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High lipolytic activity and dyslipidemia in a Spontaneous Hypertensive/NIH Corpulent (SHR/N-*cp*) rat: a genetic model of obesity and type 2 diabetes mellitus

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In order to better understand the link between obesity and type 2 diabetes, lipolysis and its adrenergic regulation was investigated in various adipose depots of obese adult females SHR/N-*cp* rats. Serum insulin, glucose, free fatty acids (FFA), triglycerides (TG) and glycerol were measured. Adipocytes were isolated from subcutaneous (SC), parametrial (PM) and retroperitoneal (RP) fat pads. Total cell number and size, basal lipolysis or stimulated by norepinephrine (NE) and BRL 37344 were measured in each depot. Obese rats were hyperinsulinemic and hyperglycemic, suggesting high insulin resistance. They presented a marked dyslipidemia, attested by increased serum FFA and TG levels. High serum glycerol levels also suggest a strong lipolytic rate. Obese rats showed an excessive development of all fat pads although a more pronounced effect was observed in the SC one. The cellularity of this depot was increased 8 fold when compared to lean rats, but these fat cells were only 1.5 to 2-fold larger. SC adipocytes showed a marked increase in their basal lipolytic activity but a lack of change in responsiveness to NE or BRL 37344. The association between high basal lipolysis and increased cellularity yields to a marked adipose cell lipolytic rate, especially from the SC region. SHR/N-*cp* rats were characterized by a hyperplasic type of obesity with an excessive development of the SC depot. The dyslipidemia, attested by an altered serum lipid profile could be attributed to excessive lipolysis that contributes to increased FFA levels, and to early development of insulin resistance through a lipotoxicity effect.

Key words: Genetic obesity, Type 2 diabetes, Adipose tissue, Lipolysis, Norepinephrine.

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Numerous studies performed on various rodent models of genetic obesity such as the ob/ob mouse or the Zucker fa/fa rat because of their similarities to early onset human obesity have attempted to identify the mechanism underlying the development of obesity and its related metabolic disorders (9, 18). On the other hand, evidence has also been presented that obese subjects, and more particularly those displaying an excessive visceral adiposity, share an abnormal metabolic profile characterized by insulin resistance, hyperinsulinemia and hyperleptinemia that predisposes to type 2 diabetes and cardiovascular disease (5, 7, 22). Insulin resistance is a characteristic feature of numerous animal models of obesity (9, 18), and more particularly of the obese SHR/N- *cp* (SHR = spontaneous hypertensive rat; *cp* = corpulent; NIH inbred strain). This strain has been obtained twenty years ago by crossing a SHR/N-rat (which was hypertensive) with a Koletsky rats, followed by an extensive backcrossing to SHR rats in order to achieve congenicity (10, 16, 21). Frank diabetes appears at a very early age in obese SHR/N-*cp* rats (two months-old) and animals remain markedly hyperinsulinemic and insulin resistant until they die from cardiovascular complications associated with diabetes. Therefore, the obese SHR/N-*cp* rat represents an interesting rat model of type 2 diabetes.

In addition, we have already found that the thermogenic capacity of brown adipose tissue was markedly defective in corpulent diabetic SHR-N-*cp* rats (2, 14, 15) and postulated that this defect may contribute to the development and the maintenance of their obesity. It therefore appeared of interest to accurately characterize the lipolytic activity of white adipose tissues, since experiments conducted on isolated fat cells from various depots have not yet been performed in obese SHR/N-*cp* rats. Therefore, specific aims of the present study were i) to examine structural changes in fat mass at a cellular level, by measuring fat cell number and size in both the corpulent diabetic SHR-N-*cp* rats and their lean littermates, ii) to compare adipose cell lipolytic activity and global lipolytic rate in both corpulent diabetic SHR/N-*cp* rats and their lean littermates, iii) to assess whether putative alterations could explain the increase in serum lipids which reflects a dyslipidemic state leading to the development of insulin resistance and type 2 diabetes. Results clearly indicate the presence of an increase in basal adipocyte lipolysis, especially at the subcutaneous level, which is highly associated to a marked adipose tissue mass expansion.

Materials and Methods

Materials and animals.– Collagenase (type II) and enzymes for glycerol assays, (-)-norepinephrine bitartrate, bovin serum albumin (BSA) were obtained from Sigma Chemical. All other chemicals were reagent grade of the best quality available. BRL 37344 (a β3-adrenergic agonist) (4-[-[(2-hydroxy-(3-chloro-phenyl)ethyl-amino]propyl]-phenoxyacetate] was obtained from Smith Kline Beecham Pharmaceutical, Epson, England.

Adults female SHR/N-*cp* rats (6-7 mo) obese (*cp/cp*), body weight: 457 ± 13 g and lean (+/cp), body weight: 240 ± 5 g, were kept at 25 ± 1 °C in metal cages with a 12/12 light schedule and were fed *ad libitum* with Purina Laboratory chow (UAR 004). The animals were killed by decapitation and blood samples were taken frozen at –80 °C until use.

Serum biological parameters.– Serum glucose levels were measured with a glu-

cose analyser (Beckman). Insulin levels were determined by radioimmunoassay (Incstar). FFA and glycerols levels were determined using a NEFA Kit (Wako Chemicals). Triglycerides (TG) concentrations were determinated by an enzymatic method, using reagent kits from Boehringer Mannheim.

Isolation of white adipocytes and lipolysis measurements.– For each cell preparation and each fat deposit, two lean or one obese SHR/N-*cp* rats were used. Adipocytes were isolated according to the method of Rodbell (17) with only minor modifications (3). SC, RP and PM fat deposits were incubated with collagenase (1mg/ml), in Krebs-Ringer bicarbonate buffer containing BSA 4%. Cellular suspensions were counted in Neubauer's hematocytometer. The mean cell diameter of at least 100 adipocytes was determined using a graduated eyepiece micrometer. Fat cell volume was calculated according to the following formula: $V = \pi ([3 \cdot SD^2 \cdot d] + d^3)/6$, where d is the mean fat cell diameter and SD is the standard deviation. Fat cell weight was obtained using the assumption that the density of the fat is 0.915.

Lipolytic activity was measured by incubating 1 to 2 x 10^5 isolated adipocytes in KRBA (BSA 4%) containing the agent to be tested. After 15 min of incubation, 200µl aliquots of infranatant were removed for enzymatic determination of glycerol released in the incubation medium, by the method of Wieland (23). The lipid content was determined gravimetrically after extraction, according to the method of Dole and Meinertz (8).

Statistical analysis.– Values presented in tables and figures are means ± standard error (SE) Analysis of variance was used to test significant main and cross effects of site and phenotype. Significant differences were located using the Fischer protected least significance difference test and considered significant when $P < 0.05$.

Results

Serum biological parameters of lean and obese animals.– As shown in Table I, *cp/cp* rats were markedly hyperinsulinemic (55-fold increase in plasma levels, $p < 0.01$) and hyperglycaemic (1.5-fold), compared to their lean littermates (+/*cp*). Obese animals were also characterized by higher serum FFA levels (7-fold), and displayed significantly higher TG (9-fold) and glycerol (3.5-fold) concentrations.

Phenotype and site effects in fat pad weights, fat cell number and size .– Total adipose weight in all three locations (PM, RP and SC depots) was markedly increased in obese compared to lean rats ($p < 0.01$). In the *cp/cp* group, the weight of the SC adipose depot was much greater than of the two internal ones (PM and RP) (Fig. 1A). The cellularity (total number of cells) of each depot was increased, particularly in the SC depot (8-fold increase) (Fig. 1B). Obese fat cells were also 1.5 to 2-fold larger ($p < 0.01$) than lean fat cells

Table I. *Metabolic characteristics of lean and obese SHR/N-*cp *rats.*
(INS, insulin, FFA free fatty acids ; TG triglycerides). Results are mean ± SEM. *p<0.05; **p< 0.01 *vs.* lean rats.

	Lean (n = 7)	Obese (n = 7)
Glucose (mM)	9.2±0.4	14.2±1.7**
INS (µU/ml)	48.8±13.80	2664±191**
FFA (mM)	0.69±0.04	4.65±0.43**
Glycerol (mM)	0.20±0.02	0.70±0.33**
TG (mM)	1.40±0.20	13.2±1.40**

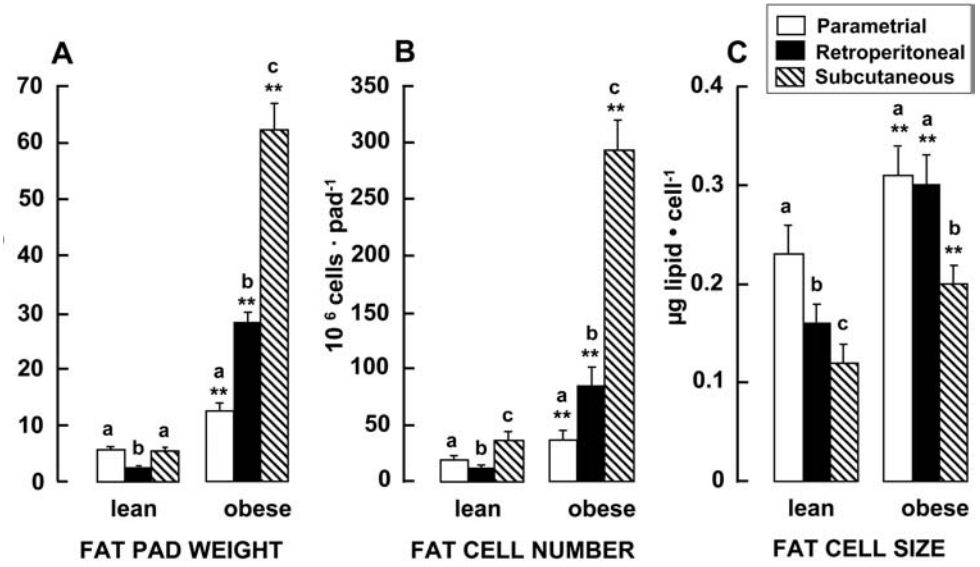

Fig. 1. *Adipose tissue characteristics in lean and obese SHR/N-cp rats.*
Values are means ± SEM of 5 to 6 experiments. p< 0.05 *, p< 0.01 ** *vs.* corresponding lean values. Means not sharing the same superscript are significantly different (p < 0.05).

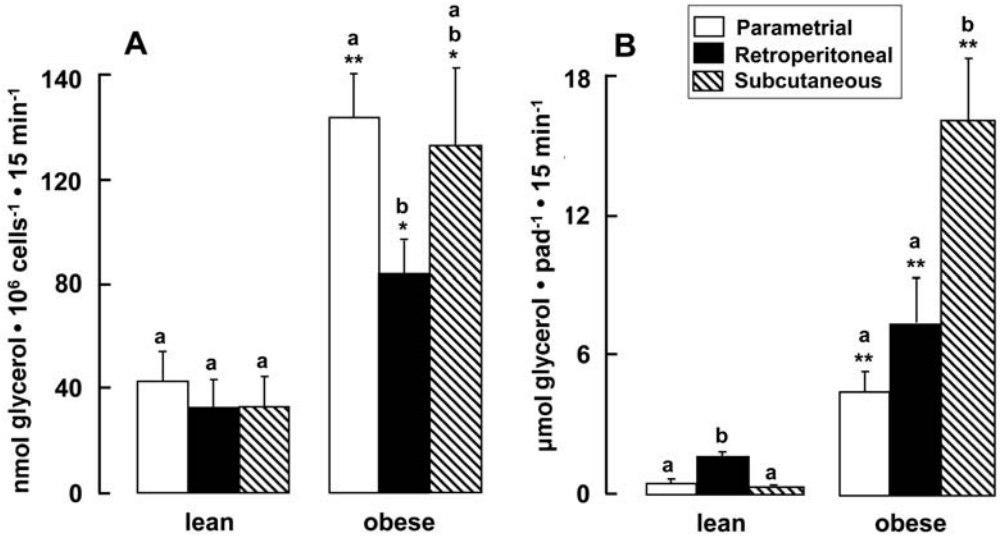

Fig. 2. *Basal lypolysis in the different fat depots from lean and obese SHR/N-cp rats.*
Values are means ± SEM of 5 to 6 experiments performed in duplicate and glycerol is expressed either per cell number (panel A) or per total fat pad (panel B). The symbol * and ** represents significant differences from lean control values (p<0.05 *, p<0.01**). Means not sharing the same superscript are significantly different (p<0.05).

(Fig. 1C). SC adipocytes were slightly but significantly lower than the cells from the two other depots. Finally, these observations are in good accordance with the highly increased fat pad weight noted in obese animal (Fig. 1A).

Phenotype and site effects in basal lipolysis.– In all three adipose depots, basal lipolysis was higher in obese than in lean animals ($p < 0.01$), whether results were expressed per cell number (Fig. 2A) or calculated per total fat pad weight (Fig. 2B). The higher lipolytic rate of all depots in obese rats results from an increased basal lipolysis of adipocytes combined with augmented cellularity. This was particularly evident in the SC adipose depot where basal lipolysis quadrupled (from 35 ± 5 to 130 ± 20 nmol glycerol • 10^6 cells^{-1} • 15min^{-1}, $p < 0.01$) and total cellularity increased 8-fold (from 35 ± 5 to 280 ± 20 • 10^6 cells • pad^{-1}, $p < 0.01$) for lean and obese rats respectively, yielding a remarkable increase in the lipolytic rate of the entire SC fat depot (Fig. 2B).

Phenotype and site effects in adrenergic responsivenesses.– Although NE responsiveness tended to be lower in obese internal adipocytes as compared to the corresponding lean adipose cells, no clear differences between phenotypes were observed in the maximal lipoytic response to this neuro-hormone. In addition, no regional variation was noted in the maximal lipolysis promoted by the catecholamine, irrespective of the phenotype considered (Fig. 3A). However, BRL 37344, a potent β3-adrenoceptor agonist promoted a similar lipolytic effect regardless of the anatomic location of fat, in both

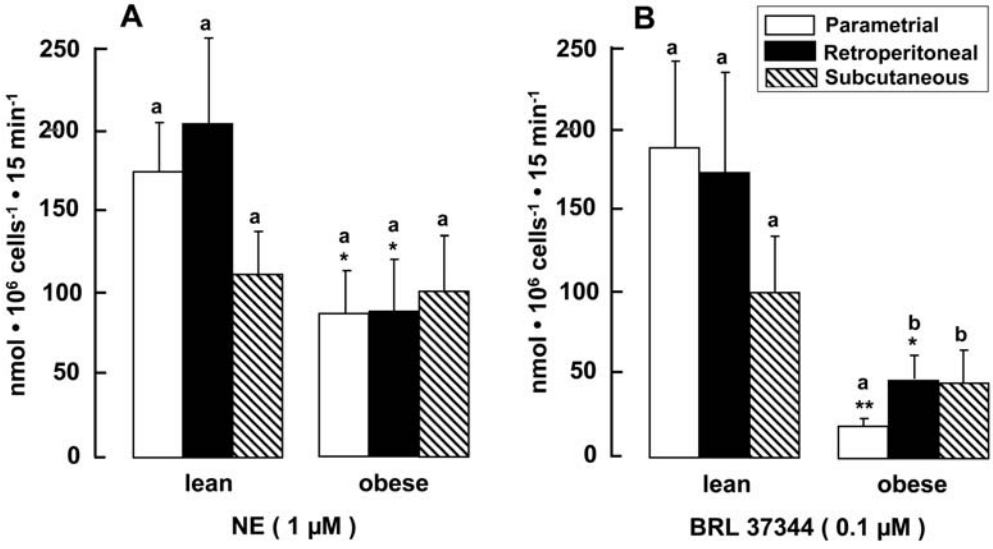

Fig. 3. *Lipolytic responsivenesses to NE and BRL 37344 of isolated adipocytes from lean and obese SHR/N-cp rats.*

Fat cells were isolated from different fat depots of 2 lean or one obese animals. Values are means ± SEM of 5 to 6 experiments performed in duplicate with 1 µM NE (panel A) or 0.1 µM BRL37344 (panel B). The symbol * and ** represents significant differences from lean control values ($p<0.05$*, $p<0.01$**). Means not sharing the same superscript are significantly different ($p<0.05$).

phenotypes. Moreover, maximal lipolysis in the presence of the β3-agonist was significantly reduced in obese PM adipocytes, as compared to the corresponding lean cells (approximately 10-fold decrease, $p < 0.01$).

Discussion

Obese SHR/N-*cp* were markedly hyperinsulinemic (Table I), a finding consistent with previous studies performed on this model (2, 14, 15). However, in contrast to other rat models of obesity such as *fa/fa* Zucker rats (4), hyperinsulinemia in obese SHR/N-*cp* rats was associated with a significant hyperglycemia. In addition, serum FFA, triglycerides and glycerol were all markedly increased in these obese rats. Similar abnormalities in the lipid profile associated with obesity, insulin resistance, glucose intolerance and type 2 diabetes have already been described in other models of obesity, although they were less important than those noted in obese SHR/N- *cp* rats (4, 9, 18). The severity of the lipid abnormalities in the obese corpulent rat that presumably contributes to the development of diabetes may explain the fact that this particular strain dies at very early age (about one year-old), mainly from cardiovascular complications resulting for atherosclerotic lesions (16, 21). It should be pointed out that the presence of such metabolic disorders in corpulent diabetic SHR/N- *cp* rat is similar to the well known lipid perturbations that are frequently observed in obese humans (5, 7, 13). Therefore, it appears important to more accurately investigate whether such metabolic complications which are often associated with obesity could be partly related to some alterations in the regulation of lipolysis from different fat depots.

In all three adipose locations investigated in *cp/cp* rats, adipocytes were larger and numerous than in lean controls, thus indicating the presence of an hypertrophic-hyperplasic type of obesity. Data clearly showed that increased cellularity, rather than augmented fat cell size, is the principal cause of increased adipose tissue mass in the cp/cp rat in contrast to what we have previously observed in the fa/fa Zucker rat (4). Fat cell size significantly increased, but much less than fat cell number. This was particularly evident in the SC adipose tissue where total cell number was 7.6 times greater in obese than in lean rats. In comparison to RP and SC fat depots, cell size and number barely increased in the PM adipose region of *cp/cp* rats. Thus, both adipocyte hypertrophy and hyperplasia during the development of obesity display a remarkable site-specificity. These results also agree with the concept that during the development of massive obesity, maximal fat cell size is limited and obesity mainly occurs because fat cells proliferate (12). However, futher studies addressing these issues are clearly warranted to confirm this hypothesis. It should also be noted that corpulent SHR/N-*cp* rats are rather characterized by a hyperplasic type of obesity whereas obese Zucker rats show a hypertrophic adiposity of all the depots (4). Basal lipolytic capacity in obese rats was significantly elevated in the different adipose regions investigated. It is likely that such an increase contributes to enhance serum FFA levels, especially because the number of cells present in each fat depot is also significantly augmented However, although the enhanced in basal lipolysis may be partly due to an enlargement in adipose cell size (1), it may also result from alterations in the regulation of the hormone-sensitive lipase (19). Indeed, changes in basal lipolysis that we observed

did not exactly parallel those in fat cell weight, suggesting that other factors than adipocyte size contribute to enhance basal lipolytic activity. Another factor explaining the remarkable increase in serum FFA concentrations would be a decreased fatty acid oxidation (or re-esterification into triglycerides). Indeed, we have previously reported that the capacity of brown adipocytes for oxidizing fatty acids (in the presence or absence of norepinephrine) was markedly decreased in obese rats (2, 14, 15). This resulted from a decrease in brown adipose tissue mitochondrial content, uncoupling protein concentration and thermogenic capacity. However, further experiments are required to determine whether fatty acid metabolism is altered in other sites of fatty acid utilization such as skeletal muscle or the liver.

Since an obese state has been associated with a reduced activity of the sympathetic nervous system (9, 18), the stimulatory effects of norepinephrine on lipolysis in subcutaneous and internal adipocytes were therefore examined and compared to a direct stimulation by a selective β3-adrenoceptor agonist (BRL 37344). The fact that the β3- agonist was less effective than NE for stimulation lipolysis in PM (and to a minor extent in RP) obese adipocyte than in the lean corresponding cells suggests that changes occurring at some receptor or post-receptor levels were mainly responsible for the decreased lipolysis. This observation agrees with a recent study which describes a drastic impaired expression of the β3-adrenoceptor in adipose tissue of the SHR/NDmc-*cp* rats, a model similar to the one we used (11). Moreover, this alteration in the β-adrenergic pathway appeared to be more severe in the PM than in the RP adipose tissue, while it did not occur in the SC fat region. Indeed, other authors (19) have already reported site differences in mRNA levels of adipose tissue hormone-sensitive lipase, a finding which has been further confirmed in another strain of rats (20).

Although the main metabolic changes associated with the (neuro)-hormonal regulation of lipolysis were found in internal depots (and more particularly in the PM one), from a quantitative point of view, the SC adipose pad is probably the tissue that contributes to the increase in serum FFA levels observed in obese rats. First, it represents the most abundant fat depot in obese rats. Second, the increase in basal lipolysis expressed per total fat pad weight is much higher in the SC than in the two internal adipose regions. Finally, the SC fat depot is the one that grows the most during the development of obesity. The fact that regional variation in adipose tissue lipolysis was highly associated with the well known metabolic complications of obesity in humans (5, 6, 13), could also be extended to such an animal model of genetic obesity and type 2 diabetes, as differences were found between lean and obese SHR/N-*cp* rats in the lipolytic activity of internal adipocytes.

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La rata SHR/N-*cp* es un modelo genético que presenta obesidad y diabetes de tipo 2. A fin de comprender mejor el vínculo entre la

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