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ORIGINAL ARTICLE

Lipid metabolism in adipose tissue and liver from diet-induced obese rats: a comparison between *Wistar* and *Sprague-Dawley* strains

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Abstract

Some researchers have proposed important variations in adipose tissue among different strains of rats and mice in response to a high-caloric (hc) diet, but data concerning the mechanisms underlying these differences are scarce. The aim of the present research was to characterize different aspects of triacylglycerol (TG) metabolism and clock genes between *Sprague-Dawley* and *Wistar* rats. For this purpose, 16 male *Sprague-Dawley* and 16 male *Wistar* rats were divided into four experimental groups ($n = 8$) and fed either a normal-caloric (nc) diet or a hc diet for 6 weeks. After sacrifice, liver and epididymal, perirenal, mesenteric, and subcutaneous adipose tissue depots were dissected, weighed and immediately frozen. Liver TG content was quantified, RNA extracted for gene expression analysis and fatty acid synthase enzyme activity measured. Two-way ANOVA and Student's *t* test were used to perform the statistical analyses. Under hc feeding conditions, *Wistar* rats were more prone to fat accumulation in adipose tissue, especially in the epididymal fat depot, due to their increased lipogenesis and fatty acid uptake. By contrast, both strains of rats showed similarly fatty livers after hc feeding. Peripheral clock machinery seems to be a potential explanatory mechanism for *Wistar* and *Sprague-Dawley* strain differences. In conclusion, *Wistar* strain seems to be the best choice as animal model in dietary-induced obesity studies.

Keywords High-caloric feeding · Obesity · Strain · *Wistar* · *Sprague-Dawley* · Rats

Introduction

Nowadays, obesity is considered the most prevalent nutritional disease around the world [18]. The widespread occurrence of this health problem in humans means that there is a need to study its causes and physiopathology. To carry out these studies, it is important to find useful animal models that sufficiently mimic all aspects of this human disease.

There are different approaches to obesity induction in laboratory animals, such as genetic manipulation, dietary treatments, or neuroendocrine alteration induction [11]. In the case of dietary treatments, high-fat diets that entail an increase in caloric intake are widely used. In addition to their obesogenic effects, these diets induce metabolic alterations which are very similar to those found in obese humans [31]. Regarding the choice of animals, rodents are considered as essential tools for obesity studies because they are well characterized in terms of metabolic pathways and display a great similarity and homology with the human genome [25]. Mice and rats are the most common rodents used for this purpose. In the case of rats, their larger size eases their handling, sampling, and performing procedures. Another important aspect in the choice of the animal model is the strain. In this context, some researchers have demonstrated physiological variations within different strains in response to high-fat diets. Analysis of strain-dependent susceptibility to diet has been performed more extensively in mice. Thus, C57BL/6, AKR, and DBA/2J mice are more susceptible to develop obesity than A/J, SWR/J, and 129S6 strains, whose tendency is to be resistant to obesity [4, 7]. In addition, strains that show similar levels of obesity may have modified outcomes, such as different lipid metabolism or circadian clock gene expression. Indeed, circadian machinery recently have been linked not only to sleep arousal disorder,

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but also to a wide variety of common metabolic alterations, as insulin resistance and liver steatosis, two frequent obesity co-morbidities [24, 32].

As far as rats are concerned, *Wistar* and *Sprague-Dawley* strains have been used in the vast majority of the reported studies devoted to inducing dietary obesity and comorbidities. However, these two strains of rats are not equally prone to develop obesity and related alterations [15, 20, 29, 30, 34]. In some of the abovementioned studies, a direct comparison was made between *Wistar* and *Sprague-Dawley* strains, but unfortunately, very few data which explain differences among them have been provided to date.

Bearing all this in mind, the aim of the present research was to evaluate differences between *Wistar* and *Sprague-Dawley* strains in lipid metabolism in epididymal and subcutaneous adipose tissues and liver after hc diet feeding. Peripheral clock gene machinery was also characterized in these tissues, because it adds a level of complexity to the comprehension of the risk factors that trigger obesity in rodents.

Materials and methods

Animals, diets, and experimental design

The experiment was conducted with 16 male *Sprague-Dawley* rats (initial body weight 180 ± 5 g) and 16 male *Wistar* rats (initial body weight 185 ± 3 g) purchased from Harlan Ibérica (Barcelona, Spain), and took place in accordance with the institution's guide for the care and use of laboratory animals, with the approval of our internal animal ethics committee (Reference protocol approval CUEID CEBA/30/2010), following European Community Council Directive. Animals were individually housed in polycarbonate metabolic cages (Techniplast Gazzada, Guguggiate, Italy) and placed in an airconditioned room (22 ± 2 °C) with a 12-h light-dark cycle. After a 6-day adaptation period, rats from each strain were randomly divided into two groups ($n = 8$) and fed either anc or hc diet (high-fat diet), supplied by Harlan Ibérica for 6 weeks. The nc diet (TD.06416, 3.7 kcal/g) was offered to control groups (SDnc, Wnc), and the hc diet (TD.06415; 4.6 kcal/g) was offered to the other two groups in order to promote obesity development (SDhc, Whc). The diet was provided to rats at 10.00 p.m., when the dark phase started in the animal facility room (inversed light cycle in the room). All animals had free access to the diet and water.

Body weight and food intake were measured daily. At the end of the experimental period, rats were sacrificed after 12 h of fasting under anesthesia (chloral hydrate) by cardiac exsanguination. Liver and adipose tissues from epididymal, perirenal, mesenteric, and subcutaneous regions were dissected and weighed, and then immediately frozen. All samples were stored at − 80 °C until analysis.

RNA extraction and quantitative real-time polymerase chain reaction in adipose tissue and liver

Total RNA was extracted using Trizol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. After DNAse treatment (Ambion; The RNA Company, Applied Biosystems, Foster City, CA, USA), 1.5 μg of total RNA was reverse transcribed into complementary DNA (cDNA) (iScript cDNA Synthesis Kit, Bio-Rad, Hercules, CA, USA). qRT-PCR analysis was performed with iCycler-MyiQ Real-Time PCR Detection System (Bio-Rad) in the presence of SYBRGreen master mix (Applied Biosystems) and specific primers(Tib Molbiol, Berlin, Germany and Eurogentec, Liège, Belgium). Primers for sterol regulatory element-binding factor 1 (*srebf1*), acetyl-CoA carboxylase (*acc*), fatty acid synthase (*fasn*), peroxisome proliferator-activated receptor γ (*pparγ*), lipoprotein lipase (*lpl*), cluster of differentiation 36 (*cd36*), adipose triglyceride lipase (*atgl*), hormone sensitive lipase (*hsl*), cryptochrome (*cry1*), clock homolog (*clock*), period homolog 2 (*per2*), aryl hydrocarbon receptor nuclear translocator-like 1 (*bmal1*), and *β-actin* were previously described [9, 16, 23]. Stearoyl-CoA desaturase (*scd*) primers were as follows: (sense 5′-CCGTGGCTTTTTCTTCTCTCA-3′; antisense 5′-CTTTCCGCCCTTCTCTTTGA-3′). The expression of *β-actin* was not modified among groups, validating its use as reference gene. Thus, mRNA levels in all samples were normalized to the values of *β-actin* and the results expressed as fold changes of threshold cycle (Ct) value relative to controls using the $2^{-\Delta\Delta Ct}$ method [17].

Fatty acid synthase activity in adipose tissue

For the analysis of fatty acid synthase (FAS) activity, 1 g of epididymal or subcutaneous adipose tissue was homogenized in 5 mL of buffer (pH 7.6) containing 150 mM KCl, 1 mM $MgCl_2$, 10 mM *N*-acetyl-cysteine, and 0.5 mM dithiothreitol. After centrifugation at 100,000 *g* for 40 min at 4 °C, the supernatant fraction was used for analysis. Enzyme activity was measured by spectrophotometry as previously described [22], and expressed as nmol NADPH consumed per minute per mg of protein.

Liver triacylglycerol content

Total hepatic lipids were extracted following the method described by Folch et al. [6]. The lipid extract was dissolved in isopropanol and TG content was measured using a commercial kit (BioSystems, Barcelona, Spain).

Statistical analysis

Results are presented as mean ± standard error of the mean (SEM). All the parameters were normally distributed according to the Shapiro-Wilks test. Two-way ANOVA test was used to

determine the effects of factors (strain and diet). Comparisons between nc and hc groups for each strain, as well as between both strains in rats fed each diet (SDnc vs. Wnc and SDhc vs. Whc) were assessed by Student's *t* test. Significance was assessed at the $P < 0.05$ level.

Results

Food intake, body weight, adipose tissue and liver weights and hepatic triacylglcyerol content

Diet × strain interaction was observed in energy intake, final body weight, and adipose tissue weights, but not in liver weight or hepatic TG content (Table 1). There were significant differences in liver and perirenal, epididymal, and pooledadipose tissue weights, but not in energy intake, final body weight, subcutaneous, and mesenteric adipose tissue weights and hepatic TG content among rats fed the nc diet from both strains (SDnc vs. Wnc). Hc diet feeding increased energy intake in both strains of rats, although this effect was greater in *Wistar* (+ 25.4%) than in *Sprague-Dawley* rats (+ 8.5%). As far as final body weight and pooled adipose tissue weights after hc diet were concerned, these parameters were significantly increased only in *Wistar* strain, with the exception of epididymal adipose tissue (Table 1). Hc diet feeding raised liver weight and hepatic TG content in the *Wistar* but not the *Sprague-Dawley* strain (Table 1).

Lipid metabolism genes expression and activity in epididymal adipose tissue

Figure 1a shows mRNA levels of genes involved in lipid uptake (*pparγ*, *lpl*, and *cd36*), in epididymal adipose tissue. The three genes were significantly increased in *Wistar* rats fed the hc diet when compared with their controls. Similar pattern of response was observed for *lpl* and *cd36*, but not for *pparγ*, in *Sprague-Dawley* rats. An interaction diet × strain was observed for *pparγ*, and *lpl* in this adipose tissue (Fig. 1a). Genes involved in lipid uptake were more highly expressed in *Wistar* than in *Sprague-Dawley* rats after hc diet feeding (SDhc vs. Whc in Table 2).

With regard to lipogenesis-related genes, hc diet feeding modified the expression of *srebf1*, *acc*, *fasn*, and *scd*, but with different patterns in each rat strain. While the expression of *acc*, *fasn,* and *scd* significantly decreased in *Sprague-Dawley* rats, *srebf1*, *acc*, and *scd* significantly increased in *Wistar* rats. An interaction diet × strain was observed for the four genes analyzed (Fig. 1b). With the exception of *acc*, no differences in gene expression were observed between both strains of rats fed a nc diet (Wnc vs. SDnc in Table 2). By contrast, *Wistar* rats fed the hc diet presented higher mRNA levels of all the lipogenic genes analyzed than did *Sprague-Dawley* rats (Table 2). FAS activity was only increased in *Wistar* strain fed hc diet compared with those fed nc diet (Fig. 1c). No differences were found in enzyme activity between Whc and SDhc groups, but Wnc had lower FAS activity than SDnc group (Table 2).

In relation to lipolytic genes, *atgl* mRNA levels were not modified by hc diet feeding. However, mRNA levels of *hsl* were decreased in *Sprague-Dawley* rats and increased in *Wistar* rats (Fig. 1d), showing diet × strain interactive effect. *Wistar* rats fed the hc diet presented higher mRNA levels of both lipases than *Sprague-Dawley* rats did (Table 2).

Lipid metabolism genes expression and activity in subcutaneous adipose tissue

In subcutaneous adipose tissue, hc diet feeding led to higher expression of *lpl* and *cd36* in *Sprague-Dawley* rats, and to

Table 1 Final body weight, energy intake, adipose tissues, and liver weights and hepatic triacylglycerol content in *Wistar* and *Sprague-Dawley* rats fed a normal-caloric or a high-caloric diet for 6 weeks

	SDncn = 8	SDhcn = 8	Wncn = 8	Whcn = 8	Strain	Diet	Diet × strain
Energy intake (Kcal/d)	59 ± 1	64 ± 2*	63 ± 1	79 ± 2***	*< 0.001*	*< 0.001*	*< 0.001*
Final body weight (g)	326 ± 5	348 ± 10	323 ± 2	395 ± 6***	*0.001*	*< 0.001*	*< 0.001*
White adipose tissue weights (g)							
Perirenal	2.63 ± 0.16	3.54 ± 0.36	5.61 ± 0.60	15.34 ± 1.17***	*< 0.001*	*< 0.001*	*< 0.001*
Epididymal	4.29 ± 0.08	5.12 ± 0.32*	6.55 ± 0.57	15.02 ± 1.23***	*< 0.001*	*< 0.001*	*< 0.001*
Mesenteric	2.64 ± 0.17	2.57 ± 0.18	2.95 ± 0.16	5.43 ± 0.33***	*< 0.001*	*< 0.001*	*< 0.001*
Subcutaneous	6.85 ± 0.59	8.34 ± 0.83	6.35 ± 0.50	16.70 ± 1.97***	*0.004*	*< 0.001*	*0.002*
Σ adipose tissues	16.26 ± 0.67	19.58 ± 1.59	21.46 ± 1.14	52.55 ± 4.18***	*< 0.001*	*< 0.001*	*< 0.001*
Liver weight (g)	10.34 ± 0.70	11.32 ± 0.37	8.42 ± 0.30	10.16 ± 0.42***	*0.005*	*0.011*	0.449
Triacylglycerols (mg/g)	8.13 ± 2.05	10.42 ± 0.86	8.25 ± 0.85	12.83 ± 0.91 *	0.836	*0.012*	0.695

SDnc Sprague-Dawley normal-caloric diet, *SDhc Sprague-Dawley* high-caloric diet, *Wnc Wistar* normal-caloric diet, *Whc Wistar* high-caloric diet. Values are means ± SEM. Comparison between SDnc vs. SDhc and Wnc vs. Whc was analyzed by Student *t* test (* $P < 0.05$; *** $P < 0.001$).The interaction of diet, strain and diet x strain was analyzed by a two-way ANOVA (italic indicate differences $P < 0.05$)

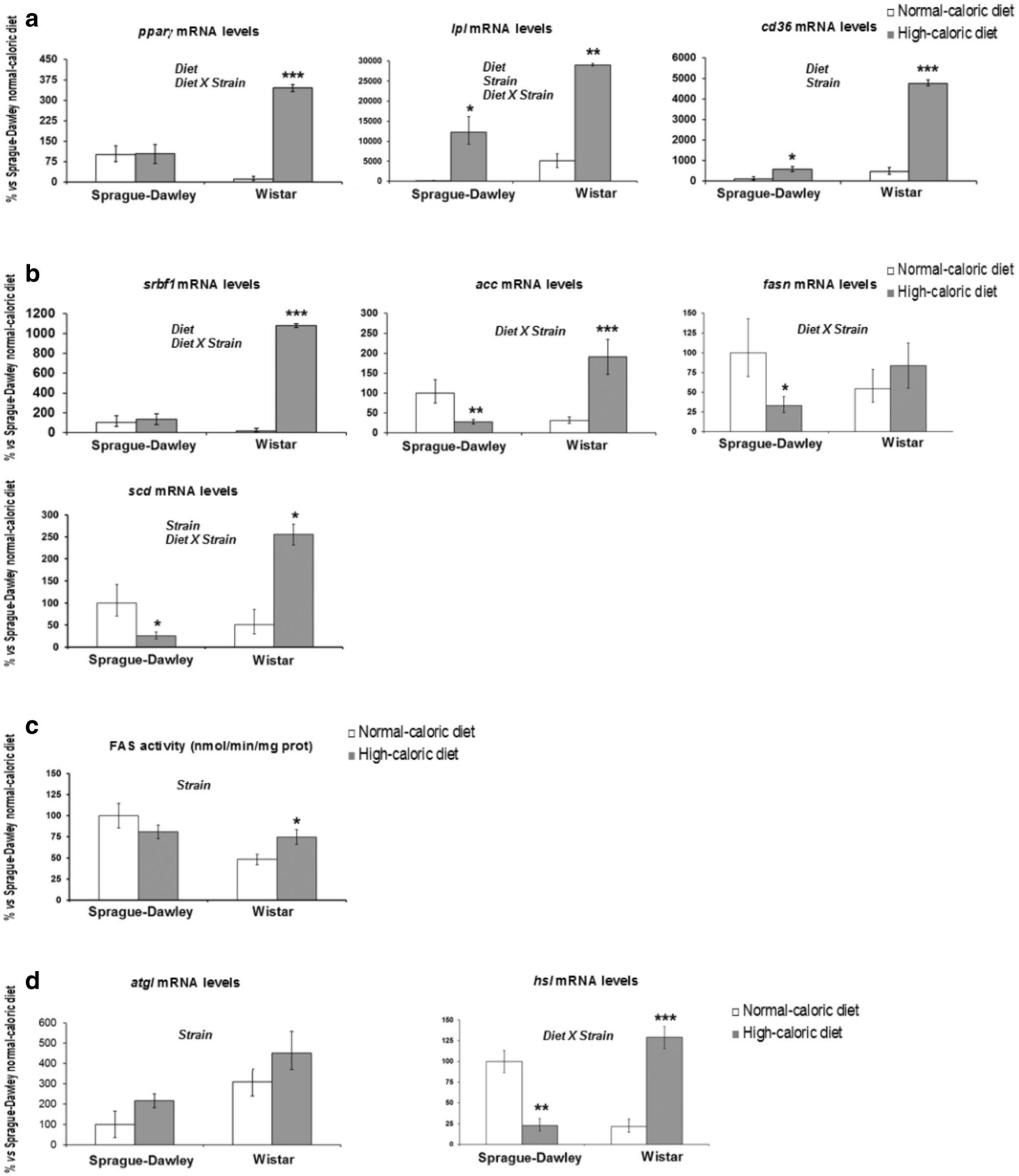


Fig. 1 mRNA levels of *pparγ*, *lpl*, and *cd36* (**a**), mRNA levels of *srebf1*, *acc*, *fasn*, and *scd* (**b**), FAS activity (**c**), and mRNA levels of *atgl* and *hsl* (**d**) in epididymal adipose tissue from *Wistar* and *Sprague-Dawley* rats fed a normal-caloric (nc) or high-caloric (hc) diet. Values are means ± SEM. Diet, strain, or diet × strain indicate interactions analyzed by two-way ANOVA ($P < 0.05$). Asterisks indicate differences between nc and hc groups for each rat strain, analyzed by the Student *t* test (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$)

Table 2 Summary table of results of gene expression and enzyme activities involved in lipid uptake, lipogenesis, lipolysis, fatty acid oxidation, and circadian rhythm between rats fed a normal-caloric or a high-caloric diet analyzed by Student's *t* test ($P < 0.05$)

	Lipid uptake			Lipogenesis					Lipolysis		Fatty acid oxidation			Circadian rhythm			
	Gene expression			Gene expression				Activity	Gene expression		Gene expression		Activity	Gene expression			
Gene/enzyme	*pparγ*	*cd36*	*lpl*	*srebf1*	*acc*	*scd*	*fasn*	FAS	*atgl*	*hsl*	*pparα*	*cpt1a*	CPT1a	*bmal1*	*clock*	*cry1*	*per2*
Epididymal																	
Whc vs. SDhc	↑	↑	↑	↑	↑	↑	↑	NS	↑	↑	NA	NA	NA	↓	NS	↑	NS
Wnc vs. SDnc	↓	NS	↑ ($P = 0.06$)	NS	↓	NS	NS	↓	NS	↓	NA	NA	NA	↑ ($P = 0.05$)	NS	↓	↓
Subcutaneous																	
Whc vs. SDhc	NS	↑	NS	NS	NS	↑	NS	NS	NS	NS	NA	NA	NA	NS	NS	NS	NS
Wnc vs. SDnc	NS	NS	NS	NS	NS	NS	↓ ($P = 0.08$)	↓	↑	NS	NA	NA	NA	NS	NS	NS	↓
Liver																	
Whc vs. SDhc	NA	NS	NA	↓	↓	NA	↓	↓ ($P = 0.07$)	NA	NA	↑	↑	↑	NS	NS	NS	NS
Wnc vs. SDnc	NA	NS	NA	↓	↓	NA	↓	NS	NA	NA	↓	NS	↑	↑	NS	NS	↓

↑: higher expression/activity in Whc or Wnc group; ↓: lower expression/activity in Whc or Wnc group; *NA* not analyzed, *NS* no significance ($P > 0.1$), *SDnc Sprague-Dawley* normal-caloric diet, *SDhc Sprague-Dawley* high-caloric diet, *Wnc Wistar* normal-caloric diet, *Whc Wistar* high-caloric diet

increased expression of *pparγ* and *lpl* in *Wistar* rats (Fig. 2a). In the case of lipogenic genes, hc diet feeding induced a significant reduction in the expression of *fasn* in *Sprague-Dawley* strain and significant increases in the expression of *srebf1*and *scd* in *Wistar* strain (Fig. 2B). No differences in gene expression were observed between both strains of rats (Wnc *vs.* SDnc in Table 2). By comparing SDhc and Whc groups, higher expression of *scd* was found in *Wistar* strain (Table 2), but no diet × strain interactive effect was observed in this tissue. As in epididymal adipose tissue, FAS activity was only increased in *Wistar* strain fed hcdiet (Fig. 2c). There were no differences between Whc and SDhc groups, and Wnc had lower FAS activity than SDnc group (Table 2).

As far as lipolytic genes are concerned, *atgl* expression was increased by hc diet feeding in *Sprague-Dawley* rats, but not in *Wistar* rats. In the case of *hsl*, its expression was increased by hc diet feeding, but this increase only reached statistical significance in *Wistar* rats (Fig. 2d). In this adipose tissue, and unlike observations made in epididymal, there were no differences between mRNA levels of both lipases in *Wistar* and *Sprague-Dawley* rats fed the hc diet (Table 2).

Lipid metabolism genes expression and activity in liver

No changes in *cd36* expression were induced by hc diet feeding in *Sprague-Dawley* or *Wistar* rats (Fig. 3a). SDhc and Whc groups had similar mRNA levels of *cd36* (Table 2).

As far as lipogenic genes were concerned, no significant changes were induced by hc diet feeding in *Sprague-Dawley* or *Wistar* rats (Fig. 3b), and there was no diet × strain interactive effect. In sharp contrast with adipose tissues, *Wistar* rats showed lower expression of lipogenic genes than *Sprague-Dawley* rats after following a nc diet (Table 2). A similar situation was observed when Whc and SDhc groups were compared (Table 2). Moreover, FAS activity was raised by hc diet feeding in both strains (Fig. 3c) and thus, no diet × strain interaction was observed in liver.

In relation to oxidative genes, even though changes in *pparα* gene expression were observed, these differences did not affect *cpt1a* gene expression and activity (Fig. 3d, e). Diet × strain interactive effect was observed for mRNA levels of *cpt1a* gene expression (Fig. 3d). *Wistar* rats showed higher expression of oxidative genes and higher activity of CPT1a than *Sprague-Dawley* rats after following a hc diet (SDhc vs. Whc in Table 2).

Clock genes and clock-controlled genes in adipose tissue and liver

Figure 4 shows mRNA levels of genes included in the clock machinery of adipose tissues and liver: the positive elements of the clock (*bmal1* and *clock*) and the negative ones (*cry1* and *per2*). As far as epididymal adipose tissue is concerned, in

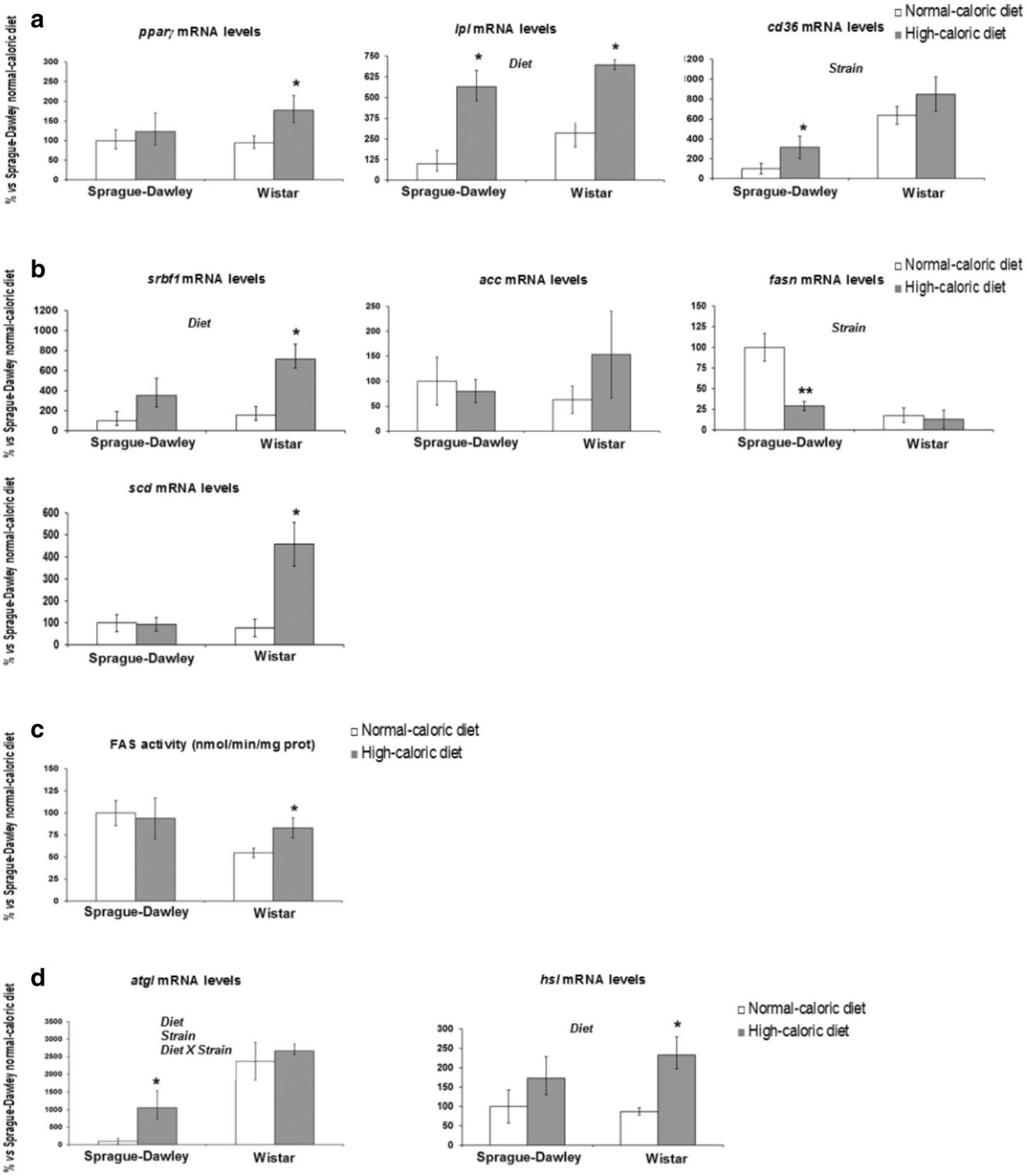


Fig. 2 mRNA levels of *pparγ*, *lpl*, and *cd36* (**a**), mRNA levels of *srebf1*, *acc*, *fasn*, and *scd* (**b**), FAS activity (**c**), and mRNA levels of *atgl* and *hsl* (**d**) in subcutaneous adipose tissue from *Wistar* and *Sprague-Dawley* rats fed a normal-caloric (nc) or high-caloric (hc) diet. Values are means ± SEM. Diet, strain, or diet × strain indicate interactions analyzed by two-way ANOVA ($P < 0.05$). Asterisks indicate differences between nc and hc groups for each rat strain, analyzed by the Student *t* test (*$P < 0.05$; **$P < 0.01$)

Sprague-Dawley rats hc diet feeding increased *clock* and *per2* expressions (Fig. 4a). In *Wistar* rats, the expression of *bmal1* was decreased and that of *clock*, *cry1*, and *per2* was increased (Fig. 4a). In *clock* gene expression only diet interacted,

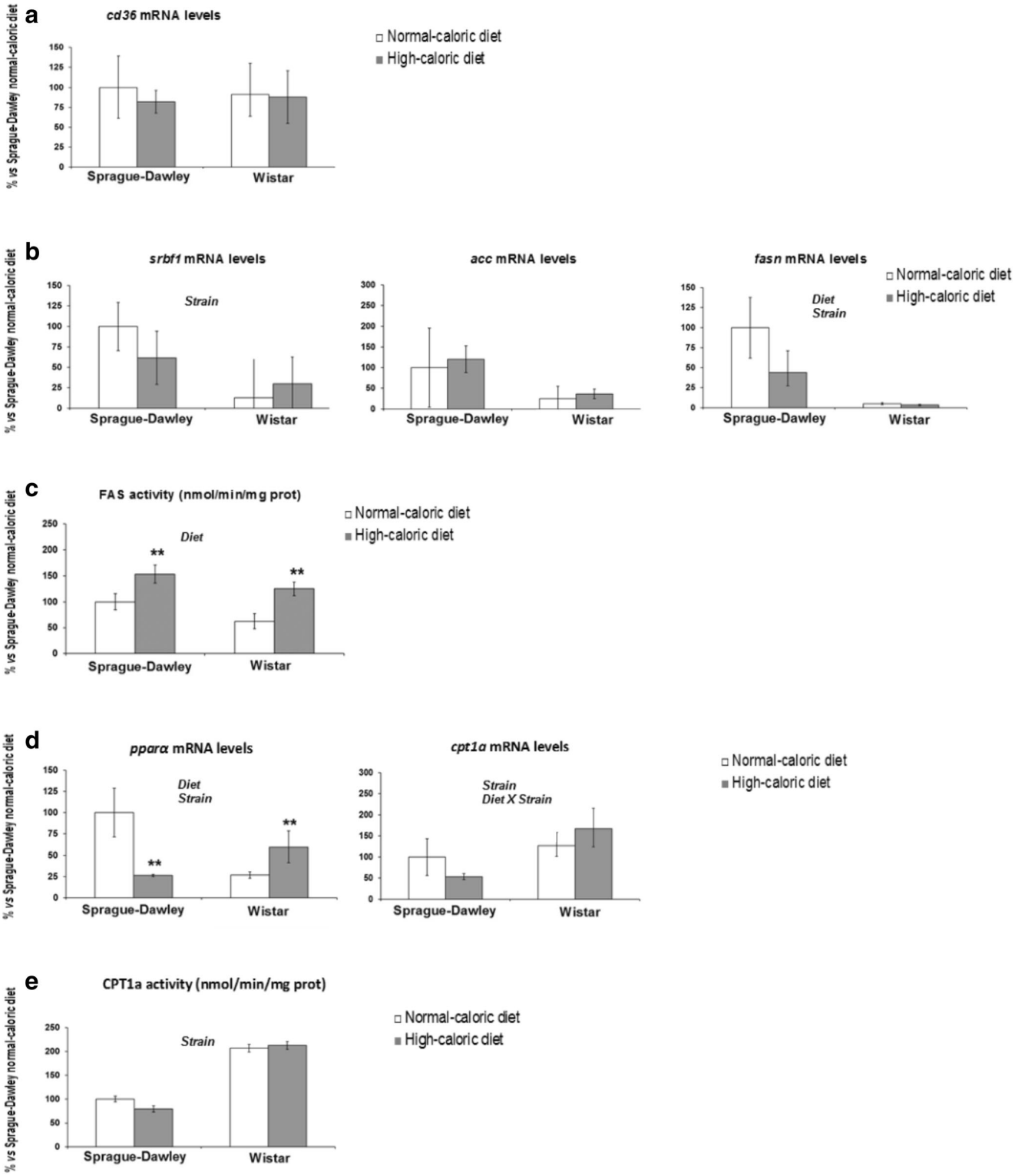
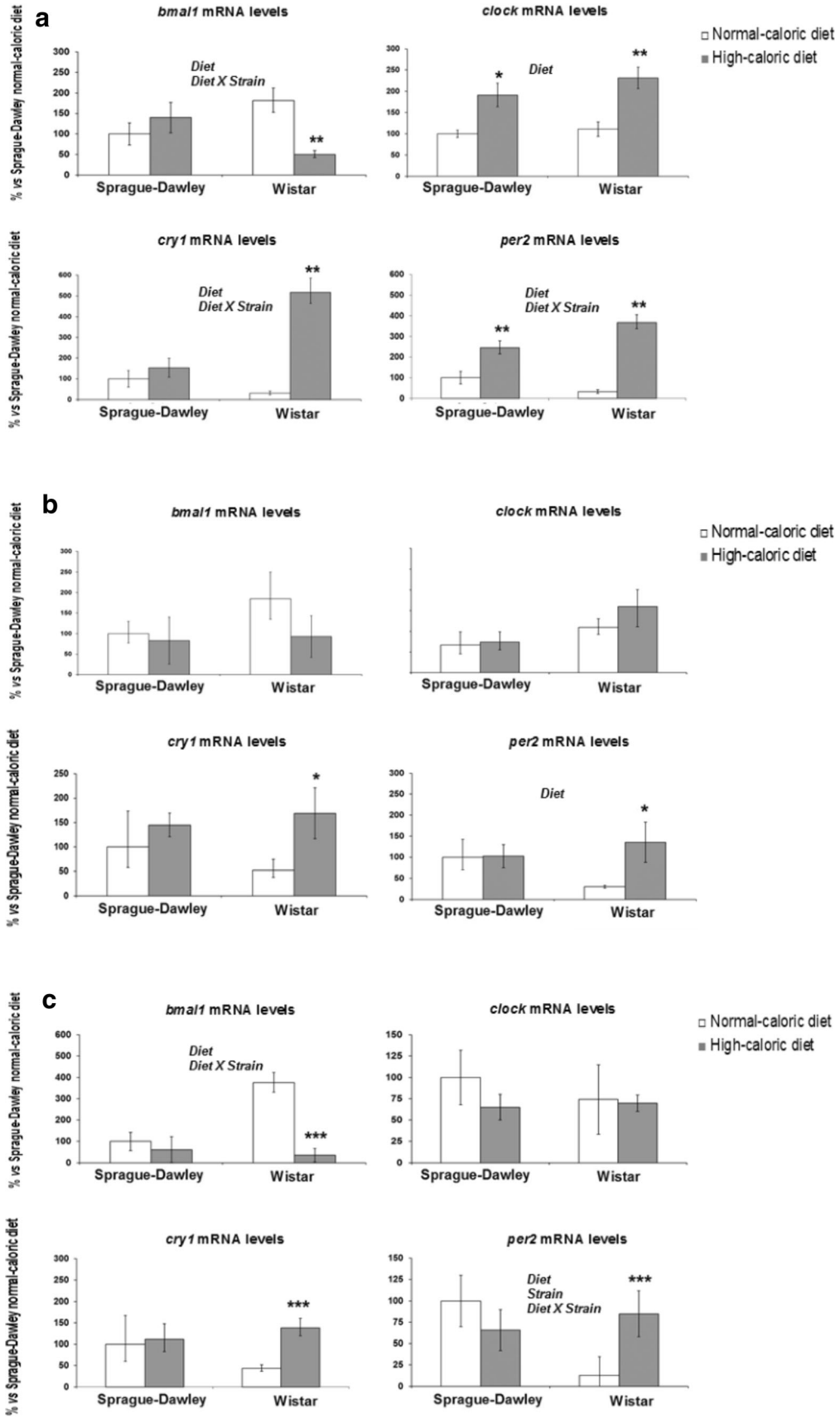


Fig. 3 mRNA levels of *cd36* (**a**), mRNA levels of *srebf1*, *acc*, *fasn* (**b**), FAS activity (**c**), mRNA levels of *pparα*, *cpt1a* (**d**), and CPT1a activity (**e**) in liver of *Wistar* and *Sprague-Dawley* rats fed a normal-caloric (nc) or high-caloric (hc) diet. Values are means ± SEM. Diet, strain, or diet × strain indicate interactions analyzed by two-way ANOVA ($P < 0.05$). Asterisks indicate differences between nc and hc groups for each rat strain, analyzed by the Student *t* test (**$P < 0.01$)

whereas in the other three genes an interaction diet × strain was observed. *Wistar* rats fed the hc diet showed lower mRNA levels of *bmal1* and higher mRNA levels of *cry1* than *Sprague-Dawley* rats in epydidimal adipose tissue (Table 2).


a
bmal1 mRNA levels
clock mRNA levels
cry1 mRNA levels
per2 mRNA levels
Diet
Diet X Strain
Strain
Sprague-Dawley
Wistar
% vs Sprague-Dawley normal-caloric diet
Normal-caloric diet
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per2 mRNA levels
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Sprague-Dawley
Wistar
% vs Sprague-Dawley normal-caloric diet
Normal-caloric diet
High-caloric diet

◀ **Fig. 4** mRNA levels of *bmal1*, *clock*, *cry1*, and *per2* in epididymal (**a**) and subcutaneous (**b**) adipose tissue, and liver (**c**) of *Wistar* and *Sprague-Dawley* rats fed a normal-caloric (nc) or high-caloric (hc) diet. Values are means ± SEM. Diet, strain, or diet × strain indicate interactions analyzed by two-way ANOVA ($P < 0.05$). Asterisks indicate differences between nc and hc groups for each rat strain, analyzed by the Student's *t* test (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

A similar pattern of response for *Wistar* rats was found for *per2* and *cry1* gene expression in subcutaneous adipose tissue (Fig. 4b).

In liver from *Sprague-Dawley* rats, no significant changes were observed in the expression of clock machinery genes. By contrast, in *Wistar* rats the expression of *cry1* and *per2* was increased, and that of *bmal1* was reduced (Fig. 4c). Interactive effect diet × strain was found for *bmal1* and diet for *per2*. Although different gene expression was observed for *bmal1* and *per2* after nc diet feeding between both strains, no differences between Whc and SDhc groups were found in liver (Table 2).

Discussion

Sprague-Dawley and *Wistar* rats have been widely used and are accepted as good rat models for high-fat obesity induction [4, 20]. It has been reported that one of the mechanisms which explains the fattening effects of high-fat diets is increased energy intake. Due to the fact that the composition of the standard diet provided to the experimental group used as control could affect high-fat diet research outcomes, its careful selection is a crucial matter [3].

In the present study, both strains of rats showed significantly increased food intake, but this effect was higher in *Wistar* rats. Although in both strains of rats fed the hc diet final body weight and adipose tissue weights were greater than those showed by their counterparts fed a nc diet, the increases only reached statistical significance in *Wistar* rats. In a study reported by Marques et al., a comparison between *Sprague-Dawley* and *Wistar* was also carried out [20]. These authors observed increased energy ingestion, weight gain, and fat in both strains of rats after 17 weeks of high-fat feeding. However, these effects were more pronounced or detected earlier in *Wistar* than in *Sprague-Dawley* rats. Reno and Fehn [29] also observed more rapid and robust weight gain after 11 weeks of high-fat feeding in *Wistar* than in *Sprague-Dawley* rats. Taken as a whole, both our results and those reported by other authors suggest that the *Wistar* rat is more susceptible to developing obesity under overfeeding conditions than the *Sprague-Dawley* rat. Hypothalamic appetite-related neuropeptide formation, leptin sensitivity, or sympathetic stimulation, leading to differences in food intake, could explain phenotypic variations between the two strains [20]. Other authors have also described differences in adipose tissue weight, without food intake modification, between these two strains of rats when animals were fed an iron-deficient diet [12].

As indicated in the Introduction section, very few data concerning differences in lipid metabolism features between these two strains of rats are available to date. In order to better understand why both strains show different sensitivity to the same hypercaloric diet, we checked the literature to consider distinct explanations. Regarding catabolic pathways, Reno and Fehn observed that while *Wistar* rats showed a respiratory quotient of 1.1 under high-fat feeding, meaning that they preferentially catabolized carbohydrates or enhanced lipogenesis, *Sprague-Dawley* rats showed a respiratory quotient of 0.75, meaning enhanced lipid oxidation[29]. Furthermore, several authors have proposed that differences in the gut microbial ecology may account for inter-individual variety in response to different dietary patterns [20, 26].

In the present study, we intended to gain more insight into this issue. Taking into account that body fat accumulation depends on the interplay between various metabolic pathways, such as de novo lipogenesis, lipid uptake, and lipolysis among others, and that interaction of genes with the diet is crucial for the induction of obesity in rodents, we analyzed key players in these metabolic pathways.

FAS and ACC are two key enzymes in adipose tissue de novo lipogenesis. These enzymes are regulated by the transcriptional factor SREBP1c. In addition, SCD is an important enzyme involved in TG assembly. In the present study, hc diet feeding induced a response which fits well with an increase in lipogenesis in epididymal adipose tissue from *Wistar* rats. By contrast, in general terms, the response in *Sprague-Dawley* rats was a reduction in this metabolic pathway. Moreover, the fatty acid transporter CD36 and the enzyme LPL are important proteins for fatty acid uptake. The results obtained concerning these parameters, as well as the transcriptional factor PPARγ in epididymal fat depot, suggest that hc diet feeding led to increased fatty acid uptake in both rat strains, even though this effect was more pronounced in *Wistar* rats. Finally, lipolysis is mainly controlled by two lipases, ATGL and HSL. The most sensitive to hc diet feeding was HSL, which was decreased in *Sprague-Dawley* rats and increased in *Wistar* rats. When a statistical comparison was carried out between both, diet × strain effect was observed for almost all the parameters analyzed. This suggests that *Wistar* rats showed greater sensitivity to hc diet-induced metabolic changes. Taken as a whole, these results show that increased lipogenesis and fatty acid uptake induced by hc diet in *Wistar* rats, although in all likelihood counteracted in part by greater lipid mobilization, can account for the greater adipose tissue size shown by these animals when compared with *Sprague-Dawley* rats. Nevertheless, it is important to point out that the increase in fat mass is not only the result of lipid uptake and

storage in adipose tissues. In fact, modifications in white adipose tissue cellularity were proposed by others to justify variability between diet-induced obese and diet-resistant *Sprague-Dawley* rats [33].

In subcutaneous adipose tissue, the expression of genes codifying for SREBP1cand SCD were significantly increased in *Wistar* rats fed the hc diet, but not in *Sprague-Dawley* rats. Gene expression of *acc* was also increased in *Wistar* rats, although the increase did not reach statistical significance, and *fasn* gene expression was reduced after hc diet only in *Sprague-Dawley* rats. By contrast, *cd36* was increased in *Sprague-Dawley* but not in *Wistar* rats. In both strains of rats, hc diet feeding led to increased expression of *lpl*. Therefore, an important difference between both strains is that while the main pathway involved in TG storage in *Wistar* rats is de novo lipogenesis and TG assembly, in *Sprague-Dawley* rats TG storage mainly depends on fatty acid uptake. Finally, only a clear diet × strain effect was found in *atgl* expression. All these effects could be responsible for the subcutaneous size differences between both strains after hc diet feeding.

Gender influence on perigonadic white adipose tissue lipid uptake has been proved, relating to LPL activity, in *Wistar* but not in *Sprague-Dawley* rats [8].Several lines of evidence have revealed that both circulating levels of sex hormones as well as glucocorticoids control internal fat mass distribution and expansion [19]. In humans, while glucocorticoid activity promotes LPL expression in visceral adipocytes and estrogens act to boost the LPL activity of the gluteofemoral depot, testosterone decreases LPL activity in visceral, and subcutaneous abdominal adipocytes, but not in subcutaneous femoral adipocytes [21]. Therefore, potential differences in plasma levels of those hormones between strains cannot be discarded as a justification, at least in part, for differences in perigonadic fat depot after hc diet feeding.

Taking into account that a circadian clock is present in adipose tissue [13], and considering that relationships between alterations in adipose tissue clock and the development of obesity have been revealed [5, 35, 37], the influence of several clock genes on the diet and strain effect was analyzed. Three of the four clock elements studied (*cry1*, *per2*, and *bmal1*), showed diet and strain interaction in epididymal adipose tissue. By contrast, neither diet nor strain effects were observed in subcutaneous localization. These results are in line with the higher power of diet and strain effects observed in epididymal than in subcutaneous adipose tissue on genes related to lipid uptake, lipogenesis, and lipolysis [14, 36]. The effect of strain was previously reported by other authors in obese mice, revealing different impact on adipose tissue clock machinery between KK and C57BL/6J strains [1, 36].

Ectopic fat accumulation in liver is a very common comorbidity associated with obesity [28]. For this reason, we were interested in assessing potential differences between *Sprague-Dawley* and *Wistar* rats in terms of sensitivity to steatosis development under hc diet feeding. Increased amounts of TG were found in both *Sprague-Dawley* and *Wistar* rats when compared with their respective control groups, but these differences only reached statistical significance in *Wistar* rats. When the expression of lipogenic genes was analyzed, it was observed that *Wistar* rats showed lower values than *Sprague-Dawley* rats. Moreover, although FAS activity raised after hc diet feeding in both strains, the effect was more prominent in *Wistar* strain (+ 53% vs. + 98%). Hepatic fatty acid oxidation also revealed strain differences when rats were fed the hc diet. Under our experimental conditions, fatty acid oxidation in *Wistar* rats was increased when compared to *Sprague-Dawley* rats, probably as a compensatory mechanism. Our results show that in addition to differences in amino acid uptake rate, urea production, or glycolytic and tricarboxylic acid cycle activities between *Wistar* and *Sprague-Dawley* rats reported by other authors [1], clear differences in hepatic lipogenesis and fatty acid oxidation exist between these two strain of rats.

With regard to clock genes, we observed changes in the hepatic gene expression of *bmal*, *per2*, and *cry1* of *Wistar* rats after hc diet feeding. By contrast, no modification was described for *Sprague-Dawley* strain, as this mainly took place in epididymal and subcutaneous adipose tissue. It is important to point out that a limitation of our study is that we measured a unique point, instead of measuring gene expression at different time phases. Additionally, in order to stabilize feeding time for all animals, the unique point measurement was performed after an overnight fasting period.

Other authors have also reported changes in clock genes in rodents fed hc diets. Kohsakaet al. conducted an extensive research and concluded that hc diet altered mammalian circadian clock function in liver, among other peripheral tissues, with consequences on lipogenesis [14]. Moreover, Pendergast et al. found that a high-fat diet altered liver rhythm in mice, thus affecting their eating distributing behavior across the day and night [27]. More recently, it has been observed that *clock-bmal1* and *fasn* expression were synchronized in the liver of mice fed a nc diet, and that this association was disrupted in mice fed a high-fat diet [10]. In the case of *pparγ*, Barnea *et. al.* demonstrated that it follows a circadian rhythm in adipose tissue [2].

In conclusion, the present study shows that the *Wistar* strain tends to accumulate more fat in adipose tissue under hc diet feeding than the *Sprague-Dawley* strain does. This is due, at least in part, to its increased lipogenesis and fatty acid uptake. Thus, it represents the best choice as animal model in dietary-induced obesity studies. These differences are especially relevant in the case of epididymal adipose tissue. By contrast, both strains of rats show similar fatty liver after the hc diet feeding. Furthermore, although more studies are needed, peripheral clock machinery seems to be involved in the lipid metabolism differences between *Wistar* and *Sprague-Dawley* rats.

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Compliance with ethical standards

Ethical approval All animal experimental protocols were reviewed and approved by the ethics committee on animal welfare of our institution (Comité Ético de Experimentación Animal de la Universidad del País Vasco, CEEA-UPV/EHU).

Conflict of interest The authors declare that there are no conflicts of interest.

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