JSA 2015;1:14-19

Giovanni Ceccarini, Alessio Basolo, Margherita Maffei, Paolo Vitti, Ferruccio Santini

Obesity Center - Endocrinology Unit, Pisa University Hospital

Energy homeostasis and locomotor activity: the role of leptin and of the melanocortin system

Abstract

For the purposes of proper control of body weight, nutritional intake and physical activity are integrated through a common regulatory system in which the leptin-melanocortin system plays a major role. This system is responsible for controlling the caloric balance through a feedback mechanism, that signals to the central nervous system the amount of stocks contained in the adipose tissue, so as to optimize dietary intake and energy expenditure. In rodents, the decrease in leptin levels that occurs following food deprivation or fat mass reduction is associated to an appetite stimulus, to a decrease in overall locomotor activity and to a simultaneous increase in food anticipatory activity, a specific motor activity that reflects the inclination to search for nutritional resources. This activity can be modulated through the administration of leptin and is mediated, at least partially, by the neurons of the melanocortin system. In humans, the studies on twins have attributed to genetic factors at least 50% of the variability in the propensity for physical activity. In particular, some polymorphisms of the melanocortin and leptin 4 receptor are associated with variations in the levels of motor activity. Clarifying the complex mechanisms involved in the regulation of physical activity and the connections between the different pathways involved in energy homeostasis could help to understand the nature of the differences existing between individuals in terms of inclination for physical activity and energy expenditure associated with it. Given the historic difficulties in the development of anti-obesity drugs that are both safe and effective, the exploitation of the multiple beneficial effects produced by physical activity is an essential component in the context of a multidimensional behavioural strategy aimed at tackling obesity and its related diseases.

Key words: locomotor activity - leptin - physical activity - obesity - melanocortin system - anorexia nervosa

Riassunto

Ai fini di un adeguato controllo del peso corporeo, l'apporto nutrizionale e l'attività fisica sono integrate attraverso un sistema regolatorio comune all'interno del quale il sistema leptina-melanocortina riveste un ruolo di grande rilievo. Questo sistema è deputato a controllare l'equilibrio calorico attraverso un meccanismo a feed-back che segnala al sistema nervoso centrale l'entità delle scorte contenute nel tessuto adiposo al fine di ottimizzare l'introito alimentare e la spesa energetica. Nei roditori, la riduzione dei livelli di leptina che si verifica in seguito alla deprivazione di cibo o alla riduzione della massa grassa è associata a uno stimolo sull'appetito, a una diminuzione dell'attività locomotoria complessiva e a un simultaneo aumento dell'attività anticipatoria del pasto, una specifica attività motoria che riflette l'attitudine alla ricerca di risorse nutrizionali. Questa attività può essere modulata dalla somministrazione di leptina ed è almeno in parte mediata dai neuroni del sistema melanocortinico. Nell'uomo, gli studi sui gemelli hanno attribuito a fattori genetici almeno il 50% della variabilità nella propensione all'attività fisica. In particolare, alcuni polimorfismi del recettore 4 della melanocortina e della leptina sono associati a variazioni dei livelli di attività motoria. Chiarire la complessità dei meccanismi che intervengono nella regolazione dell'attività fisica e le connessioni tra le diverse vie coinvolte nell'omeostasi dell'energia potrebbe consentire di comprendere la natura delle differenze che esistono tra individuo e individuo in termini di propensione all'attività fisica e di dispendio energetico a essa associato. In considerazione delle storiche difficoltà incontrate nello sviluppo di farmaci contro l'obesità che siano nel contempo sicuri ed efficaci, lo sfruttamento dei molteplici effetti favorevoli prodotti dall'attività fisica rappresenta una componente imprescindibile nel contesto di una strategia comportamentale multidimensionale finalizzata al contrasto dell'obesità e delle malattie ad essa associate.

Parole chiave: attività locomotoria - leptina - attività fisica - obesità - sistema melanocortinico - anoressia nervosa

Introduction

Under natural conditions, energy homeostasis and body weight are regulated through the harmonization of caloric intake and consumption. The latter is mainly determined by resting energy expenditure, motor activity not linked to physical exercise and voluntary physical activity (1).
Appetite and metabolism are variables regulated by very efficient neurobiological mechanisms. The mutations of individual genes at the basis of this homeostatic system are responsible for about 5% of the causes of early obesity (2). These mutations affect proteins which are mainly expressed in the central nervous system.
Studies conducted on adopted twins and their families, both biological and adoptive, have established that in 50-70% of cases the body mass index is genetically determined (3) (4)**.** But it is still not clear to what extent genetics influences the caloric intake and the energy expended through physical activity. In particular, the fact that motor behaviour and the inclination for physical activity are modulated by neurohormonal regulators is very often underestimated.
Locomotor activity is a complex behavior influenced by social, demographic and environmental factors (5). Experimental data obtained both in animals and in humans also show that locomotor activity can also vary on a genetic basis. The recognition of these regulatory mechanisms is becoming increasingly important in the face of a real obesity epidemic. When it comes to physical activity, two components are usually referred to: voluntary exercise and spontaneous physical activity. Voluntary exercise is defined as a locomotor activity not directly required for survival and not directly motivated by external events (6): in other words, voluntary exercise consists in sport and similar activities.
Spontaneous physical activity consists of all the remaining activities in everyday life (e.g. maintenance of posture, gesticulation) (6) (7). It is also true that many physical activities fall into a grey area between voluntary activities and spontaneous activities. In mammals, complex regulatory mechanisms have developed that allow for an optimal integration of physical activity with the maintenance energy homeostasis. A short-term caloric restriction and fasting decrease overall locomotor activity, while a modest caloric but prolonged restriction increases such activity, both in mice and non-human primates (8) (9). The variants of certain genes (peroxisome proliferator-activated receptor-γ, hypocretin, beta-2 adrenergic receptors, uncoupling protein 3, fat mass and obesity (FTO) gene) are associated with different levels of physical activity (10) (11). In addition, several hormonal gastrointestinal peptides, such as ghrelin, PYY, cholecystokinin, incretin and insulin appear to contribute significantly to the regulation of locomotor behavior. Certain components of the 'reward' system,such as dopamine receptors, endogenous opioids and endocannabinoids may significantly affect voluntary exercise. The main purpose of this article is to provide an overview on the influence of the melanocortin-leptin system on the different components of locomotor activity.

Rodent studies

Natural selection has led to continuous evolutionary adaptations to facilitate the acquisition of the necessary nutrients for reproduction and survival. Several studies have shown that it is possible to select mouse lines characterized by high or low levels of locomotor activity (12) (13). Rodent models have also allowed the analysis of the major factors involved in the regulation of locomotor activity, thanks to methods of objective evaluation of the activity and to the absence of confounding factors that are inevitably present in studies conducted on humans. Mouse models have provided important information through the study of knockout mice, knockdown mice or through the assessment of gene over-expression, thus allowing the successful study of the neurobiological mechanisms underlying locomotor behavior. The voluntary locomotor activity is usually measured by the number of rotations of the wheel in the cage ("running wheel activity", RWA) and expresses a rewarding activity and a self-motivated behaviour similar to voluntary physical activity in human beings. The "home cage activity" (HCA) describes spontaneous locomotor activity (7) and can be measured by infrared rays or through video-recording. In this respect, it is worth noting that the resistance to the exercise (influenced by the quality of the components of muscle fibers) and the total levels of physical activity did not probably develop synergistically and are not correlated (14).

The effect of leptin and of the melanocortin system on locomotor activity

Leptin is a hormone secreted by adipocytes in amounts proportional to their mass and regulates body weight homeostasis by inhibiting food intake and increasing energy expenditure (15) (16). Leptin-deficient (ob / ob) mice are obese and hypoactive (17); administration of leptin normalizes their body weight and physical activity levels (18). This finding would seem in line with the prevailing idea that hypoactivity is secondary to obesity. However, experimental results exist which indicate that low doses of leptin can increase both RWA and HCA during the first day of treatment, i.e. before the weight loss occurs, thus demonstrating the fact that the action of leptin on motor activity is not a minor effect with respect to weight loss (19)(20). It is worth noting that in a normal mouse, peripheral administration of over-physiological doses of leptin does not increase locomotor activity (18) (19). In line with this effect of leptin, lean mice that overexpress transgenic leptin do not show variations in motor activity; this is however reduced when the hormone secretion is interrupted (20). In mice exposed to a low-calorie diet in which serum concentrations of leptin are kept normal through the

continuous administration of the hormone, the sudden interruption of the infusion causes a 50% reduction in motor activity. Such decrease in the activity is not observed when leptin levels are restored through free access to food (21). Taken together, these data indicate that a reduction of the physiological concentrations of leptin, like the one occurring during fasting, can be one of the mechanisms that mediate the reduction of physical activity observed in subjects showing a marked weight loss. Restoring the physiological levels of leptin means encouraging an increase in locomotor activity, while over-physiological levels of leptin do not have additional effects. The lack of leptin, reflecting a negative energy balance, is therefore responsible for a reduction of total motor activity that seems to be aimed at energy saving and body weight maintenance. However, this effect of reduction in motor activity could be counterproductive in case it causes an inhibition in the search for food. It is worth noting, in this regard, that when the animal is accustomed to receiving a meal at fixed times, the levels of RWA increase immediately before this event. Such phenomenon is commonly referred to as a food anticipatory activity (22). The behaviour of rodents in which the availability of food is reduced and limited to certain hours of the day is characterized by weight loss, hypothermia and increased anticipatory activity (23). The way the administration of leptin influences food anticipatory activity has been the object of specific studies which have shown an inhibitory effect (24). In accordance with this action of leptin, ob / ob mice in which the hormone is completely absent show, despite a reduced total motor activity, a marked anticipatory activity that is abolished by the administration of the hormone (19). All the actions of leptin on physical activities can be reproduced when this is administered at low doses directly into cerebral ventricles, and this suggests that the effects of leptin on motor activity are mediated at the level of the central nervous system (19) (25). The melanocortin system includes various effectors: neuropeptide Y (NPY), agouti gene-related protein (AgRP), proopiomelanocortin (POMC) and α-melanocyte stimulating hormone (αMSH) with its specific receptors (MC3R and MC4R). Leptin receptors (LepR) are widely expressed in the central nervous system, particularly in the hypothalamus, where leptin regulates feeding and energy expenditure. In the arcuate nucleus, leptin stimulates the POMC neurons which perform an αMSH-mediated anorectic action. At the same time, leptin inhibits the neurons that express the powerful anorectic peptides NPY and AgRP. Alpha-MSH is an agonist of MC4R and MC3R, while AgRP is a high affinity antagonist for both receptors (26). In the mouse model obesity can be caused by a faulty POMC gene, by AgRP over-expression or by a reduced function of MC4R (27). The restoration of the leptin-receptor signal transduction in the arcuate nucleus of db / db mice, genetically lacking LePR and therefore obese as ob / ob mice, normalizes their locomotor activity before effects on weight appear (28). The same action is obtained when the signal from the leptin receptor is restored only in the arcuate nucleus of POMC neurons. This indicates that such neurons are major mediators of the effects of leptin on locomotor behaviour (29). The STAT-3 signal transducer is one of the major intracellular effectors of the action of leptin. Mice that show a constitutive activation of STAT-3 in AgRP neurons are lean and hyperactive (30). By contrast, mice with inactivation of the STAT-3 signal in LePR neurons show a reduced locomotor activity (31). The administration of NPY during a food restriction period increases the food anticipatory activity (32) but does not modify the total activity in normal mice. These data emphasize the behavioural effects of NPY, which are modulated by changes in energy, and identify the NPY as a possible mediator of leptin activity in food anticipatory activity. It is unclear which additional downstream centers regulate locomotor responses to leptin, but probably the signal converges on neural networks such as those of the mesolimbic-dopamine system, which is involved in the processes of reward and motivation (33) and those of the sympathetic nervous system (34). Male MC4R knockout mice have lower locomotor activity in the dark phase than normal mice (35). The administration of MC4R antagonists decreases locomotor activity in rats (36). It is interesting to notice that MC4R knockout mice have less total activity and increased fat mass compared to normal mice (37). The POMC neurons are estrogen targets (38) and provide synaptic inputs to neurons that express the hormone stimulating gonadotropins (GnRH). This could be an explanation of the effect that estrogens have on locomotor activity and of the dimorphism between sexes which is sometimes observed in mouse models. MC3R knockout mice show an attenuated food anticipatory activity associated with reduced expression of AgRP and NPY in the arcuate nucleus (39). These observations reinforce the impression that AgRP and NPY influence the anticipatory activity. In this regard, it is interesting to observe how NHLH-2 transcription factor knockout mice (nescient helix loop helix 2) present a late-onset obesity due to a reduction in spontaneous physical activity.

These animals further reduce their activity after caloric restriction, an effect which is not reversible even after restoration of normal access to food (40). If documented in humans, this phenomenon could be one of the mechanisms that contribute to regain weight after discontinuation of a diet (41). It is interesting to notice that the human homolog of NHLH-2 is implicated in the transcriptional control of MC4R. It is also assumed that the BDNF factor (brain derived neurotrophic factor), one of the major regulators of neuronal plasticity, is one of the effectors of the leptin / melanocortin system (42). Mice exposed to environmental enrichment through special cages that encourage physical activity and increase

sensory stimulation, cognitive and social activities, show increased sensitivity to leptin and an increased expression of hypothalamic BDNF, increased stimulation of POMC anorectic neurons and inhibition of NPY orexigenic neurons (43) (44). In other words, it seems that when the animal is exposed to a more natural lifestyle, its hypothalamus is affected by modifications which produce a lowering of the leptin set point and promote a restructuring of synaptic connections, arranged by BDNF, with a consequent strengthening of the systems inhibiting appetite and of those promoting motor activity. In a natural environment, therefore, the system would be geared to limit an excessive accumulation of body fat, which would hinder the animals in the exercise of their natural functions (competition for food, escape, territorial expansion). It is conceivable that environmental enrichment, translated into human physiology, may have an important role in promoting spontaneous motor activity and represents an additional means to tackle the obesity epidemic. In conclusion, the reduction in leptin levels that follows the deprivation of food or a reduction in fat mass is associated with a decrease in total motor activity and an increase in total food anticipatory activity. When food availability is limited, such actions would be aimed at minimizing energy waste, while stimulating the motor behaviour related to the search for food and to the acquisition of the necessary resources to survive (Fig. 1). These aspects are mediated, at least in part, by the melanocortin system.

Possible mechanisms of regulation of physical activity in humans

In humans, the assessment of voluntary exercise can be carried out through tools such as accelerometers, self-reports, questionnaires, direct observation, continuous assessment of cardiac activity, calorie counters. A study in which multisensory devices were used for the monitoring of motor activity (45) has shown that, compared to normal weight controls, moderately obese subjects were spending an average of two hours more per day in a sitting position and consequently two hours less in the upright position or walking. This motor behaviour did not change when obese volunteers lost weight or lean subjects gained weight. Based on these observations it is therefore conceivable that a reduced spontaneous motor activity precedes the onset of obesity and represents a predisposing factor. Other studies have shown that acute overeating causes a reduction in locomotor activity (46). This effect would be more pronounced in individuals predisposed to obesity (47). In humans, hyperactivity can be associated with caloric restriction in some extreme conditions, such as anorexia nervosa (48). It is conceivable that this phenomenon involves mechanisms implicated in food anticipation. In some situations, the stimulatory effect produced by the reduction in leptin levels on food anticipation may be dominant with respect to the inhibitory effect produced

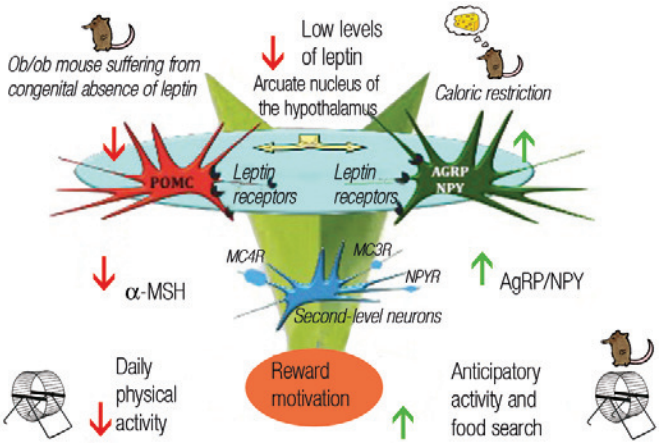

Figure 1. In a state of caloric restriction, the circulating levels of leptin are reduced in order to signal to the hypothalamus a condition of negative energy balance, resulting in the activation of the AgRP / NPY neurons and inhibition of POMC neurons. These effects leads to reduced locomotor activity and increased food anticipatory activity, in order to counter the effects of energy deprivation in famine situations. From [1], as amended.

on total motor activity. A study using the Swedish registry of twins showed that within couples, the levels of physical activity are much more similar when it comes to monozygotic twins compared with heterozygotic ones (49), showing an influence of genetic factors on motor activity. It is estimated that the variability of motor activity attributable to genetic factors is between 50% and 78% (49-51).

Numerous studies have assessed the genetic bases of physical activity (intended as intensity and duration) using different approaches: linkage studies, association studies and wide genome scan studies (52). In the Quebec Family Study (53), a polymorphism (C-2745T) located near the MC4R gene was associated to the intensity of physical activity. A limit of these studies consists in an impossibility to establish a causal link between the variables examined, and in the tools used to assess the nature and intensity of physical activity; many of them are in fact based on questionnaires completed independently. Two genetic linkage studies have also confirmed a correlation between physical activity and loci that are localized in MC4R 54 55. Another study identified a polymorphism at position 1704 in the 3 'region of MC4R, which interferes with a connection site binding a micro-RNA with marked effect on motor activity evaluated through the use of accelerometers (56). The Pima Indians, homozygous for the Arg223 polymorphism of the leptin receptor, show low levels of physical activity, calculated as ratio between total energy expenditure and baseline metabolism, using a metabolic chamber (57) (58). The levels of leptin explain 37% of

the variation of motor activity in patients suffering from anorexia nervosa (59). It is interesting to observe how physical exercise affects in turn the secretion of leptin, leading to an acute decrease in circulating levels (60), even in the absence of changes in body weight (61). In humans, the candidate genes to explain the marked inter-individual variability in the levels of physical activity are the ones that control the systems of reward and motivation, as the dopamine receptor D2 gene (62). In conclusion, to clarify the complexity of the mechanisms involved in the regulation of physical activity and the connections between the different pathways involved in energy homeostasis could help understand the nature of the differences existing between individuals in terms of inclination to physical activity and energy expenditure associated with it. Given the historic difficulties encountered in the development of anti-obesity drugs that are both safe and effective, the exploitation of the many favorable effects produced by physical activity is an essential component in the context of a multi-dimensional behavioral strategy aimed at tackling obesity and the diseases associated with it.

References

1 Ceccarini G, Maffei M, Vitti P, et al. *Fuel homeostasis and locomotor behavior: role of leptin and melanocortin pathways.* J Endocrinol Invest 2014 Dec 13 [Epub ahead of print].

2 Farooqi S, O'Rahilly S. *Genetics of obesity in humans.* Endocr Rev 2006;27:710-18.

3 Borjeson M. *The aetiology of obesity in children. A study of 101 twin pairs.* Acta Paediatr Scand 1976;65:279-87.

4 Stunkard AJ, Harris JR, Pedersen NL, et al. *The body-mass index of twins who have been reared apart.* N Engl J Med 1990;322:1483-7.

5 Bauman AE, Reis RS, Sallis JF, et al. *Correlates of physical activity: why are some people physically active and others not?* Lancet 2012;380:258-71.

6 Garland T Jr, Schutz H, Chappell MA, et al. *The biological control of voluntary exercise, spontaneous physical activity and daily energy expenditure in relation to obesity: human and rodent perspectives.* J Exp Biol 2011;214:206-29.

7 Levine JA, Eberhardt NL, Jensen MD. *Role of nonexercise activity thermogenesis in resistance to fat gain in humans.* Science 1999;283:212-4.

8 Goodrick CL, Ingram DK, Reynolds MA, et al. *Effects of intermittent feeding upon growth, activity, and lifespan in rats allowed voluntary exercise.* Exp Aging Res 1983;9:203-9.

9 Yamada Y, Colman RJ, Kemnitz WB, et al. *Long-term calorie restriction decreases metabolic cost of movement and prevents decrease of physical activity during aging in rhesus monkeys.* Exp Gerontol 2013;48:1226-35.

10 Kotz CM. *Integration of feeding and spontaneous physical activity: role for orexin.* Physiol Behav 2006;88:294-301.

11 Qi L, Cho YA. *Gene-environment interaction and obesity.* Nutr Rev 2008;66:684-94.

12 Swallow JG, Carter PA, Garland T Jr. *Artificial selection for increased wheel-running behavior in house mice.* Behav Genet 1998;28:227-37.

13 Lightfoot JT, Turner MJ, Daves M, et al. *Genetic influence on daily wheel running activity level.* Physiol Genomics 2004;19:270-6.

14 Lambert MI, et al. *Tests of running performance do not predict subsequent spontaneous running in rats.* Physiol Behav 1996;60:171-6.

15 Zhang Y, Proenca R., Maffei M, et al. *Positional cloning of the mouse obese gene and its human homologue.* Nature 1994;372:425-32.

16 Friedman JM, Halaas JL. *Leptin and the regulation of body weight in mammals.* Nature 1998;395:763-70.

17 Mayer J. *Decreased activity and energy balance in the hereditary obesity-diabetes syndrome of mice.* Science 1953;117:504-5.

18 Pelleymounter MA, Cullen MJ, Baker MB, et al. *Effects of the obese gene product on body weight regulation in ob/ob mice.* Science 1995;269:540-3.

19 Ribeiro AC, Ceccarini G, Dupré C, et al. *Contrasting effects of leptin on food anticipatory and total locomotor activity.* PLoS One 2011;6:e23364.

20 Morton GJ, Kaiyala K, Fisher JD, et al. *Identification of a physiological role for leptin in the regulation of ambulatory activity and wheel running in mice.* Am J Physiol Endocrinol Metab 2011;300:E392-401.

21 Montez JM, Soukas A, Asilmaz E, et al. *Acute leptin deficiency, leptin resistance, and the physiologic response to leptin withdrawal.* Proc Natl Acad Sci USA 2005;102:2537-42.

22 Mistlberger RE. *Circadian food-anticipatory activity: formal models and physiological mechanisms.* Neurosci Biobehav Rev 1994;18:171-95.

23 Hillebrand JJ, Kas MJ, van Elburg AA, et al. *Leptin's effect on hyperactivity: potential downstream effector mechanisms.* Physiol Behav 2008;94:689-95.

24 Exner C, Hebebrand J, Remschmidt H, et al. *Leptin suppresses semi-starvation induced hyperactivity in rats: implications for anorexia nervosa.* Mol Psychiatry 2000;5:476-81.

25 Hillebrand JJ, Koeners MP, de Rijke CE, et al. *Leptin treatment in activity-based anorexia.* Biol Psychiatry 2005;58:165-71.

26 Cone RD. *Anatomy and regulation of the central melanocortin system.* Nat Neurosci 2005;8:571-8.

27 Arch JR. *Lessons in obesity from transgenic animals.* J Endocrinol Invest 2002;25:867-75.

28 Coppari R, Ichinose M, Lee CE, et al. *The hypothalamic arcuate nucleus: a key site for mediating leptin's effects on glucose homeostasis and locomotor activity.* Cell Metab 2005;1:63-72.

29 Huo L, Gamber K, Greeley S, et al. *Leptin-dependent control of glucose balance and locomotor activity by POMC neurons.* Cell Metab, 2009;9:537-47.

30 Mesaros A, Koralv SB, Rother E, et al. *Activation of Stat3 signaling in AgRP neurons promotes locomotor activity.* Cell Metab 2008;7:236-48.

31 Bates SH, Dundon TA, Seifert M, et al. *LRb-STAT3 signaling is required for the neuroendocrine regulation of energy expenditure by leptin.* Diabetes 2004;53:3067-73.

32 Nergardh R, Ammar A, Brodin U, et al. *Neuropeptide Y facilitates activity-based-anorexia.* Psychoneuroendocrinology 2007;32:493-502.

33 Hommel JD, Trinko R, Sears RM, et al. *Leptin receptor signaling in midbrain dopamine neurons regulates feeding.* Neuron 2006;51:801-10.

34 Haynes WG, Morgan DA, Walsh SA, et al. *Receptor-mediated regional sympathetic nerve activation by leptin.* J Clin Invest 1997;100:270-8.

35 Ste Marie L, Miura GI, MArsh DJ, et al. *A metabolic defect promotes obesity in mice lacking melanocortin-4 receptors.* Proc Natl Acad Sci USA 2000;97:12339-44.

36 Adage T, Scheurink AJ, de Boer SF, et al. *Hypothalamic, metabolic, and behavioral responses to pharmacological inhibition of CNS melanocortin signaling in rats.* J Neurosci 2001;21:3639-45.

[37] Butler AA, Kesterson RA, Lhong K, et al. *A unique metabolic syndrome causes obesity in the melanocortin-3 receptor-deficient mouse.* Endocrinology 2000;141:3518-21.

[38] Morgan MA, Schulkin J, Pfaff WD. *Estrogens and non-reproductive behaviors related to activity and fear.* Neurosci Biobehav Rev 2004;28:55-63.

[39] Girardet C, Mavrikaki M, Southern MR, et al. *Assessing interactions between Ghsr and Mc3r reveals a role for AgRP in the expression of food anticipatory activity in male mice.* Endocrinology 2014;155:4843-55.

[40] Good DJ, Coyle CA, Fox DL. *Nhlh2: a basic helix-loop-helix transcription factor controlling physical activity.* Exerc Sport Sci Rev 2008;36:187-92.

[41] Crujeiras AB, Diaz-Lagares A, Abete I, et al. *Pre-treatment circulating leptin/ghrelin ratio as a non-invasive marker to identify patients likely to regain the lost weight after an energy restriction treatment.* J Endocrinol Invest 2014;37:119-26.

[42] Xu B, Goulding EH, Zang K, et al. *Brain-derived neurotrophic factor regulates energy balance downstream of melanocortin-4 receptor.* Nat Neurosci 2003;6:736-42.

[43] Mainardi M, Scabia G, Vottari T, et al. *A sensitive period for environmental regulation of eating behavior and leptin sensitivity.* Proc Natl Acad Sci USA 2010;107:16673-8.

[44] Mainardi M, Pizzorusso T, Maffei M. *Environment, leptin sensitivity, and hypothalamic plasticity.* Neural Plast 2013;2013:438072.

[45] Levine JA, Lanningham-Foster LM, McCrady SK, et al. *Interindividual variation in posture allocation: possible role in human obesity.* Science 2005;307:584-6.

[46] Levine JA, McCrady SK, Lanningham-Foster LM, et al. *The role of free-living daily walking in human weight gain and obesity.* Diabetes 2008;57:548-54.

[47] Schmidt SL, Harmon KA, Sharp TA, et al. *The effects of overfeeding on spontaneous physical activity in obesity prone and obesity resistant humans.* Obesity (Silver Spring) 2012;20:2186-93.

[48] Torsello A, Brambilla F, Tamiazzo L, et al. *Central dysregulations in the control of energy homeostasis and endocrine alterations in anorexia and bulimia nervosa.* J Endocrinol Invest 2007;30:962-76.

[49] Carlsson S, Andersson T, Lichtenstein P, et al. *Genetic effects on physical activity: results from the Swedish Twin Registry.* Med Sci Sports Exerc 2006;38:1396-401.

[50] Stubbe JH, Boomsma DI, Vink LM, et al. *Genetic influences on exercise participation in 37,051 twin pairs from seven countries.* PLoS One 2006;1:e22.

[51] Joosen AM, Gielen M, Vlietinck R, et al. *Genetic analysis of physical activity in twins.* Am J Clin Nutr 2005;82:1253-9.

[52] de Vilhena e Santos DM, Katzmarzyk PT, Seabra AF, et al. *Genetics of physical activity and physical inactivity in humans.* Behav Genet 2012;42:559-78.

[53] Loos RJ, Rankinen T, Tremblay A, et al. *Melanocortin-4 receptor gene and physical activity in the Quebec Family Study.* Int J Obes (Lond) 2005;29:420-8.

[54] Cai G, Cole SA, Butte N, et al. *A quantitative trait locus on chromosome 18q for physical activity and dietary intake in Hispanic children.* Obesity (Silver Spring) 2006;14:1596-604.

[55] Simonen RL, Rankinen T, Perusse L, et al. *Genome-wide linkage scan for physical activity levels in the Quebec Family study.* Med Sci Sports Exerc 2003;35:1355-9.

[56] Cole SA, Butte NF, Voruganti VS, et al. *Evidence that multiple genetic variants of MC4R play a functional role in the regulation of energy expenditure and appetite in Hispanic children.* Am J Clin Nutr 2010;91:191-9.

[57] Stefan N, Vozarova B, Del Parigi A, et al. *The Gln223Arg polymorphism of the leptin receptor in Pima Indians: influence on energy expenditure, physical activity and lipid metabolism.* Int J Obes Relat Metab Disord 2002;26:1629-32.

[58] De Moor MH, Liu YJ, Boomsma DI, et al. *Genome-wide association study of exercise behavior in Dutch and American adults.* Med Sci Sports Exerc 2009;41:1887-95.

[59] Holtkamp K, Herpertz-Dahlmann B, Mika C, et al. *Elevated physical activity and low leptin levels co-occur in patients with anorexia nervosa.* J Clin Endocrinol Metab 2003;88:5169-74.

[60] Zaccaria M, Ermolao A, Brugin E, et al. *Plasma leptin and energy expenditure during prolonged, moderate intensity, treadmill exercise.* J Endocrinol Invest 2013;36:396-401.

[61] Plinta R, Olszanecka-Glinianowicz M, et al. *The effect of three-month pre-season preparatory period and short-term exercise on plasma leptin, adiponectin, visfatin, and ghrelin levels in young female handball and basketball players.* J Endocrinol Invest 2012;35:595-601.

[62] Simonen RL, Rankinen T, Pérusse L, et al. *A dopamine D2 receptor gene polymorphism and physical activity in two family studies.* Physiol Behav 2003;78:751-7.

CORRESPONDENCE

Giovanni Ceccarini
ceccarg@mail.rockefeller.edu