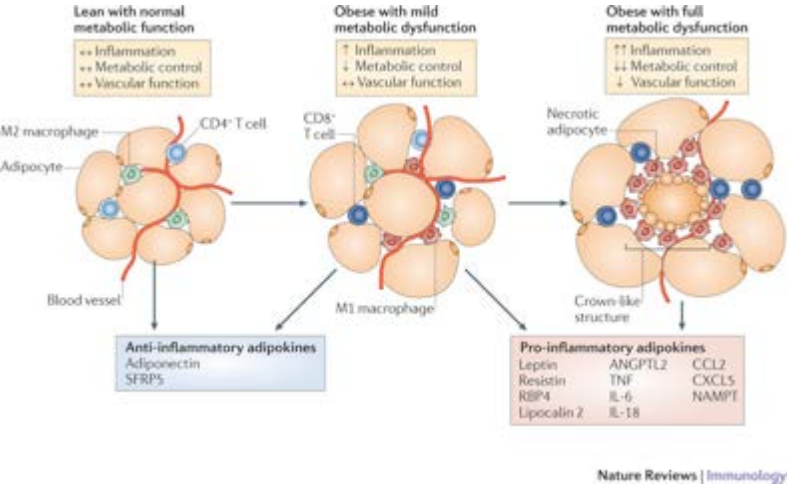
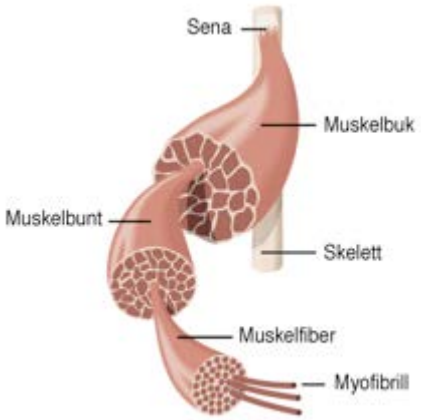
Adipokines and myokines are important for several biological effects of adipose tissue and skeletal muscle as well as health

NuGO week 2017, Varna, Bulgaria

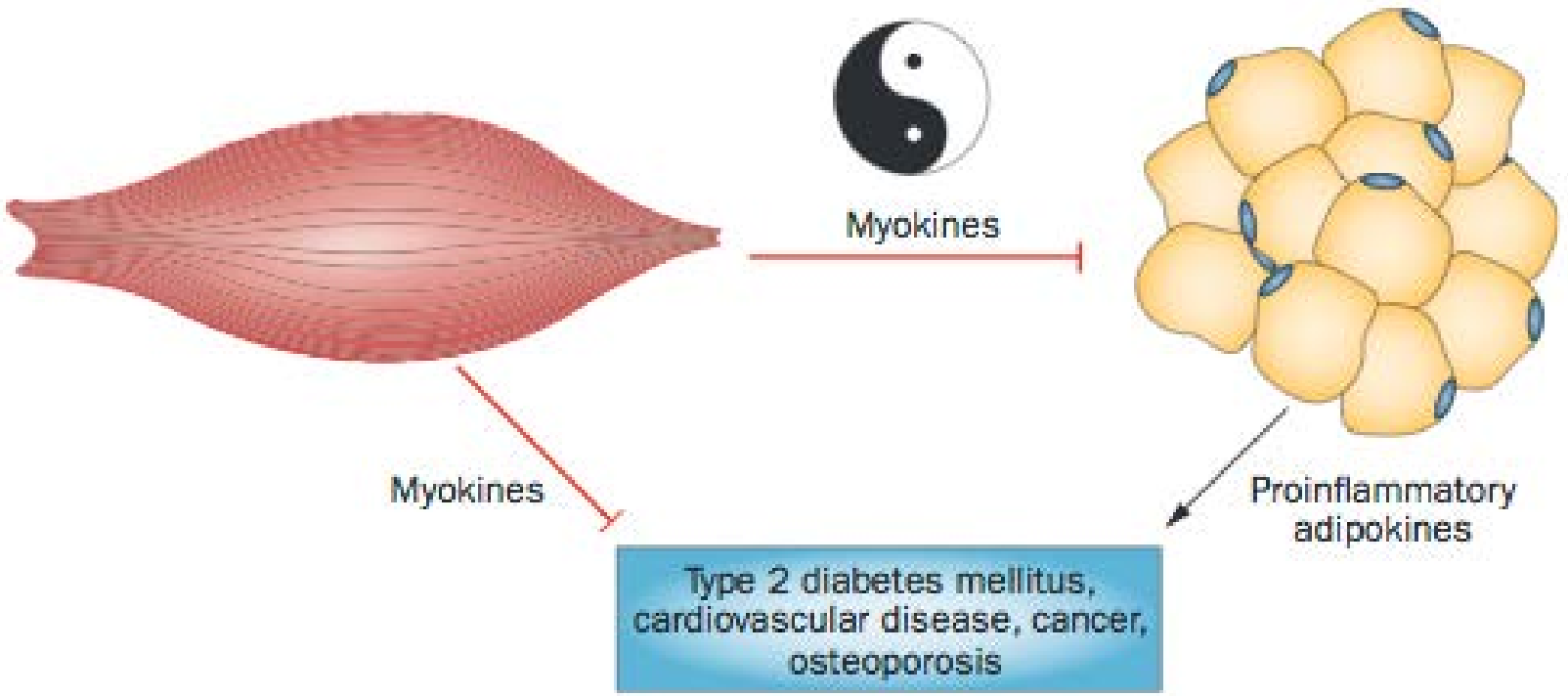
Christian A. Drevon
Department of Nutrition,
Institue of basic medical sciences
UiO, Norway
c.a.drevon@medisin.uio.no

Public health
- Diet
- Physical activity
- Non-smoking





Myokines & adipokines → health & disease



Pedersen & Febbraio. *Nat Rev Endocrinol*. 2012, 8, 457-65

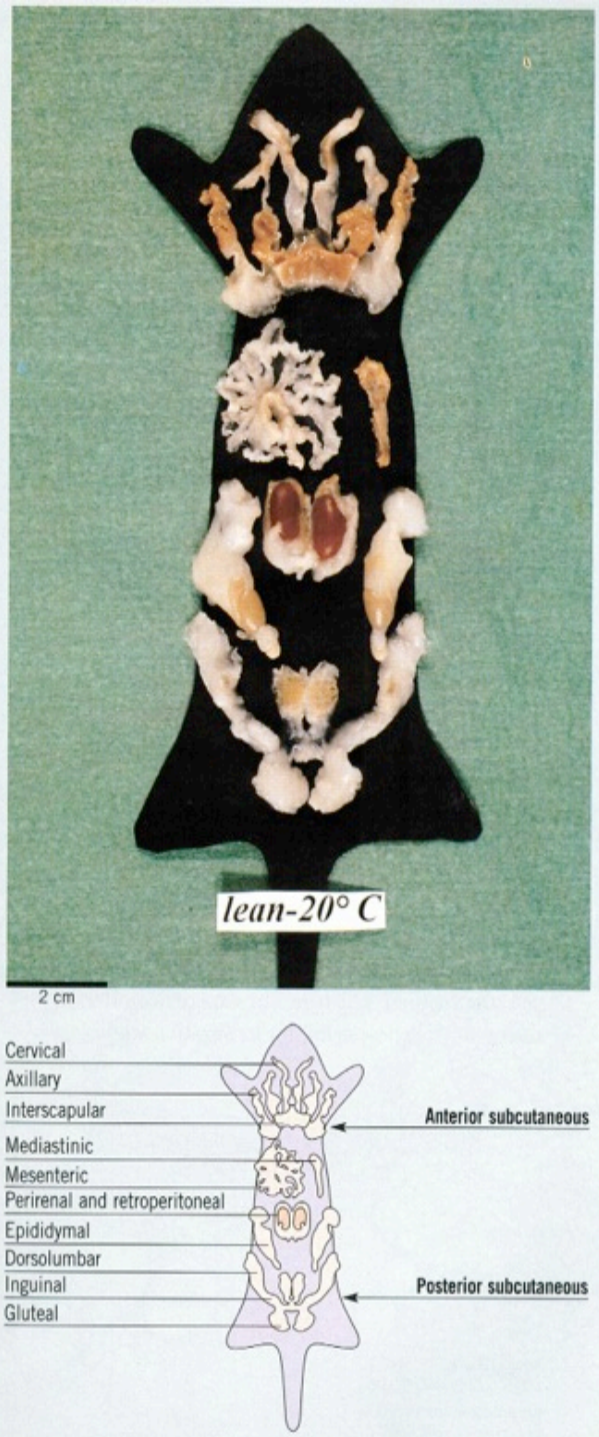
The adipose organ

Cinti S: The adipose organ, 1999
Editrice Kurtis, Milano Italy

C57BL mouse; 44 % of the adipose organ is anterior (dorsal subcutaneous interscapular +) & ventral (dorsolumbar, inguinal & gluteal)

Most **WAT**

BAT in interscapular, inguinal, mediastinal & perirenal regions



DMM Disease Models & Mechanisms
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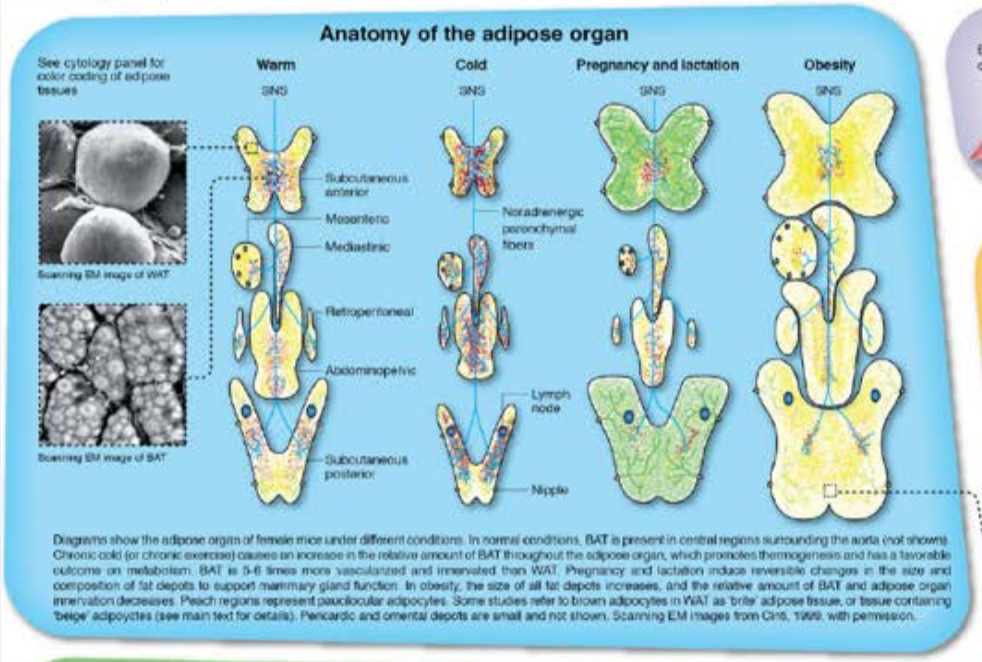
The adipose organ at a glance

Saverio Cinti

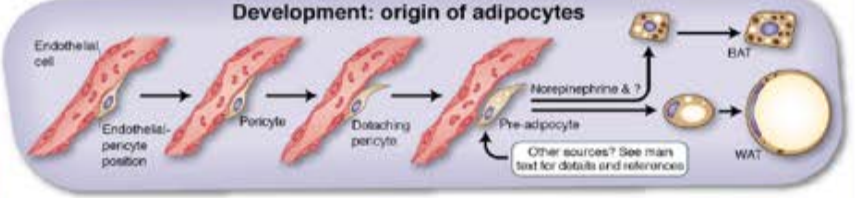
Takeda

Anatomy of the adipose organ



Diagrams show the adipose organ of female mice under different conditions. In normal conditions, BAT is present in central regions surrounding the aorta (not shown). Chronic cold (or chronic exercise) causes an increase in the relative amount of BAT throughout the adipose organ, which promotes thermogenesis and has a favorable outcome on metabolism. BAT is 5-6 times more vascularized and innervated than WAT. Pregnancy and lactation induce reversible changes in the size and composition of fat depots to support mammary gland function. In obesity, the size of all fat depots increases, and the relative amount of BAT and adipose organ innervation decreases. Peach regions represent paucilocular adipocytes. Some studies refer to brown adipocytes in WAT as 'brite' adipose tissue, or tissue containing 'beige' adipocytes (see main text for details). Pericardic and omental depots are small and not shown. Scanning EM images from Cinti, 1999, with permission.

Development: origin of adipocytes

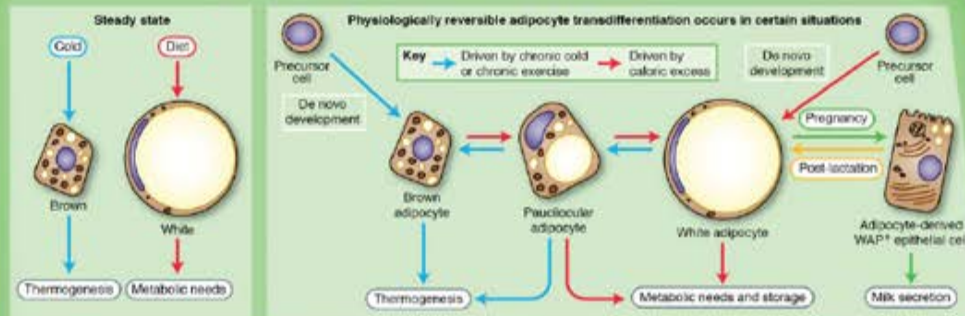


Cytology: the adipose organ contains many cell types

Corresponding color in Anatomy panel	Cell type	Markers	Relative size of mitochondria
Adipocytes			
	Brown	UCP1⁺ leptin⁻ S100-B⁻	
	Paucilocular*	UCP1$^{+/-}$ leptin$^{+/-}$ S100-B⁺	
	Small white	UCP1⁻ leptin⁺ S100-B⁺	
	Large white	UCP1⁻ leptin⁺ S100-B⁺	
	Adipocyte-derived epithelial cell	UCP1⁻ leptin⁺ WAP⁺	
Immune cells			
	Lymphocyte	Various	
Not shown	Macrophage	Various (those in CLS are MAC2⁺)	

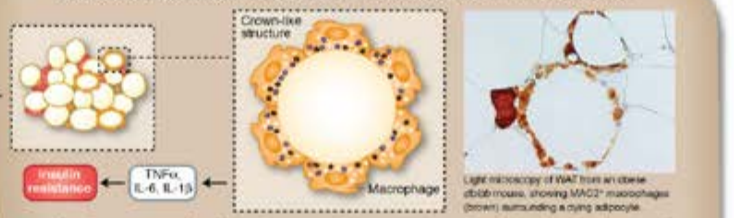
* Paucilocular adipocytes have variable expression of UCP1 and leptin, depending on the stage of differentiation between white and brown adipocytes

Adipose organ composition is dynamic



Evidence suggests that adipocyte transdifferentiation might underlie the changes in adipose organ composition that are observed in response to chronic cold or exercise, caloric excess or pregnancy/lactation. De novo adipocyte development is also stimulated by these conditions (see top right panel for details). The factors driving these transdifferentiation pathways are under investigation, but might include those detailed in Box 1 (see main text).

Adipose organ pathology contributes to insulin resistance



Obesity causes expansion of the adipose organ by increasing the number and size of white adipocytes. On reaching a critical size, white adipocytes are prone to cell death. The obese adipose organ, and particularly visceral fat, is infiltrated with macrophages, which form crown-like structures surrounding dead and dying adipocytes. These macrophages produce pro-inflammatory cytokines that enter the systemic circulation and contribute to the development of insulin resistance. Microscopy image from Cinti et al., 2005, with permission.

This article is part of a special issue on obesity: see related articles in Vol. 5, issue 5 of *Dis. Model. Mech.* at http://dmm.biologists.org/content/5/5.toc

Disease Models & Mechanisms **(2012) 5, 588-594**

Abbreviations: BAT, brown adipose tissue; IL-1β, interleukin-1β; IL-6, interleukin-6; MAC2, also known as LGALS3 (galectin-3); S100-B, S-100 protein beta chain; SNS, sympathetic nervous system; TNFα, tumor necrosis factor-α; UCP1, uncoupling protein 1; WAP, whey acidic protein; WAT, white adipose tissue.

Adipose tissue size & location

- Very large 3 - 70 % of body mass
  - May store energy for several months fasting
- Location is important
  - *Subcutanous*; protect, insulate
  - *Omental*; ectopic, insulin resistance
  - *Perirenal*; protect
  - *Epididymal*; essential fatty acids
  - *Interscapular*; BAT, heat
  - *Inguinal, axillar*; essential fatty acids
  - *Retrobulbar*; support

Adipose tissue (AT) depots

- ~ 85% of total AT is subcutaneous, in lean or obese humans
- The remaining 15% is intra-abdominal, including visceral & retroperitoneal depots
- Visceral AT (mesenteric and omental), only constitutes ~ 10% of total body fat, with the highest risk for metabolic dysregulation

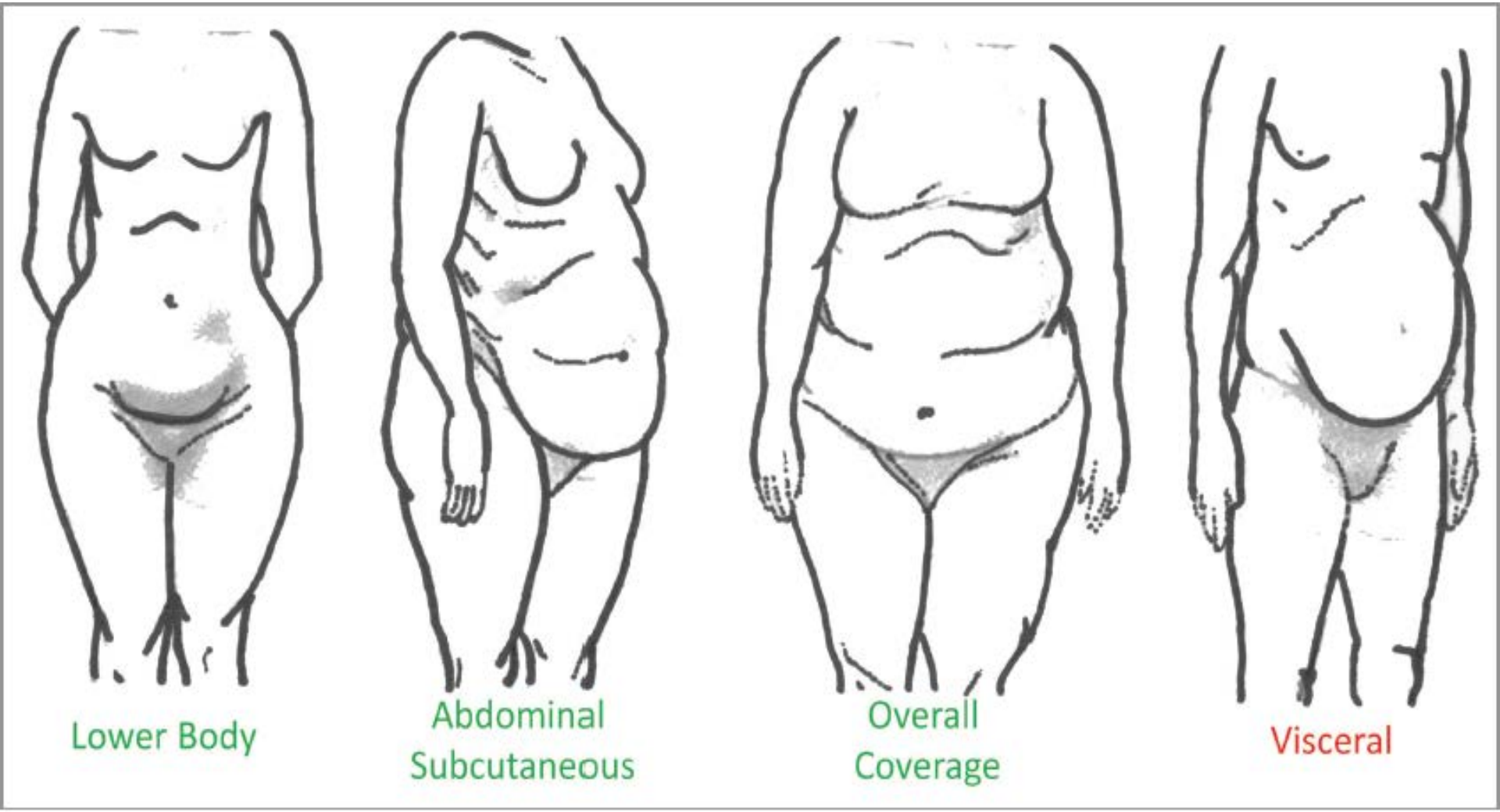


Figure 1. Description of body fat distribution in humans. Lower body: fat storage around the buttocks, hips and thighs. Abdominal subcutaneous: subcutaneous fat storage around the stomach and chest. Overall coverage: fat accumulation in the arms, breast, thighs, buttocks, lower back and breast. Visceral: intra-abdominal fat deposition among organs such as the intestines, stomach, liver and pancreas. Fat distributed within the visceral cavity is highly associated with obesity-related health consequences whereas other fat distribution is not.

CAD7

Foster, Pagliassotti. *Adipocyte* 2012, 1, 192-9

Metabolic alterations following visceral fat removal and expansion

Foster & Pagliassott. *Adipocyte* 2012, 1, 192-9

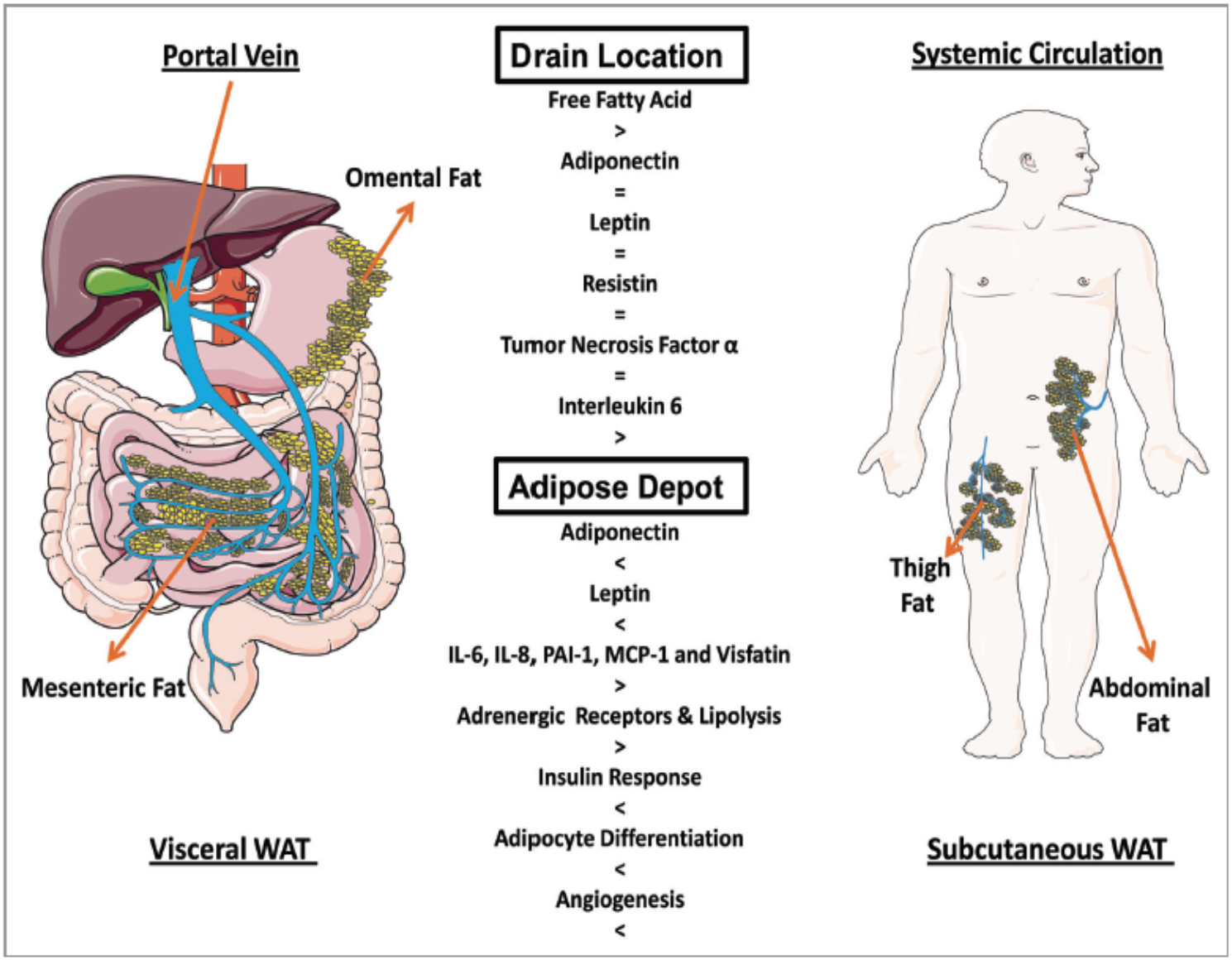
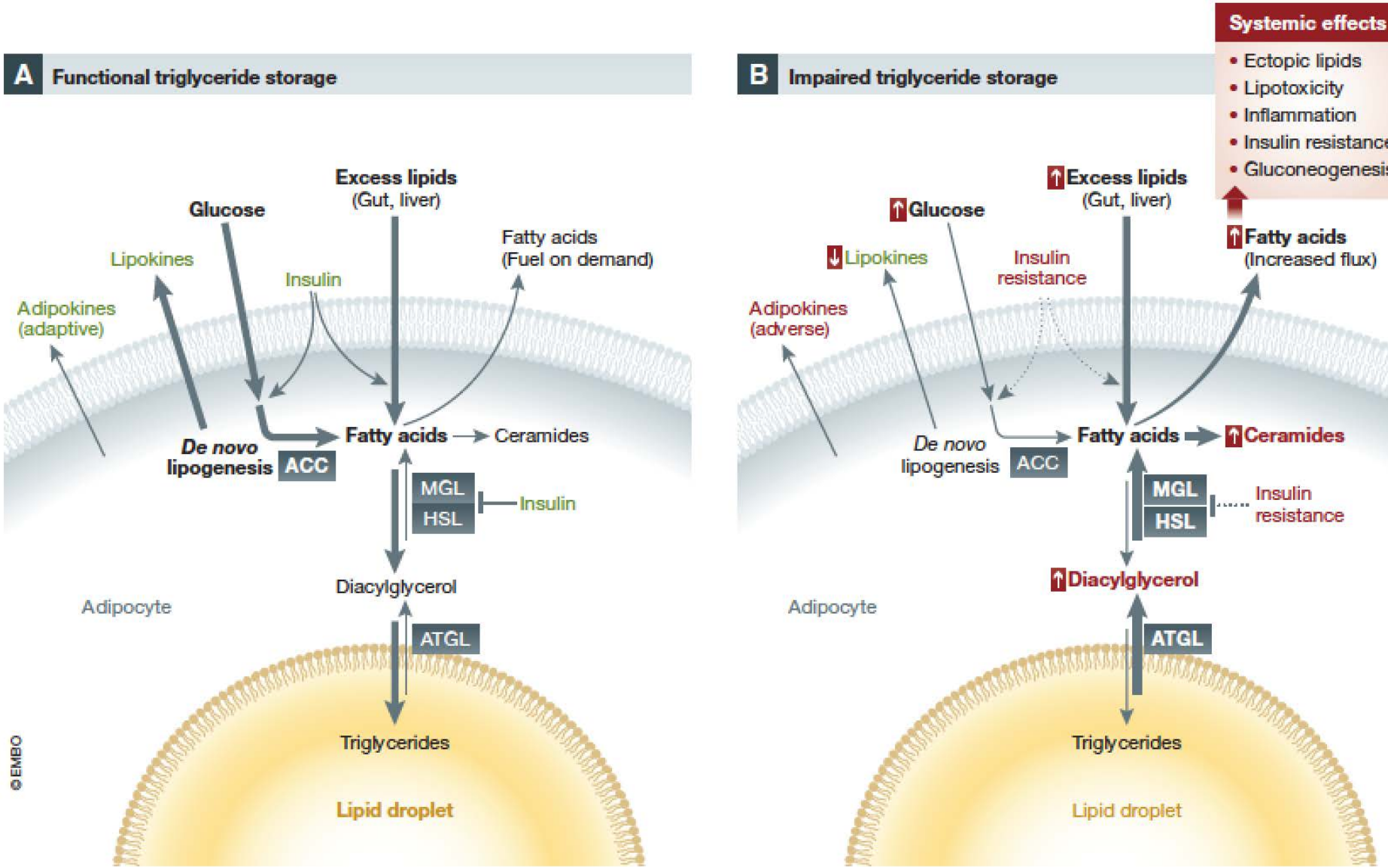


Figure 2. Differences between visceral and subcutaneous adipose tissue depots. Drain location: the visceral depot (left) releases products into the portal vein, while the subcutaneous depot (right) releases products into the systemic circulation. In obesity, portal vein effluent to the liver contains higher concentrations of free fatty acids and interleukin-6 compared with the systemic circulation. Adipose depot: Visceral and subcutaneous fat are characterized by inherent differences. When compared with subcutaneous fat, visceral fat is characterized by reduced adiponectin and leptin, increased inflammatory adipo/cytokines, enhanced lipolysis, a reduced response to insulin and reduced differentiation and angiogenesis.

Adipose tissue functions

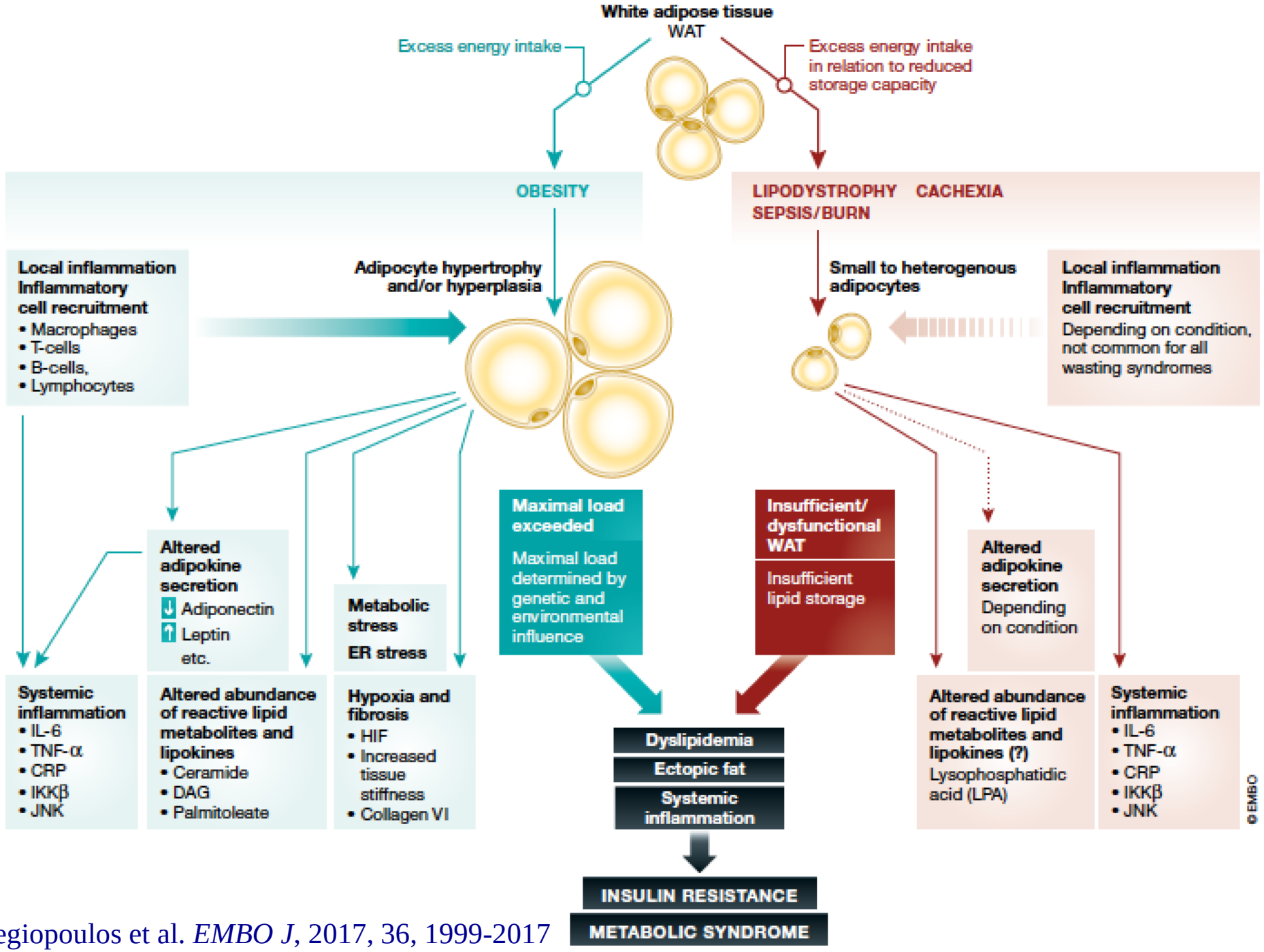
- *Storage of*
  - energy - very large & efficient
  - cholesterol, vitamin D & E
- *"Insulation"* - thermic, mechanical & (electrical)
- *Regulation of metabolism* - white & brown
- *Adipokines* – auto-/para-/endocrine

Energy excess

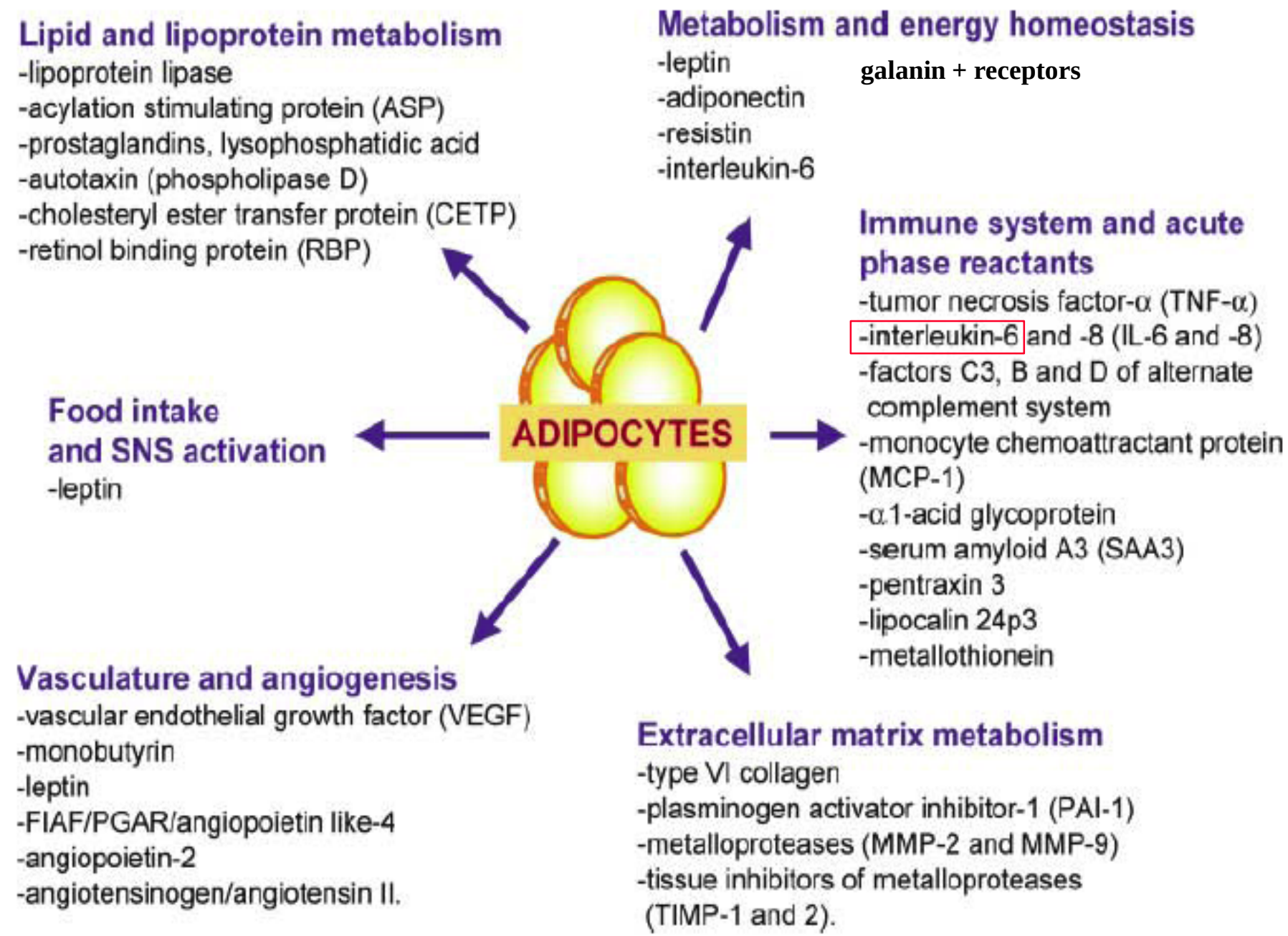


CAD10

Vegiopoulos et al. *EMBO J*, 2017, 36, 1999-2017



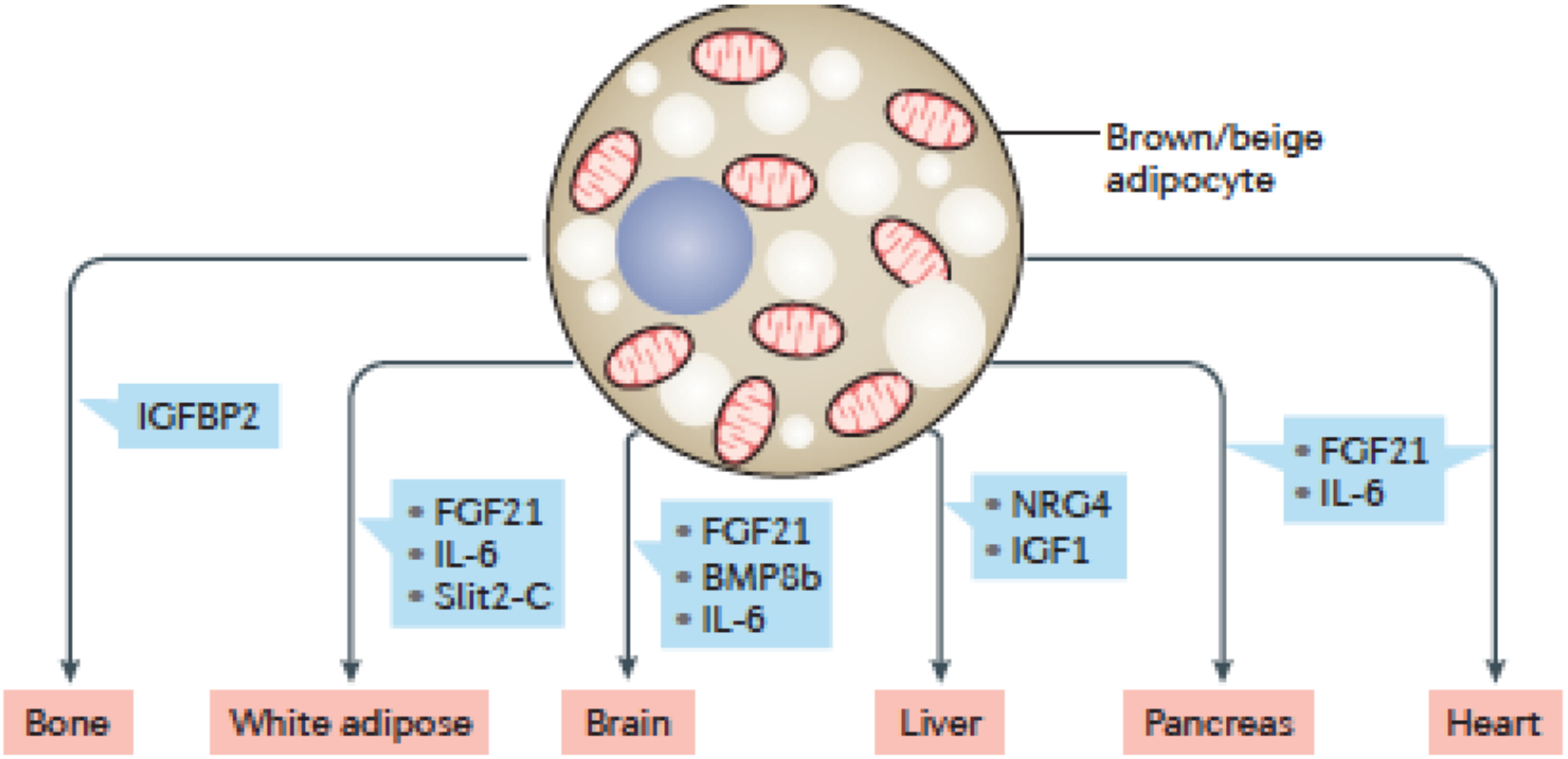
Vegiopoulos et al. *EMBO J*, 2017, 36, 1999-2017



Modified after Lafontan, *Annu Rev Pharmacol Toxicol*. 2004

Putative **batokines** and target organs

Villarroya et al. *Nat Rev Endocrinol*. 2017, 13, 26-35



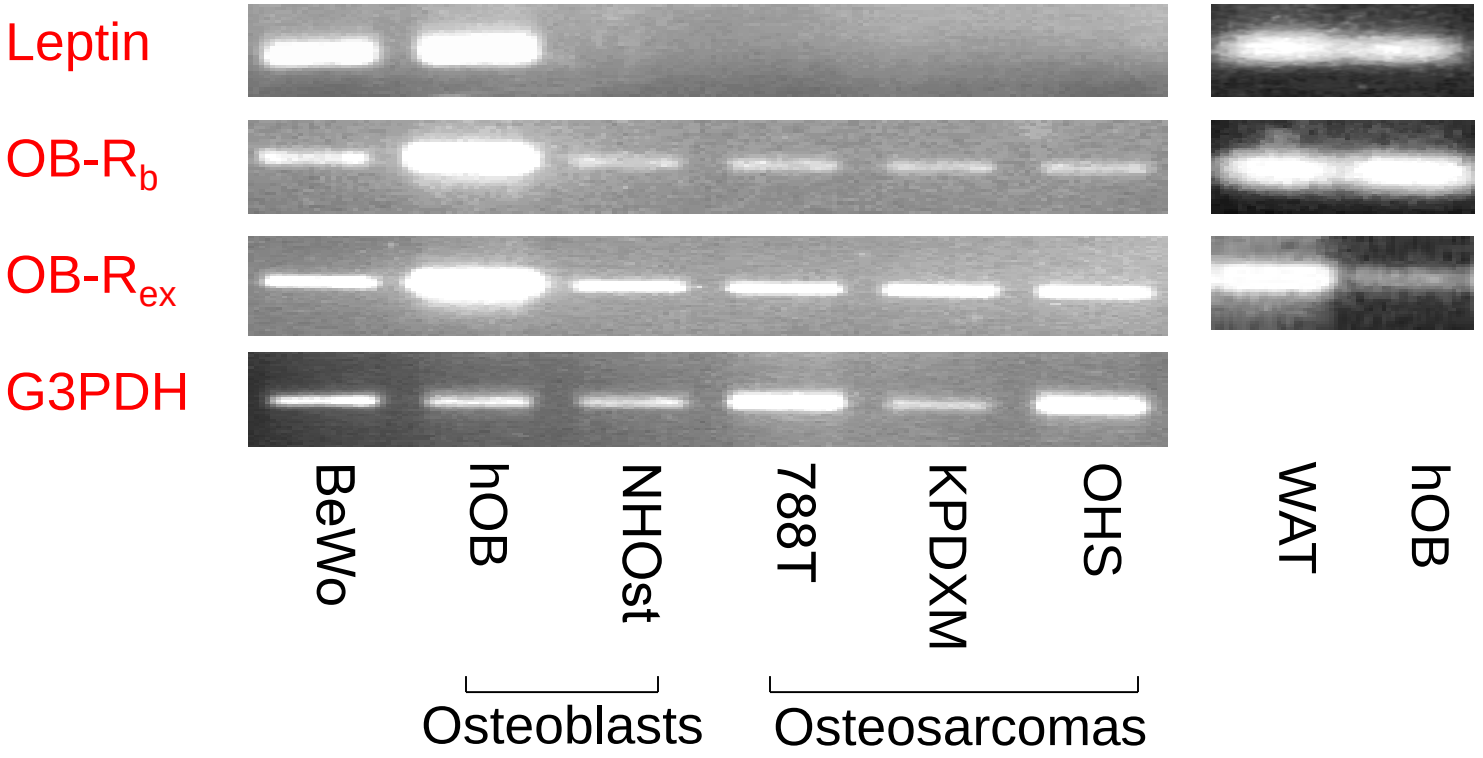
FGF21 and Slit2-C may target WAT browning WAT. FGF21, IL-6 (and ANGPTL8) may improve insulin secretion and β-cell function. NRG4 attenuates hepatic lipogenesis; insulin-like growth factor binding protein 2 (IGFBP2) promotes bone formation; FGF21 and IL-6 may increase cardiac substrate oxidation. BATokines might modulate systemic metabolism indirectly through the CNS; FGF21, IL-6 (and BMP8b) may influence sympathetic activity, feeding, circadian behaviour and female endocrine function. IGF1, insulin-like growth factor 1; NRG4, neuregulin 4.

Leptin expression

- Adipose tissue - white & brown
- Placenta (Hassink et al Pediatrics. 1997; 100 (1):E1)
- Fetus - hair follicles, bone/cartilage (Hoggard et al PNAS. 1997; 94:11073-11078)
- Gastric epithelium (Bado et al Nature. 1998, 394, 790-793)
- Breast gland epithelium (Casabiell et al. J Clin Endocrinol Metab. 1997, 82, 4270-4273)
- Skeletal muscle (Wang et al Nature. 1998, 393, 684-648)
- Bone cells (Reseland et al J Bone & Mineral Res. 2001, 16, 1426-1433

Expression of leptin & leptin receptor (OB-R) mRNA in human osteoblasts

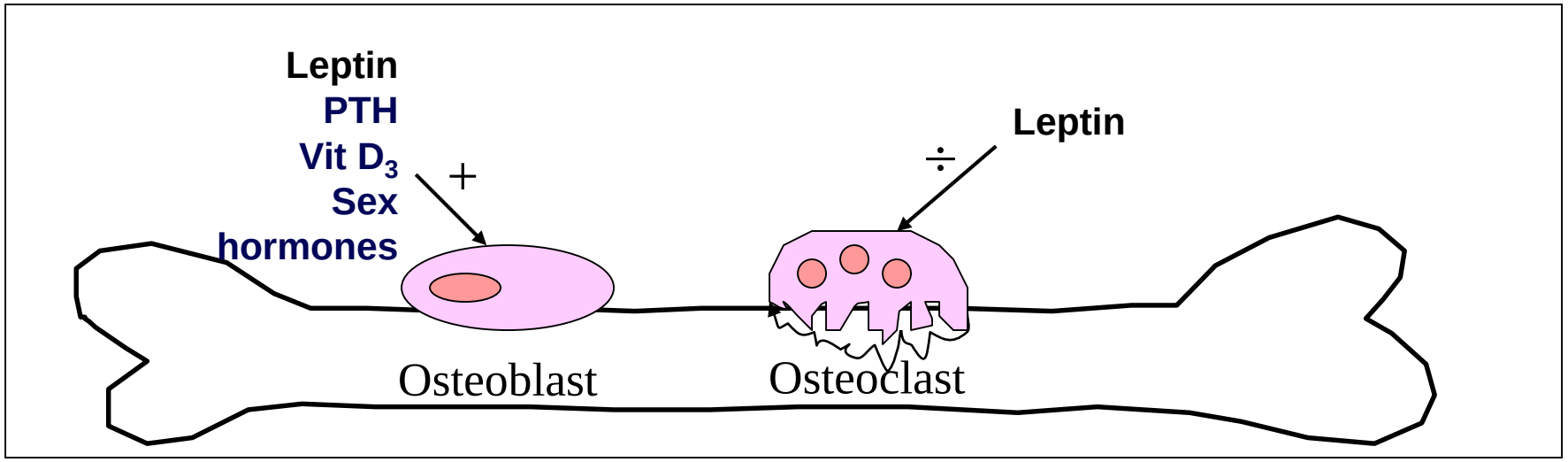
RT-PCR products



Reseland et al. *J Bone Mineral Res*, 2001, 16, 1426-33

Endocrine effects of leptin on bone metabolism

↑ Proliferation
↑ Bone mineralization
↑ Collagen deposition
≈ Leptin
≈ Leptin receptor



Bone tissue adapts to the amount of adipose tissue

Gordeladze et al. *J Cell Biochem*. 2002, 85, 825-36

Effect of energy restriction (diet) & physical exercise on mRNA from adipose tissue and whole body MRI

Lee et al. *Phys Report* 2016, 4 (21) e13019

- E**xercise** group used 15,800 kcal/w, increasing to 18,400 kcal during intervention (~17% increase; MyoGlu)
- **Diet** group used 14,500 kcal/w; loss in body weight (75% AT & 25% fat free mass → 2600 kcal/w (18% reduction; NutriTech)
- Thus, similar energy alterations

Table 3. Changes in fat depots after 12 weeks exercise and energy restriction (diet).[1]

	Exercise Change (%)	Diet[2] Change (%)	Exercise, control Change (%)	Control[2] Change (%)
MRI				
Total AT	−10.9 ± 5.1*	−8.9 ± 3.3*	−8.5 ± 2.7*	3.7 ± 1.4
Subcutaneous AT	−7.3 ± 6.0*	−8.6 ± 3.7*	−6.6 ± 2.6*	2.0 ± 1.0
Intra-abdominal AT	−19.4 ± 10.8*	−11.4 ± 6.2*	−16.9 ± 4.2*	7.7 ± 3.3
MRS fat				
Pancreas[3]	−28.5 ± 62.9	−20.8 ± 49.7	−30.3 ± 21.7	21.3 ± 20.6
Liver[4]	−27.4 ± 15.7*	−7.4 ± 2.4*	−23.3 ± 14.1[5]	−6.8 ± 1.9*

[1]Data represent mean ± SEM. Only relative values are presented due to slight differences in protocols and units calculated in the two cohorts.
[2]Only data from six subjects in the diet group were available.
[3]$n = 7$ in the exercise group.
[4]$n = 9$ in the exercise group.
[5]The reduction in the control group is significant using the Wilcoxon test (Langleite et al. 2016).
*$P < 0.05$ (baseline vs. 12 weeks).

CAD18 Markedly more AT loss with exercise

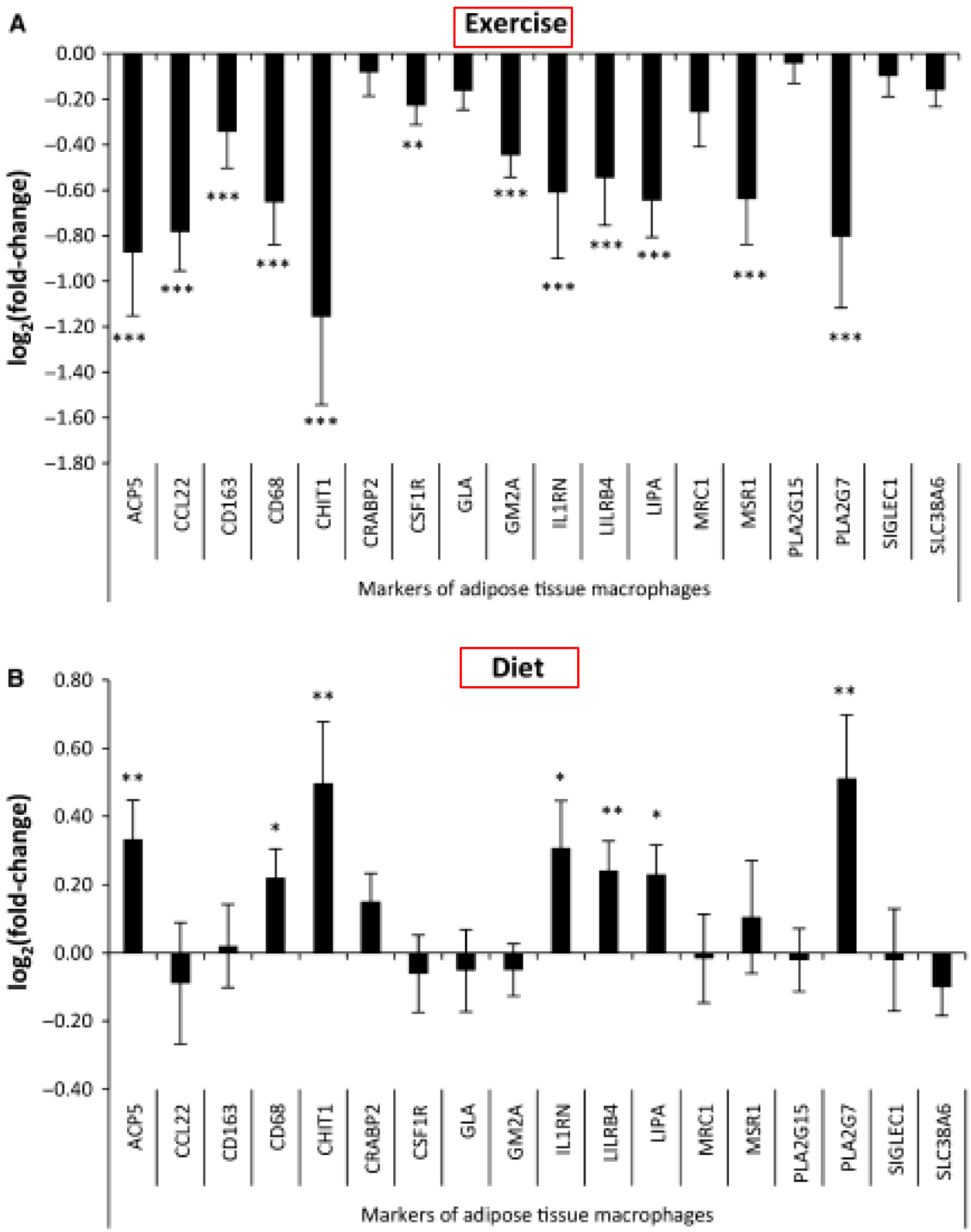
Table 4. Enrichment analysis of pathways altered in adipose tissue after exercise and energy restriction (diet).[1]

	Exercise		Diet	
	Up/down	*P*-value	Up/down	*P*-value
Immune-related pathways				
Chemokine signaling pathway	−5.1	2.4E-07	1.7	6.0E-06
Osteoclast differentiation	−5.4	7.5E-08	2.1	2.7E-08
Complement and coagulation cascades	−3.3	6.3E-04	1.3	4.5E-03
Toll-like receptor signaling pathway	−3.8	1.0E-04	1.9	3.0E-03
NOD-like receptor signaling pathway	−2.5	7.4E-03	1.0	6.4E-03
Jak-STAT signaling pathway	−2.3	1.1E-02	1.2	9.2E-03
Hematopoietic cell lineage	−4.1	2.7E-05	2.7	2.1E-12
Natural killer cell mediated cytotoxicity	−5.4	8.3E-08	2.6	6.5E-12
T-cell receptor signaling pathway	−3.4	3.8E-04	1.9	3.1E-07
B-cell receptor signaling pathway	−4.1	3.2E-05	1.3	2.7E-04
Leukocyte transendothelial migration	−4.7	1.8E-06	1.3	2.1E-04
Energy-related pathways				
Glycolysis/gluconeogenesis	0.5	0.040	−8.4	0.014
Citrate cycle (TCA cycle)	0.9	0.002	−1.6	3.7E-05
Fatty acid metabolism	0.9	0.002	−0.9	7.0E-03
Alanine, aspartate and glutamate metabolism	0.5	0.047	−0.8	0.019
Pyruvate metabolism	0.7	0.013	−1.1	0.004
Peroxisome	0.6	0.031	−1.2	9.2E-04
Insulin signaling pathway	0.7	0.011	−0.8	0.014

CAD19

A) Reduced mRNA expression of 12 of 18 markers of adipose tissue M2-like macrophages after **exercise**

B) Increased mRNA expression of 7 of 18 markers of adipose tissue M1-like macrophages after **energy restriction**



CAD20

Energy restriction vs exercise

Lee et al. *Phys Report* 2016, 4 (21) e13019

- Energy restriction
  - **Increased lipolysis**
  - **Increased expression of markers of M1-like macrophages in AT**
    - M1 "killer" macrophages activated by LPS and IFN-g, secrete high amounts of IL-12 & low amounts of IL-10
- Exercise
  - **Reduce expression of markers of M2-like macrophages & T cells**
    - M2 → wound healing & tissue repair; turn off immune activation via anti-inflammatory cytokines like IL-10
    - Resident tissue macrophages can be further elevated by IL-4
    - High levels of IL-10, TGF-b & low levels of IL-12
    - Tumor-associated macrophages are mainly M2, promote tumor growth

Exercise and Regulation of Adipokine and Myokine Production

Prog Mol Biol Transl Sci. 2015, 135, 313-36

Sven W. Görgens*, Kristin Eckardt†, Jørgen Jensen‡, Christian A. Drevon†, Jürgen Eckel*,§,1

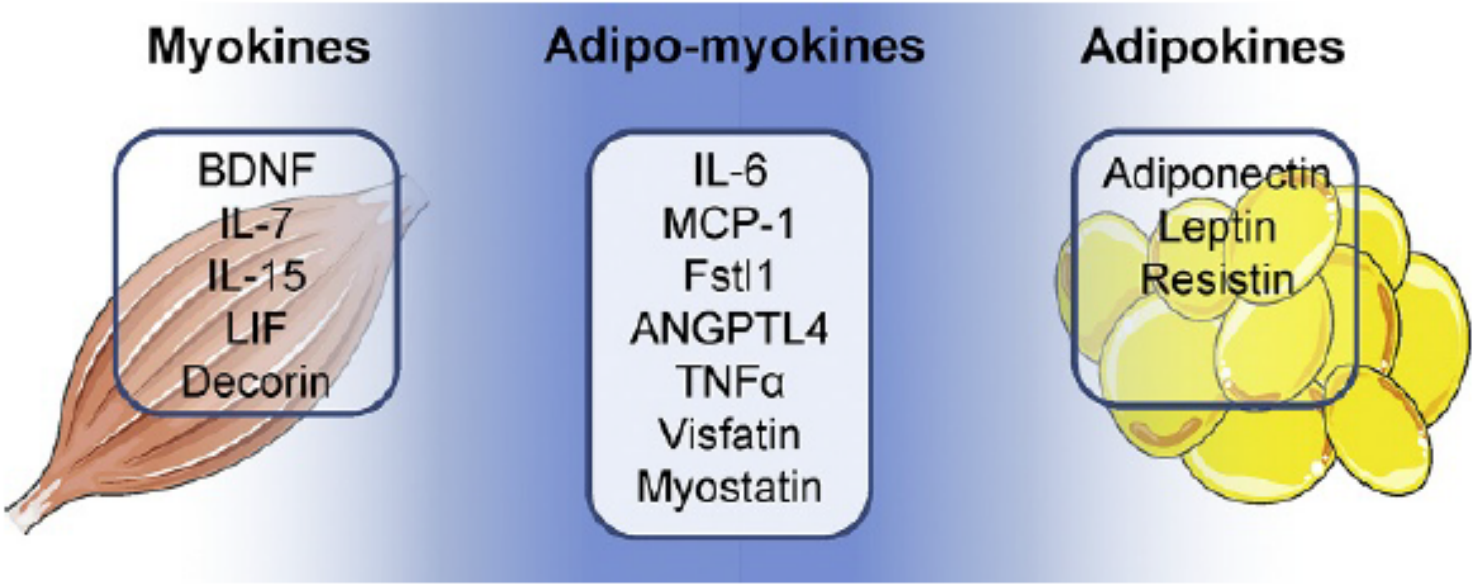
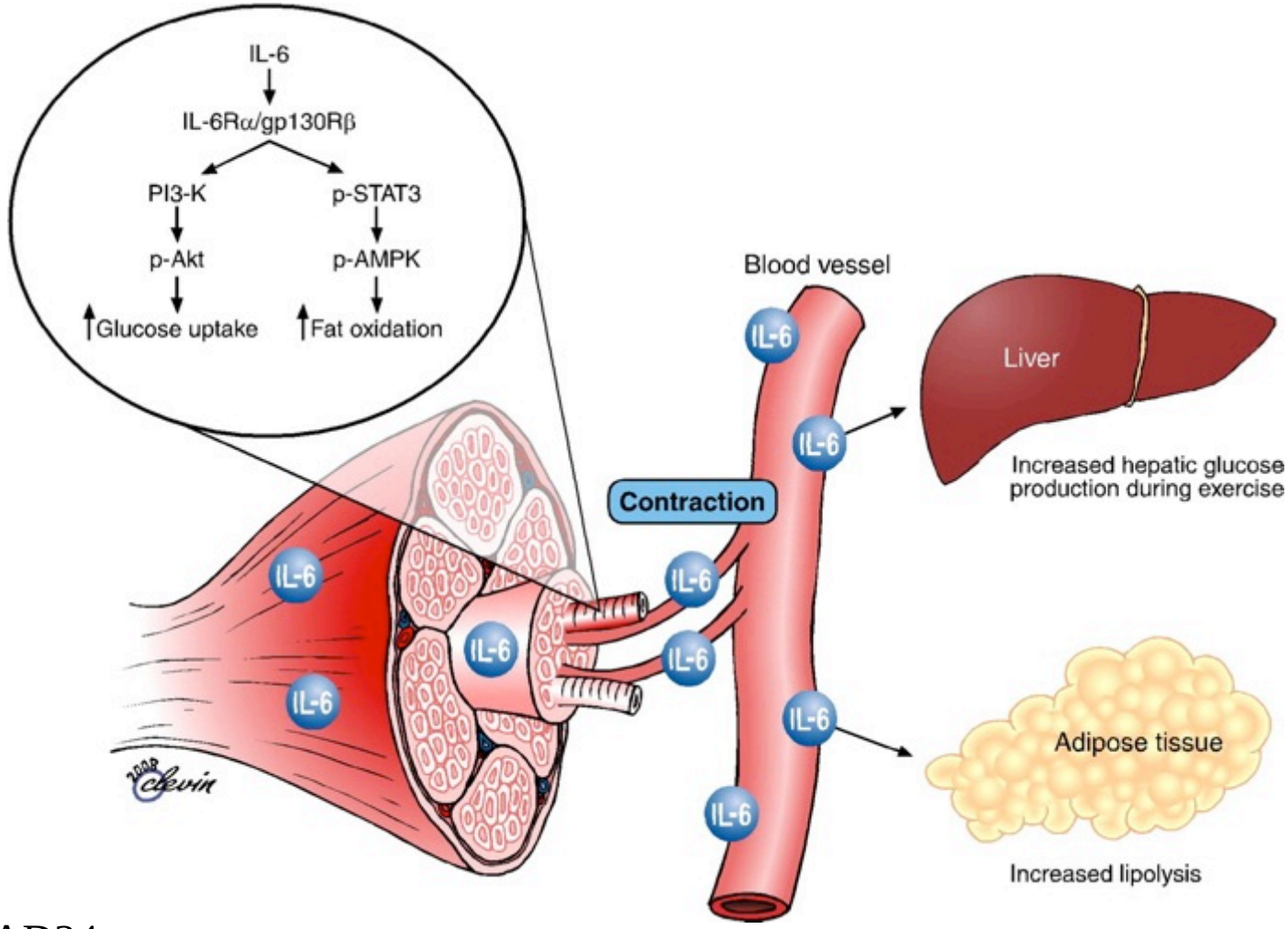


Figure 1 The adipo-myokine concept. A search of original articles in PubMed was performed for the major exercise-regulated myokines and adipokines to identify molecules that were produced and secreted in both tissues. The term adipo-myokines was used for proteins fulfilling both of these criteria. The search terms we used were "skeletal muscle" or "adipose tissue," "myokine" or "adipokine," and "exercise."

Physical activity protects against chronic disorders

- **CVD**; Thompson *ATVB* 2003, **23,** 1319-21
- **Obesity**; Catenacci & Wyatt *Nat Clin Pract Endocrinol. Metab* 2007, **3,** 518-29
- **T2D**; Knowler et al. *NEJM* 2002, **346,** 393-403
- **Osteoporosis;** Gass & Dawson-Hughes *Am J Med* 2006, **119,** S3-11
- **Dementia**; Lautenschlager et al. *JAMA* 2003, **300,** 1027-37
- **Depression**; Martinsen *Acta Psychiatr Scand Suppl* 1994, **377,** 23-7
- **Cancer**; *WCRF report* 2007, colorectal, breast, prostate

Contraction-induced signals like IL-6

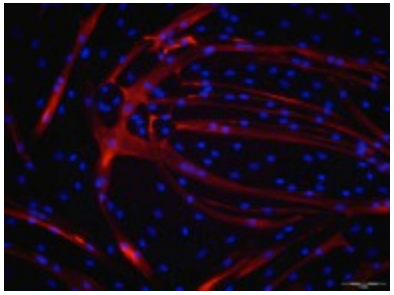


IL-6 from AT different skeletal muscle?

CAD24

Pedersen BK et al. *Physiol Rev*. 2008

Our strategy for identifying novel myokines



Human grown myotubes in culture; 6 h - FCS

Targeted immunoassay

LUMINEX multiplex assay

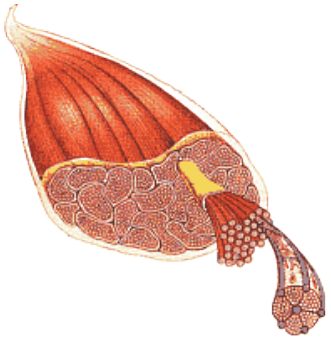
Proteomic analyses of secretome

Concentration by membrane cut-off 3 kD
Separation by 1-D SDS-PAGE
Collection of 10 “bands”, Trypsine
MALDI-TOF MS
Data sorting

Hit list including novel myokines

Hit list including ~ 20 most abundant proteins

Validity/ Physiological relevance?



Time-course
Expression detected by RT-PCR & Western
Expression during differentiation of satellite cells to myotubes
Regulated expression/ secretion?
Effect of recombinant peptide in vitro? Paracrine?
Expressed in skeletal muscle tissue?
Expression altered by exercise/ inactivity/ wasting in vivo?

CAD25

Proteomic identification of secreted proteins from human skeletal muscle cells and expression in response to strength training

Norheim et al. *Am J Physiol. Endocrinology & Metabolism*, 2011, 301, E1013-21

- **236 proteins** detected by proteomics in medium from cultured human myotubes
- **18 classically secreted proteins expressed in skeletal muscle**, using the SignalP 3.0 and Human Genome Expression Profile databases together with a published mRNA-based reconstruction of the human skeletal muscle secretome
- **17 of the secreted proteins** exhibited mRNA expression in cultured human myotubes and skeletal muscles biopsies
- **15** of theses had significantly **enhanced mRNA expression** in *m. vastus lateralis* and/or *m. trapezius* after 11 wk of strength training

Table 1. *"Classically" secreted proteins identified in media conditioned by cultured human muscle cells*

Protein Name	QM[a]	Score[b]	MW[c]	Myotubes: mRNA[d]	VL: mRNA[e]
		Three Donors			
Secreted protein, acidic and rich in cysteine	28, 16, 11	418, 251, 188	35	3.2502	0.1498
Collagen alpha-1(I) chain	11, 5, 3	214, 74, 50	139	0.5368	0.0022
Lactotransferrin	7, 4, 3	105, 58, 51	78	0.0005	0.0011
Alpha-2-macroglobulin	7, 2, 2	185, 59, 53	163	0.0196	0.0817
Lumican	7, 2, 2	131, 54, 33	38	0.0966	0.0190
Gelsolin	6, 3, 2	212, 48, 68	86	0.3438	0.2232
Cathepsin H	1, 1, 1	71, 65, 50	37	0.0303	0.0045
		Two Donors			
Pigment epithelium-derived factor	8, 2	239, 41	46	0.2952	0.1836
Plasminogen activator inhibitor 1	6, 4	149, 155	45	0.7050	0.0006
Cathepsin D	5, 2	171, 39	28	1.2771	0.1332
Tissue inhibitor of metalloproteinase 1	2, 2	117, 63	23	0.6781	0.0127
Fibronectin 1	2, 1	92, 58	262	0.6384	0.0356
Complement C1 s subcomponent	1, 1	69, 45	77	0.1409	0.0452
Cathepsin L1	1, 1	55, 50	38	0.1179	0.0545
		One Donor			
Cathepsin B	5	69	38	0.7021	0.0396
Salivary acidic proline-rich phosphoprotein 1/2	5	149	17	0.0001	ND
Follistatin-like 1	3	38	35	0.1642	0.0192
Extracellular matrix protein 1	1	39	61	0.0846	0.0028

[a]QM denotes the number matched queries. [b]Score denotes the proteins' MASCOT scores. [c]Theoretical molecular mass of proteins is displayed according to the UniProtKB/ Swiss-Prot entry (mass; kDa). [d]Mean mRNA expression from three donors is presented as normalized to RPLP0. [e]Mean mRNA expression in m. vastus lateralis biopsies from 10 healthy male subjects normalized to RPLP0

CAD27

Norheim et al. *Am J Physiol Endocrinology Metabolism*, 2011, 301, E1013-21

Table 2. *Changes in skeletal muscle mRNA levels of secreted muscle proteins in strength-training individuals*

Protein Name	M. vastus lateralis	M. trapezius
Collagen alpha-1(I) chain	5.2 (3.7–14.9)*	43.4 (3.9–139.5)*
Secreted protein, acidic and rich in cysteine	2.9 (1.7–4.9)*	9.6 (3.2–18.8)*
Plasminogen activator inhibitor 1	2.6 (1.1–7.8)	4.7 (2.5–18.5)*
Lumican	2.5 (1.7–3.7)*	4.3 (1.1–11.4)*
Tissue inhibitor of metalloproteinase 1	2.1 (1.3–3.6)*	3.0 (1.1–10.3)*
Follistatin-like 1	1.7 (1.2–3.1)*	2.6 (1.0–6.3)*
Fibronectin 1	1.8 (1.4–2.9)*	2.5 (1.2–8.0)
Complement C1 s subcomponent	1.8 (1.0–2.2)*	1.7 (1.2–6.5)
Extracellular matrix protein 1	1.8 (1.0–2.4)*	1.9 (1.4–4.6)*
Alpha-2-macroglobulin	1.8 (0.9–2.2)*	1.9 (1.1–3.0)
Gelsolin	1.5 (0.9–2.1)*	1.7 (1.4–2.9)
Pigment epithelium-derived factor	1.4 (1.1–1.7)	1.8 (1.7–3.0)*
Cathepsin B	1.3 (1.1–1.7)*	1.5 (1.0–2.4)*
Lactotransferrin	1.5 (0.7–2.3)	1.7 (0.8–2.2)
Cathepsin D	1.3 (1.1–1.7)	1.8 (1.1–2.2)*
Cathepsin L1	1.2 (0.8–1.3)	1.5 (1.1–1.8)*
Cathepsin H	1.1 (0.6–1.8)	1.2 (1.0–2.0)

CAD28

Norheim et al. *Am J Physiol Endocrinology Metabolism*, 2011, 301, E1013-21

Single exercise bout

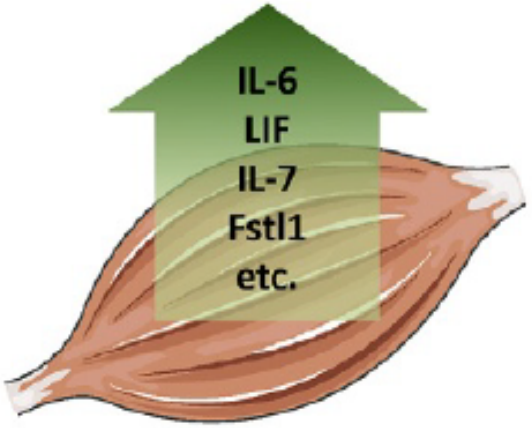
< 3 h

vs.

Chronic exercise training

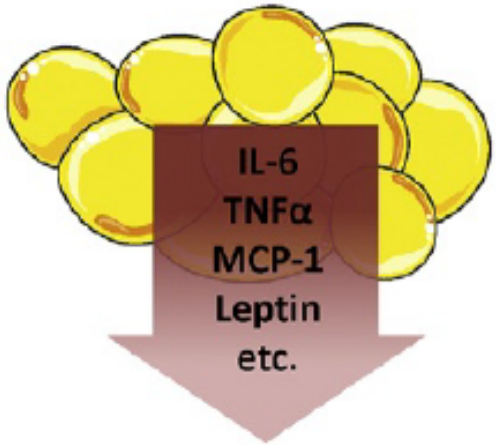
> 8 weeks

Secretion of muscle-derived adipo-myokines



- ↑ Muscle repair and growth
- ↑ Endothelia function and angiogenesis
- ↑ Adipocyte lipolysis
- ↑ Hepatic glucose release into circulation
- ↓ Inflammation

Reduction of adipose tissue-derived pro-inflammatory adipo-myokines

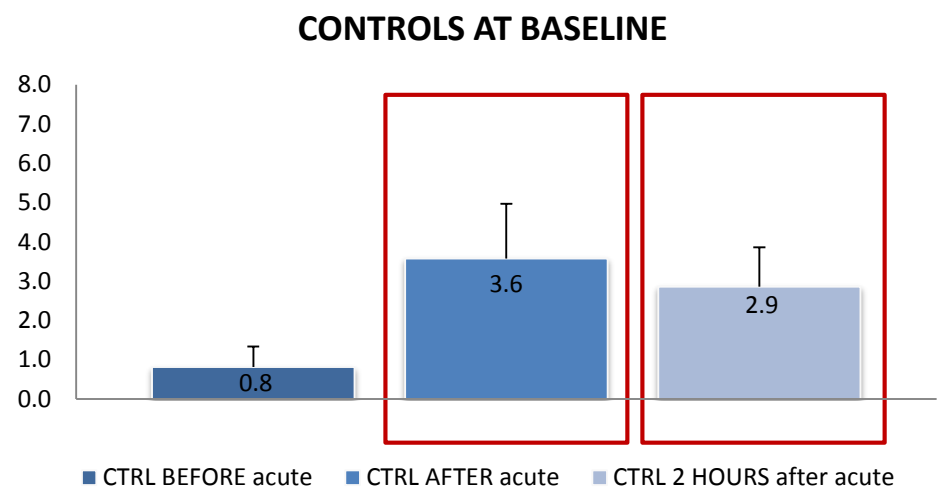


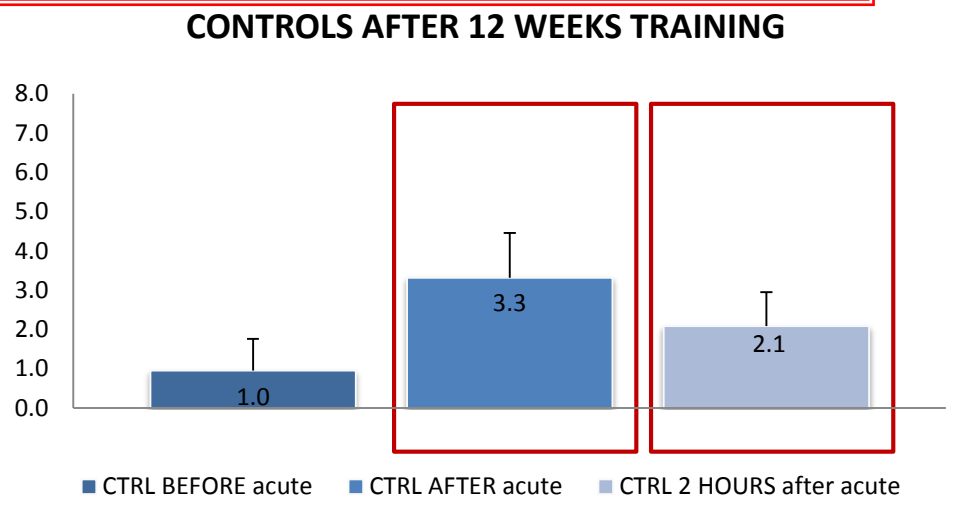
- ↑ Insulin sensitivity
- ↑ Physical fitness
- ↓ Visceral fat mass
- ↓ Inflammation

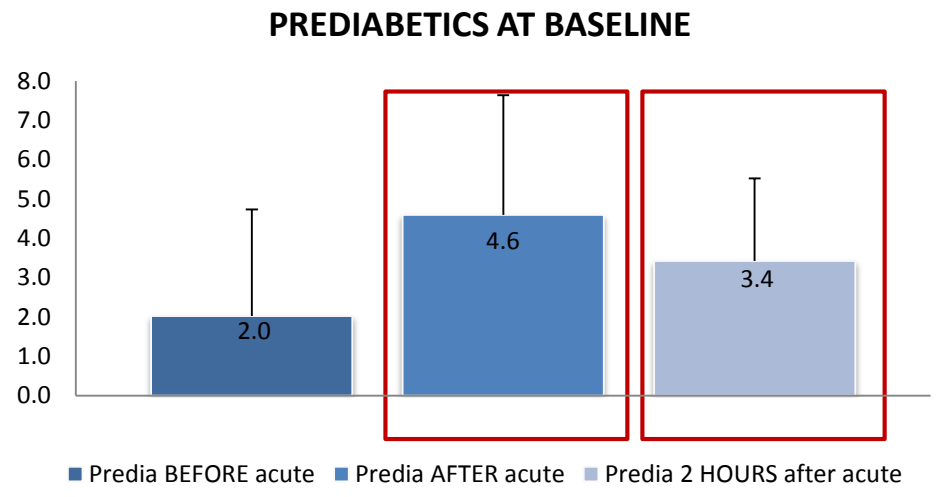
CA

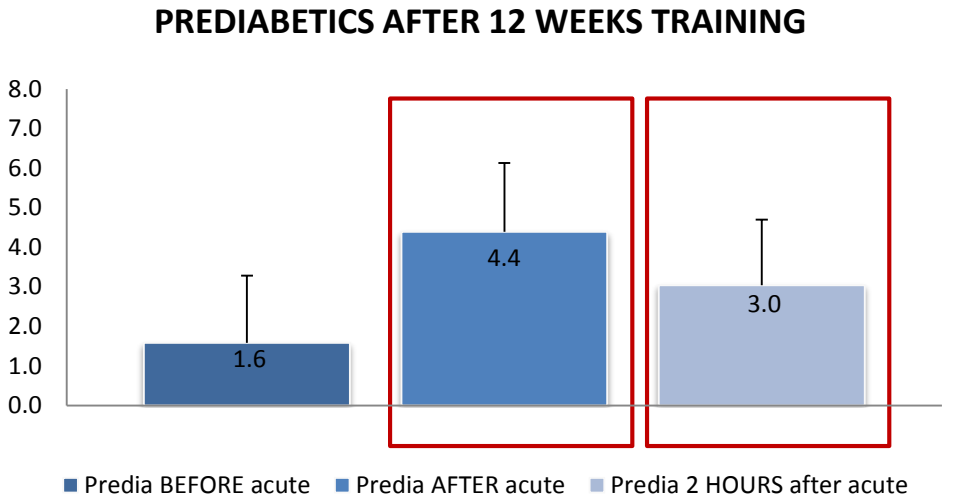
Plasma concentration of IL-6 (pg/mL)

Langleite et al. *Arch Physiol Biochem*. 2016, 122, 167-79









Significant increase in IL-6 after ACUTE exercise (both at 0 and 2 h) $p < 0,01$, but NOT after 12 w training intervention

Extracellular matrix & exercise

Hjorth et al. *Physiol Rep*. 2015 Aug;3(8). pii: e12473

- After 45 min cycling ~ **550** genes were upregulated
  - 28 genes (5%) were directly related to ECM
- Long-term exercise (12 w) enhanced expression of **289** genes >50%
  - 20% were ECM related
- > 50% of the proteoglycans in muscle were significantly enhanced after 12 w
- Secretion of the PG serglycin for the first time from SKM
- SRGN KO → enhanced expression & secretion of serpin E1 (SERPINE1; serine proteinase inhibitor superfamily. Inhibitor of tissue plasminogen activator (tPA) and urokinase (uPA) → inhibitor of fibrinolysis)

Belgian blue – mutated gene encoding myostatin inhibiting muscle growth

Myostatin & exercise in humans

Hjorth et al. *Acta Physiolog*, 2016, 217, 45–60

- Myostatin mRNA expression **reduced** in SKM after acute & long-term PA
- Even further reduced by acute exercise on top of 12 w training
- Expression of myostatin at baseline correlated negatively with insulin sensitivity
- Myostatin expression in AT **increased** after 12 w training
  - correlated positively with insulin sensitivity markers
- In cultured SKM cells but not in SGBS cells, myostatin promoted insulin-independent increase of glucose uptake
- SKM cells incubated with myostatin enhanced glucose oxidation & lactate production
- **Myostatin differentially expressed in muscle (-) and AT (+) in relation to PA and dysglycaemia. Recombinant myostatin increased consumption of glucose in human skeletal muscle cells, suggesting a role of myostatin in skeletal muscle glucose metabolism**

Table 2 SignalP-positive genes encoding secretory proteins that were downregulated more than 1.5 times (FC < 0.667) in the skeletal muscle during 12 weeks of training (n = 26)

Genes	Gene symbol	FPKM*	Fold change†	P-value‡	q-value§
Neuronal pentraxin 1	NPTX1	0.2	0.26	2E−08	3E−07
Cadherin 22, type 2	CDH22	0.2	0.39	3E−07	4E−06
Gremlin 2, DAN family BMP antagonist	GREM2	2.6	0.46	2E−05	1E−04
Protease, serine, 50	PRSS50	1.7	0.53	9E−07	9E−06
Olfactomedin 1	OLFM1	4.6	0.58	1E−11	4E−10
Myostatin	MSTN	3.8	0.58	3E−06	2E−05
Toll-like receptor 9	TLR9	0.6	0.59	4E−13	2E−11
Leucine-rich repeat containing 3B	LRRC3B	4.2	0.62	4E−03	1E−02
CD274 molecule	CD274	2.2	0.63	3E−13	1E−11
Shisa family member 3	SHISA3	0.6	0.63	3E−02	6E−02
LAMB3	LAMB3	2.6	0.64	5E−06	4E−05
Carboxylesterase 1	CES1	0.9	0.65	1E−05	8E−05
Na+/K+-transporting ATPase interacting 1	NKAIN1	7.6	0.65	5E−16	4E−14
Rho GDP dissociation inhibitor (GDI) gamma	ARHGDIG	0.7	0.66	2E−02	6E−02
Chondroadherin	CHAD	6.1	0.66	8E−07	9E−06

*Gene expression level at baseline, measured by mRNA sequencing and expressed as fragments per kilobase of transcript per million mapped reads (FPKM).

†mRNA expression after 12 weeks of training as compared to baseline of the intervention.

‡P-value generated in EdgeR.

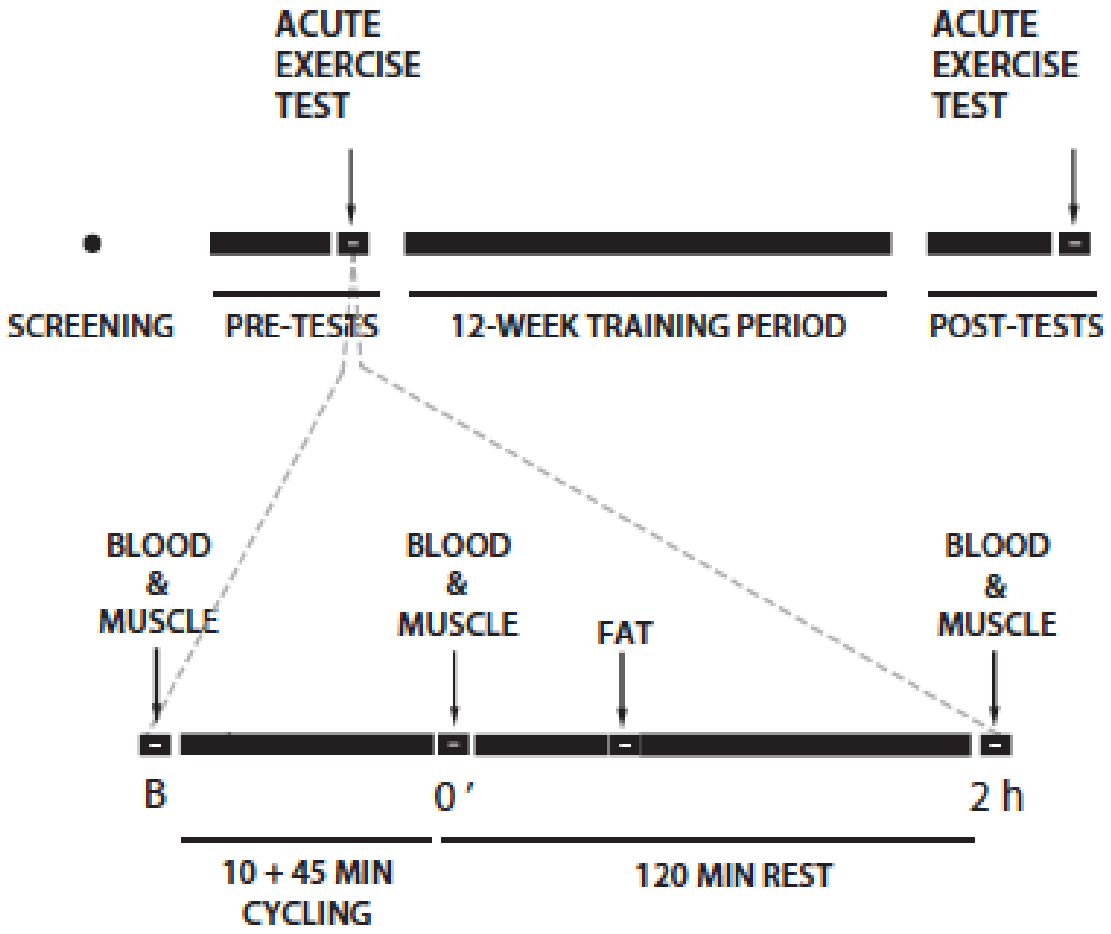
§False discovery rate.

CAD34

Another strategy is to use whole body intervention to discover new myokines

- Tests before and after training (top)
- Acute 45 min bicycle test at 70% of VO_2max (bottom)
- Blood & muscle samples taken before (B), just after (0'), and 2 h after the acute bout (2 h)
- Subcutaneous adipose tissue biopsies taken 30–60 min after acute exercise

26 middle-aged, sedentary
→ endurance and strength training for 12 w



CAD35

Global mRNA sequencing of human skeletal muscle: Search for novel exercise-regulated myokines

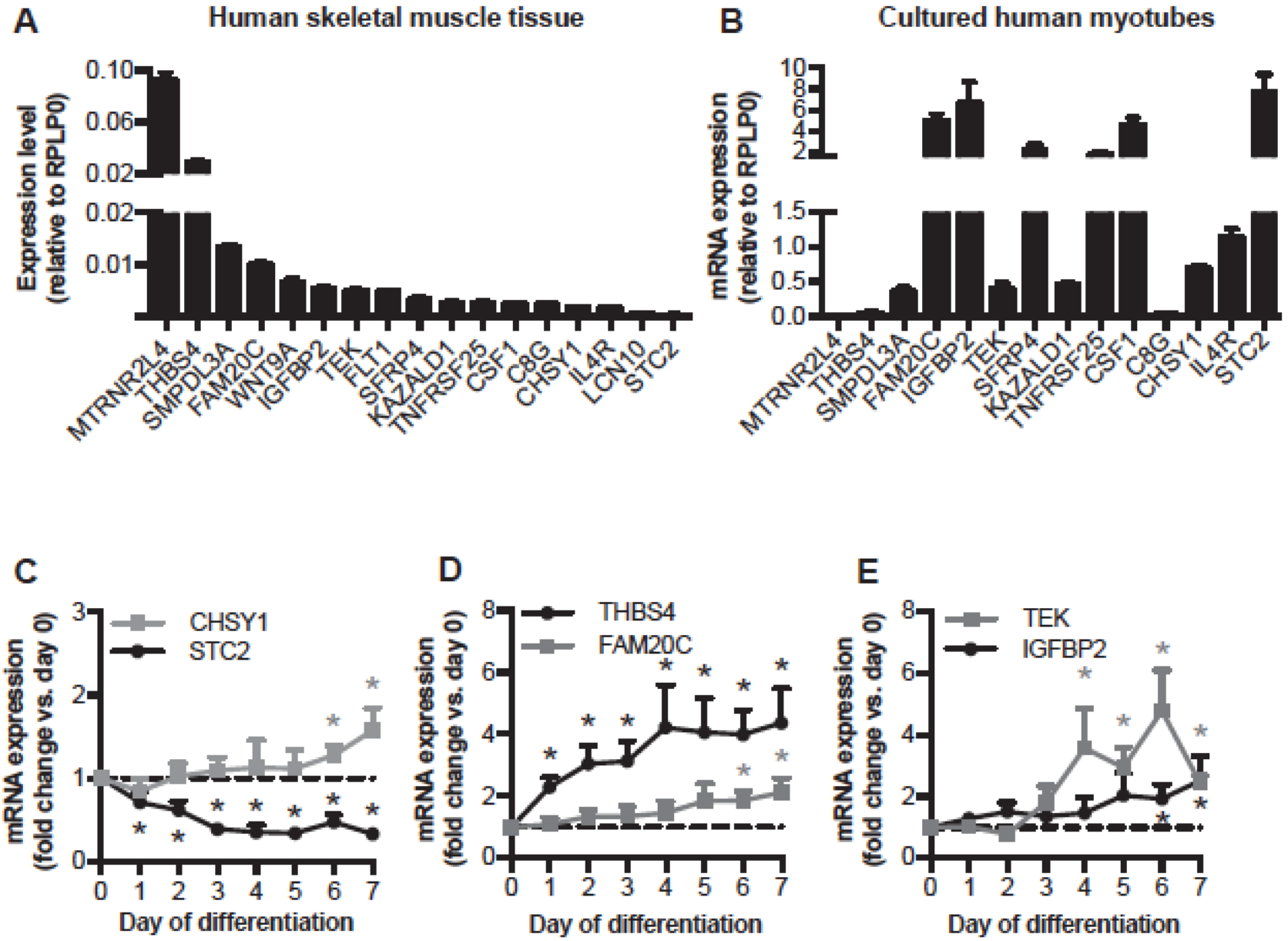
Pourteymour et al. *Mol Metab*. 2017, 6, 352-65

- **161** secretory transcripts enhanced (>1.5-fold) after acute exercise & **99** increased after 12 w
- **92** secretory transcripts were reduced after acute and/or long-term physical activity
- Selected **17 unknown** myokines sensitive to short- and/or long-term exercise
- Expression also in cultured human skeletal muscle cells
- One of the 17 candidates was macrophage colony-stimulating factor-1 (CSF1)
- CSF1 mRNA increased in skeletal muscle after acute and long-term exercise, accompanied by a rise in plasma CSF1 protein
- In cultured muscle cells, electrical pulse stimulation (EPS) increased expression and secretion of CSF1
- **Conclusion**: 17 new exercise-responsive myokines. **CSF1** responded to EPS in cultured muscle cells; up-regulated in muscle and plasma after acute & long-term exercise. **Marker of exercise?**

Venn diagrams showing the number of secretory genes that were up- or down-regulated >1.5-fold

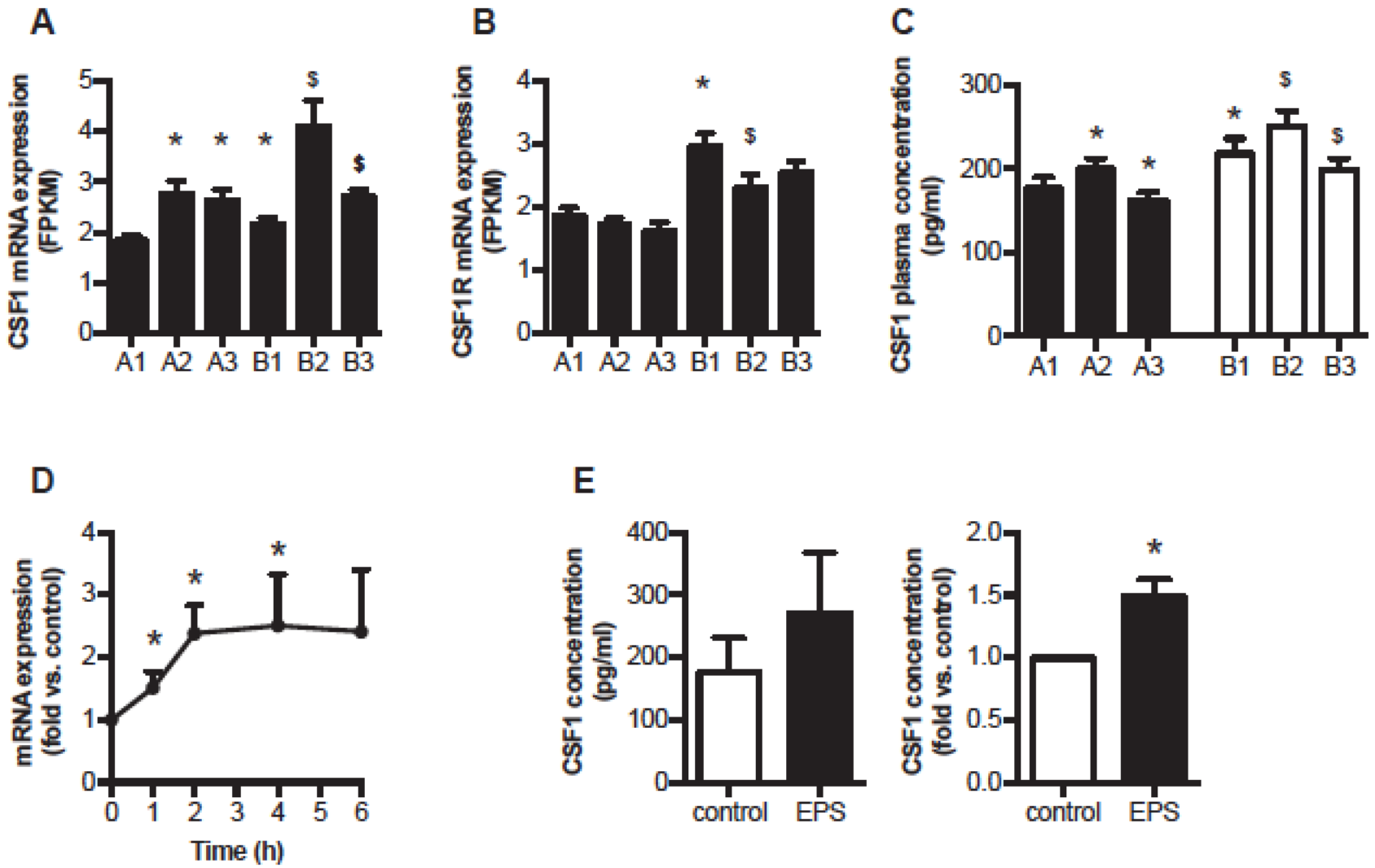
Pourteymour et al. *Mol Metab*. 2017, 6, 352-65

mRNA expression of selected genes in skeletal muscle biopsies (in A1) or cultured human myotubes. mRNA expression in biopsies was determined with RNAseq (n=26), and in myotubes by RT-PCR (n=5-6)



CA

A) mRNA expression of CSF1 and B) CSF1 receptor (CSF1R) in skeletal muscle biopsies at baseline (A1/A3) and after 12 w (B1**e**B3), *p < 0.05 vs. A1, $ p < 0.05 vs. B1. C) Plasma CSF1 before and after 12 w intervention. D) Differentiating human skeletal muscle cells. E) CSF1 conc in culture medium. Pourteymour et al. *Mol Metab*. 2017, 6, 352-65



C.

Major findings after 12 weeks training

Langleite et al. *Arch Physiol Biochem*. 2016, 122, 167-79

- Increased VO_2max ~15 %
- Increased GIR ~30 %
- Dysglycemics reduced body weight (-1.7 kg; $p<0.05$) and waist circumference (-3.7 cm; $p<0.01$)
- Visceral fat preferentially lost compared to other ATdepots
- Hepatic fat was 5-fold higher in dysglycemics than controls, and was reduced after training (29%, $p<0.01$)
- Muscle fat reduced 57% in dysglycemics; 27% in controls
- Change of VO_2max correlated strongly with change of GIR

	Controls		Dysglycemia	
	Baseline	Δ	Baseline	Δ
Age (years)	53 (17)		53 (10)	
Anthropometry				
Height (cm)	185.3 (9.3)		178.6 (5.2)*	
Weight (kg)	73.5 (16.5)	0.7 (2.5)	94.1 (14.1)*	−1.1 (1.9)
BMI (kg/m^2)	23.3 (3.1)	0.2 (0.5)	27.8 (5.3)*	−0.4 (1.2)
Waist circumference (cm)[a]	88 (9)	−0.3 (1.0)	104 (16)*	−3.5 (5.3)^*
MRI				
Thigh muscle area (mm^2/kg)	244 (62)	25 (11)^	264 (55)	26 (20)^
Adipose depots (mL)				
Supraclavicular	78 (37)	−3 (11)	118 (60)*	−3 (19)
Axillary	166 (60)	2 (52)	276 (212)*	−3 (46)
Pericardial	113 (86)	1 (23)	166 (79)	−2 (30)
Subcutaneous	4776 (896)	−340 (548)	8487 (2878)*	−439 (554)^
Intraperitoneal	694 (807)	−215 (396)^	2236 (984)*	−332 (446)^*
Retroperitoneal	851 (621)	−104 (200)^	1991 (921)*	−131 (203)^
Inguinal	66 (21)	−2 (16)	106 (59)*	−6 (21)
Epididymal	6 (3)	1 (2)	9 (3)*	1 (2)
Popliteal	132 (56)	0 (22)	174 (124)	2 (23)
Aerobic capacity				
VO_2max (mL·kg^{-1}·min^{-1})	43.4 (6.9)	6.3 (4)^	38.7 (8.1)*	4.7 (4)^
Maximum strength				
Leg press 1-RM (kg)	188 (58)	25 (20)^	250 (33)*	23 (30)^
Cable pulldown 1-RM (kg)	70 (18)	10 (9)^	75 (18)	10 (10)^
Chest press 1-RM (kg)	60 (20)	11 (11)^	63 (23)	8 (8)^
Blood parameters				
F-B-HbA$_{1C}$ (%)[b]	5.2 (0.8)		5.6 (0.6)*	
F-P-Glucose (mmol/L)[c]	5.3 (1)	0.2 (0.4)	5.8 (0.7)*	0.0 (0.6)
F-S-Insulin (pmol/L)	37.7 (23)	2 (28)	64 (45)*	−0.4 (25)
F-S-C peptide (pmol/L)	568 (129)	63 (273)	944 (351)*	−46 (425)
S-CRP (mg/L)	0.8 (1.5)	0.1 (0.7)	1.6 (2.4)*	0.0 (2.2)

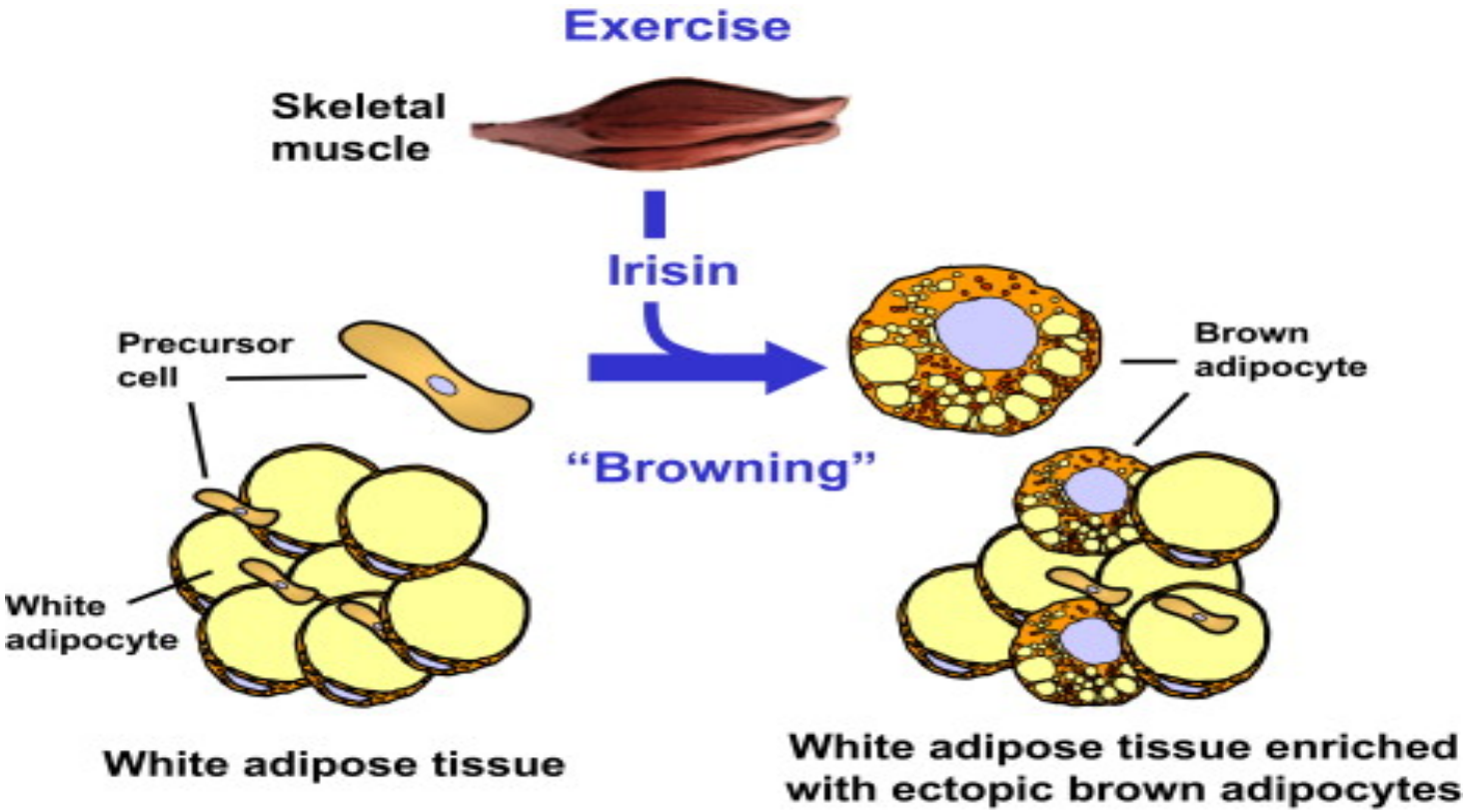
*Different from control group $p < 0.05$.
^Pre vs. post within group difference $p < 0.05$.
[a]From 17 subjects (control $n = 8$, dysglycemic $n = 9$).
[b]Screening values (post and Δ are unavailable).
[c]Not screening values.

CA

Langleite et al. *Arch Physiol Biochem*. 2016, 122, 167-79

A PGC1-a-dependent myokine that drives brown-fat-like development of white fat and thermogenesis

Boström et el. *Nature*, 2012, 481: 463-8



Francesc Villarroya **Irisin, Turning Up the Heat** *Cell Metabolism* 2012, 15, 277 - 8

Evidence against a beneficial effect of irisin in humans

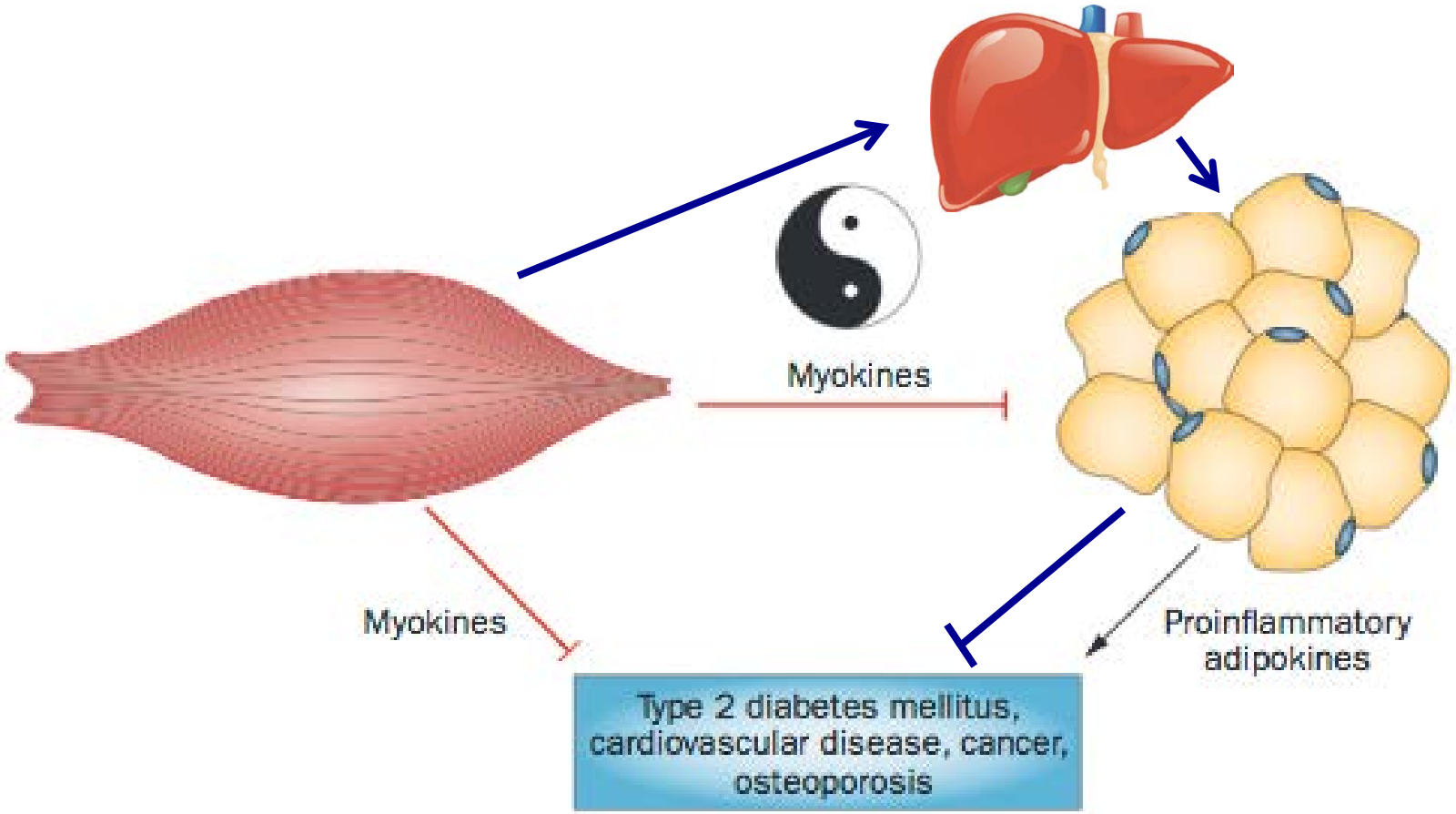
- Raschke et al. *PlosOne*, 2013, 8(9):e73680
  - Mutation in the start codon ATA in stead of ATG, very little transcription of irisin
- Norheim F et al. *FEBS J*. 2014, 281, 739-4
  - No brownin g of WAT with long-term training
- Albrecht et al. *Sci Rep*. 2015 Mar 9;5:8889
  - All 4 antibodies used in ~ 100 papers are unspecific

Interaction between plasma fetuin-A (hepatokine) and free fatty acids predicts changes in insulin sensitivity in response to long-term exercise

Lee et al. *Physiol Rep*. 2017, Mar;5(5)

- Exercise 12 w reduced plasma fetuin-A conc. (~11%, $P < 0.01$), slightly changed FFAs concentration, and improved glucose infusion rate (GIR) (~30%, $P < 0.01$)
- Changes in plasma fetuin-A & FFAs interacted to predict some of the change in GIR ($b = 42.16$, $P = 0.030$), AT insulin resistance ($b = 0.579$, $P = 0.003$), gene expression of TLR-signaling in AT & AT macrophage mRNA ($b = 94.10$, $P = 0.034$) after exercise
- The relation between FFA levels and insulin sensitivity was specific for fetuin-A in AT
- **Some effect of exercise on insulin sensitivity may be due to changes in plasma hepatokine fetuin-A and FFAs, → less TLR4 signaling in AT perhaps by modulating AT macrophages**

Myokines & adipokines → health & disease



CAD45

Modified after Pedersen & Febbraio. *Nat Rev Endocrinol*. 2012, 8, 457-65

Collaborators

Ph Ds/postdocs/researchers

- Ingrid MF Fange
- Kathrine Vinknes
- Helga Refsum
- Thomas Olsen
- Frode Norheim
- Torgrim Langleite
- Anders Kielland
- Marit Hjort
- Torgeir Holen
- Sindre Lee
- Shirin Pourteymour
- Kristin Eckardt
- Yuchuan Li
- Per O. Iversen

External collaborators

- Arild C. Rustan
- Jørgen Jensen
- Kåre I. Birkeland/Hanne L. Gulseth
- Bernd Thiede
- Per K. Hol et al.
- Britt Nakstad, Arild Rønnestad, Ola D. Saugstad,
- Jens P. Berg
- Thomas E. Gundersen, Vitas
- Willem de Vos
- **NuGO** - B van Ommen, H Daniel, M Müller, Robert Caesar
- **Food4Me** - The Gibneys et al
- **NutriTech** - B van Ommen et al
- **MyoGlu** - Birkeland et al.
- **Lifebrain** - Kristine Walhovd/Anders Fjell

Myokines & adipokines – sum up

- Several 100
- Many are important
  - Irisin is not
- Often expressed in many tissues
- Cooperation between many tissues
- *The truth is rarely pure & never simple*

  Oscar Wilde
- Thanks for your attention!