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Influence of dietary macronutrient composition on adiposity and cellularity of different fat depots in Wistar rats

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The aim of this study was to investigate the role of dietary macronutrient content on adiposity parameters and adipocyte hypertrophy/hyperplasia in subcutaneous and visceral fat depots from Wistar rats using combined histological and computational approaches. For this purpose, male Wistar rats were distributed into 4 groups and were assigned to different nutritional interventions: Control group (chow diet); high-fat group, HF (60% E from fat); high-fat-sucrose group, HFS (45% E from fat and 17% from sucrose); and high-sucrose group, HS (42% E from sucrose). At day 35, rats were sacrificed, blood was collected, tissues were weighed and fragments of different fat depots were kept for histological analyses with the new software *Adiposoft*. Rats fed with HF, HFS and HS diets increased significantly body weight and total body fat against Control rats, being metabolic impairments more pronounced on HS rats than in the other groups. Cellularity analyses using *Adiposoft* revealed that retroperitoneal adipose tissue is histologically different than mesenteric and subcutaneous ones, in relation to bigger adipocytes. The subcutaneous fat pad was the most sensitive to the diet, presenting adipocyte hypertrophy induced by HF diet and adipocyte hyperplasia induced by HS diet. The mesenteric fat pad had a similar but attenuated response in comparison to the subcutaneous adipose tissue, while retroperitoneal fat pad only presented adipocyte hyperplasia induced by the HS diet intake after 35 days of intervention. These findings provide new insights into the role of macronutrients in the development of hyperplastic obesity, which is characterized by the severity of the clinical features. Finally, a new tool for analyzing histological adipose samples is presented.

Key words: Diet-induced obesity, Macronutrient content, Hypertrophy, Hyperplasia, White adipose tissue.

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Obesity is the result of an augmented adipose tissue mass. The enlargement of this fat depot may be the result of an increase in the number of adipocytes (hyperplasia) or an increase in adipocyte size by lipid accumulation (hypertrophy). Moreover, hyperplasia can be due to the presence of new preadipocytes or to an induction of its differentiation (8). It is known that the most severe form of obesity is characterized by an adipose tissue enlargement as a consequence of adipocyte hyperplasia (18) and that it is related with an early age of onset (10). In addition, adipocyte number is apparently unafected in individuals with hyperplastic obesity following a hypocaloric diet (35). On the other hand, adipocyte cell size can influence insulin sensitivity (1, 32), glucose tolerance (37) and adipose tissue metabolism (22). Also, it has been shown that enlarged adipocytes secrete growth factors that induce adipocyte preadipocyte proliferation (28) as fat cells do not have an unlimited capacity for expansion. There are also regional differences between different fat depots, having the mesenteric region the major growth capacity and the subcutaneous the major proliferation capacity (9, 20). In these sense, a previous study of our group showed this proliferative capacity of the subcutaneous adipose tissue in rats fed with a high-fat diet (3). Furthermore, it is known that it could be due to a differential tissue perfussion or to the innervation density of adipose tissue by the sympathetic nervous system (6, 7).

On the other hand, specific dietary constituents may promote the development of insulin resistance, diabetes and obesity independently of an increased energy intake. In this sense, macronutrient profile can affect diet-induced thermogenesis (17), gene expression (5) or the level of some hormones (13, 33). Moreover, the source and amount of energy can modify adipose tissue growth by hypertrophy and hyperplasia in Holstein Steers, being hypertrophy most affected by the amount of energy and hyperplasia by the source of energy (34). Furthermore, it has been shown that a high-fat diet can modulate the proliferation of adipogenic progenitors in adult mice in a fat-depot depending manner, resulting in adipose tissue hyperplasia (20). Since there are evidence that a dietary treatment can modify adipocytes number and size, the aim of this study was to investigate the role of dietary macronutrient content on adiposity markers and adipocyte hypertrophy/hyperplasia measurements in subcutaneous and visceral fat depots of Wistar rats fed on diets with different proportions of lipids and sugars.

Material and Methods

Animals.– Experiments were performed with forty-five male Wistar rats from CIFA (Centre of Pharmaceutical Aplicated Investigation) of the University of Navarra with an initial weight of 250 g. Animals were kept in an isolated room with a constantly regulated temperature between 21 and 23 ºC, and controlled (50±10%) humidity in a 12h:12h artificial light/dark cycle. They were distributed into 4 groups and were assigned to different nutritional interventions during 35 days. Thus, Control group (n=10) was fed with a standard pelleted chow diet from Harlan Ibérica (Barcelona, Spain) and the other 3 groups were fed with different hypercaloric diets: a high-fat diet (HF, n=12), a high-fat-sucrose diet (HFS, n=12) and a high-sucrose diet (HS, n=11), whose composition is reported in Table I (23, 27). The HF diet (288 Kcal/100g)

Table I. *Composition of the experimental diets.*

		Lipids	Protein	Total CHO	Simple sugars
C	(n =10)	17%	10%	73%	7%
HF	(n =12)	60%	16%	24%	4.5%
HFS	(n =12)	45%	20%	35%	17%
HS	(n =11)	19%	12%	69%	42%

Values are represented as percentage of total energy. CHO, carbohydrates.

consisted in a mix of pate, ham, bacon, biscuits and a standard chow diet; the HFS diet (267 Kcal/100g) consisted in a mix of pate, ham, bacon, biscuits, sweetened condensed milk and a standard chow diet; and the HS diet (339 kcal/100g) consisted in pelleted chow and sweetened condensed milk.

The different groups of rats had *ad libitum* water and food access, while body weight and food intake were recorded 3 times per week. After finishing the experimental feeding period (day 35) rats were sacrificed, blood was collected from the trunk and serum stored at -20 ºC, while tissue samples of liver and retroperitoneal, mesenteric, epididymal and subcutaneous (inguinal) WAT were isolated, weighted and stored immediately at -80 ºC. All the procedures performed agreed with the national and institutional guidelines of the Animal Care and Use Committee at the University of Navarra.

Serum measurements.– Serum triglycerides were determined with the RANDOX kit for the *in vitro* diagnostic of triglycerides (Randox LTD Laboratories, Ardmore Road, UK), glucose was measured using the HK-CP kit (ABX Pentra, Montpellier, France), total cholesterol was measured using the Cholesterol-CP kit (ABX Pentra, Montpellier, France) and HDL-cholesterol with the HDL direct-CP kit (ABX Pentra, Montpellier, France) adapted for a COBAS MIRA (Rochel, Basel, Switzerland) equipment. Leptin and insulin quantification was performed by specific Elisas Kits following the protocols described by the manufacturer (Linco Research, Missouri, USA). Finally, the homeostatic model assessment (HOMA), as an insulin resistance index, was calculated using the formula: (fasting plasma insulin x plasma glucose)/22.5.

Histological analyses.– Small pieces of different fat depots were kept in formaldehyde for histological analyses. These tissues were fixed and stained with hematoxylin/eosin, and acquired using AxioVision Zeiss Imaging software (AxioVision controls via software an Axio Imager M1 Zeiss microscope and an Insight AxioCamm ICc3 camera). The magnification in this case was 20x. The acquired images were stored in uncompressed 24 bit color TIFF format. Finally, these images were analyzed with a new software (*Adiposoft* from CIMA, University of Navarra) in order to determine adipocyte diameters and adipose tissue cellularity. Adipocyte number in each tissue was estimated according to formulas from LEMONNIER *et al.* (25).

Statistical analyses.– All results are expressed as the average mean ± standard deviation. Means comparisons were tested for metabolic parameters by Anova test and Student's T test for all the variables. For frequency distribution cell sizes <25

μm were considered debris or very small adipocytes (16) and removed from the analysis. After that, cells were grouped by sizes according to adipocyte diameter (25-50 μm, small adipocytes; 50-90 μm, normal adipocytes; 90-130 μm, big adipocytes; >130 μm, very big adipocytes) and analyzed statistical differences by a repeated measures Anova test. Finally, statistical differences of Lemonnier estimations were performed using Mann-Whitney U test. A level of probability up at $p<0.05$ was set up as statistically significant and $p<0.01$ as very statistically significant. For the statistical tests, GraphPad Prism Software 4.02 version and SPSS v13.0 was used (San Diego, CA, USA).

Results

Body weight and adiposity.– During the experimental period, rats fed with the 3 obesigenic dietary treatments showed hyperphagia (Table II). At the end of the experiment, rats fed with HF and HS diets significantly increased body weight and total body fat (reflected as the sum of retroperitoneal, mesenteric, retroperitoneal and epididymal fat pads) in comparison to Control rats (Table II). HFS diet increased also total body fat; however it did not modify body weight significantly. Liver weight (Table II) was also higher in HF, HFS and HS rats than in Control rats, suggesting diet-induced steatosis.

Table II. *Weight related parameters and plasma values of male Wistar rats fed vith 4 different dietary treatments during 35 days.*

	Group			
	C (n=10)	HF (n=12)	HFS (n=12)	HS (n=11)
Weight related parameters				
Food intake (Kcal/day)	79.7±27.5^{a}	196.4±18.3^{b}	181.8±27.5^{b,c}	103.1±5.6^{d}
Food efficiency (g/Kcal)	0.023±0.007^{a}	0.014±0.005^{b}	0.012±0.005^{b}	0.029±0.006^{a,c}
Final body weight (g)	388±23^{a}	420±25^{b}	413±39^{a,b}	426±24^{b}
Total fat mass (g)	22.61±4.75^{a}	32.09±6.92^{b}	32.84±6.11^{b}	35.42±7.11^{b}
Liver weight (g)	8.85±1.18^{a}	11.19±1.01^{b}	10.38±1.53^{b}	10.4±1.14^{b}
Plasma values				
Glucose (mmol/l)	5.77±0.81^{a}	6.22±1.03^{a}	6.50±1.17^{a}	5.98±1.04^{a}
Total cholesterol (mg/dl)	63.6±7.9^{a}	58.3±12.0^{a}	56.8±11.7^{a,b}	48.4±7.2^{b}
HDL cholesterol (mg/dl)	22.4±2.7^{a}	21.9±6.5^{a,b}	20.8±4.6^{a,b}	19.3±.2^{b}
Triglycerides (mg/dl)	86.2±28.5^{a}	70.6±21.2^{a}	69.7±20.7^{a}	132.9±57.6^{b}
FFA (mg/dl)	0.78±0.12^{a}	0.69±0.08^{a,b}	0.63±0.09^{b,c}	0.91±0.22^{a,d}
Leptin (ng/dl)	2.76±1.52^{a}	5.97±2.65^{b}	7.33±3.99^{b,c}	9.45±3.66^{c}
Insulin (μU/ml)	7.18±2.94^{a}	14.78±10.92^{b}	19.62±14.65^{c}	31.54±10.13^{d}
HOMA IR	1.92±1.01^{a}	4.36±3.67^{a,b}	6.17±5.81^{b,c}	8.51±3.51^{c,d}

All the results are expressed as the mean ± SD. Statistical analyses were performed using Anova test and Student's *t* test was used to test differences in the means of each group. Different letter (a, b, c) indicated significative differences between groups of at least $p<0.05$. Total fat mass indicates the sum of the subcutaneous, retroperitoneal, mesenteric and epididymal fat pads weights. C, Control; HF, High-Fat; HFS, High-Fat-Sugar; HS, High-Sugar; FFA, Free Fatty Acids.

Metabolic parameters and hormone profile.– The three diets tested in this study induced marked hyperleptinemia and hyperinsulinemia after 35 days of experimental treatment. Regarding lipid profile, HS diet was the only one that increased free fatty acid and triglycerides levels in Wistar rats, while it caused a decrease in total cholesterol and HDL cholesterol levels (Table II). Finally, HOMA-IR, an indicator of insulin resistance, was higher in HFS and HS groups against control rats.

Histological analyses.– The results of adipose tissue cellularity obtained from *Adiposoft* program showed fat depot differences in control rats. Thus, retroperitoneal fat pad had a higher frequency of big adipocytes than subcutaneous ($p<0.01$) and mesenteric ($p<0.001$) fat pads (Rp: 15.46%±2.27; Sc: 6.21%±2.55; Mes: 3.26%±1.27) and a lower frequency of small fat cells ($p<0.001$) in comparison to these two depots (Rp: 31.16±2.54; Sc: 42.63±4.56; Mes: 49.04±0.70). Regarding the effects of obesigenic diets, adipocyte frequency analyses in the subcutaneous adipose tissue showed that HF rats had a reduced frequency of small fat cells ($p<0.001$) and a higher frequency of big adipocytes ($p<0.001$), revealing that HF diet induced an increase in subcutaneous adipocytes size compared with chow diet (Fig. 1A). This fact was not observed in HFS and HS diets. In the mesenteric fat depot, it was found exactly the same pattern of adipocyte frequency distribution that in the subcutaneous fat depot (Fig. 1B), reaching significance only for small ones ($p<0.01$). Finally, the effects of the dietary treatments on the retroperitoneal fat depot (Fig. 1C) resulted in a decrease in the percentage of big fat cells by HS diet in comparison to control diet ($p<0.01$), with no changes in the frequen-

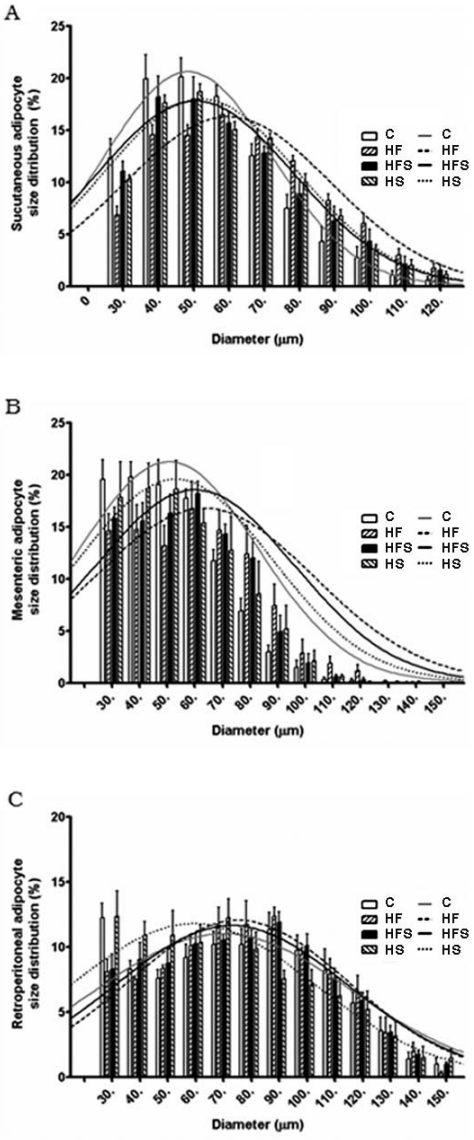

Fig. 1. *Adipocyte frequency distribution in subcutaneous (A), mesenteric (B) and retroperitoneal (C) adipose tissue of male Wistar rats fed with chow diet (C), high-fat diet (HF), high-fat-sugar diet (HFS) and high-sugar diet (HS) during 35 days (n= 3,4).* Non-lineal regression curves (Gauss) are shown in each figure.

cy of the small and normal adipocytes by any of the treatments.

Finally, we studied how the dietary treatments could affect different markers of adipose cellularity (cell volume, cells per gram and cells per total fat pad) from each depot (Fig. 2 and Table III) using *Adiposoft* histogram data and the algorithm published by LEMNONIER *et al.* (24). First of all, cell volume (Fig. 2A, 2D), as a measure of cell hypertrophia, was higher in the subcutaneous fat depot from rats fed with HF diet (p=0.055) in comparison with control fed rats. Neither the two other diets nor the HF diet affected this index in mesenteric and retroperitoneal fat depot. On the other hand (Table III), all dietary treatments increased statistically each fat depot weight analyzed (apart from for HFS and mesenteric pad). Remarkably, adipocyte number per gram of fat was not affected by the diets except for HF in the subcutaneous fat pad, which showed lower number of cells than HS fed rats ($p<0.05$), showing again a hypertrophic effect dependent on the macronutrient type (fat vs. carbohydrates). Total adipocyte number per fat pad (parameter of hyperplasia) was not changed by HF diet in all the three fat depots (Table III). However, HS diet induced an increase in this total population of adipocytes in the subcutaneous (46%, $p<0.05$) and retroperitoneal (104%, $p<0.05$) fat depots. Finally, HFS diet had an intermediate effect on adipose tissue cellularity comparing fat diet against the carbohydrate enriched diet, with much more heterogeneity between rats than in the other 3 dietary groups.

Table III. *Cellularity of three adipose depots of Wistar rats fed with 4 different dietary treatments during 35 days.*

	Group			
	C (n=4)	HF (n=4)	HFS (n=4)	HS (n=4)
Subcutaneous pad				
Fat pad weight (g)	5.67±1.25^{a}	7.41±1.98^{b}	8.16±1.54^{b}	9.17±2.41^{b}
Adipocyte number/g (x10^{6})	11.47±8.97^{a,b}	6.60±1.82^{a}	9.51±4.23^{a,b}	9.16±1.77^{b}
Adipocyte number/fat pad (x10^{6})	63.20±24.78^{a}	58.20±11.19^{a}	92.50±48.72^{a,b}	92.18±11.41^{b}
Mesenteric pad				
Fat pad weight (g)	2.98±0.95^{a}	4.11±1.05^{b}	3.78±0.94^{a,b}	4.63±1.04^{b}
Adipocyte number/g (x10^{6})	11.78±2.16^{a}	8.37±3.68^{a}	9.47±2.38^{a}	11.28±5.14^{a}
Adipocyte number/fat pad (x10^{6})	40.76±1.14^{a,b}	35.55±11.02^{a,b}	33.80±5.28^{a}	54.01±14.89^{b}
Retroperitoneal pad				
Fat pad weight (g)	7.57±2.08^{a}	10.31±2.50^{b}	10.46±2.34^{b}	10.86±2.51^{b}
Adipocyte number/g (x10^{6})	3.27±0.81^{a}	3.43±0.59^{a}	3.46±0.54^{a}	4.33±1.51^{a}
Adipocyte number/fat pad (x10^{6})	23.89±2.91^{a}	35.52±8.56^{a,b}	38.75±11.24^{b}	48.88±8.70^{b}

All the results are expressed as the mean ± SD. Statistical analyses were performed using Mann Whitney U test. Different letter (a, b, c) indicated significative differences between groups of at least $p<0.05$. C, Control; HF, High-Fat; HFS, High-Fat-Sugar; HS, High-Sugar.

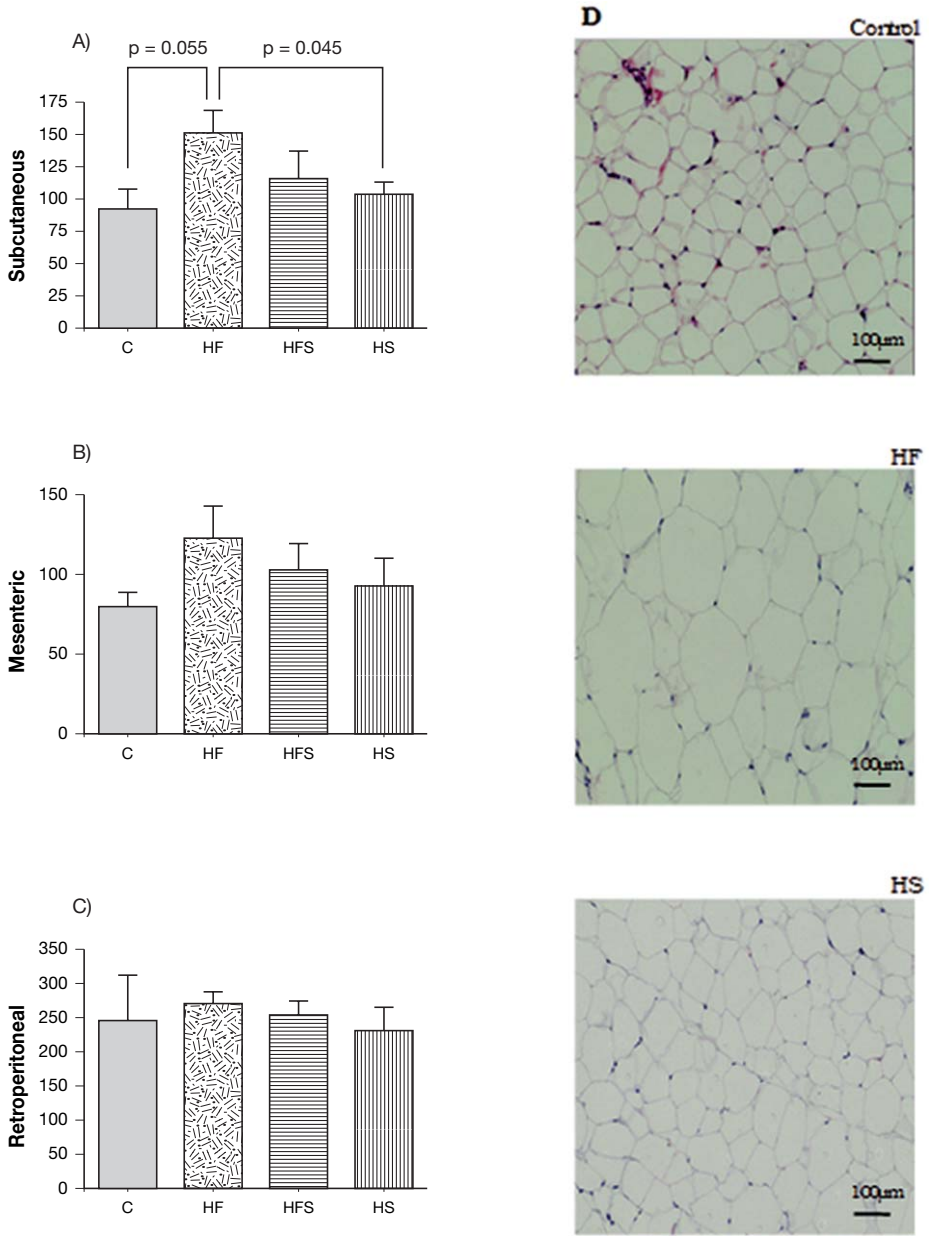

Fig. 2. *Effect of chow diet (C), high-fat diet (HF), high-fat-sugar diet (HFS) and high-sugar (HS) diet on adipocytesize of Wistar rats.*
(A) Mean adipocyte volume (pl) in subcutaneous fat pad. (B) Mean adipocyte volume (pl) in mesenteric fat pad. (C) Mean adipocyte volume (pl) in retroperitoneal fat pad. Three representative images (D) from hematoxylin and eosin stained paraffin sections of subcutaneous fat pads of Wistar rats fed with chow diet, high-fat diet and high-sugar diet.

Discussion

The effect of dietary composition on adiposity and cellularity was studied in the current investigation through the new software *Adiposoft*. For this purpose, three diets with different macronutrient distribution were used to induce obesity in male Wistar rats. Taking into account that obesity is characterized by an increased adipose mass, the three obesigenic diets were able to establish this overweight model. Moreover, the three diets increased liver weight, suggesting the induction of hepatic steatosis (29).

Diet-induced metabolic alterations in HS group were more pronounced than in the other groups. The rise in serum triglycerides and FFA has been explained because diets high in simple sugars markedly stimulate fatty acid synthesis from carbohydrates (19, 21). However, total cholesterol levels decreased with HS dietary treatment. This reduction could be the result of the lower HDL cholesterol levels, as has been previously reported by KAMGANG *et al.* (21). These rats showed also hyperinsulinemia, as described by other authors (31). The absence of changes in insulin resistance parameters in HF group could be due to the short experimental period, as other authors had reported changes in glucose, insulin and HOMA index in Wistar rats fed with a HF diet (15, 29).

Regarding histological analyses, the present investigation showed that retroperitoneal adipose tissue is histologically different than mesenteric and subcutaneous ones, with greater adipocytes in basal conditions (26). On the other hand, rats fed with HF diet developed adipocyte hypertrophy in the subcutaneous adipose tissue without changes in adipocyte number. These results agree with those of LEMONNIER *et al.* (24), as a high-fat diet induced an increase in fat cell size in the subcutaneous fat depot of old female Zucker rats. However, in a previous study from our laboratory (3), rats fed with HF diet during 56 days presented an increase in the proliferation of subcutaneous adipocytes, estimated by DNA quantification. It could be explained by the different experimental feeding length and different technical approaches. Thus, according to the theory of the critical fat cell size (11), these differences can be due to lack of time of these adipocytes to achieve a maximal adipocyte size and thereby stimulate new cell production or differentiation. Taking into account that age is a significant factor in diet-induced changes in adipocyte morphology (30), other studies using young adult female rats (aged 2 month, as in our study) fed with a high-fat diet, reported adipocyte hyperplasia in both subcutaneous and visceral fat depots (2, 14), in addition to adipocyte hypertrophy. Likewise, it has been shown in male mice that dietary fats of different origin affect adipose tissue cellularity in a different manner (4).

On the other hand, the HS diet induced an increase in the number of new fat cells in the subcutaneous and retroperitoneal depots of Wistar rats. This has been previously reported by TULP *et al.* (36). Despite that the present study has not been found a clear adipocyte hypertrophy induced by the HFS diet, other investigations revealed an effect of the same diet on fat cells, increasing subcutaneous and retroperitoneal adipocyte sizes (12).

Regarding fat-depot differences in cellularity, it has been evidenced that mice fed with high-fat diet during 60 days had a greater proportion of new adipocytes in subcutaneous than visceral fat, while adipocyte hypertrophy was more pronounced in visceral adipose tissue (20).

Although we have not found this depot-specific action, there are some differences in the methodology of both studies, as the high-fat diet used in such study (20) had only 45% of energy as fat while we used a diet with 60% of energy as fat. Moreover, the experimental period was longer.

Indeed, our findings suggest that rats fed on diets with different rate of fat/sugar develop obesity with different adiposity characteristics in a time dependent manner. Thus, a high-fat intake led mainly to adipocyte hypertrophy in subcutaneous adipose tissue, while a high sugar intake led to an increase of subcutaneous and retroperitoneal fat due to adipocyte hyperplasia. These findings provide new insights into the role of macronutrients, such fat and sugar, in the development of hyperplastic obesity, characterized by the formation of new fat cells and the severity of the clinical features. Finally, the results of this study demonstrated that this new software, *Adiposoft*, is a very useful tool to analyze adipose tissue cellularity, facilitating the determination of adipocytes diameters, volume, area and number in a great number of images, avoiding the relatively complex and delicate protocol for isolating adipocytes from the adipose tissue.

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References

1. Arner, E., P.O. Westermark, K.L. Spalding, T. Britton *et al.* (2010): Adipocyte Turnover: Relevance to Human Adipose Tissue Morphology. *Diabetes*, **59**, 105-109.
2. Aslan, H., B.Z. Altunkaynak, M.E. Altunkaynak, O. Vuraler, S. Kaplan, and B. Unal. (2006): Effect of a high fat diet on quantitative features of adipocytes in the omentum: an experimental, stereological and ultrastructural study. *Obes Sur,* **16**, 1526-1534.
3. Boqué, N., Campión, J., Milagro, F.I., Moreno-Aliaga, M.J., Martinez, J.A. (2009): Some cyclin-dependent kinase inhibitors-related genes are regulated by vitamin C in a model of diet-induced obesity. *Biol Pharm Bull,* **32**, 1462-1468.
4. Bourgeois, F., A. Alexiu, and D. Lemonnier. (1983): Dietary-induced obesity: effect of dietary fats on adipose tissue cellularity in mice. *Br J Nutr,* **49**, 17-26.
5. Capel, F., N. Viguerie, N. Vega, S. Dejean *et al.* (2008): Contribution of energy restriction and macronutrient composition to changes in adipose tissue gene expression during dietary weight-loss programs in obese women. *J Clin Endocrinol Metab,* **93**, 4315-4322.
6. Cousin, B., L. Casteilla, M. Lafontan, L. Ambid *et al.* (1993): Local sympathetic denervation of white adipose tissue in rats induces preadipocyte proliferation without noticeable changes in metabolism. *Endocrinology,* **133**, 2255-2262.
7. Crandall, D.L. and M. DiGirolamo. (1990): Hemodynamic and metabolic correlates in adipose tissue: pathophysiologic considerations. *Faseb J,* **4**, 141-147.
8. Chen, W.P., B.Y. Ho, C.L. Lee, C.H. Lee, and T.M. Pan. (2008): Red mold rice prevents the development of obesity, dyslipidemia and hyperinsulinemia induced by high-fat diet. *Int J Obes (Lond),* **32**, 1694-1704.
9. DiGirolamo, M., J.B. Fine, K. Tagra, and R. Rossmanith. (1998): Qualitative regional differences in adipose tissue growth and cellularity in male Wistar rats fed ad libitum. *Am J Physiol,* **274**, R1460-1467.
10. Dugail, I., Quignard-Boulangé, A., Dupuy, F. (1986): Role of adipocyte precursors in the onset of obesity induced by overfeeding in suckling rats. *J Nutr*, **116**, 524-535.
11. Faust, I.M. (1984): Role of the fat cell in energy balance physiology. *Res Publ Assoc Res Nerv Ment Dis,* **62**, 97-107.
12. Fernandes-Santos, C., R.E. Carneiro, L. de Souza Mendonca, M.B. Aguila, and C.A. Mandarim-de-Lacerda. (2009): Pan-PPAR agonist beneficial effects in overweight mice fed a high-fat high-sucrose diet. *Nutrition,* **25**, 818-827.
13. Fernández-Quintela, A., I. Churruca, and M.P. Portillo. (2007): The role of dietary fat in adipose

tissue metabolism. *Public Health Nutr,* **10**, 1126-1131.
14. Gale, S.K., T.B. Van Itallie, and I.M. Faust. (1981): Effects of palatable diets on body weight and adipose tissue cellularity in the adult obese female Zucker rat (fa/fa). *Metabolism,* **30**, 105-110.
15. García-Díaz, D.F., Campion, J., Milagro, F.I., Lomba, A. *et al.* (2007): Chronic mild stress induces variations in locomotive behavior and metabolic rates in high fat fed rats. *J Physiol Biochem,* **63**, 337-346.
16. Hausman, D.B., J.B. Fine, K. Tagra, S.S. Fleming *et al.* (2003): Regional fat pad growth and cellularity in obese zucker rats: modulation by caloric restriction. *Obes Res,* **11**, 674-682.
17. Hermsdorff, H.H., A.C. Volp, and J. Bressan. (2007): [Macronutrient profile affects diet-induced thermogenesis and energy intake]. *Arch Latinoam Nutr,* **57**, 33-42.
18. Hirsch, J. and B. Batchelor. (1976): Adipose tissue cellularity in human obesity. *Clin Endocrinol Metab,* **5**, 299-311.
19. Hudgins, L.C., M.K. Hellerstein, C.E. Seidman, R.A. *et al.* (2000): Relationship between carbohydrate-induced hypertriglyceridemia and fatty acid synthesis in lean and obese subjects. *J Lipid Res,* **41**, 595-604.
20. Joe, A.W., L. Yi, Y. Even, A.W. Vogl, and F.M. Rossi. (2009): Depot-Specific Differences in Adipogenic Progenitor Abundance and Proliferative Response to High-Fat Diet. *Stem Cells,* **27**, 2563-2570.
21. Kamgang, R., R.Y. Mboumi, P. N'Dille G, and J.N. Yonkeu. (2005): Cameroon local diet-induced glucose intolerance and dyslipidemia in adult Wistar rat. *Diabetes Res Clin Pract,* **69**, 224-230.
22. Koska, J., N. Stefan, S. Dubois, C. Trinidad *et al.* (2009): mRNA concentrations of MIF in subcutaneous abdominal adipose cells are associated with adipocyte size and insulin action. *Int J Obes (Lond),* **33**, 842-850.
23. Kuo, L.E., J.B. Kitlinska, J.U. Tilan, L. Li *et al.* (2007): Neuropeptide Y acts directly in the periphery on fat tissue and mediates stress-induced obesity and metabolic syndrome. *Nat Med,* **13**, 803-811.
24. Lemmonier, D. (1972): Effect of age, sex, and site on the cellularity of the adipose tissue in mice and rats rendered obese by a high-fat diet. *J Clin Invest,* **51**, 2907-2915.
25. Lemonnier, D. (1972): Effect of age, sex, and sites on the cellularity of the adipose tissue in mice and rats rendered obese by a high-fat diet. *J Clin Invest,* **51**, 2907-2915.
26. Lopes, P.A., S.V. Martins, M.S. Pinho, C.M. Alfaia *et al.* (2008): Diet supplementation with the cis-9,trans-11 conjugated linoleic acid isomer affects the size of adipocytes in Wistar rats. *Nutr Res,* **28**, 480-486.
27. Llado, I., A. Pons, and A. Palou. (1999): Effects of fasting on lipoprotein lipase activity in different depots of white and brown adipose tissues in diet-induced overweight rats. *J Nutr Biochem,* **10**, 609-614.
28. Marques, B.G., D.B. Hausman, and R.J. Martin. (1998): Association of fat cell size and paracrine growth factors in development of hyperplastic obesity. *Am J Physiol,* **275**, R1898-1908.
29. Milagro, F.I., J. Campion, and J.A. Martinez. (2006): Weight gain induced by high-fat feeding involves increased liver oxidative stress. *Obesity (Silver Spring),* **14**, 1118-1123.
30. Pouteau, E., S. Turner, O. Aprikian, M. Hellerstein *et al.* (2008): Time course and dynamics of adipose tissue development in obese and lean Zucker rat pups. *Int J Obes (Lond),* **32**, 648-657.
31. Preuss, H.G., M. Zein, P. MacArthy, D. Dipette *et al.* (1998): Sugar-induced blood pressure elevations over the lifespan of three substrains of Wistar rats. J *Am Coll Nutr,* **17**, 36-47.
32. Roberts, R., L. Hodson, A.L. Dennis, M.J. Neville *et al.* (2009): Markers of de novo lipogenesis in adipose tissue: associations with small adipocytes and insulin sensitivity in humans. *Diabetologia,* **52**, 882-890.
33. Sánchez, J., Oliver, P., Palou, A., Picó, C. (2004): The inhibition of gastric ghrelin production by food intake in rats is dependent on the type of macronutrient. *Endocrinology,* **145**, 5049-5055.
34. Schoonmaker, J.P., F.L. Fluharty, and S.C. Loerch. (2004): Effect of source and amount of energy and rate of growth in the growing phase on adipocyte cellularity and lipogenic enzyme activity in the intramuscular and subcutaneous fat depots of Holstein steers. *J Anim Sci,* **82**, 137-148.
35. Shi, H., S. Akunuru, J.C. Bierman, K.M. Hodge *et al.* (2009): Diet-induced obese mice are leptin insufficient after weight reduction. *Obesity (Silver Spring),* **17**, 1702-1709.
36. Tulp, O.L., S.P. DeBolt, C.T. Hansen, and O.E.t. Michaelis. (1991): Effects of dietary carbohydrate and phenotype on adipose cellularity in female SHR/N-cp rats. *Comp Biochem Physiol A Comp Physiol,* **99**, 229-234.
37. Weyer, C., Foley, J.E., Bogardus, C., Tataranni, P.A., Pratley, R.E. (2000): Enlarged subcutaneous abdominal adipocyte size, but not obesity itself, predicts type II diabetes independent of insulin resistance. *Diabetologia,* **43**, 1498-1506.