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Variable cartilage degradation in mice with diet-induced metabolic dysfunction: food for thought

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SUMMARY

Objective: Human cohort studies have demonstrated a role for systemic metabolic dysfunction in osteoarthritis (OA) pathogenesis in obese patients. To explore the mechanisms underlying this metabolic phenotype of OA, we examined cartilage degradation in the knees of mice from different genetic backgrounds in which a metabolic phenotype was established by various dietary approaches.
Design: Wild-type C57BL/6J mice and genetically modified mice (hCRP, LDLr$^{-/-}$. Leiden and ApoE*3-Leiden.CETP mice) based on C57BL/6J background were used to investigate the contribution of inflammation and altered lipoprotein handling on diet-induced cartilage degradation. High-caloric diets of different macronutrient composition (i.e., high-carbohydrate or high-fat) were given in regimens of varying duration to induce a metabolic phenotype with aggravated cartilage degradation relative to controls.
Results: Metabolic phenotypes were confirmed in all studies as mice developed obesity, hypercholesteremia, glucose intolerance and/or insulin resistance. Aggravated cartilage degradation was only observed in two out of the twelve experimental setups, specifically in long-term studies in male hCRP and female ApoE*3Leiden.CETP mice. C57BL/6J and LDLr$^{-/-}$. Leiden mice did not develop HFD-induced OA under the conditions studied. Osteophyte formation and synovitis scores showed variable results between studies, but also between strains and gender.
Conclusions: Long-term feeding of high-caloric diets consistently induced a metabolic phenotype in various C57BL/6J (-based) mouse strains. In contrast, the induction of articular cartilage degradation proved variable, which suggests that an additional trigger might be necessary to accelerate diet-induced OA progression. Gender and genetic modifications that result in a humanized pro-inflammatory state (human CRP) or lipoprotein metabolism (human-E3L.CETP) were identified as important contributing factors.

Introduction

Osteoarthritis (OA) is a progressive joint disease that is characterised by focal loss of articular cartilage, which impedes smooth joint movement and causes stiffness and pain. The most important risk factors for OA are age, gender and obesity. The latter being of specific interest in developed countries, where prolonged life expectancy and a progressive sedentary lifestyle in combination with a high caloric diet is predicted to exponentially increase the number of obese individuals and hence the prevalence of OA[1]. The most common subtype of OA in obese individuals is metabolically induced OA[2], here referred to as 'metabolic OA'.

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The association between knee OA and obesity has been comprehensively studied in humans. Weight loss was found to significantly reduce pain and increase mobility in knee OA patients[3] and reduced the risk of onset of the disease[4]. In obese adults, weight loss combined with exercise appears to be the most promising treatment and is therefore recommended by several international guidelines on the management of metabolic OA[5,6]. Moreover, overweight was found to be associated with hand OA as well, indicating a possible underlying systemic factor in disease pathogenesis[7]. The involvement of both metabolic and systemic aspects was demonstrated in cohort studies, where knee OA development did not depend on weight or BMI but was strongly related to concurrent dysregulation of glucose and lipid metabolism[8].

Even though the association between OA and obesity is evident, the underlying mechanisms have yet to be resolved. Animal models can help fill this knowledge gap. Combining all observations in humans, a translational animal model for metabolic OA should display obesity in concurrence with metabolic dysfunction. Various species have been used to examine metabolic OA, all relying on diet-induced obesity, with the mouse being the most comprehensively studied. Silberberg *et al.* laid the foundation of the current diet-induced metabolic OA mouse model in the 1950s by linking a high-fat diet with accelerated OA onset and progression[9]. Literature on metabolic OA models has been scarce after this promising start[10], with the majority of reports dating from the past decade.

It can be ascertained from literature that there is no consensus on the best species or the optimal study design for *in vivo* metabolic OA models. The animal model most thoroughly examined in the context of metabolic OA is the C57BL/6J mouse strain, or genetic variants based on this background, as the readily obtained obesity observed in C57BL/6J mice closely parallels the metabolic adaptations seen in human disease pathogenesis[11]. Most researchers have employed the very high-fat diet (VHFD), a high-fat diet with a supraphysiological fat content of 60 kcal% energy from fat, to induce metabolic OA in male mice – occasionally combined with an increased mechanical burden through surgery or exercise[12,13]. In contrast, published experimental designs vary greatly in study duration and age at start, which are important determinants of the capacity for metabolic adaptation and ultimately of the severity of metabolic OA. The methodology used for determination of OA severity presents another striking difference between publications. Despite international initiatives for a common scoring system, many groups employ their own scoring system or use one of many adaptations to the Mankin scoring system[14]. Taken together, although in humans the link between obesity and OA is confirmed and appropriate animal models for both diseases are available, research on metabolic OA in animal models is still in its infancy.

Here we aim to explore the suitability of the mouse as a preclinical model for metabolic OA and to define important contributing factors to disease development. To this end, we examined OA features in the knees of mice that received various high-caloric dietary regimens at variable duration. All evaluated mouse strains were C57BL/6J or based on this background, bearing genetic modifications that humanize the strains to increase translatability to the human situation. High-caloric diets ranged in fat content from more physiological to supraphysiological, with a focus on the former for translatability purposes.

Methods

A detailed methods section is available in the online supplemental file.

Mice and diets

Twelve experiments were performed in which a high-caloric diet was used to induce overweight and metabolic dysfunction in wild-type C57BL/6J mice and genetically modified mice (hCRP, LDLr$^{-/-}$. Leiden and ApoE*3Leiden.CETP mice) based on a C57BL/6J background. The high-caloric diets applied in our studies differed in macronutrient composition (Table I).

Evaluated studies

The experiments were designed to examine various diet-induced metabolic disorders and therefore differed in original research question and design (Table II).

Analysis of metabolic dysfunction and osteoarthritis

Metabolic dysfunction is defined as a significant increase in either body weight and fasted plasma cholesterol, glucose and/or insulin levels compared with normal values as observed in chow- or low fat diet (LFD, 10 kcal% energy from fat)-fed controls.

Articular cartilage degradation, osteophyte formation and synovitis were scored on coronal 5 μm knee joint sections, stained with Haematoxylin, Fast Green and Safranin-O, according to OARSI histopathology initiative recommendations for the mouse[15].

Statistical analysis

Statistical analysis was performed using IBM SPSS software (v23.0, IBM SPSS Inc., Chicago, IL, USA) or GraphPad Prism (v7.01, GraphPad, San Diego, USA). Data analysis revealed that the assumptions of normality and homoscedasticity could not be satisfied in any of our studies. Therefore, depending on study design, non-parametric Mann-Whitney *U*-test for comparison of two groups or Kruskal–Wallis test for >2 groups was used for both the metabolic parameters and histopathological scores. A probability value < 0.05 was considered statistically significant. Data are presented as median with interquartile ranges (IQR). For the OA scores, mean ± standard deviations (SD) are reported as well.

Results

Variability in diet-induced osteoarthritis severity

Ten out of the twelve diet-induced metabolic dysfunction approaches did not result in an aggravated development of OA compared with matched controls (Table III; representative images in Fig. 1). The higher OA severity scores in these studies are accompanied by relatively large SD, indicating large biological variation among mice (see also online Supplemental Fig. 1).

Severity scores demonstrated clear mouse-strain-dependent effects: in our hands, C57BL/6J mice on a high-fat diet (HFD, 45 kcal% energy from fat) regimen up to 52 weeks did not develop aggravated cartilage degradation [Fig. 1(A)]. Severity scores did increase over time, but were comparable to chow and LFD controls at each time point.

The human C-reactive protein (hCRP) knock-in mouse strain (Study 4) had previously shown diet-induced aggravation of OA[16]. Mice of both genders received either chow or HFD for 38 weeks. At endpoint, HFD-fed male hCRP mice had developed significantly more OA compared with chow controls [Fig. 1(B)]. In female hCRP mice HFD feeding had no effect on OA severity, which was comparable to males on chow.

LDLr$^{-/-}$. Leiden mice predominantly received lard-based synthetic diets, for a period of 20–31 weeks. In studies 6 and 7 the diets

Table I
Main composition of the experimental diets without any supplementations

		Type of diet§					
		Chow	LFD	WTD	MFD	HFD	VHFD
	Supplier	Ssniff GmbH	Research Diets, Inc	ABdiets	Research Diets, Inc	Research Diets, Inc	Research Diets, Inc
	Catalogue number	V1534	D12450B	4021.04	D03101604	D12451	D12492
	Used in Study	1, 2, 4, 8, 11, 12	3, 5	12	5	1-8*,†	9–11
Energy source (kcal%)	Diet components (g/kg)						
Fat		**9**	**10**	**16**	**30**	**45**	**60**
	Crude fat	33	–	–	–	–	–
	Lard	–	19.0	–	116.7	206.8	316.6
	Cacao butter	–	–	150.0	–	–	–
	Corn oil	–	–	10.0	–	–	–
	Soybean oil	–	23.7	–	26.5	29.1	32.3
	Cholesterol‡	–	0.05	0.07	0.08	0.20	0.30
Protein		**33**	**20**	**20**	**20**	**20**	**20**
	Crude protein	190	–	–	–	–	–
	Casein	–	189.6	200.0	212.2	233.1	258.4
	L-Cystine	–	2.8	0.5	3.2	3.5	3.9
Carbohydrate		**58**	**70**	**56**	**50**	**35**	**20**
	Corn starch	365	298.6	100.0	226.8	84.8	0
	Sucrose	–	331.7	405.0	264.0	201.4	88.9
	Sugar	47	–	–	–	–	–
	Maltodextrin 10	–	33.2	–	37.1	116.5	161.5
Fibre							
	Crude fibre	49	–	–	–	–	–
	Cellulose	–	47.4	62.0	53.0	58.3	64.6

* Study 4: the HFD was supplied via BioServices, which resembles the HFD from Research Diets, Inc.
† Study 6: D12451 was modified by the supplier for the intervention groups: soybean oil was changed into corn oil and 43.5 gm% of lard was replaced by either of the oils of interest (to a total of 15 gm% lard and 9 gm% oil).
‡ Natural cholesterol content of the basic diet, additional cholesterol supplementation is specified in Table II.
§ LFD, low-fat diet; WTD, Western-type diet; MFD, mid-fat diet; HFD, high-fat diet; VHFD, very high-fat diet.

were adjusted by replacing dietary lard partly with specific oils or by supplementing the diet with fructose or cholesterol, respectively. Although an overall reduction in proteoglycan content was noticeable [Fig. 1(C)], none of these dietary approaches aggravated OA development compared with chow- or LFD-fed mice. No diet-induced cartilage degradation was observed in LDLr$^{-/-}$. Leiden mice up to 31 weeks.

Male ApoE*3Leiden.CETP mice displayed high OA severity scores in general, independent of diet [Fig. 1(E)]. Compartmental sub-scores demonstrated a proportional distribution across all compartments, showing no preference for the medial or lateral side (see online Supplemental Table S1). Female ApoE*3Leiden.CETP mice demonstrated less joint damage in the chow control compared with males and an accelerated OA development in the 0.3% but not 0.1% cholesterol-supplemented group [Study 12, Fig. 1(D)].

Presence of metabolic dysfunction

To monitor the development and extent of the diet-induced metabolic dysfunction, time-dependent effects of all dietary interventions on body weight and fasted plasma levels of cholesterol, glucose and insulin were regularly measured over the course of each study (Figs. 2 and 3). Obesity was established by all high-fat diet regimens, in combination with at least one of the following comorbidities: hypercholesterolemia, glucose intolerance or insulin resistance. ApoE*3Leiden.CETP mice fed a WTD (Study 12) remained lean. Nonetheless, metabolic dysfunction in the form of hypercholesteremia was clearly observed in these mice, especially in females receiving additional 0.3% dietary cholesterol.

C57BL/6J mice (Study 1–2) rapidly responded to HFD and became obese within 12 weeks compared with age-matched chow controls. Cholesterol levels gradually increased up to three-fold compared with chow-fed controls over the course of 12 weeks and remained at this level until the end of the study. While blood glucose of HFD-fed mice remained at a constant high level, insulin levels continued to increase, indicative of insulin resistance. In Study 1, development of insulin resistance and glucose intolerance in HFD-fed mice was confirmed by insulin and glucose tolerance tests at 24 weeks (data not shown). Study 3 showed that this insulin sensitivity was attenuated upon prolonged HFD feeding, as the HFD group initially showed a similar increase in insulin levels but then recuperation to LFD-fed control levels as of week 40[17]. Also, HFD-fed mice steadily increased their body weight during the first 40 weeks, after which body weight stabilised until the end of the study. Cholesterol levels remained significantly elevated compared with control during the entire study period.

hCRP transgenic mice (Study 4) reached obesity after 18 weeks on a HFD, with modest to no changes in glucose and insulin levels up to 10 weeks. At week 36, a glucose tolerance test showed a delayed clearance of glucose in HFD-mice of both sexes compared with their chow controls (data not shown), indicative of glucose intolerance. Males exhibited higher absolute levels of glucose and insulin than females.

In LDLr$^{-/-}$. Leiden mice (Study 5), the fat percentage in a synthetic diet positively associated with the observed increase in body weight, plasma glucose and insulin levels. Cholesterol levels changed significantly on a HFD but not on a mid-fat diet (MFD, 30 kcal% energy from fat) compared with LFD controls, suggesting that a synthetic diet needs to provide in more than 30 kcal% energy from fat to induce hypercholesterolemia. Modifications to the dietary fatty acid composition (Study 6) induced metabolic changes over a period of 20 weeks. Refined soybean oil lowered all metabolic parameters except plasma glucose. Palm oil greatly induced all measured plasma parameters, with additional increases in insulin levels upon feeding unrefined palm oil.

Fructose supplementation for 20 weeks (Study 7) slightly reduced body weight increase, marginally increased cholesterol levels, but drastically increased insulin levels compared with

Table II
Overview of the evaluated mouse studies ranked by mouse strain and study duration

	Study	Original design	Age at start (weeks)	Weeks on study diet	Mice/group	Diet intervention groups§	Gender‖
C57BL/6J							
	1	Early detection of type II diabetes and its complications	12	0	12	chow	M
				6	12	chow	
					12	HFD	
				12	12	chow	
					12	HFD	
				24	12	chow	
					12	HFD	
	2[59]	The role of adipose tissue inflammation in NAFLD	12	24	10	chow	M
					15	HFD	
	3[17]	Aging	12	52	10–15	LFD	M
						HFD	
hCRP							
	4[16]‡	Metabolic syndrome	10–14	38	10	chow	M
					10	HFD	
					10	chow	F
					10	HFD	
LDLr$^{-/-}$.Leiden							
	5	Diabetic nephropathy	8	20	10	LFD	M
					10	MFD	
					10	HFD	
	6	Type II diabetes	12	20	15	HFD	M
					15	HFD + refined soybean oil	
					15	HFD + unrefined soybean oil	
					15	HFD + refined palm oil	
					15	HFD + unrefined palm oil	
	7	Diabetic nephropathy	12	20	10	HFD	M
					10	HFD + fructose	
					10	HFD + 0,2% cholesterol	
					10	HFD + 1,0% cholesterol 20w	
				31	10	HFD + 1,0% cholesterol 31w	
	8	Metabolic syndrome	13–15	30	6	chow	M
					15	HFD	
ApoE*3Leiden.CETP							
	9	Metabolic syndrome	10–16	26*	10	VHFD + fructose	M
	10	Insulin resistance and dyslipidemia	10–12	32†	8	VHFD + fructose	M
	11	Osteoarthritis	15	32	12	chow	M
					12	VHFD	
					12	VHFD + chow	
					12	VHFD low-MetS	
	12[54]‡	Atherosclerosis and OA	8–12	38	12	Chow group	M
					12	WTD + 0,4% cholesterol	
					17	WTD + 1,0% cholesterol	
					12	Chow group	F
					12	WTD + 0,1% cholesterol	
					17	WTD + 0,3% cholesterol	

* Study 9: during the final 16 weeks 10% fructose was added to the drinking water.
† Study 10: during the final 24 weeks 10% fructose was added to the drinking water.
‡ Previously published OA data.
§ LFD, low-fat diet (10 kcal% energy from fat); WTD, Western-type diet (16 kcal% energy from fat); MFD, mid-fat diet (30 kcal% energy from fat); HFD, high-fat diet (45 kcal% energy from fat); VHFD, very high-fat diet (60 kcal% energy from fat); low-MetS, mice showing inexplicably low metabolic adaptation to the VHFD.
‖ M, male and F, female.

controls. Cholesterol supplementation dose-dependently increased plasma cholesterol levels compared with controls, changing the metabolic state towards a more hypercholesterolemic phenotype without insulin resistance. In Study 8 HFD-fed male LDLr$^{-/-}$. Leiden mice continuously gained weight, reaching markedly higher weights than chow controls. Cholesterol levels increased almost 4-fold over time compared with controls. Insulin levels varied over time, but were significantly higher compared with chow control mice as of week 6.

Studies performed in ApoE*3Leiden.CETP mice were of longer duration than aforementioned studies, the shortest being 26 weeks (Study 9, Table II). In both Studies 9 and 10, fructose was added to the drinking water on top of a very high-fat diet (VHFD, 60 kcal% energy from fat). On this regimen, male ApoE*3Leiden.CETP mice rapidly gained weight during the first 16 weeks and stabilized from then onwards. Fasted plasma cholesterol levels were tripled within 12 weeks compared with baseline values. Fructose treatment provoked a steep insulin increase in Study 9, though this was not as apparent in Study 10. Insulin and glucose tolerance tests at endpoint confirmed dysregulation of glucose metabolism in both studies (data not shown).

Male ApoE*3Leiden.CETP mice demonstrated similar increases in weight and cholesterol levels on a VHFD without fructose supplementation (Study 11). Cholesterol levels showed a 2.5-fold increase compared with chow controls at endpoint. When switched from VHFD to chow diet ('VHFD + chow'), body weight and cholesterol levels decreased to near chow control levels within 12 weeks. Low-MetS mice – ApoE*3Leiden.CETP mice with low metabolic adaptations to VHFD – indeed presented low body weight gain and cholesterol levels on a VHFD, albeit consistently elevated compared with chow controls.

Table III
Overview of knee OA severity scores from twelve independent mouse studies with various approaches for diet-induced metabolic dysfunction

	Study	Age at start (weeks)	Weeks on study diet	Diet intervention groups§	Gender‖	Total OA severity score	
						Median [IQR]	Mean ± SD
C57BL/6J							
	1	12	0	chow	M	1.25 [0.75–1.50]	1.31 ± 0.70
			6	chow		2.19 [1.34–2.46]	2.06 ± 1.05
				HFD		2.00 [1.13–3.29]	2.18 ± 1.07
			12	chow		3.25 [2.42–4.50]	4.53 ± 4.26
				HFD		2.82 [1.97–4.28]	3.25 ± 1.45
			24	chow		4.94 [3.94–5.72]	5.51 ± 3.22
				HFD		4.40 [3.60–5.43]	4.38 ± 1.29
	2[59]	12	24	chow	M	3.69 [3.29–5.60]	4.19 ± 1.37
				HFD		4.00 [3.50–5.00]	4.22 ± 1.15
	3[17]	12	52	LFD	M	4.69 [3.88–6.22]	4.12 ± 1.65
				HFD		5.88 [5.00–7.50]	5.20 ± 1.58
hCRP							
	4[16]‡	10–14	38	chow	M	4.83 [3.17–5.17]	4.45 ± 1.33
				HFD		6.33 [4.42–12.25]	7.81 ± 4.54
				chow	F	3.33 [3.25–4.75]	3.70 ± 0.86
				HFD		3.50 [2.50–4.75]	3.69 ± 1.20
LDLr$^{-/-}$ Leiden							
	5	8	20	LFD	M	3.50 [3.20–4.25]	3.55 ± 0.67
				MFD		4.00 [2.90–4.64]	3.70 ± 1.36
				HFD		3.75 [2.63–5.02]	3.80 ± 1.49
	6	12	20	HFD	M	3.00 [2.25–4.00]	3.43 ± 1.30
				HFD + refined soybean oil		3.50 [2.63–4.38]	3.83 ± 0.58
				HFD + unrefined soybean oil		3.75 [2.37–4.75]	2.78 ± 1.84
				HFD + refined palm oil		3.50 [2.75–4.00]	3.50 ± 0.87
				HFD + unrefined palm oil		3.25 [2.50–3.75]	2.88 ± 0.70
	7	12	20	HFD	M	3.29 [2.67–4.27]	3.45 ± 0.88
				HFD + fructose		3.67 [2.81–4.93]	4.22 ± 2.39
				HFD + 0.2% cholesterol		3.38 [2.77–3.81]	3.90 ± 2.05
				HFD + 1.0% cholesterol 20w		3.33 [2.42–3.49]	3.09 ± 0.99
			31	HFD + 1.0% cholesterol 31w		4.08 [2.89–4.48]	4.20 ± 1.71
	8	13–15	30	chow	M	3.50 [2.59–5.67]	4.06 ± 1.47
				HFD		4.75 [4.00–5.83]	6.17 ± 4.31
ApoE*3Leiden.CETP							
	9	10–16	**26***	VHFD + fructose	M	5.88 [4.38–7.94]	7.39 ± 5.21
	10	10–12	32†	VHFD + fructose	M	5.00 [4.00–14.75]	8.32 ± 5.23
	11	15	32	chow	M	6.38 [5.00–17.63]	8.81 ± 4.89
				VHFD		5.50 [3.63–8.50]	6.53 ± 2.20
				VHFD + chow		5.50 [4.25–8.50]	7.80 ± 3.63
				VHFD, low-MetS		7.63 [5.00–12.25]	9.97 ± 5.07
	12[54]‡	8–12	38	Chow group	M	7.82 [5.94–9.81]	8.26 ± 2.29
				WTD + 0.4% cholesterol		6.88 [5.19–7.63]	7.37 ± 3.48
				WTD + 1.0% cholesterol		7.75 [6.03–8.72]	8.45 ± 3.87
				Chow group	F	5.01 [4.69–6.04]	5.35 ± 1.16
				WTD + 0.1% cholesterol		6.13 [5.63–6.88]	5.98 ± 1.04
				WTD + 0.3% cholesterol		**6.69 [5.13–7.81]**	**6.81 ± 2.25**†

Total OA severity scores presented here are group medians with interquartile ranges and group averages with standard deviation of the averaged sum scores for the tibiofemoral knee compartments (max. 24, OARSI histopathology recommendations for the mouse[15]). Bold font indicates statistically significant changed OA severity compared with chow controls. Statistical significance level was set to $P < 0.05$.

* Study 9: during the final 16 weeks 10% fructose was added to the drinking water.

† Study 10: during the final 24 weeks 10% fructose was added to the drinking water.

‡ Previously published OA data.

§ LFD, low-fat diet (10 kcal% energy from fat); WTD, Western-type diet (16 kcal% energy from fat); MFD, mid-fat diet (30 kcal% energy from fat); HFD, high-fat diet (45 kcal% energy from fat); VHFD, very high-fat diet (60 kcal% energy from fat); low-MetS, mice showing inexplicably low metabolic adaptation to the VHFD.

‖ M, male and F, female.

Cholesterol supplementation continuously increased plasma cholesterol levels in both ApoE*3Leiden.CETP genders over time without effect on weight gain (Study 12). Females were more susceptible to dietary cholesterol than their male counterparts, showing plasma cholesterol increases of 7.5-fold and 4.5-fold compared with chow controls, respectively. Blood sugar regulation parameters were not available for the latter two studies.

Variability in induction of inflammatory OA features on HFD

To better understand how metabolic dysfunction impacts OA pathology, osteophyte formation and synovitis were additionally scored. A HFD is known to increase these inflammatory OA features in mice[18,19], which can be distinguished before changes in cartilage structure become visible[20]. In general, both features showed high variability in our studies (Figs. 4 and 5).

In wild-type C57BL/6J mice, synovitis seemed to increase due to HFD feeding from 12 to 24 weeks in Study 1, as compared with chow controls. Study 2 confirmed a significant increase in synovitis scores between these two diets at 24 weeks. This difference in synovial inflammation is not visible after 52 weeks of HFD feeding (Study 3), suggesting a return to LFD control levels. The presence of osteophytes increased over time in C57BL/6J mice, but HFD feeding only aggravated this process in Study 1.

Male hCRP mice from Study 4, demonstrating significantly increased OA severity, did not show corresponding significant

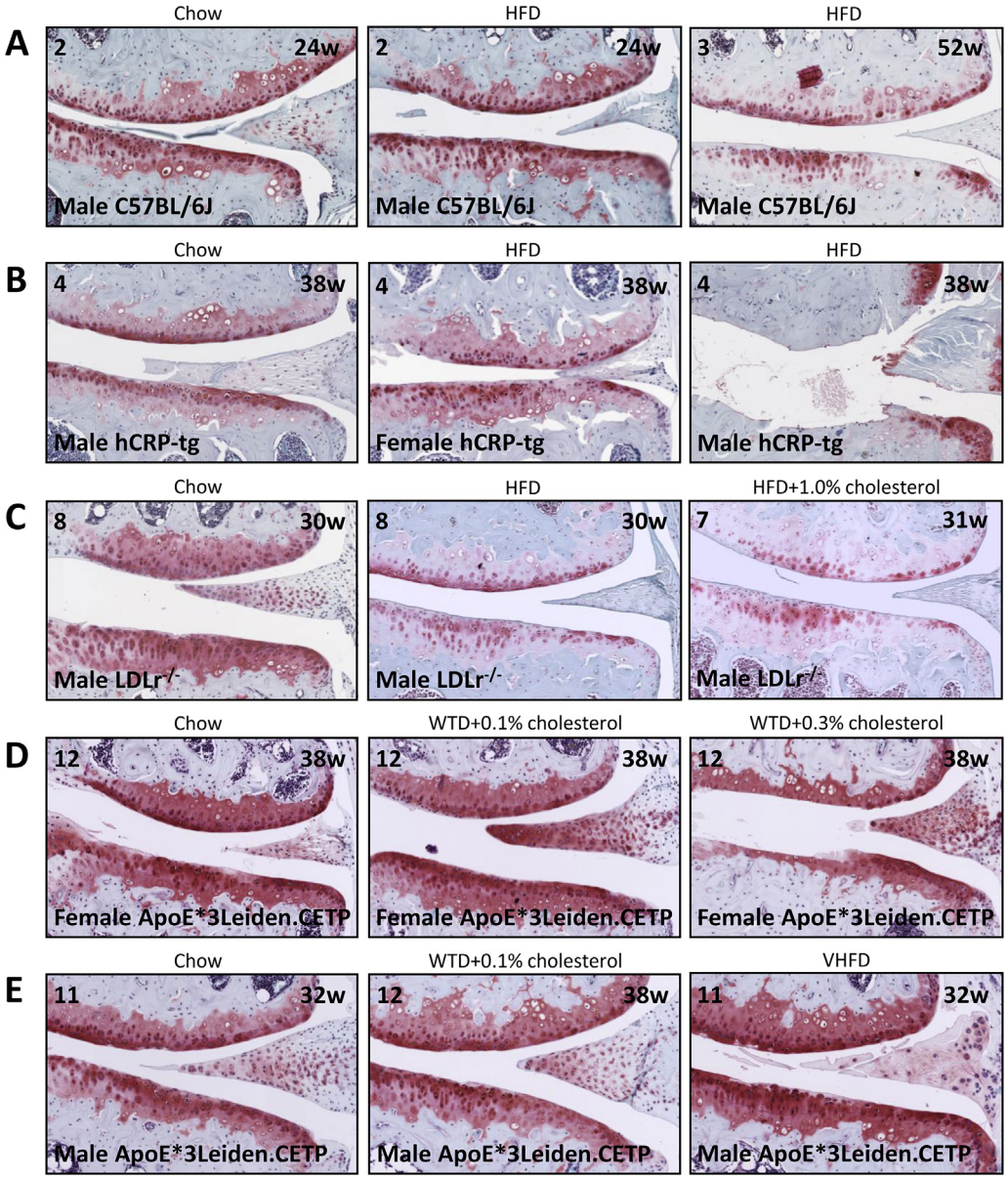

Fig. 1. Representative coronal sections of the medial tibiofemoral compartments, stained with Fast-Green/Safranin-O. Additional information for each image: study number in upper left corner, duration (weeks) in upper right corner, gender and strain in lower left corner. Magnification for all microphotographs was 40×. (A) HFD does not induce aggravated cartilage degradation at 24 or 52 weeks of feeding in male wild-type C57BL/6J mice. (B) Male hCRP transgenic mice developed aggravated cartilage degradation when fed a HFD compared with chow diet for 38 weeks, while female hCRP transgenic mice did not. (C) HFD without or with additional cholesterol did not accelerate cartilage degradation in male LDLr$^{-/-}$ mice compared to chow-fed controls. (D) Aggravated diet-induced cartilage degradation was observed in female ApoE*3Leiden.CETP mice fed a WTD with additional dietary cholesterol for 38 weeks, showing a cholesterol-dependent increase in severity. (E) Male ApoE*3Leiden.CETP mice showed surface fibrillation and loss of surface lamina, independent of the dietary interventions investigated.

increases in osteophyte formation and synovitis. Although the mice with the highest OA scores also showed the highest osteophyte and synovitis scores, this association was not applicable in general. Female hCRP mice on a HFD showed a non-significant trend ($P = 0.087$) towards an increase in synovitis score compared with chow controls, but did not differ from controls in osteophyte formation.

In LDLr$^{-/-}$ mice, additional scoring of osteophyte formation and synovitis provided a profounder picture of disease pathogenesis. Osteophyte formation increased significantly on a HFD compared with chow in Studies 5 and 8. Synovitis seemed to increase as well in these mice, with a significant difference observed between HFD- and chow-fed mice in Study 5. This difference was less clear in Study 8, where the HFD-fed mice showed an aberrant, lower synovitis scores compared to HFD-fed mice in Studies 5–7 – despite longer study duration.

Male ApoE*3Leiden.CETP mice, as with OA severity, showed overall high scores for both osteophyte formation and synovitis, independent of diet. Interestingly, female ApoE*3Leiden.CETP mice showed a discrepancy in these two features, with high synovitis scores – to the level of their male counterparts – but almost no osteophyte formation.

Discussion

We report on the variable induction of diet-induced articular cartilage degradation in twelve mouse experiments originally designed to examine various diet-induced metabolic disorders. The

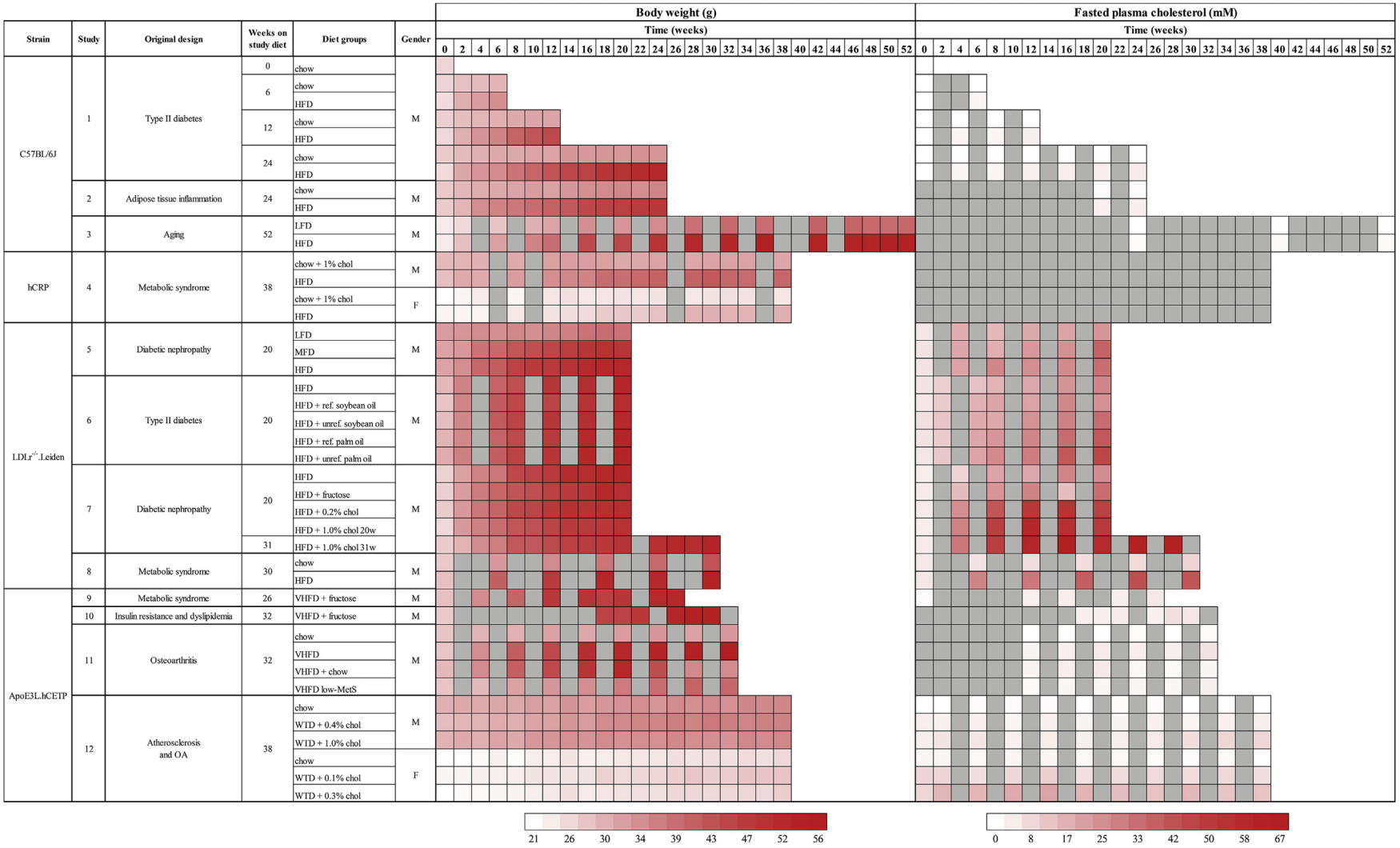

Fig. 2. Biweekly overview of the changes in body weight and total fasting cholesterol plasma levels for diet interventions of all studies investigated, ranked by mouse strain. Colour intensity matches the measured concentration for each parameter, as visualized by the scale at the bottom of each heat map. Grey indicates no data available. Supplemental Table 2 bears the actual values of the group medians with interquartile ranges (IQR) for selected time points.

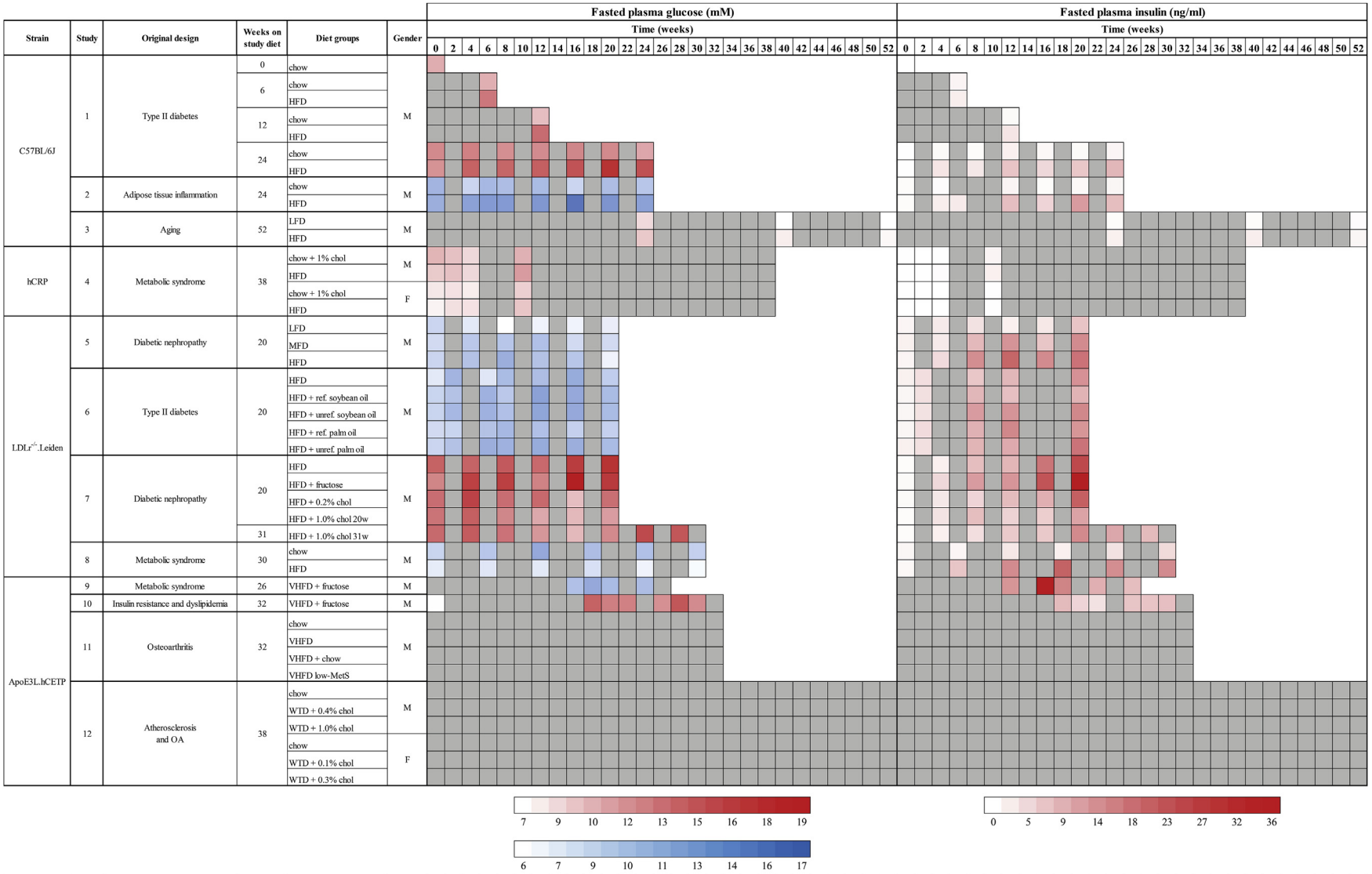

Fig. 3. Biweekly overview of the changes in fasting glucose and insulin plasma levels for diet interventions of all studies investigated, ranked by mouse strain. For the glucose heatmap, red indicates results that were immediately measured using a hand-held glucose analyser (blood glucose), while blue represents values measured by enzymatic assay at the end of the study (plasma glucose). Colour intensity matches the measured concentration for each parameter, as visualized by the scale at the bottom of each heat map. Grey indicates no data available. Supplemental Table 3 bears the actual values of the group medians with IQR for selected time points.

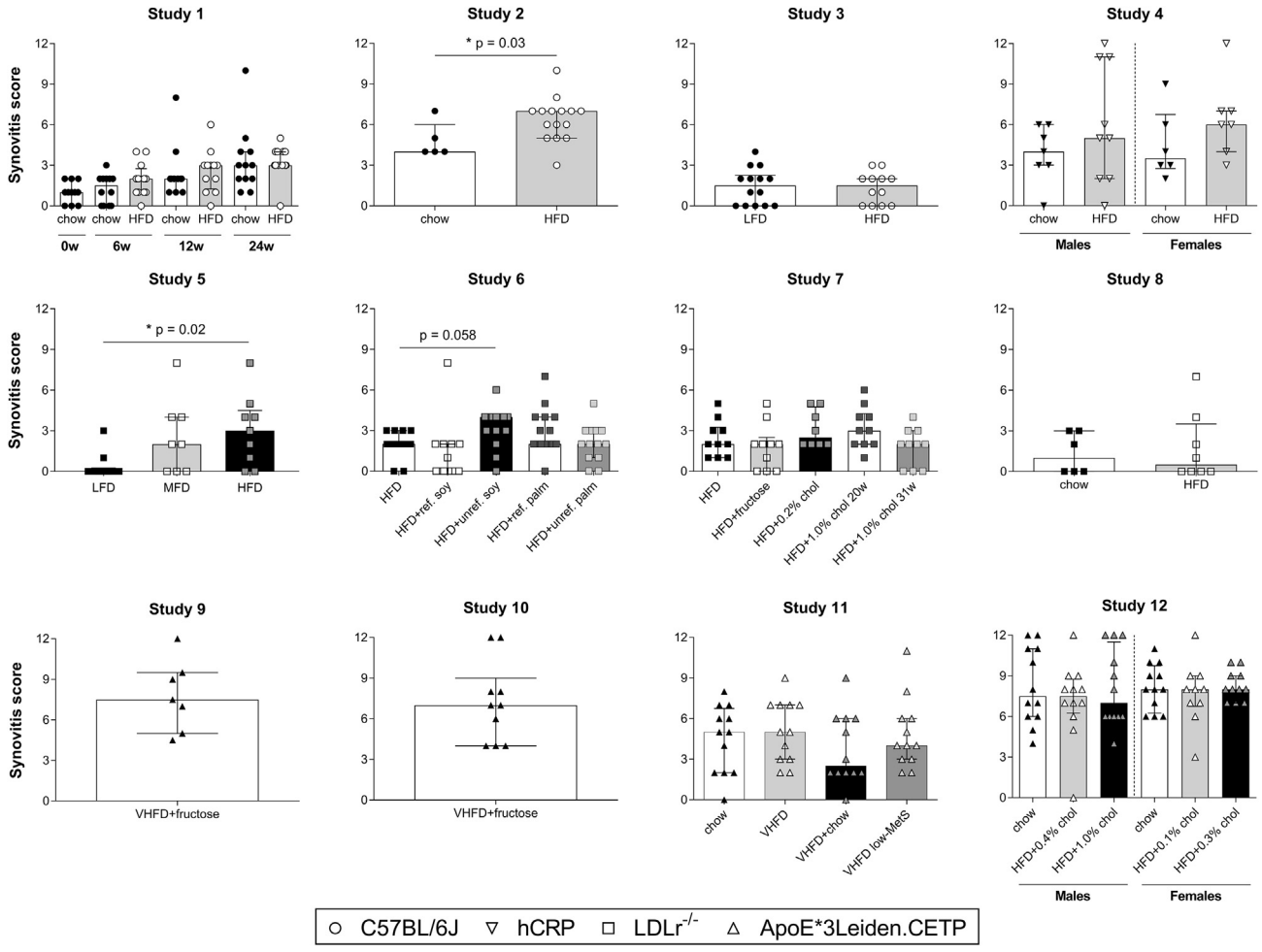

Fig. 4. Total synovitis scores presented per study in separate scatterplots showing the individual summed score for the tibiofemoral knee compartments for each animal per study group (max. 12, OARSI histopathology recommendations for the mouse[13]). Group medians (indicated by bars) and IQR are also shown. *indicates statistical significance relative to control at the same time point ($P < 0.05$). LFD, low-fat diet (10 kcal% energy from fat); WTD, Western-type diet (16 kcal% energy from fat); MFD, mid-fat diet (30 kcal% energy from fat); HFD, high-fat diet (45 kcal% energy from fat); VHFD, very high-fat diet (60 kcal% energy from fat); w, time in weeks; ref., refined oil; unref., unrefined oil; low-MetS, mice showing inexplicably low metabolic adaptation to the VHFD.

link between obesity and OA has become evident in human cohort studies as well as obesity-induced animal models. We postulated that a translational animal model for metabolic OA should display obesity in concurrence with hallmarks of metabolic dysfunction. All our studies confirmed the manifestation of a metabolic phenotype, leaving the absence of accelerated cartilage degradation unexplained. Osteophyte formation and synovitis showed variable results as well, both between HFD-fed mice and controls as between HFD-fed groups within each strain. Rather, our results suggest that an additional trigger – on top of a high-caloric dietary stressor – is necessary to evoke metabolic OA. We found that diet-induced cartilage degradation developed in two mouse models that express human genes (i.e., hCRP and ApoE*3Leiden.CETP mice), suggesting that the corresponding gene products contribute to disease development. These results challenge the general consensus that HFD feeding *per se* is sufficient to evoke OA development.

To improve the translatability of our results, we made use of relevant humanized mouse strains[21,22] and high-caloric diets with more physiological fat content. We acknowledge that our options to explore all contributing factors and perform further mechanistic research was limited due to the broad variation in experimental design and the fact that most of our studies were not preconceived to assess OA development. Also, the OARSI 2010 scoring system we employed, specifically designed for the comparison of OA severity across the various murine OA models, focuses primarily on the condition of articular cartilage. Being a more mild form of OA, diet-induced OA has been evaluated using alternative scoring systems that are more sensitive at discriminating the depth and breadth of mild to moderate OA pathology. To compensate for the cartilage-centered approach, osteophyte formation and synovitis were scored as additional OA features to examine the impact of metabolic dysfunction on different aspects of OA pathology. There may be many explanations for the discrepancy between our results and current literature[23] on diet-induced OA in small animal models, like differences between experiments and laboratories as reviewed in detail by van der Kraan[24]. Also the type of scoring system applied can affect interpretation. Nevertheless, we have encountered several other research groups for which diet-induced metabolic overload in mice did not result in OA development either. Hence, it is certainly possible that OA literature has a publication bias concerning this model; a known issue in the OA field[25] and research in general[26].

The large number of studies – with substantial group sizes – and the consistently applied methodology in our studies enabled us to deduce major confounding factors like strain and gender. Firstly, the C57BL/6J background is a relevant factor shared across all

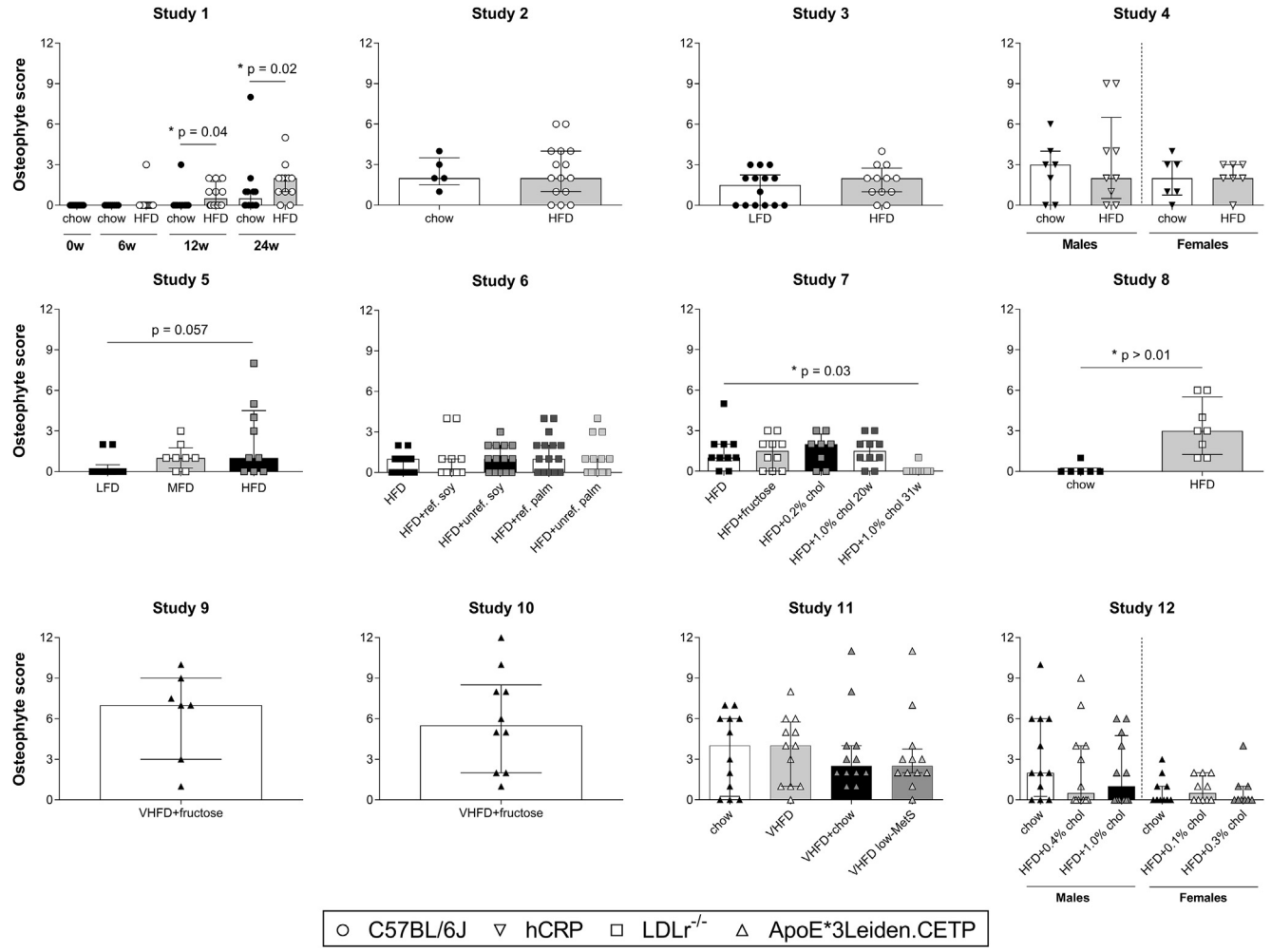

Fig. 5. Total osteophyte scores presented per study in separate scatterplots showing the individual summed score for the tibiofemoral knee compartments for each animal per study group (max. 12, OARSI histopathology recommendations for the mouse[13]). Group medians (indicated by bars) and IQR are also shown. *indicates statistical significance relative to control at the same time point ($P < 0.05$). LFD, low-fat diet (10 kcal% energy from fat); WTD, Western-type diet (16 kcal% energy from fat); MFD, mid-fat diet (30 kcal% energy from fat); HFD, high-fat diet (45 kcal% energy from fat); VHFD, very high-fat diet (60 kcal% energy from fat); w, time in weeks; ref., refined oil; unref., unrefined oil; low-MetS, mice showing inexplicably low metabolic adaptation to the VHFD.

studies. Secondly, most of our studies were conducted in male mice, mainly because males are more prone to develop metabolic dysfunction than females. Thirdly, while duration varied, dietary exposure exceeded 12 weeks in all experiments – a commonly described end point for diet-induced OA in C57BL/6J mice[19,27–29]. However, diet-induced OA studies typically employ VHFDs that contain supraphysiological quantities of fat (i.e., 60 kcal% energy from fat). In contrast, we chose to predominantly use high-fat diets in our studies with 45 kcal% energy from fat, which better approximate the fat content observed in certain human diets[30]. Moreover, in a comprehensive review on high-fat diets, Buettner *et al.*[31] considered it appropriate to state that semi-purified diets with a fat content of more than 40% energy based on animal fats and ω-6/ω-9 fatty acid-containing plant oils will lead to metabolic dysregulation in rodents. The fat fractions of the diets in our studies were all based on lard, in one study partially replaced by soybean or palm oils rich in ω-6/ω-9 fatty acids. Taken together, we believe that all studies described here employed vindicated approaches for diet-induced metabolic dysfunction.

One of the humanized mouse strains used here expresses the human transgene for C-reactive protein (hCRP). As an acute-phase protein, hCRP is able to exert proinflammatory effects through complement activation[32]. Although hCRP is not directly linked to a metabolic pathway, the protein was found to have great impact on the murine metabolic state and cartilage degradation. Obesity development on a HFD was delayed in transgenic hCRP compared with wild-type C57BL/6J mice, with the average body weight after 38 weeks of HFD feeding equating to 12 weeks of HFD feeding in C57BL/6J. Induction of insulin resistance was also delayed in hCRP mice. Together with the absence of diet-induced OA in C57BL/6J and LDLr$^{-/-}$. Leiden mice, this indicates that the severity of metabolic dysfunction is not a major determinant of OA development in the models investigated. Despite the delayed metabolic dysfunction male hCRP mice showed significant OA aggravation upon HFD feeding, whereas females gained notably less weight and did not develop diet-induced OA. Accordingly, male hCRP mice expressed 50-fold higher levels of hCRP than females[33]. A role for hCRP in OA pathogenesis is further supported by an earlier finding by our group that two different types of drugs with anti-inflammatory properties prevented OA development in hCRP male mice[16]. These results suggest that the general inflammatory status associates with the onset and progression of metabolic OA. Interestingly, the inflammatory OA features osteophyte formation and synovitis were not very pronounced in these mice. Many cohort studies have

investigated the relationship between CRP and OA, but reports are contradictory and the role of CRP is still under debate[34–37], making further investigation into this link necessary. A meta-analysis of 32 reported clinical studies by Jin and colleagues further complicates the role of CRP in OA pathogenesis, as it was concluded that low-grade systemic inflammation may play a more prominent role in symptoms of OA[38].

In line with expectations[39], ApoE*3Leiden.CETP mice, a model with humanized lipoprotein metabolism, rapidly developed obesity and metabolic dysfunction on a VHFD. In general, ApoE*3Leiden.CETP mice were highly prone to cartilage degradation, independent of the level of diet-induced metabolic dysfunction. Specifically in males, OA scores were consistently higher than in other strains investigated, even on a chow diet. The same overall high scores were found for both osteophyte formation and synovitis, again independent of the level of diet-induced metabolic dysfunction. The ApoE*3Leiden.CETP mouse expresses three human transgenes when compared with the wild-type C57BL/6J mouse (i.e., ApoE*3Leiden, CETP and APOC1)[40], suggesting that one or more of these gene products contribute to OA development. For instance, we and others have reported that the apoCI apolipoprotein may augment the general inflammatory state[41–44]. ApoE has also been implicated in systemic inflammatory processes, although it can exert both anti- and proinflammatory actions depending on the specific isoform[45,46]. Alternatively, the higher male susceptibility may also be explained by higher androgen levels, which exacerbate OA in mice[47]. Human studies also support a role of sex hormones in OA, showing increased OA prevalence and severity in postmenopausal women compared with premenopausal women and age-matched men[48,49]. Moreover, androgens and ApoE isoforms have been shown to be functionally intertwined in inflammatory processes[46,50], providing a possible rationale as to why ApoE*3Leiden.CETP males show more cartilage degradation in response to metabolic challenges compared with females.

Contrary to the males, female ApoE*3Leiden.CETP transgenic mice showed less cartilage degradation on chow but were susceptible to diet-induced OA. The inflammatory OA features showed deviant results, as ApoE*3Leiden.CETP females developed almost no osteophytes but demonstrated overall high synovitis scores independent of diet. These results contradict current literature in which excessive bone formation and synovitis due to high LDL cholesterol levels is described[51]. The gender differences may partly be explained by the significantly higher diet-induced plasma cholesterol levels observed in ApoE*3Leiden.CETP females, a discrepancy driven by sex hormones[52]. Together with the unfavourable LDL/HDL ratio in these mice, this observation is in agreement with higher OA incidence in women with elevated waist circumference and low HDL cholesterol[53]. Moreover, we previously reported suppression of WTD-induced OA development in ApoE*3Leiden.CETP females upon preventive treatment with the cholesterol-lowering anti-inflammatory drug atorvastatin (Study 12), while cholesterol-lowering alone by ezetimibe did not show this effect[54]. Again, this advocates a role for inflammation in OA development, as previously argued for the hCRP strain. For OA patients the benefit of statin therapy is unclear at present, as human studies investigating the effect of statins on OA development are scarce and a beneficial effect is not observed in all studies[55]. Potential beneficial effects might however be missed due to underdosing, as we have previously shown that the optimal lipid-lowering statin dose is much lower than the threshold for the anti-inflammatory effects[56]. Accordingly, in a population-based longitudinal study, Kadam *et al.* demonstrated that a higher mean daily statin dose was significantly associated with a decreased likelihood of clinical OA during a 10-year follow-up[57].

In conclusion, we have demonstrated that diet-induced metabolic dysfunction *per se* does not necessarily lead to aggravated articular cartilage degradation in mice on a C57BL/6J background. Whereas most of the studies evaluated here were not designed specifically for OA research, there are reports using diet-induced metabolic dysregulation as the only trigger to induce OA in small animal models[23]. In light of our results and the relatively small amount of publications since the 1950s, it is likely that the OA literature has a bias and tends to selective reporting on this issue. Nonetheless, our results support the current concept that metabolic OA is driven by low-grade inflammation[58], although metabolic factors alone might not be enough to generate progressive OA. We suggest that an additional trigger, other than high-caloric feeding alone, is necessary to evoke metabolic OA. In addition to mechanical stressors, as described in literature, we showed that gender and inflammatory factors encoded by the human transgenes in hCRP and ApoE*3Leiden.CETP mice might also trigger metabolic OA development.

Contributors

AEK, LMG, PM, MCM, SK, MPS, AVK, EJP, AMH, RK, HMGP, AMZ and RS have designed the experiments. AEK, LMG, FH, PM, MCM and SK have carried out experimental procedures. AEK has been the primary person responsible for writing the manuscript. All authors were involved in revising the manuscript critically for important intellectual content and approved the final version to be published.

Competing interests

The authors declare that they have no conflict of interest.

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Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.joca.2017.10.010.

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