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

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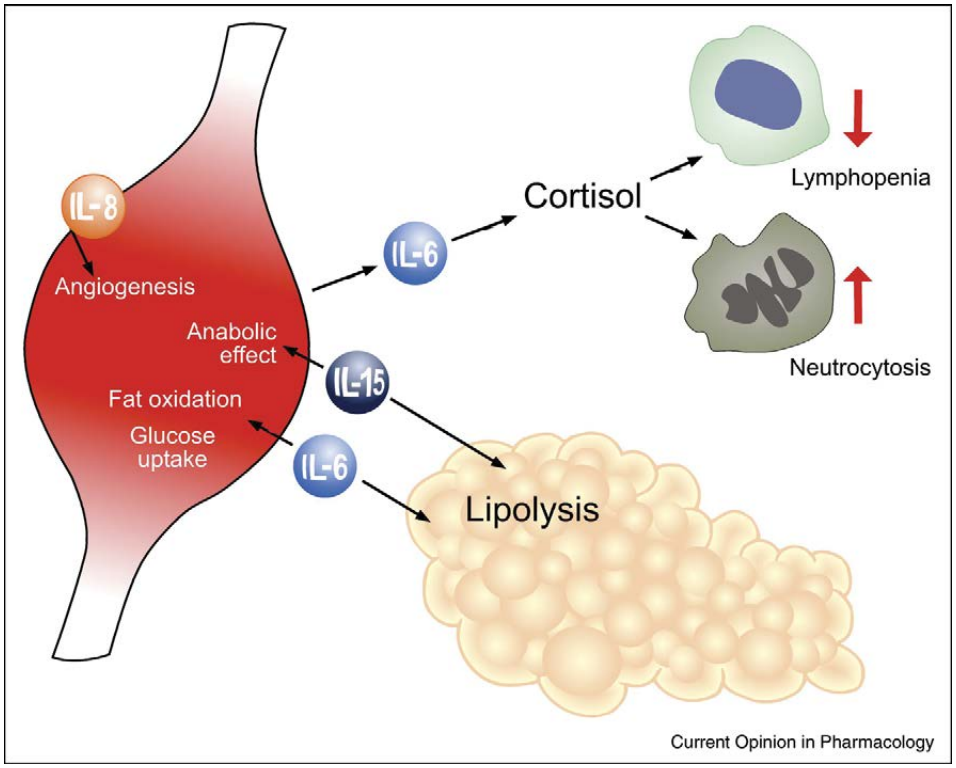
Physical activity & health

Exercise offers protection against chronic disorders like:

- **CVD**; Thompson *ATVB* 2003, **23,** 1319-1321
- **Obesity**; Catenacci & Wyatt *Nat. Clin. Pract. Endocrinol. Metab* 2007, **3,** 518-529
- **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,** 1027-1037
- **Depression**; Martinsen *Acta Psychiatr Scand Suppl* 1994, **377,** 23-27
- **Cancer**; *WCRF report* 2007

Proteins secreted from the muscle



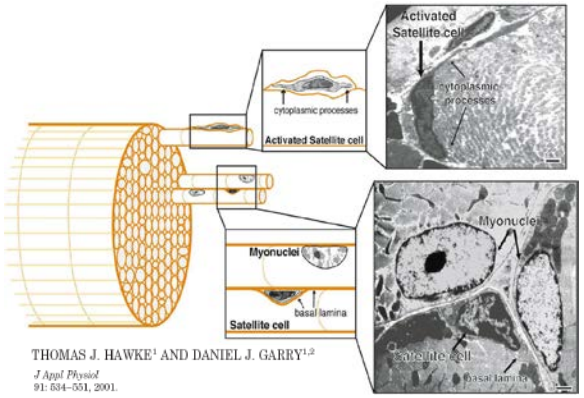
Nielsen R et al, Curr Opin Pharmacol, 2008

Aims:

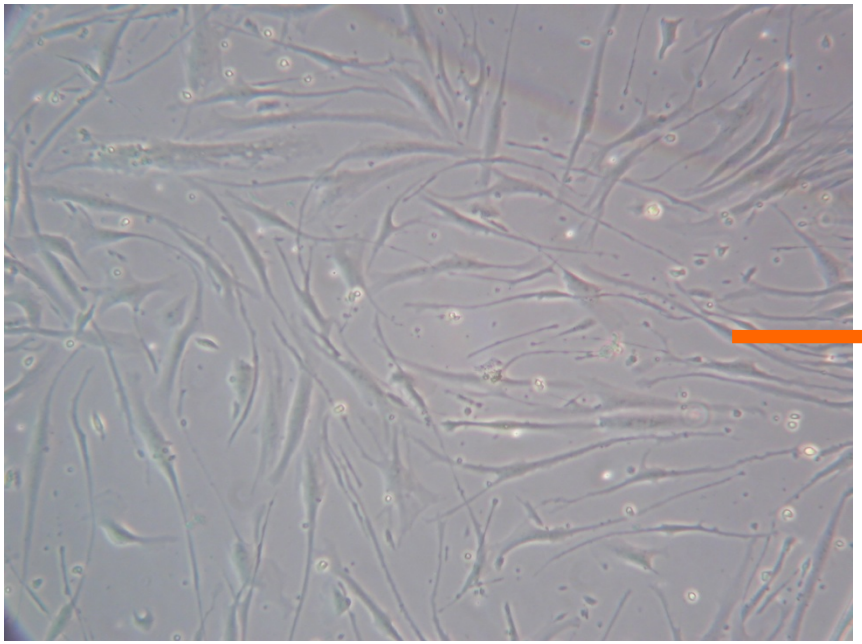
1) Characterize proteins released from cultured human myotubes

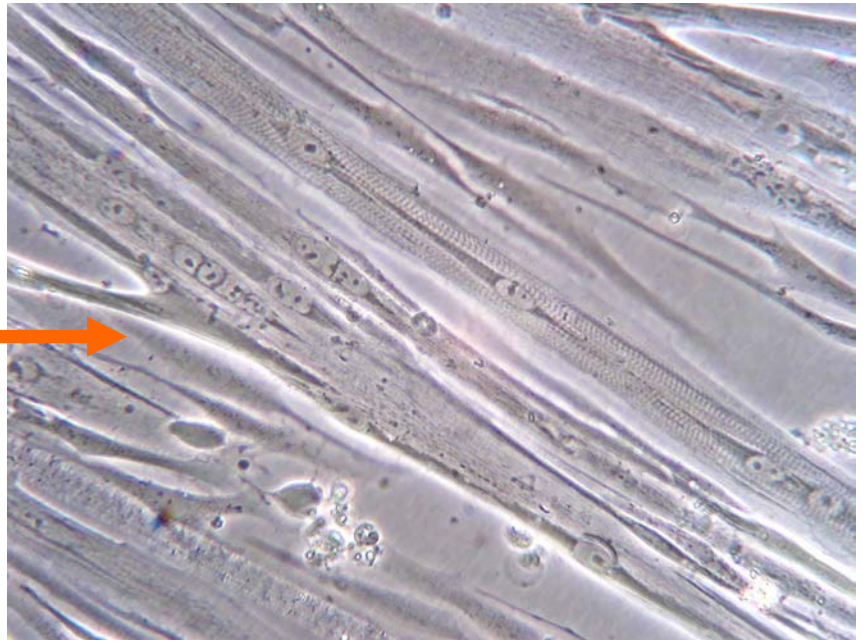
2) Examine the effect of strength training on expression of secreted muscle proteins

Differentiation of cultured human myotubes



7 days of differentiation

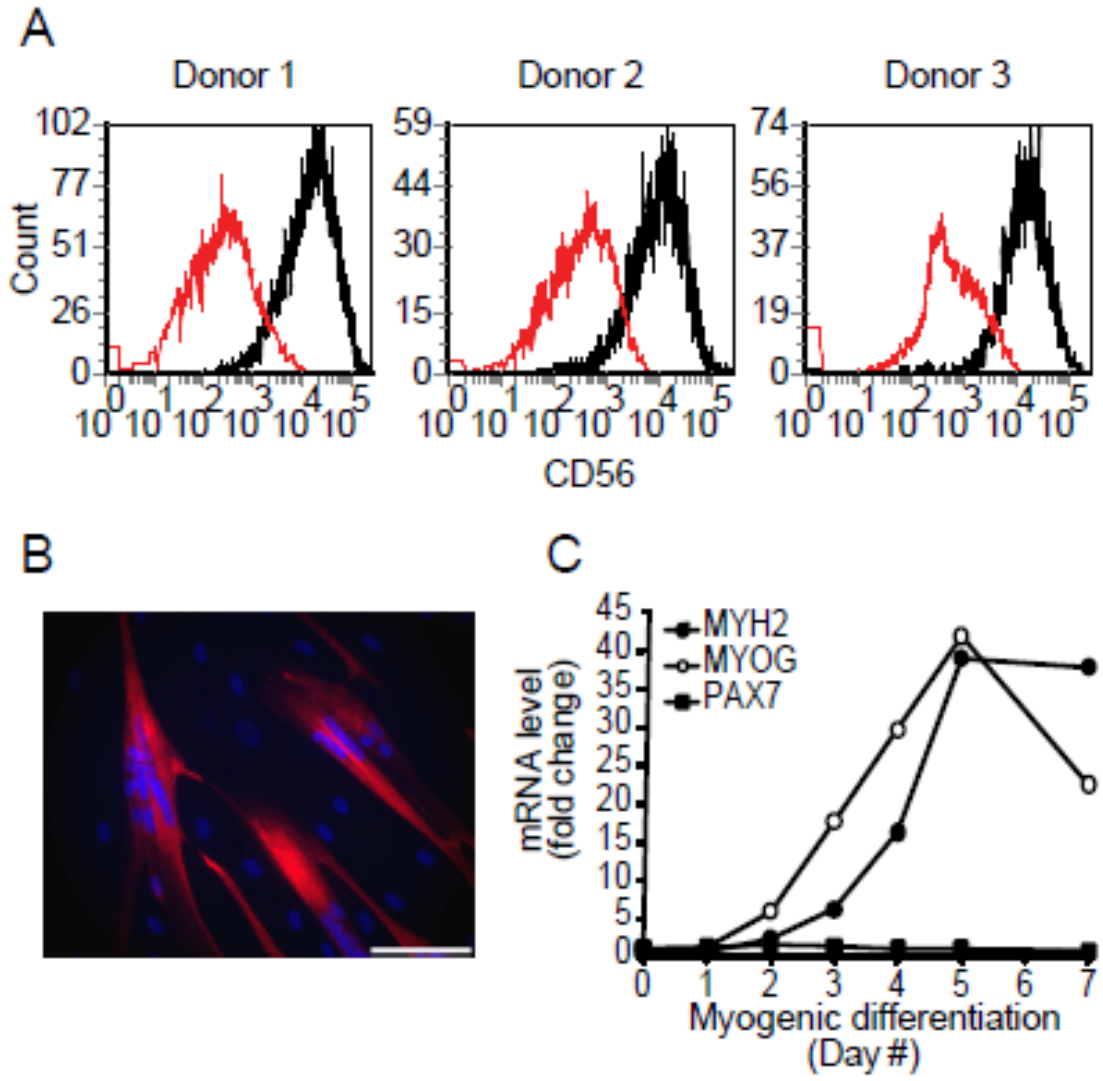




Myoblasts

Myotubes

Characterization of isolated human myoblasts



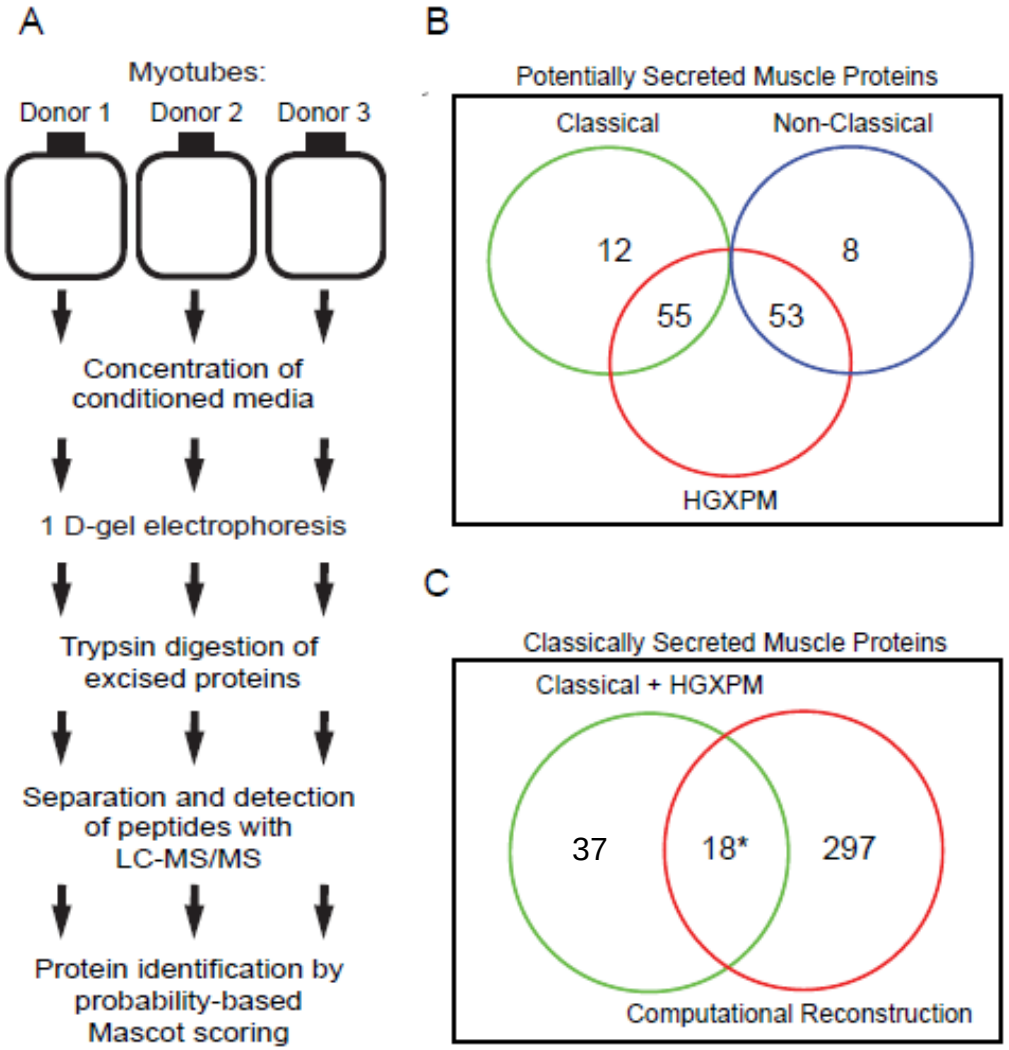
Black: CD56^{+}
Red: neg. Control

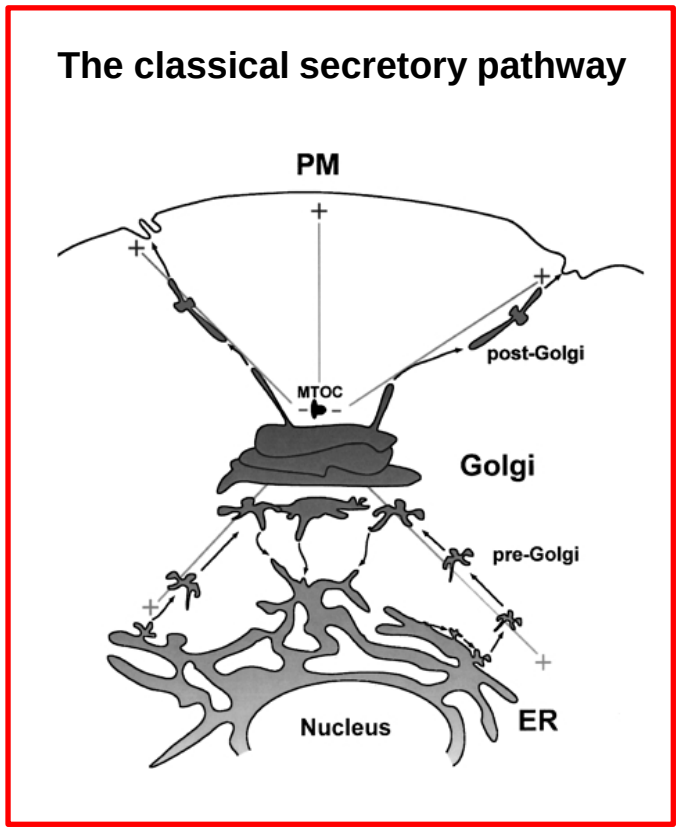
Proteome analysis of conditioned media

X 3 donors

- Three 175 cm^2 flasks confluent with myotubes
- Collected media after 6 hours incubation
- Concentrated media by spin colums
- Analyzed by 1-D gel and Mass spectrometry

Identification of proteins secreted from cultured human skeletal muscle cells

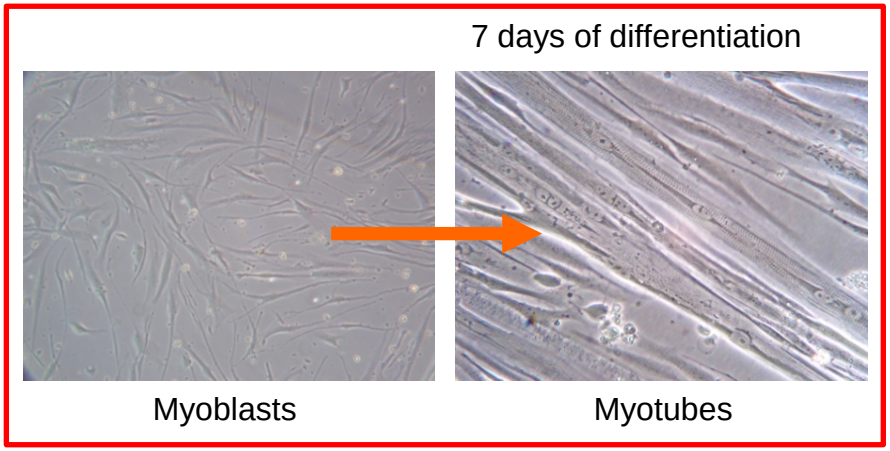


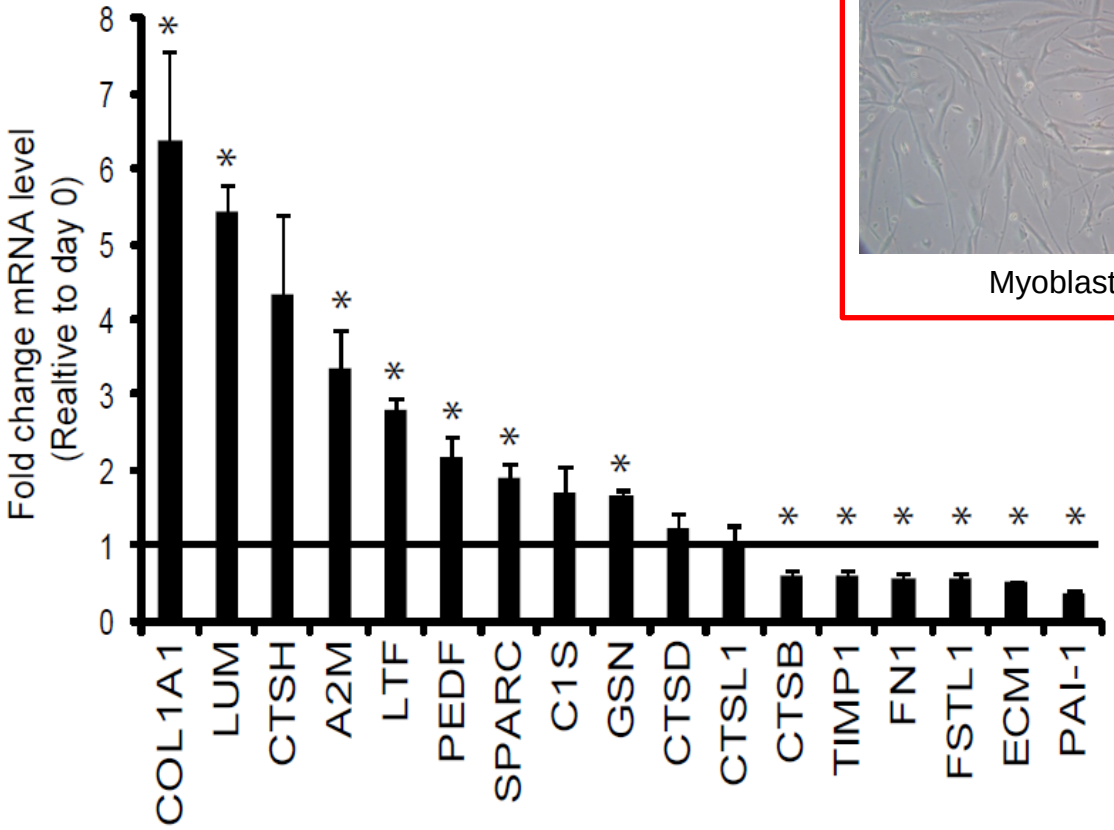


“Classically” secreted proteins identified – mRNA expression

Protein name	QM[a]	Score[b]	MW[c]	Myotubes: mRNA[d]	VL: mRNA[e]
3 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
2 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 C1s subcomponent	1, 1	69, 45	77	0.1409	0.0452
Cathepsin L1	1, 1	55, 50	38	0.1179	0.0545
1 donor					
Cathepsin B	5	69	38	0.7021	0.0396
Salivary acidic proline-rich phosphoprotein ½	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

mRNA expression of secreted proteins during myogenesis





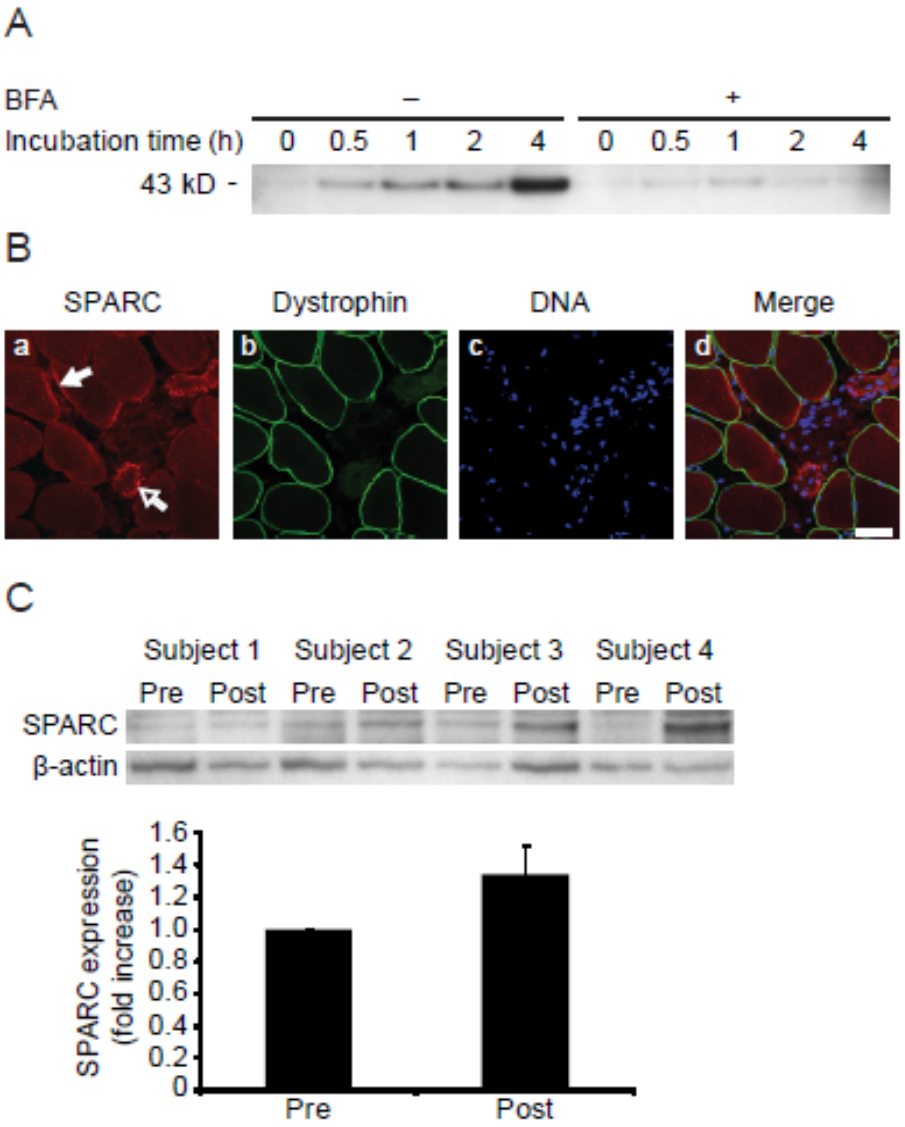
Strength training

- Strength training intervention lasting 11 weeks
  - Muscle biopsies from vastus lateralis (n = 10) and trapezius (n = 7): baseline and 11 weeks
- RT-PCR
- RPLP0 was used as endogenous control
- We compared the relative gene expression after 11 weeks of strength training with the expression on baseline: Fold Change

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 C1s 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)

SPARC is a secreted muscle protein regulated by strength training



Conclusions

- We have identified 17 muscle secretory proteins which is secreted from cultures of human muscle cells and expressed in human muscle biopsies (*m. vastus lateralis and m. trapezius*)
- Compared to baseline, strength-training significantly increase expression of several different muscle secretory proteins
- By defining the human skeletal muscle secretome *in vitro*, novel responses of skeletal muscle to strength training can be identified *in vivo*

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