

# Early molecular events of adipose tissue development during overfeeding and weight gain in humans

**Hubert VIDAL**

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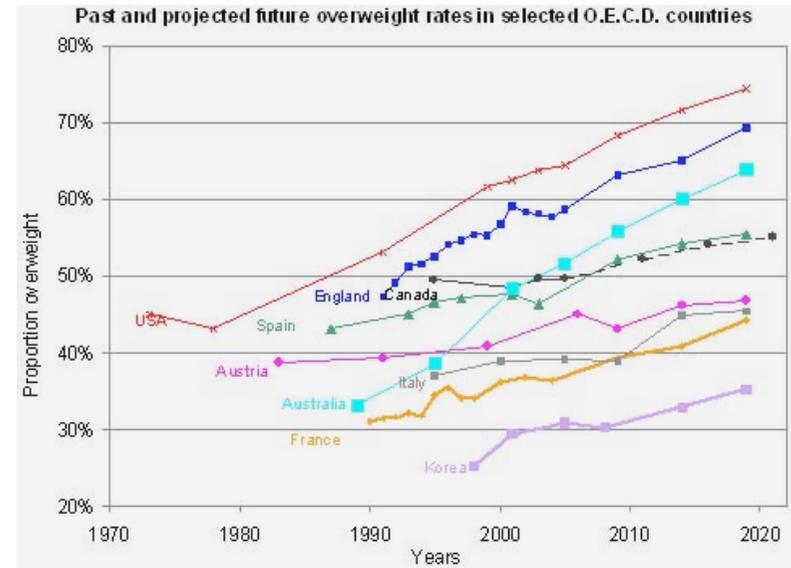
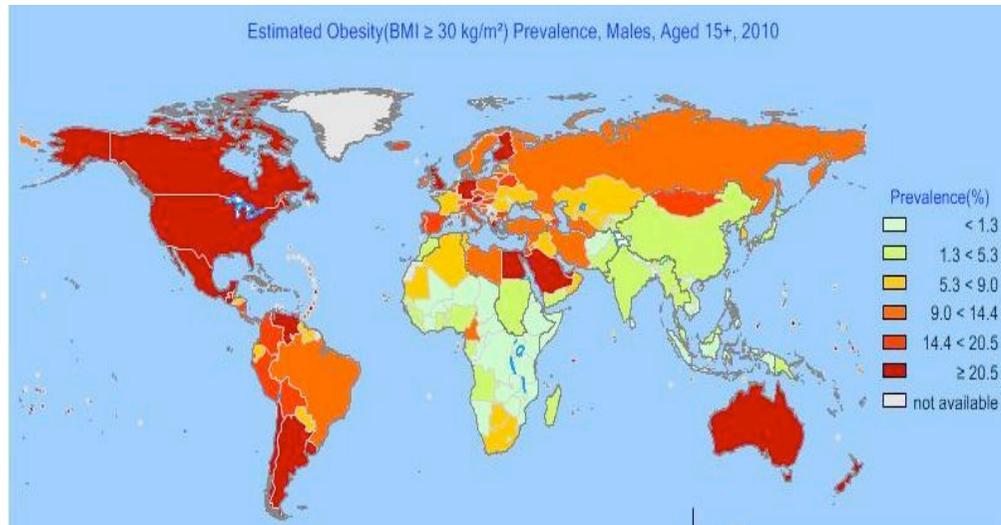
***and***

***Rhône-Alpes Human Nutrition Research Centre (CRNH-RA)***

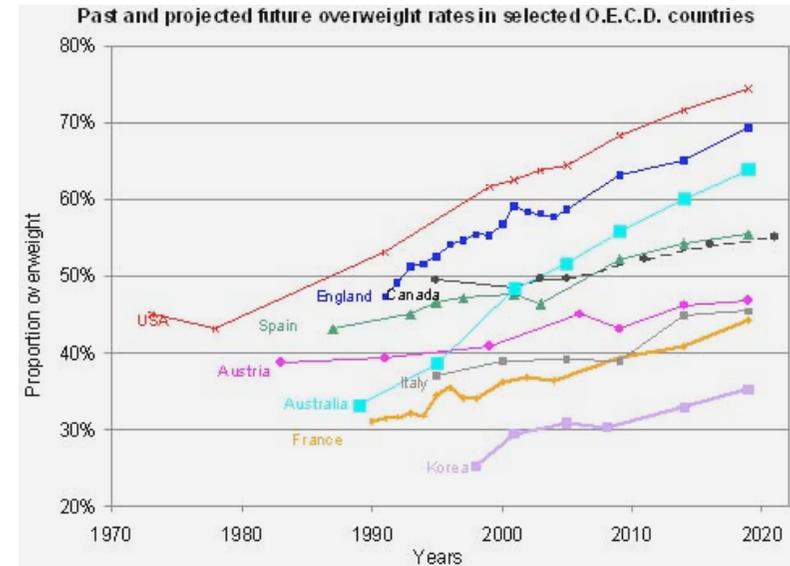
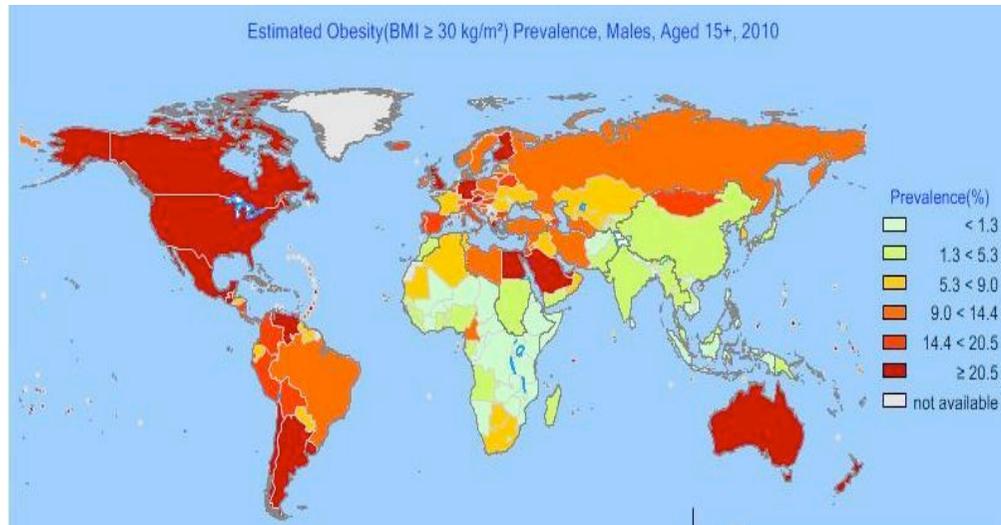
***Lyon- France***

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# OBESITY : a worldwide epidemic



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## Obesity-related complications:

Type 2 diabetes

Cardio-vascular diseases

Cancers

Sleep apnea

Reduction of life expectancy

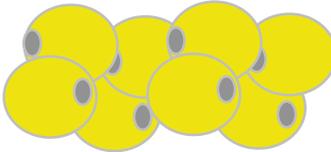
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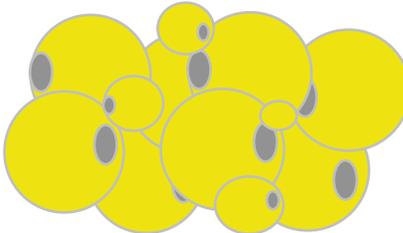
**Positive Energy Balance**

Storage of excess energy in adipose tissue (subcutaneous)



**Hypertrophy**  
Filling of existing adipocytes  
Fat cell size increase

**Hyperplasia**  
Preadipocyte recruitment  
Adipocyte differentiation  
Tissue remodeling



**Weight gain**  
**Increased fat mass**  
**Obesity**



Caloric Intake

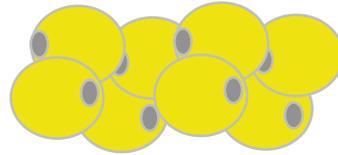
and/or



Energy Expenditure

**Positive Energy Balance**

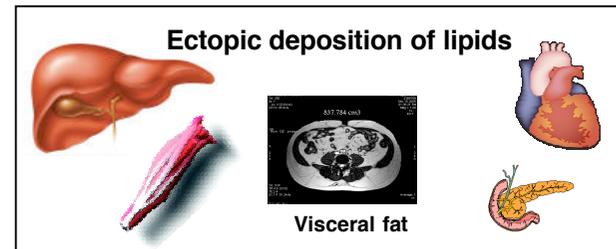
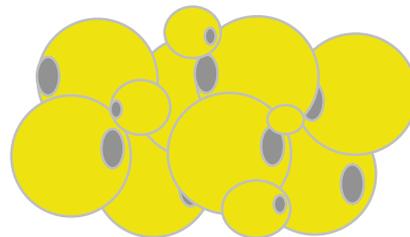
Storage of excess energy in adipose tissue (subcutaneous)



*“unlimited” storage capacity*

*limited expandability*

Low incidence of metabolic complications

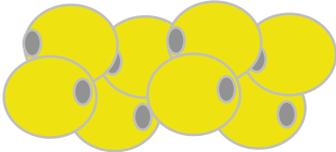


**Complications**  
(T2D, CVD, MS, etc...)



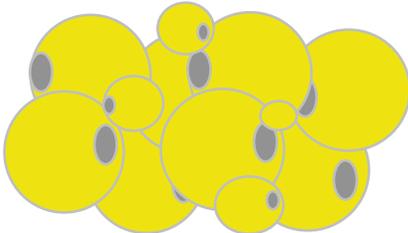
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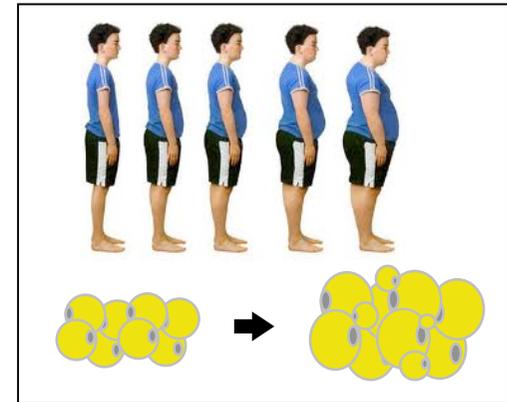
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**Weight gain**  
**Increased fat mass**  
**Obesity**

**How to study the development of adipose tissue during normal life in humans, using an experimentally feasible protocol and under ethical conditions?**





# Lipid overfeeding protocol

- **Objective** : to characterize the mechanisms of subcutaneous development during the early phase of weight gain induced by an overfeeding period with a lipid enriched-diet providing about 30% (760 kcal/d) of daily energy excess
- **Duration** : 8 weeks (56 days)
- **Subjects** : 44 volunteers (healthy men,  $33 \pm 1$  years,  $BMI = 25 \pm 1$ )

**Asked to maintain their normal lifestyle and feeding behaviour and to add each day:**

20 g (150 kcal) of butter



100 g (380 kcal) of cheese



40 g (230 kcal) of almonds



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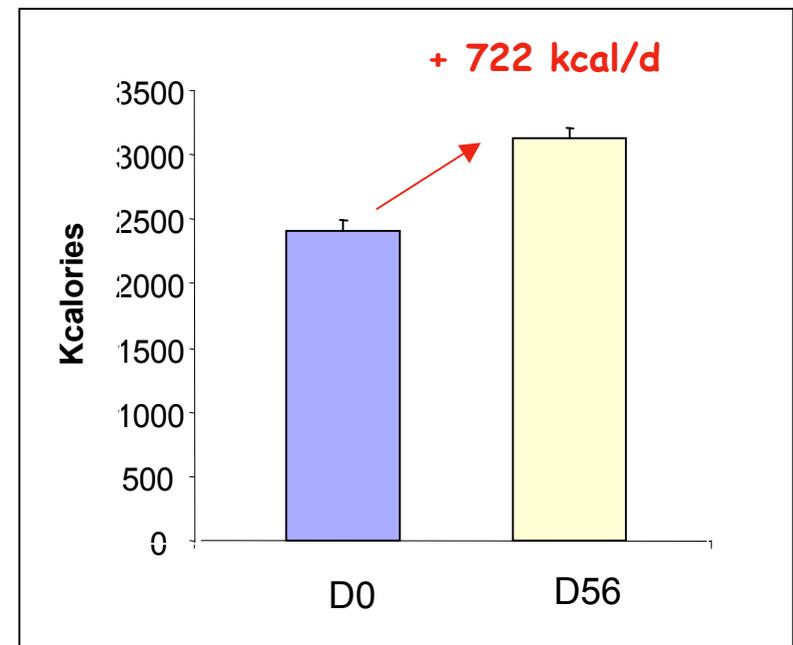
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## Anthropometric and metabolic parameters

	D0	D14	D56
n	44	44	44
Age (years)	33 ± 1	-	-

### ***Anthropometric parameters***

Body weight (kg)	79 .1 ± 1 .8	79 .9 ± 1 .8 ***	81 .6 ± 1 .8 ***
Weight gain (kg)	-	0 .76 ± 0 .14	2 .51 ± 0 .21
Waist circumference (cm)	89 .3 ± 1 .5	-	92 .4 ± 1 .5 ***
Fat mass (%)	19 .6 ± 0 .8	-	20 .3 ± 0 .8 ***

### ***Metabolic parameters***

Fasting glucose (mM)	5 .11 ± 0 .06	5 .10 ± 0 .09	5 .21 ± 0 .08
Fasting insulin (mU/L)	10 .1 ± 0 .6	11 .6 ± 0 .7 ***	10 .5 ± 0 .6
HOMA	2 .29 ± 0 .16	2 .63 ± 0 .17 **	2 .44 ± 0 .15 *

\*: p<0.05

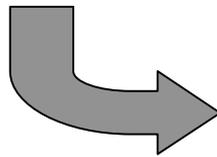
\*\* : p<0.01

\*\*\*: p<0.001

## Transcriptomic analysis in subcutaneous adipose tissue



D0  
D14  
D56



HG U133 Plus 2.0 arrays  
(47,401 transcripts)

**N = 13 subjects**

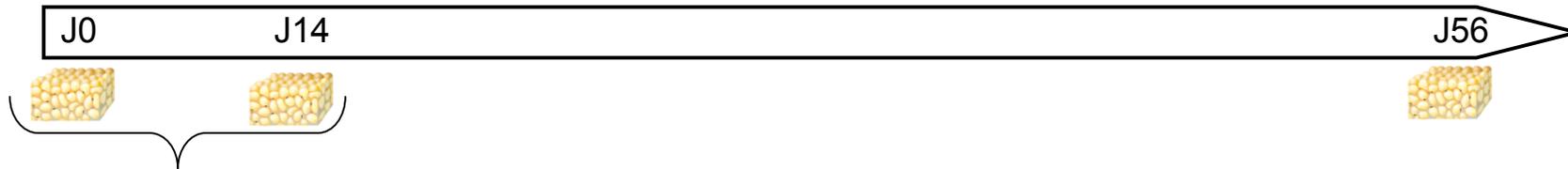
**Statistical analysis using Limma test**

**Probes with p-value < 0.05 and fold change > |1.25| at D14 and D56 were considered as differentially expressed during overfeeding**

**Biological functions and pathways identified using DAVID**

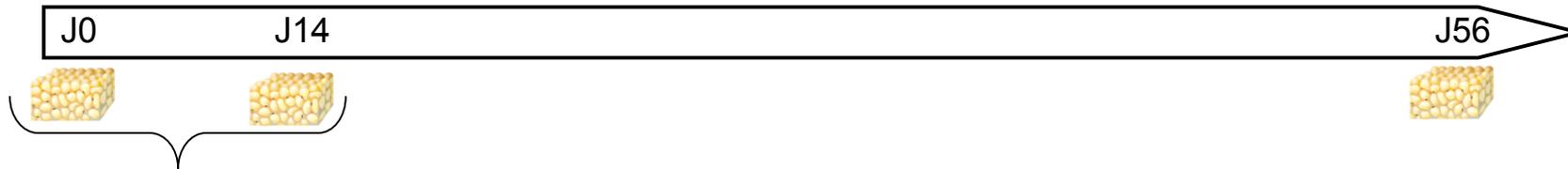
**(Database for Annotations, Visualization and Integrated Discovery)**

## Changes in gene expression at D14



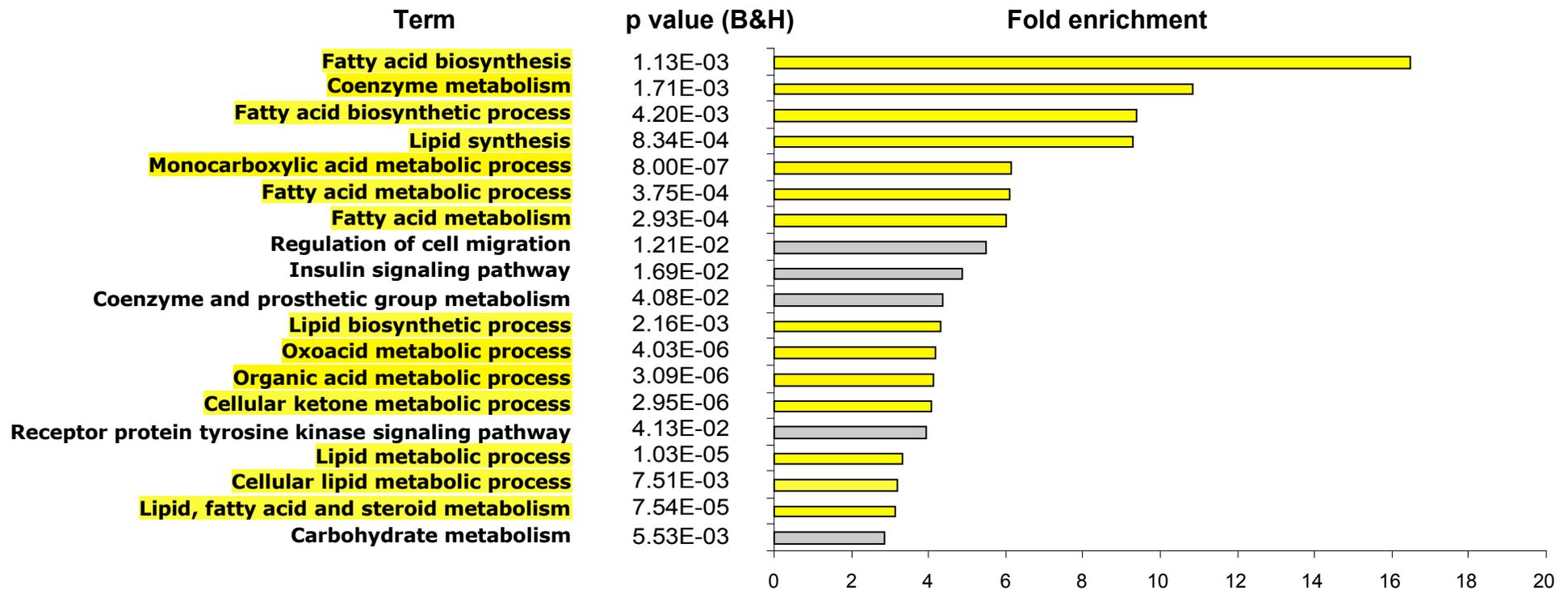
➤ **180 regulated genes between D0 and D14** (p-value<0.05/ FC>1.25)

## Changes in gene expression at D14

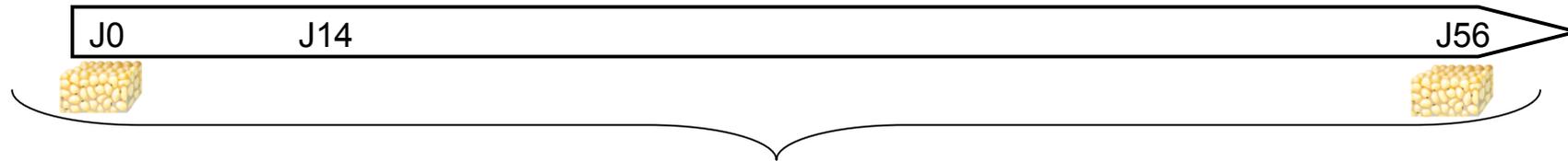


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### Pathway enrichment (DAVID)

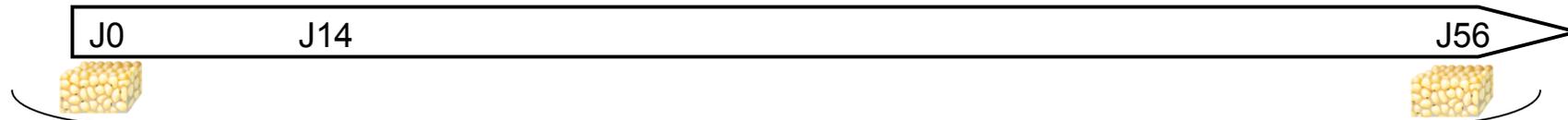


## Changes in gene expression at D56



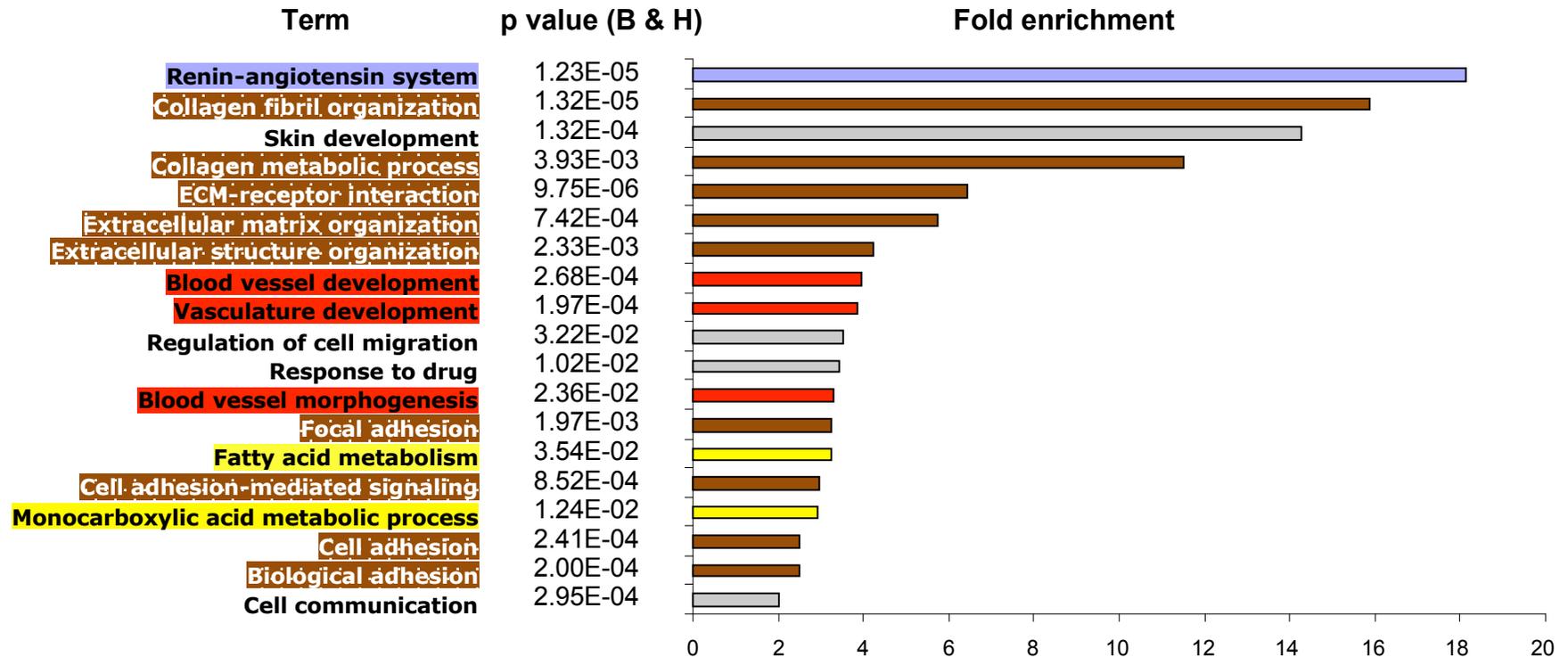
➤ **486 regulated genes between D0 and D56** (p-value<0.05/ FC>1.25).

## Changes in gene expression at D56

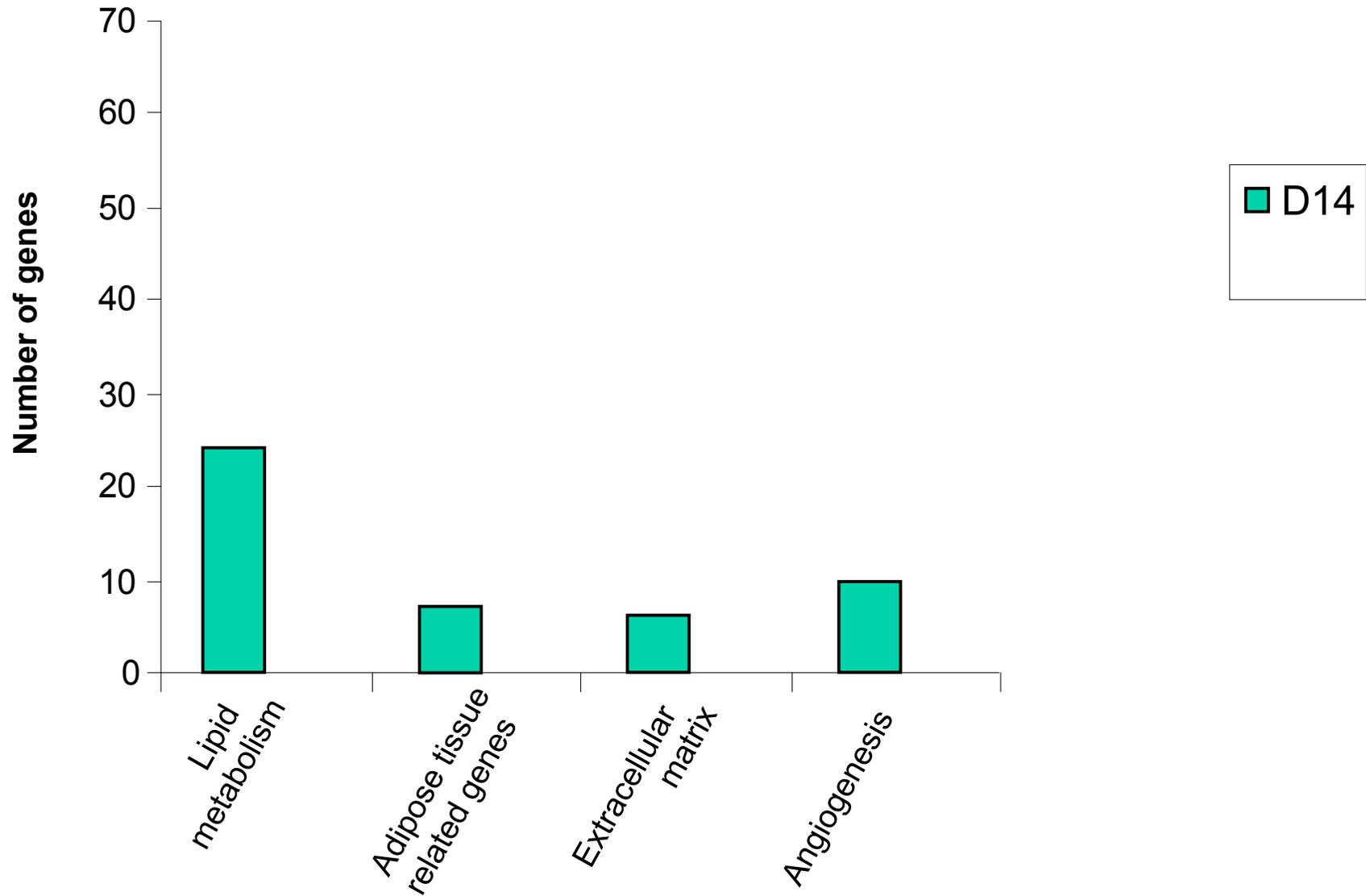


➤ **486 regulated genes between D0 and D56** (p-value<0.05/ FC>1.25).

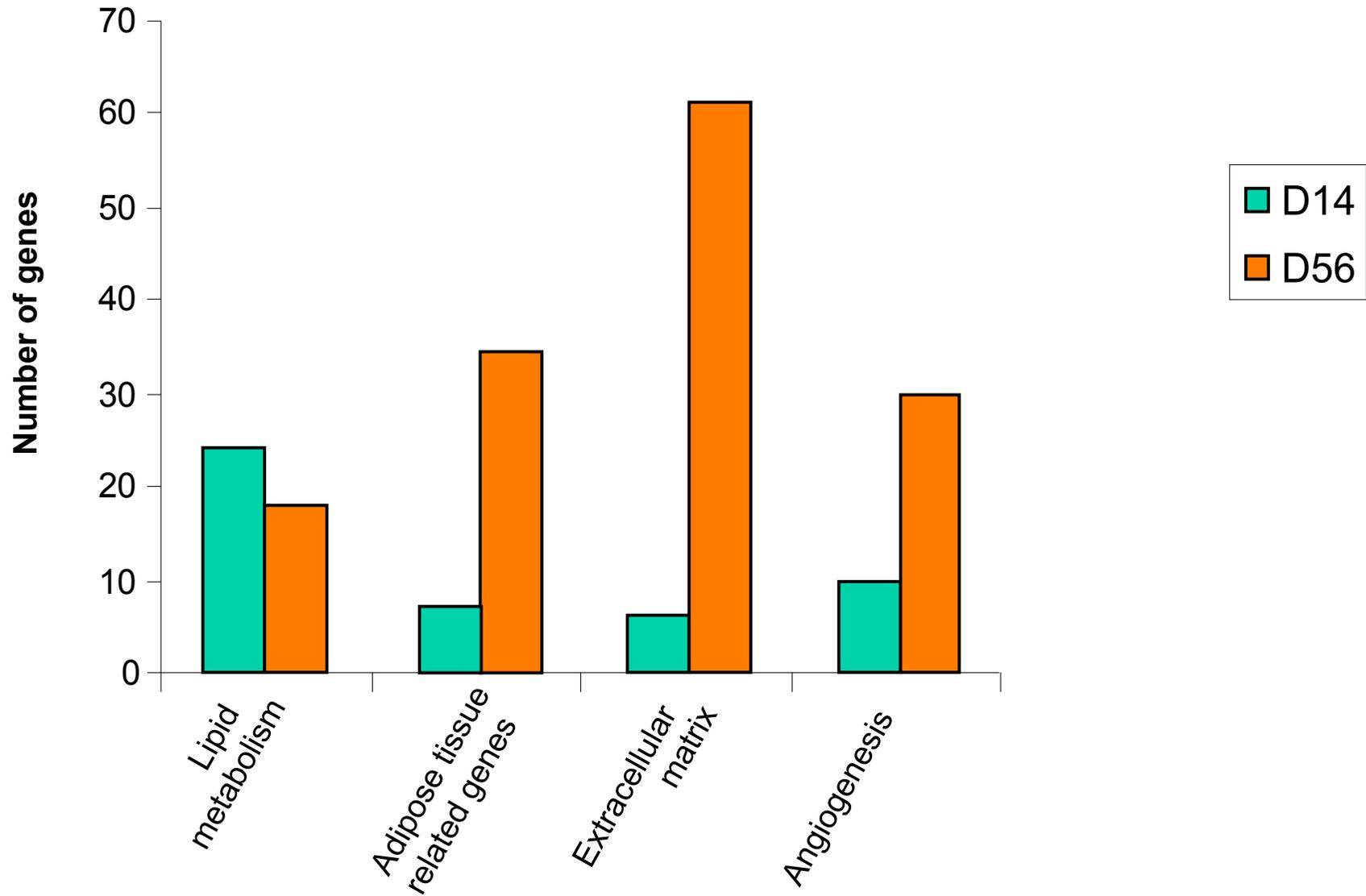
### Pathway enrichment (DAVID)



## Evolution of gene clusters in subcutaneous adipose during weight gain



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## Validation of microarray data using real-time PCR (24 subjects)

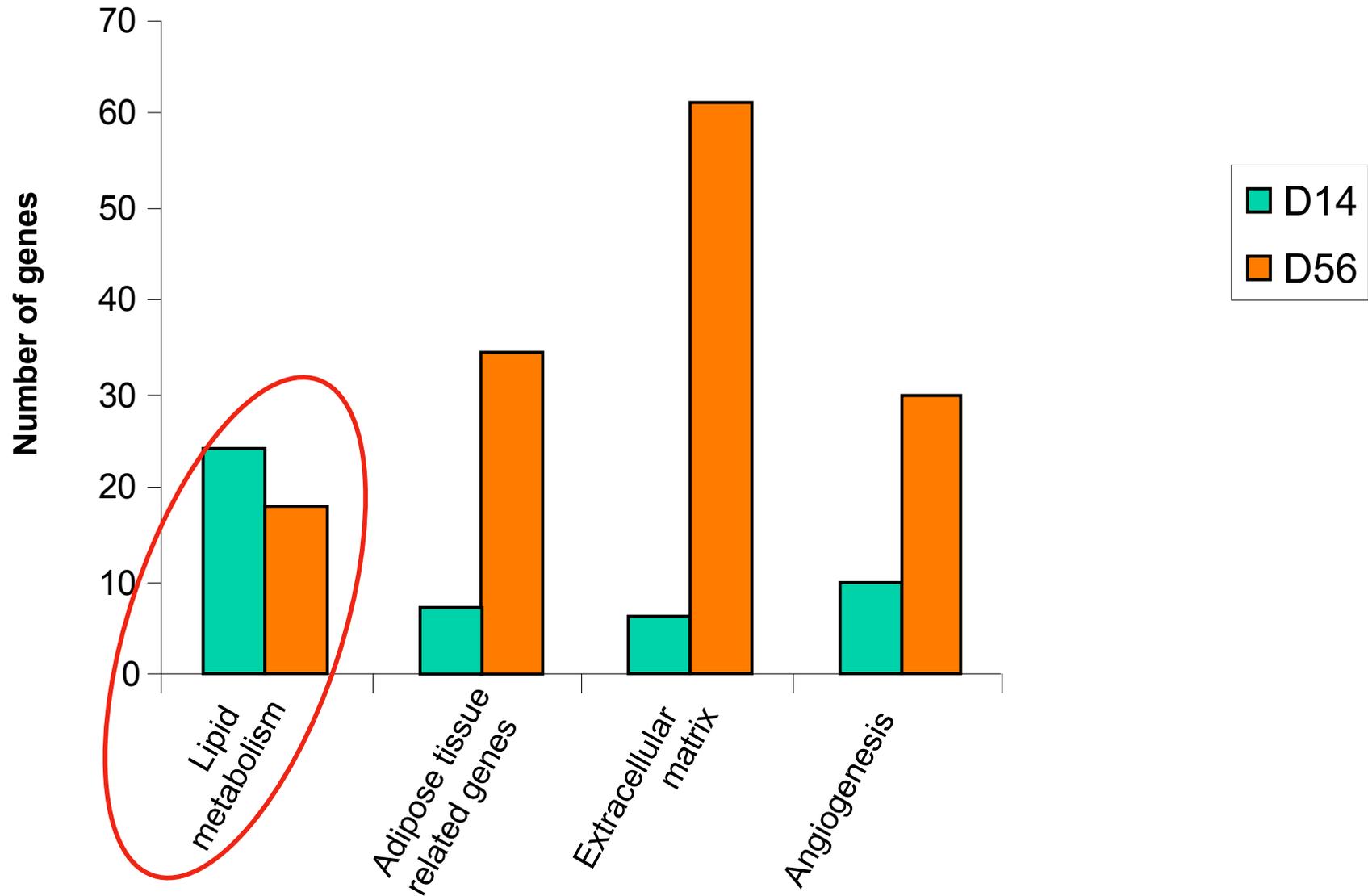
Gene Symbol	Entrez Gene	Fold change Microarray		Fold change RT-qPCR	
		D14	D56	D14	D56
<b>Lipid metabolism</b>					
AACS	65985	1.4 ± 0.1	1.6 ± 0.3	1.7 ± 0.2	2.5 ± 0.4
ACLY	47	1.8 ± 0.3	2.2 ± 0.7	1.8 ± 0.3	2.6 ± 0.4
CETP	1071	1.3 ± 0.2	3.2 ± 0.7	1.7 ± 0.3*	2.7 ± 0.6
DGAT2	84649	2.0 ± 0.4	1.7 ± 0.2	2.8 ± 0.6	1.9 ± 0.2
LPIN1	23175	1.4 ± 0.2	1.1 ± 0.1	1.5 ± 0.4	1.4 ± 0.1 *
SCD	6319	1.2 ± 0.1	2.4 ± 0.7	2.0 ± 0.4 *	2.5 ± 0.3
SLC2A5	6518	2.2 ± 0.5	4.0 ± 1.9	1.2 ± 0.4 *	2.4 ± 0.3
SREBF1	6720	1.5 ± 0.2	1.4 ± 0.1	2.0 ± 0.4	1.7 ± 0.2
VLDLR	7436	1.4 ± 0.2	1.2 ± 0.1	1.6 ± 0.3	1.5 ± 0.2 *
CIDEA	1149	0.9 ± 0.3	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1
<b>Extracellular matrix</b>					
COL6A3	1293	1.0 ± 0.1	1.3 ± 0.1	1.0 ± 0.1	1.3 ± 0.1
<b>Angiogenesis</b>					
APLNR	187	1.0 ± 0.1	1.3 ± 0.1	0.9 ± 0.1	1.4 ± 0.1
ANGPTL4	51129	0.9 ± 0.1	0.8 ± 0.1	0.9 ± 0.2	0.9 ± 0.1 *
EDN1	1906	0.8 ± 0.1	1.0 ± 0.1	0.7 ± 0.1	1.4 ± 0.2 *
<b>Renin-angiotensin system</b>					
ACE	1636	1.2 ± 0.1	1.5 ± 0.6	1.2 ± 0.1	1.4 ± 0.1
AGT	183	1.5 ± 0.2	1.4 ± 0.1	2.5 ± 0.5	2.2 ± 0.3
ENPEP	2028	1.1 ± 0.1	1.5 ± 0.1	1.2 ± 0.2	1.7 ± 0.2
MME	4311	1.4 ± 0.1	1.4 ± 0.1	1.4 ± 0.2	1.5 ± 0.2

Data are presented as mean ± SE.

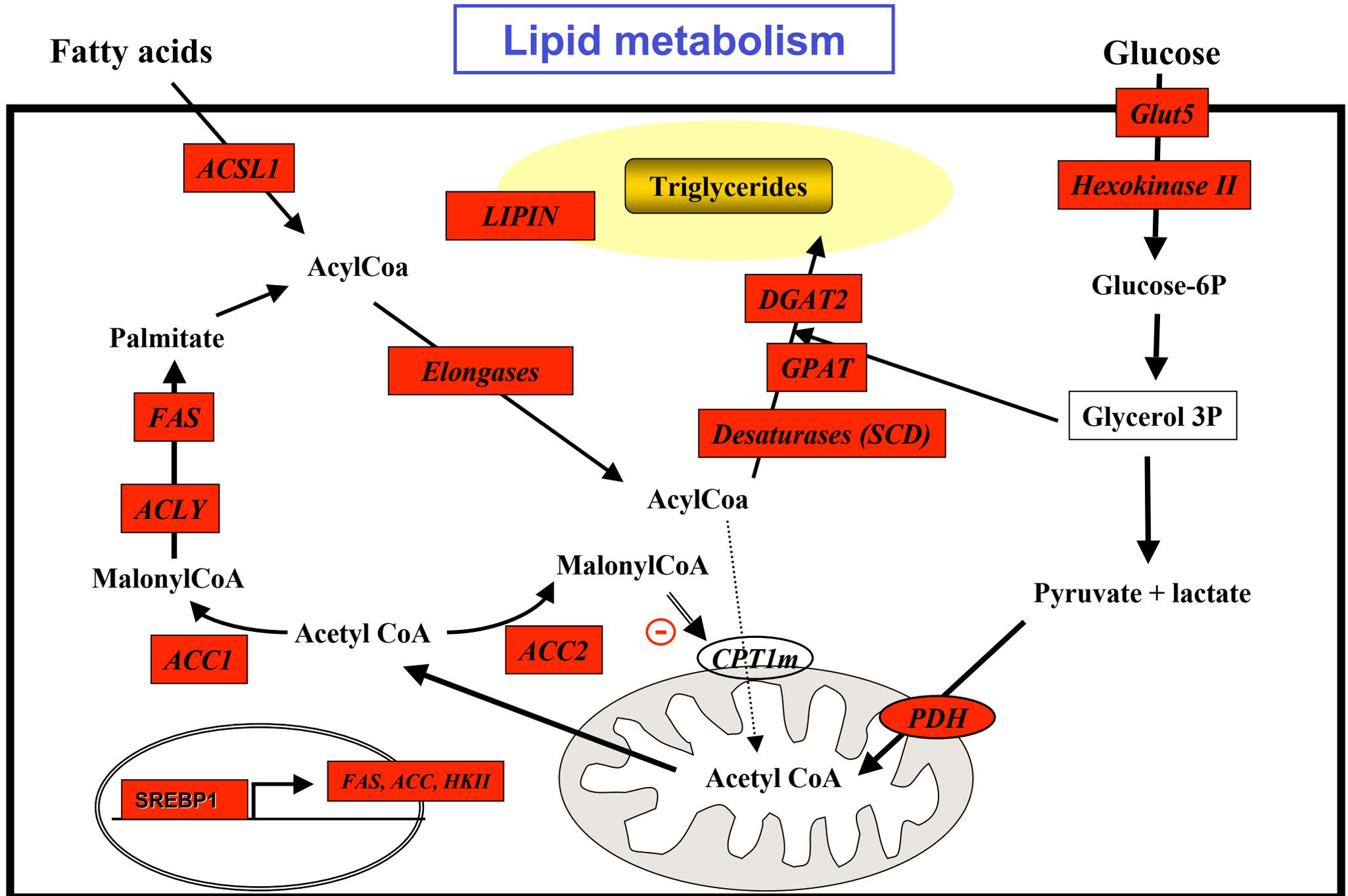
Underlined values indicate significant changes at D14 or D56 with respect to D0 (p <0.05 with paired t-test for qPCR, p< 0.05 with Limma for microarray).

\* Indicates different result between microarray and RT-PCR

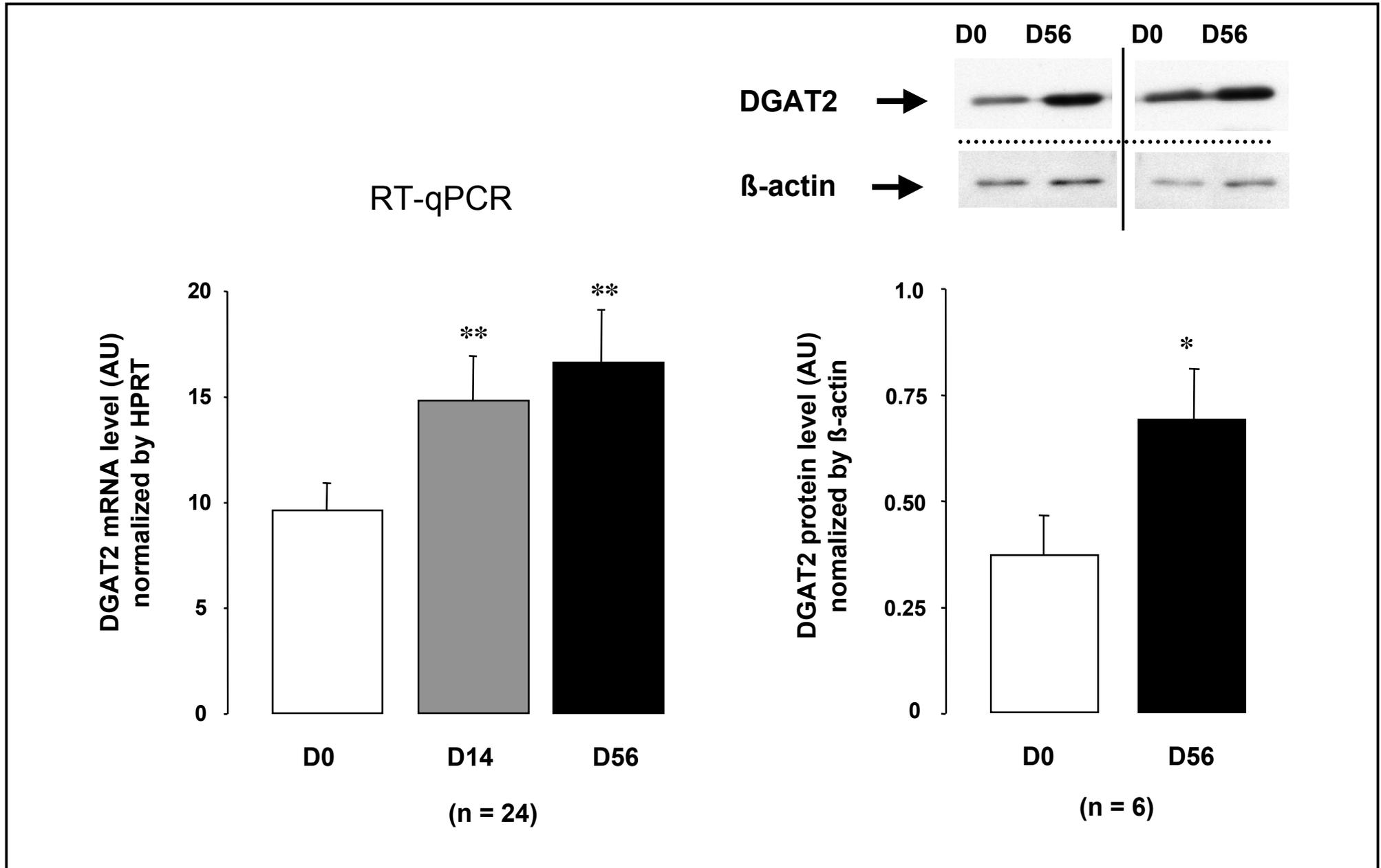
## Evolution of gene clusters in subcutaneous adipose during weight gain



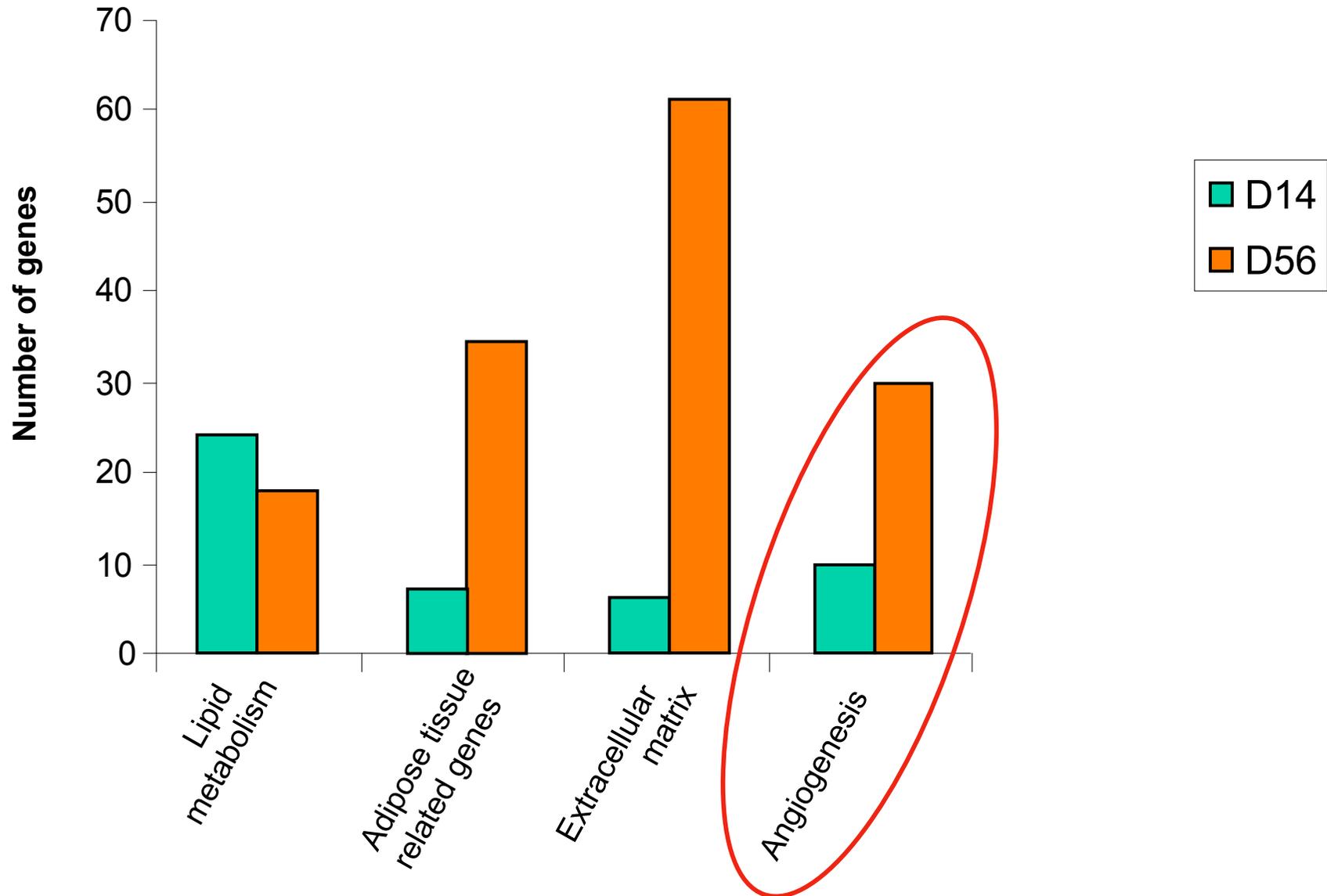
# Lipid metabolism



# Validation of DGAT2 gene expression change by RT-qPCR and Western-blot

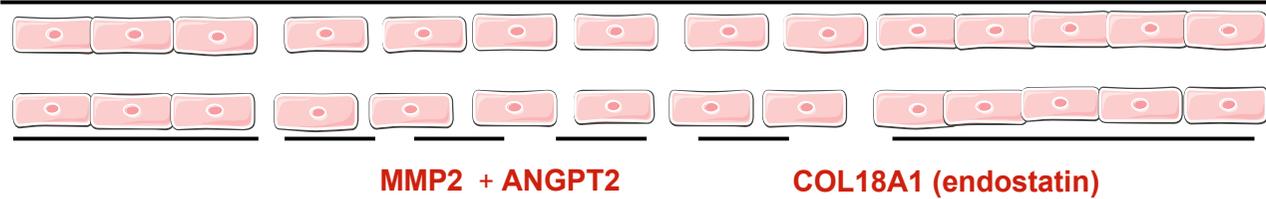


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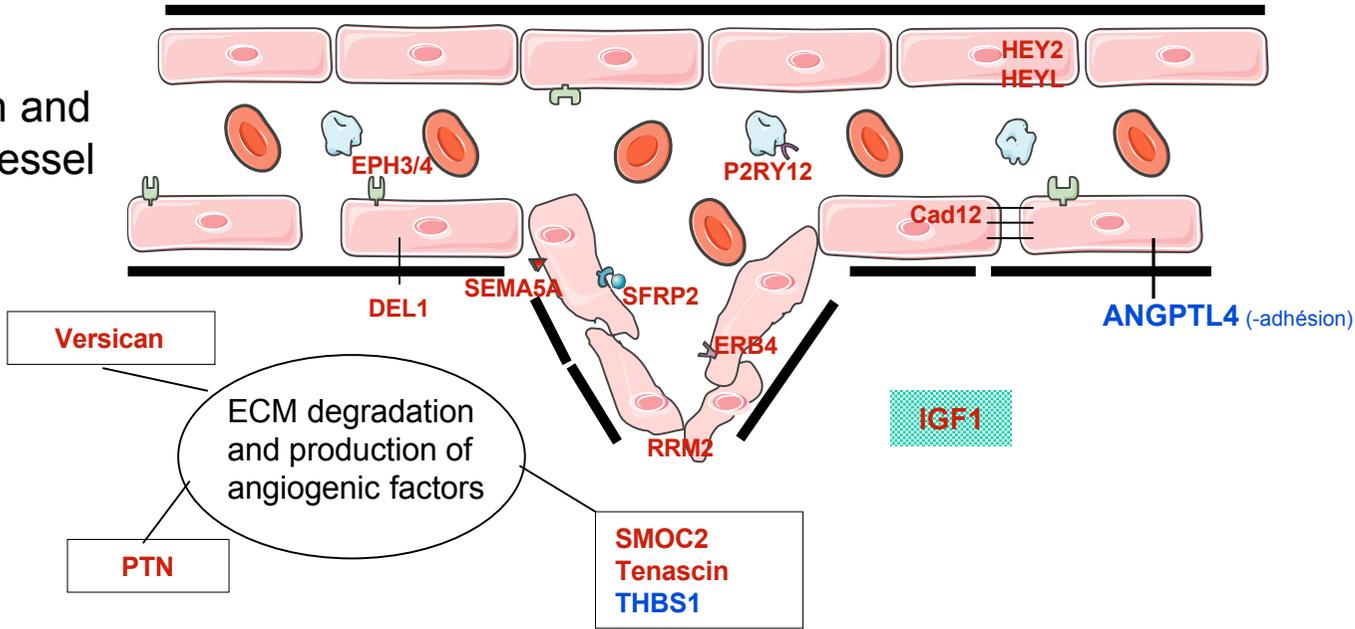


# Angiogenesis and neovascularization

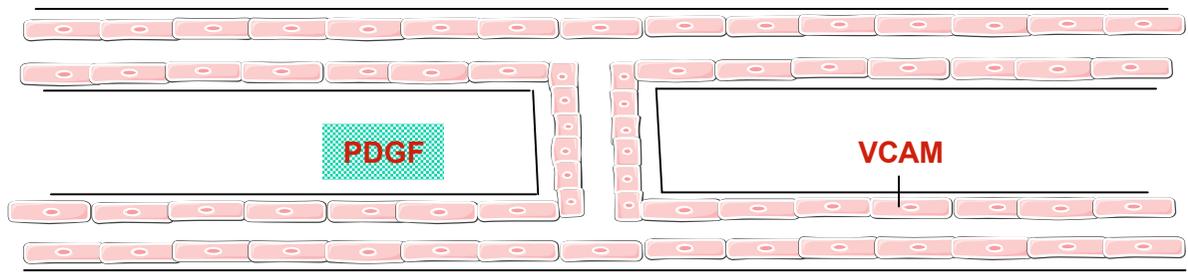
Blood vessel vasodilation  
and LB degradation



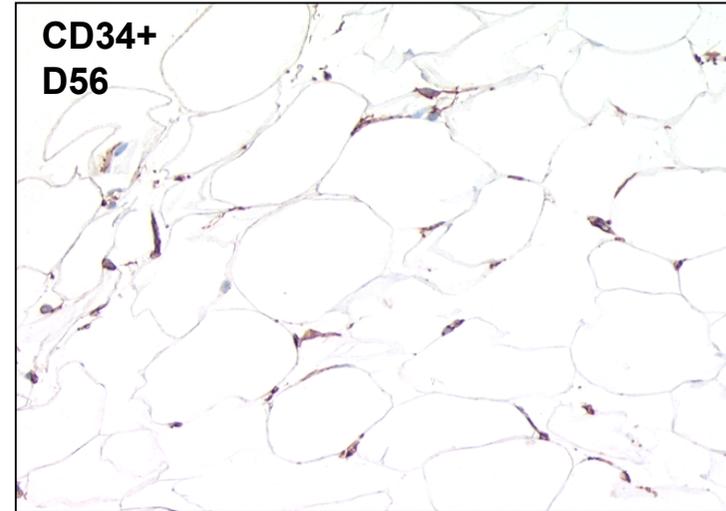
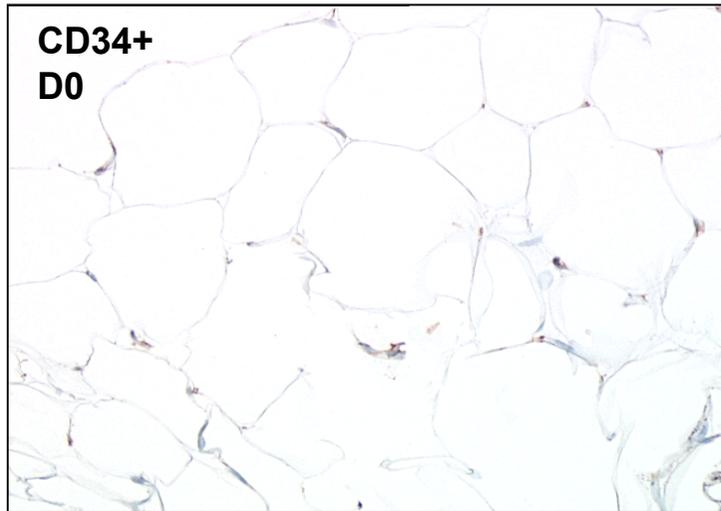
Migration, proliferation and  
growth of new blood vessel



Tubule formation and  
maturation



## Increased angiogenesis and vascularization in human subcutaneous adipose tissue during overfeeding

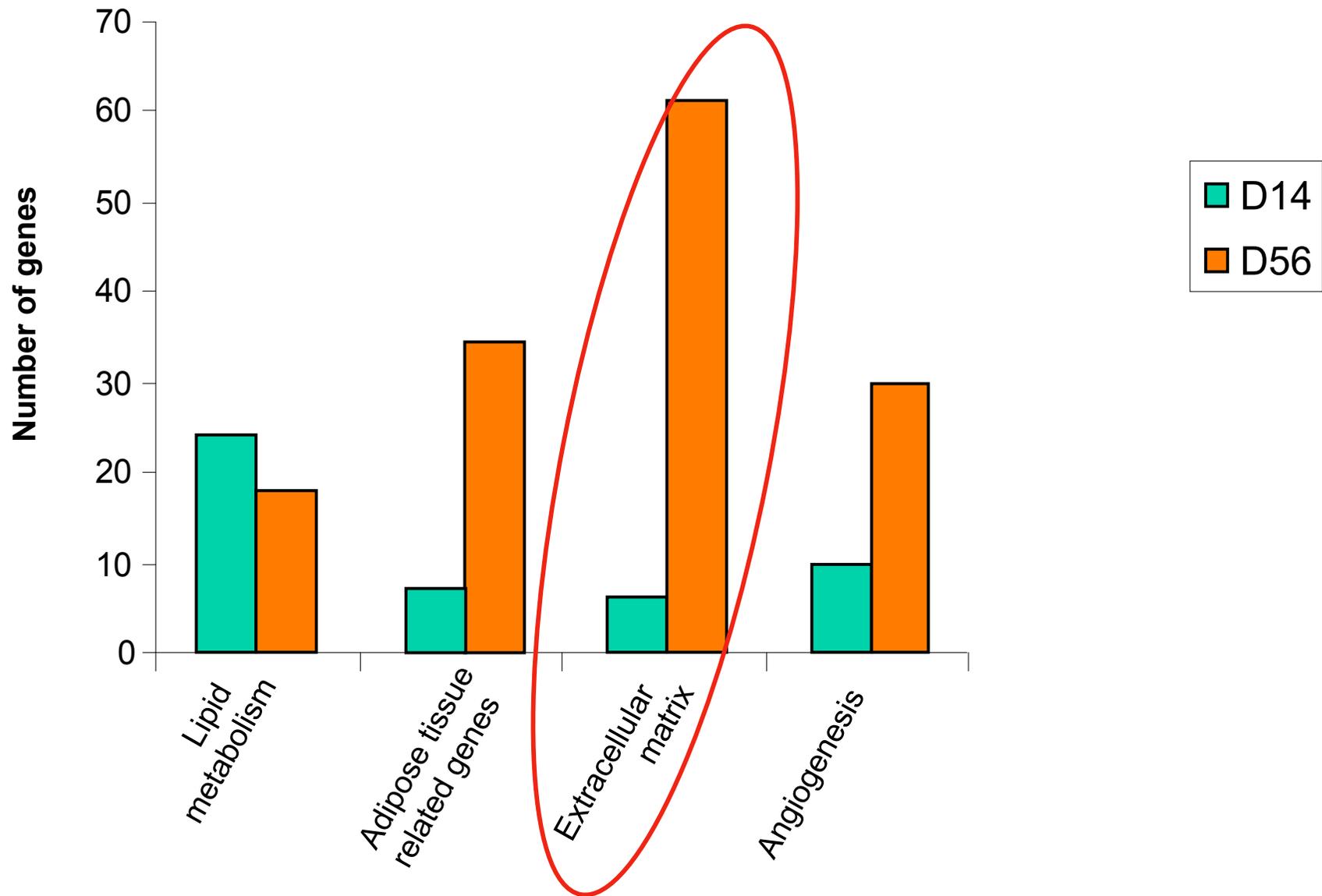


Microvascular density :  $21.2 \pm 9.7 / \text{mm}^2$

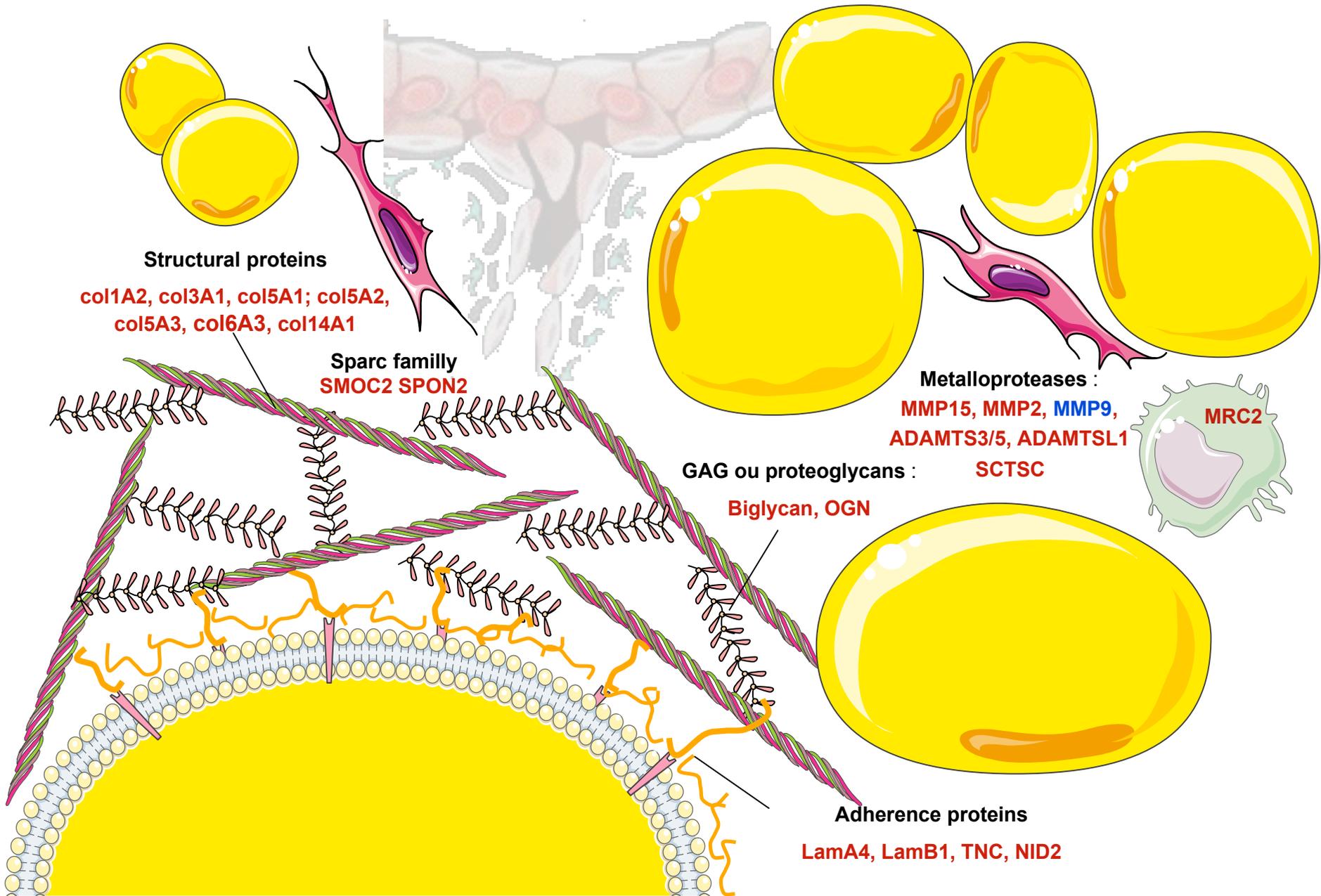
$32.1 \pm 14.9 / \text{mm}^2$

( $p < 0.01$ ,  $n = 12$ )

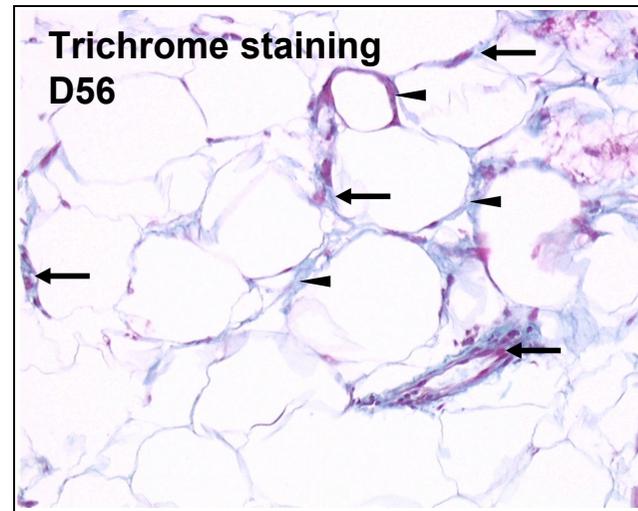
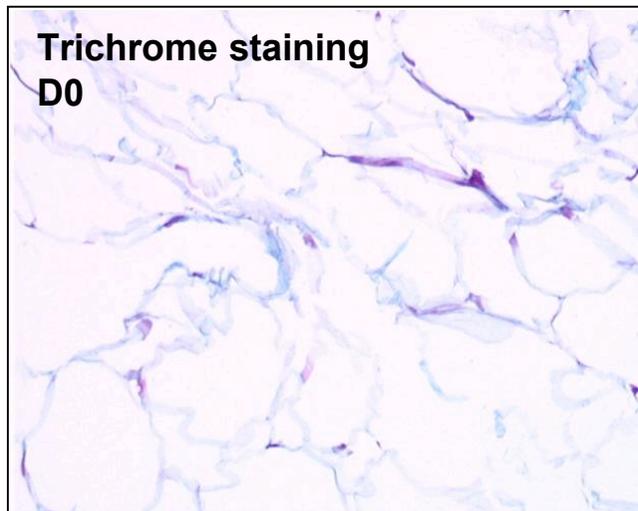
## Evolution of gene clusters in subcutaneous adipose during weight gain



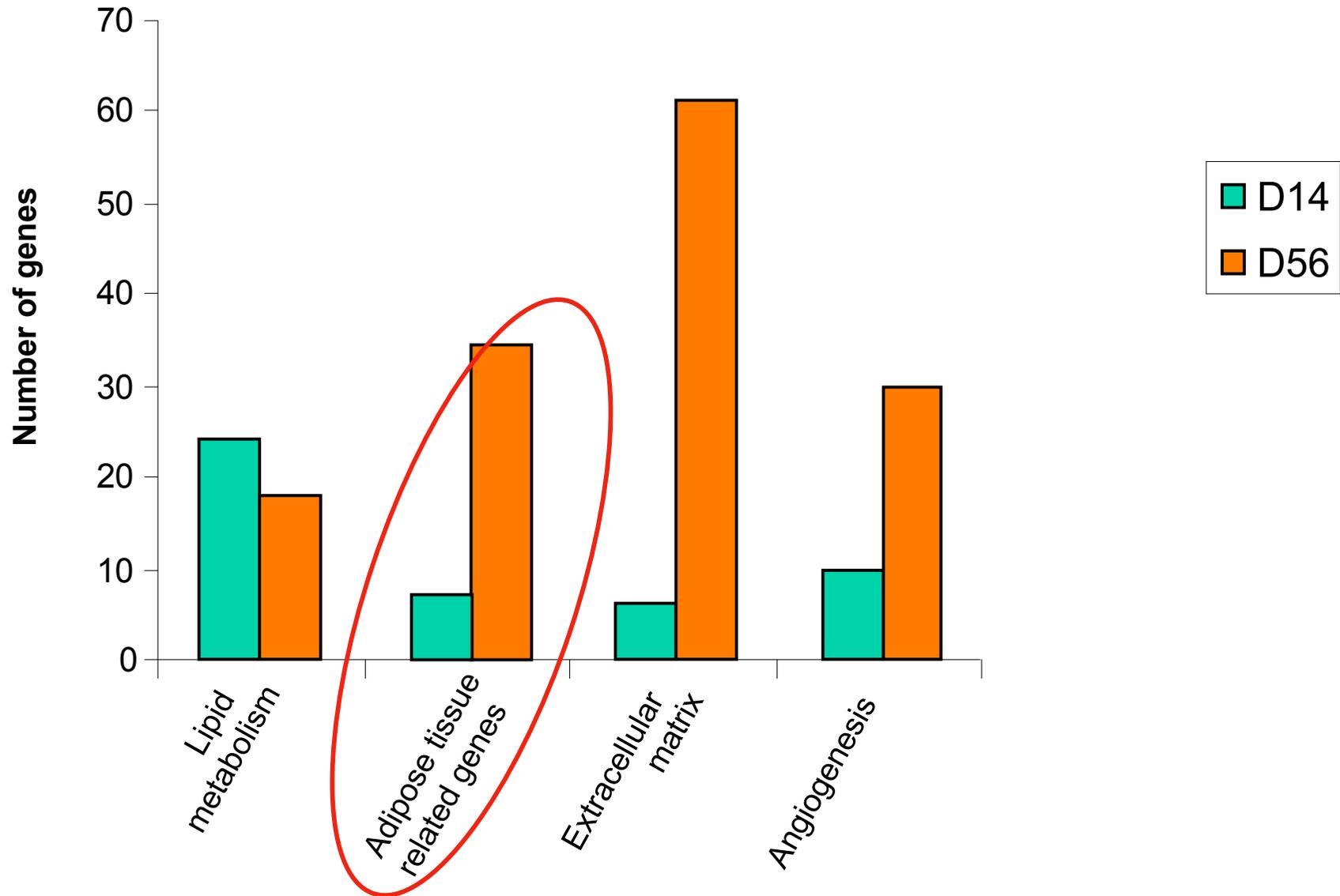
# Extracellular Matrix



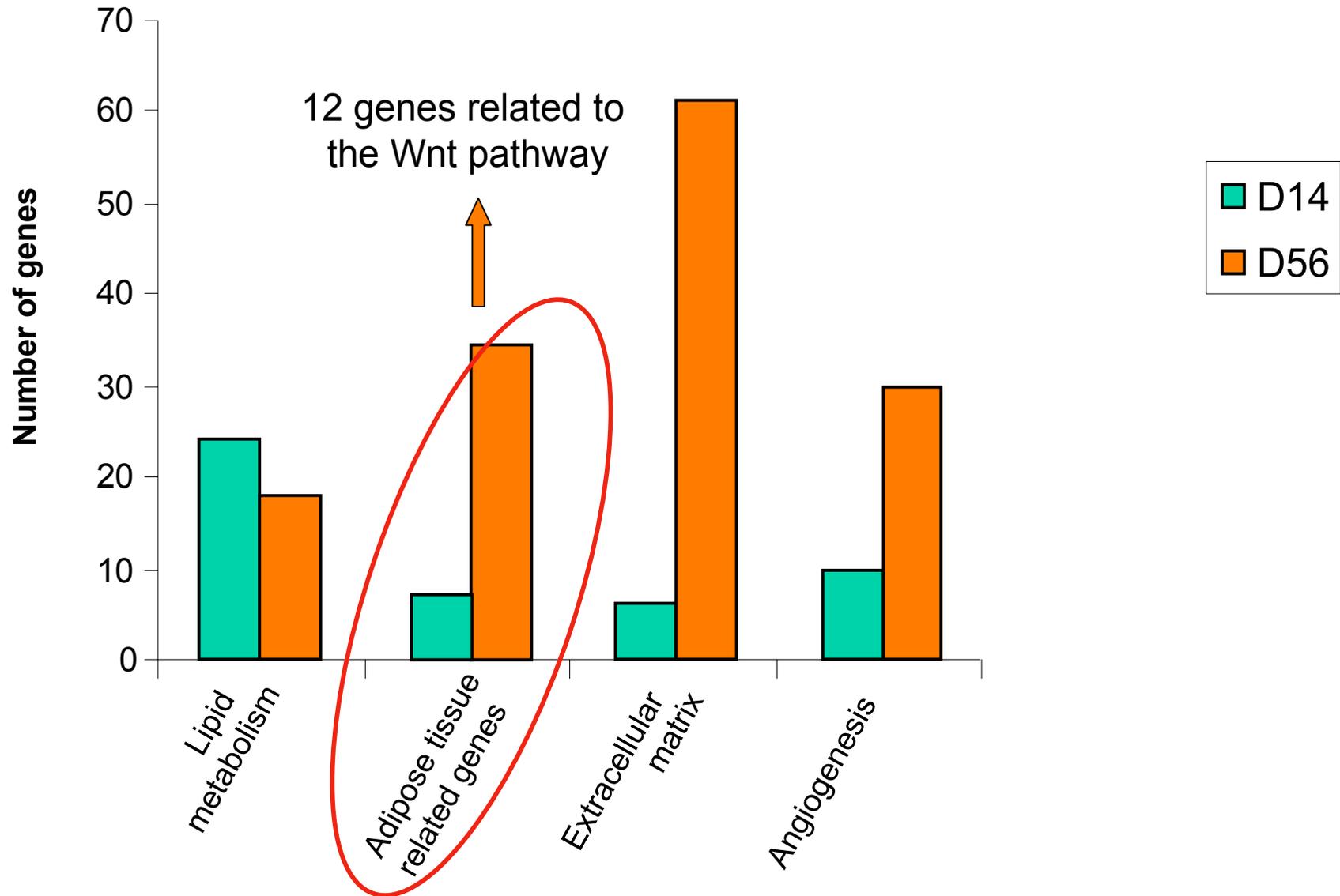
**Remodeling of subcutaneous adipose tissue during overfeeding:  
Increased extracellular matrix and conjonctive tissue deposits**



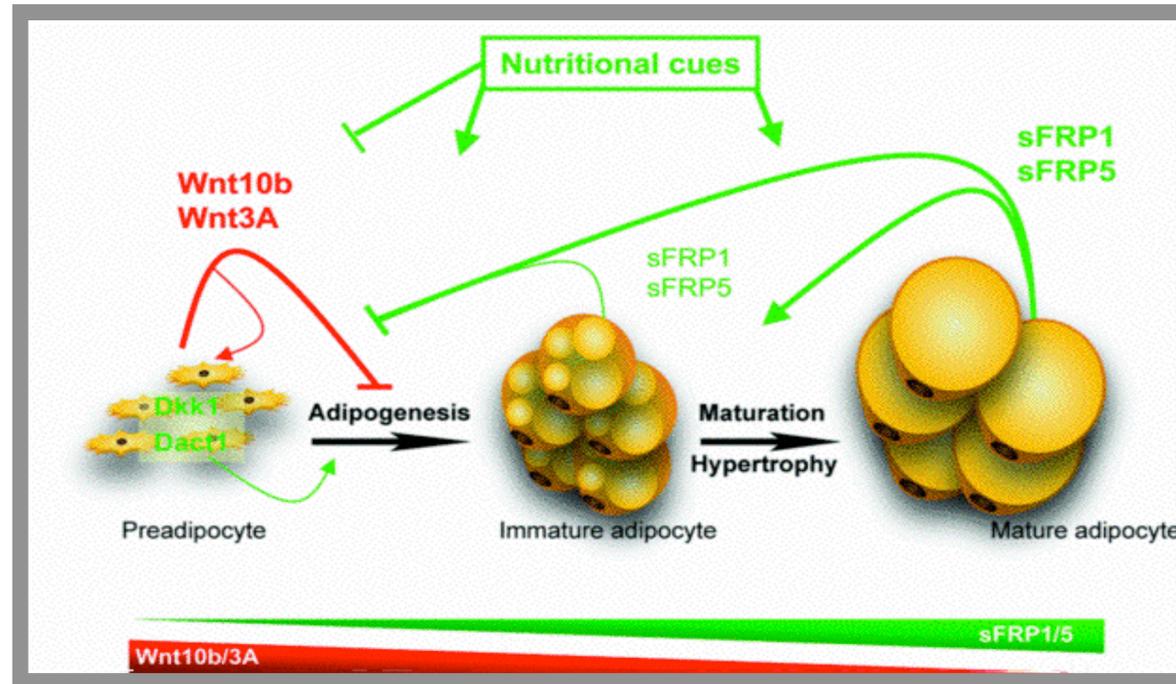
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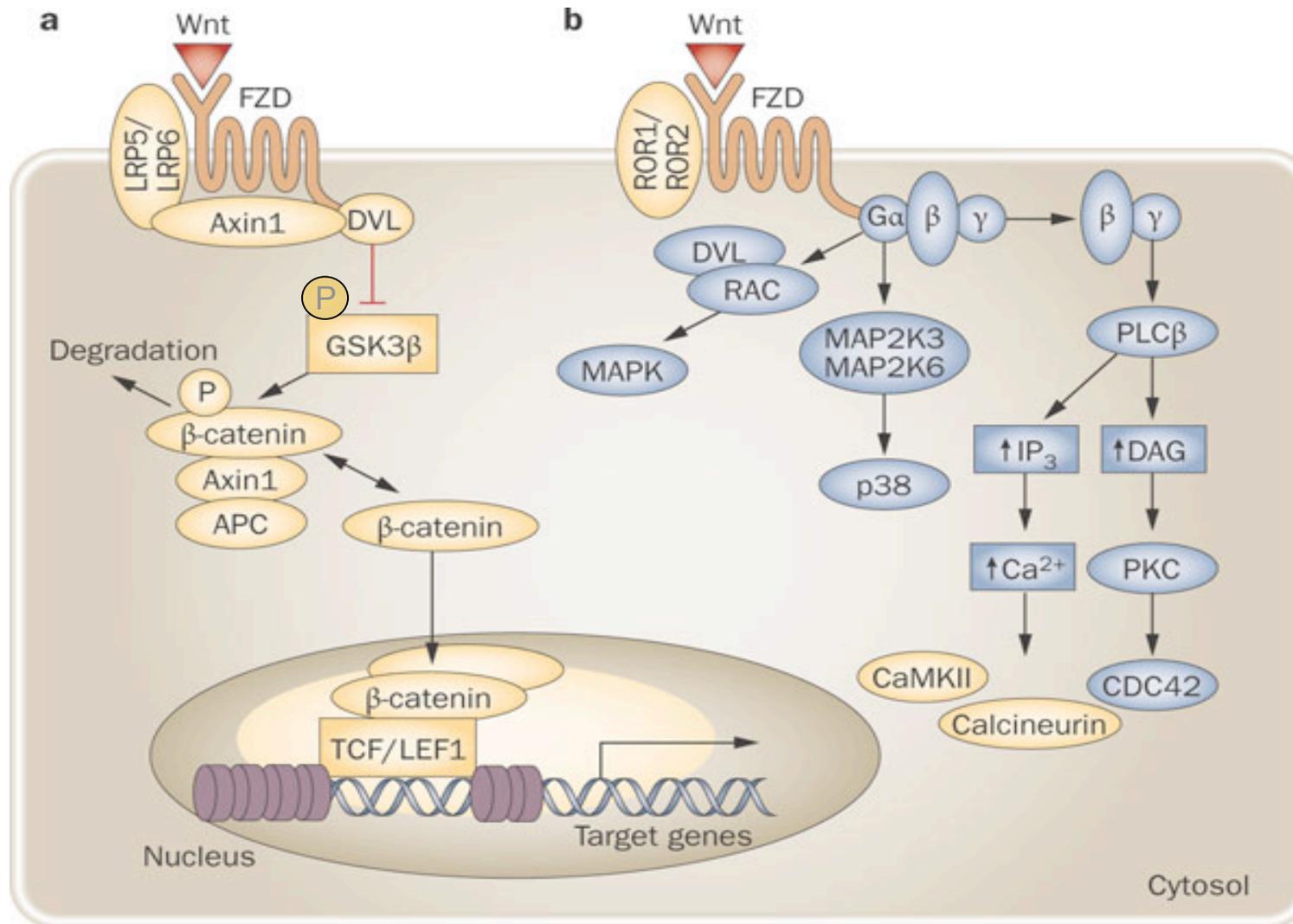
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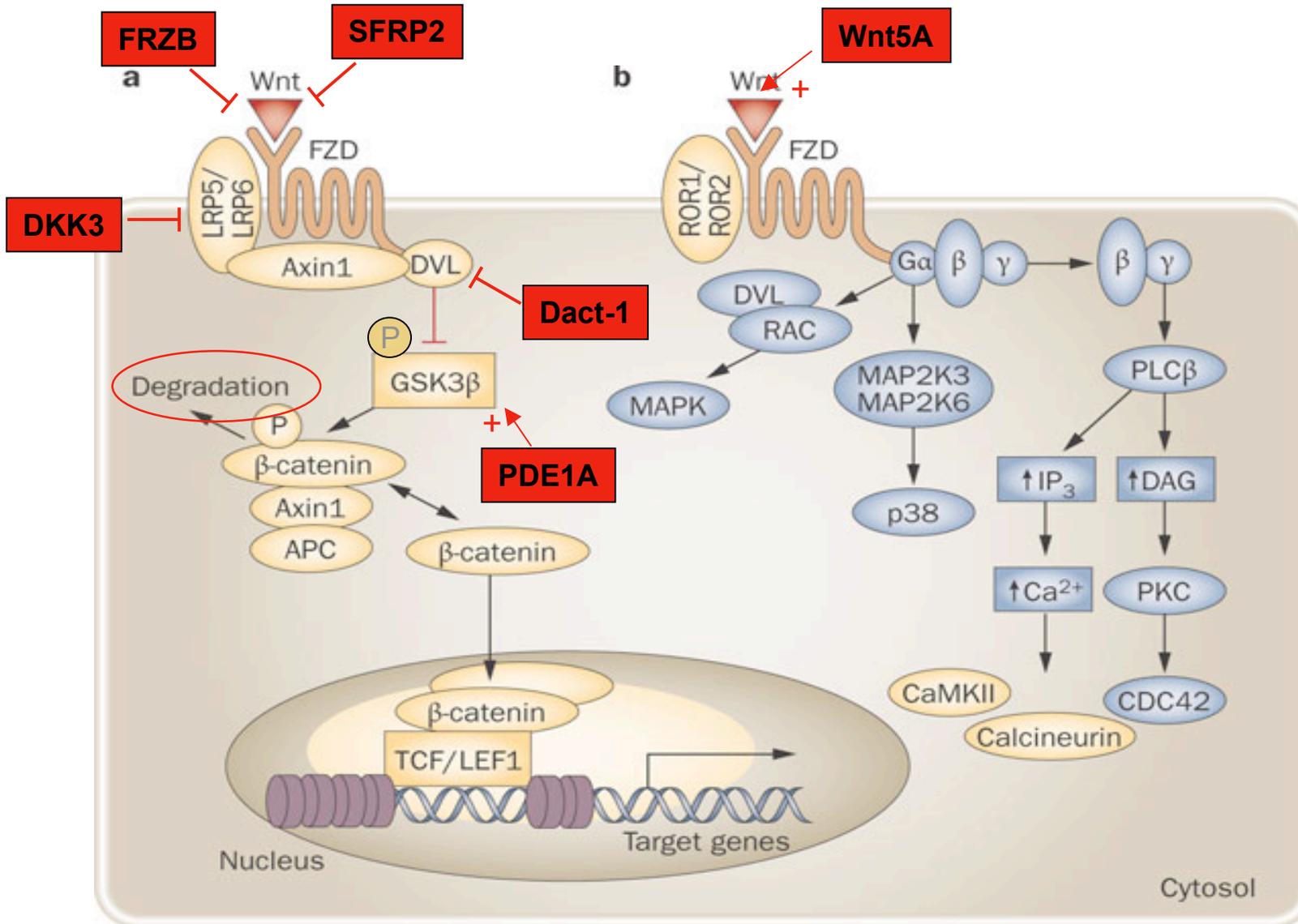
# The Wnt signalling networks is a putative paracrine regulator of adipogenesis



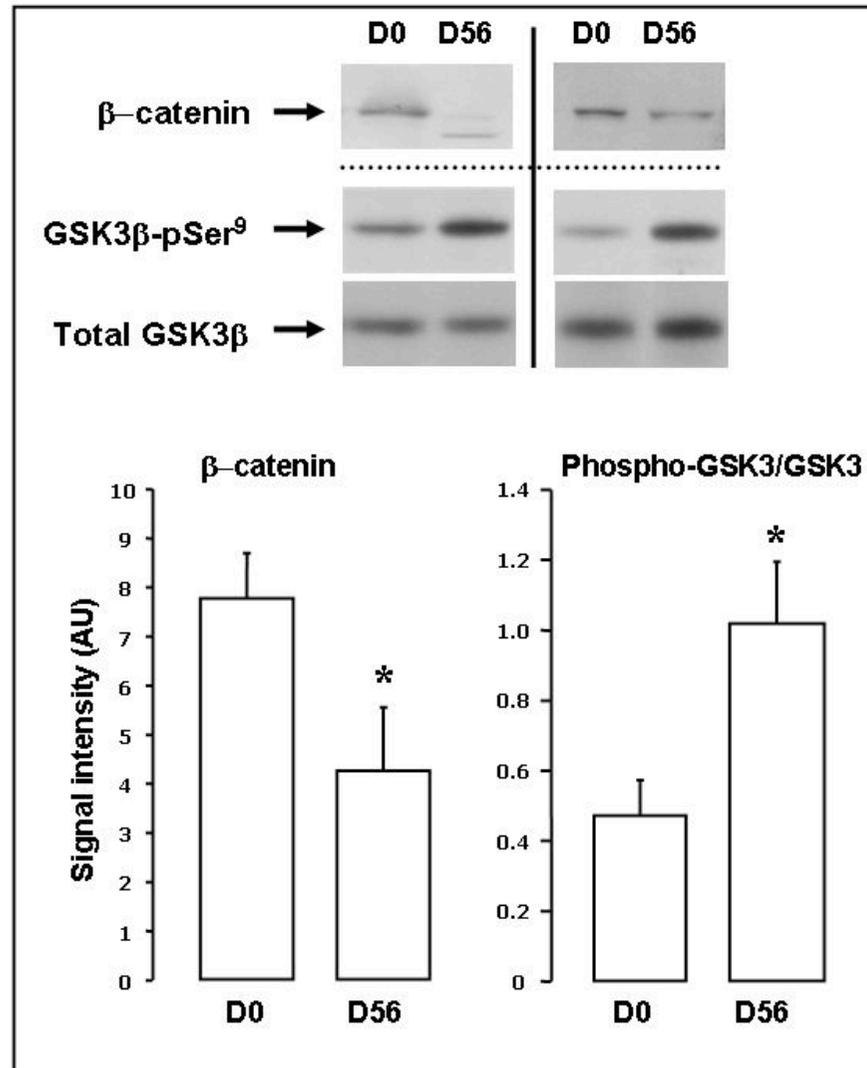
## The canonical Wnt/ $\beta$ -catenin signaling pathways must be inhibited to allow adipogenesis



The expression of several inhibitors of the canonical pathway is up-regulated during overfeeding

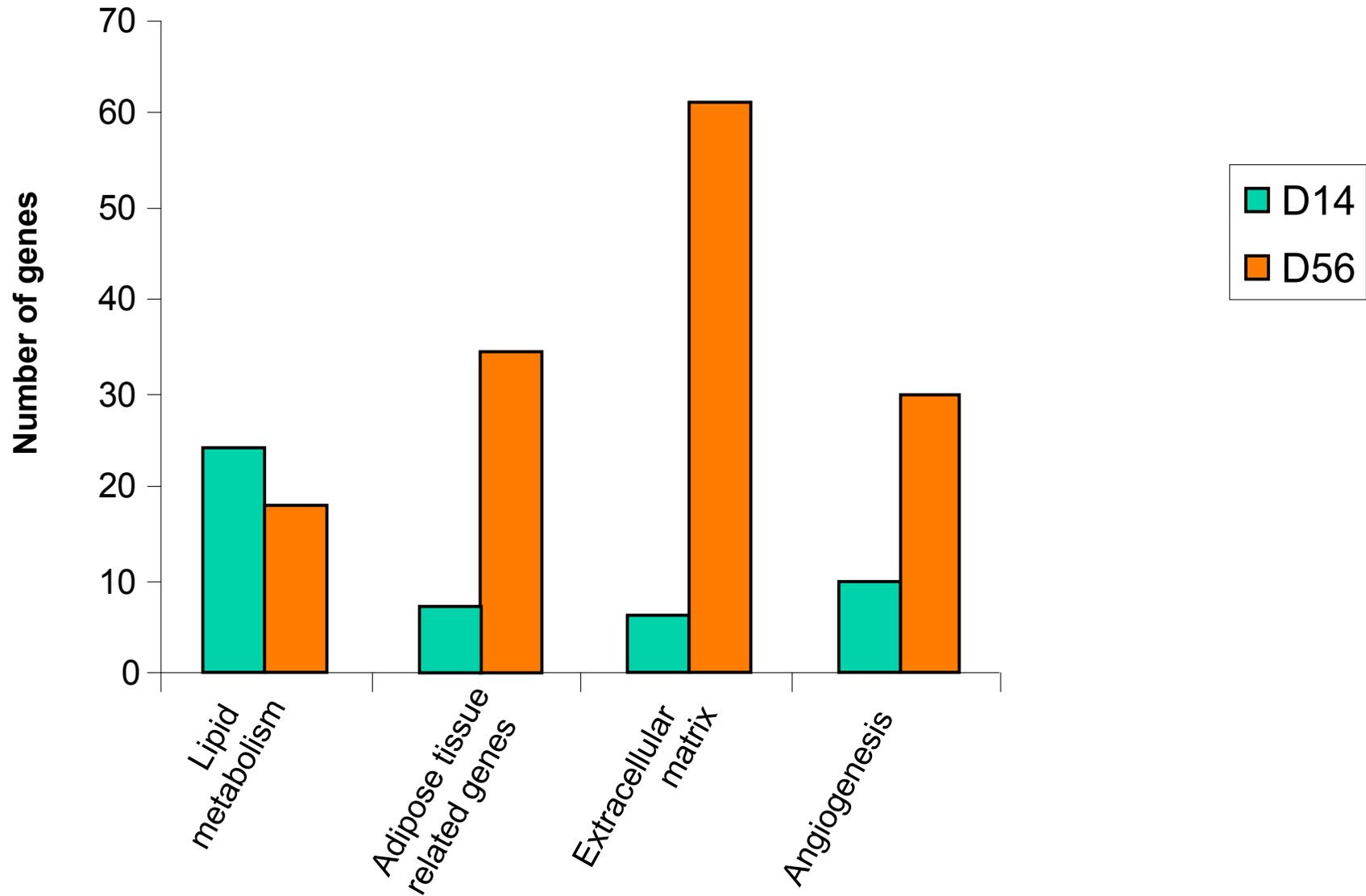


## Inhibition of the canonical Wnt/ $\beta$ -catenin pathways in human subcutaneous adipose tissue during overfeeding

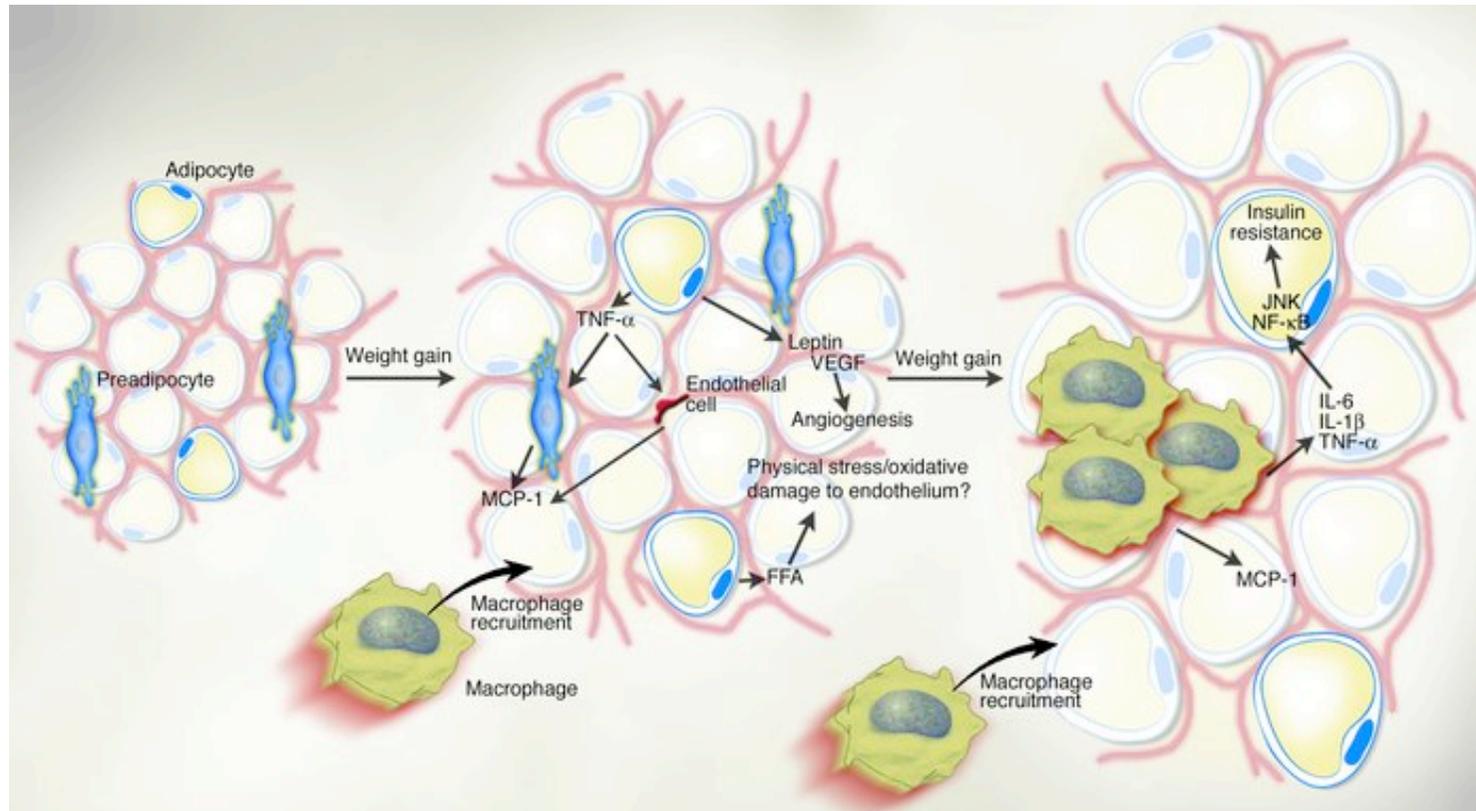


Western-blot analysis,  
n= 8  
\* p < 0.05

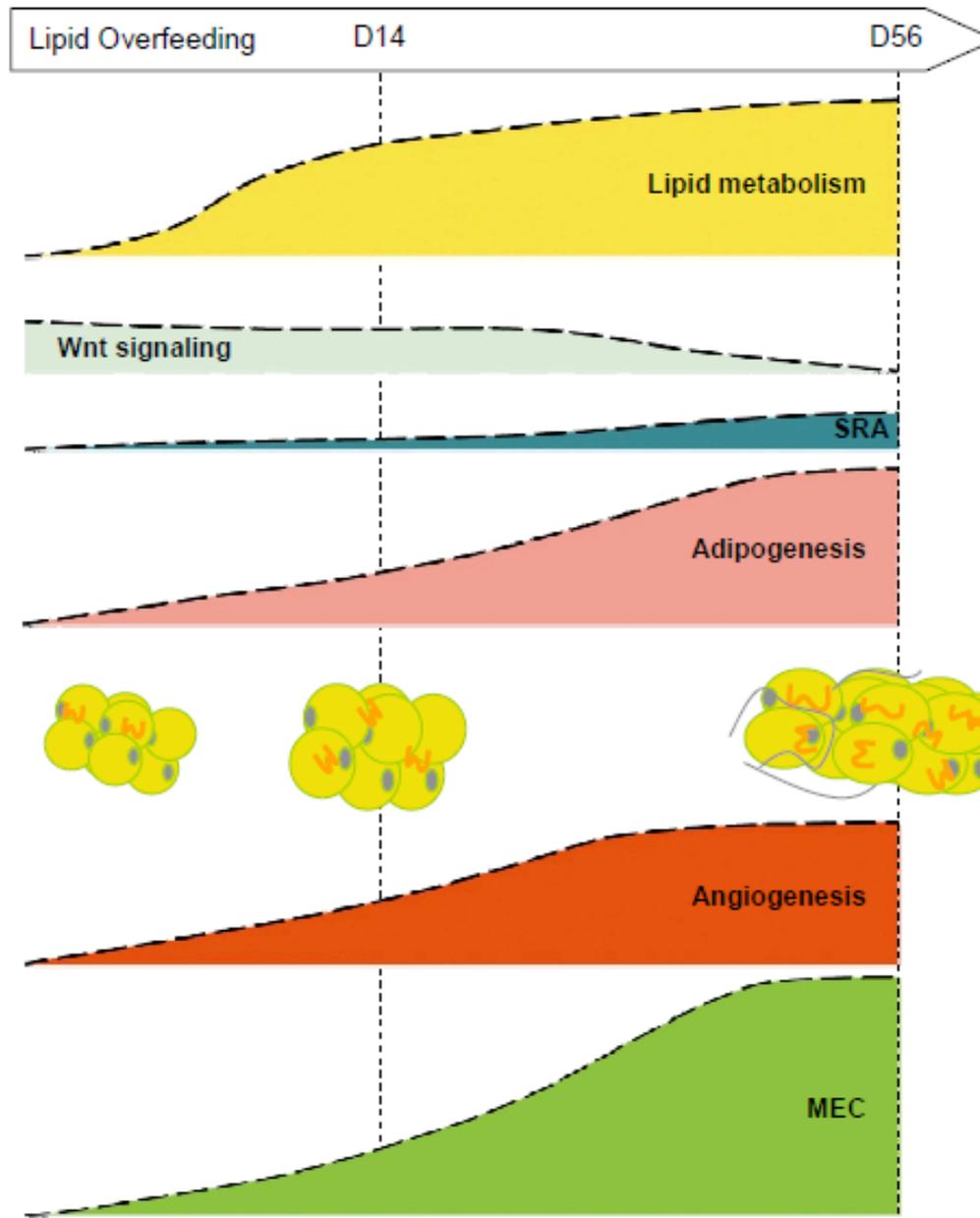
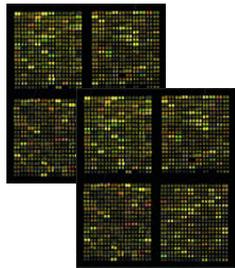
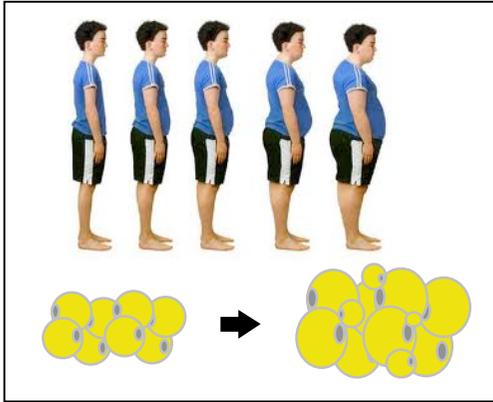
## Evolution of gene clusters in subcutaneous adipose during weight gain



# Inflammation ?



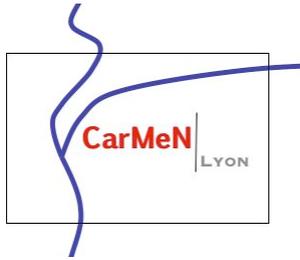
**No evidence for immune cells (macrophages) recruitment during lipid overfeeding**



Lipid filling  
Hypertrophy

Tissue remodeling

Hyperplasia



**INSERM U-1060/INRA U-1235**

**Centre de Recherche en Nutrition Humaine  
(Martine LAVILLE)**

**Maud ALLIGIER**

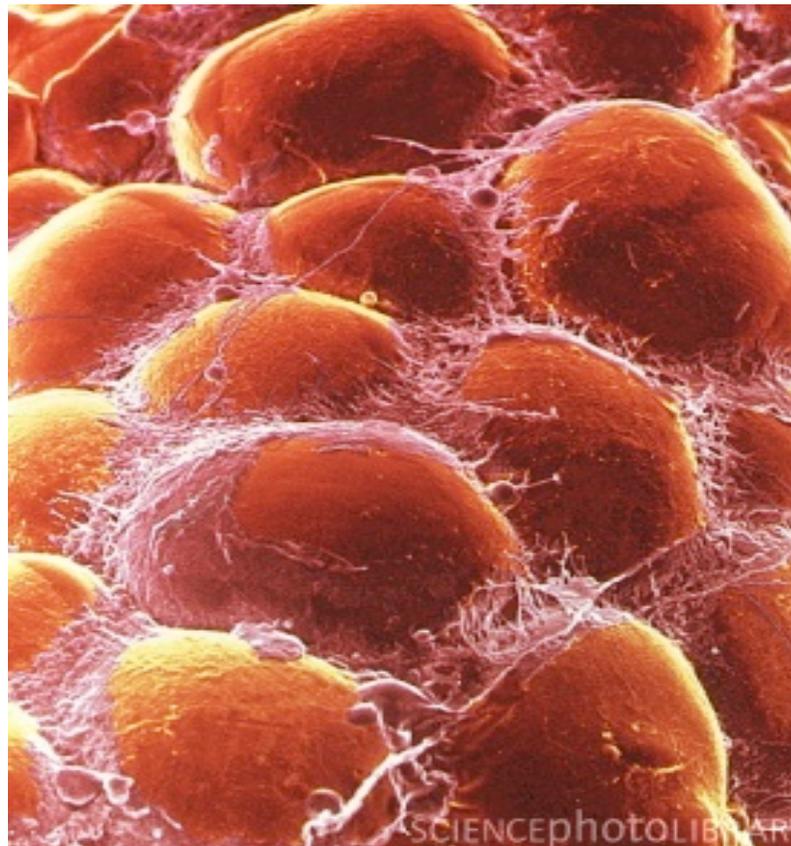


**Emmanuelle MEUGNIER  
Cyrille DEBARD  
Emmanuelle LOIZON  
Myriam OLIEL  
Cécile BOSSU  
Monique SOTHIER  
Jocelyne PEYRAT**

**Jean-Yves SCOAZEC**

**Béatrice MORIO  
Emilie CHANSEAUME**

**John BROZEK**



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**Programme hospitalier de  
recherche clinique (PHRC)**

