Available online at www.sciencedirect.com

ScienceDirect

Journal of Nutritional Biochemistry 48 (2017) 74–82

Journal of Nutritional Biochemistry

Obesity and malnutrition similarly alter the renin–angiotensin system and inflammation in mice and human adipose☆,☆☆

Thales de Almeida Pinheiro[a,b,1], Antônio Sérgio Barcala-Jorge[a,1], João Marcus Oliveira Andrade[a,c], Thaisa de Almeida Pinheiro[a,b], Emíllio César Neves Ferreira[b], Thaisa Soares Crespo[a], Gislaine Candida Batista-Jorge[a], Cássio André Vieira[a], Deborah de Farias Lelis[a], Alanna Fernandes Paraíso[a], Ugo Borges Pinheiro[a], Mariane Bertagnolli[d], Carlos Juliano Brant Albuquerque[e], André Luiz Sena Guimarães[a], Alfredo Mauricio Batista de Paula[a], Antônio Prates Caldeira[a,b], Sérgio Henrique Sousa Santos[a,e,*]

[a]*Laboratory of Health Science, Postgraduate Program in Health Sciences, Universidade Estadual de Montes Claros (Unimontes), Montes Claros, MG, Brazil*
[b]*Integrated Colleges Pythagorean of the Montes Claros (FIP), Montes Claros, Minas Gerais, Brazil*
[c]*Faculdades Santo Agostinho - FASA, Montes Claros, MG, Brazil*
[d]*Sainte-Justine University Hospital Research Center, Montreal, QC, Canada*
[e]*Institute of Agricultural Science. Food Engineering College, Universidade Federal de Minas Gerais (UFMG), Montes Claros, MG, Brazil*

Received 15 March 2017; received in revised form 9 June 2017; accepted 19 June 2017

Abstract

The main goal of the present study was to evaluate the metabolic profile, inflammatory markers and the gene expression of the renin–angiotensin system (RAS) components in the visceral adipose tissue of eutrophic, obese and malnourished individuals and mice models of obesity and food restriction. Male Swiss mice were divided into eight groups and fed different levels of food restriction (20%, 40%, or 60%) using standard or high-fat diet. Metabolic profile and adipose tissues were assessed. The expression of AGT (Angiotensinogen), ACE (Angiotensin-converting enzyme), ACE2 (Angiotensin-converting enzyme 2), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) in the mice epididymal adipose tissue and the human visceral adipose tissue was assessed. The main findings showed reduced body weight, improved metabolism, decreased adipose tissues weight and reduced adipocyte area in mice submitted to food restriction. Diminished expression of IL-6, TNF-α, AGT, AT1 and ACE was detected in the 20% and 40% food restriction animal groups, although they were increased in the 60% malnourished group. Increased expression of IL-6, TNF-α, AGT and ACE in obese and malnourished individuals was observed. Adipocytes size was increased in obese individuals and reduced in malnutrition. In conclusion, we found that food restriction of 20% and 40% improved the metabolic profile, ameliorated the inflammatory status and down-regulated the RAS in mice. Severe 60% food restriction (malnutrition), however, stimulated a proinflammatory state and increased AGT and ACE expression in the adipose tissue of mice. A similar profile was observed in the adipose tissue of obese and malnourished humans, supporting the critical role of inflammation and RAS as mediators of metabolic disorders.

Keywords: Food restriction; Malnutrition; Obesity; Inflammatory mediators; Renin–angiotensin system

☆ Conflict of interests: The authors have nothing to disclose.

☆☆ Funding: This work was supported by grants from the Coordenadoria de Aperfeiçoamento do Pessoal de Nível Superior, Conselho Nacional de Desenvolvimento Científico e Tecnológico, Fundação de Amparo à Pesquisa de Minas Gerais, and Faculdades Integradas Pitágoras, Montes Claros, Montes Claros, Minas Gerais, Brazil.

* Corresponding author at: Instituteof Agricultural Sciences, Food Engineering College, Universidade Federal de Minas Gerais (UFMG), Avenida Universitária, 1.000 – Universitário, 39.404-547, Montes Claros, MG, Brazil. Tel.: +55 38 32248327.

E-mail addresses: thalesalmeidap@yahoo.com.br (T.A. Pinheiro), antoniosergiobjorge@gmail.com (A.S. Barcala-Jorge), joao_marcus13@hotmail.com (J.M.O. Andrade), thaisafarma@yahoo.com.br (T.A. Pinheiro), emillyosalinas@yahoo.com.br (E.C.N. Ferreira), thaisacrespo@yahoo.com.br (T.S. Crespo), gislainejorge@bol.com.br (G.C. Batista-Jorge), cassiocir@yahoo.com.br (C.A. Vieira), dehlelisfarias@gmail.com (D.F. Lelis), alannaenf1989@hotmail.com (A.F. Paraíso), borgespinheiro@gmail.com (U.B. Pinheiro), mariane.b@gmail.com (M. Bertagnolli), carlosjulianobrant@gmail.com (C.J.B. Albuquerque), andreluizguimaraes@gmail.com (A.L.S. Guimarães), ambpatologi@gmail.com (A.M.B. de Paula), antoniop@fip-moc.edu.br (A.P. Caldeira), sergiosousas@hotmail.com (S.H.S. Santos).

[1] Equally contributed to this study.

http://dx.doi.org/10.1016/j.jnutbio.2017.06.008
0955-2863/

1. Introduction

The increasing prevalence of obesity [1,2] is a matter of great concern worldwide. Obesity is considered a risk factor for cardiovascular diseases, and it is one of the key features of the metabolic syndrome (MetS) [3]. The main comorbidities of MetS include hypertension, dyslipidemia, stroke, type 2 diabetes mellitus and some types of cancer [4]. Obesity is characterized by the accumulation of body fat resulting from an imbalance between food intake and energy expenditure [3,5]. Recently, obesity has been described as a proinflammatory state associated with elevation of tissue and circulating levels of proinflammatory enzymes, procoagulant factors, cytokines and chemokines, demonstrating that the adipose tissue modulates not only its biology but also the reproductive and endocrine systems, immunity, inflammation and insulin sensitivity [6]. There are evidence suggesting that the white adipose tissue (WAT) becomes hypertrophied due to macrophage infiltration that secretes proinflammatory cytokines, including tumor necrosis factor-alpha (TNF-α) and some interleukins, such as interleukin-6 (IL-6) [7].

A growing number of studies describe the importance of the renin–angiotensin system (RAS) in regulating the metabolism and the development of cardiovascular and inflammatory diseases [8–10]. Various components of the RAS have been identified in the adipose tissue [11]. Recent studies have shown that RAS significantly modulates the metabolism and endocrine function of adipocytes [12]. Further RAS components may be modulated according to different obesity degrees [13].

Caloric restriction, on the other hand, is characterized by a low-caloric diet regimen without causing malnutrition. Experimental studies in rodents and rhesus monkeys showed that partial caloric restriction increases longevity and prevents or delays the occurrence of chronic diseases such as diabetes, atherosclerosis, cardiomyopathy, tumors, autoimmune diseases, and renal and respiratory problems [14–19]. Some evidence have shown that the maximum positive effect occurs with restrictions of 55% to 60% compared to baseline intake [20,21]. Clinical studies further showed that weight loss diminishes the inflammatory status in obesity and subsequent comorbidities by decreasing the number of circulating inflammatory molecules [22]. However, a significant loss in body weight can induce a state of malnutrition. Malnourished individuals may present inflammatory, hypermetabolic and hypercatabolic conditions, in addition to reduced albumin levels [23–26].

We, therefore, hypothesize that different levels of food restriction, associated or not with a high-fat diet, may differently modulate adipose tissue inflammatory state and RAS regulation, preventing metabolic alterations as observed in obesity. Thus, the purpose of the present study was to evaluate the metabolic profile and expression of inflammatory markers and RAS components in adipose tissue of mice submitted to different food restriction degrees and to validate such profile in the visceral adipose tissue of eutrophic, obese and malnourished humans.

2. Methods

2.1. Animal study

The experiment was conducted with 64 Swiss mice (male, 4 weeks old) divided into 8 groups (n=8 each) and fed with the following respective experimental diets for 8 weeks. The groups were divided into the following: standard diet (ST) *ad libitum*, ST-20% food restriction, ST-40% food restriction, ST-60% food restriction, high-fat diet (HFD) *ad libitum*, HFD-20% food restriction, HFD-40% food restriction and HFD-60% food restriction. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution (CEEBEA - Universidade Estadual de Montes Claros). The animals were maintained under controlled light and temperature conditions.

2.2. Food restriction protocol and diet

The mice groups fed with standard and high-fat diet given *ad libitum* had their food intake measured on a daily basis. From the food intake of these groups, the food restriction of 20%, 40% and 60% was then calculated. Standard diet (Purina; Labina) used for regular mice maintenance is composed of 66% carbohydrate, 23% protein and 11% fat, representing a total of 3.95 kcal per 1 g of diet. The high-fat diet was composed of cornstarch (40.57%), casein (14%), dextrinized starch (15.5%), sucrose (10%), soybean oil (10%), cellulose fiber (5%), mineral mix AIN-93M (3.5%), vitamin mix AIN-93 (1%), L-cysteine (0.18%), choline bitartrate (0.25%) and *tert*-butylhydroquinone (0.0008%), (24% of carbohydrate, 15% of protein, and 61% of fat), representing a total of 5.28 kcal per 1 g of diet [27]. The high-fat diet was prepared according to the standards of the Official Analytical Chemists Association as described previously [28,29]. All of the high-fat diet components were purchased from Rhoster LTDA (São Paulo, SP, Brazil).

2.3. Glucose tolerance and insulin sensitivity tests (GTT and IST)

For the GTT, D-glucose (2 mg/g of body weight) was intraperitoneally injected into overnight-fasted mice. Glucose levels from tail blood samples were monitored at 0, 15, 30, 60 and 120 min after injection. ISTs were performed with the animals in the fed state after intraperitoneal injection of insulin (0.75 U/kg body weight), where tail's blood samples were taken at the time points 0, 15, 30 and 60 min after injection for the measurement of blood glucose levels.

2.4. Measurements of body weight, food intake and tissue collection

Food intake and body weight in mice were measured every day during the treatment period. Overnight-fasted mice were killed by decapitation and blood, and WAT (epididymal, retroperitoneal and mesenteric) samples were collected, weighed and frozen in dry ice and stored at −80°C for posterior analysis.

2.5. Human study

Samples of the visceral adipose tissue were collected from patients divided into the following groups: control group (eutrophic patients group), obese patients group and malnourished patients group. The control group was composed of adult patients, clinically healthy, who submitted to abdominal surgical procedures due to esthetic reasons. The obese patients were selected during the screening procedure for bariatric surgery. The bariatric procedures consisted of Roux-en-Y gastric bypass, and the biopsies of the WAT were performed during surgery. Malnourished patients (being excluded patients with cancer in this study) composed the third group. All malnourished patients were categorized using previous criteria. The biopsies were obtained during gastrointestinal surgery. During the surgeries, samples of visceral WAT and serum were collected, immediately frozen and stored at −80°C. Additional information of the individuals is presented in Table 1. All procedures performed in studies involving human participants were in agreement with the ethical standards of the institutional committee (Plataforma Brazil, 85742/13-07-2012) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

Table 1
Characterization of the individuals included in the study

	Eutrophic	Obese	Malnourished
Age (mean/S.D.)	50.01±16.42	41.55±12.44	72.14±8.03
Sex (*n*)			
Female	11	18	3
Male	8	3	11
Marital status (*n*)			
Married	12	13	3
Single	6	4	1
Other	2	4	9

2.6. Determination of blood measurements

Serum samples were obtained after centrifugation (4000×*g* for 7 min at 4°C). Total serum cholesterol, triglycerides, high-density protein (HDL), glucose and albumin were assayed using enzymatic kits (Wiener, Argentina). Measurements were made in Wiener BT-3000 Plus Chemistry Analyzer (Wiener, Argentina).

2.7. Reverse transcription and real-time polymerase chain reaction (RT-PCR)

Total RNA from the epididymal adipose tissue (Swiss mice) and visceral adipose tissue (human) samples was prepared using TRIzol reagent (Invitrogen Corp., San Diego, CA, USA), treated with DNAse and reverse transcribed with M-MLV (Invitrogen Corp.) using random hexamer primers. Levels of AGT, ACE, ACE2, IL-6 (Interleukin 6) and TNF-α mRNA were determined by RT-PCR using SYBR Green reagent (Applied Biosystems, USA) in a PlusOne platform (Applied Biosystems), with the following primers for mice: AGT, ACE, ACE2, AT1, IL-6, TNF-α and the endogenous GAPDH and β-actin. The respective primers sequences are found in the Supplementary Data. The relative comparative CT method of Livak and Schmittgen [30] was applied to compare gene expression levels between groups using the equation $2^{-\Delta\Delta CT}$.

2.8. Hematoxylin and eosin staining

Epididymal adipose tissue samples from mice and visceral adipose tissue samples from humans were fixed in 10% neutral-buffered formalin at 4°C overnight; dehydrated through a graded alcohol series, xylene and paraffin; and then embedded in paraffin. Sections of 5 μm were prepared for H&E. Images (×10 ocular and ×40 objective lenses) were captured with Evolution LC Color light camera (Media Cybernetics, USA). A total area of 1.84 mm^2, containing at least 100 fat cells for each sample, was measured using the Image-Pro Plus Software (Media Cybernetics, Rockville, MD, USA).

2.9. Statistical analysis

All data were transferred to *GraphPad* Prism software (Version 5.0, San Diego, California, USA) and analyzed with 95% confidence ($P<.05$). Data are expressed as the mean±SEM. One-way analysis of variance (ANOVA) followed by Bonferroni posttest assessed the statistical significance of the differences in mean values between the mice and human groups. Two-way ANOVA tests followed by Bonferroni posttest were applied to the data obtained from GST and IST.

3. Results

Food intake (Fig. 1A); energy intake (Fig. 1B); the body weight (Fig. 1C and D); epididymal (Fig. 1E), retroperitoneal (Fig. 1F) and mesenteric (Fig. 1G) WAT weights; and adipocyte area (Fig. 1H and I) were reduced in mice fed a standard diet and submitted to food restriction. For mice fed a high-fat diet, food intake; body weight; epididymal, retroperitoneal and mesenteric adipose tissues weight; and adipocyte surface area were also decreased in all groups under restriction as compared to control (Fig. 2A–I).

The blood glucose levels were reduced in all groups submitted to different levels of food restriction as compared to ST group (Fig. 3A and B). Also, a significant reduction of the blood glucose levels was observed in the 60% food-restricted group compared to the group with 20% of food restriction. In mice fed a high-fat diet, the IST evidenced reduced blood glucose levels in the HFD-40% and HFD-60% groups as compared to HFD (Fig. 3C). The GST (Fig. 3D) showed a reduction of the glucose levels in all restricted groups when compared to its respective control group (HFD).

The glucose and triglyceride levels were shown to be reduced in all restricted groups as compared to ST (Table 2). Additionally, a significant reduction was observed in the ST-60% group in relation to its control group for total cholesterol and HDL-cholesterol. The albumin levels were also decreased in the ST-60% FR group as compared to ST. For the high-fat-fed mice, plasma analysis also showed reduced glucose levels in all restricted groups as compared to HFD (Table 2). Total cholesterol was reduced in the groups HFD-40% and HFD-60%. Triglycerides, and albumin levels were also reduced in HFD-60% when compared to control (HFD). The biochemical parameters of the human individuals included in the study are fully described on Table 3. No statistical differences were observed between groups.

Quantitative RT-PCR analysis showed decreased expression of AGT and ACE in the ST-20% and ST-40% groups *versus* control ST mice and no difference between ST-60% and ST, although a significant increase in the ST-60% group as compared to ST-40% was observed (Fig. 4A and B). No significant differences in ACE2 and AT1 mRNA expression were observed between groups (Fig. 4C and D).

The expression levels of IL-6 and TNF-a were decreased in ST-40% FR mice as compared to ST (Fig. 4E and F), although no significant difference was observed between the ST-60% and ST groups, and a significant increase was found in IL-6 in ST-60% as compared to ST-40%.

For the high-fat-fed mice, the AGT expression levels were decreased in HFD-40% FR as compared to control (HFD) (Fig. 4G) and increased in HFD-60% as compared to HFD-20% and HFD-40%. The ACE expression levels (Fig. 4H) were significantly reduced in all restricted groups as compared to HFD. In contrast, no significant differences were found for the ACE2 expression levels between groups (Fig. 4I). The mRNA levels of AT1 receptor were increased in the HFD and HFD-60%FR groups as compared to ST (Fig. 4J). The inflammatory markers' expression showed decreased IL-6 expression in the HFD-40% group as compared to HFD mice, and no significant difference between HFD-60% and HFD was evidenced. Additionally, increased expression levels of IL- 6 in the HFD-60% FR group as compared to HFD-40% FR group were found (Fig. 4K). For the TNF-α expression, a significant decrease was found in HFD-20% and HFD-40% groups as compared to HFD (Fig. 4L).

For humans, expression of AGT (Fig. 5A) and ACE mRNA (Fig. 5B) was significantly higher in obese and malnourished individuals as compared to the eutrophic. For the ACE2 expression, no significant difference was observed between groups (Fig. 5C). Expression levels of IL-6 (Fig. 5D) and TNF-α (Fig. 5E) were significantly higher in obese and malnourished as compared to eutrophic individuals. In addition, it was observed that the adipocytes from obese individuals were significantly increased compared to eutrophic individuals, whereas in malnourished individuals, adipocyte area is significantly decreased compared to eutrophic and obese individuals (Fig. 5F and G).

4. Discussion

This study describes for the first time that although mild to moderate (20%–40%) restriction can improve the metabolic and

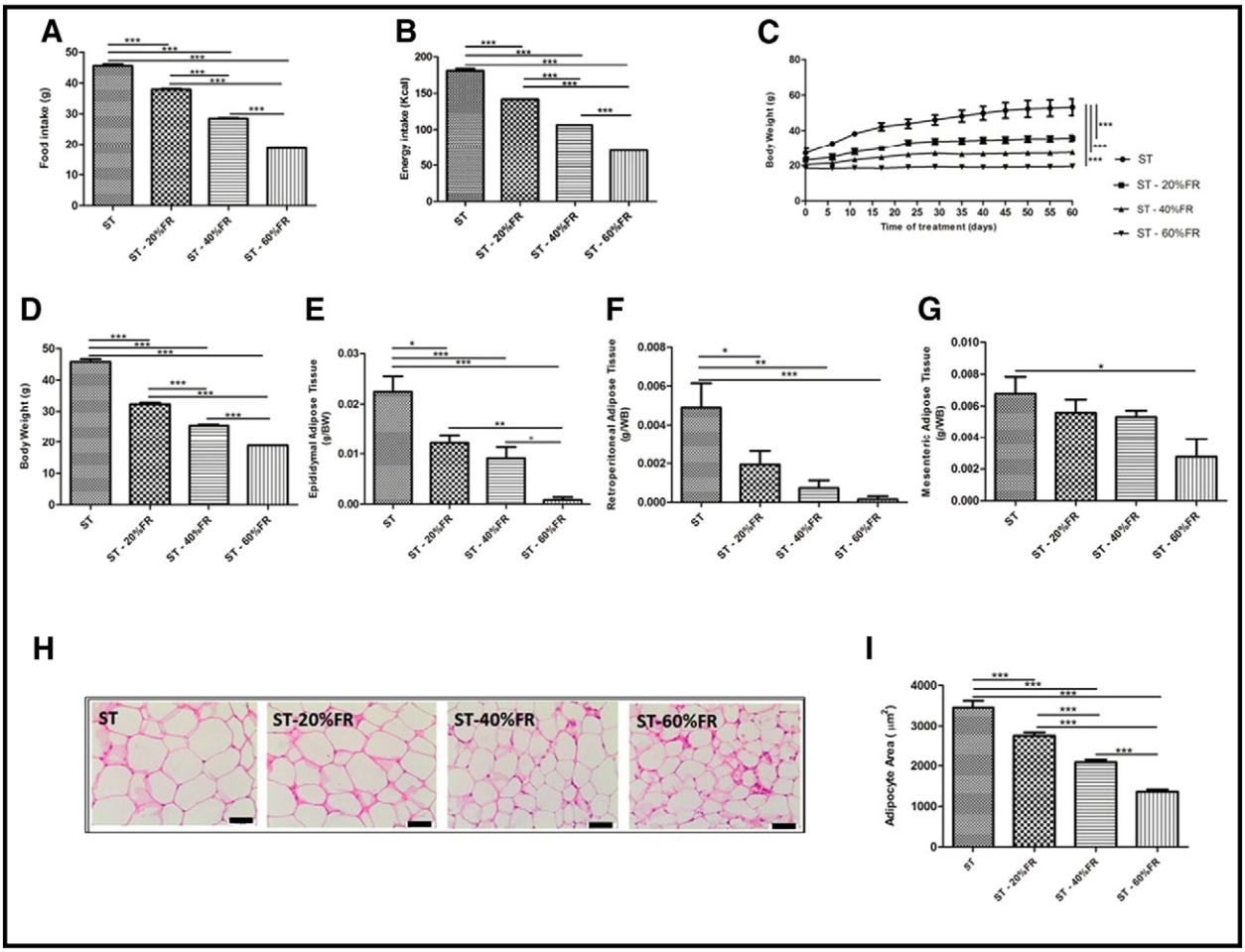

Fig. 1. Body and lipid profile of standard-diet-fed mice. Food intake, energy intake, body weight, fat weight, adipocyte area and hematoxylin/eosin staining in mice subjected to food restriction standard diet (ST, ST-20%FR, ST-40%FR and ST-60%FR). Food intake (A), energy intake (B), daily body weight (C), total body weight (D), epididymal adipose tissue weight (E), retroperitoneal adipose tissue weight (F), mesenteric adipose tissue weight (G), hematoxylin and eosin staining (H) and epididymal adipocyte area (I). Scale bar indicates 50 μm (h). Data are presented as mean±S.E.M.; *P<.05, **P<.01, ***P<.001 *versus* group indicated.

inflammatory status, a more severe restriction (60%) does not present the same protective effect in mice. Also, decreased inflammatory status in mild to moderate restriction in mice was associated with down-regulation of key RAS components, such as AGT and ACE in the adipose tissue. Interestingly, we found a similar profile in obese and malnourished humans, also presenting impaired metabolic and inflammatory profiles associated with increased AGT and ACE compared to eutrophic individuals. These results suggest that an enhanced inflammation in the adipose tissue is associated with RAS up-regulation which may significantly contribute to establishing metabolic disorders as observed in obesity and malnutrition states, and mild to moderate food restriction prevents those metabolic changes potentially through down-regulating these key mechanisms.

The decrease of the body weight in different groups of mice submitted to different levels of food restriction under standard or high-fat diets can be associated with a reduction of food intake imposed on those animals. Several studies have shown that body weight is associated with food intake [31,32] and that the imbalance between food intake and energy expenditure is a predisposing factor to obesity [3,5]. The weight reduction of epididymal, retroperitoneal and mesenteric adipose tissues with increased food restriction may be related to the progressive reduction of food intake imposed on the animals. There are many pieces of evidence in the literature demonstrating that food restriction decreases the accumulation of body fat [17,27,32] independently of the diet composition [31]. The reduction of the adipose tissue weight may also be associated with the hypertrophy process that took place since we observed a significant reduction in the area of adipocytes according to the level of food restriction, under treatment with either a standard or high-fat diet [33,34].

The results of the ISTs and GSTs showed beneficial results with the gradual increase of food restriction on standard and high-fat diets. Similar results were also found in the levels of fasting glucose, corroborating with several published studies [16,27,31,32]. Interestingly, the glucose levels of ST animals at time 0 of the tests were significantly high as compared to treatment groups, which may be explained by the energy supply that the food-restricted animals present in contrast with the ST group. Regarding the lipid profile, reduced levels of total cholesterol, HDL-cholesterol and triglycerides were found with the gradual increase of food restriction, corroborating with the results from other studies [31,34]. According to the "Consensus Statement of the Academy of Nutrition and Dietetics/American Society for Parenteral and Enteral Nutrition," malnutrition may be related to low albumin plasma levels which were observed in both groups of mice treated with 60% food restriction in both treatments [24].

Experimental studies suggest that the regulation of the RAS is influenced by the obesity degree [13]. Therefore, weight reduction

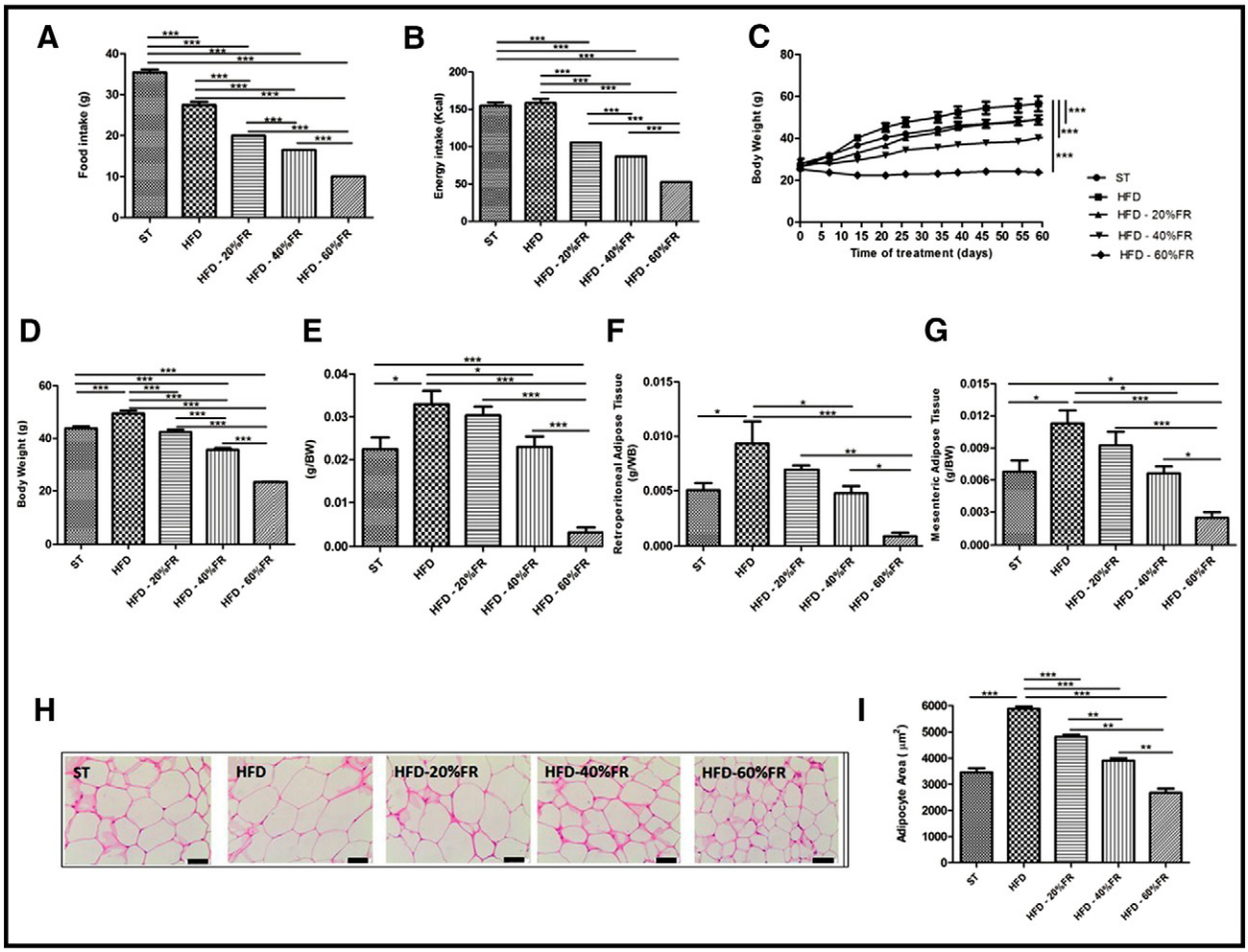

Fig. 2. Body and lipid profile of high-fat diet fed mice. Food intake, energy intake, body weight, fat weight, adipocyte area and hematoxylin/eosin staining in mice subjected to food restriction high-fat diet (ST, HFD, HFD-20%FR, HFD-40%FR, HFD-60%FR). Food intake (A), energy intake (B), daily body weight (C), total body weight (D), epididymal adipose tissue weight (E), retroperitoneal adipose tissue weight (F), mesenteric adipose tissue weight (G), hematoxylin and eosin staining (H) and epididymal adipocyte area (I). Scale bar indicates 50 μm (h). Data are presented as mean±S.E.M.; $^{*}P<.05$, $^{**}P<.01$, $^{***}P<.001$ *versus* group indicated.

induced by food restriction may exert positive effects on RAS regulation. In this work, a decreased expression of AGT and ACE in epididymal adipose tissue of mice with the gradual increase of food restriction of up to 40% of food intake baseline for standard and high-fat diets was evidenced. The present study also showed an AT1 increased expression in the HFD and HFD+60% groups. The AT1 receptor overexpression is directly allied with the Ang II deleterious effects, such as vasoconstriction, salt retention and stimulation of the sympathetic nervous system [35]. However, the AT1 reduction was not observed in the groups submitted to 60% food restriction, which, on the contrary, revealed a significant increase in the expression of this gene when compared to the groups who had reduced food intake by 40% baseline. These results suggest that food restriction of up to 40% over the basal diet, regardless of whether standard or high fat, is associated with improved regulation of the RAS, whereas values of 60% food restriction do not have the same protective effect. These results are coherent with several studies that demonstrate that food restriction can beneficially regulate the RAS on controlling the rise of the plasma renin activity, AGT plasmatic levels and decrease of the ACE activity [12]. Few studies determine the maximum limit of food restriction that animals may be subjected to without causing malnutrition; a study of longevity indicates that this limit stays between 55% and 60%, corroborating the results found in this study [27]. The expression of inflammatory markers in the epididymal adipose tissue decreased with the increase in the food restriction imposed to the animals, which was expected since the fatty tissue of animals decreased along with the food restriction degree [36,37].

Traditionally, the WAT was defined as only an energetic reservoir, but new studies have been considering this tissue as an endocrine organ and the main one responsible for the production of cytokines (adipokines) that act as mediators and regulators of immune and inflammatory responses [4,38,39]. There are several pieces of evidence suggesting that hypertrophied WAT in obesity may be associated with an increase in the infiltration of macrophages that secrete proinflammatory cytokines, such as TNF-α and some interleukins such as IL-6, thus contributing to the inflammatory state established in obesity [7]. This excessive production of proinflammatory cytokines in obese individuals has been associated with cardiometabolic diseases as it alters the balance between the anti- and proinflammatory adipokines in WAT [4,38,39]. The WAT is constituted mainly of adipocytes, although other cell types are essential for its growth and function, including preadipocytes, macrophages, lymphocytes, fibroblasts and vascular cells [4,40]. The accumulation of macrophages in the WAT is proportional to the adiposity in obese individuals and obese animal models, as well as the weight loss results in a sustained reduction in macrophages number in the adipose tissue, which is accompanied by an attenuation of the inflammatory profile [40–42]. Additionally, the macrophages are more abundant in the visceral adipose tissue than in

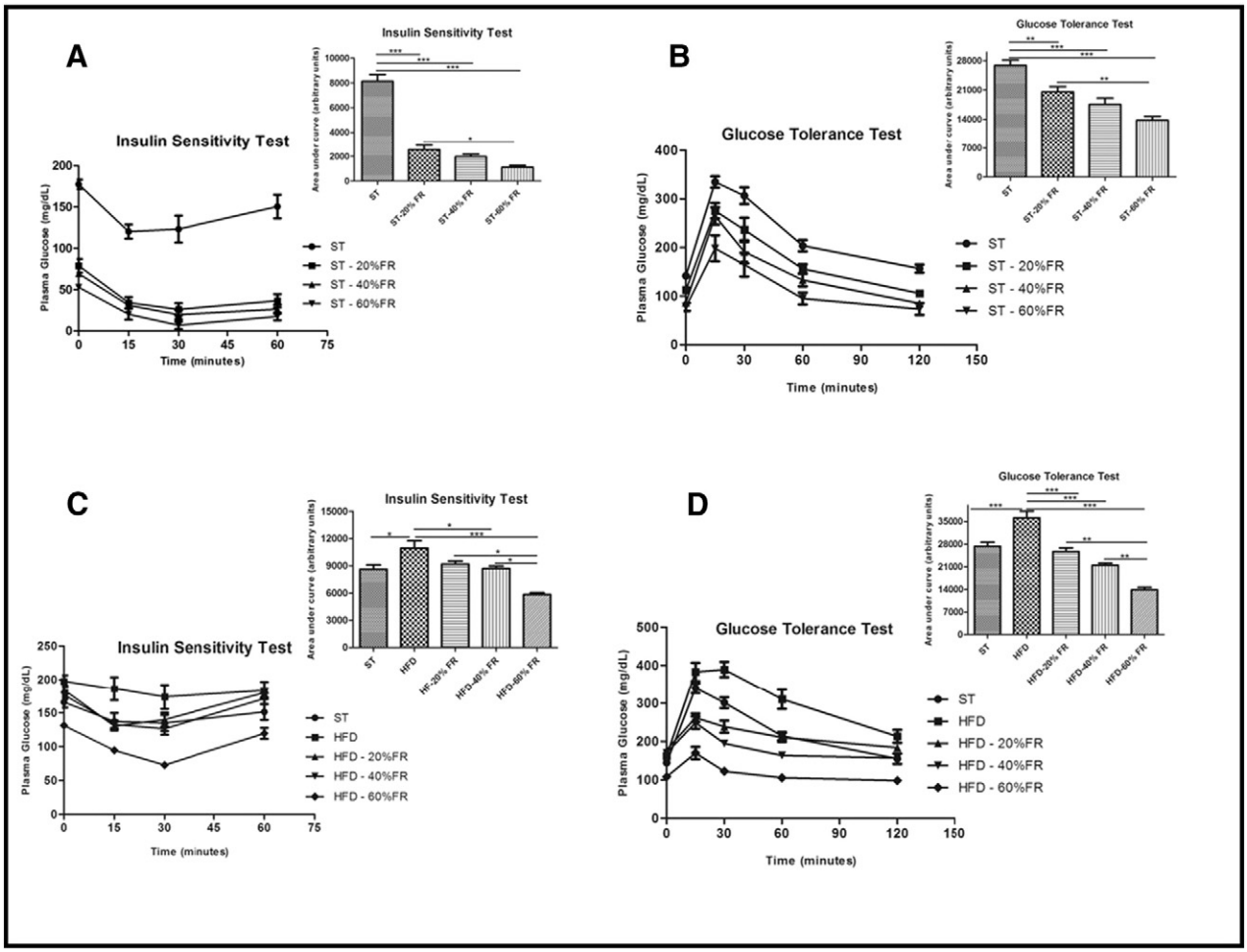

Fig. 3. Glycemic profile of mice fed standard and high-fat diet. ISTs and GSTs in mice subjected to food restriction standard (A and B) and high-fat (C and D) diets. (A) IST between treatment groups (ST-20%FR, ST-40%FR, ST-60%FR) and ST. (B) GST test between treatment groups (ST-20%FR, ST-40%FR, ST-60%FR) and ST. (C) IST between treatment groups (HFD-20%FR, HFD-40%FR, HFD-60%FR) and HFD. (D) GST test between treatment groups (HFD-20%FR, HFD-40%FR, HFD-60%FR) and HFD. Data are presented as mean±S.E.M.; $^{*}P<.05$, $^{**}P<.01$, $^{***}P<.001$ *versus* group indicated.

the subcutaneous, which is associated with a higher risk to develop insulin resistance [43].

Since the animals showed a decrease in body weight and adipose tissue (epididymal, retroperitoneal and mesenteric) with increased food restriction, a decreased expression of inflammatory markers was expected. However, restriction groups of 60% did not have this effect similarly to what is observed in obese and malnourished individuals [24,27].

Table 2
Biochemical parameters in mice subjected to food restriction

	ST	ST-20%FR	ST-40%FR	ST-60%FR
Glucose (mg/dl)	133.6±27.6	81.4±18.2***	62.8±19.12***	35.71±17.2***
Total cholesterol (mg/dl)	110.0±11.7	100.0±9.3	95.8±14.3	82.1±23.2*
HDL (mg/dl)	76.0±12.9	67.8±5.9	64.4±7.4	60.15±6.9*
Triglycerides (mg/dl)	113.9±8.8	82.8±14.7*	62.1±22.1***	49.29±19.7***
Albumin (g/dl)	5.5±0.6	5.7±0.3	5.5±0.3	4.6±0.4*
	HFD	HFD-20%FR	HFD-40%FR	HFD-60%FR
Glucose (mg/dl)	163.9±40.9	101.5±30.2$^{\#\#\#}$	94.5±23.7$^{\#\#\#}$	45.0±15.1$^{\#\#\#}$
Total cholesterol (mg/dl)	137.5±24.4	111.8±37.1	89.4±12.7$^{\#\#}$	83.1±17.7$^{\#\#}$
HDL (mg/dl)	87.0±17.2	76.8±8.4	74.2±8.2	69.4±9.9$^{\#}$
Triglycerides (mg/dl)	118.3±27.8	111.9±32.7	94.2±9.3	75.2±16.4$^{\#\#}$
Albumin (g/dl)	5.8±0.2	5.6±0.1	5.5±0.2	5.1±0.3$^{\#\#\#}$

Plasma glucose, lipid parameters and albumin in mice subjected to food restriction fed standard (ST, ST-20%FR, ST-40%FR, ST-60%FR) and high-fat diets (HFD, HFD-20%FR, HFD-40%FR, HFD-60%FR). Data are presented as mean±S.E.M.; $^{*}P<.05$, $^{**}P<.01$, $^{***}P<.001$. *Between treatment groups (ST-20%FR, ST-40%FR, ST-60%FR) and ST. $^{\#}P<.05$, $^{\#\#}P<.01$, $^{\#\#\#}P<.001$. $^{\#}$Between treatment groups (HFD-20%FR; HFD-40%FR; HFD-60%FR) and HFD.

Table 3
Biochemical parameters of humans included in the study

	Eutrophic	Obese	Malnourished
Glucose (mg/dl)	88.24±14.54	97.33±15.68	104.90±27.78
Total cholesterol (mg/dl)	189.78±47.29	194.67±37.12	213.50±131.74
High-density lipoprotein (mg/dl)	45.13±9.26	47.90±17.33	48.0±21.02
Triglycerides (mg/dl)	112.33±43.47	172.15±86.55	89.6±30.58
Albumin (g/dl)	4.0±0.51	4.0±0.54	3.95±0.07

Plasma glucose, lipid parameters and albumin of humans included in the study (eutrophic, obese and malnourished). Data are presented as mean±S.D.; no statistical differences were found between groups.

In conclusion, the present study shows that 20% and 40% food restriction prevents deterioration of glucose and lipid metabolic profile and reestablishes body weight and the weight of epididymal, retroperitoneal and mesenteric adipose tissues in mice subjected to standard and high-fat diets. Twenty percent and 40% food restriction improved the metabolism, modulating some of the RAS components and cytokine expressions in mice, while a severe 60% food restriction (malnutrition) did not protect and produced a proinflammatory state with increased AGT, ACE and AT1 expression similar to high-fat obese animals. We also conclude that obese and malnourished human individuals present a similar inflammatory profile that might be modulated, in part, by the RAS. These results support mild to moderate food restriction as a therapeutic alternative tool for preventing obesity-related disorders associated with a proinflammatory status and RAS activation in the adipose tissue. However, it is noteworthy to mention the mean age of the malnourished patients included in the study, which is due to the difficulty in recruiting malnourished individuals who submitted to elective surgeries that do not have cancer or other important diseases.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jnutbio.2017.06.008.

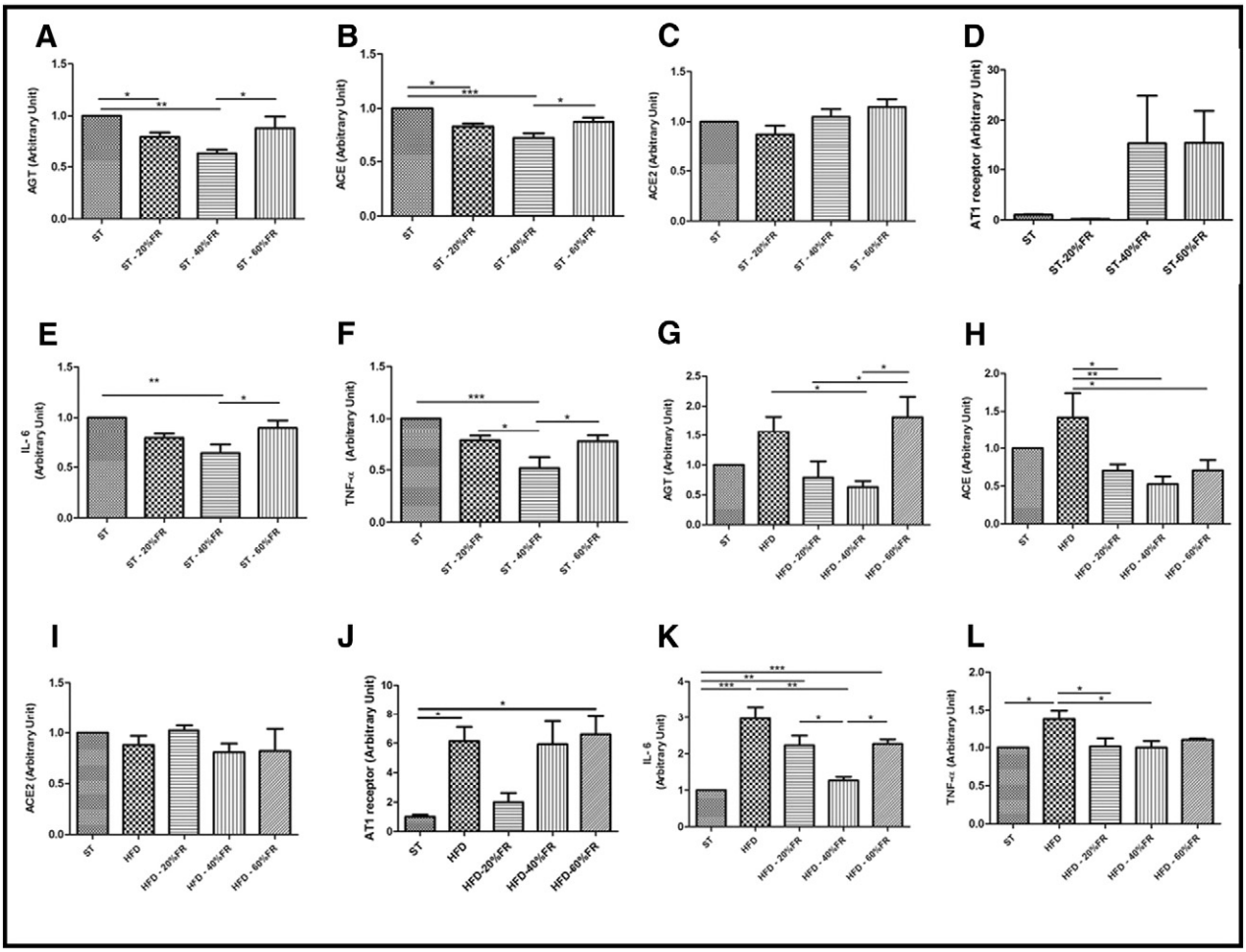

Fig. 4. AGT, ACE, ACE2, AT1, IL-6 and TNF-α mRNA expression levels in mice. Effects of 8-week food restriction on gene expression levels of components of the renin–angiotensin and inflammatory markers in epididymal adipose tissue of mice fed a standard diet and high-fat diet, respectively (A–J). AGT (A), ACE (B), ACE2 (C), AT1 (D), IL-6 (E), TNF-α (F), AGT (G), ACE (H), ACE2 (I), AT1 (J), IL-6 (K) and TNF-α (L). Data are presented as mean±S.E.M.; *P<.05, **P<.01, ***P<.001 *versus* group indicated.

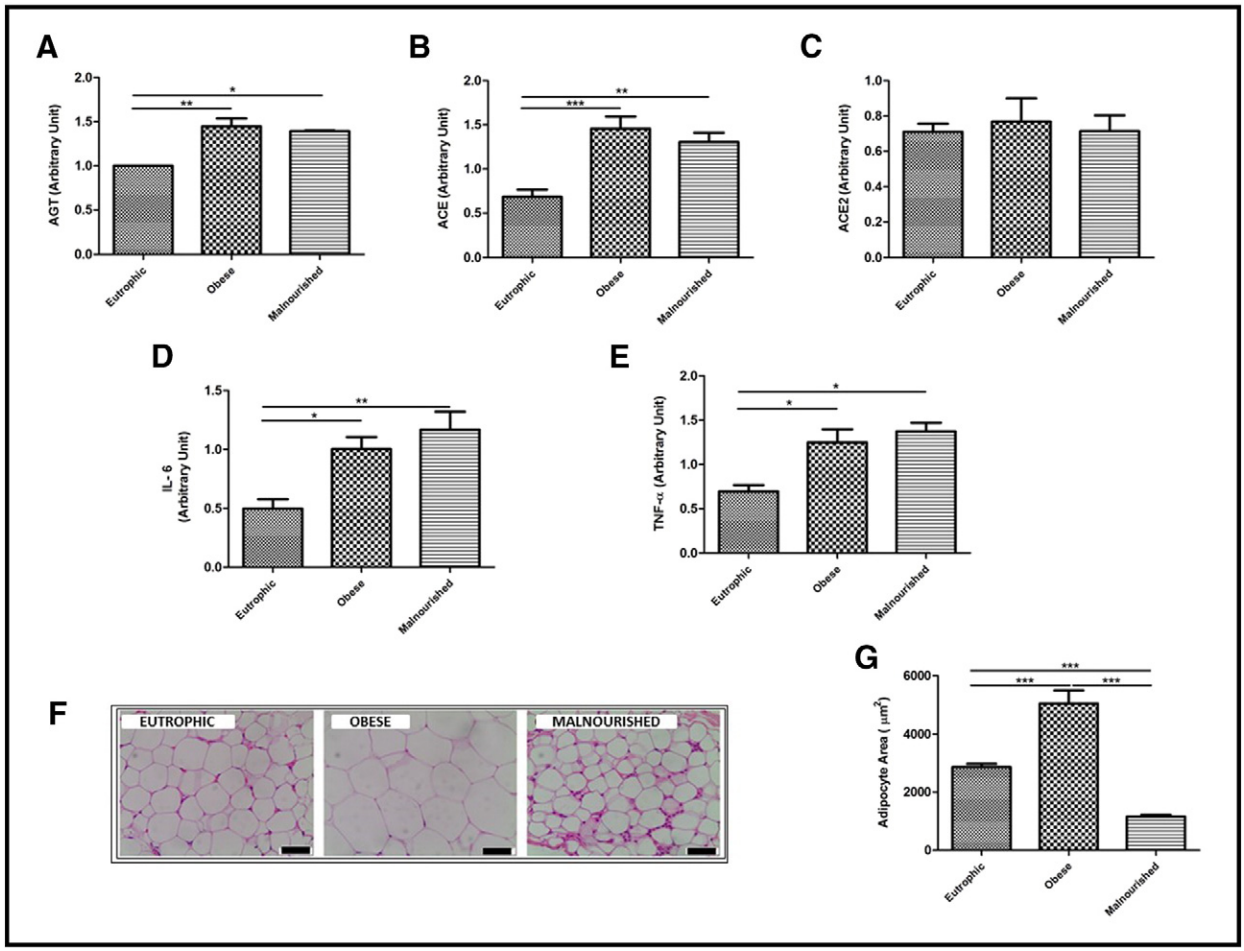

Fig. 5. AGT, ACE, ACE2, IL-6 and TNF-α mRNA expression levels in humans. RAS components' and inflammatory markers' gene expression levels in visceral adipose tissue of eutrophic, obese and malnourished human patients (A–E). AGT (A), ACE (B), ACE2 (C), IL-6 (D) and TNF-α (E). Adipocyte area and hematoxylin/eosin staining of eutrophic, obese and malnourished human patients (F–G). Hematoxylin and eosin staining (F) and adipocyte area (G). Data are presented as mean±S.E.M.; $^{*}P<.05$, $^{**}P<.01$, $^{***}P<.001$.

References

[1] de Ferranti S, Mozaffarian D. The perfect storm: obesity, adipocyte dysfunction, and metabolic consequences. Clin Chem 2008;54:945–55.
[2] Jackson AW, Lee DC, Sui X, Morrow Jr JR, Church TS, Maslow AL, et al. Muscular strength is inversely related to prevalence and incidence of obesity in adult men. Obesity 2010;18:1988–95.
[3] Hill JO, Melanson EL, Wyatt HT. Dietary fat intake and regulation of energy balance: implications for obesity. J Nutr 2000;130:284S–8S.
[4] Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. Nat Rev Immunol 2011;11:85–97.
[5] Haslam DW, James WP. Obesity. Lancet 2005;366:1197–209.
[6] Hotamisligil GS. Inflammation and metabolic disorders. Nature 2006;444:860–7.
[7] Engstrom G, Hedblad B, Stavenow L, Lind P, Janzon L, Lindgarde F. Inflammation-sensitive plasma proteins are associated with future weight gain. Diabetes 2003; 52:2097–101.
[8] Santos SH, Braga JF, Mario EG, Porto LC, Rodrigues-Machado Mda G, Murari A, et al. Improved lipid and glucose metabolism in transgenic rats with increased circulating angiotensin-(1–7). Arterioscler Thromb Vasc Biol 2010;30:953–61.
[9] 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;57:340–7.
[10] Santos SH, Fernandes LR, Pereira CS, Guimaraes AL, de Paula AM, Campagnole-Santos MJ, et al. Increased circulating angiotensin-(1–7) protects white adipose tissue against development of a proinflammatory state stimulated by a high-fat diet. Regul Pept 2012;178:64–70.
[11] Massiera F, Seydoux J, Geloen A, Quignard-Boulange A, Turban S, Saint-Marc P, et al. Angiotensinogen-deficient mice exhibit impairment of diet-induced weight gain with alteration in adipose tissue development and increased locomotor activity. Endocrinology 2001;142:5220–5.
[12] Engeli S, Negrel R, Sharma AM. Physiology and pathophysiology of the adipose tissue renin–angiotensin system. Hypertension 2000;35:1270–7.
[13] Shinozaki K, Ayajiki K, Nishio Y, Sugaya T, Kashiwagi A, Okamura T. Evidence for a causal role of the renin–angiotensin system in vascular dysfunction associated with insulin resistance. Hypertension 2004;43:255–62.
[14] Engeli S, Schling P, Gorzelniak K, Boschmann M, Janke J, Ailhaud G, et al. The adipose-tissue renin–angiotensin–aldosterone system: role in the metabolic syndrome? Int J Biochem Cell Biol 2003;35:807–25.
[15] Ingram DK, Cutler RG, Weindruch R, Renquist DM, Knapka JJ, April M, et al. Dietary restriction and aging: the initiation of a primate study. J Gerontol 1990;45: B148–63.
[16] Mattson MP. Energy intake, meal frequency, and health: a neurobiological perspective. Annu Rev Nutr 2005;25:237–60.
[17] Ramsey JJ, Colman RJ, Binkley NC, Christensen JD, Gresl TA, Kemnitz JW, et al. Dietary restriction and aging in rhesus monkeys: the University of Wisconsin study. Exp Gerontol 2000;35:1131–49.
[18] Weindruch R, Sohal RS. Caloric intake and aging. N Engl J Med 1997;337:986–94.
[19] Weindruch R, Walford RL. The retardation of aging and disease by dietary restriction. J Nutr 1990;120:1139.
[20] Merry BJ. Molecular mechanisms linking calorie restriction and longevity. Int J Biochem Cell Biol 2002;34:1340–54.
[21] Speakman JR, Hambly C. Starving for life: what animal studies can and cannot tell us about the use of caloric restriction to prolong human lifespan. J Nutr 2007;137:1078–86.
[22] Cottam DR, Mattar SG, Barinas-Mitchell E, Eid G, Kuller L, Kelley DE, et al. The chronic inflammatory hypothesis for the morbidity associated with morbid obesity: implications and effects of weight loss. Obes Surg 2004;14:589–600.
[23] Blum D, Omlin A, Baracos VE, Solheim TS, Tan BH, Stone P, et al. Cancer cachexia: a systematic literature review of items and domains associated with involuntary weight loss in cancer. Crit Rev Oncol Hematol 2011;80:114–44.
[24] Kemnitz JW, Roecker EB, Weindruch R, Elson DF, Baum ST, Bergman RN. Dietary restriction increases insulin sensitivity and lowers blood glucose in rhesus monkeys. Am J Physiol 1994;266:E540–7.
[25] Kumar NB, Kazi A, Smith T, Crocker T, Yu D, Reich RR, et al. Cancer cachexia: traditional therapies and novel molecular mechanism-based approaches to treatment. Curr Treat Options Oncol 2010;11:107–17.

[26] Penet MF, Winnard Jr PT, Jacobs MA, Bhujwalla ZM. Understanding cancer-induced cachexia: imaging the flame and its fuel. Curr Opin Support Palliat Care 2011;5:327–33.
[27] Lane MA, Ingram DK, Roth GS. Calorie restriction in nonhuman primates: effects on diabetes and cardiovascular disease risk. Toxicol Sci 1999;52:41–8.
[28] Speakman JR, Mitchell SE. Caloric restriction. Mol Aspects Med 2011;32:159–221.
[29] White JV, Guenter P, Jensen G, Malone A, Schofield M, Academy of N, et al. Consensus statement of the Academy of Nutrition and Dietetics/American Society for Parenteral and Enteral Nutrition: characteristics recommended for the identification and documentation of adult malnutrition (undernutrition). J Acad Nutr Diet 2012;112:730–8.
[30] Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(−Delta Delta C(T)) method. Methods 2001;25:402–8.
[31] Sharp S, Poglitsch M, Zilla P, Davies NH, Sturrock ED. Pharmacodynamic effects of C-domain-specific ACE inhibitors on the renin–angiotensin system in myocardial infarcted rats. J Renin Angiotensin Aldosterone Syst 2015;16:1149–58.
[32] Souza AP, Sobrinho DB, Almeida JF, Alves GM, Macedo LM, Porto JE, et al. Angiotensin II type 1 receptor blockade restores angiotensin-(1–7)-induced coronary vasodilation in hypertrophic rat hearts. Clin Sci 2013;125:449–59.
[33] Kirchner H, Hofmann SM, Fischer-Rosinsky A, Hembree J, Abplanalp W, Ottaway N, et al. Caloric restriction chronically impairs metabolic programming in mice. Diabetes 2012;61:2734–42.
[34] Moura LP, Figueredo GA, Bertolini NO, Ceccato M, Pereira JR, Sponton AC, et al. Dietary restriction, caloric value and the accumulation of hepatic fat. Lipids Health Dis 2012;11:2.
[35] Benigni A, Cassis P, Remuzzi G. Angiotensin II revisited: new roles in inflammation, immunology and aging. EMBO Mol Med 2010;2:247–57.
[36] Lijnen HR, Van Hul M, Hemmeryckx B. Caloric restriction improves coagulation and inflammation profile in obese mice. Thromb Res 2012;129:74–9.
[37] Chujo Y, Fujii N, Okita N, Konishi T, Narita T, Yamada A, et al. Caloric restriction-associated remodeling of rat white adipose tissue: effects on the growth hormone/insulin-like growth factor-1 axis, sterol regulatory element binding protein-1, and macrophage infiltration. Age 2013;35:1143–56.
[38] Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. J Clin Endocrinol Metab 2004;89:2548–56.
[39] Vazquez-Vela ME, Torres N, Tovar AR. White adipose tissue as endocrine organ and its role in obesity. Arch Med Res 2008;39:715–28.
[40] 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;112:1821–30.
[41] Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante Jr AW. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 2003;112:1796–808.
[42] Cancello R, Henegar C, Viguerie N, Taleb S, Poitou C, Rouault C, et al. Reduction of macrophage infiltration and chemoattractant gene expression changes in white adipose tissue of morbidly obese subjects after surgery-induced weight loss. Diabetes 2005;54:2277–86.
[43] Bruun JM, Lihn AS, Pedersen SB, Richelsen B. Monocyte chemoattractant protein-1 release is higher in visceral than subcutaneous human adipose tissue (AT): implication of macrophages resident in the AT. J Clin Endocrinol Metab 2005;90:2282–9.