Effects of iNOS inhibition in High Fat Diet-induced obesity

B. Martin[1], AE. Declèves[2], V. Colombaro[1], I. Jadot[1], C. Depommier[1], G. Federici[1], I.Habsch[1], N. Caron[1]

[1] Molecular Physiology Research Unit-URPhyM, University of Namur (UNamur), 61, rue de Bruxelles, B-5000 Namur, Belgium

[2] Laboratory of Molecular Biology, University of MONS (UMons), 6, avenue du champ de Mars, B-7000 Mons, Belgium

BACKGROUND

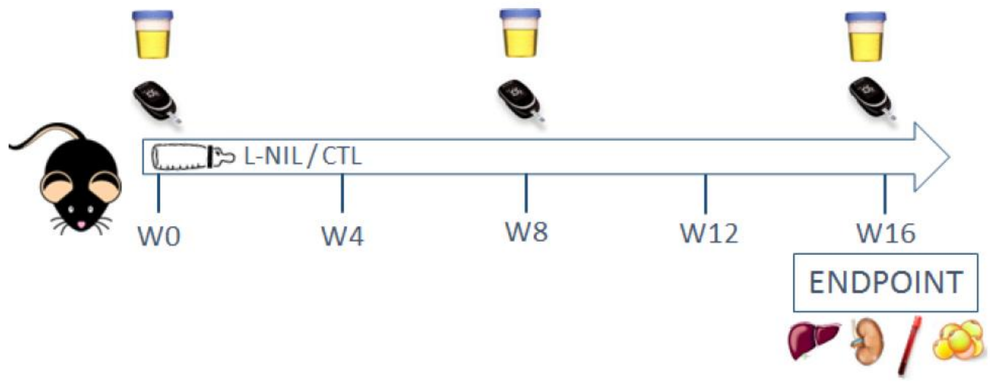
Obesity is a worldwide problem due to a sedentary lifestyle and imbalanced diet, as observed in most developed countries. This disease is caused by caloric excess promoting deleterious cellular responses. Endothelial dysfunction, impaired vasodilation and insulin resistance are considered as key features of obesity.

Interactions between metabolic and hemodynamic factors activate intracellular signalling pathways leading to oxidative stress and production of pro-inflammatory cytokines and fibrotic factors. Among vasoactive factors, **nitric oxide** (NO) has been identified as playing a critical role in the pathogenesis of metabolic diseases. NO produced by the inducible nitric oxide synthase (iNOS) has particularly cytotoxic and pro-inflammatory roles.

Therefore, the aim of this study was to investigate the involvement of **iNOS** in the development of progressive renal dysfunction leading to obesity-induced **kidney** disease as well as its impact in **liver** and **adipose tissue**.

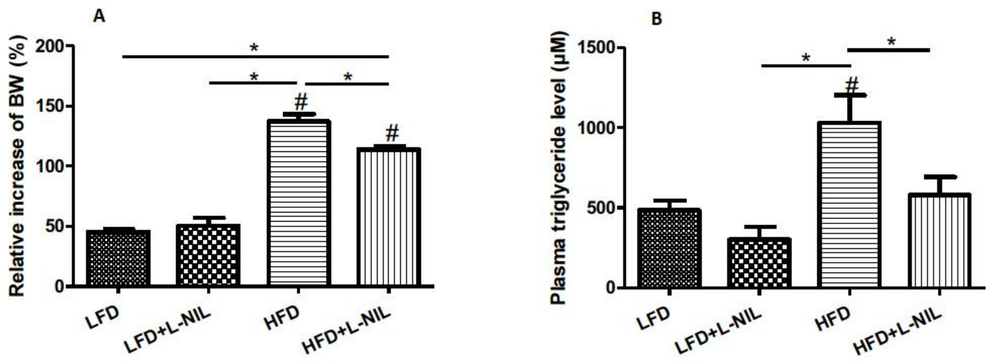
METHODS

C57BL/6 male mice were randomized to a Low Fat Diet (LFD) or a High Fat Diet (HFD) during 16 weeks and treated with pharmacological agent, L-NIL (specific inhibitor of iNOS) in drinking water, or vehicle. Four experimental groups were designed: LFD, LFD+L-NIL, HFD and HFD+L-NIL (n=8 in each group).



RESULTS

1. Effects of iNOS inhibition on metabolic parameters



A and B) Data are means ± SEM. Statistical analysis: # p< 0.05 vs own CTL. * p< 0.05 between the two groups (one way ANOVA followed by Newman-Keuls test).

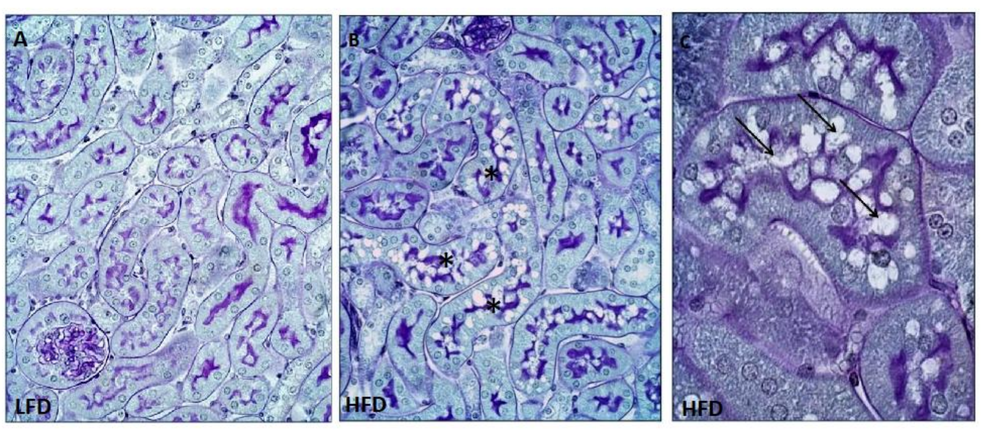
16 Weeks on diet	LFD	LFD+L-NIL	HFD	HFD+L-NIL
Kidney weight (g/mm of tibia length)	0.0140 ± 0.0006	0.0139 ± 0.0005	0.0183 ± 0.0003 #*	0.0177 ± 0.0014 #*
Liver weight (g/mm of tibia length)	0.0664 ± 0.0042	0.0597 ± 0.0065	0.1156 ± 0.0053 #*	0.1415 ± 0.0085 #*
Fasting plasma glucose level (mg/dl)	130 ± 7	116 ± 9	218 ± 12 #*	181 ± 10 #*+
Plasma insulin (ng/ml)	0.73 ± 0.07	0.73 ± 0.06	2.33 ± 0.55 #*	0.98 ± 0.07 +
Plasma NEFA level (nM)	1463 ± 135	1410 ± 226	2379 ± 201 #*	1568 ± 207 +

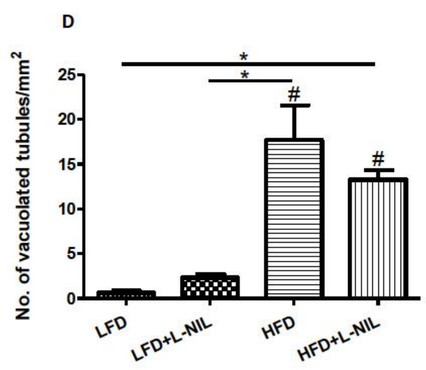
Data are means ± SEM. Statistical analysis: # p< 0.05 vs LFD. * p< 0.05 vs LFD+L-NIL. + p< 0.05 vs HFD (one way ANOVA followed by Newman-Keuls test).

2. Effects of iNOS inhibition on kidney structure and function

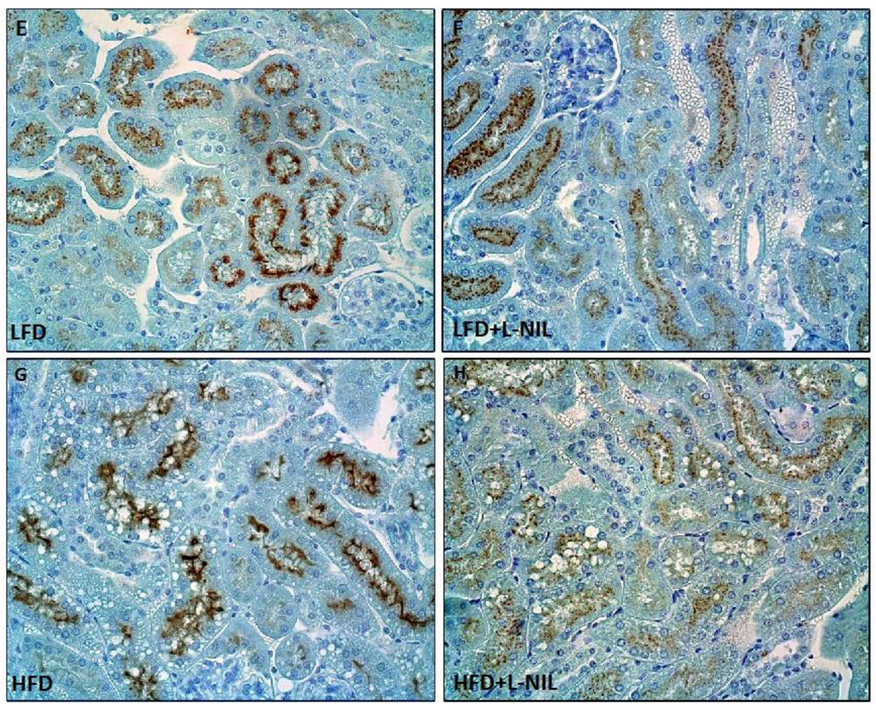
16 Weeks on diet	LFD	LFD+L-NIL	HFD	HFD+L-NIL
Urinary protein level (mg/mg cre)	1.3 ± 0.09	1.9 ± 0.3	2.2 ± 0.2 #	1.6 ± 0.07 +
Urinary albumin level (µg/mg cre)	11.04 ± 1.2	19.3 ± 5.6	31.4 ± 3.8 #	21.7 ± 2.4
Urinary glucose level (mg/mg cre)	0.21 ± 0.03	0.31 ± 0.07	0.78 ± 0.05 # *	0.43 ± 0.05 #+
Glomerular area (mean grid No)	549.8 ± 15.4	550.9 ± 22.1	732.8 ± 17.6 #*	773.3 ±2 4.5 #*
PAS-positive matrix (mean grid No)	336.4 ± 7.7	350.4 ± 18.7	434.4 ± 16.6 #*	440.9 ± 15.9 #*

Data are means ± SEM. Statistical analysis: # p< 0.05 vs LFD. + p< 0.05 vs HFD. * p< 0.5 vs LFD+L-NIL (one way ANOVA followed by Newman-Keuls test).



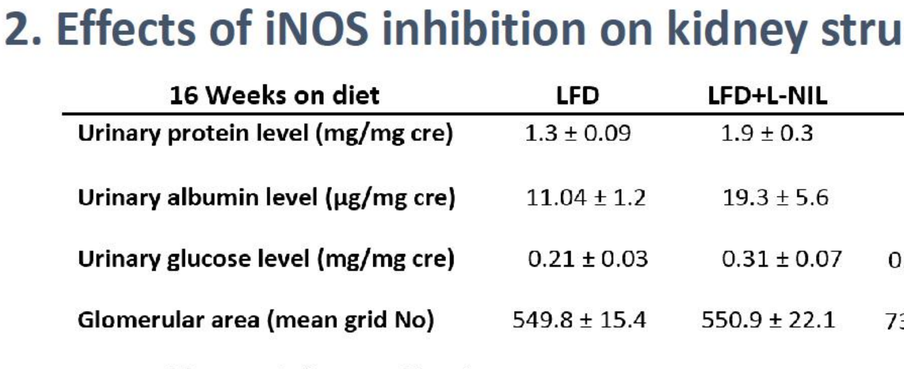


A, B and C) Tubular cells in kidney. * shows vacuolated tubules and → shows impaired brush border. Magnification 400x (A and b) and 600x (C). .
D) Data are means ± SEM. Statistical analysis: # p< 0.05 vs own CTL. * p< 0.05 between the two groups (one way ANOVA followed by Newman-Keuls test).



E, F, G, and H) iNOS immunostaining of the renal tissue in LFD (E), LFD+L-NIL (F), HFD (G) and HFD+L-NIL (H). Magnification 400x.

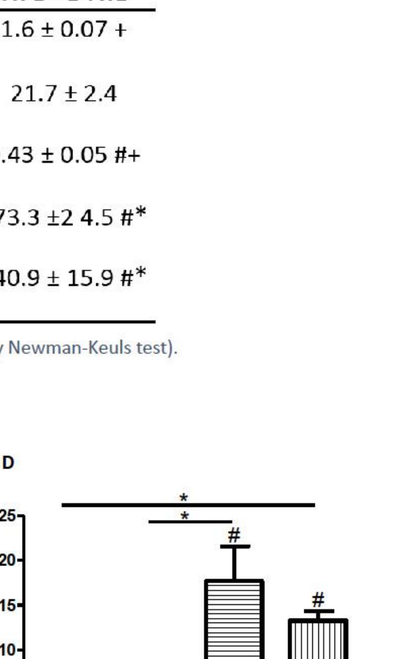
3. Effects of iNOS inhibition on adipose tissue



A, B and C) Adipose tissue structure in LFD, HFD and HFD+L-NIL mice. Magnfication 400x .

D, E) Data are means ± SEM. Statistical analysis: # p< 0.05 vs own CTL. * p<0,5 between the two groups (one way ANOVA followed by Newman-Keuls test).

4. Effects of iNOS inhibition on hepatic tissue



A and B) Data are means ± SEM. Statistical analysis: # p< 0.05 vs own CTL. * p< 0.05 between the two groups (one way ANOVA followed by Newman-Keuls test).

SUMMARY

- L-NIL treatment prevents the increase of body weight, plasma insulin concentration, plasma fasting glucose, NEFA and triglycerides levels.
- iNOS inhibition seems to play a role in obesity-induced kidney disease as attested by a decrease of urinary protein level but has less effects on kidney structure. Moreover, iNOS immunostaining is weaker in HFD+L-NIL and LFD+L-NIL in comparison to LFD and HFD groups.
- L-NIL treatment also decreases adipocyte size and inflammation process in adipose tissue.
- Finally, L-NIL treatment prevents the increase of liver triglyceride level but has less impact on inflammation in liver.

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