The FXR agonist obeticholic acid normalizes lipid droplet and triglyceride handling in visceral adipose tissue preadipocytes from a non-genomic rabbit model of metabolic syndrome

E. Maneschi, L. Vignozzi, A. Morelli, T. Mello, S. Filippi, I. Cellai, P. Comeglio, E. Sarchielli, A. Calcagno, R. Vettor, GB. Vannelli, L. Adorini and M. Maggi.

Department of Experimental and Clinical Biomedical Sciences, University of Florence, Italy

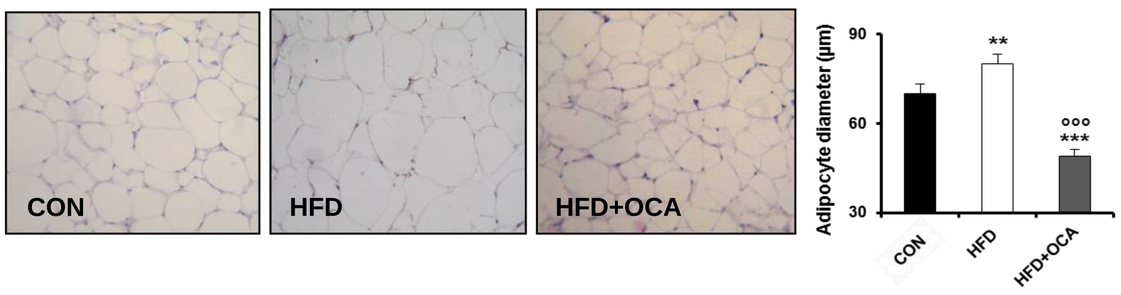
Introduction: Adipose tissue (AT) dysfunction is characterized by ectopic fat deposition in the abdominal viscera and liver, inflammatory and adipokine dysregulation, and insulin resistance and may be a more important mediator than total fat mass of type 2 diabetes, hypertension and dyslipidaemia development, all these features clustering in the metabolic syndrome (MetS). We recently demonstrated that the selective FXR agonist obeticholic acid (OCA) ameliorates the metabolic profile and reduces visceral AT (VAT) in a high-fat diet (HFD)-induced rabbit model of MetS (1).

Aim: We studied the effects of *in vivo* OCA dosing on the adipogenic capacity of isolated VAT preadipocytes (rPAD) from MetS rabbits, compared to control diet (CON).

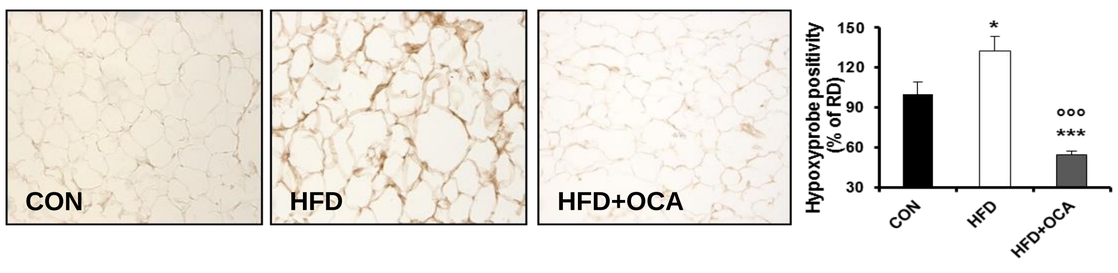
Methods: VAT and liver were studied by immunohistochemistry, western blot, and RT-PCR. Isolated rPAD were exposed to adipocyte differentiating mixture (DIM) (0.5 Mm 3-isobutyl-1-methylxanthine, 5µg/ml insulin, 1µM dexamethasone) for 10 days to evaluate adipogenic potential.

Analysis of adipocyte size, hypoxia and GLUT4 membrane translocation in VAT from experimental rabbits

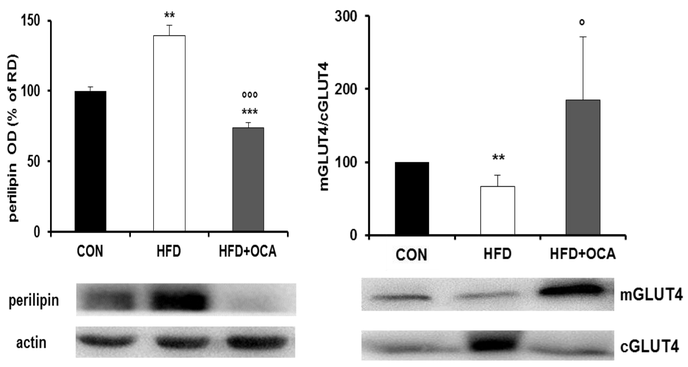
Histomorphometric analysis



Immunohistochemical staining



Western Blot analysis



Adipocyte size, hypoxia, expression of perilipin and cytosolic GLUT4 were significantly **increased** in HFD VAT and **normalized** by OCA.

**p<0.01, ***p<0.0001 vs. CON; °p<0.05, °°p<0.01, °°°p<0.0001 vs. HFD.

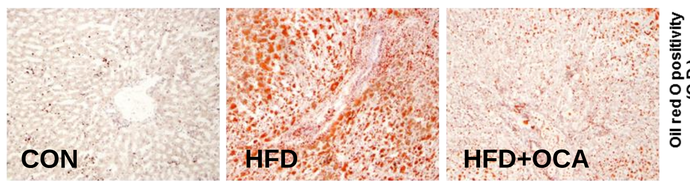
Effect of OCA treatment on mRNA expression of VAT-specific genes

Genes	% variation, HFD+OCA vs. HFD
SHP	274.3±92.6 **
FABP4	- 47±11.3 **
c/EBPα	- 61.2±12.3 ***
LPL	- 49.6±7.9 *
leptin	- 58.2±23 *
GLUT4	- 31.7±8.7 *
IRS-1	- 32±3.9 **
RhoA	- 37±8.2 **
Rock1	- 34.8±7.8 **
Rock2	- 56±16.1 **
DGAT2	- 63.5±17.3 *
PR	- 42.3±8.1 *
VIM	- 17.7±2.3
αSMA	- 48.8±15.8
MCP1	- 13.7±5.1
eNOS	- 4.8±1
ERα	- 22±5.8
PKG1	- 21.4±4.7

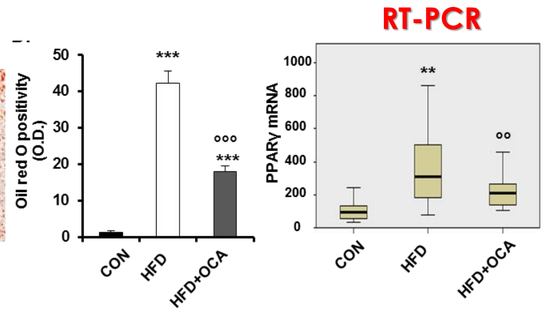
Data are expressed as percentage of variation vs. HFD. *p<0.05; **p<0.01; ***p<0.001 vs. HFD

OCA ameliorates HFD-induced liver steatosis and inflammation

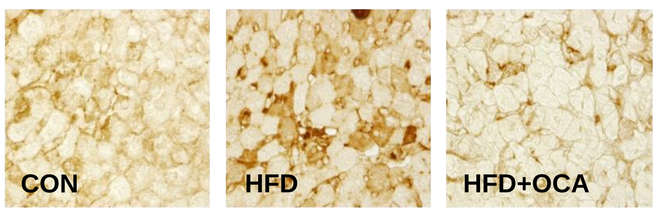
Oil Red O staining



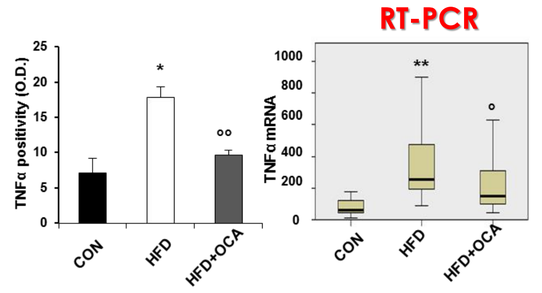
RT-PCR



Immunohistochemistry for TNFα



RT-PCR

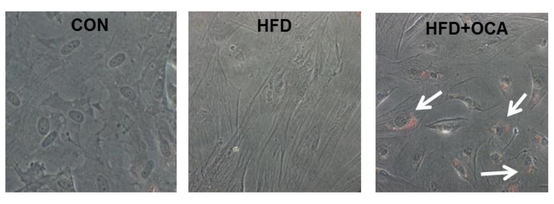


*p<0.01 **p<0.001 *** p<0.0001 vs. RD; °p<0.05, °°p<0.01, °°°P<0.0001 vs. HFD

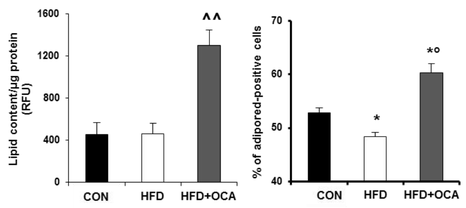
Effects of OCA treatment on adipogenic capacity in rPAD from all groups

OCA ameliorates spontaneous adipogenic differentiation in untreated rPAD

Oil Red O staining



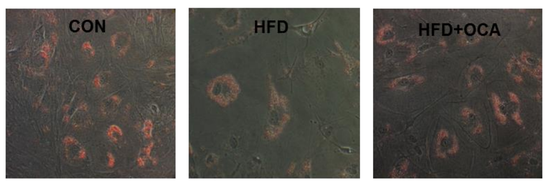
Adipored assay



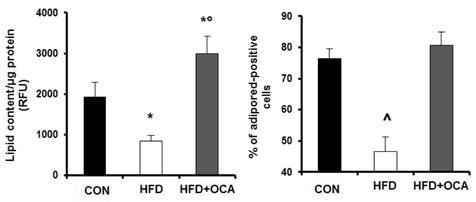
^p<0.01; ^^p<0.001 vs. all other groups *p<0.01 vs. RD; °p<0.01 vs. HFD.

OCA ameliorates DIM-induced adipogenic differentiation in rPAD

Oil Red O staining



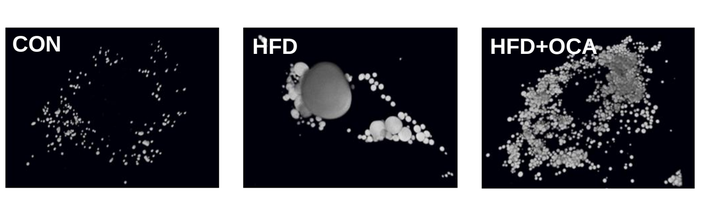
Adipored assay



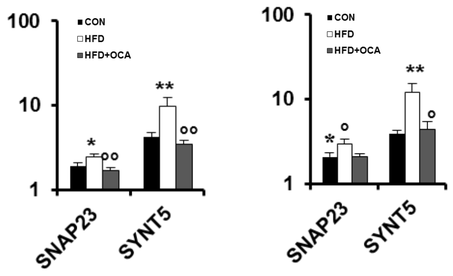
^p<0.01; ^^p<0.001 vs. all other groups *p<0.01 vs. RD; °p<0.01 vs. HFD.

OCA positively affects the lipid droplets fusion processes

Confocal microscopy

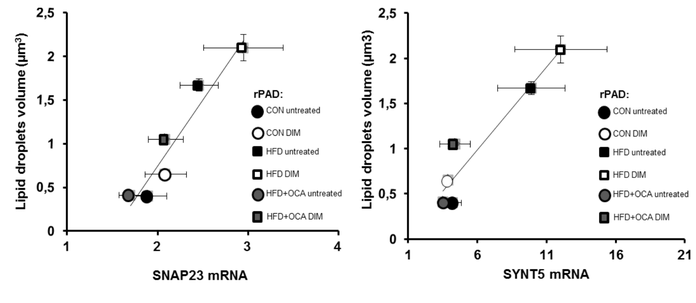


untreated rPAD
(relative expression profile)



DIM rPAD
(relative expression profile)

Relationship between the lipid droplet volume and the SNAP23 or SYNT5 mRNA expression



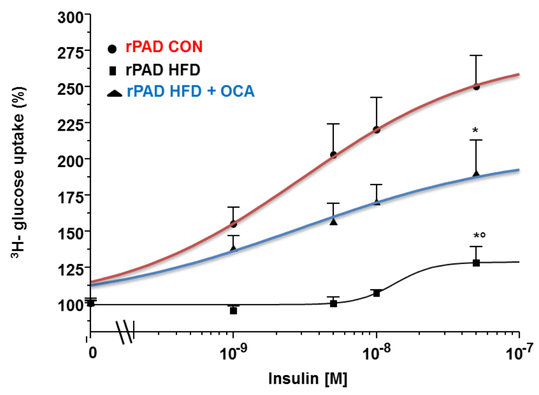
*p<0.05 **p<0.01 ***p<0.0001vs. RD; °p<0.05, °°p<0.01, °°°p<0.0001 vs. HFD

Quantitative real time RT-PCR of adipocyte-specific genes in rPAD

	CON	HFD	HFD+OCA
Adipocyte-related genes			
DKK1	6.4±2**	1.5±0.3°	13.6±2.1**
c/EBPα	2.3±0.5**	1.2±0.3°	2.5±0.5**
PPARγ	2.5±0.5**	1.1±0.3°	1.7±0.1**
FABP4	20.6±7**	5.3±1.1**	10.9±3.6**
adiponectin	9.5±4.3**	0.9±0.1°	2.6±0.7**
leptin	8.7±2.6**°	0.7±0.2	1.8±0.4
CCND1	0.8±0.3	2.6±0.7*	1.1±0.1
CCND3	2.3±0.5*^	1.2±0.3	1.9±0.3**^^

*p<0.05;**p<0.01 vs. relative time 0; °p<0.01 vs. all other groups; ^p<0.05; ^^p<0.01 vs. relative CCND1.

Insulin sensitivity of DIM-exposed rPAD (glucose uptake)



***p<0.01 vs. RD;**
°p<0.01 vs. HFD+OCA

CON and HFD+OCA shared EC_{50}= 2.96±0.51 nM;
HFD EC_{50}= 13.5±6.09 nM

CON E_{max} = 273±3%; HFD E_{max} = 128±4%

HFD+OCA E_{max}= 205±3%

Conclusions

Overall, OCA dosing in a MetS rabbit model ameliorates liver and VAT functions. This could reflect the ability of OCA to restore insulin sensitivity in AT unable to finalize its storage function, counteracting MetS-induced metabolic alterations and pathological AT deposition.

1. Vignozzi L. et al. Farnesoid X receptor activation improves erectile function in animal model of metabolic syndrome and diabetes , The Journal of Sexual Medicine, 2011