GG2

Implications of mitochondrial skeletal muscle metabolism on diabetes and obesity before and after weight loss

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GG_SFEFS_ 19.10.2009

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HUG Hôpitaux Universitaires de Genève

Folie 1

GG2 19.10.2009

GG_PC; 12.10.2009

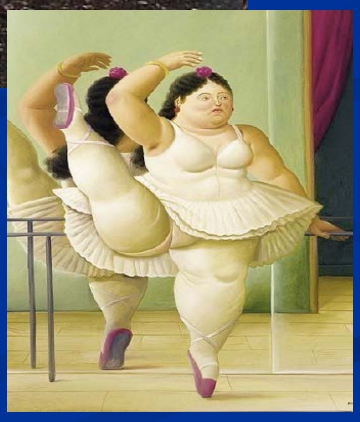
Plan

- Introduction
  - Insulin resistance and mitochondrial function
- Study 1
  - Lipid peroxidation in skeletal muscle of obese as compared to endurance-trained humans: a case of good vs. bad lipids?
- Study 2

  Upregulation of peroxisome proliferator-activated receptor gamma coactivator gene (PGC1A) during weight loss is related to insulin sensitivity but not to energy expenditure
- Perspectives

To start up

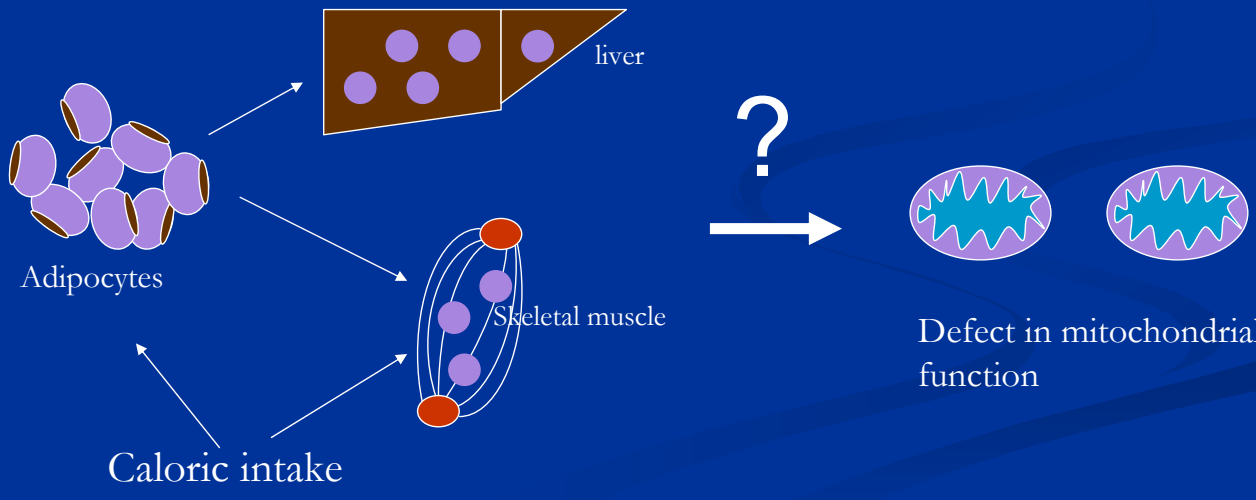
Course de l'escalade, Geneva



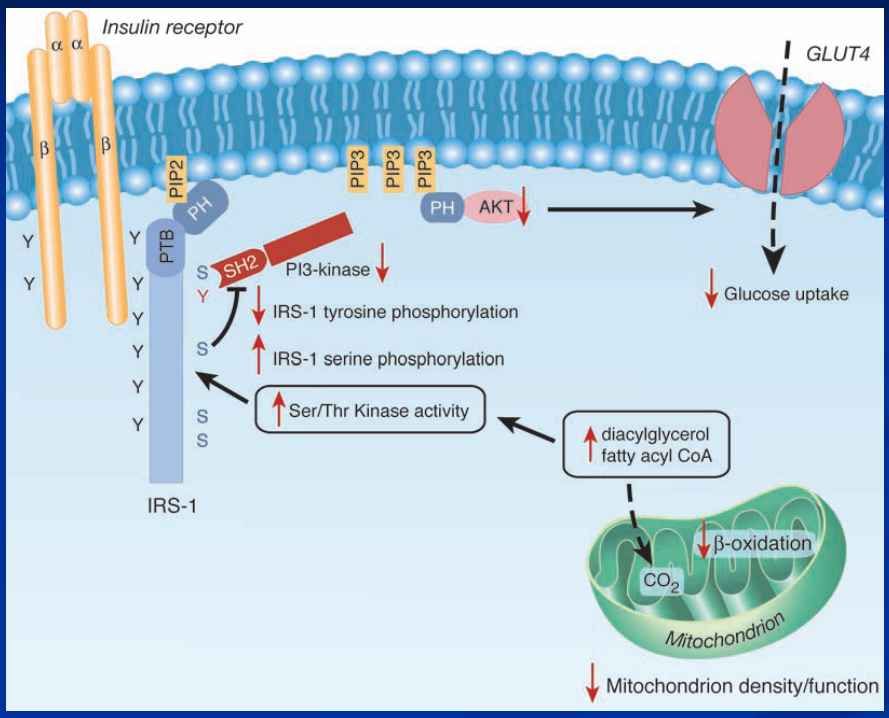
Botero : the dancer

Insulin resistance (IR)

A diminished response of a target cell or organ to a physiological concentration of insulin.



Mitochondrial dysfunction or decreased mitochondrial density are candidates mediators of obesity-related insulin resistance in skeletal muscle.



Lowell, Science 2005

Mitochondrial hypothesis

↑ Insulin Resistance

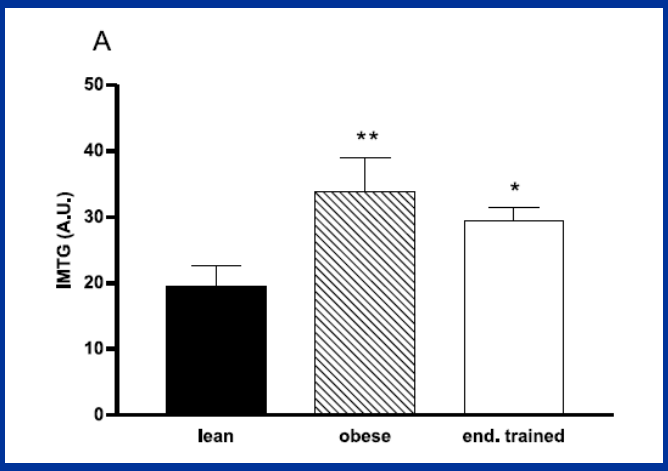
↑ Intra-myocellular triglyceride content (IMTG)

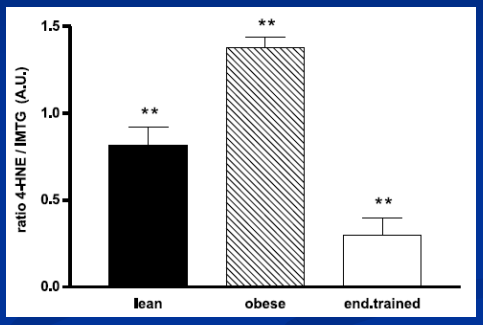
↓ Oxydative phosphorylation

FEBS Letters 551 (2003) 104–106 FEBS 27574

Lipid peroxidation in skeletal muscle of obese as compared to endurance-trained humans: a case of good vs. bad lipids?

Aaron P. Russell[a,b,*], Giacomo Gastaldi[b,c], Elisabetta Bobbioni-Harsch[c], Patrizia Arboit[b], Charles Gobelet[a], Olivier Dériaz[a], Alain Golay[c], Joseph L. Witztum[d], Jean-Paul Giacobino[b]





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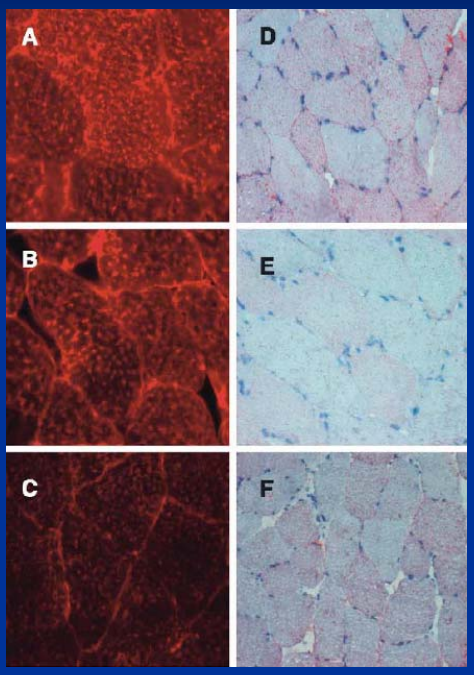
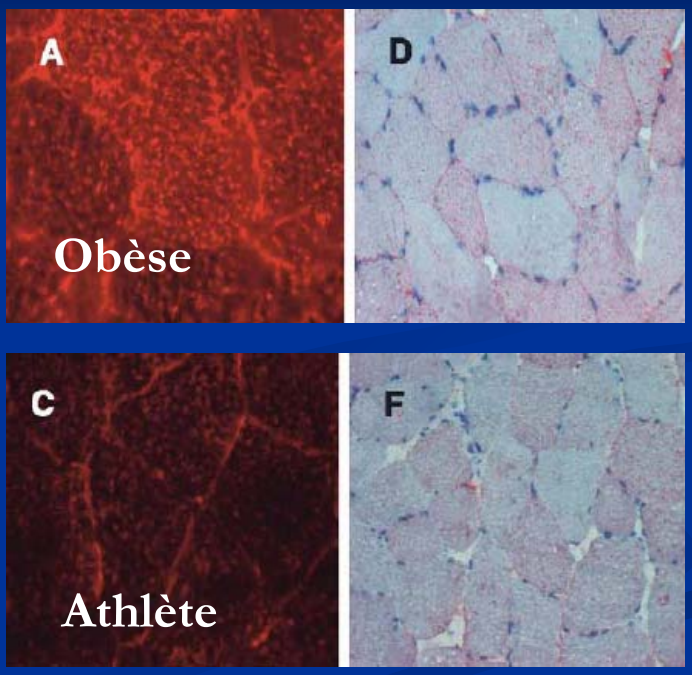
Peroxidation des lipides intramusclaires





4-HNE IMTG

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Conclusion

- IMTG content was the same in the obese and endurance trained (ET) subjects.
- The lipid peroxidation/IMTG ratio was 4.2-fold higher in the obese subjects.
- Obesity results in an increased level of IMTG peroxidation while ET has a protective efect on IMTG peroxidation.
- This suggests a link between the lipid peroxidation/IMTG ratio and insulin resistance.

Insulin action in skeletal muscle couples directly to mitochondrial energetics and substrate selection

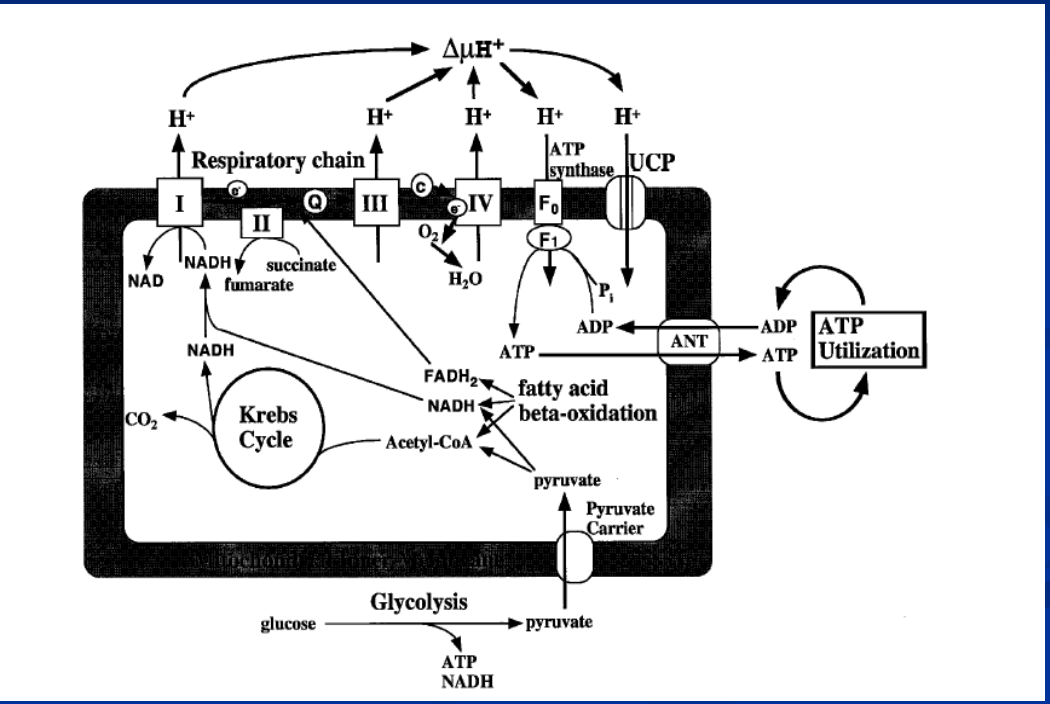
Insulin resistance and mitochondria

- 4 potential mitochondrial targets :
  - Uncoupling protein 3 (UCP3)
  - Carnitine palmitoyltransferase-1(CPT1)
  - Peroxisome proliferator-activated receptor gamma Co-activator 1alpha (PGC-1alpha)
  - Mitofusin-2 (Mfn-2)

Uncoupling protein 3 (UCP3)

- UCP3 is selectively expressed in skeletal muscle and brown adipose tissue
- UCP3 is a member of the mitochondrial anion carrier super family
- High homology with UCP1, UCP3 was proposed to be an uncoupling protein (cold thermogenesis)
- Role and function of UCP3 (?):
  - Regulation of energy expenditure
  - Regulation of fatty acid metabolism
  - Prevention of reactive oxygen species production (ROS)

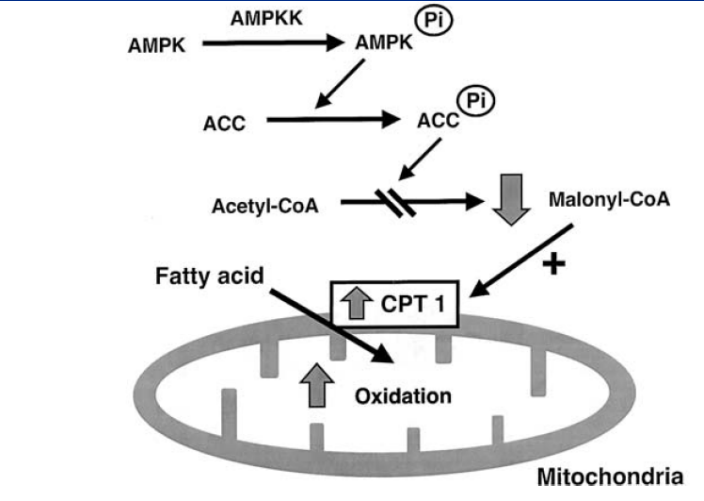
UCP3



Carnitine palmitoyltransferase-1 (CPT-1)

- CPT-1 is the rate limiting enzyme for transport of cytosolic long-chain acyl CoA molecules into the mitochondria for oxidation
- Malonyl CoA is an allosteric inhibitor of fatty acid oxidation via direct binding of CPT1

Carnitine palmitoyltransferase-1

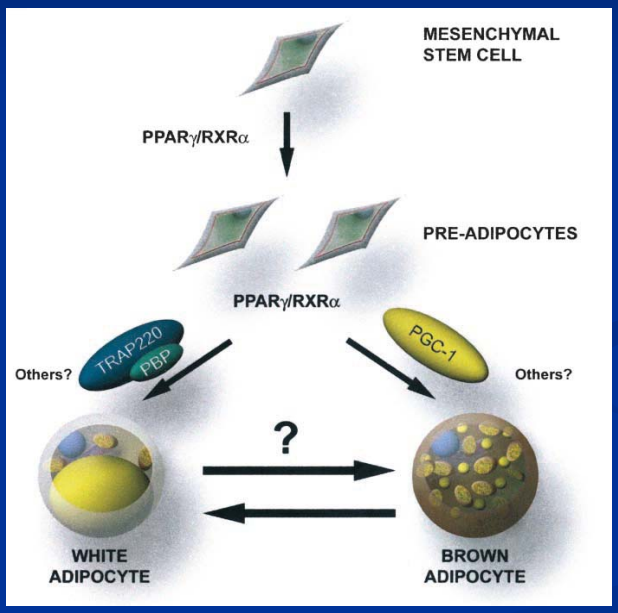


Y. Minokoshi and B. Kahn

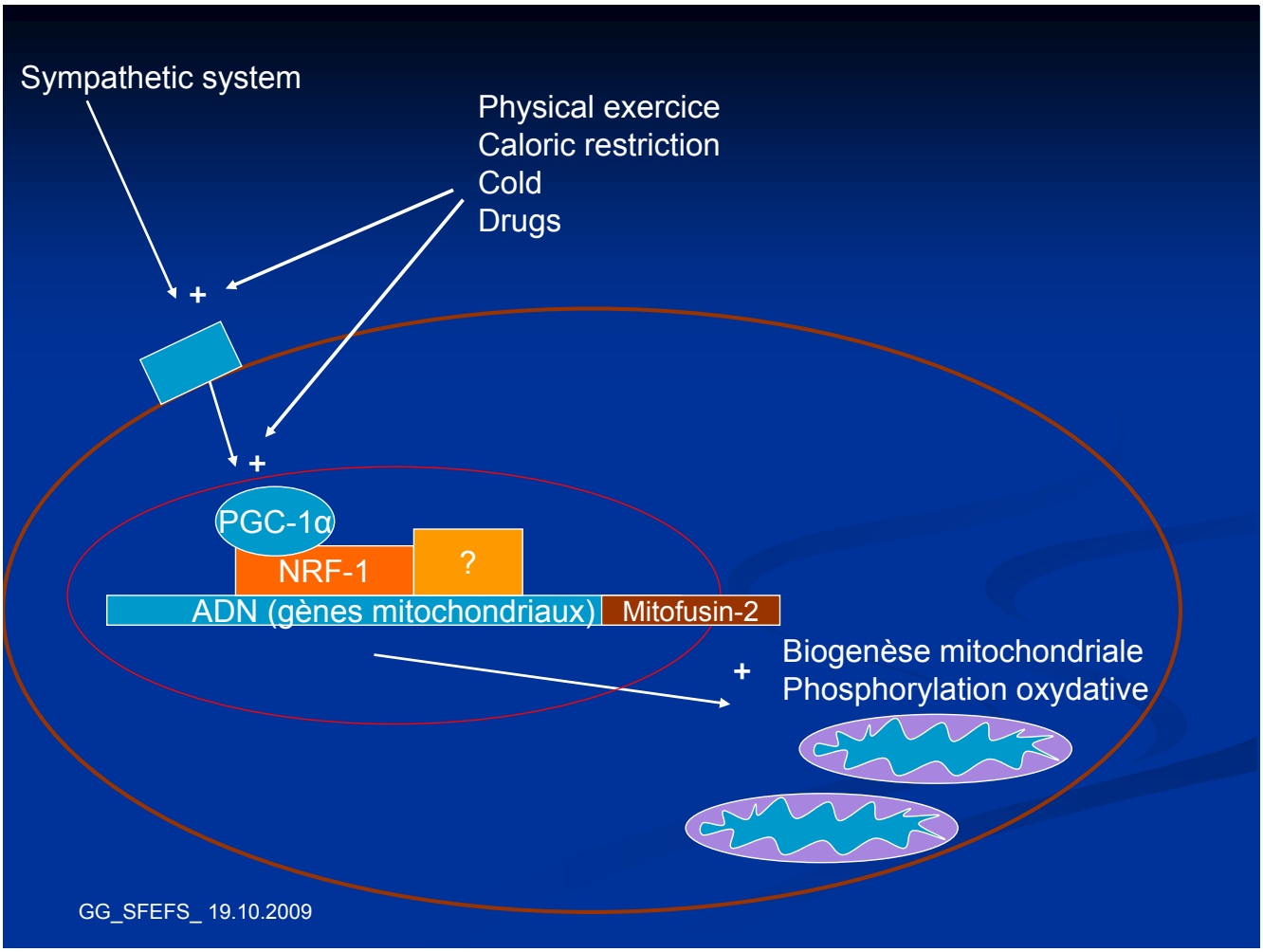
PPAR γ (Peroxisome proliferator-activated receptor) Co-activator 1 α

- A nuclear transcriptional coactivator implicated in :
  - Mitochondrial biogenesis
  - Adaptive thermogenesis
  - Insulin secretion
  - Gluconeogenesis

PPAR γ (Peroxisome proliferator-activated receptor) Co-activator 1 α(PGC-1 α)



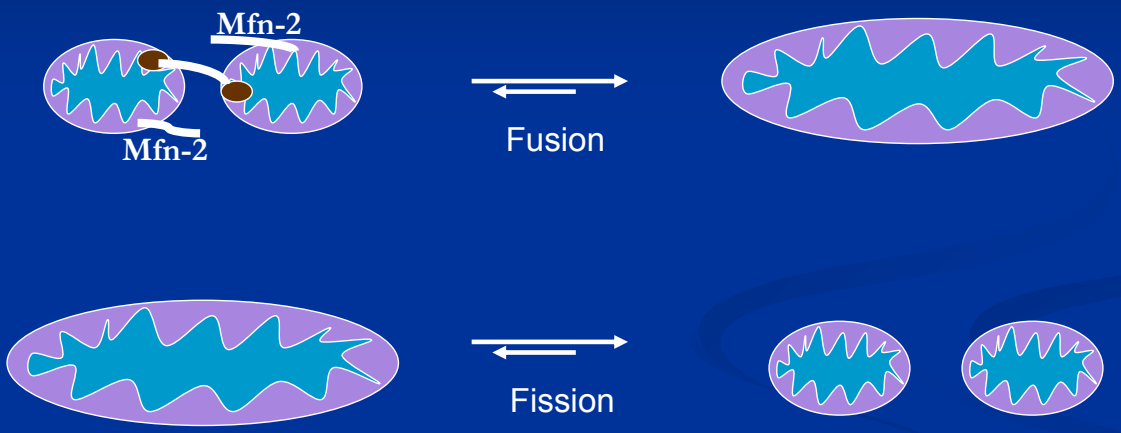
Puigserver, P. Endocr Rev, 2003. **24**(1): p. 78-90.



Mitofusin-2 (Mfn-2)

- Mfn-2 is a GTP-ase protein involved in mitochondrial fusion
- Mfn-2 increases the expression of GLUT4 and the mitochondrial oxidative capacity.

Mitofusin-2 (Mfn-2)



Upregulation of PGC1A during weight loss is related to insulin sensitivity but not to energy expenditure

Gastaldi et al., Diabetologia, 2007

The Aim of the Study

- To investigate the metabolic modifications accompanying surgically-induced body weight loss
- To better understand the regulation of genes which encode proteins involved in lipid oxidation such as Uncoupling protein 3 (UCP3) and PPAR-γ coactivator-1α (PGC-1α) in muscle.

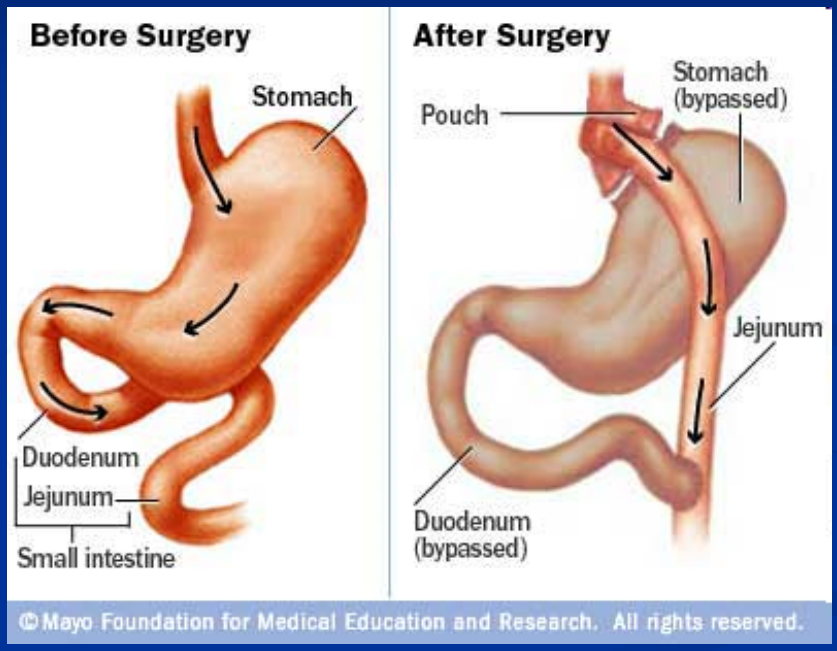
Subjects

- 17 Morbidly obese women
  - BMI (basal) 39 ± 2 kg/m2
  - Age 41 ± 2 years
- Before, 3 and 12 months post Roux-en-Y gastric bypass
  - 17 patients before and after 3months
  - 11 patients at 12 months

Methods and Measurements

- Body weight
- Glucose uptake (hyperinsulemic euglycemic clamp)
- Energy expenditure (indirect calorimetry)
- Plasma measurements :
  - Glucose (enzymatically)
  - Free Fatty Acids (FFA) (enzymatically)
  - Insulin (RIA)
- Muscle biopsy
  - UCP3 mRNA measurements (Quantitative PCR)
  - PGC-1α mRNA measurements (Quantitative PCR)

Roux-en-Y gastric bypass (RYGB)



Restrictive and malabsorptive surgical procedure

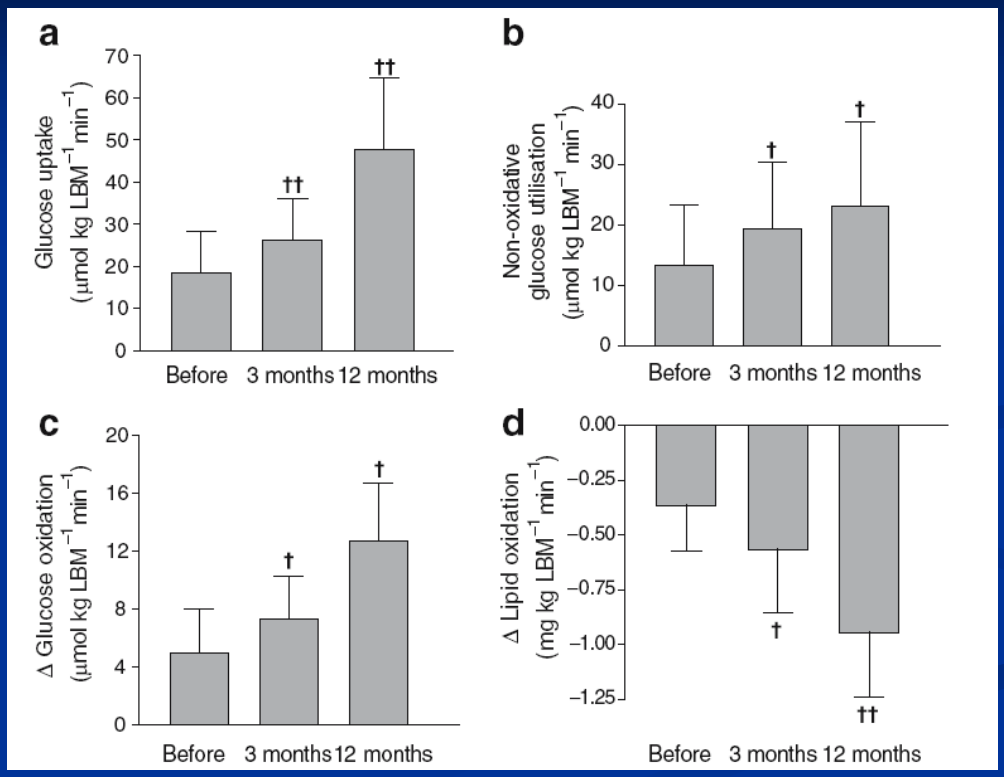
Provides large and Durable weight loss

Well known to improve Insulin resistance and cardiometabolic outcomes

Upregulation of PGC1A during weight loss is related to insulin sensitivity but not to energy expenditure

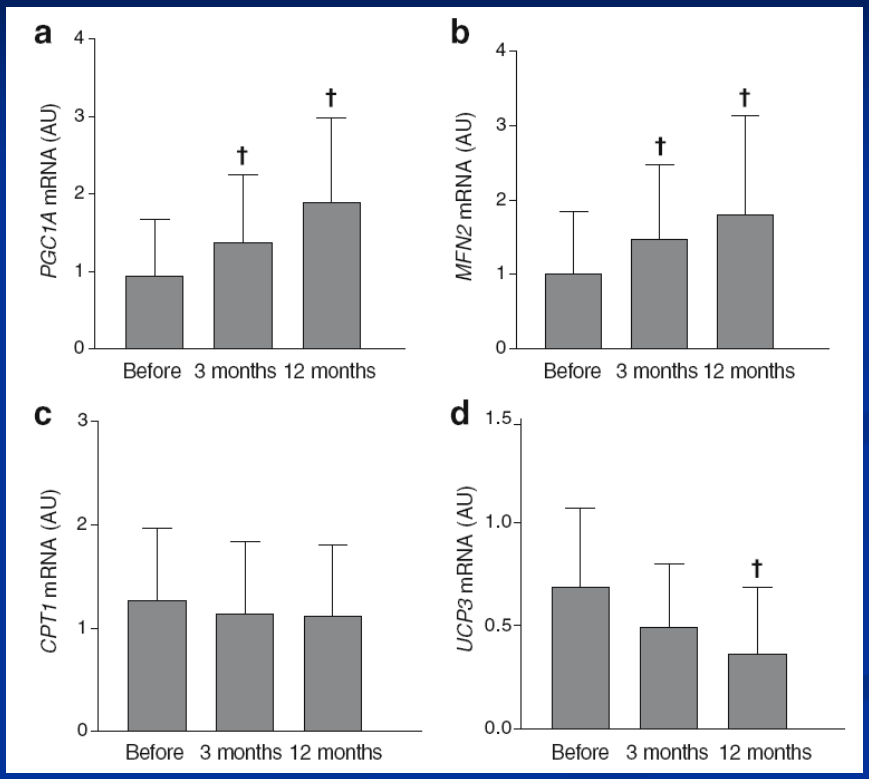
	Before RYGB (n=17)	3 months (n=17)	12 months (n=11)
Body weight (kg)	124.4±13 (127.8±12)	102.2±12[c]	82.5±13[c]
BMI (kg/m^2)	45.9±4 (46.1±3)	37.7±4[c]	30.5±5[c]
Plasma glucose (mmol/l)	5.8±1.1 (5.9±1.2)	4.9±0.4[a]	4.5±0.4[a]
Plasma insulin (pmol/l)	168.6±86 (170.3±86)	84.3±34[c]	56.8±34[b]
Energy expenditure (MJ/day)	8.28±0.4 (8.10±0.7)	6.80±0.8[b]	6.90±0.9[b]
Protein oxidation (mg kg LMB^{-1} min^{-1})	0.79±0.3 (0.84±0.2)	0.46±0.13[b]	0.74±0.3
Glucose oxidation (μmol kg LMB^{-1} min^{-1})	5.25±4.4 (5.45 ±2.8)	1.93±4.4	1.54±4.9[a]
Lipid oxidation (mg kg LMB^{-1} min^{-1})	1.66±0.5 (1.52±0.3)	1.88±0.5	1.98±0.3

Upregulation of PGC1A during weight loss is related to insulin sensitivity but not to energy expenditure



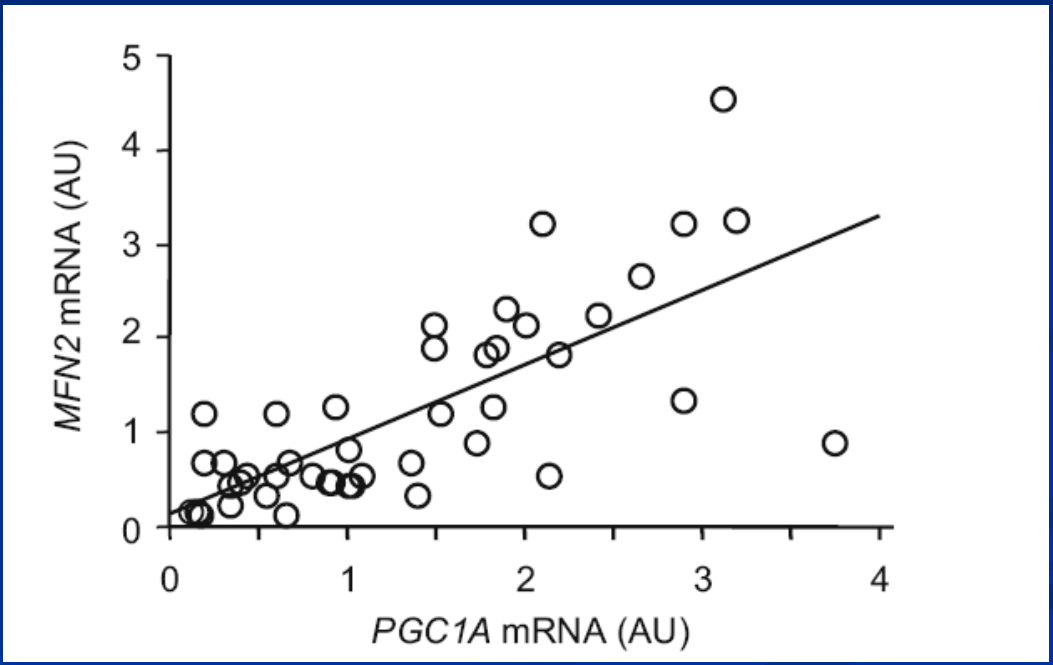
Gastaldi et al., Diabetologia, 2007

Upregulation of PGC1A during weight loss is related to insulin sensitivity but not to energy expenditure



Gastaldi et al., Diabetologia, 2007

Upregulation of PGC1A during weight loss is related to insulin sensitivity but not to energy expenditure



Gastaldi et al., Diabetologia, 2007

Simple regression

Table 3 Synthesis of simple regression analyses of BMI and muscle molecular parameters vs insulin sensitivity measured before and during RYGB-induced weight loss

Independent variable	Dependent variable	
	Glucose uptake	
	p value	r^2
PGC1A	0.005	0.17
MFN2	0.0001	0.29
UCP3	0.03	–0.10
BMI	0.0001	–0.29

Gastaldi et al., Diabetologia, 2007

Multiple regression

Table 4 Multiple regression analysis of factors influencing insulin sensitivity, i.e. glucose uptake

Independent variable	Dependent variable	
	Glucose uptake	
	p value	R^2
PGC1A	NS	0.48
MFN2	0.006	0.48
UCP3	NS	0.48
BMI	0.002	0.48

There was a significant and independent relationship of BMI (p= 0.002) and *MFN2* (p=0.006) with insulin sensitivity measured in morbidly obese women before and during RYGB-induced weight loss

Gastaldi et al., Diabetologia, 2007

Summary

- Surgically-induced body weight loss leads to :
  - ↑ glucose uptake
  - ↑ lipid oxidation
  - ↑ PGC-1α mRNA (at 3 and 12months), similar for NG and DT2 patients
  - ↓ UCP3 mRNA at 12months

Conclusion

- Massive weight loss upregulates PGC1A mRNA expression in skeletal muscle.
- This increase is associated with enhanced MFN2 expression, which contributes to the amelioration of insulin sensitivity.
- CPT1 or UCP3 mRNA expression does not show any significant impact on the modifications of insulin sensitivity or on the regulation of energy expenditure.

Perspectives

- To further analyse the link between lipid peroxidation, mitochondrial dysfunction and insulin sensitivity.
- To identify co-factors that have a permissive role for PGC1A and stimulates Mfn-2 and to determine whether these co-factors are activated in different physiological conditions

Acknowledgements

- Dr PD Elisabetta Bobbioni-Harsch
- Dr PhD Aaron Russell
- Prof. Alain Golay
- Prof. Jean-Paul Giacobino

Mes meilleurs remerciements à la fondation suisse pour l'encouragement de la recherche sur la nutrition en Suisse pour son soutien et sa générosité.

GG_SFEFS_ 19.10.2009

QUESTIONS

&

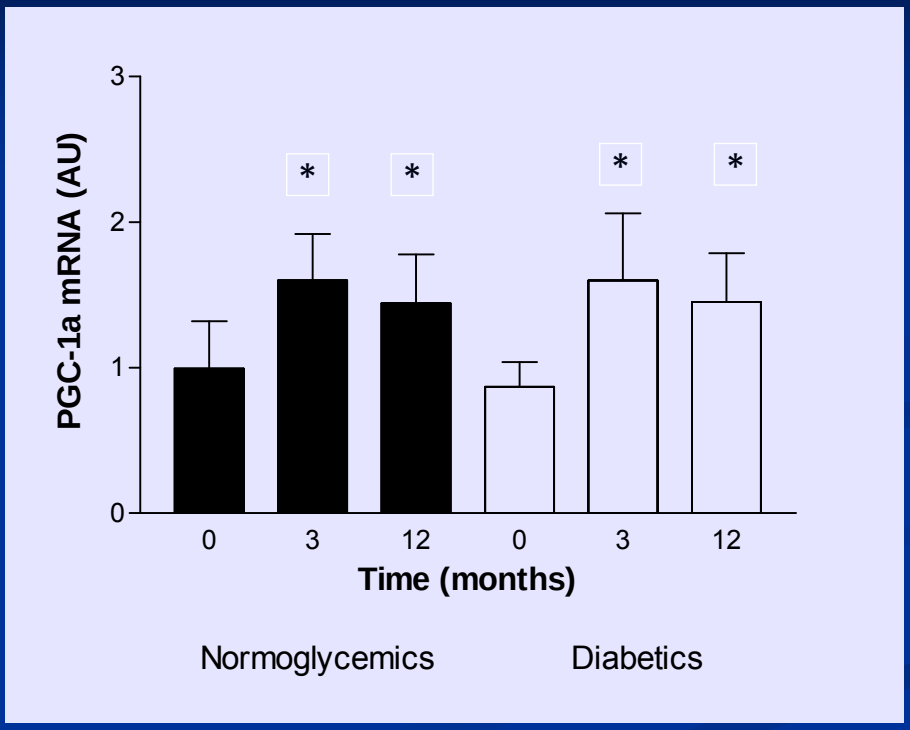
DISCUSSION

Subgroups analysis

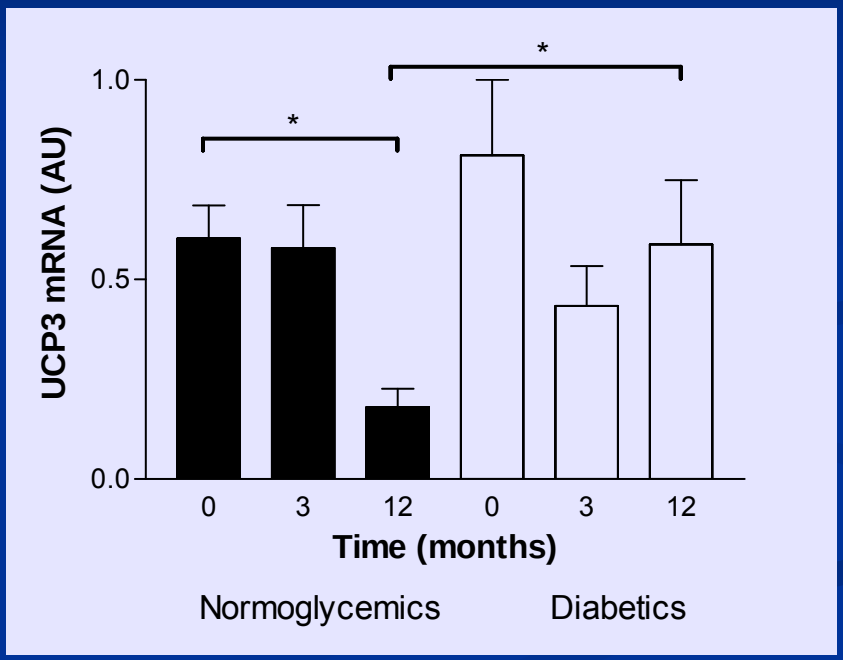
Before surgery:

- Normoglycemic patients (NG, n=10)
- Diabetic patients (D, n=7)

PGC-1α mRNA



UCP3 mRNA



*** = p< 0.04**

Subgroups Conclusions

- In morbidly obese stable body weight subjects diabetes is not associated to a lower PGC-1α mRNA expression in muscle
- Prolonged caloric restriction is accompanied to a sustained increased in PGC-1α mRNA
- During body weight loss the regulation of PGC-1α is similar in the NG and D obese subjects.

- Fatty acids increase the expression of UCP2 and UCP3 mRNA, implying that these UCPs are somehow involved in fatty-acid metabolism (Nedergaard and Cannon, 2003).
- UCP2 and UCP3 catalyze net proton conductance, but only when activated by fatty acids and free radical-derived alkenals (Brand et al., 2004a, 2004b).
- UCP2 and UCP3 can probably export fatty acids and other anions (Echtay et al., 1999; Jaburek et al., 1999).
- Mice in which UCP2 or UCP3 are knocked out show only weak phenotypes in the laboratory (Harper and Himms-Hagen, 2001).

Mitochondrie et IR

→Rotenone-sensitive NADH:02 oxidoreductase is 40% decreased in type 2 diabetic patients

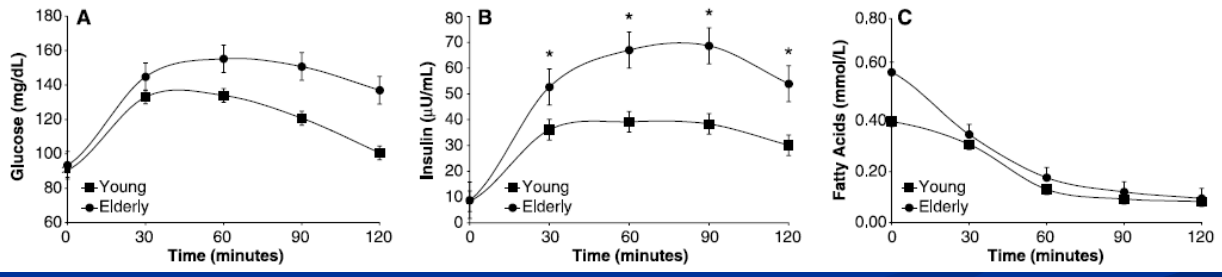
→Mitochondrial morphology is modified (small and spaced)

Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes

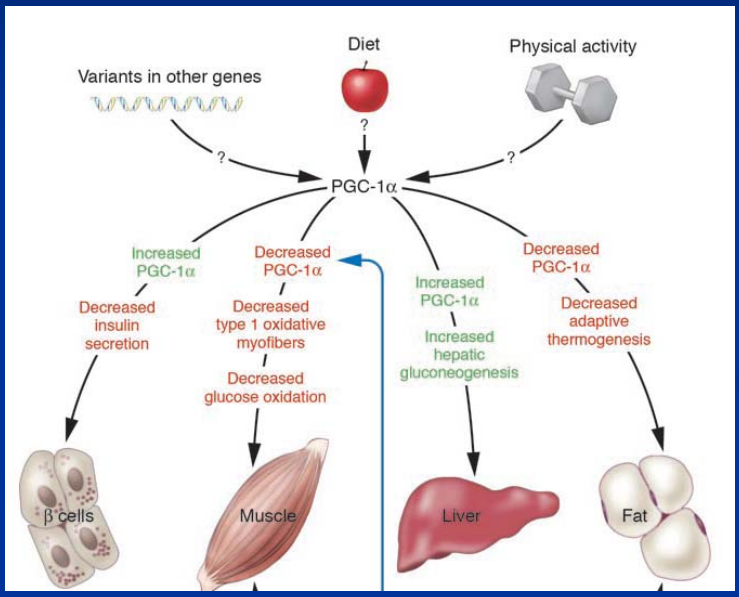
Kelley et al., Diabetes, 2002

Mitochondrial Dysfunction in the Elderly: Possible Role in Insulin Resistance

Kitt Falk Petersen,[1] Douglas Befroy,[1,7] Sylvie Dufour,[1,7] James Dziura,[1] Charlotte Ariyan,[3] Douglas L. Rothman,[4] Loretta DiPietro,[5,6] Gary W. Cline,[1] Gerald I. Shulman[1,2,7]*



Modulation de l'expression de PGC-1



↑ PGC-1alpha
Améliore performance physique
Switch expression fibres musc. type IIx en IIa et I

PGC-1alpha_KO
↓ Mitochondrie et perf. Physique

DT et pré-DT
↓ PGC-1alpha

Mitochondrial Dysfunction in the Elderly: Possible Role in Insulin Resistance

Table 1. Body composition of study participants.

	Age (years)	Body weight (kg)	Fat mass (kg)	% Fat mass (% body weight)	LBM (kg)	BMI (kg/m²)
Young (n = 13)	27 ± 2	71 ± 4	19.9 ± 2.5	28 ± 3	54 ± 5	23.8 ± 1.1
Elderly (n = 15)	70 ± 2	70 ± 3	20.1 ± 1.7	29 ± 2	49 ± 3	25.1 ± 0.5
P value	<0.0001	0.69	0.93	0.77	0.28	0.28

↑ **IR**

Table 2. Metabolic rates and tissue lipid content of participants (*24*).

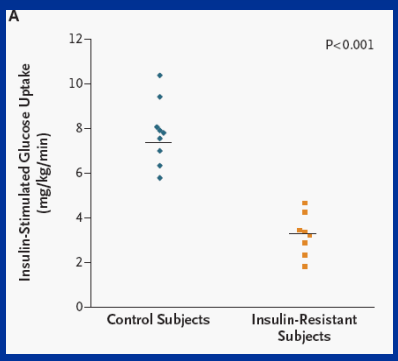
	Basal rates of glucose production (mg/kg of LBM/min)	Clamp peripheral glucose metabolism rate (mg/kg of LBM/min)	Intramyocellular lipid content (%)	Intrahepatic lipid content (%)	Mitochondrial TCA flux rate (nmol/g of muscle/min)	Mitochondrial ATP synthesis rate (μmol/g of muscle/min)
Young	2.3 ± 0.1	6.2 ± 0.6	0.96 ± 0.08	0.49 ± 0.10	96 ± 10	7.50 ± 0.77
Elderly	2.4 ± 0.1	4.0 ± 0.4	1.39 ± 0.15	1.61 ± 0.38	62 ± 5	4.06 ± 0.65
P value	0.34	<0.002	0.035	0.036	<0.006	<0.004

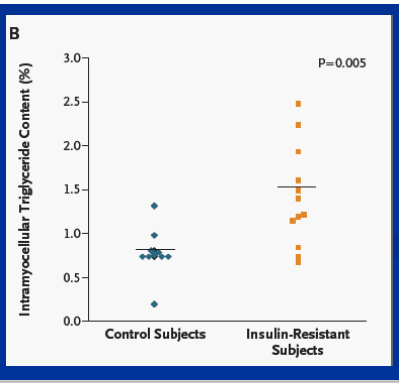
↑ **IMLC**

↓ **OX et PHOS**

Impaired Mitochondrial Activity in the Insulin-Resistant Offspring of Patients with Type 2 Diabetes

Kitt Falk Petersen, M.D., Sylvie Dufour, Ph.D., Douglas Befroy, Ph.D., Rina Garcia, B.A., and Gerald I. Shulman, M.D., Ph.D.





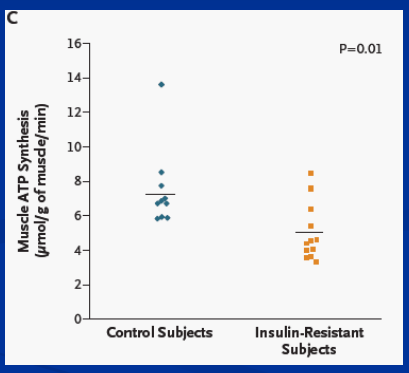


Table 1. Characteristics of the Two Groups of Subjects.*

Characteristic	Insulin-Sensitive Controls	Insulin-Resistant Subjects
Age (yr)	28±7	26±7
Weight (kg)	60±13	64±9
Height (m)	1.69±0.11	1.65±0.09
Body-mass index	21±2	23±2
Activity index†	2.6±0.5	2.4±0.4
Glycosylated hemoglobin (%)‡	5.1±0.3	5.2±0.4
Adipocyte-derived factors		
Adiponectin (μg/ml)	12±4	11±4
Tumor necrosis factor α (pg/ml)	1.5±0.3	1.8±0.9
Interleukin-6 (pg/ml)	0.52±0.31	0.68±0.42
Resistin (ng/ml)	0.77±0.24	0.79±0.24

lipolysis

- Fasting : low insulin, low catecholamines, low glucose
- Stress : high catecholamine
- Obesity : high insulin, high FFA, high glucose

- Fatty acids increase the expression of UCP2 and UCP3 mRNA, implying that these UCPs are somehow involved in fatty-acid metabolism (Nedergaard and Cannon, 2003).
- UCP2 and UCP3 catalyze net proton conductance, but only when activated by fatty acids and free radical-derived alkenals (Brand et al., 2004a, 2004b).
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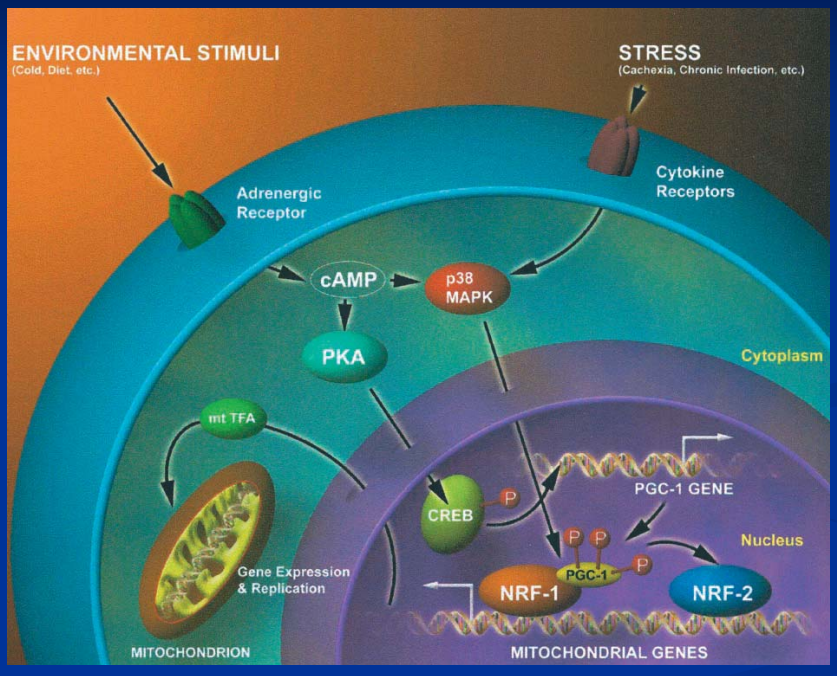
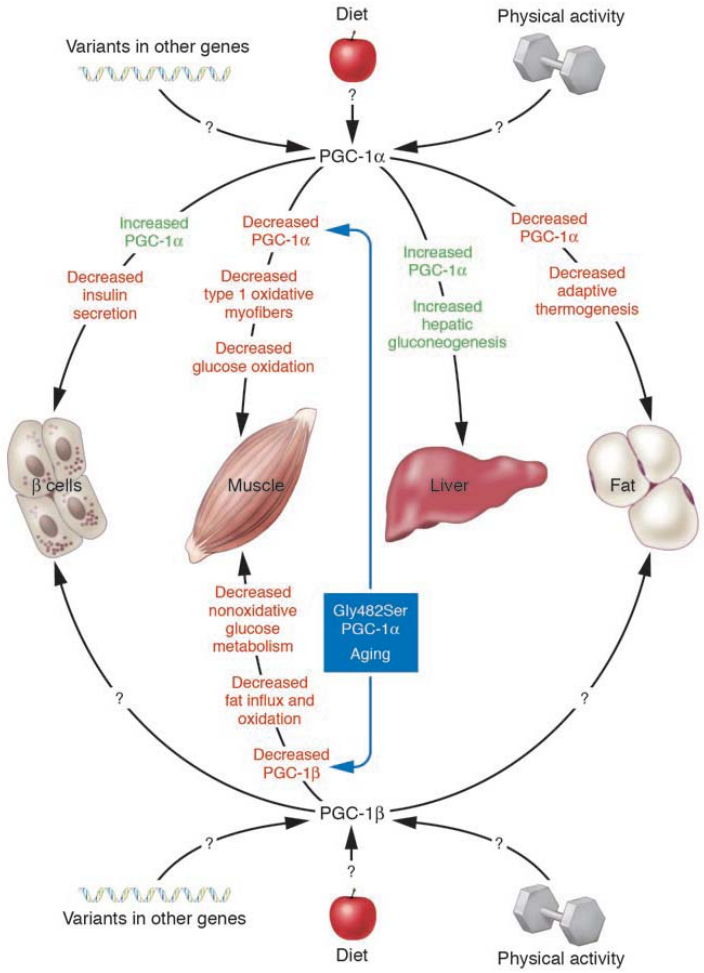
- Mice in which UCP2 or UCP3 are knocked out show only weak phenotypes in the laboratory (Harper and Himms-Hagen, 2001).

PGC-1



- Biogenèse Mitochondriale
- Thermogenèse adaptative
- Secretion insulin
- Néoglucogenèse.

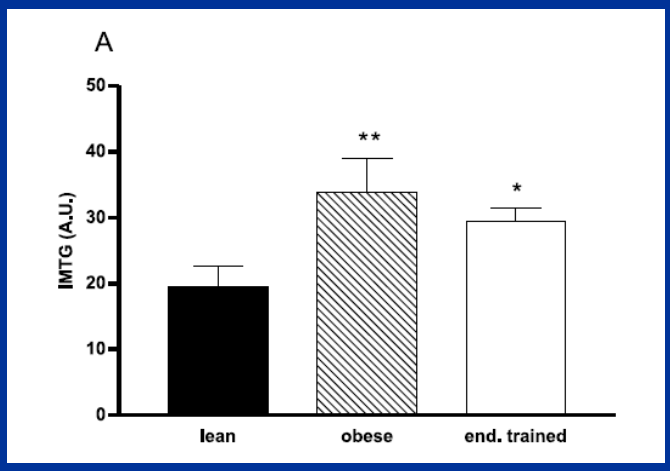
Puigserver, P. Endocr Rev, 2003. **24**(1): p. 78-90.

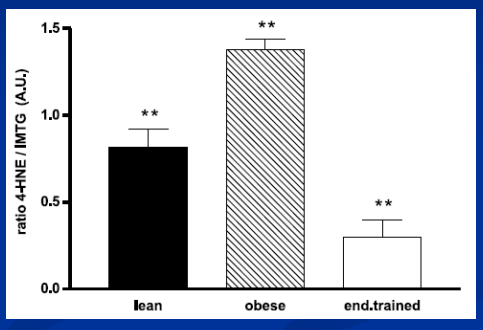


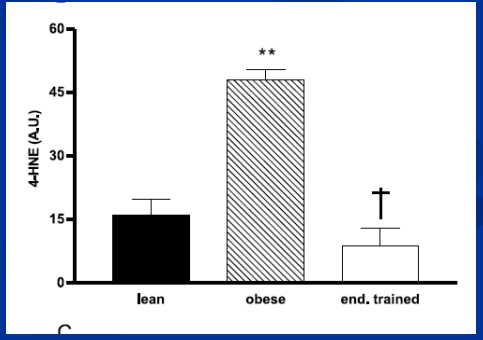
FEBS Letters 551 (2003) 104–106 FEBS 27574

Lipid peroxidation in skeletal muscle of obese as compared to endurance-trained humans: a case of good vs. bad lipids?

Aaron P. Russell[a,b,*], Giacomo Gastaldi[b,c], Elisabetta Bobbioni-Harsch[c], Patrizia Arboit[b], Charles Gobelet[a], Olivier Dériaz[a], Alain Golay[c], Joseph L. Witztum[d], Jean-Paul Giacobino[b]







Impaired in vivo mitochondrial function but similar intramyocellular lipid content in patients with type 2 diabetes mellitus and BMI-matched control subjects

V. B. Schrauwen-Hinderling · M. E. Kooi · M. K. C. Hesselink · J. A. L. Jeneson · W. H. Backes · C. J. A. van Echteld · J. M. A. van Engelshoven · M. Mensink · P. Schrauwen

Diabetologia (2007) 50:113–120
DOI 10.1007/s00125-006-0475-1

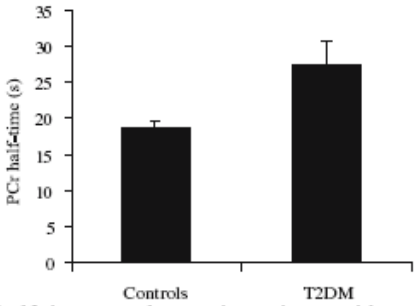


Fig. 4 PCr half-time was longer in patients with type 2 diabetes mellitus (T2DM) than BMI-matched controls ($p<0.05$)

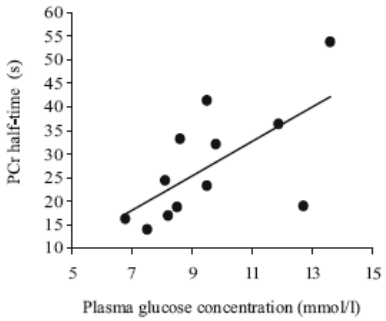


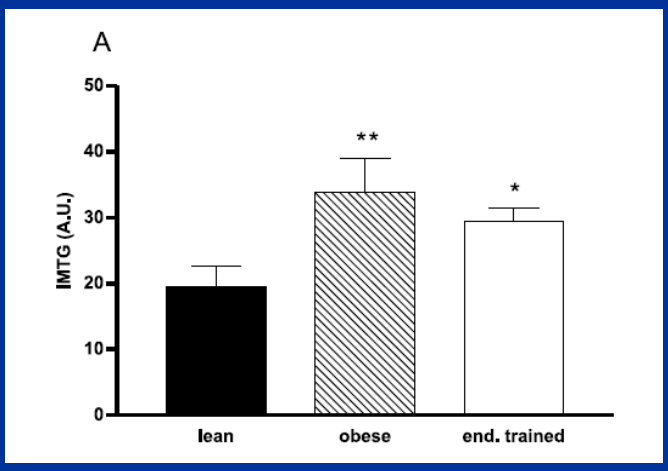
Fig. 5 Plasma glucose concentration correlates with the PCr half-time in diabetes patients ($r^2=0.42$, $p<0.01$)

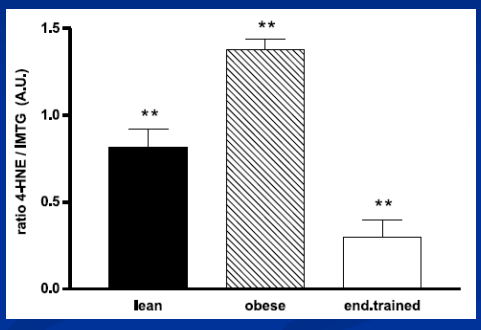
GG_SFEFS_ 19.10.2009

FEBS Letters 551 (2003) 104–106 FEBS 27574

Lipid peroxidation in skeletal muscle of obese as compared to endurance-trained humans: a case of good vs. bad lipids?

Aaron P. Russell[a,b,*], Giacomo Gastaldi[b,c], Elisabetta Bobbioni-Harsch[c], Patrizia Arboit[b], Charles Gobelet[a], Olivier Dériaz[a], Alain Golay[c], Joseph L. Witztum[d], Jean-Paul Giacobino[b]





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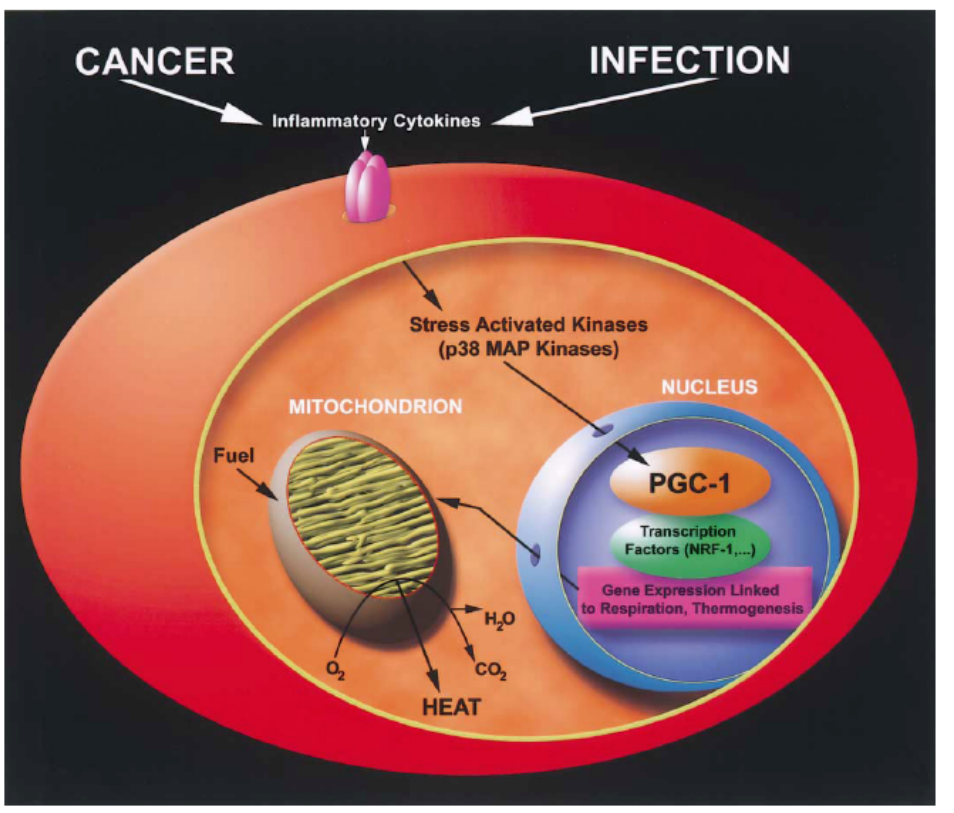
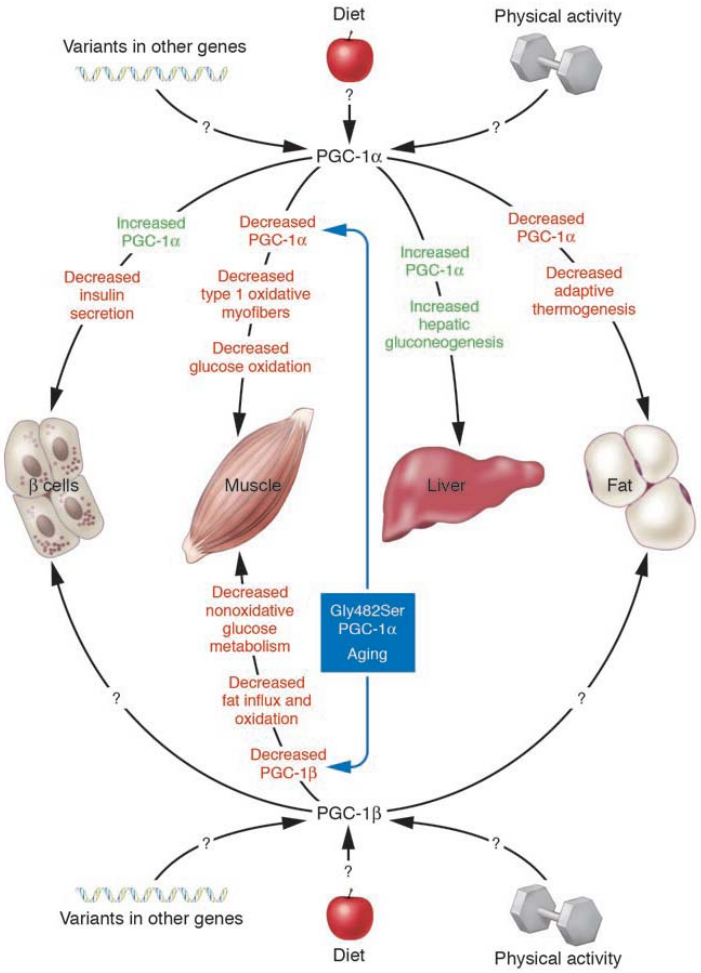
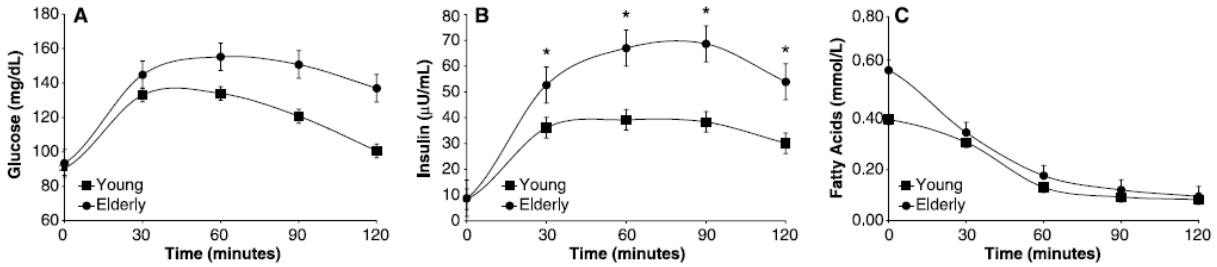


Molecular Cell, Vol. 8, 971–982, November, 2001, Cytokine Stimulation of Energy Expenditure through p38 MAP Kinase Activation of PPAR Coactivator-1



Mitochondrial Dysfunction in the Elderly: Possible Role in Insulin Resistance

Kitt Falk Petersen,[1] Douglas Befroy,[1,7] Sylvie Dufour,[1,7] James Dziura,[1] Charlotte Ariyan,[3] Douglas L. Rothman,[4] Loretta DiPietro,[5,6] Gary W. Cline,[1] Gerald I. Shulman[1,2,7]*



Mitochondrial Dysfunction in the Elderly: Possible Role in Insulin Resistance

Table 1. Body composition of study participants.

	Age (years)	Body weight (kg)	Fat mass (kg)	% Fat mass (% body weight)	LBM (kg)	BMI (kg/m^2)
Young (n = 13)	27 ± 2	71 ± 4	19.9 ± 2.5	28 ± 3	54 ± 5	23.8 ± 1.1
Elderly (n = 15)	70 ± 2	70 ± 3	20.1 ± 1.7	29 ± 2	49 ± 3	25.1 ± 0.5
P value	<0.0001	0.69	0.93	0.77	0.28	0.28

Table 2. Metabolic rates and tissue lipid content of participants (*24*).

	Basal rates of glucose production (mg/kg of LBM/min)	Clamp peripheral glucose metabolism rate (mg/kg of LBM/min)	Intramyocellular lipid content (%)	Intrahepatic lipid content (%)	Mitochondrial TCA flux rate (nmol/g of muscle/min)	Mitochondrial ATP synthesis rate (μmol/g of muscle/min)
Young	2.3 ± 0.1	6.2 ± 0.6	0.96 ± 0.08	0.49 ± 0.10	96 ± 10	7.50 ± 0.77
Elderly	2.4 ± 0.1	4.0 ± 0.4	1.39 ± 0.15	1.61 ± 0.38	62 ± 5	4.06 ± 0.65
P value	0.34	<0.002	0.035	0.036	<0.006	<0.004

↑ IR

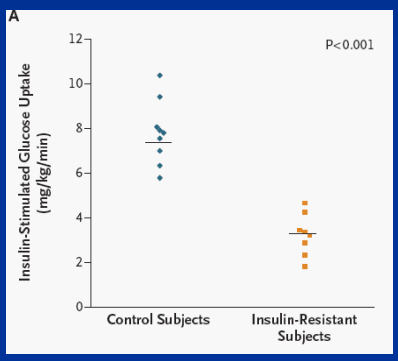
↑ IMLC

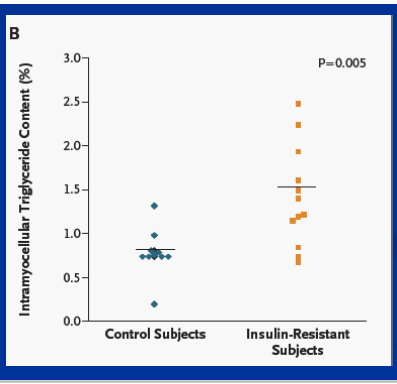
↓ OX et PHOS

GG_SFEFS_ 19.10.2009

Impaired Mitochondrial Activity in the Insulin-Resistant Offspring of Patients with Type 2 Diabetes

Kitt Falk Petersen, M.D., Sylvie Dufour, Ph.D., Douglas Befroy, Ph.D., Rina Garcia, B.A., and Gerald I. Shulman, M.D., Ph.D.





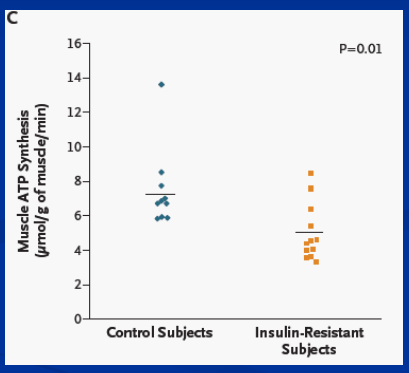


Table 1. Characteristics of the Two Groups of Subjects.*

Characteristic	Insulin-Sensitive Controls	Insulin-Resistant Subjects
Age (yr)	28±7	26±7
Weight (kg)	60±13	64±9
Height (m)	1.69±0.11	1.65±0.09
Body-mass index	21±2	23±2
Activity index†	2.6±0.5	2.4±0.4
Glycosylated hemoglobin (%)‡	5.1±0.3	5.2±0.4
Adipocyte-derived factors		
Adiponectin (μg/ml)	12±4	11±4
Tumor necrosis factor α (pg/ml)	1.5±0.3	1.8±0.9
Interleukin-6 (pg/ml)	0.52±0.31	0.68±0.42
Resistin (ng/ml)	0.77±0.24	0.79±0.24

Mitochondria and IR

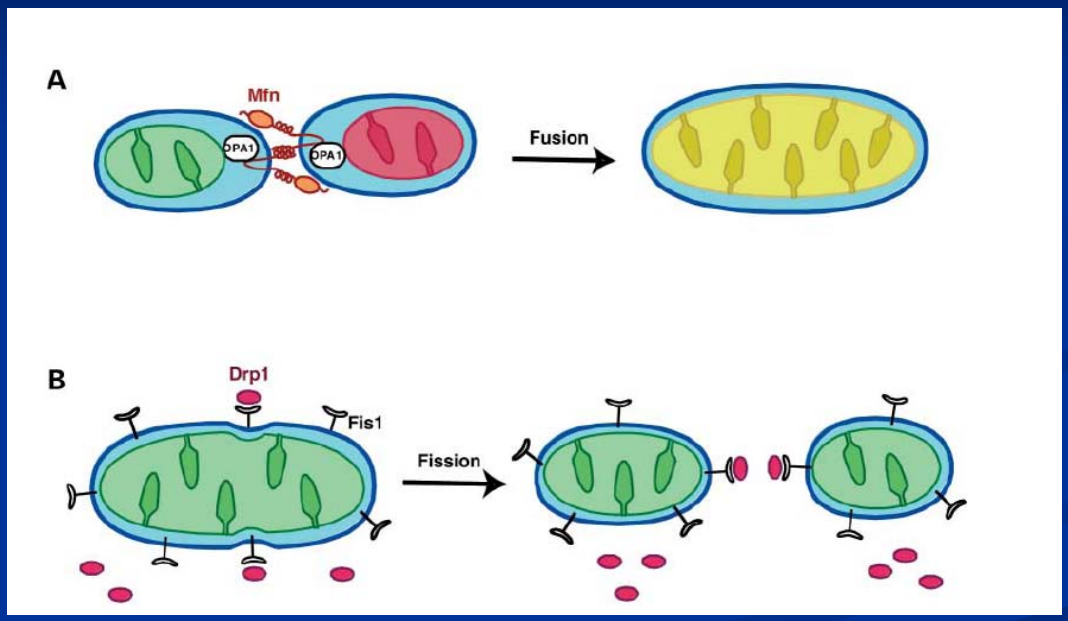
Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes

Kelley et al., Diabetes, 2002

→ Type 2 diabetic patients show a reduction of 40% in NADH:02 oxidoreductase

→ Changes in mitochondrial morphology (small and separated)

Mitofusin-2 (Mfn-2)



Chen H and David Chan, Human molecular Genetics, 2005