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ORIGINAL CONTRIBUTION

Mice chronically fed a westernized experimental diet as a model of obesity, metabolic syndrome and osteoporosis

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Abstract *Background* Most studies in animals use diets with several features (for example low-fat, rich in micronutriments), likely to be strongly protective against chronic diseases. *Aim of the study* The present study, performed in wild type outbred mice, was designed to evaluate the validity of a model of 'westernized' (W) diet reproducing, as closely as possible, the overall composition of an average human regime in western countries *Results* In contrast to the standard (S) diet, the W diet triggered a marked increase in adiposity with some characteristics of metabolic syndrome (hypercholesterolemia, hyperinsulinemia...). There was an heterogeneity in the propensity to become obese upon exposure to the W diet in female mice. Overweight mice also presented some disturbances of renal function, such as hyperalbuminuria and hypocitraturia. Mice adapted to the W diet showed a reduction of bone mineral density, especially the non-obese ones. *Conclusion* These data suggest that a model of westernized diet could be appropriate for exploring the effects of mutations, drugs, or specific nutritional factors in animals and could be more relevant for human situations.

Key words mice – westernized diet – metabolic syndrome – bone mineral density

Introduction

Animal models have been useful for investigating chronic diseases that currently plague public health in most countries: obesity, type 2 diabetes, hypertension, atherosclerosis, cancer or osteoporosis [1–4]. All of these are multi-factorial diseases that arise from the combined action of many genes, environmental and behavioral factors, each exerting typically a small effect on disease risk [5]. In order to investigate the effects of nutritional factors, various types of experimental diets have been used in animal studies. Most of these diets, however, focused on a specific nutrient or a specific category of nutrients (fat, fructose, sodium, for example) but remains a poor representative of average human westernized diet (as determined by national dietary surveys [6–7]). Frequently, their global composition is derived from that of the standard diets originally developed for optimizing the growth and the reproduction of the animals [8]. Although the

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composition of these diets can vary from one supplier to another, their overall dietary profile integrates features reminiscent of the hunter–gatherer diets, which are thought to be strongly protective against the development of chronic diseases [9].

The present study was thus designed to compare the long-term effects of a regime that reproduces the major features of human westernized diet with that of a classical chow-diet, taking into account intakes of macronutrients, fatty acids, minerals and vitamins. This approach also differs from the strategy of feeding laboratory animals with palatable human food items (cafeteria diet), whose aim is to promote energy intake rather than to reproduce the average composition of the westernized diet [10].

Materials and methods

Animals, experimental design and diets

The experiments were performed in OF1 female mice obtained from Charles River Laboratories (l'Arbresle, France). These outbred albino mice derive from the CF1 line that was bred by Carworth Farms since 1935. The progenitors were fed either a standard or a westernized diet just before mating and were maintained on each of these diets during pregnancy and until weaning of the pups. The offspring was fed the same diets until their sacrifice at 30 weeks of age. Except for body weight, measured regularly after weaning, all the physiological, biochemical, and histological investigations in the offspring have been made immediately before the sacrifice. During all the protocol, the animals were housed at a constant temperature (21°C) and were allowed free access to food and distilled water in full compliance with the French Government animal welfare policy.

The diets in pellet form were produced at the Institut National de la Recherche Agronomique (UPAE, Jouy-en-Josas, France), with adequate control of nutriment (macro- and micro-) composition.

Plasma and urine analysis

Blood was collected into heparin-treated capillary tubes by puncture of the retro-bulbar venous plexus in non-anesthetized mice. Plasma creatinine and glucose concentrations were measured by enzymatic methods (Kodak Biolyzer, Eastman Kodak, Rochester NY). Sodium, potassium, calcium, magnesium, proton and bicarbonate concentrations were determined by atomic absorption spectrophotometry (model 3110, Perkin Elmer, Norwalk CT) and with a pH/blood-gas analyzer (Compact 1, AVL Instruments Médicaux, Eragny-sur-Oise, France). Cholesterol and triglyceride concentrations were determined by enzymatic assays (RTU and PAP kits BioMérieux, Charbonnière-les-Bains, France). Insulin concentration was analyzed by using a radioimmunoassay kit (SRI-13K Linco Research, Saint Charles MO). A 24-h urine sample was collected using individual metabolic cages specially designed for mouse housing (Marty Technology, Paris, France) and after an adaptation period of 5 days. Urinary sodium, potassium, calcium, magnesium and creatinine concentrations were analyzed with the same techniques used for plasma samples. Osmolality and albumin concentration were determined, respectively, with a freezing point osmometer (Roebling, Berlin, Germany) and by laser immunonephelometry using a rabbit anti-mouse albumin antibody. The analysis of chloride, phosphate, sulfate and citrate concentrations was performed by ionic chromatography (DX320 Dionex, Sunnyvale CA). Urea concentration was determined by a colorimetric assay (kit 535 Sigma Chemical Co., St. Louis MO).

Tissues analysis

Bone densitometry

Whole body, tibia, and caudal vertebrae bone mineral measurements were performed in anaesthetized mice by dual-energy X-ray absorptiometry (PIXImus, Lunar Corporation, Madison WI), as previously described [11]. The PIXImus device was also used to measure total body fat.

Histology of the aorta

Sections (10 μm thick) of the aortic root, performed every 200 μm along the first 800 μm of the ascending aorta, were analyzed for the presence of intimal lesions, using Oil Red O-staining as already described [12].

Statistics

Data were compared by ANOVA with a single factor design or a mixed factorial design with repeated measures on the second factor, followed by a Student's *t*-test using Bonferroni correction for multiple group comparisons. Statistical significance was accepted for $P < 0.05$.

Results

Diet composition

As shown in Table 1, the total carbohydrate content was similar in the standard and westernized diets but with very different quantities of starch (454 g/kg vs.

Table 1 Diet composition[a]

	Standard diet (g/kg)	Westernized diet (g/kg)
Soluble Fish Protein Concentrate[b]	55	55
Casein[b]	0	55
Soy protein isolate[b]	70	0
Brewer yeast (dried)[b]	30	0
Cereals[b]	650	430
Sucrose	40	210
Corn oil	50	0
Lard	0	160
Cellulose[b]	40	40
NaCl	8	18
K_2HPO_4	14	8
$CaHPO_4$	3	3
$CaCO_3$	18	0
MgO	2.1	0.5
Trace elements/Vitamins[b]	20	20

[a]The standard or Westernized diet provided, respectively, (in mg/g diet): animal protein 49 or 102, plant protein 165 or 41, starch 454 or 299, lipid 51 or 154, cholesterol 0 or 1.1, sodium 3.04 or 7.10, potassium 9.17 or 5.48, calcium 9.23 or 1.57, magnesium 1.71 or 0.59, chloride 4.6 or 11.0, phosphorus 6.2 or 2.8; (in μg/g) Mn 82 or 6, Cu 22 or 3, Fe 280 or 25, and Zn 64 or 18, and vitamins (also in μg/g) A 4.1 or 1.8, D 0.04 or 0.01, B_1 18 or 2.4, B_2 7.4 or 4.9, B_6 9.5 or 4.7, B_9 3.9 or 0.6, and B_{12} 0.04 or 0.01. The fatty acid composition of the lipid fraction of the Standard and Westernized diets was, respectively: $C_{16:0}$ 29.1 or 41.4%, $C_{16:1}$ 1.8 or 2.7%, $C_{18:0}$ 9.0 or 20.2%, $C_{18:1}$ 26.7 or 16.3%, $C_{18:2}$ 31.9 or 17.1%, $C_{18:3}$ 1.4 or 2.3%.

[b]Soluble Fish Protein Concentrate (SFPC90): Sopropêche, Boulogne-sur-Mer, France; Casein: Louis François, Saint-Maur, France; Soy Protein Isolate (SPI): Archer Daniels Midland, Decatur, IL, USA; Brewer Yeast: NutriProcess, Serqueux, France; Cellulose: Sigma, L'Isle d'Abeau, France; Cereals (wheat 62%, maize 14% and barley 24%), Moulins de Massagettes, Rochefort-Montagne, France; Trace elements and vitamins (corresponding to the AO3 rodents diet): UAR, Villemoisson/Orge, France.

All the other ingredients (sucrose, corn oil, lard) were purchased from a local supermarket and the minerals were obtained from Sigma

279 g/kg) and sucrose (39 g/kg vs. 210 g/kg). The lipid content was higher in the westernized diet (154 g/kg vs. 51 g/kg) with greater amounts of some fatty acids ($C_{16:0}$, $C_{18:0}$) and of cholesterol (the standard diet being practically devoid of cholesterol) and a relatively low proportion of polyunsaturated fatty acids. The protein content was lower in the westernized diet (14.3 vs. 21.4% in the standard diet) with an inversion of the animal protein/plant protein ratio (2.29 vs. 0.30 in the standard diet). All the minerals were in lower amounts in the westernized diet than in the standard diet (ratio ranging from 0.1 to 0.6) except sodium and chloride, more abundant in the former. All the vitamins were also in lower amounts in the westernized diet than in the standard diet, with a ratio ranging from 0.1 to 0.6 (Table 1). In parallel, the energy density of the westernized diet was higher than that of the standard diet (4960 kcal/kg vs. 3280 kcal/kg).

The 24-h dietary intakes was lower ($P < 0.01$) in the westernized group (4.3 ± 0.3 g) compared to the standard group (5.9 ± 0.3 g): as a result, energy intake was similar for all the groups of animals, given the lower energetic content of the westernized diet. The decreased food intake in the westernized group was accompanied by a corresponding decrease in water intake (4.3 ± 0.3 ml vs. 6.3 ± 0.4 ml, $P < 0.01$).

Growth curves

Mice fed either a westernized or a standard diet gained weight similarly, up to 12 weeks of age (Fig. 1a). After 12 weeks, the group of mice fed a westernized diet became progressively heavier ($P < 0.001$) compared to the group fed a standard diet. The average body weight reached 51.9 ± 2.1 g for the westernized group versus 35.4 ± 0.7 g for the standard group at 27 weeks of age. The animals fed a westernized diet were still growing at an appreciable rate at this age (1.1 g per week on average). The distribution of body weight shows that the minimal value recorded was not different in the westernized and standard groups, whatever the age of the animals (Fig. 1b). In contrast, the value defining the lower quartile became progressively higher ($P < 0.01$) in the westernized group after 12 weeks. At 27 weeks, the value reached 46.2 g in the westernized group compared to 32.5 g in the standard group. The shift in the distribution of body weight in mice fed a westernized diet was also apparent in the value defining the higher quartile and in the maximal body weight that became progressively higher ($P < 0.001$) after 12 weeks, reaching, respectively, 54.3 g and 76.5 g at 27 weeks versus 38.5 g and 44.7 g in animals fed a standard diet. Despite the shift of the distribution of body weight in mice fed a westernized diet toward higher values compared to mice fed a standard diet, a significant overlap still persisted between the two groups. At 27 weeks of age, 50% of the animals in the westernized group had still a body weight comprised in the range observed in the standard group (30.1–44.7 g). To take into account this variability, two groups of mice fed a westernized diet were defined thereafter according to their body weight: a non-obese group composed of animals weighing less than 45 g and an obese group formed of animals weighing more than 50 g.

Morphometric parameters

At 30 weeks of age (Table 2), the mean body weight of the obese westernized group was by definition much higher (59.4 ± 1.5 g; $P < 0.0001$) than those of the standard and non-obese westernized groups that were not statistically different from each other (around 36 g). Body length was also similar in the standard group and non-obese westernized group (around

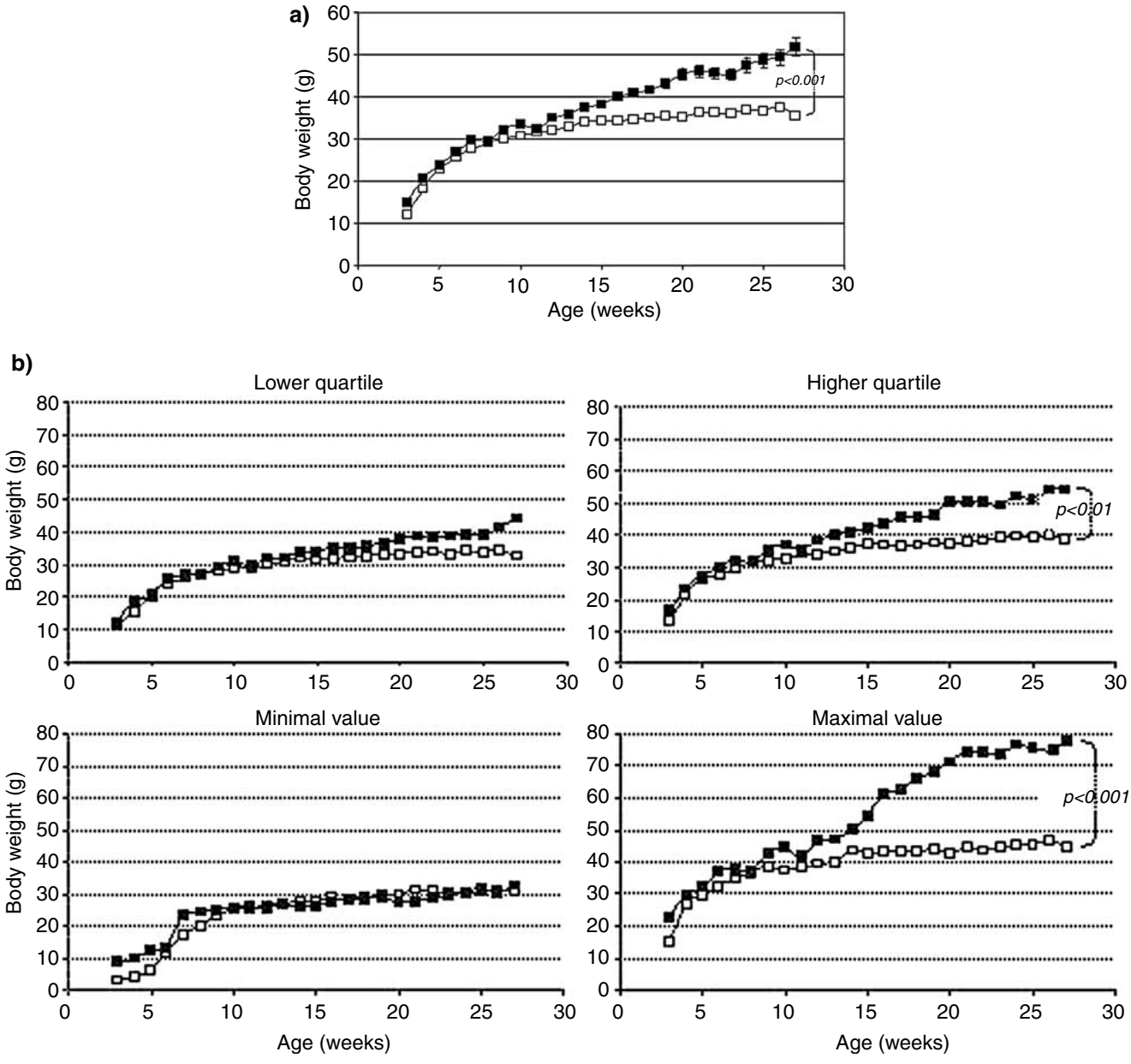

Fig. 1 (**a**) Growth curve of mice fed a standard (empty squares) or a westernized (filled squares) diet. After 12 weeks of age, the animals eating a westernized diet ($n = 102$) become progressively overweight compared to those eating a standard diet ($n = 95$). (**b**) Body weight distribution according to the age of mice fed a standard or a westernized diet. The minimal body weight is similar in the two groups of mice up to 27 weeks of age. In contrast, the body weight defining the lower quartile becomes progressively different between the two groups after 12 weeks of age. The effect is even more pronounced for the body weight defining the higher quartile and for the maximal body weight reached

10.6 cm) whereas it was significantly higher in the obese westernized group (11.4 cm; $P < 0.0001$). Total body fat was also markedly increased in the obese westernized group compared to the standard group and non-obese westernized group (32.6 vs. 12.8% and 14.1%: $P < 0.0001$).

Bone mineral density was reduced in 30-week-old mice fed a westernized diet compared to mice fed a standard diet. The reduction was observed ($P < 0.0001$) at the whole body (54 ± 1 and 58 ± 1, vs. 69 ± 1 mg/cm^2), femoral (83 ± 3 and 93 ± 2, vs. 111 ± 2 mg/cm^2) and vertebral (62 ± 2 and 72 ± 1, vs. 81 ± 1 mg/cm^2) levels in the non-obese and obese westernized groups. Nevertheless, the effect was less pronounced in the obese group that had significantly higher values than the non-obese group ($P < 0.02$, $P < 0.006$ and $P < 0.0008$, respectively). Similar differences were observed with the bone mineral content.

Table 2 Morphometric characteristics[a]

	Standard diet (n = 25)	Western diet non-obese (n = 14)	Western diet obese (n = 23)	$P<$
Body weight (g)	35.6 ± 1.5	36.5 ± 1.7	59.4 ± 1.5[c,d]	0.0001
Body lenght[b] (cm)	10.7 ± 0.1	10.5 ± 0.1	11.4 ± 0.1[c,d]	0.0001
Body fat (% body weight)	12.8 ± 1.2	14.1 ± 1.6	32.6 ± 1.2[c,d]	0.0001
Whole body bone mineral				
Content (mg)	725 ± 15	494 ± 20[c]	551 ± 15[c,d]	0.0001
Density (mg/cm^2)	69 ± 1	54 ± 1[c]	58 ± 1[c,d]	0.0001
Femur mineral				
Content (g)	52 ± 1	37 ± 2[c]	45 ± 1[c,d]	0.0001
Density (mg/cm^2)	111 ± 2	83 ± 3[c]	93 ± 2[c,d]	0.0001
Vertebra mineral				
Content (g)	18 ± 1	12 ± 1[c]	14 ± 1[c]	0.0001
Density (mg/cm^2)	81 ± 1	62 ± 2[c]	72 ± 1[c,d]	0.0001

[a]Values are means ± SEM
[b]measured between the root of the tail and the extremity of the noose while the animals were lying on the back under anesthesia
[c]Significantly different from standard diet
[d]Significantly different from westernized diet non-obese

Table 3 Twenty-four-h urinary excretions[a]

	Standard diet (n = 24)	Westernized diet non-obese (n = 15)	Westernized diet obese (n = 23)	$P<$
Water (ml)	1.6 ± 0.2	1.2 ± 0.2[c]	1.1 ± 0.2[c]	0.03
Osmoles (μOsmoles)	4930 ± 365	2937 ± 377[c]	3315 ± 370[c]	0.001
Creatinine (μmoles)	10.2 ± 0.6	6.3 ± 0.8[c]	7.6 ± 0.6[c]	0.006
Albumin (μg)	36 ± 8	26 ± 10	76 ± 17[c,d]	0.005
Sodium[b]	31 ± 5	111 ± 7[c]	100 ± 5[c]	0.0001
Potassium[b]	67 ± 5	47 ± 3[c]	46 ± 3[c]	0.001
Calcium[b]	0.4 ± 0.1	0.1 ± 0.1[c]	0.1 ± 0.1[c]	0.0001
Magnesium[b]	4.8 ± 0.5	1.4 ± 0.2[c]	1.2 ± 0.2[c]	0.0003
Chloride[b]	40 ± 9	126 ± 12[c]	115 ± 17[c]	0.0001
Phosphate[b]	19 ± 2	9.8 ± 0.8[c]	8.7 ± 0.7[c]	0.0001
Sulfate[b]	11.4 ± 0.6	8.2 ± 0.7[c]	7.6 ± 0.6[c]	0.01
Citrate[b]	5.6 ± 0.3	0.1 ± 0.4[c]	0.1 ± 0.3[c]	0.0001
Urea[b]	15.1 ± 0.7	9.8 ± 0.7[c]	9.3 ± 0.8[c]	0.0001

[a]Values are means ± SEM. The recovery rate of the metabolic cages used for the collection of urine was 25% for water and 55% for monovalent ions
[b]Data for cations, anions and urea excretion are expressed as μmoles/μmoles creatinine
[c]Significantly different from standard diet
[d]Significantly different from westernized diet non-obese

Urinary excretion

The 24-h urinary excretions were similar in 30-week-old non-obese and obese mice fed a westernized diet (Table 3), except the excretion of albumin that was increased in the obese group compared to the non-obese and standard groups (76 ± 17 vs. 26 ± 10 μg and 36 ± 8 μg, $P < 0.006$). All the excretion values were significantly reduced in non-obese and obese animals fed a westernized diet compared to those fed a standard diet, except the excretion of sodium (702 ± 79 and 758 ± 64 vs. 319 ± 43 μmol, $P < 0.0001$) and chloride (793 ± 81 and 879 ± 75 vs. 404 ± 47 μmol, $P < 0.0005$) that were increased (Table 4). Although creatinine excretion was also reduced in the westernized groups compared to the standard group (6.3 ± 0.8 and 7.6 ± 0.6 vs. 10.2 ± 0.6 μmol, $P < 0.006$), all the differences between the groups remained statistically significant when the excretion values were normalized to creatinine excretion.

Plasma parameters and aortic lesions

At 30 weeks of age, no difference was observed between the three groups of mice in plasma concentration of creatinine, sodium, potassium, calcium and magnesium (data not presented). The pH was not different between the groups, but the concentration of bicarbonate was lower in the non-obese and obese westernized groups compared to the standard group (20.8 ± 0.7 and 20.7 ± 0.7 vs. 23.1 ± 0.7 mM, $P < 0.02$) (Table 4). The concentration of glucose and insulin was similar in the non-obese westernized group and standard group (6.28 ± 0.25 mM and 6.17 ± 0.24 mM and 0.51 ± 0.16 ng/ml and 0.49 ± 0.16 ng/ml, respectively) and was higher in the obese westernized group

Table 4 Plasma parameters

	Standard diet (n = 17)	Westernized diet non-obese (n = 16)	Westernized diet obese (n = 16)	$P<$
Creatinine (μM)	18.7 ± 0.8	21.0 ± 0.9	21.1 ± 0.8	NS
pH	7.30 ± 0.01	7.31 ± 0.01	7.27 ± 0.01	NS
Bicarbonate (mM)	23.1 ± 0.7	20.8 ± 0.7[a]	20.7 ± 0.7[a]	0.02
Glucose (mM)	6.17 ± 0.24	6.21 ± 0.26	7.03 ± 0.24[a,b]	0.01
Cholesterol (mM)	2.55 ± 0.21	3.49 ± 0.25[a]	3.84 ± 0.25[a,b]	0.0003
Triglyceride (mM)	0.70 ± 0.07	0.65 ± 0.07	0.73 ± 0.07	NS
Insulin (ng/ml)	0.49 ± 0.16	0.51 ± 0.16	0.97 ± 0.15[a,b]	0.03

Values are means ± SEM
[a]Significantly different from standard diet
[b]Significantly different from westernized diet non-obese

(7.03 ± 0.24 mM, $P < 0.01$ and 0.97 ± 0.15 ng/ml, $P < 0.03$, respectively). The concentration of triglyceride was not different between the groups whereas the concentration of cholesterol was higher in the non-obese and obese westernized groups (3.49 ± 0.25 mM and 3.84 ± 0.25 mM, $P < 0.0003$) compared to the standard group (2.55 ± 0.21 mM). The hypercholesterolemia was accompanied by the presence of fatty streaks that were observed at the root of the aorta in 75% of non-obese and obese mice fed a westernized diet, but never in mice fed a standard diet (Fig. 2).

Discussion

The westernized diet has been formulated with the objective of reproducing as far as possible the human diet consumed in industrialized and urbanized countries. Several lines of evidence indicate that this diet is highly discordant with the genetically determined nutritional needs of human beings, thus possibly fostering the development of chronic diseases [9, 13, 14]. Largely based on refined cereals, sugars and oils, as well as on dairy products, meat and salty processed foods, the westernized diet is thought to induce sub-deficiencies in minerals (iron, magnesium, calcium, potassium, phosphorus, zinc) and vitamins (A, C, B12, B6, B1, B2, B9) in sizeable fractions of the populations. It also results in an excess of saturated fatty acids, a relative lack of polyunsaturated fatty acids, a lack of fibers and a large excess of sodium together with a high potential to generate net acid load [9]. The formulation of the westernized diet used in the present study was elaborated from data obtained by recent dietary surveys performed in different countries [13, 14]. The westernized rodent diet present major differences with the commercially available standard diets classically used in laboratories. Standard diets are globally alike and present several features reminiscent of those ascribed to the hunter–gatherer diets, supposed to fulfill the physiological human needs and to protect against the development of chronic diseases [9]. Compared to a westernized diet, they are significantly richer in minerals and vitamins and contain smaller amounts of sucrose, saturated fatty acids and salt. Curiously, these diets also have less mono and polyunsaturated fatty acids with a greater ratio $n-6/n-3$, and they are almost completely devoid of cholesterol.

To evaluate the long-term effect of westernized diet in mice, compared to a standard diet, the choice of an outbred strain was made to avoid any peculiar genetic feature that could be attached to a given inbred strain. Mice can react very differently to dietary changes, depending on their genetic background. For example, a high fat intake during 7 weeks has been shown to increase adiposity in some inbred strains (AKR/J, C57L/J, A/J, C3H/HeJ, DBA/2J, C57BL/6J), but not in others (SJL/J, I/STN, SWR/J) [15]. Similarly, a high-fat diet during 26 weeks produces obesity in both A/J and C57BL/6J mice but, whereas obesity was associated with only moderate glucose intolerance and insulin resistance in A/J mice, obese C57BL/6J mice showed clear-cut hyperglycemia and hyperinsulinemia [16]. Various inbred mouse strains also present large differences in the susceptibility to atherosclerosis when fed a high-cholesterol diet during 14 weeks [17]. Even basal food and water intakes can vary significantly from one strain to another [18]. The use of an outbred strain has a correlate: all the mice do not react in the same way to the stimulus. In the present study, only 50% of the animals exposed to the westernized diet developed adiposity and body weight higher than the range of values observed in the animals fed a standard diet.

Continuous exposure to the westernized regime, from fetal and early postnatal periods up to the adult age, intended to mimic as closely as possible a human situation. In mice, a low protein diet that impairs fetal or early postnatal growth has been reported to affect life expectancy and susceptibility to the effects of an obesity-promoting cafeteria diet after weaning [19, 20]. In rats, manipulation of dietary sodium intake during the perinatal period has been shown to influ-

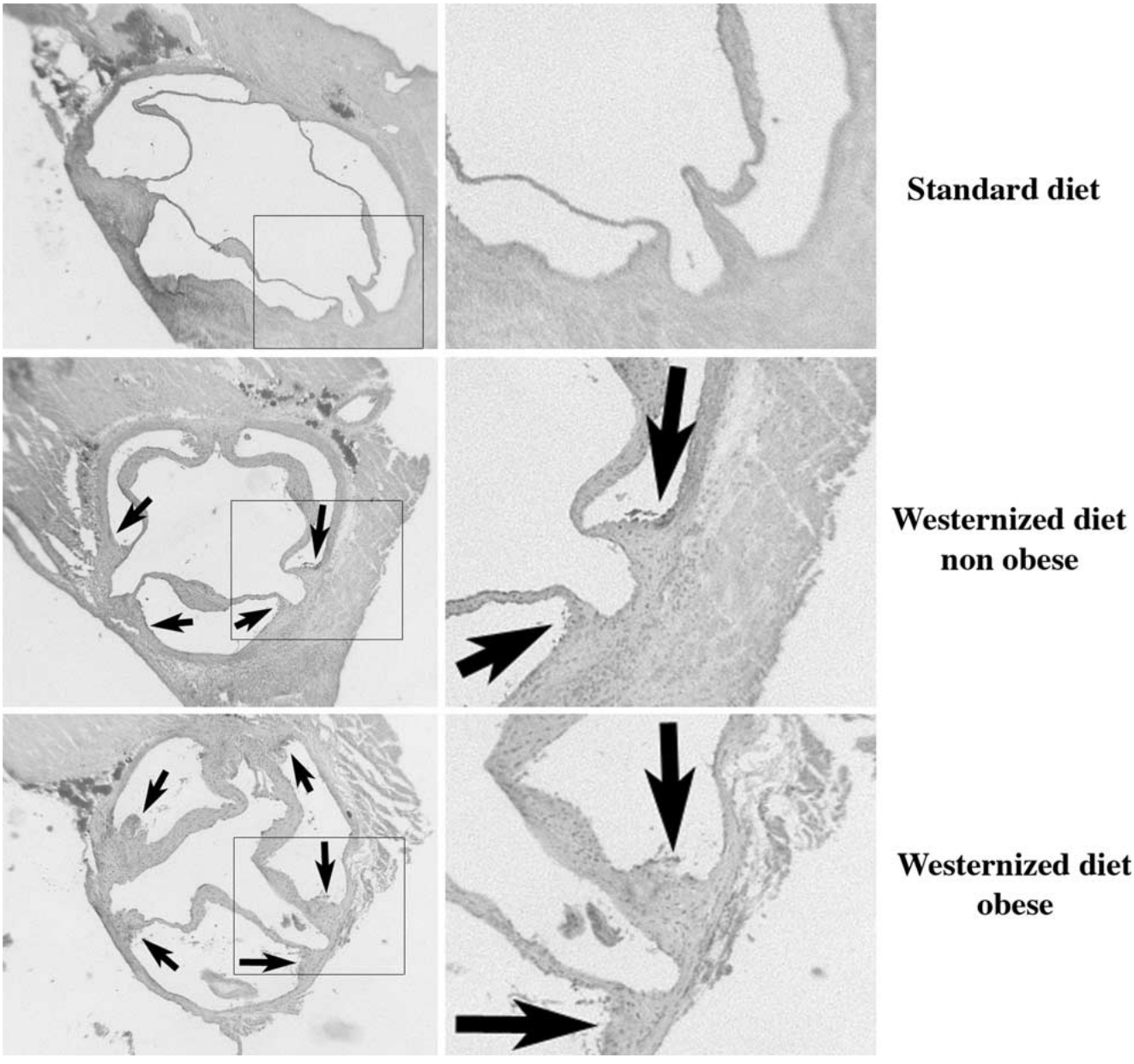

Fig. 2 Aortic structure in mice fed a standard or a westernized diet. Fatty streaks (arrows) are present in 75% of non-obese and obese mice fed a westernized diet, while none is observed in mice fed a standard diet. Oil Red O-staining sections of aortic roots are taken at 400 μm from aortic cusps, magnification 40× (100× for the enlarged sections)

ence blood pressure and insulin sensitivity at the adult age [21, 22]. In humans, poor fetal growth has also been linked to detrimental effects in adulthood, including obesity, metabolic syndrome and type 2 diabetes [23].

In 50% of the mice, chronic exposure to the westernized diet induced a marked increase in adiposity accompanied by hypercholesterolemia, hyperglycemia and hyperinsulinemia, with signs of vascular lesions. Compensated metabolic acidosis and bone loss were also observed. Interestingly, some of these features were shared between non-obese and obese mice (hypercholesterolemia, vascular fatty streaks, acidosis, bone loss) whereas others were found only in obese mice (hyperglycemia, hyperinsulinemia, albuminuria). Bone loss was less pronounced in obese than non-obese mice, as expected from the known protective effect of overweight and obesity [24, 25]. Although hypertension and cardiac hypertrophy were absent in both non-obese and obese mice (data not presented), the

phenotype clearly mimics pathological states, such as 'diabesity' and osteoporosis, frequently observed in human westernized populations [26]. The absence of hypertension (data not presented) may be partly explained by the relatively small difference between the sodium content of the westernized and standard diets (2.3 ratio) combined to the fact that mice have a high capacity to maintain sodium and fluid homeostasis [27].

The development of a metabolic-like syndrome together with a marked bone loss is noteworthy because when a single nutritional parameter is modified in mice, it is usually necessary to reach extreme non-physiological values to get similar phenotypes to those obtained in this study. For example, fatty streaks and hypercholesterolemia have been induced by the 0.1% cholesterol westernized diet, far below the special regimes classically used to obtain the same phenotype, containing up to 1.25–2.0% of cholesterol [28]. It is also notable that the increase in adiposity, glycemia and insulinemia was induced by a diet with 30% of the energy coming from fat, a value at the low end of the range (32–58%) habitually used to trigger obesity and diabetes in mice [15, 29, 30] and with a near-complete digestion of fat as assessed by measurement of free fatty acid in the cecum (data not shown). Likewise, a marked bone loss has been obtained with a mildly calcium-depleted diet (0.15%) in comparison to other studies [31]. The role of proteins on bone metabolism is equivocal since, on the one hand, excess dietary proteins increase the acid load and promote urinary calcium losses but, on the other hand, relatively low protein intakes have been shown to be sub-optimal for calcium retention [32]. The present data indicate that a moderately calcium-depleted diet containing a low protein level (15%), with a low animal protein/plant protein ratio, is globally detrimental for bone metabolism compared to a calcium-rich diet containing a high protein level (21%) together with a high animal protein/plant protein ratio. Overall, these data demonstrate that the phenotype is determined by the combination of nutriments, minerals and vitamins rather than by the effect of a single component.

The fact that mice develop phenotypes reminiscent of human disturbances when exposed *ad libitum* to the same "non-physiological" diet suggest an evolutionary conservation of the mechanisms underlying the development of obesity, metabolic syndrome and osteoporosis [33, 34], despite obvious metabolic differences and physiopathological characteristics (lack of development of pancreatic amyloid). Some discrepancies reported in the literature between mice and humans, as in the case of diabetes, could be related to the use of a standard (protective) diet that would impair the accurate analysis of the genetic effects by inhibiting the development of the diseases [35]. It would be interesting to test whether the average westernized human diet would affect in the same way the other animal species habitually used in laboratories.

In conclusion, these results show that chronic exposure to an average human westernized regime triggers a development of obesity, with features of metabolic syndrome and signs of osteoporosis in some but not all wild type mice from an outbred strain. This suggests that this type of diet would be more appropriate as a maintenance diet than the standard diets currently used in laboratories for exploring the effects of mutations and drugs. It would also present the advantage of facilitating the extrapolation of the data to human situations.

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