Tweak: a new cytokine within the context of obesity

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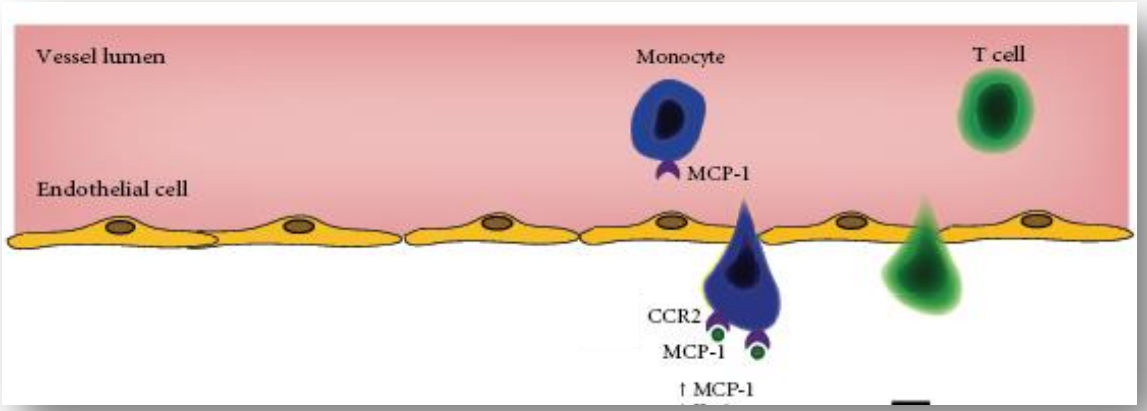
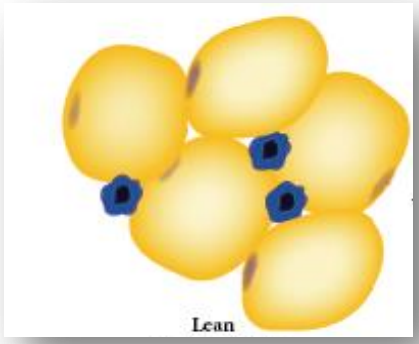
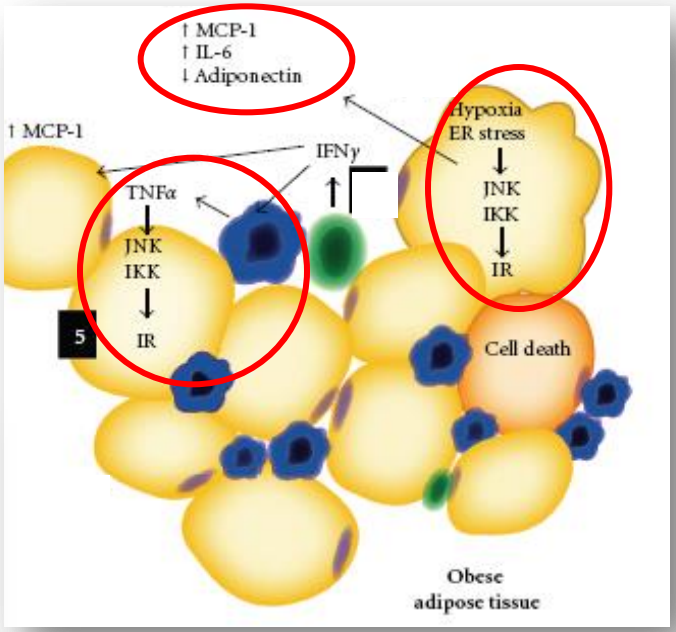
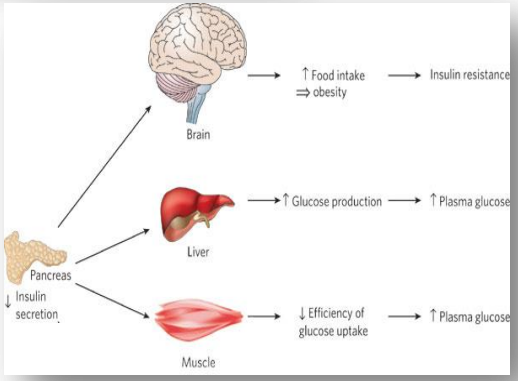
HJ23 Hospital Joan XXIII

inflammation in obesity



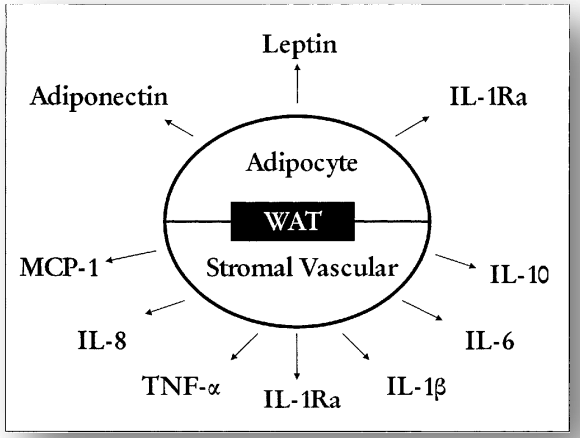






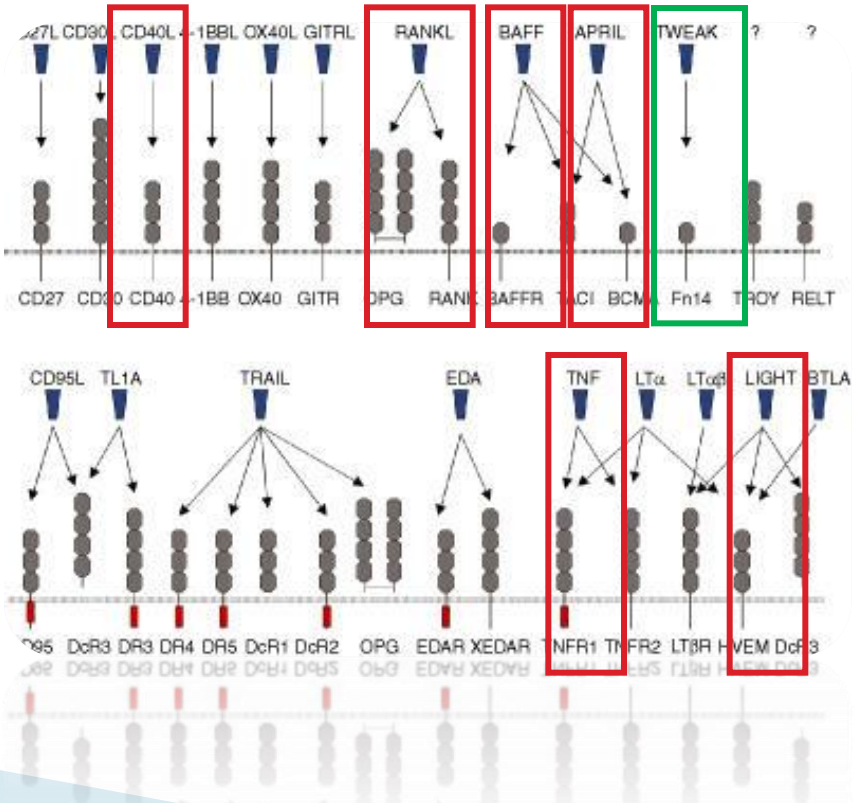
Inflammatory cytokines

Altered cytokines /adipokines/ chemokines in obesity



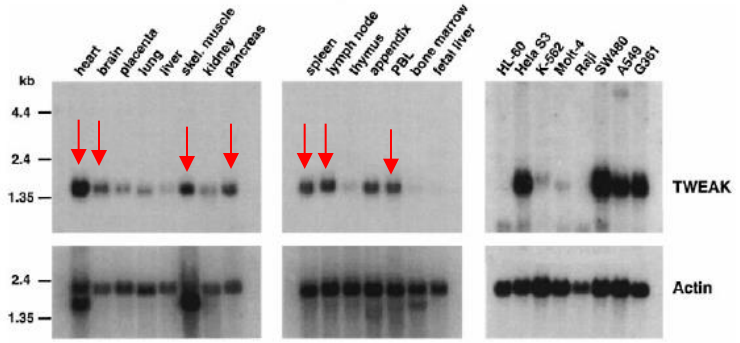
TNF-superfamily

Members of the tumor necrosis factor (TNF) superfamiliy are potent regulators of inflammation and cell survival and consist of 20 ligands that signal through 29 different receptors.



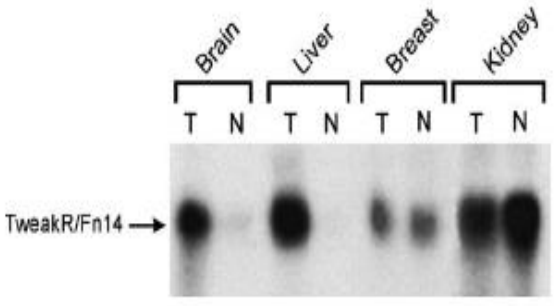
Tweak (Tumor necrosis factor like weak inducer of apoptosis)

- TWEAK is a widely expressed cytokine

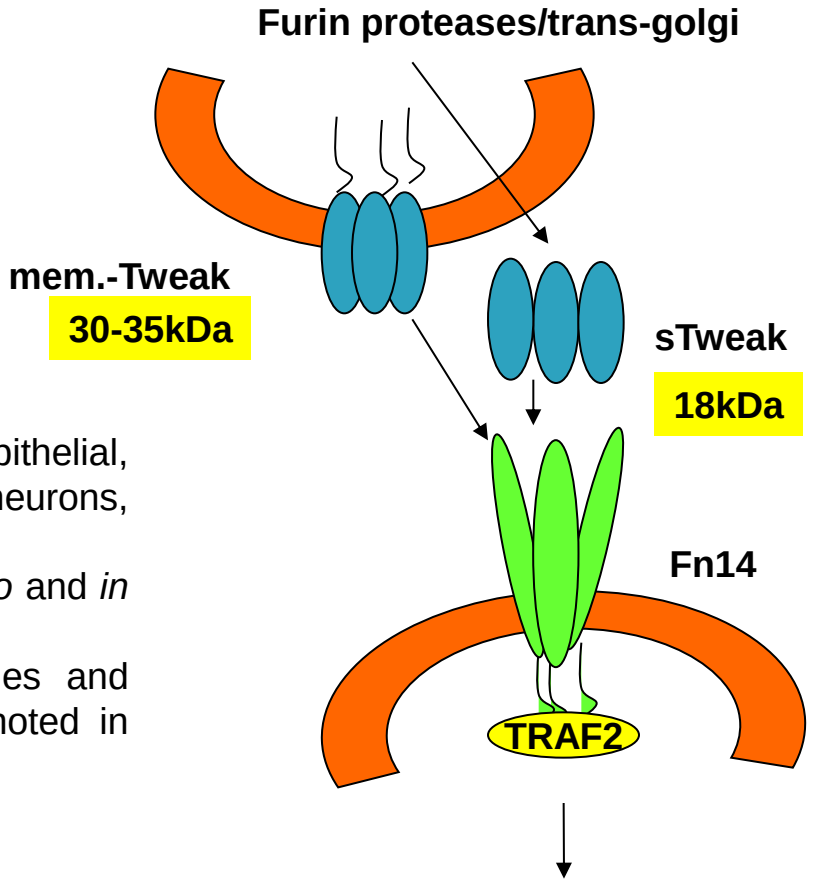


Chicheportiche et al.. *JBC* 19 (1997), 32401–32410,

- Fn14 is expressed by many cell types including epithelial, mesenchymal and endothelial cells, as well as neurons, astrocytes etc..
- *Fn14* gene expression is highly regulated both *in vitro* and *in vivo*.
- Fn14 is expressed at low levels in normal tissues and significantly higher levels of expression have been noted in multiple tumor specimens.



Wiley, J.A. Willis / Cytokine & Growth Factor Reviews 14 (2003), 241–249



Signal transduction/pathway activation
NF-κB can, non-can, MAPK

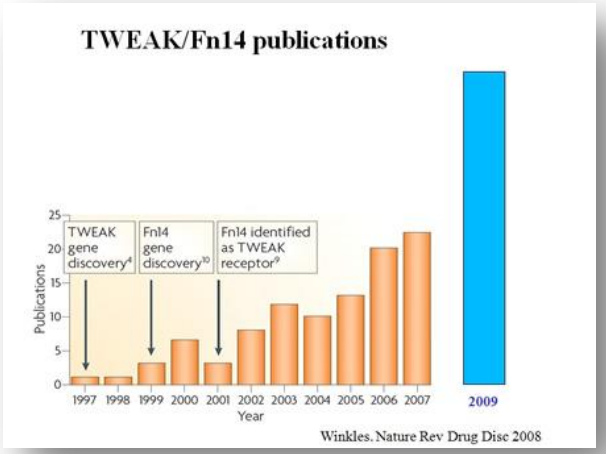
Inflammation, Cell growth, angiogenesis
Cell death, Cell survival etc..

Pathogenic role of TWEAK in human diseases

- TWEAK-dependent Fn14 signaling can contribute to the clinical severity of autoimmune inflammatory diseases: rheumatoid arthritis, SLE (lupus nephritis), multiple sclerosis, atopic dermatitis, autoimmune encephalomyelitis.

Others:

- chronic renal failure: Kidney injury
- Cancer: prostate cancer, renal carcinomas, breast tumors, esophageal adenocarcinoma, endometrial cancer, Glial tumors
- Ulcerative collitis
- Periodontitis
- Cardivascular component (Heart failure, cardiomyopathy, atherosclerosis, abdominal aortic aneurysm, non-dialysis Chronic Kidney Disease, pulmonary artery hypertension and acute myocardial infarction.
- ischemic stroke (cerebral ischemia)



Tweak circulating levels as potential biomarker

❖Autoimmune disease ↑
- rheumatoid arthritis
- SLE
- multiple sclerosis

❖Cardivascular component ↓
- Heart failure
- Cardiomyopathy
- Atherosclerosis
- Abdominal aortic aneurysm
- Non-dialysis chronic kidney disease
- Pulmonary artery hypertension
- Acute myocardial infarction

TWEAK/Fn14 IN OBESITY

1. Selected Cohorts

Clinical and anthropometrical characteristics of the studied cohorts

	Obesity study				Type 2 diabetes study			Severe obesity study		
	Lean BMI<25 n=19	**Overweight 25=<BMI<30 n=28**	**Obese 30=<BMI<40 n=15**	***p***	**Control n=36**	**Type 2 diabetic n=11**	***p***	**Control n=35**	**Severe obesity n=23**	***p***
Age (years)	51.7 ± 16.0	57.1 ± 15.0	57.4 ± 12.8	ns	61.6 ± 10.6	66.1 ± 8.6	ns	44.5±8.3	40±0.4	ns
Gender (n, male/female)	13/6	16/12	9/6	ns	21/15	5/6	ns	23/12	9/14	ns
BMI (kg/m^2)	23.6 (24.2)	27.2 (27.9)	32.1 (33.6)	<0.001	28.6 (31.5)	28.7 (30.4)	ns	26.2±3.6	57.4±7.3	<0.001
Waist circumference (cm)	83.0 (90.0)	97.0 (100.0)	107.0 (117.2)	<0.001	100.0 (107.0)	97.0 (102.0)	ns	90.8±13	146.2±23.5	<0.001
FFA (mM)	0.8 (1.2)	0.7 (0.9)	0.8 (1.0)	ns	0.8 ± 0.3	0.9 ± 0.4	ns	0.9±0.5	0.6±0.4	ns
Cholesterol (mM)	5.2 ± 1.2	4.9 ± 1.0	5.2 ± 0.8	ns	5.1 ± 0.9	4.7 ± 1.2	ns	4.8 (5.6)	5.2 (5.5)	ns
HDL-chol (mM)	1.5 ± 0.5	1.3 ± 0.3	1.4 ± 0.3	ns	1.4 (1.6)	1.2 (1.9)	ns	1.3 (1.5)	1 (1.5)	ns
Triglycerides (mM)	1.0 (1.6)	1.1 (1.5)	1.0 (1.3)	ns	1.0 (1.5)	1.7 (2.3)	<0.001	0.9 (1.5)	1.2 (1.6)	ns
Glucose (mM)	4.8 ± 0.7	5.5 ± 0.5[a]	5.6 ± 0.5[b]	<0.001	5.6 (5.8)	8.3 (10.1)	<0.001	5 (5.7)	5.4 (6.1)	ns
Insulin (µIU/ml)	3.4 (6.7)	4.0 (7.2)	6.6 (16.5)	ns	4.5 (7.7)	10.2 (21.4)	ns	4 (7.6)	23 (51)	<0.001
HOMA-IR	0.75 (1.83)	1.01 (2.09)	1.60 (4.79)	0.028	1.22 (2.10)	3.66 (23.66)	<0.001	1 (2)	5.4 (13)	<0.001
sIL-6 (pg/ml)	1.4 (2.5)	1.0 (2.2)	2.5 (5.2)	0.022	1.4 (2.6)	1.5 (2.4)	ns	1.3 (2.6)	5.5 (8.9)	<0.001

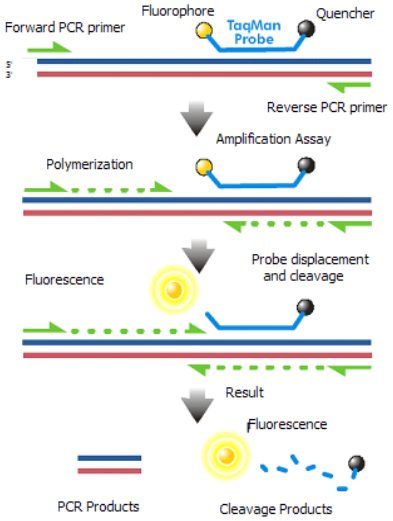
[a] Differences vs lean
[b] Differences vs overweight
ns: not significant
ND: not determined

Chacón *et al*. Cytokine 2006, 33 129-37
Vendrell *et al*. JCEM. 2010; 95:2983-92

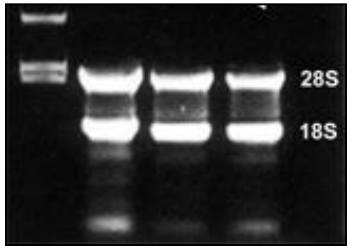
Applied Biosystems Fast Real Time

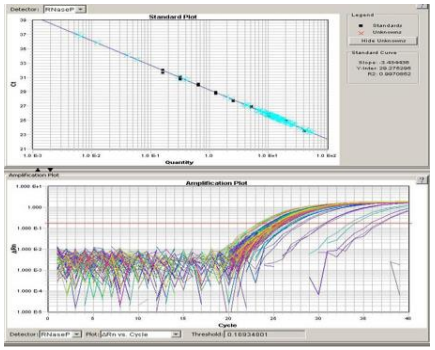
SAT and VAT

500mg



RNA isolation by RNAsy® Mini Kit (Qiagen)





Measured gene expression TWEAK and Fn14

2. TWEAK/Fn14 expression study in SAT-VAT paired samples

Differential expression levels of TWEAK and Fn14 in the studied cohorts (A). Depot specific analysis (B)

A.

	Obesity study				Type 2 diabetes study			Severe obesity study		
	Lean BMI<25 n=19	Overweight 25=<BMI<30 n=28	Obese 30=<BMI<40 n=15	*p*	Control n=36	Type 2 diabetes n=11	*p*	Control n=35	Severe obesity n=23	*p*
Gene expression levels (arbitrary units)										
SAT *TWEAK*	0.94±0.21	1.06±0.22	0.92±0.33	ns	1.01 ±0.3	1.16±0.3	ns	0.98±0.3	1.35±0.43	0.001
VAT *TWEAK*	0.80±0.18	0.90±0.18	0.89±0.38	ns	0.89±0.25	0.89±1.16	ns	0.87±0.2	1.34±0.7	0.004
SAT *Fn14*	0.78±0.46	0.67±0.30	0.93±0.53	ns	0.8±0.4	1.6±1.3	ns	0.64 (1.03)	2.6 (13.3)	<0.001
VAT *Fn14*	1.4 (3.9)	0.8 (1.7)	0.8 (1.4)	ns	0.9 (1.6)	1.4 (1.7)	ns	0.84 (1.7)	5.7 (35.1)	<0.001

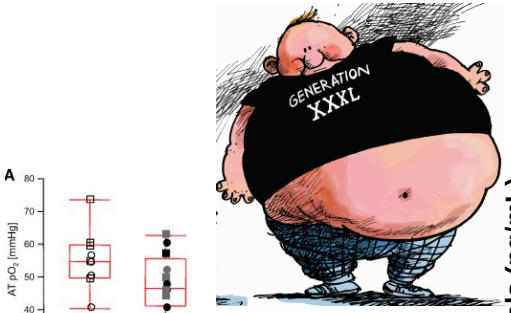
B.

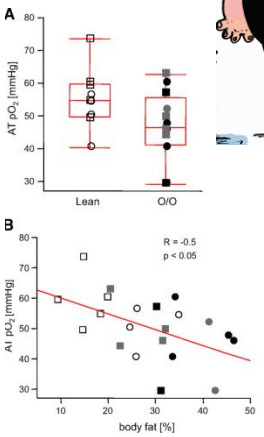
	Lean	Overweight	Obese	Severely obese	Type 2 Diabetes
TWEAK	SAT>VAT p=0.015	SAT>VAT p=0.02	SAT=VAT p=0.578	SAT=VAT p=0.848	SAT>VAT p=0.06
Fn14	SAT<VAT p=0.002	SAT<VAT p=0.024	SAT=VAT p=0.609	SAT<VAT p=0.033	SAT=VAT p=0.477

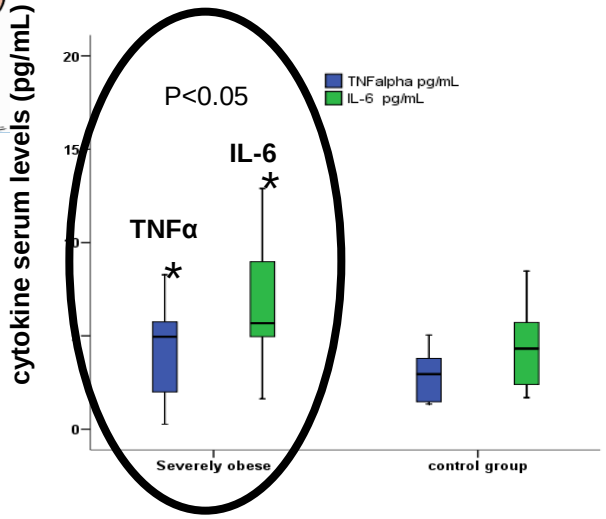
Vendrell *et al*. JCEM. 2010; 95:2983-92

What is inducing the over-expression of Tweak/Fn14 in severely obese subjects?

'hypoxia hypothesis' suggested that the dysregulation of the production of inflammation-related adipokines in obesity, linked to the development of the metabolic syndrome and other obesity-associated disorders, is a specific response to relative hypoxia in clusters of adipocytes that become distant from the vasculature as adipose tissue mass expands **(Trayhurn P & Wood IS Br J Nutr (2008) 92, 347–355)**

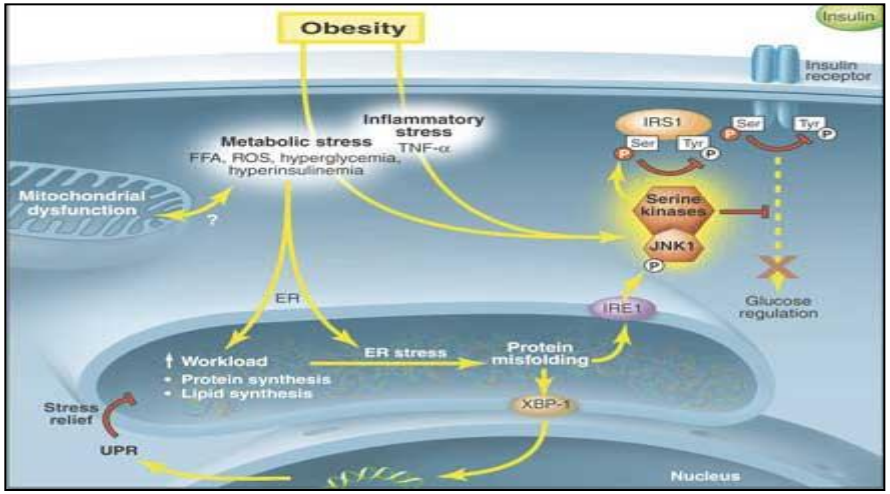






***Pasarica et al. Diabetes* 58:718–725, 2009**

endoplasmic reticulum (ER) stress In the transformation of a healthy functional adipocyte to a hypertrophic, dysfunctional adipocyte, mitochondrial function is lost, and multiple stress-signaling cascades are initiated from the ER **(Hotamisligil GS. Cell. (2010) 140,900-17).**



Selected candidate genes

	Gene	Function
Hypoxia regulated	*VISFATIN*	Pro-inflammatory and potentially insulin-mimetic adipokine. HIF-1 hypoxia regulated gene.
	FIAF	Lipid metabolism. Highly up-regulated during hypoxia
	HIF-1α	Key transcription factor in hypoxia
	HYOU1	Oxygen-regulated protein (150 kDa) has been reported to be linked to type 2 diabetes and obesity.
	GLUT1	Glucose transporter 1 gene in human adipocytes. It has been found to be increased under hypoxia.
	VEGF	Mediator of inflammation and an angiogenic factor and it is hypoxia regulated.
ER stress	*GRP*78	Protein strongly related with glucose metabolism and ER stress.
	XBP-1	Transcription factor that modulates the ER stress response.

Have our studied subjects altered the expression of hypoxia and ER regulated genes?

Gene expression analysis of hypoxia and ER genes in SAT and VAT samples in obesity (arbitrary units)

	Obesity study						Severe obesity study			
	Lean n=19		Overweight n=28		Obese n=15		Overweight control group n=35		Severe Obese n=23	
Depot	**SAT**	**VAT**	**SAT**	**VAT**	**SAT**	**VAT**	**SAT**	**VAT**	**SAT**	**VAT**
Adipokynes										
VISFATIN	1.2 (2.7)	0.8 (1.3)	0.9 (1.4)	0.8 (1.1)	1 (1.9)	0.8 (1.1)	1.2 (2.5)	0.9 (1.3)	1.6 (7.2)*	6.6 (27.8)*#
Hypoxia										
FIAF	2±0.9#	1.1±0.6	1.8±0.6#	0.9±0.6	1.5±0.5	1.3±0.9	1.7±0.72#	1.14±0.7	2.8±1.54*	1.8±1.34*#
HIF-1α	0.9 (19	1 (1.2)	0.9 (1)	0.9 (1)	1 (1.3)	1 (1.3)	0.9 (1.1)	1 (1.3)#	1.8 (4.5)*	3.2 (5.3)*#
HYOU1	0.8±0.2	1±0.2#	0.9±0.1	0.9±0.2	0.9±0.2	0.9±0.1	0.8 (1)	0.9 (1)	1.4 (1.6)*	1.4 (1.7)*
GLUT1	1 (1.8)	1.6 (2.3)#	0.9 (1.2)	1.1 (1.6)#	0.9 (1.2)	1.3 (1.7)	1 (1.1)	1.1 (1.7)#	2.8 (4.8)	5.1 (9.4)*#
VEGF	1.4 (2)*	1.5 (1.7)*	1 (1.3)	1 (1.5)	1 (1.5)	1 (1.2)	1.3 (1.8)	1.2 (1.5)	2.5 (4.3)*	6.5 (9)*#
ER stress										
GRP78	0.9±0.2	1.1±0.2#	0.8±0.1	1±0.3#	0.9±0.3	1±0.2	0.9±0.2	1±0.2#	1.4±0.6*	1.8±1*
XBP-1	0.8±0.2	1±0.3#	0.8±0.1	1±0.2#	0.8±0.2	0.8±0.1	0.8±0.2	1±0.3#	0.9±0.5*	1.6±0.9*#

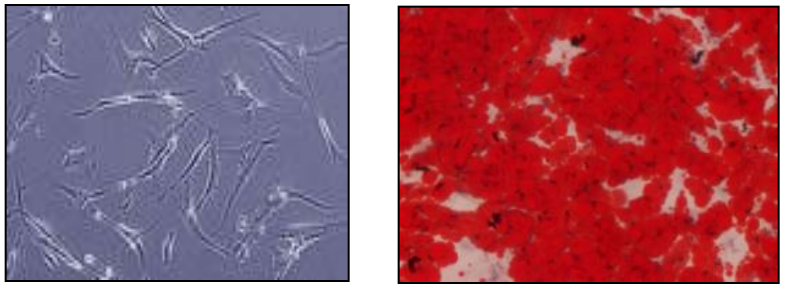
*differences when comparing same depot between obesity group study $p<0.05$

#differences when comparing depots within same obesity group $p<0.05$

Vendrell *et al*. JCEM. 2010; 95:2983-92

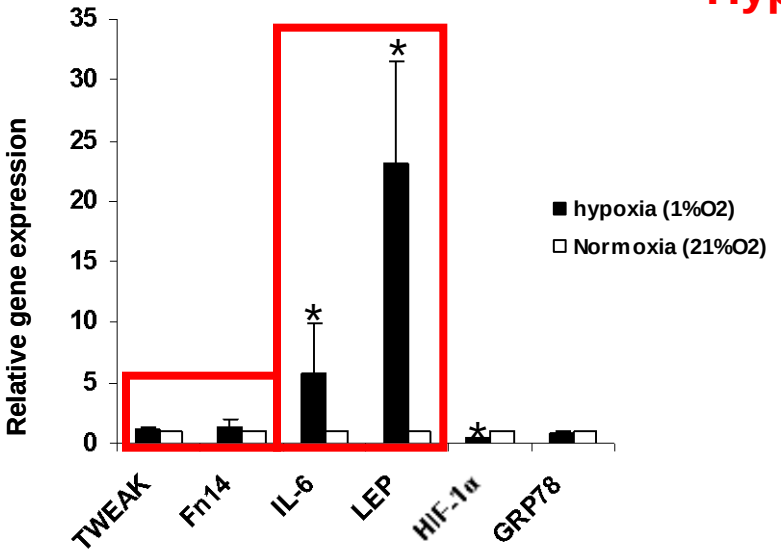
Study of effect of hypoxia, inflammation and ER stress on *TWEAK* and *Fn14* gene expression in human SGBS adipocytes

Simpson-Golabi-Behmel syndrome

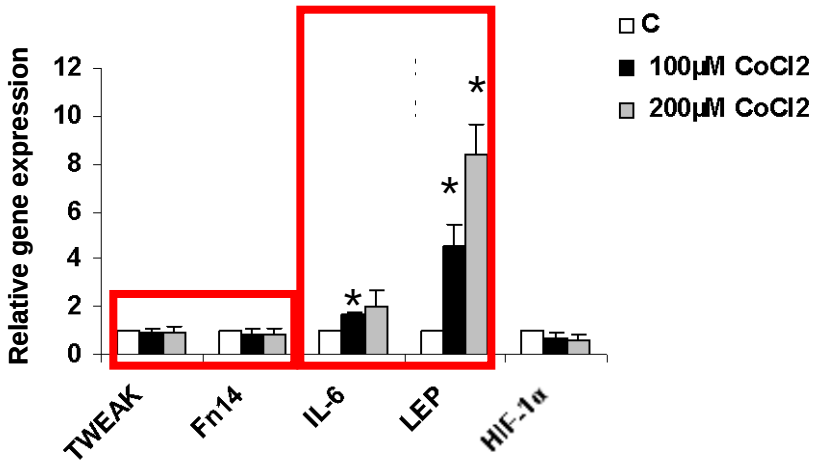


d0 d14

Hypoxia



1% O2



$CoCl_2$

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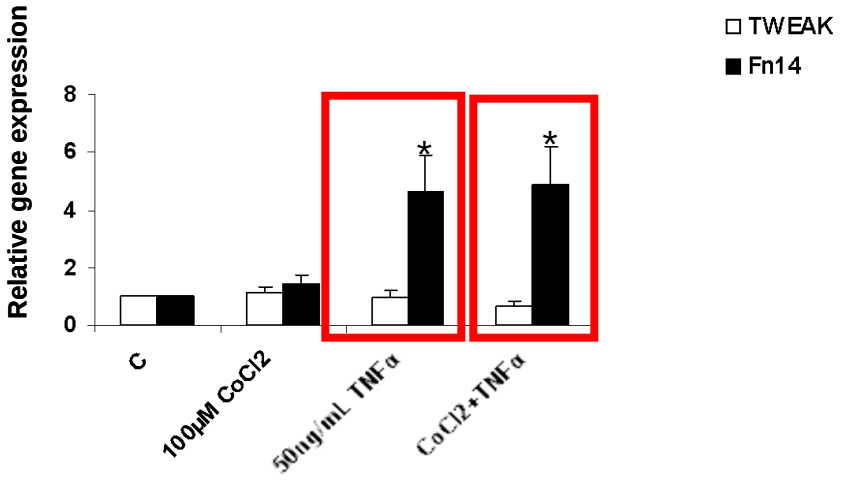
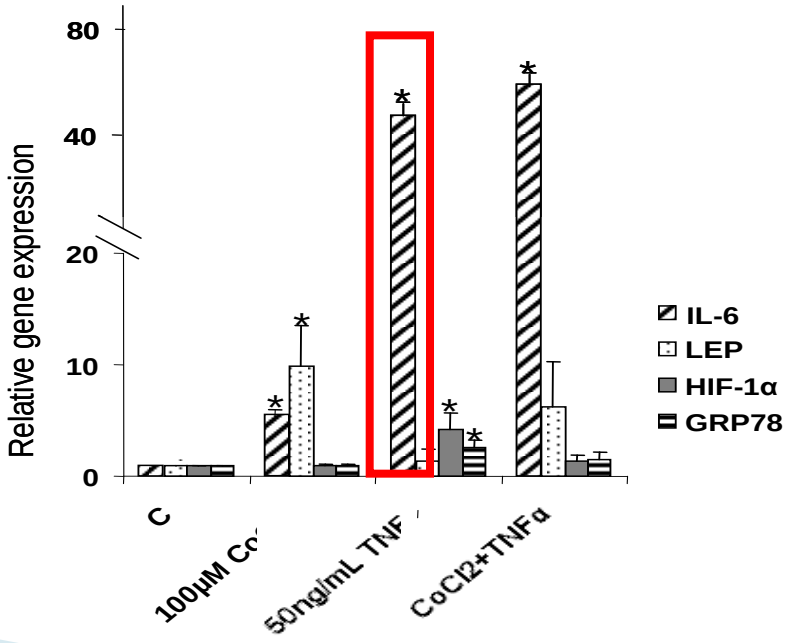
Infammatory stimulus (TNFα) **Infammation + Hypoxia**

Infammatory stimulus





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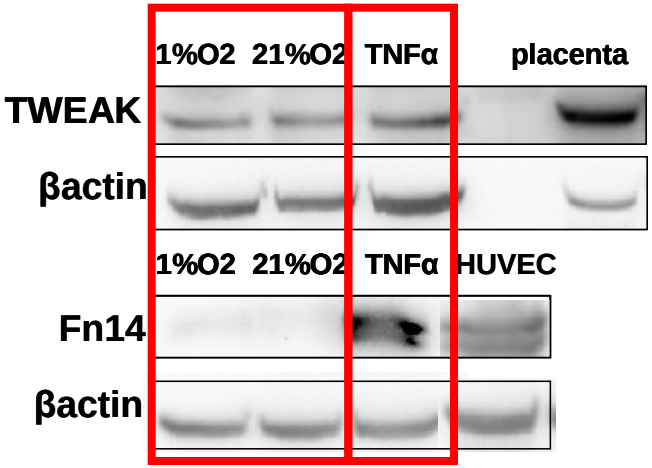
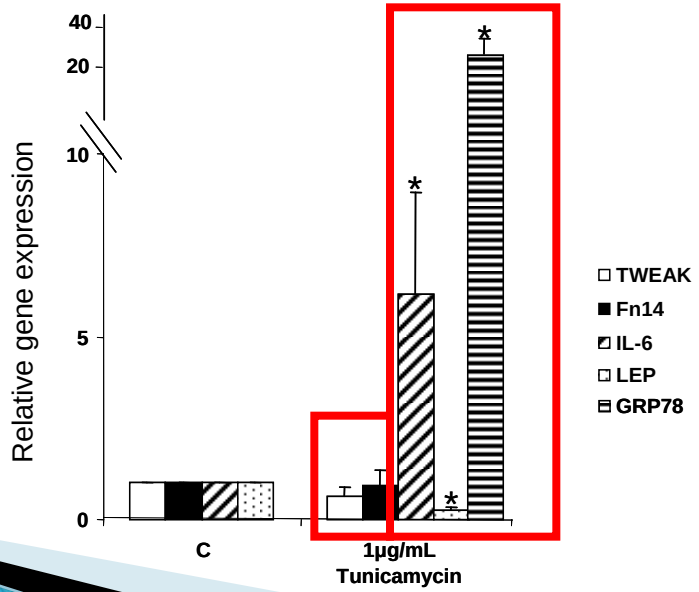
Hypoxia and inflammation on TWEAK and Fn14 protein expression in human SGBS adipocytes



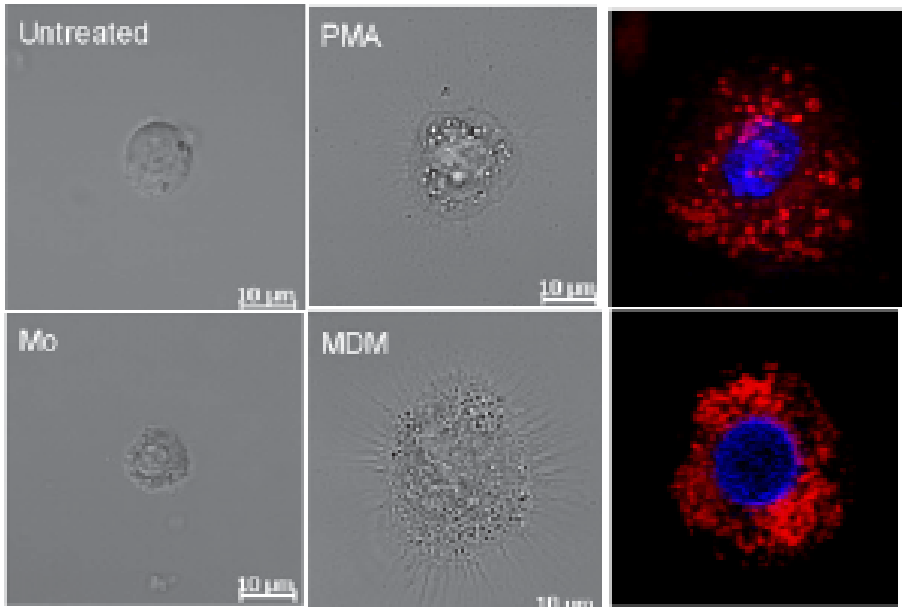
Effect of ER stress on *TWEAK* and *Fn14* gene expression in human SGBS adipocytes



Study the of hypoxia, inflammation and ER stress on *TWEAK* and *Fn14* gene expression in THP-1 human macrophages

THP-1

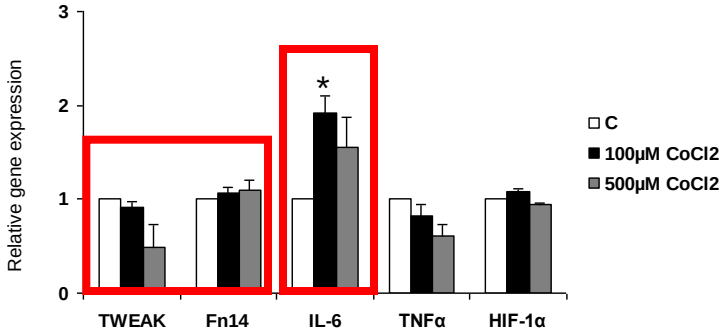
Human Monocytes



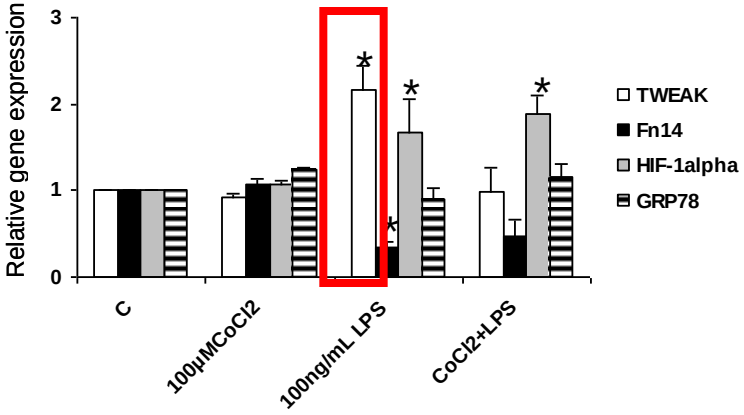
- Macrophage differentiation is associated with a reduction in the nucleocytoplasmic ratio due to an increase in cytoplasmic volume
- Increasing numbers of lysosomes and mitochondria with macrophage differentiation

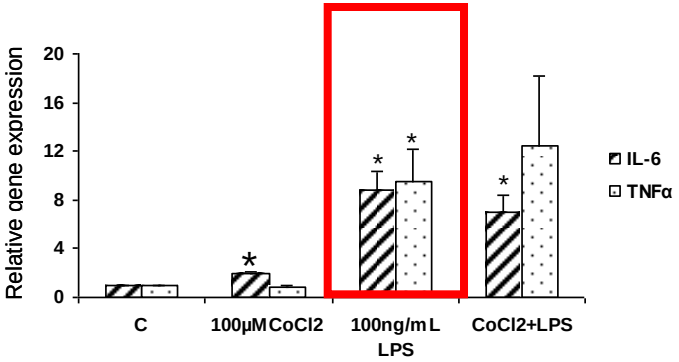
Study the of hypoxia, inflammation and ER stress on *TWEAK* and *Fn14* gene expression in THP-1 human macrophages

Hypoxia

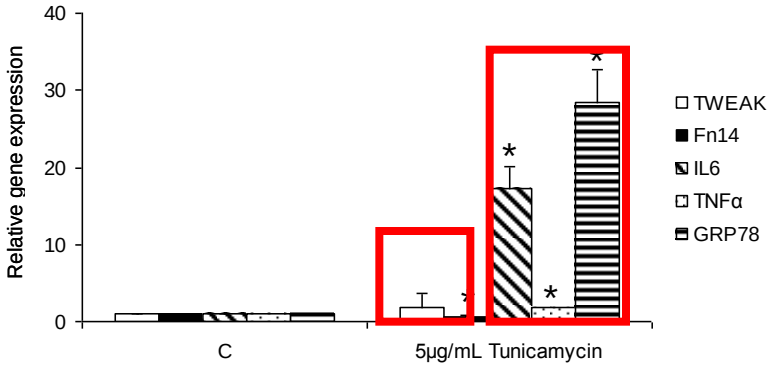


Inflammation

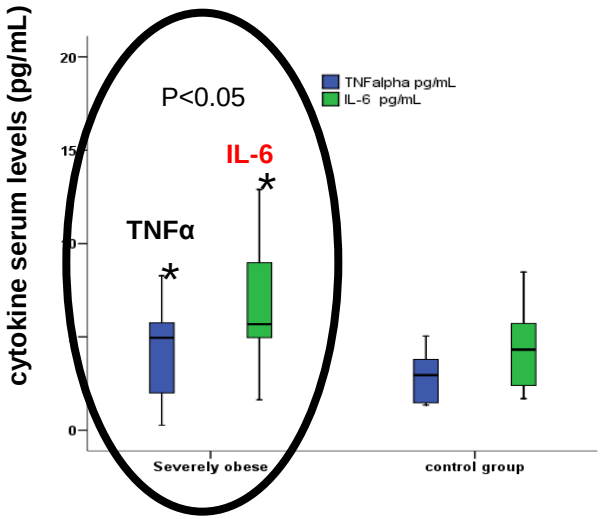




ER stress



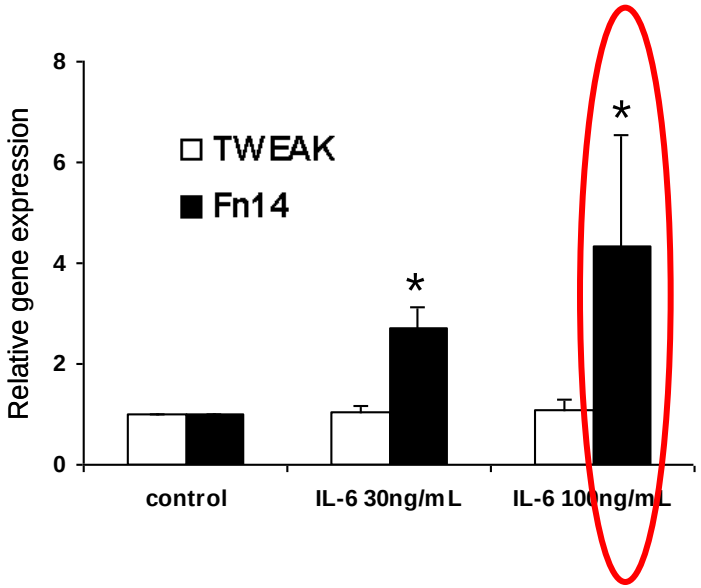
Vendrell *et al*. JCEM. 2010; 95:2983-92



- **Adipose Tissue of severely obese subjects have altered expression levels of Hypoxia and ER stress genes**
- **Hypoxia, ER and inflammatory stimuli strongly increases IL-6 levels in adipocytes and macrophages**
- **IL-6 is a potent inflammatory cytokine**

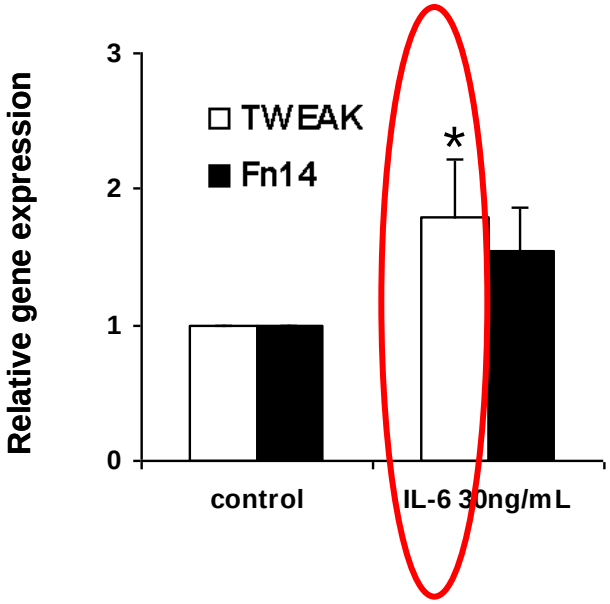
Is IL-6 altering *TWEAK* and *Fn14* expression levels?

Human SGBS adipocytes



Fn14

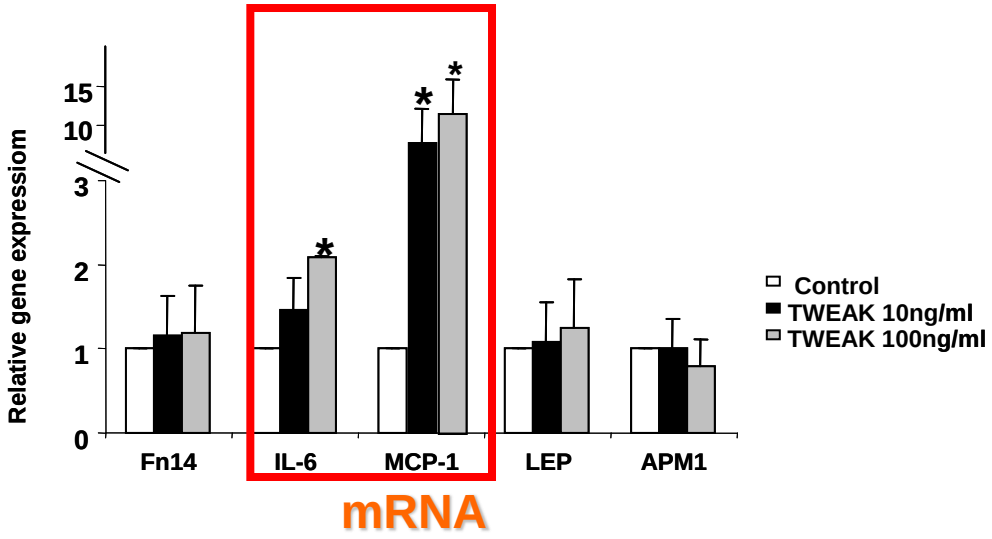
Human THP-1 macrophages

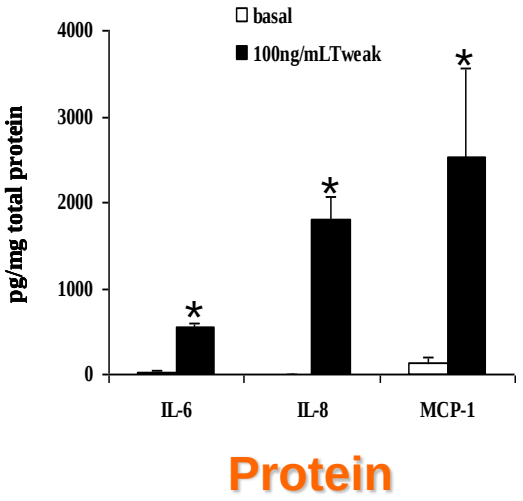


TWEAK

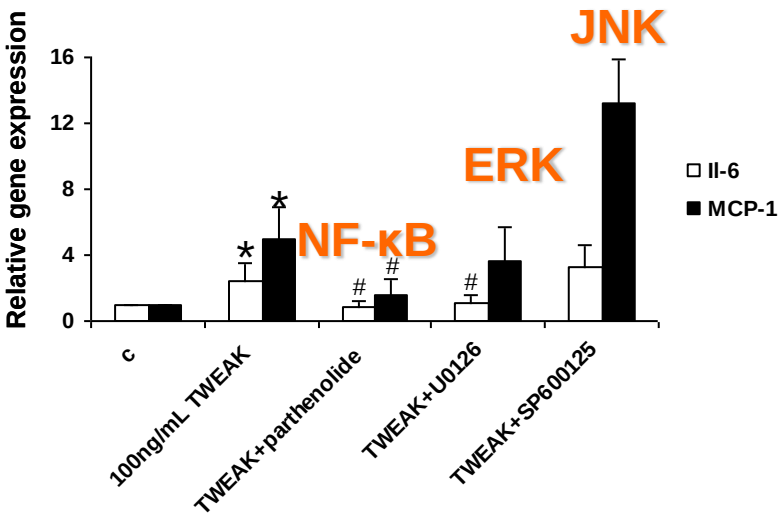
Vendrell *et al*. JCEM. 2010; 95:2983-92

Is Tweak *a* Pro-inflammatory cytokine on human adipocytes





How is Tweak signaling in human adipocytes?



Vendrell *et al*. JCEM. 2010; 95:2983-92

Summary

✓*TWEAK/Fn14* is expressed in SAT and VAT tissue

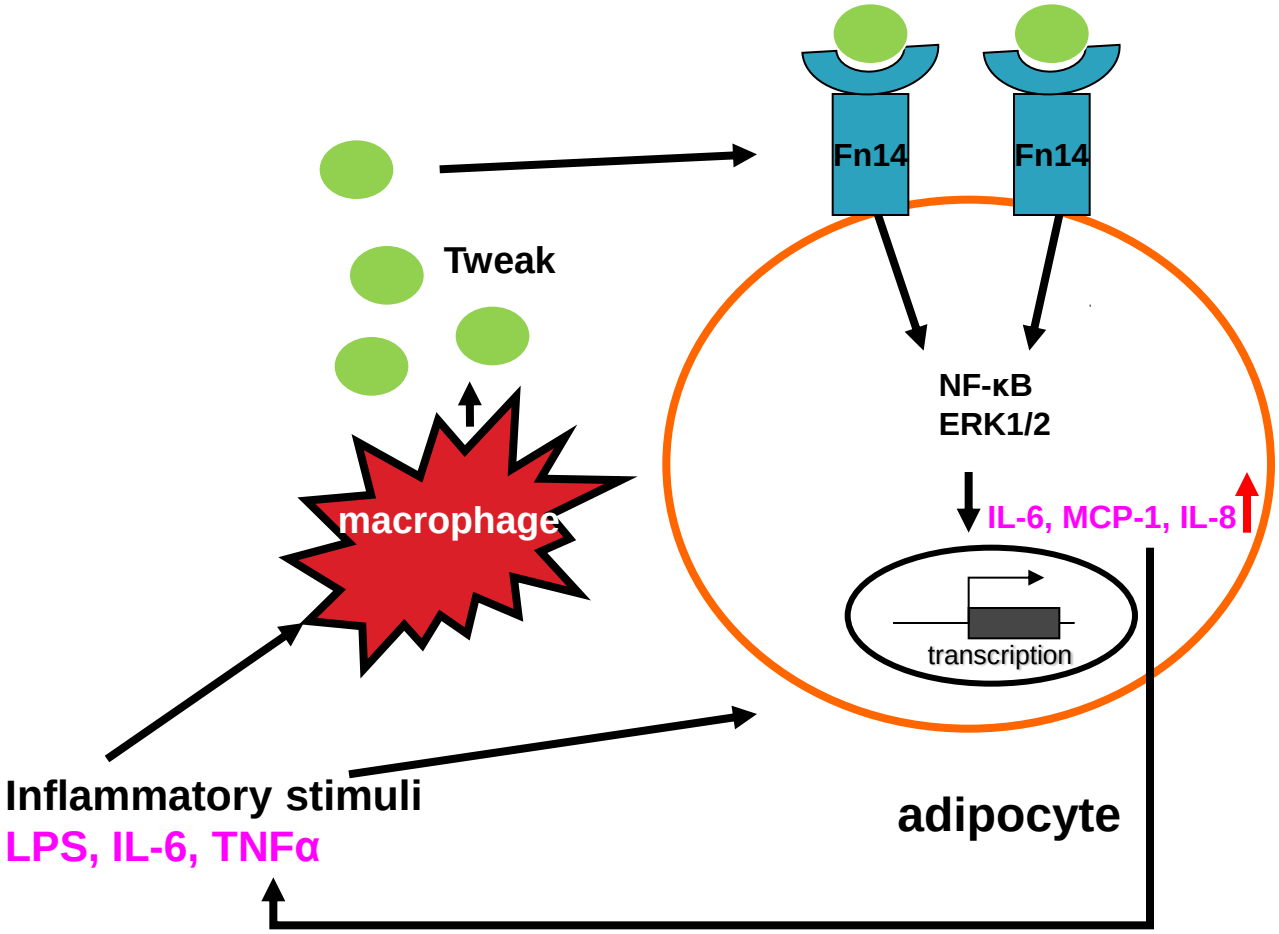
✓TWEAK acts as a pro-inflammatory cytokine in human adipocyte cells

✓ We report an over expression of *TWEAK*/*Fn14*, hypoxia and ER characteristic genes in both SAT and VAT adipose tissue depots of severely obese patients

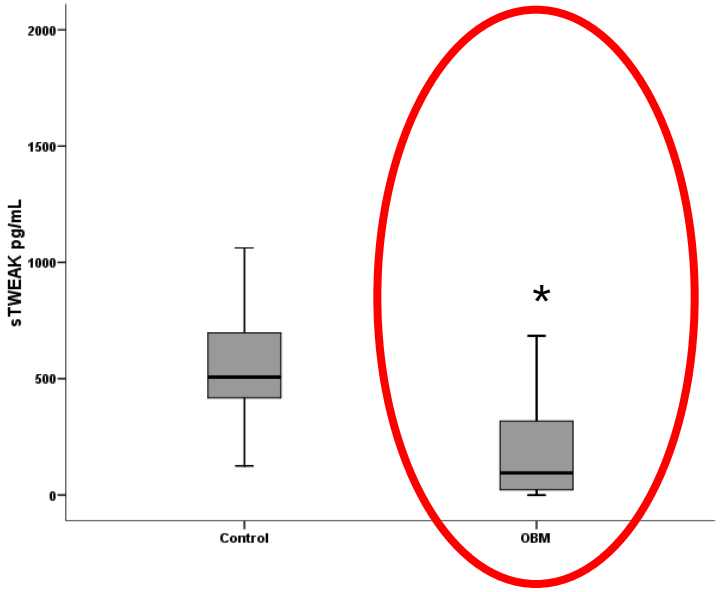
✓However both *TWEAK* and *Fn14* genes were neither hypoxia nor ER stress sensitive but inflammation regulated

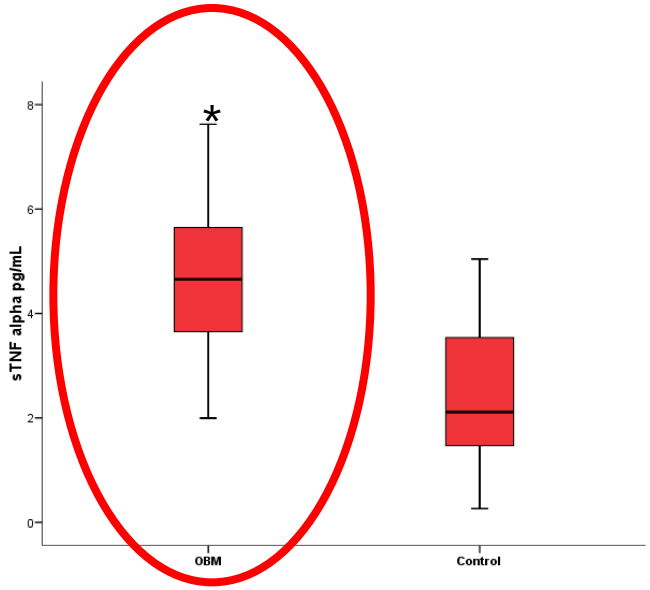
✓The increment of *TWEAK* and *Fn14* expression in severe obesity may be due to the inflammatory environment, in particular that caused by the presence of IL-6, a cytokine that could be playing a key role in *TWEAK/Fn14* regulation

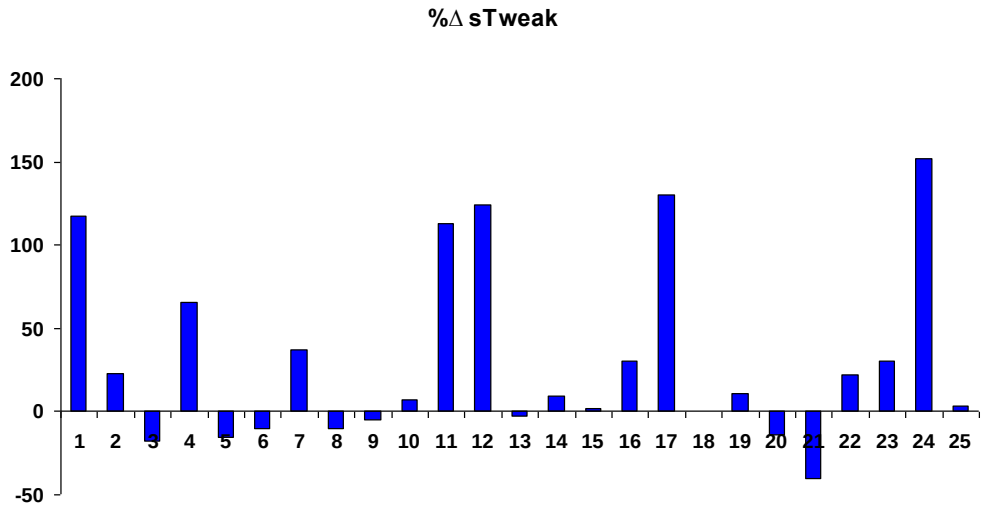
MODEL OF ACTION OF TWEAK-Fn14 IN ADIPOSE TISSUE



Circulating Tweak in severely obese subjects



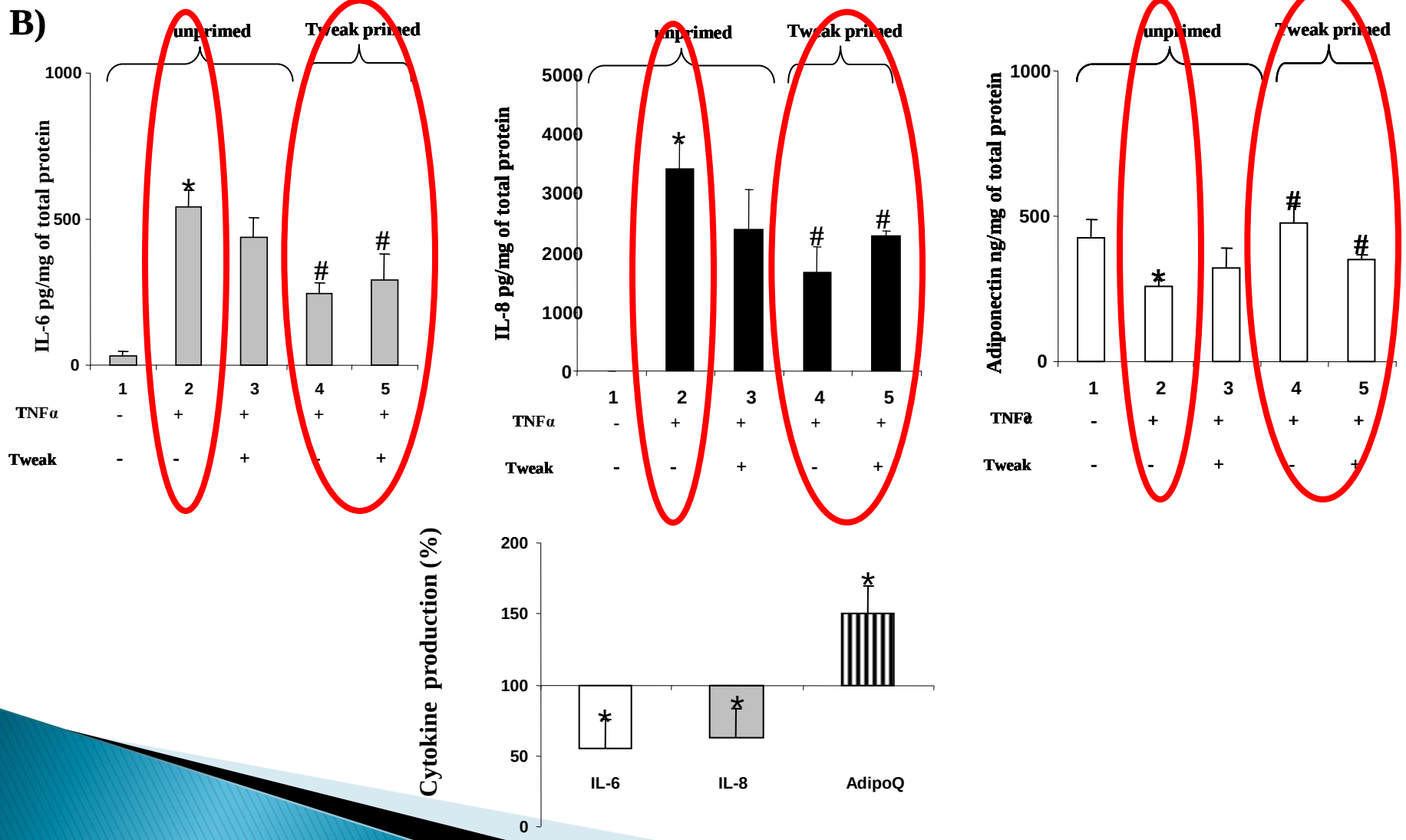




Could fluctuations in circulating Tweak have an impact on TNFα-induced inflammatory response?

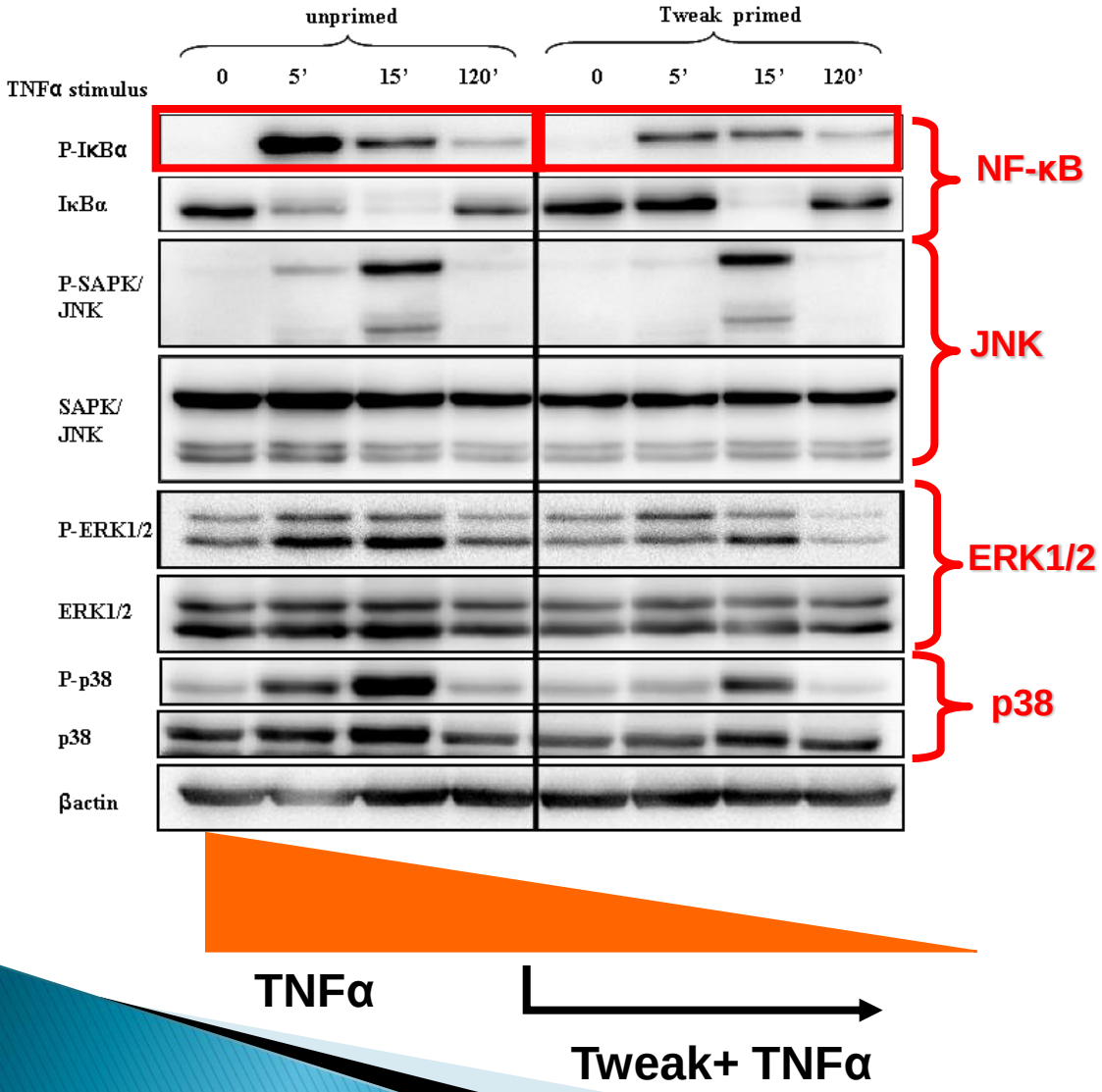
How the adipocyte responds in an environment where Tweak and TNFα are present?

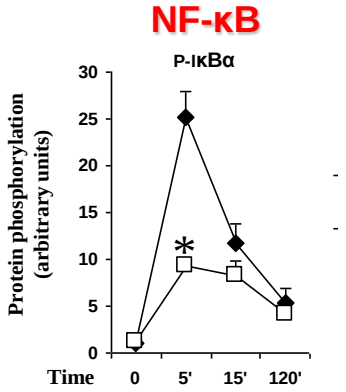
Effect of Tweak in TNFα-induced cytokine secretion

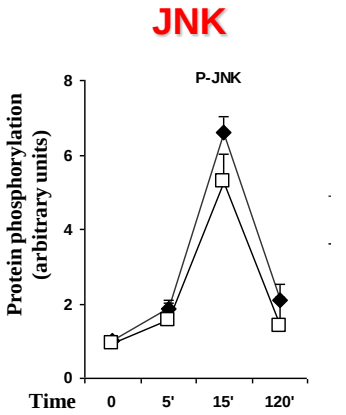


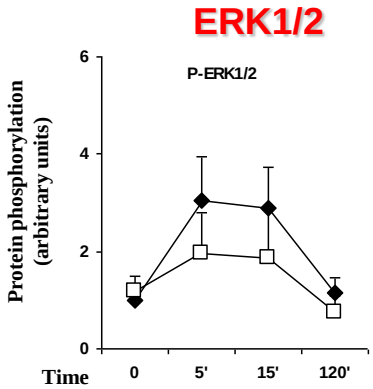
Regulation of TNFα signaling pathways after Tweak-priming

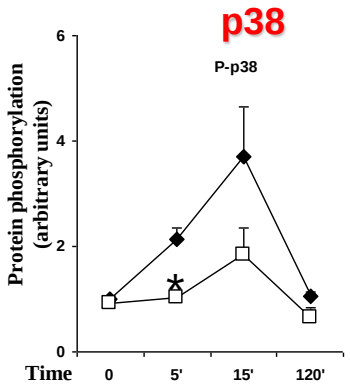
Mature human SGBS adipocytes



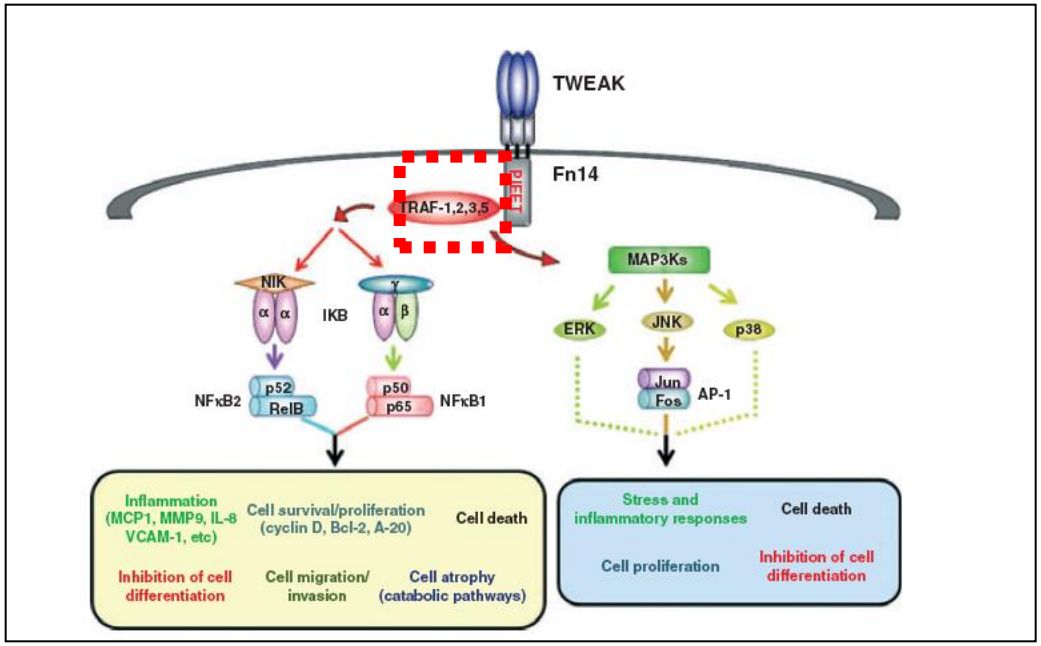




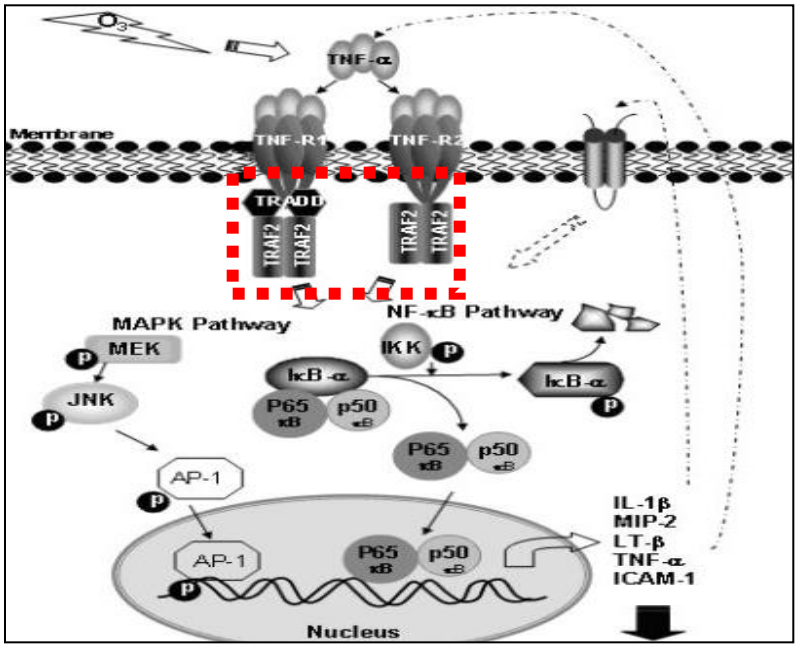




Tweak signaling

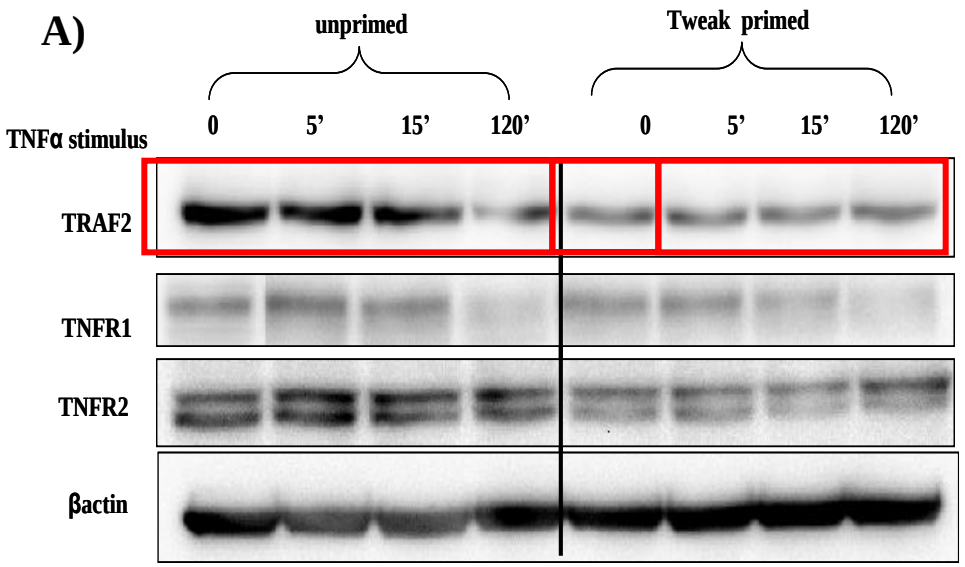


TNFα signaling



Adipocyte Tweak priming reduced TRAF2 protein levels

Mature human SGBS adipocytes



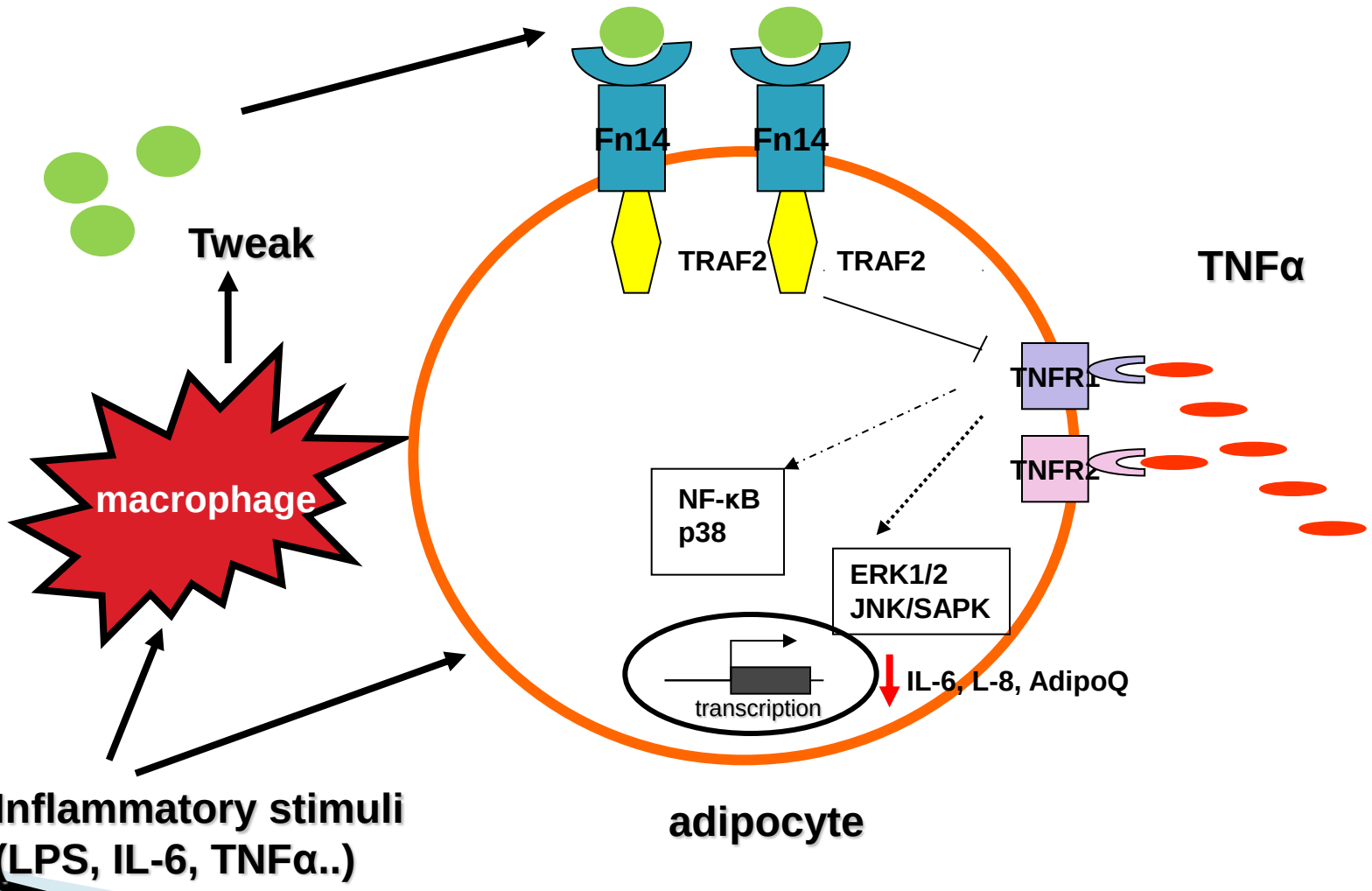
Conclusion

✓Tweak modulates IL-6, IL-8 and Adiponectin production induced by TNFα in adipocytes

✓ Tweak modulates TNFα signalling in part by TRAF2 cellular protein levels

✓Our observation of lower Tweak circulating levels in severely obese subjects could indicate that these subjects are prone to having a more potent inflammatory TNFα outcome

Hypothesis for the mechanism of interaction between Tweak and TNFα



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