Endocrinology. 2014; 155(5): 1838-1850

Perturbation of hypothalamic MicroRNA expression patterns in male rats after metabolic distress: impact of obesity and conditions of negative energy balance

Susana Sangiao-Alvarellos, Lara Pena-Bello, María Manfredi-Lozano, Manuel Tena-Sempere, and Fernando Cordido

Abstract

The hypothalamus plays a crucial role in body weight homeostasis through an intricate network of neuronal circuits that are under the precise regulation of peripheral hormones and central transmitters. Although deregulated function of such circuits might be a major contributing factor in obesity, the molecular mechanisms responsible for the hypothalamic control of energy balance remain partially unknown. MicroRNAs (miRNAs) have been recognized as key regulators of different biological processes, including insulin sensitivity and glucose metabolism. However, the roles of miRNA pathways in the control of metabolism have been mostly addressed in peripheral tissues, whereas the potential deregulation of miRNA expression in the hypothalamus in conditions of metabolic distress remains as yet unexplored. In this work, we used high-throughput screening to define to what extent the hypothalamic profiles of miRNA expression are perturbed in two extreme conditions of nutritional stress in male rats, namely chronic caloric restriction and high-fat diet–induced obesity. Our analyses allowed the identification of sets of miRNAs, including let-7a, mir-9*, mir-30e, mir-132, mir-145, mir-200a, and mir-218, whose expression patterns in the hypothalamus were jointly altered by caloric restriction and/or a high-fat diet. The predicted targets of these miRNAs include several elements of key inflammatory and metabolic pathways, including insulin and leptin. Our study is the first to disclose the impact of nutritional challenges on the hypothalamic miRNA expression profiles. These data will help to characterize the molecular miRNA signature of the hypothalamus in extreme metabolic conditions and pave the way for targeted mechanistic analyses of the involvement of deregulated central miRNAs pathways in the pathogenesis of obesity and related disorders.

Metabolic syndrome is defined by a series of related disorders, including obesity, insulin resistance, glucose intolerance, and hyperlipidemia. Obesity is the major factor for the development of metabolic syndrome and has emerged as one of the most important medical problems of the 21st century, being also a major risk factor for the development of cardiovascular diseases and type 2 diabetes. According to World Health Organization estimates, type 2 diabetes affects 347 million people worldwide (1), and its prevalence continues to increase due to advancing age, inactivity, and increasing obesity rates (2).

Contrary to the idea that obesity is triggered by the influence of isolated metabolic insults and/or merely by enhanced food intake, it has now become clear that obesity is a multifaceted condition in which genetic load, developmental programming, and environment are major contributing factors, together with specific gene-diet interactions (3, 4). During the past few decades, much of the effort in obesity research has been focused on the study of peripheral metabolic tissues, such as the white adipose tissue (WAT), liver, and muscle, because they function as the main sites of use and storage of nutrients and energy excess. However, data gleaned in recent years have highlighted the putative important role of neurohormonal deregulation in the pathogenesis of obesity (5–7), based on the essential roles that the central nervous system in general and the hypothalamus in particular play in energy homeostasis. Thus, discrete hypothalamic nuclei and circuits are essential for the control of food intake and sensing of multiple circulating peripheral signals such as leptin, insulin, adiponectin, gut hormones, and nutrients. These various signals function as metabolic “gauges” that interact with specific hypothalamic regions to modulate (or eventually perturb) energy balance both peripherally and centrally (8–10). Deregulation of such neuroendocrine homeostasis is likely to play a key role in the onset and progression of obesity.

In particular, by signaling adiposity to the hypothalamus, insulin and leptin have been proven essential for the central inhibitory control of feeding; an action that is conducted in part by reducing the expression of orexigenic neuropeptides and increasing the expression of anorexigenic neuropeptides (11, 12). Leptin

deficiency results in morbid obesity in both animals and humans (11, 13–15), and the central effects of leptin and insulin are very relevant for the regulation of circulating glucose levels (11, 16). However, obesity is associated with enhanced levels of leptin and insulin but decreased responses to endogenous and exogenous leptin and insulin, a phenomenon of *resistance* whose molecular basis has not been fully clarified. In addition, other peripheral hormones play a central role with leptin and insulin in the fine control of energy balance and metabolism (5, 17).

In recent years, the functional importance of noncoding RNAs in the control of diverse biological processes has been recognized. In particular, microRNAs (miRNAs) are well-known regulators of the mammalian cell phenotype (18, 19). miRNAs are small, non–protein-coding RNAs that negatively regulate gene expression by promoting degradation and/or inhibiting translation of target mRNAs (20). miRNAs have been implicated in many biological processes, including development, cell proliferation and neoplasia, glucose homeostasis, and lipid metabolism (21–25). miRNA-dependent regulation has been reported in pancreatic islets, liver, and adipose tissue in various model systems. For example, *miR-375* regulates pancreatic insulin secretion (26, 27), *mir-103* and *mir-107* influence insulin sensitivity in peripheral tissues (28), and *mir-143* and *mir-145* are up-regulated in the livers of genetic and dietary mouse models of obesity, whereas deficiency in the *mir-143/145* cluster protects from the development of obesity-associated insulin resistance (29, 30). In addition, miRNAs of the *let-7* family have been shown to participate in the regulation of glucose metabolism. Mice overexpressing *let-7* had decreased fat mass and body weight (BW), as well as reduced body size. Global and pancreas-specific overexpression of *let-7* in mice also resulted in impaired glucose tolerance and reduced glucose-induced pancreatic insulin secretion (31). In the same way, global knockdown of the *let-7* family prevented and treated impaired glucose tolerance in mice with diet-induced obesity, at least in part by improving the insulin sensitivity in liver and muscle (24).

As mentioned above, the hypothalamus is key in the regulation of energy and BW homeostasis. However, despite recent evidence suggesting the deregulation of various miRNA systems in peripheral tissues in conditions of metabolic disturbance, the possibility of perturbed expression of hypothalamic miRNAs in conditions of nutritional or metabolic stress, neither the potential alterations of hypothalamic miRNA expression in situations of leptin nor insulin resistance, has not been thoroughly addressed. In the present work, we performed an analysis of the miRNA expression profiles using a TaqMan Low Density miRNA array in the hypothalamus of rats reared with a normal diet (ND), a high-fat diet (HFD), and caloric restriction (CR) during 3 months after weaning. Based on high-throughput screening and bioinformatics predictions of their involvement in insulin-, leptin- and adiponectin-signaling pathways, the expression profiles of *let-7a*, *mir-9**, *mir-30e*, *mir-132*, *mir-145*, *mir-200a*, and *mir-218* were further analyzed in several models of altered metabolism.

Materials and Methods

Animals

All experiments and animal protocols involved in this study were reviewed and approved by the ethics committees of the University of A Coruña and Complexo Hospitalario Universitario de A Coruña, in accordance with European Union Normative for the use of experimental animals. Male Wistar rats of different ages were used in this study and housed in a temperature-controlled room with a 12-hour light and 12-hour dark cycle (lights on from 8:00 am to 8:00 pm). Groups of rats born on the same day were used; on the day of birth, the animals were pooled at random and were evenly distributed between the dams to be bred in litters of 11 to 12 pups per dam. All rats were provided with ad libitum access to water.

Body composition and tissue dissection

Three days before killing, rat BW and composition were analyzed. Body fat and lean mass were measured by nuclear magnetic resonance with the EchoMRI method (Echomedical System). In addition, liver and visceral and epididymal WAT were dissected and weighed. Somatic indices were calculated as the ratio between tissue weight and BW and are expressed as a percentage. Rats were killed by cervical dislocation, and trunk blood was extracted. The hypothalami were dissected and stored at −80°C until further processing for real-time PCR or microarray assays. The hypothalamus was defined by the posterior margin of the optic chiasm and the anterior margin of the mammillary bodies to the depth of approximately 2 mm, following previous references (32).

Bioinformatics analysis

miRNAs are small endogenous RNAs that pair to sites in mRNAs to direct posttranscriptional repression. Many sites that match the miRNA seed (nucleotides 2–7), particularly those in 3′ untranslated regions, are preferentially conserved (33). Computational methods play important roles in the identification of new miRNAs and their targets. To search potential targets for a specific miRNA or potential regulators for a specific protein, we used five bioinformatics algorithms jointly: TargetScan, http://www.targetscan.org/ (34); miRanda, http://www.microrna.org/microrna/home.do (35); PicTar, http://pictar.mdc-berlin.de/ (36); Diana Lab, http://diana.cslab.ece.ntua.gr/microT/ (37); and miRWalk http://www.umm.uni-heidelberg.de/apps/zmf/mirwalk/ (38). Subsequently, pathway analysis was performed using the putative target gene list derived from the above cited bioinformatics algorithms. This analysis was performed using Ingenuity Pathway Analysis software (Ingenuity Systems, http://www.ingenuity.com/), generating a canonical pathway analysis. The networks are given a score based on the probability of inclusion of the number of molecules in the generated networks over the probability of a network being generated by chance with random molecules. This score is generated as a negative log *P* value. Canonical pathway analysis is performed by comparing the data set of interest against known canonical (signaling and metabolic) pathways within the database.

Quantitative real-time PCR

For assays of miRNAs, total RNA was extracted with an mirVana miRNA Isolation Kit (Ambion, Inc). Quality and concentration of the RNA were determined by agarose gel and a NanoDrop spectrophotometer ND-1000 (Thermo Scientific), respectively. Real-time PCR was performed on a Roche Light Cycler 480 real-time PCR detection system. cDNA was synthesized by using 10 ng of total RNA with TaqMan-specific RT primers and a TaqMan miRNA reverse transcription kit (Applied Biosystems). Thereafter, quantitative real-time PCR was performed using predesigned assays (Applied Biosystems). PCR reactions were performed as follows: 50°C for 2 minutes, 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. For data analysis, relative standard curves were constructed from serial dilutions of one reference sample cDNA, and the input value of the target gene was standardized to *RNU6* levels in each sample. The samples and standard curves were analyzed in duplicate.

miRNA microarray assays

miRNA microarray assays were performed with TaqMan Array Rodent MicroRNA Cards (Applied Biosystems). Comprehensive coverage of Sanger miRBase v15 is enabled across a 2-card set of TaqMan Array MicroRNA Cards for a total of 641 miRNAs. In addition, each card contains 5 candidate endogenous control assays and 1 negative control assay. Extraction of RNA and synthesis and amplification of cDNA were performed according to the manufacturer's instructions (http://www3.appliedbiosystems.com/cms/groups/mcb_support/documents/generaldocuments/cms_042326.pdf).

Plasma measurements

Insulin (Millipore), leptin, and adiponectin (Abcam) levels were measured by commercially available ELISAs, according to the specifications of the manufacturers. Plasma glucose and triglyceride levels were assessed using a commercial kit based on a colorimetric method (Spinreact).

Experimental designs

Hypothalamic miRNA profiling in rats reared with different diets: long-term experiments (experiment 1)

To define the hypothalamic miRNA profiles and their possible variation with different BW, percent fat mass, insulin sensitivity, and other obesity-related factors, 30 male Wistar rats were used. At weaning day (postnatal day 21), with a mean BW of 98.1 ± 1.57 g, the animals were divided into 3 groups: (1) control group, with ad libitum access to standard chow diet (3.85 kcal/g; 10% kcal percent fat; Research Diets, Inc) (n = 10); (2) diet-induced obesity (DIO) group, with ad libitum access to an HFD diet (4.73 kcal/g; 45% kcal percent fat; Research Diets, Inc) (n = 10); and (3) CR group (n = 10), with a standard

chow diet, but with food restricted to 65% of the daily amount ingested by the control group. To this CR group, a fixed amount of food was provided daily, and the animals ate all the food offered. This feeding regimen was chosen for obtaining animals with lower percentages of body fat and increased sensitivity to insulin. The animals were housed under these feeding regimens until they were killed after 3 months. Animals were killed at 10:00 am, 2 hours after the beginning of the light phase. miRNA microarray assays were performed for 4 animals of each group.

The array results were confirmed by individual assays for selected miRNAs. Once array results were confirmed and validated, we selected a small group of miRNAs to analyze in the subsequent studies, taking into account their degree of expression in the hypothalamus, their possible targets (obtained by computational approximations), and previous studies in other tissues.

Alterations in hypothalamic miRNAs by fasting and different diets: short-term and midterm experiments (experiment 2)

It is well known that an HFD is an important inducer of insulin resistance, and in rats, different manipulations of feeding have been shown to have various impacts on hypothalamic metabolism, although the nature of such changes depends on the type and timing of the nutritional challenge (39, 40). In experiment 2, we evaluated the impact of the HFD during 2 weeks or 1 month on a set of hypothalamic miRNAs. To this end, adult rats (BW of 260 ± 4 g, 11 weeks of age) were fed with the HFD or ND. The rats were killed 15 and 30 days after the beginning of the feeding with the HFD.

Effects of leptin on hypothalamic miRNAs in fed and fasted rats (experiment 3)

To monitor changes in hypothalamic miRNA expression in conditions of altered leptin signaling, we performed experiment 3, in which one group of adult rats (189 g ± 9 g, 9 weeks of age) was fed ad libitum and another group was deprived of food for 48 hours. Fed (with normal values of insulin, leptin, and glucose) and fasted (state associated with low levels of insulin, glucose, and leptin) rats received 2 ip injections of leptin (ProSpec, 1 mg/kg dissolved in 200 μL of saline) or vehicle. Injections were given at 24 and 48 hours after the beginning of fasting. Treatments were started at 8:00 am and were performed in the light phase. Animals were killed 2 hours after the second injection (10:00 am).

Statistical analysis

Data were analyzed using SigmaStat 3.1 (Systat Software, Inc) and are expressed as a percentage of the control group in each experiment (means ± SEM). Statistical significance was determined by the Student *t* test (experiments with 2 groups), one-way ANOVA with a post hoc Tukey test (experiments with more than 2 groups and 1 variable), or two-way ANOVA with a post hoc Tukey test (experiments with 2 or more groups and 2 variables). A value of $P \leq .05$ was considered significant. Different superscript letters or asterisks indicate statistical significance.

Results

Hypothalamic miRNA profiling in rats reared with different diets: long-term experiments

Effect of different diets on BW and composition

Major body parameters, including percent fat and lean body mass as well as BW gain during the 3-month period of nutritional manipulation, are shown in Figure 1, A to C. The percentage of fat mass was very low in animals subjected to CR (scarcely 2%) but was 9.5% in the control group and rose to 18.8% in HFD animals (Figure 1A). On the contrary, the percentage of lean mass decreased as an inverse function of the feeding regimen; the highest percentage was seen in CR rats and the lowest in HFD animals, although net differences were not as obvious (Figure 1B). As expected, total BW gain was maximal in HFD rats, whereas CR animals displayed the lowest BW gain during the study period (Figure 1C).

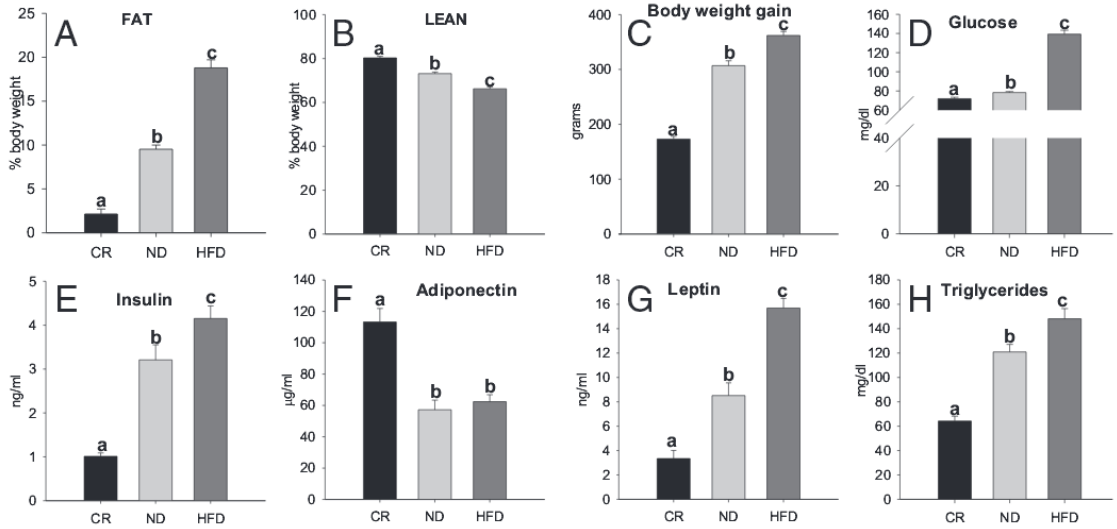

Figure 1. Effect of the HFD and CR during 3 months on percent body fat mass (A), percent body lean mass (B), body weight gain (C) and plasma glucose (D), insulin (E), adiponectin (F), leptin (G), and triglyceride (H) levels. Different letters above the bars indicate statistical differences. A value of $P \leq .05$ was considered statistically significant (one-way ANOVA with a post hoc Tukey test). CR, n = 10; ND, n = 10; and HFD, n = 10.

Plasma parameters

To monitor the potential metabolic alterations induced by the different diets, we analyzed different plasma parameters. As shown in Figure 1, D to H, animals subjected to CR displayed signs of increased insulin sensitivity because they had lower basal glucose (Figure 1D) and insulin (Figure 1E) levels and enhanced adiponectin concentrations (Figure 1F) compared with those of the control (ND) and HFD groups. In contrast, HFD rats presented signs of insulin resistance and metabolic deregulation, as evidenced by higher basal glucose, insulin, and leptin (Figure 1G) levels, together with increased plasma triglyceride concentrations (Figure 1H) and decreased adiponectin levels.

Microarray profiling of hypothalamic miRNA expression and changes related to different diets

To investigate the global hypothalamic profiles of miRNAs and their potential changes under different nutritional manipulations (HFD and CR), we used a high-throughput approach based on TaqMan Low Density miRNA Array technology. Of the 641 miRNAs analyzed, 326 were found to be expressed in the hypothalamus. The miRNA profiles obtained in normal rats are shown in Figure 2A, organized according to relative expression levels, from the lowest to the highest expression. For a more detailed presentation and analysis of data, see Supplemental Table 1 published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org. In addition, comparative expression profiling of miRNA signatures in hypothalami from rats subjected to CR or the HFD was conducted. In at least one nutritional manipulation, 74 miRNAs were deregulated. Fold change and statistical significance are reported in Figure 2B and Supplemental Table 2. These miRNAs belong to 63 different miRNA families, of which 29 are broadly conserved, 15 are conserved, and 19 are poorly conserved across mammalian species. Among the widely conserved families that were affected by diet regimens, it is worth noting that 6 of 10 members of the *mir-30* family were altered by nutritional manipulations, whereas 4 of 5 members of the *mir-200*bc family and several *let-7* miRNAs were deregulated after persistent nutritional challenge.

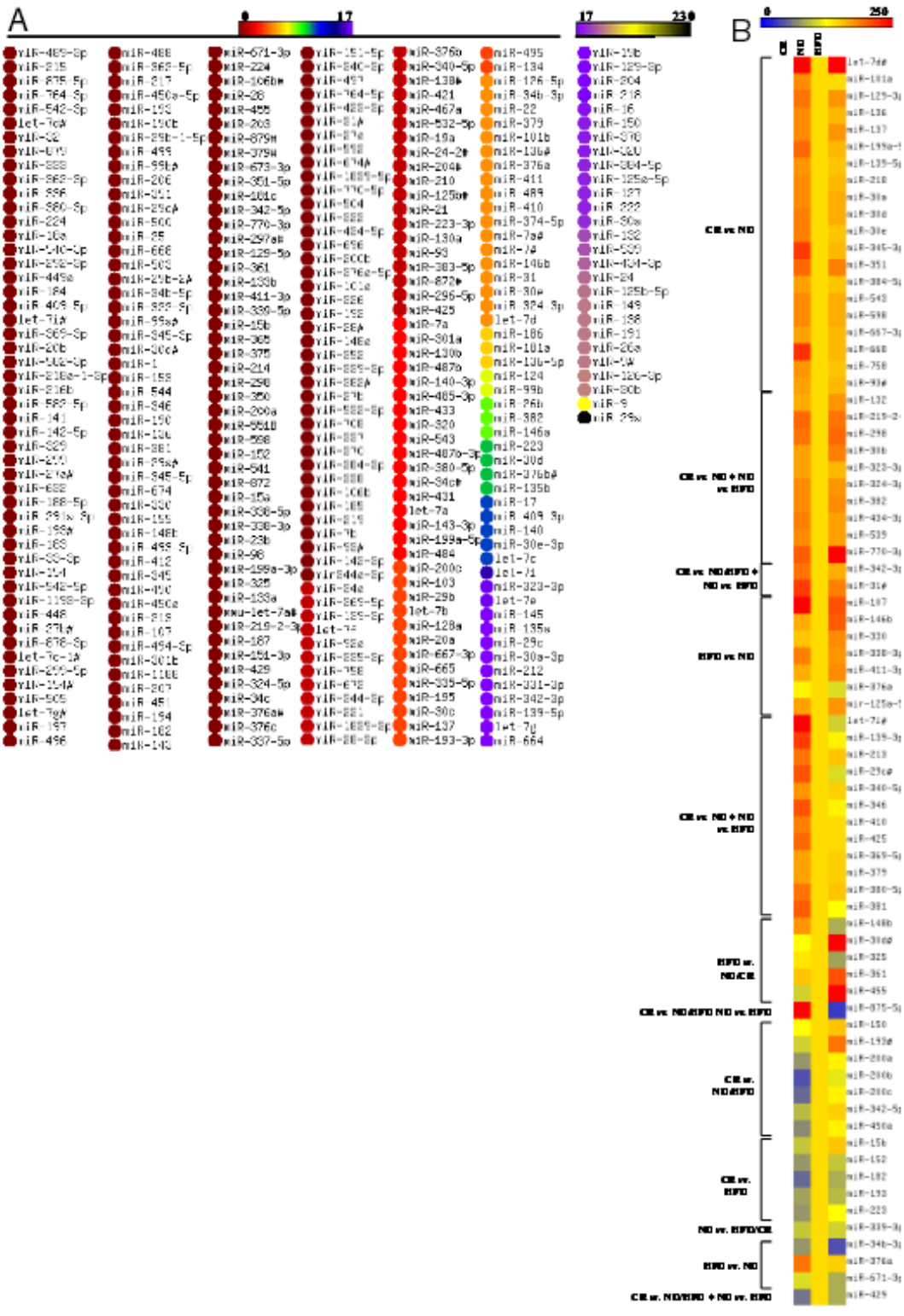

Figure 2. Heat map of miRNA microarray data displaying expression profiles in the adult male rat hypothalamus. A, Expression of each miRNA was calculated by the ΔΔCt method, using the RNU6 gene as internal reference. Expression is given in arbitrary units and is the mean of four samples. To facilitate interpretation of the results, the first 5 columns have a scale different from that for the last column. B, Overview of the miRNAs that show statistically significant differences in at least one treatment in the adult male rat hypothalamus reared with different diets. The expression intensity of each miRNA varies from blue to red. Expression is given in arbitrary units and is the mean of four samples, where ND = 100%. Row labels on the left side of the image indicate significant different types. A value of $P \leq .05$ was considered statistically significant (one-way ANOVA with a post hoc Tukey test). In all cases, the animals were reared with feeding of these diets during the 3 months after weaning. Data were plotted using matrix2png 1.2.2 (http://www.chibi.ubc.ca/matrix2png/).

After high-throughput screening, individual quantitative PCR validation was implemented using the same samples, an approach that demonstrated high reproducibility of our expression assays. Of note, however, the actual magnitude of the (fold) increase or decrease displayed some variance between individual quantitative PCR and the TaqMan Low Density miRNA array technology. In particular, mir-145 and mir-9* showed statistically significant differences when the analysis was conducted individually, with higher values in HFD rats compared with ND/CR animals. Likewise, let-7a was up-regulated in HFD vs ND rats. Results from targeted miRNA assays are shown in Figure 3. Note that, for the sake of simplicity, presentation of analyses is grouped into categories, depending on the type and magnitude of changes after nutritional manipulation. Thus, in Figure 3, top panel, miRNAs whose expression levels increase only with HFD (let-7a, mir-9*, mir-30b, mir-100a, and mir-145) are shown. In Figure 3, middle panels, we display miRNAs whose expression levels increase only with CR (mir-29a, mir-30e, mir-323-3p, and mir-374-5p). Furthermore, miRNAs that are increased in expression with either HFD or CR (mir-132, mir-218, and mir-539) are presented Figure 3, middle panels. Finally, in Figure 3, bottom panel, miRNAs that are down-regulated at the hypothalamus after CR (mir-200a, mir-200b, and mir200c) is presented.

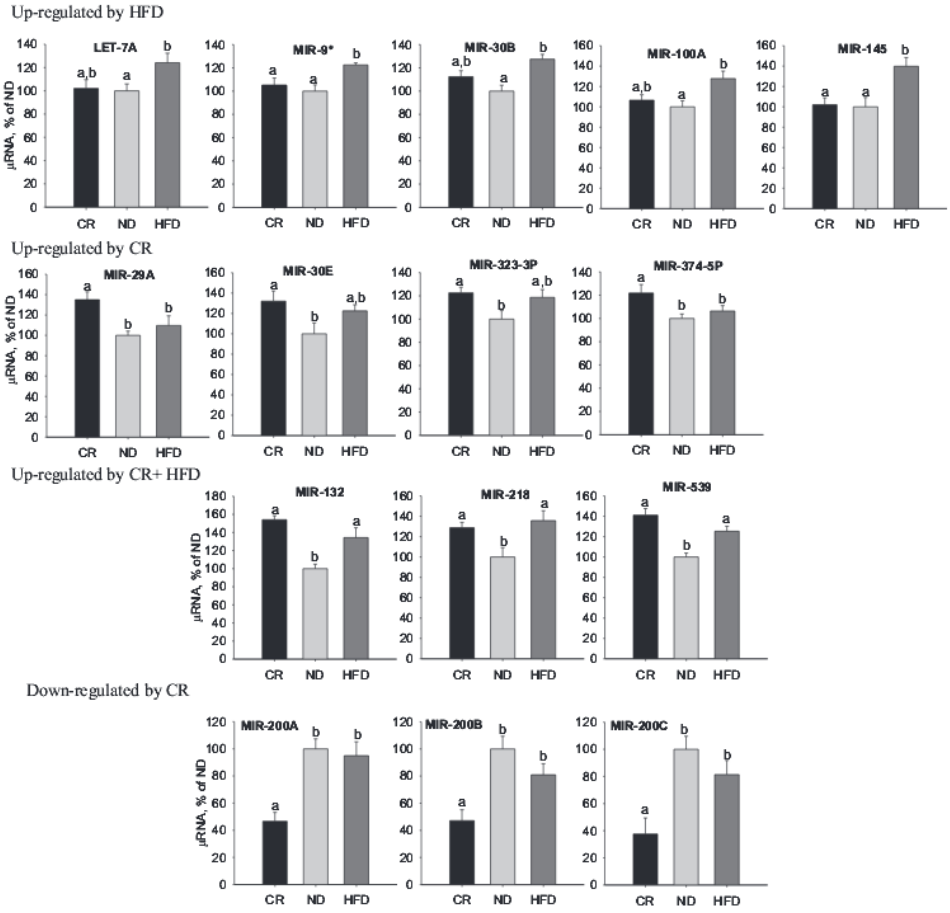

Figure 3. Real-time PCR validation of several miRNAs of interest. Values are expressed in arbitrary units (means ± SEM), where ND = 100%. Different letters above the bars indicate statistical differences (ANOVA with a post hoc Tukey test). CR, n = 5; ND, n = 5; and HFD, n = 5. The animals were reared with consumption of these diets during 3 months after weaning.

For further studies, we chose 7 miRNAs among those significantly deregulated by CR and/or HFD (let-7a, mir-9*, mir-30e, mir-132, mir-145, mir-200a, and mir-218). We selected these miRNAs for 3 reasons: (1) because their hypothalamic expression is high; (2) because previous studies in other tissues linked them with metabolic alterations; and (3) because of data from informatics models, which suggested that they are involved in the regulation of the signaling pathways of insulin, leptin, or adiponectin, fatty acid or lipid metabolism, and/or inflammation (Figure 4, Supplemental Figure 1, and Supplemental Table 3).

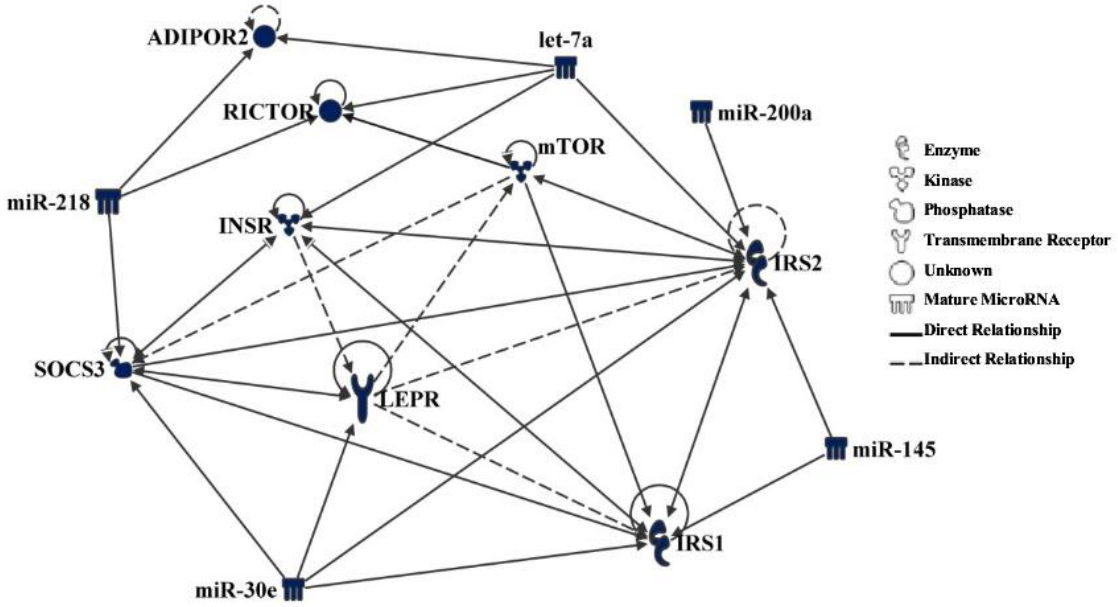

Figure 4. Schematic representation of the interrelation between let-7a, mir-9*, mir-30e, mir-132, mir-145, mir-200a, and mir-218 and genes related to insulin, leptin, and adiponectin signaling pathways. Analysis was performed with ingenuity pathway analysis software. ADIPOR2, adiponectin receptor 2; INSR, insulin receptor; IRS1, insulin receptor substrate 1; IRS2, insulin receptor substrate 2; LEPR, leptin receptor; mTOR, mammalian target of rapamycin; RICTOR, rapamycin-insensitive companion of mTOR; SOCS3, suppressor of cytokine signaling 3. Arrows indicate positive interactions, whereas arrows with T-bars indicate negative interactions. Solid lines indicate direct interactions, and dotted lines indicate indirect interactions.

Bioinformatics analysis

For the set of miRNAs indicated above (let-7a, mir-9*, mir-30e, mir-132, mir-145, mir-200a, and mir-218), we conducted analyses using up to 5 different bioinformatics algorithms to predict putative target genes/pathways. Of note, the various bioinformatics tools identified slightly different targets. Nonetheless, comparison between analyses and algorithms allowed us to select gene targets identified by at least three different programs; results are shown in Supplemental Table 3. Finally, the top scoring canonical pathways corresponding to identified targets for let-7a, mir-9*, mir-30e, mir-132, mir-145, mir-200a, and mir-218 are shown in Supplemental Figure 1. For all the miRNAs studied, except mir-200a, some of the pathways with the highest score are related to neuronal development, such as axonal guidance signaling, semaphorin signaling, ephrin receptor signaling, and dendritic cell maturation. For mir-132 and mir-218, neuronal cAMP-response element binding protein (CREB) signaling also displayed high scores. Notably, CREB-dependent gene expression has been involved in many different aspects of nervous system function (41, 42). Moreover, signaling pathways disturbed in obesity, such as nuclear factor κβ, ILs, phosphatidylinositol 3-kinase/serine-threonine protein kinase (AKT), ceramide, insulin receptor, p70S6K, and Janus tyrosine kinase/signal transducer and activator of transcription, were also found among the top scoring canonical pathway targets of the selected miRNAs (Supplemental Figure 1).

Diet-induced alterations in hypothalamic miRNAs: short-term and midterm experiments

Effect of different diets on BW and composition and plasma parameters

Major body parameters and plasma levels of key factors in rats fed during 2 weeks and 1 month with different diets are shown in Table 1. As expected, BW gain was greater in animals fed with the HFD. In turn, whereas glucose levels were higher in HFD rats after 2 weeks of diet, at the end of the 1-month period circulating glucose concentrations were lower than in controls, probably due to the trend for higher levels of insulin in HFD animals, which, nonetheless, was not statistically significant. The HFD did not alter the liver somatic index, but significantly increased the visceral and epididymal somatic indices at the 2 time points studied (Table 1).

Table 1. Effects of a HFD During 2 Weeks or 1 Month on Body Weight Gain, Plasma Glucose, Insulin, and Triglyceride Levels and Somatic Indices

					Somatic Index, %		
	Body Weight Gain, g	Glucose, mg/dL	Insulin, ng/mL	Triglycerides, mg/dL	Liver	Epididymal	Visceral
ND, 2 wk	41.2 ± 2.5	120 ± 1.5	2.3 ± 0.3	162 ± 13	3.7 ± 0.1	0.96 ± 0.05	0.68 ± 0.04
HFD, 2 wk	69.8 ± 2.1[a]	134 ± 4.2[a]	2.9 ± 0.4	129 ± 24	3.7 ± 0.1	1.43 ± 0.08[a]	0.97 ± 0.04[a]
ND, 1-mo	192.3 ± 6.6	138.42 ± 1.9	3.5 ± 0.5	151 ± 9	4.18 ± 0.08	1.28 ± 0.09	1.01 ± 0.05
HFD, 1-mo	228.5 ± 6.9[a]	124.5 ± 5.4[a]	4.3 ± 0.4	146 ± 14	3.99 ± 0.09	2.49 ± 0.21[a]	1.60 ± 0.09[a]

[a], $P < .05$ vs ND. Somatic index was calculated as the ratio between tissues weight and body weight and was expressed as a percentage.

Hypothalamic miRNA expression

Based on results from long-term nutritional manipulations (including the HFD), the hypothalamic expression levels of the set of miRNAs selected from long-term experiments (let-7a, mir-9*, mir-30e, mir-132, mir-145, mir-200a, and mir-218) were also assayed in male rats subjected to the HFD for 2 weeks or 1 month and compared with data obtained in rats exposed to the HFD for 3 months in the previous experiment. The time course of the HFD-induced changes in those miRNA targets is shown in Figure 5. Our analyses showed that mir-30e, mir-145, and mir-218 miRNA levels in the hypothalamus were similarly deregulated by the HFD, with an initial suppression followed by a variable increase in hypothalamic expression, which become significant after 1 month for mir-218 and after 3 months for mir-145. On the other hand, hypothalamic let-7a and mir-9* expression levels were consistently elevated by the HFD, whereas mir-132 expression was enhanced only at 3 months after the HFD. In contrast, mir-200a expression in the hypothalamus decreased in animals fed with the HFD for 2 weeks and 1 month, but its miRNA levels returned to control values at 3 months after HFD exposure (Figure 5).

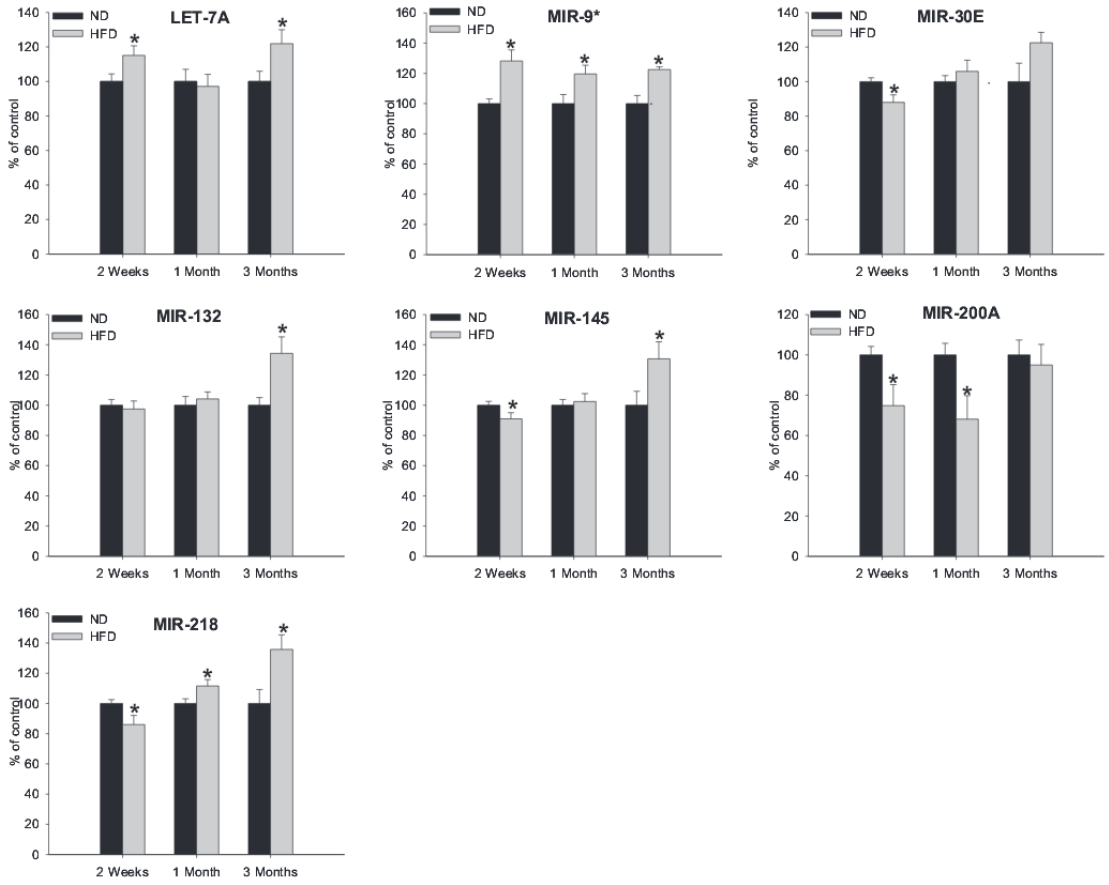

Figure 5. Effect of HFD during 2 weeks (n = 9), 1 month (n = 8), and 3 months (n = 5) on let-7a, mir-9*, mir-30e, mir-132, mir-145, mir-200a, and mir-218 miRNA expression levels in the hypothalamus of adult male rats. Values are expressed in arbitrary units as means ± SEM. For each time point, HFD values are expressed as a percentage of animals fed with the ND (100%, dotted line). Asterisk above the bars indicate statistical differences (Student t test). In all cases, the animals were killed between 3 and 4 months of age.

Effect of leptin on hypothalamic expression of selected miRNAs and mRNAs in fed and fasting rats

Effect of leptin on body weight/composition and plasma parameters

The impact of leptin on various somatic and metabolic parameters was evaluated in rats fed ad libitum or subjected to a 48-hour fast. Food deprivation evoked the expected decreases in BW, glucose, insulin, triglycerides, and the liver somatic index. Fed rats treated with leptin for 48 hours (2 boluses, 24 hours apart) displayed a significant decrease in food intake (Figure 6A), so that, in contrast with control animals, leptin-injected animals fed ad libitum lost weight during the treatment period (Figure 6B). In addition, leptin-treated rats fed ad libitum had significantly lower glucose levels than the control group (Figure 6C), whereas their insulin levels tended to be higher (Figure 6D). Leptin treatment diminished triglyceride levels both in fed and fasted animals (Figure 6E). Finally, although liver and visceral somatic indices were not altered by leptin, leptin tended to diminish the epididymal somatic index, which was further decreased by leptin in fasted animals (Figure 6, F–H).

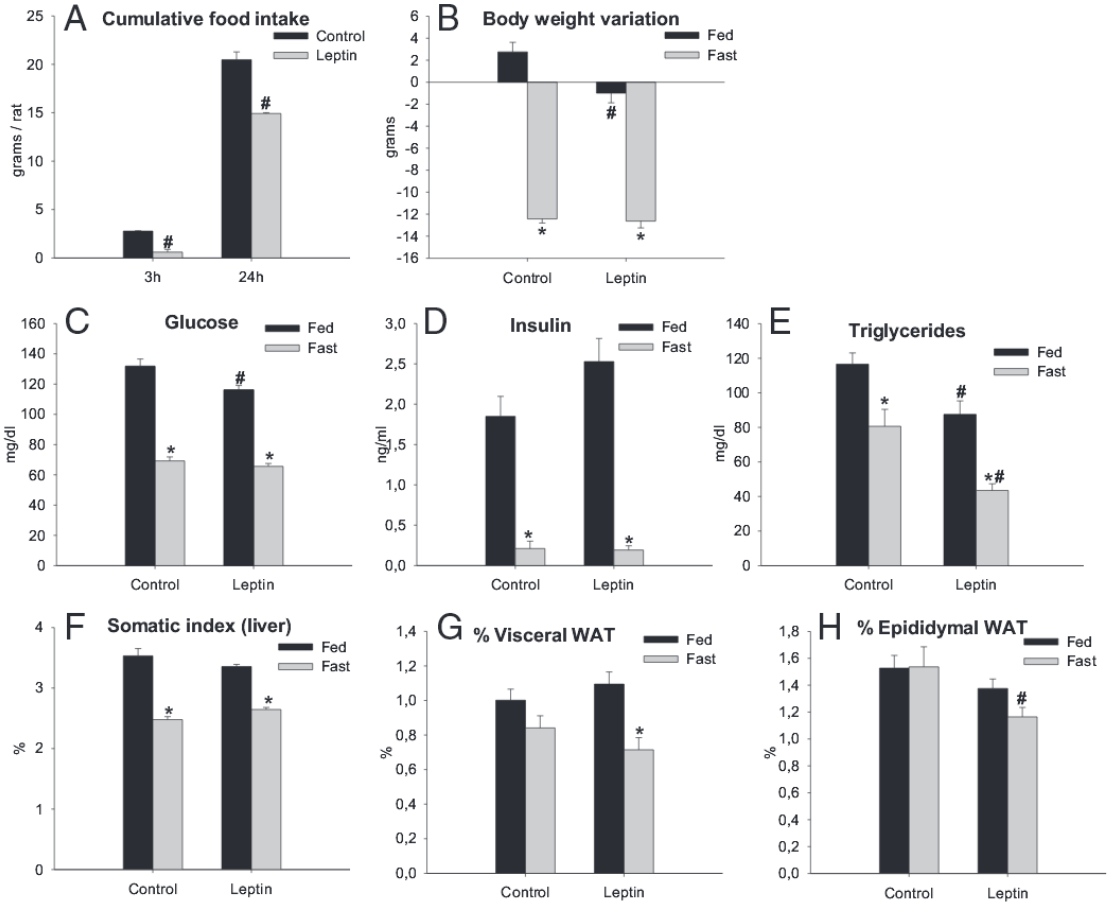

Figure 6. Effect of a single ip leptin injection on cumulative food intake during 3 and 24 hours after treatment (A) and the effect of 2-day ip leptin treatment in fed and fasted animals on body weight (B), plasma glucose (C), insulin (D), and triglyceride (E) levels, and liver (F), visceral WAT (G), and epididymal WAT (H) somatic indexes. *, $P \leq .05$ vs fed; #, $P \leq .05$ vs. control (rats injected with saline). Values are expressed as means ± SEM; n = 7 to 8 (two-way ANOVA with a post hoc Tukey test). Somatic indexes were calculated as the ratio between tissue weight and body weight and was expressed as a percentage.

Effect of leptin on selected hypothalamic miRNA expression

Fasting caused an increase in *let-7a*, *mir-132*, *mir-145*, and *mir-9** miRNA expression levels in the hypothalamus, an effect that was completely reversed by treatment with leptin (Figure 7). In turn, leptin treatment increased *mir-218* and *mir-9** miRNA expression levels in rats fed ad libitum, whereas the effect of leptin was the opposite in rats subjected to fasting. Likewise, *mir-30e* expression levels remained unchanged after treatment with leptin in fed rats but decreased after leptin administration to fasted rats (Figure 7). There were no changes of any type for *mir-200a*, *mir-29a*, and *mir-31* (Figure 7).

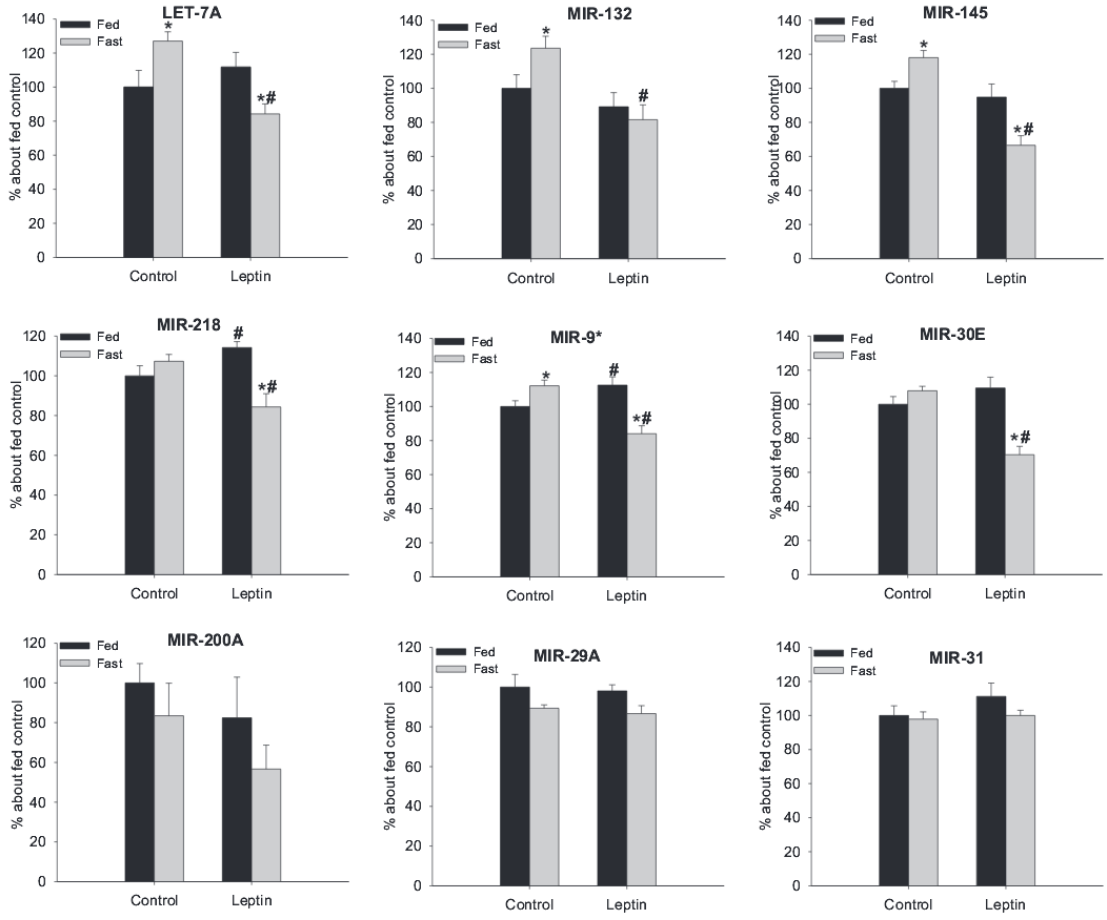

Figure 7. Effect of 2-day leptin treatment on let-7a, mir-9*, mir-30e, mir-132, mir-145, mir-200a, and mir-218 miRNA expression levels in hypothalamus of adult male rats in fed and fasted conditions. Values are expressed in arbitrary units as means ± SEM (n = 7–8) and as percentage of fed controls (fed animals treated with saline = 100%). *, $P \leq .05$ vs fed; #, $P \leq .05$ vs control (rats injected with saline) (two-way ANOVA with a post hoc Tukey test). - See more at: http://press.endocrine.org/doi/full/10.1210/en.2013-1770#sthash.UuCnX4Gk.dpuf

Discussion

Obesity is a major health problem and is reaching pandemic proportions in both developed and developing countries. The hypothalamus plays a key role in energy homeostasis by controlling food intake and sensing multiple peripheral metabolic signals, such as leptin, insulin, adiponectin, gut hormones, and nutrients that interact with specific hypothalamic regions to modulate central and peripheral metabolic pathways (8–10). However, the ultimate molecular mechanisms whereby such hypothalamic integration is conducted remain largely unknown. In this scenario, the possibility that miRNA regulatory pathways participate in the central control of energy homeostasis is appealing but has yet to be characterized. In fact, although different miRNAs have been related to the control of metabolism in vertebrates (43–46), their involvement in such function at hypothalamic levels remains unexplored. By the use of microarray analysis followed by quantitative PCR validation, in this study we intended to characterize changes in the hypothalamic miRNA signature in different nutritional and metabolic conditions, including HFD-induced obesity, chronic CR, and leptin treatments.

Our analyses documented the expression of 327 miRNAs in the hypothalamus, with up to 74 being deregulated by chronic CR and/or the HFD. Of note, recent studies in rats identified strong expression of miRNAs of the *let-7*, *mir-7*, *mir-9*, and *mir-30* families in discrete hypothalamic nuclei, such as the arcuate and paraventricular nuclei (47, 48). These miRNAs were also highly expressed in whole hypothalamic fragments in our study; however, the greatest expression levels in present analyses were detected for *mir-29a* and *mir-9*, followed by *mir-30b*, *mir-126-3p*, *mir-9**, *mir-26a*, *mir-191*, *mir-138*, *mir-149*, *mir-125b-5p*, *mir-24*, *mir-434-3p*, *mir-539*, and *mir-132*.

The miRNAs that were deregulated by long-term nutritional challenges (HFD or/and CR) belong to 63 different families, 29 of which are broadly conserved across mammalian species. For operational reasons, of the total set of altered targets, we focused further analyses on a subset of 7 miRNAs; these included (1) *mir-30e*, whose family showed 6 of its 10 members altered in our array studies; (2) *let-7a*, which belongs to a family with several members deregulated and previously related with the control of glucose metabolism and insulin sensitivity (24, 31); (3) *mir-9** and *mir-132*, which are brain-specific miRNAs with high hypothalamic expression; (4) *mir-145*, which related with insulin and glucose metabolism in peripheral tissues in obesity (30); (5) *mir-218*, which has been linked to the inhibition of AKT phosphorylation (49, 50); and (6) *mir-200a*, which, together with *mir-200b* and *mir-200c*, was the miRNA that displayed a greater fold change in our arrays and whose alteration has been linked to predisposition to develop overweight in rats after early leptin blockade (51).

In addition to the reasons listed above, different bioinformatic models pointed out that these miRNAs target and putatively regulate elements of pathways related with neuronal development, neuronal CREB signaling, and, more importantly, insulin, adiponectin, leptin, and inflammation signaling pathways. For instance, all miRNAs studied individually in this work, except *mir-145*, are seemingly involved in some (or several) steps of neurogenesis (52–59). Similarly, participation of these miRNAs in inflammatory signaling provides a tenable link with obesity, because this is now considered an inflammatory disease that results from the consolidation of a state of low-grade inflammation in the hypothalamus. Such an inflammatory state would contribute to the resistance to the effects of leptin and insulin (60, 61) and to alteration of neuronal circuits in the hypothalamus (for a review, see Ref. 6).

miRNA expression analyses together with metabolic profiling in our models of metabolic stress allowed us to identify 2 stages during the progression of diet-induced obesity: (1) an early phase during the first few weeks of the HFD; and (2) a chronic period, when obesity and associated metabolic alterations were clearly established. During the initial stage, several miRNAs showed a decline in their expression levels (*mir-30e*, *mir-145*, *mir-200a*, and *mir-218*), which were normalized over time (*mir-200a*) or even displayed an increase at later periods (*mir-218*, *mir-145*, and *mir-30e*). These late responses may be reactive or secondary to the primary metabolic insult, as an attempt to restore metabolic homeostasis against the metabolic deregulation that occurs when obesity gets consolidated and hypothalamic insulin, leptin, or inflammation signaling is primarily disturbed (62–65). Different manipulations of feeding have been shown to have a variable impact on hypothalamic metabolism, although the nature of such changes depends on the type and timing of the nutritional challenger (39, 40). Therefore, we cannot rule out the possibility that some of the differences observed during the development of DIO were due to the age at which the animals were exposure to the HFD rather than to the duration of this diet. Nonetheless, because we have previously shown that some of our miRNA targets vary their hypothalamic expression levels along postnatal development (32), HFD exposures in the different DIO groups were adjusted in such a way that that all animals were sampled in adulthood.

Although our high-throughput data did not permit us to demonstrate a direct causal link between the observed changes in hypothalamic expression of the selected miRNAs and the malfunction of central pathways, potentially altered in extreme metabolic conditions, bioinformatics predictions allowed us to set educated hypothesis for tenable hubs where this pathogenic connection may take place. For instance, compelling experimental evidence has demonstrated that hypothalamic insulin and leptin resistance is associated with decreased AKT activity (66, 67) and high levels of suppressor of cytokine signaling-3 (SOCS3) (68, 69). In this context, algorithm predictions suggest that *miR-218* and *mir-30 miRNA* families are potential regulators of SOCS3, whereas *mir-143/145*, *mir-132*, *mir-30*, and *mir-218* families are possible regulators of oxysterol binding protein-like 8, a protein that promotes insulin-stimulated AKT activation and is expressed at high levels in the brain (30). Furthermore, *mir-218* and *let-7* miRNAs have been proposed to target the mammalian target of rapamycin component, RICTOR, thereby inhibiting AKT phosphorylation (49, 50), whereas *let-7* miRNAs regulate glucose metabolism in peripheral tissues by decreasing the protein expression levels of insulin receptor, insulin receptor substrate 2, TORC1, IGF-I, and IGF-I receptor (24, 31). Therefore, the observed increase in the expression levels of these miRNAs during development of obesity might induce a decline in the activation of AKT (and hence leptin/insulin resistance) by regulating RICTOR and/or oxysterol binding protein-like 8.

Similarly, it is interesting to note that our models of high hypothalamic expression of SOCS3, such as long-term HFD or leptin treatment (our unpublished observations), displayed increased *mir-218* and *mir-30e* miRNA expression levels in the hypothalamus. Assuming a repressor role of these miRNAs on SOCS3 expression, it is tempting to speculate that these responses may be set to decrease SOCS3 mRNA levels as a means to relieve its negative effects on leptin/insulin signaling in the long term. On the other hand, *mir-218* has been shown to inactivate the proinflammatory nuclear factor κβ pathway in glioma cells (70, 71), whereas brain inflammation up-regulated *mir-132*, which in turn promoted anti-inflammatory responses (72). On this basis, it is tenable that the increases in *mir-218* and *mir-132*

expression after long-term HFD exposure might be a mechanism to alleviate excessive inflammation that is likely to occur in conditions of persistent obesity.

Admittedly, the magnitude of some of the changes in hypothalamic miRNA expression reported here, although statistically significant, are of moderate magnitude, a feature that may call for caution when the functional relevance of such modifications is interpreted. We believe, however, that part of such modest changes are due to our analytic approach, in which large-scale microarray analyses were applied to whole hypothalamic fragments. Thus, notable changes in discrete hypothalamic nuclei/areas might be partially obscured or compensated. Although this is a limitation of our approach, it is fair to assume that the observed changes are genuine and point out specific deregulated pathways, whose precise location and functional relevance would require targeted characterization in subsequent studies. In any event, we do believe that our array data followed by targeted expression analyses in different preclinical models of metabolic distress has translational value and permit initial identification of appealing candidates for such follow-up studies.

In conclusion, by the use of a combination of preclinical models of severe metabolic distress, our present study is the first to document changes in hypothalamic miRNA expression profiles in conditions of overweight, chronic CR, and leptin treatment. Notably, the deregulated miRNAs displayed different time-course alterations in their expression patterns after potent obesogenic insults (such as an HFD) and were identified by different computational algorithms as putative regulators of genes encoding key factors in insulin and leptin signaling, as well as in neurogenesis and inflammation. Although further functional analyses of such pathophysiological connections is still pending, our study brings new insights into the potential roles of several miRNAs in the mechanisms whereby the hypothalamus primarily controls and adapts to dynamic fluctuations in energy homeostasis and highlights the potential role of miRNA regulatory pathways as a possible link (and eventual therapeutic target) for obesity, inflammation, and neurodegenerative diseases.

Abbreviations:

AKT, serine-threonine protein kinase; BW, body weight; CR, caloric restriction; CREB, cAMP-response element binding protein; DIO, diet-induced obesity; HFD, high-fat diet; ND, normal diet; SOCS3, suppressor of cytokine signaling-3

Acknowledgments

This work was supported by Instituto de Salud Carlos III (cofunded by the Fondo Europeo de Desarrollo Regional [FEDER] Program from the European Union) Research Grants PI10/00088 and PI13/00322FIS and by Ministerio de Economia y Competitividad, Spain (cofunded by FEDER) Projects IN845B-2010/187 and 10CSA916014PR (Xunta de Galicia, Spain) (to F.C.), EM2013/011 (Xunta de Galicia, Spain) (to S.S.A.), and BFU 2011-25021 (to M.T.S.).

Disclosure Summary: The authors have nothing to disclose.

References

1. World Health Organization. Diabetes fact sheet no. 312. Geneva, Switzerland: World Health Organization; 2013. http://www.who.int/mediacentre/factsheets/fs312/en/. Accessed February 21, 2014.
2. Travers ME, McCarthy MI. Type 2 diabetes and obesity: genomics and the clinic. Hum Genet. 2011;130:41–58.
3. Remmers F, Delemarre-van de Waal HA. Developmental programming of energy balance and its hypothalamic regulation. Endocr Rev. 2011;32:272–311.
4. Bouret SG, Simerly RB. Developmental programming of hypothalamic feeding circuits. Clin Genet. 2006;70:295–301.
5. Williams LM. Hypothalamic dysfunction in obesity. Proc Nutr Soc. 2012;71:521–533.
6. Cai D. Neuroinflammation and neurodegeneration in overnutrition-induced diseases. Trends Endocrinol Metab. 2013;24:40–47.
7. Thaler JP, Yi CX, Schur EA, et al. Obesity is associated with hypothalamic injury in rodents and humans. J Clin Invest. 2012;122:153–162.
8. Ahima RS, Lazar MA. Adipokines and the peripheral and neural control of energy balance. Mol Endocrinol. 2008;22:1023–1031.
9. Ahima RS, Qi Y, Singhal NS, Jackson MB, Scherer PE. Brain adipocytokine action and metabolic regulation. Diabetes. 2006;55(suppl 2):S145–S154.

10. Chen HC, Roth JD, Schroeder BE, Weyer C. Role of islet-, gut-, and adipocyte-derived hormones in the central control of food intake and body weight: implications for an integrated neurohormonal approach to obesity pharmacotherapy. Curr Diabetes Rev. 2008;4:79–91.
11. Belgardt BF, Brüning JC. CNS leptin and insulin action in the control of energy homeostasis. Ann NY Acad Sci. 2010;1212:97–113.
12. Carvalheira JB, Torsoni MA, Ueno M, et al. Cross-talk between the insulin and leptin signaling systems in rat hypothalamus. Obes Res. 2005;13:48–57
13. Bjørbaek C. Central leptin receptor action and resistance in obesity. J Investig Med. 2009;57:789–794.
14. van den Hoek AM, Teusink B, Voshol PJ, Havekes LM, Romijn JA, Pijl H. Leptin deficiency per se dictates body composition and insulin action in *ob/ob* mice. J Neuroendocrinol. 2008;20:120–127.
15. Montague CT, Farooqi IS, Whitehead JP, et al. Congenital leptin deficiency is associated with severe early-onset obesity in humans. Nature. 1997;387:903–908.
16. Hedbacker K, Birsoy K, Wysocki RW, et al. Antidiabetic effects of IGFBP2, a leptin-regulated gene. Cell Metab. 2010;11:11–22.
17. Dridi S, Taouis M. Adiponectin and energy homeostasis: consensus and controversy. J Nutr Biochem. 2009;20:831–839.
18. Baek D, Villén J, Shin C, Camargo FD, Gygi SP, Bartel DP. The impact of microRNAs on protein output. Nature. 2008;455:64–71.
19. Grimson A, Farh KK, Johnston WK, Garrett-Engele P, Lim LP, Bartel DP. MicroRNA targeting specificity in mammals: determinants beyond seed pairing. Mol Cell. 2007;27:91–105.
20. Zhao S, Liu MF. Mechanisms of microRNA-mediated gene regulation. Sci China C Life Sci. 2009;52:1111–1116.
21. Kloosterman WP, Plasterk RH. The diverse functions of microRNAs in animal development and disease. Dev Cell. 2006;11:441–450.
22. Krichevsky AM, King KS, Donahue CP, Khrapko K, Kosik KS. A microRNA array reveals extensive regulation of microRNAs during brain development. RNA. 2003;9:1274–1281.
23. Alexander R, Lodish H, Sun L. MicroRNAs in adipogenesis and as therapeutic targets for obesity. Expert Opin Ther Targets. 2011;15:623–636.
24. Frost RJ, Olson EN. Control of glucose homeostasis and insulin sensitivity by the Let-7 family of microRNAs. Proc Natl Acad Sci USA. 2011;108:21075–21080.
25. Heneghan HM, Miller N, Kerin MJ. Role of microRNAs in obesity and the metabolic syndrome. Obes Rev. 2010;11:354–361.
26. Bolmeson C, Esguerra JL, Salehi A, Speidel D, Eliasson L, Cilio CM. Differences in islet-enriched miRNAs in healthy and glucose intolerant human subjects. Biochem Biophys Res Commun. 2011;404:16–22.
27. Poy MN, Hausser J, Trajkovski M, et al. miR-375 maintains normal pancreatic α- and β-cell mass. Proc Natl Acad Sci USA. 2009;106:5813–5818.
28. Trajkovski M, Hausser J, Soutschek J, et al. MicroRNAs 103 and 107 regulate insulin sensitivity. Nature. 2011;474:649–653.
29. Takanabe R, Ono K, Abe Y, et al. Up-regulated expression of microRNA-143 in association with obesity in adipose tissue of mice fed high-fat diet. Biochem Biophys Res Commun. 2008;376:728–732.
30. Jordan SD, Krüger M, Willmes DM, et al. Obesity-induced overexpression of miRNA-143 inhibits insulin-stimulated AKT activation and impairs glucose metabolism. Nat Cell Biol. 2011;13:434–446.
31. Zhu H, Shyh-Chang N, Segrè AV, et al. The Lin28/let-7 axis regulates glucose metabolism. Cell. 2011;147:81–94.
32. Sangiao-Alvarellos S, Manfredi-Lozano M, Ruiz-Pino F, et al. Changes in hypothalamic expression of the Lin28/let-7 system and related microRNAs during postnatal maturation and after experimental manipulations of puberty. Endocrinology. 2013;154:942–955.
33. Krol J, Loedige I, Filipowicz W. The widespread regulation of microRNA biogenesis, function and decay. Nat Rev Genet. 2010;11:597–610.
34. Lewis BP, Shih IH, Jones-Rhoades MW, Bartel DP, Burge CB. Prediction of mammalian microRNA targets. Cell. 2003;115:787–798.
35. John B, Enright AJ, Aravin A, Tuschl T, Sander C, Marks DS. Human MicroRNA targets. PLoS Biol. 2004;2:e363.
36. Krek A, Grün D, Poy MN, et al. Combinatorial microRNA target predictions. Nat Genet. 2005;37:495–500.
37. Maragkakis M, Reczko M, Simossis VA, et al. DIANA-microT web server: elucidating microRNA functions through target prediction. Nucleic Acids Res. 2009;37:W273–W276.
38. Dweep H, Sticht C, Pandey P, Gretz N. miRWalk—database: prediction of possible miRNA binding sites by "walking" the genes of three genomes. J Biomed Inform. 2011;44:839–847.
39. Chang GQ, Gaysinskaya V, Karatayev O, Leibowitz SF. Maternal high-fat diet and fetal programming: increased proliferation of hypothalamic peptide-producing neurons that increase risk for overeating and obesity. J Neurosci. 2008;28:12107–12119.
40. Page KC, Malik RE, Ripple JA, Anday EK. Maternal and postweaning diet interaction alters hypothalamic gene expression and modulates response to a high-fat diet in male offspring. Am J Physiol Regul Integr Comp Physiol. 2009;297:R1049–R1057.
41. Yamashima T. 'PUFA-GPR40-CREB signaling' hypothesis for the adult primate neurogenesis. Prog Lipid Res. 2012;51:221–231

42. Barco A, Marie H. Genetic approaches to investigate the role of CREB in neuronal plasticity and memory. Mol Neurobiol. 2011;44:330–349.
43. Poy MN, Spranger M, Stoffel M. microRNAs and the regulation of glucose and lipid metabolism. Diabetes Obes Metab. 2007;9(suppl 2):67–73.
44. Guay C, Roggli E, Nesca V, Jacovetti C, Regazzi R. Diabetes mellitus, a microRNA-related disease? Transl Res. 2011;157:253–264.
45. McGregor RA, Choi MS. microRNAs in the regulation of adipogenesis and obesity. Curr Mol Med. 2011;11:304–316.
46. Sacco J, Adeli K. MicroRNAs: emerging roles in lipid and lipoprotein metabolism. Curr Opin Lipidol. 2012;23:220–225.
47. Amar L, Benoit C, Beaumont G, et al. MicroRNA expression profiling of hypothalamic arcuate and paraventricular nuclei from single rats using Illumina sequencing technology. J Neurosci Methods. 2012;209:134–143.
48. Herzer S, Silahtaroglu A, Meister B. Locked nucleic acid-based in situ hybridisation reveals miR-7a as a hypothalamus-enriched microRNA with a distinct expression pattern. J Neuroendocrinol. 2012;24:1492–1504.
49. Uesugi A, Kozaki K, Tsuruta T, et al. The tumor suppressive microRNA miR-218 targets the mTOR component Rictor and inhibits AKT phosphorylation in oral cancer. Cancer Res. 2011;71:5765–5778.
50. Venkataraman S, Birks DK, Balakrishnan I, et al. MicroRNA 218 acts as a tumor suppressor by targeting multiple cancer phenotype-associated genes in medulloblastoma. J Biol Chem. 2013;288:1918–1928.
51. Benoit C, Ould-Hamouda H, Crepin D, et al. Early leptin blockade predisposes fat-fed rats to overweight and modifies hypothalamic microRNAs. J Endocrinol. 2013;218:35–47.
52. Coolen M, Thieffry D, Drivenes Ø, Becker TS, Bally-Cuif L. miR-9 controls the timing of neurogenesis through the direct inhibition of antagonistic factors. Dev Cell. 2012;22:1052–1064.
53. Kawahara H, Imai T, Okano H. MicroRNAs in neural stem cells and neurogenesis. Front Neurosci. 2012;6:30.
54. Pathania M, Torres-Reveron J, Yan L, et al. A miR-132 enhances dendritic morphogenesis, spine density, synaptic integration, and survival of newborn olfactory bulb neurons. PLoS One. 2012;7:e38174.
55. Yoo AS, Sun AX, Li L, et al. MicroRNA-mediated conversion of human fibroblasts to neurons. Nature. 2011;476:228–231.
56. Nigro A, Menon R, Bergamaschi A, et al. MiR-30e and miR-181d control radial glia cell proliferation via HtrA1 modulation. Cell Death Dis. 2012;3:e360.
57. Liu C, Zhao X. MicroRNAs in adult and embryonic neurogenesis. Neuromolecular Med. 2009;11:141–152.
58. Lang MF, Shi Y. Dynamic roles of microRNAs in neurogenesis. Front Neurosci. 2012;6:71.
59. Choi PS, Zakhary L, Choi WY, et al. Members of the miRNA-200 family regulate olfactory neurogenesis. Neuron. 2008;57:41–55.
60. Milanski M, Arruda AP, Coope A, et al. Inhibition of hypothalamic inflammation reverses diet-induced insulin resistance in the liver. Diabetes. 2012;61:1455–1462.
61. Zhang X, Zhang G, Zhang H, Karin M, Bai H, Cai D. Hypothalamic IKKβ/NF-κB and ER stress link overnutrition to energy imbalance and obesity. Cell. 2008;135:61–73.
62. Koch L, Wunderlich FT, Seibler J, et al. Central insulin action regulates peripheral glucose and fat metabolism in mice. J Clin Invest. 2008;118:2132–2147.
63. Buettner C, Muse ED, Cheng A, et al. Leptin controls adipose tissue lipogenesis via central, STAT3-independent mechanisms. Nat Med. 2008;14:667–675.
64. Obici S, Feng Z, Karkanias G, Baskin DG, Rossetti L. Decreasing hypothalamic insulin receptors causes hyperphagia and insulin resistance in rats. Nat Neurosci. 2002;5:566–572.
65. Ring LE, Zeltser LM. Disruption of hypothalamic leptin signaling in mice leads to early-onset obesity, but physiological adaptations in mature animals stabilize adiposity levels. J Clin Invest. 2010;120:2931–2941.
66. Clegg DJ, Gotoh K, Kemp C, et al. Consumption of a high-fat diet induces central insulin resistance independent of adiposity. Physiol Behav. 2011;103:10–16.
67. Caricilli AM, Penteado E, de Abreu LL, et al. Topiramate treatment improves hypothalamic insulin and leptin signaling and action and reduces obesity in mice. Endocrinology. 2012;153:4401–4411.
68. Bjørbaek C, El-Haschimi K, Frantz JD, Flier JS. The role of SOCS-3 in leptin signaling and leptin resistance. J Biol Chem. 1999;274:30059–30065.
69. de Backer MW, Brans MA, van Rozen AJ, et al. Suppressor of cytokine signaling 3 knockdown in the mediobasal hypothalamus: counterintuitive effects on energy balance. J Mol Endocrinol. 2010;45:341–353.
70. Song L, Huang Q, Chen K, et al. miR-218 inhibits the invasive ability of glioma cells by direct downregulation of IKK-β. Biochem Biophys Res Commun. 2010;402:135–140.
71. Xia H, Yan Y, Hu M, et al. MiR-218 sensitizes glioma cells to apoptosis and inhibits tumorigenicity by regulating ECOP-mediated suppression of NF-κB activity. Neuro Oncol. 2013;15:413–422.
72. Shaked I, Meerson A, Wolf Y, et al. MicroRNA-132 potentiates cholinergic anti-inflammatory signaling by targeting acetylcholinesterase. Immunity. 2009;31:965–973.