ACITON OF NICOTINC ACID ON ADIPONECTIN, LEPTIN AND PLASMINOGEN ACTIVATOR INHIBITOR 1 SECRETION AND EXPRESSION IN 3T3-L1 ADIPOCYTES UNDER OXIGEN DEPRIVATION

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Introduction: Obesity has been shown to play crucial role in the development of cardiovascular disease (CVD). Deysregulated production of adiponectin and leptin is partly responsable obesity-linked CVD.
Recent studies have revealed that hypoxia ambient plays a role in the alteration of adipokines expression and secretion, increasing leptin and plasminogen activator inhibitior 1 (PAI-1) produciton and decreasing adiponectin production.
The nicotinic acid (NA), a pharmacological agent for the treatment of dyslipidemia, stimulates adiponectin production while also reduces serium leptin and PAI-1 contributing to reduce the CVD risks.

Objective: Investigate the effect of nicotinic acid on adipokines expression and secretion in adipocytes before and after hypoxia.

Methods: 3T3-L1 cells were grown to confluence and induced to differentiated adipocytes. The mature adipocytes culture was divided into 4 groups:

1. Control: adipocytes were maintained in high glucose DMEM medium, 10% FBS;
2. Drug: adipocytes were treatmented with NA (10µmol/L);
3. Hypoxia: adipocytes were induced by hypoxia (1%O_2, 94%N_2, 5% CO_2) in different periods (4, 8 and 12 hours);
4. Drug + Hypoxia: adipocytes were pretreated with NA during 24 hours and induced by hypoxia for different periods (4, 8, 12 hours);

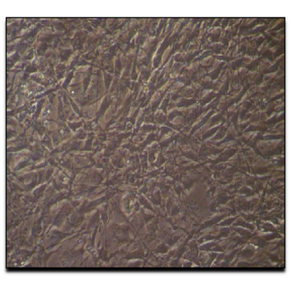
✓ Levels of adipokines and HIF-1α in medium were quantified using immunoassay (ELISA). Adipokines expression was analyzed by real time PCR.

✓ HIF-1α expression was analyzed by Western blot .

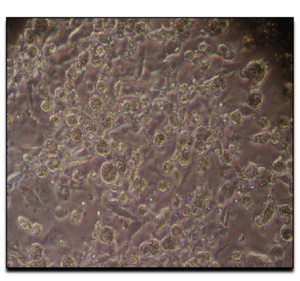
✓ Statical analysis: The statical analysis was performed using SPSS 17.0 software. The results were expressed as mean values S.D. Difference groups were analysed by unparied Student's *t* test. *p* -Values <0.05 were considered significant.

Results

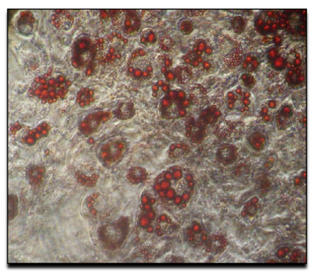
Cell Differentiation



3T3-L1 Fibroblast



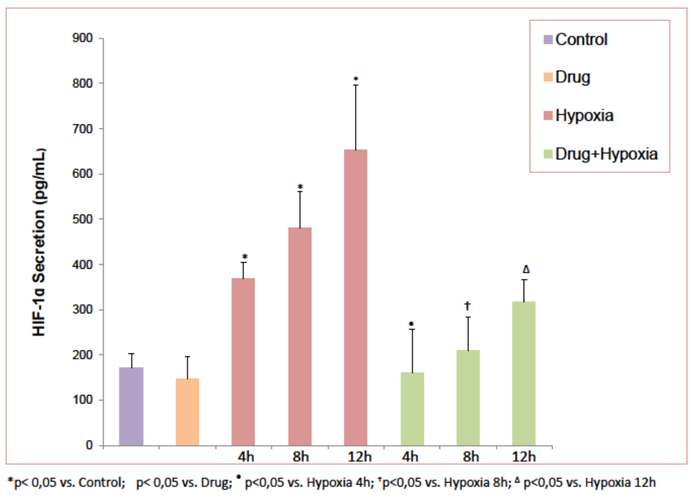
Fibroblast differentiated into 3T3-L1 adipocyte



3T3-L1 adipocyte stained with *Oil Red*

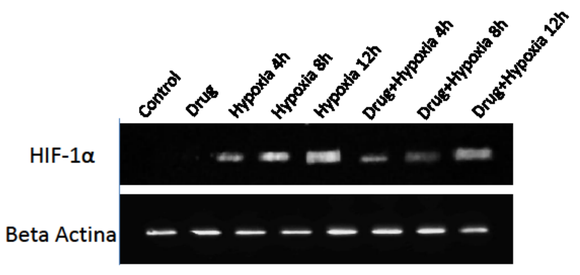
Measureament of HIF-1α in cellular medium

❖ Figure 1: Effect of nicotinc acid on HIF-1α secretion in medium adipocytes under hypoxia (n=10)



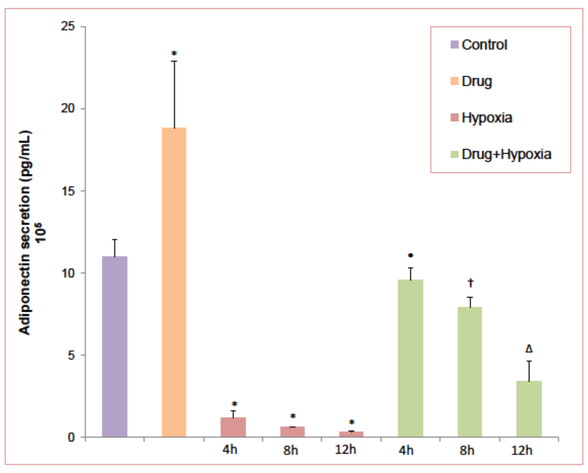
*p< 0,05 vs. Control; p< 0,05 vs. Drug; * p<0,05 vs. Hypoxia 4h; †p<0,05 vs. Hypoxia 8h; Δ p<0,05 vs. Hypoxia 12h

Analyze of HIF-1α expression in 3T3-L1 adipocytes by Western blot



Measureament of adipokines in cellular medium

❖ Figure 2: Effect of nicotinc acid on adiponectin secretion in medium adipocytes under hypoxia (n=10)



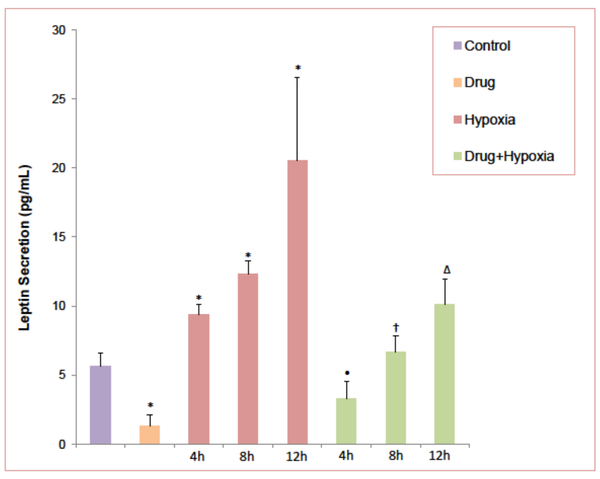
*p< 0,05 vs. Control
p< 0,05 vs. Drug
*p< 0,05 vs. Hypoxia 4h
† p< 0,05 vs.Hypoxia 8h
Δ p< 0,05 vs. Hypoxia 12h

Analysis of adipokines expression

❖ Table 1: Effect of nicotinc acid on adiponectin expression in 3T3-L1 adipocytes under hypoxia (n=10)

GROUPS	ADIPONECTIN EXPRESSION (mRNA relative HPRT / adiponectin)
Control	255 ± 130,7
Drug	1195,7 ± 418,7*
Hypoxia 4 hours	2,95 ± 2*
Hypoxia 8 hours	0,62 ± 0,25*
Hypoxia 12 hours	0,08 ± 0,02*
Drug + Hypoxia 4 hours	278,7 ± 146,7
Drug + Hypoxia 8 hours	214,3 ± 62,8†
Drug + Hypoxia 12 hours	22,2 ± 9,8Δ

❖ Figure 3: Effect of nicotinc acid on leptin secretion in medium adipocytes under hypoxia (n=10)

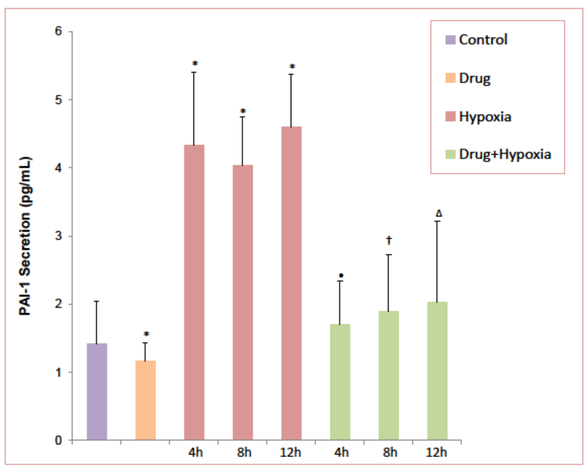


*p< 0,05 vs. Control
p< 0,05 vs. Drug
*p< 0,05 vs. Hypoxia 4h
† p< 0,05 vs.Hypoxia 8h
Δ p< 0,05 vs. Hypoxia 12h

❖ Table 2: Effect of nicotinc acid on leptin expression in 3T3-L1 adipocytes under hypoxia (n=10)

GROUPS	LEPTIN EXPRESSION (mRNA relative HPRT / leptin)
Control	0,196 ± 0,34
Drug	0,36 ± 0,007
Hypoxia 4 hours	136,4 ± 17*
Hypoxia 8 hours	320,7 ± 72,3*
Hypoxia 12 hours	571,7 ± 53,63*
Drug + Hypoxia 4 hours	5,06 ± 3,125
Drug + Hypoxia 8 hours	26,8 ± 9,4†
Drug + Hypoxia 12 hours	74,7 ± 14,1Δ

❖ Figure 3: Effect of nicotinc acid on PAI-1 secretion in medium adipocytes under hypoxia (n=10)



*p< 0,05 vs. Control
p< 0,05 vs. Drug
*p< 0,05 vs. Hypoxia 4h
† p< 0,05 vs.Hypoxia 8h
Δ p< 0,05 vs. Hypoxia 12h

❖ Table 3: Effect of nicotinc acid on PAI-1 expression in 3T3-L1 adipocytes under hypoxia (n=10)

GROUPS	PAI-1 EXPRESSION (mRNA relative HPRT / PAI-1)
Control	1,41 ± 0,44
Drug	0,036 ± 0,044
Hypoxia 4 hours	7,31 ± 2,66*
Hypoxia 8 hours	16 ± 2,7*
Hypoxia 12 hours	30,6 ± 7,44*
Drug + Hypoxia 4 hours	0,24 ± 0,32
Drug + Hypoxia 8 hours	0,65 ± 0,41†
Drug + Hypoxia 12 hours	8 ± 3,5Δ

Conclusion

The present study suggests:

❖ Hypoxia and nicotinic acid are associated with alterations of adiponectin ,lepitn and PAI-1 expression and secretion.

❖ Deprivation oxygen decreased adiponectin expression and secretion while also increased leptin and PAI-1 expression and secretion, these effects can contribute to cardiovascular diseases.

❖ Nicotinic acid increased adiponectin expression and secretion whilst decreased leptin and PAI-1 secretion. It suggests that the drug helps to reduce the risk of cardiovascular diseases.

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