VISCERAL OBESITY INDUCED BY A HIGH-CALORIE DIET LEADS TO DYSLIPIDEMIA, INSULIN RESISTANCE AND IMPAIRS ADRENAL FUNCTION IN MALE RABBITS

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ABSTRACT

The nutritional status and obesity alter the HPA axis function. The aim was to study the impact of a diet induced obesity on hormonal pituitary- adrenal axis, as well as structural changes that appeared in affect the adrenal gland. Two groups of adult male New Zealand White rabbits (n = 16) were maintained on high-calorie (454 Kcal/day) and standard diets (258 kcal/day) for 15 weeks. Plasma triglycerides (TG), total cholesterol (Tchol), HDL-c, LDL-c, glucose ACTH, cortisol, insulin and levels cortisol within adrenal and periadrenal fat (PAF) were measured. We show that high-calorie diet induced a significant increase of body weight, visceral adipose tissue (VAT) and adrenal. Increased TG, Tchol an LDL-c and decreased LDL-c were associated to hyperglycemia (HG) and hyperinsulinemia (HI) in obese rabbits. Plasma ACTH was increased in obese compared to controls rabbits, but PAF cortisol increases more significantly than within adrenal and plasma. Adrenal gland of obese rabbits shows a reduction in the thickness of the zona reticularis, while fasciculata and medullary zones were enlarged, with a proliferation of pyknotic nuclei; the medulla shows a vasodilatation and a high density of connective fibers that grew through the cortex until they reach the glomerulosa zone. We suggested that high-calorie diet induced visceral obesity and led to disrupt metabolic parameters that onset dyslipidemia and development of insulin resistance. High-calorie diet seems also to induce a nutritional stress that stimulates the secretion of ACTH and cortisol by the adrenal axis and an overproduction of cortisol from PAF, probably by stimulating 11β-HSD1 activity. These disturbances may be involved in the fibrosis of the medulla and the intense pyknosis in the fasciculata and medulla in obese rabbits which could be related to an apoptotic process.

Key words: Obesity, dyslipidemia, insulin resistance, adrenal axis.

INTRODUCTION

The obese phenotype in patients with Cushing's syndrome shows that the HPA axis is affected (Orth, 1995). In the adipose tissue (AT), overexpression of the enzyme 11β-HSD1 leads to increase synthesis of the major glucocorticoid, (cortisol in humans and corticosterone in rodents), which induces the proliferation of preadipocytes and their differentiation into adipocytes (Thomson *et al.,* 2007). Over the past decade the obesity-inducing diet in rabbits was used in obesity research (Carroll *et al.,* 2004). It was reported that young rabbits fed *ad libitum* rapidly gain weight, 86% of which is made up of adipose tissue (Carroll *e tal.,*1996). Obese rabbits showed hemodynamic and neurohumoral changes similar to those observed in obese human subjects and the model was used to study disorders associated with obesity (Carroll *et al.,* 1996, Carroll *et al.,* 2004). Other studies have focused on fat dysfunction in New Zealand White (Rong *et al.,* 1999; Zhang *et al.,* 2009) and Angora rabbits (Dhungel *et al.,* 2009) or on the development of visceral obesity and metabolic syndrome (Vignozzi *et al.,* 2011). The aim of the present study was to evaluate the impact of obesity on adrenal morphology and function.

MATERIALS AND METHODS

Animal and experimental design

Sixteen adult New Zealand White male rabbits were used housed two animals per cage. Room temperature and relative humidity were 5°c and 65-75% respectively with 12h light *per* day. After one week acclimatization, rabbits were divided in two groups, one with free access to control feed (22.5% fat, 48% soluble sugars and 258 Kcal *per* day) and the other with free access to a commercial feed with high fat-sucrose from STCO (12% fat, 22.5% soluble sugars and 454 Kcal *per* day). Both groups were provided water *ad libitum*. Body weight changes were estimated weekly. After 15 weeks, all rabbits were euthanized by bleeding in accordance with the principles and guidelines established by the Algerian Ministry of Agriculture for the care and use of laboratory animals, veterinary medicine and surgery. Visceral adipose tissue (VAT), adrenals and peri adrenal fat (PAF) were removed then weighted. All biological samples were taken between 9 and 10 am. PAF and the left adrenal were immediately frozen in liquid nitrogen and ground to powder, diluted in an appropriate volume of distilled water and then frozen at -80°C until assays will be performed.

Biochemical parameters

Plasma glucose, TG, Tchol, HDL-c, LDL-c were determined using Biomaghreb, Elitech and Spinreact kits. Plasma, adrenal and PAF levels of cortisol, and plasma ACTH and insulin were estimated using RIA and IRMA kit protocols (Immunotech, Marseille, France). Cross-reactivity with ACTH analogues was very low and sensitivity was of 1.2 pg/mL. Anti-cortisol also shows a negligible cross reaction with other analogue steroids. Interval for sensitivity was 10-2000 nM.

Histological parameters

Structural modifications were analyzed on adrenal section of 4µm stained using Masson's trichrome, following a Bouin-Hollande fixative step. Differences between control and obese groups (M±sem) were analyzed using Statistica 6.0 software for Student t and Anova tests and were considered significant if $p < 0.05$.

RESULTS AND DISCUSSION

Variations of body weight: We observe a concomitant increase in both control and rabbit fed high calorie diet group until the fifth week, without significant difference with 2.94±0.077 vs 2.98 ± 0.098 kg respectively. This increase became more significant from 6weeks and reached 3.69 ± 0.112 vs 3.09 ± 0.072 kg (17.4% and 35.16%) respectively after 15 weeks (fig1).

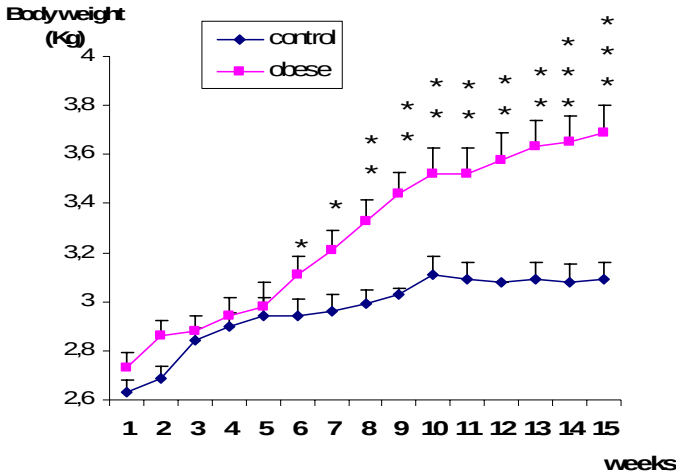


Figure 1: Variations in body weight (kg±esm) in two groups of rabbits fed with standard and high calorie diet.

Variations of tissues weight

Table 1: Variations in absolute and relative in VAT and adrenal weights in rabbits fed with control and high fat calorie diet during 15 weeks **(*:** p<0.05; **: p<0.01;***: p<0.001)

Weights tissues: Absolute (g±esm) and relative (g/kg bw±esm)				
	Visceral adipose tissue VAT		Adrenal	
	Control n=8)	Obese (n=7)	Control (n=8)	Obese (n=7)
Absolute	80.11 ± 5,14	122.86± 13.26 (***)	0.294 ± 0.009	0.339 ±0.01 (**)
Relative	26.2± 1.85	33.89± 2.59 (*)	0.09 ± 0.002	0.09±0.005

The concomitant increase of body weight, VAT and adrenal in rabbits fed with high-calorie diet during 15 weeks was consistent with a development of visceral obesity. Similar results were reported in New Zealand White rabbit maintained for 36 weeks under high fat sucrose diet (HFSD) (Zhao et al., 2008) and Angora rabbits with high fat diet (HFD) for 10 weeks (Dungel et al., 2009; Singha et al., 2009). The weight gain would be a consequence of a default control due to a disruption of energy balance (Basdevant and Guy-Grand, 2004). Thus, differentiated adipocytes accumulate triglycerides until they reach a critical size beyond which it recruits new adipocytes, contributing to gradual increased number of adipocytes and lead to hyperplasia (Basdevant, 2006). We suggest that fat levels in the chow (22.5%) compared to standard (12%), contributed to affect mechanisms that control proliferation and differentiation of adipocyte lineage, by accelerating one and/or both processes.

Variations in metabolic parameters

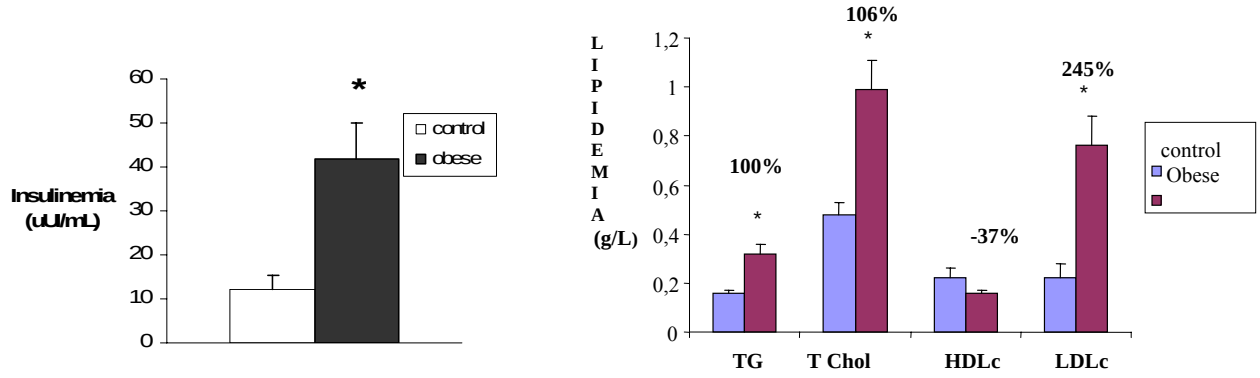


Figure 2 : Plasma insulin levels in control and obese rabbit after 15 weeks of standard and high calorie diet (*: p<0.05).

Visceral obesity disrupted metabolic parameters and resulted in HG (1.85±0.41vs1.23±0.13g/L, p<0.05), HI and dyslipidemia which is characterized by increased TG and Tchol levels and decreased of HDL-c and increased LDL-c (table 2, fig2). Our results were confirmed in New Zealand White rabbits maintained under HFSD (Zhao *et al*., 2007) and HFD for 36 weeks and 10 weeks respectively (Zhang *et al.*, 2008). Dyslipidemia was observed in humans after a high carbohydrate diet (Fried and Rao, 2003) and related to increased catabolism, partly stimulated by activity of hepatic lipase (Duvillard *et al*, 2000). As this enzyme is deficient in rabbits (Clay *et al.*,1989) degradation is attributed to the hydrolysis of TG stored in the hepatocytes. Indeed, Zhang *et al.* (2008) reported that only 59% of no esterified fatty acid were solicited in obese rabbits to synthesize TG, whereas uptake of free fatty acids from excess lipolysis of TG contribute to develop insulin resistance and metabolic syndrome (Sonnenberg *et al.*, 2004) We suggest that concomitant HI and HG in obese rabbit reflect an impaired insulin sensitivity and contributes to develop resistance by consumption of high carbohydrate rate from fat and sugars.

Impact on pituitary-adrenal axis

Hormonal analysis showed no significant increase in plasma levels of ACTH and cortisol in obese rabbit compared to control, but cortisol levels in the adrenal gland and the PAF were significantly increased. Indeed, excess of systemic GC and Cushing's syndrome leads to accumulation of visceral fat, that of TG in the liver, HG and insulin resistance through a pathophysiological pathways (Ghatercole and Stewart, 2010). Administration of C-terminal ACTH fragments to fasted rats for 16 hours, increases food intake while N-terminal α-MSH fragment induces an increase of food intake, but not

ACTH1-10. Transgenic mice overexpressing 11β HSD1 show hypertension, HG, HI, glucose intolerance, insulin resistance and increased FFA and TG (Masuzaki *et al.*, 2001). We suggest that nutritional status in obese rabbits causes metabolic stress which disrupt homeostasis through dyslipidemia, insulin resistance and activate the pituitary-adrenal axis by releasing ACTH and cortisol. The increased cortisol levels in the PAF indicates a local stress, probably due to overexpression of 11β-HSD1.

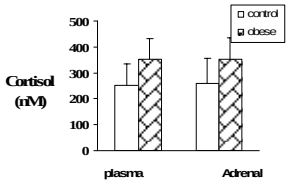
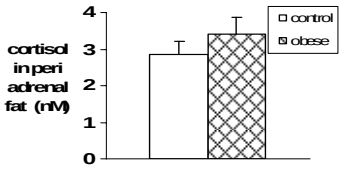
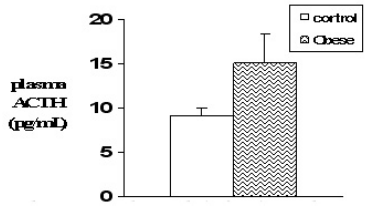


Figure 3: Cortisol levels in plasma and adrenal in control and obese rabbit



Figures 4 Cortisol levels in peri-adrenal fat in control and obese rabbit



Figures 5: Plasma ACTH in control and obese rabbit

Impact on adrenal structure

Our results show cell hypertrophy of fasciculata and glomerulosa zones with a highly significant regression of zona reticularis in adrenal of obese rabbit (photos 1-6, table 2). Hypertrophy of zona glomerulosa and arrangement as a cellular cords would be associated with a stimulatory action of ACTH on aldosterone production (Malendowicz et al.,1992). Indeed, in obese rabbits the pituitary-adrenal axis was stimulated in response to nutritional stress due to the composition of diet. The presence of optically empty spaces could probably correspond to translucent vacuoles (Marchetti and Marchetti, 2009). Dilation of blood capillaries within ZF of control rabbit and heterogeneous appearance of nuclei during pyknosis process, while the number of pyknotic nuclei was also increased compared to control, while structure of medulla was more altered.

These results were corroborated in rats stressed by high temperature (Koko et al, 2004), but zona glomerulosa regresses in diabetic Wistar rat (Liu et Shu,1996). Adrenal of obese rabbit show an intense fibrosis in the medulla due to excessive development of connective fibers, with presence of blood capillaries. We note a massive 'migration' of connective fibers and medullar cells towards ZR (photo 4), ZF (photo 4-5-6), ZG (photo 6) then basal connective capsule Adrenal medulla of obese rabbits show a significant cell proliferation that invade the zona_reticularis and asciculata as spans of chromaffin tissue that extend into the thickness of the zona glomerulosa. This phenomenon was consecutive to the intense fibrosis process.

Table 2: Thickness variations in different zone of adrenal gland of control and obese rabbit

Adrenal zones	Zona thickness (µm ± esm)	
	Control (n=4)	Obese (n=4)
Connective tissue	54 ± 12,72	121,5 ± 9 (*)
Zona glomerulosa	108 ± 12,72	158,62 ± 9,80 (NS)
Fasciculata	1350 ± 63,63	2396,25 ± 98,07 (**)
Zona reticularis	520 ± 49,02	137,75 ± 20,43 (***)
Medulla	1518,75 ± 74,62	2497,5 ± 149,24 (**)

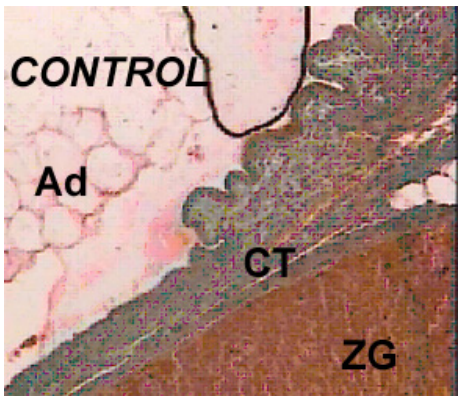


Photo 1: Masson's trichrome staining shows that the basal connective tissue (CT) in adrenal of control rabbit was developed, with presence of arterioles (Ar - Photo 2) and adipocytes (Ad). (G x 100)

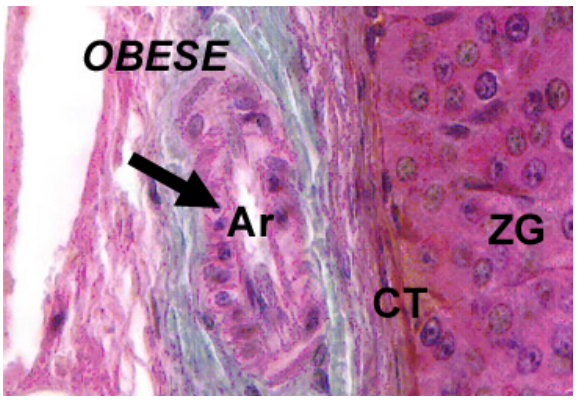


Photo 2: In obese rabbit , basal connective tissue was highly developed, vascularized, with abundant nuclei f fibroblasts characterizing excessive collagen secretion (G x 100)

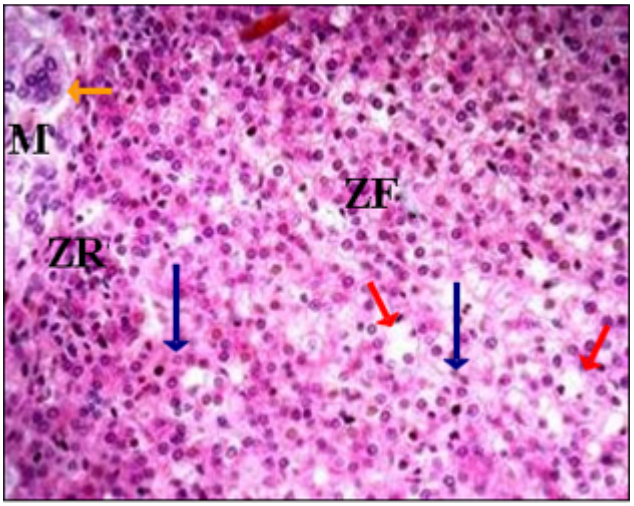


Photo 3: Dilation of blood capillaries within ZF of control rabbit and heterogeneous appearance of nuclei during pyknotic process (Gx100)

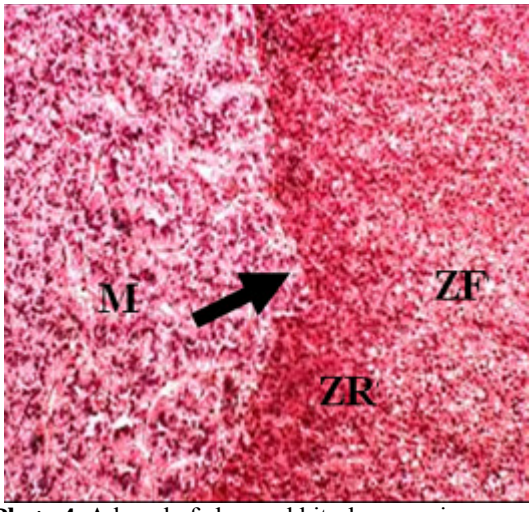


Photo 4: Adrenal of obese rabbit, shows an increase and reduction in the thickness of ZF and ZR respectively Number of pyknotic nuclei was also increased compared to the control. Medulla shows a beginning of evagination in ZR (Gx100)

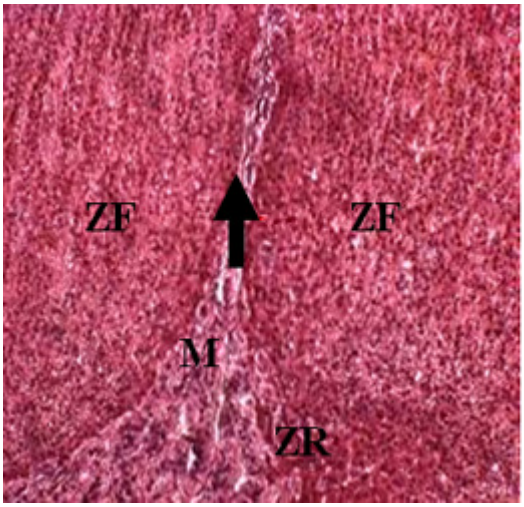


Photo 5: Adrenal of obese rabbit shows an intense fibrosis in the medulla due to excessive development of connective fibers. We note a massive 'migration' of connective fibers and medullar cells towards ZF (Gx100)

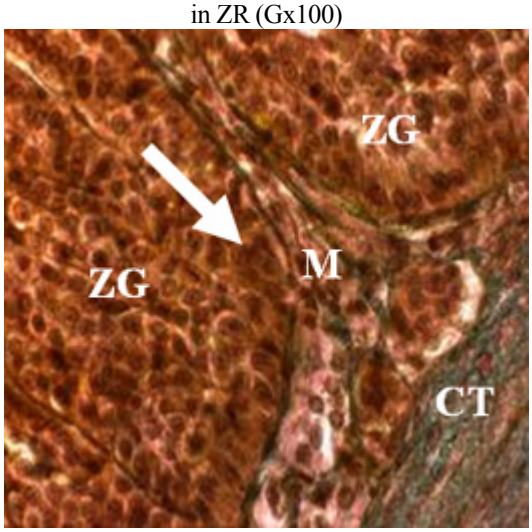


Photo 6: Adrenal of obese rabbit shows an intense fibrosis in the medulla due to excessive development of connective fibers. We note a massive 'migration' of connective fibers and medullar cells towards ZG and basal connective capsule (Gx100)

CONCLUSIONS

Visceral obesity induced by high calorie diet causes a nutritional stress that stimulates the secretion of ACTH and cortisol by the adrenal axis and an overproduction of cortisol from PAF, probably by stimulating 11β-HSD1 activity. These disturbances may be involved in the fibrosis in the medulla and the intense pyknosis in the fasciculata and medulla in obese rabbits which could be correlated with an apoptotic process. This need to be confirmed by estimating caspase 3 activity and biomakers of the inflammatory process, such as C-reactive protein and interleukins.

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