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Fat-enriched rather than high-fructose diets promote whitening of adipose tissue in a sex-dependent manner☆

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Abstract

Adipose tissue is a critical regulator of energy metabolism and an effector organ of excessive caloric intake. We studied the effects of high-fructose (HFruD), high-fat (HFD) and mixed high-sucrose and high-fat diet (HFHSD) on adipocyte morphology and biology and consecutive metabolic effects in male and female C57BL/6 mice. Forty male and 40 female mice were randomly assigned to one of four dietary groups and fed for 10 weeks *ad libitum*. After 10 weeks of feeding, mice were analyzed in regard to glucose metabolism, insulin sensitivity and alteration in adipocyte morphology and function. Weight gain and diminished insulin sensitivity in HFD- and HFHSD-fed mice were accompanied by increased adipocyte size and a shift in size distribution towards larger adipocytes in all mice. The latter effect was also found but less pronounced in HFruD-fed mice, while insulin sensitivity and body weight remained unaffected. In male mice, expansion of white adipocytes along with decreased uncoupling protein 1 (UCP-1) expression and alterations of mitochondrial biogenesis was found after HFD and HFHSD feeding, while in female mice, UCP-1 expression was also reduced in the HFruD dietary group. Diet-induced cellular alterations were less pronounced in female mice. Our data demonstrate that high-fat rather than high fructose consumption drives metabolically disadvantageous alterations of adipocyte differentiation involving whitening and insulin resistance in a sex-dependent manner with most deleterious effects seen upon administration of combined sucrose and fat-enriched diet in male mice.

Keywords: Adipocyte; Fructose; High fat; Insulin resistance; Sex difference; Mitochondrion; Lipid droplet

1. Introduction

Rates of obesity have reached epidemic proportions worldwide in the past decades [1]. In parallel the prevalence of chronic disorders such as the metabolic syndrome, type 2 diabetes and cardiovascular disease have increased dramatically [2]. Not only excess caloric intake alone, but also the composition of food plays a pivotal role in developing obesity and impaired glucose and insulin metabolism [3]. Both high fructose and fat intake have been accused to be the main drivers of obesity and type 2 diabetes [4,5]. Eating behavior on the other hand associated with beneficial diet is postulated to reduce the risk of obesity [6].

Adipocytes are essential players in maintaining energy equilibrium through metabolizing glucose and other substrates [7] and storing excess calories as triacylglycerol thus preventing the organism from lipotoxicity [8]. For a long time only seen as an energy storage compartment, adipose tissue today is recognized as a major endocrine organ with multiple signaling and regulatory properties towards organs and tissues throughout the body [9]. Two to three distinct types of adipose tissue exist: classical white adipose tissue (WAT) is either localized subcutaneously (subcutaneous adipose tissue (SAT)) or intra-abdominally as visceral adipose tissue (VAT) depots which interacts with several metabolically active tissues through adipocytokines, cytokines and free fatty acids [10]. Thermogenic brown adipose tissue (BAT) is clustered in defined anatomical regions. So-called "beige" or "inducible" adipocytes share characteristics of brown adipocytes but are found in WAT depots [11].

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Due to the variety of adipocytokines and plasticity of adipose tissue in general, many mechanisms are discussed by which it negatively affects insulin sensitivity. In states of obesity both possibilities of increased adipocyte number (hyperplasia) and increased adipocyte size (hypertrophy) contribute to excess adipose tissue accumulation [12], eventually leading to adipocyte hypoxia and subsequent altered production of adipocytokines and inflammatory cytokines [13,14]. Adipose tissue inflammation was found to directly determine hepatic glucose output providing another mechanism by which adipose tissue can be linked to systemic glucose metabolism [15], emphasizing the interplay between these two tissues.

In this study we aimed to clearly characterize diet-specific effects on adipocyte morphology and function and also to investigate whether these nutritional effects are sex-specific.

2. Material and methods

2.1. Animals and diets

All animal experiments were performed according to the guidelines of the Education based on the Austrian Animal Testing Act of 1988 (BMWF-66.011/0065/—WF/V/3b/2016). Forty female and 40 male 7 weeks old C57BL/6 mice (Charles River Laboratories, Sulzfeld, Germany) were either fed a standard diet (SD, TD.05075, 66.9%/kcal carbohydrate, 20.2%/kcal protein, 12.9%/kcal fat), a high-fat diet (HFD, TD.06414, 21.3%/kcal carbohydrate, 18.4%/kcal protein, 60.3%/kcal fat, fatty acid profile: 37% saturated, 47% monounsaturated, 16% polyunsaturated fatty acids), a fructose rich diet (HFruD, TD.89247, 66.8%/kcal carbohydrate from fructose, 20.2%/kcal protein, 12.9%/kcal fat) or a mixed sucrose and fat rich diet (HFHSD, TD.88137, 42.7%/kcal carbohydrate containing 34% sucrose/g, 15.2%/kcal protein, 42%/kcal, 0.2% cholesterol, fatty acid profile: 61.8% saturated, 27.3% monounsaturated, 4.7% polyunsaturated fatty acids) *ad libitum*. All diets were purchased from Harlan/Envigo (United Kingdom). Mice were housed in groups of 6 at 12 h light/dark cycle at a temperature range between 20 and 24 °C.

After 10 weeks, oral gavage glucose tolerance test (oGTT, 1 mg glucose/g bodyweight) and intraperitoneal insulin tolerance test (ipITT, 0.75 mU insulin/g bodyweight) were performed [16]. Areas under the curve (AUC) above baselines were calculated by the linear trapezoidal method. Finally, mice were anesthetized and sacrificed *via* harvest of vital organs and removal of cardiac blood.

2.2. Hematology

Whole blood samples were taken and heparin was used as an anticoagulant. Hematological parameters were analyzed using the scil VET abc 2003 (scil animal care company GmbH, Viernheim, Germany). Plasma blood samples were taken using EDTA as an anticoagulant and free fatty acids measured with the Hitachi 902 B analyzer (Roche, Mannheim, Germany). Murine high sensitive (hs) CRP levels were measured in plasma samples using a commercially available Quantikine ELISA mouse CRP kit (R&D, Minneapolis, USA).

2.3. RNA extraction and quantitative RT-PCR analysis

Forty to 70 mg of frozen adipose tissue samples were homogenized in 500 μl TriZol reagent (Qiagen, Venlo, Netherlands) and total RNA was extracted using RNeasy Lipid Tissue Mini Kit (Qiagen) according to the manufacturer's instruction.

After reverse transcription using Omniscript RT Kit (Qiagen), TaqMan probe based quantitative PCR of glucose-uptake transporter 4 (GLUT4), leptin, adiponectin, caveolin-1 (CAV1), peroxisome proliferator-activated receptor-γ (PPARγ), peroxisome proliferator-activated receptor γ coactivator 1-alpha (PGC1α), hypoxia inducible factor subunit 1α (HIF1α) and uncoupling protein 1 (UCP-1) was performed using intron-spanning primers. Commercially available predesigned or custom designed primers/probes are summarized in Table 1S. Steady-state mRNA expression data were normalized to reference gene hypoxanthine-guanine phosphoribosyltransferase (HPRT). All expression analyses were performed in duplicates.

2.4. Immunohistochemistry and adipocyte size quantification

For immunohistochemistry (IHC), samples from subcutaneous and visceral adipose tissue depots were dissected, fixed in 10% neutral buffered formalin, dehydrated, embedded in paraffin, sectioned and stained with hematoxylin and eosin (H&E). Adipocyte size was calculated using the ImageJ plugin Adiposoft [17]. Sections were stained with primary antibody for UCP-1 (Abcam, Cambridge, UK) and visualized with VectaStain Elite ABC HRP kit (Vector Laboratories, Burlingame, USA).

2.5. Transmission electron microscopy

For transmission electron microscopy, 2×2 mm subcutaneous and visceral adipose tissue depot samples were fixed at 4°C with 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer at pH 7.3 for 72 to 96 h, washed and post-fixed with 1% osmium tetroxide in 0.1 M sodium cacodylate buffer for 2 h at room temperature. Samples were washed, dehydrated with an ascending acetone series and embedded in PolyBed medium epoxy resin (Ted Pella Inc. Redding, CA, USA. Ultra-thin sections (80 nm) were cut with a diamond knife (Diatome, Switzerland), mounted on copper grids, stained for 2 min with lead citrate and examined with the EFTEM Zeiss Libra 120 using a 2×2K camera (Tröndle, Germany) and iTEM software (Olympus, Japan). TEM imaging of AT was performed in a limited number of representative mice per dietary group; accordingly, no statistical analysis was performed and only non-quantitative data are provided.

2.6. Statistical analysis

All statistical analyses were performed with the statistical analysis software package (SPSS version 17.0; SPSS, Chicago, IL, USA). Descriptive data are expressed as means ± S.D. Unpaired two-tailed Student's *t* test for parametric data and Mann–Whitney *U* test for nonparametric data were used to test for differences between HFD, HFruD, HFHSD and SD feeding control groups. One-way ANOVA analysis with Bonferroni correction (homogenous variance) or Games-Howell (heterogenous variances) was used to compare data between dietary groups (supplementary material). For all calculations statistical significance was inferred at a two-tailed P of ≤.05. To reduce the influence of extreme outliers, they were determined based on z-scores ≥±3 and winsorized by replacing the values with the next highest non-outlier value.

3. Results

3.1. Divergent effects of HFruD, HFD and HFHSD on systemic and adipose tissue inflammation, body weight, glucose tolerance and insulin sensitivity of C57BL/6 mice

After 10 weeks of feeding, HFD- and HFHSD- but not HFruD-fed mice of both sexes gained significantly more weight than SD-fed mice (Table 1). When compared to SD-fed mice, average caloric intake estimated by quantification of total food consumption per dietary group was increased by 39%, 175% and 136% in HFruD-, HFD- and HFHSD-fed mice, respectively.

Fasting glucose was significantly elevated in HFruD-, HFD- and HFHSD-fed female mice when compared to SD-fed female mice while in male mice fasting glucose levels did not differ between any dietary groups (Table 1). Non-esterified fatty acids (NEFAs) were significantly increased in HFD- and HFHSD-fed mice of both sexes compared to SD-fed mice (Table 1). Systemic inflammation estimated by high-sensitive CRP was markedly elevated in HFHSD-fed mice of both sexes (Table 1). Adipose tissue inflammation as determined by macrophage marker F4/80 steady-state mRNA expression analysis was significantly increased in VAT of both male and female HFD- and HFHSD-fed mice compared to SD-fed mice, while F4/80 mRNA expression was increased in SAT of male HFruD- and HFD-fed mice only (Figs. 4 and 5S).

To assess glucose metabolism, mice were starved for 6 h and oGTT was performed. Blood glucose levels and AUC of glucose excursion are shown in Fig. 1 A-C.

In female mice, HFD and HFruD feeding significantly diminished glucose tolerance while HFD and HFHSD but not HFruD feeding led to significantly increased AUC levels in male mice (Fig. 1C).

To assess insulin sensitivity, ipITT was performed after starvation for 6 h. Blood glucose levels and AUC of glucose excursion in response to insulin injections are shown in Fig. 1 D–F. As determined by ipITT, insulin sensitivity was significantly impaired in HFD- and HFHSD-fed female and male mice when compared to SD-fed mice (Fig. 1F). HFruD feeding resulted in worsening of insulin sensitivity by trend but without statistical significance (Fig. 1F).

3.2. HFD and HFHSD but not HFruD affect adipocyte morphology and promote whitening of subcutaneous adipose tissue in male mice

In male mice, mean adipocyte size of visceral adipose tissue (VAT) was significantly increased after HFD and increased by trend after HFHSD feeding when compared to SD (Fig. 2). Adipocyte size of subcutaneous adipose tissue (SAT) was significantly increased in both HFD- and HFHSD-fed mice when compared to SD-fed mice. In contrast,

Table 1
Effects of HFruD, HFD and HFHSD on body weight, fasting glucose, NEFAs and CRP

Diet	SD (n=10/group)	HFruD (n=10/group)	HFD (n=10/group)	HFHSD (n=10/group)
Female C57BL/6 mice				
Weight wk. 0 (g)	18.5±0.6	18.7±0.8	18.5±0.6	18.8±1
Weight wk. 10 (g)	21.7±0.9	22.2±1.1	23.7±1.4†	26.2±4.2†
Fasting glucose (mg/dl)	114.8±15.7	147.2±28.3†	136.8±22.6*	155.8±30†
NEFA (mmol/L)	0.27±0.19	0.4±0.33	0.55±0.27*	0.54±0.16†
CRP (ng/L)	6.1±2	7.7±3.8	8.2±3.9	9.5±3.4*
Male C57BL/6 mice				
Weight wk. 0 (g)	22.9±0.9	22.3±1	23.2±1.3	23.4±1.3
Weight wk. 10 (g)	28±2.6	26.8±1	34.6±2.9‡	37.2±2.4‡
Fasting glucose (mg/dl)	157.2±26.8	167.7±39.1	164.3±36	179.3±35.7
NEFA (mmol/L)	0.21±0.1	0.23±0.09	0.46±0.27*	0.42±0.14‡
CRP (ng/L)	6.7±3.4	6.2±1.9	7.7±2.7	11.5±3.3†

Values are reported as means ± S.D. Significance is reported in comparison to SD-fed control mice.
SD = standard diet, HFruD = high-fructose diet, HFD = high-fat diet, HFHSD = high-fat, high-sucrose diet, wk. = weeks of feeding study.
* P≤.05,
† P≤.01,
‡ P≤.001.

adipocyte size was not significantly altered in HFruD-fed male mice neither in visceral nor in subcutaneous adipose tissue in comparison with SD-fed male mice.

Histological assessment of adipose tissue and adipocyte size of female mice which gave similar results as found in male mice is shown in the supplement.

Adipocyte size distributions (≤30 μm, 30–50 μm, 50–100 μm and ≥100 μm diameter) are shown in Fig. 2. Relative adipocyte size distribution in VAT and SAT of male mice was tremendously shifted towards large adipocytes after HFD and HFHSD feeding, while a shift towards larger adipocytes was markedly less pronounced in HFruD-fed male mice when compared to SD-fed male mice.

Changes of relative adipocyte size distributions were similar but less pronounced in HFD- and HFHSD-fed female mice. Remarkably, HFruD-fed female mice had more adipocytes with a diameter of less than 30 μm in VAT compared to SD-fed female mice (Fig. 1S).

TEM imaging of adipocytes of SAT and VAT from a limited number of representative mice per dietary group displayed occurrence of multiple microlipid droplets and a high proportion of round-shaped mitochondria in SD-fed mice only. Additionally, images suggested a markedly reduced amount of mitochondria per μm perimeter cytoplasm in HFD- or HFHSD-fed mice and a moderately reduced amount in HFruD-fed mice when compared to SD mice. Limited data further suggested that smaller mean adipocyte sizes were associated with the occurrence of beige adipose tissue like morphology in FFPE sections, increased occurrence of mitochondria of round shape and additional lipid droplets in the cytoplasm (Supplementary, Figs. 2S and 3S).

In order to confirm our non-quantitative data suggesting a beige adipocyte phenotype in SD and HFruD and a mainly white adipocyte phenotype in fat-enriched diet-fed mice, UCP-1 expression was determined in SAT samples.

Supporting our qualitative data, in male mice UCP-1 mRNA expression was significantly reduced in SAT after HFD and HFHSD but not after HFruD feeding in comparison with SD-fed mice (Fig. 3). UCP-1 expression data were further supported by IHC staining confirming UCP-1 positive beige adipocytes in SAT of representative histological samples (Fig. 3).

In contrast, in female mice UCP-1 mRNA expression was significantly reduced in SAT extracts of HFruD- and HFD-fed and by trend in HFHSD-fed mice when compared to SD-fed female mice (Fig. 4S).

Accordingly, expression of mitochondrial biogenesis marker PGC1α was significantly decreased in HFD- and HFHSD-fed male mice when compared to SD-fed male mice. M-RNA expression of HIF1α was significantly increased in HFruD- and HFD- and by trend in HFHSD-fed male mice when compared to SD-fed male mice (Fig. 4).

3.3. High-fat, high-fat/high-sucrose and fructose-enriched diet is associated with altered steady-state mRNA expression levels in SAT and VAT

Steady-state mRNA expression data of SAT and VAT extracts are shown in Fig. 4. In VAT, GLUT4 mRNA expression was significantly reduced in HFruD-, HFD- and HFHSD-fed male mice while PPARγ mRNA expression was only significantly reduced when HFHSD-fed male mice were compared to SD-fed male mice. Adiponectin mRNA expression was significantly reduced in HFHSD-fed male mice compared to SD-fed male mice. Leptin mRNA expression was significantly higher in HFD- and HFHSD- and by trend also in HFruD-fed male mice. In contrast to VAT, CAV1 mRNA expression was significantly up-regulated in SAT of HFD- and HFHSD- and by trend in HFruD-fed male mice when compared to SD-fed male mice. Similar to VAT, PPAR-γ mRNA expression was significantly down-regulated in HFHSD-fed males and leptin was significantly increased in HFD- and HFHSD- and by trend HFruD-fed male mice when compared to SD-fed male mice.

In contrast to VAT, PGC1α expression did not significantly differ between the groups and HIF1α was significantly increased in HFruD-fed male mice only when compared to SD male mice. Steady-state mRNA expression analysis of adipose tissue depots of female mice is shown in the supplement (Fig. 5S).

3.4. Metabolic phenotypes in female C57BL/6 mice are less pronounced than in male mice

Relative weight gain in female mice fed either HFD or HFHSD was significantly less pronounced than in male mice (Table 2). Fasting glucose levels were lower in SD-fed female mice than in SD-fed male mice, while no differences in fasting glucose levels between any other dietary groups were found between male and female mice. In male but not female mice, granulocyte count was significantly increased after HFruD, HFD or HFHSD feeding (Table 2).

HFD feeding resulted in significantly more pronounced postprandial hyperglycemia as determined by oGTT in male mice than in female mice. Insulin sensitivity was significantly lower in SD-, HFD- and HFHSD- and by trend also in HFruD-fed male mice when compared to female mice (Table 2).

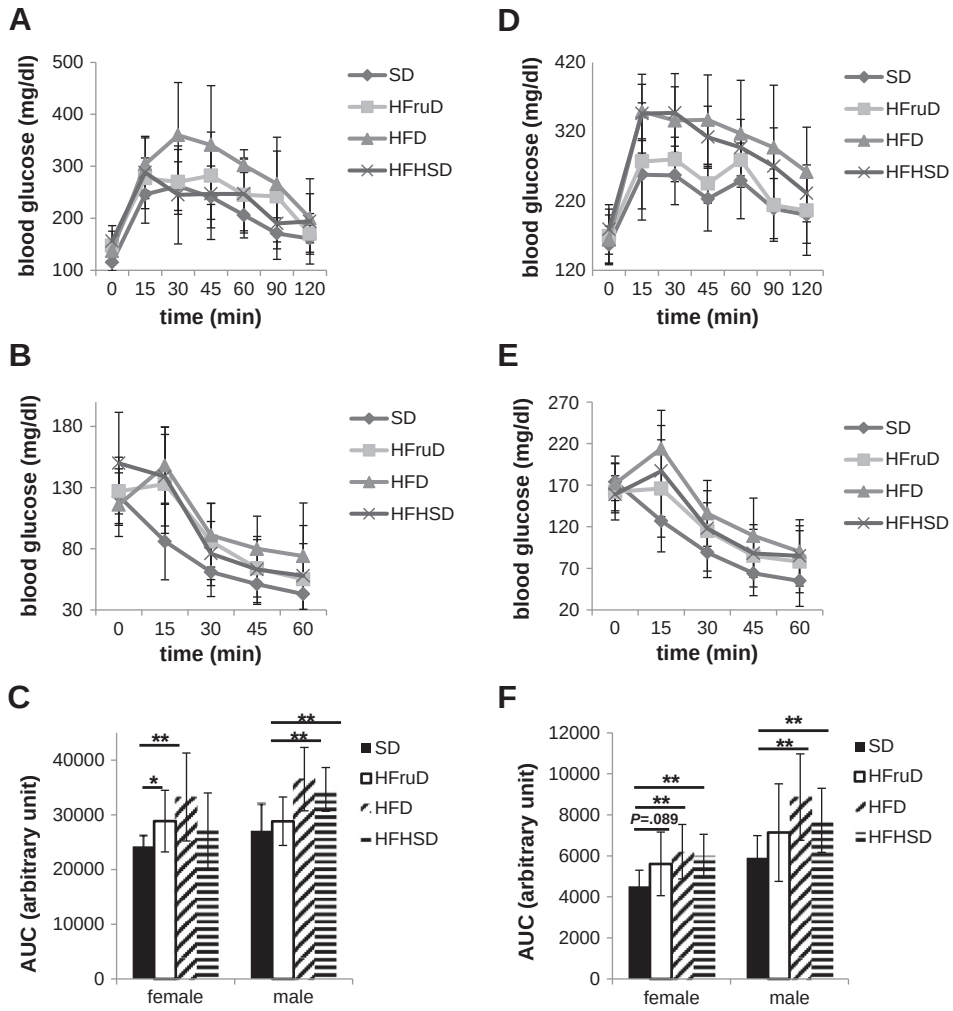

Fig. 1. Effects of HFruD, HFD or HFHSD feeding on systemic glucose tolerance and insulin sensitivity. Female (A) or male (B) C57BL/6 mice were orally gavaged with 1 mg/g body weight glucose and glucose clearance determined by repeated measurements of blood glucose levels. AUC of glucose excursion (C) was calculated and each diet compared to the SD-fed control group. Intraperitoneal ITT were performed in female (D) and male (E) C57BL/6 mice *via* intraperitoneal insulin injection. AUC of glucose excursion was calculated for each group (F) and compared to the SD-fed control group. Average glucose concentrations of all mice per dietary group are shown. AUC = area under the curve. $n=10$ for each group. AUC values are reported as means ± S.D. $^{*}P\leq.05$, $^{**}P\leq.01$, $^{***}P\leq.001$. SD = standard diet, HFruD = high-fructose diet, HFD = high-fat diet, HFHSD = high-fat, high-sucrose diet.

4. Discussion

Adipose tissue has turned out as a major determining factor in whole body glucose homeostasis and body weight control in the past years [18]. Not only quantity but also quality of food tremendously affects metabolic health. Both high fructose and fat intake have been blamed for dramatically increasing rates of obesity and type 2 diabetes. While fructose is thought to primarily act *via* activation of hepatic *de novo* lipogenesis [19], high-fat diet causes harmful alterations in several peripheral organs and tissues [20] but also the central nervous system [21]. The latter might contribute to frustrating long-term results of lifestyle intervention based weight reducing strategies in overweight and obesity.

In this study we set out to define effects of various frequently used diets on adipocyte morphology and function and associated consecutive metabolic effects. Further, we also investigated whether these effects are sex-dependent. We studied the consequences of high fructose intake and high-fat diet feeding in order to clearly characterize and compare systemic and cellular effects of dietary components that are typically accused to drive the global obesity epidemic. Additionally, a diet enriched with fat including cholesterol and sucrose was investigated. Latter probably best resembles a typical Western diet with a high content of saturated fatty acids and also carbohydrates.

In contrast to HFD and HFHSD, HFruD *ad libitum* feeding did not lead to a significant weight gain in our study which is in agreement with recent data demonstrating that 3 months of HFruD diet was associated with increased metabolic rate but preserved leptin sensitivity thus preventing weight gain despite increased food intake [22]. In contrast, increased leptin expression in HFD- and HFHSD-fed mice reflects blunted leptin signaling in the central nervous system leading to reduced sensitivity to satiation signals [21,23]. Remarkably, we found that consumption of HFHSD was associated with even more pronounced weight gain than intake of a HFD. We speculate, that this might be explained by sucrose-induced interference with the central homeostatic circuitry by modulating the synaptic abundance of AMPA receptors in the nucleus accumbens [24,25] or the activity of recently described glucose-responsive neurons in the paraventricular thalamus [26]. Alternatively, this result might be explained by a failure of leptin to suppress the reward value of sucrose in the brain in a state of leptin resistance [27].

Significant weight gain in mice fed with fat-enriched diets is partly explainable by increased caloric intake and most likely also decreased energy expenditure in response to these diets.

Expectedly, feeding any fat-enriched diet resulted in reduced insulin sensitivity in male and female mice while consumption of a

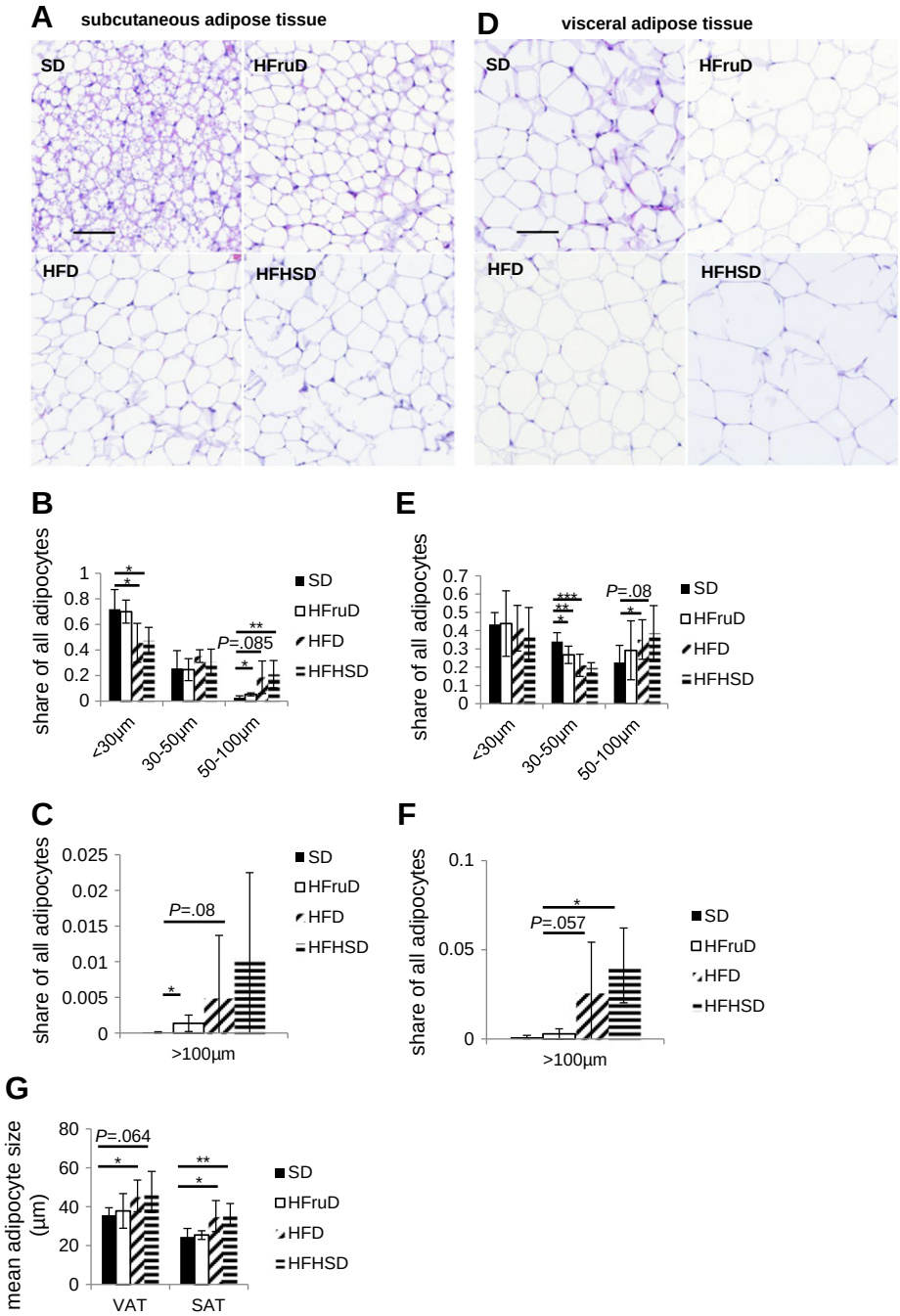

Fig. 2. Effects of HFruD, HFD and HFHSD on adipocyte size and distribution in SAT and VAT depots. Representative images of formalin-fixed, paraffin-embedded hematoxylin and eosin stained sections of male SAT (A) and VAT (D) are shown. Sections were analyzed in regards to adipocyte size (G) and distributional pattern containing adipocytes of distinct sizes (B-F). Analyses were made with Adiposoft ImageJ plugin and adipocyte size distribution compared to SD-fed controls. Scale bar represents 100 µm length. Approximately 4000 adipocytes per histological image obtained from 6 mice per dietary group were analyzed.Relative shares and adipocyte sizes are reported as means ± S.D. *$P \leq .05$, **$P \leq .01$, ***$P \leq .001$. SD = standard diet, HFruD = high-fructose diet, HFD = high-fat diet, HFHSD = high-fat, high-sucrose diet.

high-fructose diet did not diminish systemic insulin sensitivity as determined by ipITT. These findings are in accordance with recent results from diet-intervention trials showing that short-term fructose consumption is highly associated with hepatic insulin resistance, but does not affect systemic insulin sensitivity [28]. In our study, insulin sensitivity measured by ipITT and expressed as AUC above baseline might rather reflect peripheral than hepatic insulin sensitivity. However, HFruD feeding was associated with aggravated glucose tolerance in female but not in male mice. This finding might best be explained by deleterious effects of even short term high fructose intake on beta cell mass [29] and well-known increased susceptibility of glucose intolerance in females [30]. On a cellular basis we found that feeding a diet with high fat content increases the average adipocyte size by shifting cells from smaller to larger populations. Distinctly different adipocyte size distribution led us to the hypothesis that the distributional pattern could directly be linked to metabolic dysfunction as shown in earlier studies [31]. Actually, GLUT4 expression reflecting cellular insulin signaling was significantly reduced in VAT depots and not skeletal muscle (data not shown) of male mice fed a fat-enriched diet supporting the notion that impaired

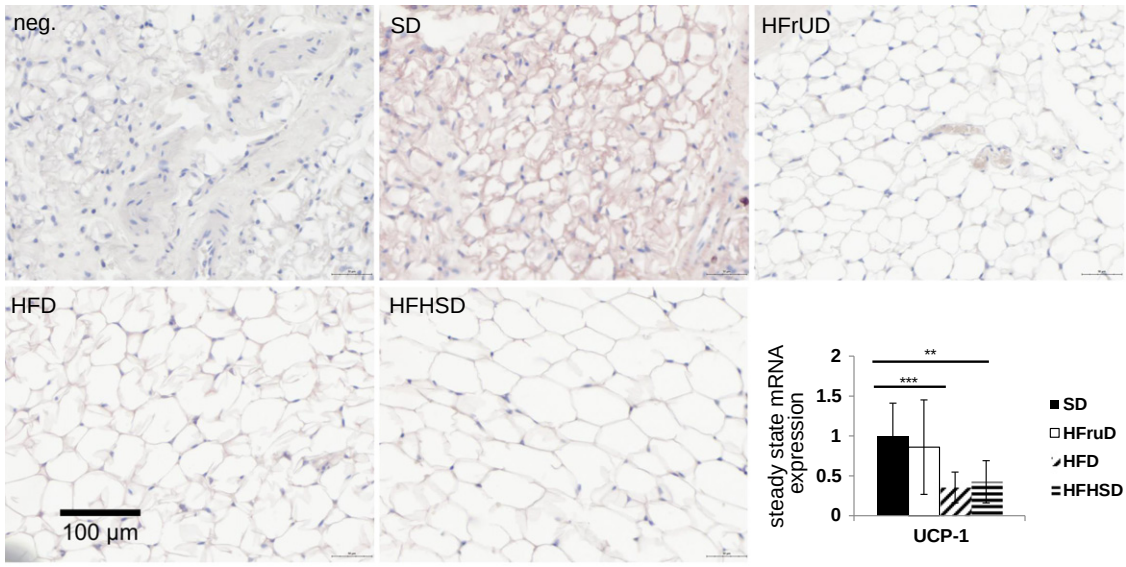

Fig. 3. UCP-1 immunohistochemical staining and UCP-1 steady-state mRNA expression of subcutaneous adipose tissue extracts of male mice. Representative images of IHC staining of SAT with antibody specific for beige adipose tissue marker UCP-1 confirms identity of beige adipocytes in WAT depot of SD-fed male mice. Positive staining is less apparent in HFruD-, HFD- and HFHSD-fed male mice. Diagram shows UCP-1 steady-state mRNA expression of SAT of male mice relative to HPRT. HFD- and HFHSD-fed male mice exhibit less UCP-1 IHC staining and significantly lower UCP-1 steady-state mRNA expression compared to SD-fed male mice. Steady-state mRNA expression data are reported as means ± S.D. $^{*}P \leq .05$, $^{**}P \leq .01$, $^{***}P \leq .001$. SD = standard diet, HFruD = high-fructose diet, HFD = high-fat diet, HFHSD = high-fat, high-sucrose diet. SAT = subcutaneous adipose tissue, WAT = white adipose tissue.

glucose metabolism and insulin sensitivity as determined by oGTT and ipITT are primarily due to diminished insulin resistance of adipocytes. As body-weight-based glucose concentrations were used for oGTT in our experiments, mild overestimations of glucose intolerance in obese mice cannot fully be excluded [32].

Reductions of GLUT4 expression were less pronounced after HFruD feeding in VAT of male mice. In female mice, fat-enriched diets only led to borderline reductions of GLUT4 expressions. Data on altered metabolic function and size distribution were completed by a predominantly white phenotype of adipocytes in HFD- and HFHSD-fed mice as determined visually by IHC, TEM and also mRNA expression of BAT/beige AT specific marker UCP-1 [33]. In contrast, a predominantly beige phenotype of subcutaneous adipocytes was found in SD- and HFruD-fed male mice, while in female HFruD-fed mice UCP-1 mRNA expression was significantly reduced when compared to SD-fed mice. Interestingly, mean SAT adipocyte size and distribution pattern were similar in HFruD-fed and SD-fed female mice indicating the presence of a larger population of adipocyte precursor cells supporting previous findings in rats [34].

As beige AT and BAT are positively associated with increased energy expenditure due to the uncoupling of oxidative phosphorylation from energy need and insulin sensitivity [35], the whitening of SAT in HFD- and HFSHSD-fed mice fits well to the significant weight gain and impaired glucose/insulin metabolism observed in these mice. Besides discussed effects of sucrose in the central nervous system, we hypothesize that the most pronounced weight gain in the HFHSD feeding group might partly result from sucrose stimulated increase in food intake while simultaneous increase in energy expenditure is impeded through the high fat content of this diet. Noteworthy, only HFHSD-fed male mice revealed down–regulated mRNA expression of

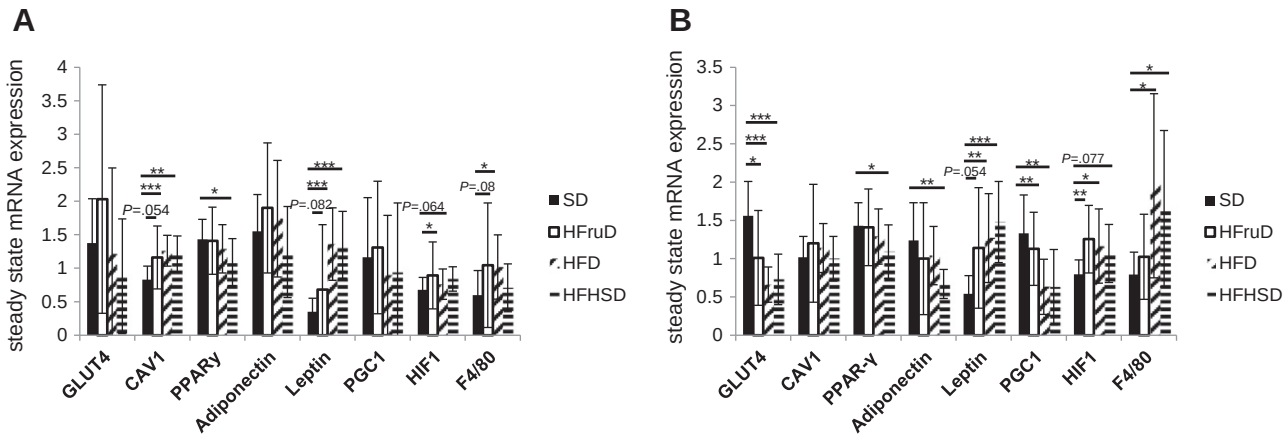

Fig. 4. Steady-state mRNA expression analysis of SAT (A) and VAT (B) depots of male mice fed with SD, HFruD, HFD or HFHSD. Steady-state mRNA expression of insulin/glucose metabolism (GLUT4), adipocyte function (CAV1, PPARγ), adipocytokines (adiponectin, leptin), mitochondrial biogenesis (PGC1), hypoxic stress response (HIF1) and inflammation (F4/80) was analyzed. Steady-state mRNA expression data are normalized to HPRT and reported as means ± S.D. $^{*}P \leq .05$, $^{**}P \leq .01$, $^{***}P \leq .001$. SD = standard diet, HFruD = high-fructose diet, HFD = high-fat diet, HFHSD = high-fat, high-sucrose diet. SAT = subcutaneous adipose tissue, VAT = visceral adipose tissue.

Table 2
Sex specific effects of HFruD, HFD and HFHSD in C57BL/6 mice

	Female	Male
Standard diet (SD) feeding		
Weight gain (%)	17±4	22±7
AUC oGTT (arbitrary unit)	24,245±1972	27,118±4885
AUC ipITT (arbitrary unit)	4513±787	5910±1079†
Granulocyte %	28.8±7.5	19.9±4.2†
High-fructose (HFruD) diet feeding		
Weight gain (%)	18.7±3	20±6
AUC oGTT (arbitrary unit)	28,867±5636	28,819±4429
AUC ipITT (arbitrary unit)	5610±1555	7136±2383
Granulocyte %	31.7±7.7	27±6.9
High-fat diet (HFD) feeding		
Weight gain (%)	28.5±9	49.1±9‡
AUC oGTT (arbitrary unit)	33,876±9190	36,524±5800
AUC ipITT (arbitrary unit)	6202±1332	8868±2105†
Granulocyte %	25.8±13.5	27±7.7
High-fat, high-sucrose diet (HFHSD) feeding		
Weight gain (%)	39.1±18	59.4±9‡
AUC oGTT (arbitrary unit)	27,036±6967	34,655±3990†
AUC ipITT (arbitrary unit)	5999±1059	7736±1563*
Granulocyte %	23.4±7.6	26±4.8

Values are reported as means ± S.D. Significance is reported in comparison to female mice. SD = standard diet, HFruD = high-fructose diet, HFD = high-fat diet, HFHSD = high-fat, high-sucrose diet.
* $P \leq .05$,
† $P \leq .01$,
‡ $P \leq .001$.

master adipogenic regulator PPARγ [36] and insulin sensitizing adipocytokine adiponectin [37] further supporting the notion of an attenuated metabolic phenotype of mice fed with this diet. Steady-state mRNA expression analysis of PGC1α and UCP-1 also revealed that high expression of mitochondrial biogenesis/activity markers were associated with lower body weight and improved glucose homeostasis and insulin sensitivity. Altered mRNA expression of critical adipocytokines, important molecules in the insulin signaling pathway and proteins involved in adipocyte differentiation and function was associated with larger adipocytes in both SAT and VAT, linking normal adipocyte size directly to proper functionality [38]. Interestingly, not only hypertrophic adipocytes of HFD- and HFHSD-fed male mice [39,40], but also HFruD-fed male mice displayed increased HIF1α mRNA levels in VAT suggesting adipose tissue hypoxia and increased oxidative stress despite relatively regular adipocyte size and distribution after high fructose intake. Additionally, SAT of HFruD- and HFHSD- but not HFD-fed male mice showed increased HIF1α mRNA expression implicating that fructose contributes to oxidative stress the in AT.

In this study and consistent with published data [41,42], effects of fat-enriched diets on body weight and glucose tolerance as well as insulin sensitivity were less pronounced in female than in male mice. Also, overall harmful effects on adipocyte morphology and function were found to be less distinct in female mice, suggesting sex-specific effects of high-fat diets on energy and glucose metabolism. Our findings fit well to studies showing that weight gain tendentially expands through hyperplasia in females and by hypertrophy in males [43–45]. Remarkably, fructose feeding was associated with decreased UCP-1 expression in SAT of female but not in male mice, further supporting sex-specific differences upon dietary intake in male and female mice. Sex-specific metabolic consequences might be explained by direct effects of estrogens as ovariectomized mice were reported to be significantly more susceptible to diet induced obesity than control mice [42]. In accordance with these data, Hamilton and colleagues [46] recently reported that selective activation of estrogen receptor alpha (ERα) increases oxygen consumption, core body temperature and expression of critical transcription factors such as PGC1α in ovariectomized mice and as a consequence prevented weight gain and improved insulin action.

While the percentage of granulocytes of all leukocytes (GRA%) was significantly lower in SD-fed male mice compared to SD-fed females, high-fructose and fat-rich diets led to a significant increase in the GRA% in male but not in female mice, suggesting that diets might exert sex-specific effects on systemic inflammation. Mechanistically, these findings might be explained by less pronounced diet-induced AT inflammation in female mice as determined by steady-state mRNA expression levels of macrophage marker F4/80. In contrast to male mice, increased F4/80 expression was found exclusively in VAT of HFHSD-fed female mice.

4.1. Conclusions

Taken together, our results suggest that combined sucrose and fat-enriched diets resembling a typical Western diet exert more deleterious effects on body weight and insulin sensitivity than diets enriched with fructose or fat alone. The higher content of saturated fatty acids in HFHSD compared with HFD might also contribute to the potentially more harmful effects on glucose homeostasis and insulin sensitivity observed after HFHSD feeding [47]. On a cellular basis, feeding of any fat-enriched diet is associated with tremendous alterations in adipocyte size, morphology and function, while effects of fructose-rich diets on adipocyte morphology are far less pronounced and primarily characterized by increased oxidative stress. From our results we further hypothesize that female sex is associated with decreased susceptibility to diet-induced alterations in adipocyte morphology and function and as a consequence systemic insulin resistance.

Author contributions

J.D. performed the experiments and wrote the manuscript. C.R., K.R., K.S., P.M., S.F. performed the experiments, G.G. and J.D. performed statistical analysis. All authors contributed the analysis and discussion of experimental data. S.K. designed the study and wrote the manuscript. S.K. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Duality of interest

No potential conflicts of interest relevant to this article were reported.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jnutbio.2017.07.009.

References

[1] Ross SE, Flynn JI, Pate RR. What is really causing the obesity epidemic? A review of reviews in children and adults. J Sports Sci 2015;414:1–6. http://dx.doi.org/10.1080/02640414.2015.1093650.
[2] Reis JP, Loria CM, Sorlie PD, Park Y, Hollenbeck A, Schatzkin A. Lifestyle factors and risk for new-onset diabetes: a population-based cohort study. Ann Intern Med 2011;155:292–9. http://dx.doi.org/10.7326/0003-4819-155-5-201109060-00006.

[3] Wells JCK. Obesity as malnutrition: the dimensions beyond energy balance. Eur J Clin Nutr 2013;67:507–12. http://dx.doi.org/10.1038/ejcn.2013.31.
[4] Rutledge AC, Adeli K. Fructose and the metabolic Syndrome : pathophysiology and molecular mechanisms. Nutrition 2007;65:13–23. http://dx.doi.org/10.1301/nr.2007.jun.S13.
[5] Liu Z, Patil IY, Jiang T, Sancheti H, Walsh JP, Stiles BL, et al. High-fat diet induces hepatic insulin resistance and impairment of synaptic plasticity. PLoS One 2015;10:e0128274.
[6] Ley SH, Pan A, Li Y, Manson JE, Willett WC, Sun Q, et al. Changes in overall diet quality and subsequent Type 2 diabetes risk: three U.S. prospective cohorts. Diabetes Care 2016;39:2011–8.
[7] Rosen ED, Spiegelman BM. Adipocytes as regulators of energy balance and glucose homeostasis. Nature 2006;444:847–53.
[8] Lelliott C, Vidal-Puig a J. Lipotoxicity, an imbalance between lipogenesis de novo and fatty acid oxidation. Int J Obes Relat Metab Disord 2004;28(Suppl. 4):S22–8. http://dx.doi.org/10.1038/sj.ijo.0802854.
[9] Galic S, Oakhill JS, Steinberg GR. Adipose tissue as an endocrine organ. Mol Cell Endocrinol 2010;316:129–39. http://dx.doi.org/10.1016/j.mce.2009.08.018.
[10] Tilg H, Moschen AR. Adipocytokines: mediators linking adipose tissue, inflammation and immunity. Nat Rev Immunol 2006;6:772–83. http://dx.doi.org/10.1038/nri1937.
[11] Giralt M, Villarroya F. White, brown, beige/brite: different adipose cells for different functions? Endocrinology 2013;154:2992–3000. http://dx.doi.org/10.1210/en.2013-1403.
[12] Jo J, Gavrilova O, Pack S, Jou W, Mullen S, Sumner AE, et al. Hypertrophy and/or hyperplasia: dynamics of adipose tissue growth. PLoS Comput Biol 2009;5. http://dx.doi.org/10.1371/journal.pcbi.1000324.
[13] Rajkovic N, Zamaklar M, Lalic K, Jotic A, Lukic L, Milicic T, et al. Relationship between obesity, adipocytokines and inflammatory markers in Type 2 diabetes: relevance for cardiovascular risk prevention. Int J Environ Res Public Health 2014;11:4049–65. http://dx.doi.org/10.3390/ijerph110404049.
[14] Hosogai N, Fukuhara A, Oshima K, Miyata Y, Tanaka S, Segawa K, et al. Adipose tissue hypoxia in obesity and its impact on adipocytokine dysregulation. Diabetes 2007;56:901–11. http://dx.doi.org/10.2337/db06-0911.
[15] Perry RJ, Camporez J-PG, Kursawe R, Titchenell PM, Zhang D, Perry CJ, et al. Hepatic acetyl CoA links adipose tissue inflammation to hepatic insulin resistance and Type 2 diabetes. Cell 2015;160:745–58. http://dx.doi.org/10.1016/j.cell.2015.01.012.
[16] Ayala JE, Samuel VT, Morton GJ, Obici S, Croniger CM, Shulman GI, et al. Standard operating procedures for describing and performing metabolic tests of glucose homeostasis in mice. Dis Model Mech 2010;3:525–34. http://dx.doi.org/10.1242/dmm.006239.
[17] Galarraga M, Campion J, Munoz-Barrutia A, Boque N, Moreno H, Martinez JA, et al. Adiposoft: automated software for the analysis of white adipose tissue cellularity in histological sections. J Lipid Res 2012;53:2791–6. http://dx.doi.org/10.1194/jlr.D023788.
[18] Guilherme A, Virbasius JV, Puri V, Czech MP. Adipocyte dysfunctions linking obesity to insulin resistance and Type 2 diabetes. Nat Rev Mol Cell Biol 2008;9:367–77.
[19] Tappy L, Lê KA, Tran C, Paquot N. Fructose and metabolic diseases : new findings, new questions. Nutrition 2010;26:1044–9. http://dx.doi.org/10.1016/j.nut.2010.02.014.
[20] Rutkowski JM, Stern JH, Scherer PE. The cell biology of fat expansion. J Cell Biol 2015;208:501–12.
[21] Ryan KK, Woods SC, Seeley RJ. Central nervous system mechanisms linking the consumption of palatable high-fat diets to the defense of greater adiposity. Cell Metab 2012;15:137–49. http://dx.doi.org/10.1016/j.cmet.2011.12.013.
[22] Tillman EJ, Morgan DA, Rahmouni K, Swoap SJ. Three months of high-fructose feeding fails to induce excessive weight gain or leptin resistance in mice. PLoS One 2014;9:e107206. http://dx.doi.org/10.1371/journal.pone.0107206.
[23] Friedman JM, Best C, Collip JB. A tale of two hormones. Nat Publ Gr 2010;16:1100–6. http://dx.doi.org/10.1038/nm1010-1100.
[24] Peng XX, Lister A, Rabinowitsch A, Kolaric R, Cabeza de Vaca S, Ziff EB, et al. Episodic sucrose intake during food restriction increases synaptic abundance of AMPA receptors in nucleus accumbens and augments intake of sucrose following restoration of ad libitum feeding. Neuroscience 2015;295:58–71. http://dx.doi.org/10.1016/j.neuroscience.2015.03.025.
[25] Peng X-X, Ziff EB, Carr KD. Effects of food restriction and sucrose intake on synaptic delivery of AMPA receptors in nucleus Accumbens. Synapse 2011;65:1024–31. http://dx.doi.org/10.1002/syn.20931.
[26] Labouebe G, Boutrel B, Tarussio D, Thorens B. Glucose-responsive neurons of the paraventricular thalamus control sucrose-seeking behavior. Nat Neurosci 2016;19:999–1002.
[27] Domingos AI, Vaynshteyn J, Sordillo A, Friedman JM. The reward value of sucrose in leptin-deficient obese mice. Mol Metab 2014;3:73–80. http://dx.doi.org/10.1016/j.molmet.2013.10.007.
[28] Ter Horst KW, Schene MR, Holman R, Romijn JA, Serlie MJ. Effect of fructose consumption on insulin sensitivity in nondiabetic subjects: a systematic review and meta-analysis of diet-intervention trials. Am J Clin Nutr 2016;104:1562–76. http://dx.doi.org/10.3945/ajcn.116.137786.
[29] Maiztegui B, Borelli M, Raschia M, Del Zotto H, Gagliardino J. Islet adaptive changes to fructose-induced insulin resistance: β-cell mass, glucokinase, glucose metabolism and insulin secretion. J Endocrinol 2008. http://dx.doi.org/10.1677/JOE-08-0386.
[30] Hanefeld M, Koehler C, Fuecker K, Henkel E, Schaper F, Temelkova-Kurktschiev T. Insulin secretion and insulin sensitivity pattern is different in isolated impaired glucose tolerance and impaired fasting glucose. Diabetes Care 2003;26:868–74.
[31] Blüher M, Michael MD, Peroni OD, Ueki K, Carter N, Kahn BB, et al. Adipose tissue selective insulin receptor knockout protects against obesity and obesity-related glucose intolerance. Dev Cell 2002;3:25–38. http://dx.doi.org/10.1016/S1534-5807(02)00199-5.
[32] McGuinness OP, Ayala JE, Laughlin MR, Wasserman DH. NIH experiment in centralized mouse phenotyping: the Vanderbilt experience and recommendations for evaluating glucose homeostasis in the mouse. Am J Physiol Endocrinol Metab 2009;297:E849-5. http://dx.doi.org/10.1152/ajpendo.90996.2008.
[33] Shabalina I, Petrovic N, deJong JA, Kalinovich A, Cannon B, Nedergaard J. UCP1 in Brite/beige adipose tissue mitochondria is functionally thermogenic. Cell Rep 2013;5:1196–203. http://dx.doi.org/10.1016/j.celrep.2013.10.044.
[34] Zubiría GM, Alzamendi A, Moreno G, Rey AM, Spinedi E, Giovambattista A. Long-term fructose intake increases Adipogenic potential: evidence of direct effects of fructose on adipocyte precursor cells. Nutrients 2016;8. http://dx.doi.org/10.3390/nu8040198.
[35] Harms M, Seale P. Brown and beige fat: development, function and therapeutic potential. Nat Med 2013;19:1252–63. http://dx.doi.org/10.1038/nm.3361.
[36] Tontonoz P, Spiegelman BM. Fat and beyond: the diverse biology of PPARgamma. Annu Rev Biochem 2008;77:289–312. http://dx.doi.org/10.1146/annurev.biochem.77.061307.091829.
[37] Kawano J, Arora R. The role of adiponectin in obesity, diabetes, and cardiovascular disease. J Cardiometab Syndr 2009;4:44–9. http://dx.doi.org/10.1111/j.1559-4572.2008.00030.x.
[38] Farnier C, Krief S, Blache M, Diot-Dupuy F, Mory G, Ferre P, et al. Adipocyte functions are modulated by cell size change: potential involvement of an integrin/ERK signalling pathway. Int J Obes Relat Metab Disord 2003;27:1178–86. http://dx.doi.org/10.1038/sj.ijo.0802399.
[39] Trayhurn P. Hypoxia and adipocyte physiology: implications for adipose tissue dysfunction in obesity. Annu Rev Nutr 2014;34:207–36. http://dx.doi.org/10.1146/annurev-nutr-071812-161156.
[40] He Q, Gao Z, Yin J, Zhang J, Yun Z, Ye J. Regulation of HIF-1{alpha} activity in adipose tissue by obesity-associated factors: adipogenesis, insulin, and hypoxia. Am J Physiol Endocrinol Metab 2011;300:E877-5. http://dx.doi.org/10.1152/ajpendo.00626.2010.
[41] Bégin-Heick N. Of mice and women: the beta 3-adrenergic receptor leptin and obesity. Biochem Cell Biol 1996;74:615–22.
[42] Hong J, Stubbins RE, Smith RR, Harvey AE, Núñez NP. Differential susceptibility to obesity between male, female and ovariectomized female mice. Nutr J 2009;8:11. http://dx.doi.org/10.1186/1475-2891-8-11.
[43] Tchernof A, Després J-P. Pathophysiology of human visceral obesity: an update. Physiol Rev 2013;93:359–404.
[44] Tchoukalova YD, Koutsari C, Karpyak MV, Votruba SB, Wendland E, Jensen MD. Subcutaneous adipocyte size and body fat distribution. Am J Clin Nutr 2008;87:56–63.
[45] Pradhan AD. Sex differences in the metabolic syndrome: implications for cardiovascular health in women. Clin Chem 2013;60:44–52.
[46] Hamilton DJ, Minze LJ, Kumar T, Cao TN, Lyon CJ, Geiger PC, et al. Estrogen receptor alpha activation enhances mitochondrial function and systemic metabolism in high-fat-fed ovariectomized mice. Physiol Rep 2016;4.
[47] Lottenberg AM, Afonso Mda S, Lavrador MS, Machado RM, Nakandakare ER. The role of dietary fatty acids in the pathology of metabolic syndrome. J Nutr Biochem 2012;23:1027–40. http://dx.doi.org/10.1016/j.jnutbio.2012.03.004.