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High-fat diet induced adiposity and insulin resistance in mice lacking the myotonic dystrophy protein kinase

Esther Llagostera [a], Mari Carmen Carmona [b], Meritxell Vicente [b], Rosa María Escorihuela [c], Perla Kaliman [b,*]

[a] Departament de Bioquímica i Biologia Molecular, Facultat de Biologia, Universitat de Barcelona, Barcelona, Spain
[b] Diabetes and Obesity Laboratory, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS) Hospital Clínic, Universitat de Barcelona, Barcelona, Spain
[c] Medical Psychology Unit, Department of Psychiatry and Forensic Medicine, School of Medicine, Institute of Neurosciences, Autonomous University of Barcelona, E-08193 Bellaterra, Barcelona, Spain

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ABSTRACT

Myotonic dystrophy 1 (MD1) is caused by a CTG expansion in the 3′-unstranslated region of the myotonic dystrophy protein kinase (DMPK) gene. MD1 patients frequently present insulin resistance and increased visceral adiposity. We examined whether DMPK deficiency is a genetic risk factor for high-fat diet-induced adiposity and insulin resistance using the DMPK knockout mouse model. We found that high-fat fed DMPK knockout mice had significantly increased body weights, hypertrophic adipocytes and whole-body insulin resistance compared with wild-type mice. This nutrient–genome interaction should be considered by physicians given the cardiometabolic risks and sedentary life-style associated with MD1 patients.
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1. Introduction

Myotonic dystrophy 1 (MD1), an autosomal dominant neuromuscular disorder, is the most common form of adult muscular dystrophy [1]. The MD1 mutation has been identified as the expansion of an unstable CTG-repeat in the 3′-untranslated region of a gene encoding the serine/threonine protein kinase myotonic dystrophy protein kinase (DMPK) [2–5].

Studies in mice have indicated that several mechanisms may simultaneously contribute to the wide spectrum of symptoms observed in MD1 [6,7]. One of the mechanisms proposed is that the repeat expansion affects the level of DMPK expression in *cis* by altering its transcription or by retaining DMPK transcripts in the cell nucleus. This results in a decrease in the amount of DMPK protein expression, or haploinsufficiency. Results from DMPK knockout mice – which develop mild myopathy, cardiac abnormalities and metabolic alterations – give experimental support to this mechanism [8–11].

Insulin resistance, which is a major factor in the development of type 2 diabetes [12], is a metabolic feature in MD1 patients, who frequently exhibit normal basal insulin levels but excessive insulin release after a glucose load [13,14]. Whole-body glucose disposal in MD1 patients is reduced by 15–25% following insulin infusion [15] and experiments with forearm muscle indicate a 70% decrease in insulin sensitivity in skeletal muscle [16]. In MD1 patients, fat mass is significantly increased and lean body mass is decreased [17–20]. Computerized tomography (CT) scanning at the umbilical level revealed increased visceral fat area in MD1 patients compared with healthy controls. Visceral fat area in MD1 patients correlated positively with plasma glucose levels in the glucose tolerance test, and with serum total cholesterol and triglycerides [21]. Moreover, comparison between MD1 patients with long and short CTG repeat expansions did not reveal significant differences in body mass index or body fat mass [22], indicating that their fat accumulation is not exclusively related with the degree of toxicity of the long repeats.

Here we explored whether DMPK deficiency is a genetic risk factor for adiposity and systemic insulin resistance, by examining the metabolic phenotype of DMPK KO mice after a 6-week high-fat diet (HFD). We found that under the dietary stress of a high-fat regime, DMPK knockout mice developed more overweight and whole-body insulin resistance than wild type mice. These data indicate that in MD1 patients, DMPK underexpression may interact with environmental factors to induce a phenotype of prediabetes and increased adiposity.

* Corresponding author.
E-mail address: pkaliman@clinic.ub.es (P. Kaliman).

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2. Materials and methods

2.1. Mouse experiments

All animal studies were performed in accordance with the guidelines and under approval of the Institutional Review Committee for the Animal Care and Use of the University of Barcelona. The $dmpk^{-/+}$ heterozygous mice on 129SV background were generated by Reddy et al. [8]. Mice were backcrossed at least 9 times maintaining them as congenic in the colony.

The normal diet contained 4% fat by weight (11% of calories from fat) (Teklad Global 14% Protein Rodent Maintenance Diet, Harlan Teklad, Madison, WI), while the high-fat diet had 34.9% fat by weight (60% of calories from fat) (D12492, Research Diets; New Brunswick, NJ). For diet treatments, 2-month old male mice were individually fed a normal or a fat-adjusted diet for 6 weeks (two cohorts of DMPK knockout and wild-type mice, each containing at least 10 animals/genotype/diet). Weight and food intake were measured daily.

2.2. Home cage activity

Home cage activity was automatically recorded by video tracking in specially designed individual cages for automated recordings (Med Associates, Inc.). Mice were monitored by an infra-red-sensitive camera mounted above the cages, which was connected to a computer running the video tracking software programmed to measure two types of activity: ambulation (horizontal displacements) and small movements (grooming behaviour, small rearings and stereotyped movements). The session lasted 24 h, starting between 2:00 and 3:00 pm. Lights were automatically turned off at 8:00 pm and on at 8:00 am.

2.3. Metabolic measurements

Blood glucose levels in whole venous blood were measured using an automatic glucose monitor (AccuChek®, Roche Diagnostics One Touch Basic, Lifescan). Plasma insulin levels were measured by ELISA, using rat insulin as a standard (Crystal Chem). Insulin tolerance tests (ITT) were performed on animals that had been fasted overnight and injected with 0.75 unit/kg body weight of human regular insulin (Lilly). LINCO Research Mouse Adiponectin ELISA Kit (Cat.#EZMADP-60K) was used to determine adiponectin levels in plasma samples from fed male mice. Liver lipid was extracted as described [23]. Free fatty acids, cholesterol and triglyceride levels were measured using kits from Wako Chemicals.

2.4. Histological studies

Adipose tissues were fixed in 95% ethanol, embedded in paraffin and cut into 8 μm sections. Hematoxylin and eosin staining was performed following standard protocols. Image areas were determined using the MacBiophotonics Image J software. Average adipocyte size was determined in 15 randomly selected fields per animal, from a total of 5 animals per condition. A relative estimation of the total number of cells was obtained as cell number/mm^2 × total tissue weight (mg).

Pancreas were fixed in phosphate buffered paraformaldehyde (4% w/v), dehydrated, embedded in paraffin and cut into 5 μm sections. Insulin immunostaining was performed using a peroxidase indirect labeling technique, as previously reported [24]. Islet area was determined by manually circumscribing the outline of all the islets in a section from a total of three tissue sections per animal (n = 5 animals/group).

2.5. Statistical analysis

Data are presented as mean ± S.E. Statistical analysis was performed using a two-tailed unpaired t-test. Values of $P < 0.05$ were considered as statistically significant.

3. Results

Body weight of DMPK knockout (KO) and wild type (WT) mice fed normal chow or high-fat (HF) diet was monitored. Absolute body weight and body weight increase were already significantly higher after 2 weeks of HF diet in the DMPK KO mice and the difference persisted over time (Fig. 1A). This difference was not due to increased food intake, as both KO and WT mice consumed comparable amounts of food during this period (Fig. 1B). Body weights of KO on a normal diet were similar to those of control mice (Fig. 1A). Overweight in KO compared to WT mice could not be attributed to mobility impairment as assessed in home cage activity studies (Fig. 1C). Therefore, the increased weight gain of KO compared to WT mice, despite equal energy intake and physical activity, implicates DMPK in the regulation of energy balance.

No differences in the weight of liver, heart, skeletal muscle, brown adipose tissue, or pancreas were observed between WT and KO mice on high fat diet (Fig. 2A). However, the weights of epididymal and subcutaneous white adipose tissues (WAT) were

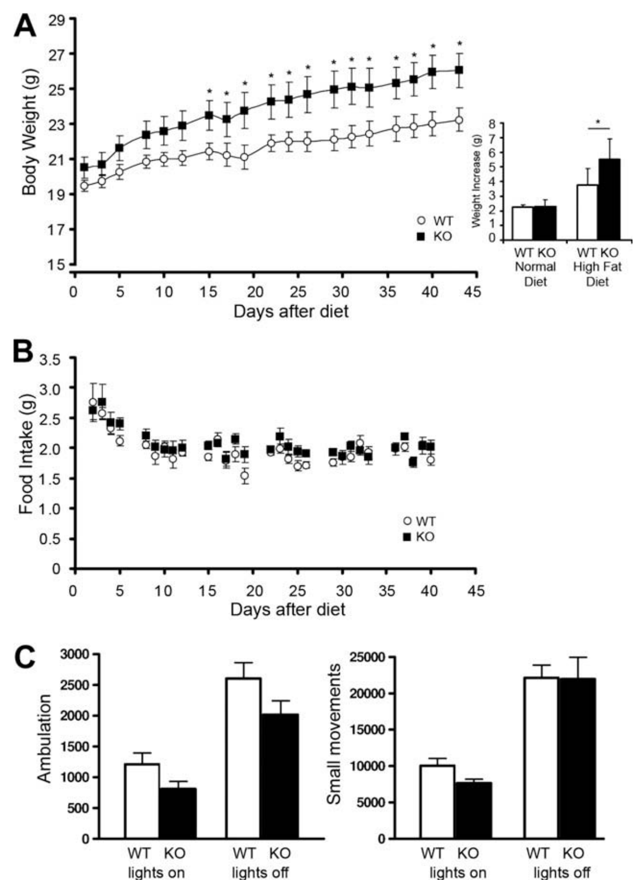

Fig. 1. (A) Changes in body weight of DMPK knockout (KO) and wild type (WT) mice (n = 10 animals/group) during a 6-week high-fat diet. The box shows body weight increases from mice fed a standard (St) or high-fat (HF) diet for 6 weeks. (B) Food intake of $DMPK^{-/-}$ and wild type mice during the 6-week high fat diet. (C) Home cage activity was automatically recorded by video tracking in specially designed home cages for automated recordings. Ambulation (horizontal displacements) and small movements (grooming behaviour, small rearings and stereotyped movements) were measured (n = 8 animals/group). Data are means ± S.E.M. $^{*}P < 0.05$, $^{**}P < 0.01$ versus the corresponding wild-type value.

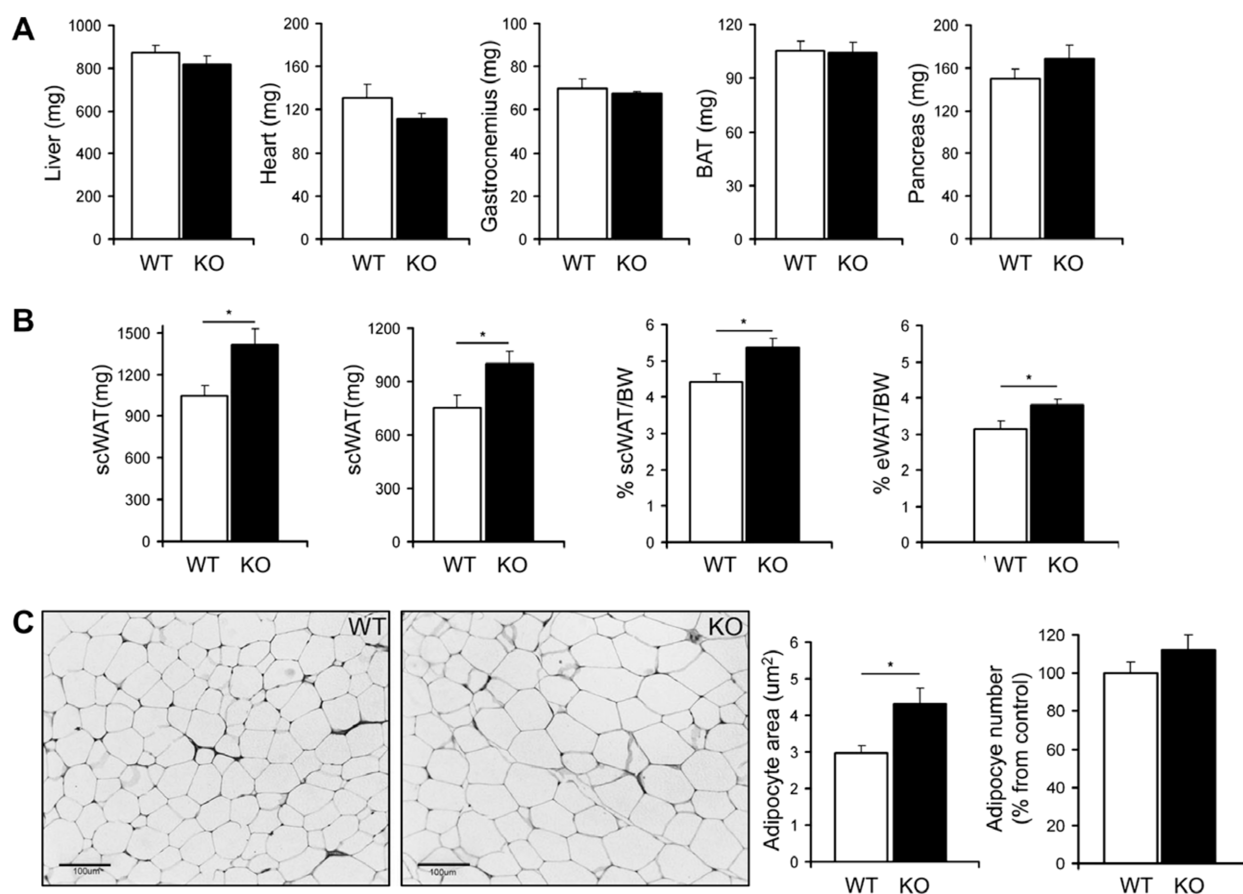

Fig. 2. (A) Weight of various tissues (from left to right: liver, heart, gastrocnemius, brown adipose tissue and pancreas) from DMPK knockout (KO) and wild type (WT) mice (n = 10 animals/group) at the end of the 6-week high-fat diet. (B) Weight of subcutaneous (sc) and epididymal (e) white adipose tissue (WAT) from KO and WT mice at the end of the 6-week high fat diet (n = 10 animals/group). (C) Representative hematoxylin and eosin staining of epididymal WAT from KO and WT mice at the end of the 6-week high-fat diet (n = 5 animals/group). Scale bar, 100 μm. (D) Distribution of adipocyte size in epididymal WAT from KO and WT mice at the end of the 6-week high fat diet (n = 5 animals/group). (E) Estimation of adipocyte number in epididymal WAT from KO and WT mice at the end of the 6-week high-fat diet (n = 5 animals/group). Data are means ± S.E.M. $^{*}P < 0.05$, $^{**}P < 0.01$ versus the corresponding wild-type value.

significantly increased in DMPK KO mice (Fig. 2B). WAT weight was similar in wild type and DMPK KO mice fed a normal diet (data not shown). A significant increase in the size of adipocytes in epididymal WAT was observed in the KO mice fed a HF diet compared with WT controls (45 ± 10% increase, n = 5 per condition analyzed, P = 0.014) (Fig. 2C and D); this effect was not accompanied by a significant difference in cell number (Fig. 2E). We did not detect significant differences in the expression of PPARγ, PGC-1α or adiponectin in WAT from KO compared with WT mice after the HF diet (data not shown).

Table 1 summarizes metabolic parameters of KO and WT mice on normal and HF diet. While the insulin plasma levels remained similar in both groups after the HF diet, fast glycaemia in HF-fed KO mice was significantly higher than that of WT mice (P = 0.008). Plasma adiponectin was comparable between WT and DMPK KO mice. Similar plasma lipid levels were found in KO and WT mice fed high fat diets. Triglyceride and cholesterol accumulation in liver was similar in KO and WT mice after the HF diet, indicating that DMPK did not influence hepatic lipid storage.

We examined the effect of *dmpk* ablation on whole-body insulin sensitivity after 6 weeks on HF diet. Insulin tolerance tests (ITT) revealed no differences in the blood glucose levels between KO and WT mice on normal chow (Fig. 3A). Both groups showed a decreased response to exogenous insulin after the HF diet intervention, reflecting increased insulin resistance at these pharmacological levels of hormone. However, DMPK KO mice developed a

Table 1
Metabolic parameters from mice fed a normal or high-fat diet for 6 weeks. All data are means ± S.E.M.

	Normal diet		High-fat diet	
	WT	KO	WT	KO
Glycaemia (mg/dL) (fed)	114.2 ± 4.26	109.2 ± 3.86	122.5 ± 3.78	119.4 ± 3.91
Glycaemia (mg/dL) (fast)	64.00 ± 2.58	65.07 ± 2.16	74.43 ± 5.06	96.29 ± 4.88*
Insulinemia (μg/mL) (fed)	N.D	N.D	1.36 ± 0.14	1.06 ± 0.10
Insulinemia (μg/mL) (fast	N.D	N.D	0.20 ± 0.05	0.12 ± 0.02
Plasma adiponectin (ng/mL)	11.55 ± 0.81	11.47 ± 1.04	13.19 ± 0.79	13.29 ± 0.72
Plasma TG (g/L)	N.D	N.D	0.53 ± 0.04	0.59 ± 0.05
Plasma NEFA (mM)	N.D	N.D	1.11 ± 0.12	1.21 ± 0.13
Hepatic TAG (mg/g)	21.30 ± 1.16	23.96 ± 3.04	86.94 ± 22.91	68.33 ± 12.00
Hepatic CHOL (mg/g)	1.36 ± 0.08	1.34 ± 0.08	5.05 ± 0.59	3.89 ± 0.22

* $P < 0.05$ (n = 10 animals/group).

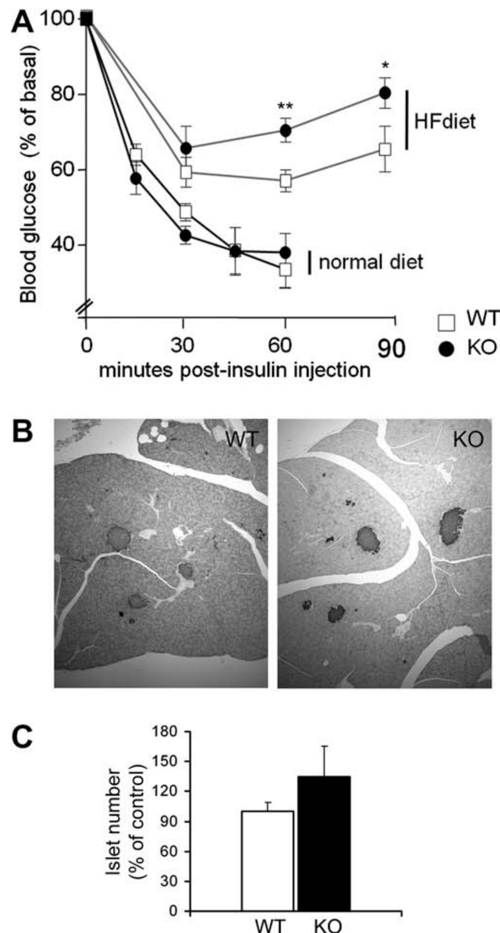

Fig. 3. (A) Insulin tolerance test. Blood glucose levels in KO and WT mice ($n = 10$/group) fed a normal or high-fat diet for 6 weeks were determined at the indicated times after intraperitoneal injection with a bolus of insulin. (B) Peroxidase immunostaining of pancreatic sections with insulin antibodies. Representative sections of pancreas from KO and WT mice at the end of the 6-week high fat-diet are shown ($n = 5$ animals/group). (C) The number of islets containing insulin positive cells per pancreas area (mm^2) was measured in pancreas sections from KO and WT mice at the end of the 6-week high fat-diet, as indicated in Methods ($n = 5$ animals/group). All data are means ± S.E.M. $^{*}P < 0.05$, $^{**}P < 0.01$ versus the corresponding wild-type value.

more severe systemic insulin resistance than the WT mice (Fig. 3A).

In order to establish whether insulin resistance in the DMPK KO mice affected the insulin-producing cells, we immunostained pancreatic tissue sections to specifically analyze insulin expression. (Fig. 3B and C). No differences were observed in the measurement of pancreatic beta cell mass in the KO mice compared with the WT mice after the high fat diet.

4. Discussion

We have recently provided in vivo and in vitro evidence for the role of DMPK in the regulation of insulin action and glucose homeostasis [25]. Those findings indicated that reduced DMPK expression may directly influence the onset of insulin-resistance in MD1 patients and suggested that DMPK could represent a susceptibility gene to type 2-diabetes. Here we demonstrate that a high-fat (HF) diet leads to adverse metabolic outcomes (i.e. systemic insulin resistance and visceral fat accumulation) in a genetic context of DMPK deficiency.

Our data show that a HF diet aggravates the already altered metabolic phenotype of DMPK KO mice fed a normal diet. Indeed, compared with the WT control mice, the DMPK KO mice on the HF diet exhibited increased body weight and fat mass. Interestingly, when fed a normal diet, the KO mice showed no alterations in the insulin tolerance tests. However, after the HF diet, systemic insulin sensitivity was significantly lower in KO compared with WT mice. After 6 weeks on a HF diet, KO mice also showed increased white adipose tissue mass compared with WTs, in the absence of any detectable change in pancreas structure, adipocyte marker expression, or adiponectin secretion. The increase in adipose tissue mass correlated with adipocyte hypertrophy. After the HF regime, DMPK KO mice showed a slight but significant increase in fast glycaemia but did not show differences in fed blood glucose, plasma insulin or plasma lipid levels compared with WT mice.

It is well established that accumulation of lipids in non-adipose cells leads to cell dysfunction, i.e. insulin resistance in skeletal muscle [26] and impaired insulin secretion and apoptosis in pancreatic β-cells [27,28]. The finding that serum lipid levels were not elevated in DMPK KO mice as compared with WT mice, and that both groups had similar triglyceride content in liver, indicates that the increase in lipid storage in adipose tissue in DMPK KO mice may have protected these mice from systemic lipotoxicity and further impairment of glucose homeostasis and metabolic alterations.

In MD1 patients, the primary muscular disease usually leads to inactivity and sedentary lifestyles, which in turn may contribute to overweight and obesity, as well as cardiometabolic risk. Moreover, overweight and obesity often lead to prediabetes which confers a 6-fold increased risk of diabetes compared with normal glucose tolerance [29]. In this context, our data showing that DMPK KO mice present significantly higher metabolic risks under a HF diet than the WT mice (i.e. insulin resistance and visceral adiposity), point to a nutrient–genome interaction which physicians should take into account for early prevention and management of co-morbid cardiometabolic risks in Myotonic Dystrophy 1 patients.

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