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ARTICLE

Mouse strain-dependent variation in obesity and glucose homeostasis in response to high-fat feeding

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Abstract
Aims/hypothesis Metabolic disorders are commonly investigated using knockout and transgenic mouse models. A variety of mouse strains have been used for this purpose. However, mouse strains can differ in their inherent propensities to develop metabolic disease, which may affect the experimental outcomes of metabolic studies. We have investigated strain-dependent differences in the susceptibility to diet-induced obesity and insulin resistance in five commonly used inbred mouse strains (C57BL/6J, 129X1/SvJ, BALB/c, DBA/2 and FVB/N).
Methods Mice were fed either a low-fat or a high-fat diet (HFD) for 8 weeks. Whole-body energy expenditure and body composition were then determined. Tissues were used to measure markers of mitochondrial metabolism, inflammation, oxidative stress and lipid accumulation.
Results BL6, 129X1, DBA/2 and FVB/N mice were all susceptible to varying degrees to HFD-induced obesity, glucose intolerance and insulin resistance, but BALB/c mice exhibited some protection from these detrimental effects. This protection could not be explained by differences in mitochondrial metabolism or oxidative stress in liver or muscle, or inflammation in adipose tissue. Interestingly, in contrast with the other strains, BALB/c mice did not accumulate excess lipid (triacylglycerols and diacylglycerols) in the liver; this is potentially related to lower fatty acid uptake rather than differences in lipogenesis or lipid oxidation.
Conclusions/interpretation Collectively, our findings indicate that most mouse strains develop metabolic defects on an HFD. However, there are inherent differences between strains, and thus the genetic background needs to be considered carefully in metabolic studies.

Keywords Carbohydrate metabolism · Insulin sensitivity and resistance · Lipid metabolism · Weight regulation and obesity

Abbreviations

ACC	Acetyl-CoA carboxylase
CPT-1	Carnitine palmitoyltransferase 1
CS	Citrate synthase
DAG	Diacylglycerol
DXA	Dual-energy x-ray absorptiometry
ETC	Electron-transport chain
FAS	Fatty acid synthase
FATP	Fatty acid transport protein
GTT	Glucose tolerance test
βHAD	β-Hydroxyacyl-CoA dehydrogenase
HFD	High-fat diet
ISI	Insulin sensitivity index
ITT	Insulin tolerance test
LFD	Low-fat diet
LOOH	Lipid hydroperoxide
MHO	Metabolically healthy obese
PDK4	Pyruvate dehydrogenase lipoamide kinase isozyme 4

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PGC1α	Peroxisome proliferator-activated receptor γ coactivator-1α
RER	Respiratory exchange ratio
SCD-1	Stearoyl-CoA desaturase 1
TAG	Triacylglycerol
TBARS	Thiobarbituric acid reactive substances
UCP3	Uncoupling protein 3

Introduction

In the past 30 years, the use of transgenic and knockout mice to investigate the role of specific proteins in metabolic disease has become commonplace in medical research. However, when creating genetically manipulated mice, consideration must be given to the genetic background on which the mouse is created and differences in metabolic phenotype that might be associated with gene manipulation on mixed genetic backgrounds. Several previous studies have shown that mouse strains can differ substantially in their metabolic phenotype under normal low-fat diet (LFD) conditions and in response to a high-fat diet (HFD) [1–5]. Some problems with these and other previous strain comparisons are the use of diverse HFDs varying in lipid and carbohydrate content and the investigation of limited variables that may underpin the development of metabolic disease and importantly some contradictory findings. For example, FVB/N mice have been characterised as both obesity-prone [6] and obesity-resistant [3], with similar contradictory observations reported for DBA/2 mice [1, 7].

The C57BL/6 mouse strain is generally suggested to be the best strain for studying metabolic disease. However, it is worth noting that BL6 mice have also been described as 'diabetes-prone' or 'diabetes-resistant' depending on which other mouse strain was used in the comparison [8]. The preference for the use of C57BL/6 mice probably stems from the early studies of Surwit and colleagues, where it was shown that high-fat/high-sucrose diets caused marked increases in fasting glucose and insulin levels [9, 10], indicating obesity-induced insulin resistance in this strain. Later studies also suggested the potential of insulin secretory defects in C57BL/6 mice [11]. Because of the utility of using 129X1/SvJ mouse embryonic stem cells for the generation of gene-manipulated strains, much of the original metabolic phenotyping in knockout and transgenic mice was performed using mice produced on this strain or more commonly using mice backcrossed to C57BL/6 for a defined or undefined number of generations [12]. There is, however, clear evidence that genetically manipulated mice on a mixed 129/BL6 background can display a different phenotype from mice where the same genetic manipulation is performed on a pure genetic background [13].

Several mechanisms have been proposed to be responsible for obesity-related insulin resistance, including lipid accumulation in non-adipose tissues, inflammation, mitochondrial dysfunction, and/or oxidative stress [14–18]. Much of the evidence supporting these various theories is based on studies in normal or genetically manipulated mice fed HFDs. However, to what extent these factors may be related to the genetic background of the mice is still not completely resolved. Here, we describe a comprehensive study on the response of five commonly used mouse strains (C57BL/6, 129X1/SvJ, BALB/c, DBA/2 and FVB/N) to the same HFD, examining glucose tolerance, whole-body and tissue-specific lipid accumulation, and aspects of tissue inflammation, mitochondrial function and oxidative stress.

Methods

Eight-week old male C57BL/6J, 129X1/SvJ, BALB/c, DBA/2 and FVB/N mice were purchased from the Australian Resource Centre (Perth, Australia). Mice were maintained in a temperature-controlled room (22±1°C) with a 12 h light/dark cycle and ad libitum access to food and water. After 1 week on a standard low-fat chow diet (LFD; 8% of calories from fat, 21% of calories from protein, 71% of calories from carbohydrate; Gordon's Specialty Stock Feeds, Yanderra, NSW, Australia), mice were randomly allocated to remain on the LFD or to receive an HFD ad libitum for 8 weeks. The HFD (45% of calories from fat (lard), 20% of calories from protein, 35% of calories from carbohydrate) was made in-house as described elsewhere [19] and contained similar micronutrient and mineral content to the LFD. During the 8-week feeding period, body weight and food intake were monitored on a biweekly basis. Mice were kept in cages of four animals, and food intake per cage was averaged and is expressed in kJ mouse^{-1} day^{-1}. Plasma and tissue samples were collected from mice at 09:00–10:00 hours without any prior fasting period. All experiments were approved by the Garvan Institute/St Vincent's Hospital Animal Experimentation Ethics Committee, and followed guidelines issued by the National Health and Medical Research Council of Australia.

Determination of body composition and energy expenditure Fat and lean body mass were measured in mice using dual-energy x-ray absorptiometry (DXA) (Lunar PIXImus2 densitometer; GE Healthcare, Little Chalfont, UK) in accordance with the manufacturer's instructions. The rate of oxygen consumption ($\dot{V}O_2$) and respiratory exchange ratio (RER) of individual mice were measured using an Oxymax indirect calorimeter (Columbus Instruments, Columbus,

OH, USA) as previously described [19]. Energy expenditure (kJ heat produced) was calculated as calorific value × $\dot{V}O_2$, where calorific value is 3.815+(1.232×RER) [20].

Glucose and insulin tolerance Mice were fasted for 6 h and injected i.p. with glucose (2 g/kg) or insulin (0.75 U/kg), and blood glucose levels were monitored using an Accu-check II glucometer (Roche Diagnostics, Castle Hill, NSW, Australia). Plasma insulin levels were determined by radio-immunoassay (Linco Research, St Charles, MO, USA).

Tissue lipid analyses Triacylglycerol (TAG) content was determined using a colorimetric assay kit (Triglycerides GPO-PAP; Roche Diagnostics, Indianapolis, IN, USA) as previously described [21]. Plasma NEFAs were measured using a colorimetric kit (Wako Diagnostics, Osaka, Japan), and adiponectin was determined by radioimmunoassay (Linco Research). For diacylglycerol (DAG) and ceramide measurements, lipids were extracted from muscle and liver in solvents containing 2 nmol ceramide (17:0) and 10 nmol DAG (17:0/17:0) [22]. DAG and ceramide levels were measured using a hybrid linear ion trap–triple quadrupole mass spectrometer (QTRAP 5500: AB Sciex, Foster City, CA, USA). Ceramide molecular lipids were analysed by precursor-ion scanning for protonated dehydrated sphingosine at *m/z* 264.3. DAG molecular lipids were analysed by multiple neutral-loss scanning for ammoniated fatty acids. Data were analysed and quantified with LipidView (AB Sciex) version 1.1 after isotope correction.

Immunoblotting Whole-tissue lysates were prepared from powdered muscle and liver by manual homogenisation in RIPA buffer [23]. Proteins were resolved by SDS-PAGE electrophoresis, and immunoblot analysis was conducted as described elsewhere [19, 24, 25]. Immunolabelled bands were quantified using ImageJ 1.44p software.

Measurement of palmitate oxidation, enzyme activity and oxidative damage Palmitate oxidation and enzyme activities were measured in muscle and liver homogenates as described previously [19, 26]. Similarly, thiobarbituric acid reactive substances (TBARS), lipid hydroperoxide (LOOH) and protein carbonyls were measured in homogenates as described previously [27–29]. Homogenate protein content was measured using the Bradford method (Bio-Rad Laboratories, Regents Park, NSW, Australia).

Analysis of gene expression RNA was extracted using TRI-Reagent (Sigma-Aldrich, Castle Hill, NSW, Australia) according to the manufacturer's protocol, followed by DNase treatment (RQ1 RNase-free DNase; Promega, Arundel, QLD, Australia) and synthesis of complementary DNA using Random primer 9 (New England Biolabs, Arundel, QLD, Australia) and Superscript III reverse transcriptase (Invitrogen, Mulgrave, VIC, Australia) according to the manufacturer's instructions. Real-time PCR was performed using the Lightcycler 480 Probes Master mix on a real-time PCR System (7900HT; Applied Biosystems, Foster City, CA, USA). The value obtained for each specific product was normalised to a control gene (hypoxanthine–guanine phosphoribosyltransferase). Primer sequences are shown in electronic supplementary material (ESM) Table 1.

Statistical analysis All results are presented as mean ± SEM. Data were analysed with an unpaired Student's *t* test. Comparisons of energy expenditure (kJ/h) were carried out by analysis of covariance with [lean body mass+(0.2×fat mass)] as the covariate (see Even and Nadkarni [30]). Statistical significance was accepted at $p<0.05$.

Results

Body weight, fat mass and food intake Compared with animals on an LFD, body weight was significantly increased in BL6, 129X1, DBA/2 and FVB/N mice fed an HFD, but remained unchanged in BALB/c mice. HFD-fed DBA/2 and 129X1 mice showed the largest increase in body weight (~25%) in comparison with other strains (Fig. 1). Whole-body adiposity measured by DXA was increased in all mouse strains on the HFD, including the BALB/c mice, as was the size of the epididymal and inguinal fat depots (Table 1).

Food intake was measured biweekly as an average of two cages with four mice per cage for each strain. The energy intake (shown in kJ/day) during the 8-week feeding period was higher in HFD-fed 129X1, DBA/2 and FVB/N mice than LFD-fed controls, but was not different in BL6 and BALB/c mice (Table 1).

Energy expenditure and fuel selection Energy expenditure was ~5–10% higher in HFD-fed animals, and analysis of covariance using [lean body mass+(0.2)×fat mass)] as a covariate [30] indicated that this difference was significant in 129X1, BALB/c and FVB/N mice. The RER was decreased in all five strains, consistent with increased lipid intake and catabolism (Table 1 and ESM Fig. 1).

Glucose tolerance and insulin action Whole-body glucose clearance during an i.p. glucose tolerance test (GTT) was determined (Fig. 1), and, in four of the mouse strains, HFD-fed animals displayed significant impairment in glucose tolerance, as evidenced by a substantial increase in the incremental glucose AUC (BL6, +97%; 129X1, +284%; DBA/2, +130%; FVB/N, +37%). In contrast, BALB/c mice exhibited no deterioration in glucose tolerance on the HFD. It should be noted that glucose bolus injections were

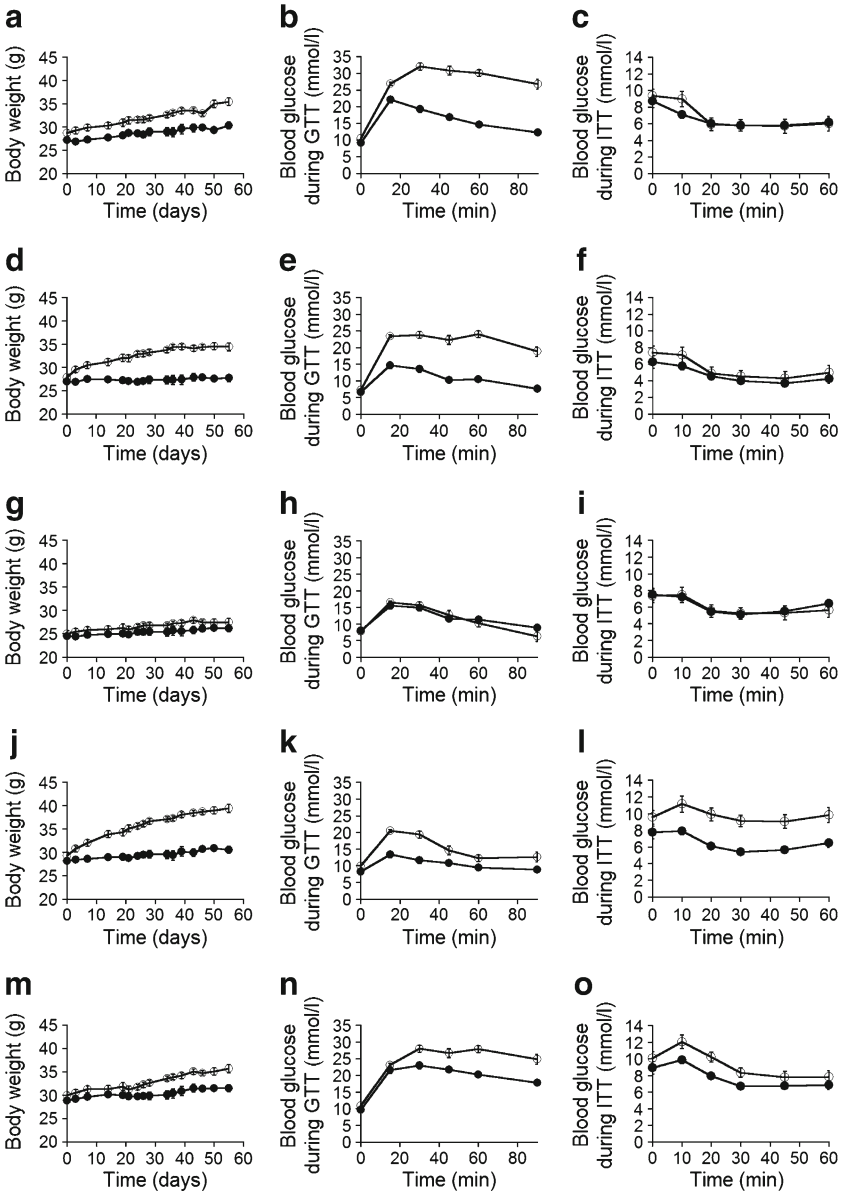

Fig. 1 Body weight during the 8 week feeding period, i.p. glucose tolerance test (GTT) and insulin tolerance test (ITT) in LFD-fed (black circles) and HFD-fed (white circles) C57BL/6 (**a**–**c**), 129X1 (**d**–**f**), BALB/c (**g**–**i**), DBA/2 (**j**–**l**) and FVB/N (**m**–**o**) mice. Glucose (2 g/kg) or insulin (0.75 U/kg) was injected at the 0 time point, and blood glucose levels were monitored for 90 or 60 min after injection, respectively. Inverse area under curve (iAUC) for ITT was calculated as described previously [31]. Endpoint body weight $p<0.0001$ for BL6, 129X1, DBA/2 and FVB/N; AUC (GTT) $p<0.001$ for BL6, 129X1 and DBA/2 and $p<0.05$ for FVB/N; iAUC (ITT) $p<0.05$ for BL6, DBA/2 and FVB/N; $n=6$–8 for each strain and diet group

calculated according to the animals' body weight, and this may have contributed, in part, to the observed differences in glucose tolerance across the strains.

Fasting blood glucose and fasting plasma insulin were significantly increased in all strains on the HFD, except for BALB/c (Table 2). As a surrogate measure of insulin sensitivity, 'fasting glucose' was multiplied by 'fasting insulin' to calculate an insulin sensitivity index (ISI). The ISI indicated substantial impairment in insulin sensitivity in all strains, except BALB/c (increase in ISI value vs LFD group: BL6, +96%; 129X1, +128%; BALB/c, +4%; DBA/2, +234%; FVB/N, +70%). An i.p. insulin tolerance test (ITT) was also used as a second measure of insulin action (Fig. 1). Similar to the ISI, the inverse AUC for the i.p. ITT during the first 20 min [31] indicated the development of insulin resistance in BL6, DBA/2 and FVB/N mice, while HFD-fed 129X1 and BALB/c were not different from LFD controls.

Circulating factors Plasma adiponectin and NEFA levels were unchanged by the HFD across all strains. Plasma TAG levels were increased in 129X1 mice, decreased in FVB/N mice, and unchanged in the other strains (Table 2).

Lipid accumulation in non-adipose tissues All HFD-fed mouse strains had increased TAG deposition in their muscles, while only FVB/N mice also displayed elevated DAG and ceramide content (Fig. 2). In the liver, TAG and DAG levels were significantly increased in BL6, 129X1, DBA/2 and FVB/N mice, with decreased ceramides also observed in liver from BL6 mice (Fig. 2). Intriguingly, HFD-fed BALB/c mice did not accumulate any excess

Table 1 Animal characteristics, including tissue weight, food intake and energy expenditure, separated by mouse strain and diet group

Characteristic	Diet type	C57Bl/6	129X1	BALB/c	DBA/2	FVB/N
Body weight (g)	LFD	30.3±0.4	27.6±0.4	26.0±0.6	31.2±0.7	31.1±0.5
	HFD	35.4±0.9***	34.7±0.6***	27.8±0.5	39.0±0.8***	34.5±0.6***
Fat mass (g)	LFD	4.7±0.3	6.1±0.5	5.3±0.1	7.4±0.2	5.7±0.4
	HFD	10.4±0.5***	12.7±0.5***	8.1±0.4***	15.1±0.7***	10.4±0.5***
Tissue weight (g)						
eWAT	LFD	0.34±0.02	0.33±0.01	0.54±0.02	0.79±0.07	0.39±0.06
	HFD	1.42±0.15***	1.38±0.05***	1.09±0.10***	1.82±0.10***	1.17±0.06***
iWAT	LFD	0.24±0.01	0.34±0.04	0.36±0.04	0.56±0.06	0.33±0.03
	HFD	0.74±0.14***	0.94±0.07***	0.54±0.03**	1.55±0.11***	0.63±0.03***
BAT	LFD	0.12±0.01	0.12±0.01	0.15±0.03	0.17±0.01	0.17±0.02
	HFD	0.13±0.01	0.21±0.02**	0.15±0.01	0.34±0.04***	0.23±0.01**
Liver	LFD	1.48±0.07	1.20±0.02	1.31±0.05	1.63±0.05	1.70±0.04
	HFD	1.21±0.08	1.25±0.07	1.17±0.02*	1.64±0.04	1.71±0.04
Food intake (kJ/day)	LFD	56.40±0.96	44.98±3.30	46.77±2.80	48.91±1.13	40.67±4.51
	HFD	58.14±1.84	61.90±5.06	44.56±0.08	60.73±0.67	52.58±1.55
Energy expenditure						
kJ/h	LFD	2.13±0.04	1.96±0.04	1.92±0.04	2.17±0.04	2.21±0.04
	HFD	2.17±0.04	2.17±0.04*	2.05±0.04**	2.47±0.08	2.42±0.04**
RER	LFD	0.97±0.03	0.94±0.01	0.97±0.02	0.88±0.02	0.95±0.01
	HFD	0.84±0.01**	0.87±0.01*	0.86±0.03***	0.83±0.01	0.84±0.02***

Food intake was calculated for two cages with four mice per cage for each strain and diet group. Whole-body energy expenditure was analysed with analysis of covariance using [lean mass+(0.2×fat mass)] as a covariate

LFD vs HFD: *$p<0.05$, **$p<0.01$ and ***$p<0.001$, n=6–8 for each strain and diet group

BAT, brown adipose tissue; eWAT, epididymal white adipose tissue; iWAT, inguinal white adipose tissue

TAG or DAG in their livers compared with LFD-fed controls, and also displayed a 25% reduction in ceramide levels (Fig. 2).

Markers of mitochondrial oxidative metabolism To determine if differences in glucose homeostasis and lipid accumulation across the different strains were related to alterations in mitochondrial fuel use, we examined several markers of mitochondrial function and lipid metabolism in muscle and liver (Figs 3 and 4, respectively). In muscle, there was a significant increase in protein content of peroxisome proliferator-activated receptor γ coactivator-1α (PGC1α), uncoupling protein 3 (UCP3), pyruvate dehydrogenase lipoamide kinase isozyme 4 (PDK4) and subunits of the complexes of the electron-transport chain (ETC) in all mouse strains on the HFD, except BALB/c (Fig. 3a). In

Table 2 Plasma characteristics

Characteristic	Diet type	C57Bl/6	129X1	BALB/c	DBA/2	FVB/N
Glucose (mmol/l)	LFD	9.14±0.36	6.59±0.28	7.96±0.34	8.21±0.36	9.67±0.36
	HFD	10.51±0.29**	7.31±0.25**	7.74±0.16	9.90±0.50*	10.95±0.43*
Insulin (pmol/l)	LFD	124.0±6.9	93.0±8.6	108.5±10.3	227.3±18.9	154.9±8.6
	HFD	216.9±24.1**	185.9±15.5***	110.2±8.6	414.9±55.1**	204.9±13.8*
Adiponectin (mg/l)	LFD	3.84±0.36	4.17±0.36	3.99±0.47	10.24±1.00	7.59±1.02
	HFD	3.59±0.50	4.62±0.48	3.97±0.35	9.45±0.54	7.48±0.42
TAG (mmol/l)	LFD	4.39±0.34	5.84±0.17	6.13±0.44	10.76±0.82	10.14±0.92
	HFD	4.52±0.27	7.22±0.40**	6.82±0.42	13.20±1.03	6.57±0.70*
NEFA (mmol/l)	LFD	0.76±0.05	0.87±0.05	0.79±0.05	0.97±0.10	0.71±0.04
	HFD	0.79±0.02	0.85±0.08	0.85±0.06	1.01±0.08	0.66±0.04

LFD vs HFD: *$p<0.05$, **$p<0.01$ and ***$p<0.001$, n=8 for each strain and diet group

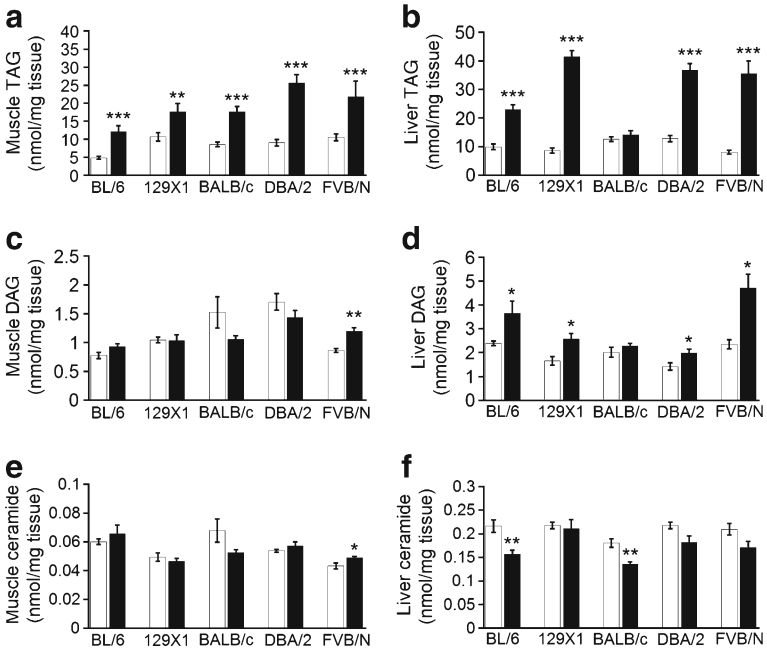

Fig. 2 Muscle and liver TAG (**a**, **b**), DAG (**c**, **d**) and ceramide (**e**, **f**) levels in LFD-fed (white bars) and HFD-fed (black bars) mice. *$p<0.05$, **$p<0.01$, ***$p<0.001$; $n=6$–8 for each strain and diet group

BALB/c muscle, mitochondrial proteins were either unchanged or decreased in the HFD-fed animals. A similar trend was observed with citrate synthase (CS) and β-hydroxyacyl-CoA dehydrogenase (βHAD) activity in skeletal muscle, with BALB/c being the only strain that showed no increase in enzyme activity on the HFD (Fig. 3b, c). Furthermore, to determine the effect of HFD on fatty acid oxidative capacity, we measured the rate of palmitate oxidation. Skeletal muscle from fat-fed mice of all strains, including BALB/c, displayed an increased capacity for palmitate oxidation (ESM Fig. 2). Although there was some increase in fatty acid oxidation in BALB/c muscle, the collective findings suggest that an HFD in BL6, 129X1, DBA/2 and FVB/N mice leads to stimulation of mitochondrial oxidative pathways, whereas HFD-fed BALB/c mice show less change in the same muscle variables.

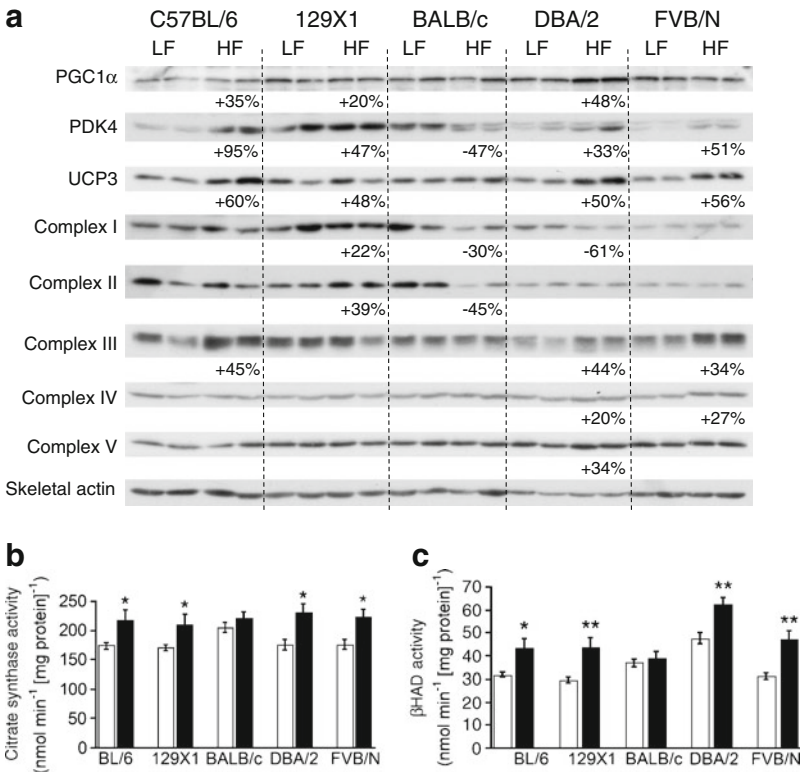

Fig. 3 Markers of mitochondrial oxidative metabolism in skeletal muscle. (**a**) Representative immunoblotting results on muscle oxidative proteins in low-fat (LF) and high-fat (HF) fed mice. Shown is $n=2$, but percentage differences underneath the corresponding lanes represent $n=6$–8 for each strain and diet group. Only significant ($p<0.05$) differences between the diet groups are shown. Complex I–V represent subunits of the complexes of the ETC. Skeletal actin in muscle was used as loading control and shows similar distribution in LF and HF groups in each strain. (**b**) Citrate synthase and (**c**) βHAD activity in LFD-fed (white bars) and HFD-fed (black bars) mice. *$p<0.05$, **$p<0.01$; $n=6$–8 for each strain and diet group

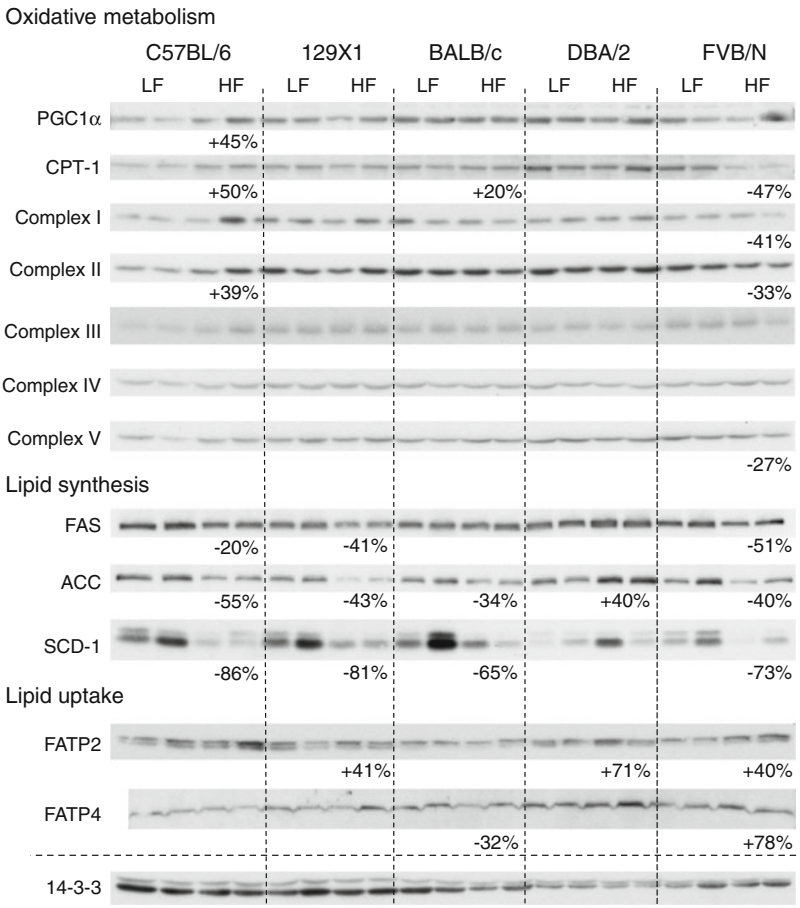

Fig. 4 Markers of oxidative metabolism, lipid synthesis and lipid uptake in the liver. Representative immunoblotting results on oxidative and lipogenic enzymes and fatty acid transporters in low-fat (LF) and high-fat (HF) fed mice. Shown is $n=2$, but percentage differences underneath the corresponding lanes represent $n=6–8$ for each strain and diet group. Only significant ($p<0.05$) differences between the diet groups are shown. 14-3-3 was used as loading control and shows similar distribution between LF and HF groups in each strain

In the liver, CS and βHAD activity was significantly increased in 129X1, DBA/2 and FVB/N mice, but not in BL6 and BALB/c mice (ESM Fig. 3). Western blot analysis in the liver showed a significant increase in PGC1α, carnitine palmitoyltransferase 1(CPT-1) and complex II of the ETC in HFD-fed BL6 mice, a decrease in several mitochondrial markers in FVB/N mice, and no change in any other strain (Fig. 4). Furthermore, the palmitate oxidation rate was unchanged in all mouse strains on the HFD (ESM Fig. 2). These findings suggest that the absence of lipid accumulation in liver of HFD-fed BALB/c mice is not due to an increase in mitochondrial lipid use.

Lipid synthesis and uptake in the liver Because BALB/c mice did not accumulate liver lipid on an HFD or exhibit increased mitochondrial oxidative capacity, we examined the protein levels of several markers of lipogenesis and fatty acid uptake. The protein content of fatty acid synthase (FAS), acetyl-CoA carboxylase (ACC) and stearoyl-CoA desaturase 1 (SCD-1) in liver was decreased in all strains, except DBA/2, which showed unchanged FAS and SCD-1 levels and increased ACC protein on the HFD (Fig. 4). HFD-fed BALB/c mice showed a similar reduction in lipogenic enzymes to other mouse strains, suggesting that a decrease in lipogenesis was unlikely to be the reason for the lower liver lipid content in BALB/c mice.

In addition, we measured protein levels of the major hepatic fatty acid transport proteins (FATPs) 2 and 4 as markers of fatty acid uptake capacity. HFD-fed BALB/c and BL6 mice were the only strains that did not show a consistent increase in liver FATP in comparison with their LFD-fed counterparts. In HFD-fed BALB/c mice, protein levels of FATP2 remained unchanged, whereas FATP4 was significantly reduced compared with the LFD-fed mice (Fig. 4). These results suggest that lipid accumulation in BALB/c liver on an HFD may, in part, be explained by a reduced capacity for fatty acid uptake.

Diet-induced changes in inflammation in adipose tissue The reduced insulin action associated with diet-induced obesity has often been linked to increased inflammation in adipose and other tissues [32]. To determine if differences in glucose tolerance and insulin action in the mouse strains were associated with macrophage infiltration in adipose tissue, we examined the gene expression of *F4/80* (also known as *Emr1*), *Cd68* and *Cd11c* (also known as *Itgax*), which are surface markers of M1 and M2 macrophages, as well as *Tnfa*, *mcp1* and *Il6* as markers of cytokine production by

macrophages and adipocytes. *F4/80*, *Cd68* and *Cd11c* mRNA increased in adipose tissue of all HFD-fed mouse strains (Fig. 5), indicating that leucocyte and macrophage infiltration into adipose tissue is already present after 8 weeks of HFD feeding in all strains, including BALB/c mice (Fig. 5a–c). Interestingly, increased *Tnfa*, *mcp1* and *Il6* mRNA levels were only present in BALB/c, DBA/2 and FVB/N mice, demonstrating that not all strains respond to increased macrophage infiltration with increased cytokine expression after 8 weeks on the HFD (Fig. 5d–f). Together, the gene expression analysis does not support reduced adipose tissue inflammation as a major reason for the different glucose handling observed in HFD-fed BALB/c mice. In addition, we measured protein levels of c-Jun N-terminal kinase, inhibitors of NF-κB kinases α and β, and IκB (total and phosphorylated for all except IκB) as markers of inflammation in the livers of LFD- and HFD-fed mice and observed no major changes in the different strains after 8 weeks of high-fat feeding (data not shown).

Markers of oxidative stress To determine if lipid accumulation in muscle and liver correlates with oxidative damage to lipids and to examine if lower oxidative stress levels could partly explain the differences observed in BALB/c mice, we measured LOOH and TBARS. In muscle, LOOH and TBARS levels were significantly decreased in HFD-fed BALB/c mice, whereas there was a trend to increased oxidative damage in the other strains (Fig. 6a, b). In the liver, however, HFD-fed BALB/c mice were the only strain that displayed increased lipo-oxidative damage compared with LFD-fed counterparts (Fig. 6c, d). In addition to lower LOOH and TBARS levels in muscle of HFD-fed BALB/c mice, protein carbonylation and glutathione peroxidase activity were also significantly decreased (ESM Fig. 4), suggesting decreased oxidative stress in muscle of HFD-fed BALB/c mice, but not in the other strains. Low oxidative stress in muscle, but not in the liver, may potentially contribute to the preserved glucose tolerance and insulin action observed in BALB/c mice fed the HFD.

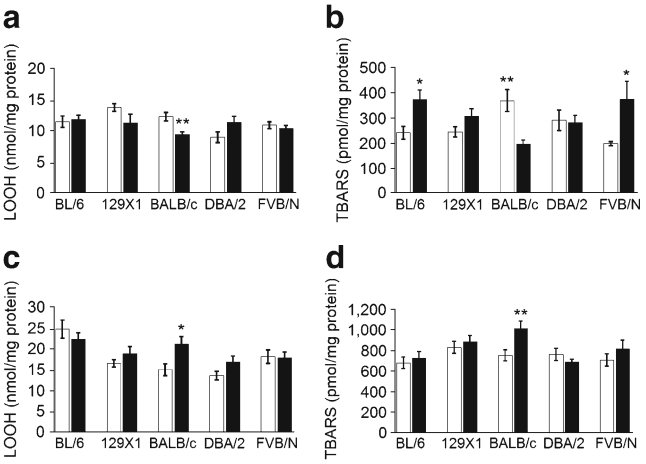

Fig. 6 Markers of lipid peroxidative damage in muscle (**a**, **b**) and liver (**c**, **d**). LOOH and TBARS were measured in LFD-fed (white bars) and HFD-fed (black bars) mice as markers of oxidative stress. *$p<0.05$, **$p<0.01$; $n=6$–8 for each strain and diet group

Discussion

The data obtained in this study gives a clear indication of the similarities and differences in a number of metabolic variables in five commonly used mouse strains in response to 8 weeks of high-fat feeding. On an HFD, all strains of mice gained a significant amount of body fat. All strains, except BALB/c, also exhibited a significant but variable deterioration in glucose tolerance. Intriguingly, BALB/c mice maintained normal glucose tolerance despite increased adiposity, increased muscle TAG accumulation, oxidative stress in liver, and elevated levels of adipose tissue inflammation.

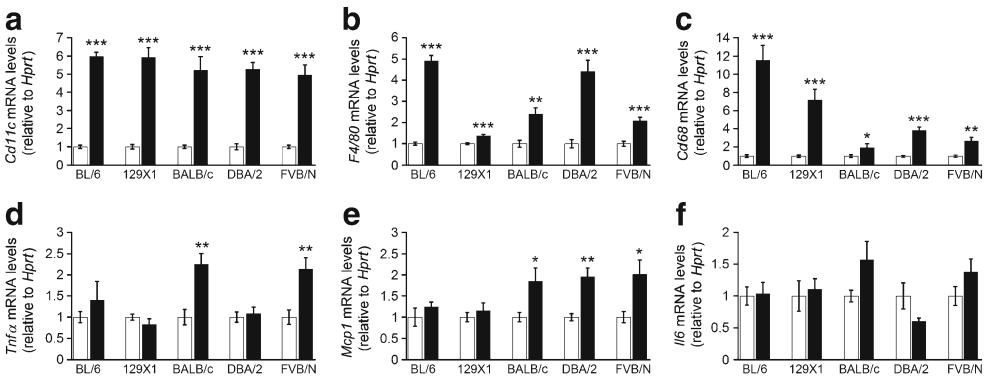

Fig. 5 Markers of inflammation in adipose tissue. Gene expression analysis of (**a**) *Cd11c*, (**b**) *F4/80*, (**c**) *Cd68*, (**d**) *Tnfa*, (**e**) *mcp1* and (**f**) *Il6* in epididymal adipose tissue of LFD-fed (white bars) and HFD-fed (black bars) mice. Results are expressed as fold change in the HFD-fed mice vs the LFD-fed mice for each strain. *$p<0.05$, **$p<0.01$, ***$p<0.001$; $n=5$ for each strain and diet group. HPRT, hypoxanthine–guanine phosphoribosyltransferase

This is somewhat surprising because increases in many of these variables have been suggested as key abnormalities predisposing to insulin resistance [32–34]. The most obvious difference in BALB/c mice that might explain their ability to maintain glucose tolerance and insulin action on an HFD was the lack of hepatic lipid accumulation. All other strains displayed substantial accumulation of TAGs and DAGs in liver on the HFD and significant deterioration in glucose intolerance and insulin action.

Lipid accumulation in non-adipose tissues is closely related to the development of glucose intolerance and insulin resistance [16, 35]. BALB/c mice displayed a similar pattern of lipid accumulation in muscle to the other strains. Thus, excess muscle lipid deposition could not explain variations in glucose homeostasis across the different strains in the present investigation. Interestingly, fat-fed BALB/c mice did display a disparate lipid profile to the other strains in the liver, with no excess TAG or DAG accumulation, and reduced ceramide content. This difference in liver lipid accumulation may partly explain the maintained glucose tolerance and insulin action in the HFD-fed BALB/c mice and is potentially due to lower rates of fatty acid uptake in BALB/c liver. In BALB/c mice, the levels of FATP2 and FATP4, which are the most abundant fatty acid transporters in liver [36], remained either unchanged (FATP2) or were decreased (FATP4), whereas in most other strains these transporters increased when fed an HFD. The BALB/c mice phenotype of increased fat mass but normal glucose tolerance and insulin action seems similar to the recently described metabolically healthy obese (MHO) humans. MHO humans, despite having severe adiposity, remain relatively insulin sensitive, as indicated by HOMA-IR and euglycaemic–hyperinsulinaemic clamps [37]. Interestingly, one of the observed metabolic differences between MHO and obese insulin-resistant humans was liver lipid content, with the MHO individuals displaying low liver lipid accumulation, despite visceral adiposity [38]. Obesity in fat-fed BALB/c mice might provide an opportunity to further investigate the links between hepatic lipid metabolism and insulin action.

Each of the five mouse strains exhibited defined but different physiological responses to an HFD. This difference in metabolic response was clearly apparent on comparison of the GTT curves. Although HFD-fed BL6, 129X1, DBA2 and FVB/N mice all became glucose intolerant, the response of each strain to a glucose bolus differed substantially, on both LFD and HFD. For example, FVB/N mice on a standard LFD are relatively glucose intolerant in comparison with other strains, and have similar glucose tolerance to BL6 mice on an HFD. BL6 mice are the most common mouse strain used in the study of metabolic disease, as they have been suggested to be the most susceptible to the development of diet-induced obesity and insulin resistance [9–11, 39, 40]. In the present investigation, BL6 mice displayed intermediate adiposity, insulin resistance and lipid accumulation in muscle and liver when compared with the other four mouse strains investigated. The strain that accumulated the most fat (37% of total body weight) and exhibited the greatest glucose intolerance on an HFD (threefold increase in AUC) was the 129X1 strain. A large number of genetically manipulated mice are produced on the 129X1 background and then backcrossed to BL6 for varying generations. The clear difference in glucose tolerance and other metabolic variables between BL6 and 129X1 suggests it is extremely important to know the extent of a mixed background in genetically manipulated mice before drawing conclusions about the influence of a specific gene on metabolic homeostasis.

DBA/2 mice also displayed an interesting response to the HFD. Although they showed the largest increase in body weight and also gained substantial amounts of fat mass, they became only marginally glucose intolerant on the HFD, as reported previously [1]. They exhibited similar differences in lipid accumulation, mitochondrial metabolism, inflammation and oxidative stress to other strains. However, they did exhibit a large increase in fasting insulin, suggesting that compensatory hyperinsulinaemia reduces HFD-induced glucose intolerance in this strain [1, 4].

Mitochondrial dysfunction, oxidative stress and inflammation are commonly suggested as mediators of insulin resistance and glucose intolerance in obese animals and humans [16, 18, 41]. In the present study, all strains (except BALB/c) responded to the HFD in the same way as BL6 mice did in our previous study [19], with a modest increase in the expression and activity of mitochondrial proteins, and an increase in their capacity to oxidise fat. BALB/c was the only mouse strain in which markers of mitochondrial oxidative capacity remained unchanged (enzyme activities) or were even decreased (protein content) in HFD-fed animals, and glucose tolerance and insulin action were preserved in this strain. These results suggest that the HFD-induced increases in muscle mitochondrial capacity are not associated with improved glucose tolerance and insulin action in these mouse strains. Oxidative stress markers were assessed in liver and muscle of all strains. The only consistent effect was that HFD increased markers of lipid peroxidation in liver of BALB/c mice, but decreased these markers in muscle of the same animals, which again is difficult to reconcile with the observation that BALB/c mice maintain normal glucose tolerance and insulin action on an HFD. Investigation of the inflammatory state of adipose tissue in the different strains of HFD-fed mice indicated substantial evidence of macrophage infiltration (increased *F4/80* and *Cd11c* gene expression) in all strains and evidence of increased cytokine expression (*Tnfa* and

mcp1) in three of the five strains including BALB/c. These results demonstrate that adipose tissue inflammation can be observed within 8 weeks of starting an HFD, even in the presence of normal glucose tolerance and insulin action in BALB/c mice, and thus support the notion that inflammation may only contribute to glucose intolerance and insulin resistance after long-term high-fat feeding [42, 43]. It should be noted that this study examined specific biomarkers that have often been suggested as important factors influencing the development of metabolic disease. However, the measurement of individual markers within pathways may not necessarily describe how these pathways respond as a system, with compensatory changes after high-fat feeding possibly differing between mouse strains.

The susceptibility of different mouse strains to diet-induced obesity and insulin resistance will be partly related to genetic difference in variables linked to energy balance and glucose homeostasis [7, 44, 45, www.phenome.jax.org]. In addition, the response of different mouse strains will be highly dependent on feeding duration, diet composition and housing conditions. Variations in these experimental parameters may explain why previous studies have characterised the 129 and DBA/2 strains as either prone to obesity and insulin resistance, as shown in our comparison [1, 7], or relatively resistant to the effects accompanying an HFD [1, 7, 46]. Similarly, BALB/c mice have been previously reported as being either prone [47] or resistant [48] to diet-induced hepatic lipid accumulation. The strength of the present study is that all strains of mice were studied at the same time, under the same experimental conditions. We do, however, acknowledge that this comparison was carried out with male mice only and there may be sex differences between strains [49].

Altogether, this extensive comparison of the effects of an HFD on different inbred mouse strains demonstrates that all strains accumulate body fat and show signs of adipose tissue inflammation and muscle lipid accumulation. However, the effect of fat accumulation on markers of glucose homeostasis is variable and strain-dependent. In particular, BALB/c mice display preserved glucose tolerance and insulin action on an HFD, and this seems to be directly related to the lack of accumulation of fat in the liver of these mice, despite lipid accumulation in muscle. The results suggest that liver lipid content is a major determinant of glucose tolerance, and highlight the need for caution when comparing results of dietary interventions in studies involving different or mixed strains of mice.

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Contribution statement MKM designed and performed experiments, analysed and interpreted data, and wrote the manuscript. NLH performed experiments, interpreted data and drafted the manuscript. SHB performed experiments, interpreted data and drafted the manuscript. ML performed experiments, interpreted data and drafted the manuscript. TWM performed experiments, interpreted data and drafted the manuscript. GJC performed experiments, interpreted data and drafted the manuscript. NT conceived the study, analysed and interpreted data, and wrote the manuscript. All the authors gave their final approval for submission of the manuscript.

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