The dual FXR/TGR5 agonist INT-767 reduces visceral fat mass, promoting preadipocyte brown differentiation, mitochondrial function and insulin sensitivity in a rabbit model of high fat diet-induced metabolic syndrome

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Introduction and objectives

Expanding brown adipose tissue is a potential therapeutic strategy to counteract insulin resistance and metabolic syndrome (MetS). **Farnesoid X receptor** (FXR) and **takeda G protein-coupled receptor 5** (TGR5) activation enhances insulin sensitivity, suggesting the capacity of FXR/TGR5 agonists to promote brown differentiation in adipose tissue.

The aim of this study is to investigate the effect of a FXR/TGR5 agonist on visceral adipose tissue (VAT) by using a rabbit model of high fat diet (HFD)-induced MetS.

Methods

We employed a recently established **animal model of high fat diet (HFD)-induced MetS**, characterized by insulin resistance, hypertension, atherogenic dyslipidemia and VAT accumulation (Filippi et al., 2009; doi: 10.1111/j.1743-6109.2009.01467.x.). **Subgroups of MetS rabbits were treated with increasing doses of the dual FXR/TGR5 agonist INT-767 (3, 10, 30mg/Kg, orally, daily, 5 days a week for 12 weeks)**. Rabbits fed with a regular diet (RD) were taken as control. We studied the effects of HFD and *in vivo* INT-767 treatments on VAT function and the adipogenic potential of rabbit preadipocytes (rPAD) isolated from VAT of regular diet (RD), HFD, and INT-767-treated HFD rabbits. VAT was studied by immunohistochemistry, western blot, and RT-PCR. Isolated rPADs were cultured for 10 days in a standard growth medium (to evaluate the spontaneous adipogenic potential) or were exposed *in vitro* to a differentiating mixture (insulin, dexamethazone, isobutylmethylxantine, DIM), known to favor white adipogenic phenotyping.

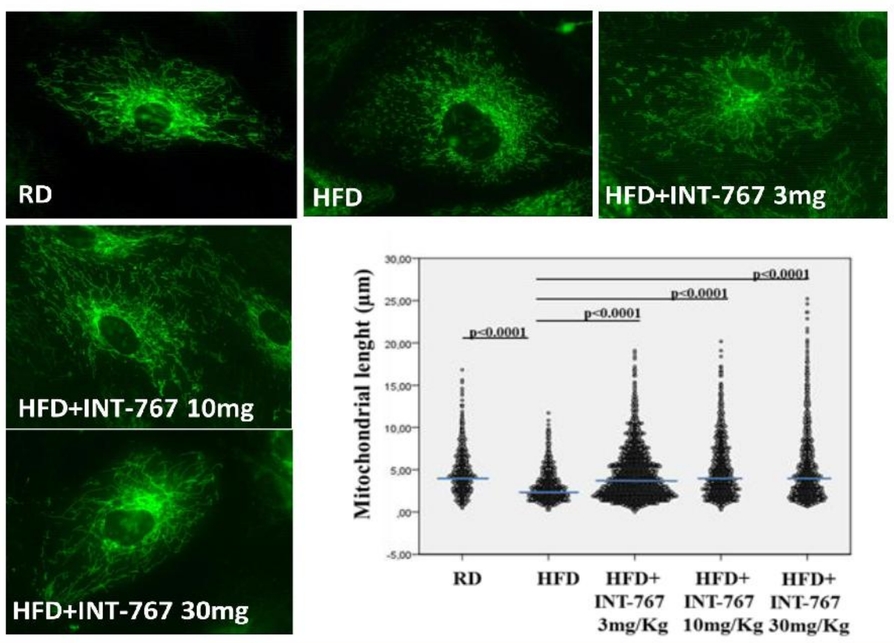
Results

Beneficial metabolic effects of INT-767 treatments in HFD-induced MetS model

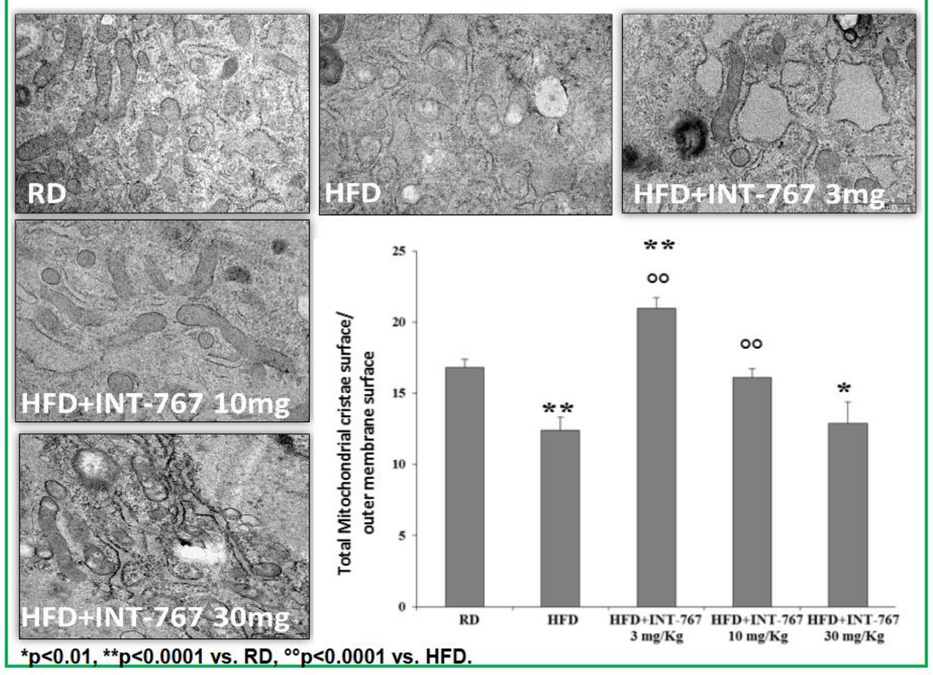
INT-767, at all doses tested, significantly **reduced VAT mass**, even below the RD level. INT-767 reduced dose dependently several parameters related to MetS, such as **hyperglycemia**, **glucose intolerance** and **hypercholesterolemia**. At all tested doses of INT-767 we found **increased HDL levels**, compared to either RD or HFD groups. **HFD-induced hypertriglyceridemia was reduced by all INT-767** treatments, without reaching statistical significance when compared to HFD. However, INT-767 restored in all the treated groups triglyceride levels up to the RD level . In contrast, HFD-induced increase in MAP was not affected by any treatment. **Prevalence of MetS** (three or more factors higher than two standard deviations of values recorded in RD rabbits) was decreased from 63.6% in HFD to 40%, 0% and 0% in INT-767 3, 10, 30 mg/Kg, respectively

INT-767 treatment improves mitochondrial function and enhances in preadipocytes expression of genes involved in brown adipogenesis and mitochondrial biogenesis.

INT-767 treatment improves mitochondrial function. The mitochondrial function was visualized by incubation with the mitochondria-targeted fluorescent probe (MitoTracker) in rPAD

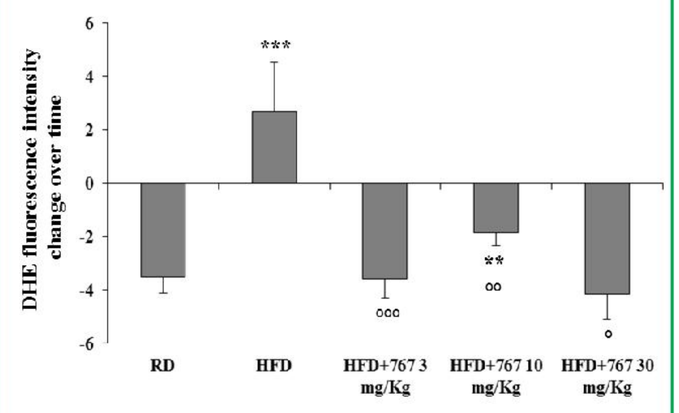


Effect of in vivo INT-767 treatment on mitochondrial ultrastructure in rPAD. This analysis was performed using transmission electron microscopy.



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

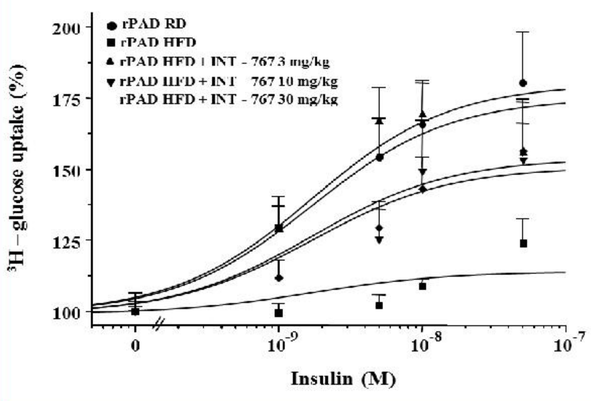
Effect of in vivo INT-767 treatment on superoxide production in rPAD stained with 10 µM dihydroethidium (DHE) and imaged for 3 minutes. (f) The bar-graph show changes in integrated fluorescence intensity measured in the nuclei of rPAD cells during time lapse imaging.



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

Quantitative RT-PCR analysis

Moreover, *in vivo* treatments of HFD rabbits with different doses of **INT-767 increased** mRNA **expression** of several **genes involved in brown adipogenesis** (*UCP1*, *CIDEA*, *BMP4*, *BMP7*, *HOXC9*, *TMEM26*, *LHX8*), **mitochondrial biogenesis** (*Tfam*, *NRF1*), **membrane respiratory chain** (*SLC25A12*, *NDUFB3*, *NDUFB5*, *SDHB*), **pro-fusion** (*MFN2*), **pro-fission** (*Fis1*) **proteins of mitochondria** and **cGMP signaling** (*GCa1*, *GCb1*, *PKG1*).

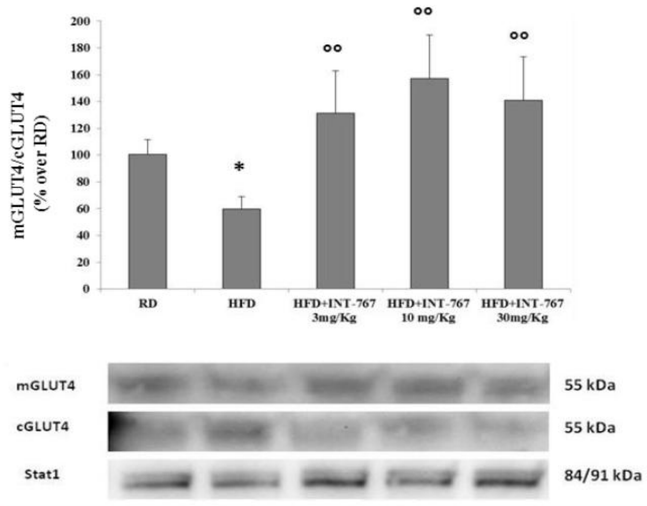


Insulin sensitivity in DIM-induced rPADs.

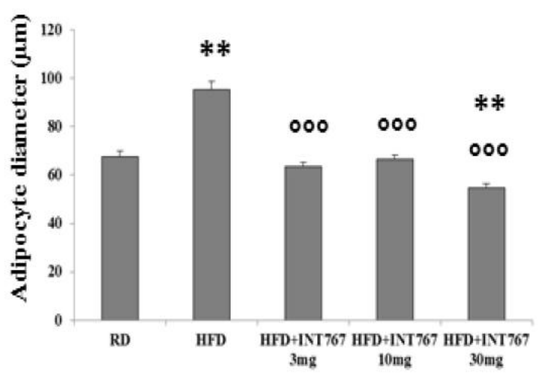
Glucose uptake in response to insulin increased dose dependently, with the expected EC_{50} in all rPADs (shared **EC_{50}=1.6±0.6 nM**). However, the maximal effect of insulin was significantly reduced in HFD rPADs (E_{max}=114.2±5.8%), as compared with RD (E_{max}=180.1±9.5%, p=0.002). Interestingly, **all INT-767 treatments increased insulin E_{max}**, when compared to HFD (E_{max} INT-767 3 mg/Kg=174.9±9.1%, p=0.027; E_{max} INT-767 10mg/Kg=153.6±7.6, p=0.01; E_{max} INT-767 30mg/Kg=150.9±7.4, p=0.01), although the E_{max} of both INT-767 10 mg/Kg and INT-767 30 mg/Kg groups was still lower than that of the RD rabbits (p=0.006).

INT-767 treatment counteracts HFD-induced VAT remodelling

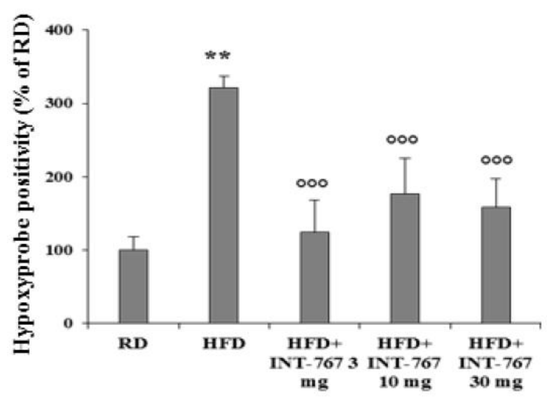
GLUT4 membrane translocation. Western Blot analysis



Adipocyte diameter. Hematoxylin/eosin-staining of VAT sections

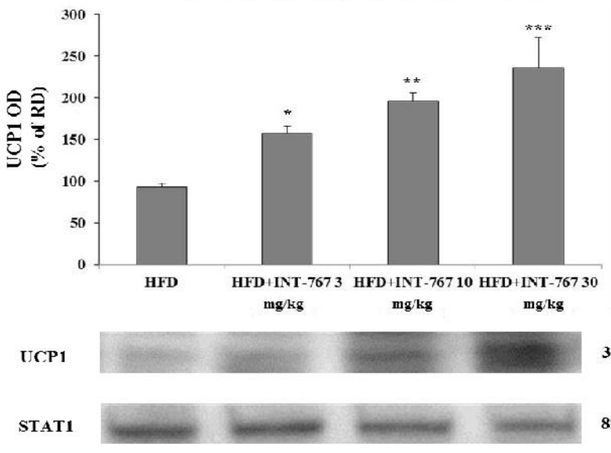


Immunohistochemical staining of hypoxyprobe adducts in VAT sections from experimental rabbits



Protein expression of UCP1 in VAT extracts. Western Blot analysis

*p<0.05, **p<0.01, ***p<0.0001 vs. HFD.



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

Conclusions

The dual FXR/TGR5 agonist INT-767 ameliorates the metabolic profile and reduces visceral adiposity by improving insulin sensitivity and promoting brown differentiation in visceral adipose tissue.

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