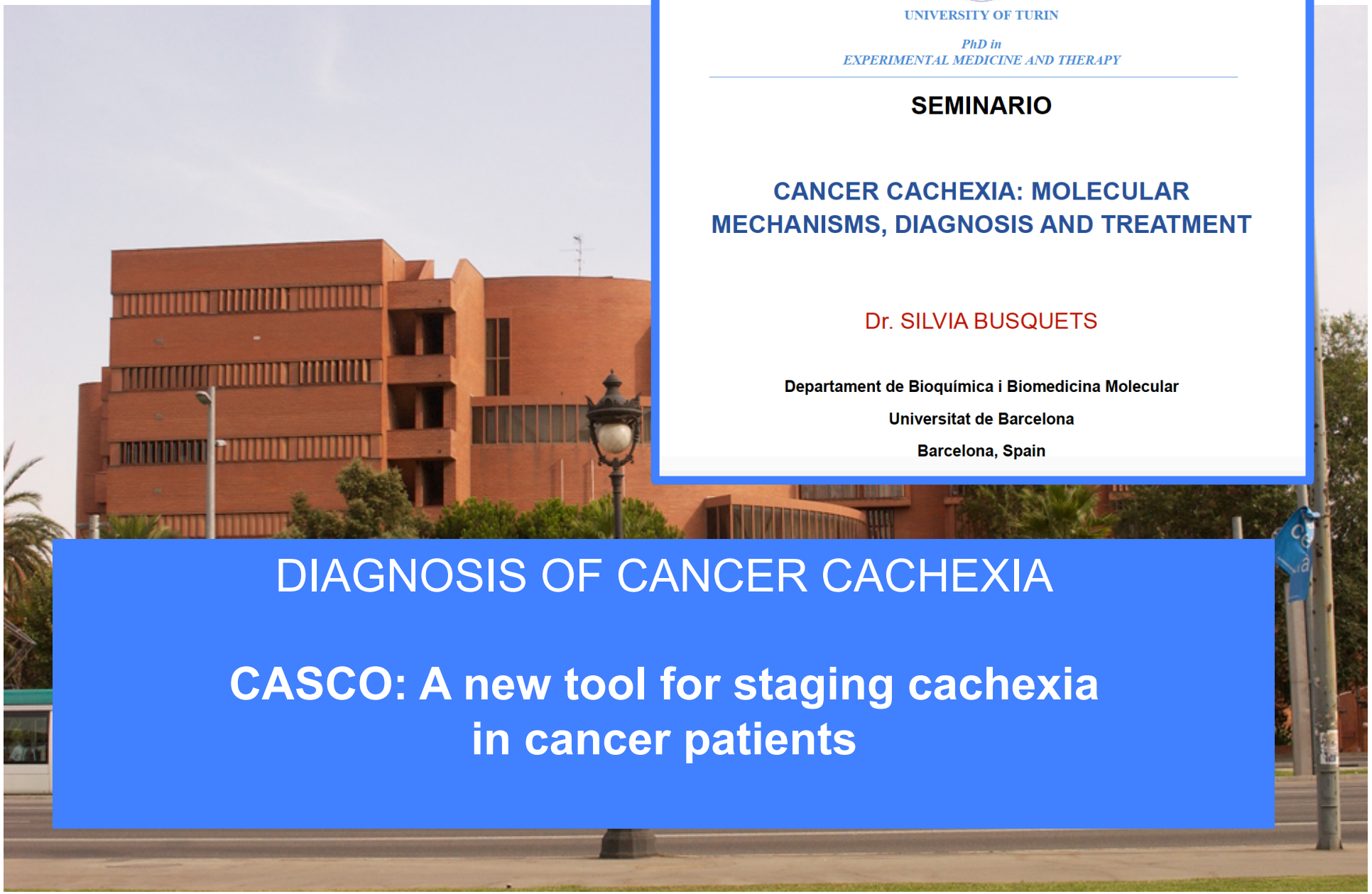
Conclusions and perspectives

Knowledge of the molecular mechanisms

underlying cancer cachexia may allow

for the design of distinctive therapeutic approaches

that ameliorate or even successfully cure this syndrome.



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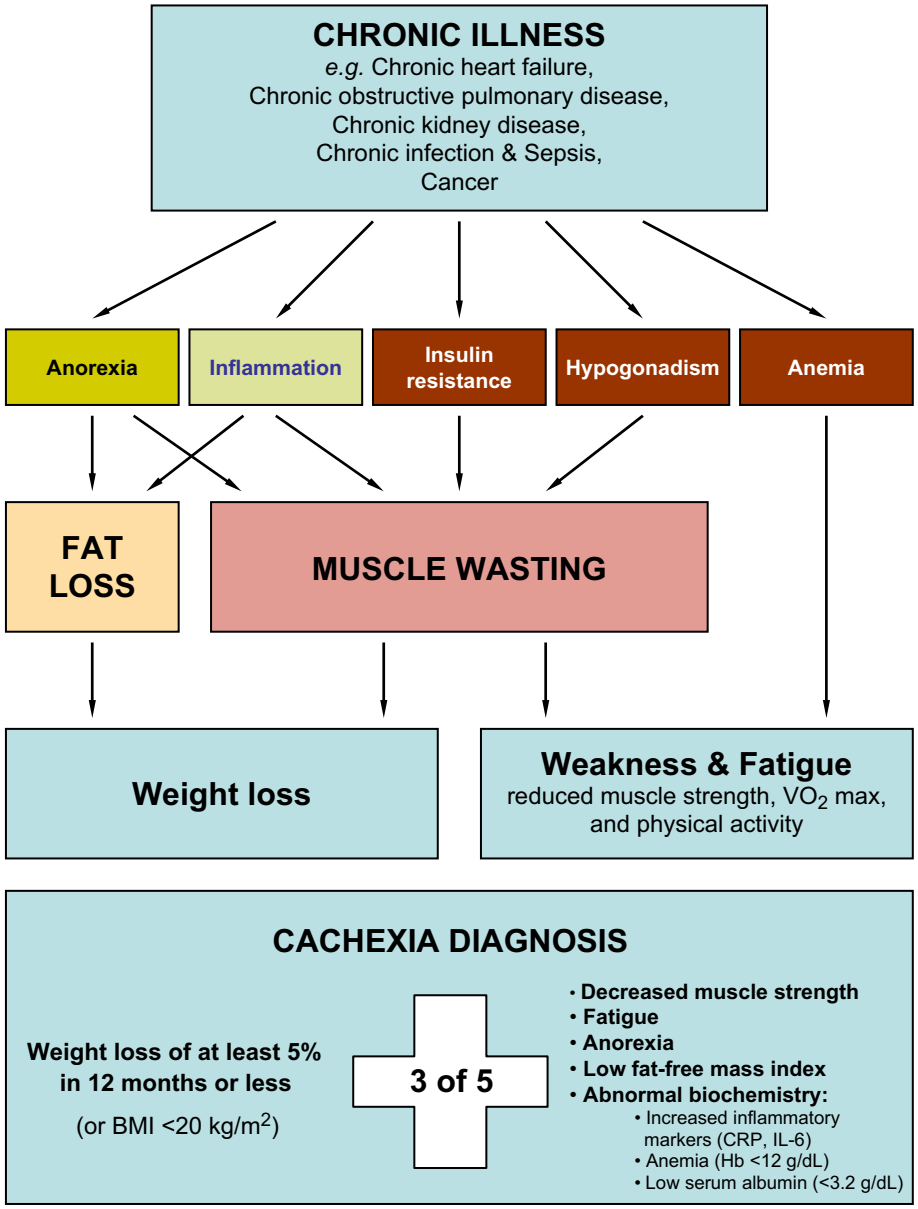
DIAGNOSIS OF CANCER CACHEXIA

CASCO: A new tool for staging cachexia in cancer patients



Cachexia, is a complex metabolic syndrome associated with underlying illness and characterized by **loss of muscle with or without loss of fat mass.**

Evans et al. Cachexia a new definition. Clinical Nutrition 2008, 27:793

Definition and classification of cancer cachexia: an international consensus

Kenneth Fearon, Florian Strasser*, Stefan D Anker, Ingvar Bosaeus, Eduardo Bruera, Robin L Fainsinger, Aminah Jatoi, Charles Loprinzi, Neil MacDonald, Giovanni Mantovani, Mellar Davis, Maurizio Muscaritoli, Faith Ottery, Lukas Radbruch, Paula Ravasco, Declan Walsh, Andrew Wilcock, Stein Kaasa, Vickie E Baracos*

Lancet Oncol **2011; 12: 489–95**

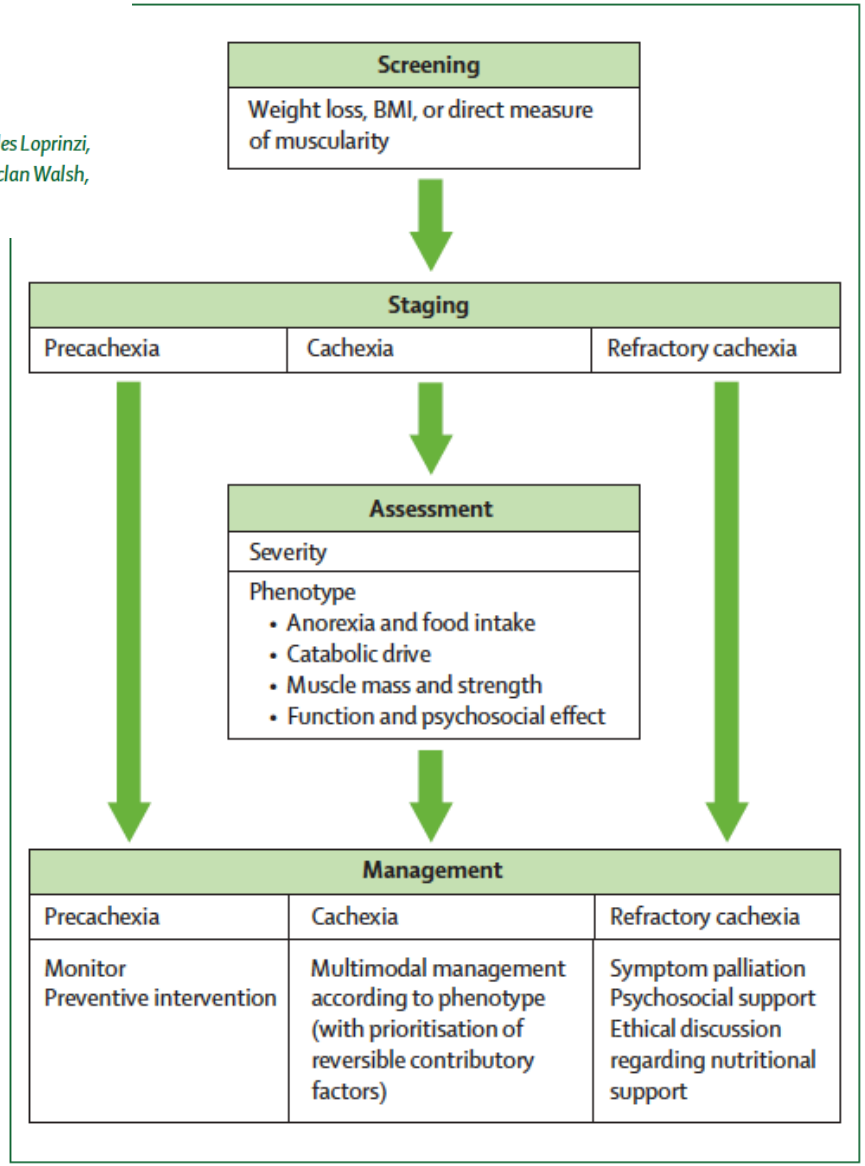


Figure 3: **Management algorithm for cancer cachexia**
Patients should be screened for cachexia, then undergo detailed assessment. All patients require optimum oncological and general medical management. Once patients with cachexia have been phenotyped, a detailed multimodal management plan (including nutrition, exercise, anti-inflammatory strategies, and other adjuncts) can be established. BMI=body-mass index.

Staging cachexia

- Cachexia has been defined but the definition does not consider the problem of staging it. **Classification of patients is important when considering therapy**.

- The objective of the **CAchexia SCOre** (**CASCO**) is to fulfill the existing gap in the classification of cachectic cancer patients.

CAchexia SCOre (CASCO)

J Cachexia Sarcopenia Muscle
DOI 10.1007/s13539-011-0027-5

ORIGINAL ARTICLE

The cachexia score (CASCO): a new tool for staging cachectic cancer patients

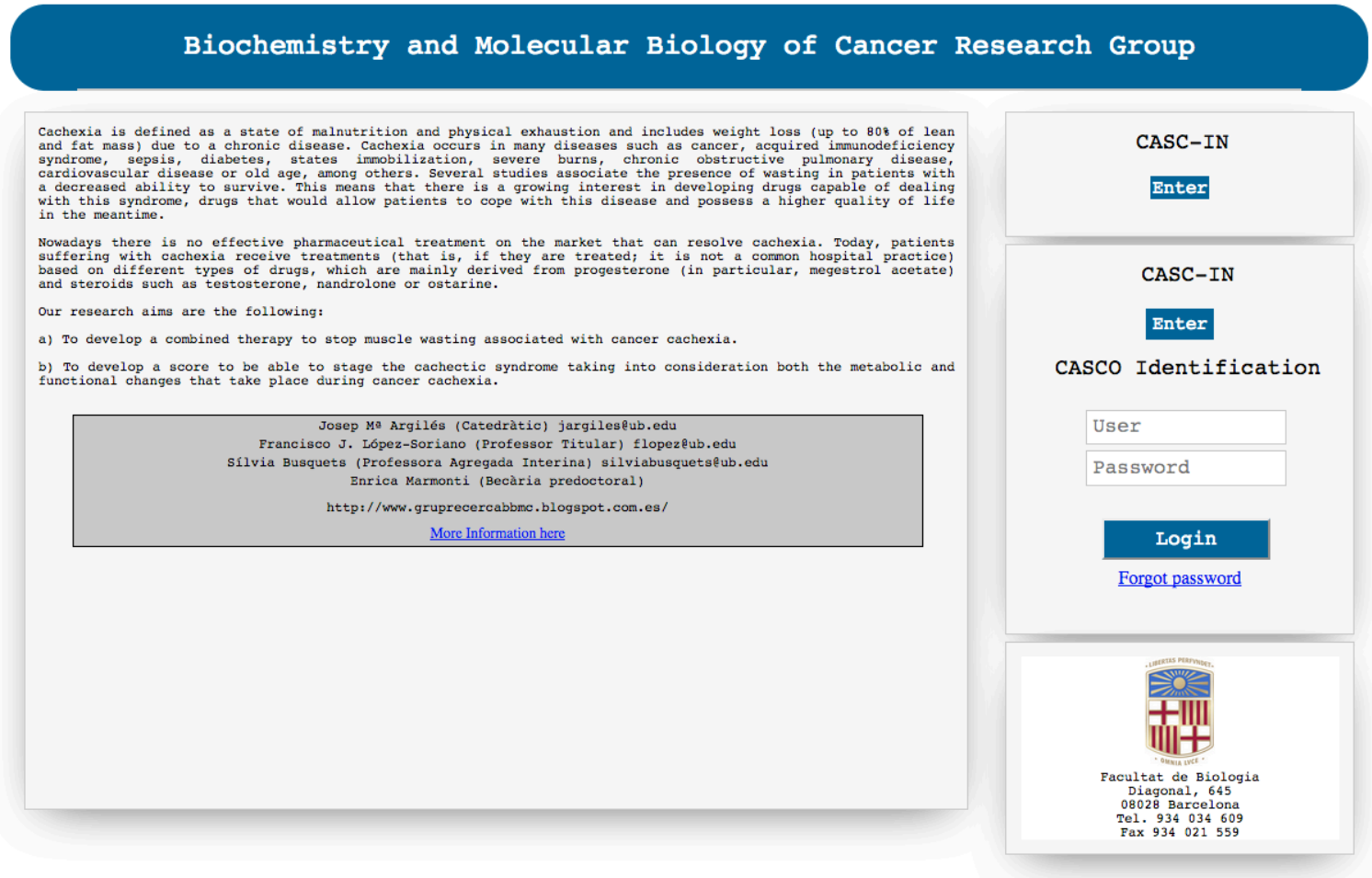
Josep M. Argilés · Francisco J. López-Soriano · Míriam Toledo · Angelica Betancourt · Roberto Serpe · Silvia Busquets

CASCO takes into consideration five components:

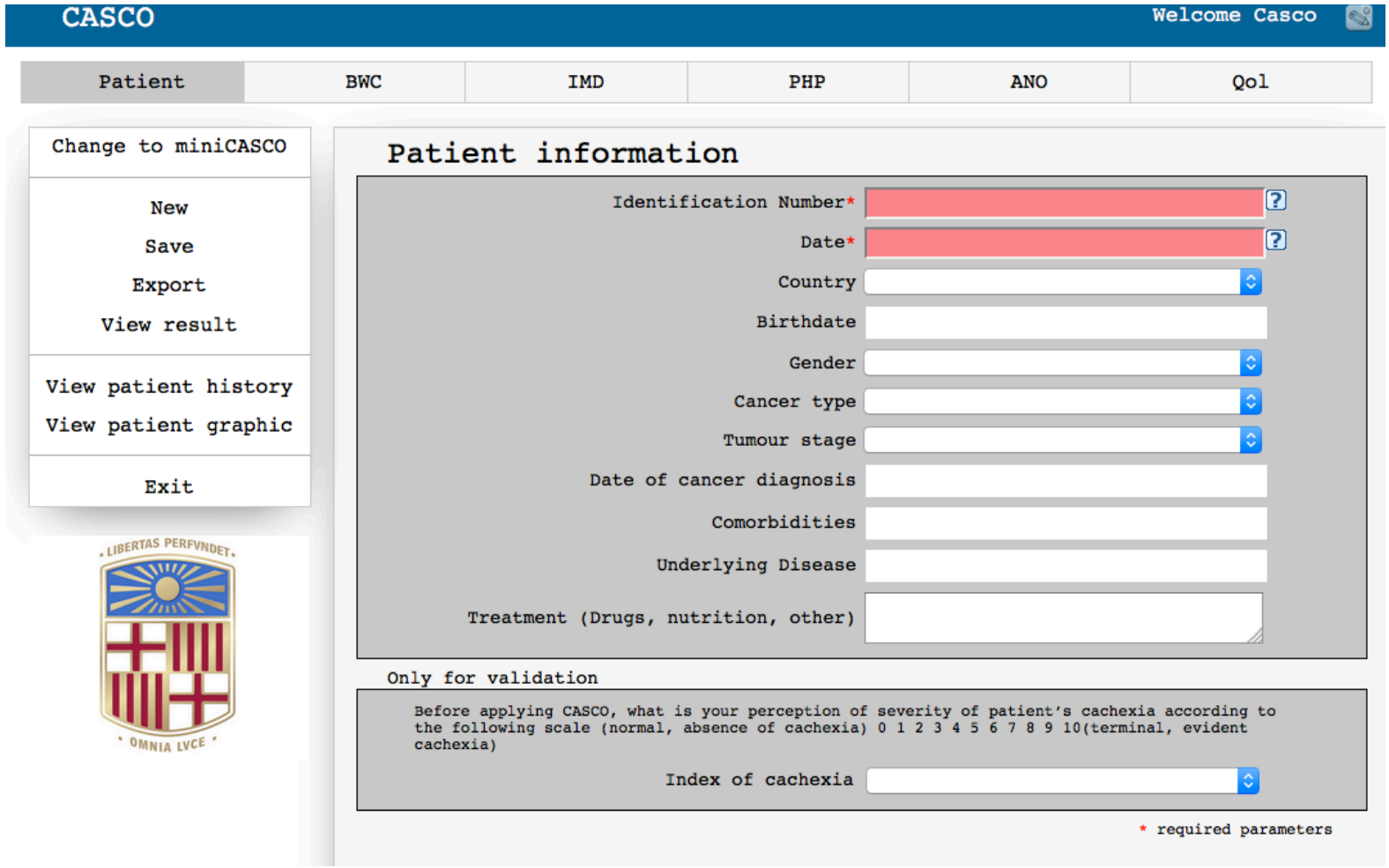
1. Body weight and lean body mass loss (BWC)
2. Inflammatory, immunological, and metabolic disturbances (IMD)
3. Physical performance (PHP)
4. Anorexia (ANO)
5. Quality of life (QoL)

Casco web page

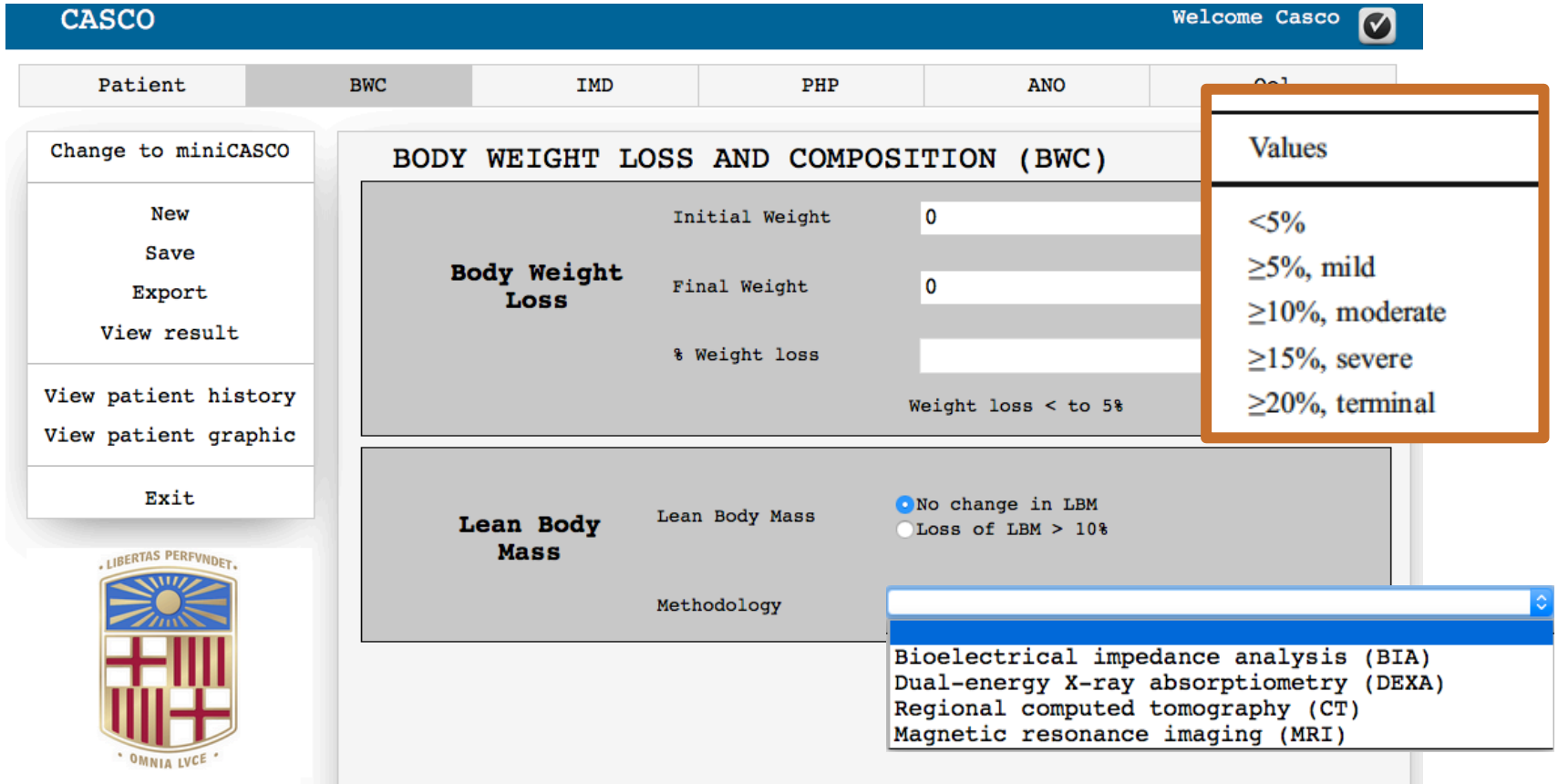
https://www.ub.edu/cancerresearchgroup/



PATIENT INFORMATION



Body weight and lean body mass loss

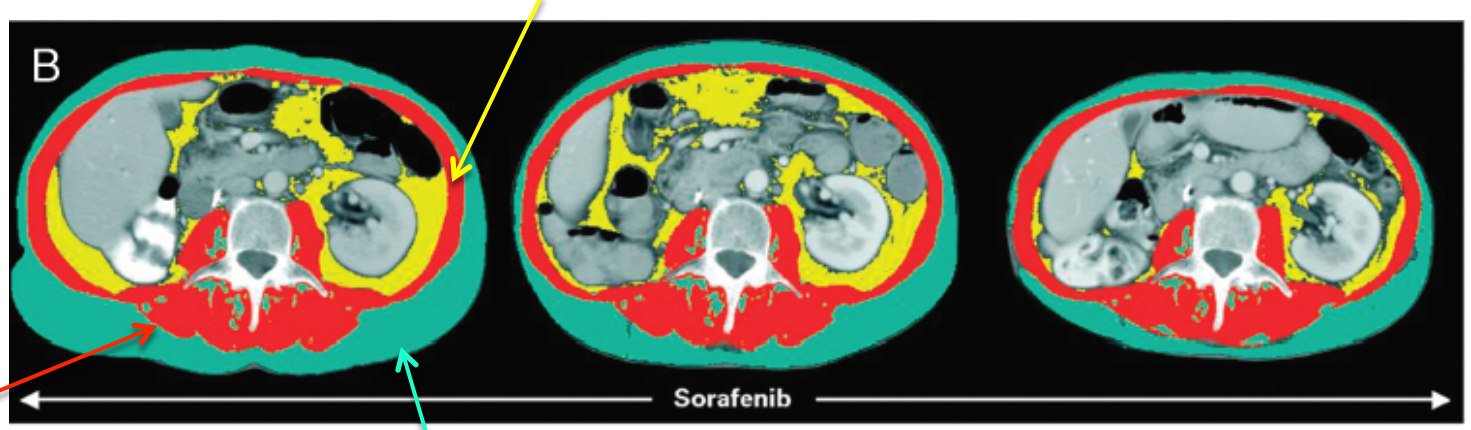


Body weight and lean body mass loss (BWC)

40%

Body weight and lean body mass loss

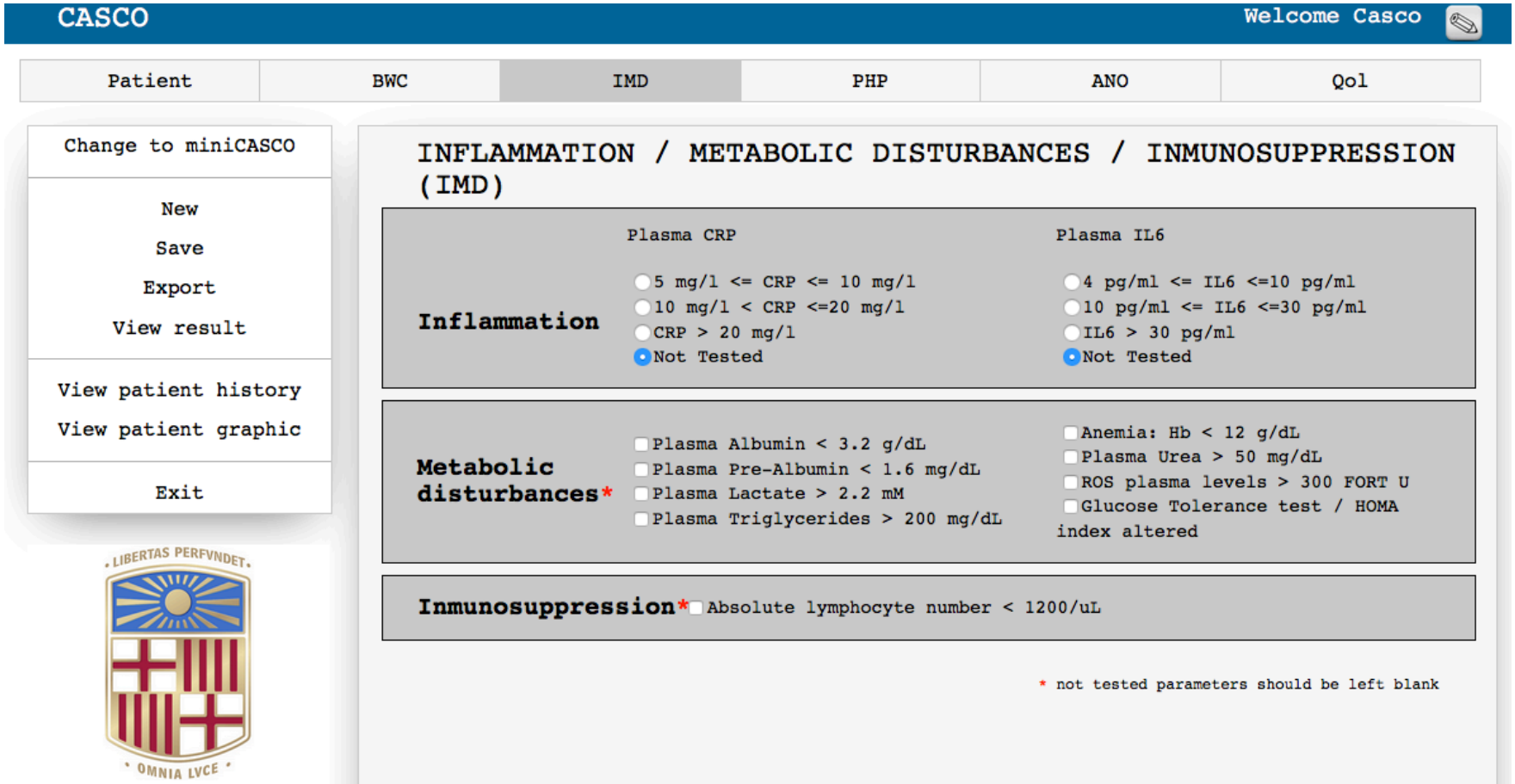
- Bioelectrical impedance analysis (BIA)
- Dual X-ray absorptiometry (DEXA)
- Cross-sectional imaging: **computed tomography (CT)** or magnetic resonance imaging (MRI).



computed tomography (CT)

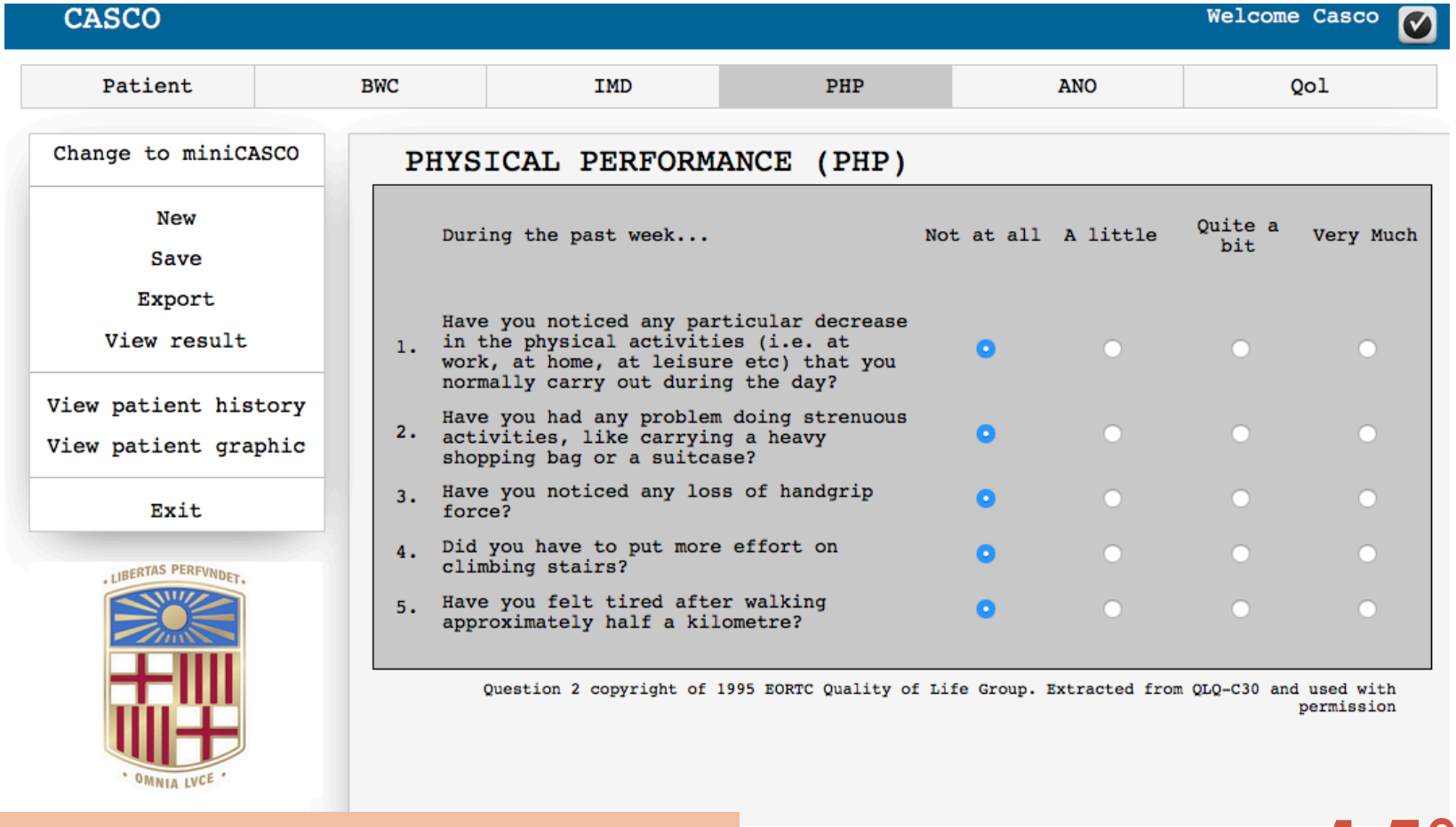
Antoun et al J Clin Oncol. 2010; 28:1054–1060.

Inflammatory, immunological and metabolic disturbances



Inflammatory, immunological, and metabolic disturbances (IMD) 20%

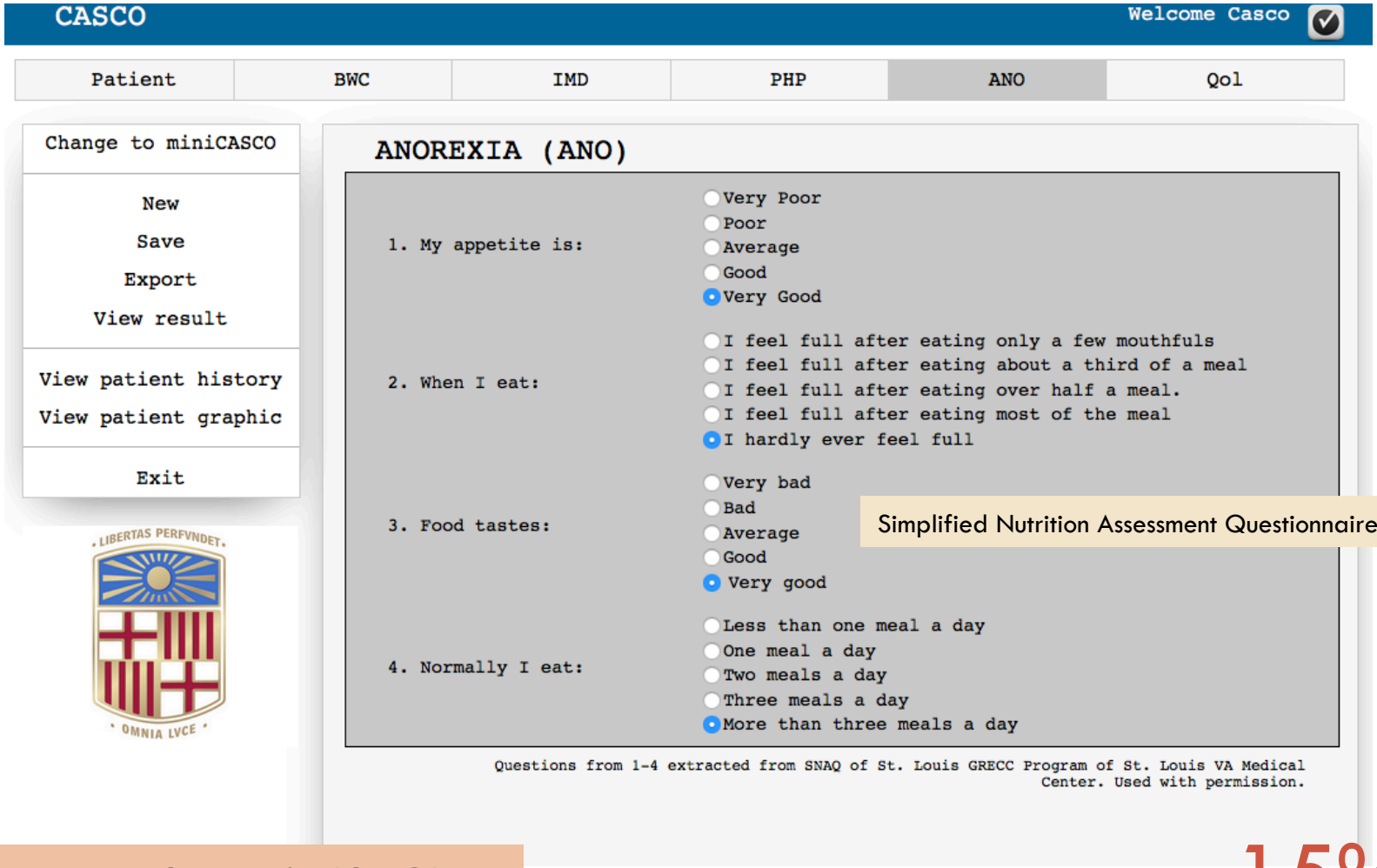
Physical performance



Physical performance (PHP)

15%

Anorexia

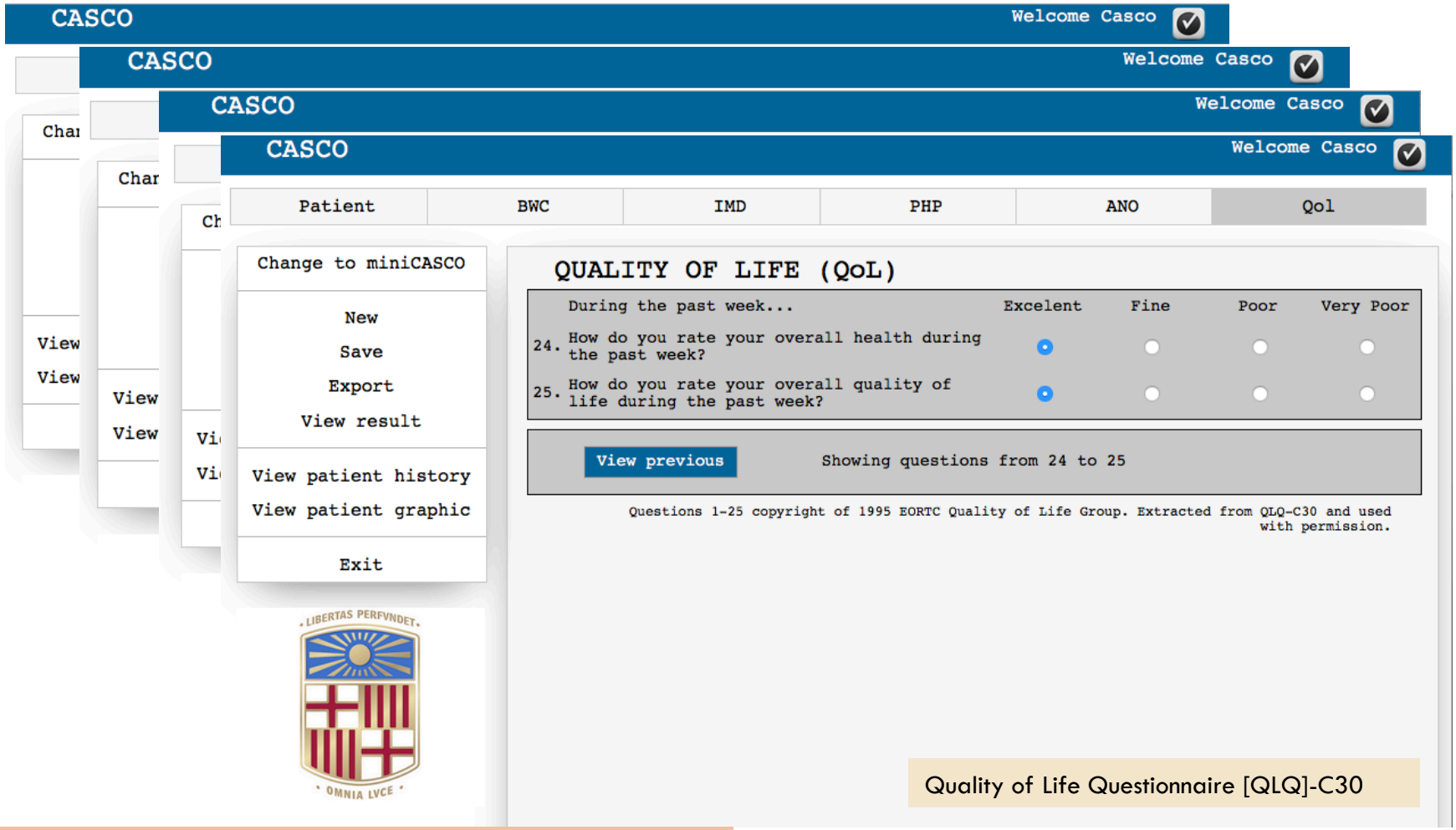


Simplified Nutrition Assessment Questionnaire (SNAQ)

Anorexia (ANO)

15%

Quality of life



Quality of life (QoL)

10%

Validation of CASCO

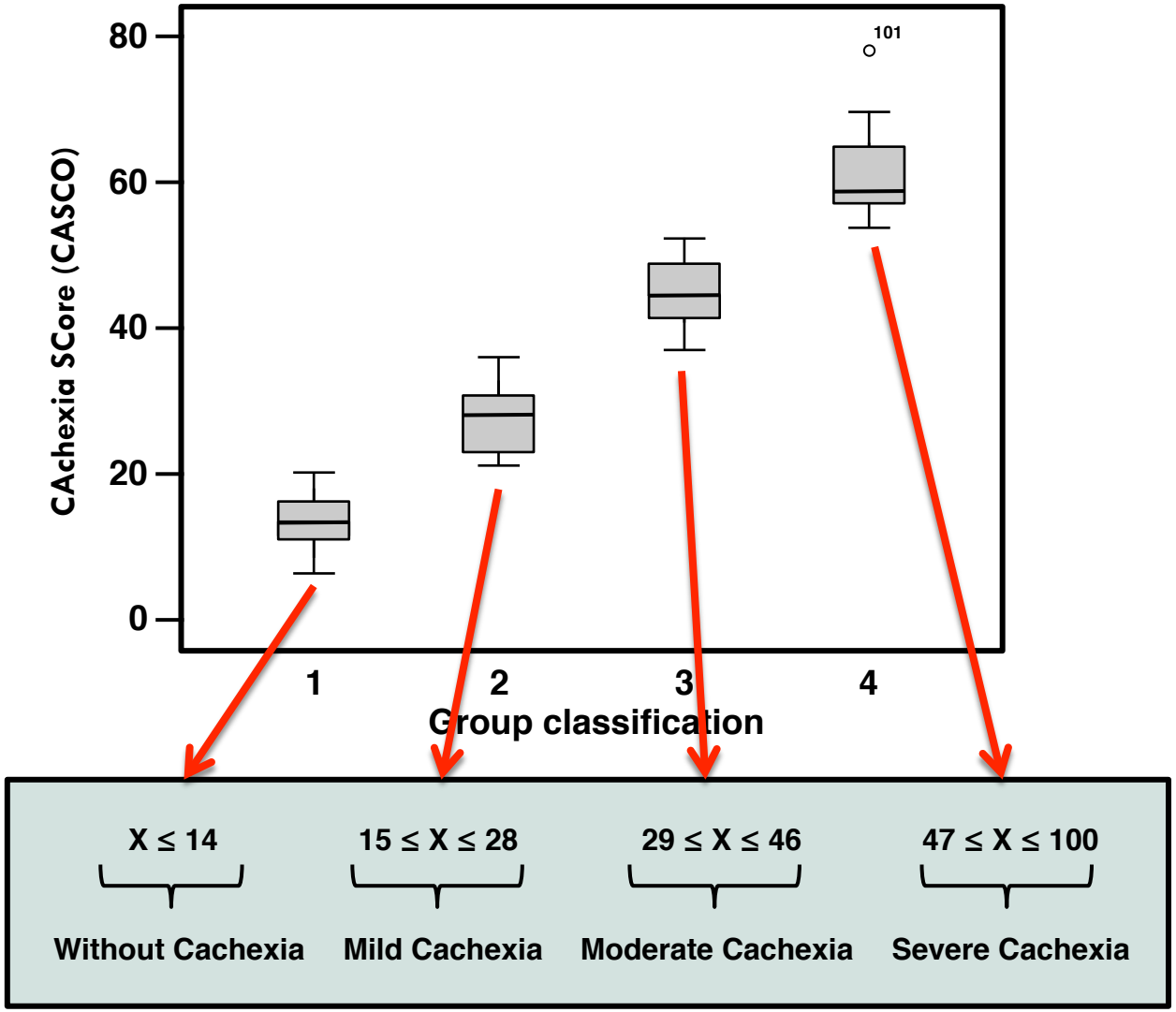
frontiers in Physiology

ORIGINAL RESEARCH
published: 17 February 2017
doi: 10.3389/fphys.2017.00092

Validation of the CAchexia SCOre (CASCO). Staging Cancer Patients: The Use of miniCASCO as a Simplified Tool

Josep M. Argilés[1,2†], Angelica Betancourt[1†], Joan Guàrdia-Olmos[3,4], Maribel Peró-Cebollero[3,4], Francisco J. López-Soriano[1,2], Clelia Madeddu[5], Roberto Serpe[5] and Silvia Busquets[1,2]*

Degrees of cachexia

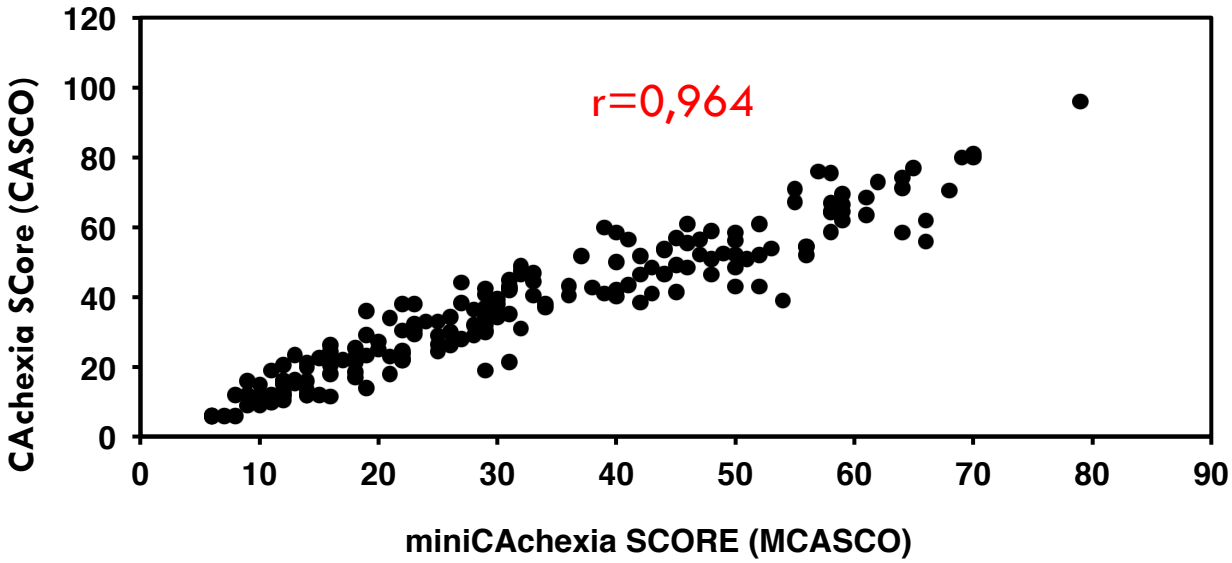


miniCASCO (CASCO's short form)

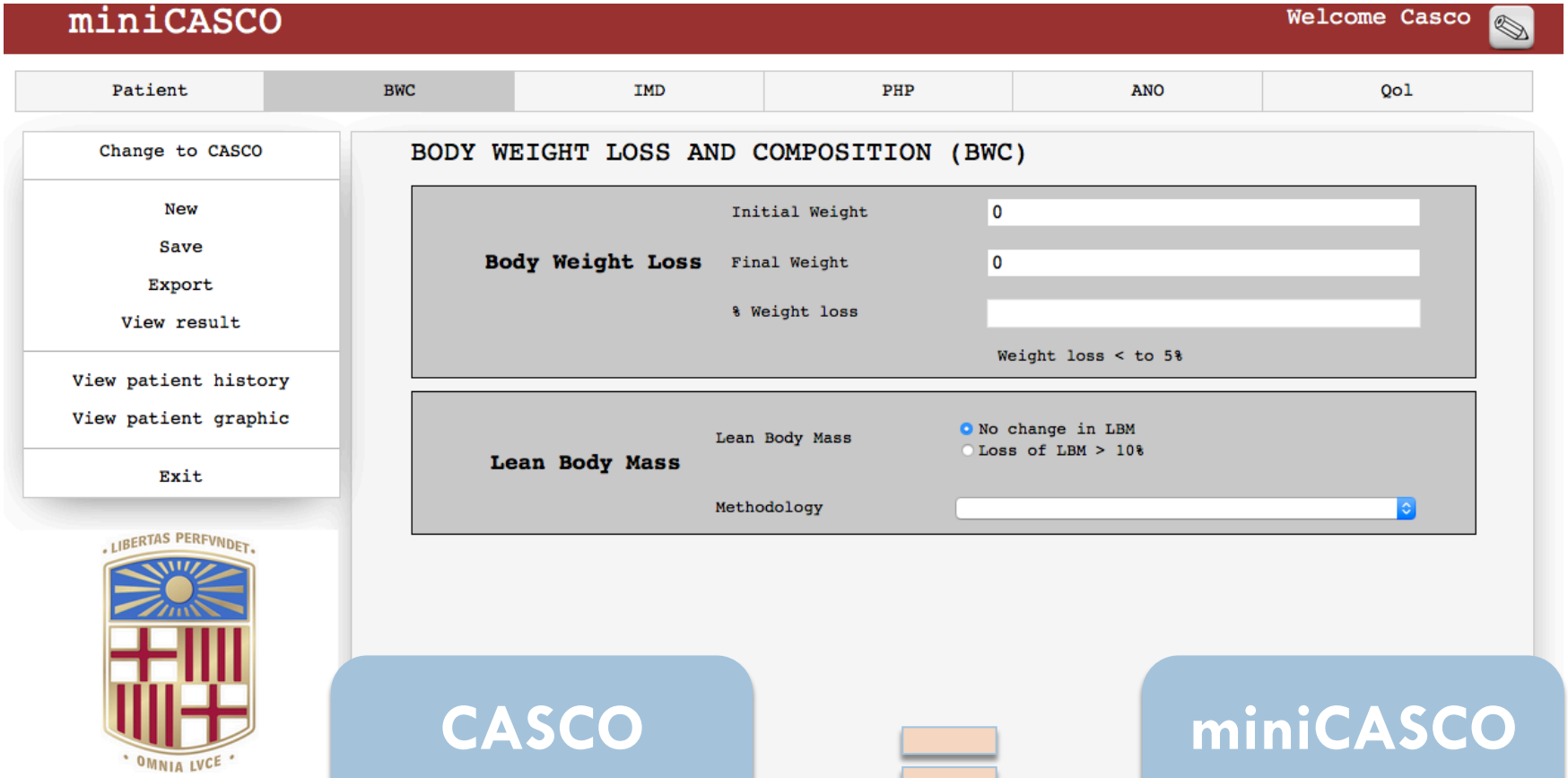
CASCO (47 items) → **miniCASCO (21 items)**

Reduction of the number of items

(component analysis: based on factorial loadings of the items in the component and the discrimination index)



miniCASCO (CASCO's short form)

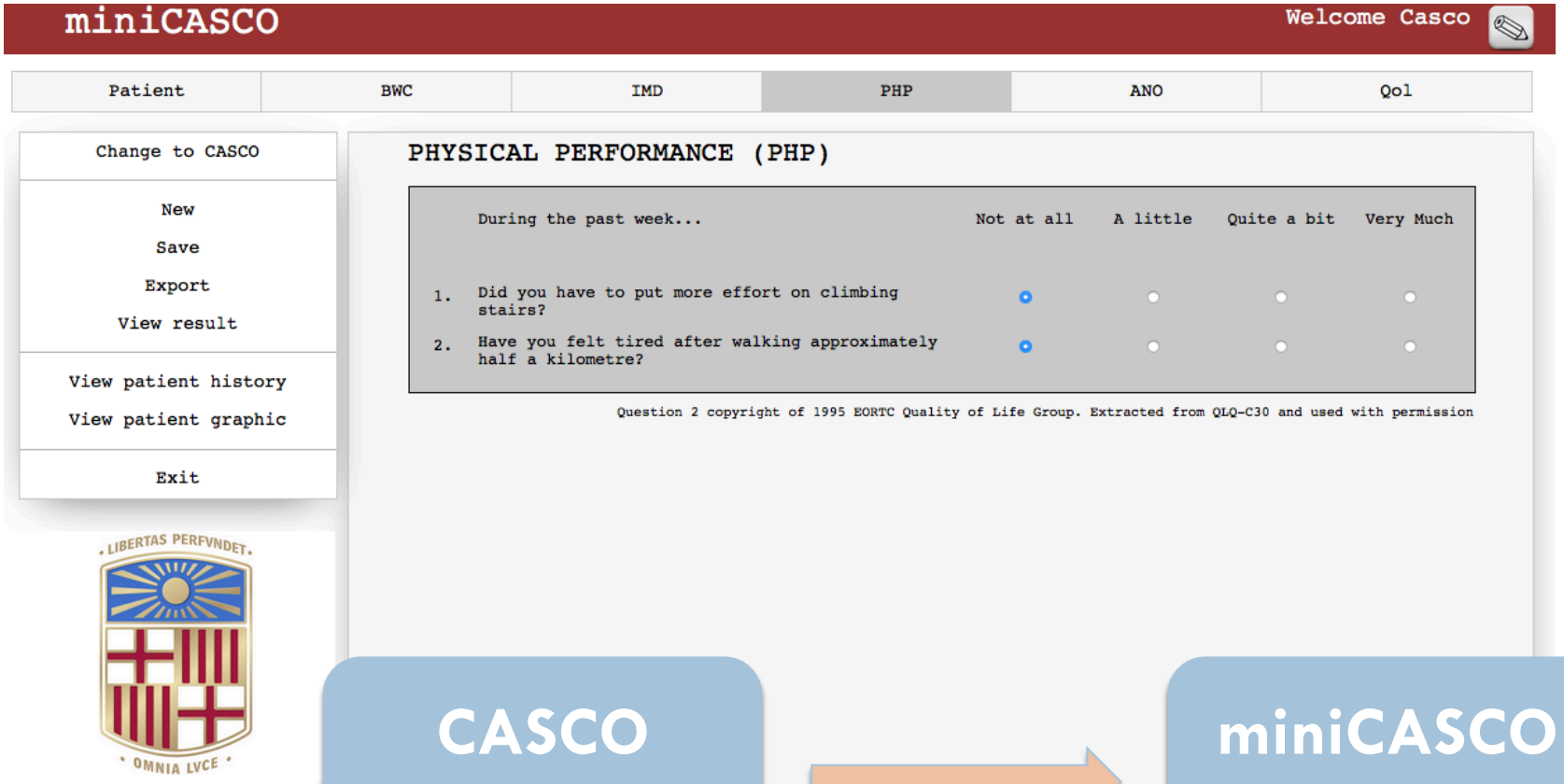


CASCO (2 items) = **miniCASCO (2 items)**

miniCASCO (CASCO's short form)

CASCO (11 items) → **miniCASCO (4 items)**

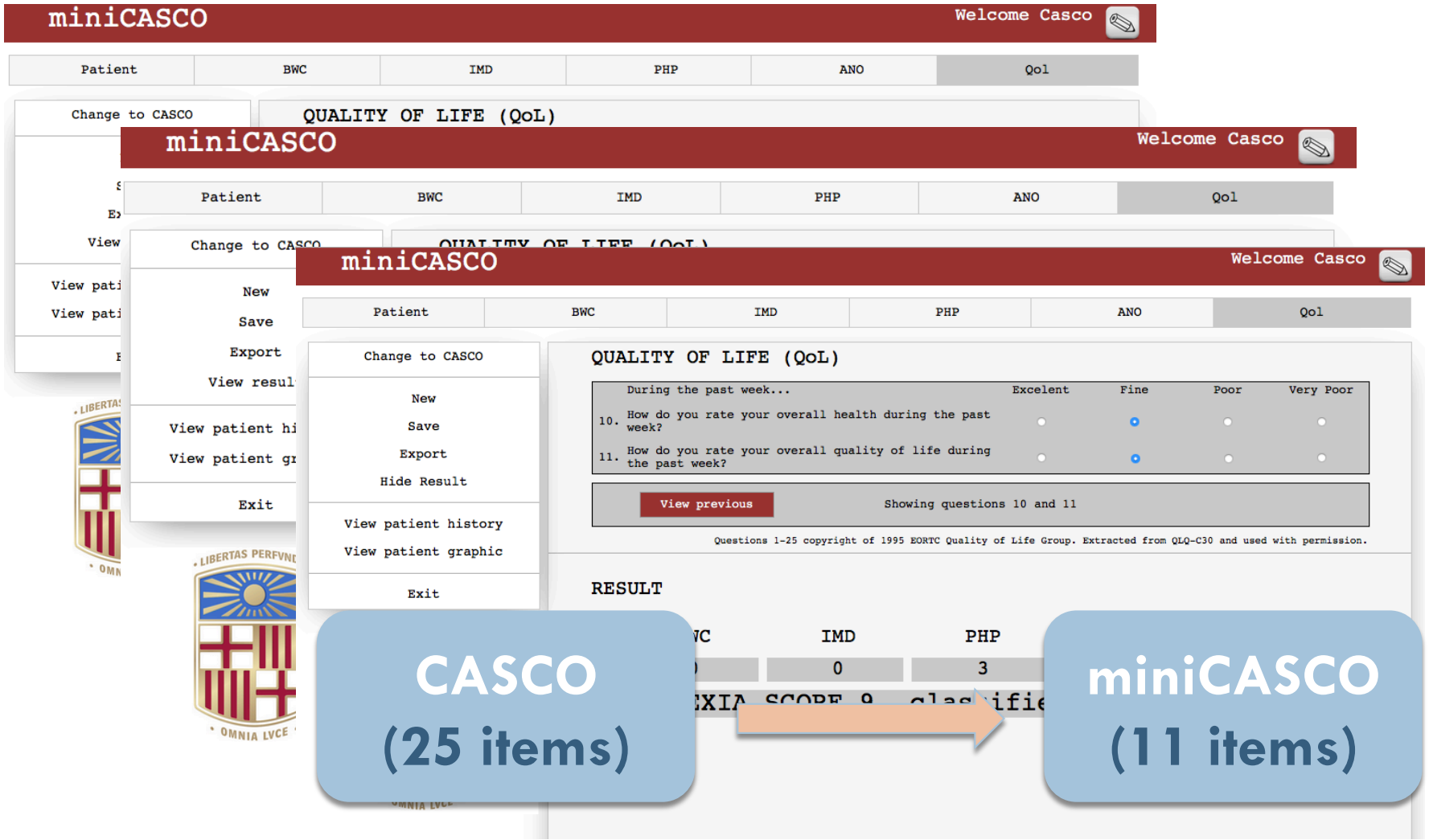
miniCASCO (CASCO's short form)



CASCO (5 items) → **miniCASCO (2 items)**

miniCASCO (CASCO's short form)

miniCASCO (CASCO's short form)



CONCLUSIONS

CASCO is a useful tool for the classification of cachexia according to 5 components:

- Body weight and lean body mass loss
- Inflammatory, immunological, and metabolic disturbances
- Physical performance
- Anorexia
- Quality of life

CASCO and miniCASCO could be also **useful tools for the treatment and nutritional recommendations of cachectic cancer patients** and will therefore **allow for a more adequate therapy depending of the cachexia classification.**

UNIVERSITY OF TURIN

PhD in
EXPERIMENTAL MEDICINE AND THERAPY

SEMINARIO

CANCER CACHEXIA: MOLECULAR MECHANISMS, DIAGNOSIS AND TREATMENT

Dr. SILVIA BUSQUETS

Departament de Bioquímica i Biomedicina Molecular

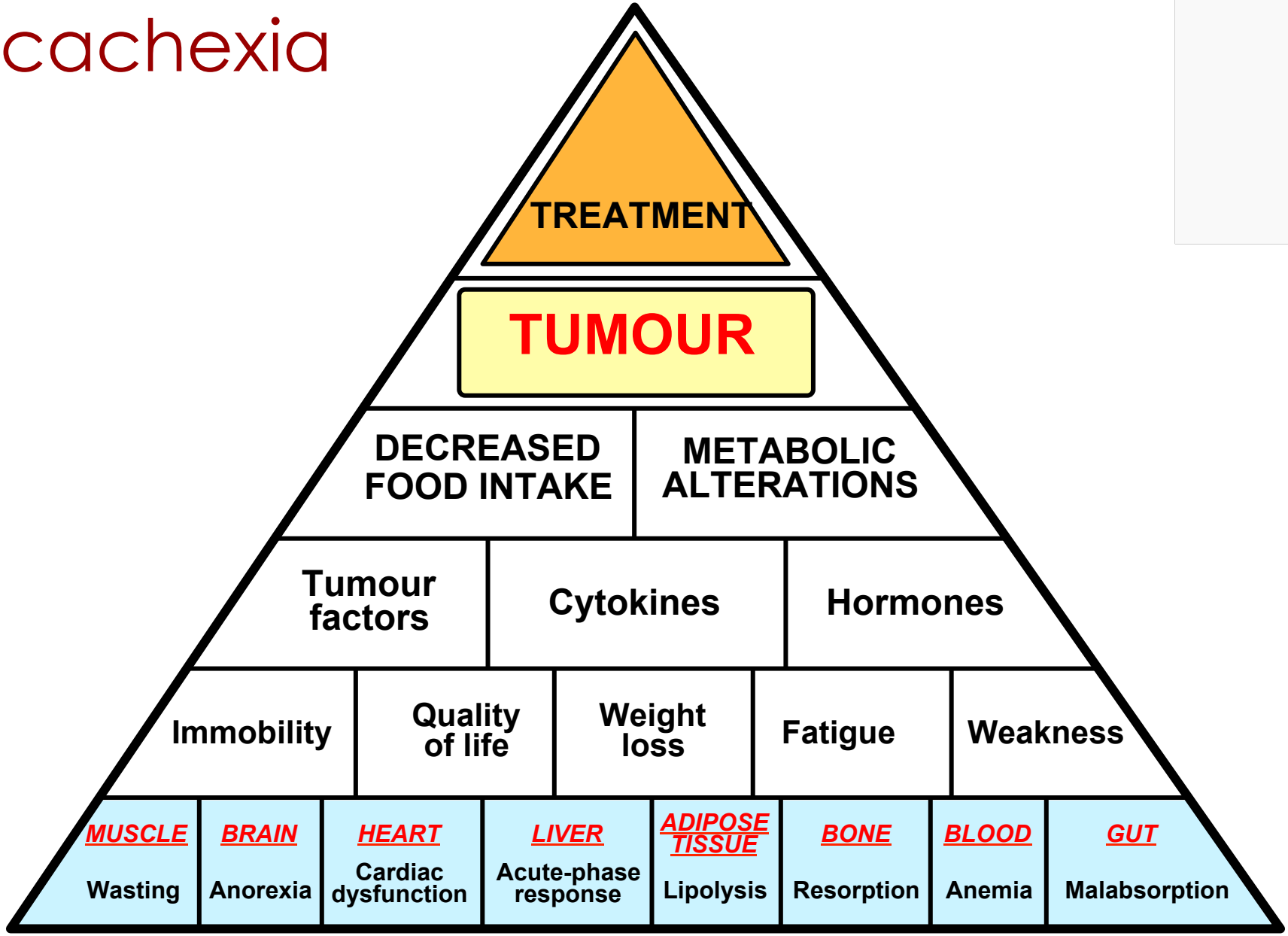
Universitat de Barcelona

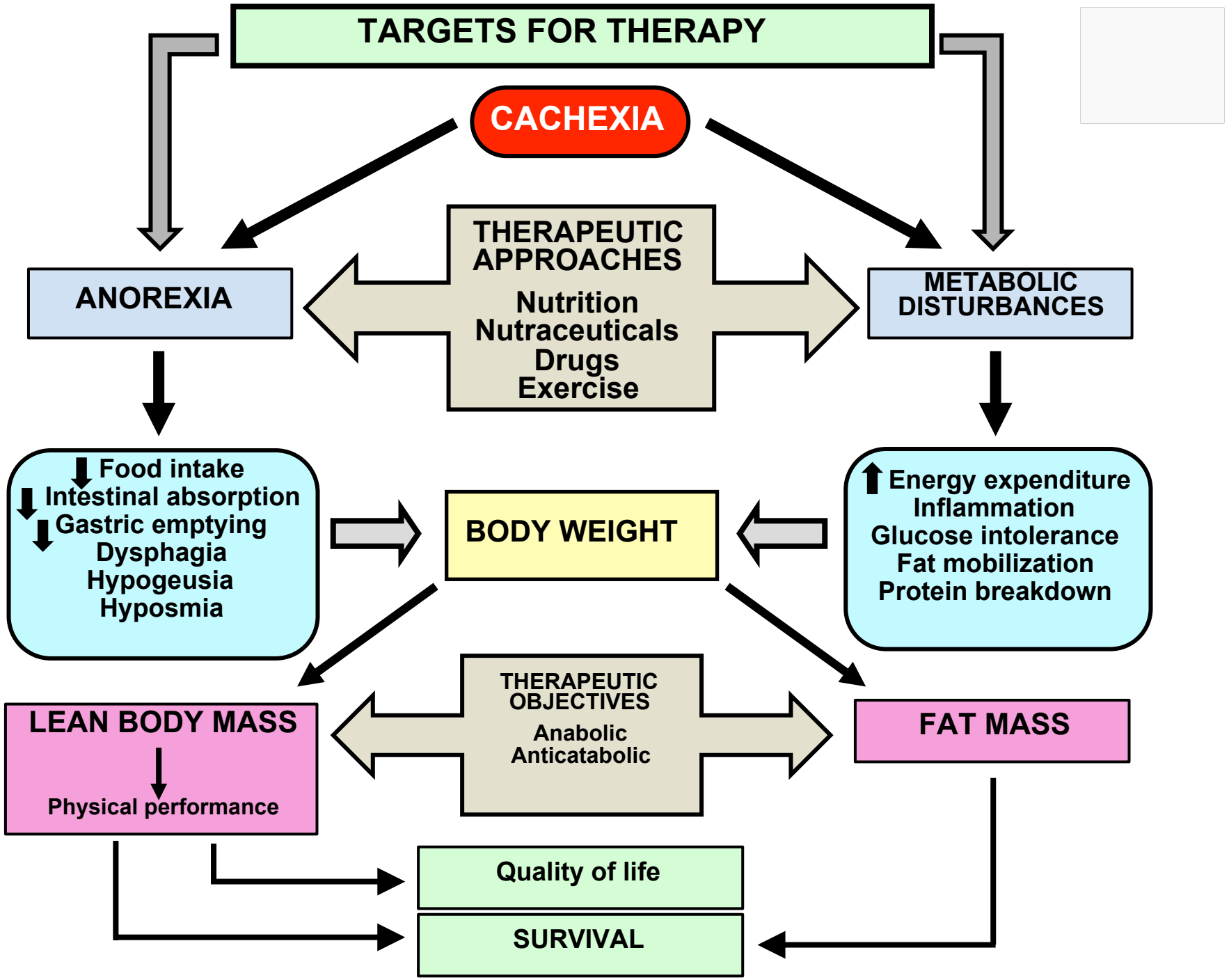
Barcelona, Spain

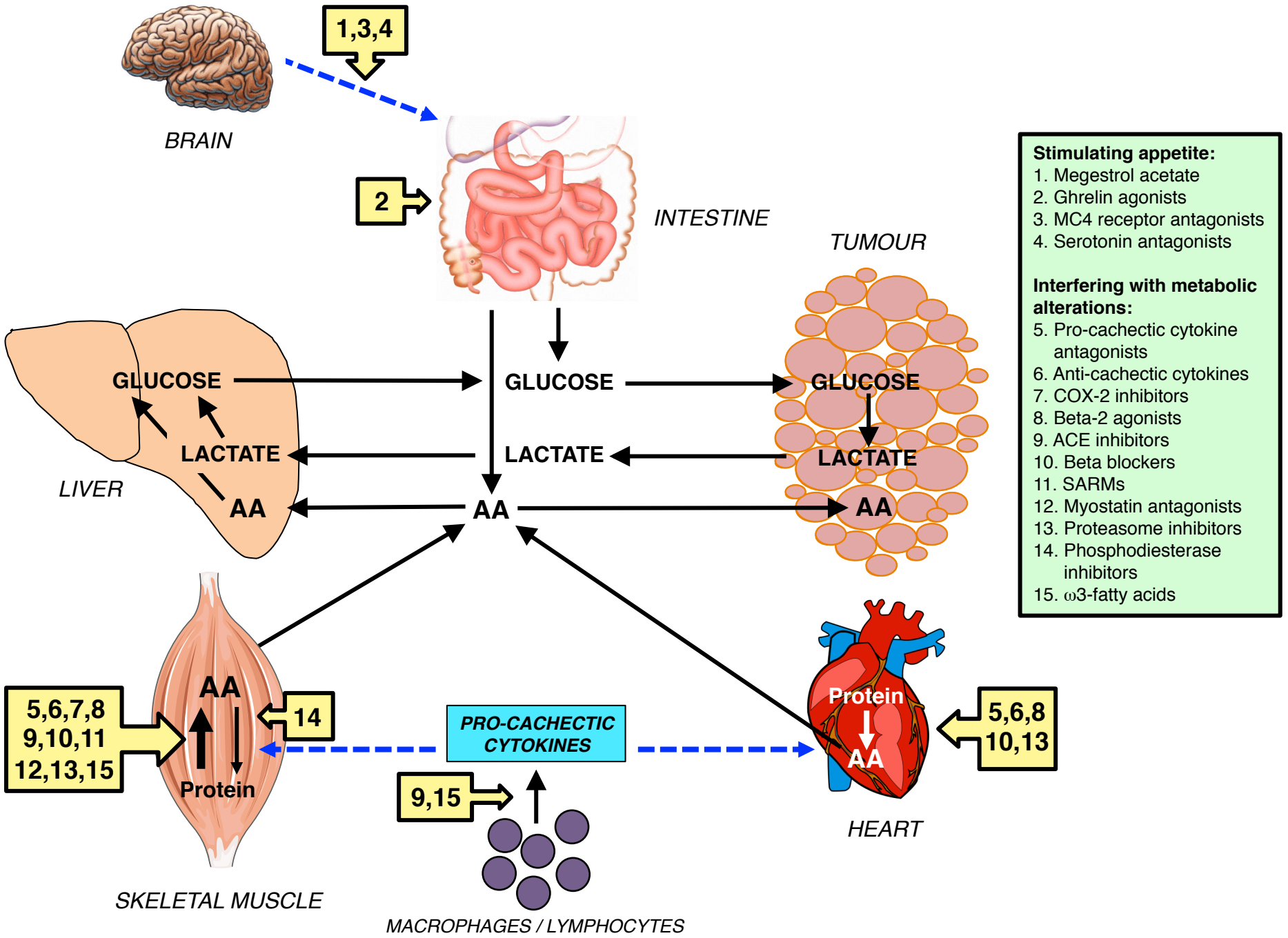
TREATMENT OF CANCER CACHEXIA

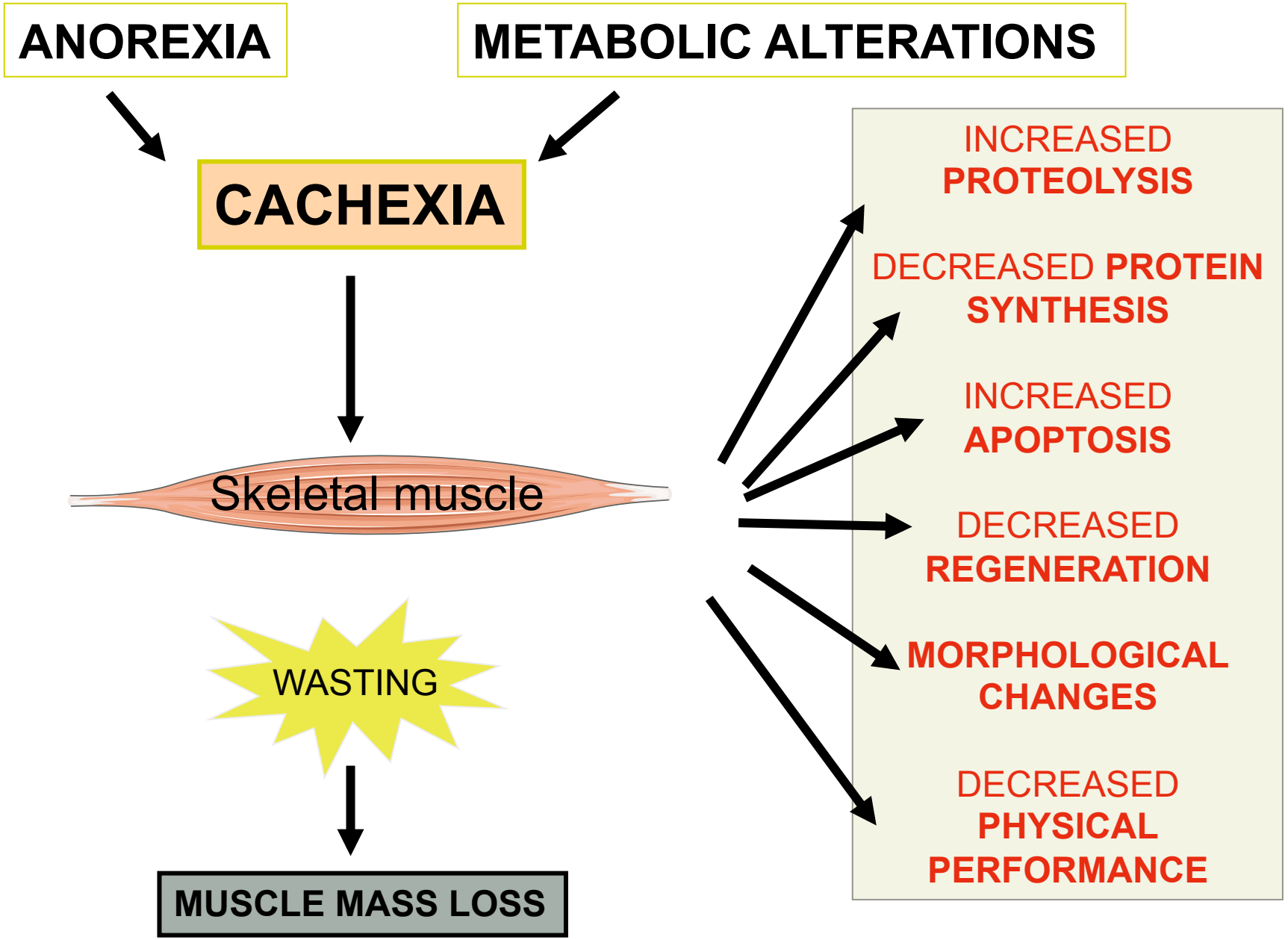
Complete reversal on muscle wasting in an animal model of cancer cachexia: additive effects of myostatin inhibition and beta-2 agonist treatment

Therapies to counteract cachexia

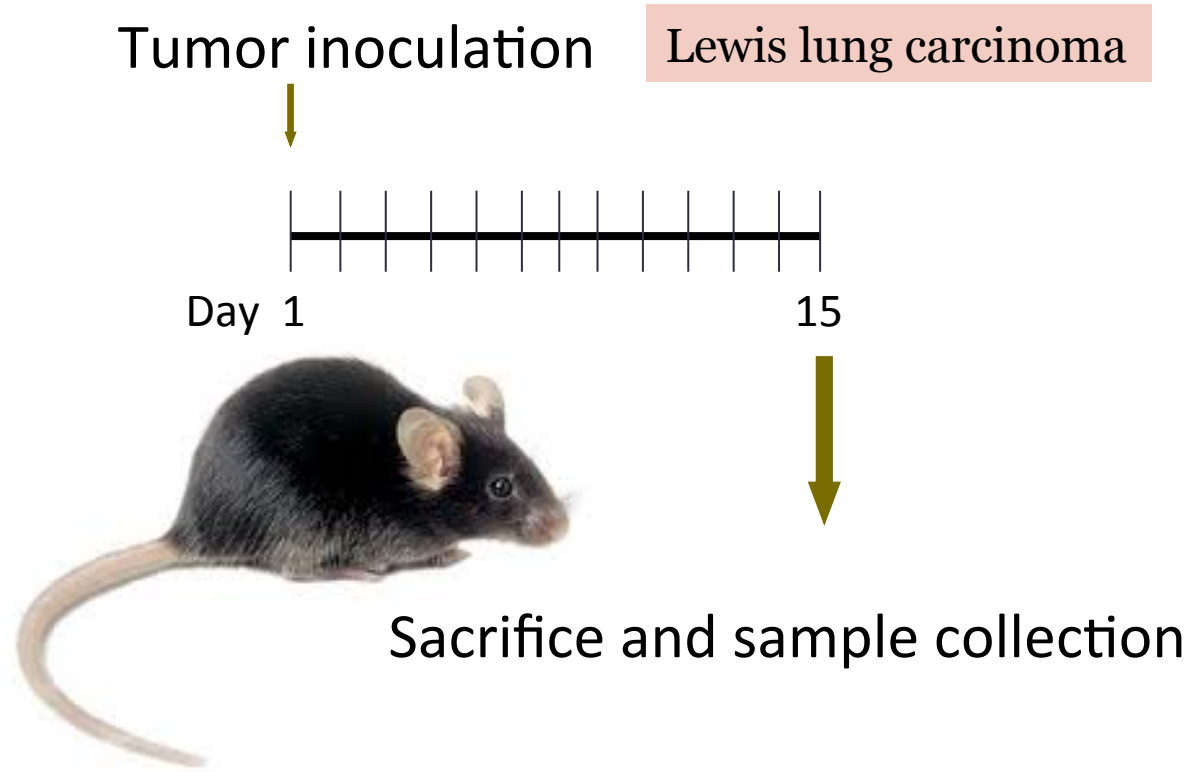



TARGETS FOR THERAPY
CACHEXIA
ANOREXIA
THERAPEUTIC APPROACHES
Nutrition
Nutraceuticals
Drugs
Exercise
METABOLIC DISTURBANCES
Food intake
Intestinal absorption
Gastric emptying
Dysphagia
Hypogeusia
Hyposmia
BODY WEIGHT
Energy expenditure
Inflammation
Glucose intolerance
Fat mobilization
Protein breakdown
LEAN BODY MASS
Physical performance
THERAPEUTIC OBJECTIVES
Anabolic
Anticatabolic
FAT MASS
Quality of life
SURVIVAL


1,3,4
BRAIN
2
INTESTINE
TUMOUR
GLUCOSE
LACTATE
AA
LIVER
GLUCOSE
LACTATE
AA
GLUCOSE
LACTATE
AA
5,6,7,8
9,10,11
12,13,15
AA
Protein
14
SKELETAL MUSCLE
PRO-CACHECTIC
CYTOKINES
9,15
MACROPHAGES / LYMPHOCYTES
Protein
AA
5,6,8
10,13
HEART
Stimulating appetite:
1. Megestrol acetate
2. Ghrelin agonists
3. MC4 receptor antagonists
4. Serotonin antagonists
Interfering with metabolic alterations:
5. Pro-cachectic cytokine antagonists
6. Anti-cachectic cytokines
7. COX-2 inhibitors
8. Beta-2 agonists
9. ACE inhibitors
10. Beta blockers
11. SARMs
12. Myostatin antagonists
13. Proteasome inhibitors
14. Phosphodiesterase inhibitors
15. ω3-fatty acids


ANOREXIA
METABOLIC ALTERATIONS
CACHEXIA
Skeletal muscle
WASTING
MUSCLE MASS LOSS
INCREASED PROTEOLYSIS
DECREASED PROTEIN SYNTHESIS
INCREASED APOPTOSIS
DECREASED REGENERATION
MORPHOLOGICAL CHANGES
DECREASED PHYSICAL PERFORMANCE

Experimental Cachexia model



0 1 2 3 4 5 6 7 8 9 10 11 12 13 14

TUMOUR INOCULATION

Lewis lung carcinoma

SACRIFICE AND
TISSUE COLLECTION

Dra. Sílvia Busquets

silviabusquets@ub.edu

Experimental Cachexia model

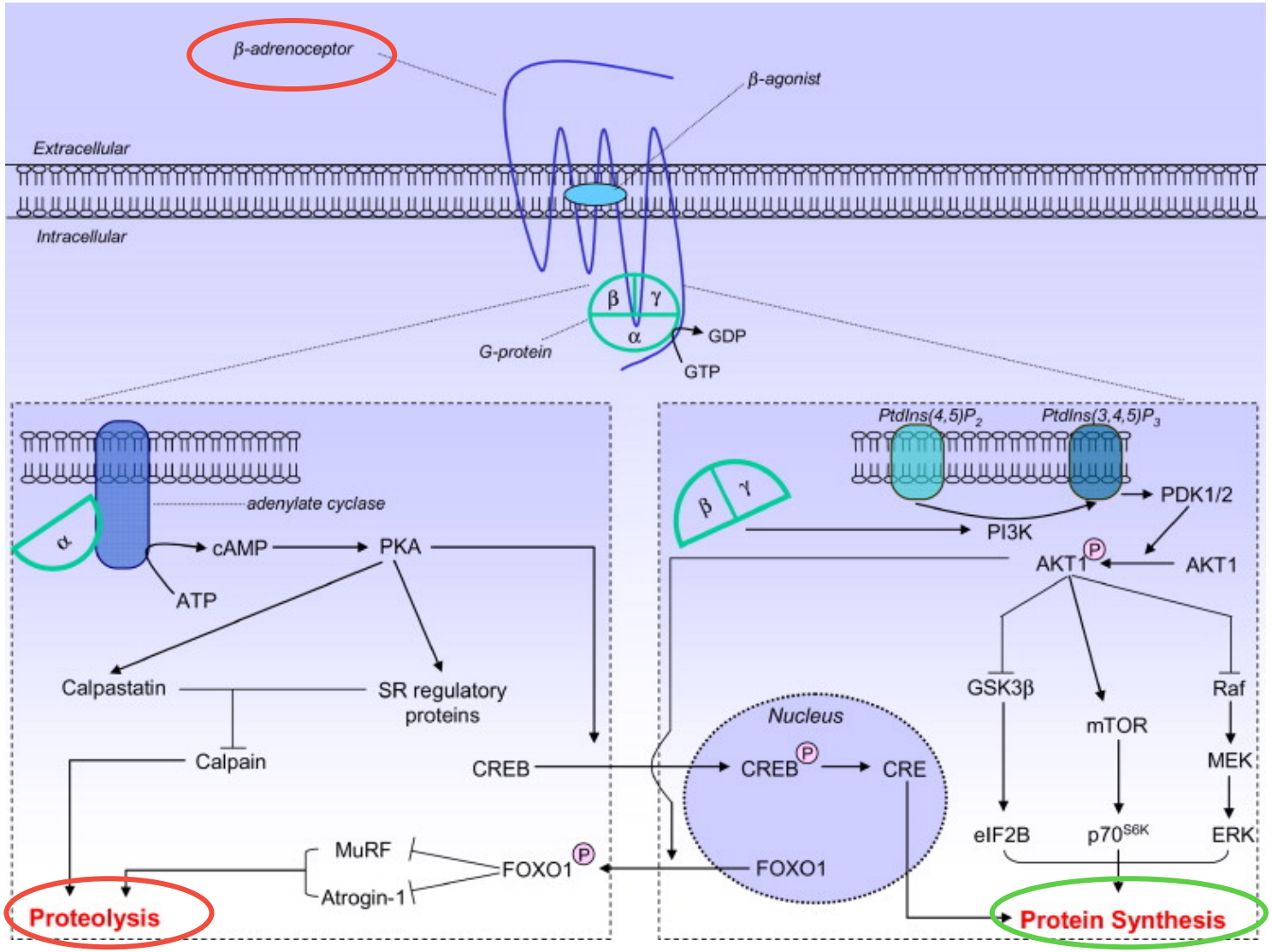
Lewis lung carcinoma

	Control	Tumor	P -value
Initial Body Weight (g)	17 ± 0.2	17 ± 0.5	NS
Final Body Weight (g)	22 ± 1	14 ± 1	<0.001
Weight Increase (%)	29%	-17%	
Muscles (mg/100g Initial Body Weight)			
GASTROCNEMIUS	764 ± 16	**381 ± 6**	<0.001
TIBIALIS	241 ± 8	**123 ± 7**	<0.001
SOLEUS	42 ± 1	**37 ± 3**	NS
EDL	58 ± 4	**39 ± 3**	<0.01
HEART	656 ± 10	**530 ± 6**	<0.001

P-value: Student *t*-test

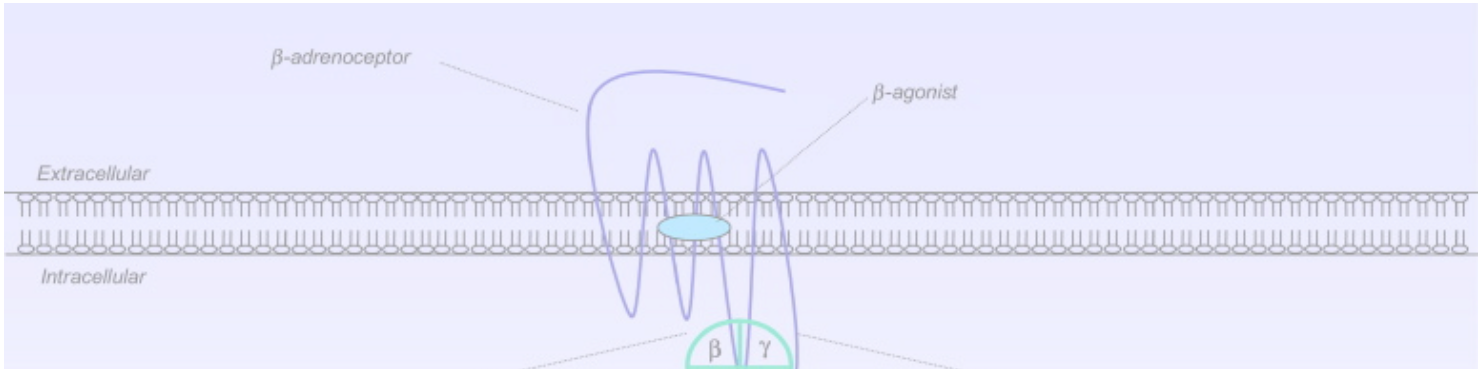
Lewis lung carcinoma (LLC)
vehicle (saline)–treated animals
(placebo control)

LLC-treated animals with:
soluble receptor antagonist of myostatin
(10 mg/kg BW s.c. twice a week)
+
Formoterol
(1 mg/ kg BW s.c. once a day)



Possible β2-adrenergic signaling pathways involved in skeletal muscle hypertrophy following administration of a β2-agonist.

Figure obtained from: Lynch GS, Schertzer JD, Ryall JG. **Therapeutic approaches for muscle wasting disorders.** Pharmacol Ther. 2007 Mar;113(3):461-87.

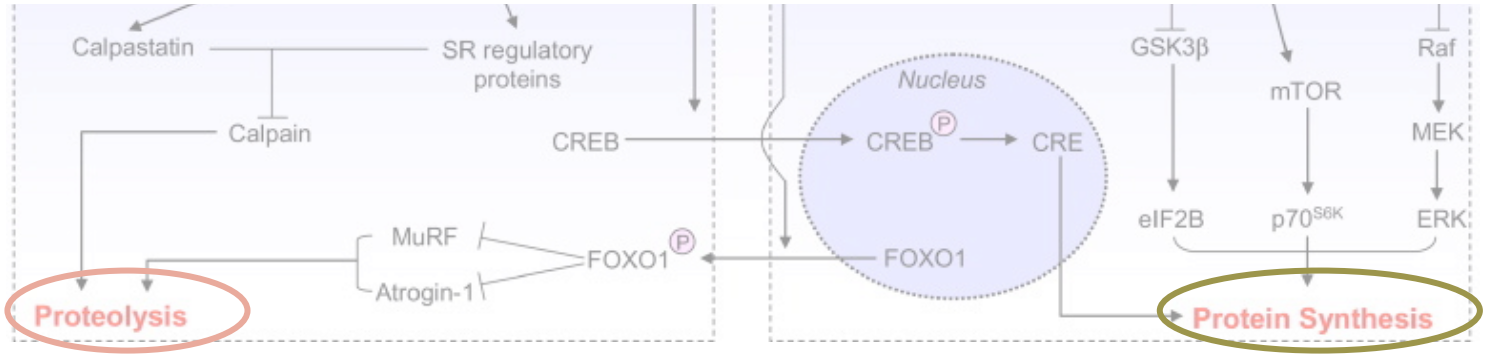


[CANCER RESEARCH 64, 6725–6731, September 15, 2004]

Anticachectic Effects of Formoterol: A Drug for Potential Treatment of Muscle Wasting

Sílvia Busquets, Maria T. Figueras, Gemma Fuster, Vanessa Almendro, Rodrigo Moore-Carrasco, Elisabet Ametller, Josep M. Argilés, and Francisco J. López-Soriano

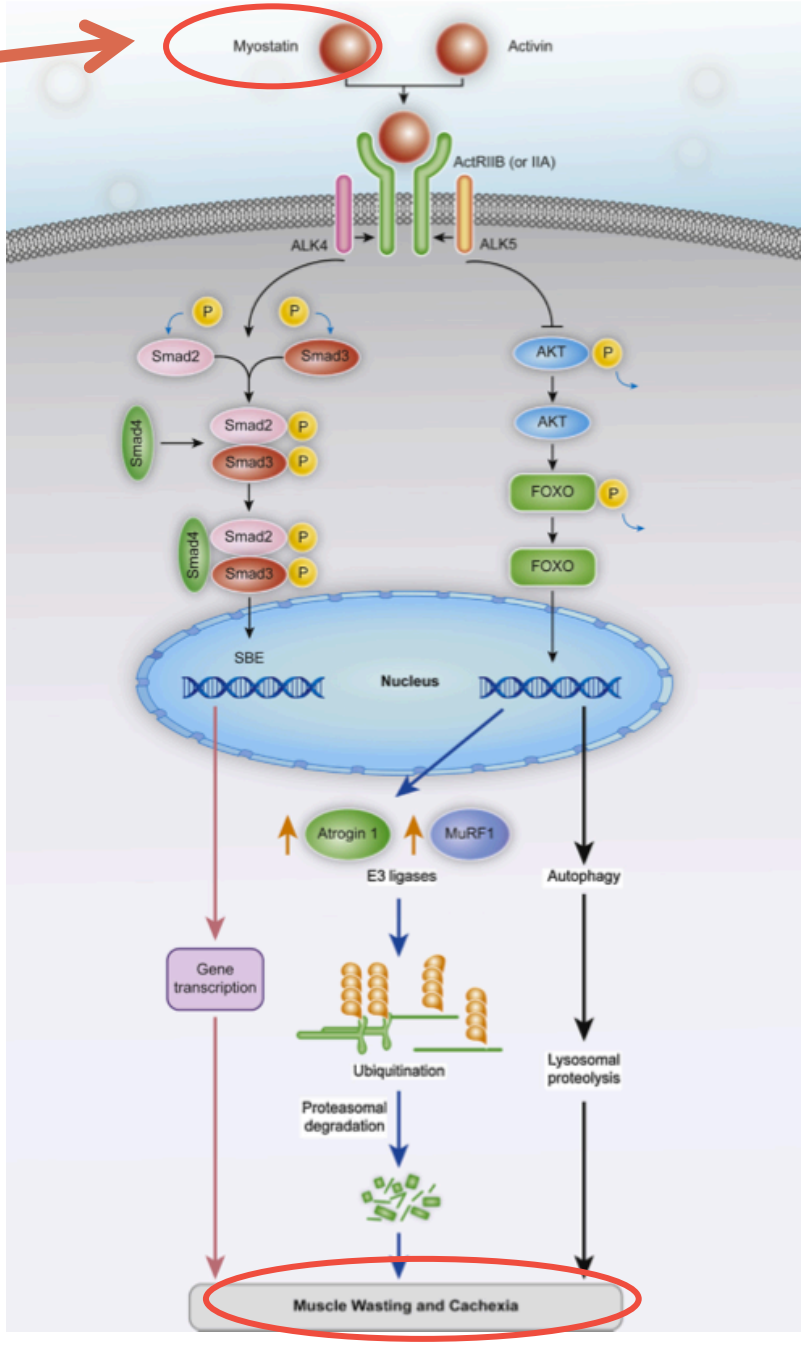
Cancer Research Group, Departament de Bioquímica i Biologia Molecular, Facultat de Biologia, Universitat de Barcelona, Barcelona, Spain



Possible β2-adrenergic signaling pathways involved in skeletal muscle hypertrophy following administration of a β2-agonist.

Figure obtained from: Lynch GS, Schertzer JD, Ryall JG. **Therapeutic approaches for muscle wasting disorders.** Pharmacol Ther. 2007 Mar;113(3):461-87.

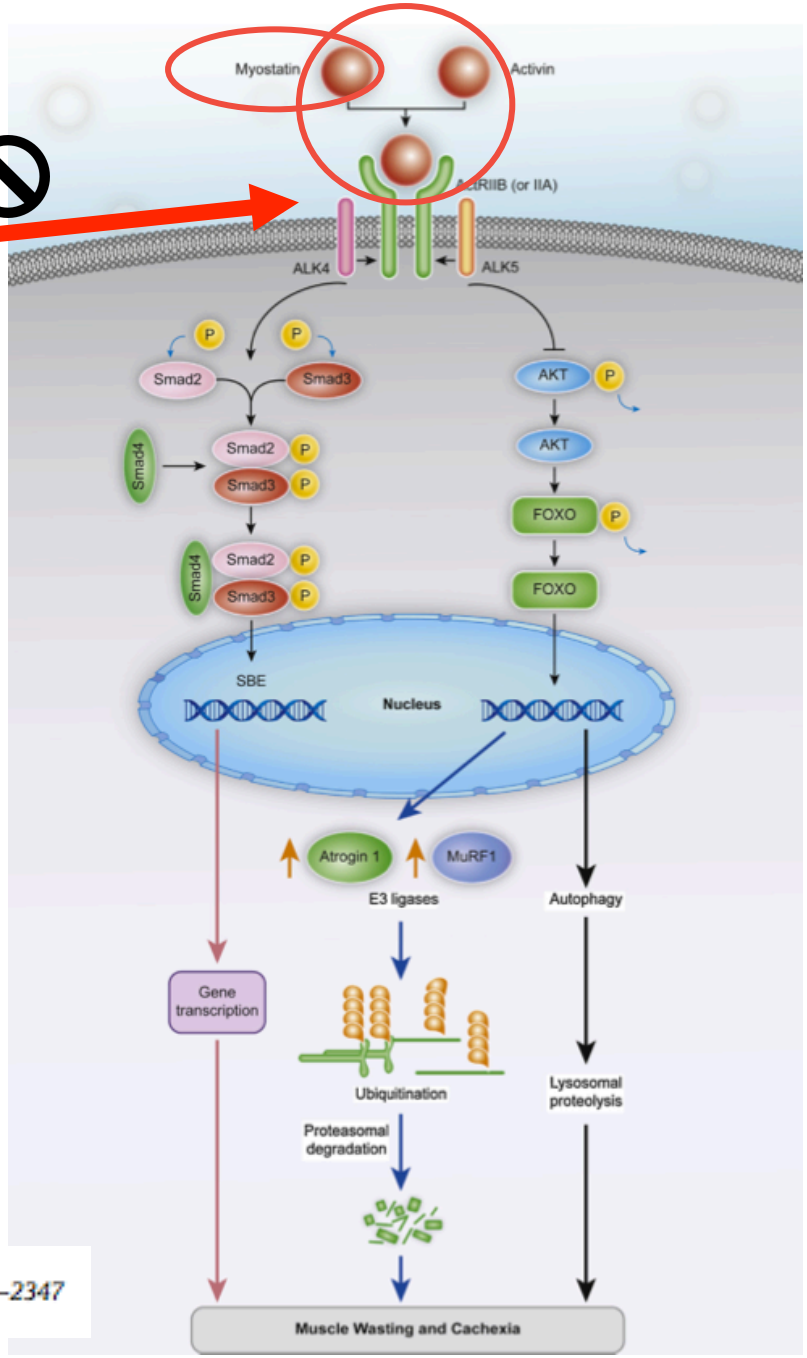
Myostatin acts as a negatively muscle growth regulator



H.Q. Han et al. / The International Journal of Biochemistry & Cell Biology 45 (2013) 2333–2347

sActRIIB

soluble receptor antagonist of myostatin

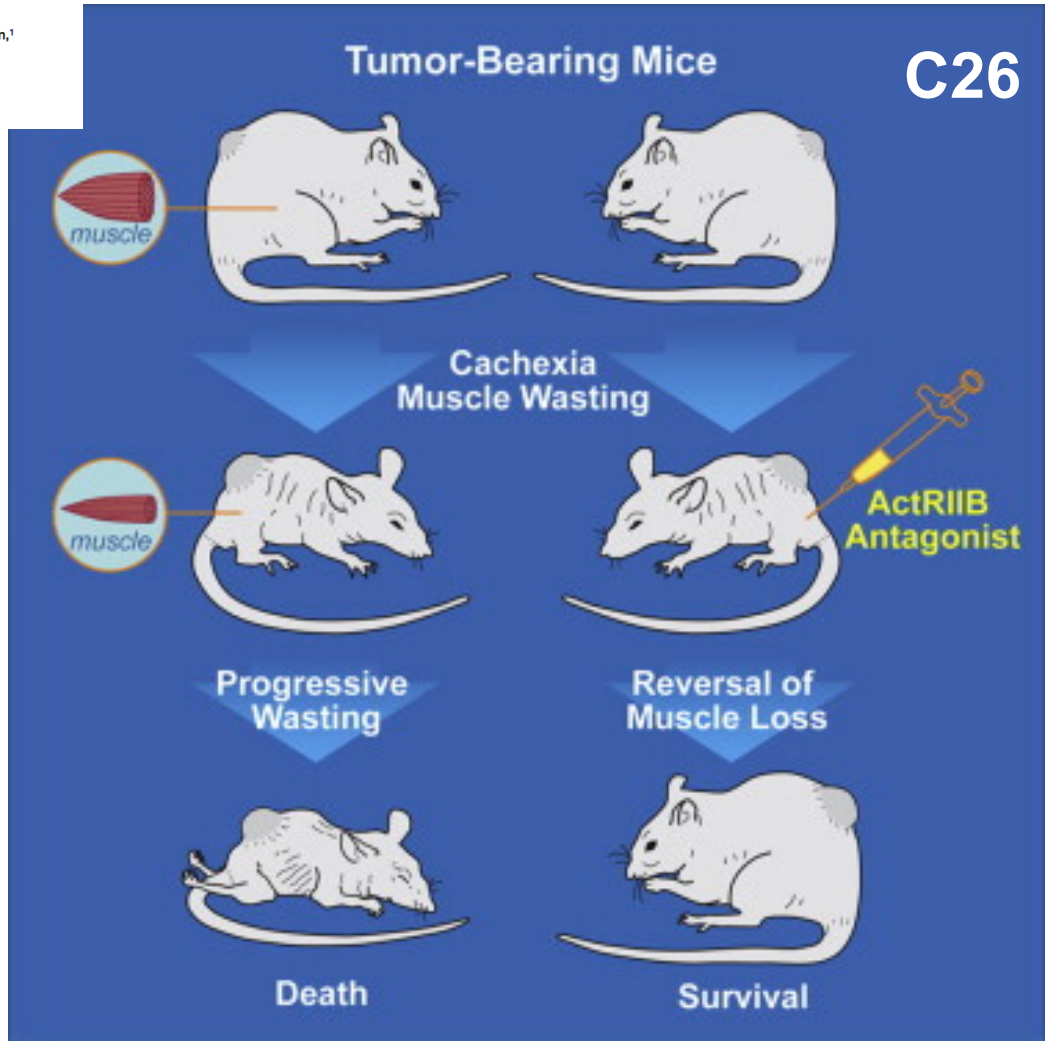


H.Q. Han et al. / The International Journal of Biochemistry & Cell Biology 45 (2013) 2333–2347

Cell

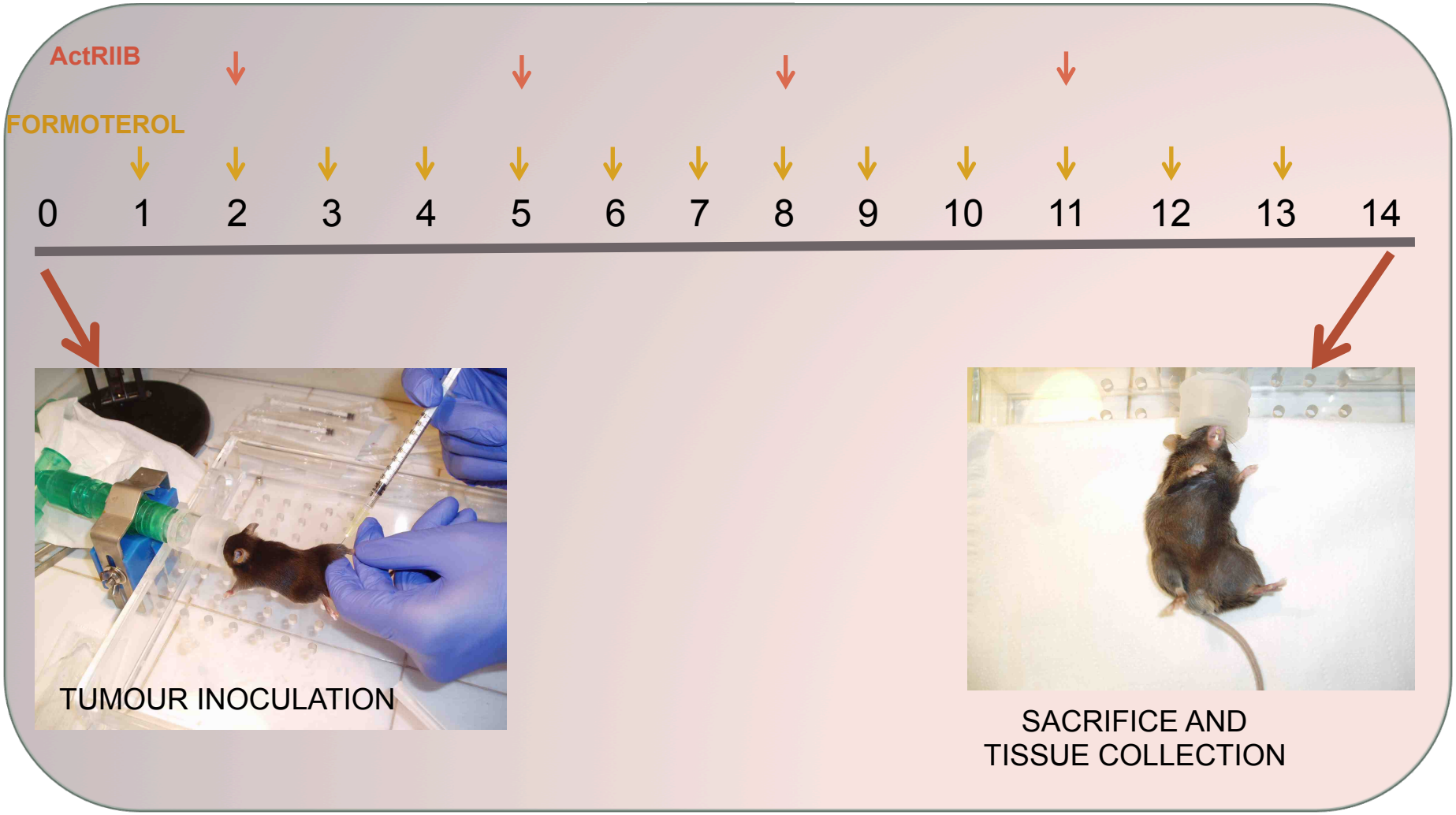
Reversal of Cancer Cachexia and Muscle Wasting by ActRIIB Antagonism Leads to Prolonged Survival

Xiaolan Zhou,[1] Jin Lin Wang,[1] John Lu,[1] Yanping Song,[1] Keith S. Kwak,[1] Qingsheng Jiao,[1] Robert Rosenfeld,[1] Qing Chen,[1] Thomas Boone,[1] W. Scott Simonet,[1] David L. Lacey,[1] Alfred L. Goldberg,[2] and H.Q. Han[1,*]
[1]Departments of Metabolic Disorders and Protein Science, Amgen Research, Thousand Oaks, CA 91320, USA
[2]Department of Cell Biology, Harvard Medical School, Boston, MA 02115, USA
*Correspondence: hqhan@amgen.com
DOI 10.1016/j.cell.2010.07.011



Control
Control + ActRIIB
Control + Formoterol
Control + ActRIIB + Formoterol

LLC
LLC + ActRIIB
LLC + Formoterol
LLC + ActRIIB + Formoterol



Dra. Sílvia Busquets
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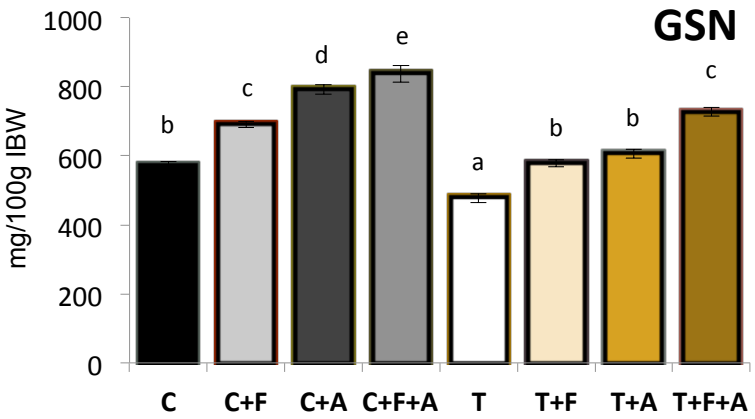
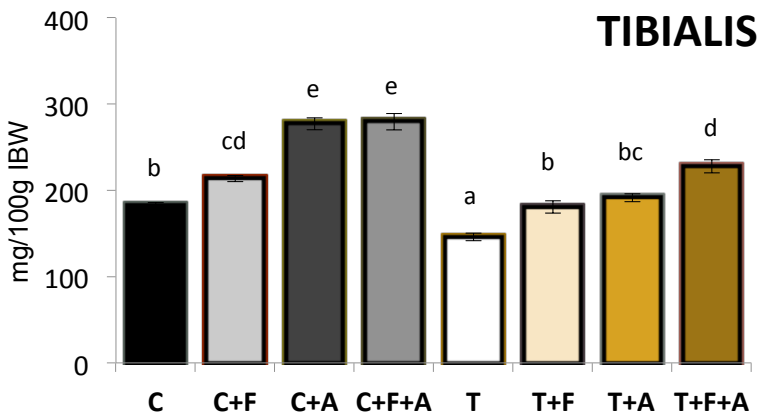
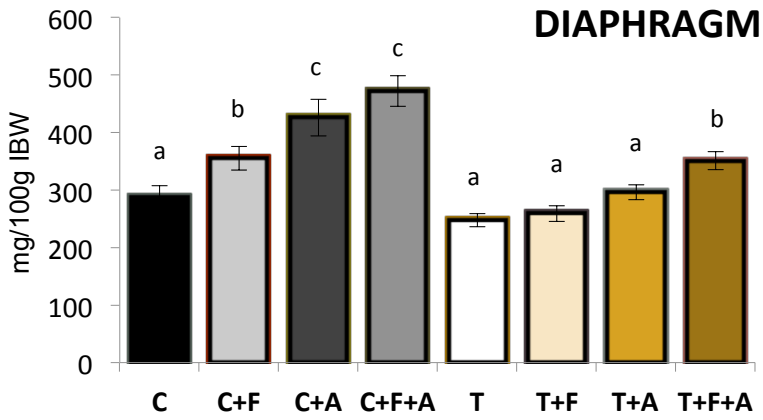
MUSCLE WEIGHTS





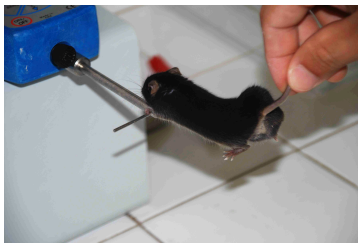


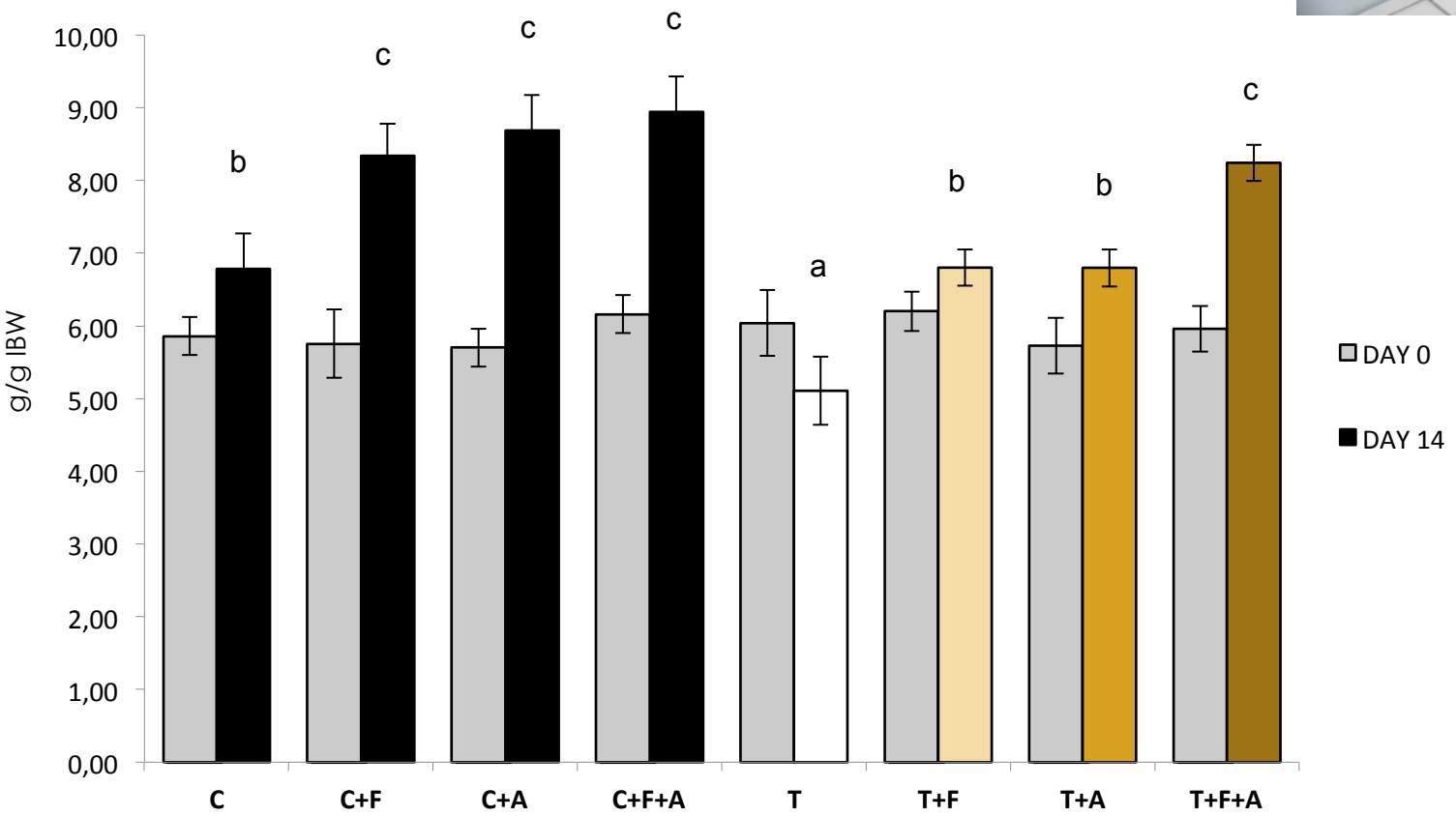
Results are mean ± S.E.M. for the number of animals indicated in parentheses. Muscle weights are expressed as mg/100 g of initial body weight. GSN: gastrocnemius muscle. Statistical significance of the results by **full factorial three-way ANOVA** (fixed factors: tumour, formoterol treatment, and soluble receptor antagonist of myostatin treatment). Statistically significant difference by *post hoc* Duncan test. Different superscripts indicate significant differences between groups.

SKELETAL MUSCLE STRENGTH

Dra. Sílvia Busquets
silviabusquets@ub.edu

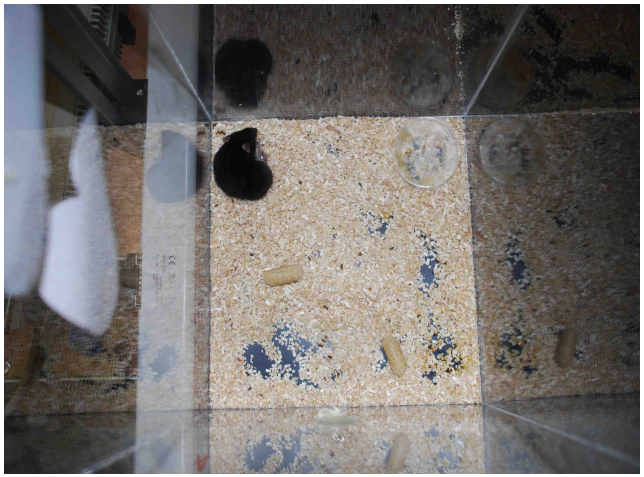
SKELETAL MUSCLE STRENGTH

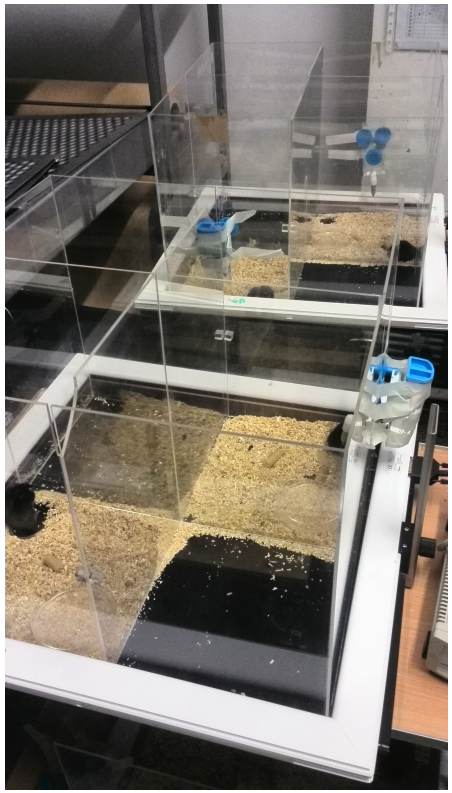




Grip force is expressed as g/g initial body weight. Statistical significance of the results by full factorial three-way ANOVA (fixed factors: tumour, formoterol treatment, and soluble receptor antagonist of myostatin treatment).
Statistically significant difference by *post hoc* Duncan test. Different superscripts indicate significant differences between groups.

PHYSICAL ACTIVITY



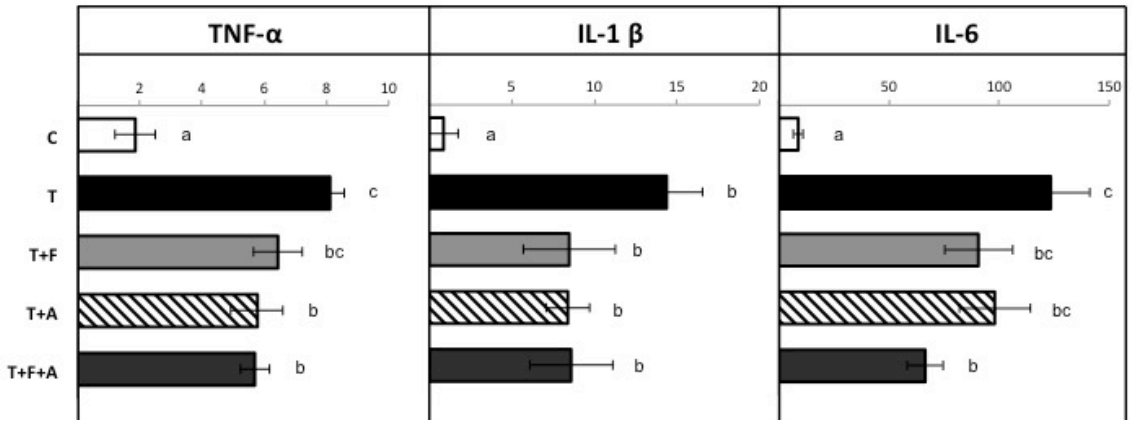


IR ACTIMETER System and ACTITRAK software from PANLAB

PHYSICAL ACTIVITY

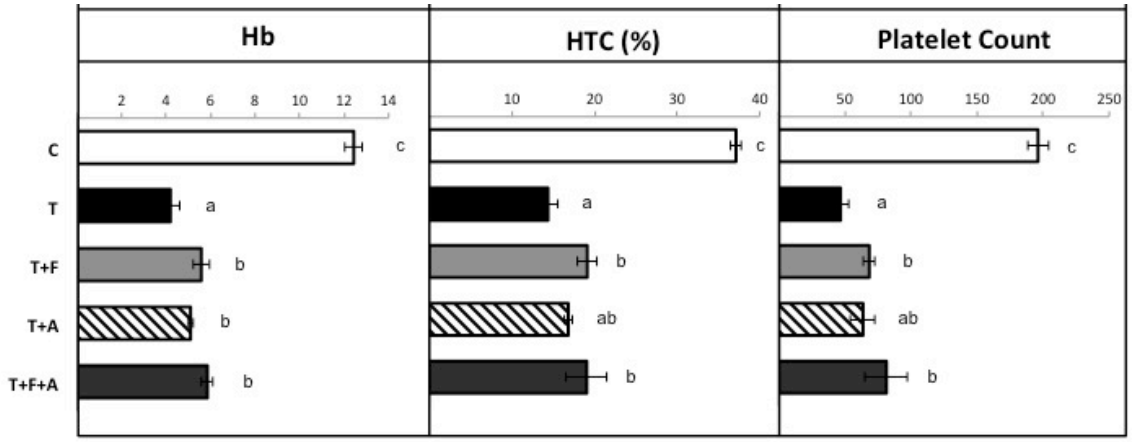
	C (10)	**T** (10)	**T + F** (10)	**T + A** (10)	**T + A + F** (7)	ANOVA
Physical activity						p-value
Total activity	65321 ± 2643 ^{b}	15451 ± 1152 ^{a}	19627 ± 1085 ^{a}	17041 ± 1383 ^{a}	19900 ± 631 ^{a}	0,000
Stereotyped movement	5312 ± 300 ^{b}	1893 ± 327 ^{a}	1545 ± 118 ^{a}	1769 ± 157 ^{a}	1888 ± 154 ^{a}	0,000
Locomotor movements	60010 ± 2636 ^{b}	13558 ± 1060 ^{a}	18082 ± 1019 ^{a}	15272 ± 1326 ^{a}	18012 ± 689 ^{a}	0,000
Velocity and distance						
Mean Velocity (cm/s)	0,64 ± 0,03 ^{c}	0,10 ± 0,03 ^{a}	0,14 ± 0,01 ^{ab}	0,13 ± 0,01 ^{ab}	0,17 ± 0,01 ^{b}	0,000
Travelled distance (cm)	46443 ± 990 ^{c}	7190 ± 737 ^{a}	10623 ± 1062 ^{ab}	9324 ± 784 ^{ab}	12172 ± 768 ^{b}	0,000
Time (%)						
Resting	73,3 ± 1,4 ^{a}	93,4 ± 0,6 ^{c}	89,7 ± 1,5 ^{bc}	90,3 ± 1,1 ^{bc}	87,3 ± 1,9 ^{b}	0,000
Slow movements	17,9 ± 1,2 ^{c}	6,2 ± 0,5 ^{a}	9,4 ± 1,3 ^{ab}	9,0 ± 1,0 ^{ab}	11,7 ± 1,7 ^{b}	0,000
Fast movements	8,8 ± 0,03 ^{b}	0,4 ± 0,08 ^{a}	0,9 ± 0,20 ^{a}	0,6 ± 0,11 ^{a}	1,0 ± 0,16 ^{a}	0,000

PLASMA CYTOKINE LEVELS



Results are mean ± S.E.M. for the number of animals indicated in parentheses. Cytokines are expressed as pg/ml plasma. Statistical significance of the results by one-way ANOVA. Statistically significant difference by *post hoc* Duncan test. Different superscripts indicate significant differences between groups.

BLOOD MARKERS



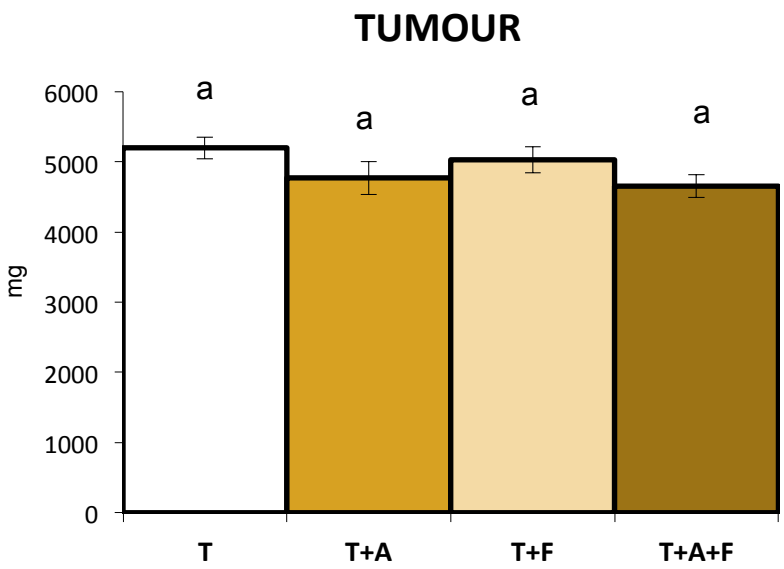
Results are mean ± S.E.M. for the number of animals indicated in parentheses. Cytokines are expressed as pg/ml plasma. Statistical significance of the results by one-way ANOVA. Statistically significant difference by *post hoc* Duncan test. Different superscripts indicate significant differences between groups.

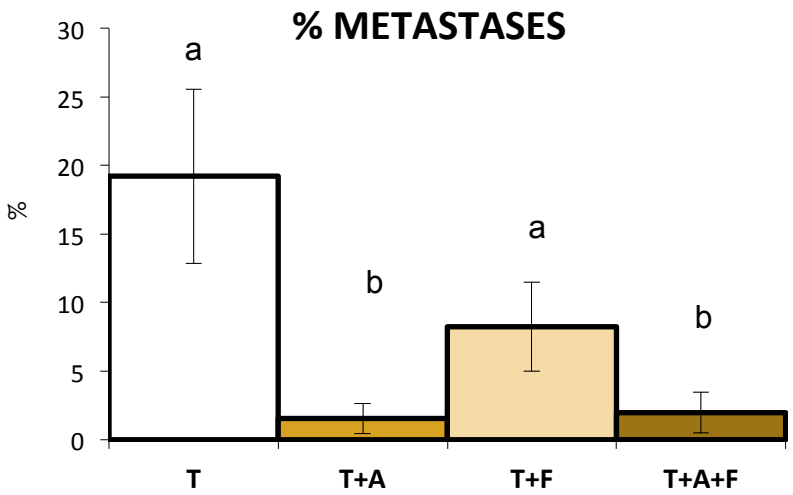
FOOD INTAKE

Food Intake	C (7)	C + F (7)	C + A (7)	C + A + F (7)
g/100 g IBW	226 ± 7 [ab]	258 ± 4 [c]	260 ± 8 [c]	256 ± 13 [c]
	T (8)	**T + F (8)**	**T + A (8)**	**T + A + F (8)**
g/100 g IBW	216 ± 7 [a]	221 ± 3 [a]	215 ± 3 [a]	240 ± 4 [b]

Statistical significance of the results by full factorial three-way ANOVA (fixed factors: tumour (T), formoterol treatment (F), and soluble receptor antagonist of myostatin treatment (A)).
Results are mean ± S.E.M. for the number of animals indicated in parentheses.
Food intake is expressed in g/100g of initial body weight and refers to the ingestion during the period of the experiment prior to sacrifice that took place 14 days after tumour inoculation.

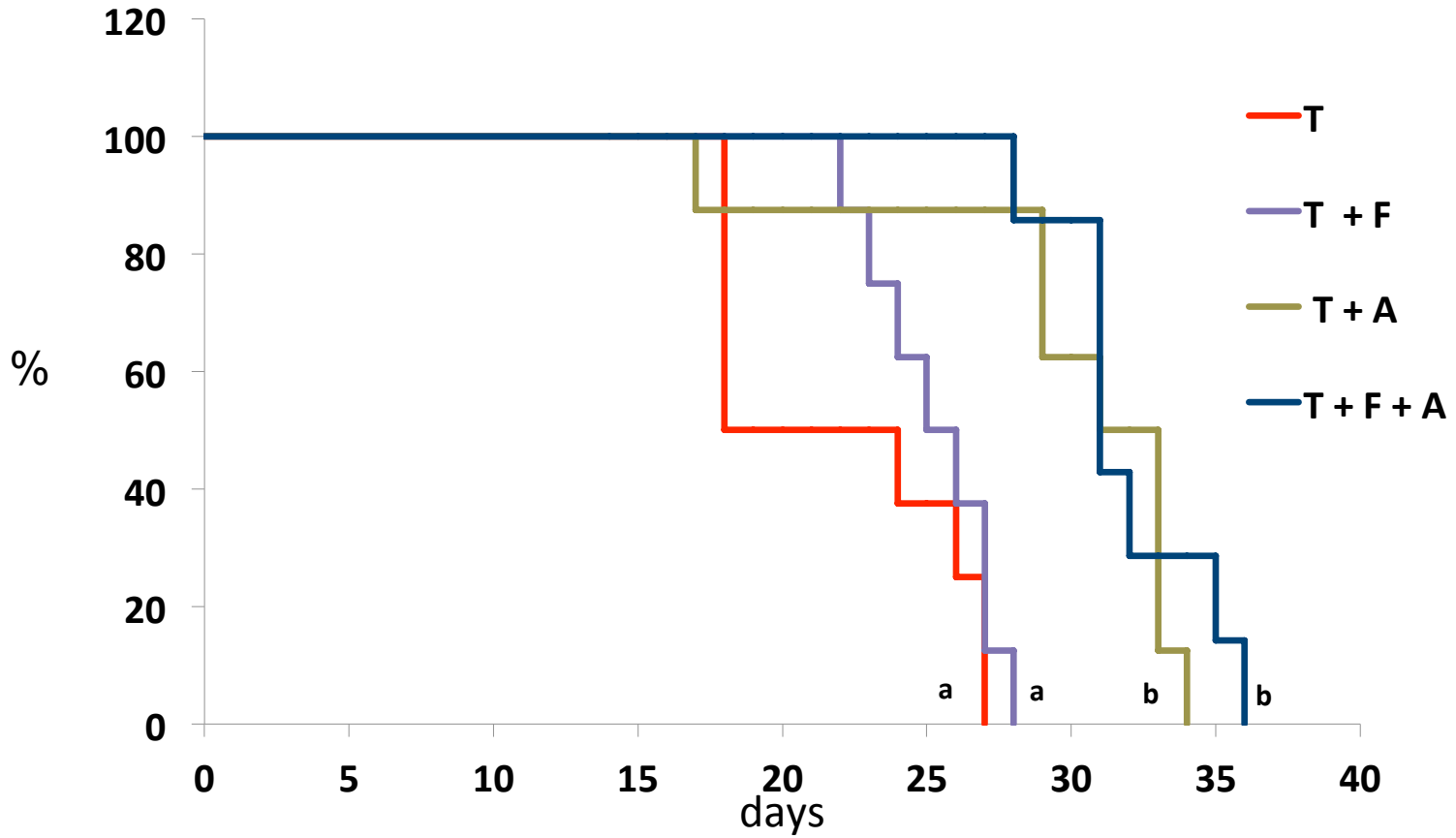
TUMOUR MASS AND METASTASES





Tumor mass is expressed in g and metastases are expressed as percentage of the lung volume. Statistical significance of the results by full factorial **two-way ANOVA** (fixed factors: formoterol treatment (F), and soluble receptor antagonist of myostatin treatment (A). Statistically significant difference by *post hoc* Duncan test. Different superscripts indicate significant differences between groups.

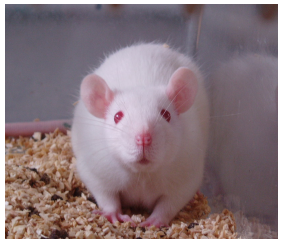
SURVIVAL



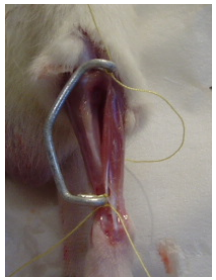
Kaplan-Meier survival analysis. Comparison of Survival curves were analyzed by Log-rank test (Mantel-Cox).
The global comparison for the treatments has a P value=0.000.
Different subscripts means significant differences detected by pairwise comparisons (Bonferroni correction).
P values < 0.05 were considered significant.

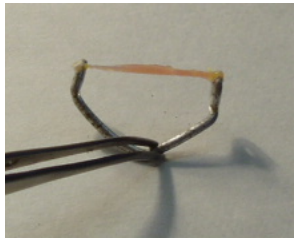
PROTEIN DEGRADATION:

MEASURED *EX VIVO* AS TYROSINE RELEASE, USING INCUBATED ISOLATED EDL MUSCLES



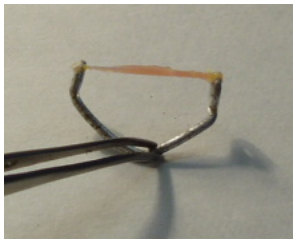
EDL isolation

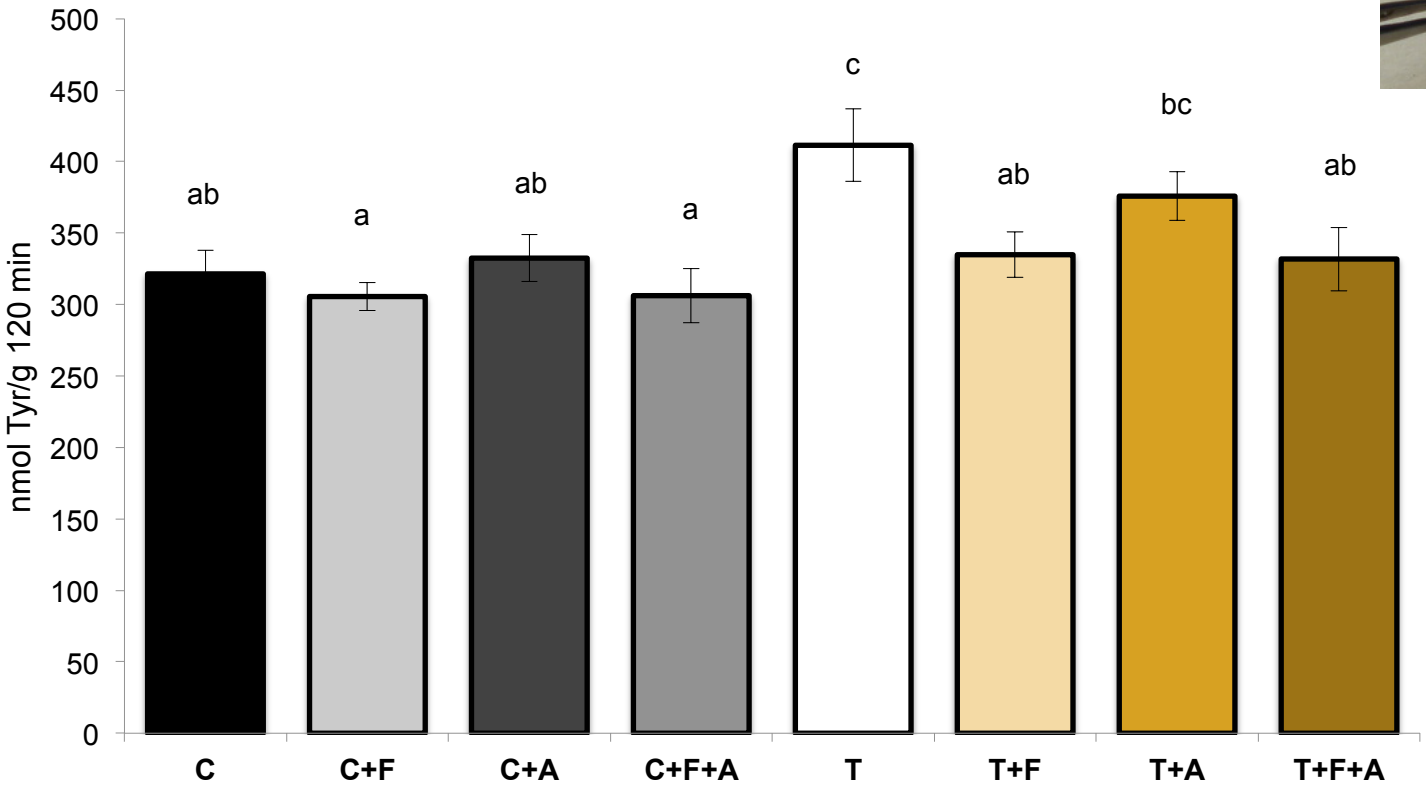




EDL incubated in a shaking-thermostatized water bath at 35 °C for 3 hours in 2 ml of Krebs-Henseleit buffer (shaking rate of 45 cycles/min and gassed with O2/CO2 19:1)

PROTEIN DEGRADATION: MEASURED *EX VIVO* AS TYROSINE RELEASE, USING INCUBATED ISOLATED EDL MUSCLES





Proteolytic rates were measured in the presence of cycloheximide (0.5 mmol/L) and are expressed as nanomoles tyrosine per gram and 2 hours of incubation. Statistical significance of the results by full factorial three-way ANOVA (fixed factors: tumour (T), formoterol treatment (F), and soluble receptor antagonist of myostatin treatment (A)). Statistically significant difference by *post hoc* Duncan test. Different superscripts indicate significant differences between groups.

PROTEIN DEGRADATION: PROTEOLYTIC SYSTEMS

PROTEOLYTIC SYSTEMS IN MUSCLE

Lysosomal	Non-Lysosomal
Cathepsins	Ca^{2+}-dependent system (calpains)
	ATP-ubiquitin-dependent system

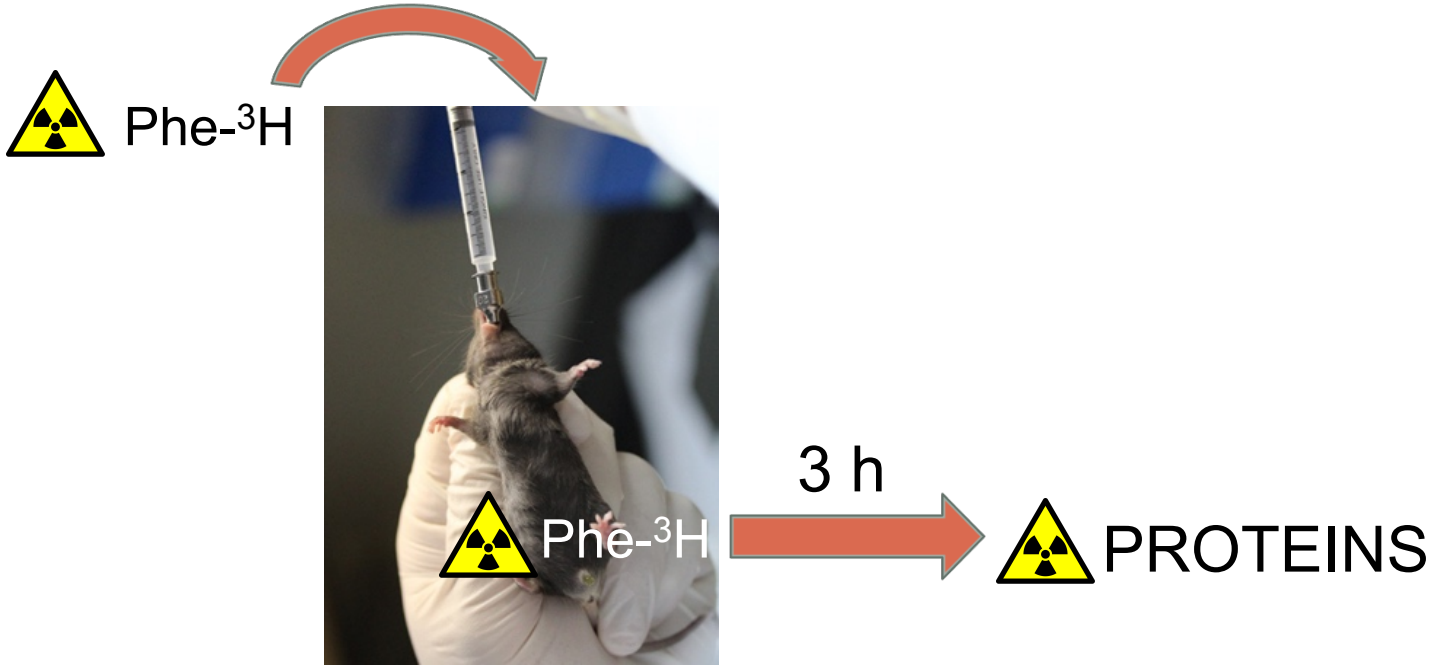
PROTEIN DEGRADATION: GENE EXPRESSION OF PROTEOLYTIC SYSTEMS IN TIBIALIS MUSCLE

Table 3. Effects of the combination of formoterol and sActRIIB treatment on tibialis muscle gene expression in mice bearing the Lewis lung carcinoma

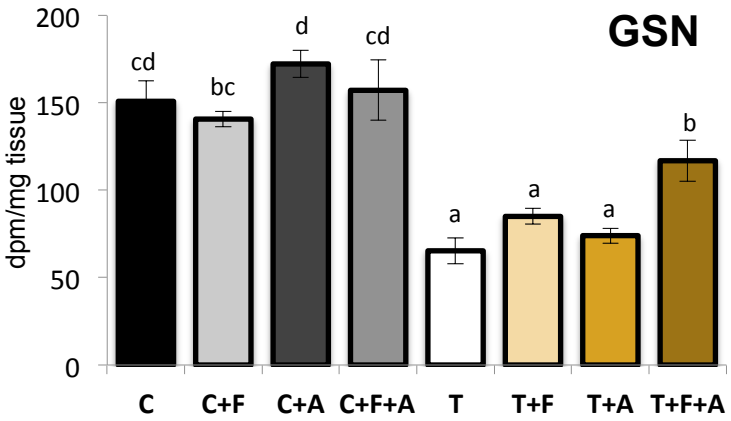
Proteolytic system	C (6)	T (7)	T + F (7)	T + A (6)	T + A + F (7)	ANOVA *p* values
Ubiquitin-dependent						
Atrogin-1	100 ± 23^{a}	264 ± 20^{c}	196 ± 21^{bc}	250 ± 34^{bc}	173 ± 33^{ab}	0.000
MuRF-1	100 ± 22^{a}	206 ± 14^{b}	167 ± 29^{ab}	206 ± 45^{b}	178 ± 34^{ab}	0.000
Ubiquitin	100 ± 6^{a}	158 ± 8^{b}	118 ± 10^{a}	153 ± 9^{b}	109 ± 15^{a}	0.000
E2	100 ± 5^{ab}	118 ± 6^{c}	103 ± 6^{ab}	108 ± 7^{bc}	89 ± 4^{a}	0.000
C8 proteasome subunit	100 ± 6^{a}	160 ± 8^{c}	135 ± 7^{b}	145 ± 8^{bc}	120 ± 10^{ab}	0.000
Calcium-dependent						
m-Calpain	100 ± 8^{ab}	147 ± 7^{c}	116 ± 6^{b}	107 ± 6^{b}	86 ± 8^{a}	0.000
Lysosomal						
Cathepsin B	100 ± 6^{b}	126 ± 8^{b}	35 ± 20^{a}	92 ± 8^{b}	7 ± 2^{a}	0.000

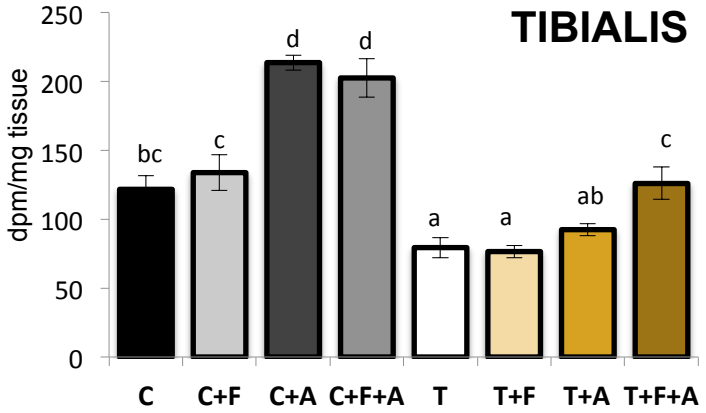
Results are mean ± SEM for the number of animals indicated in parentheses. C: mice without tumor; T: tumor-bearing mice; T + F: treated with formoterol; T + A: treated with sActRIIB; T + A + F: treated with both sActRIIB and formoterol. Statistical significance of the results by one-way ANOVA following a *post hoc* Duncan test. Different superscripts indicate significant differences between groups.

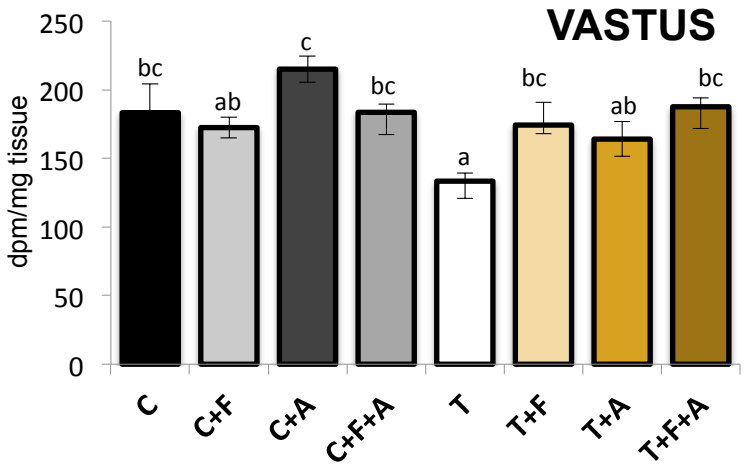
PROTEIN SYNTHESIS: MEASURED *IN VIVO* USING TRITIATED PHENYLALANINE



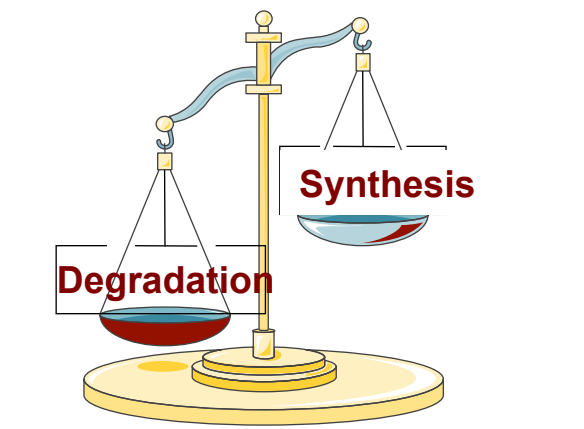
PROTEIN SYNTHESIS: MEASURED *IN VIVO* USING TRITIATED PHENYLALANINE

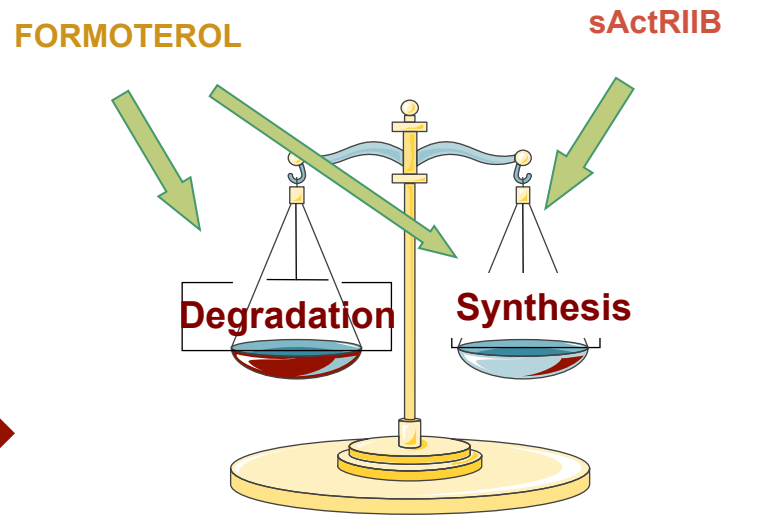


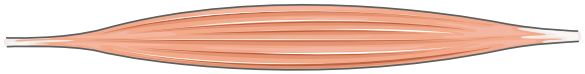


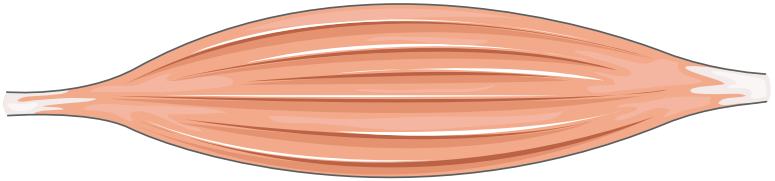


Statistical significance of the results by full factorial three-way ANOVA (fixed factors: tumour (T), formoterol treatment (F), and soluble receptor antagonist of myostatin treatment (A)).
Statistically significant difference by *post hoc* Duncan test. Different superscripts indicate significant differences between groups.







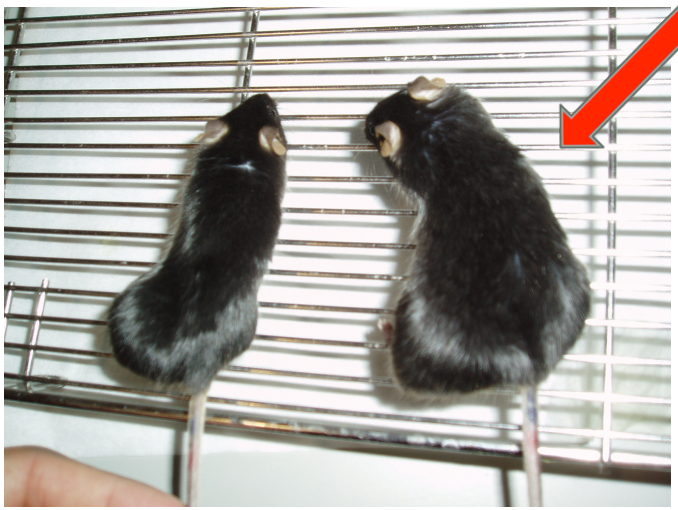


Muscle wasting

Combining formoterol and the soluble ActRIIB seems to be a very promising treatment for experimental cancer cachexia.

Lewis lung carcinoma (LLC)

LLC-treated animals with:
soluble receptor antagonist of myostatin
+
Formoterol



IJC
International Journal of Cancer

Complete reversal of muscle wasting in experimental cancer cachexia: Additive effects of activin type II receptor inhibition and β-2 agonist

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Conclusions and perspectives

- **Treatment of cancer cahexia**:
  - because cachexia is a multifactorial syndrome, a **multimodal approach** is needed.
  - Multimodal therapy should incorporate at least a double strategy, both **anticatabolic and anabolic.**
  - Treatment should be started right from the moment of cachexia diagnosis (need of a diagnosis tool).

Conclusions and perspectives

CANCER CACHEXIA TREATMENT

anabolic/anticatabolic drugs
+
adequate nutritional support

moderate-to-high endurance exercise

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Thanks to Prof. Paola Costelli