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Gluten-free diet reduces adiposity, inflammation and insulin resistance associated with the induction of PPAR-alpha and PPAR-gamma expression☆,☆☆

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Abstract

Gluten exclusion (protein complex present in many cereals) has been proposed as an option for the prevention of diseases other than coeliac disease. However, the effects of gluten-free diets on obesity and its mechanisms of action have not been studied. Thus, our objective was to assess whether gluten exclusion can prevent adipose tissue expansion and its consequences. C57BL/6 mice were fed a high-fat diet containing 4.5% gluten (Control) or no gluten (GF). Body weight and adiposity gains, leukocyte rolling and adhesion, macrophage infiltration and cytokine production in adipose tissue were assessed. Blood lipid profiles, glycaemia, insulin resistance and adipokines were measured. Expression of the PPAR-α and γ, lipoprotein lipase (LPL), hormone sensitive lipase (HSL), carnitine palmitoyl acyltransferase-1 (CPT-1), insulin receptor, GLUT-4 and adipokines were assessed in epididymal fat. Gluten-free animals showed a reduction in body weight gain and adiposity, without changes in food intake or lipid excretion. These results were associated with up-regulation of PPAR-α, LPL, HSL and CPT-1, which are related to lipolysis and fatty acid oxidation. There was an improvement in glucose homeostasis and pro-inflammatory profile-related overexpression of PPAR-γ. Moreover, intravital microscopy showed a lower number of adhered cells in the adipose tissue microvasculature. The overexpression of PPAR-γ is related to the increase of adiponectin and GLUT-4. Our data support the beneficial effects of gluten-free diets in reducing adiposity gain, inflammation and insulin resistance. The data suggests that diet gluten exclusion should be tested as a new dietary approach to prevent the development of obesity and metabolic disorders.

Keywords: Gluten-free diet; Obesity; Inflammation; Insulin resistance; PPAR

1. Introduction

Gluten is a protein complex consisting of glutenins and prolamins, which may be present in several cereals, such as wheat, rye and barley [1].

Although a gluten-free diet is a well-established treatment for coeliac disease, nowadays gluten-free diets have been proposed to be used for the prevention and treatment of diseases such as rheumatoid arthritis [2], Type 1 diabetes mellitus [3–5], obesity and insulin resistance (IR) [6].

Obesity is associated with important comorbidities that compromise an individual's health in many countries around the world [7,8]. In particular, the accumulation of visceral fat is the common link to metabolic syndrome, atherosclerosis and Type 2 diabetes mellitus [9–11]. Diet, as a part of lifestyle modification, is the primary strategy for the prevention and treatment of obesity. For this reason, gluten-free diets have been used as an anti-obesity, anti- inflammatory and anti-diabetic strategy. However, well-controlled in vivo studies evaluating the benefice of such dietary approaches are rare in the literature.

The aim of this study was to evaluate the effect of a gluten-free diet on body weight and adiposity gains, the inflammatory profile of adipose tissue and glucose homeostasis using an experimental model of diet-induced obesity.

2. Methods and materials

This project was approved by the Ethics Committee for Animal Experimentation at the Federal University of Minas Gerais (protocols #222/08 and #161/2010).

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☆☆ Conflict of interest: The authors declare that there are no conflicts of interest in regard to this study.

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2.1. Animals and diets

Eight-week-old male C57BL/6 mice were group housed in an environment with light cycles of 12 hours (7:00–19:00) and controlled temperature (20–24°C). The animals were divided into a control group, which was fed a high-fat diet contained 4.5% wheat gluten (Granotec, Curitiba, Brazil) to induce obesity, and a gluten-free (GF) group, which received the same diet without gluten supplementation for 8 weeks. The high-fat diet was composed of 25%, 61% and 15% of the total kcal from carbohydrate, fat and protein, respectively, and had a caloric density of 22.11 kJ/g [12]. All of the mice had free access to food and tap water.

Body weight and food intake were measured individually once a week. At the end of experimental period the animals were overnight fasted and euthanized under anaesthesia. Fasting is necessary since food intake (that occurs mainly at night in mice) interferes with biochemical determinations such as lipid profile, glucose, insulin and leptin. Moreover, fasting was also necessary to keep intestine and liver free of the influence of food intake. Blood, visceral fat (epididymal, retroperitoneal, peri-renal, mesenteric and omental sites), liver, the gastrocnemius muscle and the faeces (directly in the distal colon) were collected immediately after euthanasia for subsequent analyses. Fat-free mass was calculated as follows: body weight (g)−sum of epididymal, retroperitoneal, peri-renal, mesenteric and omental fat (g). Subcutaneous fat was omitted from the calculation because it contribute minimally to total body fat.

2.2. Blood analyses and lipid content

Lipid profile, total protein, glucose, insulin, leptin, adiponectin and resistin concentrations were measured in the serum or plasma. Blood glucose, triacylglycerol and total cholesterol were determined using enzymatic colorimetric kits (Labtest, Brazil). Protein was determinate according to the Lowry method [13]. ELISA kits were used to measure the concentrations of insulin, leptin, adiponectin and resistin, according to the manufacturer's instructions (Millipore, MO, USA, for insulin and R&D Systems, MN, USA, for others). The insulin sensitivity test was performed as previously described [14]. The Homeostasis Model Assessment of Basal Insulin Resistance (HOMA-IR) was calculated as the product of fasting plasma glucose (mM) multiples insulin (mU/L) divided by a constant (22.5), as previously described [15].

The total lipid content of the adipose tissue, faeces, liver and the gastrocnemius muscle was determined according to the Folch method [16].

2.3. Histological analyses

For the analysis of adipocyte size, samples of adipose tissue were fixed in Bouin's solution, embedded in paraffin, cut and stained with hematoxylin and eosin and analysed using Image Pro Plus software (Bethesda, MD, USA). The adipocyte diameter of each animal was calculated by median of 100 adipocyte measurements. Crown-like structures (CLS) were calculated as the average of the number of CLS found in 10 representative fields.

2.4. Cytokine concentrations in adipose tissue

Aliquots of epididymal adipose tissue were homogenised with an extraction solution of cytokines (bovine serum albumin 0.05%; aprotinin 0.02 μl/ml; benzochloride 0.05 mg/ml; NaCl 0.023 mg/ml; DMSO 1 μL/ml; EDTA 0.37 mg/ml; PMSF 0.02 mg/ml; Tween 20 0.5 μL/ml in phosphate-buffered saline). After centrifugation, the infranatants were used for the determination of monocyte chemotactic protein-1 (MCP-1/CCL-2), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α) and interleukin-10 (IL-10) concentrations, using ELISA kits and according to the manufacturer's instructions (BD-Pharmigen, CA, USA).

2.5. Intravital microscopy

Intravital microscopy was performed on the epididymal adipose tissue microvasculature. Mice were anaesthetised with 10 mg/kg xylazine and 100 mg/kg ketamine hydrochloride, injected intraperitoneally. The right jugular vein was cannulated, and rhodamine 6G (Sigma, St. Louis, MO, USA) was injected intravenously (0.15 mg/kg) to visualise the leukocyte/endothelial cell interactions. Rhodamine epi-illumination was achieved with a 150-W variable HBO mercury lamp in conjunction with a Zeiss filter set 15 (546/12 nm band–pass filter, 580 nm Fourier transforms, 590 nm late potentials; Zeiss, Wetzlar, Germany). Microscopic images were captured using a Nikon Eclipse 50i microscope (Nikon Instruments, Japan) (×20 objective) with a video camera (5100 HS; Panasonic, Secaucus, NJ, USA), and consecutive digital recordings were made using both filters. The data analysis was performed off-line. Rolling leukocytes were defined as the cells moving slower than the cells showing a regular flux in a given vessel. The flux of the rolling cells was measured as the number of rolling cells passing by a given point in the venule per minute, with the results expressed as cells per minute. A leukocyte was considered to be adhered if it remained stationary for at least 30 s, and total leukocyte adhesion was quantified as the number of adhered cells within a 100 μm length of venule, with the results expressed as cells/100 μm [17].

2.6. Measurement of gene expression by real-time polymerase chain reaction (PCR)

The epididymal adipose tissue was removed for the evaluation of leptin, lipoprotein lipase (LPL), acetylCoA carboxylase (ACC), peroxisome proliferator-activated receptor alpha (PPAR-α), hormone sensitive lipase (HSL), carnitine palmitoyl transferase-1 (CPT-1), MCP-1/CCL-2, IL-6, TNF-α, IL-10, PPAR-γ, insulin receptor, glucose transporter GLUT-4, adiponectin and resistin expression. The samples were transferred to Trizol solution (Invitrogen #15596–026) for RNA extraction as previously described [18]. To obtain cDNA, the samples were placed in the thermocycler at 72°C for 5 min for annealing, followed by a cycle of 42°C for 3 h and 72°C for 15 min for transcription. Real-time semi-quantitative PCR was performed using the Power SYBR MasterMix (Applied Biosystems, CA, USA) and specific primers in an ABI 7900 HT fast real-time PCR System (Applied Biosystems, CA, USA). The results were expressed relative to β-actin expression (fold-increased) or the ΔΔCt method [18].

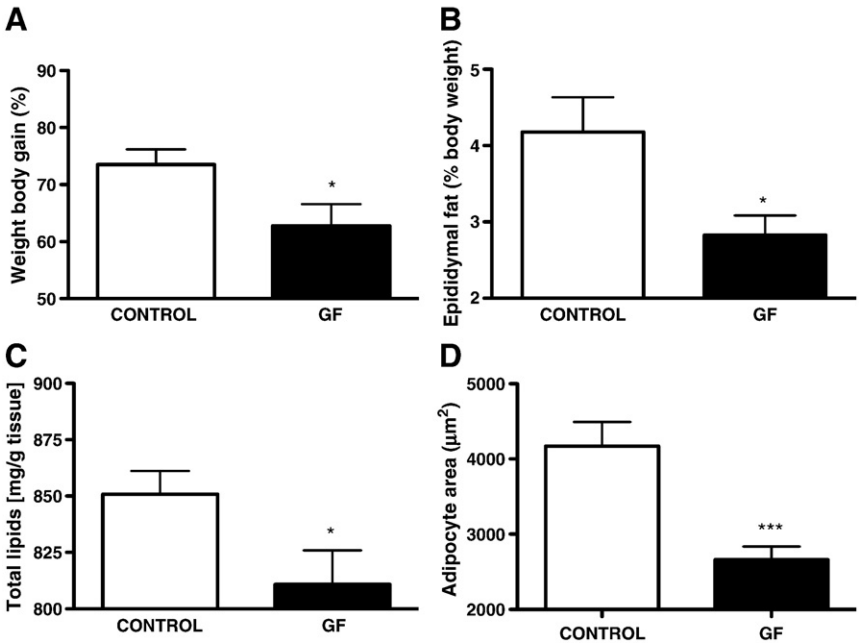

Fig. 1. (A) Weight gain (% of initial weight). (B) Relative weight of epididymal fat (as % of total body weight). (C) lipid content and (D) adipocyte area in the visceral tissue of C57BL/6 mice fed a high-fat diet containing 4.5% wheat gluten (control) or a high-fat diet without gluten (GF) for 8 weeks. n=9/group (A); 23–24/group (B); 19–20/group (C) and 4–5/group (D). The bars represent the mean, and the lines represent the S.E.M. *P<.05; ***P<.001.

Table 1
Food intake, fat-free mass, fecal lipids excretion, blood lipid profile, blood total protein and ectopic (liver and muscle) lipid concentration in mice fed control or gluten-free diet

Parameter	Control	GF	P
*Total food intake (g)	166.9±3.6	163.6±3.9	.533
Fat-free mass (g)	31.28±0.98	29.95±0.69	.286
Fecal lipids (mg/g)	225.2±39.0	234.7±69.6	.904
Blood triacylglycerol (mmol/L)	0.97± 0,14	1.07±0.15	.631
Blood cholesterol (mmol/L)	2.47±0.37	2.35±0.27	.794
Blood total protein (g/L)	21.23±0.31	21.22±0.30	.973
Liver lipids (mg/g)	89.5±16.0	85.0±9.7	.815
Muscle lipids (mg/g)	113.5±19.3	102.7±7.3	.609

* Total food intake during the 8 experimental weeks. Average±S.E.M. n=4–14 per group.

The primers used in this study were:

ACC: 5′TCCGCACTGACTGTAACCACAT, 3′TGCTCCGCACAGATTCTTCA
Adiponectin: 5′AGGTTGGATGGCAGGC, 3′GTCTCACCCTTAGGACCAAGAA
CPT-1: 5′TCCCAGGCAAAGAGACAGACTTGC, 3′GCAGGCGCGAGCCCTCATAG
GLUT-4: 5′CTGCAAAGCGTAGGTACCAA, 3′CCTCCCGCCCTTAGTTG
HSL: 5′ACCGAGACAGGCCTCAGTGTG, 3′GAATCGGCCACCGGTAAAGAG
IL-10: 5′GGTTGCCAAGCCTTATCGGA, 3′ACCTGCTCCACTGCCTTGCT
IL-6: 5′ACAACCACGGCCTTCCCTACTT, 3′CACGATTTCCCAGAGAACATGTG
Insulin receptor: 5′ATGAGGCCAACCTTCCTGGAA, 3′ACGGGACATTCTCCATGTCT
Leptin: 5′CCTGTGGCTTTGGTCCTATCTG, 3′AGGCAAGCTGGTGAGGATCTG
LPL: 5′AGTCTGGCCTCGAACTAAACTATGTAT, 3′TCCCAGGACACAGGAAGCTAA
MCP-1/CCL-2: 5′CCACTCACCTGCTGCTACTCAT, 3′TGGTGATCCTCTTGTAGCTCTCC
PPAR-α: 5′TACCACTATGGAGTCCACGCATGT, 3′TTGCAGCTTCGATCACACTTGTCG
PPAR-γ: 5′ACAGACAAGATTTGAAAGAAGCGGTGA, 3′TCCGAAGTTGGTGGGCCAGA
Resistin: 5′AGACTGCTGTGCCTTCTGGG, 3′CCCTCCTTTTCCTTTTCTTCCTTG
TNF-α: 5′CGTCGTAGCAAACCACCAAG, 3′GAGATAGCAAATCGGCTGACG
B-actin: 5′CTGCCTGACGGCCAAGTC, 3′CAAGAAGGAAGGCTGGAAAAGA

2.7. Statistical analysis

The data were expressed as mean±S.E. (for parametric data) or the median with the interquartile range (for non-parametric data) and analysed using the software Prism 5.0 (GraphPad Software, CA, USA). To test the distribution of the data, we used the Kolmogorov-Smirnov test, and for detecting outliers, we used Grubbs tests and box-plots (for parametric and non-parametric data, respectively). To verify statistical differences between groups, we applied Student's t test and the Mann–Whitney U test (for parametric and non-parametric data, respectively). $P \leq .05$ was considered statistically significant.

3. Results

The weight gain and the epididymal adiposity were significantly lower in mice fed a gluten-free diet (Fig. 1A, B). These results were supported by the lower lipid concentration (Fig. 1C) and adipocyte size (Fig. 1D) in the epididymal fat of GF animals. No differences were seen between groups regarding fat-free mass and serum total proteins (Table 1), suggesting that the relative lack of digestion of gluten did not affect mice development. The reduction of weight gain and epididymal fat was not associated with a lower food intake or increased lipid excretion, as they were similar in both groups (Table 1). The lower weight gain did not affect the levels of blood triacylglycerols or cholesterol, and the liver and muscle lipid depositions remained unaltered between the groups (Table 1).

Plasma leptin was lower in the GF animals, reflecting its lower expression in adipose tissue (Fig. 2A). The circulating levels of resistin were also reduced after gluten exclusion, although the gene expression of this adipokine was similar between the groups (Fig. 2B). As can be seen in Fig. 2C adiponectin gene expression in adipose tissue and adiponectin serum levels were increased in mice fed GF diet.

Glucose homeostasis was also influenced by gluten exclusion, with a reduction in fasting glycaemia and insulinaemia (Fig. 3A and B). The improvements in the HOMA-IR index and the insulin sensitivity test confirmed the attenuation of insulin resistance after gluten exclusion (Fig. 3C, D).

These results are consistent with the higher number of insulin receptors and GLUT-4 glucose transporters in the GF group (Fig. 3E). The expression of nuclear transcription factor PPAR-γ, which is involved in the induction of the GLUT-4 transporter, was more highly expressed in mice fed the gluten-free diet (Fig. 3F).

Our next step was to analyse the changes in lipid metabolism that could be related to those effects. We first analysed the expression of LPL, HSL, ACC and CPT-1, which are related to the degradation of lipoprotein-derived triacylglycerol, the lipolysis of triacylglycerols and the synthesis and oxidation of fatty acids, respectively (Fig. 4). The results showed an increase in LPL, HSL and CPT-1 expression, with no change in ACC expression (Fig. 4A, B). The data for LPL and HSL are consistent with a higher intracellular concentration of free fatty acids (FFA) in the adipocytes of GF

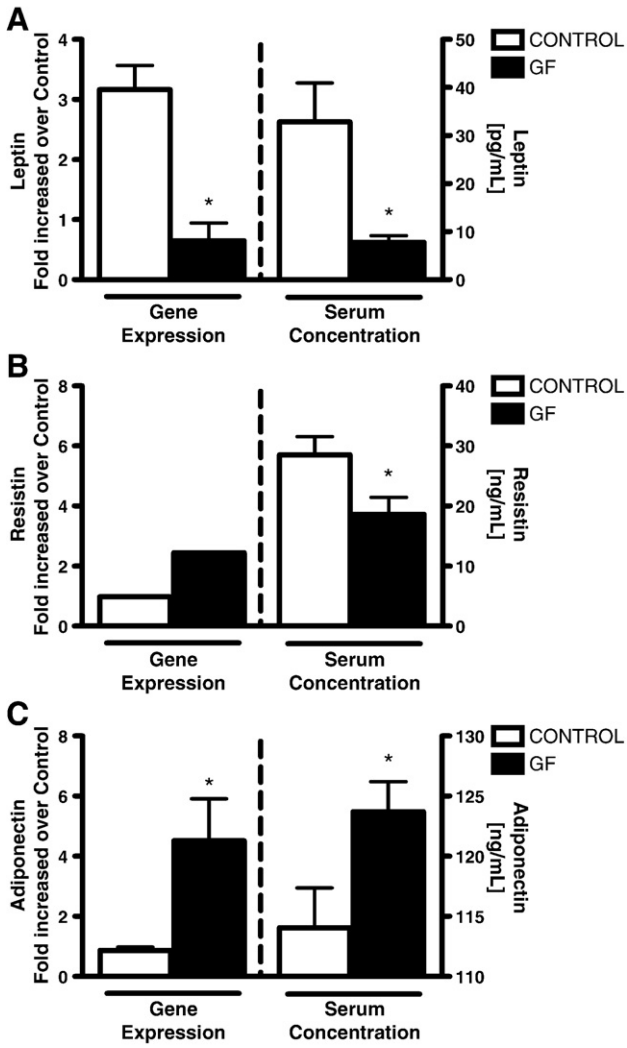

Fig. 2. Expression (in the epididymal fat) and concentration (in the serum) of the adipokines (A) leptin, (B) resistin and (C) adiponectin of C57BL/6 mice fed a high-fat diet containing 4.5% wheat gluten (control) or a high-fat diet without gluten (GF) for 8 weeks. n=5–10/group for concentration data and 3–4/group for expression data. The bars represent the mean, and the lines represent the S.E.M. (for adipokines expression, the bars represent the median, and the lines represent the interquartile range). *$P \leq .05$.

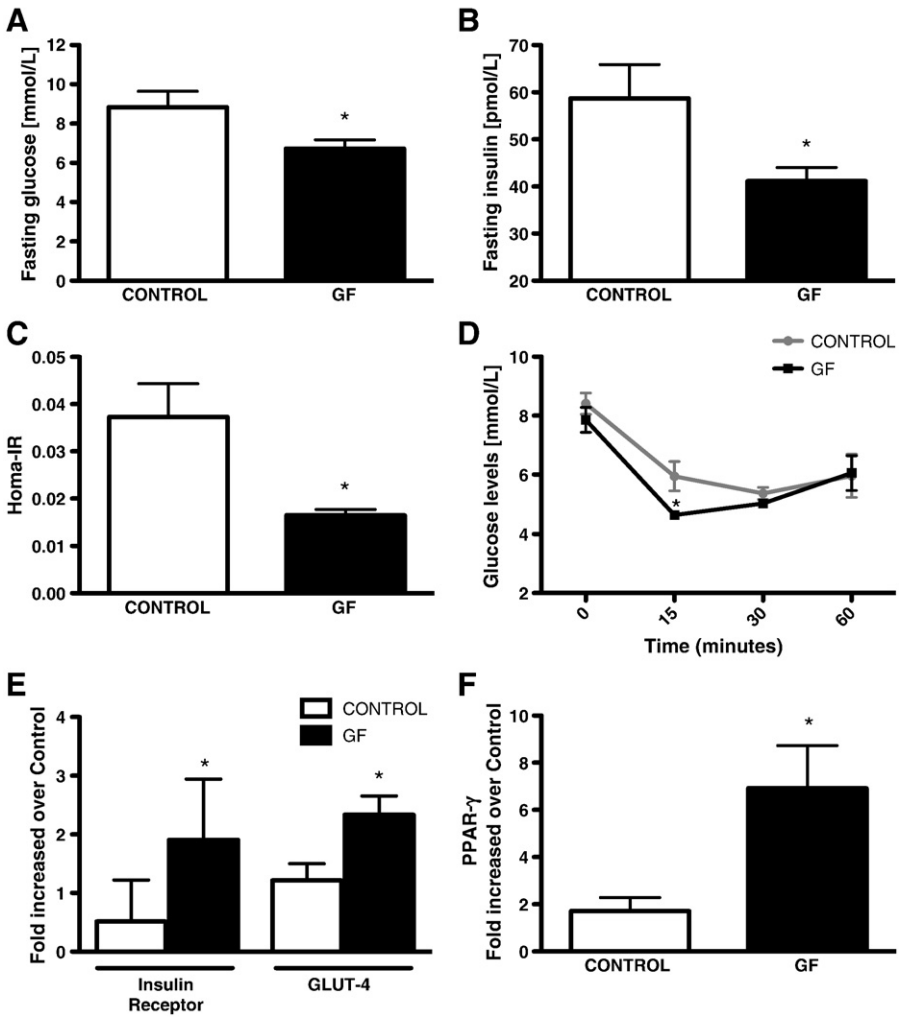

Fig. 3. Glucose homeostasis in C57BL/6 mice fed a high-fat diet containing 4.5% wheat gluten (control) or a high-fat diet without gluten (GF) for 8 weeks. (A) Fasting glycaemia, (B) insulinaemia, (C) HOMA-IR index, (D) Insulin sensitivity test, (E) insulin receptor and GLUT-4 and (F) expression of the nuclear transcription factor PPAR-γ in the epididymal fat. n=15–18/group (A), 8/group (B, C); 9–10 (D) and 3–7/group (E and F). The bars represent the mean, and the lines represent the SEM (for insulin receptor and PPAR-γ expression, the bars represent the median, and the lines represent the interquartile range). *$P \leq .05$.

animals and the rapid mitochondrial oxidation of the FFA. Because FFA activate the nuclear receptor PPAR-α [19], which in turn influences lipid metabolism, the expression of this receptor was assessed. PPAR-α was expressed at higher levels in the GF animals (Fig. 4C).

We analysed leukocyte rolling and adhesion in vivo through intravital microscopy (supplementary video). Although there was no difference in rolling, we found that leukocyte adhesion was reduced in the GF group (Fig. 5A). We also evaluate the presence of activated macrophages in the visceral adipose tissue and the consequent production of cytokines. Although differences in the expression of MCP-1/CCL-2 were not detected, the concentration of this chemotactic protein was reduced in mice fed the gluten-free diet (Fig. 5B). As a result, we found a lower number of CLSs composed of activated macrophages in mice from the GF group (Fig. 5C, G–H). Among the cytokines produced by activated macrophages, we investigated the expression and concentrations of TNF-α, IL-6 and IL-10 in adipose tissue. The expression and concentration of these cytokines decreased after the exclusion of gluten suggesting the attenuation of the chronic inflammation generated by the expanded adipose tissue (Fig. 5D–F). Together, these data indicate that gluten exclusion attenuates the inflammatory state induced by a high-fat diet.

4. Discussion

Some indirect evidence suggests that gluten exclusion provides benefits not only to coeliac patients, but also to those suffering from certain chronic diseases, such as obesity [2–6]. However, to our knowledge, this is the first study investigating the effects and impact of a gluten-free diet on obesity and lipid and glucose metabolism.

The gluten-free diet slowed body weight gain, thereby reducing visceral adiposity and adipocyte size. Because adipocyte hypertrophy is one of the major factors responsible for adipose tissue inflammation and glucose homeostasis imbalance, dietary approaches that restrict adipocyte expansion are also likely to improve insulin resistance [9,20].

Since our results did not reveal any differences in food intake (P=.533), fecal lipid excretion (P=.904) or lipid content in blood, muscle and liver, we believe that the differences found between groups on fat content in adipose tissue are the consequence of

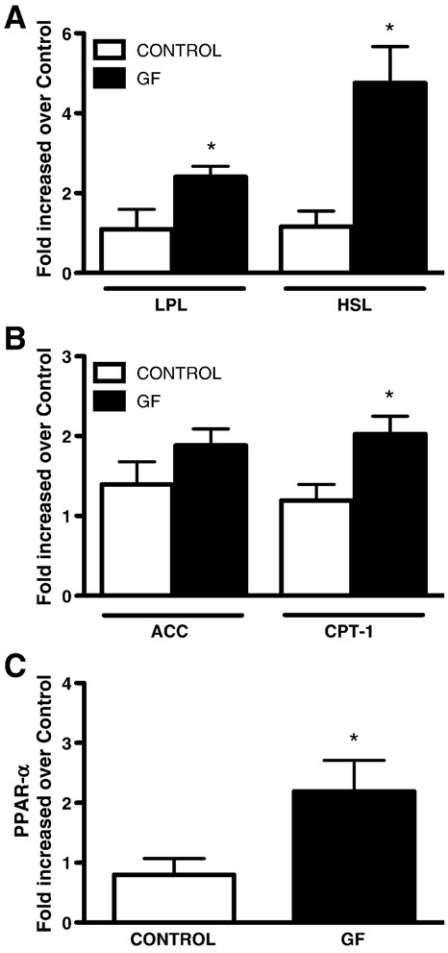

Fig. 4. Gene expression of (A) LPL and HSL, (B) ACC and CPT-1, (C) nuclear receptor PPAR-α in the epididymal fat of C57BL/6 mice fed high-fat diet containing 4.5% wheat gluten (control) or a high-fat diet without gluten (GF) for 8 weeks. n=3-6/group. The bars represent the mean, and the vertical lines represent the S.E.M. (for HSL and ACC expression, the bars represent the median, and the lines represent the interquartile range). *$P \leq .05$.

metabolic alterations in lipid and glucose metabolism in GF group instead reduction of lipid absorption.

Therefore, the lower adiposity in the GF animals occurred despite the unchanged food intake, faeces lipid excretion and lipogenesis in adipose tissue. Reduced adiposity in mice fed GF diet seem to be mediated, at least in part, by the increased expression of the lipolytic enzyme and mitochondrial oxidative, such as HSL and CPT-1, as well as increased expression of PPAR-α and γ, well known transcription factors involved in the adipose tissue remodelling.

PPARs play an important role in lipid and glucose homeostasis. PPAR-α agonists are related to increased fat oxidation and the improvement of insulin sensitivity [21]. PPAR-α agonists also decrease intracellular fatty acyl CoA and malonyl CoA and increase fatty acid oxidation [22,23]. The latter is favoured by the induction of CPT-1 expression by PPAR-α, which permits the influx of fatty acids into mitochondria [24]. Our results suggest that a gluten-free diet could inhibit the accumulation of triacylglycerol in adipose tissue by inducing concomitantly lipolysis of intracellular triglycerides (increased expression of HSL) and increasing mitochondrial oxidation of the fatty acids (released after HSL action) as suggested by the increased expression of CPT-1. Corroborating those results are the lower lipid accumulation in epididymal fat of GF mice and the similar lipid concentration in blood and liver.

Our results also showed the protective effects of a gluten-free diet on glucose homeostasis. Glycaemia, insulinaemia, HOMA-IR and insulin sensitivity were all improved in the GF mice. These effects may be related to the lower accumulation of visceral adiposity [9–12] in the GF mice, among other factors. Gluten exclusion has been also related to a reduction in the incidence of type 1 diabetes mellitus [3–5]. Our results are supported by a previous study showing that pigs fed a cereal-free diet had improved insulin sensitivity associated with lower concentrations of C-reactive protein [6].

We also showed that animals fed a gluten-free diet have lower leukocyte adhesion, macrophage infiltration and pro-inflammatory cytokine production, e.g., MCP-1/CCL-2, TNF-α and IL-6. These results are consistent with a gliadin-macrophage interaction, which triggers an inflammatory response, inducing the expression and production of pro-inflammatory cytokines [25].

Several studies have highlighted the relationship between excess adiposity, inflammation and insulin resistance [11,26–28]. Macrophage infiltration is correlated with an increase in adiposity [27], especially at the visceral site [28]. The expansion of visceral adipose tissue releases chemokines involved in macrophage recruitment [11,29]. Both activated macrophages and hypertrophic adipocytes are able to produce large amount adipokines, which act negatively on insulin signalling [9]. Our results confirm the macrophage-adipocyte connection because the exclusion of gluten reduced adipocyte size, leukocyte adhesion and macrophage infiltration in adipose tissue, resulting in lower concentrations of pro-inflammatory cytokines and adipokines, including TNF-α, IL-6, leptin and resistin, which are known to induce IR [7,29–34].

In addition to adiposity, the gluten-free diet played an important role in reducing insulin resistance by increasing the expression of PPAR-γ, insulin receptor, GLUT-4 and adiponectin [33–35]. PPAR-γ is abundantly expressed in adipose tissue, and it is the target of anti-diabetic drugs, such as the glitazones. PPAR-γ agonists improve insulin sensitivity and glucose metabolism, reduce inflammation and promote the differentiation of pre-adipocytes into adipocytes [24,36], resulting in smaller and more insulin-sensitive adipocytes [31].

It is known that PPAR-γ stimulates GLUT-4 and adiponectin gene expression in adipose tissue [33,37]. In contrast, TNF-α is related to the down-regulation and degradation of PPAR-γ [29]. Therefore, our data suggest that the reduction of TNF-α production after gluten exclusion leads to increased PPAR-γ levels, triggering the higher expression of adiponectin and GLUT-4, which contributes to the improvement in insulin sensitivity in the GF group.

It has been previously demonstrated that gluten products may accumulate in the lysosomes of intestinal cells, leading to metabolic reactions that culminate in the proteosomal degradation of PPAR-γ [38]. This may represent another mechanism by which gluten exclusion attenuates inflammation and glucose homeostasis.

Taken together, our data suggest that the improvement in glucose homeostasis noted with the gluten-free diet primarily involves a reduction of the inflammatory profile, leading to an increase in the transcription of PPAR-γ and the metabolic modulation linked to PPAR-γ viability.

In conclusion, our data show that the removal of wheat gluten from the diet exerts a protective effect against body weight and adiposity gains through, at least in part, by provoke lipid mobilization and oxidation in adipose tissue, causing a lower accumulation of lipid. In addition, a gluten-free diet improves insulin sensitivity through the suppression of the inflammatory profile, leading to an increase in PPAR-γ expression that culminates in systemic increased of adiponectin levels and adipose GLUT-4 expression concomitant with reduced resistin concentrations. Our data support the beneficial effects of gluten exclusion in reducing body weight and adiposity gain, inflammation and insulin resistance.

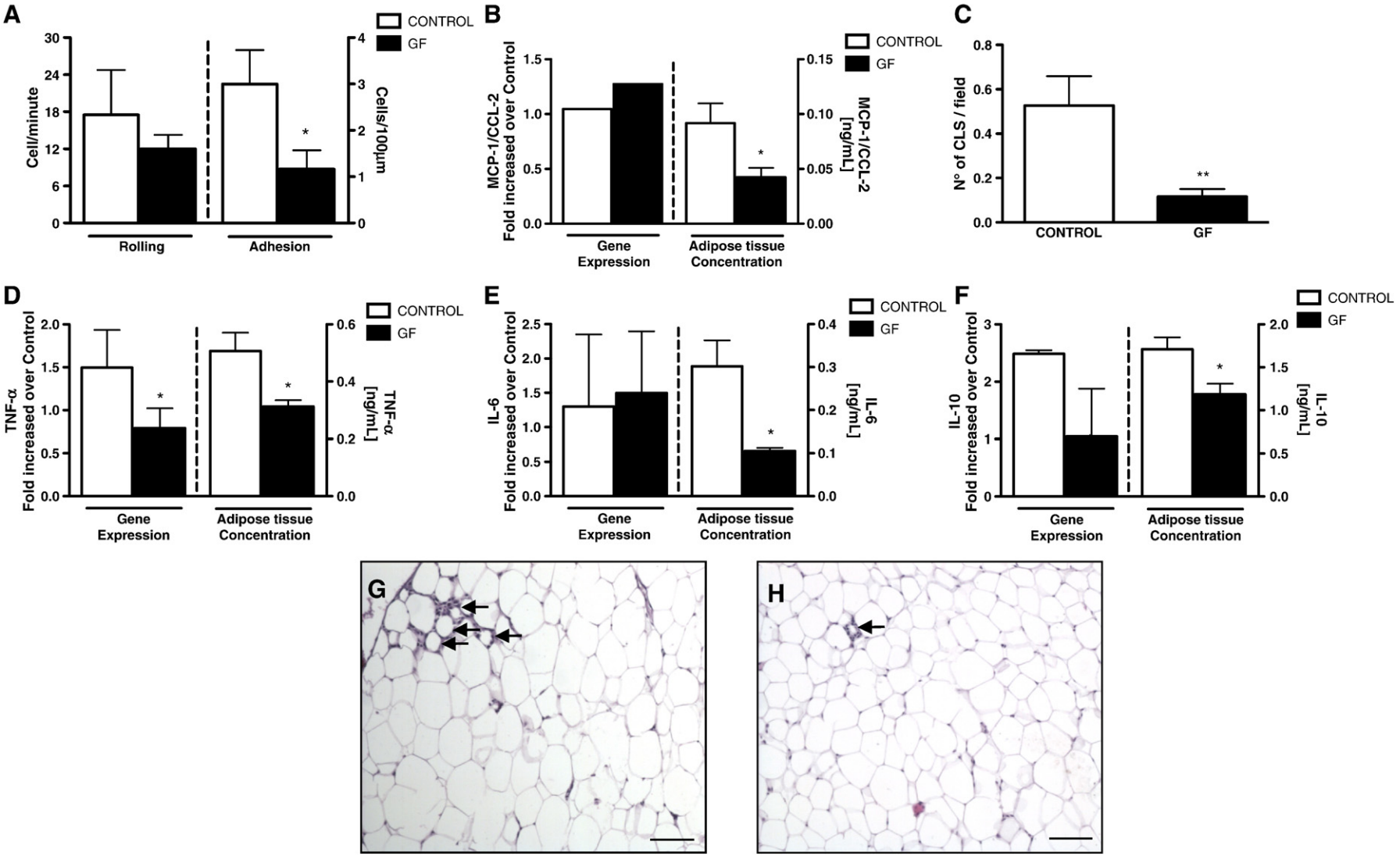

Fig. 5. Measurements from the epididymal adipose tissue of C57BL/6 mice fed a high-fat diet containing 4.5% wheat gluten (control group) or a high-fat diet without gluten (GF group) for 8 weeks. (A) Leukocyte rolling and adhesion in the microcirculation. (B) Gene expression and concentration of MCP-1/CCL-2. (C) Macrophage-forming crown-like structures (CLS). Gene expression and concentration of (D) TNF-α, (E) IL-6, (F) IL-10 and histology of the control (G) and gluten-free (H) groups. The arrows indicate the CLS; horizontal bars=100 µm. n=3–7/group (A, C, D, E, F), 18–19/group (B). The bars represent the mean, and the vertical lines represent the S.E.M. (for MCP-1/CCL-2, TNF-α, IL-6 and IL-10 expression, the bars represent the median, and the lines represent the interquartile range). *P≤.05; **P<.01.

Supplementary materials related to this article can be found online at http://dx.doi.org/10.1016/j.jnutbio.2012.08.009.

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References

[1] Green PH, Cellier C. Celiac disease. N Engl J Med 2007;357(17):1731-43.
[2] Hafstrom I, Ringertz B, Spangberg A, von Zweigbergk L, Brannemark S, Nylander I, et al. A vegan diet free of gluten improves the signs and symptoms of rheumatoid arthritis: the effects on arthritis correlate with a reduction in antibodies to food antigens. Rheumatology (Oxford) 2001;40(10):1175-9.
[3] Ejsing-Duun M, Josephsen J, Aasted B, Buschard K, Hansen AK. Dietary gluten reduces the number of intestinal regulatory T cells in mice. Scand J Immunol 2008;67(6):553-9.
[4] Mojibian M, Chakir H, Lefebvre DE, Crookshank JA, Sonier B, Keely E, et al. Diabetes-specific HLA-DR-restricted proinflammatory T-cell response to wheat polypeptides in tissue transglutaminase antibody-negative patients with type 1 diabetes. Diabetes 2009;58(8):1789-96.
[5] Pastore M-R, Bazzigaluppi E, Belloni C, Arcovio C, Bonifacio E, Bosi E. Six months of gluten-free diet do not influence autoantibody titers, but improve insulin secretion in subjects at high risk for type 1 diabetes. J Clin Endocrinol Metab 2003;88(1):162-5.
[6] Jönsson T, Olsson S, Ahrén B, Bøg-hansen TC, Dole A, Lindeberg S. Agrarian diet and diseases of affluence — do evolutionary novel dietary lectins cause leptin resistence? BMC Endocrine Disorders 2005;5(10):1-7.
[7] Galic S, Oakhill JS, Steinberg GR. Adipose tissue as an endocrine organ. Mol Cell Endocrinol 2010;316(2):129-39.
[8] WHO. World Health Organization. Obesity. [cited 2009 21/12]; Available from: http://www.who.int/topics/obesity/en/2009.
[9] Hajer GR, van Haeften TW, Visseren FL. Adipose tissue dysfunction in obesity, diabetes, and vascular diseases. Eur Heart J 2008;29(24):2959-71.
[10] Kim CS, Lee SC, Kim YM, Kim BS, Choi HS, Kawada T, et al. Visceral fat accumulation induced by a high-fat diet causes the atrophy of mesenteric lymph nodes in obese mice. Obesity (Silver Spring) 2008;16(6):1261-9.
[11] Lee HS, Park JH, Kang JH, Kawada T, Yu R, Han IS. Chemokine and chemokine receptor gene expression in the mesenteric adipose tissue of KKAy mice. Cytokine 2009;46(2):160-5.
[12] Akagiri S, Naito Y, Ichikawa H, Mizushima K, Takagi T, Handa O, et al. A mouse model of metabolic syndrome; increase in visceral adipose tissue precedes the development of fatty liver and insulin resistance in high-fat diet-fed male KK/Ta mice. J Clin Biochem Nutr 2008;42(2):150-7.
[13] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193(1):265-75.
[14] Santos SH, Fernandes LR, Mario EG, Ferreira AV, Porto LC, Alvarez-Leite JI, et al. Mas deficiency in FVB/N mice produces marked changes in lipid and glycemic metabolism. Diabetes 2008;57(2):340-7.
[15] Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28(7):412-9.
[16] Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 1957;226(1):497-509.
[17] Dourado LP, Noviello MD, Alvarenga DM, Menezes Z, Perez DA, Batista NV, et al. Experimental food allergy leads to adipose tissue inflammation, systemic metabolic alterations and weight loss in mice. Cellular Immunology 2011;270(2):198-206.
[18] Heneghan HM, Miller N, McAnena OJ, O'Brien T, Kerin MJ. Differential miRNA expression in omental adipose tissue and in the circulation of obese patients identifies novel metabolic biomarkers. J Clin Endocrinol Metab 2011;96(5):E846-50.
[19] Ruby MA, Goldenson B, Orasanu G, Johnston TP, Plutzky J, Krauss RM. VLDL hydrolysis by LPL activates PPAR-alpha through generation of unbound fatty acids. J Lipid Res 2010;51(8):2275-81.
[20] Maury E, Brichard SM. Adipokine dysregulation, adipose tissue inflammation and metabolic syndrome. Mol Cell Endocrinol 2010;314(1):1–16.
[21] Lee JY, Hashizaki H, Goto T, Sakamoto T, Takahashi N, Kawada T. Activation of peroxisome proliferator-activated receptor-alpha enhances fatty acid oxidation in human adipocytes. Biochem Biophys Res Commun 2011;407(4):818-22.
[22] Furuhashi M, Ura N, Murakami H, Hyakukoku M, Yamaguchi K, Higashiura K, et al. Fenofibrate improves insulin sensitivity in connection with intramuscular lipid content, muscle fatty acid-binding protein, and beta-oxidation in skeletal muscle. J Endocrinol 2002;174(2):321-9.
[23] Young ME, Goodwin GW, Ying J, Guthrie P, Wilson CR, Laws FA, et al. Regulation of cardiac and skeletal muscle malonyl-CoA decarboxylase by fatty acids. Am J Physiol Endocrinol Metab 2001;280(3):E471-9.
[24] Abranches MV, Oliveira FCE, Bressan J. Peroxisome proliferator-activated receptor: effects on nutritional homeostasis, obesity and diabetes mellitus. Nutr Hosp 2011;26(2):271-9.
[25] Thomas KE, Sapone A, Fasano A, Vogel SN. Gliadin stimulation of murine macrophage inflammatory gene expression and intestinal permeability are MyD88-dependent: role of the innate immune response in Celiac disease. J Immunol 2006;176(4):2512-21.
[26] Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, et al. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J Clin Invest 2003;112(12):1821-30.
[27] Coenen KR, Gruen ML, Chait A, Hasty AH. Diet-induced increases in adiposity, but not plasma lipids, promote macrophage infiltration into white adipose tissue. Diabetes 2007;56(3):564-73.
[28] Murano I, Barbatelli G, Parisani V, Latini C, Muzzonigro G, Castellucci M, et al. Dead adipocytes, detected as crown-like structures, are prevalent in visceral fat depots of genetically obese mice. J Lipid Res 2008;49(7):1562-8.
[29] Guilherme A, Virbasius JV, Puri V, Czech MP. Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. Nat Rev Mol Cell Biol 2008;9(5):367-77.
[30] Hennige AM, Stefan N, Kapp K, Lehmann R, Weigert C, Beck A, et al. Leptin down-regulates insulin action through phosphorylation of serine-318 in insulin receptor substrate 1. FASEB J 2006;20(8):1206-8.
[31] Kang J-H, Tsuyoshi G, Han I-S, Kawada T, Kim YM, Yu R. Dietary capsaicin reduces obesity-induced insulin resistance and hepatic steatosis in obese mice fed a high-fat diet. Obesity 2010;18(4):780-7.
[32] Lago F, Dieguez C, Gomez-Reino J, Gualillo O. The emerging role of adipokines as mediators of inflammation and immune responses. Cytokine Growth Factor Rev 2007;18(3–4):313-25.
[33] Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. Nat Rev Immunol 2011;11(2):85-97.
[34] Zou C, Shao J. Role of adipocytokines in obesity-associated insulin resistance. J Nutr Biochem 2008;19(5):277-86.
[35] Lago R, Gomez R, Lago F, Gomez-Reino J, Gualillo O. Leptin beyond body weight regulation — current concepts concerning its role in immune function and inflammation. Cell Immunol 2008;252(1–2):139-45.
[36] Martin H. Role of PPAR-gamma in inflammation. Prospects for therapeutic intervention by food components Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis 2010;669(1–2):57-63.
[37] Wu Z, Xie Y, Morrinson RF, Bucher NLR, Farmer SR. PPARgamma induces the insulin-dependent glucose transporter GLUT4 in the absence of C/EBPalpha during the conversion of 3T3 fibroblasts Into adipocytes. J Clin Invest 1998;101(1):22-32.
[38] Maiuri L, Luciani A, Villella VR, Vasaturo A, Giardino I, Pettoello-Mantovani M, et al. Lysosomal accumulation of gliadin p31-43 peptide induces oxidative stress and tissue transglutaminase-mediated PPARgamma downregulation in intestinal epithelial cells and coeliac mucosa. Gut 2010;59(3):311-9.